# The roles of the *Saccharomyces cerevisiae* Paf1 complex in regulating transcriptional repression

by

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The conserved Paf1 complex is important for proper gene expression in both yeast and humans. The Paf1 complex has been shown to repress the transcription of numerous genes. However, the mechanism by which the Paf1 complex mediates transcriptional repression remains largely unstudied. Here I use *ARG1* as a model gene to investigate transcriptional repression by the Paf1 complex in *Saccharomyces cerevisiae*. Interestingly, I found that Paf1 complex-dependent histone modifications that are normally associated with active transcription are enriched on the *ARG1* coding region and contribute to repression. The Rtf1 subunit of the Paf1 complex appears to mediate *ARG1* repression primarily through histone H2B ubiquitylation and histone H3 K4 methylation. However, Paf1 has repressive functions aside from these histone modifications. Interestingly, occupancy of the activator Gcn4 is increased at the *ARG1* promoter in *paf1Δ* cells, resulting in *ARG1* derepression that is dependent on the histone acetyltransferase Gcn5 and histone H3 acetylation sites. Together my results suggest that Paf1 mediates *ARG1* repression by preventing Gcn4 recruitment to the *ARG1* promoter and subsequent histone H3 acetylation. I found that Paf1 does not alter nucleosome occupancy at the *ARG1* promoter. However, I detect antisense transcription in the *ARG1* promoter that positively correlates with *ARG1* sense transcription. Interestingly, Paf1 prevents antisense transcription from traversing the *ARG1* promoter, representing a potential mechanism by which the Paf1 complex controls promoter accessibility and ultimately *ARG1* expression. Given these results, I hypothesize that

the Paf1 complex mediates *ARG1* repression partially by facilitating histone modifications that are refractory to *ARG1* transcription and partially by inhibiting antisense transcription which controls promoter accessibility. Importantly, events that I observed at my model gene, *ARG1*, are demonstrated at other Paf1 complex-repressed genes.

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# PREFACE

I would like to thank my committee members for their insight and guidance. I would also like to thank everyone in the Department of Biological Sciences, especially members of the Martens and Brodsky lab, for their help with methods and reagents and for good conversation in the lunch room. I would especially like to thank my fellow members of the Arndt lab for their help and support. I would also like to thank my family for their support and their belief that I could accomplish anything as long as I did my best. And most importantly, I would like to thank my advisor and mentor, Karen Arndt, for her careful and purposeful guidance, and unwavering support.

# 1.0 INTRODUCTION

In eukaryotes, RNA Polymerase II (Pol II) transcription can be divided into several stages, including initiation, elongation, and termination. During initiation, the transcription initiation machinery, which includes the multi-subunit polymerase and several general transcription factors, assembles at the promoter. During elongation, multiple elongation factors associate with the polymerase to facilitate transcription of a chromatin template. Finally, several transcription termination factors regulate termination, which leads to the release and recycling of the transcription machinery. Each stage of the transcription cycle is regulated by a plethora of proteins to ensure proper gene expression. Because my thesis research is focused on the repressive functions of the Paf1 transcription elongation complex, this chapter primarily focuses on the regulatory events during the elongation stage of transcription, with an emphasis on transcriptional repression mechanisms.

## 1.1 EFFICIENT TRANSCRIPTION ELONGATION IS AN IMPORTANT DETERMINANT OF GENE EXPRESSION.

During transcription initiation, the binding of the TATA-binding protein (TBP) subunit of TFIID to the consensus sequence TATA(A/T)A(A/T)(A/G) triggers the assembly of the pre-initiation complex at the promoter, which includes RNA Pol II and general transcription factors, TFIIA,

TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (reviewed in SIKORSKI and BURATOWSKI 2009). The general transcription factors position the polymerase at the transcription start site. TFIIE and an ATP-dependent helicase within TFIIH unwind the DNA. *In vitro* transcription of a DNA template by RNA Pol II minimally requires the general transcription factors (reviewed in SIKORSKI and BURATOWSKI 2009). However, transcription of a chromatin template requires a multitude of accessory factors. The regulation of transcription initiation is an important aspect of controlling gene expression and has thus been studied for many years. More recently, the regulation of post-initiation stages has been shown to be equally important for ensuring proper gene expression. In particular, a multitude of factors coordinate to regulate transcription elongation.

In *Drosophila*, it has been shown that, in non-heat-shock conditions, the polymerase begins transcribing the *HSP70* gene, but stalls about 20-40 nucleotides downstream of the transcription start site (GIARDINA *et al.* 1992; RASMUSSEN and LIS 1993; RASMUSSEN and LIS 1995; ROUGVIE and LIS 1988). However, RNA Pol II rapidly resumes transcription in inducing conditions (GIARDINA *et al.* 1992; RASMUSSEN and LIS 1993; RASMUSSEN and LIS 1995; ROUGVIE and LIS 1988). Consequently, the promoter-proximal pausing of RNA Pol II facilities rapid induction of *HSP70* expression. At one time this was considered to be a unique phenomenon. However, more recent analyses in multiple organisms suggest that promoter-proximal pausing of RNA Pol II is an important regulatory mechanism at numerous genes. Genome-wide studies in human and *Drosophila* cells identified thousands of genes that exhibit an accumulation of RNA Pol II at their 5' coding regions (GUENTHER *et al.* 2007; MUSE *et al.* 2007). Closer examinations of a subset of genes revealed that, similar to what was observed at *HSP70*, RNA Pol II had stalled during transcription elongation at a promoter-proximal region

30-50 nucleotides downstream of the transcription start site (GILCHRIST *et al.* 2008; LEE *et al.* 2008b; MUSE *et al.* 2007). Wide-spread pausing of RNA polymerase has also been observed in *Saccharomyces cerevisiae*, suggesting that the regulatory mechanism of promoter-proximal pausing is conserved throughout eukaryotes (ALEXANDER *et al.* 2010; RADONJIC *et al.* 2005). The paused polymerase appears to poise a gene for rapid induction. Consistent with this idea, many genes exhibiting paused RNA Pol II are those that respond to environmental stimuli (MUSE *et al.* 2007) or are briefly induced during development (WANG *et al.* 2007; ZEITLINGER *et al.* 2007). For example, in yeast, paused polymerase is detected at the majority of genes poised for rapid activation upon exit from stationary phase (RADONJIC *et al.* 2005). However, paused polymerase has also been shown to inhibit the transcription of some genes (ZEITLINGER *et al.* 2007). Additionally, polymerase pausing has been proposed to facilitate co-transcriptional events, such as RNA splicing (ALEXANDER *et al.* 2010).

### 1.1.1 The release of paused and arrested RNA Pol II

A major player in the regulation of the pausing of RNA Pol II during early elongation is the Negative Elongation Factor (NELF) complex. NELF, which is absent in yeast, but conserved in higher eukaryotes, is composed of 4 subunits, NELF-A, B, C/D, and E (NARITA *et al.* 2003). The NELF complex inhibits elongation both *in vitro* (PRICE *et al.* 2007; RENNER *et al.* 2001) and *in vivo* (AIDA *et al.* 2006; MUSE *et al.* 2007; WU *et al.* 2005; WU *et al.* 2003). Its recruitment to RNA Pol II requires DRB Sensitivity-Inducing Factor (DSIF) and together NELF and DSIF promote polymerase pausing (RENNER *et al.* 2001). The mechanism by which NELF inhibits transcription elongation is still unclear. However, it has been proposed that NELF may bind to the clamp domain of RNA Pol II, inducing changes to the active site. This hypothesis is based

on the knowledge that the NELF-A subunit has similarity to a viral protein, HDAg, which binds RNA Pol II in this manner (YAMAGUCHI *et al.* 2001; YAMAGUCHI *et al.* 2007). Consistent with this hypothesis, NELF has been shown to associate with RNA Pol II (NARITA *et al.* 2003). An alternative hypothesis is that NELF binds the nascent mRNA as it emerges from RNA Pol II, to prevent further elongation. Consistent with this idea, the NELF-E subunit contains an RNA recognition motif and its RNA binding activity is required for NELF to inhibit elongation *in vitro* (RAO *et al.* 2008; YAMAGUCHI *et al.* 2002).

Release of paused RNA Pol II into productive elongation is triggered by the phosphorylation of NELF and DSIF by Positive Transcription Elongation Factor b (P-TEFb) (IVANOV *et al.* 2000; KIM and SHARP 2001; PETERLIN and PRICE 2006). This event causes the release of NELF from the elongation complex (IVANOV *et al.* 2000; KIM and SHARP 2001; PETERLIN and PRICE 2006). DSIF, however, remains associated with RNA Pol II after the detachment of NELF (ANDRULIS *et al.* 2000; KAPLAN *et al.* 2000) and influences later events in elongation (WADA *et al.* 1998).

P-TEFb also phosphorylates the C-terminal domain (CTD) of Rpb1, the largest subunit of RNA Pol II. The RNA Pol II CTD contains a heptapeptide repeat (YSPTSPS) that is phosphorylated on serines at positions 2, 5, and 7 in the repeat. Importantly, the phosphorylation state of the CTD changes throughout the transcription cycle and is important for recruiting the appropriate regulatory factors during each stage of transcription. During initiation, the RNA Pol II CTD is hypophosphorylated. Upon the transition from initiation to early elongation, the CTD becomes phosphorylated on serine 5 by CDK7 (Kin28 in yeast) of the general transcription factor TFIIH (KOMARNITSKY *et al.* 2000). Phosphorylated serine 5 is recognized by the mRNA capping enzyme, thus coordinating mRNA 5' end capping and early transcription elongation

(FABREGA *et al.* 2003). In yeast, phosphorylation of serine 5 can be reversed by Ssu72 (KRISHNAMURTHY *et al.* 2004) and tends to decline as elongation proceeds (Figure 1). Later in the elongation stage, serine 2 of the CTD becomes phosphorylated. Serine 2 is phosphorylated mainly by Ctk1 in yeast (KEOGH *et al.* 2003; PATTURAJAN *et al.* 1999) (Figure 1). Additionally, *in vitro* evidence suggests that Bur1 may also contribute to serine 2 phosphorylation (MURRAY *et al.* 2001). Yeast Ctk1 and Bur1 closely resemble P-TEFb in humans, which phosphorylates serine 2 (PRICE 2000). Serine 2 phosphorylation promotes the recruitment of cleavage and polyadenylation factors, connecting the later stage of elongation with RNA 3' end processing (AHN *et al.* 2004; NI *et al.* 2004). In yeast, serine 2 is dephosphorylated by Fcp1 (KOBOR *et al.* 1999). Serine 7 in the CTD is also phosphorylated by Kin28 of TFIIH (KIM *et al.* 2009b). Patterns of serine 7 phosphorylation overlap with serine 5 across genes; however, its functions are not well understood (KIM *et al.* 2009b).

Paused polymerases can resume transcription elongation with the help of factors such as P-TEFb. However, a paused polymerase may backtrack such that the 3' end of the nascent mRNA becomes misaligned with the RNA Pol II active site, resulting in polymerase arrest. TFIIS promotes the release of arrested polymerase by eliciting cleavage of the nascent transcript by the elongation complex (IZBAN and LUSE 1993; REINES *et al.* 1992). Specifically, the transcript is cleaved in a 3' to 5' direction such that the RNA can realign with the RNA Pol II active site (IZBAN and LUSE 1993; REINES *et al.* 1992). In this manner, TFIIS promotes read-through of arrest sites, such as unusual DNA sequences and DNA-bound proteins, among others (KERPPOLA and KANE 1990; MOTE *et al.* 1994; REINES and MOTE 1993; WIND and REINES 2000). Importantly, TFIIS also facilitates transcription through nucleosomes. This has been demonstrated by several *in vitro* studies (BONDARENKO *et al.* 2006; GUERMAH *et al.* 2006; HSIEH

*et al.* 2010; IZBAN and LUSE 1992; KIM *et al.* 2010; KIREEVA *et al.* 2005; UJVARI *et al.* 2008). Additionally, TFIIS has been shown to cooperate with TFIIF to facilitate nucleosome traversal *in vivo* (LUSE *et al.* 2011). The effects of chromatin on transcription are discussed in more detail below.

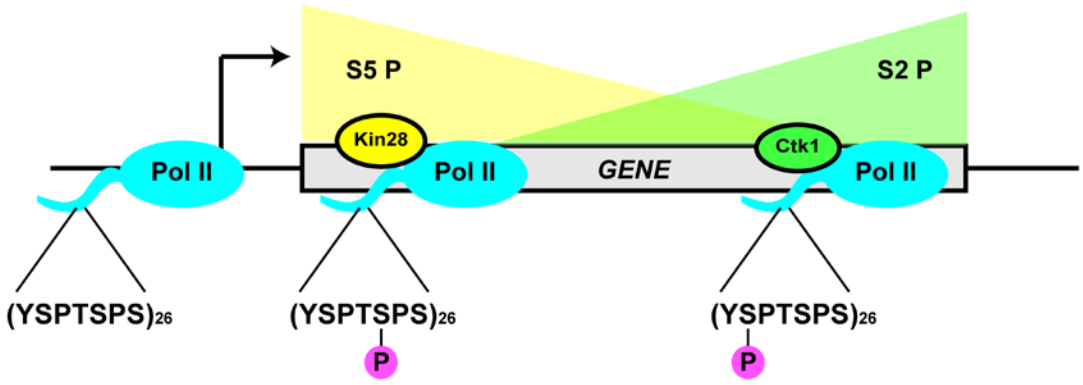


**Figure 1: The phosphorylation state of the CTD of RNA Pol II during transcription**

The C-terminal domain (CTD) of Rpb1, the largest subunit of RNA Pol II, is comprised of 26 repeats of the sequence YSPTSPS. During transcription initiation, the CTD is hypophosphorylated. Early in transcription elongation, serine 5 within the heptapeptide repeat is phosphorylated by Kin28 of TFIID (KOMARNITSKY *et al.* 2000). Serine 5 phosphorylation declines toward the 3' end of the gene due to the action of the phosphatase, Ssu72 (KRISHNAMURTHY *et al.* 2004). Later in elongation, serine 2 becomes phosphorylated by Ctk1 (KEOGH *et al.* 2003; PATTURAJAN *et al.* 1999).

### 1.1.2 Chromatin structure inhibits transcription.

The role of TFIIS in facilitating transcription through nucleosomes is very important, because the incorporation of DNA into chromatin severely inhibits transcription. Eukaryotic DNA is highly compacted within the nucleus in the form of chromatin. The basic unit of chromatin is the nucleosome, which consists of two of each of the four histone proteins, H2A, H2B, H3, and H4, in a globular arrangement, wrapped by 147 base pairs of DNA (KORNBERG 1974; LUGER *et al.* 1997). A large amount of evidence indicates that nucleosomes impede transcription elongation. For example, elongation efficiency is severely reduced during transcription of reconstituted chromatin templates compared to naked DNA *in vitro* (IZBAN and LUSE 1991; ORPHANIDES *et al.* 1998). Furthermore, besides RNA Pol II and the general transcription factors, additional factors are required to prevent nucleosome-induced pausing and promote productive elongation (ORPHANIDES *et al.* 1998). *In vivo*, at yeast *GAL* genes, transcriptional induction is associated with the loss of nucleosomes in both the promoter and coding region, suggesting that efficient elongation requires chromatin disassembly (KRISTJUHAN and SVEJSTRUP 2004; SCHWABISH and STRUHL 2004). Furthermore, transcription rates have been shown to be inversely correlated with nucleosome occupancy within open reading frames (ORFs) globally (LEE *et al.* 2004). In fact, a recent study in yeast that used a deep-sequencing based method to determine the positions of all active RNA Pol II revealed extensive pausing and backtracking of the polymerase throughout the body of transcripts (CHURCHMAN and WEISSMAN 2011). Paused polymerase was particularly noticeable at the positions of the first four nucleosomes, confirming that nucleosomes act as a barrier to transcription elongation *in vivo*.

## 1.2 THE FUNCTIONS OF HISTONE MODIFICATIONS AND ELONGATION FACTORS

Eukaryotic cells have evolved many mechanisms to overcome the barriers imposed by chromatin to ensure proper gene expression, including the posttranslational modification of histone proteins, transcription elongation factors, histone chaperones, and chromatin remodeling enzymes. Those with connections to the Paf1 complex are discussed in detail below.

### 1.2.1 Histone modifications influence transcription elongation.

Histone proteins are subject to a wide variety of posttranslational modifications, most of which occur on the unstructured, N-terminal tails that extend beyond the nucleosome (LUGER *et al.* 1997). Arguably, the best understood histone modifications include acetylation, ubiquitylation, and methylation of histone residues. Histone acetylation occurs on multiple sites, including several lysines on the N-terminal tails of histone H3 and H4. Acetylation is thought to loosen DNA-histone contacts by neutralizing the basic charge of the lysine. At gene promoters, histone acetylation usually correlates with active transcription (POKHOLOK *et al.* 2005). Histone acetyltransferases (HATs) and histone deaceylases (HDACs) localize to ORFs, resulting in rapid turnover of acetylation (CARROZZA *et al.* 2005; CLOSE *et al.* 2006; GILBERT *et al.* 2004; GOVIND *et al.* 2007; KEOGH *et al.* 2005; WANG *et al.* 2002). Consistent with a positive role in transcription elongation, in vitro transcription reactions with purified factors requires HAT activity for elongation through a chromatin template (CAREY *et al.* 2006; GUERMAH *et al.* 2006). Furthermore, in yeast, the HAT Gcn5 has been shown to promote nucleosome eviction and transcription elongation through its HAT activity (GOVIND *et al.* 2005; GOVIND *et al.* 2007;

KRISTJUHAN and SVEJSTRUP 2004; KRISTJUHAN *et al.* 2002). Additionally, a recent study using chemically defined nucleosome arrays demonstrated that histone acetylation interferes with the formation of higher order chromatin structure (FIERZ *et al.* 2011).

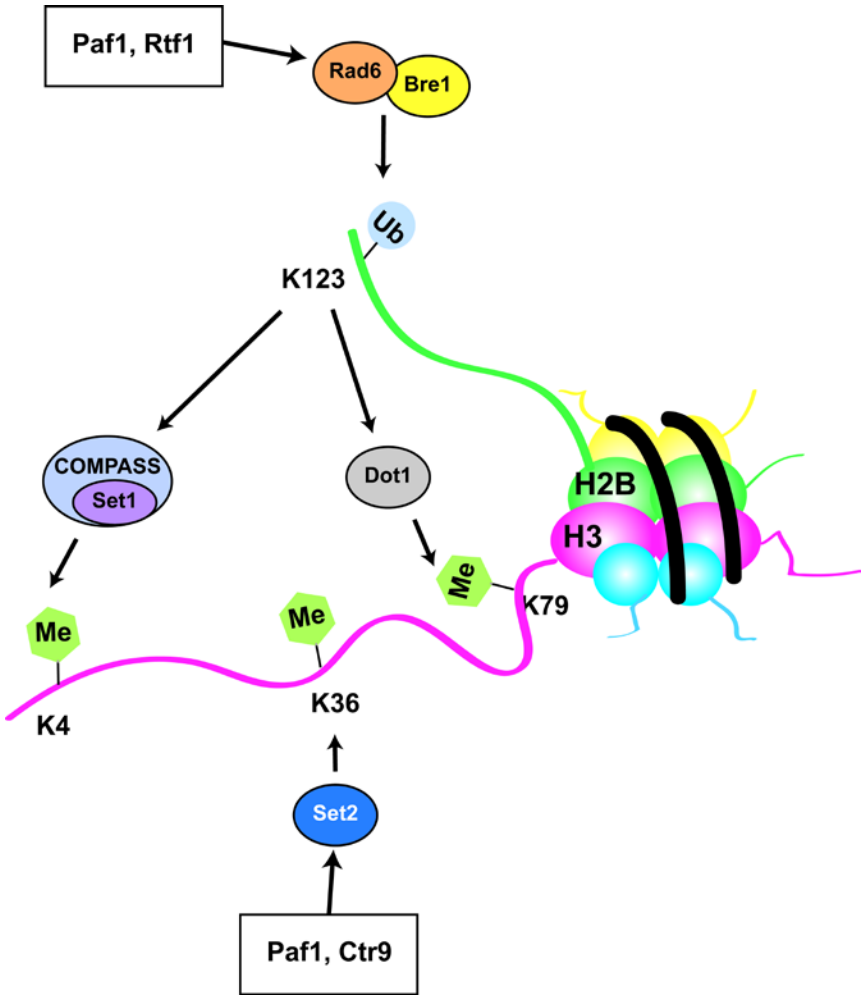


**Figure 2: Histone H2B monoubiquitylation and histone H3 K4, K36, and K79 methylation**

In yeast, the ubiquitin conjugating enzyme, Rad6, and the ubiquitin ligase, Bre1, monoubiquitylate histone H2B K123 (HWANG *et al.* 2003; ROBZYK *et al.* 2000; WOOD *et al.* 2003a). Histone H2B monoubiquitylation is a prerequisite for di- and trimethylation of histone H3 K4 and K79 by the histone methyltransferases Set1 and Dot1, respectively (BRIGGS *et al.* 2002; DOVER *et al.* 2002; NG *et al.* 2002b; SUN and ALLIS 2002). Histone H3 is methylated on K36 by the methyltransferase, Set2 (STRAHL *et al.* 2002). Paf1 and Rtf1 subunits of the Paf1 complex are required for histone H2B K123 monoubiquitylation and the downstream di- and trimethylation of histone H3 K4 and K79 (KROGAN *et al.* 2003a; NG *et al.* 2003a; NG *et al.* 2003b; WOOD *et al.* 2003b). Paf1 and Ctr9 are required for trimethylation of K36 on histone H3 (CHU *et al.* 2007).

Mono-ubiquitylation of histone H2B on lysine (K) 123 (K120 in humans) is another modification that has been shown to inhibit chromatin compaction (FIERZ *et al.* 2011). In yeast, the ubiquitin conjugase, Rad6, and the ubiquitin ligase, Bre1, are responsible for histone H2B ubiquitylation (HWANG *et al.* 2003; ROBZYK *et al.* 2000; WOOD *et al.* 2003a) (Figure 2). Histone H2B ubiquitylation is found on gene promoters and coding regions (HENRY *et al.* 2003; KAO *et al.* 2004; XIAO *et al.* 2005). Interestingly, this modification has both positive and negative effects on transcription, which are discussed in more detail below. Histone H2B ubiquitylation is a prerequisite for di- and trimethylation of histone H3 K4 and K79 by the histone methyltransferases Set1 and Dot1, respectively (BRIGGS *et al.* 2002; DOVER *et al.* 2002; NG *et al.* 2002b; SUN and ALLIS 2002) (Figure 2). The nature of this histone crosstalk is not completely understood. However, a subunit of the Set1-containing COMPASS complex (complex proteins associated with Set1) has been shown to be affected by histone H2B ubiquitylation. Cps35 (also called Swd2) is a COMPASS subunit that is required for histone H3 K4 di- and trimethylation and K79 methylation. Histone H2B ubiquitylation is required for Cps35 to associate with the complex, thus connecting histone H2B ubiquitylation and histone H3 methylation on K4 and K79 (LEE *et al.* 2007). The human homolog of Set1 is mixed lineage leukemia (MLL), which exists in a COMPASS-like complex and regulates the expression of developmental genes through histone H3 K4 methylation (reviewed in COSGROVE and PATEL 2010; SMITH *et al.* 2011). In addition to its important role in development, MLL influences the pathogenesis of a subset of human leukemias, as chromosomal translocations that result in oncogenic MLL-fusion proteins are associated with acute lymphoblastic or myelogenous leukemias (reviewed in COSGROVE and PATEL 2010; SMITH *et al.* 2011).

Histone H3 is also methylated on K36 by the methyltransferase, Set2 (STRAHL *et al.* 2002) (Figure 2). Both histone H3 K4 and K36 methylation occur across most genes in a distinct pattern that is influenced by the phosphorylation state of the RNA Pol II CTD. Serine 5 phosphorylation by Kin28 recruits Set1 to RNA Pol II early in elongation, resulting in a peak of histone H3 K4 trimethylation near promoters (NG *et al.* 2003b). Just downstream, K4 dimethylation peaks in 5' coding regions, whereas K4 monomethylation occurs across the gene (LIU *et al.* 2005; POKHOLOK *et al.* 2005). Later in elongation, serine 2 phosphorylation of the RNA Pol II CTD recruits Set2, resulting in histone H3 K36 methylation toward the 3' end of the coding region (KROGAN *et al.* 2003b; LI *et al.* 2003; SCHAFT *et al.* 2003; XIAO *et al.* 2003) (Figure 3). Interestingly, these histone modifications modulate histone acetylation through the recruitment of HATs and HDACs. Histone H3 K4 trimethylation recruits the NuA3 histone acetyltransferase (HAT) complex, resulting in increased histone H3 K14 acetylation (MARTIN *et al.* 2006; TAVERNA *et al.* 2006). Dimethylation of histone H3 K4 activates the Set3 histone deacetylase complex (HDAC) (GOVIND *et al.* 2010; KIM and BURATOWSKI 2009). Histone H3 K36 dimethylation promotes the activity of the Rpd3S HDAC (CARROZZA *et al.* 2005; KEOGH *et al.* 2005; LI *et al.* 2007; LI *et al.* 2009) (Figure 3). Through these pathways, histone H3 methylation restricts histone acetylation to promoters to prevent inappropriate transcription from cryptic start sites internal to coding regions and restore chromatin in the wake of the polymerase.

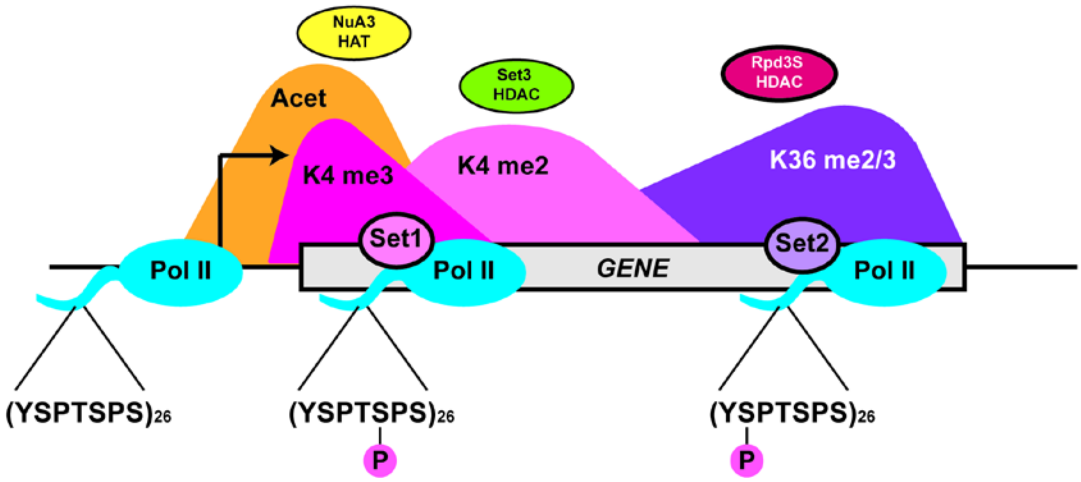


**Figure 3. Typical distribution of histone modifications across a gene**

Serine 5 phosphorylation on the RNA Pol II CTD recruits Set1 to RNA Pol II early in elongation, resulting in a peak of histone H3 K4 trimethylation near promoters (NG *et al.* 2003b). Just downstream, K4 dimethylation peaks in 5' coding regions (LIU *et al.* 2005; POKHOLOK *et al.* 2005). Later in elongation, serine 2 phosphorylation of the RNA Pol II CTD recruits Set2, resulting in histone H3 K36 methylation toward the 3' end of the coding region (KROGAN *et al.* 2003b; LI *et al.* 2003; SCHAFT *et al.* 2003; XIAO *et al.* 2003). Histone H3 K4 trimethylation recruits the NuA3 histone acetyltransferase (HAT) complex, resulting in increased histone H3 K14 acetylation near the promoter (MARTIN *et al.* 2006; TAVERNA *et al.* 2006). Within the coding region, dimethylation of histone H3 K4 activates the Set3 histone deacetylase complex (HDAC) (GOVIND *et al.* 2010; KIM and BURATOWSKI 2009). At the 3' coding region, histone H3 K36 dimethylation promotes the activity of the Rpd3S HDAC (CARROZZA *et al.* 2005; KEOGH *et al.* 2005; LI *et al.* 2007; LI *et al.* 2009). Therefore, the recruitment of HDACs to coding regions restricts acetylation to a region near the promoter.

### 1.2.2 DSIF/Spt4-Spt5 functions during transcription elongation.

In addition to its role in inhibiting transcription elongation in promoter-proximal regions in cooperation with NELF, DSIF influences later events in transcription elongation. Spt4 and Spt5 are yeast homologues of DSIF (HARTZOG *et al.* 2002). In yeast, Spt4 and Spt5 were identified in a genetic screen for mutations that suppress defects in gene expression caused by promoter mutations (WINSTON *et al.* 1984). Both yeast and human factors have been implicated in regulating chromatin during transcription elongation. Biochemical experiments in yeast and human cells revealed that DSIF/Spt4-Spt5 interacts with RNA Pol II during elongation and has both positive and negative effects on elongation (BOURGEOIS *et al.* 2002; HARTZOG *et al.* 2002; KROGAN *et al.* 2002b; LINDSTROM *et al.* 2003). Although the functions of DSIF/Spt4-Spt5 are not completely understood, several genetic interactions with elongation and chromatin-related factors suggest that DSIF/Spt4-Spt5 regulates transcription elongation through the modulation of chromatin structure. For example, in yeast Spt4-Spt5 genetically interacts with the ATP-dependent chromatin remodeler, Chd1 (SIMIC *et al.* 2003), and kinases and phosphatases that modify the CTD of RNA Pol II (LINDSTROM and HARTZOG 2001). Consistent with its genetic interactions with CTD modifying enzymes, Spt4-Spt5 has been shown to recruit the Rpd3S HDAC to active genes in cooperation with CTD kinases, Kin28 and Ctk1(DROUIN *et al.* 2010). Rpd3S has been shown to prevent transcription from cryptic sites within coding region through

its HDAC activity (CARROZZA *et al.* 2005; KEOGH *et al.* 2005; LI *et al.* 2007; LI *et al.* 2009). Therefore, Spt4-Spt5 may indirectly affect chromatin by recruiting Rpd3S HDAC. Importantly, Spt4-Spt5 genetically and physically interacts with the Paf1 complex, suggesting that they cooperate to control transcription elongation (COSTA and ARNDT 2000; SQUAZZO *et al.* 2002). Moreover, Spt5 is required for the recruitment of the Paf1 complex to the elongation complex and for ubiquitylation of histone H2B on K123 (LIU *et al.* 2009; ZHOU *et al.* 2009).

### 1.2.3 FACT/Spt16-Pob3 functions as a histone chaperone.

As its name implies, FACT (Facilitate Chromatin Transcription) was originally identified as a factor that stimulates transcription of a reconstituted chromatin template (ORPHANIDES *et al.* 1998; ORPHANIDES *et al.* 1999). Beyond this, many physical and genetic interactions suggest that FACT (Spt16-Pob3 in yeast) has important roles during transcription (COSTA and ARNDT 2000; FORMOSA *et al.* 2002; ORPHANIDES *et al.* 1999; SIMIC *et al.* 2003; SQUAZZO *et al.* 2002). It is now known that FACT directly participates in the reorganization of nucleosomes within the ORFs of actively transcribed genes (ORPHANIDES and REINBERG 2000) and reassembles chromatin in the wake of RNA Pol II (FORMOSA *et al.* 2002; MASON and STRUHL 2003). There are two current proposed models of FACT function. In one model, FACT displaces a single H2A-H2B dimer to allow RNA Pol II to pass (BELOTSERKOVSKAYA *et al.* 2003; ORPHANIDES *et al.* 1999; REINBERG and SIMS 2006). In support of this model, FACT binds H2A-H2B dimer *in vitro* (ORPHANIDES *et al.* 1999). However, *in vivo*, chromatin reorganization is not associated with the H2A-H2B dimer loss that this model predicts (RHOADES *et al.* 2004; XIN *et al.* 2009). Instead, analysis of *in vitro* hydroxyl radical accessibility and endonuclease cleavage showed that FACT creates more accessibility than could be explained by the loss of an H2A-H2B dimer,

yet partially protects nucleosomal DNA (XIN *et al.* 2009). These results have lead to the second model, in which FACT performs a more dramatic reorganization of the nucleosome without histone eviction by tethering histones to the DNA. Future studies will likely shed more light on the functions of FACT. Interestingly, FACT genetically interacts with the Paf1 complex, indicating cooperative functions during transcription elongation (COSTA and ARNDT 2000; SQUAZZO *et al.* 2002). Consistent with this, Paf1 augments FACT-mediated *in vitro* transcription of a chromatin template (PAVRI *et al.* 2006).

### 1.2.4 Chd1 is a chromatin remodeling enzyme involved in transcription elongation.

Chd1 is a conserved ATP-dependent chromatin remodeling enzyme (STOCKDALE *et al.* 2006; TRAN *et al.* 2000). It associates with regions of active transcription (SIMIC *et al.* 2003; STOKES *et al.* 1996) and physically interacts with DSIF/Spt4-Spt5, FACT, and the Paf1 complex (KELLEY *et al.* 1999; KROGAN *et al.* 2002b; SIMIC *et al.* 2003), pointing to an important role during transcription. The mechanistic details of chromatin remodeling by Chd1 are not well understood. However, Chd1 has been shown to create a chromatin structure that inhibits cryptic transcription initiation (QUAN and HARTZOG 2009). Chd1 contains a chromo domain which can bind methylated lysines (BANNISTER *et al.* 2001). It has been shown that the human homolog of Chd1 associates with chromatin by recognition of histone H3 K4 methylation (FLANAGAN *et al.* 2005; SIMS *et al.* 2005), but there are differing reports as to whether this occurs in yeast (FLANAGAN *et al.* 2005; PRAY-GRANT *et al.* 2005; SIMS *et al.* 2005). Instead, the Rtf1 subunit of the Paf1 complex in yeast has been shown to recruit Chd1 to chromatin (SIMIC *et al.* 2003). Additionally, the 30 N-terminal amino acids of Rtf1 have been defined as a region required for Chd1 interaction (WARNER *et al.* 2007).

## 1.3 THE PAF1 TRANSCRIPTION ELONGATION COMPLEX

### 1.3.1 Identification of the Paf1 complex

The search for accessory proteins besides general transcription factors that are required for transcription initiation lead to the identification of Paf1 (polymerase associated factor 1) in *Saccharomyces cerevisiae* (SHI *et al.* 1996; WADE *et al.* 1996). As the name implies, Paf1 was found to associate with RNA Pol II by affinity chromatography (SHI *et al.* 1996). It was subsequently demonstrated that Paf1 exists in a nuclear complex with Ctr9, Cdc73, Rtf1, and Leo1 (KROGAN *et al.* 2002b; MUELLER and JAEHNING 2002; SHI *et al.* 1997; SQUAZZO *et al.* 2002). Initial characterization of the Paf1 complex by several labs revealed phenotypes associated with transcriptional defects, genetic interactions with other transcription factors, and effects on the expression of select genes upon disruption of Paf1 complex members, implicating the Paf1 complex in transcriptional regulation (BETZ *et al.* 2002; COSTA and ARNDT 2000; KROGAN *et al.* 2002b; POKHOLOK *et al.* 2002; PORTER *et al.* 2002; SHI *et al.* 1997; SHI *et al.* 1996; SQUAZZO *et al.* 2002). Later studies demonstrated critical functions of the Paf1 complex during all stages of transcription and revealed Paf1 complex-dependent gene expression throughout the yeast genome. Importantly, the Paf1 complex and its functions are conserved throughout eukaryotes and defects in the Paf1 complex are associated with human diseases. Since studies of the Paf1 complex in budding yeast will likely provide insight into the molecular basis of several human diseases, here, I review functions of the Paf1 complex in transcription and gene expression, primarily focusing on studies performed in *Saccharomyces cerevisiae*.

### 1.3.2 The association of the Paf1 complex with RNA Pol II and chromatin

Consistent with roles during multiple stages of transcription, the Paf1 complex accompanies RNA Pol II during transcription from the transcription start site to the poly (A) site (KIM *et al.* 2004; MAYER *et al.* 2010). Rtf1 and Cdc73 are required for RNA Pol II-association, because, although the other complex members remain associated with each other, they become dissociated from the polymerase and chromatin upon the loss of either subunit (MUELLER *et al.* 2004; NORDICK *et al.* 2008; QIU *et al.* 2006). Deletion analysis used to map regions of Rtf1 required for chromatin association defined a central region of Rtf1 (amino acids 201 to 395), now termed the ORF association region (OAR) (WARNER *et al.* 2007). Rtf1 has been shown to genetically and physically interact with Spt5, a transcription elongation factor (SQUAZZO *et al.* 2002). Interestingly, phosphorylation of Spt5 by the Bur1 kinase and its regulatory partner, Bur2, promotes recruitment of the Paf1 complex to chromatin (LARIBEE *et al.* 2005; LIU *et al.* 2009; ZHOU *et al.* 2009). Therefore, the Rtf1 OAR may associate with RNA Pol II indirectly through an interaction with Spt5. In contrast, recombinant Cdc73 can interact with purified RNA Pol II, suggesting that Cdc73 may directly contact RNA Pol II *in vivo* (SHI *et al.* 1997). Beyond the interactions of Paf1 complex subunits with RNA Pol II, Leo1 has been shown to contribute to Paf1 complex recruitment through its RNA binding activity (DERMODY and BURATOWSKI 2010). Consequently, Leo1 may stabilize the association of the Paf1 complex with chromatin by interacting with the nascent mRNA (DERMODY and BURATOWSKI 2010).

### 1.3.3 The Paf1 complex regulates transcription elongation

Paf1 complex subunits have been implicated in initiation by influencing DNA-binding specificity of TATA-binding protein (TBP) (STOLINSKI *et al.* 1997) and in termination and 3' end formation by mediating recruitment of 3' end processing factors (MUELLER *et al.* 2004; PENHEITER *et al.* 2005; SHELDON *et al.* 2005). However, the Paf1 complex is currently best characterized for its critical roles during transcription elongation. Several phenotypes and interactions initially implicated the Paf1 complex in regulating the elongation stage of transcription. For example, strains lacking Paf1 complex subunits exhibit phenotypes associated with transcription elongation defects, such as sensitivity to 6-azauracil and mycophenolic acid (COSTA and ARNDT 2000; SQUAZZO *et al.* 2002). Additionally, Paf1 complex members genetically and physically interact with elongation factors such as the Spt4-Spt5 (yDSIF) and Spt16-Pob3 (yFACT) complexes, suggesting that these complexes function in parallel to modulate transcription elongation (COSTA and ARNDT 2000; KROGAN *et al.* 2002b; SQUAZZO *et al.* 2002). Further characterization of the functions of the Paf1 complex has shown that the Paf1 complex is required for phosphorylation of serine 2 of the RNA Pol II CTD during elongation (MUELLER *et al.* 2004; NORDICK *et al.* 2008). Additionally, the Rtf1 subunit associates with and recruits a chromatin remodeling enzyme, Chd1, to ORFs (SIMIC *et al.* 2003). The Paf1 complex also regulates several co-transcriptional histone modifications that influence gene expression, including histone H2B K123 monoubiquitylation and methylation of histone H3 on K4, K36, and K79 (CHU *et al.* 2007; KROGAN *et al.* 2003a; NG *et al.* 2003a; NG *et al.* 2003b; WOOD *et al.* 2003b). Together, these observations suggest that the Paf1 complex influences gene expression through multiple functions during transcription elongation.

### 1.3.4 The Paf1 complex influences gene expression through histone H2B K123 monoubiquitylation and histone H3 methylation on K4 and K79

The Paf1 complex is required for several histone modifications, including histone H2B ubiquitylation (NG *et al.* 2003a; WOOD *et al.* 2003b). Histone H2B is mono-ubiquitylated on K123 by the ubiquitin conjugase, Rad6, and the ubiquitin ligase, Bre1 (HWANG *et al.* 2003; ROBZYK *et al.* 2000; WOOD *et al.* 2003a). Histone H2B ubiquitylation is required for di- and trimethylation of histone H3 K4 and K79 by the histone methyltransferases Set1 and Dot1, respectively (BRIGGS *et al.* 2002; DOVER *et al.* 2002; NG *et al.* 2002b; SUN and ALLIS 2002). Paf1 and Rtf1 promote histone H2B ubiquitylation by facilitating the association of Rad6 and Set1 with RNA Pol II during transcription elongation (KROGAN *et al.* 2003a; NG *et al.* 2003a; NG *et al.* 2003b; WOOD *et al.* 2003b; XIAO *et al.* 2005). Consequently, the loss of Paf1 or Rtf1 abolishes histone H2B ubiquitylation and di- and trimethylation of histone H3 on K4 and K79 (KROGAN *et al.* 2003a; NG *et al.* 2003a; NG *et al.* 2003b; WOOD *et al.* 2003b) (Figure 2). Since Paf1 is required for normal Rtf1 protein levels, Rtf1 is probably the primary subunit that regulates histone H2B ubiquitylation and subsequent methylation of histone H3 on K4 and K79 (MUELLER *et al.* 2004). In fact, the Arndt lab has shown that Rtf1 amino acids 62-152 are required for these histone modifications, leading us to define this region as the Rtf1 histone modification domain (HMD) (TOMSON *et al.* 2011b; WARNER *et al.* 2007).

Histone H2B ubiquitylation and histone H3 K4 and K79 methylation are enriched on the coding regions of active genes, indicating that these modifications are associated with active transcription (BERNSTEIN *et al.* 2002; SANTOS-ROSA *et al.* 2002; XIAO *et al.* 2005). Consistent with a positive role, histone H2B ubiquitylation has been shown to enhance the transcription elongation rate of a chromatin template *in vitro* (PAVRI *et al.* 2006). *In vivo,* histone H2B

ubiquitylation facilitates transcription of galactose-inducible genes by promoting nucleosome-reassembly in the wake of RNA Pol II in cooperation with the histone chaperone, Spt16 (FLEMING *et al.* 2008). Additionally, a recent study using chemically defined nucleosome arrays demonstrated that histone H2B ubiquitylation interferes with chromatin compaction, which may facilitate transcription (FIERZ *et al.* 2011).

Despite its connections to active transcription, histone H2B ubiquitylation also negatively influences transcription. For example, histone H2B ubiquitylation and the downstream methylation of histone H3 on K4 and K79 regulate telomeric silencing (HUANG *et al.* 1997; KROGAN *et al.* 2002a; NG *et al.* 2003a; NG *et al.* 2002a; NISLOW *et al.* 1997; SUN and ALLIS 2002; VAN LEEUWEN *et al.* 2002). In *Saccharomyces cerevisiae*, heterochromatic silencing occurs at three locations through similar mechanisms (APARICIO *et al.* 1991; KAYNE *et al.* 1988): the mating-type loci (*HMR* and *HML*) (KLAR *et al.* 1981; NASMYTH *et al.* 1981), the rDNA loci (BRYK *et al.* 1997; SMITH and BOEKE 1997), and telomeric regions (GOTTSCHLING *et al.* 1990). Telomeric silencing is mediated by silent information regulator (Sir) proteins by preferentially binding to hypomethylated histones in telomeric regions (reviewed in SHAHBAZIAN and GRUNSTEIN 2007). The genome-wide loss of histone H3 K4 and K79 methylation is thought to cause a redistribution of Sir proteins from telomeric regions, resulting in the loss of silencing of telomere-adjacent genes (reviewed in RUSCHE *et al.* 2003). Consequently, complete deletion of *PAF1* or *RTF1* (KROGAN *et al.* 2003a; NG *et al.* 2003a) or disruption of the Rtf1 HMD indirectly results in telomeric silencing defects (TOMSON *et al.* 2011b; WARNER *et al.* 2007).

In yeast, silencing defects are often detected with a growth assay using strains in which *URA3* is integrated at a telomere-adjacent locus. If proper telomeric silencing occurs, the *URA3* gene is silenced. Consequently, these cells can grow on medium containing 5-fluoroorotic acid

(5-FOA), which is converted to a toxin by the *URA3* gene product (BOEKE *et al.* 1984). However, it has recently been shown that, in some mutants, expression of *URA3* in this assay does not result from the loss of telomeric silencing per se, but instead from the exacerbation of metabolic changes induced by 5-FOA (ROSSMANN *et al.* 2011; TAKAHASHI *et al.* 2011). Therefore, although Paf1 complex-dependent histone modifications have been implicated in telomeric silencing, additional work is required to determine whether these modifications do, in fact, regulate natural telomeric silencing. However, Rad6, the E2 mediating histone H2B ubiquitylation, is required for silencing of the natural mating-type loci, supporting a role for histone H2B ubiquitylation in gene silencing (HUANG *et al.* 1997). Furthermore, aside from its potential effects on Sir protein binding, histone H2B ubiquitylation may also prevent heterochromatic silencing through its inhibition of chromatin compaction (FIERZ *et al.* 2011).

In addition to the silencing of subtelomeric genes, microarray analysis of transcript levels in cells in which the histone H2B ubiquitylation site was mutated (*htb1-K123R*) has demonstrated that histone H2B ubiquitylation represses many genes throughout the genome (MUTIU *et al.* 2007). In fact, the majority of affected genes exhibited increased expression in *htb1-K123R* cells, indicating that histone H2B ubiquitylation predominantly acts to repress transcription (MUTIU *et al.* 2007). Consistent with repressive functions, histone H2B ubiquitylation has been shown to increase nucleosome stability at the promoters of repressed genes (CHANDRASEKHARAN *et al.* 2009). Furthermore, removal of histone H2B ubiquitylation by the de-ubiquitylating enzyme, Ubp8, is required for full expression of a subset of inducible genes, including *GAL1*, *GAL10*, and *SUC2* (DANIEL *et al.* 2004; HENRY *et al.* 2003; KAO *et al.* 2004). Although these observations suggest that histone H2B ubiquitylation has important

functions for repression of global transcription, the mechanism by which histone H2B ubiquitylation represses transcription is not well-understood.

Importantly, like its yeast counterpart, the human Paf1 complex controls gene expression through histone H2B ubiquitylation and subsequent histone H3 methylation on K4 and K79 (DING *et al.* 2009; KIM *et al.* 2009a; MINSKY *et al.* 2008; PAVRI *et al.* 2006; ZHU *et al.* 2005b). Furthermore, histone H2B ubiquitylation in humans also has both positive and negative effects on transcription. For example, histone H2B ubiquitylation is preferentially associated with highly expressed genes (MINSKY *et al.* 2008). In particular, histone H2B ubiquitylation has been shown to stimulate proper *HOX* gene expression in human cells (ZHU *et al.* 2005b) and the transcription of pluripotency genes in embryonic stem cells (DING *et al.* 2009), thus promoting proper development and stem cell identity, respectively. However, de-ubiquitylation by Usp22, the human homolog of yeast Ubp8, inhibits heterochromatic silencing and promotes gene activation (ZHANG *et al.* 2008; ZHAO *et al.* 2008b). Human Bre1/RNF20 acts as a tumor suppressor by promoting transcription of tumor suppressor genes and repressing proto-oncogenes, underscoring the importance of both positive and negative gene regulation by histone H2B ubiquitylation (SHEMA *et al.* 2008).

### 1.3.5 Paf1 complex-dependent histone methylation influences histone acetylation

In addition to its regulation by histone H2B ubiquitylation, histone H3 K4 methylation participates in pathways of histone crosstalk that specify either histone acetylation or deacetylation. Histone H3 K4 methylation recruits the NuA3 HAT complex, resulting in increased histone H3 K14 acetylation and gene expression (MARTIN *et al.* 2006; TAVERNA *et al.* 2006). Interestingly, histone H3 K4 dimethylation also lowers histone acetylation levels at the 5'

ends of genes through activation of the Set3 HDAC (GOVIND *et al.* 2010; KIM and BURATOWSKI 2009). Consistent with this pathway of histone H3 K4 methylation-directed deacetylation, the loss of Paf1 results in increased acetylation at 5' coding regions (CHU *et al.* 2007).

In addition to methylation of K4 and K79 on histone H3, Paf1 and Ctr9 are required for trimethylation of K36 on histone H3 by the histone methyltransferase, Set2 (CHU *et al.* 2007) (Figure 2). Set2 associates with the elongating form of RNA Pol II in the body of actively transcribed genes in a Paf1 complex-dependent manner (CHU *et al.* 2007; KROGAN *et al.* 2003b; XIAO *et al.* 2003). Since the association of Set2 with RNA Pol II requires phosphorylation of the CTD on serine 2 (KIZER *et al.* 2005), the Paf1 complex may influence Set2 recruitment indirectly through its effects on CTD phosphorylation (MUELLER *et al.* 2004; NORDICK *et al.* 2008). Interestingly, histone H3 K36 dimethylation promotes the activity of the Rpd3S HDAC, which reduces histone acetylation on transcribed genes and inhibits transcription from cryptic promoters within coding regions (CARROZZA *et al.* 2005; KEOGH *et al.* 2005; LI *et al.* 2007; LI *et al.* 2009). This pathway of methylation-driven deacetylation is thought to restore chromatin after passage of RNA Pol II on active genes. Analysis of *paf1Δ set2Δ* double mutant strains suggests that Paf1 and Set2 function separately to reduce cryptic initiation and histone acetylation at 3' coding regions (CHU *et al.* 2007). These results may not be surprising since Paf1 is selectively required for histone H3 K36 trimethylation (CHU *et al.* 2007), yet dimethylation is sufficient for Rpd3 HDAC activity (LI *et al.* 2009). Therefore, at 5' coding regions, the Paf1 complex reduces histone acetylation, possibly through histone H3 K4 methylation-mediated deacetylation by Set3. However, at 3' coding regions, the Paf1 complex reduces acetylation through a mechanism that is parallel to the established Set2-Rpd3 pathway.

Given its essential roles in modulating several histone modifications, the Paf1 complex likely regulates gene expression by promoting histone modifications. However, while genome-wide analysis identifies numerous genes that require the Paf1 complex for proper expression (PENHEITER *et al.* 2005), only a subset of Paf1 complex-responsive genes exhibit altered expression in the absence of these same histone modifications (MUTIU *et al.* 2007). Therefore, the Paf1 complex likely has roles aside from facilitating histone modifications that control gene expression. Consistent with this hypothesis, the human Paf1 complex has recently been shown to stimulate *in vitro* transcription of a chromatin template independently of histone modifications (KIM *et al.* 2010). Further investigation is required to elucidate critical histone modification-independent functions of the Paf1 complex.

### 1.3.6 The Paf1 complex coordinates transcription with termination and 3' end processing

In addition to its critical functions during transcription elongation, the Paf1 complex is important for proper transcription termination (SHELDON *et al.* 2005) and RNA 3' end formation (MUELLER *et al.* 2004; NORDICK *et al.* 2008; PENHEITER *et al.* 2005). The loss of Paf1 complex members results in shorter poly(A) tail lengths (MUELLER *et al.* 2004). Additionally, the Paf1 complex has been shown to modulate expression of a subset of genes, not by regulating elongation, but by controlling poly(A) site usage (PENHEITER *et al.* 2005). Specifically, the loss of Paf1 results in the read-through of poly(A) sites, producing 3'-extended transcripts that are subject to nonsense-mediated decay (PENHEITER *et al.* 2005). Termination and 3' end processing defects that occur in the absence of Paf1 can be attributed to the reduced recruitment of 3' end processing factors to chromatin. In the absence of Paf1 complex members, altered poly(A) site usage is associated with reduced chromatin association of the cleavage and polyadenylation factor, Pcf11 (MUELLER

*et al.* 2004). Additionally, Cft1, another 3' end processing factor, associates with RNA Pol II in a Paf1 complex-dependent manner (NORDICK *et al.* 2008). The recruitment of cleavage and polyadenylation factors to RNA Pol II and chromatin requires the serine 2-phosphorylated form of the RNA Pol II CTD (AHN *et al.* 2004; LICATALOSI *et al.* 2002). Therefore, the Paf1 complex may regulate the recruitment of 3' end processing factors indirectly through its effects on CTD phosphorylation. However, direct interactions between the Paf1 complex and 3' end processing factors have been demonstrated in yeast and humans (NORDICK *et al.* 2008; ROZENBLATT-ROSEN *et al.* 2009). Therefore, the Paf1 complex may recruit 3' end processing factors through a mechanism that does not rely on RNA Pol II CTD phosphorylation. Consistent with a mechanism that is independent of CTD phosphorylation, it has recently been shown that the human Paf1 complex is required for activator-stimulated mRNA polyadenylation (NAGAIKE *et al.* 2011).

Together, these observations suggest that the Paf1 complex plays an important role in coordinating transcription with 3' end processing. Given that the Paf1 complex is required for the recruitment of 3' end processing factors to chromatin (MUELLER *et al.* 2004; NORDICK *et al.* 2008), yet it dissociates from RNA Pol II shortly after the poly (A) site has been transcribed (KIM *et al.* 2004; MAYER *et al.* 2010), the Paf1 complex may participate in an exchange of elongation factors for 3' end processing factors during transcription termination. Consistent with this hypothesis, when dissociated from chromatin, the Paf1 complex associates with RNA processing factors (NORDICK *et al.* 2008). However, the exact mechanism by which the Paf1 complex regulates termination and 3' end processing of polyadenylated transcripts remains unclear.

The Paf1 complex is also required for proper termination and 3' end formation of non-polyadenylated transcripts (SHELDON *et al.* 2005). The loss of Paf1 complex members or Paf1 complex-dependent histone modifications results in 3'-extended non-polyadenylated small nucleolar RNAs (snoRNAs) (SHELDON *et al.* 2005; TOMSON *et al.* 2011b). snoRNA termination defects in the absence of Paf1 complex members are associated with reduced recruitment of the 3' end processing factor, Nrd1 (SHELDON *et al.* 2005). Therefore, similar to its effects on the termination of polyadenylated transcripts, the Paf1 complex may mediate snoRNA termination by promoting recruitment of 3' end processing factors. Interestingly, it has recently been shown that the termination function of the Paf1 complex can be inhibited through an interaction with an activator (KIM and LEVIN 2011). Specifically, a physical interaction between Mpk1 MAPK and Paf1 prevents premature termination by inhibiting recruitment of the Sen1-Nrd1-Nab3 complex (KIM and LEVIN 2011). However, the mechanism by which the Paf1 complex recruits 3' end processing factors for termination remains to be revealed. Additionally, disruption of the Rtf1 HMD results in snoRNA termination defects, implicating histone H2B ubiquitylation in the regulation transcription termination (TOMSON *et al.* 2011b). Interestingly, nucleosome depletion in terminator regions has been shown to require Pol II transcription (FAN *et al.* 2010). Therefore, aside from facilitating recruitment of 3' end processing factors, the Paf1 complex may promote proper transcription termination through histone H2B ubiquitylation and its effects on chromatin structure (CHANDRASEKHARAN *et al.* 2009; FIERZ *et al.* 2011).

The prevalence of Paf1 complex-dependent termination and 3' end formation throughout the genome has not yet been assessed. However, given the important roles of transcription termination, which include regulating transcript stability and RNA Pol II recycling (reviewed in GILMOUR and FAN 2008; KUEHNER *et al.* 2011; RICHARD and MANLEY 2009; ROSONINA *et al.*

2006), Paf1 complex-dependent termination may have wide-spread effects on gene expression. Importantly, the functions of the Paf1 complex in regulating termination and 3' end formation are conserved from yeast to humans, as the human Paf1 complex also promotes proper RNA 3' end formation (NAGAIKE *et al.* 2011; ROZENBLATT-ROSEN *et al.* 2009).

### 1.3.7 The Paf1 complex has essential functions in metazoans

As mentioned above, the critical functions of the Paf1 complex, including RNA Pol II-association (ROZENBLATT-ROSEN *et al.* 2005) and roles in transcription elongation (CHEN *et al.* 2009; KIM *et al.* 2010), histone modifications (DING *et al.* 2009; KIM *et al.* 2009a; ROZENBLATT-ROSEN *et al.* 2009; ZHU *et al.* 2005b), and RNA 3' end formation (NAGAIKE *et al.* 2011; ROZENBLATT-ROSEN *et al.* 2009), are conserved between yeast and humans. However, there are some differences in complex composition in yeast and higher eukaryotes. In humans, the Paf1 complex is minimally composed of Paf1, Ctr9, Cdc73, Leo1, and the higher eukaryote-specific subunit, Ski8, which is involved in mRNA surveillance (ROZENBLATT-ROSEN *et al.* 2005; YART *et al.* 2005; ZHU *et al.* 2005a). A few reports differ on whether human Rtf1 is absent from (ROZENBLATT-ROSEN *et al.* 2005; YART *et al.* 2005; ZHU *et al.* 2005b) or present in (KIM *et al.* 2010) the human complex. Therefore, human Rtf1 appears to be less stably associated with the Paf1 complex. Consistent with this, Rtf1 is not stably associated with the *Drosophila* Paf1 complex (ADELMAN *et al.* 2006). However, despite its less stable association with the Paf1 complex, human Rtf1 still influences gene expression (DING *et al.* 2009; MUNTEAN *et al.* 2010).

Additionally, the Paf1 complex has evolved critical roles in important signal transduction pathways in higher eukaryotes. Rtf1 regulates the transcription of Notch target genes in *Drosophila* and zebrafish (ADELMAN *et al.* 2006; AKANUMA *et al.* 2007; TENNEY *et al.* 2006).

The human and *Drosophila* homologs of Cdc73, Hyrax and Parafibromin, respectively, bind directly to β-catenin/Armadillo for proper transcription of Wnt/Wg target genes (MOSIMANN *et al.* 2006). Furthermore, human Paf1 complex subunits modulate the transcription of *HOX* genes (ZHU *et al.* 2005b), interleukin-6 responsive inflammatory genes (YOUN *et al.* 2007), and pluripotency genes (DING *et al.* 2009). Beyond these important functions, members of the human Paf1 complex have been implicated in cancer. Pancreatic differentiation factor 2/Paf1 is overexpressed in pancreatic cancer cell lines and overexpression in cell culture results in transformation (MONIAUX *et al.* 2006). Additionally, the gene encoding human Paf1 is amplified in many cancers, including breast and uterine cancers (HESELMEYER *et al.* 1997; KALLIONIEMI *et al.* 1994). Furthermore, parafibromin/Cdc73 is a tumor suppressor encoded by HRPT2, a gene that is mutated in hyperparathyroidism-jaw tumor syndrome (BRADLEY *et al.* 2006; CARPTEN *et al.* 2002; HOWELL *et al.* 2003). Given the conservation of the multiple functions of the Paf1 complex from yeast to humans, cumulatively, these observations indicate that the study of the Paf1 complex in yeast may elucidate the underlying mechanisms of several human diseases.

## 1.4 MECHANISMS OF GENE REPRESSION

Transcriptional repression can be accomplished through a wide variety of mechanisms. Transcriptional repressors may counteract gene activation by inhibiting the activator, interfering with assembly of the transcription machinery, or by establishing a repressive chromatin structure. Additionally, repressors can perform these functions directly or indirectly by recruiting co-repressors. In addition to the actions of repressors and co-repressor proteins, the transcription of non-coding RNA can effectively regulate gene expression. The multiple ways in which

repressors, co-repressors, and the transcription of non-coding RNA can mediate gene repression are discussed below.

### 1.4.1 Gene-specific transcriptional repressors

DNA-binding transcriptional activators are critical for recruiting TBP and the pre-initiation complex for transcription, often by creating an accessible chromatin environment at a gene's promoter. Repressors, therefore, promote repression by opposing the actions of the activator, which can be accomplished in many ways. For example, repressors can modulate expression or localization of an activator. In yeast, Pho80-Pho85 represses phosphate-responsive genes by phosphorylating the activator, Pho4, resulting in its relocalization to the cytosol (KAFFMAN *et al.* 1994; O'NEILL *et al.* 1996). Srb10, a subunit of the Mediator complex, promotes repression of Gcn4-regulated genes by phosphorylating Gcn4, targeting it for ubiquitylation and degradation (CHI *et al.* 2001). Similar mechanisms are employed in higher eukaryotes as well. For example, Mdm2 ubiquitylates the tumor suppressor p53, marking it for nuclear export and degradation (FUCHS *et al.* 1998; INOUE *et al.* 2001). Alternatively, repressors can inhibit the association of activators with general transcription factors. For example, in yeast, Gal80 prevents the activation of galactose metabolic genes by binding to the activator, Gal4, preventing association of the transcription machinery (MA and PTASHNE 1987; SALMERON *et al.* 1990). Therefore, through specific interactions with certain activators, repressors can strongly repress transcription in a gene-specific manner.

In addition to eliciting repression by altering activator binding, repressors can promote repression by binding to DNA elements within the promoters of their target genes. For example, in *Drosophila*, the repressor Engrailed competes with the activator, Fushi tarazu, for a common

promoter binding site (JAYNES and O'FARRELL 1988). Therefore, binding of the repressor to the promoter interferes with activator binding. Alternatively, repressors can prevent TBP binding or inhibit the assembly of the pre-initiation complex. For example, LBP-1 inhibits binding of TBP/TFIID on the long terminal repeats of HIV (KATO *et al.* 1991). Additionally, *Drosophila* even-skipped (AUSTIN and BIGGIN 1995; HAN and MANLEY 1993) and kruppel (AUBLE *et al.* 1997; LICHT *et al.* 1994; SAUER *et al.* 1995) inhibit the assembly of the pre-initiation complex at specific genes involved in development.

### 1.4.2 General transcriptional repressors

In addition to gene-specific repressors, some repressors have a more global role by controlling TBP function at many genes. TBP binds to the minor groove of DNA, which provides limited sequence specificity. Consequently, TBP has a high affinity for non-specific DNA (COLEMAN *et al.* 1995). Therefore, not surprisingly, the interaction of TBP with DNA must be tightly regulated. Mot1 (modifier of transcription 1) is a conserved ATPase that regulates TBP-DNA binding (EISEN *et al.* 1995). In yeast, Mot1 is essential for viability (DAVIS *et al.* 1992). The N-terminal 800 residues of Mot1 are both necessary and sufficient for TBP-binding (ADAMKEWICZ *et al.* 2000; AUBLE *et al.* 1997). The last C-terminal residues contain the ATPase domain (AUBLE *et al.* 1997). Mot1 was also identified as Bur3 in a genetic screen for mutations that promote transcription of a *SUC2* allele that lacks an upstream activating sequence (UAS) (PRELICH 1997; PRELICH and WINSTON 1993). Interestingly, Mot1 has both positive and negative effects on the transcription of numerous genes (ANDRAU *et al.* 2002; DASGUPTA *et al.* 2002; GEISBERG *et al.* 2002). Its negative influence on transcription can be explained by its ability to use ATP-hydrolysis to remove TBP from promoter DNA (AUBLE *et al.* 1994; SPROUSE

*et al.* 2008a; VAN WERVEN *et al.* 2009). Conversely, Mot1 is thought to promote transcription by facilitating productive TBP-binding through the removal of non-specifically bound TBP. Consistent with this idea, a recent genome-wide analysis of TBP binding showed that expression of a Mot1 mutant lacking the Mot1 ATPase domain using a transient replacement strategy resulted in dramatic redistribution of TBP (VENTERS *et al.* 2011). Therefore, Mot1 may be important for placing Mot1 at promoters that are otherwise less preferred by TBP. Additionally, even within the same promoter, Mot1 is important for establishing the correct orientation of TBP binding. For example, Mot1 promotes transcription of *URA3* by removing a non-productive TBP bound in the reverse orientation (SPROUSE *et al.* 2008b). The detection of unstable transcripts produced in the opposite direction and multiple TBP sites at many promoters, suggests that promoters are inherently bidirectional in yeast (NEIL *et al.* 2009; XU *et al.* 2009) and humans cells (CORE *et al.* 2008; DENISSOV *et al.* 2007; HE *et al.* 2008; PREKER *et al.* 2008). Therefore, Mot1 may play an important wide-spread role in establishing productive transcription by regulating TBP binding.

Mot1 functionally interacts with another factor that regulates TBP binding, NC2. NC2 is composed of 2 subunits each of Dr1 and Drap1, which interact via a histone fold-like domain (GOPPELT *et al.* 1996; INOSTROZA *et al.* 1992; YEUNG *et al.* 1997). Like Mot1, Drap1 was also identified in a search for mutations that bypassed the requirement for the UAS in *SUC2* transcription (PRELICH 1997). Therefore, the gene encoding Drap1 is also known as *BUR6* (Bypass UAS Requirement 6). NC2 interacts physically with TBP *in vitro* and genetically *in vivo* (CANG *et al.* 1999). Its colocalization with Mot1 and TBP suggests that NC2 cooperates with Mot1 to regulate transcription (DASGUPTA *et al.* 2002; GEISBERG *et al.* 2001; GEISBERG *et al.* 2002; GEISBERG and STRUHL 2004; VAN WERVEN *et al.* 2008; ZANTON and PUGH 2006). In

fact, Mot1 and NC2 physically interact, further supporting cooperative function (KLEJMAN *et al.* 2004). NC2 does not alter TBP-DNA binding, however. Instead, it prevents TFIIA and TFIIB from associating with the TBP-DNA complex (GOPPELT *et al.* 1996; KIM *et al.* 1995). Similar to NC2, the NOT complex prevents assembly of the pre-initiation complex. The NOT complex consists of Not1, Not2, Not3, Not4, Not5, Caf1, and Ccr4 (BAI *et al.* 1999; COLLART and STRUHL 1994; LIU *et al.* 1998). The NOT complex prevents recruitment of the RNA Pol II holoenzyme by preventing the interaction between Spt3 and DNA-bound TBP (reviewed in HAMPSEY 1998).

### 1.4.3 Corepressors

Corepressors do not bind to DNA themselves, but associate with DNA-binding repressors to promote transcriptional repression. Often, corepressors are recruited to genes by a wide-variety of DNA-binding repressors, allowing then to have wide-spread repressive effects. Additionally, corepressors recruit additional protein complexes that have repressive functions, including histone modifying enzymes and chromatin remodelers. One of the first corepressor complexes identified, Tup1-Ssn6, exhibits all of these features that are critical to corepressor function. Tup1-Ssn6 was shown to be necessary for repression of mating type a-specific genes by the DNA binding repressor complex α2-Mcm1 in *Saccharomyces cerevisiae* (KELEHER *et al.* 1992). In addition to the repression of a-specific genes, genome-wide expression studies indicated that Tup1-Ssn6 is required for the repression of more than 180 genes in yeast (GREEN and JOHNSON 2004; SMITH and JOHNSON 2000). Tup1-Ssn6 is recruited to genes by a variety of gene-specific DNA binding repressors, including Mig1, which regulates glucose-repressed genes (NEHLIN *et al.* 1991), Crt1, which represses DNA-damage response genes (HUANG *et al.* 1998), and Rox1,

which represses anaerobic response genes (BALASUBRAMANIAN *et al.* 1993). Tup1-Ssn6 consists of 3 or 4 molecules of Tup1 and 1 of Ssn6 (also called Cyc8). Tup1 contains an N-terminal helical region (JABET *et al.* 2000) and a C-terminal region containing 7 WD40 repeats that fold into a seven-bladed propeller which is important for interacting with repressors (GREEN and JOHNSON 2005; SPRAGUE *et al.* 2000). Ssn6 contains 10 tetratricopeptide repeats (TPRs) (SCHULTZ *et al.* 1990). Different TPRs are required for the repression of certain genes (TZAMARIAS and STRUHL 1995) and for interaction with different HDACs (DAVIE *et al.* 2003; DAVIE *et al.* 2002). Therefore, the multiple sites of interaction allow Tup1-Ssn6 to coordinate with a variety of binding partners to elicit gene-specific repression.

Once recruited to genes, Tup1-Ssn6 mediates repression through interactions with components of the transcription machinery and regulation of chromatin structure. Mediator is a multi-subunit complex that has multiple roles in transcription, including facilitating assembly of the transcription machinery during initiation and mediating interactions between gene-specific regulators and RNA Pol II (reviewed in SIKORSKI and BURATOWSKI 2009). Tup1-Ssn6 is thought to repress transcription by interfering with mediator-activator interactions. For example, for the repression of some genes, Tup1-Ssn6 has been shown to bind and sequester Srb7, a subunit of mediator (GROMOLLER and LEHMING 2000). Since the association of Srb7 with mediator is required for activation, the interaction of Srb7 with Tup1-Ssn6 prevents transcriptional activation (GROMOLLER and LEHMING 2000).

Tup1-Ssn6 also promotes transcriptional repression through effects on histone acetylation. Tup1 preferentially interacts with hypoacetylated histone H3 and H4 via its helical N-terminal region and this interaction is required for repression of many genes (EDMONDSON *et al.* 1996). Additionally, Tup1-Ssn6 physically interacts with multiple HDACs, including Rpd3,

Hos1, and Hos2 (DAVIE *et al.* 2003). Therefore, Tup1-Ssn6 mediates repression through the recruitment of HDACs. In accordance with this idea, Tup1-Ssn6 localizes to regions of hypoacetylated chromatin (BONE and ROTH 2001; DAVIE *et al.* 2002). Furthermore, the combined loss of Rpd3, Hos1, and Hos2 result in the derepression of several Tup1-Ssn6-repressed genes (DAVIE *et al.* 2003; DAVIE *et al.* 2002; WATSON *et al.* 2000). Consistent with its role in chromatin regulation, Tup1-Ssn6 also appears to contribute to nucleosome repositioning at some promoters in cooperation with the ISW2 chromatin remodeler (ZHANG and REESE 2004). Interestingly, Tup1-Ssn6 also has a less-understood role in gene activation through the recruitment of SAGA, the Gcn5-containing HAT complex, and the Swi/Snf chromatin remodeler (PAPAMICHOS-CHRONAKIS *et al.* 2002; PROFT and STRUHL 2002).

Although Tup1 and Ssn6 have no sequence homologs in higher eukaryotes, homologs exist in *Schizosaccharomyces pombe* and *Caenorhabditis elegans*. Importantly, structurally and functionally related corepressors are found in higher eukaryotes (reviewed in COUREY and JIA 2001), including *Drosophila* Groucho and mammalian Transducin beta-like and transducin beta-like related (TBL/TBLR) proteins. Groucho plays an important role in many developmental processes, including sex determination, pattern formation, and eye development. TBL/TBLR proteins function with a wide variety of mammalian repressors. Both Groucho and TBL/TBLR proteins have protein domains with similar structure and function as Tup1, including a glutamine-rich N-terminal tetramerization domain (LI 2000) and a C-terminal propeller structure consisting of WD repeat domains (PICKLES *et al.* 2002). Additionally, both Groucho and TBL/TBLR bind histones and interact with HDACs, suggesting that they mediate repression by regulating chromatin structure similar to Tup1-Ssn6 (BRANTJES *et al.* 2001; CHEN *et al.* 1999;

EDMONDSON *et al.* 1996; FLORES-SAAIB and COUREY 2000; PALAPARTI *et al.* 1997; YOON *et al.* 2003a).

### 1.4.4 Regulation of gene expression by non-coding RNAs

Recently developed technologies, such as high-density microarrays and high-throughput sequencing, have revealed that the eukaryotic transcriptome is much more complex than previously thought. In addition to genome-wide chromatin immunoprecipitation (ChIP) analyses unexpectedly localizing RNA Pol II to intergenic regions (STEINMETZ *et al.* 2006), genome-wide transcription analyses discovered that up to 85% of the yeast genome is transcribed (DAVID *et al.* 2006; NAGALAKSHMI *et al.* 2008). Similar results were obtained with multiple organisms, including humans (FEJES-TOTH *et al.* 2009; KIM *et al.* 2005b). Non-coding RNAs (ncRNAs) account for a large portion of the transcription observed (DAVID *et al.* 2006; FEJES-TOTH *et al.* 2009; NAGALAKSHMI *et al.* 2008). Interestingly, many ncRNAs arise from start sites within intergenic regions and overlap with coding genes. Furthermore, the majority of ncRNAs divergently arise from the promoters of coding genes, demonstrating the bidirectional nature of promoters in yeast and higher eukaryotes (NEIL *et al.* 2009; XU *et al.* 2009).

In human cells, ncRNAs can be categorized based on their location. Many of the ncRNAs observed were identified as promoter-associated short or long RNAs (PASRs and PALRs) (FEJES-TOTH *et al.* 2009; KAPRANOV *et al.* 2007). Most promoter-associated ncRNAs are transcribed in the same direction as overlapping coding regions. Others, however, arise from start sites within the 3' untranslated regions (UTR) of coding genes and are designated termini-associated short RNAs (TASRs) (FEJES-TOTH *et al.* 2009; KAPRANOV *et al.* 2007). Promoter-

associated ncRNAs are most prevalent, accounting for nearly 20% of all sequences (FEJES-TOTH *et al.* 2009). Importantly, given the wide-spread overlap with coding genes, ncRNAs clearly have the potential to regulate gene expression.

#### 1.4.4.1 SUTs and CUTs

In yeast, ncRNAs can be divided into two groups based on their stability. Stable unannotated transcripts (SUTs) are detected in wild-type strains (NEIL *et al.* 2009; XU *et al.* 2009). Cryptic unstable transcripts (CUTs), which are degraded by the nuclear exosome, can be observed in strains lacking Rrp6, the catalytic subunit of the exosome (NEIL *et al.* 2009; XU *et al.* 2009). CUT RNA biogenesis has been somewhat defined. Transcription termination of CUTs requires Nab3 and Nrd1, which also direct termination of non-polyadenylated transcripts (ARIGO *et al.* 2006; THIEBAUT *et al.* 2006). Interestingly, Nab3 and Nrd1 recruit the TRAMP polyadenylation complex to CUTs (THIEBAUT *et al.* 2006). Polyadenylation by TRAMP appears to target CUTs for 3' to 5' degradation by the nuclear exosome (LACAVA *et al.* 2005; VANACOVA *et al.* 2005; WYERS *et al.* 2005). Additionally, many CUTs are subject to decapping and 5' to 3' cytoplasmic decay by Xrn1 (LEE *et al.* 2008a; THOMPSON and PARKER 2007).

SUTs and CUTs can overlap coding regions in either the sense or antisense direction (NEIL *et al.* 2009; XU *et al.* 2009). SUTs and CUTs account for about 12% of transcripts in yeast (XU *et al.* 2009). Approximately 70% of SUTs and CUTs in yeast exhibit a transcription start site that is located within the 5' nucleosome free region within a gene's promoter (MAVRICH *et al.* 2008; NEIL *et al.* 2009; XU *et al.* 2009). Of these, the majority are divergently transcribed relative to the mRNA (NEIL *et al.* 2009; XU *et al.* 2009). Almost 30% of SUTs and CUTs arise from the 3' nucleosome free region of a gene (MAVRICH *et al.* 2008; NEIL *et al.* 2009; XU *et al.*

2009). The majority of these transcripts are transcribed in the antisense direction relative to the mRNA (NEIL *et al.* 2009; XU *et al.* 2009).

#### 1.4.4.2 ncRNAs in X-inactivation

While the pervasiveness of regulatory ncRNAs is not known, close examination of a growing number of genes indicates that ncRNAs have an important role in regulating gene expression at a subset of loci. X-inactivation by Xist may be one of the best understood examples of gene regulation by ncRNAs. Xist RNA is transcribed from the X-inactivation center on the inactive X chromosome (BROCKDORFF *et al.* 1992; BROWN *et al.* 1992) (Figure 4A). It mediates repression by coating the inactive X chromosome and recruiting the Polycomb repressive complex 2 (PRC2), which methylates histone H3 K27, a repressive chromatin mark (CLEMSON *et al.* 1996; MARAHRENS *et al.* 1997; PENNY *et al.* 1996). Repeat A (RepA) is another ncRNA that is transcribed from the 5' region of Xist in the same direction. RepA recruits PRC2 to the X-inactivation center to induce transcription of Xist and promote X-inactivation (ZHAO *et al.* 2008a) (Figure 4A). However, on the active X chromosome, transcription of Tsix, a ncRNA in the opposite orientation as Xist, prevents transcription of Xist (LEE 2000; LEE *et al.* 1999; LEE and LU 1999; LUIKENHUIS *et al.* 2001; SADO *et al.* 2001) (Figure 4A). Therefore, transcription of Tsix in the antisense direction inhibits transcription of Xist. Tsix transcription is also regulated by another ncRNA, Xite, which acts as an enhancer to increase Tsix transcription on the active X chromosome (OGAWA and LEE 2003; STAVROPOULOS *et al.* 2005). This complex network of regulatory ncRNAs is not completely understood. However, the regulation of overlapping transcripts and the recruitment of chromatin-related factors by ncRNAs are events that have also been observed in yeast.

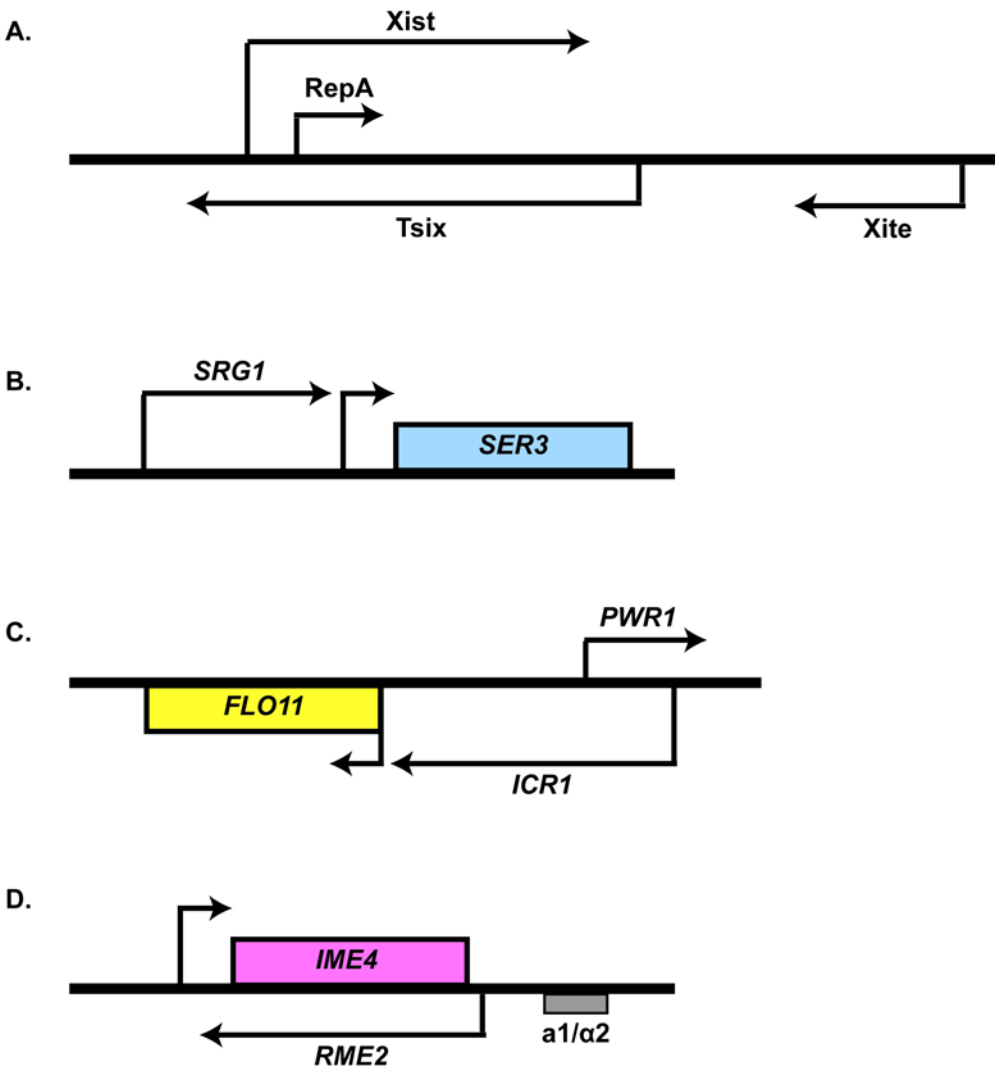


**Figure 4: Schematic of several loci with ncRNAs that regulate gene expression**

Diagram of genes and/or ncRNAs transcribed from the X-inactivation center (A), the *SER3* locus (B), the *FLO11* locus, and the *IME4* locus (D). Genes are represented by colored blocks. ncRNAs are represented by labeled arrows. In (D), the grey bar represents a binding site for the a1/α2 repressor.

#### 1.4.4.3 ncRNAs transcribed in the sense direction relative to overlapping genes

Several examples of regulation of gene expression by ncRNAs have been described in yeast. Probably the best characterized example is the repression of *SRG1* by *SER3*. *SRG1* is a ncRNA whose transcription start site lies upstream of *SER3*, a gene involved in serine biosynthesis (Figure 4B). Unlike Xist and Tsix, *SRG1* and *SER3* are transcribed in the same direction, such that *SRG1* overlaps with the *SER3* promoter (MARTENS *et al.* 2004; THOMPSON and PARKER 2007) (Figure 4B). *SRG1* transcription is required for repression of *SER3* (MARTENS *et al.* 2004; MARTENS *et al.* 2005). The ncRNA product does not mediate repression. However, the act of transcribing *SRG1* promotes repression through a transcriptional interference mechanism that prevents activator binding (MARTENS *et al.* 2004; MARTENS *et al.* 2005). Interestingly, *SRG1* transcription maintains nucleosome occupancy over the *SER3* promoter in a manner that is dependent on the chromatin reassembly factors, FACT and Spt6 (HAINER *et al.* 2011). Therefore, the transcription of a ncRNA obstructs activator binding by establishing a repressive chromatin environment at the promoter of a coding gene.

*FLO11* is also regulated by the transcription of a ncRNA. This discovery was prompted, partly, by the finding that an HDAC, Rpd3L, promotes *FLO11* gene expression (BUMGARNER *et al.* 2009). Since histone deacetylation is generally associated with transcriptional repression, it was predicted that Rpd3L might promote *FLO11* expression indirectly by inhibiting an interfering ncRNA. However, like *SER3*, *FLO11* is repressed by a ncRNA through transcriptional interference (BUMGARNER *et al.* 2009). The transcription start site for the ncRNA, *ICR1*, lies upstream of *FLO11* and both *FLO11* and *ICR1* are expressed from the same strand (BUMGARNER *et al.* 2009) (Figure 4C). Interestingly, *ICR1* is also regulated by a ncRNA

(BUMGARNER *et al.* 2009). *PWR1* encodes a ncRNA that overlaps with *ICR1* in the antisense orientation relative to *ICR1* and represses *ICR1* transcription (BUMGARNER *et al.* 2009) (Figure 4C). It appears that Rpd3L HDAC promotes recruitment of an activator, Flo8. Flo8 induces transcription of *PWR1*, which, in turn, inhibits *ICR1* transcription, relieving transcriptional interference of *FLO11* (BUMGARNER *et al.* 2009).

#### 1.4.4.4 ncRNAs transcribed in the antisense direction relative to overlapping genes

*IME4* regulates entry into meiosis in diploid yeast cells. At the *IME4* locus, a ncRNA, *RME2*, is transcribed in the antisense direction relative to *IME4* (Figure 4D). *RME2* is repressed by a1/α2, which binds to a conserved site located downstream of the *IME4* ORF (HONGAY *et al.* 2006) (Figure 4D). Increased *RME2* transcription is associated with low *IME4* transcription and vice versa (HONGAY *et al.* 2006). The anti-correlation between sense and antisense expression and the fact that *RME2* traverses the *IME4* coding region and promoter suggested a transcriptional interference mechanism of repression. However, it has recently been shown that antisense transcription across the *IME4* promoter is not required for *IME4* repression (GELFAND *et al.* 2011). Instead, regions within the *IME4* coding region are required to confer repression by *RME2* (GELFAND *et al.* 2011). It is now proposed that transcription of *RME2* does not prevent initiation of *IME4* transcription, but does prevent production of full length *IME4* mRNA.

Additionally, the transcription of antisense ncRNAs can regulate gene expression by modulating the chromatin environment. For example, antisense transcription at the *GAL10* locus leads to repression by recruiting the histone H3 K36 methyltransferase, Set2, and subsequent HDAC activity (HOUSELEY *et al.* 2008). Alternatively, antisense transcription has also been shown to positively regulate gene expression. For example, antisense transcription at *PHO5* has been shown to promote transcriptional activation by stimulating chromatin remodeling at the

promoter and promote recruitment of the transcription machinery (UHLER *et al.* 2007). These observations indicate that ncRNAs have both positive and negative effects on the expression of numerous genes, through multiple mechanisms. Therefore, transcription factors that modulate the transcription of ncRNAs may have dramatic and wide-spread effects on gene expression.

## 1.5 *ARG1* AS A MODEL GENE FOR TRANSCRIPTIONAL STUDIES

The *ARG1* gene encodes argininosuccinate synthetase, an enzyme required for arginine biosynthesis. *ARG1* transcription is subject to arginine repression and general amino acid control, and has therefore served as a model gene for the study of transcriptional regulation by these pathways. Additionally, *ARG1* has been used to examine how co-activators, chromatin remodeling enzymes, and histone modifying enzymes contribute to gene activation in inducing conditions. However, much less is known about how *ARG1* transcription is repressed in non-inducing conditions. Importantly, *ARG1* was identified by microarray analysis to be a gene negatively regulated by Ctr9 (Kathryn Sheldon and Karen Arndt, unpublished data) and Paf1 in rich media (PENHEITER *et al.* 2005). With my thesis work, I aim to elucidate the role of the Paf1 complex in transcriptional repression in yeast. To this end, *ARG1* serves as a model locus of Paf1 complex-dependent transcriptional repression.

### 1.5.1 *ARG1* transcription is regulated by several *trans*-acting factors

Arginine metabolism is highly regulated by environmental signals (reviewed in MESSENGUY and DUBOIS 2000). *ARG1* is an arginine anabolic gene that, along with *ARG3* and *ARG4*, is required

for the conversion of ornithine to arginine. To ensure appropriate arginine biosynthesis, *ARG1* transcription is regulated by several *trans*-acting factors that respond to the availability of arginine and other amino acids. In the presence of arginine, the ArgR/Mcm1 complex, consisting of Arg80, Arg81, Arg82, and Mcm1, binds to arginine control (ARC) elements in the *ARG1* promoter and represses *ARG1* transcription (AMAR *et al.* 2000; BECHET *et al.* 1970; CRABEEL *et al.* 1995; CRABEEL *et al.* 1990; DELFORGE *et al.* 1975; DUBOIS *et al.* 1987; EL BAKKOURY *et al.* 2000; QIU *et al.* 1990). In conditions of nutrient starvation, Gcn4 activates *ARG1* transcription by binding to sites within the *ARG1* promoter (DELFORGE *et al.* 1975; HINNEBUSCH 1986). In addition to ARC elements and Gcn4-binding sites, the *ARG1* promoter contains a binding site for the general transcription activator, Abf1 (CRABEEL *et al.* 1995; CRABEEL *et al.* 1988). Although, very little is known about the role of Abf1 in *ARG1* activation, at other genes it has been shown to promote gene expression by altering chromatin structure (GANAPATHI *et al.* 2011; LASCARIS *et al.* 2000; YARRAGUDI *et al.* 2004).

### 1.5.2 Transcriptional activation of *ARG1*

In response to starvation for any amino acid, Gcn4 is induced at the translation level and activates the transcription of amino acid biosynthetic genes, including *ARG1* (reviewed in HINNEBUSCH 2005). Upon binding to the *ARG1* promoter, Gcn4 quickly recruits several co-activators, including SRB/Mediator complex, the HAT-containing complex SAGA, and the chromatin remodeling complexes, SWI/SNF and RSC (SWANSON *et al.* 2003). While the SRB/Mediator complex appears to be recruited independently of other co-activators, RSC is required for SAGA recruitment, and SAGA is required for SWI/SNF recruitment (GOVIND *et al.* 2005; YOON *et al.* 2003b). However, all co-activators stimulate the recruitment of TBP and

RNA Pol II and chromatin immunoprecipitation analyses indicate that all co-activators are recruited at a similar time following gene induction (GOVIND *et al.* 2005; QIU *et al.* 2004). ChIP experiments indicate that Gcn5, the HAT component of SAGA, promotes histone H3 acetylation and nucleosome eviction in the *ARG1* promoter (GOVIND *et al.* 2010; GOVIND *et al.* 2007). However, multiple HDACs, including Rpd3, Hos3, Hos2, and Hda1, prevent an increase in histone acetylation at the 3' end of *ARG1* (GOVIND *et al.* 2010; GOVIND *et al.* 2007). Interestingly, in inducing conditions, Gcn4 constitutively recruits two subunits of the ArgR/Mcm1 repressor complex, Arg80 and Mcm1, to facilitate swift repression upon the availability of arginine (YOON *et al.* 2004). Furthermore, Gcn4 and these subunits of the ArgR/Mcm1 repressor complex stimulate each other's binding to the *ARG1* promoter (HONG and YOON 2011; YOON and HINNEBUSCH 2009). However, the complete ArgR/Mcm1 repressor complex only assembles in the presence of arginine.

### 1.5.3 Transcriptional repression of *ARG1*

In the presence of arginine, ArgR/Mcm1 complex induces transcription of arginine catabolic genes, *CAR1* and *CAR2*, and represses transcription of four anabolic genes, *ARG5,6*, *ARG3*, *ARG8*, and *ARG1* (reviewed in MESSENGUY and DUBOIS 2000). Of the four subunits of the ArgR/Mcm1 repressor complex, Arg80 and Arg81 are specific regulators, while Mcm1 and Arg82 are pleiotropic factors, as they are also involved in mating and sporulation (DUBOIS *et al.* 1987; DUBOIS and MESSENGUY 1994; ELBLE and TYE 1991). Arg82 also has inositol polyphosphate multikinase activity; however, this function is not required for *ARG1* repression (ODOM *et al.* 2000; SAIARDI *et al.* 2000; SAIARDI *et al.* 1999). Arg82 functions in the ArgR/Mcm1 complex by binding to and stabilizing Arg80 and Mcm1 (EL BAKKOURY *et al.*

2000). Mutational analysis suggests that the N-terminal region of Arg81 binds to arginine (AMAR *et al.* 2000), which stimulates binding of Arg81, along with Arg80 and Mcm1, to ARC elements within the *ARG1*promoter (DUBOIS and MESSENGUY 1991). However, little is known about the downstream effects of the assembly of the ArgR/Mcm1 repressor complex.

Besides the ArgR/Mcm1, several chromatin-related factors have been implicated in *ARG1* repression. Paradoxically, some of the factors required for *ARG1* repression in rich media, such as the SRB/Mediator, SAGA, SWI/SNF, and RSC complexes, also promote *ARG1* expression in inducing conditions (RICCI *et al.* 2002; SWANSON *et al.* 2003). However, neither their positive nor their negative roles in *ARG1* transcription have been well-defined. Interestingly, histone H2B ubiquitylation also appears to have opposing regulatory roles in repressing and inducing condtions. Specifically, the loss of histone H2B ubiquitylation results in *ARG1* derepression in non-inducing conditions and reduced transcription in inducing conditions, suggesting that the correct balance of histone H2B ubiquitylation is required for proper *ARG1* expression (LEE *et al.* 2005; MUTIU *et al.* 2007; TURNER *et al.* 2002). However, the mechanistic details of how histone H2B ubiquitylation modulates *ARG1* expression are still unknown.

## 1.6 THESIS AIMS

The conserved Paf1 complex, consisting of Paf1, Rtf1, Ctr9, Cdc73, and Leo1 subunits, is important for proper gene expression in eukaryotes. The Paf1 complex has both positive and negative effects on transcription. The Paf1 complex has been shown to positively influence transcription by modulating several histone modifications. However, the mechanism by which the Paf1 complex mediates transcriptional repression is unknown. The goal of my thesis

research was to elucidate mechanisms by which the Paf1 complex mediates transcriptional repression in *Saccharomyces cerevisiae*. I used a well-characterized gene, *ARG1*, as a model gene of Paf1 complex-dependent repression to answer several critical questions. For example, is the Paf1 complex a direct or indirect repressor of transcription? Are there similarities between positive and negative regulation by the Paf1 complex? More specifically, do known functions of the Paf1 complex contribute to repression? Conversely, does the Paf1 complex have previously unrecognized functions that contribute to repression? And finally, how does the Paf1 complex affect transcription throughout the yeast genome? Fortunately, I was able to address all of these questions with my thesis research.

Interestingly, I found that Paf1 complex-dependent histone modifications that are normally associated with active transcription are enriched on the *ARG1* coding region and contribute to repression. Analyses focusing on the Rtf1 subunit of the Paf1 complex indicate that Rtf1 mediates *ARG1* repression primarily through histone H2B ubiquitylation and histone H3 K4 methylation. However, Paf1 has repressive functions aside from these histone modifications. To further understand the repressive functions of Paf1, I examined the combinatorial effects of multiple gene deletions on *ARG1* expression. These analyses suggest that Paf1 functions independently of the gene-specific repressor complex, the ArgR/Mcm1 complex. However, Paf1 mediates *ARG1* repression partially through the gene-specific activator, Gcn4. Additionally, several genetic and biochemical analyses suggest that Paf1 mediates *ARG1* repression by preventing Gcn4 recruitment to the *ARG1* promoter and subsequent histone H3 acetylation.

Additionally, I found that Paf1 does not alter nucleosome occupancy at the *ARG1* promoter. However, Paf1 appears to prevent antisense transcription from traversing the *ARG1* promoter, which may influence Gcn4 recruitment and *ARG1* expression. Importantly, events

that I observed at my model gene, *ARG1*, occur at other Paf1 complex-repressed genes, suggesting that the Paf1 complex promotes transcriptional repression of a subset of genes through similar mechanisms.

Beyond examining specific Paf1 complex-repressed genes, I used high density tiled microarray analysis to investigate Paf1 complex-dependent transcription throughout the yeast genome. The high resolution data confirmed that numerous genes require the Paf1 complex for proper expression and revealed wide-spread transcription termination defects. Cumulatively, my thesis research is the first detailed investigation into the repressive functions of the Paf1 complex and provides insight into the multiple functions of the Paf1 complex. Additionally, my studies of the yeast Paf1 complex will provide insights into the analogous human complex, which has multiple connections to human health.

# 2.0 THE PAF1 COMPLEX REPRESSES *ARG1* TRANSCRIPTION IN SACCHAROMYCES CEREVISIAE BY PROMOTING HISTONE MODIFICATIONS

## 2.1 INTRODUCTION

The organization of eukaryotic DNA into chromatin presents a significant obstacle to transcription by RNA polymerase II (Pol II). To allow proper gene expression, a multitude of accessory factors associate with RNA Pol II to facilitate transcription of a chromatin template. A conserved, multifunctional protein complex that enables proper RNA Pol II transcription is the Paf1 complex. In *Saccharomyces cerevisiae*, the Paf1 complex consists of Paf1, Ctr9, Cdc73, Rtf1, and Leo1 (KROGAN *et al.* 2002b; MUELLER and JAEHNING 2002; SHI *et al.* 1997; SQUAZZO *et al.* 2002). Many physical and genetic interactions and phenotypes implicate the Paf1 complex in regulating the elongation stage of transcription. Specifically, strains lacking Paf1 complex members exhibit phenotypes associated with transcription elongation defects, such as sensitivity to 6-azauracil and mycophenolic acid (COSTA and ARNDT 2000; SQUAZZO *et al.* 2002). During transcription elongation, the Paf1 complex associates with RNA Pol II on open reading frames (ORFs) (KROGAN *et al.* 2002b; POKHOLOK *et al.* 2002) where it orchestrates modifications to the chromatin template (CHU *et al.* 2007; KROGAN *et al.* 2003a; NG *et al.* 2003a; NG *et al.* 2003b; WOOD *et al.* 2003b) and influences the phosphorylation state of the RNA Pol II carboxy-terminal domain (CTD) (MUELLER *et al.* 2004; NORDICK *et al.* 2008). In addition, the Paf1 complex

genetically and physically interacts with elongation factors such as the Spt4-Spt5 (yDSIF) and Spt16-Pob3 (yFACT) complexes, suggesting coordinated functions of these elongation factors during transcription (COSTA and ARNDT 2000; KROGAN *et al.* 2002b; SQUAZZO *et al.* 2002).

Appropriate transcription by RNA Pol II depends on the dynamic regulation of chromatin structure, which is modulated by histone modifications. Members of the Paf1 complex are required for the establishment of several histone modifications that are associated with active genes. Specifically, Paf1 and Ctr9 are required for histone H3 lysine (K) 36 trimethylation by the histone methyltransferase Set2 (CHU *et al.* 2007), and Paf1 and Rtf1 are needed for methylation of histone H3 K4 and K79 by the histone methyltransferases Set1 and Dot1, respectively (KROGAN *et al.* 2003a; NG *et al.* 2003a; NG *et al.* 2003b). Di- and trimethylation of histone H3 K4 and K79 is dependent on the mono-ubiquitylation of histone H2B K123 by the ubiquitin conjugating enzyme Rad6 and the ubiquitin ligase Bre1 (BRIGGS *et al.* 2002; SUN and ALLIS 2002). Because Paf1 and Rtf1 are also required for histone H2B ubiquitylation, the Paf1 complex most likely regulates histone H3 K4 and K79 methylation indirectly through histone H2B ubiquitylation (NG *et al.* 2003a; WOOD *et al.* 2003b). Both histone H3 K4 methylation and H2B ubiquitylation correlate with active transcription. These modifications are enriched on the coding regions of active genes (BERNSTEIN *et al.* 2002; SANTOS-ROSA *et al.* 2002; XIAO *et al.* 2005) and the necessary histone modifying enzymes are recruited to active genes in a Paf1 complex-dependent manner (KROGAN *et al.* 2003a; NG *et al.* 2003b; XIAO *et al.* 2005). Importantly, Rad6 and Bre1 are evolutionarily conserved and the interconnections between the Paf1 complex, histone H2B ubiquitylation, and gene expression observed in yeast extend to other eukaryotes, including humans (ZHU *et al.* 2005b).

Due to its multiple roles during transcription elongation, it is not surprising that the Paf1 complex is required for the proper expression of many genes (PENHEITER *et al.* 2005). However, it is unclear how the Paf1 complex regulates the expression of most genes or whether the complex employs similar mechanisms to effect gene activation and repression. To investigate the repressive function of the Paf1 complex, I focused on *ARG1*, a gene whose expression is negatively regulated by Paf1 (PENHEITER *et al.* 2005) and whose *cis*- and *trans*-regulatory factors are well characterized. *ARG1* transcription is repressed by the ArgR/Mcm1 complex in rich media (AMAR *et al.* 2000; BECHET *et al.* 1970; CRABEEL *et al.* 1995; CRABEEL *et al.* 1990; DELFORGE *et al.* 1975; DUBOIS *et al.* 1987; EL BAKKOURY *et al.* 2000; QIU *et al.* 1990) and induced by Gcn4 in conditions of amino acid starvation (DELFORGE *et al.* 1975; HINNEBUSCH 1986). Interestingly, although histone H2B ubiquitylation is generally associated with active transcription, this modification has been implicated in *ARG1* repression. Deletion of *RAD6*, mutation of the Rad6 ubiquitin conjugation site, or mutation of histone H2B K123 results in derepression of an *ARG1-lacZ* reporter construct (TURNER *et al.* 2002). Consistent with these observations, both gene-specific and genome-wide studies found increased *ARG1* expression in *htb1-K123R* cells (LEE *et al.* 2005; MUTIU *et al.* 2007; ZHANG *et al.* 2005b). Therefore, it is possible that the Paf1 complex may promote transcriptional activation and repression through the very same histone modifications. However, it is unknown whether the Paf1 complex or Paf1 complex-dependent modifications are enriched at repressed loci such as *ARG1* or contribute to their repression. Therefore, I investigated the role of the Paf1 complex in transcriptional repression, with a particular focus on characterizing the contributions of Paf1 complex-dependent histone modifications to repression. My results indicate that the Paf1 complex associates with and determines the histone modification state at *ARG1* under repressing

conditions, that Rtf1-dependent histone H2B ubiquitylation can both activate and repress transcription, and that Paf1 has roles in *ARG1* repression beyond its known roles in facilitating histone modifications.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Yeast Strains and Media

Rich (YPD) and synthetic complete (SC) media were prepared as described (ROSE 1990). Yeast strains used in these studies are isogenic with FY2, a *GAL2*$^{+}$ derivative of S288C, and listed in Table 1 (WINSTON *et al.* 1995). Because certain cellular auxotrophies influence the level of *ARG1* repression (E. Crisucci and K. Arndt, unpublished observations), experiments were performed with prototrophic strains where possible. Mating types of prototrophic strains were assigned through visual examination of mating with *MAT***a** and *MAT*α tester strains. Gene disruptions were created through PCR-mediated gene replacement via transformation and/or mating, sporulation, and tetrad dissection and confirmed by PCR or Southern analysis (AUSUBEL 1988; ROSE 1990). PCR fragments for gene replacement with *KanMX* were generated by amplification of the *KanMX* cassette on pRS400 (BRACHMANN *et al.* 1998). Strains containing an integrated copy of *htb1-K123R* as the only source of H2B, were constructed and verified as described (TOMSON *et al.* 2011b). Strains containing *rtf1* internal deletion mutations were created through a two-step gene replacement method in which constructs encoding the N-terminally triple HA-tagged Rtf1 derivatives were integrated to replace endogenous *RTF1* (ROTHSTEIN 1991). Comparisons between strains expressing HA-tagged and untagged Rtf1

derivatives revealed that the HA tag did not alter Rtf1 function or interfere with *ARG1* repression. A yeast strain containing an integrated, tagged copy of *RPB1*, *RPB1-13xMYC::KanMX*, was constructed as previously described and generously provided by Joe Martens (HAINER *et al.* 2011).

### 2.2.2 Northern Analysis

Unless stated otherwise, 10 μg of total RNA, isolated from cells grown in YPD at 30°C to a density of 1-2 x $10^7$ cells/ml, were subjected to Northern analysis with random-prime-labeled, PCR-amplified DNA probes for *ARG1* (+34 to +1201), *SNZ1* (+79 to +890), *GAP1* (+133 to +1213), and *SCR1* (-242 to +283) as described previously (SWANSON *et al.* 1991). Signals were quantified using phosphorimager and ImageQuant software. *ARG1* signals were normalized to the loading control *SCR1*. To facilitate comparisons between samples and avoid introducing errors from the very low *ARG1* transcript levels in wild-type strains, normalized *ARG1* transcript levels in experimental samples are presented relative to normalized *ARG1* transcript levels in an *arg80*Δ control strain, which was processed in parallel. The normalized *ARG1* transcript levels in *arg80*Δ samples (not shown) were set equal to one. Relative signals for at least three independent samples were averaged and plotted with standard deviation.

### 2.2.3 Western Analysis

Whole cell extracts were prepared by a rapid boiling method as described (STOLINSKI *et al.* 1997). Briefly, cells were grown in YPD to a density of approximately 4x$10^7$ cells/ml. 1.5 ml culture was harvested by centrifugation and resuspended in 20 μl sample buffer (80mM Tris pH

6.8, 2% SDS, 1% beta-mercaptoethanol, 10% glycerol, 2mM phenylmethanesulfonylfluoride (PMSF), and 0.1% bromophenol blue), and immediately boiled for 2 min at 100°C. After glass bead lysis, an additional 80 µl sample buffer was added, and 20 µl of this lysate were separated on a SDS-10% polyacrylamide gel. Membranes were probed with a 1:2,500 dilution of anti-HA antibody (Roche #11666606001), followed by a 1:5,000 dilution of sheep anti-mouse horseradish peroxidase-coupled secondary antibody (GE Healthcare). As a loading control, membranes were probed with a 1:100,000 dilution of anti-glucose-6-phosphate dehydrogenase antibody (G6PDH, Sigma A9521), followed by a 1:5,000 dilution of donkey anti-rabbit horseradish peroxidase-coupled secondary antibody (GE Healthcare).

### 2.2.4 Chromatin Immunoprecipitation Assays

Cells were grown in YPD to a density of ~1 x $10^7$ cell/ml and harvested or washed and resuspended in minimal media and incubated for an additional 30 minutes. Chromatin was prepared as described previously (SHIRRA *et al.* 2005). Sonicated chromatin was incubated with antibodies at 4°C overnight. Agarose-conjugated anti-HA (Santa Cruz Biotechnology sc-7392 AC) or anti-MYC (Santa Cruz Biotechnology sc-40 AC) was used to precipitate HA-Paf1 or Rpb1-Myc, respectively. Polyclonal anti-Rtf1 antibody (SQUAZZO *et al.* 2002), anti-H3 trimethyl K36 (Abcam ab9050), anti-H3 trimethyl K4 (Active Motif 39159), anti-H3 dimethyl K4 (Millipore 07-030), or anti-H3 (Abcam ab1791), followed by incubation with protein A-coupled Sepharose beads (GE Healthcare 17-5280-01), were used to precipitate Rtf1 or the appropriate histone protein. Precipitated DNA was purified using PCR purification columns (Qiagen). For HA-Paf1 and Rtf1 ChIP assays, two dilutions of input and immunoprecipitated (IP) DNA from three independent chromatin preparations were amplified by PCR in the presence of [α-

$^{32}$P]dATP. PCR products were separated on 6% native polyacrylamide gels and signals were quantified using a phosphorimager and ImageQuant software. After signals were multiplied by their dilution factor, the average input was divided by the average IP signal. IP/input signals for *ARG1* were normalized to a subtelomeric control region on chromosome VI (VOGELAUER *et al.* 2000). For ChIP assays examining histone modification levels, immunoprecipitated DNA from three independent chromatin preparations was used in quantitative real-time PCR with SYBR green detection (Fermentas). IP/input values for the histone modifications were normalized to those for total histone H3. Error bars represent standard error of the mean.

## 2.3 RESULTS

### 2.3.1 Members of the Paf1 complex repress *ARG1* transcription.

The Paf1 complex was first implicated in *ARG1* repression by microarray analyses investigating changes in gene expression in *paf1*Δ cells (PENHEITER *et al.* 2005). To determine if other members of the Paf1 complex are required for *ARG1* repression in rich media (YPD), *ARG1* transcript levels were examined by Northern analysis in wild-type strains and strains lacking individual members of the Paf1 complex (Figure 5A and B). Early in our analysis, I discovered that certain cellular auxotrophies influence the degree of *ARG1* repression in otherwise wild-type cells, even when grown in rich media (data not shown). Therefore, to eliminate any effects of auxotrophies on my measurements, I analyzed *ARG1* transcript levels in prototrophic strains wherever possible. Under these conditions, wild-type cells had very low *ARG1* transcript levels (Figure 5A and B). In contrast, strains lacking Paf1, Ctr9, Cdc73, or Rtf1 exhibited high levels

of *ARG1* expression relative to the isogenic wild-type control strain. Of these strains, *paf1*Δ and *ctr9*Δ mutants were most defective in *ARG1* repression. These results indicate that although Leo1 does not appear to have a very significant role, Paf1, Ctr9, and to a lesser extent Cdc73 and Rtf1 each contribute to *ARG1* repression in nutrient-rich conditions.

To determine if the repressive functions of the Paf1 complex are specific to nutrient-rich conditions or if the Paf1 complex also negatively regulates *ARG1* expression in nutrient-limiting conditions, when *ARG1* is activated, I examined the effects of deleting Paf1 complex members on *ARG1* transcript levels in minimal media. First, I examined the timing of *ARG1* induction in wild-type cells and found that *ARG1* expression was fully induced 30 minutes after the cells were transferred to minimal media from rich media (Figure 5C). Consequently, I measured *ARG1* expression in Paf1 complex mutant strains that were grown in YPD and shifted to minimal media for 30 minutes. I found that deletion of genes encoding Paf1 complex members resulted in slightly higher *ARG1* expression in inducing conditions (Figure 5D and E). Consistent with my results, it was shown previously that *paf1*Δ strains exhibit more *ARG1* expression than wild-type cells when *ARG1* transcription is induced with sulfometuron methyl (SM), which increases cellular levels of the *ARG1* activator Gcn4 (SWANSON *et al.* 2003). SM functions by inhibiting acetolactase synthase, an enzyme that catalyzes the first common step in leucine, isoleucine, and valine biosynthesis (LAROSSA and SCHLOSS 1984). The resulting amino acid deficiencies derepress translation of *GCN4* (HINNEBUSCH 2005). These results suggest that the Paf1 complex acts as a transcriptional repressor of *ARG1* in both repressing and inducing conditions.

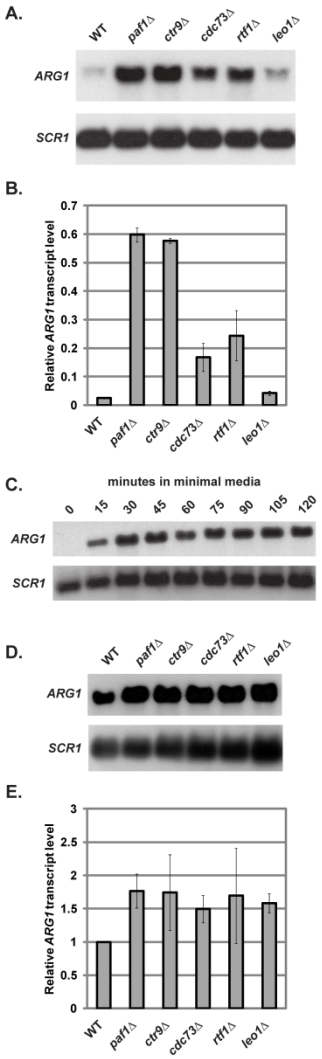


**Figure 5: Members of the Paf1 complex are required for repression of *ARG1* in both repressing and inducing conditions.**

Representative Northern analysis (A) and quantitation (B) of *ARG1* transcript levels in wild-type (KY1699), *paf1*Δ (KY1700), *ctr9*Δ (KY1705), *cdc73*Δ (KY1706), *rtf1*Δ (KY1704), and *leo1*Δ (KY1805) strains. *SCR1* serves as a loading control. Transcript levels were quantified and normalized to the levels detected in an *arg80Δ* (KY1709) control strain (not shown) as described in Materials and Methods. Values shown are the means of three independent

experiments. Error bars represent one standard deviation of the mean. (C) Northern analysis of *ARG1* transcript levels in cells shifted from YPD to minimal media for various times. Wild-type (KY1699) cells were grown to log phase in YPD, washed in water, resuspended in minimal media, and harvested at various time points. Representative Northern analysis (D) and quantitation (E) of *ARG1* transcript levels in wild-type (KY1699), *paf1*Δ (KY1700), *ctr9*Δ (KY1705), *cdc73*Δ (KY1706), *rtf1*Δ (KY1704), and *leo1*Δ (KY1805) strains that were grown to log phase in YPD then shifted to minimal media for 30 minutes prior to harvesting for RNA. Relative signal in wild-type cells was set equal to one. The means of three independent experiments are shown. Error bars represent one standard deviation of the mean.

### 2.3.2 Paf1 and Rtf1 are present at *ARG1* in repressing conditions.

While it is known that the Paf1 complex associates with RNA Pol II during transcription elongation (KROGAN *et al.* 2002b; POKHOLOK *et al.* 2002), it is unclear whether the Paf1 complex localizes to repressed genes. Therefore, I examined whether the Paf1 complex localizes to *ARG1* in repressing conditions by performing chromatin immunoprecipitation (ChIP) analysis using PCR primers that amplify the promoter, 5', middle, and 3' coding region of *ARG1* (Figure 6A). Relative to the untagged control strain, I reproducibly detected a low level of HA-Paf1 occupancy at the *ARG1* coding region in repressing conditions (Figure 6B). Rtf1 occupancy, detected with polyclonal antisera against Rtf1, mirrored that of HA-Paf1 and was enriched over an *rtf1*Δ control strain (Figure 6C). These results indicate that members of the Paf1 complex localize to the *ARG1* coding region in repressing conditions. When cells were shifted to minimal media, HA-Paf1 and Rtf1 occupancy increased across the *ARG1* coding region indicating that,

similar to other active genes, Paf1 complex occupancy correlates with gene expression levels at *ARG1* (Figure 6B and C) (MAYER *et al.* 2010).

Since the Paf1 complex associates with RNA Pol II during transcription elongation (KROGAN *et al.* 2002b; POKHOLOK *et al.* 2002), Paf1 complex occupancy correlates with RNA Pol II levels on active genes (MAYER *et al.* 2010). To determine whether Paf1 complex occupancy also correlates with RNA Pol II occupancy on a Paf1 complex-repressed gene, ChIP analysis was performed to examine Rpb1-Myc levels at *ARG1* in strains grown in repressing or inducing conditions (Figure 6D). In repressing conditions, Rpb1-Myc was enriched at the *ARG1* promoter and coding region compared to the untagged control strain, indicating that low levels of RNA Pol II are present at *ARG1* in repressing conditions (Figure 6D). This is consistent with my finding that long exposures of Northern blots revealed low levels of *ARG1* transcription in wild-type cells grown in rich media (data not shown). As expected, Rpb1-Myc occupancy increased across the *ARG1* coding region when cells were shifted to minimal media (Figure 6D). Therefore, similar to its association with activated genes, the Paf1 complex likely associates with *ARG1* through its interaction with RNA Pol II even under repressing conditions.

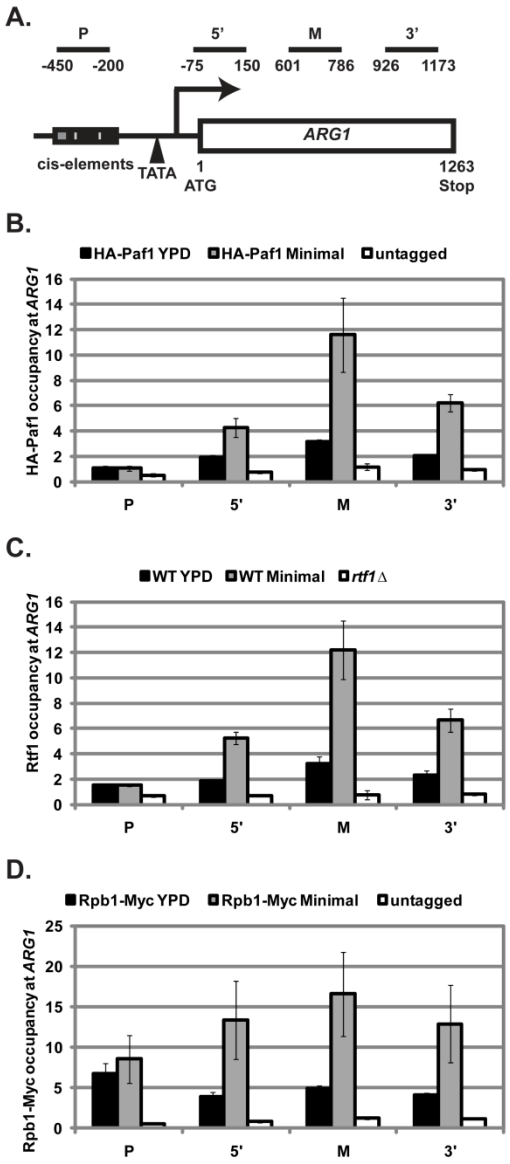


**Figure 6: The Paf1 complex localizes to the *ARG1* promoter and coding region in repressing conditions.**

(A) Locations of PCR products used for ChIP analysis of the *ARG1* locus. (B) ChIP analysis of HA-Paf1 occupancy at promoter (P), 5', middle (M), and 3' regions of *ARG1* in cells expressing HA-tagged Paf1 (KY1721) or untagged Paf1 (KY1699) grown in rich media (YPD) or shifted to minimal media for 30 minutes (Minimal). (C) ChIP analysis of Rtf1 association with *ARG1* in wild-type (KY1699) and *rtf1*Δ (KY1704) strains grown in rich media (YPD) or shifted to minimal media for 30 minutes (Minimal). (D) ChIP analysis of Rpb1-Myc occupancy at the *ARG1* locus in an untagged control strain (KY1699) and cells expressing Myc-tagged Rpb1 (KY1302) grown in

YPD or shifted to minimal media for 30 minutes. ChIP data were quantified and normalized as described in Materials and Methods. Shown are the means of three independent experiments. Error bars represent standard error of the mean.

### 2.3.3 Histone H3 methylation contributes to Paf1 complex-mediated *ARG1* repression.

The presence of the Paf1 complex at the *ARG1* locus in repressing conditions suggests that the Paf1 complex may regulate the histone modification state at *ARG1* under these conditions. To determine if histone H3 methylation is present at the promoter, 5', middle, or 3' coding region of *ARG1* in repressing conditions, I performed ChIP assays using antibodies that detect histone H3 K4 trimethylation, H3 K4 dimethylation, and H3 K36 trimethylation. While total histone H3 levels were similar in all strains examined (Figure 7D), changes in the histone modification pattern at the *ARG1* locus were detected in the absence of Paf1 complex members. Specifically, both histone H3 K4 di- and trimethylation marks were detected in wild-type strains at all four regions examined but were lost in strains deleted for Paf1, Rtf1, or Set1 (Figure 7A and B). Similarly, whereas histone H3 K36 trimethylation was detected at all four locations in the wild-type strain, H3 K36 trimethylation was undetectable in the *paf1*Δ and *set2*Δ strains (Figure 7C). Furthermore, histone H3 K36 trimethylation was specifically reduced at the promoter in the absence of Rtf1 (Figure 7C). These results indicate that the histone H3 K4 and K36 methylation marks are present at the *ARG1* locus in repressing conditions in a Paf1 complex-dependent manner.

To determine to what extent Paf1 complex-dependent histone H3 methylation is required for *ARG1* repression, I performed Northern analysis of *ARG1* transcript levels in strains lacking Set1, Set2, or Dot1. While previous work showed that deletion of *SET2* or *DOT1* caused increased expression of an *ARG1-lacZ* reporter construct (MUTIU *et al.* 2007), these mutations did not lead to a change in repression of the native *ARG1* gene that was statistically different from wild-type (Figure 7E and F). The differing results may be due to increased sensitivity of the *ARG1* expression reporter or the presence of auxotrophies in the previously analyzed strains. In contrast, *set1*Δ strains exhibited an increase in *ARG1* expression (Figure 7E and F). This result is consistent with the finding that loss of Bre2 or Swd3, components of the Set1-containing COMPASS complex, results in increased expression of an *ARG1-lacZ* reporter construct (MUTIU *et al.* 2007). However the increase in endogenous *ARG1* transcript levels in *set1*Δ cells was not as high as in *paf1*Δ cells (Figure 7E and F). Together these results suggest that none of the methyltransferases examined individually are as important for *ARG1* repression as Paf1. Since the Paf1 complex is important for multiple methylation marks, I tested whether the combined loss of multiple methyltransferases might derepress *ARG1* to the same degree as deleting *PAF1*. Surprisingly, no combination of double or triple mutations caused any more than a ~2.6-fold increase in *ARG1* transcript levels, whereas deletion of *PAF1* resulted in a ~12 fold increase in *ARG1* transcript levels (Figure 7E and F). Together, these results demonstrate that Paf1-dependent histone H3 K4 and K36 methylation are present at *ARG1* in repressing conditions and histone H3 K4 methylation contributes to *ARG1* repression; however the Paf1 subunit has repressive functions in addition to facilitating histone H3 methylation.

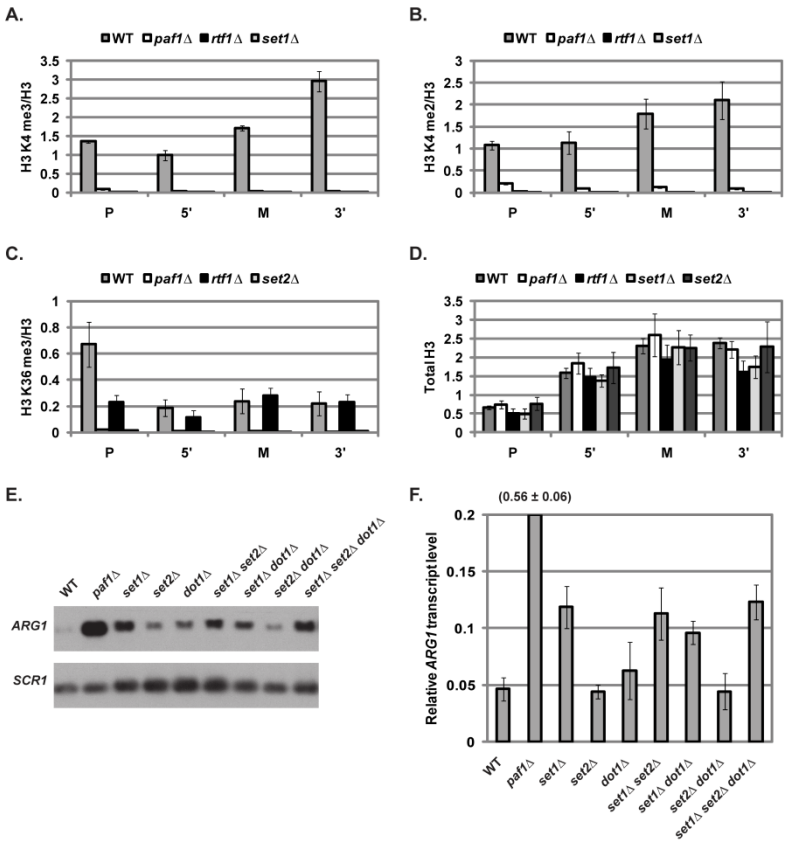


**Figure 7: Paf1 complex-dependent histone modifications are present at *ARG1* in repressing conditions and contribute to transcriptional repression.**

Paf1 complex-dependent histone modifications are present at *ARG1* in repressing conditions and contribute to transcriptional repression. ChIP analysis of relative H3 K4 trimethylation (me3) (A), H3 K4 dimethylation (me2) (B), H3 K36 me3 (C), and total H3 (D) levels in wild-type (KY1699), *paf1*Δ (KY1700), *rtf1*Δ (KY1704), *set1*Δ (KY1755), and *set2*Δ (KY1716) strains. ChIP data were quantified and normalized as described in Materials and Methods. Histone H3 K4 me3, K4 me2, and K36 me3 levels are presented relative to total H3 levels. The means of three independent experiments are shown. Error bars represent standard error of the mean. Representative Northern analysis (E) and quantitation (F) of *ARG1* mRNA levels in wild-type (KY1699), *set1*Δ (KY1715), *set2*Δ (KY1716), *dot1*Δ (KY1717), *set1*Δ *set2*Δ (KY1821), *set1*Δ *dot1*Δ (KY1826), *set2*Δ *dot1*Δ (KY1832), and *set1*Δ *set2*Δ *dot1*Δ (KY1847) strains. Values shown are the means of three independent experiments, quantified and normalized to the levels detected in an *arg80Δ* (KY1709) control strain (not shown) as described in Materials and Methods. Error bars

represent one standard deviation of the mean. The y axis was cropped to allow for comparisons between lower values. The value for *paf1*Δ is indicated above the appropriate bar.

### 2.3.4 Rtf1 represses *ARG1* by promoting histone modifications.

My results demonstrate that histone H3 K4 methylation is present at *ARG1* in cells grown in rich media and can contribute to *ARG1* repression. Because histone H3 K4 methylation is dependent on histone H2B ubiquitylation (SUN and ALLIS 2002; WOOD *et al.* 2003b), I tested whether the Paf1 complex mediates *ARG1* repression by promoting histone H2B K123 ubiquitylation. Indeed, as previously reported, I found that *rad6*Δ and *bre1*Δ strains and strains in which the histone H2B ubiquitylation site is mutated (*htb1-K123R*) exhibited *ARG1* derepression (Figure 8A and B) (HOSSAIN *et al.* 2009; LEE *et al.* 2005; MUTIU *et al.* 2007; TURNER *et al.* 2002; ZHANG *et al.* 2005b). Note that *rad6*Δ cells exhibited higher levels of *ARG1* derepression than either *bre1*Δ or *htb1-K123R* strains. These results are consistent with reports that Rad6 may function with another ubiquitin ligase that is required for *ARG1* repression and further suggest that Rad6 has additional targets important for *ARG1* repression (TURNER *et al.* 2002). Together, these results suggest that histone H2B ubiquitylation and downstream histone H3 K4 methylation are important for *ARG1* repression. The Arndt lab previously identified a region within Rtf1 that is essential for these histone modifications (WARNER *et al.* 2007); therefore, I decided to further examine the role of Rtf1 in *ARG1* repression.

In addition to defining the region of Rtf1 required for histone modifications, the Arndt lab assigned other Rtf1 functions, including ORF association, Paf1 complex assembly, and interaction with the chromatin remodeling factor Chd1, to specific regions of the Rtf1 protein using deletion analysis (WARNER *et al.* 2007). To determine which region and thus which function of Rtf1 is important for *ARG1* repression, *ARG1* transcript levels were examined in *rtf1* deletion strains that define different functional classes (Figure 9C-E). Mutations were chosen because they delete a region of Rtf1 with a known function (*rtf1 Δ1, Δ3, Δ5, Δ7, Δ12,* and *Δ13*) or because they cause a phenotype that indicates a defect in transcription (*rtf1 Δ5*). While Western analysis confirmed that the internal Rtf1 deletion mutant proteins were expressed to similar levels as full-length Rtf1 (Figure 8D), Northern analysis indicated that the internal *rtf1* deletions had differential effects on *ARG1* transcript levels. Deletion of Rtf1 region 1 (amino acids 3-30), which is required for an interaction between Rtf1 and Chd1 (WARNER *et al.* 2007), did not cause *ARG1* derepression, suggesting that Rtf1-dependent recruitment of Chd1 is not required for *ARG1* repression (Figure 8E and F). Consistent with this result, a *chd1*Δ mutation did not alter *ARG1* repression (data not shown). Similarly, cells lacking Rtf1 region 7 (amino acids 251-300), which is required for the association of Rtf1 with ORFs (WARNER *et al.* 2007), showed only a slight increase in *ARG1* transcription under repressing conditions (Figure 8E and F). This result suggests that stable association with the *ARG1* coding region may not be required for full repression of *ARG1* by Rtf1. Furthermore, deletion of Rtf1 regions 12 (amino acids 491-535) or 13 (536-558), which are required for the interaction between Rtf1 and other Paf1 complex members, Paf1 and Ctr9 (WARNER *et al.* 2007), did not result in *ARG1* derepression, suggesting that a stable interaction between Rtf1 and other Paf1 complex members is not required for *ARG1* repression (Figure 8E and F).

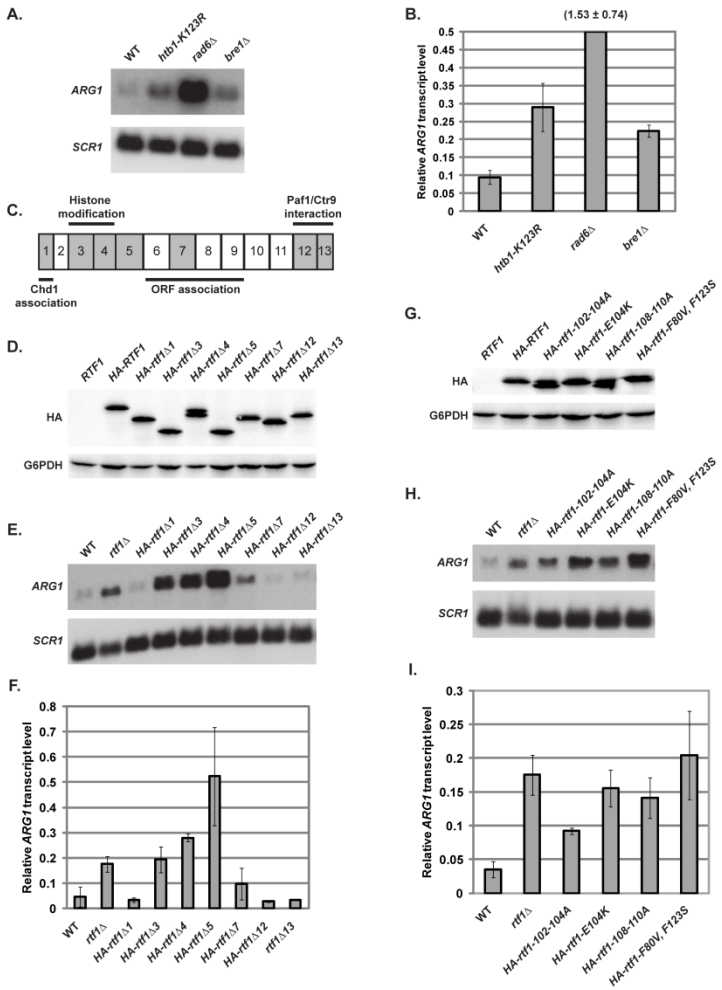


**Figure 8: Rtf1 mediates *ARG1* repression primarily through histone H2B ubiquitylation.**

Representative Northern analysis (A) and quantitation (B) measuring *ARG1* transcript levels in wild-type (KY1698), *htb-K123R* (KY1732), *rad6*Δ (KY1711), and *bre1*Δ (KY1713) strains. The y axis was shortened to facilitate comparison between lower values. The value for *rad6*Δ is indicated above the bar. (C) Schematic of the 13 regions of Rtf1 defined by internal deletion mutations and their associated function (62). In grey are regions that were examined for effects on *ARG1* repression. (D) Western analysis of wild type and mutant Rtf1 protein levels using an anti-HA antibody in strains expressing untagged Rtf1 (KY1698), HA-Rtf1 (KY2082), HA-rtf1Δ1 (KY1722), HA-rtf1Δ3 (KY1723), HA-rtf1Δ4 (KY1724), HA-rtf1Δ5 (KY1980), HA-rtf1Δ7 (KY1725), HA-rtf1Δ12 (KY1726), or HA-rtf1Δ13 (KY1727). G6PDH serves as a loading control. Note that a faster-migrating band observed for HA-rtf1Δ4 is likely a degradation product, which has been reproducibly observed with several forms of Rtf1 (TOMSON *et al.* 2011b; WARNER *et al.* 2007). Representative Northern analysis (E) and quantitation (F) of relative *ARG1*

transcript levels in wild-type (KY1698), *rtf1*Δ (KY1703), *HA-rtf1*Δ*1* (KY1722), *HA-rtf1*Δ*3* (KY1723), *HA-rtf1*Δ*4* (KY1724), *HA-rtf1*Δ*5* (KY1980), *HA-rtf1*Δ*7* (KY1725), *HA-rtf1*Δ*12* (KY1726), and *HA-rtf1*Δ*13* (KY1727) strains. The means of three independent experiments are shown, quantified and normalized to the levels detected in an *arg80Δ* (KY1709) control strain (not shown) as described in Materials and Methods. Error bars represent one standard deviation of the mean. (G) Western analysis of wild type and mutant Rtf1 protein levels using an anti-HA antibody in strains expressing untagged Rtf1 (KY1698), HA-Rtf1 (KY2082), HA-rtf1-102-104A (KY1981), HA-rtf1-E104K (KY1982), HA-rtf1-108-110A (KY1983), and HA-rtf1-F80V, F123S (KY1984). G6PDH serves as a loading control. The faster-migrating band for HA-rtf1-102-104A and HA-rtf1-108-110A has been previously observed and is likely a product of proteolysis (TOMSON *et al.* 2011b; WARNER *et al.* 2007). Representative Northern analysis (H) and quantitation (I) of relative *ARG1* transcript levels in wild-type (KY1698), *rtf1*Δ (KY1703), *HA-rtf1-102-104A* (KY1981), *HA-rtf1-E104K* (KY1982), *HA-rtf1-108-110A* (KY1983), and *HA-rtf1-F80V, F123S* (KY1984) strains. Graphs depict the means of three independent experiments, quantified and normalized to the levels detected in an *arg80Δ* (KY1709) control strain (not shown) as described in Materials and Methods. Error bars represent one standard deviation of the mean.

Rtf1 regions 3 (amino acids 62-109) and 4 (amino acids 112-152) are required for Rtf1-dependent histone modifications, leading us to define these regions collectively as the Rtf1 histone modification domain (HMD) (TOMSON *et al.* 2011b; WARNER *et al.* 2007). Interestingly, deletion of Rtf1 region 3 or 4 resulted in significant *ARG1* derepression (Figure 8E and F). Furthermore, disruption of the Rtf1 HMD derepressed *ARG1* to the same degree as completely deleting the *RTF1* gene, suggesting that Rtf1 mediates *ARG1* repression primarily through promoting histone modifications (Figure 8E and F). The Arndt lab recently identified a set of specific amino acid substitutions within the Rtf1 HMD that impair its histone modification functions (TOMSON *et al.* 2011b). Therefore, I examined whether these substitutions, which

greatly diminish histone H2B K123 ubiquitylation, also result in *ARG1* derepression. While Western analysis demonstrated that wild-type Rtf1 and Rtf1 point mutant proteins were expressed to similar levels (Figure 8G), cells expressing the Rtf1 point mutants, Rtf1-102-104A, Rtf1-E104K, Rtf1-108-110A, and Rtf1-F80V, F123S, exhibited *ARG1* derepression similar to strains lacking Rtf1 entirely (Figure 8H and I). My results strongly suggest that Rtf1 mediates *ARG1* repression by promoting histone H2B ubiquitylation and subsequent H3 K4 methylation.

Complete deletion of *RTF1* causes a suppressor-of-Ty ($Spt^-$) phenotype, indicating that deletion of *RTF1* suppresses defects in transcription caused by the insertion of Ty transposons or their long terminal repeats within the promoters or 5' ends of genes (STOLINSKI *et al.* 1997). Strains lacking any of Rtf1 regions 3-9 (spanning amino acids 62-395) individually have an $Spt^-$ phenotype suggesting that regions 3-9 are each important for transcriptional regulation (WARNER *et al.* 2007). Of these regions, only region 5 has yet to be assigned a specific function. I found that *rtf1Δ5* strains exhibited levels of *ARG1* derepression that were higher than an *rtf1Δ* strain (Figure 8E and F), indicating that region 5 may have a negative effect on the function of the rest of the protein. Interestingly, although region 5 is not required for histone modifications, secondary structure predictions performed by the VanDemark lab suggest that a portion of Rtf1 region 5 may fold as a domain proximal to the HMD. Furthermore, a portion of region 5 confers RNA binding activity to HMD-containing fragments of Rtf1 (Anthony Piro, unpublished data). Therefore, RNA binding by Rtf1 may play a role in *ARG1* repression. Future analysis of this region may reveal new insights on the regulation or functions of the Paf1 complex.

### 2.3.5 Histone H2B K123 is required for full derepression in *paf1*Δ cells.

My results suggest that Rtf1 mediates *ARG1* repression primarily through histone H2B ubiquitylation and H3 K4 methylation. To test this hypothesis, I examined the effect of mutating the histone H2B ubiquitylation site, alone or in combination with deletion of *RTF1*. I found that *rtf1Δ* and *rtf1Δ htb1-K123R* cells had similar levels of *ARG1* derepression (Fig. 5A and B), consistent with Rtf1 and histone H2B ubiquitylation functioning in the same pathway for *ARG1* repression. However, *rtf1*Δ cells reproducibly showed significantly lower levels of *ARG1* derepression than *paf1*Δ cells, suggesting that Paf1 has repressive functions aside from its role in promoting histone H2B ubiquitylation (Figure 5A and B). To test whether Paf1 and H2B ubiquitylation have independent roles in *ARG1* repression, I performed Northern analysis on *paf1*Δ *htb1-K123R* double mutant cells. If Paf1 and H2B ubiquitylation have completely independent effects on *ARG1* repression, *paf1*Δ *htb1-K123R* double mutant strains should exhibit an elevated level of *ARG1* derepression compared to *paf1*Δ and *htb1-K123R* single mutant strains. In contrast to this prediction, *htb1-K123R* significantly reduced the level of *ARG1* derepression in *paf1*Δ cells (Figure 9C and D). This result suggests that histone H2B ubiquitylation is required for full *ARG1* derepression in *paf1*Δ cells and argues that this modification can have both positive and negative effects on the same gene.

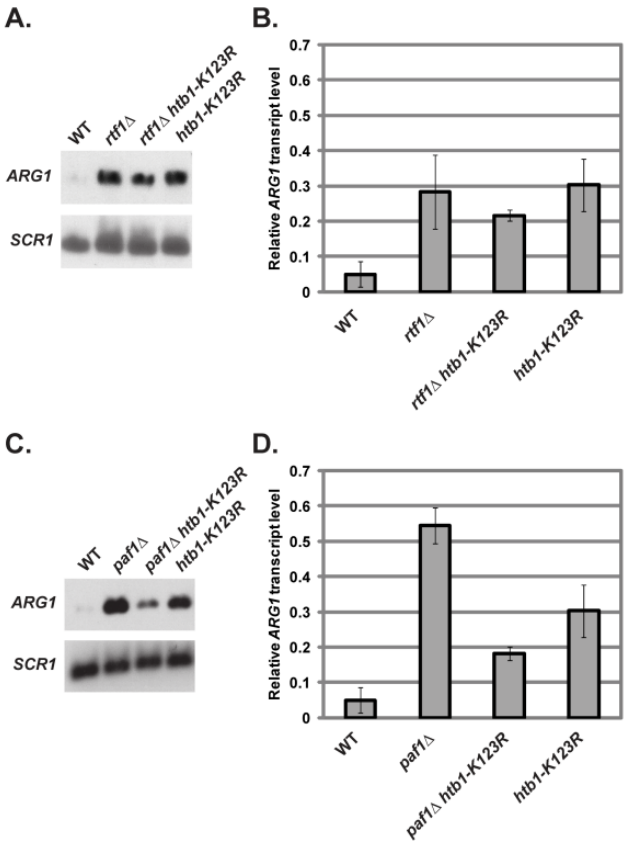


**Figure 9: *ARG1* derepression in *paf1*Δ cells partially requires histone H2B ubiquitylation.**

Representative Northern analysis (A) and quantitation (B) examining *ARG1* transcript levels in wild-type (KY1699), *rtf1*Δ (KY1703), *rtf1*Δ *htb1-K123R* (KY2074), and *htb1-K123R* (KY1732) strains. Representative Northern analysis (C) and quantitation (D) examining *ARG1* transcript levels in wild-type (KY1699), *paf1*Δ (KY1700), *paf1*Δ *htb1-K123R* (KY1731), and *htb1-K123R* (KY1732) strains. The means of three independent experiments are shown, quantified and normalized to the levels detected in an *arg80Δ* (KY1709) control strain (not shown) as described in Materials and Methods. Error bars represent one standard deviation of the mean.

### 2.3.6 The Paf1 complex uses similar mechanisms to repress other genes.

To determine if the manner in which the Paf1 complex mediates repression of *ARG1* extends to other genes, I examined the effects of deleting *PAF1* and *RTF1* on the expression of *SNZ1* and *GAP1*, which encode a protein involved in vitamin B biosynthesis and a general amino acid permease, respectively. I chose to examine these genes because, like *ARG1*, *GAP1* and *SNZ1* have been shown by genome-wide expression studies to be derepressed in *paf1*Δ and *htb1-K123R* strains (MUTIU *et al.* 2007; PENHEITER *et al.* 2005; ZHANG *et al.* 2005b). Using Northern analysis, I found that *SNZ1* and *GAP1* were repressed in wild-type cells and derepressed in the absence of Paf1 or Rtf1 (Figure 10A). Similar to *ARG1*, *paf1*Δ cells exhibited higher derepression of these genes than *rtf1*Δ cells, suggesting that Paf1 and Rtf1 may function in a similar manner at all three genes.

To further test the requirements for *SNZ1* and *GAP1* repression, I performed Northern analyses of these genes in strains expressing the *rtf1* internal deletion mutations. I found that the expression profile of *SNZ1* mirrored that of *ARG1* with *rtf1*Δ*3, rtf1*Δ*4* and *rtf1*Δ*5* cells exhibiting high levels of *SNZ1* derepression (Figure 10B). Consistent with a requirement for the Rtf1 HMD in repressing *SNZ1* transcription, *rtf1* point mutations within the HMD-coding region also caused *SNZ1* derepression (Figure 10C). Furthermore, the levels of *SNZ1* derepression that occurred in these mutants closely mimicked the effects I observed at *ARG1* (Figure 8H and I), with *rtf1-102-104A* cells exhibiting the least dramatic derepression and *rtf1-F80V, F123S* cells exhibiting the most dramatic derepression (Figure 10C).

Similar to both *ARG1* and *SNZ1*, repression of *GAP1* requires a functional Rtf1 HMD, as *rtf1*Δ4 cells or strains expressing the *rtf1* HMD point mutations showed significant derepression of *GAP1* (Figure 10D and E). However, unlike *ARG1* and *SNZ1*, high levels of *GAP1*

derepression did not occur in *rtf1*Δ3 or *rtf1*Δ5, suggesting that Rtf1 regions 3 and 4 are not equivalent in all cases (Figure 10D and E). Furthermore, while amino acid substitutions within the HMD resulted in *GAP1* derepression, the relative levels of derepression caused by these substitutions differed from those observed at *ARG1* and *SNZ1*. Specifically, *rtf1-102-104A* cells exhibited a high level of *GAP1* derepression and *rtf1-F80V, F123S* cells exhibited a low level of *GAP1* derepression (Figure 10E). While the differences between *GAP1* and the other genes examined will likely enrich further studies of the functions of the Paf1 complex, the overall similarities point toward a common mechanism of gene repression by the Paf1 complex in which Rtf1-dependent histone modifications play a prominent role.

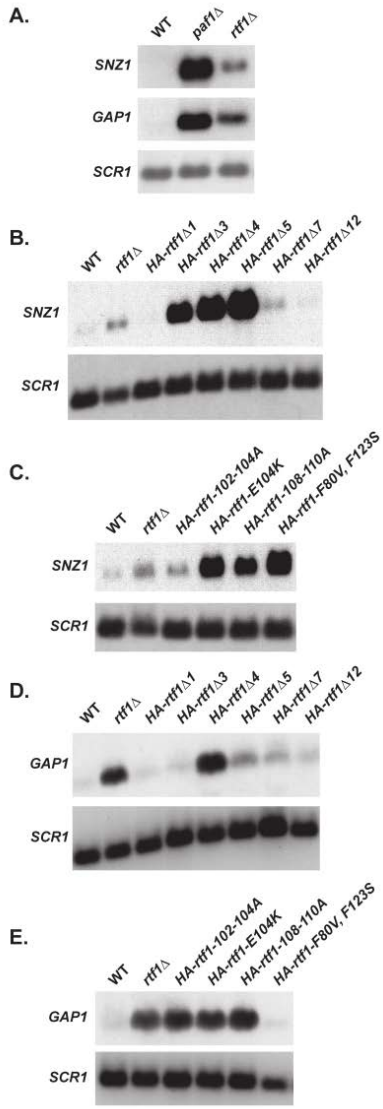


**Figure 10: The Paf1 complex has similar repression mechanisms at other genes.**

Northern analysis of *SNZ1* and *GAP1* transcript levels in wild-type (KY1699), *paf1*Δ (KY1700), and *rtf1*Δ (KY1704) strains. Northern analysis of *SNZ1* (B and C) or *GAP1* (D and E) in wild-type (KY1698), *rtf1*Δ (KY1703), *HA-rtf1Δ1* (KY1722), *HA-rtf1Δ3* (KY1723), *HA-rtf1Δ4* (KY1724), *HA-rtf1Δ5* (KY1980), *HA-rtf1Δ7* (KY1725), *HA-rtf1Δ12* (KY1726), *HA-rtf1-102-104A* (KY1981), *HA-rtf1-E104K* (KY1982), *HA-rtf1-108-110A* (KY1983), and *HA-rtf1-F80V, F123S* (KY1984) strains. Northern blots are representative of at least two independent experiments.

## 2.4 DISCUSSION

In this study, I investigate the mechanisms by which the yeast Paf1 complex negatively regulates transcription, using the well-characterized *ARG1* gene as a framework for my studies. While genome-wide expression patterns indicate that the repressive effects of the Paf1 complex are widespread (PENHEITER *et al.* 2005), an analysis of how the Paf1 complex mediates gene repression has not been previously described. Here, I report that the Paf1, Rtf1, Ctr9, and Cdc73 subunits of the Paf1 complex contribute to *ARG1* repression. Consistent with a direct repressive role, the Paf1 complex is present at the *ARG1* coding region when cells are grown in conditions that strongly repress *ARG1* transcription. Under these conditions, histone modifications primarily controlled by Rtf1 are present at *ARG1* and contribute to repression. Interestingly, Paf1 appears to have repressive functions beyond its role in mediating known Paf1 complex-dependent histone modifications. Finally, an analysis of two additional genes, *SNZ1* and *GAP1*, indicates that the characteristics of Paf1 complex-mediated transcriptional repression observed at *ARG1* extend to other genes.

The correlation between Paf1 complex occupancy and gene activity (MAYER *et al.* 2010) raises the question of how the Paf1 complex is recruited to a gene in repressing conditions. My data indicate a modest but significant occupancy of both the Paf1 complex and RNA Pol II at the *ARG1* coding region in nutrient-rich media. In these conditions, a very low level of transcriptional activity can be detected by my Northern blot assays. Therefore, consistent with its known association with RNA Pol II during transcription elongation (KROGAN *et al.* 2002b; POKHOLOK *et al.* 2002), I hypothesize that the low levels of transcription occurring in repressing conditions are sufficient to result in enrichment of the Paf1 complex across the *ARG1* locus. Interestingly, an antisense transcript traversing the *ARG1* coding region was detected by

Steinmetz and coworkers (DAVID *et al.* 2006; XU *et al.* 2009), raising the possibility that antisense transcription could contribute to RNA Pol II occupancy at *ARG1*. Consistent with transcriptional activity in the antisense direction, histone H3 K4 methylation and K36 methylation at *ARG1* were highest at 3' and 5' locations, respectively, a histone methylation pattern that is opposite of the typical distribution (KIZER *et al.* 2005; KROGAN *et al.* 2003a; KROGAN *et al.* 2003b; LI *et al.* 2003; NG *et al.* 2003b; SCHAFT *et al.* 2003; XIAO *et al.* 2003). A reversed histone modification pattern has been observed at *GAL10* (HOUSELEY *et al.* 2008), one of several genes recently shown to be regulated by antisense transcription (HONGAY *et al.* 2006; HOUSELEY *et al.* 2008; UHLER *et al.* 2007; XU *et al.* 2011). Whether the Paf1 complex and its associated histone modifications repress *ARG1* expression by impacting antisense transcription at the *ARG1* locus remains to be determined.

In accordance with the localization of the Paf1 complex to *ARG1* in repressing conditions, ChIP analysis demonstrated that histone H3 K4 and K36 methylation are significantly enriched at *ARG1* in a Paf1 complex-dependent manner. Both histone H3 K4 and K36 methylation have been shown to impact the levels of histone acetylation on genes through several established pathways of histone crosstalk. In one well-studied pathway, histone H3 K36 dimethylation is required for the activity of the Rpd3S histone deacetylase complex (HDAC), which reduces histone acetylation on transcribed genes and inhibits transcription from cryptic promoters within coding regions (CARROZZA *et al.* 2005; KEOGH *et al.* 2005; LI *et al.* 2007; LI *et al.* 2009). I found that eliminating histone H3 K36 methylation by deleting *SET2* had little impact on *ARG1* expression in repressing conditions, suggesting that Set2-dependent histone deacetylation is unlikely to be involved in maintaining *ARG1* repression. I also found no

indications that the histone H3 K79 methyltransferase, Dot1, plays an important role in *ARG1* repression.

In contrast to the effects of deleting *SET2* and *DOT1*, deletion of *SET1*, the gene encoding the histone H3 K4 methyltransferase, caused a significant reduction in *ARG1* repression. Interestingly, histone H3 K4 methylation has been implicated in pathways that direct either the acetylation or deacetylation of histones. By recruiting the NuA3 histone acetyltransferase (HAT) complex, histone H3 K4 methylation increases histone H3 K14 acetylation levels and gene activation (MARTIN *et al.* 2006; TAVERNA *et al.* 2006). By activating the Set3 HDAC, histone H3 K4 dimethylation lowers histone acetylation levels at the 5' ends of genes (GOVIND *et al.* 2010; KIM and BURATOWSKI 2009). Because histone deacetylation has well-established links to gene repression, including the silencing of genes near telomeres (reviewed in reference SHAHBAZIAN and GRUNSTEIN 2007), it is possible that histone deacetylation driven by histone H3 K4 methylation and the Set3 HDAC could be involved in repressing *ARG1* and other loci. However, I did not observe a loss of *ARG1* repression in *set3Δ* cells (data not shown). Therefore, although I cannot rule out the possibility that histone H3 K4 methylation leads to the recruitment of other HDACs, I currently have no experimental support for a model in which this modification represses *ARG1* through activation of the Set3 complex.

Because Rtf1 is essential for histone H3 K4 di- and trimethylation, I chose to investigate further the role of this Paf1 complex subunit in gene repression. The Arndt lab previously showed that disruption of the Rtf1 HMD, either through deletion or substitution of conserved residues, dramatically reduces global levels of histone H2B K123 ubiquitylation and histone H3 K4 tri- and dimethylation (TOMSON *et al.* 2011b; WARNER *et al.* 2007). Because these same *rtf1* mutations alleviate *ARG1* repression to approximately the same degree as an *rtf1* null allele, I

conclude that Rtf1 mediates *ARG1* repression primarily through its histone modification functions. In support of this idea, a comparison of *rtf1*Δ cells and *rtf1*Δ *htb1-K123R* cells revealed approximately the same levels of *ARG1* depression, strongly suggesting that Rtf1 and histone H2B ubiquitylation function in the same pathway for *ARG1* repression. Therefore, I conclude that Rtf1 mediates repression by promoting histone H2B ubiquitylation and downstream H3 K4 methylation. Similar effects of the *rtf1* mutations were obtained for two other genes, *SNZ1* and *GAP1*, suggesting that Rtf1 can repress a subset of genes through similar mechanisms.

Microarray analysis of transcript levels in *htb1-K123R* cells revealed that the majority of affected genes exhibited increased expression, indicating that the repressive functions of histone H2B ubiquitylation are required at many genes (MUTIU *et al.* 2007). Providing a possible mechanism for gene repression by H2B K123 ubiquitylation, a study revealed that this modification enhances nucleosome stability at the promoters of repressed genes (CHANDRASEKHARAN *et al.* 2009). Although I did not detect a reduction in histone H3 occupancy at the *ARG1* promoter or coding region in *rtf1*Δ cells, it remains possible that my ChIP assays lacked the sensitivity to detect subtle changes in nucleosome stability. In addition to its role in nucleosome stability, histone H2B ubiquitylation is required for proper telomeric silencing (HUANG *et al.* 1997; SUN and ALLIS 2002). Consequently, complete deletion of *RTF1* (KROGAN *et al.* 2003a; NG *et al.* 2003a) or disruption of the Rtf1 HMD results in telomeric silencing defects (TOMSON *et al.* 2011b; WARNER *et al.* 2007). The genome-wide loss of histone H3 K4 and K79 methylation in these cells has been proposed to cause a redistribution of telomeric silencing factors from their normal sites of action (reviewed in reference RUSCHE *et al.*

2003). Whether similar mechanisms can influence the occupancy of regulatory factors at genes such as *ARG1* remains to be determined.

In addition to its repressive role, histone H2B K123 also positively regulates *ARG1* expression under certain circumstances. For example, derepression of *ARG1* in a *paf1*Δ strain is partially suppressed by the *htb1-K123R* substitution (Figure 9). The histone H2B K123 residue itself may be important for full *ARG1* derepression in *paf1Δ* cells through effects on nucleosome structure. Alternatively, the finding of an effect of *htb1-K123R* in a *paf1Δ* cells suggests that in *paf1Δ* cells, a low level of histone H2B ubiquitylation occurs that is required for full levels of *ARG1* derepression. In support of this idea, a *bre1Δ* mutation also partially suppresses *ARG1* transcription in *paf1Δ* strains (data not shown). Another possibility is that histone H2B ubiquitylation and subsequent deubiquitylation, which is important for full expression of inducible genes, such as *GAL1* and *SUC2* (DANIEL *et al.* 2004; HENRY *et al.* 2003; KAO *et al.* 2004), may be required for full *ARG1* expression in the absence of Paf1. Consistent with this possibility, the loss of Ubp8, which deubiquitylates histone H2B, somewhat reduces *ARG1* expression in inducing conditions (LEE *et al.* 2005). Histone H2B ubiquitylation in humans has also been shown to have both positive and negative influences on transcription. For example, histone H2B ubiquitylation facilitates transcription elongation *in vitro* (PAVRI *et al.* 2006) and preferentially associates with sites of active transcription *in vivo* (MINSKY *et al.* 2008). However, removal of histone H2B ubiquitylation by Usp22, the human homolog of Ubp8, inhibits heterochromatic silencing and facilitates gene activation (ZHANG *et al.* 2008; ZHAO *et al.* 2008b). Importantly, histone H2B ubiquitylation in human cells promotes transcription of tumor suppressor genes and represses several proto-oncogenes, indicating that both the negative and

positive transcriptional effects of histone H2B ubiquitylation are critical for cancer prevention (SHEMA *et al.* 2008).

In contrast to my observations on histone modifications, my data do not indicate strong repressive roles for other Rtf1 functions, including Chd1 interaction, ORF association, and Paf1 complex association. In agreement with previous studies (MUELLER *et al.* 2004; WARNER *et al.* 2007), these observations suggest that members of the Paf1 complex retain some functionality when their stable interactions with each other or elongating RNA Pol II are disrupted. Differing reports on whether human Rtf1 is absent from (ROZENBLATT-ROSEN *et al.* 2005; YART *et al.* 2005; ZHU *et al.* 2005b) or present in (KIM *et al.* 2010) the human Paf1 complex has lead to the conclusion that, like *Drosophila* Rtf1 (ADELMAN *et al.* 2006), human Rtf1 is a less stably associated member of the complex. However, despite its less stable association with the Paf1 complex, human Rtf1 retains its effects on gene expression (DING *et al.* 2009; MUNTEAN *et al.* 2010). Therefore, it may not be surprising that, in yeast, repression of a subset of genes by Rtf1 does not require stable association with other Paf1 complex members.

While Rtf1 mediates repression primarily through histone H2B ubiquitylation and its downstream modifications, my results suggest that Paf1 has repressive functions aside from histone H2B ubiquitylation and other known Paf1-dependent histone modifications. As most known roles for the Paf1 complex are intimately connected to histone modifications (reviewed in JAEHNING 2010), it will be important to explore histone modification-independent functions of the complex. Interestingly, in a recent study, the human Paf1 complex was shown to stimulate *in vitro* transcription of a chromatin template independently of histone modifications (KIM *et al.* 2010). The extensive functional conservation between the yeast and human Paf1 complexes (reviewed in JAEHNING 2010) strongly suggests that mechanistic studies of Paf1 complex-

mediated gene repression in yeast will yield insights on the human complex, defects in which are associated with cancers (reviewed in CHAUDHARY *et al.* 2007) and the loss of stem cell identity (DING *et al.* 2009).

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

| Strain | Genotype |
|---|---|
| KY1302 | *MATα RPB1-13xMYC*::*KanMX* |
| KY1698 | *MAT***a** |
| KY1699 | *MATα* |
| KY1700 | *MATα paf1Δ::KanMX* |
| KY1703 | *MAT***a** *rtf1Δ::KanMX* |
| KY1704 | *MATα rtf1Δ::KanMX* |
| KY1705 | *MAT***a** *ctr9Δ::KanMX* |
| KY1706 | *MATα cdc73Δ::KanMX* |
| KY1709 | *MATα arg80Δ::KanMX* |
| KY1711 | *MAT***a** *rad6Δ::KanMX* |
| KY1713 | *MAT***a** *bre1Δ::KanMX* |
| KY1714 | *MAT***a** *bre1Δ::KanMX ura3Δ0* |
| KY1715 | *MAT***a** *set1Δ::KanMX* |
| KY1716 | *MAT***a** *set2Δ::KanMX* |
| KY1717 | *MAT***a** *dot1Δ::KanMX* |
| KY1721 | *MATα 3xHA-PAF1* |
| KY1722 | *MAT***a** *3xHA-rtf1Δ1* |
| KY1723 | *MAT***a** *3xHA-rtf1Δ3* |
| KY1724 | *MAT***a** *3xHA-rtf1Δ4* |
| KY1725 | *MATα 3xHA-rtf1Δ7* |
| KY1726 | *MAT***a** *3xHA-rtf1Δ12* |
| KY1727 | *MAT***a** *3xHA-rtf1Δ13* |
| KY1731 | *MATα HTA1-htb1-K123R (hta2-htb2)Δ::KanMX paf1Δ::KanMX* |
| KY1732 | *MATα HTA1-htb1-K123R (hta2-htb2)Δ::KanMX ura3Δ0* |
| KY1755 | *MATα set1Δ::KanMX* |
| KY1805 | *MATα leo1Δ::KanMX* |
| KY1821 | *MAT***a** *set1Δ::KanMX set2Δ::KanMX* |
| KY1826 | *MATα set1Δ::KanMX dot1Δ::KanMX* |
| KY1832 | *MATα set2Δ::KanMX dot1Δ::KanMX* |
| KY1847 | *MAT***a** *set1Δ::KanMX set2Δ::KanMX dot1Δ::KanMX* |
| KY1980 | *MAT***a** *3xHA-rtf1Δ5* |
| KY1981 | *MAT***a** *3xHA-rtf1-102-104A* |
| KY1982 | *MATα 3xHA-rtf1-E104K* |
| KY1983 | *MAT***a** *3xHA-rtf1-108-110A* |
| KY1984 | *MAT***a** *3xHA-rtf1-F80V, F123S* |
| KY2074 | *MAT***a** *HTA1-htb1-K123R (hta2-htb2)Δ::KanMX rtf1Δ::KanMX* |
| KY2082 | *MATα 3xHA-RTF1 leu2Δ1 trp1Δ63 lys2-128δ ura3-52* |

# 3.0 PAF1 INHIBITS GCN4 RECRUITMENT AND ANTISENSE TRANSCRIPTION AT THE PROMOTERS OF REPRESSED GENES.

## 3.1 INTRODUCTION

Eukaryotic organisms employ several mechanisms to repress gene expression. In one mechanism, DNA binding transcriptional repressors recruit co-repressors that inhibit the basal transcriptional machinery, interfere with activator binding, or recruit histone modifying proteins, such as histone deacetylase complexes (HDACs) (reviewed in PAYANKAULAM *et al.* 2010). The removal of histone acetylation by HDACs is associated with transcriptional repression. For example, in *Saccharomyces cerevisiae*, histone deacetylation by Sir2 mediates the silencing of telomere-adjacent genes (reviewed in SHAHBAZIAN and GRUNSTEIN 2007). Additionally, histone deacetylation by the Rpd3S complex prevents aberrant transcription initiation from cryptic sites within coding regions (CARROZZA *et al.* 2005; KEOGH *et al.* 2005; LI *et al.* 2007; LI *et al.* 2009). Furthermore, the recruitment and/or activity of HDAC complexes has been shown to be regulated by histone methylation in various histone crosstalk pathways. Specifically, histone H3 lysine (K) 36 dimethylation is required for the activity of the Rpd3S complex (CARROZZA *et al.* 2005; KEOGH *et al.* 2005; LI *et al.* 2007; LI *et al.* 2009). Additionally, histone H3 K4 dimethylation activates the Set3 HDAC, which reduces acetylation levels at the 5' ends of genes (GOVIND *et al.* 2010; KIM and BURATOWSKI 2009).

More recently, antisense transcription has been shown to repress transcription. For example, antisense transcription across the *IME4* promoter inhibits *IME4* expression through a transcription-interference mechanism (HONGAY *et al.* 2006). Additionally, antisense transcription can mediate repression by recruiting histone modifying enzymes that create a repressive chromatin structure. For example, antisense transcription at the *GAL10* locus promotes repression by recruiting Set2, the histone H3 K36 methyltransferase, and subsequent histone deacetylase (HDAC) activity (HOUSELEY *et al.* 2008). Interestingly, antisense transcription has also been shown to positively regulate gene expression. For example, antisense transcription at *PHO5* stimulates transcriptional activation by promoting chromatin remodeling and RNA Pol II recruitment at the promoter (UHLER *et al.* 2007).

Through microarray studies, the Paf1 complex, which consists of Paf1, Ctr9, Cdc73, Rtf1, and Leo1 in yeast (KROGAN *et al.* 2002b; MUELLER and JAEHNING 2002; SHI *et al.* 1997; SQUAZZO *et al.* 2002), was also found to play a role in transcriptional repression. How the Paf1 complex functions in gene repression is of great interest because of the many connections between this complex and human disease (reviewed in CHAUDHARY *et al.* 2007; MONIAUX *et al.* 2006; NEWEY *et al.* 2009). The Paf1 complex associates with RNA polymerase II (Pol II) on open reading frames (ORFs) during transcription elongation (KROGAN *et al.* 2002b; MAYER *et al.* 2010; POKHOLOK *et al.* 2002; WADE *et al.* 1996) and regulates the phosphorylation state of the RNA Pol II carboxy-terminal domain (CTD) (MUELLER *et al.* 2004; NORDICK *et al.* 2008). In addition to its functions during transcription elongation, the Paf1 complex is important for proper transcription termination and RNA 3' end formation (MUELLER *et al.* 2004; PENHEITER *et al.* 2005; SHELDON *et al.* 2005). Importantly, like the yeast Paf1 complex, the human Paf1 complex associates with RNA Pol II on actively transcribed genes (ROZENBLATT-ROSEN *et al.* 2005),

promotes proper RNA 3' end formation (NAGAIKE *et al.* 2011; ROZENBLATT-ROSEN *et al.* 2009), and facilitates transcription elongation of a chromatin template (CHEN *et al.* 2009; KIM *et al.* 2010), indicating that the functions of the Paf1 complex are conserved throughout eukaryotes.

The Paf1 complex is also required for several histone modifications (CHU *et al.* 2007; KROGAN *et al.* 2003a; NG *et al.* 2003a; NG *et al.* 2003b; WOOD *et al.* 2003b). The Arndt has previously shown that Paf1 inhibits histone acetylation on the coding region of active genes (CHU *et al.* 2007). Additionally, Paf1 and Rtf1 are required for ubiquitylation of histone H2B (LARIBEE *et al.* 2005; WOOD *et al.* 2003b; XIAO *et al.* 2005) and the subsequent methylation of histone H3 K4 and K79 (BRIGGS *et al.* 2002; KROGAN *et al.* 2003a; NG *et al.* 2003a; NG *et al.* 2003b; SUN and ALLIS 2002). Furthermore, Paf1 and Ctr9 are required for histone H3 K36 trimethylation (CHU *et al.* 2007). As in yeast, the human Paf1 complex is required for these same histone modifications (DING *et al.* 2009; KIM *et al.* 2009a; ROZENBLATT-ROSEN *et al.* 2009; ZHU *et al.* 2005b), which control the expression of many genes, including *HOX* genes (ZHU *et al.* 2005b) and genes that maintain embryonic stem cell identity (DING *et al.* 2009). Therefore, the important roles of the Paf1 complex in transcription may explain its connections to human diseases, such as pancreatic, breast, and renal cancer, among others (reviewed in CHAUDHARY *et al.* 2007; MONIAUX *et al.* 2006; NEWEY *et al.* 2009).

My thesis work aims to understand the role of the Paf1 complex in transcriptional repression in yeast. To this end, *ARG1*, which encodes arginosuccinate synthetase, an enzyme required for arginine biosynthesis, serves as a model locus of Paf1 complex-dependent transcriptional repression. *ARG1* is a valuable model gene because its transcription is modulated by well-characterized pathways, including arginine repression and general amino acid control pathways, mediated by the ArgR/Mcm1 complex and Gcn4, respectively. The ArgR/Mcm1

complex, consisting of Arg80, Arg81, Arg82, and Mcm1, binds to arginine control elements in the *ARG1* promoter and represses *ARG1* transcription in the presence of arginine (AMAR *et al.* 2000; BECHET *et al.* 1970; CRABEEL *et al.* 1995; CRABEEL *et al.* 1990; DELFORGE *et al.* 1975; DUBOIS *et al.* 1987; EL BAKKOURY *et al.* 2000; QIU *et al.* 1990). In conditions of nutrient starvation, Gcn4 activates *ARG1* transcription by binding to sites within the *ARG1* promoter (DELFORGE *et al.* 1975; HINNEBUSCH 1986).

*ARG1* was identified by microarray analysis to be a gene negatively regulated by Paf1 in rich media (PENHEITER *et al.* 2005). I have since found that, in addition to Paf1, other members of the Paf1 complex, including Ctr9, and to a lesser extent, Rtf1 and Cdc73, contribute to *ARG1* repression (CRISUCCI and ARNDT 2011). Furthermore, I have demonstrated that, while Rtf1 mediates *ARG1* repression by promoting histone H2B ubiquitylation and subsequent H3 K4 methylation, Paf1 appears to have repressive functions apart from these histone modifications (CRISUCCI and ARNDT 2011). Therefore, in this chapter, I focus my investigation on the Paf1 subunit of the complex. I found that *ARG1* derepression in *paf1Δ* cells partially requires the *ARG1* coding region. Paf1 mediates *ARG1* repression independently of the ArgR/Mcm1 complex. In contrast, the *ARG1* derepression that occurs in *paf1Δ* strains is associated with increased promoter occupancy of the activator Gcn4, resulting in *ARG1* derepression in a manner that is partially dependent on Gcn4, the histone acetyltransferase Gcn5, and histone H3 acetylation sites. Interestingly, I detect antisense transcription at the *ARG1* locus that traverses the *ARG1* promoter in *paf1Δ* cells. Since this antisense transcription positively correlates with *ARG1* sense transcription, it may regulate *ARG1* sense transcription by increasing promoter accessibility for *ARG1* activators.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Yeast Strains and Media

Rich (YPD) and synthetic complete (SC) media were prepared as described (ROSE 1990). Where indicated, sulfometuron methyl (SM) was added to SC media lacking isoleucine and valine (SC-ILV) at a final concentration of 0.6 μg/ml. Yeast strains used in these studies are isogenic with FY2, a *GAL2+* derivative of S288C, and listed in Table 1 (WINSTON *et al.* 1995). Strains containing *ARG80-13xMYC::HIS3, ARG81-13xMYC::HIS3,* and *ARG82-13xMYC::HIS3* were derived from matings between *paf1Δ::KanMX* cells and strains previously described and generously provided by Alan Hinnebusch (YOON *et al.* 2004). Histone H3 and H4 mutant strains were derived from matings between *paf1Δ::KanMX* cells and strains constructed and generously provided by Jef Boeke (DAI *et al.* 2008). Gene deletions and insertions were created by transforming diploid yeast strains with the appropriate PCR fragment (AUSUBEL 1988; ROSE 1990). Following sporulation and tetrad dissection, genotypes were confirmed by PCR analysis (AUSUBEL 1988; ROSE 1990). The *NatMX* cassette on pAG25 was amplified by PCR for gene replacement with *NatMX* (GOLDSTEIN and MCCUSKER 1999). To generate *gcn4Δ::KanMX* strains, the *gcn4Δ::KanMX* locus in the yeast deletion collection was amplified by PCR as described (SHIRRA *et al.* 2008; WINZELER *et al.* 1999). All other PCR fragments for gene replacement with *KanMX* were generated by PCR amplification of the *KanMX* cassette on pRS400 (BRACHMANN *et al.* 1998).

To generate strains in which a complete *ARG1* gene, with its 5' and 3' regulatory sequences, was inserted at the *LYP1* locus in the opposite orientation of *LYP1*, genomic DNA was used as a template in PCR reactions to amplify *ARG1* (-497 to +1553 relative to the

translation start site), using primers that permitted replacement of the *LYP1* locus (-44 to +2035) (Figure 11). To generate strains in which the *ARG1* promoter and coding region were integrated at the *LYP1* locus in the same orientation as *LYP1*, *ARG1* (-497 to +1263) was amplified by PCR with primers that permitted replacement of *LYP1* (-290 to +1836), such that the *ARG1* promoter and coding region were adjacent to the *LYP1* 3' UTR (Figure 21). For strains in which the *LYP1* promoter, coding region, and 3' UTR were replaced with those of *ARG1* in the same orientation, *ARG1* (-497 to +1553) was amplified by PCR to allow replacement of *LYP1* (-290 to +2059) (Figure 21). PCR products were transformed into *arg1Δ::NatMX* haploid strains. Transformants were selected on SC medium lacking arginine (SC-R) and proper integration was confirmed using PCR analysis and resistance to thialysine. The resulting strains were mated to *paf1Δ::KanMX* strains to obtain *lyp1Δ::ARG1 arg1Δ::NatMX paf1Δ::KanMX.*

To create strains containing the *HIS3* coding region under the control of the *ARG1* promoter and 3' UTR, the *HIS3* coding region (+1 to +663) was amplified from wild-type genomic DNA with primers that permitted replacement of the *ARG1* coding region with that of *HIS3*. The purified PCR product was transformed into a *his3Δ::NatMX* haploid strain. Transformants were selected on SC medium lacking histidine (SC-H). The resulting strain was mated to *paf1Δ::KanMX* strains to obtain *ARG1p-HIS3*$_{1\text{-}663}$ *his3Δ::NatMX paf1Δ::KanMX* strains.

To generate strains containing *HIS3* and its 3' UTR under the control of the *ARG1* promoter, *HIS3* (+1 to +822) was amplified by PCR with primers that permitted replacement of the *ARG1* coding region and 3' UTR (+1 to +1449) with that of *HIS3*. The PCR product was transformed into a *his3Δ200* haploid strain in which *PET56* was integrated at the *LEU2* locus, to prevent petite formation. Transformants were selected on SC-H medium. Integration was

confirmed by PCR analysis. The resulting strains were mated with *his3Δ::NatMX* strains to obtain *ARG1p-HIS3*$_{1-822}$ *his3Δ::NatMX* strains.

Strains containing a terminator element integrated in the antisense direction in the *ARG1* coding region were generated using pDW1, a plasmid generously provided by Joe Martens. Plasmid pDW1 served as a template in PCR reactions to amplify a DNA fragment containing *URA3* flanked by *HIS3* terminator elements. The primers used in the PCR reactions introduced sequences that permitted integration of the *HIS3* terminator at *ARG1* position +420 relative to the translation start site. The resulting PCR fragment was transformed into *ura3Δ0* haploid strains. Transformants were selected on SC medium lacking uracil (SC-U). Transformants were spread on medium containing 5-floroorotic acid to counter-select against *URA3*. Strains that lost *URA3* but retained one integrated terminator element were identified and confirmed by PCR analysis.

A strain containing a temperature sensitive mutation in the gene encoding the largest subunit of RNA polymerase II, *rpb1-1*, was generously provided by Fred Winston and was crossed by a *paf1Δ::KanMX* strain to generate an *rpb1-1 paf1Δ::KanMX* strain (NONET *et al.* 1987). Strains were transformed with a *URA3*-marked vector that expressed a triple HA-tagged form of Paf1 or an empty vector, pRS316. To analyze mRNA stability, strains were grown at 25°C in SC-U media to a cell density of 1-2x$10^7$ cells/ml. Each culture was diluted with an equal volume of SC-U media that was pre-warmed to 49°C and immediately shifted to 37°C. Cells were harvested for RNA isolation at various time points.

### 3.2.2 Northern Analysis

Total RNA was isolated from cells grown in YPD at 30°C to a density of 1-2 x $10^7$ cells/ml. Unless otherwise stated, 10 μg of total RNA was subjected to Northern analysis with random-

prime-labeled DNA probes for *ARG1* (+34 to +1201), *HIS3* (-27 to +376) and *SCR1* (-242 to +283) as described previously (SWANSON *et al.* 1991). A phosphoimager and ImageQuant software were used to quantify signals. *ARG1* or *HIS3* signals were normalized to *SCR1*, which serves as a loading control. The relative *ARG1* signal in *arg80Δ* samples (not always shown), which were grown and processed in parallel with experimental samples, was set equal to one. The relative *HIS3* signal in wild-type samples was set equal to one. Quantitations of Northern analyses represent the mean of at least three independent experiments. Error bars represent standard deviation of the mean.

### 3.2.3 Chromatin Immunoprecipitation Assays

YPD cultures were grown to a cell density of 1-2 x $10^7$ cell/ml and harvested for the isolation of chromatin as described (SHIRRA *et al.* 2005). Immunoprecipitation (IP) of sonicated chromatin was performed as described previously (SHIRRA *et al.* 2005). Agarose-conjugated anti-HA (Santa Cruz Biotechnology) was used to IP a C-terminally triple HA-tagged form of Gcn4. Agarose-conjugated anti-MYC (Santa Cruz Biotechnology) was used to IP a C-terminally 13xMYC-tagged form of Arg80, Arg81, or Arg82. Anti-diacetyl H3 (Millipore, 06-599) or anti-H3 (Abcam ab1791), followed by incubation with protein A-coupled Sepharose beads (GE Healthcare, 17-5280-01) were used to IP the appropriate histone proteins. Input and IP DNA were used as templates in quantitative PCR reactions containing [α-$^{32}$P]dATP. After PCR products were resolved on 6% native polyacrylamide gels, and signals were quantified using a phosphorimager and ImageQuant software. For analysis of epitope-tagged protein occupancy at *ARG1*, IP/input signals for *ARG1* were normalized to a subtelomeric control region on chromosome VI, where, as expected, no occupancy was observed (VOGELAUER *et al.* 2000). For

analysis of histone acetylation levels at *ARG1*, acetylated histone H3 levels were normalized to total H3 levels. The means of three independent experiments were plotted with standard error.

### 3.2.4 β-Galactosidase Assays

*ura3Δ0* strains were transformed with p180 or p227, which express Gcn4-LacZ or a constitutively expressed Gcn4-LacZ, respectively (HINNEBUSCH 1985; MUELLER and HINNEBUSCH 1986). Extracts were prepared and β-galactosidase assays were performed and quantified as previously described (ROSE and BOTSTEIN 1983).

### 3.2.5 Nucleosome Scanning Assays

Cells were grown to a density of 2 x $10^7$ cells/ml in YPD or SC-ILV with 0.6 μg/ml sulfometuron methyl (SM) or DMSO as a control. Nucleosome scanning assays were performed as described (HAINER *et al.* 2011). Control genomic DNA and mononucleosomal DNA, which was generated by digestion with micrococcal nuclease (MNase), was used as a template in real-time PCR using SYBR Green (Fermentas) detection. PCR primers were designed to tile a region containing the *ARG1* promoter, from the translation stop codon of *GPD2* to approximately 500 bases into the *ARG1* coding region. PCR amplicons were approximately 100 base pairs in length with approximately 70 base pairs of overlap with neighboring amplicons. *ARG1* signals were normalized to a well-positioned nucleosome in the *GAL1-10* promoter, GAL NB (BRICKNER *et al.* 2007; HAINER *et al.* 2011; LOHR 1984). Nucleosome scanning assay results were plotted as the relative MNase protection for at least three independent experiments. Error bars represent standard deviation of the mean.

### 3.2.6 Western Analysis

Whole cell extracts were prepared from cells grown in YPD to a density of approximately 4 x$10^7$ cells/ml as described previously (SHIRRA *et al.* 2005). 50 µg of extract were separated on a 15% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. To examine levels of MYC-tagged proteins, membranes were incubated with a 1:1000 dilution of anti-MYC primary antibody (Santa Cruz, sc-40) followed by a 1:5000 dilution of sheep anti-mouse horseradish peroxidase (HRP)-coupled IgG secondary antibody (GE Healthcare, NA931). A 1:2000 dilution of anti-Sse1 antiserum, generously provided by Jeff Brodsky (GOECKELER *et al.* 2002), followed by a 1:5000 dilution of sheep anti-rabbit HRP-coupled IgG secondary antibody (GE Healthcare, NA934) was used to probe for the loading control, Sse1. Alternatively, membranes were probed with a 1:100,000 dilution of anti-glucose-6-phosphate dehydrogenase antibody (G6PDH, Sigma A9521), followed by a 1:5,000 dilution of donkey anti-rabbit horseradish peroxidase-coupled secondary antibody (GE Healthcare) as a loading control.

### 3.2.7 Strand-Specific Reverse Transcription (RT) PCR

Strand-Specific RT-PCR was performed as described (PEROCCHI *et al.* 2007). Briefly, total RNA was DNase-treated with TURBO DNase I (Ambion) at 37°C for 30 minutes and purified with the QIAGEN RNeasy clean-up kit. 2 µg RNA was used in a strand-specific cDNA synthesis reaction with SuperScript II RT (Invitrogen) or a no RT control reaction, both of which contained a primer against the transcript of interest and a primer against the *ACT1* mRNA, as described (PEROCCHI *et al.* 2007). 0.5 to 5 µl of the cDNA synthesis or no RT control reaction was PCR amplified with GoTaq Polymerase (Promega). PCR conditions were as follows: 94°C

for 3 minutes followed by a sequence of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, which was repeated 24 times, followed by incubation at 72°C for 10 minutes.

### 3.2.8 RNA 3' End Mapping

cDNA corresponding to antisense transcripts detected at the *ARG1* locus was prepared using FirstChoice RNA Ligase Mediated-Rapid Amplification of cDNA Ends (RLM-RACE) kit (Applied Biosystems) according to the manufacturer's protocol. Briefly, total RNA from wild-type and *paf1Δ* cells was used in reverse transcription reactions containing a 3' RACE adapter provided by the manufacturer. The 3' RACE adapter contains a defined sequence upstream of $T_{12}$, which anneals to poly(A) tails. After reverse transcription, two sequential PCR reactions were performed using nested primers that were complementary to the antisense transcript and the 3' RACE adapter sequence. For the first PCR reaction (outer PCR), the mRNA-specific primer was designed to anneal to the antisense strand at position +505 relative to the *ARG1* translation start site. The second PCR reaction (inner PCR) was performed with a primer that was designed to anneal to the antisense strand at position *ARG1* +470. Nested PCR primers complementary to the 3' RACE adapter were provided by the manufacturer. The resulting cDNA was inserted into a cloning vector using TOPO TA Cloning Kit (Invitrogen) and transformed into One Shot MAX Efficiency DH5αT1$^R$ *E. coli* (Invitrogen). Plasmid DNA was isolated from the transformed bacteria using the Qiagen mini-prep kit. Plasmid inserts were examined by sequence analysis.

## 3.3 RESULTS

### 3.3.1 Transcriptional read-through of the *GPD2* terminator is not the cause of *ARG1* derepression in *paf1*Δ.

I have previously demonstrated that Rtf1 mediates *ARG1* repression through histone H2B ubiquitylation and histone H4 methylation (CRISUCCI and ARNDT 2011). However, in the absence of Paf1, the high level of *ARG1* mRNA is only partly explained by the loss of histone modifications (CRISUCCI and ARNDT 2011). Therefore, Paf1 has repressive functions aside from these histone modifications. Interestingly, widespread transcriptional defects, such as cryptic initiation within coding regions and transcriptional read-through, occur in the absence of Paf1 (CHU *et al.* 2007, Chapter 4; SHELDON *et al.* 2005; TOMSON *et al.* 2011b). Therefore, it is possible that such defects could indirectly result in *ARG1* derepression. For example, transcriptional read-through of the upstream *GPD2* terminator might regulate *ARG1* expression through a transcriptional interference mechanism in a manner similar to that occurring at the native *SER3* locus (MARTENS *et al.* 2004). To determine whether *ARG1* repression involved the upstream *GPD2* gene, *ARG1* was moved to a different chromosomal location, divorcing it from possible transcriptional read-through of *GPD2*. Specifically, a chromosomal region containing *ARG1* and its 5' and 3' regulatory sequences (-497 to +1553) was inserted at the *LYP1* locus in an orientation opposite to that of *LYP1* (Figure 11A). Northern analysis was performed to examine *ARG1* expression in *PAF1* or *paf1Δ* cells in which the only copy of *ARG1* was expressed from its endogenous locus or the *LYP1* locus. I found that in the presence of Paf1, *ARG1* transcript levels were similarly repressed at either chromosomal location (Figure 11A and B). Additionally, deletion of *PAF1* resulted in similar levels of *ARG1* derepression at both

locations (Figure 11A and B). These results suggest that *ARG1* expression is not influenced by the upstream gene, *GPD2*. Additionally, these results define a chromosomal region that retains Paf1-mediated *ARG1* repression: -497 to +1553.

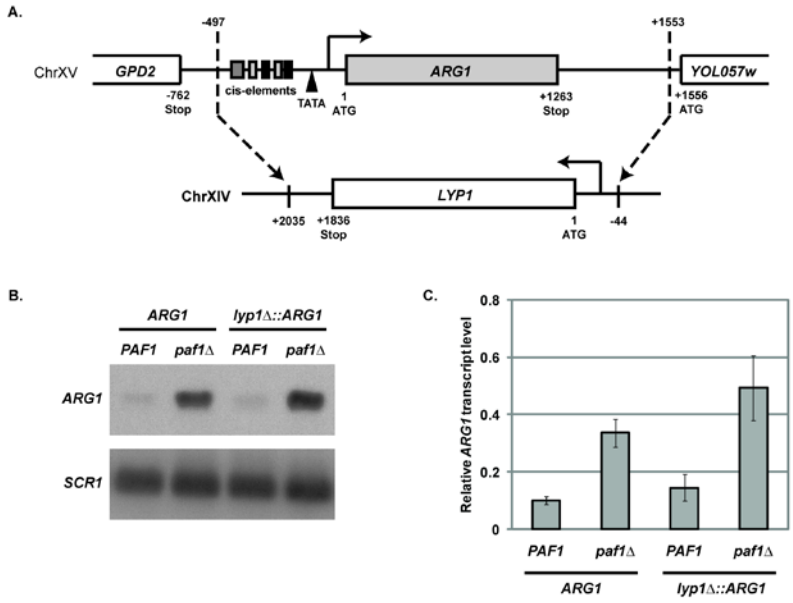


**Figure 11: *ARG1* retains Paf1-mediated repression when expressed from an ectopic chromosomal location.**

(A) Schematic of the boundaries of the *ARG1* locus integrated at the *LYP1* locus. In *arg1Δ::NatMX* strains, *ARG1* -497 to +1553 (relative to the translation start site) was integrated at the *LYP1* locus, replacing *LYP1* -44 to +2035 in the opposite direction as described in Materials and Methods. Representative Northern analysis (B) and quantitation (C) of *ARG1* transcript levels in wild-type (KY1698) and *paf1Δ* (KY1700) cells in which *ARG1* is expressed from its endogenous location or wild-type (KY1734) and *paf1Δ* (KY1735) cells in which the only copy of *ARG1* is integrated at the *LYP1* locus (*lyp1Δ::ARG1*). *SCR1* serves as a loading control. Transcript levels were quantified and normalized to the levels detected in an *arg80Δ* (KY1709) control strain (not shown) as described in Materials and Methods. Values shown are the means of three independent experiments. Error bars represent one standard deviation of the mean.

### 3.3.2 The *ARG1* coding region is required for the full derepression that occurs in the absence of Paf1.

To further localize regions of the *ARG1* locus that confer Paf1-mediated repression, I constructed strains in which the *ARG1* coding region was replaced with that of *HIS3*, leaving the *ARG1* promoter and 3' untranslated region (UTR) intact (Figure 12A). I then examined *HIS3* transcript levels by Northern analysis in strains containing *HIS3* at its endogenous location or integrated at the *ARG1* locus (*ARG1p-HIS3*). Native *HIS3* was expressed in wild-type cells, with a slight reduction or increase in native *HIS3* levels observed in the absence of Paf1 or Rtf1, respectively (Figure 12B and C). However, when the *HIS3* coding region was under the control of the *ARG1* promoter, *HIS3* was repressed in wild-type cells and derepressed in the absence of Paf1 and Rtf1 (Figure 12B and D). Because the *HIS3* coding region behaved similarly to the *ARG1* coding region when under the control of the *ARG1* promoter and 3' UTR, my results suggest that the mechanism of Paf1-mediated repression of *ARG1* is partially independent of the coding region. However, transcript levels from the native *ARG1* gene increase more than 10-fold in *paf1Δ* cells and approximately 4-fold in *rtf1Δ* cells (CRISUCCI and ARNDT 2011), but both *paf1Δ* and *rtf1Δ* cells showed an approximately 3-fold increase in *ARG1p-HIS3* expression (Figure 12B and D). Since the loss of Paf1 results in less derepression when the *ARG1* coding region is replaced that that of *HIS3*, these results suggest that the *ARG1* coding region is required for the full derepression observed in *paf1Δ* cells. To determine whether the increased *ARG1p-HIS3* expression in *paf1Δ* cells is dependent on Gcn4, a transcriptional activator of *ARG1*, I examined *ARG1p-HIS3* transcript levels in *gcn4Δ* and *paf1Δ gcn4Δ* cells. As expected, *HIS3* transcription was reduced in *gcn4Δ* cells (Figure 12B and D). Interestingly, *HIS3* was derepressed to similar levels in both *paf1Δ* and *paf1Δ gcn4Δ* cells (Figure 12B and D), indicating that when *HIS3* is

under the control of the *ARG1* promoter, derepression of *HIS3* in the absence of Paf1 is independent of Gcn4. Together, these results suggest that Paf1 has some repressive functions that require the *ARG1* coding region and some that may require the *ARG1* promoter or 3' UTR. Furthermore, the repressive functions that do not require the *ARG1* coding region are not dependent on Gcn4.

Together, these results suggest that, in addition to having coding-region specific functions, Paf1 has some repressive functions conferred by the *ARG1* promoter and/or 3' UTR. To determine whether the *ARG1* 3' UTR is required for regulation by Paf1, I replaced the *ARG1* coding region and 3' UTR with that of *HIS3* and examined *HIS3* transcript levels by Northern analysis in the presence or absence of Paf1 (Figure 12B and E). Surprisingly, in wild-type cells, *ARG1p-HIS3*, now with the *HIS3* 3' UTR, was expressed at higher levels than *ARG1p-HIS3* fused to the *ARG1* 3' UTR (Figure 12B and E). Therefore, replacing the *ARG1* 3' UTR with the *HIS3* 3' UTR resulted in increased transcript levels, possibly by conferring increased mRNA stability. Importantly, despite the increased expression conferred by the *HIS3* 3' UTR, deletion of *PAF1* resulted in additional derepression, indicating that some repressive functions of Paf1 are independent of the *ARG1* 3' UTR (Figure 12B and E). Therefore, these results suggest that the *ARG1* 3' UTR is not required for *ARG1p-HIS3* repression by Paf1 and additionally suggest that Paf1 has repressive functions at the *ARG1* promoter.

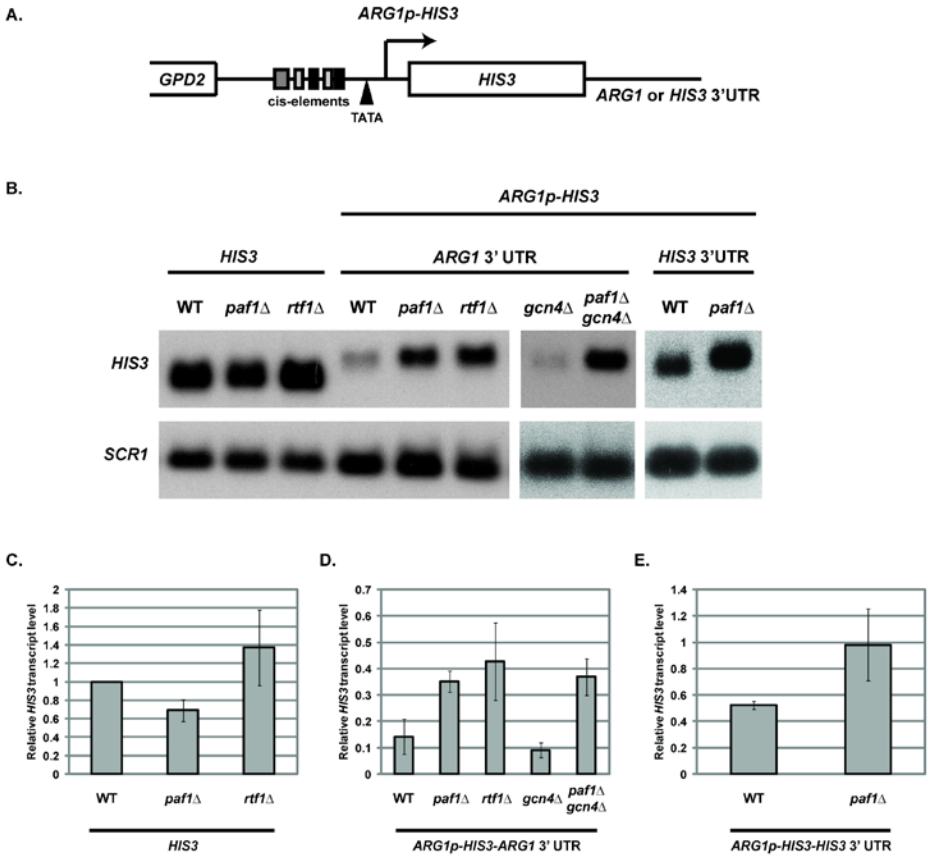


**Figure 12: The *ARG1* coding region is required for the full derepression that occurs in *paf1Δ* cells.**

(A) A representation of *ARG1p-HIS3* where the *ARG1* coding region or coding region and 3' UTR was replaced with that of *HIS3* as described in Materials and Methods. (B) A representative Northern blot examining *HIS3* mRNA levels when expressed from the endogenous *HIS3* chromosomal location in wild-type (KY1699), *paf1*Δ (KY1700), and *rtf1Δ* (KY1703) cells, when *HIS3* is fused to the *ARG1* promoter (*ARG1p-HIS3*) in wild-type (KY1736), *paf1*Δ (KY1737), *rtf1Δ* (KY2175), *gcn4Δ* (KY1739), and *paf1Δ gcn4Δ* (KY1740) cells, and when the *HIS3* coding region and 3' UTR are fused to the *ARG1* promoter in wild-type (KY1871) and *paf1Δ* (KY1874) cells. (C) Quantitation of *HIS3* transcript levels in wild-type (KY1699), *paf1*Δ (KY1700), and *rtf1Δ* (KY1703) cells in which *HIS3* is expressed from its normal chromosomal location. (D) Quantitation of *HIS3* transcript levels in wild-type (KY1736), *paf1*Δ (KY1737), *rtf1Δ* (KY2175), *gcn4Δ* (KY1739), and *paf1Δ gcn4Δ* (KY1740) cells, in which the *HIS3* coding region is under the control of the *ARG1* promoter (*ARG1p-HIS3*). (E) Quantitation of *HIS3* transcript levels in wild-type (KY1871) and *paf1Δ* (KY1874) cells in which the *HIS3* coding region and 3' UTR are under the control of the *ARG1* promoter. (C-E) Transcript levels were quantified and normalized to the levels

detected in a wild-type (KY1699) strain as described in Materials and Methods. The means of three independent experiments are shown. Error bars represent one standard deviation of the mean. Note that different Y-axes were used to facilitate comparison between samples.

### 3.3.3 *ARG1* repression by Paf1 is independent of the gene-specific repressor Arg80.

My results suggest that Paf1 has repressive functions conferred by the *ARG1* promoter. Therefore, I next turned to examining whether there are Paf1-dependent changes that occur at the *ARG1* promoter. I hypothesized that Paf1 might mediate *ARG1* repression by facilitating promoter-association of the repressor, Arg80. Therefore, I performed chromatin immunoprecipitation (ChIP) analysis of Arg80 recruitment using strains expressing Myc-tagged Arg80 or an untagged control strain, to ask whether Arg80 occupancy at the *ARG1* promoter is influenced by Paf1. Unexpectedly, I found that Arg80 occupancy at the *ARG1* promoter was increased in *paf1Δ* strains, suggesting that Paf1 actually inhibits promoter-association of Arg80 (Figure 13A). To determine if Gcn4 also influences Arg80 occupancy at the *ARG1* promoter, I examined Arg80 enrichment at the *ARG1* promoter in strains lacking Gcn4. However, deletion of *GCN4* did not have additional effects on Arg80 occupancy, suggesting that in repressing conditions Gcn4 does not regulate Arg80 recruitment (Figure 13A).

The increased Arg80 occupancy observed in *paf1Δ* cells was not due to increased protein levels. Western analysis of Myc-tagged Arg80 protein levels indicated that deletion of *PAF1* had no effect on Arg80 expression (Figure 13B). Additionally, Western analysis of Myc-tagged Arg81 and Arg82 protein levels indicated that the loss of Paf1 resulted in only a slight increase

in Arg81and Arg82 protein levels (Figure 13C). Furthermore, despite increased Arg80 promoter occupancy, significant *ARG1* derepression occurs in *paf1Δ* strains, suggesting that a functional repressor complex does not assemble at the *ARG1* promoter in these conditions.

To test genetically if Paf1 mediates *ARG1* repression in the same pathway as Arg80, I examined *ARG1* mRNA levels by Northern analysis in single and double mutant strains grown in rich media. As expected, deletion of *PAF1* or *ARG80* individually resulted in *ARG1* derepression (Figure 13D and E). Interestingly, *ARG1* transcript levels in *paf1Δ arg80Δ* strains were higher than in either single deletion strain, suggesting that Paf1 and Arg80 function in independent pathways for *ARG1* repression (Figure 13D and E). Together, these observations suggest that, although Paf1 inhibits promoter-association of Arg80, Paf1 mediates *ARG1* repression independently of Arg80.

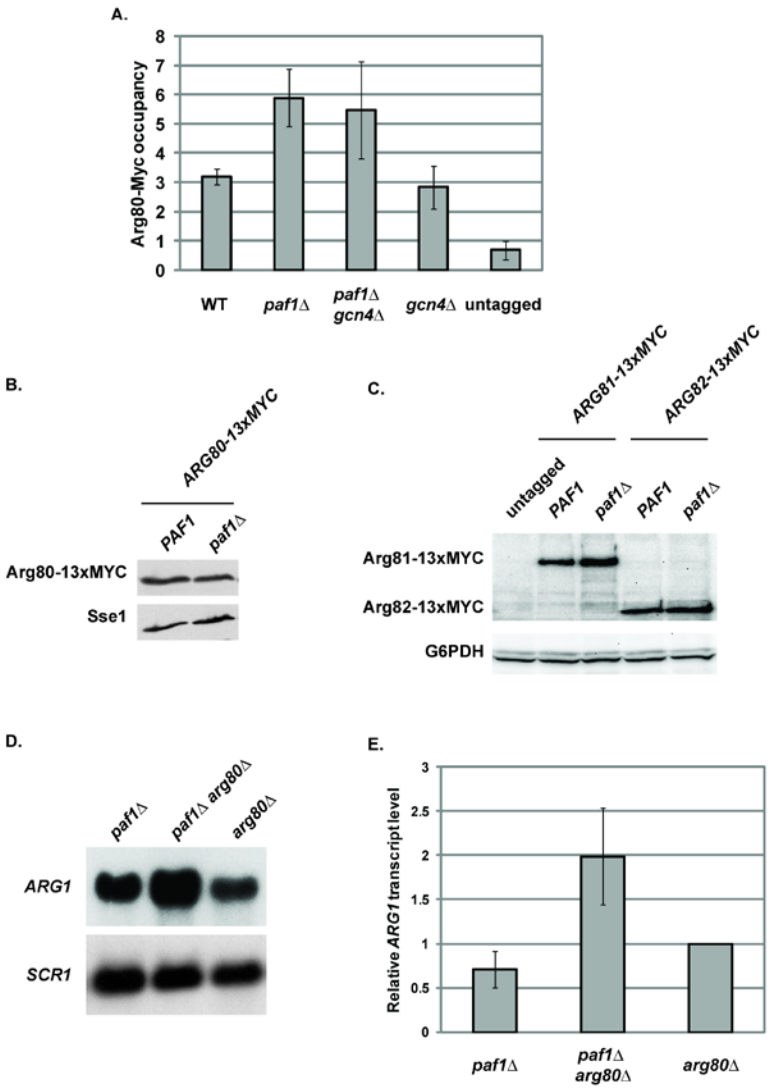


**Figure 13: Regulation of *ARG1* expression by Paf1 is independent of the gene-specific repressor Arg80.**

(A) ChIP analysis of Arg80 binding to the *ARG1* promoter in wild-type (KA119), *paf1*Δ (KA116), *paf1*Δ *gcn4*Δ (KA118), and *gcn4*Δ (KA117) strains expressing MYC-tagged Arg80 and an untagged control strain (KY1728). PCR primers used in ChIP analysis amplified the *ARG1* promoter region (-450 to -200 relative to the translation start site). ChIP signals were quantified and normalized as described in Materials and Methods. Shown are the means of three independent experiments. Error bars represent standard error of the mean. (B) Western analysis of Arg80 protein levels in wild-type (OKA127) and *paf1*Δ (OKA145) strains containing MYC-tagged Arg80 compared to loading control Sse1. (C) Western analysis of Arg81 and Arg82 protein levels in an untagged control strain

(KY1699), *PAF1* (KA139) and *paf1Δ* (KA142) cells expressing MYC-tagged Arg81, and *PAF1* (KA143) and *paf1Δ* (KA146) cells expressing MYC-tagged Arg82. G6PDH serves as a loading control. Representative Northern analysis (D) and quantitation (E) of *ARG1* transcript levels in *paf1*Δ (KY1702), *paf1*Δ *arg80*Δ (KY1720), and *arg80*Δ (KY1709) strains. *SCR1* serves as loading control. Transcript levels were quantified and normalized to the levels detected in an *arg80Δ* (KY1709) strain as described in Materials and Methods. The means of three independent experiments are shown. Error bars represent one standard deviation of the mean.

### 3.3.4 Paf1 inhibits promoter-association of Gcn4 in repressing conditions.

My data suggest that Paf1 does not mediate *ARG1* repression indirectly through the repressor Arg80. However, Paf1 might mediate *ARG1* repression by inhibiting the activator, Gcn4. To determine whether Paf1 influences recruitment of Gcn4 to the *ARG1* promoter, I performed ChIP analyses using strains expressing HA-tagged Gcn4 or an untagged control strain. Gcn4 protein is expressed at very low levels in cells grown in rich media, but ChIP analysis was sensitive enough to detect HA-tagged Gcn4 at the *ARG1* promoter in repressing conditions (Figure 14A). Interestingly, like Arg80, Gcn4 occupancy was modestly increased in *paf1Δ* strains, suggesting that Paf1 inhibits recruitment of Gcn4 to the *ARG1* promoter in repressing conditions (Figure 14A). It is also not known whether Arg80 impacts Gcn4 promoter-occupancy in repressing conditions. Therefore, I examined HA-Gcn4 occupancy in *arg80Δ* strains. Gcn4 occupancy was also increased in *arg80Δ* strains, suggesting that both Paf1 and Arg80 inhibit Gcn4 recruitment to the *ARG1* promoter (Figure 14A). If Paf1 and Arg80 function independently to inhibit Gcn4 recruitment to the *ARG1* promoter, I would expect that the loss of both Paf1 and Arg80 would

result in a higher increase in Gcn4 recruitment than the loss of either protein individually. Indeed, *paf1Δ arg80Δ* strains exhibited higher Gcn4 occupancy than either single mutant strain, suggesting that Paf1 and Arg80 independently inhibit the association of Gcn4 with the *ARG1* promoter (Figure 14A). These results show that that, in *paf1Δ* strains, *ARG1* expression is associated with increased promoter-association of Gcn4 and further support the conclusion that Paf1 functions independently of Arg80.

The increased Gcn4 occupancy at the *ARG1* promoter in *paf1Δ* strains could be due to an increase in Gcn4 protein levels. However, Gcn4 protein levels are so low in cells grown in rich media that they are undetectable by Western analysis in both wild-type and *paf1Δ* cells (data not shown). Therefore, I examined Gcn4 expression in other ways. First, I used plasmid-expressed *GCN4-lacz* fusion constructs to examine Gcn4 expression by measuring β-galactosidase activity. Interestingly, I detected increased β-galactosidase activity in *paf1Δ* cells (Figure 14B). These results could indicate that Paf1 inhibits *GCN4* transcription or translation. Therefore, to determine if Paf1 inhibits Gcn4 translation, I examined β-galactosidase activity in wild-type and *paf1Δ* cells expressing a constitutive *GCN4-lacz* fusion construct, in which translational regulation was eliminated by deletion of several upstream regulatory micro-ORFs (Gcn4c) (MUELLER and HINNEBUSCH 1986). With cells expressing the constitutive *GCN4-lacz*, I also detected increased β-galactosidase activity in *paf1Δ* cells compared to wild-type cells (Figure 14C). Since increased *GCN4-lacz* expression occured in *paf1Δ* cells, even in the absence of translational control, these results would suggest that Paf1 increases Gcn4 expression, not by inhibiting translation, but by repressing *GCN4* transcription. However, strand-specific RT-PCR indicated that Paf1 has no effect on transcription of endogenous *GCN4* (Figure 14D). I predict that Paf1 may regulate *GCN4-lacz* expression by regulating plasmid copy number or by

influencing protein stability. Therefore, I currently have no conclusive evidence to suggest that Paf1 regulates promoter-occupancy of Gcn4 at *ARG1* by modulating Gcn4 expression.

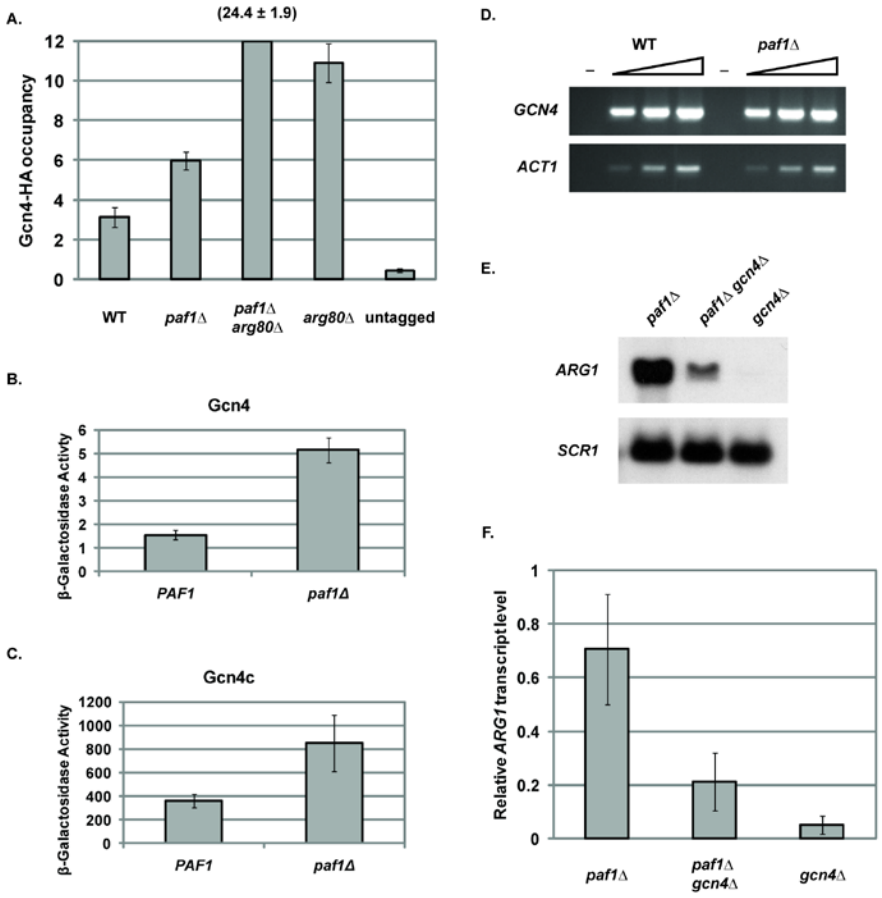


**Figure 14: Paf1 inhibits recruitment of Gcn4 to the *ARG1* promoter in repressing conditions.**

(A) ChIP analysis of Gcn4 occupancy at the *ARG1* promoter in wild-type (KY1728), *paf1*Δ (KA116), *paf1*Δ *arg80*Δ (KY1730), and *arg80*Δ (KY1729) strains expressing HA-tagged Gcn4 and untagged Gcn4 (KA119). PCR primers used in ChIP analysis amplified the *ARG1* promoter region (-450 to -200 relative to the translation start site). ChIP signals were quantified and normalized as described in Materials and Methods. Shown are the means of three independent experiments. Error bars represent standard error. The y-axis was truncated to facilitate comparison of lower values. The value for *paf1*Δ *arg80*Δ is indicated above the bar. (B) Quantitation of β-galactosidase activity in wild-type (ECY155) and *paf1Δ* (ECY410) cells transformed with a plasmid expressing *GCN4-lacZ* (p180). (C) Quantitation of β-galactosidase activity in wild-type (ECY155) and *paf1Δ* (ECY410) cells transformed with a plasmid expressing constitutive *GCN4-lacZ* (Gcn4c) (p227). (D) Strand-specific RT-PCR examining *GCN4* transcript levels in wild-type (KY1699) and *paf1Δ* (KY1700) cells. 4 µl of no RT reactions (-) and 0.5 µl, 2 µl, and

4 µl volumes of undiluted or 1:2 cDNA were used in PCR reactions to amplify *GCN4* and *ACT1*, respectively. Representative Northern analysis (C) and quantitation (D) of *ARG1* transcript levels in wild-type (KY1699), *paf1*Δ (KY1702), *paf1*Δ *gcn4*Δ (KY1719), and *gcn4*Δ (KY1708) strains. Transcript levels were quantified and normalized as described in Materials and Methods. Shown are the means of three independent experiments. Error bars represent one standard deviation.

### 3.3.5 The derepression of native *ARG1* in *paf1Δ* cells is partially Gcn4-dependent.

In *paf1Δ* cells *ARG1* derepression is associated with increased promoter occupancy of Gcn4, which may contribute to increased *ARG1* transcription. I found that the low level of *HIS3* derepression that occurs in *paf1Δ* cells when the *HIS3* coding region is fused to the *ARG1* promoter and 3' UTR is not dependent on Gcn4. However, I wanted to examine whether the additional repressive functions conferred by the *ARG1* coding region are Gcn4-dependent. Therefore, I examined transcript levels of native *ARG1* in the absence of Paf1 and/or Gcn4. I found that *ARG1* mRNA levels in *paf1Δ gcn4Δ* strains were reduced compared to *paf1Δ* strains, suggesting that the *ARG1* derepression that occurs in the absence of Paf1 is highly Gcn4-dependent (Figure 14E and F). However, *ARG1* transcript levels in *paf1Δ gcn4Δ* cells were still higher than in wild-type cells (Figure 14E and F), supporting my conclusion that Paf1 has both Gcn4-dependent and –independent roles. Furthermore, the fact that the *ARG1* derepression that occurs in *paf1Δ* cells was suppressed by deletion of *GCN4*, supports the idea that Paf1 mediates *ARG1* repression partially by inhibiting recruitment and/or activity of the activator, Gcn4.

### 3.3.6 Paf1 does not regulate *ARG1* expression by regulating mRNA stability.

Thus far, my results suggest that Paf1 mediates *ARG1* repression partially through a Gcn4-dependent mechanism that requires the *ARG1* promoter and coding region and partially through an additional Gcn4-independent mechanism at the *ARG1* promoter. I hypothesized that Paf1 regulates *ARG1* expression at the level of transcription, given the important roles described for the Paf1 complex in this process. However, a post-transcriptional role has not been investigated. For example, Paf1 could reduce *ARG1* expression by promoting mRNA instability. Therefore, to determine if Paf1 affects *ARG1* mRNA stability, I examined *ARG1* transcript levels in strains expressing a temperature sensitive version of *RPB1*, the gene encoding the largest subunit of RNA Pol II. Specifically, *rpb1-1* cells were harvested for RNA isolation and Northern analysis at several time points after shifting cultures to the restrictive temperature. Interestingly, *ARG1* mRNA was actually lost at a quicker rate in *paf1Δ* strains than in strains expressing Paf1, indicating that *paf1Δ* cells have increased *ARG1* expression despite reduced *ARG1* mRNA stability (Figure 15A and B). Consistent with these results, deletion of *PAF1* has been shown to alter poly(A) tail length and poly(A) site usage, sometimes generating transcripts that are subject to nonsense-mediated decay (MUELLER *et al.* 2004; PENHEITER *et al.* 2005). Since Paf1 appears to promote *ARG1* mRNA stability, these results do not support a post-transcriptional role for Paf1 in repressing *ARG1* expression.

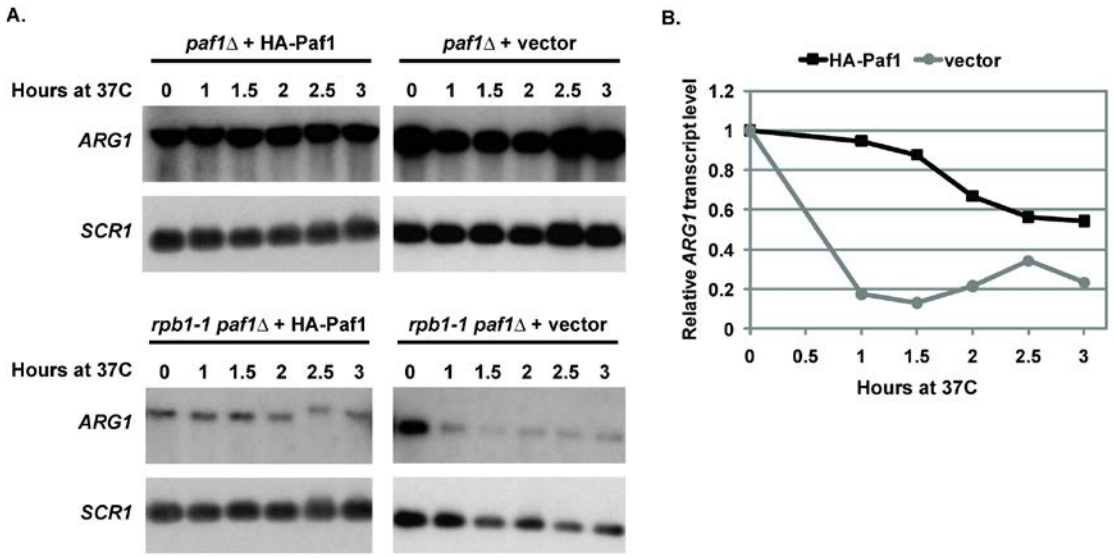


**Figure 15: *ARG1* mRNA stability in the presence and absence of Paf1.**

(A) Representative Northern analysis examining *ARG1* mRNA levels in *paf1*Δ (KY2026) (top panels) and *rpb1-1 paf1*Δ (KA155) strains (bottom panels) containing a plasmid expressing HA-Paf1 (pRS316-HA-Paf1) or empty vector (pRS316). Cultures grown in SC media lacking uracil at 30°C, diluted with an equal volume of pre-warmed media, and immediately shifted to 37°C. Cells were harvested for RNA immediately prior to and at several time points after temperature shift. 30 μg RNA was analyzed for cells expressing HA-Paf1 due to the low expression of *ARG1* in these cells, while only 10 μg RNA was analyzed for cells lacking Paf1. (E) Quantitation of relative *ARG1* transcript levels. Results are representative of two independent experiments. For each strain, transcript levels prior to temperature shift were set equal to one.

### 3.3.7 Histone H3 acetylation is required for the derepression that occurs in the absence of Paf1.

Since my results suggest that Paf1 does not regulate *ARG1* expression by promoting mRNA instability, I returned to examining transcriptional regulation of *ARG1* by Paf1. I have shown that the increase in *ARG1* transcription in *paf1Δ* strains is associated with increased Gcn4 occupancy at the *ARG1* promoter. One possible explanation for this correlation could be that in *paf1Δ* cells, the *ARG1* promoter adopts a histone modification state that allows increased binding of trans-acting factors. Since I have shown that Paf1 mediates *ARG1* repression in a partially Gcn4-dependent manner, I chose to examine the role of histone acetylation, which is regulated by both Paf1 and Gcn4, in modulating *ARG1* transcription. The Prelich and Arndt labs previously showed that Paf1 inhibits histone acetylation in the coding region of active genes (CHU *et al.* 2007). In contrast, Gcn4 has been shown to promote histone acetylation by recruiting the histone acetyltransferase (HAT) Gcn5 to the promoter of Gcn4-activated genes (GOVIND *et al.* 2005; KUO *et al.* 2000). Therefore, I hypothesized that Paf1 might oppose the actions of Gcn4, thus promoting *ARG1* repression by preventing histone acetylation by Gcn5.

Regulation of *ARG1* transcription by Gcn5 is complex, however, as Gcn5 has both positive and negative effects on *ARG1* transcription. Specifically, in inducing conditions, Gcn5 promotes *ARG1* activation by promoting histone acetylation and recruitment of SWI/SNF, TBP, and RNA Pol II (GOVIND *et al.* 2005; GOVIND *et al.* 2007; KUO *et al.* 1996; KUO *et al.* 2000; QIU *et al.* 2004; SWANSON *et al.* 2003). However, in repressing conditions, Gcn5 promotes *ARG1* repression through a less-understood mechanism that requires its acetyltransferase activity (RICCI *et al.* 2002). To determine if the *ARG1* derepression in *paf1Δ* strains requires Gcn5, I examined the combinatorial effect of Paf1 and Gcn5 on *ARG1* expression by Northern analysis. Consistent

with their known role in *ARG1* repression, both *paf1Δ* and *gcn5Δ* cells exhibited *ARG1* derepression (Figure 16A). Furthermore, *ARG1* transcript levels in *paf1Δ gcn5Δ* double mutant strains were higher than in wild-type strains, suggesting that Paf1 and Gcn5 have some overlapping repressive functions (Figure 16A). However, *paf1Δ gcn5Δ* strains exhibited less derepression than either single mutant strain, suggesting that the full level of derepression that occurs in the absence of Paf1 requires Gcn5 and vice versa (Figure 16A).

The fact that Gcn5 is required for full *ARG1* derepression in *paf1Δ* strains is consistent with the hypothesis that in *paf1Δ* strains, increased recruitment of Gcn4 results in increased histone acetylation by Gcn5 and ultimately *ARG1* derepression. Therefore, I performed ChIP analysis to determine if Paf1 affects histone H3 acetylation levels at *ARG1*. I found that although *gcn5Δ* strains exhibited reduced histone H3 acetylation at the *ARG1* promoter and coding region, *paf1Δ* strains exhibited histone H3 acetylation levels similar to wild-type strains, indicating that Paf1 has no effect on histone H3 acetylation levels at *ARG1* (Figure 16B). However, this may not be surprising, because it has been shown that the action of several histone deacetylases (HDACs) prevents an increase in histone acetylation upon transcriptional induction (GOVIND *et al.* 2007).

Because HDACs may oppose increases in histone acetylation caused by the deletion of *PAF1*, I chose to determine if Gcn5 acetylation sites on histone H3 are required for proper *ARG1* expression by examining *ARG1* transcript levels in strains in which Gcn5-target lysines in histone H3 were changed to alanine. *ARG1* transcript levels in strains expressing histone H3 mutant proteins, H3-K9A, H3-K14A, and H3-K4, 9, 14, 16A, were similar to wild-type histone H3 strains, suggesting that the acetylation of these histone H3 residues is not required for *ARG1* repression (Figure 16C). Interestingly, these histone H3 substitutions partially restored

repression in *paf1Δ* strains, suggesting that the full *ARG1* expression that occurs in the absence of Paf1 requires histone H3 acetylation (Figure 16C). Mutation of histone H4 acetylation sites did not restore repression in *paf1Δ* strains (Figure 16D), suggesting that the *ARG1* derepression in *paf1Δ* cells requires histone H3 acetylation but not histone H4 acetylation.

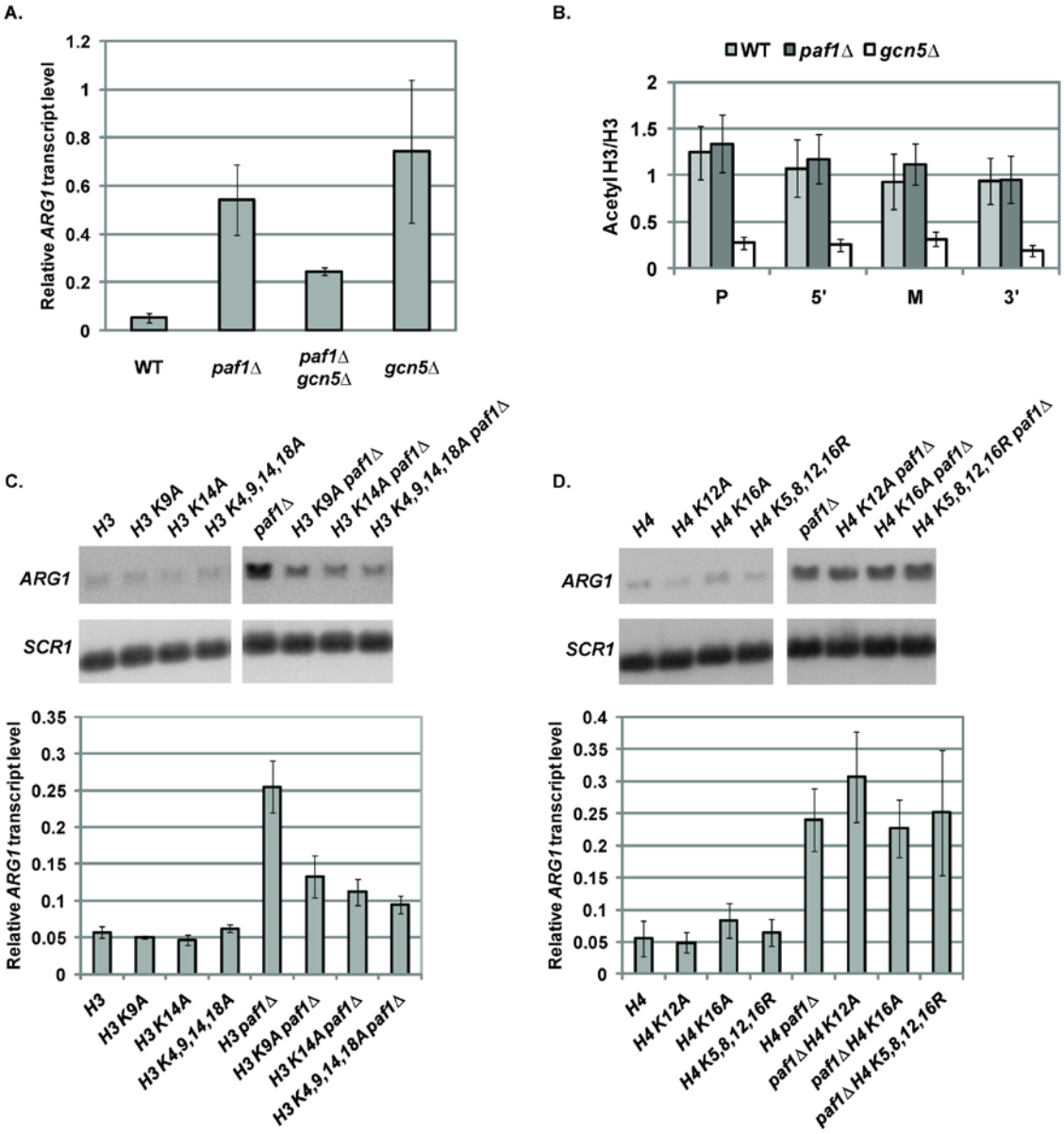


**Figure 16: Mutation of histone H3 acetylation sites partially restores *ARG1* repression in the absence of Paf1.**

(A) Quantitation of Northern analysis of relative *ARG1* expression in wild-type (KY205), *paf1*Δ (KY803), *paf1*Δ *gcn5*Δ (KY1348), and *gcn5*Δ (KY1343) strains. Transcript levels were quantified and normalized as described in Materials and Methods. Shown are the means of three independent experiments. Error bars represent one standard deviation. (B) ChIP analysis of Acetyl H3 relative to total H3 levels in wild-type (KY1699), *paf1*Δ (KY1700), and *gcn5*Δ (KY1741-43) strains. ChIP signals were quantified and normalized as described in Materials and Methods.

Shown are the means of three independent experiments. Error bars represent standard error. (C) Representative Northern analysis (top) and quantitation (bottom) of *ARG1* transcript levels in wild-type (KA120), *H3 K9A* (KA122), *H3 K14A* (KA124), *H3 K4, 9, 14, 18A* (KA126), *paf1*Δ (KA121), *paf1*Δ *H3 K9A* (KA123), *paf1*Δ *H3 K14A* (KA125), and *paf1*Δ *H3 K4, 9, 14, 18A* (KA127) strains. (D) Representative Northern analysis (top) and quantitation (bottom) of *ARG1* transcript levels in wild-type (KA128), *H4 K12A* (KA130), *H4 K16A* (KA132), *H4 K5, 8, 12, 16A* (KA134), *paf1*Δ (KA129), *paf1*Δ *H4 K12A* (KA131), *paf1*Δ *H4 K16A* (KA133), and *paf1*Δ *H4 K5, 8, 12, 16A* (KA135) strains. Transcript levels in (C and D) were quantified and normalized as described in Materials and Methods. Shown are the means of three independent experiments. Error bars represent one standard deviation.

### 3.3.8 Deletion of *PAF1* does not alter nucleosome occupancy at the *ARG1* promoter and 5' coding region.

Histone H3 acetylation by Gcn5 has been shown to result in nucleosome eviction (GOVIND *et al.* 2007). Furthermore, Gcn4 has been shown to recruit several co-activators to the *ARG1* promoter, including SWI/SNF and RSC chromatin remodeling complexes (SWANSON *et al.* 2003). Since *ARG1* derepression in *paf1Δ* cells partially requires Gcn4, Gcn5, and histone H3 acetylation sites, I examined the possibility that Paf1 regulates nucleosome occupancy at the *ARG1* promoter. Furthermore, *ARG1* is a well-studied model gene, yet little is known about its chromatin structure in repressing and activating conditions. To examine the chromatin structure of the *ARG1* promoter, I performed nucleosome scanning assays using PCR primers that tile across the *ARG1* promoter and 5' coding region.

To examine changes in nucleosome occupancy that occur upon transcriptional induction, wild-type strains were grown to log phase in SC-ILV media and treated with sulfometuron

methyl (SM), which increases cellular levels of Gcn4 (HINNEBUSCH 1988), or mock-treated with DMSO for two hours. In mock-treated wild-type strains, the nucleosome scanning assay revealed a region of low relative MNase protection in the *ARG1* promoter, flanked by several peaks of high MNase protection (Figure 17A). These results suggest that the *ARG1* regulatory elements are located within a nucleosome-free region, which is surrounded by several well-positioned nucleosomes. Notably, these results also match well with genome-wide analyses of nucleosome occupancy in wild-type cells (JIANG and PUGH 2009). The peaks of MNase protection were reduced in wild-type strains treated with SM, suggesting that transcriptional induction is associated with reduced nucleosome occupancy at the *ARG1* promoter (Figure 17A). Furthermore, these results define the nucleosome architecture at the *ARG1* promoter and 5' coding region in repressing and inducing conditions and confirm that the assay can detect changes in nucleosome occupancy that occur with transcriptional induction.

To determine whether Paf1 influences nucleosome occupancy at the *ARG1* promoter and 5' coding region in repressing conditions, wild-type and *paf1Δ* strains were grown to log phase in YPD and harvested for the nucleosome scanning assay. The relative MNase protection profile for wild-type cells grown in YPD (Figure 17B) was nearly identical to wild-type cells grown in SC-ILV media (Figure 17B). Interestingly, although loss of Paf1 results in derepression of *ARG1*, the relative MNase protection profile in *paf1Δ* strains closely resembled that of wild-type, except that a very slight reduction in occupancy and forward-shifting of the nucleosome positioned over the TATA element occurs in *paf1Δ* cells (Figure 17B). Consistent with these results, ChIP analysis detected similar levels of histone H3 levels in wild-type and *paf1Δ* cells at the *ARG1* promoter and coding region (CRISUCCI and ARNDT 2011). Together, these results suggest that Paf1 does not dramatically affect nucleosome occupancy at the *ARG1* promoter or 5'

region. Furthermore, my results suggest that a significant level of *ARG1* expression can occur without changes in nucleosome occupancy.

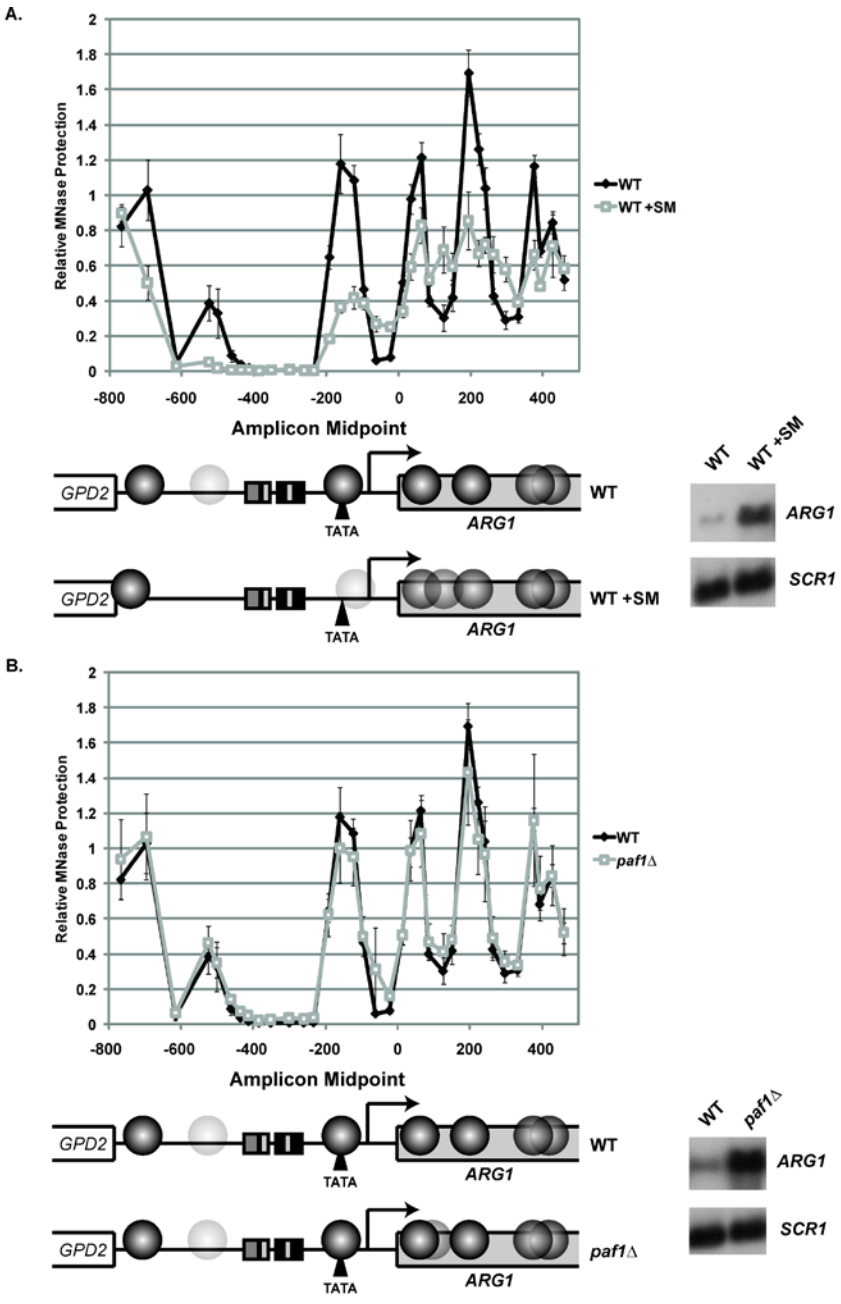


**Figure 17: Loss of Paf1 does not significantly alter nucleosome occupancy at the *ARG1* promoter and 5' coding region.**

(A) Nucleosome scanning assay examining nucleosome occupancy at the *ARG1* promoter and 5' region in wild-type strains in repressing and inducing conditions. Wild-type (KY1699) cells were grown to log phase in SC-ILV media and treated with 0.6 μg/ml SM or mock-treated with DMSO for two hours prior to crosslinking. Mononucleosomal DNA was prepared and subjected to real-time PCR analysis using primers that tile a chromosomal region containing

the *ARG1* promoter. The midpoint position of each PCR fragment relative to the translation start site is plotted on the X-axis. Relative MNase protection is plotted on the Y-axis. Bottom left: Schematic representation of predicted nucleosome occupancy. Shaded circles represent potential nucleosomes. Bottom right: Representative Northern blot examining *ARG1* transcript levels in strains harvested for RNA prior to crosslinking. (B) Nucleosome occupancy at the *ARG1* promoter region in wild-type (KY1699) and *paf1*Δ (KY1700 and KY1701) strains grown in YPD. Samples were processed and relative MNase protection calculated as in (A). Bottom left: Schematic representation of predicted nucleosome occupancy. Bottom right: Representative Northern analysis of *ARG1* mRNA levels immediately prior to crosslinking.

### 3.3.9 Paf1 prevents antisense transcription from traversing the *ARG1* promoter.

Histone H3 K4 methylation is usually highest at 5' coding regions (KROGAN *et al.* 2003a; NG *et al.* 2003b), while K36 methylation is generally highest at 3' coding regions (KIZER *et al.* 2005; KROGAN *et al.* 2003b; LI *et al.* 2003; SCHAFT *et al.* 2003; XIAO *et al.* 2003). However, I previously examined enrichment of these modifications at the *ARG1* locus and observed a reversed histone modification pattern (CRISUCCI and ARNDT 2011). I hypothesized that, similar to what has been described at *GAL10* (HOUSELEY *et al.* 2008), antisense transcription at the *ARG1* locus could contribute to the reversed pattern of histone modifications. Interestingly, previous genome-wide studies reported antisense transcripts arising from the *ARG1* 3' UTR and traversing the coding region in wild-type cells (DAVID *et al.* 2006; XU *et al.* 2009). As of yet, it is not clear if antisense transcripts have any effect on the expression of *ARG1*. However, based

on results described earlier in this chapter, I hypothesized that Paf1 might regulate antisense transcription at or near the *ARG1* promoter, which could, in turn, alter Gcn4 occupancy.

To both confirm the presence of antisense transcription at the *ARG1* locus and to determine whether it is regulated by Paf1, I performed strand-specific cDNA synthesis followed by PCR. As expected, a cDNA synthesis primer complementary to the *ARG1* mRNA detects the derepression of *ARG1* sense transcription that occurs in *paf1Δ* strains (Figure 18A and B). To detect antisense transcripts at the *ARG1* locus, I used several different cDNA synthesis primers within the promoter and coding region (Figure 18A and C). Using cDNA synthesis primers downstream of the *ARG1* transcription start site (primers E and F), I detected antisense transcription that traverses the *ARG1* coding region in both wild-type and *paf1Δ* strains at similar levels (Figure 18A and C). This confirms that antisense transcription traverses the *ARG1* coding region, but suggests that levels of antisense transcription across the *ARG1* coding region are not regulated by Paf1. However, using cDNA synthesis primers upstream of the *ARG1* transcription start site (primers A-D), I found very low levels of antisense transcription within the *ARG1* promoter in wild-type cells (Figure 18A and C). Interestingly, antisense transcription within the *ARG1* promoter was dramatically increased in *paf1Δ* cells, suggesting that Paf1 prevents antisense transcription within the *ARG1* promoter (Figure 18A and C).

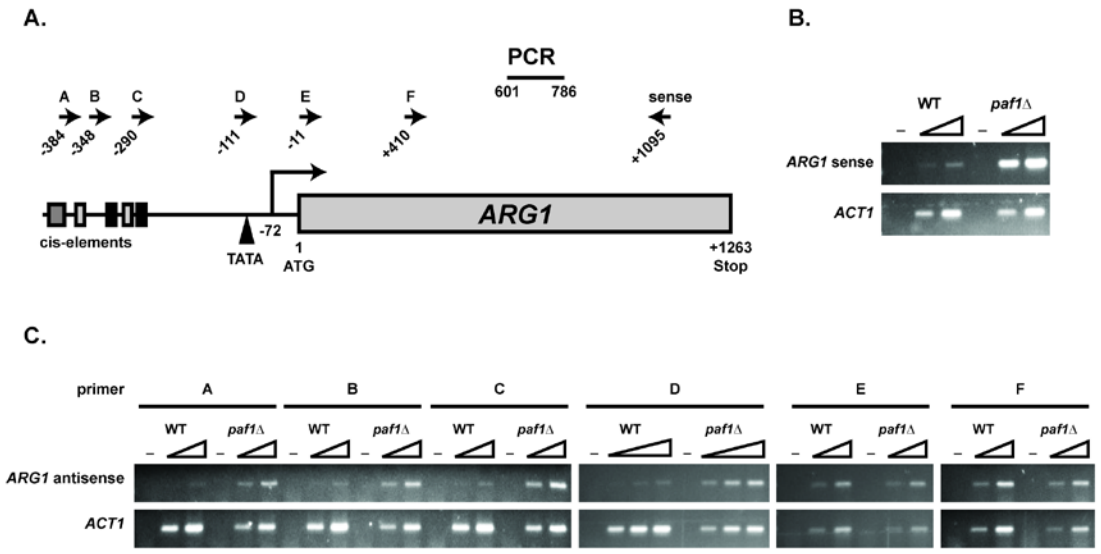


**Figure 18: Paf1 inhibits antisense transcription across the *ARG1* promoter.**

(A) Picture showing the locations of primers used in cDNA synthesis (arrows) and PCR (bar) for strand-specific RT-PCR. (B) Strand-specific RT-PCR examining *ARG1* sense transcription in wild-type (KY1699) and *paf1*Δ (KY1700) strains. 3 µl of no RT reactions (-) and 1 µl and 3 µl volumes of 1:2 cDNA were used in PCR reactions to amplify *ARG1* and *ACT1*. Results shown are representative of more than three independent experiments. (C) Strand-specific RT-PCR examining antisense transcription within the *ARG1* promoter and coding region in wild-type (KY1699) and *paf1*Δ (KY1700) strains. cDNA synthesis primers used are noted above wild-type and *paf1Δ* samples and correspond to those depicted in (A). 3 µl of no RT reactions (-) and 1 and 3 µl or 1 µl, 2 µl, and 3 µl volumes of undiluted or 1:2 cDNA were used in PCR reactions to amplify *ARG1* and *ACT1*, respectively. Reactions with cDNA synthesis primers A, C, D, and F were performed only once. Results shown for cDNA synthesis primer E are representative of two independent experiments. Results shown with cDNA synthesis primer B are representative of more than 3 independent experiments.

### 3.3.10 3’ end mapping of antisense transcripts at the *ARG1* locus.

Levels of antisense transcription occurring within the *ARG1* coding region do not increase in the absence of Paf1. However, Paf1 appears to prevent antisense transcription from traversing the promoter. Therefore, I predicted that 3’-extended antisense transcripts occur in *paf1Δ* cells. To map the 3’ ends of antisense transcripts occurring in wild-type and *paf1Δ* cells, I performed 3’ RACE. In 3’ RACE, the antisense transcripts were copied into cDNA in a poly(A)-dependent manner. The resulting cDNA was cloned into a bacterial vector and transformed into *E. coli*. After isolation from bacteria, the vector inserts were examined by sequence analysis. 17 wild-type and 20 *paf1Δ* clones were analyzed. Multiple 3’ ends were detected by this method (Figure 19, Table 2). In wild-type cells, 7 out of 17 clones exhibited 3’ ends located at +352 relative to the *ARG1* translation start site (Figure 19, Table 2). An additional 3 clones exhibited 3’ ends located at +335 or +424. The remaining 7 clones exhibited 3’ ends at -65, -66, or -69, which is very close to the *ARG1* sense transcription start site (-72) (Figure 19, Table 2). Therefore, in wild-type cells, slightly more than half of the antisense transcripts detected terminate upstream of the ARG1 translation start site (ATG) at a location within the *ARG1* coding region. The remaining antisense transcripts traversed the ATG and terminated immediately before the *ARG1* transcription start site. Interestingly, in *paf1Δ* samples, all 20 clones examined exhibited 3’ ends located close to the *ARG1* transcription start site (-49, -64, -65, -66, -67, -69). Therefore, this analysis indicates that while shorter and longer antisense transcripts occur in wild-type cells, deletion of Paf1 results in an increase in longer antisense transcripts that terminate very close to the *ARG1* transcription start site. These results are consistent with my hypothesis that Paf1 prevents the occurrence of antisense transcripts that extend beyond the *ARG1* coding region. However, antisense transcripts traversing the *cis*-elements within the *ARG1* promoter, which

were detected by strand-specific RT-PCR, were not detected by 3' RACE. Because 3' RACE specifically detects polyadenylated transcripts, the longer antisense transcripts traversing the *ARG1 cis*-elements may be non-polyadenylated.

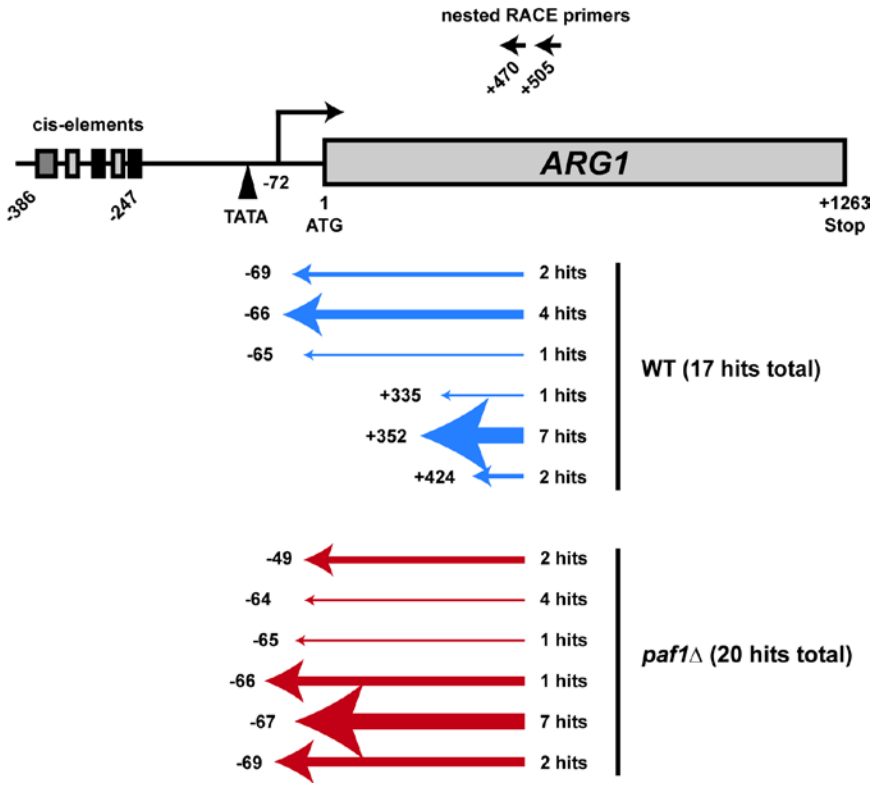


**Figure 19: Mapping 3' ends of antisense transcripts at the *ARG1* locus in wild-type and *paf1Δ* cells.**

Schematic of 3' RACE results. Shown are the locations of the mRNA-specific nested RACE primers. Blue arrows depict sequencing results for 17 cDNA clones from wild-type samples. Red arrows depict sequencing results for 17 cDNA clones from *paf1Δ* samples. 3' end positions are indicated on the left of each arrow. The number of clones with the corresponding 3' end position is indicated on the right of each arrow. The thickness of each arrow is relative to the number of clones with the corresponding 3' end position. 3' RACE results are also listed in Table 2.

### 3.3.11 Integration of a transcription terminator in the *ARG1* coding region in the antisense orientation did not block antisense transcription.

My results suggest that both polyadenylated and non-polyadenylated antisense transcripts occur at ARG1. Furthermore, in *paf1Δ* cells, there is an increase in 3'-extended antisense transcripts that may influence *ARG1* sense expression. To determine if the antisense transcription traversing the *ARG1* promoter in *paf1Δ* cells influences *ARG1* sense transcription, I inserted the *HIS3* terminator element into the *ARG1* coding region in the antisense direction, in an attempt to terminate antisense transcription (Figure 20A). The terminator element had no effect on *ARG1* sense transcription, as strand-specific RT-PCR analysis demonstrated that *ARG1* transcription is repressed in the presence of Paf1 and derepressed in the absence of Paf1 in strains containing the terminator inserted in the *ARG1* coding region (Figure 20B). Surprisingly, strand-specific RT-PCR with cDNA synthesis primers within the promoter (primer B) and ORF (primer F), indicated that the terminator element did not successfully terminate antisense transcription (Figure 20C).

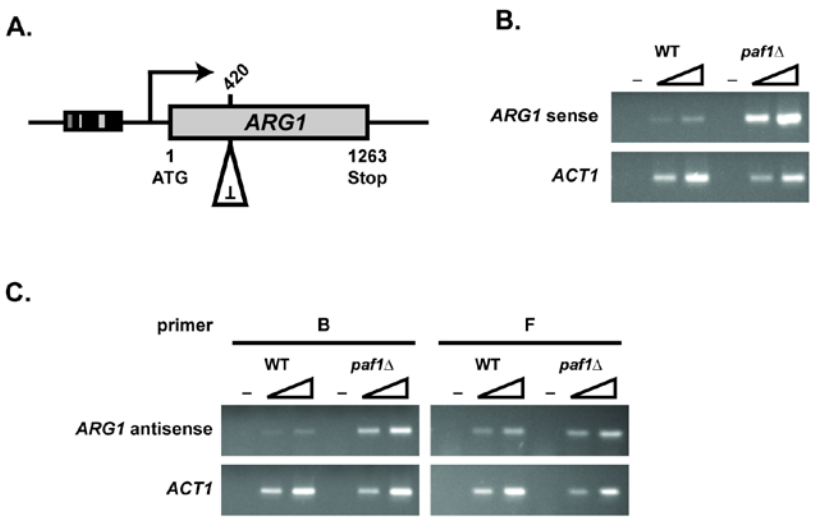


**Figure 20: Integration of a terminator element does not terminate antisense transcription at *ARG1*.**

(A) Schematic of the location of the terminator element inserted in the *ARG1* coding region in the antisense direction as described in Materials and Methods. (B) Strand-specific RT-PCR examining *ARG1* sense transcription in wild-type (KY2144) and *paf1*Δ (KY2147) strains containing a terminator element integrated in the *ARG1* coding region. 3 µl of no RT reactions (-) and 1 µl and 3 µl volumes of 1:2 cDNA were used in PCR reactions to amplify *ARG1* and *ACT1*. This experiment was performed only once. (C) Strand-specific RT-PCR examining antisense transcription in wild-type (KY2144) and *paf1*Δ (KY2147) strains containing a terminator element integrated in the *ARG1* coding region. cDNA synthesis primers used are noted above wild-type and *paf1Δ* samples and correspond to those depicted in (Fig. 8A). 5 µl of no RT reactions (-) and 2 and 5 µl cDNA were used in PCR reactions to amplify *ARG1*. 3 µl of no RT reactions (-) and 1 µl and 3 µl volumes of 1:2 cDNA were used in PCR reactions to amplify *ACT1*. This experiment was performed only once.

### 3.3.12 The *ARG1* 3' UTR is not required for antisense transcription.

Because integration of a strong, defined terminator element failed to terminate the antisense transcription at the *ARG1* locus, I decided to block the antisense transcript by eliminating the antisense transcript start site. Previous genome-wide studies identified antisense transcripts arising from the *ARG1* 3' UTR in wild-type cells (DAVID *et al.* 2006; XU *et al.* 2009). Therefore, I decided to move the *ARG1* promoter and coding region to the *LYP1* locus, this time in the same direction as *LYP1*. In this way, the *ARG1* promoter and coding region were fused to the *LYP1* 3' UTR, which does not contain known start sites for antisense transcripts (DAVID *et al.* 2006; XU *et al.* 2009) (Figure 21A). As a control, I created a strain in which the *ARG1* promoter, coding region, and 3' UTR were integrated at the *LYP1* locus (Figure 21A). Therefore, I would expect to detect the antisense transcript when the *ARG1* 3' UTR, but not the *LYP1* 3' UTR, is adjacent to the *ARG1* promoter and coding region. Surprisingly, strand-specific RT-PCR using primers within the coding region (primer F) detected antisense transcription in both wild-type and *paf1Δ* cells when the *ARG1* promoter and coding region were adjacent to both the *ARG1* and *LYP1* 3' UTR (Figure 21C). Furthermore, antisense transcription occurred within the *ARG1* promoter, with increased antisense transcription in *paf1Δ* cells, regardless of the 3' UTR (Figure 21C). Additionally, the *LYP1* 3' UTR had no effect on *ARG1* sense transcription (Figure 21B). Since replacing the *ARG1* 3' UTR with that of *LYP1* did not eliminate antisense transcription across the *ARG1* promoter and coding region, I was not able to examine the effect of antisense transcription on *ARG1* sense transcription. These results suggest that antisense transcription may arise from start sites within the *ARG1* coding region.

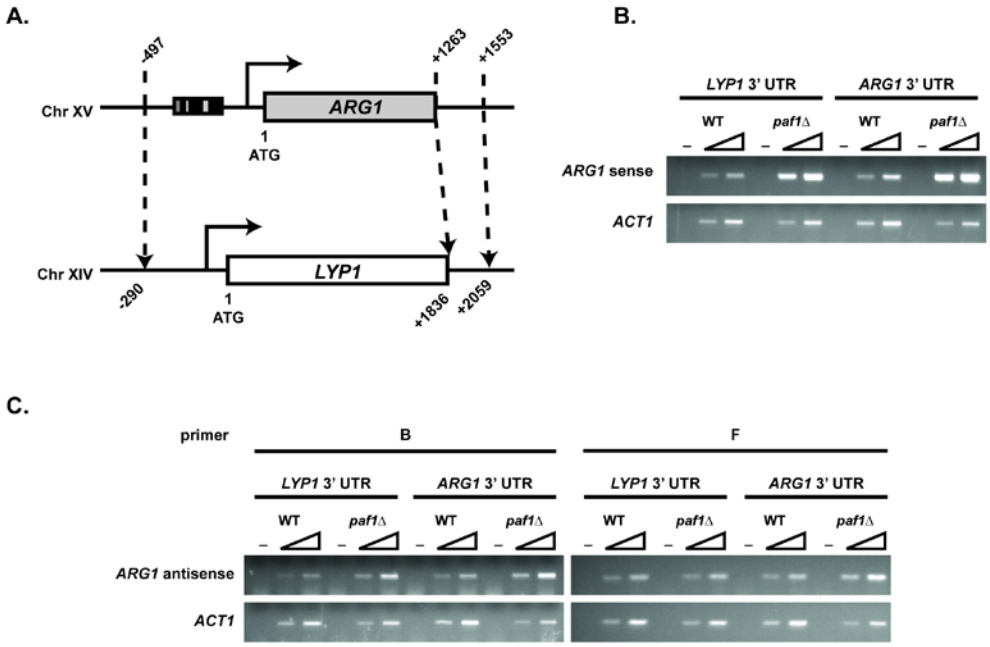


**Figure 21: The *ARG1* 3' UTR is not required for antisense transcription. (A) Schematic of the *ARG1* regions integrated at the *LYP1* locus in the same orientation as *LYP1*.**

To fuse the *ARG1* promoter and coding region with the *LYP1* 3' UTR, *ARG1* -497 to +1263 was integrated at *LYP1* -290 to +1836. As a control, the *ARG1* promoter, coding region, and 3' UTR (*ARG1* -497 to +1553) was integrated at *LYP1* -290 to +2059. Methods of strain construction are described in Materials and Methods. (B) Strand-specific RT-PCR examining *ARG1* sense (B) and antisense (C) transcription in wild-type (KY2112) and *paf1*Δ (KY2115) cells in which the *ARG1* promoter and coding region are fused to the *LYP1* 3' UTR and wild-type (KY2118) and *paf1*Δ (KY2121) cells in which the *ARG1* promoter, coding region, and 3' UTR are integrated at the *LYP1* locus. cDNA synthesis primers used are noted above wild-type and *paf1Δ* samples and correspond to those depicted in (Fig. 8A). For analysis of *ARG1* sense transcription, 3 µl of no RT reactions (-) and 1 µl and 3 µl volumes of 1:2 cDNA were used in PCR reactions to amplify *ARG1*. For analysis of antisense transcription, 6 µl of no RT reactions (-) and 2 µl and 6 µl volumes of cDNA were used in PCR reactions to amplify *ARG1*. 3 µl of no RT reactions (-) and 1 µl and 3 µl volumes of 1:2 cDNA were used in PCR reactions to amplify *ACT*. This experiment was performed only once.

### 3.3.13 Antisense transcription across the *ARG1* promoter positively correlates with *ARG1* sense transcription.

My results suggest that antisense transcription at the *ARG1* locus may arise from start sites within the *ARG1* coding region, yet integration of a terminator element in the *ARG1* coding region failed to terminate antisense transcription. Therefore, I decided to begin investigating a potential regulatory role for antisense transcription at the *ARG1* promoter by determining if antisense transcription positively or negatively correlates with *ARG1* sense transcription. To determine the correlation between *ARG1* sense and antisense transcription, I performed strand-specific RT-PCR with RNA isolated from wild-type cells grown in repressing and activating conditions. *ARG1* induction upon SM treatment resulted in increased *ARG1* mRNA levels as expected (Figure 22A). Interestingly, SM treatment also resulted in increased antisense transcription across the *ARG1* promoter (Figure 22B). The positive correlation between *ARG1* sense and antisense transcription may indicate that antisense transcription promotes *ARG1* sense transcription. Alternatively, antisense transcription may be a result of *ARG1* sense transcription.

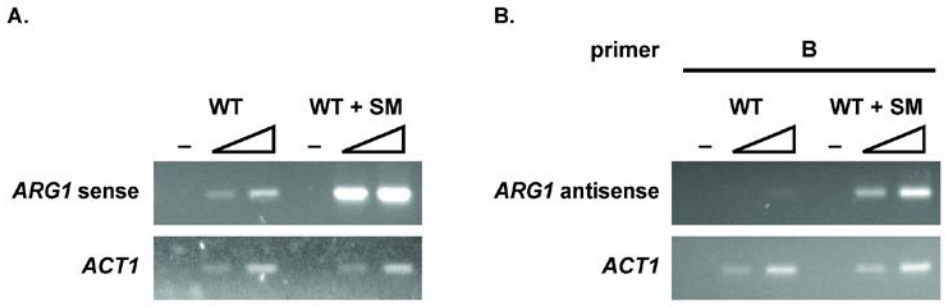


**Figure 22: Antisense transcription traversing the *ARG1* promoter positively correlates with *ARG1* sense transcription in wild-type cells.**

Strand-specific RT-PCR examining *ARG1* sense (A) and antisense (B) transcription in wild-type (KY1699) strains grown in SC-ILV media and mock-treated with DMSO or treated for two hours with 0.6 μg/ml sulfometuron methyl (+SM). cDNA synthesis primers used are noted above samples and correspond to those depicted in (Fig. 8A). For analysis of *ARG1* sense transcription, 3 μl of no RT reactions (-) and 1 μl and 3 μl volumes of 1:2 cDNA were used in PCR reactions to amplify *ARG1* and *ACT1*. For analysis of antisense transcription, 3 μl of no RT reactions (-) and 1 μl and 3 μl volumes of undiluted cDNA were used in PCR reactions to amplify *ARG1* and 3 μl of no RT reactions (-) and 1 μl and 3 μl volumes of 1:2 cDNA were used in PCR reactions to amplify *ACT*. This experiment was performed only once.

To determine if other mutations that cause *ARG1* sense derepression besides *paf1Δ* also cause an increase in antisense transcription across the *ARG1* promoter, I performed strand-specific RT-PCR examining *ARG1* sense and antisense transcript levels in several mutant strains that I have previously found to result in various levels of *ARG1* derepression, including *htb1-K123R, arg80Δ, rtf1Δ, gcn5Δ, set1Δ,* and *set2Δ* (CRISUCCI and ARNDT 2011). For all mutant strains examined, increased *ARG1* sense transcription was accompanied by an increase in antisense transcription across the *ARG1* promoter (Figure 23A-C). Although I have not

quantified these results, it appears that in these mutants, antisense transcription increases proportionally to the increase in sense transcription. These results support my finding that antisense transcription traversing the *ARG1* promoter positively correlates with *ARG1* sense transcription. Interestingly, deletion of *PAF1* and *ARG80*, which resulted in increased Gcn4 binding at the *ARG1* promoter (Figure 23A and C), caused the highest levels of antisense transcription across the *ARG1* promoter (Figure 14A).

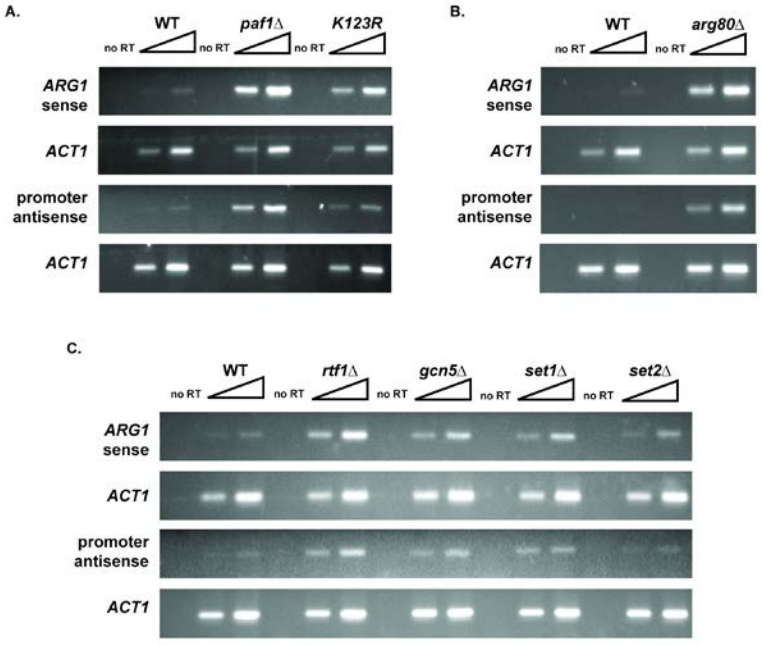


**Figure 23: Antisense transcription traversing the *ARG1* promoter positively correlates with *ARG1* sense transcription in mutant cells.**

(A) Strand-specific RT-PCR examining *ARG1* sense and antisense transcription in wild-type (KY1699), *paf1Δ* (KY1700), and *htb1-K123R* (KY1732) strains. (B) Analysis of *ARG1* sense and antisense transcription in wild-type (KY1699) and *arg80Δ* (KY1709) cells by strand-specific RT-PCR. (C) Strand-specific RT-PCR examining *ARG1*

sense and antisense transcription in wild-type (KY1699), *rtf1Δ* (KY1704), *gcn5Δ* (KY1743), *set1Δ* (KY1755), and *set2Δ* (KY1716) cells. (A-C) cDNA synthesis primer B (Fig. 8A) was used in cDNA synthesis reactions to detect antisense transcription traversing the *ARG1* promoter. For analysis of *ARG1* sense transcription, 3 µl of no RT reactions (-) and 1 µl and 3 µl volumes of 1:2 cDNA were used in PCR reactions to amplify *ARG1* and *ACT1*. For analysis of antisense transcription, 3 µl of no RT reactions (-) and 1 µl and 3 µl volumes of undiluted cDNA were used in PCR reactions to amplify *ARG1* and 3 µl of no RT reactions (-) and 1 µl and 3 µl volumes of 1:2 cDNA were used in PCR reactions to amplify *ACT*. This experiment was performed only once.

### 3.3.14 Paf1 inhibits antisense transcription and Gcn4 recruitment at the promoters of other Paf1-repressed genes.

I have found that, in the absence of Paf1, increased antisense transcription and Gcn4 recruitment at the *ARG1* promoter is accompanied by *ARG1* derepression, suggesting that Paf1 mediates *ARG1* repression by inhibiting Gcn4 recruitment and antisense transcription. To determine if similar events occur at other Paf1-repressed genes, I examined antisense transcription and activator recruitment at the promoters of two Gcn4-regulated genes, *ARG3* and *SNZ1*, which have been shown by microarray analyses to be negatively regulated by Paf1 (PENHEITER *et al.* 2005). As expected, strand-specific RT-PCR analysis detected increased *SNZ1* and *ARG3* sense transcription in *paf1Δ* cells (Figure 24A and B). Interestingly, similar to *ARG1*, there is increased antisense transcription across the Gcn4 binding sites within the *SNZ1* and *ARG3* promoters in *paf1Δ* cells (Figure 24A and B). To determine if antisense transcription across the *SNZ1* and *ARG3* promoters is associated with increased Gcn4 occupancy, I performed ChIP

analyses using strains expressing HA-tagged Gcn4 or an untagged control strain. At the *SNZ1* promoter, Gcn4 was not detected in either wild-type or *paf1Δ* strains at levels above the untagged control strain (Figure 24C). However, Gcn4 could be detected at the *ARG3* promoter in wild-type cells at levels higher than the untagged control (Figure 24D). Interestingly, deletion of *PAF1* resulted in increased Gcn4 occupancy at the *ARG3* promoter (Figure 24D). Therefore, similar to what I observed at the *ARG1* gene, deletion of *PAF1* resulted in increased *SNZ1* and *ARG3* sense transcription and increased antisense transcription traversing Gcn4 binding sites within the promoter. Additionally, at *ARG1* and *ARG3*, this is accompanied by increased Gcn4 recruitment to the promoter. These results suggest that Paf1 may repress *ARG1*, *ARG3*, and *SNZ1* by inhibiting antisense transcription and/or Gcn4-recruitment at promoters.

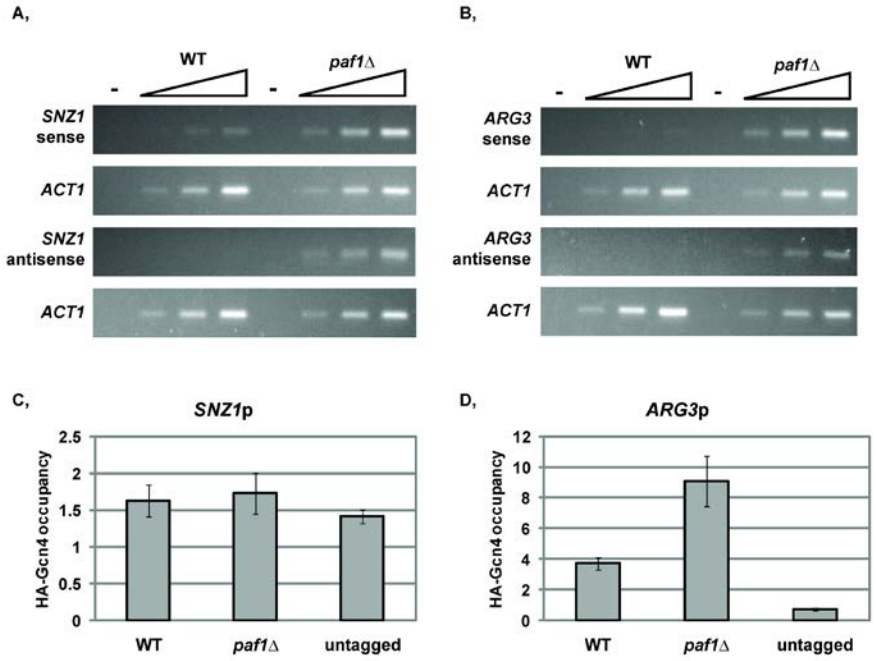


**Figure 24: Paf1 inhibits antisense transcription and Gcn4 recruitment at the promoters of other Paf1-repressed genes.**

(A) Strand-specific RT-PCR examining *SNZ1* sense transcription and antisense transcription across the promoter in wild-type (KY1699) and *paf1Δ* (KY1700) strains. 4 µl of no RT reactions (-) and 0.5 µl, 2 µl, and 4 µl volumes of undiluted or 1:2 cDNA were used in PCR reactions to examine *SNZ1* sense and *ACT1*, respectively. 8 µl of no RT reactions (-) and 2 µl, 4 µl, and 8 µl volumes of undiluted cDNA were used in PCR reactions to examine *SNZ1* antisense transcript levels. The data shown are representative of two independent experiments. (B) Strand-specific RT-PCR examining *ARG3* sense transcription and antisense transcription across the promoter in wild-type (KY1699) and *paf1Δ* (KY1700) strains. 6 µl of no RT reactions (-) and 1 µl, 3 µl, and 6 µl volumes of 1:2 cDNA were used in PCR reactions to examine *ARG3* sense and *ACT1*. The same volumes of undiluted no RT reactions and cDNA were used in PCR reactions to examine *ARG3* antisense. The data shown are representative of two independent experiments. ChIP analysis of HA-Gcn4 occupancy at the *SNZ1* (C) and *ARG3* (D) promoter in wild-type (OKA178) and *paf1Δ* (OKA193) cells expressing HA-Gcn4 and a strain expressing untagged Gcn4 (OKA192). PCR primers used in ChIP analysis amplified *SNZ1* -325 to -222 and *ARG3* -371 to -242, relative to their translation start site. ChIP signals were quantified and normalized as described in Materials and Methods. Shown are the means of three independent experiments. Error bars represent standard error.

## 3.4 DISCUSSION

Here I report the detailed investigation of transcriptional repression by Paf1, using the model gene, *ARG1*. My results define regions of the *ARG1* locus that confer Paf1-mediated repression. Specifically, I found that *ARG1* expression is not influenced by the upstream gene, *GPD2*. Furthermore, the derepression that occurs in the absence of Paf1 partially requires the *ARG1* coding region. Additionally, Paf1 mediates *ARG1* repression independently of the ArgR/Mcm1 repressor complex. Interestingly, at the *ARG1* promoter, Gcn4 occupancy is increased in *paf1Δ* cells, resulting in *ARG1* derepression that is partially suppressed by deletion of *GCN4* or *GCN5*, or mutation of histone H3 acetylation sites. Together my results support a model in which Paf1 mediates *ARG1* repression by preventing Gcn4 recruitment to the *ARG1* promoter and subsequent histone H3 acetylation. Interestingly, I found that Paf1 prevents antisense transcription, which positively correlates with *ARG1* sense transcription, from traversing the *ARG1* promoter, representing a potential mechanism by which the Paf1 complex controls promoter accessibility and ultimately *ARG1* expression.

While investigating the effect of Paf1 on promoter occupancy of Gcn4 and Arg80, I also examined how Gcn4 affects Arg80 binding and vice versa. In repressing conditions I found that deletion of *GCN4* had no effect on Arg80 occupancy at the *ARG1* promoter. Additionally, I found that deletion of *ARG80* resulted in increased Gcn4 recruitment, suggesting that Arg80 inhibits Gcn4 recruitment in repressing conditions, possibly to prevent inappropriate gene

activation. These observations in repressing conditions are the opposite of observations made in inducing conditions, in which Gcn4 and the ArgR/Mcm1 complex reciprocally stimulate each other's promoter-binding (YOON *et al.* 2004; YOON and HINNEBUSCH 2009). Specifically, in inducing conditions, Gcn4 promoter-binding is augmented by Mcm1 binding and Gcn4 constitutively recruits a Mcm1/Arg80 heterodimer (YOON *et al.* 2004). Excess arginine stimulates recruitment of Arg81 and Arg82, resulting in the assembly of a functional repressor complex (YOON *et al.* 2004). In this manner, *ARG1* activation by Gcn4 is quickly abrogated upon the addition of excess arginine. My results suggest that once repressive conditions are established, Arg80 inhibits Gcn4 recruitment.

By examining *ARG1* expression when integrated at an ectopic chromosomal location, I have defined a region of the *ARG1* locus that retains Paf1-mediated repression. Within this region, I examined whether the *ARG1* coding region and/or 3' UTR is required for regulation by Paf1. By replacing the *ARG1* coding region with that of *HIS3*, I found that the derepression observed in the absence of Paf1 partially requires the *ARG1* coding region. Interestingly, the derepression that does not require the *ARG1* coding region is also independent of Gcn4. However, derepression of native *ARG1* in *paf1Δ* cells is partially Gcn4-dependent. Together, these results suggest that Paf1 has Gcn4-independent functions that do not specifically require the *ARG1* coding region and Gcn4-dependent roles that are conferred by the *ARG1* coding region. I previously demonstrated that Paf1mediates *ARG1* repression partially by promoting histone modifications and partially through another mechanism. Because Gcn4 has not been shown to influence Paf1-dependent histone modifications, this may be the Gcn4-independent function of Paf1. My results suggest that antisense transcription that traverses the *ARG1*

promoter in the absence of Paf1 arises from start sites within the *ARG1* coding region. Therefore, Gcn4-dependent functions of Paf1 may include inhibiting antisense transcription.

*ARG1* derepression in *paf1Δ* cells is associated with increased promoter occupancy of Gcn4. Additionally, the *ARG1* derepression that occurs in the absence of Paf1 is suppressed by deletion of *GCN5* or mutation of histone H3 acetylation sites. In activating conditions, Gcn4 recruits several co-activators to the *ARG1* promoter, including the HAT complex SAGA (SWANSON *et al.* 2003). The catalytic subunit of the SAGA complex, Gcn5, acetylates lysine 14 and other residues on histone H3 (GRANT *et al.* 1997; KUO *et al.* 1996). Together with SWI/SNF and RSC, SAGA promotes TBP- and RNA Pol II-recruitment and subsequent transcription elongation (GOVIND *et al.* 2005; KUO *et al.* 2000; SWANSON *et al.* 2003). Therefore, by reducing Gcn4 occupancy at the *ARG1* promoter, Paf1 may inhibit a previously defined pathway in which Gcn4 recruits Gcn5, resulting in histone H3 acetylation and *ARG1* expression.

In addition to its role in promoting *ARG1* expression during transcriptional activation, Gcn5 is also required for *ARG1* repression in rich media in a manner that is dependent on its acetyltransferase activity (RICCI *et al.* 2002). Interestingly, in the presence of Paf1, mutation of histone H3 acetylation sites did not result in *ARG1* derepression, suggesting that acetylation of the histone H3 residues is not required for *ARG1* repression. Therefore, Gcn5 may acetylate another histone residue or a non-histone protein that is required for *ARG1* repression. For example, Gcn5 has been shown to inhibit chromatin remodeling by the RSC complex by acetylating K25 of the Rsc4 subunit (VANDEMARK *et al.* 2007). Since RSC subunits have been shown to contribute to *ARG1* repression (SWANSON *et al.* 2003), Gcn5 may mediate *ARG1* repression by acetylating Rsc4.

The unexpected patterns of histone modifications previously observed at *ARG1* lead us to investigate the possibility of antisense transcription at the *ARG1* locus (CRISUCCI and ARNDT 2011). In accordance with genome-wide studies reporting antisense transcripts arising from the *ARG1* 3' UTR and traversing the coding region in wild-type cells (DAVID *et al.* 2006; XU *et al.* 2009), I detected antisense transcripts crossing the *ARG1* coding region. Therefore, the histone modifications observed at the *ARG1* locus may be laid down co-transcriptionally during antisense transcription; however, basal levels of *ARG1* sense transcription may also contribute to the histone modification pattern observed at *ARG1*. Additionally, although genome-wide analyses identified antisense transcripts arising from the *ARG1* 3' UTR (DAVID *et al.* 2006; XU *et al.* 2009), replacement of the *ARG1* 3' UTR did not eliminate antisense transcription. Therefore, antisense transcription may be capable of arising from multiple start sites within the *ARG1* 3' UTR and coding region.

Interestingly, Paf1 appears to prevent antisense transcription from traversing the *ARG1* promoter. Since I found that antisense transcription across the *ARG1* promoter positively correlates with *ARG1* sense transcription, it is possible that Paf1 represses *ARG1* by preventing antisense transcription across the promoter. Since I was unable to block antisense transcription at the *ARG1* locus by inserting a strong defined terminator sequence within the *ARG1* coding region, I have not yet been able to directly test this hypothesis. While antisense transcription has been shown to negatively regulate the transcription of coding genes (HONGAY *et al.* 2006; HOUSELEY *et al.* 2008; XU *et al.* 2011), it can also positively regulate gene expression. For example, antisense transcription at *PHO5* has been shown to promote transcriptional activation by stimulating chromatin remodeling at the promoter and subsequent RNA Pol II recruitment (UHLER *et al.* 2007). Since I found that antisense transcription across the *ARG1* promoter

positively correlates with *ARG1* sense transcription and Gcn4 occupancy, I hypothesize that, similar to *PHO5*, antisense transcription may support promoter accessibility at *ARG1*, which could result in increased recruitment of Gcn4 and RNA Pol II in *paf1Δ* strains. Unlike *PHO5*, I did not observe chromatin remodeling in the absence of Paf1. Therefore, antisense transcription may promote Gcn4 recruitment by another method, such as histone modifications. Alternatively, *ARG1* sense transcription may promote antisense transcription in the absence of Paf1. In this case, antisense transcription may not influence sense transcription or may participate in a feed forward mechanism to subsequently promote sense transcription.

It would also be interesting to understand how Paf1 prevents antisense transcription from traversing the *ARG1* promoter and whether this function of Paf1 is connected to known roles in transcription termination. The Paf1 complex has been shown to be important for proper transcription termination of both polyadenylated and non-polyadenylated transcripts. Specifically, the loss of Paf1 results in altered polyadenylation site utilization and enrichment of 3' extended transcripts (PENHEITER *et al.* 2005). Additionally, the Arndt lab has previously shown that deletion of *PAF1* results in small nucleolar RNAs (snoRNAs) with extended 3' ends (SHELDON *et al.* 2005). Therefore, the Paf1 complex could prevent antisense transcription from traversing the *ARG1* promoter by promoting proper termination of the antisense transcripts at the *ARG1* locus. Consistent with this hypothesis, a combination of 3' RACE and strand-specific RT-PCR identified longer polyadenylated and presumably non-polyadenylated transcripts in *paf1Δ* cells. Finally, since the conserved Paf1 complex is important for human health, additional studies of the multiple functions of the Paf1 complex in yeast may explain some of its roles in transcription and disease in higher eukaryotes.

**Table 2: 3' RACE Results**

| | **WT** | | ***paf1Δ*** | |
|---|---|---|---|---|
| | Number of Clones | 3' End Position | Number of Clones | 3' End Position |
| | 2 | -69 | 3 | -49 |
| | 4 | -66 | 1 | -64 |
| | 1 | -65 | 1 | -65 |
| | 1 | 335 | 4 | -66 |
| | 7 | 352 | 7 | -67 |
| | 2 | 424 | 4 | -69 |
| Total Clones | 17 | | 20 | |

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

| Strain | Genotype |
|---|---|
| ECY155 | *MAT***a** *ura3Δ0* |
| ECY410 | *MAT***a** *ura3Δ0 paf1Δ::KanMX* |
| KA117 | *MAT***a** *ARG80-13xMYC::HIS3 gcn4Δ::KanMX his3Δ0* |
| KA118 | *MAT***a** *ARG80-13xMYC::HIS3 paf1Δ::KanMX gcn4Δ::KanMX his3Δ0* |
| KA119 | *MAT***a** *ARG80-13xMYC::HIS3 his3Δ0* |
| KA120 | *MATα (hht2-hhf2)::HHTS-URA3/HHFS (hht1-hhf1)Δ::NatMX ura3-52* |
| KA121 | *MATα (hht2-hhf2)::HHTS-URA3/HHFS (hht1-hhf1)Δ::NatMX paf1Δ::KanMX ura3Δ0* |
| KA122 | *MAT***a** *(hht2-hhf2)::hhts K9A-URA3/HHFS (hht1-hhf1)Δ::NatMX ura3Δ0* |
| KA123 | *MAT***a** *(hht2-hhf2)::hhts K9A-URA3/HHFS (hht1-hhf1)Δ::NatMX paf1Δ::KanMX ura3Δ0* |
| KA124 | *MATα (hht2-hhf2)::hhts K14A-URA3/HHFS (hht1-hhf1)Δ::NatMX ura3Δ0* |
| KA125 | *MATα (hht2-hhf2)::hhts K14A-URA3/HHFS (hht1-hhf1)Δ::NatMX paf1Δ::KanMX ura3-52* |
| KA126 | *MATα (hht2-hhf2)::hhts K4,9,14,18A-URA3/HHFS (hht1-hhf1)Δ::NatMX ura3-52* |
| KA127 | *MATα (hht2-hhf2)::hhts K4,9,14,18A-URA3/HHFS (hht1-hhf1)Δ::NatMX paf1Δ::KanMX ura3-52* |
| KA128 | *MATα (hht2-hhf2)::HHTS/HHFS-URA3 (hht1-hhf1)Δ::NatMX ura3-52* |
| KA129 | *MATα (hht2-hhf2)::HHTS/HHFS-URA3 (hht1-hhf1)Δ::NatMX paf1Δ::KanMX ura3-52* |
| KA130 | *MATα (hht2-hhf2)::HHTS/hhfs K12A-URA3 (hht1-hhf1)Δ::NatMX ura3-52* |
| KA131 | *MATα (hht2-hhf2)::HHTS/hhfs K12A-URA3 (hht1-hhf1)Δ::NatMX paf1Δ::KanMX ura3Δ0* |
| KA132 | *MAT***a** *(hht2-hhf2)::HHTS/hhfs K16A-URA3 (hht1-hhf1)Δ::NatMX ura3Δ0* |
| KA133 | *MAT***a** *(hht2-hhf2)::HHTS/hhfs K16A-URA3 (hht1-hhf1)Δ::NatMX paf1Δ::KanMX ura3Δ0* |
| KA134 | *MATα (hht2-hhf2)::HHTS/hhfs K5,8,12,16R-URA3 (hht1-hhf1)Δ::NatMX ura3-52* |
| KA135 | *MAT***a** *(hht2-hhf2)::HHTS/hhfs K5,8,12,16R-URA3 (hht1-hhf1)Δ::NatMX paf1Δ::KanMX ura3Δ0* |

KA139 *MAT***a** *ARG81-13xMYC::HIS3 his3Δ1*

KA142 *MAT***a** *ARG81-13xMYC::HIS3 paf1Δ::KanMX his3Δ1*

KA143 *MAT***a** *ARG82-13xMYC::HIS3 his3Δ1*

KA146 *MAT*α *ARG82-13xMYC::HIS3 paf1Δ::KanMX his3Δ1*

KA155 *MAT*? *rpb1-1 paf1Δ::KanMX ura3-52*

KY803 *MAT*α *his3Δ200 lys2-173R2 leu2*Δ(*0* or *1*) *ura3*(Δ*0* or *52*)

KY1343 *MAT*α *gcn5*Δ*::HIS3 his3Δ200 leu2Δ1 ura3-52 trp1Δ63*

KY1348 *MAT*α *gcn5*Δ*::HIS3 paf1*Δ*::URA3 his3Δ200 leu2Δ1 ura3-52*

KY1698 *MAT***a**

KY1699 *MAT*α

KY1700 *MAT*α *paf1Δ::KanMX*

KY1702 *MAT***a** *paf1Δ::KanMX leu2Δ0 ura3Δ0*

KY1703 *MAT***a** *rtf1Δ::KanMX*

KY1704 *MAT*α *rtf1Δ::KanMX*

KY1708 *MAT*α *gcn4Δ::KanMX*

KY1709 *MAT*α *arg80Δ::KanMX*

KY1716 *MAT***a** *set2Δ::KanMX*

KY1719 *MAT*α *paf1Δ::KanMX gcn4Δ::KanMX*

KY1720 *MAT***a** *paf1Δ::KanMX arg80Δ::KanMX*

KY1728 *MAT*α *GCN4-3xHA::KanMX*

KY1729 *MAT***a** *GCN4-3xHA::KanMX arg80Δ::KanMX*

KY1730 *MAT***a** *GCN4-3xHA::KanMX paf1Δ::KanMX arg80Δ::KanMX*

KY1732 *MAT*α *HTA1-htb1-K123R (hta2-htb2)Δ::KanMX ura3Δ0*

KY1734 *MAT***a** *(lyp1* $_{-44-2035}$*)Δ::ARG1*$_{-497-1533}$ *arg1Δ::NatMX*

KY1735 *MAT***a** *(lyp1* $_{-44-2035}$*) Δ::ARG1*$_{-497-1533}$ *arg1Δ::NatMX paf1Δ::KanMX*

KY1736 *MAT*α *ARG1p-HIS3*$_{1-663}$ *his3Δ::NatMX*

KY1737 *MAT***a** *ARG1p-HIS3*$_{1-663}$ *his3Δ::NatMX paf1Δ::KanMX*

KY1739 *MAT***a** *ARG1p-HIS3*$_{1-663}$ *his3Δ::NatMX gcn4Δ::KanMX ura3-52*

KY1740 *MAT***a** *ARG1p-HIS3*$_{1-663}$ *his3Δ::NatMX gcn4Δ::KanMX paf1Δ::KanMX ura3-52*

KY1741 *MAT***a** *gcn5Δ::KanMX*

KY1743 *MAT*α *gcn5Δ::KanMX*

KY1755 *MAT*α *set1Δ::KanMX*

KY1871 *MAT***a** *ARG1p-HIS3*$_{1\ -822}$ *his3Δ::NatMX*

KY1874 *MAT***a** *ARG1p-HIS3*$_{1\ -822}$ *his3Δ::NatMX paf1Δ::KanMX*

KY2026 *MAT***a** *paf1Δ::KanMX ura3-52*

KY2112 *MAT***a** *(lyp1*$_{-290-1836}$*)Δ::ARG1*$_{-497-1263}$ *arg1Δ::NatMX*

KY2115 *MAT***a** *(lyp1*$_{-290-1836}$*)Δ::ARG1*$_{-497-1263}$ *arg1Δ::NatMX paf1Δ::KanMX*

KY2118 *MAT*α *(lyp1*$_{-290-2059}$*)Δ::ARG1*$_{-497-1553}$ *arg1Δ::NatMX*

KY2121 *MAT*α *(lyp1*$_{-290\ -2059}$*)Δ::ARG1*$_{-497-1553}$ *arg1Δ::NatMX paf1Δ::KanMX*

KY2144 *MAT***a** *ARG1::HIS3-T* $_{antisense\ 420}$ *ura3Δ0*

KY2147 *MAT***a** *ARG1::HIS3-T* $_{antisense\ 420}$ *paf1Δ::KanMX ura3Δ0*

OKA127 *MAT***a** *ARG80-13xMYC::HIS3 his3Δ0 leu2Δ0 ura3Δ0 met15Δ*

OKA145 *MAT***a** *ARG80-13xMYC::HIS3 paf1Δ::KanMX his3Δ0 leu2Δ0 ura3Δ0 met15Δ*

OKA178 *MAT***a** *ARG80-13xMYC::HIS3 GCN4-3xHA::KanMX his3Δ0*

OKA192 *MAT***a** *ARG80-13xMYC::HIS3 his3Δ0*

OKA193 *MAT***a** *ARG80-13xMYC::HIS3 GCN4-3xHA::KanMX paf1Δ::KanMX his3Δ0*

# 4.0 IDENTIFICATION OF TRANSCRIPTIONAL DEFECTS IN *PAF1Δ* CELLS USING STRAND-SPECIFIC HIGH DENSITY TILING MICROARRAYS

## 4.1 INTRODUCTION

Transcription termination plays important roles in regulating gene expression by promoting RNA Pol II recycling, preventing transcriptional interference of downstream promoters, and influencing transcript stability (reviewed in references GILMOUR and FAN 2008; KUEHNER *et al.* 2011; RICHARD and MANLEY 2009; ROSONINA *et al.* 2006). In addition to its functions during transcription elongation, the Paf1 complex is important for proper transcription termination (SHELDON *et al.* 2005) and RNA 3' end formation in yeast (MUELLER *et al.* 2004; NORDICK *et al.* 2008; PENHEITER *et al.* 2005). The loss of Paf1 complex members or Paf1 complex-dependent histone modifications results in 3'-extended small nucleolar RNAs (snoRNAs) (SHELDON *et al.* 2005; TOMSON *et al.* 2011b). Furthermore, loss of Paf1 complex members results in reduced RNA Pol II-association of Cft1, a cleavage and polyadenylation factor (NORDICK *et al.* 2008), and altered poly(A) tail length and polyadenylation site usage, sometimes generating transcripts that are subject to nonsense-mediated decay (MUELLER *et al.* 2004; NORDICK *et al.* 2008; PENHEITER *et al.* 2005). Therefore, the Paf1 complex likely plays a critical role in coordinating transcription with transcription termination and 3' end processing. Importantly, the human Paf1 complex also promotes proper RNA 3' end formation (NAGAIKE *et al.* 2011; ROZENBLATT-

ROSEN *et al.* 2009), indicating that the functions of the Paf1 complex in regulating 3' end formation are conserved throughout eukaryotes.

Non-coding transcription is also becoming increasingly recognized as a powerful regulator of gene expression. Such transcription can negatively regulate the transcription of coding genes. For example, transcription across promoters has been shown to inhibit transcription of coding genes through a transcription-interference mechanism in which the act of transcription prevents activator binding. Such inhibitory non-coding transcripts have been identified both in the sense direction at *SER3* and *FLO11* (BUMGARNER *et al.* 2009; MARTENS *et al.* 2004; MARTENS *et al.* 2005) and in the antisense direction at *IME4* (HONGAY *et al.* 2006). Additionally, antisense transcription can cause repression of coding transcripts by creating a repressive chromatin environment. For example, antisense transcription at the *GAL11* locus leads to repression by recruiting the histone H3 K36 methyltransferase, Set2, and subsequent histone deacetylase (HDAC) activity (HOUSELEY *et al.* 2008). Alternatively, antisense transcription has also been shown to positively regulate transcription of coding mRNAs. For example, antisense transcription at *PHO5* has been shown to promote transcriptional activation by stimulating chromatin remodeling at the promoter and subsequent RNA Pol II recruitment (UHLER *et al.* 2007).

A previous genome-wide study investigated changes in gene expression that occur in the absence of Paf1 (PENHEITER *et al.* 2005). However, the microarrays used in this study contained approximately 16 probes internal to each open reading frame (ORF) (PENHEITER *et al.* 2005) and, therefore, would not directly detect changes in transcription termination, polyadenylation site usage, or changes in transcription of novel non-coding RNAs. Therefore, to uncover such transcriptional defects that occur in the absence of Paf1throughout the yeast genome, I initiated a

project that involved the use of strand-specific high-density tiling microarray analyses. These arrays contained 6.5 million probes tiling the yeast genome that provided strand-specific transcript levels with 8 nucleotide resolution. In addition to identifying individual genes whose proper transcription requires Paf1, the high resolution and strand-specific data allowed us to appreciate changes in termination, polyadenylation site usage, and non-coding transcription that occur in the absence of Paf1.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Sample preparation

100 ml cultures grown to a cell density of 1-2x$10^7$ cell/ml were harvested for RNA preparation by hot phenol total RNA extraction as previously described (SCHMITT *et al.* 1990). 100 μg total RNA was DNase-treated for 30 min at 37°C with DNase I (GE Healthcare). DNase-treated RNA was purified using the RNeasy Mini Kit with modifications to the protocol designed to facilitate purification of small transcripts as described (JUNEAU *et al.* 2007). 25 μg purified, DNase-treated RNA was used in cDNA synthesis reactions containing SuperScript II Reverse Transcriptase (Invitrogen #18064-014), random hexamers, oligo (dT), and dNTPs as described (JUNEAU *et al.* 2007). The resulting cDNA was purified using the protocol and buffers from the QIAquick Nucleotide Removal Kit (Qiagen #28304) with the columns from the MinElute Reaction Cleanup Kit (Qiagen #28204) (JUNEAU *et al.* 2007).

### 4.2.2 Microarray Hybridization and Analysis

Three independent cDNA samples prepared from wild-type and *paf1Δ* cells were mailed to our collaborator, Corey Nislow at the University of Toronto, for additional processing and hybridization to strand-specific high-density microarrays. The arrays contain 6.5 million probes that tile each strand of the *Saccharomyces cerevisiae* genome, providing strand-specific genome-wide transcript levels with 8 nucleotide resolution (DAVID *et al.* 2006). Each sample was hybridized separately, each yielding a separate Affymetrix CEL file containing the intensity values for each probe. CEL files were processed by Kara Juneau and Curtis Palm at Stanford University, using Affymetrix Tiling Array Software (TAS). The resulting data are an average of three *paf1Δ* vs. wild-type comparisons. Curtis Palm determined intervals of significance, which are continuous regions with significant relative signal as determined by several criteria, including threshold, p value, minimum run, and maximum gap. Curtis Palm determined the threshold value based on changes in expression observed at known Paf1-regulated loci. Probes with relative intensities with p values of 0.1 or less were included in intervals. The minimum run required to specify an interval was 75 nucleotides. The maximum gap allowed in an interval was 50 nucleotides. Intervals were determined irrespective of open reading frame or gene structure. The data were binned by Kyle Tsui and Lawrence Heilser, who provided us with 80 bins of data internal to each ORF and 80 bins, each 10 nucleotides in length, for 800 nucleotides both upstream and downstream of each ORF

## 4.3 RESULTS

### 4.3.1 Identification of Paf1-regulated genes

To uncover transcriptional defects that occur in the absence of Paf1, I prepared cDNA for hybridization to high resolution microarrays. Specifically, I extracted total RNA from three independently grown wild-type and *paf1Δ* cultures, which, after DNase treatment, I used in cDNA synthesis reactions using both oligo (dT) and random hexamers. Our collaborator, Dr. Corey Nislow, hybridized the cDNA symples to the arrays and performed the initial data analysis, providing us with the average $\log_2$ ratio signal intensity in *paf1Δ* relative to wild-type cells for 6.5 million probes that tile the yeast genome. Given this large data set, one can examine the relative intensity for each probe individually or divide the data into bins to extract specific information. For example, to examine changes in transcription within coding regions of genes, the signals for probes internal to coding regions were divided into 80 bins of variable length, relative to the length of the ORF. Brett Tomson converted the log2 intensity to raw intensity for each internal bin and calculated the mean and median ratio of *paf1Δ* intensity relative to wild-type for each ORF, retaining strand-specificity. Therefore, by calculating the mean and median relative intensity across 80 bins for each ORF, we identified genes whose expression was altered in *paf1Δ* cells. We obtained the mean and median signal for 6609 open reading frames (ORFs). From this data set, mitochondrial genes and dubious ORFs were eliminated. Of the remaining 5824 ORFs, 1090 genes exhibited a two-fold or more change in transcription. Given that 19% of genes examined showed Paf1-dependent expression, Paf1 proves to be an important regulator of gene expression throughout the genome. Of the Paf1-dependent genes, 880 genes (15%) exhibited a two-fold or more reduction in transcript levels (Table 4) and 210 genes (4%)

exhibited a two-fold or more increase in transcript levels (Table 5) (Figure 25) in *paf1Δ* cells. Therefore, while Paf1 appears to function predominantly as a positive transcriptional regulator, Paf1 also has important repressive roles at numerous genes.

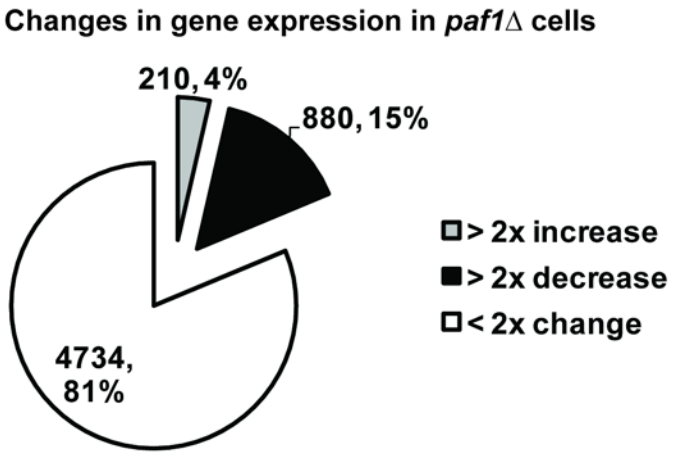


**Figure 25: Changes in transcription in *paf1Δ* cells.**

Pie chart depicting the number of genes whose expression increases 2-fold or more (> 2x increase), decreases 2-fold or more (> 2x decrease), or changes less than 2 fold (< 2x change) in the absence of Paf1. Values indicate the number and percentage of genes with the appropriate change in transcription.

Importantly, the array data match well with my analyses of gene expression by other methods, such as Northern analysis and RT-PCR. For example, the array data indicated that *ARG1*, *GAP1*, *SNZ1*, and *ARG3* were derepressed in the absence of Paf1 (Figure 26A-D). I performed Northern analysis and/or RT-PCR to examine expression of each of these genes and found that *ARG1* (Figure 26A), *GAP1* (Figure 26B), *SNZ1* (Figure 26C), and *ARG3* (Figure

26D), were, in fact, derepressed in *paf1Δ* cells. Additionally, as part of her rotation project, Sarah Hainer performed Northern analysis or RT-PCR to examine expression of five genes identified in our arrays as Paf1-activated (*YTP1*, *AGP2*, *HBT1*, *NCA3*, and *SNO1*) and six genes identified as Paf1-repressed (*ATF2*, *YOR356W*, *YOL057W*, *CWP1*, *GDT1*, and *HEM15*) (data not shown). The expression changes that she observed by these methods correlated well with those indicated by our arrays. Together, these results suggest that our array data accurately report transcription in wild-type and *paf1Δ* cells and have identified many Paf1-regulated genes.

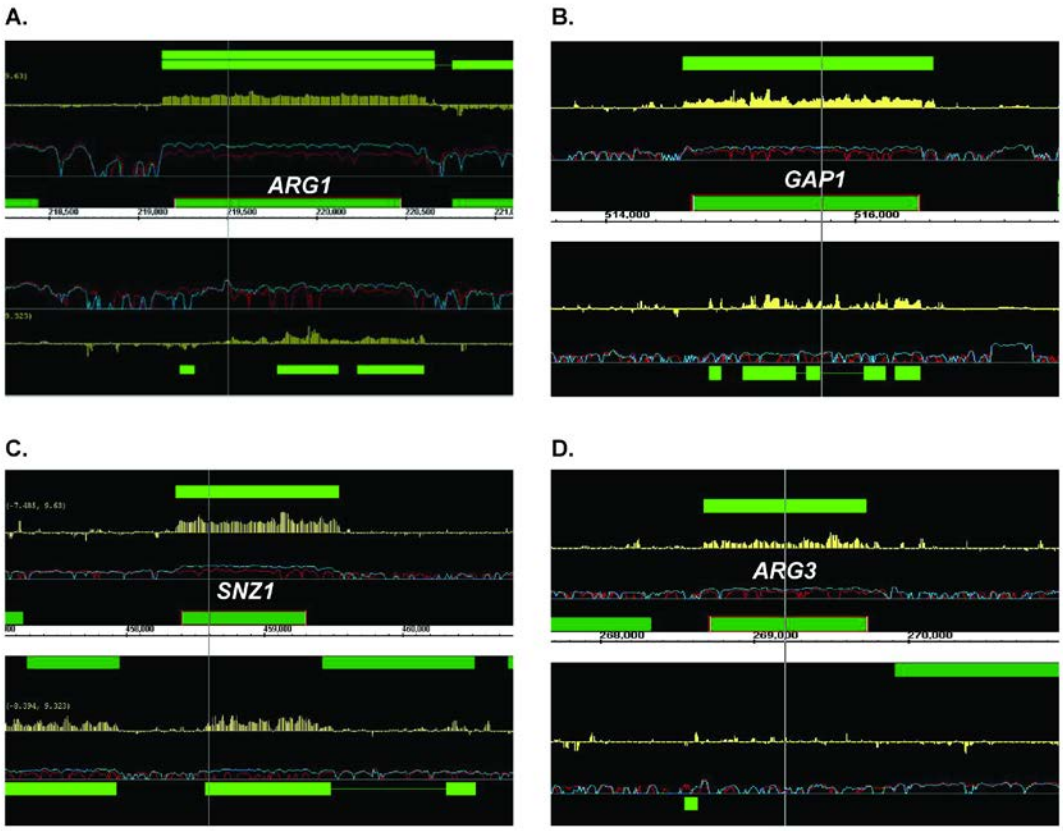


**Figure 26: Integrated Genome Browser snapshots of high density microarray data at the *ARG1*** (A), *GAP1* (B), *SNZ1* (C), and *ARG3* (D) locus. Dark green bars represent coding regions. Red line represents transcript levels in wild-type cells. Blue line represents transcript levels in *paf1Δ* cells. Yellow bars depict the $\log_2$ ratio of *paf1Δ* relative to wild-type. Light green bars indicate intervals of significance as described in Materials and Methods. Genes and the associated data for the Watson and Crick strand are shown above and below chromosomal coordinates, respectively.

### 4.3.2 Overlap between Paf1- and Gcn4-regulated genes

The goal of my thesis has been to elucidate mechanisms by which the Paf1 complex mediates transcriptional repression. To do this, I have used *ARG1* as a model gene. Interestingly, I found that Paf1 inhibits recruitment of the gene-specific activator, Gcn4, to the *ARG1* promoter (Figure 14A). Furthermore, the *ARG1* derepression that occurs in the absence of Paf1 is Gcn4-dependent (Figure 14E and F). Therefore, I hypothesize that Paf1 may mediate *ARG1* repression by inhibiting Gcn4, which raises the possibility that Paf1 might regulate other Gcn4-activated genes through a similar mechanism. Therefore, now that we have indentified numerous additional Paf1-repressed genes (Table 5), I decided to ask whether other Paf1-repressed genes are also Gcn4-activated.

If Paf1 regulates Gcn4-activated genes through a common mechanism, then I would expect many Paf1-repressed genes to also be Gcn4-activated, and vice versa. Therefore, I compared genes identified by our microarray analyses to be negatively regulated by Paf1 with two published data sets of Gcn4-regulated genes. The first data set includes 80 targets of Gcn4 binding (POKHOLOK *et al.* 2005). These genes exhibit Gcn4-dependent expression in nutrient limiting conditions, contain a conserved Gcn4 binding site in their promoter, and their promoters are bound by Gcn4 *in vitro* (POKHOLOK *et al.* 2005). The second data set includes 126 Gcn4-activated genes, which were considered Gcn4-activated if their expression was decreased at least 1.5 fold in the absence of Gcn4 (HUGHES *et al.* 2000). I found that 7 of the 80 targets of Gcn4 binding were amongst the 210 Paf1-repressed genes (Figure 27A). These results indicate that although other genes besides *ARG1* are regulated by both Paf1 and Gcn4, relatively few Paf1-repressed genes are also targets of Gcn4 binding. Consistent with these results, I found that of the 126 Gcn4-activated genes, only 20 were amongst the 210 Paf1-repressed genes (Figure 27B).

For comparison, I examined the overlap between Paf1-activated genes and the two data sets of Gcn4-regulated genes. I found that of the 880 Paf1-activated genes, 19 are targets of Gcn4 binding and 19 are activated by Gcn4, indicating that approximately 2% of Paf1-activated genes are also Gcn4-activated (Figure 27C and D). Together, these results suggest that, while Paf1 may inhibit the action of Gcn4 at *ARG1*, this is likely not a common mechanism of gene regulation by Paf1.

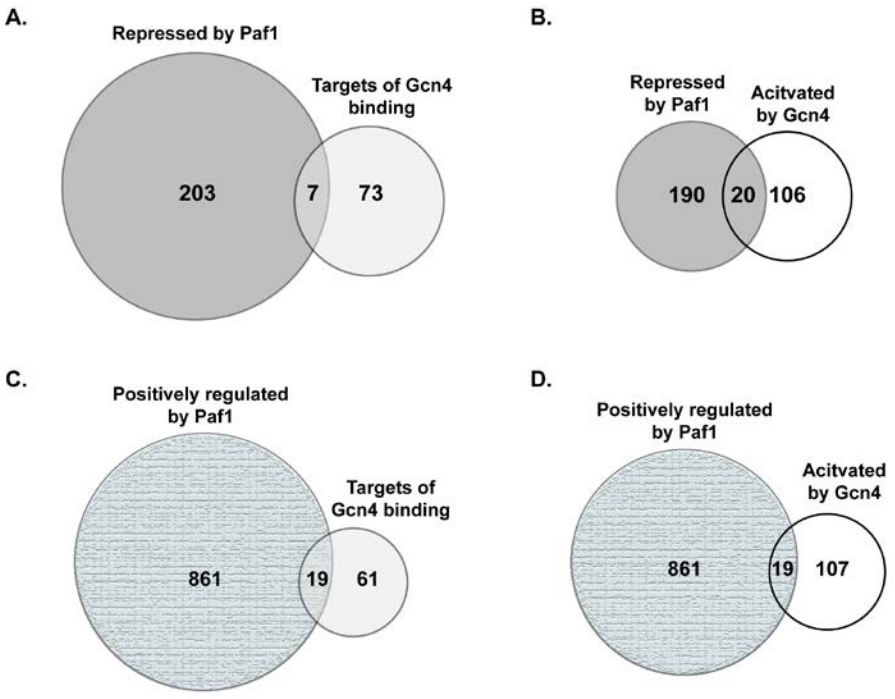


**Figure 27: Comparison of Paf1-regulated and Gcn4-activated genes**

Overlap of Paf1-repressed genes with known targets of Gcn4 binding (A) and Gcn4-activated genes (B). Overlap of Paf1-activated genes with known targets of Gcn4 binding (C) and Gcn4-activated genes (D).

### 4.3.3 Identification of Paf1-regulated antisense transcription

Given the widespread roles of the Paf1 complex in the regulation of transcription, I predicted that the Paf1 complex may regulate transcription of non-coding RNAs. Since genome-wide studies have identified an antisense transcript at the *ARG1* locus, my model locus of Paf1-dependent repression (DAVID *et al.* 2006; XU *et al.* 2009), I was particularly interested in determining whether antisense transcription occurs at other Paf1-regulated genes and whether Paf1 influences such transcription. At my model gene, *ARG1*, visual inspection of the array data using Integrated Genome Browser (NICOL *et al.* 2009) revealed that deletion of *PAF1* resulted in an increase in both sense and antisense transcription (Figure 18C). Since antisense transcription positively correlates with sense transcription, it is possible that antisense transcription facilitates sense transcription. Therefore, Paf1 could inhibit sense transcription indirectly by inhibiting antisense transcription at the *ARG1* locus. While I performed experiments to examine this hypothesis (Chapter 2), I also predicted that if Paf1 does mediate gene repression by inhibiting antisense, I would expect other Paf1-repressed genes to exhibit Paf1-repressed antisense transcription.

To determine whether Paf1-regulated genes are associated with Paf1-regulated antisense transcription, Brett Tomson calculated the mean and median relative probe intensity for both the sense and antisense strands for probes internal to coding regions. We found that of the 210 genes that displayed a 2-fold or more increase in sense expression in *paf1Δ* cells, 57 (27%) also exhibited a 2-fold or more increase in antisense transcription and 6 (3%) exhibited a 2-fold or more decrease in antisense transcription (Figure 28A). These results indicate that a large proportion of Paf1-repressed genes also have Paf1-repressed antisense transcription. In comparison, of the 880 genes whose expression was decreased 2-fold or more in the absence of Paf1, 62 (7%) displayed a 2-fold or more decrease in antisense transcription, and only 3 (0.3%)

had a 2-fold or more decrease in antisense transcription (Figure 28B). These data indicate that Paf1-dependent changes in antisense transcription usually positively correlate with changes in sense transcription. In particular, the yeast genome contains numerous loci at which Paf1 represses both sense and antisense transcription.

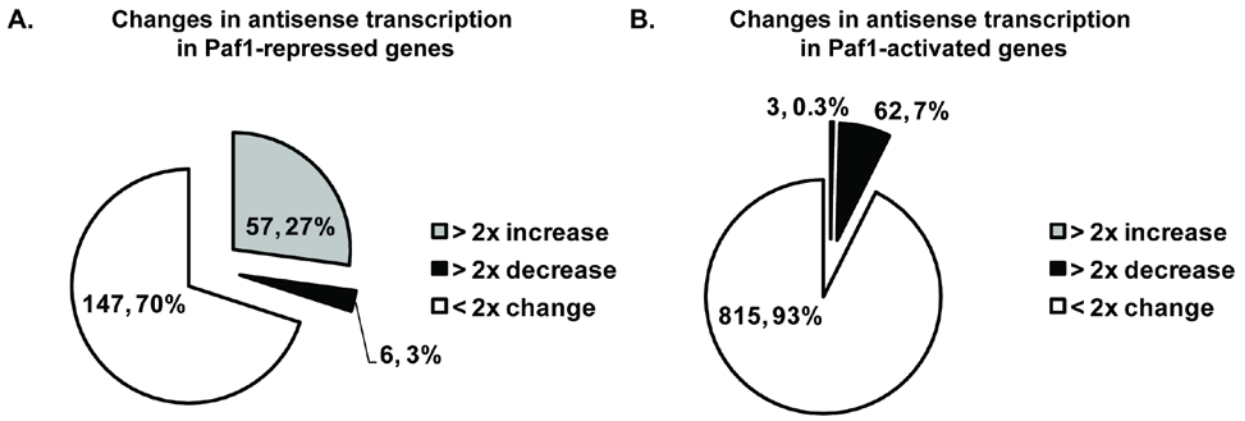


**Figure 28: Changes in antisense transcription associated with Paf1-regulated genes**

(A) Pie chart depicting the number of Paf1-regulated genes (as indicated by a 2-fold or more increase in sense transcription in *paf1Δ* cells) that are associated with antisense transcription that increases 2-fold or more (> 2x increase), decreases 2-fold or more (> 2x decrease), or exhibits a less than 2-fold change in *paf1Δ* cells (< 2x change). Values indicate the number and percentage of Paf1-repressed genes with the appropriate change in antisense transcription. (B) Pie chart depicting the number of Paf1-activated genes (as indicated by at least a 2-fold decrease in sense transcription in *paf1Δ* cells) that are associated with antisense transcription that increases 2-fold or more (> 2x increase), decreases 2-fold or more (> 2x decrease), or exhibits a less than 2 fold change in *paf1Δ* cells (< 2x change). Values indicate the number and percentage of Paf1-activated genes with the appropriate change in antisense transcription.

Despite our promising results, it has subsequently been demonstrated that some antisense transcripts detected by microarray analyses may be artifacts caused by spurious second-strand cDNA synthesis during reverse transcription reactions (PEROCCHI *et al.* 2007). Therefore, our array data may be an overrepresentation of Paf1-regulated antisense transcription. To begin examining this possibility, I performed strand-specific RT-PCR, a method which has been used to distinguish between real antisense transcripts and artifacts (PEROCCHI *et al.* 2007). I examined *ARG1* and *SNZ1*, whose mean and median relative probe intensity showed a more than 2-fold increase in both sense and antisense transcription in *paf1Δ* cells (Figure 26A and Table 6). Additionally, I examined *ARG3*, a gene that did not meet our strict criteria, because, although sense transcription increased more than 2-fold in *paf1Δ* cells (13.82 mean, 6.58 median), the median antisense change was just under a 2-fold increase (mean 2.81, median 1.95). However, we felt that since the mean relative probe intensity increased more than 2-fold and the median relative intensity was just below our cut-off value, *ARG3* transcriptional changes could be verified. Interestingly, my results using strand-specific RT-PCR matched well with our array results. As expected, strand-specific RT-PCR detected an increase in sense transcription in *paf1Δ* cells at all three loci. Interestingly, this method also detected an increase in antisense transcription over the promoters of *ARG1*, *SNZ1*, and *ARG3* in *paf1Δ* cells (Figures 18B, 24A and 24B), suggesting that antisense transcription detected at these genes is not an artifact of second-strand cDNA synthesis. Additionally, antisense artifacts resulting from second-strand cDNA synthesis would be expected to increase proportionally to increases in sense expression. However, despite the increase in *ARG1* sense transcription in *paf1Δ* cells, antisense transcription within the *ARG1* coding region does not increase in *paf1Δ* cells (Figure 18B), further supporting

my conclusion that antisense transcription at *ARG1* is not an artifact. Therefore, while the antisense transcription detected by our microarray analysis requires additional verification, these results suggest that antisense transcripts detected at these loci are not artifacts of second-strand cDNA synthesis.

### 4.3.4 Identification of snoRNA transcription termination defects in *paf1Δ* cells

The Arndt lab previously identified a role for the Paf1 complex in proper transcription termination and 3' end formation of snoRNAs (SHELDON *et al.* 2005). In the absence of Paf1, snoRNAs *SNR13* and *SNR47* exhibit extended 3' ends due to reduced recruitment of Nrd1, a 3' end processing factor (SHELDON *et al.* 2005). Given the high resolution of the array data, I predicted that termination defects at other snoRNA genes could be detected. But first, I visually examined the array data to determine whether this method revealed readthrough of the transcription termination site that was previously identified at *SNR13* and *SNR47* by Northern analysis (SHELDON *et al.* 2005). At both loci, there appears to be increased transcription downstream of the ORF in the absence of Paf1, indicative of termination defects (Figure 29A and B). However, the effect is more pronounced at *SNR47*, where in *paf1Δ* cells there is increased transcription beyond the *SNR47* termination site, which continues into the downstream gene (Figure 29A and B). While the results at *SNR13* are more subtle, the results at *SNR47* match well with previously published Northern analyses detecting a 3'-extended transcript that continues into the downstream gene (SHELDON *et al.* 2005). Therefore, although the array data may not detect subtle termination defects, the arrays do detect more robust or more highly expressed 3'-extended transcripts at loci such as *SNR47*.

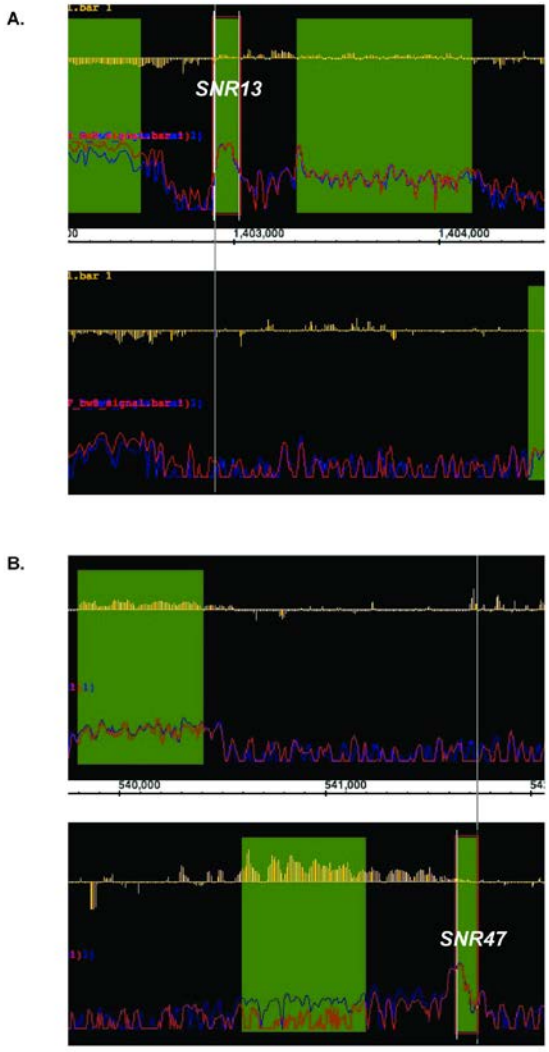


**Figure 29: Integrated Genome Browser snapshots of high density microarray data at the *SNR13* and *SNR47* loci**

Integrated Genome Browser snapshots of high density microarray data at the *SNR13* (A) and *SNR47* (B) loci. Dark green bars represent coding regions. Red line represents transcript levels in wild-type cells. Blue line represents transcript levels in *paf1Δ* cells. Yellow bars depict the $\log_2$ ratio of *paf1Δ* relative to wild-type. Genes and the associated data for the Watson and Crick strands are shown above and below chromosomal coordinates, respectively.

To identify additional snoRNA genes whose proper transcription requires Paf1, we used data that was binned in increments of 10 nucleotides downstream of each ORF. Brett Tomson calculated the mean and median *paf1Δ*/wild-type probe intensity for 20 bins (200 nucleotides) downstream of each snoRNA ORF. With this analysis, Brett was able to identify snoRNA genes with extended 3' ends, likely resulting from readthrough of the transcription termination signals in the absence of Paf1. She found that, of 75 snoRNA genes not located within introns, 17 (23%) exhibited at least a 2-fold average increase in transcription within 200 bases downstream of the gene (Table 7). Additionally, 2 of 8 snoRNA genes located in introns, *SNR191* and *SNR54*, showed a 2-fold or more increase in transcription within 150 bases downstream in *paf1Δ* cells. Of the two previously identified snoRNAs whose termination requires the Paf1 complex, *SNR47*, but not *SNR13*, showed at least a 2-fold increase in transcription within the downstream 200 nucleotides (Table 7). Therefore, while analysis of the array data in this manner may not identify subtle snoRNA transcription termination defects in the absence of Paf1, we were able to identify several novel snoRNA genes with strong Paf1-dependent effects on transcription termination. These newly identified genes will be analyzed by Brett Tomson as she uncovers the mechanism by which the Paf1 complex mediates transcription termination of snoRNAs.

## 4.4 DISCUSSION

Here, we used high-resolution strand-specific microarray analyses to examine genome-wide transcriptional defects that occur in the absence of Paf1. In addition to identifying numerous genes whose proper transcription requires Paf1, we investigated the overlap between Paf1- and

Gcn4-regulated genes, uncovered a potential role of Paf1 in regulating antisense transcription, and revealed a genome-wide role for Paf1 in the transcription termination of snoRNAs. Together, our results report the first detailed investigation of transcriptional defects that occur genome-wide in the absence of Paf1.

Our data indicate that a large portion of the yeast genome requires Paf1 for proper expression, indicating that the Paf1 complex has wide-spread effects on gene expression. Consistent with these results, strains lacking Paf1 complex members exhibit phenotypes associated with transcriptional defects, such as sensitivity to 6-azauracil and mycophenolic acid (COSTA and ARNDT 2000; SQUAZZO *et al.* 2002). Of the more than 1000 genes that exhibited a 2-fold or more change in expression in *paf1Δ* cells, most were positively regulated by Paf1, indicating that predominant function of the Paf1 complex is to promote transcription. These results are not surprising, because the Paf1 complex is well-characterized as a positive regulator of transcription. The Paf1 complex associates with RNA Pol II on ORFs during transcription elongation (KROGAN *et al.* 2002b; POKHOLOK *et al.* 2002). Additionally, members of the Paf1 complex are required for histone modifications that are associated with active transcription. For example, Paf1 and Rtf1 are required for histone H2B ubiquitylation (LARIBEE *et al.* 2005; WOOD *et al.* 2003b; XIAO *et al.* 2005) and histone H3 K4 methylation (BRIGGS *et al.* 2002; KROGAN *et al.* 2003a; NG *et al.* 2003a; NG *et al.* 2003b; SUN and ALLIS 2002), histone modifications that are enriched on the coding regions of active genes (BERNSTEIN *et al.* 2002; SANTOS-ROSA *et al.* 2002; XIAO *et al.* 2005).

Although the majority of Paf1-regulated genes are activated by Paf1, we identified numerous genes that require Paf1 for transcriptional repression, indicating that the Paf1 complex has both positive and negative effects on transcription. The repressive functions of the Paf1

complex are not completely understood. However, I have shown that Paf1-dependent histone modifications localize to Paf1-repressed genes, such as *ARG1*, *GAP1*, and *SNZ1*, and contribute to repression (CRISUCCI and ARNDT 2011), indicating that Paf1-dependent histone modifications have both positive and negative effects at different genes. Additionally, Paf1 has repressive functions aside from mediating histone modifications (CRISUCCI and ARNDT 2011). Future studies are required to uncover the repressive functions of the Paf1 complex. At *ARG1*, I found that Paf1 inhibits recruitment of the activator, Gcn4, and antisense transcription across the *ARG1* promoter. Therefore, I hypothesized that the Paf1 complex may regulate many other genes by inhibiting Gcn4-recruitment. However, I found that only 3-10% of Paf1-repressed genes are also Gcn4-regulated. This is only a small proportion, considering that ~ 30% of genes repressed by Snf1, a known repressor of Gcn4 target genes, are also Gcn4-regulated (SHIRRA *et al.* 2008). Therefore, inhibition of Gcn4 recruitment by Paf1 is probably not a wide-spread mechanism of transcriptional repression.

Interestingly, we found that 27% of Paf1-repressed genes are associated with Paf1-repressed antisense transcription. It is possible that Paf1 represses sense transcription indirectly by repressing antisense transcription. Alternatively, antisense transcription may result from increased sense transcription. I attempted to examine these possibilities by using a strong defined terminator to block antisense transcription at the *ARG1* locus. However, antisense transcription failed to terminate. Therefore, additional experiments, such as identifying and mutating antisense transcript promoter elements, are required to determine the relationship between sense and antisense transcription.

Additionally, since performing our microarray studies, it has been shown that antisense artifacts in microarray analyses can result from second-strand cDNA synthesis (PEROCCHI *et al.*

2007). Actinomycin D added to cDNA synthesis reactions has been shown to prevent antisense artifacts (PEROCCHI *et al.* 2007). Specifically, actinomycin D prevents second-strand synthesis by binding single- or double-stranded DNA (GOLDBERG *et al.* 1962), inhibiting DNA-dependent but not RNA-dependent DNA synthesis (MULLER *et al.* 1971; RUPRECHT *et al.* 1973). After learning of these potential experimental artifacts, I examined antisense transcription at several genes using strand-specific RT-PCR, a method that has been used to distinguish between real antisense transcripts and artifacts (PEROCCHI *et al.* 2007). The array data indicated that *ARG1* and *SNZ1* exhibited a 2-fold or more increase in antisense transcription in *paf1Δ* cells and a slightly more subtle increase in antisense transcription in *paf1Δ* cells at *ARG3*. I did detect an increase in antisense transcription in *paf1Δ* cells at *ARG1*, *SNZ1*, and *ARG3* with strand-specific RT-PCR, indicating that antisense transcripts detected at these loci are not experimental artifacts. However, there were inconsistencies with the 5' and 3' boundaries predicted by the two methods. For example, while the array data showed an increase in antisense transcription within the coding regions of *ARG1*, *SNZ1*, and *ARG3* in *paf1Δ* cells, RT-PCR detected an increase in antisense transcription in *paf1Δ* cells in the promoters of these genes. Additionally, while the array data showed that antisense transcription arises from the *ARG1* 3' UTR, antisense transcription was still detected by RT-PCR analysis using a strain in which the *ARG1* promoter and coding region were fused to the 3' UTR of a heterologous gene. This may indicate that antisense transcription detected by the microarrays in the *ARG1* 3' UTR is the result of experimental artifacts or, alternatively, that antisense transcription arises from multiple start sites within the 3' coding region and 3' UTR. Therefore, in addition to verifying antisense transcription by strand-specific RT-PCR, analysis of specific antisense transcripts may require mapping of both the 5' and 3' ends. In the future, to investigate genome-wide effects of the Paf1 complex on antisense

transcription, we will perform high-density tiling array analyses with cDNA prepared in the presence of actinomycin D to prevent antisense artifacts.

In addition to performing the cDNA synthesis for microarray analyses in the presence of actinomycin D, we plan to examine antisense transcription in the absence of both Paf1 and Trf4. Trf4 is a non-canonical poly(A) polymerase that stimulates RNA degradation by the nuclear exosome through its polyadenylation activity (DAVIS and ARES 2006; KADABA *et al.* 2004; LACAVA *et al.* 2005; VANACOVA *et al.* 2005; WYERS *et al.* 2005). Trf4 substrates include rRNA and snoRNA precursors, hypomodified tRNAs, and cryptic unstable transcripts (CUTs) (DAVIS and ARES 2006; KADABA *et al.* 2004; LACAVA *et al.* 2005; VANACOVA *et al.* 2005; WYERS *et al.* 2005). Importantly, CUTs are non-coding RNAs 200-600 nucleotides in length that are often transcribed from intergenic regions (NEIL *et al.* 2009; WYERS *et al.* 2005) and have been shown to regulate expression of overlapping genes. For example, *SRG1* encodes a CUT that represses *SER3* expression through transcriptional interference (MARTENS *et al.* 2005). In contrast, stabilization of antisense CUTs at the *PHO84* locus results in *PHO84* repression by targeting Hda1, a histone deacetylase, to the *PHO84* promoter (CAMBLONG *et al.* 2007). Interestingly, genome-wide analysis of CUT expression indicates that numerous CUTs overlap with coding genes and may potentially regulate their expression (NEIL *et al.* 2009). Therefore, given the important roles of the Paf1 complex in transcription, we hypothesize that the Paf1 complex may regulate expression of coding genes indirectly by modulating CUT transcription and aim to identify Paf1 complex-dependent changes in CUT expression by performing microarray analyses in the absence of Trf4.

In addition to our investigation of Paf1-dependent changes in antisense transcription, we were also interested in the role of the Paf1 complex in transcription termination of snoRNAs.

The Arndt lab previously identified a role for the Paf1 complex in termination of *SNR13* and *SNR47* (SHELDON *et al.* 2005). However, we were interested in using our high resolution array data to uncover additional Paf1-regulated snoRNA genes. We found that 23% of snoRNAs exhibited at least a 2-fold increase in transcription within 150-200 nucleotides downstream in the absence of Paf1, indicative of 3'-extended transcripts resulting from readthrough of the transcription termination signal. Although we will verify these results by Northern analysis, the fact that our analysis identified *SNR47*, a snoRNA gene that was previously shown to require the Paf1 complex for proper transcription termination (SHELDON *et al.* 2005), supports the validity of our results. However, the analysis of our array data by this method revealed only a subtle effect on termination of another known Paf1 complex-regulated snoRNA gene, *SNR13* (SHELDON *et al.* 2005), suggesting that additional snoRNA genes may require the Paf1 complex for proper transcription termination.

Together our results suggest that the Paf1 complex mediates proper transcription termination at multiple snoRNA genes. However, the mechanisms by which the Paf1 complex promotes proper termination remain unclear. The Paf1 complex associates with RNA Pol II on ORFs from transcription start site to the poly(A) site (KIM *et al.* 2004; MAYER *et al.* 2010). Additionally, when dissociated from chromatin, the Paf1 complex associates with RNA processing factors (NORDICK *et al.* 2008). Therefore, the Paf1 complex may be involved in the exchange of transcription elongation factors for transcription termination and 3' end processing factors. Consistent with this hypothesis, the Paf1 complex promotes recruitment of the 3' end processing factor, Nrd1, to *SNR47* (SHELDON *et al.* 2005). Additionally, histone H2B ubiquitylation has been implicated in regulating transcription termination (TOMSON *et al.* 2011b). Therefore, the Paf1 complex may promote proper transcription termination through histone H2B

ubiquitylation and its effects on chromatin structure (CHANDRASEKHARAN *et al.* 2009). Importantly, since the role of the Paf1 complex in regulating both histone H2B ubiquitylation and transcription termination and 3' end formation is conserved to humans (NAGAIKE *et al.* 2011; ROZENBLATT-ROSEN *et al.* 2009; ZHU *et al.* 2005b), studies of this role in yeast will provide insight into important functions of the Paf1 complex in higher eukaryotes as well.

**Table 4: Genes with at least a 2-fold decrease in expression in *paf1Δ* samples**

| | Mean | Median |
|---|---|---|
| CWP1 | 0.0276845 | 0.0194193 |
| PAF1 | 0.1412462 | 0.0340056 |
| PHM6 | 0.0689984 | 0.0376281 |
| ATF2 | 0.0851525 | 0.0552353 |
| SPL2 | 0.4884391 | 0.0870203 |
| YLR040C | 0.109791 | 0.087718 |
| ALT2 | 0.1264654 | 0.0968843 |
| MNN1 | 0.1357889 | 0.0986687 |
| ERD1 | 0.1155193 | 0.0994294 |
| FRE4 | 0.2158443 | 0.0998498 |
| YOL014W | 0.1003965 | 0.102559 |
| YNR014W | 0.1943399 | 0.1128453 |
| EST3 | 0.2277194 | 0.1251943 |
| PHO89 | 0.1251306 | 0.1273785 |
| MGA1 | 0.2313617 | 0.1273993 |
| TPO1 | 0.1717414 | 0.1300621 |
| QDR2 | 0.1571988 | 0.1335271 |
| YLR042C | 0.1494841 | 0.1359078 |
| EHT1 | 0.1495972 | 0.136154 |
| HEM15 | 0.135667 | 0.1367979 |
| ALF1 | 0.2385248 | 0.1369215 |
| YOR356W | 0.1776411 | 0.1372791 |
| BNA1 | 0.2060632 | 0.1380601 |
| ARR2 | 0.2095388 | 0.1406382 |
| TPO4 | 0.2186965 | 0.1446031 |
| PHO11 | 0.1484395 | 0.1447219 |
| YGL159W | 0.2052221 | 0.1456527 |
| PHO12 | 0.1471803 | 0.1468982 |
| PNS1 | 0.2384121 | 0.151841 |
| SCM4 | 0.1602893 | 0.1529968 |
| YJR116W | 0.2069322 | 0.1535924 |
| YJL107C | 0.3299057 | 0.1542742 |
| YPL014W | 0.1486509 | 0.1547438 |
| TIR1 | 0.1919615 | 0.1551239 |
| TNA1 | 0.156275 | 0.156412 |
| YAR066W | 0.200734 | 0.1577907 |
| YHL015W-A | 0.2733172 | 0.1585328 |
| ILV3 | 0.1664586 | 0.1621337 |
| GLT1 | 0.1626287 | 0.1636398 |
| YHB1 | 0.1610815 | 0.1638088 |
| IZH2 | 0.181694 | 0.1647138 |
| HXT1 | 0.1869366 | 0.168269 |
| SYC1 | 0.2647023 | 0.169457 |
| YHR214W | 0.21264 | 0.1712034 |
| IZH4 | 0.2277966 | 0.1722555 |
| BNA5 | 0.2129141 | 0.1730214 |
| CYC1 | 0.1839164 | 0.1735152 |
| LEU1 | 0.17445 | 0.1743968 |
| AGA2 | 0.1831127 | 0.1750497 |
| YDL241W | 0.193182 | 0.1771138 |
| SPC1 | 0.1962725 | 0.178301 |
| SUR1 | 0.1971178 | 0.1787454 |
| KAP122 | 0.1783154 | 0.1789604 |
| GDT1 | 0.1752983 | 0.1836867 |
| YPL279C | 0.2028003 | 0.1849074 |
| ELO1 | 0.1736951 | 0.1853367 |
| STP4 | 0.2226153 | 0.186007 |
| GRX8 | 0.3068276 | 0.1860536 |
| RPI1 | 0.2156256 | 0.1864631 |
| YOR390W | 0.1846687 | 0.1880227 |
| STE14 | 0.2122454 | 0.1882508 |
| KEI1 | 0.1877955 | 0.1887758 |
| ZRT2 | 0.2079439 | 0.1924463 |
| CYB5 | 0.1908644 | 0.1933469 |
| RCE1 | 0.2880789 | 0.1957069 |
| YBR196C-A | 0.3842997 | 0.1982854 |
| YPR003C | 0.33732 | 0.2008458 |
| HO | 0.2555478 | 0.2012813 |
| CLN1 | 0.254092 | 0.201834 |
| SFK1 | 0.2269646 | 0.2037569 |
| MET6 | 0.2027162 | 0.2040621 |
| FSH1 | 0.211805 | 0.20447 |
| BNA6 | 0.2302409 | 0.2062215 |

| | | |
|---|---|---|
| SEC59 | 0.2313272 | 0.2100992 |
| PHO5 | 0.2531908 | 0.2124548 |
| SEC22 | 0.2339079 | 0.2153912 |
| YIL082W | 0.2117133 | 0.2163617 |
| YOX1 | 0.2336315 | 0.2164717 |
| MFA2 | 0.2356084 | 0.2192411 |
| FAR1 | 0.2227809 | 0.2201143 |
| LOT5 | 0.242447 | 0.220118 |
| COS9 | 0.3284335 | 0.2202987 |
| PCL1 | 0.3398363 | 0.2206543 |
| YEA4 | 0.2856524 | 0.2207443 |
| MDN1 | 0.2592024 | 0.2215697 |
| YPR116W | 0.3216364 | 0.2232069 |
| HSP30 | 0.2487605 | 0.2235175 |
| YER156C | 0.2143831 | 0.224037 |
| SST2 | 0.2149167 | 0.2279353 |
| NRM1 | 0.2712633 | 0.2286857 |
| BTT1 | 0.3255846 | 0.2324715 |
| ERG7 | 0.223209 | 0.2332668 |
| PPT1 | 0.2302479 | 0.2346698 |
| GPI12 | 0.3037369 | 0.235337 |
| HTB2 | 0.2473297 | 0.2353616 |
| FUR1 | 0.2479482 | 0.2356319 |
| CLN2 | 0.2396925 | 0.2360347 |
| CIN1 | 0.3002986 | 0.2360702 |
| FUS1 | 0.3090978 | 0.2367076 |
| UTR2 | 0.2337254 | 0.2376166 |
| YHR045W | 0.2365853 | 0.2378036 |
| NMA1 | 0.244844 | 0.239952 |
| RAS1 | 0.2373103 | 0.2400333 |
| FZF1 | 0.3779679 | 0.2401741 |
| LYS4 | 0.223822 | 0.241143 |
| PEX32 | 0.311213 | 0.241496 |
| YLR036C | 0.3054706 | 0.2416298 |
| YOR316C-A | 0.3798508 | 0.2416906 |
| YJR124C | 0.2389941 | 0.241934 |
| YER071C | 0.298633 | 0.2420245 |
| YHR100C | 0.2556756 | 0.2421246 |
| SPE3 | 0.2436254 | 0.2434973 |
| COX4 | 0.2817591 | 0.2440385 |
| PRM7 | 0.2835404 | 0.2450741 |
| ARO4 | 0.2511278 | 0.2460324 |
| YDR132C | 0.4074667 | 0.2465182 |
| FCY21 | 0.2757269 | 0.246747 |
| YMR130W | 0.3481095 | 0.2473929 |
| CHA1 | 0.2441055 | 0.2474145 |
| FEN1 | 0.2535741 | 0.2479499 |
| TOS2 | 0.2903647 | 0.2486621 |
| YBR220C | 0.2502607 | 0.2495598 |
| GDE1 | 0.2853529 | 0.2496323 |
| MET22 | 0.240026 | 0.2496724 |
| COS10 | 0.2910361 | 0.2514451 |
| YPS3 | 0.2686691 | 0.2527684 |
| NRK1 | 0.4761937 | 0.2539043 |
| YBR196C-B | 0.3029127 | 0.2540645 |
| PAC1 | 0.3816495 | 0.2545596 |
| FSF1 | 0.2392422 | 0.2552996 |
| IZH1 | 0.2544628 | 0.2566465 |
| YDR531W | 0.2568528 | 0.2573123 |
| GWT1 | 0.2949378 | 0.2582889 |
| HUT1 | 0.277619 | 0.2584565 |
| YBR219C | 0.3749193 | 0.2585975 |
| CSI2 | 0.2646807 | 0.2594686 |
| LEU9 | 0.2575325 | 0.2597981 |
| YKE4 | 0.2840537 | 0.2639506 |
| APT1 | 0.2626636 | 0.2657515 |
| SLP1 | 0.3351809 | 0.2665079 |
| SAM1 | 0.2678084 | 0.2667843 |
| TSC10 | 0.2927507 | 0.2667898 |
| NNT1 | 0.2995285 | 0.2669935 |
| RKI1 | 0.2742159 | 0.2677934 |
| BAT1 | 0.2698431 | 0.2696951 |
| YAR028W | 0.3491465 | 0.2703775 |
| ERV15 | 0.2371965 | 0.2706143 |
| ARB1 | 0.2746416 | 0.2717304 |
| PEX31 | 0.3132192 | 0.2718504 |
| DIC1 | 0.3067513 | 0.2721131 |
| AIF1 | 0.2948321 | 0.2723192 |

| | | |
|---|---|---|
| HEM13 | 0.2817795 | 0.2726453 |
| CLB1 | 0.4033262 | 0.2735365 |
| OAC1 | 0.2823719 | 0.2742091 |
| MDE1 | 0.2701561 | 0.277065 |
| BAR1 | 0.2827246 | 0.2785072 |
| MMF1 | 0.2777725 | 0.2791495 |
| YPL245W | 0.306075 | 0.2794509 |
| YNR061C | 0.2883613 | 0.2795364 |
| GAT2 | 0.3177453 | 0.2797672 |
| FUS3 | 0.275832 | 0.2810107 |
| PTI1 | 0.3455491 | 0.2815244 |
| YCL047C | 0.4592248 | 0.281735 |
| POP5 | 0.3028704 | 0.2821928 |
| YLR413W | 0.2782782 | 0.2823966 |
| LSM2 | 0.2928684 | 0.2827373 |
| FMP42 | 0.2833381 | 0.2829727 |
| ALD5 | 0.2657011 | 0.283627 |
| YKL027W | 0.3259125 | 0.2849538 |
| POP8 | 0.3370326 | 0.285239 |
| AGA1 | 0.2842295 | 0.2855531 |
| YPR147C | 0.3024059 | 0.2862239 |
| HMS2 | 0.2728383 | 0.2866602 |
| GGC1 | 0.299122 | 0.2866995 |
| SAM2 | 0.2852748 | 0.2870034 |
| HMG1 | 0.2861342 | 0.2876417 |
| BNA4 | 0.4609369 | 0.2890157 |
| YMR259C | 0.3033824 | 0.2909808 |
| HXT4 | 0.3712479 | 0.2915342 |
| COG2 | 0.4873299 | 0.2916154 |
| SUT1 | 0.4297516 | 0.2926271 |
| YNL108C | 0.359491 | 0.2932931 |
| AAH1 | 0.2953568 | 0.2937087 |
| CST26 | 0.2839648 | 0.2946723 |
| EPT1 | 0.2927464 | 0.2947018 |
| PLB2 | 0.3104273 | 0.2954825 |
| HTA1 | 0.3033046 | 0.2957985 |
| VHS1 | 0.273432 | 0.2959668 |
| PMI40 | 0.2953379 | 0.2979668 |
| VTC3 | 0.2957263 | 0.2983065 |
| ERG28 | 0.3158406 | 0.2990994 |
| RMA1 | 0.4383593 | 0.2993206 |
| HIS1 | 0.293993 | 0.2996004 |
| YHR020W | 0.3027558 | 0.2999506 |
| ERG5 | 0.2994896 | 0.3008408 |
| YEH1 | 0.2951851 | 0.3018062 |
| NRT1 | 0.3156155 | 0.3036455 |
| TEC1 | 0.3064984 | 0.3038333 |
| GNA1 | 0.3445516 | 0.3045935 |
| OST6 | 0.3159629 | 0.3046453 |
| HSD1 | 0.3129315 | 0.3050277 |
| YLR073C | 0.3156501 | 0.3056671 |
| TCB2 | 0.3147161 | 0.3061979 |
| HEM3 | 0.293732 | 0.3068578 |
| ASP1 | 0.3073935 | 0.3084055 |
| YOL057W | 0.2905938 | 0.3088083 |
| SCW10 | 0.3090441 | 0.3094568 |
| GPI14 | 0.2994006 | 0.3095179 |
| MIS1 | 0.3174202 | 0.3095286 |
| SRL1 | 0.3104772 | 0.3097236 |
| ERG24 | 0.3156553 | 0.3098015 |
| SPE2 | 0.3173947 | 0.3100742 |
| SER33 | 0.4674648 | 0.3101049 |
| YML018C | 0.316628 | 0.3108218 |
| YPR063C | 0.3225297 | 0.3108928 |
| VCX1 | 0.2961439 | 0.3109271 |
| CIN2 | 0.3353855 | 0.3110647 |
| CCP1 | 0.3166894 | 0.3118437 |
| YNL058C | 0.306033 | 0.3123615 |
| YJL193W | 0.3589765 | 0.313828 |
| ECM17 | 0.3318398 | 0.3140614 |
| GUK1 | 0.3201192 | 0.3146687 |
| MVB12 | 0.3250182 | 0.3148939 |
| MAL13 | 0.4353186 | 0.315167 |
| YGR031W | 0.4399178 | 0.3155959 |
| MMP1 | 0.2979845 | 0.3161888 |
| TRM8 | 0.3272276 | 0.3169414 |
| ALG8 | 0.3180805 | 0.3170867 |
| YCL049C | 0.4049861 | 0.3176105 |

| | | |
|---|---|---|
| HTA2 | 0.3245377 | 0.3177728 |
| RGD2 | 0.3725083 | 0.3180912 |
| SUR4 | 0.3098605 | 0.3186904 |
| UTP20 | 0.3276665 | 0.3187053 |
| FSH3 | 0.5003877 | 0.3194841 |
| SVL3 | 0.3215366 | 0.3198713 |
| YBL029C-A | 0.3406953 | 0.3212919 |
| DAD3 | 0.4213625 | 0.3216099 |
| AFG2 | 0.3549013 | 0.3225205 |
| ABF1 | 0.3188424 | 0.3232045 |
| YIP3 | 0.3252131 | 0.3238245 |
| YAH1 | 0.3535515 | 0.3245756 |
| CIS3 | 0.3722841 | 0.3245863 |
| STE12 | 0.3253736 | 0.3246088 |
| REX2 | 0.3322943 | 0.3247877 |
| YOL159C | 0.4967577 | 0.3249185 |
| COX6 | 0.3210771 | 0.3253287 |
| SIZ1 | 0.3817122 | 0.325547 |
| YGK3 | 0.3670661 | 0.3255516 |
| GRE2 | 0.3171516 | 0.325899 |
| GPI8 | 0.3349582 | 0.3265693 |
| HMF1 | 0.3290881 | 0.3273351 |
| ERG10 | 0.330559 | 0.3275976 |
| CCC1 | 0.354794 | 0.3280325 |
| PCM1 | 0.3193008 | 0.3281837 |
| YVC1 | 0.3742057 | 0.3282799 |
| YLR317W | 0.4448664 | 0.3287243 |
| YOR296W | 0.4154452 | 0.3293544 |
| ERG26 | 0.334844 | 0.3297701 |
| DFR1 | 0.4644012 | 0.3302697 |
| YDR352W | 0.3159295 | 0.3304003 |
| IME1 | 0.4860354 | 0.3306994 |
| YMR122W-A | 0.3294363 | 0.3308318 |
| URH1 | 0.3299284 | 0.3311474 |
| SCS7 | 0.3481549 | 0.3313211 |
| NHP6A | 0.3116081 | 0.3315351 |
| YGL101W | 0.3323491 | 0.3315958 |
| GTR2 | 0.4187188 | 0.3316944 |
| YDL038C | 0.3260542 | 0.3317728 |
| SIM1 | 0.328209 | 0.3319842 |
| YNL010W | 0.3342008 | 0.3334635 |
| RLI1 | 0.3388752 | 0.3335579 |
| FRS2 | 0.3225385 | 0.3335731 |
| TPO2 | 0.3412951 | 0.3340118 |
| AGE1 | 0.3526841 | 0.3341429 |
| RPB7 | 0.3344054 | 0.3342331 |
| VTC2 | 0.32259 | 0.3344571 |
| SMP3 | 0.3473975 | 0.3345283 |
| YDR210W | 0.3401766 | 0.3346825 |
| YLR285C-A | 0.3694316 | 0.3350913 |
| PAU18 | 0.3292873 | 0.3361319 |
| CYT1 | 0.3936162 | 0.3361688 |
| AAC3 | 0.4001589 | 0.3364553 |
| YGL194C-A | 0.4743868 | 0.3365027 |
| STE23 | 0.309278 | 0.3366625 |
| ERG12 | 0.3368142 | 0.3370585 |
| YOR385W | 0.3326137 | 0.3374658 |
| ARR1 | 0.3956126 | 0.3376143 |
| RPB8 | 0.3442264 | 0.3376663 |
| LAG2 | 0.4834488 | 0.3380187 |
| ANT1 | 0.3287867 | 0.3381235 |
| FMP37 | 0.3159113 | 0.3383332 |
| IMD2 | 0.3321413 | 0.3383584 |
| BUD16 | 0.3565465 | 0.3393544 |
| AML1 | 0.3324044 | 0.3399461 |
| POS5 | 0.3888473 | 0.340174 |
| IMD1 | 0.3738297 | 0.3403923 |
| COX8 | 0.3458603 | 0.3408345 |
| RPS14B | 0.4137012 | 0.3417907 |
| YKL069W | 0.3982203 | 0.341834 |
| LDB17 | 0.4040324 | 0.3418743 |
| GTT3 | 0.345975 | 0.3421636 |
| YML082W | 0.3767052 | 0.3426077 |
| YCR090C | 0.4128795 | 0.3427643 |
| GPI10 | 0.4546454 | 0.3428309 |
| YUR1 | 0.4418834 | 0.3432163 |
| SMF1 | 0.3367971 | 0.3433761 |
| SAS3 | 0.3947657 | 0.3435484 |

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| HEK2 | 0.3408912 | 0.3435613 |
| GAS3 | 0.345329 | 0.3436457 |
| UBP8 | 0.3396456 | 0.3438622 |
| ACB1 | 0.3396171 | 0.3439554 |
| RAM2 | 0.3322391 | 0.3441704 |
| YMC1 | 0.3777615 | 0.3447205 |
| THI22 | 0.4281509 | 0.344907 |
| GCN1 | 0.3511883 | 0.3451065 |
| PHS1 | 0.3447449 | 0.3461104 |
| ARG80 | 0.3955717 | 0.3461257 |
| YNL300W | 0.3607353 | 0.3464486 |
| YNL097C-B | 0.4044267 | 0.3476402 |
| YGR210C | 0.3462759 | 0.3479822 |
| MRF1 | 0.4652197 | 0.3481703 |
| YPR071W | 0.3643777 | 0.3482918 |
| SAM4 | 0.336023 | 0.3483387 |
| TVP38 | 0.3651926 | 0.3485784 |
| BOR1 | 0.4400389 | 0.3486905 |
| PGM1 | 0.3452941 | 0.3491218 |
| SPE4 | 0.3810866 | 0.349158 |
| EOS1 | 0.329499 | 0.3499349 |
| PSE1 | 0.3573024 | 0.3500247 |
| RPL9A | 0.365184 | 0.3508953 |
| YDR307W | 0.3546151 | 0.351439 |
| YDL085C-A | 0.3938704 | 0.3515815 |
| GPI2 | 0.4322388 | 0.3521384 |
| YDL160C-A | 0.4009186 | 0.3524707 |
| FUN26 | 0.3680858 | 0.352918 |
| BNA7 | 0.3721866 | 0.3555699 |
| ECM3 | 0.3465288 | 0.3559352 |
| TRX1 | 0.345451 | 0.3561055 |
| MKC7 | 0.3696064 | 0.3565366 |
| CAX4 | 0.4410691 | 0.3580334 |
| RNR1 | 0.3588505 | 0.358173 |
| YMR321C | 0.3674727 | 0.3585225 |
| LYS5 | 0.4891634 | 0.3586956 |
| ERG11 | 0.3576955 | 0.3596875 |
| CLU1 | 0.3569176 | 0.3597382 |
| YNL193W | 0.5037779 | 0.3598962 |
| FLD1 | 0.4231845 | 0.3599473 |
| TUB4 | 0.4007296 | 0.3600865 |
| ERG2 | 0.3639718 | 0.3601858 |
| GPX2 | 0.3556237 | 0.3601925 |
| PAU20 | 0.4972724 | 0.3605072 |
| RHR2 | 0.3935682 | 0.361119 |
| RPL26A | 0.3993745 | 0.3611742 |
| DPM1 | 0.3633965 | 0.3615997 |
| SVS1 | 0.3460075 | 0.3624613 |
| ALG14 | 0.3871506 | 0.3624616 |
| HOM2 | 0.3648265 | 0.3625839 |
| YLR118C | 0.3797547 | 0.3627873 |
| PSA1 | 0.3665732 | 0.3630802 |
| MAK3 | 0.4690111 | 0.363492 |
| SKI8 | 0.3754976 | 0.3637478 |
| PRS1 | 0.3597489 | 0.3641329 |
| LAA1 | 0.3827566 | 0.3641896 |
| YOR051C | 0.3605392 | 0.3644426 |
| RPS21A | 0.3838668 | 0.365413 |
| RPL27B | 0.3806329 | 0.3658549 |
| OLE1 | 0.3607376 | 0.3659694 |
| POR2 | 0.3345927 | 0.3663939 |
| PRS3 | 0.3429026 | 0.3666347 |
| VPS28 | 0.5045003 | 0.3674678 |
| POA1 | 0.4569587 | 0.3674685 |
| RAM1 | 0.426984 | 0.3676238 |
| VTC1 | 0.3802188 | 0.3682524 |
| FAS1 | 0.3683849 | 0.3684735 |
| YKL033W-A | 0.3452979 | 0.3686691 |
| RIB4 | 0.3800497 | 0.3697689 |
| CSR1 | 0.3781203 | 0.3701019 |
| PRS4 | 0.3651866 | 0.3702621 |
| YPR157W | 0.4910722 | 0.3711944 |
| SUM1 | 0.3555076 | 0.3716898 |
| COX7 | 0.3455482 | 0.3719017 |
| SMM1 | 0.3831481 | 0.3738911 |
| INP52 | 0.4402673 | 0.3739924 |
| HFI1 | 0.452266 | 0.3740143 |
| DUT1 | 0.3707051 | 0.3744503 |

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| YDR341C | 0.3831342 | 0.3746544 |
| LIA1 | 0.3627152 | 0.3751817 |
| UBP5 | 0.4035837 | 0.3752467 |
| ATR1 | 0.495184 | 0.3753518 |
| LAC1 | 0.3645922 | 0.376922 |
| RKR1 | 0.3711158 | 0.3785951 |
| MST28 | 0.4978139 | 0.3786301 |
| KAP95 | 0.3640392 | 0.3790027 |
| URA7 | 0.3782356 | 0.3791118 |
| RAP1 | 0.373723 | 0.3795133 |
| CBP4 | 0.4785247 | 0.3797846 |
| ITR2 | 0.3639749 | 0.380103 |
| RPS27A | 0.3709149 | 0.3803651 |
| YDL206W | 0.4556911 | 0.3805047 |
| DPH2 | 0.3685873 | 0.3805064 |
| OLA1 | 0.3670625 | 0.3805235 |
| RPL33B | 0.4009244 | 0.3806728 |
| OAR1 | 0.4459168 | 0.3808504 |
| RPS16B | 0.3773294 | 0.3809922 |
| CDC6 | 0.413195 | 0.3810514 |
| STE2 | 0.3901694 | 0.3813446 |
| STE6 | 0.3769969 | 0.3814831 |
| MRI1 | 0.3806514 | 0.3827276 |
| HNM1 | 0.378002 | 0.382907 |
| YLR137W | 0.4643226 | 0.3829598 |
| AXL2 | 0.3742129 | 0.3834269 |
| RPC34 | 0.3889392 | 0.383437 |
| UPS1 | 0.4059957 | 0.3834512 |
| POL5 | 0.3791464 | 0.3835722 |
| IPT1 | 0.3892027 | 0.3836536 |
| CNA1 | 0.4044681 | 0.3840991 |
| ALG7 | 0.390725 | 0.3848941 |
| SNU13 | 0.3917328 | 0.3850734 |
| RPS7B | 0.409343 | 0.3863276 |
| AIM29 | 0.3722564 | 0.3868421 |
| YIL064W | 0.4007213 | 0.3869393 |
| URK1 | 0.3842238 | 0.3878872 |
| CTR86 | 0.4500514 | 0.3879878 |
| MFA1 | 0.3714503 | 0.3880283 |
| YPR1 | 0.3567686 | 0.3888034 |
| VRG4 | 0.3843251 | 0.3888342 |
| YNR048W | 0.4474399 | 0.3888678 |
| SPT14 | 0.4610666 | 0.3889207 |
| MOT3 | 0.4257575 | 0.3889922 |
| NSR1 | 0.3888954 | 0.3891588 |
| YAR075W | 0.3759147 | 0.3894113 |
| AIM1 | 0.3713901 | 0.3897867 |
| DOT1 | 0.5048121 | 0.3900382 |
| ISC1 | 0.4470212 | 0.3904954 |
| TOS1 | 0.3857853 | 0.3905198 |
| VBA4 | 0.3982554 | 0.390834 |
| SHO1 | 0.3668884 | 0.3912262 |
| SIR2 | 0.4172289 | 0.3913673 |
| SLS1 | 0.4197083 | 0.3921956 |
| SUN4 | 0.3955385 | 0.3929746 |
| LSM5 | 0.3672972 | 0.3934794 |
| MTO1 | 0.4935978 | 0.3935361 |
| SFG1 | 0.3663514 | 0.3937531 |
| YGL039W | 0.4122699 | 0.3941925 |
| IMD3 | 0.4053538 | 0.3943175 |
| IGO2 | 0.4295338 | 0.3945965 |
| UTP10 | 0.3905792 | 0.3946444 |
| ILV5 | 0.3926278 | 0.3947612 |
| MAE1 | 0.3931047 | 0.3951218 |
| ADE8 | 0.3795718 | 0.3957276 |
| ELP2 | 0.3872411 | 0.3958744 |
| SPT4 | 0.3867453 | 0.3965136 |
| GPI11 | 0.4475695 | 0.3965235 |
| RPE1 | 0.4057734 | 0.3970907 |
| NGL1 | 0.4916326 | 0.3983924 |
| YDL211C | 0.4850783 | 0.3984203 |
| GOT1 | 0.3951283 | 0.3984323 |
| HTB1 | 0.4010834 | 0.3990773 |
| RSC3 | 0.3838694 | 0.3993262 |
| ATO3 | 0.4087994 | 0.3995991 |
| PAU4 | 0.3800041 | 0.3996438 |
| RIB3 | 0.3896993 | 0.4004726 |
| HSL7 | 0.3904178 | 0.4005647 |

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| TIM18 | 0.4463786 | 0.4009063 |
| EMP70 | 0.3967502 | 0.4009519 |
| AMD1 | 0.3953453 | 0.4010466 |
| YCL021W-A | 0.4088474 | 0.4015783 |
| HIS6 | 0.4223142 | 0.4017257 |
| SDO1 | 0.4596254 | 0.4018802 |
| YOR164C | 0.4093564 | 0.4019287 |
| EFR3 | 0.397668 | 0.4021321 |
| MSC7 | 0.4141542 | 0.4021448 |
| ECM40 | 0.4532509 | 0.4030974 |
| RIM1 | 0.4024312 | 0.4032128 |
| CTF19 | 0.4856059 | 0.4033566 |
| PAP2 | 0.4833983 | 0.4033843 |
| PRS5 | 0.4002107 | 0.4037173 |
| TVP15 | 0.4858665 | 0.4043523 |
| AAT1 | 0.3962814 | 0.4048892 |
| HOG1 | 0.4065496 | 0.4049424 |
| HMO1 | 0.4112162 | 0.4049701 |
| GRX4 | 0.3881582 | 0.405047 |
| HRK1 | 0.4306757 | 0.4056065 |
| HIS2 | 0.4062212 | 0.4056594 |
| PAU10 | 0.4392928 | 0.4059752 |
| RPL34A | 0.4170683 | 0.4063862 |
| PRY2 | 0.4192822 | 0.4066163 |
| MNT3 | 0.4200038 | 0.4068917 |
| TOM7 | 0.4239857 | 0.4071588 |
| GIC1 | 0.3831366 | 0.4072451 |
| SEC14 | 0.3925951 | 0.4074097 |
| DOT6 | 0.4051473 | 0.4083889 |
| NOP1 | 0.4420433 | 0.4087045 |
| TED1 | 0.4051528 | 0.4087662 |
| ADO1 | 0.408757 | 0.4092328 |
| YOL092W | 0.417551 | 0.410007 |
| SUR7 | 0.4090755 | 0.4102476 |
| SUR2 | 0.4319758 | 0.4110942 |
| MNL1 | 0.3970632 | 0.4111906 |
| RBL2 | 0.4198715 | 0.4112844 |
| EXG2 | 0.405035 | 0.4114128 |
| PHA2 | 0.5032638 | 0.4117268 |
| TPO3 | 0.3947978 | 0.4117637 |
| SEC53 | 0.4216096 | 0.4117681 |
| WSC2 | 0.41584 | 0.4117996 |
| TYW3 | 0.4917095 | 0.4119499 |
| RPL31B | 0.4175941 | 0.4120107 |
| FAU1 | 0.4213372 | 0.4123984 |
| YMR209C | 0.4176171 | 0.4134419 |
| SAH1 | 0.4244953 | 0.4134481 |
| CWP2 | 0.4259248 | 0.4139323 |
| PTC2 | 0.4177713 | 0.4141068 |
| HPT1 | 0.408331 | 0.4143955 |
| YBL028C | 0.3836617 | 0.4144052 |
| STO1 | 0.4310564 | 0.4147232 |
| NKP2 | 0.4589536 | 0.4148093 |
| YFH1 | 0.4927266 | 0.415496 |
| MHP1 | 0.4165477 | 0.4156721 |
| PHO13 | 0.4121977 | 0.4157345 |
| OSH2 | 0.4103819 | 0.4157517 |
| BCK1 | 0.4045505 | 0.4162496 |
| ATG5 | 0.4650359 | 0.4163787 |
| GPA1 | 0.4898416 | 0.4164877 |
| SDH3 | 0.4107061 | 0.4166458 |
| VBA1 | 0.4093053 | 0.4167109 |
| RPA135 | 0.4169995 | 0.4167878 |
| RPL13A | 0.409576 | 0.4172961 |
| FAS2 | 0.4125122 | 0.4174167 |
| REV1 | 0.4606179 | 0.4176167 |
| ECM9 | 0.4235712 | 0.4181198 |
| MES1 | 0.4115569 | 0.4184033 |
| GPI18 | 0.5020085 | 0.418442 |
| EGT2 | 0.4136832 | 0.4187415 |
| PAU8 | 0.4337337 | 0.4187913 |
| YBR238C | 0.406817 | 0.4188044 |
| PCT1 | 0.4754527 | 0.4191267 |
| YOR291W | 0.4542498 | 0.4192253 |
| ALD6 | 0.422095 | 0.4193267 |
| SPI1 | 0.4879205 | 0.4196176 |
| CAF40 | 0.4109729 | 0.4196912 |
| RFC5 | 0.4780033 | 0.4200625 |

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| EXG1 | 0.4165456 | 0.4203571 |
| RPL22A | 0.4257276 | 0.420662 |
| RSN1 | 0.4130641 | 0.4207145 |
| ILM1 | 0.4218145 | 0.4210805 |
| LAG1 | 0.4328141 | 0.4211599 |
| NTF2 | 0.4148838 | 0.4212193 |
| RPL8A | 0.4199223 | 0.4223699 |
| YGR149W | 0.49259 | 0.4225312 |
| NIS1 | 0.4272403 | 0.4226985 |
| RPL21A | 0.4380963 | 0.4227275 |
| ALE1 | 0.4300072 | 0.4229993 |
| APT2 | 0.4209553 | 0.4234067 |
| DIA1 | 0.4250087 | 0.4235873 |
| SAT4 | 0.4251052 | 0.4236368 |
| PFA4 | 0.4786806 | 0.4237038 |
| ATG27 | 0.4040154 | 0.4237821 |
| RPO31 | 0.4209658 | 0.4242097 |
| ERP3 | 0.4540971 | 0.4247501 |
| TAZ1 | 0.4565143 | 0.4247554 |
| YIH1 | 0.4174074 | 0.4247801 |
| ECM22 | 0.4958408 | 0.4250523 |
| PUF4 | 0.432576 | 0.4254068 |
| FMP41 | 0.4143216 | 0.4255627 |
| GPD1 | 0.4127203 | 0.4264257 |
| FPR4 | 0.4245934 | 0.4267921 |
| HOT13 | 0.4378382 | 0.4272739 |
| TRM3 | 0.4377015 | 0.4272767 |
| TGL5 | 0.4028045 | 0.4280487 |
| SOL3 | 0.4258621 | 0.4281848 |
| ARO3 | 0.4274097 | 0.428237 |
| TMN3 | 0.432396 | 0.4282566 |
| PTK1 | 0.4915546 | 0.4282915 |
| YJR054W | 0.4709944 | 0.4283323 |
| YCP4 | 0.4238781 | 0.4286923 |
| DPP1 | 0.4239184 | 0.4293418 |
| OST5 | 0.4446828 | 0.4293551 |
| SCW11 | 0.4351105 | 0.4293954 |
| PAU6 | 0.4246409 | 0.4295853 |
| DSE2 | 0.4245081 | 0.4298731 |
| SNF4 | 0.4461794 | 0.429947 |
| APS2 | 0.4524247 | 0.4300809 |
| YER010C | 0.4330496 | 0.4301874 |
| HMG2 | 0.4203025 | 0.4313456 |
| VPS66 | 0.4226815 | 0.4315902 |
| RPS29A | 0.444156 | 0.4317695 |
| SLT2 | 0.4427095 | 0.4323129 |
| TFB2 | 0.4757281 | 0.4326298 |
| NIT3 | 0.4209813 | 0.4329947 |
| GRH1 | 0.437498 | 0.4330067 |
| ADE13 | 0.4311985 | 0.4330118 |
| RPL36A | 0.4116324 | 0.433213 |
| ATP20 | 0.437628 | 0.4332715 |
| TRM82 | 0.4373149 | 0.4333555 |
| SOH1 | 0.4437324 | 0.4337022 |
| ALG2 | 0.467762 | 0.4338952 |
| ALG6 | 0.4363441 | 0.4344004 |
| GAR1 | 0.4486699 | 0.435194 |
| LRC2 | 0.4741931 | 0.4352839 |
| KES1 | 0.4300938 | 0.4354858 |
| YHL008C | 0.4937974 | 0.4355504 |
| CLB2 | 0.4312225 | 0.4358826 |
| ANB1 | 0.5005031 | 0.4360907 |
| RRP42 | 0.3991592 | 0.4361753 |
| UTP22 | 0.4330879 | 0.4362179 |
| DED81 | 0.4429414 | 0.4362519 |
| RPS28B | 0.4277562 | 0.4364636 |
| RPS9A | 0.4654613 | 0.4366386 |
| STH1 | 0.4395418 | 0.4366391 |
| VTC4 | 0.4441164 | 0.4366832 |
| TMA108 | 0.4509389 | 0.4369891 |
| YEA6 | 0.4706289 | 0.437062 |
| RPL20A | 0.448093 | 0.4371903 |
| CPT1 | 0.4731699 | 0.4374068 |
| ERP6 | 0.467183 | 0.437446 |
| MCD4 | 0.4368007 | 0.4375058 |
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| MET13 | 0.461449 | 0.4376081 |
| ATM1 | 0.4199564 | 0.4376608 |

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| KEL3 | 0.4531397 | 0.4378394 |
| BSC1 | 0.4659086 | 0.4379131 |
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| TIF6 | 0.4379317 | 0.4380009 |
| GCD7 | 0.4423691 | 0.4381098 |
| PAN6 | 0.4303102 | 0.4385006 |
| AVT5 | 0.4393719 | 0.4385189 |
| RRP8 | 0.4617571 | 0.4387821 |
| NSG1 | 0.4474171 | 0.438894 |
| GCD1 | 0.4446986 | 0.4390921 |
| RPL18B | 0.4519712 | 0.4394624 |
| HAP4 | 0.460455 | 0.4397008 |
| USO1 | 0.4263749 | 0.4398056 |
| ARK1 | 0.4682736 | 0.4402163 |
| RPS21B | 0.4737109 | 0.4404575 |
| TMS1 | 0.4340532 | 0.4404777 |
| SWD2 | 0.4528674 | 0.440546 |
| GAL80 | 0.4215076 | 0.440763 |
| SLD5 | 0.480411 | 0.4409563 |
| SXM1 | 0.4339865 | 0.4411856 |
| YPR013C | 0.4351936 | 0.442311 |
| FCP1 | 0.451875 | 0.4424965 |
| SAD1 | 0.4736291 | 0.4426124 |
| YBR242W | 0.4496795 | 0.4434342 |
| NIP7 | 0.4294932 | 0.4434931 |
| KTR1 | 0.4447211 | 0.4435811 |
| YMR148W | 0.4650022 | 0.4436977 |
| YCL002C | 0.474065 | 0.4437027 |
| YHL039W | 0.4305673 | 0.4441324 |
| CDC4 | 0.4218328 | 0.444294 |
| RPS18B | 0.456121 | 0.4453432 |
| YLR099W-A | 0.4752623 | 0.4453701 |
| SMX2 | 0.4321613 | 0.4454535 |
| RPL43B | 0.4636869 | 0.445582 |
| PDR12 | 0.4570097 | 0.4460804 |
| RPL16A | 0.4557566 | 0.4464591 |
| PMT5 | 0.4668844 | 0.4465333 |
| SFT2 | 0.4521052 | 0.446746 |
| YKR070W | 0.4663228 | 0.4477448 |
| AKR2 | 0.4651817 | 0.4478138 |
| YSY6 | 0.4550571 | 0.4479839 |
| FSH2 | 0.4647199 | 0.4480685 |
| SCP160 | 0.443373 | 0.4481383 |
| ATP17 | 0.4398004 | 0.4482804 |
| LOS1 | 0.5027257 | 0.4483571 |
| RPL18A | 0.4609494 | 0.4485995 |
| YNL035C | 0.4541786 | 0.4487688 |
| ERG4 | 0.4518133 | 0.4488003 |
| SCY1 | 0.4860129 | 0.4489164 |
| NTE1 | 0.4503564 | 0.4490989 |
| PKR1 | 0.446831 | 0.4494679 |
| YPD1 | 0.4396939 | 0.4494793 |
| HOM3 | 0.4373003 | 0.4498203 |
| SAS5 | 0.4179012 | 0.4499928 |
| MNN2 | 0.4408805 | 0.4508343 |
| TYW1 | 0.4526965 | 0.4508669 |
| ACS2 | 0.441013 | 0.4512031 |
| RKM1 | 0.4562464 | 0.4512277 |
| PHO91 | 0.4577447 | 0.4512443 |
| RPS18A | 0.4452681 | 0.4512666 |
| YNR021W | 0.4502942 | 0.4516419 |
| CHS7 | 0.4476278 | 0.451983 |
| PNP1 | 0.463167 | 0.4521669 |
| RPS9B | 0.4614158 | 0.4521792 |
| RPS22A | 0.4382443 | 0.4521897 |
| YHR032W | 0.4489909 | 0.452267 |
| PHO84 | 0.4529356 | 0.4523663 |
| BEM2 | 0.4542347 | 0.452609 |
| RPL6B | 0.4646239 | 0.4527386 |
| PHO81 | 0.4587544 | 0.4528464 |
| NUP159 | 0.442134 | 0.4529474 |
| RPS7A | 0.4639369 | 0.4532043 |
| ACC1 | 0.4501337 | 0.4534766 |
| BDH1 | 0.4423651 | 0.4535364 |
| RTN1 | 0.4548119 | 0.4539054 |
| YCR043C | 0.4615641 | 0.4539406 |
| NOP7 | 0.448889 | 0.4543365 |
| YDR541C | 0.4635511 | 0.4544359 |

| | | |
|---|---|---|
| DAK1 | 0.469835 | 0.4545229 |
| GUA1 | 0.4577315 | 0.4547827 |
| GEF1 | 0.4919969 | 0.454808 |
| TSC3 | 0.4823391 | 0.4548481 |
| PAB1 | 0.4498306 | 0.4548804 |
| WSC3 | 0.4793051 | 0.455493 |
| RTT10 | 0.4838577 | 0.4555293 |
| COR1 | 0.4591648 | 0.4556273 |
| NIP1 | 0.4629731 | 0.4556865 |
| GCN3 | 0.4965429 | 0.4560346 |
| TRM112 | 0.4708191 | 0.4561341 |
| YLR466C-B | 0.4564884 | 0.4562553 |
| LDB16 | 0.4745156 | 0.4562927 |
| YMR155W | 0.4746449 | 0.4563414 |
| LCB3 | 0.4541843 | 0.4564708 |
| EGD1 | 0.4584756 | 0.4565991 |
| YHR113W | 0.4583579 | 0.4569788 |
| ERG8 | 0.4336582 | 0.4571514 |
| PHO8 | 0.4579106 | 0.4572103 |
| KGD1 | 0.4531386 | 0.4572594 |
| HGH1 | 0.4656439 | 0.4575452 |
| NPY1 | 0.4542963 | 0.4575597 |
| HCM1 | 0.4327611 | 0.4578244 |
| PSD1 | 0.4447183 | 0.4583098 |
| SML1 | 0.4632215 | 0.4584801 |
| RPL21B | 0.4541345 | 0.4588093 |
| PMT3 | 0.4659019 | 0.4588207 |
| SRP40 | 0.4696601 | 0.4589354 |
| RPL12A | 0.4711169 | 0.4590342 |
| INP53 | 0.4774237 | 0.4592421 |
| ARO1 | 0.4577242 | 0.4592853 |
| YIP1 | 0.4835014 | 0.4593324 |
| RPL7B | 0.4761332 | 0.4595077 |
| YMR310C | 0.4420319 | 0.459799 |
| HEM1 | 0.4657364 | 0.4599028 |
| SBH2 | 0.4606304 | 0.4600056 |
| GAS1 | 0.4549298 | 0.4602299 |
| POL30 | 0.4519556 | 0.4603804 |
| RPL26B | 0.4554427 | 0.4605618 |
| RPL33A | 0.4794188 | 0.4609794 |
| SGE1 | 0.4702471 | 0.4612326 |
| RAX1 | 0.4619269 | 0.4619219 |
| CHS3 | 0.4570383 | 0.4620568 |
| ERG1 | 0.4595111 | 0.4622247 |
| ASI1 | 0.4824285 | 0.4624121 |
| URA5 | 0.4752444 | 0.4625792 |
| NOP9 | 0.4690804 | 0.4630292 |
| RPS16A | 0.4477634 | 0.4631313 |
| PIR1 | 0.5046733 | 0.4631858 |
| DIE2 | 0.4581352 | 0.4632198 |
| VAS1 | 0.4678024 | 0.4635225 |
| YOR342C | 0.4620302 | 0.4635294 |
| RFT1 | 0.4774448 | 0.4636445 |
| RPS19A | 0.4651316 | 0.4638463 |
| MNN5 | 0.4756835 | 0.4640123 |
| YLR194C | 0.4539789 | 0.4646298 |
| SVP26 | 0.461474 | 0.4648645 |
| DUG2 | 0.4867965 | 0.4653557 |
| ACO2 | 0.4924236 | 0.4657733 |
| ASH1 | 0.4681718 | 0.4660989 |
| WRS1 | 0.4630715 | 0.4662681 |
| ERV14 | 0.4805557 | 0.4664713 |
| RPL14B | 0.5018914 | 0.4670043 |
| RFC4 | 0.4830479 | 0.4670852 |
| SYS1 | 0.4685371 | 0.4671166 |
| VMA5 | 0.4698942 | 0.467623 |
| YEL007W | 0.4884343 | 0.4676803 |
| ECM33 | 0.4784267 | 0.4682741 |
| THR1 | 0.4603865 | 0.4685064 |
| PMT4 | 0.4746176 | 0.4685447 |
| RPL27A | 0.4766745 | 0.4690239 |
| PCL2 | 0.4830651 | 0.469107 |
| SHE2 | 0.5067064 | 0.4694195 |
| LSG1 | 0.477696 | 0.4694732 |
| RPL7A | 0.4645772 | 0.470022 |
| ORM1 | 0.4555315 | 0.4706549 |
| CHS1 | 0.4586234 | 0.4708796 |
| PMD1 | 0.5011916 | 0.4713447 |

| | | |
|---|---|---|
| KTR3 | 0.456018 | 0.4714501 |
| ADD66 | 0.4997563 | 0.4715671 |
| RPS11B | 0.4775724 | 0.4718281 |
| DDP1 | 0.4575656 | 0.4724205 |
| RPL6A | 0.4962531 | 0.4728321 |
| ADE5,7 | 0.4715983 | 0.472935 |
| GMH1 | 0.4668002 | 0.4733622 |
| VMA9 | 0.4785847 | 0.4734493 |
| TBF1 | 0.4641474 | 0.4740549 |
| MET17 | 0.4641541 | 0.4740874 |
| OTU1 | 0.4946063 | 0.4744238 |
| AFG1 | 0.455973 | 0.4747309 |
| TIF4631 | 0.4705696 | 0.4755179 |
| DBP3 | 0.4622908 | 0.4758577 |
| REB1 | 0.4602914 | 0.4762133 |
| CPR5 | 0.4801533 | 0.4765744 |
| CWH41 | 0.485561 | 0.4769018 |
| RET2 | 0.4821778 | 0.4770513 |
| PEX2 | 0.4633455 | 0.4772052 |
| RPL34B | 0.476773 | 0.4774219 |
| UTR4 | 0.4622241 | 0.4776105 |
| COG1 | 0.4770072 | 0.4778229 |
| SCS2 | 0.4845449 | 0.4780726 |
| RPS24B | 0.5007784 | 0.4783151 |
| RPL14A | 0.4983811 | 0.4796321 |
| CRH1 | 0.485102 | 0.4797884 |
| QCR8 | 0.4880829 | 0.4798848 |
| RPS11A | 0.5004478 | 0.4800146 |
| RPA190 | 0.4823566 | 0.480733 |
| SEC7 | 0.4821798 | 0.4810464 |
| RER1 | 0.4894063 | 0.4814007 |
| GIS2 | 0.4819285 | 0.48151 |
| RPL16B | 0.504692 | 0.4816087 |
| MRS3 | 0.4836475 | 0.4817593 |
| PMT1 | 0.4802685 | 0.4819713 |
| LAS21 | 0.4853395 | 0.4823514 |
| RPS0A | 0.4871379 | 0.4826221 |
| MPD1 | 0.4852304 | 0.4826692 |
| TRZ1 | 0.4840373 | 0.482681 |
| FAA4 | 0.4895356 | 0.4828555 |
| LEU4 | 0.4790477 | 0.4832329 |
| KRE27 | 0.4943362 | 0.4832383 |
| ORM2 | 0.4858213 | 0.4832435 |
| RRP5 | 0.4856031 | 0.483379 |
| URB2 | 0.4884205 | 0.4837229 |
| TMA19 | 0.490883 | 0.4837723 |
| RNR2 | 0.4846058 | 0.4840307 |
| ERP4 | 0.4916645 | 0.4840719 |
| YHR078W | 0.4823711 | 0.4851821 |
| MID1 | 0.486631 | 0.485498 |
| RPS2 | 0.5062868 | 0.4856318 |
| COX5A | 0.4784618 | 0.4856582 |
| CMK2 | 0.4891345 | 0.4859698 |
| YHR175W-A | 0.4882798 | 0.4861668 |
| ARO2 | 0.482775 | 0.4862402 |
| LRC1 | 0.5040619 | 0.4865479 |
| YLR342W-A | 0.4449526 | 0.4865586 |
| YGR035W-A | 0.460374 | 0.4866387 |
| RPS26B | 0.4654814 | 0.4868773 |
| RPL36B | 0.4810615 | 0.4874903 |
| RPL20B | 0.4916371 | 0.4875966 |
| RPL8B | 0.4746629 | 0.4879403 |
| BLM10 | 0.4815797 | 0.4888318 |
| HTZ1 | 0.4953201 | 0.4891719 |
| RPL17B | 0.4959537 | 0.4898459 |
| RPS3 | 0.4927777 | 0.4900817 |
| HAS1 | 0.5052765 | 0.4905168 |
| IRC21 | 0.4934668 | 0.4908083 |
| RPL29 | 0.4859097 | 0.4909509 |
| ERI1 | 0.4845532 | 0.4912684 |
| AST1 | 0.486676 | 0.491349 |
| ADK1 | 0.4872068 | 0.4915423 |
| RPS14A | 0.5016199 | 0.492083 |
| SUP45 | 0.4817769 | 0.4923916 |
| RPL23A | 0.4938049 | 0.493675 |
| DYN2 | 0.5020944 | 0.4938712 |
| STT4 | 0.4896448 | 0.4941468 |
| RSP5 | 0.4941224 | 0.4943716 |

| | | |
|---|---|---|
| HHT2 | 0.5056277 | 0.4945883 |
| URB1 | 0.5057695 | 0.4950262 |
| YKL215C | 0.479265 | 0.4952145 |
| CLA4 | 0.5062269 | 0.4953385 |
| TMA7 | 0.4739332 | 0.495577 |
| TUB2 | 0.5009129 | 0.4963189 |
| HHF2 | 0.5035001 | 0.4963272 |
| AVT1 | 0.4844744 | 0.4964476 |
| SSH1 | 0.5000507 | 0.4966168 |
| RPL42B | 0.4842137 | 0.4966801 |
| RPL13B | 0.5021062 | 0.4968802 |
| CBF5 | 0.4974996 | 0.4970558 |
| DSE1 | 0.4939589 | 0.4972757 |
| MVD1 | 0.4878789 | 0.4973979 |
| SAN1 | 0.4846829 | 0.4974329 |
| HEM12 | 0.5001099 | 0.4978953 |
| YCR024C-B | 0.4928656 | 0.498269 |
| GDS1 | 0.5014768 | 0.498275 |
| IRC22 | 0.4984242 | 0.4987666 |
| KAP123 | 0.5023901 | 0.4992161 |
| CDC42 | 0.4918415 | 0.5008609 |
| UBC9 | 0.4986912 | 0.5008915 |
| VNX1 | 0.5017304 | 0.5019131 |
| YGR203W | 0.4724045 | 0.5023768 |
| TCB3 | 0.4985425 | 0.5024617 |
| GSH2 | 0.5010269 | 0.5035297 |
| KRE33 | 0.4945982 | 0.5036318 |

**Table 5: Genes with at least a 2-fold increase in expression in *paf1Δ* samples**

| | Mean | Median |
|---|---|---|
| DDR2 | 138.31699 | 46.04513 |
| NCA3 | 33.225957 | 25.714021 |
| SNZ1 | 35.067324 | 19.271385 |
| PUT1 | 23.224952 | 14.530925 |
| DUR3 | 20.178639 | 12.672053 |
| BOP2 | 18.429691 | 11.65312 |
| HSP12 | 38.871708 | 11.592689 |
| HSP26 | 15.222772 | 11.249152 |
| YJL133C-A | 12.209236 | 10.701516 |
| GAP1 | 15.166057 | 10.699591 |
| ARG1 | 12.518081 | 9.7348137 |
| YDR042C | 14.130253 | 9.1070049 |
| AMS1 | 11.791441 | 8.6086927 |
| SPG4 | 9.6018859 | 8.4637089 |
| FMP23 | 18.212573 | 8.2459106 |
| PDH1 | 8.7906547 | 7.6177654 |
| YOR338W | 12.886853 | 7.4962453 |
| MOH1 | 11.809309 | 7.4892781 |
| FIT1 | 15.691019 | 7.442703 |
| YKR104W | 11.093183 | 7.3058085 |
| SNO1 | 17.567334 | 7.202236 |
| YJR005C-A | 10.905793 | 6.7535061 |
| ARG3 | 13.819376 | 6.5752781 |
| NFT1 | 9.149295 | 6.2542708 |
| GSC2 | 6.3315013 | 6.242731 |
| YLR149C | 8.408691 | 6.1709539 |
| TMT1 | 7.8750407 | 5.6344007 |
| ARN1 | 6.1032578 | 5.5232879 |
| YTP1 | 12.211515 | 5.514506 |
| HXK1 | 9.4971127 | 5.486294 |
| FIT3 | 5.7066994 | 5.4846008 |
| MEP2 | 10.061923 | 5.469235 |
| FIT2 | 6.3147652 | 5.301455 |
| GPH1 | 8.4628101 | 5.2832653 |
| AGP2 | 9.3182783 | 4.9839788 |
| NCE103 | 4.9840503 | 4.9548079 |
| BIO5 | 8.8969086 | 4.8593282 |
| YGP1 | 5.0122531 | 4.8012264 |
| SER3 | 5.2381874 | 4.6784904 |
| XBP1 | 7.4894595 | 4.6538404 |
| YBL048W | 18.653788 | 4.5153881 |
| TMA10 | 7.1532913 | 4.4544995 |
| DAN1 | 8.5148917 | 4.4246676 |
| HSP78 | 5.5698621 | 4.3800949 |
| MSC1 | 8.2483889 | 4.3649036 |
| VID24 | 5.3421683 | 4.272981 |
| CRC1 | 6.1807535 | 4.2723088 |
| REC104 | 7.6202022 | 4.1884119 |
| YNR064C | 7.0934888 | 4.1720999 |
| YGR066C | 4.9376413 | 4.118305 |
| QNQ1 | 6.5343183 | 4.117359 |
| ARN2 | 4.22117 | 4.1080765 |
| HMX1 | 5.1123691 | 4.0387771 |
| YJR151W-A | 4.594219 | 3.8919593 |
| RTN2 | 7.8541578 | 3.8899906 |
| TIS11 | 3.8775429 | 3.8405724 |
| GLC3 | 5.7912028 | 3.8170847 |
| YHR007C-A | 6.6398984 | 3.7851225 |
| ZRT1 | 3.6604026 | 3.5972667 |
| YLR346C | 6.6046424 | 3.5859097 |
| YHL048C-A | 5.8620725 | 3.4802236 |
| HPF1 | 3.5268772 | 3.4281138 |
| AGP1 | 3.6538503 | 3.4164468 |
| VHT1 | 3.6763914 | 3.3996846 |
| CCC2 | 4.0998303 | 3.3878412 |
| YDL159C-B | 4.663435 | 3.3494655 |
| YER039C-A | 4.1154066 | 3.3219331 |
| YMR196W | 5.0823834 | 3.2621066 |
| CIT2 | 3.1481988 | 3.1868975 |
| LAP4 | 3.8118158 | 3.1690258 |
| HBT1 | 4.5028108 | 3.1239502 |
| YNL018C | 3.8294331 | 3.0955017 |
| HBN1 | 5.1082514 | 3.0846118 |

| | | |
|---|---|---|
| YMR090W | 7.3607522 | 3.0643528 |
| SPS19 | 5.0186973 | 3.0551178 |
| GCY1 | 5.4912184 | 3.0432511 |
| YNL162W-A | 4.5593539 | 3.0368443 |
| PCA1 | 3.8577185 | 3.0230353 |
| SAG1 | 3.6371191 | 3.0107068 |
| UBI4 | 2.9796257 | 2.9874982 |
| GOR1 | 5.1633078 | 2.9871839 |
| YHR212W-A | 7.4876201 | 2.9855913 |
| YPL056C | 4.6474027 | 2.9593325 |
| NQM1 | 5.2715403 | 2.9401299 |
| YLL053C | 4.3308016 | 2.9322267 |
| UBC8 | 5.5716397 | 2.9296466 |
| YNL034W | 3.8912194 | 2.9138372 |
| FMP45 | 4.5113419 | 2.8998138 |
| GSP2 | 2.9633722 | 2.8845957 |
| GAD1 | 4.0540435 | 2.876031 |
| YPL135C-A | 5.2431158 | 2.8710929 |
| ARO10 | 4.4959031 | 2.8709022 |
| YKL071W | 4.0608739 | 2.8457111 |
| ADR1 | 3.68638 | 2.8093319 |
| PRY1 | 2.9932988 | 2.8001234 |
| GAC1 | 3.8811518 | 2.7827099 |
| YNL019C | 6.0915676 | 2.7591051 |
| GTO3 | 4.781657 | 2.7492945 |
| ERR2 | 4.6954942 | 2.7421263 |
| YFL041W-A | 4.8747225 | 2.7146878 |
| YNR062C | 6.6946945 | 2.7137778 |
| GCV1 | 3.0243097 | 2.7136885 |
| OPI3 | 2.8063588 | 2.7078353 |
| YLR162W-A | 3.4903943 | 2.696821 |
| AIM30 | 4.2629805 | 2.6626172 |
| GDH2 | 3.0079121 | 2.6291931 |
| GTT2 | 4.1667084 | 2.6084631 |
| UGA1 | 3.1591785 | 2.6007533 |
| PRB1 | 2.6791671 | 2.5891479 |
| YHR159W | 3.7969809 | 2.5837856 |
| YOR062C | 4.6746638 | 2.5813058 |
| GPG1 | 4.527712 | 2.5810594 |
| PEX21 | 4.2684361 | 2.5809575 |
| YPL205C | 3.9237796 | 2.5478323 |
| FMP40 | 3.7925087 | 2.5450737 |
| SLF1 | 3.0771681 | 2.5345567 |
| YFL054C | 4.3350591 | 2.5242257 |
| UIP4 | 4.6301137 | 2.5235787 |
| TAR1 | 10.646738 | 2.5186081 |
| SHY1 | 3.4573669 | 2.5017694 |
| VMR1 | 2.6162073 | 2.497355 |
| UGA2 | 5.6018433 | 2.4914812 |
| ERR1 | 5.1617793 | 2.4912791 |
| YIR014W | 4.7124553 | 2.4784734 |
| ALD4 | 2.7186473 | 2.4776522 |
| YLR154C-H | 2.862514 | 2.4761166 |
| YGL117W | 4.6943457 | 2.4708334 |
| RAD34 | 4.2043374 | 2.4610549 |
| GDB1 | 2.9102455 | 2.4570973 |
| RTS3 | 7.8926552 | 2.4533238 |
| FRE3 | 3.0167088 | 2.4530066 |
| ATG3 | 3.4957632 | 2.4523643 |
| GPM2 | 5.0486291 | 2.4511391 |
| CIT1 | 2.4637186 | 2.4413557 |
| ATG8 | 3.217515 | 2.4411901 |
| STB2 | 3.4019413 | 2.4366993 |
| BSC4 | 3.941631 | 2.4339845 |
| TSL1 | 2.8452971 | 2.4269097 |
| IRC15 | 4.2747104 | 2.4263088 |
| ATG9 | 3.0099995 | 2.4213345 |
| YOR389W | 3.4586808 | 2.4189314 |
| AQY2 | 5.4269439 | 2.4188694 |
| MRPS17 | 3.8156229 | 2.4058428 |
| STF1 | 4.2755917 | 2.3968077 |
| PUG1 | 3.9367526 | 2.3949463 |
| YAT2 | 3.0592724 | 2.3921762 |
| YOR381W-A | 2.9308089 | 2.3910981 |
| GRE1 | 4.6334924 | 2.3901443 |
| NDE2 | 3.0600755 | 2.3786066 |
| PGM2 | 3.8670329 | 2.3744017 |
| YOR059C | 3.2750997 | 2.3588835 |

| | | |
|---|---|---|
| YKL100W-A | 3.8725653 | 2.3553912 |
| BIO3 | 3.4505177 | 2.3391833 |
| YKL070W | 3.2812326 | 2.3370862 |
| LIN1 | 4.0657558 | 2.3311686 |
| YIL169C | 2.5538143 | 2.324625 |
| ECM13 | 3.3880968 | 2.320223 |
| RIM8 | 2.8055877 | 2.3174904 |
| SAW1 | 3.2496478 | 2.3013074 |
| NTH1 | 2.6066364 | 2.2994305 |
| EMP46 | 3.9611436 | 2.2952805 |
| NTG1 | 3.1161343 | 2.2927277 |
| ERR3 | 4.5716276 | 2.2894245 |
| MEP1 | 3.900857 | 2.2836222 |
| RAD26 | 2.9399384 | 2.2655819 |
| DLD1 | 2.9430055 | 2.2590314 |
| CYB2 | 2.9351542 | 2.2512314 |
| SPG5 | 2.3486275 | 2.2420535 |
| FRE5 | 3.3390642 | 2.2354836 |
| FRE2 | 3.2224619 | 2.2280311 |
| CIS1 | 2.6959422 | 2.2034235 |
| BAT2 | 2.2366618 | 2.1968164 |
| RMD6 | 3.5812521 | 2.196033 |
| FMP16 | 4.8545288 | 2.1829413 |
| YOR072W-B | 5.8795071 | 2.177849 |
| YOR020W-A | 2.3430543 | 2.1743486 |
| FET5 | 2.2612086 | 2.1629999 |
| GDH3 | 4.0406395 | 2.1541461 |
| HSP32 | 2.7053519 | 2.144927 |
| DAS1 | 2.2204371 | 2.1251482 |
| YIR021W-A | 2.588517 | 2.124716 |

| | | |
|---|---|---|
| FMP46 | 2.2415187 | 2.1234301 |
| YKL151C | 3.1472755 | 2.1230992 |
| SIT1 | 2.1750787 | 2.1213435 |
| YGR110W | 3.4089735 | 2.1206411 |
| YOL131W | 2.5782685 | 2.1163624 |
| YKR011C | 2.6987109 | 2.1154905 |
| YNR073C | 3.5196715 | 2.1154611 |
| PKP1 | 3.1761737 | 2.096655 |
| SKS1 | 2.9285955 | 2.0951028 |
| YFR026C | 3.7580766 | 2.0943795 |
| YKL133C | 3.0841337 | 2.0918017 |
| YPL277C | 2.8340073 | 2.0906763 |
| GSY2 | 2.3643764 | 2.0891603 |
| YCL048W-A | 4.9175206 | 2.0864206 |
| SGA1 | 4.6547419 | 2.0783328 |
| SUE1 | 3.6065823 | 2.0667573 |
| GIP1 | 2.9249061 | 2.0611763 |
| SIP18 | 3.1209377 | 2.0588535 |
| ATG11 | 2.5092949 | 2.0532223 |
| YER053C-A | 2.8079373 | 2.0474375 |
| PDR10 | 2.696969 | 2.036653 |
| MPM1 | 2.9587711 | 2.0221513 |
| NTR2 | 5.0276437 | 2.0217209 |
| YPS5 | 3.9589012 | 2.0165057 |
| YDR034W-B | 3.0928237 | 2.016048 |
| ACS1 | 3.4421275 | 2.0110886 |
| RPM2 | 2.5873943 | 2.0096479 |
| YMR105W-A | 2.8066023 | 2.0068415 |
| YGL235W | 3.8090614 | 1.995993 |

**Table 6: Genes with both antisense and sense transcription that increases 2-fold or more in *paf1Δ* samples**

| | Sense | | Antisense | |
|---|---|---|---|---|
| | **Mean** | **Median** | **Mean** | **Median** |
| SNZ1 | 35.0673 | 19.271385 | 24.4578 | 17.8892 |
| BIO5 | 8.89691 | 4.8593282 | 25.9281 | 14.1525 |
| ARN1 | 6.10326 | 5.5232879 | 18.1717 | 9.58563 |
| FIT1 | 15.691 | 7.442703 | 17.1243 | 8.47485 |
| GSC2 | 6.3315 | 6.242731 | 11.1761 | 8.30162 |
| FIT3 | 5.7067 | 5.4846008 | 37.7441 | 7.77783 |
| SER3 | 5.23819 | 4.6784904 | 9.13471 | 6.60349 |
| HXK1 | 9.49711 | 5.486294 | 12.1304 | 6.21254 |
| UBI4 | 2.97963 | 2.9874982 | 9.8144 | 6.12185 |
| ARN2 | 4.22117 | 4.1080765 | 9.13247 | 6.06205 |
| VID24 | 5.34217 | 4.272981 | 9.45838 | 5.97539 |
| NCE103 | 4.98405 | 4.9548079 | 8.04875 | 5.82293 |
| GAP1 | 15.1661 | 10.699591 | 8.67477 | 5.77226 |
| CIT1 | 2.46372 | 2.4413557 | 6.78752 | 5.40284 |
| ZRT1 | 3.6604 | 3.5972667 | 6.70324 | 5.09684 |
| CCC2 | 4.09983 | 3.3878412 | 6.10174 | 4.53803 |
| ATG8 | 3.21751 | 2.4411901 | 5.60537 | 4.35804 |
| CIT2 | 3.1482 | 3.1868975 | 7.19608 | 4.21616 |
| TIS11 | 3.87754 | 3.8405724 | 5.76307 | 3.94378 |
| AGP2 | 9.31828 | 4.9839788 | 7.23619 | 3.69733 |
| VHT1 | 3.67639 | 3.3996846 | 7.21619 | 3.68972 |
| FIT2 | 6.31477 | 5.301455 | 13.0138 | 3.62395 |
| PRY1 | 2.9933 | 2.8001234 | 5.22176 | 3.61219 |
| PCA1 | 3.85772 | 3.0230353 | 5.10096 | 3.58492 |
| LIN1 | 4.06576 | 2.3311686 | 5.84167 | 3.56112 |
| BAT2 | 2.23666 | 2.1968164 | 5.26854 | 3.34669 |
| SAG1 | 3.63712 | 3.0107068 | 5.97305 | 3.31909 |
| FET5 | 2.26121 | 2.1629999 | 3.81419 | 3.20513 |
| ARG1 | 12.5181 | 9.7348137 | 4.50007 | 3.13606 |
| GDH2 | 3.00791 | 2.6291931 | 4.09426 | 3.11973 |
| OPI3 | 2.80636 | 2.7078353 | 5.05556 | 3.03051 |
| VMR1 | 2.61621 | 2.497355 | 4.47797 | 3.01966 |
| GCV1 | 3.02431 | 2.7136885 | 6.07017 | 2.96037 |

| | | | | |
|---|---|---|---|---|
| ALD4 | 2.71865 | 2.4776522 | 3.84931 | 2.85332 |
| YGP1 | 5.01225 | 4.8012264 | 3.97525 | 2.83087 |
| SIT1 | 2.17508 | 2.1213435 | 3.11609 | 2.8305 |
| YKR104W | 11.0932 | 7.3058085 | 4.90479 | 2.79279 |
| PRB1 | 2.67917 | 2.5891479 | 4.40813 | 2.78586 |
| DAS1 | 2.22044 | 2.1251482 | 4.18573 | 2.70718 |
| YJL133C-A | 12.2092 | 10.701516 | 5.02354 | 2.69861 |
| TSL1 | 2.8453 | 2.4269097 | 3.47295 | 2.67098 |
| YOR072W-B | 5.87951 | 2.177849 | 3.3548 | 2.55611 |
| ADR1 | 3.68638 | 2.8093319 | 3.54746 | 2.52455 |
| UGA1 | 3.15918 | 2.6007533 | 4.35325 | 2.52042 |
| YHR159W | 3.79698 | 2.5837856 | 3.22359 | 2.51946 |
| AGP1 | 3.65385 | 3.4164468 | 4.45427 | 2.47097 |
| GSP2 | 2.96337 | 2.8845957 | 3.79007 | 2.38103 |
| SPG5 | 2.34863 | 2.2420535 | 3.6919 | 2.30831 |
| NFT1 | 9.1493 | 6.2542708 | 3.73107 | 2.2696 |
| SKS1 | 2.9286 | 2.0951028 | 3.73765 | 2.25729 |
| FRE3 | 3.01671 | 2.4530066 | 3.05838 | 2.15067 |
| HSP78 | 5.56986 | 4.3800949 | 4.31024 | 2.12667 |
| BSC4 | 3.94163 | 2.4339845 | 2.82425 | 2.08866 |
| MEP2 | 10.0619 | 5.469235 | 2.86143 | 2.08457 |
| YER053C-A | 2.80794 | 2.0474375 | 1.9998 | 2.08089 |
| YOR059C | 3.2751 | 2.3588835 | 3.00299 | 2.08012 |
| ERR1 | 5.16178 | 2.4912791 | 2.96796 | 2.00714 |

**Table 7: snoRNAs exhibiting Paf1-dependent termination**

| | Mean |
|---|---|
| SNR48 | 8.60394 |
| SNR71 | 6.28089 |
| SNR161 | 4.7699 |
| SNR45 | 4.31242 |
| SNR60 | 3.85685 |
| SNR6 | 3.78078 |
| SNR81 | 3.75334 |
| SNR85 | 3.52054 |
| SNR64 | 3.05996 |
| SNR32 | 2.89057 |
| SNR79 | 2.82628 |
| SNR47 | 2.32781 |
| SNR53 | 2.30485 |
| SNR42 | 2.29291 |
| SNR33 | 2.1752 |
| SNR56 | 2.1287 |
| SNR7-L | 1.95333 |
| SNR7-S | 1.9113 |

**Table 8: *S. cerevisiae* strains used in Chapter 4**

| Strain | Genotype |
|---|---|
| ECY151 | *MAT***a** *leu2Δ0 ura3Δ03* |
| ECY58 | *MAT***a** *paf1Δ::KanMX leu2Δ0 ura3Δ03* |

# 5.0 CONCLUSIONS AND FUTURE DIRECTIONS

## 5.1 CONCLUSIONS

The Paf1 complex is a conserved transcription elongation complex that is required for proper gene expression. The *Saccharomyces cerevisiae* genome contains numerous Paf1 complex-activated genes and those that require the Paf1 complex for repression (PENHEITER *et al.* 2005). Many of the known functions of the Paf1 complex are associated with its role as a positive transcriptional regulator. For example, the Paf1 complex stimulates transcription of a chromatin template *in vitro* (PAVRI *et al.* 2006). *In vivo*, the Paf1 complex is enriched on actively transcribed coding regions (KROGAN *et al.* 2002b; MAYER *et al.* 2010; POKHOLOK *et al.* 2002; WADE *et al.* 1996) and is required for histone modifications that are associated with active transcription (CHU *et al.* 2007; KROGAN *et al.* 2003a; NG *et al.* 2003a; NG *et al.* 2003b; WOOD *et al.* 2003b). However, the role of the Paf1 complex as a negative regulator of gene expression has remained largely unstudied. Therefore, my thesis work is the first examination of the repressive functions of the Paf1 complex.

Since very little is known about the repressive role of the Paf1 complex, I decided to perform a detailed analysis of a model locus of Paf1 complex-dependent repression. It was previously shown by a genome-wide expression study that *ARG1* requires Paf1 for repression. However, I confirmed these results using Northern analysis and determined that other members

of the Paf1 complex also contribute to *ARG1* repression. Specifically, Paf1 and Ctr9, and to a lesser extent, Rtf1 and Cdc73, are required for *ARG1* repression. Using *ARG1* as a model gene, I determined which previously known roles of the Paf1 complex contribute to *ARG1* repression, examined the effects of the Paf1 complex on gene-specific regulatory proteins, and analyzed the effect of the Paf1 complex on the chromatin environment at the *ARG1* locus. Additionally, my results have implicated the Paf1 complex in the transcription of non-coding RNAs. Importantly, I observed similar effects of the Paf1 complex at other genes, suggesting that the Paf1 complex mediates repression of a subset of genes through similar mechanisms. Finally, I obtained a high-resolution view of Paf1-dependent transcription across the entire yeast genome using high-density tiling microarray analysis.

### 5.1.1 Genome-wide analysis of Paf1-dependent transcription using high-resolution microarrays

In collaboration with Corey Nislow (University of Toronto), I initiated a project that involved the use of high-resolution microarrays to examine transcriptional changes observed in the absence of Paf1. Data analysis by Brett Tomson and members of the Nislow lab enabled the identification of many genes that require the Paf1 complex for proper expression. Paf1 appears to function predominantly as a positive regulator of transcription, however many genes require Paf1 for repression. We also found that Paf1 appears to regulate antisense transcription at several genes. Furthermore, we revealed a genome-wide role for Paf1 in the transcription termination of snoRNAs. These data will likely prove useful for future analyses of the roles of the Paf1 complex in regulating the transcription of both coding and non-coding RNAs.

### 5.1.2 The role of the nascent RNA in regulating gene expression

Transcription is tightly coupled to RNA biogenesis, as proteins involved in 5' capping, splicing, and 3' end formation are recruited to the nascent RNA in a co-transcriptional manner (reviewed in RONDON *et al.* 2010). Along with RNA processing factors, a host of other proteins associate with the nascent RNA to protect it from degradation and facilitate nuclear export, forming a messenger ribonucleoparticle (mRNP) (reviewed in RONDON *et al.* 2010). Not only does transcription stimulate mRNP formation, but mRNP formation regulates transcription through a feedback mechanism (reviewed in RONDON *et al.* 2010). For example, mutations in the THO/TREX complex, which regulates mRNP biogenesis, cause defects in mRNP formation that are associated with impaired transcription elongation (CHAVEZ and AGUILERA 1997; MASON and STRUHL 2005; RONDON *et al.* 2003). The reduced transcription elongation in these mutants may be explained by the increased formation of R-loops, which are DNA:RNA hybrids formed between the DNA template strand and the nascent RNA (GOMEZ-GONZALEZ and AGUILERA 2007; HUERTAS and AGUILERA 2003). R-loops have been shown to impede transcription elongation by inhibiting the passage of the polymerase (BENTIN *et al.* 2005; TOUS and AGUILERA 2007). Additionally, it has been proposed that the malformation of mRNPs may block transcription by the preventing release of the polymerase or by triggering RNA quality control pathways (reviewed in RONDON *et al.* 2010). While it is not completely understood how malformed mRNPs are recognized, changes in transcript polyadenylation may be indicative of defects in mRNP biogenesis, as defects in mRNP formation have been shown to result in extended poly(A) tails (HILLEREN and PARKER 2001; JENSEN *et al.* 2001).

Interestingly, the Paf1 complex plays roles in RNA biogenesis which could implicate it in mRNP formation. For example, yeast strains lacking Paf1 complex members exhibit altered

poly(A) tail length and reduced recruitment of 3' end formation factors (MUELLER *et al.* 2004; PENHEITER *et al.* 2005; SHELDON *et al.* 2005; TOMSON *et al.* 2011a). Furthermore, members of the Paf1 complex exhibit RNA binding activity (DERMODY and BURATOWSKI 2010). Finally, Paf1 was found to genetically and physically interact with Hpr1, a component of the THO complex, suggesting that the two complexes may have overlapping functions (SHI *et al.* 1996). While further work is required to determine whether the Paf1 complex plays a critical role in mRNP biogenesis, it is interesting to speculate that, if so, the Paf1 complex could regulate transcription indirectly by influencing mRNP formation.

### 5.1.3 The Paf1 complex mediates *ARG1* repression in *cis*.

Several lines of evidence suggest that the Paf1 complex may be a direct repressor of *ARG1* transcription. First, analysis of *ARG1* expression in single and double mutant strains suggested that the Paf1 complex mediates *ARG1* repression independently of the gene-specific repressor, Arg80. Paf1 also had no effect on Arg80 protein levels and had only minor influence on the expression of other members of the ArgR/Mcm1 repressor complex. Therefore, it is unlikely that the Paf1 complex mediates *ARG1* repression indirectly through the ArgR/Mcm1 repressor complex. Furthermore, ChIP analysis detected the Paf1 complex on the *ARG1* coding region, which is consistent with a direct repressive function.

ChIP analysis also detected low levels of RNA Pol II at the *ARG1* promoter and coding region, possibly due to low levels of *ARG1* sense and/or antisense transcription that occurs in rich media. As expected, in inducing conditions, RNA Pol II levels increased across the *ARG1* coding region. Interestingly, at the promoter, RNA Pol II levels were unexpectedly high, especially in repressing conditions. These results may be indicative of a paused polymerase at a

promoter-proximal region. Only low levels of Paf1 and Rtf1 were observed at the *ARG1* promoter, suggesting that the majority of the polymerase at the promoter is not associated with the Paf1 complex. Conversely, the RNA Pol II levels observed at 5', middle, and 3' coding regions of *ARG1* in both repressing and inducing conditions appear to be associated with similar levels of Paf1 complex, suggesting that the majority of the polymerase within the coding region is associated with the Paf1 complex. These results may indicate that the Paf1 complex facilitates promoter escape or the release of paused polymerase at the 5' coding region.

### 5.1.4 Paf1 complex-dependent histone modifications are enriched at the *ARG1* locus and contribute to repression.

The Paf1 complex is required for the methylation of several residues on histone H3 (K4, K36, and K79) (CHU *et al.* 2007; KROGAN *et al.* 2003a; NG *et al.* 2003a; NG *et al.* 2003b; WOOD *et al.* 2003b). To determine whether the Paf1 complex mediates *ARG1* repression by promoting histone H3 methylation, I examined *ARG1* transcript levels in the absence of Set1, Set2, and Dot1, the histone H3 K4, K36, and K79 methyltransferases, respectively. I found that Set1, but not Set2 or Dot1, was required for *ARG1* repression, implicating histone H3 K4 methylation in *ARG1* repression. However, histone H3 K4 methylation is often associated with actively transcribed regions. Therefore, I performed ChIP analysis to determine whether histone H3 K4 di- and trimethylation is enriched at the *ARG1* locus. Indeed, histone H3 K4 di- and trimethylation were detected at the *ARG1* promoter and coding region, suggesting that histone H3 methylation is present at *ARG1* in repressing conditions and contributes to *ARG1* repression.

Histone H3 K4 di- and trimethylation occur downstream of histone H2B K123 monoubiquitylation by Rad6 and Bre1. Therefore, I asked whether the upstream mark is

required for *ARG1* repression. The loss of histone H2B ubiquitylation upon deletion of *RAD6* or *BRE1* or mutation of the ubiquitylation site (*htb1-K123R*) resulted in *ARG1* derepression. Therefore, the Paf1 complex mediates *ARG1* derepression through histone H2B ubiquitylation and downstream H3 K4 methylation. Consistent with these results, the analysis of several Rtf1 mutant strains indicated that the Rtf1 histone modification domain is required for *ARG1* repression.

However, the loss of histone H2B ubiquitylation and H3 K4 methylation cannot entirely explain the derepression that occurs in *paf1Δ* cells, because deletion of *PAF1* resulted in higher levels of *ARG1* mRNA than deletion of *SET1* or *BRE1* or the *htb1-K123R* mutation. Analysis of *ARG1* transcript levels in *htb1-K123R* and *htb1-K123R rtf1Δ* cells, however, suggested that Rtf1 mediates repression in the same pathway as histone H2B ubiquitylation. Therefore, while Rtf1 mediates *ARG1* repression primarily through histone H2B ubiquitylation and H3 K4 methylation, Paf1 mediates *ARG1* repression partially through histone modifications and partially through another mechanism. Importantly, analysis of several mutant strains revealed similar effects on the expression of *ARG1*, *GAP1*, and *SNZ1*, pointing toward similar mechanisms of repression at all three loci.

### 5.1.5 Paf1 complex subunits differentially contribute to *ARG1* repression.

My detailed analysis of the role of Paf1 and Rtf1 in *ARG1* repression raises the question of why different Paf1 complex subunits are required to different degrees for *ARG1* repression. I found that deletion of *RTF1* or *CDC73* results in a similar level of *ARG1* derepression. Interestingly, it has been shown that Rtf1 protein levels are reduced in the absence of Cdc73 (MUELLER *et al.* 2004; SQUAZZO *et al.* 2002). Therefore, the *ARG1* derepression observed in *cdc73Δ* cells may be

due to the reduced abundance of Rtf1. Deletion of *PAF1* or *CTR9* results in a similar level of *ARG1* derepression that is a higher level of derepression than observed in *rtf1Δ* or *cdc73Δ* cells. Both Paf1 and Ctr9 are required for proper Rtf1 protein levels (MUELLER *et al.* 2004; SQUAZZO *et al.* 2002). Therefore, a portion of the *ARG1* derepression that occurs in *paf1Δ* or *ctr9Δ* cells is likely due to a reduction in Rtf1 protein levels. Additionally, the loss of Paf1 results in reduced Ctr9 protein levels and vise versa. Therefore, individual deletion of *PAF1* or *CTR9* cannot distinguish between the functions of Paf1 and Ctr9. Similar interdependencies have been observed in the human Paf1 complex. Specifically, loss of human Ctr9, Cdc73, or the subunit specific to the human complex, Ski8, results in reduced Paf1 and Leo1 protein levels (LIN *et al.* 2008; ZHU *et al.* 2005a).

While the interdependencies among Paf1 complex members may explain some of my results obtained in strains lacking individual complex members, it is intriguing that the requirement for these complex members for *ARG1* repression mirrors their requirement for snoRNA termination. Specifically, as for *ARG1* repression, Paf1 and Ctr9, and to a lesser extent, Rtf1 and Cdc73 are required for proper snoRNA termination (SHELDON *et al.* 2005). Furthermore, of the Rtf1 point mutant strains examined for their effects on *ARG1* repression and snoRNA termination, *rtf1-F80V, F123S* cells exhibit the strongest defect in both functions (TOMSON *et al.* 2011a). As this *rtf1* mutant strain lacks histone H2B ubiquitylation, it appears that histone H2B ubiquitylation is critical for both processes (TOMSON *et al.* 2011a). Consistent with this idea, the loss of the histone H2B ubiquitylating enzymes, Rad6 or Bre1, or mutation of the ubiquitylation site (*htb1-K123R*) results in aberrant snoRNA termination (TOMSON *et al.* 2011a). It would be interesting to understand how histone H2B ubiquitylation is involved in mediating each of these processes. Importantly, these observations suggest that as we further

elucidate the roles of the Paf1 complex in *ARG1* repression, we may also gain insight into its functions in transcription termination.

### 5.1.6 Paf1 inhibits recruitment of Gcn4 to the *ARG1* promoter.

To further define the role of Paf1 in *ARG1* repression, I asked whether Paf1 influenced recruitment of Gcn4 or Arg80 to the *ARG1* promoter. ChIP analysis of Gcn4 occupancy at the *ARG1* promoter revealed that Gcn4 occupancy was increased in *paf1Δ* cells, suggesting that Paf1 inhibits recruitment of Gcn4 to the promoter. Although *ARG1* derepression in the absence of Paf1 was associated with an approximately 2-fold increase in Gcn4 recruitment, it is not clear whether this modest increase in recruitment is sufficient to stimulate the high increase in *ARG1* expression observed. Arg80 also inhibited recruitment of Gcn4 to the promoter. However, analysis of Gcn4 occupancy in *paf1Δ arg80Δ* cells indicated that Paf1 and Arg80 independently inhibit Gcn4 recruitment to the *ARG1* promoter, further confirming that Paf1 and Arg80 make separate contributions to *ARG1* repression. Importantly, I found that Gcn4 is required for the full level of *ARG1* derepression that occurs in *paf1Δ* cells. Together, these results suggest that Paf1 mediates repression partially by inhibiting recruitment of Gcn4 to the *ARG1* promoter and partially through a Gcn4-independent mechanism.

Interestingly, I also found that Paf1 inhibits recruitment of Arg80 to the *ARG1* promoter. Several lines of evidence suggest that Paf1 functions separately from Arg80 in the repression of *ARG1*. Furthermore, Gcn4 did not influence Arg80 recruitment. Therefore, I hypothesize that the increased recruitment of Arg80 in *paf1Δ* cells may be the result of a more accessible chromatin environment at the *ARG1* promoter under these conditions. It may not be surprising that Paf1 mediates *ARG1* repression independently of Arg80, considering the differences

between their mechanisms of repression. Arg80 mediates repression by interacting with DNA binding sites in the *ARG1* promoter as part of the ArgR/Mcm1 repressor complex (AMAR *et al.* 2000; BECHET *et al.* 1970; CRABEEL *et al.* 1995; CRABEEL *et al.* 1990; DELFORGE *et al.* 1975; DUBOIS *et al.* 1987; EL BAKKOURY *et al.* 2000; QIU *et al.* 1990). While the mechanistic details of gene repression by the ArgR/Mcm1 complex are not known, other DNA binding transcriptional repressors recruit co-repressors that inhibit the basal transcriptional machinery, interfere with activator binding, or recruit histone modifying proteins (reviewed in PAYANKAULAM *et al.* 2010). In contrast, Paf1 has not been shown to exhibit specific DNA-binding activity, but instead associates with RNA Pol II during transcription elongation (KROGAN *et al.* 2002b; MAYER *et al.* 2010; POKHOLOK *et al.* 2002; WADE *et al.* 1996) and regulates histone modifications (CHU *et al.* 2007; KROGAN *et al.* 2003a; NG *et al.* 2003a; NG *et al.* 2003b; WOOD *et al.* 2003b) and transcription termination (MUELLER *et al.* 2004; PENHEITER *et al.* 2005; SHELDON *et al.* 2005; TOMSON *et al.* 2011a). Therefore, Paf1 regulates transcription through more general mechanisms. Consistent with this idea, while Arg80 represses transcription of four arginine biosynthesis genes as part of the ArgR/Mcm1 complex (reviewed in MESSENGUY and DUBOIS 2000), myself and others have demonstrated that Paf1 represses several hundred genes throughout the yeast genome (PENHEITER *et al.* 2005).

### 5.1.7 The loss of histone H3 acetylation partially suppresses *ARG1* derepression in *paf1Δ* cells.

The loss of Paf1 appears to make the *ARG1* promoter more accessible to Gcn4. Given the connection between the Paf1 complex and chromatin, I hypothesized that, to prevent Gcn4 binding at the *ARG1* promoter, Paf1 may establish a “closed” chromatin environment.

Therefore, I performed a nucleosome scanning assay to examine nucleosome occupancy at the *ARG1* promoter and 5' region. I found that in wild-type cells, the *cis*-elements in the *ARG1* promoter are located within a nucleosome-depleted region and flanked by several well-positioned nucleosomes. Importantly, these data match well with genome-wide studies of nucleosome occupancy (JIANG and PUGH 2009). Induction of *ARG1* expression in wild-type cells resulted in a dramatic reduction in nucleosome occupancy at the *ARG1* promoter and early 5' region. However, deletion of *PAF1* did not result in reduced nucleosome occupancy. The only change observed in *paf1Δ* cells was a very slight forward-shifting of the nucleosome immediately downstream of the *cis*-elements. This minor change in nucleosome occupancy is unlikely to account for the increase in *ARG1* mRNA observed in *paf1Δ* cells. Consistent with these results, ChIP analysis of total histone H3 levels did not detect a decrease in histone H3 levels in *paf1Δ* cells. These results indicate that Paf1 does not regulate nucleosome occupancy at the *ARG1* promoter or 5' region. Additionally, these results indicate that derepression in *paf1Δ* cells can occur in the absence of nucleosome eviction.

Besides nucleosome eviction, histone acetylation also allows the polymerase to pass through nucleosomes. Therefore, I examined the possibility that Paf1 established *ARG1* repression by preventing histone acetylation. Consistent with this hypothesis, Gcn4 has been shown to recruit the histone acetyltransferase (HAT), Gcn5, to promote transcription of Gcn4-regulated genes (GOVIND *et al.* 2005; KUO *et al.* 2000). Conversely, the Arndt lab previously demonstrated that the Paf1 complex inhibits acetylation on the coding region of active genes (CHU *et al.* 2007). Furthermore, I performed a few key experiments to suggest that the Paf1 complex could mediate repression by inhibiting histone acetylation at *ARG1*. For example, *ARG1* derepression in *paf1Δ* cells is partially suppressed by deletion of *GCN5*. Additionally,

substitution mutation of lysines on histone H3 that are acetylated by Gcn5 also partially restored *ARG1* repression in *paf1Δ* cells. However, ChIP analysis did not detect the increase in acetylation at *ARG1* in *paf1Δ* cells that is predicted by this hypothesis. Several histone deacetylase (HDAC) complexes have been shown to maintain low histone acetylation at the *ARG1* locus. Therefore, the actions of HDACs may oppose increases in histone H3 acetylation. Alternatively, *ARG1* depression may not require an increase in acetylation per se. Instead, derepression may be facilitated by the rapid turnover of histone acetylation by rounds of acetylation and deacetylation.

### 5.1.8 Antisense transcription occurs at the *ARG1* locus.

Histone modifications occur in distinct patterns at most genes. Histone H3 K4 trimethylation is usually highest at 5' coding regions (KROGAN *et al.* 2003a; NG *et al.* 2003b), while H3 K36 trimethylation is generally highest at 3' coding regions (KIZER *et al.* 2005; KROGAN *et al.* 2003b; LI *et al.* 2003; SCHAFT *et al.* 2003; XIAO *et al.* 2003). However, the histone modification pattern detected at *ARG1* is opposite of the expected pattern. A similar phenomenon had been observed at the *GAL10* locus, where, in repressing conditions, histone H3 K4 methylation was unexpectedly enriched at the *GAL10* 3' coding region (HOUSELEY *et al.* 2008). It was determined that the inverted pattern of histone H3 K4 methylation was established by a non-coding RNA transcribed from the opposite strand, in the antisense direction relative to *GAL10* (HOUSELEY *et al.* 2008). Therefore, I hypothesized that antisense transcription at the *ARG1* locus could contribute to the reversed pattern of histone modifications. To examine this possibility, I performed strand-specific RT-PCR. Interestingly, I did detect antisense transcription that traversed the *ARG1* coding region, similar to what has been detected by global

transcription analyses (DAVID *et al.* 2006; XU *et al.* 2009). After using several cDNA synthesis primers located within the *ARG1* promoter and 5' coding region, it appears that antisense transcription traverses the *ARG1* coding region at similar levels in wild-type and *paf1Δ* cells. However, antisense transcription that traverses the promoter was more prominent in the absence of Paf1, suggesting that Paf1 prevents antisense transcription from traversing the *ARG1* promote.

3' RACE was performed to map the 3' end of the antisense transcripts at *ARG1*. Similar to what I observed with RT-PCR, 3' RACE detected an increase in 3' extended transcripts in *paf1Δ* cells. However, the longest transcripts did not reach the *cis*-elements, but instead terminated near the *ARG1* transcription start site. Since 3' RACE specifically detects polyadenylated transcripts, these results may indicated that a mixture of shorter polyadenylated and longer non-polyadenylated antisense transcripts occur at the *ARG1* locus and the loss of Paf1 results in an increase in longer transcripts.

Interestingly, antisense transcription traversing the *ARG1* promoter correlated with *ARG1* sense transcription. Therefore, I hypothesized that antisense transcription across the *ARG1* promoter may facilitate Gcn4 promoter-association. To examine this possibility, I integrated a terminator element in the antisense direction, hoping to terminate antisense transcription before it reached the *ARG1* promoter. However, the terminator failed to terminate antisense transcription. Therefore, I instead attempted to eliminate the antisense transcription start site, which was predicted by global transcription analyses to be located within the *ARG1* 3' UTR (DAVID *et al.* 2006; XU *et al.* 2009). To this end, I integrated the *ARG1* promoter and coding region at the *LYP1* locus, such that the *ARG1* promoter and coding region were fused to the *LYP1* 3' UTR. I chose the *LYP1* 3' UTR because no antisense transcripts have been observed at this locus (DAVID *et al.* 2006; XU *et al.* 2009). As a control, I integrated the *ARG1* promoter, coding region, and 3'

UTR at the *LYP1* locus to account for any change in expression that may occur simply due to the change in chromosomal context. However, antisense transcription was still observed when the *ARG1* promoter and coding region were fused to the *LYP1* 3' UTR. Furthermore, increased *ARG1* mRNA levels were detected in *paf1Δ* cells, regardless of whether the *ARG1* promoter and coding region were fused to the *ARG1* or *LYP1* 3' UTR. These results suggest that the antisense transcripts observed at the *ARG1* locus do not arise from start sites within the 3' UTR, but instead from start sites within the *ARG1* coding region.

### 5.1.9 Defining the regions of the *ARG1* locus required for Paf1-mediated repression

I next asked whether the *ARG1* coding region is required for *ARG1* repression by Paf1. To do this, I replaced the *ARG1* coding region with that of *HIS3*, such that the *HIS3* coding region was fused to the *ARG1* promoter and 3' UTR. This chimeric gene was regulated similarly to *ARG1*, in that it was repressed in wild-type cells and derepressed in the absence of Paf1 or Rtf1. However, the level of derepression that occurred in the absence of Paf1 was lower than what was observed with native *ARG1*, suggesting that Paf1-mediated repression partially requires the *ARG1* coding region. Furthermore, the low level of derepression observed in *paf1Δ* cells was independent of Gcn4. Therefore, the Gcn4-dependent functions of Paf1 require the *ARG1* coding region. Additionally, preliminary results indicated that, as expected, replacement of the *ARG1* coding region with the *HIS3* coding region eliminated antisense transcription (data not shown). Therefore, my results indicate that antisense transcription is required for the full derepression that occurs in the absence of Paf1.

To confirm that the *ARG1* 3' UTR is not required for Paf1-mediated repression, I replaced the *ARG1* coding region and 3' UTR with that of *HIS3*, such that the *ARG1* promoter

was fused to the *HIS3* coding region and 3' UTR. The *HIS3* 3' UTR appeared to confer increased expression in wild-type cells, possibly due to an increase in mRNA stability. However, similar to the previous construct, deletion of *PAF1* resulted in an increase in expression, but lower than the fold increase observed with native *ARG1*. These results confirm that the *ARG1* coding region is partially required for Paf1-mediated repression and further confirm that the *ARG1* 3' UTR is not required for Paf1-mediated repression.

### 5.1.10 Working Model of *ARG1* repression by the Paf1 complex

The repression of *ARG1* by the Paf1 complex is surprisingly complex. The Rtf1 subunit appears to mediate repression primarily by promoting histone H2B ubiquitylation and histone H3 K4 methylation. However, Paf1 appears to have roles beyond these histone modifications, which include inhibiting the recruitment of Gcn4 to the *ARG1* promoter. Paf1 also appears to inhibit histone H3 acetylation by Gcn5. Furthermore, Paf1 prevents antisense transcription from traversing the promoter, which positively influences *ARG1* sense transcription (Figure 30A).

Given these results, I hypothesize that in wild-type cells, the Paf1 complex promotes histone H2B ubiquitylation and H3 K4 methylation, which represses *ARG1* sense transcription. Also, the antisense transcript terminates upstream of the *ARG1* promoter (Figure 30B). However, in *paf1Δ* cells, the repressive histone modifications are lost, causing a minor increase in *ARG1* transcription. Transcription through the *ARG1* 3' coding region alters chromatin in such a way that promotes increased transcription of long antisense transcripts that traverse the *ARG1* promoter. Antisense transcription across the *ARG1* promoter enhances recruitment of Gcn4. Gcn4 recruits the co-activator, Gcn5, which acetylates histone H3 in nucleosomes across

the *ARG1* locus, further promoting *ARG1* sense transcription (Figure 30C). The positive influence of sense and antisense may continue through a feed-forward mechanism.

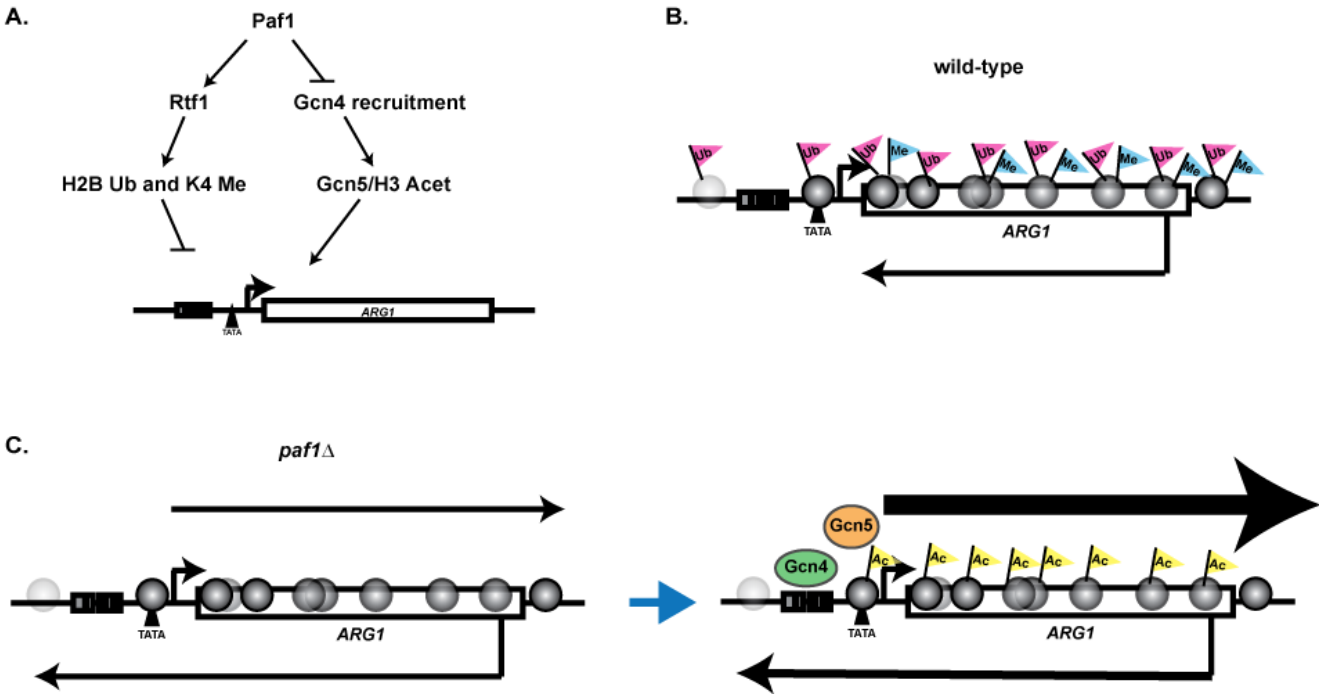


**Figure 30: Working Model of Paf1 complex-mediated *ARG1* repression**

## 5.2 FUTURE DIRECTIONS

Although my thesis work has made significant progress toward understanding the repressive functions of the Paf1 complex, many important questions remain. For example, my model makes the prediction that, as previously reported, Gcn4 recruits Gcn5, resulting in histone H3 acetylation. This prediction is easily testable by performing ChIP analysis using epitope-tagged

Gcn5. I would expect increased Gcn5 recruitment to *ARG1* in the absence of Paf1 in a Gcn4-dependent manner.

Another prediction of my model is that antisense transcription across the *ARG1* promoter enhances Gcn4 recruitment. Alternatively, increased Gcn4 recruitment may be due to the loss of Paf1-dependent histone modifications. This can be examined by performing ChIP analysis of Gcn4 in strains in which antisense transcription has been eliminated by replacing the *ARG1* coding region with that of *HIS3*. If antisense transcription enhances Gcn4 recruitment in *paf1Δ* cells, then *paf1Δ* cells should not exhibit increased Gcn4 recruitment in the absence of antisense transcription. If increased Gcn4 recruitment still occurs in *paf1Δ* cells, then it is likely due to the loss of histone modifications. If so, one would also expect to observe increased Gcn4 recruitment at the promoter of native *ARG1* in *rtf1Δ* cells. Along those lines, it would be interesting to know whether over-expression of Rtf1 in *paf1Δ* cells restores histone modifications. If so, it would help distinguish between the multiple roles of Paf1.

It would also be important to map the 5' and 3' ends of the antisense transcript(s) at the *ARG1* locus. Although I performed 3' RACE to determine the 3' end of antisense transcripts at the *ARG1* locus, this method requires a poly(A) tail. RT-PCR detected longer transcripts than were identified by 3' RACE, suggesting that the longer transcripts may not be polyadenylated. Therefore, RNase protection assays could be performed to determine the 3' end of antisense transcripts in wild-type and *paf1Δ* cells. Additionally, high resolution microarray analysis in stabilizing conditions would provide similar information on a genome-wide scale.

Furthermore, genome-wide studies predicted that the 5' end of the antisense transcript was located within the *ARG1* 3' UTR. However, my results demonstrated that replacement of the *ARG1* 3' UTR with that of *LYP1* did not eliminate antisense transcription. This may mean

that antisense transcripts arise from multiple start sites within the *ARG1* 3' coding region and 3' UTR. Therefore, 5' RACE or primer extension could be used to determine the location of the transcription start sites in both wild-type and *paf1Δ* cells. This would also determine whether the same start site(s) are used in the presence and absence of Paf1. Antisense transcription could then be eliminated by deletion of small regions containing the transcription start site, which would enable me to study the effects of antisense transcription in the context of a relatively intact *ARG1* coding region.

It would also be interesting to determine why there is increased antisense transcription across the *ARG1* promoter in *paf1Δ* cells. The loss of Paf1 results in read-through of the antisense transcript termination site, resulting in a 3'-extended transcript. This possibility is intriguing considering the Paf1 complex and histone H2B ubiquitylation have been implicated in regulating termination of both polyadenylated and non-polyadenylated transcripts (MUELLER *et al.* 2004; PENHEITER *et al.* 2005; SHELDON *et al.* 2005; TOMSON *et al.* 2011b). Conditional mutant forms of termination and 3' end formation factors, such as Nab3, Nrd1, or Pcf11, could be used to test this hypothesis.

It would also be interesting to examine whether the general repressor complex Tup1-Ssn6 is involved in Paf1-mediated *ARG1* repression. Tup1-Ssn6 was found to promote the binding of Gcn4 to the *ARG1* promoter in inducing conditions (KIM *et al.* 2005a). Interestingly, Tup1-Ssn6 was found to associate with both the *ARG1* promoter and the 3' coding region (KIM *et al.* 2005a). It was proposed that the localization of Tup1-Ssn6 to the 3' coding region was indicative of a potential role in transcription elongation (KIM *et al.* 2005a). However, in light of my results indicating that antisense transcription arises from the *ARG1* 3' coding region in wild-type cells in inducing conditions, Tup1-Ssn6 may influence antisense transcription. Interestingly, Ssn6

interacts with Set2 (TRIPIC *et al.* 2006), the histone H3 K36 methyltransferase, which could help recruit Tup1-Ssn6 to *ARG1*. Also, Tup1-Ssn6 has been shown to recruit HAT complexes to genes, including the Gcn5-containing SAGA complex (PROFT and STRUHL 2002). Alternatively, Ssn6 interacts with Rpd3 and Hos2 (DAVIE *et al.* 2003; DAVIE *et al.* 2002), HDACs that have been shown to deacetylate histones within the *ARG1* ORF(GOVIND *et al.* 2010). The interaction with both HATs and HDACs could allow Tup1-Ssn6 to effectively manage acetylation levels at the *ARG1* locus. It would be interesting to examine whether the derepression that occurs in *paf1Δ* cells requires Tup1-Ssn6, whether Paf1inhibits its recruitment, and whether its recruitment to the *ARG1* 3' coding region requires antisense transcription.

Finally, I have performed high-density microarray analysis to examine Paf1-dependent transcription and identified numerous genes that require the Paf1 complex for proper expression and proper transcription termination. However, given the Paf1-repressed antisense transcription that I observed at *ARG1*, *ARG3*, and *SNZ1*, it would be useful to perform a genome-wide analysis to examine expression of SUTs and CUTs. Therefore, the Arndt lab plans to repeat the high-density microarray analysis, with modifications to enhance accurate detection of SUTs and CUTs. Such modifications include the addition of antinomycin D to cDNA synthesis reactions to prevent antisense artifacts resulting from second strand synthesis. Additionally, including samples prepared from strains deleted for *PAF1* and *RRP6* or *TRF4* could enable the detection of CUTs.

Cumulatively, my thesis work has revealed a complex mechanism of repression by the Paf1 complex. I've found that Paf1 complex-dependent histone modifications that are normally found at sites of active transcription are enriched at *ARG1* and contribute to repression. Additionally, to my knowledge, *ARG1* and *PHO5* are the only yeast genes that have been shown

to be positively regulated by antisense transcription (UHLER *et al.* 2007). However, while antisense transcription at the *PHO5* locus promotes gene expression by stimulating nucleosome eviction (UHLER *et al.* 2007), my results suggest that antisense transcription facilitates *ARG1* expression through a unique feed-forward mechanism that does not require dramatic changes in chromatin structure. Beyond my progress, many interesting questions remain. Therefore, my work will serve as a foundation for future studies.

# 6.0 APPENDIX

## THE ROLE OF RKR1 IN UBIQUITYLATION OF HISTONE H2A.Z

*RKR1* was originally identified as a gene whose mutation causes synthetic lethality with the loss of Rtf1, suggesting that Rkr1 and Rtf1 have a common function in the cell (BRAUN *et al.* 2007). Loss of Rkr1 results in inositol auxotrophy and telomeric silencing defects (BRAUN *et al.* 2007), which are phenotypes associated with transcriptional defects. Furthermore, deletion of Rkr1 causes synthetic sickness with mutations that abolish histone H2B ubiquitylation (BRAUN *et al.* 2007). *RKR1* encodes a protein with a C-terminal RING domain (BRAUN *et al.* 2007). RING domain-containing proteins often mediate ubiquitylation (LORICK *et al.* 1999). Indeed, Rkr1 has *in vitro* ubiquitin-protein ligase activity. Together these observations suggest that Rkr1 regulates chromatin structure and transcription, likely through protein ubiquitylation.

However, the target of Rkr1 ubiquitylation is unknown. Therefore, to identify other transcription-related factors that genetically interact with Rkr1, Epistatic miniarray analysis (E-MAP) was performed in collaboration with Nevan Krogan at the University of California, San Francisco. For this analysis, a *rkr1Δ* strain was crossed with approximately 380 strains deleted for genes encoding transcription-related proteins. Double mutants were generated and assayed for growth, revealing cases of synthetic sickness or lethality. Furthermore, the genetic

interaction profile of Rkr1 was compared to other factors and the data were clustered to identify functional groups. Importantly, the genetic interaction profile of Rkr1 clustered with that of seven subunits of the Swr1 complex (*VPS71*, *VPS72*, *SWC5*, *SWC3*, *ARP6*, *SWR1*, *HTZ1*), an ATP-dependent chromatin remodeling complex that deposits the histone variant H2A.Z into chromatin (GUILLEMETTE *et al.* 2005; RAISNER *et al.* 2005).

H2A.Z, or Htz1 in yeast, is found at heterochromatic boundaries, such as near telomeres and flanking the silent HMR mating-type locus, where it prevents the spread of heterochromatin (MENEGHINI *et al.* 2003; ZHANG *et al.* 2004). Htz1 is also found at promoters throughout the genome (GUILLEMETTE *et al.* 2005; LI *et al.* 2005; RAISNER *et al.* 2005; ZHANG *et al.* 2005a). Specifically, Htz1-containing nucleosomes often flank a nucleosome-free region that contains the transcription start site (RAISNER *et al.* 2005). Proper transcriptional induction appears to require Htz1 (GUILLEMETTE *et al.* 2005; LI *et al.* 2005; ZHANG *et al.* 2005a). Importantly, a portion of histone H2A.Z is monoubiquitylated in mammalian cells. In mammalian cells, H2A.Z is excluded from constitutive pericentric heterochromatin, but is associated with facultative heterochromatin, such as the inactive X chromosome, and euchromatin. The inactive X-chromosome is enriched for monoubiquitylated H2A.Z, indicating that monoubiquitylation of H2A.Z distinguishes its association with facultative heterochromatin or euchromatin (SARCINELLA *et al.* 2007).

To examine ubiquitylation of Htz1 in yeast, *RKR1* and *rkr1Δ* strains were transformed with an overexpression plasmid expressing FLAG-tagged Htz1 (pMB82) and a plasmid expressing copper-inducible HIS-tagged (pUb221) or untagged ubiquitin (pUb175). Ubiquitylation assays to examine Htz1 ubiquitylation were performed as previously described (MURATANI *et al.* 2005). Briefly, cell lysates were prepared from induced cultures in denaturing

buffer to prevent protein de-ubiquitylation. Ubiquitylated proteins were purified by Ni-NTA chromatography, resolved by SDS-PAGE, and subjected to Western analysis with an antibody against the FLAG tag. Polyubiquitylation of FLAG-Htz1 can be seen as a ladder in the *RKR1* strain, but is reduced in the *rkr1Δ* strain (Figure 31A), indicating that in these conditions, Rkr1 is important for polyubiquitylation of Htz1 in yeast.

Additionally, I examined Htz1 ubiquitylation in strains expressing plasmid-encoded HA-tagged forms of Rkr1. Rkr1-C1508A contains a mutation in the C-terminal RING domain that abolishes *in vitro* ubiquitin-protein ligase activity (BRAUN *et al.* 2007) and Rkr1$\Delta N_{2-227}$ contains an N-terminal deletion generated by Anthony Piro. As expected, Htz1 ubiquitylation is reduced in strains expressing Rkr1-C1508A, indicating that the ubiquitin-protein ligase activity of Rkr1 is required for Htz1 ubiquitylation (Figure 31B). Interestingly, Htz1 ubiquitylation if also reduced in strains expressing Rkr1$\Delta N_{2-227}$ , indicating that N-terminus of Rkr1 is required for Htz1 ubiquitylation (Figure 31B). Since Rkr1$\Delta N_{2-227}$ is expressed to similar levels as wild-type Rkr1 (Figure 31B), this result may indicate that the N-terminus of Rkr1 is required for substrate recognition.

These experiments indicate that overexpression of Htz1 results in Htz1 polyubiquitylation in yeast in a Rkr1-dependent manner. I also performed ubiquitylation assays in strains expressing integrated *FLAG-HTZ1*. Although I could detect expression of Rkr1 and Htz1, I was unable to detect polyubiquitylation of Htz1 (data not shown). Kristin Klucevsek has also obtained similar results more recently. Therefore, Rkr1-dependent ubiquitylation of Htz1 may only occur when Htz1 is overexpressed. Additionally, since the FLAG epitope contains multiple lysines, FLAG-Htz1 contains several additional potential ubiquitylation sites compared to untagged Htz1. Therefore, it is possible that the FLAG epitope contributes to Htz1

ubiquitylation. Consistent with this idea, Rkr1 has recently been implicated in the degradation of nonstop proteins (BENGTSON and JOAZEIRO 2010; WILSON *et al.* 2007), which lack a stop codon and contain a poly-lysine track because the ribosome reads into the poly(A) tail of the nonstop transcript (reviewed in reference WAGNER and LYKKE-ANDERSEN 2002). Future work will likely elucidate the fascinating functions of Rkr1.

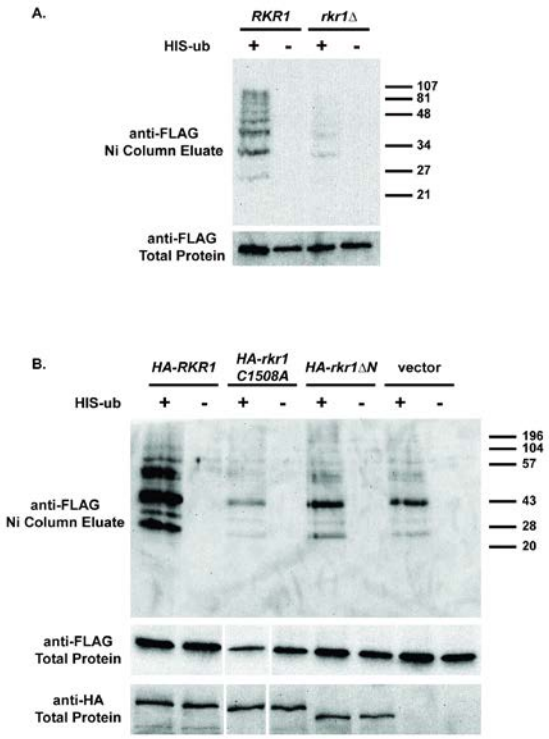


**Figure 31: Rkr1 is required for polyubiquitylation of everexpressed Htz1.**

(A) *htz1Δ* (KY1403) and *htz1Δ rkr1Δ* (KY1415) strains were transformed with a plasmid expressing FLAG-Htz1 (pMB82) and a plasmid expressing HIS-tagged ubiquitin (pUb221) or untagged ubiquitin (pUb175). Ubiquitylation assays were performed as described above. Ubiquitylated protein was purified using a Ni column. Anti-FLAG M2 (Sigma) antibody (1:1000) and sheep anti-mouse horseradish peroxidase-coupled secondary antibody (1:5000) (GE Healthcare) were used to detect FLAG-Htz1 in the Ni column eluate and total protein. (B) *htz1Δ rkr1Δ* (KY1415) cells were transformed with a plasmid expressing HA-Rkr1 (pAP2), HA-Rkr1 C1508A (pAP3), HA-Rkr1ΔN (pAP4), or empty vector (pRS313), in addition to a plasmid expressing FLAG-Htz1 (pMB82) and a plasmid expressing HIS-tagged ubiquitin (pUb221) or untagged ubiquitin (pUb175). Ubiquitylated protein was purified using a Ni column. Anti-FLAG M2 antibody(Sigma) (1:1000) and sheep anti-mouse horseradish peroxidase-coupled secondary antibody (1:5000) (GE Healthcare) were used to detect FLAG-Htz1 in the Ni column eluate and total protein. Additionally, anti-HA (Roche) antibody (1:1000) and sheep anti-mouse horseradish peroxidase-coupled secondary antibody (1:5000) (GE Healthcare) were used to detect HA-tagged wild-type Rkr1 or mutant rkr1 in total protein.

**Table 9: *S. cerevisiae* strains used in this appendix**

| Strain | Genotype |
|---|---|
| KY1403 | *MATα htz1Δ::KanMX his3Δ200 lys2Δ0 leu2Δ0 ura3Δ0* |
| KY1415 | *MAT***a** *htz1Δ::KanMX rkr1Δ::KanMX his3Δ200 leu2Δ0 ura3Δ0 trp1Δ63* |

## BIBLIOGRAPHY


ADAMKEWICZ, J. I., C. G. MUELLER, K. E. HANSEN, W. A. PRUD'HOMME and J. THORNER, 2000 Purification and enzymic properties of Mot1 ATPase, a regulator of basal transcription in the yeast Saccharomyces cerevisiae. J Biol Chem **275:** 21158-21168.

ADELMAN, K., W. WEI, M. B. ARDEHALI, J. WERNER, B. ZHU *et al.*, 2006 Drosophila Paf1 modulates chromatin structure at actively transcribed genes. Mol Cell Biol **26:** 250-260.

AHN, S. H., M. KIM and S. BURATOWSKI, 2004 Phosphorylation of serine 2 within the RNA polymerase II C-terminal domain couples transcription and 3' end processing. Mol Cell **13:** 67-76.

AIDA, M., Y. CHEN, K. NAKAJIMA, Y. YAMAGUCHI, T. WADA *et al.*, 2006 Transcriptional pausing caused by NELF plays a dual role in regulating immediate-early expression of the junB gene. Mol Cell Biol **26:** 6094-6104.

AKANUMA, T., S. KOSHIDA, A. KAWAMURA, Y. KISHIMOTO and S. TAKADA, 2007 Paf1 complex homologues are required for Notch-regulated transcription during somite segmentation. EMBO Rep **8:** 858-863.

ALEXANDER, R. D., S. A. INNOCENTE, J. D. BARRASS and J. D. BEGGS, 2010 Splicing-dependent RNA polymerase pausing in yeast. Mol Cell **40:** 582-593.

AMAR, N., F. MESSENGUY, M. EL BAKKOURY and E. DUBOIS, 2000 ArgRII, a component of the ArgR-Mcm1 complex involved in the control of arginine metabolism in Saccharomyces cerevisiae, is the sensor of arginine. Mol Cell Biol **20:** 2087-2097.

ANDRAU, J. C., C. J. VAN OEVELEN, H. A. VAN TEEFFELEN, P. A. WEIL, F. C. HOLSTEGE *et al.*, 2002 Mot1p is essential for TBP recruitment to selected promoters during in vivo gene activation. EMBO J **21:** 5173-5183.

ANDRULIS, E. D., E. GUZMAN, P. DORING, J. WERNER and J. T. LIS, 2000 High-resolution localization of Drosophila Spt5 and Spt6 at heat shock genes in vivo: roles in promoter proximal pausing and transcription elongation. Genes Dev **14:** 2635-2649.

APARICIO, O. M., B. L. BILLINGTON and D. E. GOTTSCHLING, 1991 Modifiers of position effect are shared between telomeric and silent mating-type loci in S. cerevisiae. Cell **66:** 1279-1287.

ARIGO, J. T., D. E. EYLER, K. L. CARROLL and J. L. CORDEN, 2006 Termination of cryptic unstable transcripts is directed by yeast RNA-binding proteins Nrd1 and Nab3. Mol Cell **23:** 841-851.

AUBLE, D. T., K. E. HANSEN, C. G. MUELLER, W. S. LANE, J. THORNER *et al.*, 1994 Mot1, a global repressor of RNA polymerase II transcription, inhibits TBP binding to DNA by an ATP-dependent mechanism. Genes Dev **8:** 1920-1934.

AUBLE, D. T., D. WANG, K. W. POST and S. HAHN, 1997 Molecular analysis of the SNF2/SWI2 protein family member MOT1, an ATP-driven enzyme that dissociates TATA-binding protein from DNA. Mol Cell Biol **17:** 4842-4851.

AUSTIN, R. J., and M. D. BIGGIN, 1995 A domain of the even-skipped protein represses transcription by preventing TFIID binding to a promoter: repression by cooperative blocking. Mol Cell Biol **15:** 4683-4693.

AUSUBEL, F. M., R. BRENT, R.E. KINGSTON, D.D. MOORE, J.G. SEIDMAN, J.A. SMITH, AND K. STRUHL, 1988 Current Protocols in Molecular Biology.

BAI, Y., C. SALVADORE, Y. C. CHIANG, M. A. COLLART, H. Y. LIU *et al.*, 1999 The CCR4 and CAF1 proteins of the CCR4-NOT complex are physically and functionally separated from NOT2, NOT4, and NOT5. Mol Cell Biol **19:** 6642-6651.

BALASUBRAMANIAN, B., C. V. LOWRY and R. S. ZITOMER, 1993 The Rox1 repressor of the Saccharomyces cerevisiae hypoxic genes is a specific DNA-binding protein with a high-mobility-group motif. Mol Cell Biol **13:** 6071-6078.

BANNISTER, A. J., P. ZEGERMAN, J. F. PARTRIDGE, E. A. MISKA, J. O. THOMAS *et al.*, 2001 Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature **410:** 120-124.

BECHET, J., M. GREENSON and J. M. WIAME, 1970 Mutations affecting the repressibility of arginine biosynthetic enzymes in Saccharomyces cerevisiae. Eur J Biochem **12:** 31-39.

BELOTSERKOVSKAYA, R., S. OH, V. A. BONDARENKO, G. ORPHANIDES, V. M. STUDITSKY *et al.*, 2003 FACT facilitates transcription-dependent nucleosome alteration. Science **301:** 1090-1093.

BENGTSON, M. H., and C. A. JOAZEIRO, 2010 Role of a ribosome-associated E3 ubiquitin ligase in protein quality control. Nature **467:** 470-473.

BENTIN, T., D. CHERNY, H. J. LARSEN and P. E. NIELSEN, 2005 Transcription arrest caused by long nascent RNA chains. Biochim Biophys Acta **1727:** 97-105.

BERNSTEIN, B. E., E. L. HUMPHREY, R. L. ERLICH, R. SCHNEIDER, P. BOUMAN *et al.*, 2002 Methylation of histone H3 Lys 4 in coding regions of active genes. Proc Natl Acad Sci U S A **99:** 8695-8700.

BETZ, J. L., M. CHANG, T. M. WASHBURN, S. E. PORTER, C. L. MUELLER *et al.*, 2002 Phenotypic analysis of Paf1/RNA polymerase II complex mutations reveals connections to cell cycle regulation, protein synthesis, and lipid and nucleic acid metabolism. Mol Genet Genomics **268:** 272-285.

BOEKE, J. D., F. LACROUTE and G. R. FINK, 1984 A positive selection for mutants lacking orotidine-5'-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol Gen Genet **197:** 345-346.

BONDARENKO, V. A., L. M. STEELE, A. UJVARI, D. A. GAYKALOVA, O. I. KULAEVA *et al.*, 2006 Nucleosomes can form a polar barrier to transcript elongation by RNA polymerase II. Mol Cell **24:** 469-479.

BONE, J. R., and S. Y. ROTH, 2001 Recruitment of the yeast Tup1p-Ssn6p repressor is associated with localized decreases in histone acetylation. J Biol Chem **276:** 1808-1813.

BOURGEOIS, C. F., Y. K. KIM, M. J. CHURCHER, M. J. WEST and J. KARN, 2002 Spt5 cooperates with human immunodeficiency virus type 1 Tat by preventing premature RNA release at terminator sequences. Mol Cell Biol **22:** 1079-1093.

BRACHMANN, C. B., A. DAVIES, G. J. COST, E. CAPUTO, J. LI *et al.*, 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.

BRADLEY, K. J., B. M. CAVACO, M. R. BOWL, B. HARDING, T. CRANSTON *et al.*, 2006 Parafibromin mutations in hereditary hyperparathyroidism syndromes and parathyroid tumours. Clin Endocrinol (Oxf) **64:** 299-306.

BRANTJES, H., J. ROOSE, M. VAN DE WETERING and H. CLEVERS, 2001 All Tcf HMG box transcription factors interact with Groucho-related co-repressors. Nucleic Acids Res **29:** 1410-1419.

BRAUN, M. A., P. J. COSTA, E. M. CRISUCCI and K. M. ARNDT, 2007 Identification of Rkr1, a nuclear RING domain protein with functional connections to chromatin modification in Saccharomyces cerevisiae. Mol Cell Biol **27:** 2800-2811.

BRICKNER, D. G., I. CAJIGAS, Y. FONDUFE-MITTENDORF, S. AHMED, P. C. LEE *et al.*, 2007 H2A.Z-mediated localization of genes at the nuclear periphery confers epigenetic memory of previous transcriptional state. PLoS Biol **5:** e81.

BRIGGS, S. D., T. XIAO, Z. W. SUN, J. A. CALDWELL, J. SHABANOWITZ *et al.*, 2002 Gene silencing: trans-histone regulatory pathway in chromatin. Nature **418:** 498.

BROCKDORFF, N., A. ASHWORTH, G. F. KAY, V. M. MCCABE, D. P. NORRIS *et al.*, 1992 The product of the mouse Xist gene is a 15 kb inactive X-specific transcript containing no conserved ORF and located in the nucleus. Cell **71:** 515-526.

BROWN, C. J., B. D. HENDRICH, J. L. RUPERT, R. G. LAFRENIERE, Y. XING *et al.*, 1992 The human XIST gene: analysis of a 17 kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus. Cell **71:** 527-542.

BRYK, M., M. BANERJEE, M. MURPHY, K. E. KNUDSEN, D. J. GARFINKEL *et al.*, 1997 Transcriptional silencing of Ty1 elements in the RDN1 locus of yeast. Genes Dev **11:** 255-269.

BUMGARNER, S. L., R. D. DOWELL, P. GRISAFI, D. K. GIFFORD and G. R. FINK, 2009 Toggle involving cis-interfering noncoding RNAs controls variegated gene expression in yeast. Proc Natl Acad Sci U S A **106:** 18321-18326.

CAMBLONG, J., N. IGLESIAS, C. FICKENTSCHER, G. DIEPPOIS and F. STUTZ, 2007 Antisense RNA stabilization induces transcriptional gene silencing via histone deacetylation in S. cerevisiae. Cell **131:** 706-717.

CANG, Y., D. T. AUBLE and G. PRELICH, 1999 A new regulatory domain on the TATA-binding protein. EMBO J **18:** 6662-6671.

CAREY, M., B. LI and J. L. WORKMAN, 2006 RSC exploits histone acetylation to abrogate the nucleosomal block to RNA polymerase II elongation. Mol Cell **24:** 481-487.

CARPTEN, J. D., C. M. ROBBINS, A. VILLABLANCA, L. FORSBERG, S. PRESCIUTTINI *et al.*, 2002 HRPT2, encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome. Nat Genet **32:** 676-680.

CARROZZA, M. J., B. LI, L. FLORENS, T. SUGANUMA, S. K. SWANSON *et al.*, 2005 Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. Cell **123:** 581-592.

CHANDRASEKHARAN, M. B., F. HUANG and Z. W. SUN, 2009 Ubiquitination of histone H2B regulates chromatin dynamics by enhancing nucleosome stability. Proc Natl Acad Sci U S A **106:** 16686-16691.

CHAUDHARY, K., S. DEB, N. MONIAUX, M. P. PONNUSAMY and S. K. BATRA, 2007 Human RNA polymerase II-associated factor complex: dysregulation in cancer. Oncogene **26:** 7499-7507.

CHAVEZ, S., and A. AGUILERA, 1997 The yeast HPR1 gene has a functional role in transcriptional elongation that uncovers a novel source of genome instability. Genes Dev **11:** 3459-3470.

CHEN, G., J. FERNANDEZ, S. MISCHE and A. J. COUREY, 1999 A functional interaction between the histone deacetylase Rpd3 and the corepressor groucho in Drosophila development. Genes Dev **13:** 2218-2230.

CHEN, Y., Y. YAMAGUCHI, Y. TSUGENO, J. YAMAMOTO, T. YAMADA *et al.*, 2009 DSIF, the Paf1 complex, and Tat-SF1 have nonredundant, cooperative roles in RNA polymerase II elongation. Genes Dev **23:** 2765-2777.

CHI, Y., M. J. HUDDLESTON, X. ZHANG, R. A. YOUNG, R. S. ANNAN *et al.*, 2001 Negative regulation of Gcn4 and Msn2 transcription factors by Srb10 cyclin-dependent kinase. Genes Dev **15:** 1078-1092.

CHU, Y., R. SIMIC, M. H. WARNER, K. M. ARNDT and G. PRELICH, 2007 Regulation of histone modification and cryptic transcription by the Bur1 and Paf1 complexes. Embo J **26:** 4646-4656.

CHURCHMAN, L. S., and J. S. WEISSMAN, 2011 Nascent transcript sequencing visualizes transcription at nucleotide resolution. Nature **469:** 368-373.

CLEMSON, C. M., J. A. MCNEIL, H. F. WILLARD and J. B. LAWRENCE, 1996 XIST RNA paints the inactive X chromosome at interphase: evidence for a novel RNA involved in nuclear/chromosome structure. J Cell Biol **132:** 259-275.

CLOSE, P., N. HAWKES, I. CORNEZ, C. CREPPE, C. A. LAMBERT *et al.*, 2006 Transcription impairment and cell migration defects in elongator-depleted cells: implication for familial dysautonomia. Mol Cell **22:** 521-531.

COLEMAN, R. A., A. K. TAGGART, L. R. BENJAMIN and B. F. PUGH, 1995 Dimerization of the TATA binding protein. J Biol Chem **270:** 13842-13849.

COLLART, M. A., and K. STRUHL, 1994 NOT1(CDC39), NOT2(CDC36), NOT3, and NOT4 encode a global-negative regulator of transcription that differentially affects TATA-element utilization. Genes Dev **8:** 525-537.

CORE, L. J., J. J. WATERFALL and J. T. LIS, 2008 Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. Science **322:** 1845-1848.

COSGROVE, M. S., and A. PATEL, 2010 Mixed lineage leukemia: a structure-function perspective of the MLL1 protein. FEBS J **277:** 1832-1842.

COSTA, P. J., and K. M. ARNDT, 2000 Synthetic lethal interactions suggest a role for the Saccharomyces cerevisiae Rtf1 protein in transcription elongation. Genetics **156:** 535-547.

COUREY, A. J., and S. JIA, 2001 Transcriptional repression: the long and the short of it. Genes Dev **15:** 2786-2796.

CRABEEL, M., M. DE RIJCKE, S. SENECA, H. HEIMBERG, I. PFEIFFER *et al.*, 1995 Further definition of the sequence and position requirements of the arginine control element that mediates repression and induction by arginine in Saccharomyces cerevisiae. Yeast **11:** 1367-1380.

CRABEEL, M., R. LAVALLE and N. GLANSDORFF, 1990 Arginine-specific repression in Saccharomyces cerevisiae: kinetic data on ARG1 and ARG3 mRNA transcription and stability support a transcriptional control mechanism. Mol Cell Biol **10:** 1226-1233.

CRABEEL, M., S. SENECA, K. DEVOS and N. GLANSDORFF, 1988 Arginine repression of the Saccharomyces cerevisiae ARG1 gene. Comparison of the ARG1 and ARG3 control regions. Curr Genet **13:** 113-124.

CRISUCCI, E. M., and K. M. ARNDT, 2011 The Paf1 complex represses ARG1 transcription in Saccharomyces cerevisiae by promoting histone modifications. Eukaryot Cell **10:** 712-723.

DAI, J., E. M. HYLAND, D. S. YUAN, H. HUANG, J. S. BADER *et al.*, 2008 Probing nucleosome function: a highly versatile library of synthetic histone H3 and H4 mutants. Cell **134:** 1066-1078.

DANIEL, J. A., M. S. TOROK, Z. W. SUN, D. SCHIELTZ, C. D. ALLIS *et al.*, 2004 Deubiquitination of histone H2B by a yeast acetyltransferase complex regulates transcription. J Biol Chem **279:** 1867-1871.

DASGUPTA, A., R. P. DARST, K. J. MARTIN, C. A. AFSHARI and D. T. AUBLE, 2002 Mot1 activates and represses transcription by direct, ATPase-dependent mechanisms. Proc Natl Acad Sci U S A **99:** 2666-2671.

DAVID, L., W. HUBER, M. GRANOVSKAIA, J. TOEDLING, C. J. PALM *et al.*, 2006 A high-resolution map of transcription in the yeast genome. Proc Natl Acad Sci U S A **103:** 5320-5325.

DAVIE, J. K., D. G. EDMONDSON, C. B. COCO and S. Y. DENT, 2003 Tup1-Ssn6 interacts with multiple class I histone deacetylases in vivo. J Biol Chem **278:** 50158-50162.

DAVIE, J. K., R. J. TRUMBLY and S. Y. DENT, 2002 Histone-dependent association of Tup1-Ssn6 with repressed genes in vivo. Mol Cell Biol **22:** 693-703.

DAVIS, C. A., and M. ARES, 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.

DAVIS, J. L., R. KUNISAWA and J. THORNER, 1992 A presumptive helicase (MOT1 gene product) affects gene expression and is required for viability in the yeast Saccharomyces cerevisiae. Mol Cell Biol **12:** 1879-1892.

DELFORGE, J., F. MESSENGUY and J. M. WIAME, 1975 The regulation of arginine biosynthesis in Saccharomyces cerevisiae. The specificity of argR- mutations and the general control of amino-acid biosynthesis. Eur J Biochem **57:** 231-239.

DENISSOV, S., M. VAN DRIEL, R. VOIT, M. HEKKELMAN, T. HULSEN *et al.*, 2007 Identification of novel functional TBP-binding sites and general factor repertoires. EMBO J **26:** 944-954.

DERMODY, J. L., and S. BURATOWSKI, 2010 Leo1 subunit of the yeast paf1 complex binds RNA and contributes to complex recruitment. J Biol Chem **285:** 33671-33679.

DING, L., M. PASZKOWSKI-ROGACZ, A. NITZSCHE, M. M. SLABICKI, A. K. HENINGER *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.

DOVER, J., J. SCHNEIDER, M. A. TAWIAH-BOATENG, A. WOOD, K. DEAN *et al.*, 2002 Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. J Biol Chem **277:** 28368-28371.

DROUIN, S., L. LARAMEE, P. E. JACQUES, A. FOREST, M. BERGERON *et al.*, 2010 DSIF and RNA polymerase II CTD phosphorylation coordinate the recruitment of Rpd3S to actively transcribed genes. PLoS Genet **6:** e1001173.

DUBOIS, E., J. BERCY and F. MESSENGUY, 1987 Characterization of two genes, ARGRI and ARGRIII required for specific regulation of arginine metabolism in yeast. Mol Gen Genet **207:** 142-148.

DUBOIS, E., and F. MESSENGUY, 1991 In vitro studies of the binding of the ARGR proteins to the ARG5,6 promoter. Mol Cell Biol **11:** 2162-2168.

DUBOIS, E., and F. MESSENGUY, 1994 Pleiotropic function of ArgRIIIp (Arg82p), one of the regulators of arginine metabolism in Saccharomyces cerevisiae. Role in expression of cell-type-specific genes. Mol Gen Genet **243:** 315-324.

EDMONDSON, D. G., M. M. SMITH and S. Y. ROTH, 1996 Repression domain of the yeast global repressor Tup1 interacts directly with histones H3 and H4. Genes Dev **10:** 1247-1259.

EISEN, J. A., K. S. SWEDER and P. C. HANAWALT, 1995 Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. Nucleic Acids Res **23:** 2715-2723.

EL BAKKOURY, M., E. DUBOIS and F. MESSENGUY, 2000 Recruitment of the yeast MADS-box proteins, ArgRI and Mcm1 by the pleiotropic factor ArgRIII is required for their stability. Mol Microbiol **35:** 15-31.

ELBLE, R., and B. K. TYE, 1991 Both activation and repression of a-mating-type-specific genes in yeast require transcription factor Mcm1. Proc Natl Acad Sci U S A **88:** 10966-10970.

FABREGA, C., V. SHEN, S. SHUMAN and C. D. LIMA, 2003 Structure of an mRNA capping enzyme bound to the phosphorylated carboxy-terminal domain of RNA polymerase II. Mol Cell **11:** 1549-1561.

FAN, X., Z. MOQTADERI, Y. JIN, Y. ZHANG, X. S. LIU *et al.*, 2010 Nucleosome depletion at yeast terminators is not intrinsic and can occur by a transcriptional mechanism linked to 3'-end formation. Proc Natl Acad Sci U S A **107:** 17945-17950.

FEJES-TOTH, K., V. SOTIROVA, R. SACHIDANANDAM, G. ASSAF, G. J. HANNON *et al.*, 2009 Post-transcriptional processing generates a diversity of 5'-modified long and short RNAs. Nature **457:** 1028-1032.

FIERZ, B., C. CHATTERJEE, R. K. MCGINTY, M. BAR-DAGAN, D. P. RALEIGH *et al.*, 2011 Histone H2B ubiquitylation disrupts local and higher-order chromatin compaction. Nat Chem Biol **7:** 113-119.

FLANAGAN, J. F., L. Z. MI, M. CHRUSZCZ, M. CYMBOROWSKI, K. L. CLINES *et al.*, 2005 Double chromodomains cooperate to recognize the methylated histone H3 tail. Nature **438:** 1181-1185.

FLEMING, A. B., C. F. KAO, C. HILLYER, M. PIKAART and M. A. OSLEY, 2008 H2B ubiquitylation plays a role in nucleosome dynamics during transcription elongation. Mol Cell **31:** 57-66.

FLORES-SAAIB, R. D., and A. J. COUREY, 2000 Analysis of Groucho-histone interactions suggests mechanistic similarities between Groucho- and Tup1-mediated repression. Nucleic Acids Res **28:** 4189-4196.

FORMOSA, T., S. RUONE, M. D. ADAMS, A. E. OLSEN, P. ERIKSSON *et al.*, 2002 Defects in SPT16 or POB3 (yFACT) in Saccharomyces cerevisiae cause dependence on the Hir/Hpc pathway: polymerase passage may degrade chromatin structure. Genetics **162:** 1557-1571.

FUCHS, S. Y., V. ADLER, T. BUSCHMANN, X. WU and Z. RONAI, 1998 Mdm2 association with p53 targets its ubiquitination. Oncogene **17:** 2543-2547.

GANAPATHI, M., M. J. PALUMBO, S. A. ANSARI, Q. HE, K. TSUI *et al.*, 2011 Extensive role of the general regulatory factors, Abf1 and Rap1, in determining genome-wide chromatin structure in budding yeast. Nucleic Acids Res **39:** 2032-2044.

GEISBERG, J. V., F. C. HOLSTEGE, R. A. YOUNG and K. STRUHL, 2001 Yeast NC2 associates with the RNA polymerase II preinitiation complex and selectively affects transcription in vivo. Mol Cell Biol **21:** 2736-2742.

GEISBERG, J. V., Z. MOQTADERI, L. KURAS and K. STRUHL, 2002 Mot1 associates with transcriptionally active promoters and inhibits association of NC2 in Saccharomyces cerevisiae. Mol Cell Biol **22:** 8122-8134.

GEISBERG, J. V., and K. STRUHL, 2004 Cellular stress alters the transcriptional properties of promoter-bound Mot1-TBP complexes. Mol Cell **14:** 479-489.

GELFAND, B., J. MEAD, A. BRUNING, N. APOSTOLOPOULOS, V. TADIGOTLA *et al.*, 2011 Regulated antisense transcription controls expression of cell-type-specific genes in yeast. Mol Cell Biol **31:** 1701-1709.

GIARDINA, C., M. PEREZ-RIBA and J. T. LIS, 1992 Promoter melting and TFIID complexes on Drosophila genes in vivo. Genes Dev **6:** 2190-2200.

GILBERT, C., A. KRISTJUHAN, G. S. WINKLER and J. Q. SVEJSTRUP, 2004 Elongator interactions with nascent mRNA revealed by RNA immunoprecipitation. Mol Cell **14:** 457-464.

GILCHRIST, D. A., S. NECHAEV, C. LEE, S. K. GHOSH, J. B. COLLINS *et al.*, 2008 NELF-mediated stalling of Pol II can enhance gene expression by blocking promoter-proximal nucleosome assembly. Genes Dev **22:** 1921-1933.

GILMOUR, D. S., and R. FAN, 2008 Derailing the locomotive: transcription termination. J Biol Chem **283:** 661-664.

GOECKELER, J. L., A. STEPHENS, P. LEE, A. J. CAPLAN and J. L. BRODSKY, 2002 Overexpression of yeast Hsp110 homolog Sse1p suppresses ydj1-151 thermosensitivity and restores Hsp90-dependent activity. Mol Biol Cell **13:** 2760-2770.

GOLDBERG, I. H., M. RABINOWITZ and E. REICH, 1962 Basis of actinomycin action. I. DNA binding and inhibition of RNA-polymerase synthetic reactions by actinomycin. Proc Natl Acad Sci U S A **48:** 2094-2101.

GOLDSTEIN, A. L., and J. H. MCCUSKER, 1999 Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. Yeast **15:** 1541-1553.

GOMEZ-GONZALEZ, B., and A. AGUILERA, 2007 Activation-induced cytidine deaminase action is strongly stimulated by mutations of the THO complex. Proc Natl Acad Sci U S A **104:** 8409-8414.

GOPPELT, A., G. STELZER, F. LOTTSPEICH and M. MEISTERERNST, 1996 A mechanism for repression of class II gene transcription through specific binding of NC2 to TBP-promoter complexes via heterodimeric histone fold domains. EMBO J **15:** 3105-3116.

GOTTSCHLING, D. E., O. M. APARICIO, B. L. BILLINGTON and V. A. ZAKIAN, 1990 Position effect at S. cerevisiae telomeres: reversible repression of Pol II transcription. Cell **63:** 751-762.

GOVIND, C. K., H. QIU, D. S. GINSBURG, C. RUAN, K. HOFMEYER *et al.*, 2010 Phosphorylated Pol II CTD recruits multiple HDACs, including Rpd3C(S), for methylation-dependent deacetylation of ORF nucleosomes. Mol Cell **39:** 234-246.

GOVIND, C. K., S. YOON, H. QIU, S. GOVIND and A. G. HINNEBUSCH, 2005 Simultaneous recruitment of coactivators by Gcn4p stimulates multiple steps of transcription in vivo. Mol Cell Biol **25:** 5626-5638.

GOVIND, C. K., F. ZHANG, H. QIU, K. HOFMEYER and A. G. HINNEBUSCH, 2007 Gcn5 promotes acetylation, eviction, and methylation of nucleosomes in transcribed coding regions. Mol Cell **25:** 31-42.

GRANT, P. A., L. DUGGAN, J. COTE, S. M. ROBERTS, J. E. BROWNELL *et al.*, 1997 Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes Dev **11:** 1640-1650.

GREEN, S. R., and A. D. JOHNSON, 2004 Promoter-dependent roles for the Srb10 cyclin-dependent kinase and the Hda1 deacetylase in Tup1-mediated repression in Saccharomyces cerevisiae. Mol Biol Cell **15:** 4191-4202.

GREEN, S. R., and A. D. JOHNSON, 2005 Genome-wide analysis of the functions of a conserved surface on the corepressor Tup1. Mol Biol Cell **16:** 2605-2613.

GROMOLLER, A., and N. LEHMING, 2000 Srb7p is a physical and physiological target of Tup1p. EMBO J **19:** 6845-6852.

GUENTHER, M. G., S. S. LEVINE, L. A. BOYER, R. JAENISCH and R. A. YOUNG, 2007 A chromatin landmark and transcription initiation at most promoters in human cells. Cell **130:** 77-88.

GUERMAH, M., V. B. PALHAN, A. J. TACKETT, B. T. CHAIT and R. G. ROEDER, 2006 Synergistic functions of SII and p300 in productive activator-dependent transcription of chromatin templates. Cell **125:** 275-286.

GUILLEMETTE, B., A. R. BATAILLE, N. GEVRY, M. ADAM, M. BLANCHETTE *et al.*, 2005 Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. PLoS Biol **3:** e384.

HAINER, S. J., J. A. PRUNESKI, R. D. MITCHELL, R. M. MONTEVERDE and J. A. MARTENS, 2011 Intergenic transcription causes repression by directing nucleosome assembly. Genes Dev **25:** 29-40.

HAMPSEY, M., 1998 Molecular genetics of the RNA polymerase II general transcriptional machinery. Microbiol Mol Biol Rev **62:** 465-503.

HAN, K., and J. L. MANLEY, 1993 Transcriptional repression by the Drosophila even-skipped protein: definition of a minimal repression domain. Genes Dev **7:** 491-503.

HARTZOG, G. A., J. L. SPEER and D. L. LINDSTROM, 2002 Transcript elongation on a nucleoprotein template. Biochim Biophys Acta **1577:** 276-286.

HE, Y., B. VOGELSTEIN, V. E. VELCULESCU, N. PAPADOPOULOS and K. W. KINZLER, 2008 The antisense transcriptomes of human cells. Science **322:** 1855-1857.

HENRY, K. W., A. WYCE, W. S. LO, L. J. DUGGAN, N. C. EMRE *et al.*, 2003 Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. Genes Dev **17:** 2648-2663.

HESELMEYER, K., M. MACVILLE, E. SCHROCK, H. BLEGEN, A. C. HELLSTROM *et al.*, 1997 Advanced-stage cervical carcinomas are defined by a recurrent pattern of chromosomal aberrations revealing high genetic instability and a consistent gain of chromosome arm 3q. Genes Chromosomes Cancer **19:** 233-240.

HILLEREN, P., and R. PARKER, 2001 Defects in the mRNA export factors Rat7p, Gle1p, Mex67p, and Rat8p cause hyperadenylation during 3'-end formation of nascent transcripts. RNA **7:** 753-764.

HINNEBUSCH, A. G., 1985 A hierarchy of trans-acting factors modulates translation of an activator of amino acid biosynthetic genes in Saccharomyces cerevisiae. Mol Cell Biol **5:** 2349-2360.

HINNEBUSCH, A. G., 1986 The general control of amino acid biosynthetic genes in the yeast Saccharomyces cerevisiae. CRC Crit Rev Biochem **21:** 277-317.

HINNEBUSCH, A. G., 1988 Mechanisms of gene regulation in the general control of amino acid biosynthesis in Saccharomyces cerevisiae. Microbiol Rev **52:** 248-273.

HINNEBUSCH, A. G., 2005 Translational regulation of GCN4 and the general amino acid control of yeast. Annu Rev Microbiol **59:** 407-450.

HONG, S., and S. YOON, 2011 Mcm1p binding sites in the ARG1 promoter positively regulate ARG1 transcription and S. cerevisiae growth in the absence of arginine and Gcn4p. Amino Acids **40:** 623-631.

HONGAY, C. F., P. L. GRISAFI, T. GALITSKI and G. R. FINK, 2006 Antisense transcription controls cell fate in Saccharomyces cerevisiae. Cell **127:** 735-745.

HOSSAIN, M. A., J. M. CLAGGETT, T. NGUYEN and T. L. JOHNSON, 2009 The cap binding complex influences H2B ubiquitination by facilitating splicing of the SUS1 pre-mRNA. RNA **15:** 1515-1527.

HOUSELEY, J., L. RUBBI, M. GRUNSTEIN, D. TOLLERVEY and M. VOGELAUER, 2008 A ncRNA modulates histone modification and mRNA induction in the yeast GAL gene cluster. Mol Cell **32:** 685-695.

HOWELL, V. M., C. J. HAVEN, K. KAHNOSKI, S. K. KHOO, D. PETILLO *et al.*, 2003 HRPT2 mutations are associated with malignancy in sporadic parathyroid tumours. J Med Genet **40:** 657-663.

HSIEH, F. K., M. FISHER, A. UJVARI, V. M. STUDITSKY and D. S. LUSE, 2010 Histone Sin mutations promote nucleosome traversal and histone displacement by RNA polymerase II. EMBO Rep **11:** 705-710.

HUANG, H., A. KAHANA, D. E. GOTTSCHLING, L. PRAKASH and S. W. LIEBMAN, 1997 The ubiquitin-conjugating enzyme Rad6 (Ubc2) is required for silencing in Saccharomyces cerevisiae. Mol Cell Biol **17:** 6693-6699.

HUANG, M., Z. ZHOU and S. J. ELLEDGE, 1998 The DNA replication and damage checkpoint pathways induce transcription by inhibition of the Crt1 repressor. Cell **94:** 595-605.

HUERTAS, P., and A. AGUILERA, 2003 Cotranscriptionally formed DNA:RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. Mol Cell **12:** 711-721.

HUGHES, T. R., M. J. MARTON, A. R. JONES, C. J. ROBERTS, R. STOUGHTON *et al.*, 2000 Functional discovery via a compendium of expression profiles. Cell **102:** 109-126.

HWANG, W. W., S. VENKATASUBRAHMANYAM, A. G. IANCULESCU, A. TONG, C. BOONE *et al.*, 2003 A conserved RING finger protein required for histone H2B monoubiquitination and cell size control. Mol Cell **11:** 261-266.

INOSTROZA, J. A., F. H. MERMELSTEIN, I. HA, W. S. LANE and D. REINBERG, 1992 Dr1, a TATA-binding protein-associated phosphoprotein and inhibitor of class II gene transcription. Cell **70:** 477-489.

INOUE, T., R. K. GEYER, D. HOWARD, Z. K. YU and C. G. MAKI, 2001 MDM2 can promote the ubiquitination, nuclear export, and degradation of p53 in the absence of direct binding. J Biol Chem **276:** 45255-45260.

IVANOV, D., Y. T. KWAK, J. GUO and R. B. GAYNOR, 2000 Domains in the SPT5 protein that modulate its transcriptional regulatory properties. Mol Cell Biol **20:** 2970-2983.

IZBAN, M. G., and D. S. LUSE, 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.

IZBAN, M. G., and D. S. LUSE, 1992 Factor-stimulated RNA polymerase II transcribes at physiological elongation rates on naked DNA but very poorly on chromatin templates. J Biol Chem **267:** 13647-13655.

IZBAN, M. G., and D. S. LUSE, 1993 The increment of SII-facilitated transcript cleavage varies dramatically between elongation competent and incompetent RNA polymerase II ternary complexes. J Biol Chem **268:** 12874-12885.

JABET, C., E. R. SPRAGUE, A. P. VANDEMARK and C. WOLBERGER, 2000 Characterization of the N-terminal domain of the yeast transcriptional repressor Tup1. Proposal for an association model of the repressor complex Tup1 x Ssn6. J Biol Chem **275:** 9011-9018.

JAEHNING, J. A., 2010 The Paf1 complex: platform or player in RNA polymerase II transcription? Biochim Biophys Acta **1799:** 379-388.

JAYNES, J. B., and P. H. O'FARRELL, 1988 Activation and repression of transcription by homoeodomain-containing proteins that bind a common site. Nature **336:** 744-749.

JENSEN, T. H., K. PATRICIO, T. MCCARTHY and M. ROSBASH, 2001 A block to mRNA nuclear export in S. cerevisiae leads to hyperadenylation of transcripts that accumulate at the site of transcription. Mol Cell **7:** 887-898.

JIANG, C., and B. F. PUGH, 2009 A compiled and systematic reference map of nucleosome positions across the Saccharomyces cerevisiae genome. Genome Biol **10:** R109.

JUNEAU, K., C. PALM, M. MIRANDA and R. W. DAVIS, 2007 High-density yeast-tiling array reveals previously undiscovered introns and extensive regulation of meiotic splicing. Proc Natl Acad Sci U S A **104:** 1522-1527.

KADABA, S., A. KRUEGER, T. TRICE, A. M. KRECIC, A. G. HINNEBUSCH *et al.*, 2004 Nuclear surveillance and degradation of hypomodified initiator tRNAMet in S. cerevisiae. Genes Dev **18:** 1227-1240.

KAFFMAN, A., I. HERSKOWITZ, R. TJIAN and E. K. O'SHEA, 1994 Phosphorylation of the transcription factor PHO4 by a cyclin-CDK complex, PHO80-PHO85. Science **263:** 1153-1156.

KALLIONIEMI, A., O. P. KALLIONIEMI, J. PIPER, M. TANNER, T. STOKKE *et al.*, 1994 Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. Proc Natl Acad Sci U S A **91:** 2156-2160.

KAO, C. F., C. HILLYER, T. TSUKUDA, K. HENRY, S. BERGER *et al.*, 2004 Rad6 plays a role in transcriptional activation through ubiquitylation of histone H2B. Genes Dev **18:** 184-195.

KAPLAN, C. D., J. R. MORRIS, C. WU and F. WINSTON, 2000 Spt5 and spt6 are associated with active transcription and have characteristics of general elongation factors in D. melanogaster. Genes Dev **14:** 2623-2634.

KAPRANOV, P., J. CHENG, S. DIKE, D. A. NIX, R. DUTTAGUPTA *et al.*, 2007 RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science **316:** 1484-1488.

KATO, H., M. HORIKOSHI and R. G. ROEDER, 1991 Repression of HIV-1 transcription by a cellular protein. Science **251:** 1476-1479.

KAYNE, P. S., U. J. KIM, M. HAN, J. R. MULLEN, F. YOSHIZAKI *et al.*, 1988 Extremely conserved histone H4 N terminus is dispensable for growth but essential for repressing the silent mating loci in yeast. Cell **55:** 27-39.

KELEHER, C. A., M. J. REDD, J. SCHULTZ, M. CARLSON and A. D. JOHNSON, 1992 Ssn6-Tup1 is a general repressor of transcription in yeast. Cell **68:** 709-719.

KELLEY, D. E., D. G. STOKES and R. P. PERRY, 1999 CHD1 interacts with SSRP1 and depends on both its chromodomain and its ATPase/helicase-like domain for proper association with chromatin. Chromosoma **108:** 10-25.

KEOGH, M. C., S. K. KURDISTANI, S. A. MORRIS, S. H. AHN, V. PODOLNY *et al.*, 2005 Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. Cell **123:** 593-605.

KEOGH, M. C., V. PODOLNY and S. BURATOWSKI, 2003 Bur1 kinase is required for efficient transcription elongation by RNA polymerase II. Mol Cell Biol **23:** 7005-7018.

KERPPOLA, T. K., and C. M. KANE, 1990 Analysis of the signals for transcription termination by purified RNA polymerase II. Biochemistry **29:** 269-278.

KIM, J., M. GUERMAH, R. K. MCGINTY, J. S. LEE, Z. TANG *et al.*, 2009a RAD6-Mediated transcription-coupled H2B ubiquitylation directly stimulates H3K4 methylation in human cells. Cell **137:** 459-471.

KIM, J., M. GUERMAH and R. G. ROEDER, 2010 The human PAF1 complex acts in chromatin transcription elongation both independently and cooperatively with SII/TFIIS. Cell **140:** 491-503.

KIM, J. B., and P. A. SHARP, 2001 Positive transcription elongation factor B phosphorylates hSPT5 and RNA polymerase II carboxyl-terminal domain independently of cyclin-dependent kinase-activating kinase. J Biol Chem **276:** 12317-12323.

KIM, K. Y., and D. E. LEVIN, 2011 Mpk1 MAPK association with the Paf1 complex blocks Sen1-mediated premature transcription termination. Cell **144:** 745-756.

KIM, M., S. H. AHN, N. J. KROGAN, J. F. GREENBLATT and S. BURATOWSKI, 2004 Transitions in RNA polymerase II elongation complexes at the 3' ends of genes. EMBO J **23:** 354-364.

KIM, M., H. SUH, E. J. CHO and S. BURATOWSKI, 2009b Phosphorylation of the yeast Rpb1 C-terminal domain at serines 2, 5, and 7. J Biol Chem **284:** 26421-26426.

KIM, S. J., M. J. SWANSON, H. QIU, C. K. GOVIND and A. G. HINNEBUSCH, 2005a Activator Gcn4p and Cyc8p/Tup1p are interdependent for promoter occupancy at ARG1 in vivo. Mol Cell Biol **25:** 11171-11183.

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

KIM, T. H., L. O. BARRERA, M. ZHENG, C. QU, M. A. SINGER *et al.*, 2005b A high-resolution map of active promoters in the human genome. Nature **436:** 876-880.

KIM, T. K., Y. ZHAO, H. GE, R. BERNSTEIN and R. G. ROEDER, 1995 TATA-binding protein residues implicated in a functional interplay between negative cofactor NC2 (Dr1) and general factors TFIIA and TFIIB. J Biol Chem **270:** 10976-10981.

KIREEVA, M. L., B. HANCOCK, G. H. CREMONA, W. WALTER, V. M. STUDITSKY *et al.*, 2005 Nature of the nucleosomal barrier to RNA polymerase II. Mol Cell **18:** 97-108.

KIZER, K. O., H. P. PHATNANI, Y. SHIBATA, H. HALL, A. L. GREENLEAF *et al.*, 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.

KLAR, A. J., J. N. STRATHERN, J. R. BROACH and J. B. HICKS, 1981 Regulation of transcription in expressed and unexpressed mating type cassettes of yeast. Nature **289:** 239-244.

KLEJMAN, M. P., L. A. PEREIRA, H. J. VAN ZEEBURG, S. GILFILLAN, M. MEISTERERNST *et al.*, 2004 NC2alpha interacts with BTAF1 and stimulates its ATP-dependent association with TATA-binding protein. Mol Cell Biol **24:** 10072-10082.

KOBOR, M. S., J. ARCHAMBAULT, W. LESTER, F. C. HOLSTEGE, O. GILEADI *et al.*, 1999 An unusual eukaryotic protein phosphatase required for transcription by RNA polymerase II and CTD dephosphorylation in S. cerevisiae. Mol Cell **4:** 55-62.

KOMARNITSKY, P., E. J. CHO and S. BURATOWSKI, 2000 Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes Dev **14:** 2452-2460.

KORNBERG, R. D., 1974 Chromatin structure: a repeating unit of histones and DNA. Science **184:** 868-871.

KRISHNAMURTHY, S., X. HE, M. REYES-REYES, C. MOORE and M. HAMPSEY, 2004 Ssu72 Is an RNA polymerase II CTD phosphatase. Mol Cell **14:** 387-394.

KRISTJUHAN, A., and J. Q. SVEJSTRUP, 2004 Evidence for distinct mechanisms facilitating transcript elongation through chromatin in vivo. EMBO J **23:** 4243-4252.

KRISTJUHAN, A., J. WALKER, N. SUKA, M. GRUNSTEIN, D. ROBERTS *et al.*, 2002 Transcriptional inhibition of genes with severe histone h3 hypoacetylation in the coding region. Mol Cell **10:** 925-933.

KROGAN, N. J., J. DOVER, S. KHORRAMI, J. F. GREENBLATT, J. SCHNEIDER *et al.*, 2002a COMPASS, a histone H3 (Lysine 4) methyltransferase required for telomeric silencing of gene expression. J Biol Chem **277:** 10753-10755.

KROGAN, N. J., J. DOVER, A. WOOD, J. SCHNEIDER, J. HEIDT *et al.*, 2003a 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, N. J., M. KIM, S. H. AHN, G. ZHONG, M. S. KOBOR *et al.*, 2002b RNA polymerase II elongation factors of Saccharomyces cerevisiae: a targeted proteomics approach. Mol Cell Biol **22:** 6979-6992.

KROGAN, N. J., M. KIM, A. TONG, A. GOLSHANI, G. CAGNEY *et al.*, 2003b Methylation of histone H3 by Set2 in Saccharomyces cerevisiae is linked to transcriptional elongation by RNA polymerase II. Mol Cell Biol **23:** 4207-4218.

KUEHNER, J. N., E. L. PEARSON and C. MOORE, 2011 Unravelling the means to an end: RNA polymerase II transcription termination. Nat Rev Mol Cell Biol **12:** 283-294.

KUO, M. H., J. E. BROWNELL, R. E. SOBEL, T. A. RANALLI, R. G. COOK *et al.*, 1996 Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. Nature **383:** 269-272.

KUO, M. H., E. VOM BAUR, K. STRUHL and C. D. ALLIS, 2000 Gcn4 activator targets Gcn5 histone acetyltransferase to specific promoters independently of transcription. Mol Cell **6:** 1309-1320.

LACAVA, J., J. HOUSELEY, C. SAVEANU, E. PETFALSKI, E. THOMPSON *et al.*, 2005 RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. Cell **121:** 713-724.

LARIBEE, R. N., N. J. KROGAN, T. XIAO, Y. SHIBATA, T. R. HUGHES *et al.*, 2005 BUR kinase selectively regulates H3 K4 trimethylation and H2B ubiquitylation through recruitment of the PAF elongation complex. Curr Biol **15:** 1487-1493.

LAROSSA, R. A., and J. V. SCHLOSS, 1984 The sulfonylurea herbicide sulfometuron methyl is an extremely potent and selective inhibitor of acetolactate synthase in Salmonella typhimurium. J Biol Chem **259:** 8753-8757.

LASCARIS, R. F., E. GROOT, P. B. HOEN, W. H. MAGER and R. J. PLANTA, 2000 Different roles for abf1p and a T-rich promoter element in nucleosome organization of the yeast RPS28A gene. Nucleic Acids Res **28:** 1390-1396.

LEE, A., K. D. HANSEN, J. BULLARD, S. DUDOIT and G. SHERLOCK, 2008a Novel low abundance and transient RNAs in yeast revealed by tiling microarrays and ultra high-throughput sequencing are not conserved across closely related yeast species. PLoS Genet **4:** e1000299.

LEE, C., X. LI, A. HECHMER, M. EISEN, M. D. BIGGIN *et al.*, 2008b NELF and GAGA factor are linked to promoter-proximal pausing at many genes in Drosophila. Mol Cell Biol **28:** 3290-3300.

LEE, C. K., Y. SHIBATA, B. RAO, B. D. STRAHL and J. D. LIEB, 2004 Evidence for nucleosome depletion at active regulatory regions genome-wide. Nat Genet **36:** 900-905.

LEE, J. S., A. SHUKLA, J. SCHNEIDER, S. K. SWANSON, M. P. WASHBURN *et al.*, 2007 Histone crosstalk between H2B monoubiquitination and H3 methylation mediated by COMPASS. Cell **131:** 1084-1096.

LEE, J. T., 2000 Disruption of imprinted X inactivation by parent-of-origin effects at Tsix. Cell **103:** 17-27.

LEE, J. T., L. S. DAVIDOW and D. WARSHAWSKY, 1999 Tsix, a gene antisense to Xist at the X-inactivation centre. Nat Genet **21:** 400-404.

LEE, J. T., and N. LU, 1999 Targeted mutagenesis of Tsix leads to nonrandom X inactivation. Cell **99:** 47-57.

LEE, K. K., L. FLORENS, S. K. SWANSON, M. P. WASHBURN and J. L. WORKMAN, 2005 The deubiquitylation activity of Ubp8 is dependent upon Sgf11 and its association with the SAGA complex. Mol Cell Biol **25:** 1173-1182.

LI, B., M. GOGOL, M. CAREY, D. LEE, C. SEIDEL *et al.*, 2007 Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. Science **316:** 1050-1054.

LI, B., L. HOWE, S. ANDERSON, J. R. YATES, 3RD and J. L. WORKMAN, 2003 The Set2 histone methyltransferase functions through the phosphorylated carboxyl-terminal domain of RNA polymerase II. J Biol Chem **278:** 8897-8903.

LI, B., J. JACKSON, M. D. SIMON, B. FLEHARTY, M. GOGOL *et al.*, 2009 Histone H3 lysine 36 dimethylation (H3K36me2) is sufficient to recruit the Rpd3s histone deacetylase complex and to repress spurious transcription. J Biol Chem **284:** 7970-7976.

LI, B., S. G. PATTENDEN, D. LEE, J. GUTIERREZ, J. CHEN *et al.*, 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, S. S., 2000 Structure and function of the Groucho gene family and encoded transcriptional corepressor proteins from human, mouse, rat, Xenopus, Drosophila and nematode. Proc Natl Sci Counc Repub China B **24:** 47-55.

LICATALOSI, D. D., G. GEIGER, M. MINET, S. SCHROEDER, K. CILLI *et al.*, 2002 Functional interaction of yeast pre-mRNA 3' end processing factors with RNA polymerase II. Mol Cell **9:** 1101-1111.

LICHT, J. D., W. HANNA-ROSE, J. C. REDDY, M. A. ENGLISH, M. RO *et al.*, 1994 Mapping and mutagenesis of the amino-terminal transcriptional repression domain of the Drosophila Kruppel protein. Mol Cell Biol **14:** 4057-4066.

LIN, L., J. H. ZHANG, L. M. PANICKER and W. F. SIMONDS, 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, D. L., and G. A. HARTZOG, 2001 Genetic interactions of Spt4-Spt5 and TFIIS with the RNA polymerase II CTD and CTD modifying enzymes in Saccharomyces cerevisiae. Genetics **159:** 487-497.

LINDSTROM, D. L., S. L. SQUAZZO, N. MUSTER, T. A. BURCKIN, K. C. WACHTER *et al.*, 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, C. L., T. KAPLAN, M. KIM, S. BURATOWSKI, S. L. SCHREIBER *et al.*, 2005 Single-nucleosome mapping of histone modifications in S. cerevisiae. PLoS Biol **3:** e328.

LIU, H. Y., V. BADARINARAYANA, D. C. AUDINO, J. RAPPSILBER, M. MANN *et al.*, 1998 The NOT proteins are part of the CCR4 transcriptional complex and affect gene expression both positively and negatively. EMBO J **17:** 1096-1106.

LIU, Y., L. WARFIELD, C. ZHANG, J. LUO, J. ALLEN *et al.*, 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.

LORICK, K. L., J. P. JENSEN, S. FANG, A. M. ONG, S. HATAKEYAMA *et al.*, 1999 RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. Proc Natl Acad Sci U S A **96:** 11364-11369.

LUGER, K., A. W. MADER, R. K. RICHMOND, D. F. SARGENT and T. J. RICHMOND, 1997 Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature **389:** 251-260.

LUIKENHUIS, S., A. WUTZ and R. JAENISCH, 2001 Antisense transcription through the Xist locus mediates Tsix function in embryonic stem cells. Mol Cell Biol **21:** 8512-8520.

LUSE, D. S., L. C. SPANGLER and A. UJVARI, 2011 Efficient and rapid nucleosome traversal by RNA polymerase II depends on a combination of transcript elongation factors. J Biol Chem **286:** 6040-6048.

MA, J., and M. PTASHNE, 1987 The carboxy-terminal 30 amino acids of GAL4 are recognized by GAL80. Cell **50:** 137-142.

MARAHRENS, Y., B. PANNING, J. DAUSMAN, W. STRAUSS and R. JAENISCH, 1997 Xist-deficient mice are defective in dosage compensation but not spermatogenesis. Genes Dev **11:** 156-166.

MARTENS, J. A., L. LAPRADE and F. WINSTON, 2004 Intergenic transcription is required to repress the Saccharomyces cerevisiae SER3 gene. Nature **429:** 571-574.

MARTENS, J. A., P. Y. WU and F. WINSTON, 2005 Regulation of an intergenic transcript controls adjacent gene transcription in Saccharomyces cerevisiae. Genes Dev **19:** 2695-2704.

MARTIN, D. G., K. BAETZ, X. SHI, K. L. WALTER, V. E. MACDONALD *et al.*, 2006 The Yng1p plant homeodomain finger is a methyl-histone binding module that recognizes lysine 4-methylated histone H3. Mol Cell Biol **26:** 7871-7879.

MASON, P. B., and K. STRUHL, 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.

MASON, P. B., and K. STRUHL, 2005 Distinction and relationship between elongation rate and processivity of RNA polymerase II in vivo. Mol Cell **17:** 831-840.

MAVRICH, T. N., I. P. IOSHIKHES, B. J. VENTERS, C. JIANG, L. P. TOMSHO *et al.*, 2008 A barrier nucleosome model for statistical positioning of nucleosomes throughout the yeast genome. Genome Res **18:** 1073-1083.

MAYER, A., M. LIDSCHREIBER, M. SIEBERT, K. LEIKE, J. SODING *et al.*, 2010 Uniform transitions of the general RNA polymerase II transcription complex. Nat Struct Mol Biol **17:** 1272-1278.

MENEGHINI, M. D., M. WU and H. D. MADHANI, 2003 Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. Cell **112:** 725-736.

MESSENGUY, F., and E. DUBOIS, 2000 Regulation of Arginine Metabolism in *Saccharomyces cerevisiae*: a Network of Specific and Pleiotropic Proteins in Response to Multiple Environmental Signals. Food technol biotechnol **38:** 277-285.

MINSKY, N., E. SHEMA, Y. FIELD, M. SCHUSTER, E. SEGAL *et al.*, 2008 Monoubiquitinated H2B is associated with the transcribed region of highly expressed genes in human cells. Nat Cell Biol **10:** 483-488.

MONIAUX, N., C. NEMOS, B. M. SCHMIED, S. C. CHAUHAN, S. DEB *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.

MOSIMANN, C., G. HAUSMANN and K. BASLER, 2006 Parafibromin/Hyrax activates Wnt/Wg target gene transcription by direct association with beta-catenin/Armadillo. Cell **125:** 327-341.

MOTE, J., JR., P. GHANOUNI and D. REINES, 1994 A DNA minor groove-binding ligand both potentiates and arrests transcription by RNA polymerase II. Elongation factor SII enables readthrough at arrest sites. J Mol Biol **236:** 725-737.

MUELLER, C. L., and J. A. JAEHNING, 2002 Ctr9, Rtf1, and Leo1 are components of the Paf1/RNA polymerase II complex. Mol Cell Biol **22:** 1971-1980.

MUELLER, C. L., S. E. PORTER, M. G. HOFFMAN and J. A. JAEHNING, 2004 The Paf1 complex has functions independent of actively transcribing RNA polymerase II. Mol Cell **14:** 447-456.

MUELLER, P. P., and A. G. HINNEBUSCH, 1986 Multiple upstream AUG codons mediate translational control of GCN4. Cell **45:** 201-207.

MULLER, W. E., R. K. ZAHN and H. J. SEIDEL, 1971 Inhibitors acting on nucleic acid synthesis in an oncogenic RNA virus. Nat New Biol **232:** 143-145.

MUNTEAN, A. G., J. TAN, K. SITWALA, Y. HUANG, J. BRONSTEIN *et al.*, 2010 The PAF complex synergizes with MLL fusion proteins at HOX loci to promote leukemogenesis. Cancer Cell **17:** 609-621.

MURATANI, M., C. KUNG, K. M. SHOKAT and W. P. TANSEY, 2005 The F box protein Dsg1/Mdm30 is a transcriptional coactivator that stimulates Gal4 turnover and cotranscriptional mRNA processing. Cell **120:** 887-899.

MURRAY, S., R. UDUPA, S. YAO, G. HARTZOG and G. PRELICH, 2001 Phosphorylation of the RNA polymerase II carboxy-terminal domain by the Bur1 cyclin-dependent kinase. Mol Cell Biol **21:** 4089-4096.

MUSE, G. W., D. A. GILCHRIST, S. NECHAEV, R. SHAH, J. S. PARKER *et al.*, 2007 RNA polymerase is poised for activation across the genome. Nat Genet **39:** 1507-1511.

MUTIU, A. I., S. M. HOKE, J. GENEREAUX, G. LIANG and C. J. BRANDL, 2007 The role of histone ubiquitylation and deubiquitylation in gene expression as determined by the analysis of an HTB1(K123R) Saccharomyces cerevisiae strain. Mol Genet Genomics **277:** 491-506.

NAGAIKE, T., C. LOGAN, I. HOTTA, O. ROZENBLATT-ROSEN, M. MEYERSON *et al.*, 2011 Transcriptional activators enhance polyadenylation of mRNA precursors. Mol Cell **41:** 409-418.

NAGALAKSHMI, U., Z. WANG, K. WAERN, C. SHOU, D. RAHA *et al.*, 2008 The transcriptional landscape of the yeast genome defined by RNA sequencing. Science **320:** 1344-1349.

NARITA, T., Y. YAMAGUCHI, K. YANO, S. SUGIMOTO, S. CHANARAT *et al.*, 2003 Human transcription elongation factor NELF: identification of novel subunits and reconstitution of the functionally active complex. Mol Cell Biol **23:** 1863-1873.

NASMYTH, K. A., K. TATCHELL, B. D. HALL, C. ASTELL and M. SMITH, 1981 A position effect in the control of transcription at yeast mating type loci. Nature **289:** 244-250.

NEHLIN, J. O., M. CARLBERG and H. RONNE, 1991 Control of yeast GAL genes by MIG1 repressor: a transcriptional cascade in the glucose response. EMBO J **10:** 3373-3377.

NEIL, H., C. MALABAT, Y. D'AUBENTON-CARAFA, Z. XU, L. M. STEINMETZ *et al.*, 2009 Widespread bidirectional promoters are the major source of cryptic transcripts in yeast. Nature **457:** 1038-1042.

NEWEY, P. J., M. R. BOWL and R. V. THAKKER, 2009 Parafibromin--functional insights. J Intern Med **266:** 84-98.

NG, H. H., S. DOLE and K. STRUHL, 2003a The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. J Biol Chem **278:** 33625-33628.

NG, H. H., Q. FENG, H. WANG, H. ERDJUMENT-BROMAGE, P. TEMPST *et al.*, 2002a Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. Genes Dev **16:** 1518-1527.

NG, H. H., F. ROBERT, R. A. YOUNG and K. STRUHL, 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, H. H., R. M. XU, Y. ZHANG and K. STRUHL, 2002b Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. J Biol Chem **277:** 34655-34657.

NI, Z., B. E. SCHWARTZ, J. WERNER, J. R. SUAREZ and J. T. LIS, 2004 Coordination of transcription, RNA processing, and surveillance by P-TEFb kinase on heat shock genes. Mol Cell **13:** 55-65.

NICOL, J. W., G. A. HELT, S. G. BLANCHARD, JR., A. RAJA and A. E. LORAINE, 2009 The Integrated Genome Browser: free software for distribution and exploration of genome-scale datasets. Bioinformatics **25:** 2730-2731.

NISLOW, C., E. RAY and L. PILLUS, 1997 SET1, a yeast member of the trithorax family, functions in transcriptional silencing and diverse cellular processes. Mol Biol Cell **8:** 2421-2436.

NONET, M., C. SCAFE, J. SEXTON and R. YOUNG, 1987 Eucaryotic RNA polymerase conditional mutant that rapidly ceases mRNA synthesis. Mol Cell Biol **7:** 1602-1611.

NORDICK, K., M. G. HOFFMAN, J. L. BETZ and J. A. JAEHNING, 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.

O'NEILL, E. M., A. KAFFMAN, E. R. JOLLY and E. K. O'SHEA, 1996 Regulation of PHO4 nuclear localization by the PHO80-PHO85 cyclin-CDK complex. Science **271:** 209-212.

ODOM, A. R., A. STAHLBERG, S. R. WENTE and J. D. YORK, 2000 A role for nuclear inositol 1,4,5-trisphosphate kinase in transcriptional control. Science **287:** 2026-2029.

OGAWA, Y., and J. T. LEE, 2003 Xite, X-inactivation intergenic transcription elements that regulate the probability of choice. Mol Cell **11:** 731-743.

ORPHANIDES, G., G. LEROY, C. H. CHANG, D. S. LUSE and D. REINBERG, 1998 FACT, a factor that facilitates transcript elongation through nucleosomes. Cell **92:** 105-116.

ORPHANIDES, G., and D. REINBERG, 2000 RNA polymerase II elongation through chromatin. Nature **407:** 471-475.

ORPHANIDES, G., W. H. WU, W. S. LANE, M. HAMPSEY and D. REINBERG, 1999 The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature **400:** 284-288.

PALAPARTI, A., A. BARATZ and S. STIFANI, 1997 The Groucho/transducin-like enhancer of split transcriptional repressors interact with the genetically defined amino-terminal silencing domain of histone H3. J Biol Chem **272:** 26604-26610.

PAPAMICHOS-CHRONAKIS, M., T. PETRAKIS, E. KTISTAKI, I. TOPALIDOU and D. TZAMARIAS, 2002 Cti6, a PHD domain protein, bridges the Cyc8-Tup1 corepressor and the SAGA coactivator to overcome repression at GAL1. Mol Cell **9:** 1297-1305.

PATTURAJAN, M., N. K. CONRAD, D. B. BREGMAN and J. L. CORDEN, 1999 Yeast carboxyl-terminal domain kinase I positively and negatively regulates RNA polymerase II carboxyl-terminal domain phosphorylation. J Biol Chem **274:** 27823-27828.

PAVRI, R., B. ZHU, G. LI, P. TROJER, S. MANDAL *et al.*, 2006 Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. Cell **125:** 703-717.

PAYANKAULAM, S., L. M. LI and D. N. ARNOSTI, 2010 Transcriptional repression: conserved and evolved features. Curr Biol **20:** R764-771.

PENHEITER, K. L., T. M. WASHBURN, S. E. PORTER, M. G. HOFFMAN and J. A. JAEHNING, 2005 A posttranscriptional role for the yeast Paf1-RNA polymerase II complex is revealed by identification of primary targets. Mol Cell **20:** 213-223.

PENNY, G. D., G. F. KAY, S. A. SHEARDOWN, S. RASTAN and N. BROCKDORFF, 1996 Requirement for Xist in X chromosome inactivation. Nature **379:** 131-137.

PEROCCHI, F., Z. XU, S. CLAUDER-MUNSTER and L. M. STEINMETZ, 2007 Antisense artifacts in transcriptome microarray experiments are resolved by actinomycin D. Nucleic Acids Res **35:** e128.

PETERLIN, B. M., and D. H. PRICE, 2006 Controlling the elongation phase of transcription with P-TEFb. Mol Cell **23:** 297-305.

PICKLES, L. M., S. M. ROE, E. J. HEMINGWAY, S. STIFANI and L. H. PEARL, 2002 Crystal structure of the C-terminal WD40 repeat domain of the human Groucho/TLE1 transcriptional corepressor. Structure **10:** 751-761.

POKHOLOK, D. K., N. M. HANNETT and R. A. YOUNG, 2002 Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. Mol Cell **9:** 799-809.

POKHOLOK, D. K., C. T. HARBISON, S. LEVINE, M. COLE, N. M. HANNETT *et al.*, 2005 Genome-wide map of nucleosome acetylation and methylation in yeast. Cell **122:** 517-527.

PORTER, S. E., T. M. WASHBURN, M. CHANG and J. A. JAEHNING, 2002 The yeast pafl-rNA polymerase II complex is required for full expression of a subset of cell cycle-regulated genes. Eukaryot Cell **1:** 830-842.

PRAY-GRANT, M. G., J. A. DANIEL, D. SCHIELTZ, J. R. YATES, 3RD and P. A. GRANT, 2005 Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. Nature **433:** 434-438.

PREKER, P., J. NIELSEN, S. KAMMLER, S. LYKKE-ANDERSEN, M. S. CHRISTENSEN *et al.*, 2008 RNA exosome depletion reveals transcription upstream of active human promoters. Science **322:** 1851-1854.

PRELICH, G., 1997 Saccharomyces cerevisiae BUR6 encodes a DRAP1/NC2alpha homolog that has both positive and negative roles in transcription in vivo. Mol Cell Biol **17:** 2057-2065.

PRELICH, G., and F. WINSTON, 1993 Mutations that suppress the deletion of an upstream activating sequence in yeast: involvement of a protein kinase and histone H3 in repressing transcription in vivo. Genetics **135:** 665-676.

PRICE, D. H., 2000 P-TEFb, a cyclin-dependent kinase controlling elongation by RNA polymerase II. Mol Cell Biol **20:** 2629-2634.

PRICE, K. L., K. S. MILLEN and S. C. LUMMIS, 2007 Transducing agonist binding to channel gating involves different interactions in 5-HT3 and GABAC receptors. J Biol Chem **282:** 25623-25630.

PROFT, M., and K. STRUHL, 2002 Hog1 kinase converts the Sko1-Cyc8-Tup1 repressor complex into an activator that recruits SAGA and SWI/SNF in response to osmotic stress. Mol Cell **9:** 1307-1317.

QIU, H., C. HU, C. M. WONG and A. G. HINNEBUSCH, 2006 The Spt4p subunit of yeast DSIF stimulates association of the Paf1 complex with elongating RNA polymerase II. Mol Cell Biol **26:** 3135-3148.

QIU, H., C. HU, S. YOON, K. NATARAJAN, M. J. SWANSON *et al.*, 2004 An array of coactivators is required for optimal recruitment of TATA binding protein and RNA polymerase II by promoter-bound Gcn4p. Mol Cell Biol **24:** 4104-4117.

QIU, H. F., E. DUBOIS, P. BROEN and F. MESSENGUY, 1990 Functional analysis of ARGRI and ARGRIII regulatory proteins involved in the regulation of arginine metabolism in Saccharomyces cerevisiae. Mol Gen Genet **222:** 192-200.

QUAN, T. K., and G. A. HARTZOG, 2009 Histone H3K4 and K36 methylation, Chd1 and Rpd3S oppose the functions of Saccharomyces cerevisiae Spt4-Spt5 in transcription. Genetics **184:** 321-334.

RADONJIC, M., J. C. ANDRAU, P. LIJNZAAD, P. KEMMEREN, T. T. KOCKELKORN *et al.*, 2005 Genome-wide analyses reveal RNA polymerase II located upstream of genes poised for rapid response upon S. cerevisiae stationary phase exit. Mol Cell **18:** 171-183.

RAISNER, R. M., P. D. HARTLEY, M. D. MENEGHINI, M. Z. BAO, C. L. LIU *et al.*, 2005 Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. Cell **123:** 233-248.

RAO, J. N., K. SCHWEIMER, S. WENZEL, B. M. WOHRL and P. ROSCH, 2008 NELF-E RRM undergoes major structural changes in flexible protein regions on target RNA binding. Biochemistry **47:** 3756-3761.

RASMUSSEN, E. B., and J. T. LIS, 1993 In vivo transcriptional pausing and cap formation on three Drosophila heat shock genes. Proc Natl Acad Sci U S A **90:** 7923-7927.

RASMUSSEN, E. B., and J. T. LIS, 1995 Short transcripts of the ternary complex provide insight into RNA polymerase II elongational pausing. J Mol Biol **252:** 522-535.

REINBERG, D., and R. J. SIMS, 3RD, 2006 de FACTo nucleosome dynamics. J Biol Chem **281:** 23297-23301.

REINES, D., P. GHANOUNI, Q. Q. LI and J. MOTE, JR., 1992 The RNA polymerase II elongation complex. Factor-dependent transcription elongation involves nascent RNA cleavage. J Biol Chem **267:** 15516-15522.

REINES, D., and J. MOTE, JR., 1993 Elongation factor SII-dependent transcription by RNA polymerase II through a sequence-specific DNA-binding protein. Proc Natl Acad Sci U S A **90:** 1917-1921.

RENNER, D. B., Y. YAMAGUCHI, T. WADA, H. HANDA and D. H. PRICE, 2001 A highly purified RNA polymerase II elongation control system. J Biol Chem **276:** 42601-42609.

RHOADES, A. R., S. RUONE and T. FORMOSA, 2004 Structural features of nucleosomes reorganized by yeast FACT and its HMG box component, Nhp6. Mol Cell Biol **24:** 3907-3917.

RICCI, A. R., J. GENEREAUX and C. J. BRANDL, 2002 Components of the SAGA histone acetyltransferase complex are required for repressed transcription of ARG1 in rich medium. Mol Cell Biol **22:** 4033-4042.

RICHARD, P., and J. L. MANLEY, 2009 Transcription termination by nuclear RNA polymerases. Genes Dev **23:** 1247-1269.

ROBZYK, K., J. RECHT and M. A. OSLEY, 2000 Rad6-dependent ubiquitination of histone H2B in yeast. Science **287:** 501-504.

RONDON, A. G., S. JIMENO and A. AGUILERA, 2010 The interface between transcription and mRNP export: from THO to THSC/TREX-2. Biochim Biophys Acta **1799:** 533-538.

RONDON, A. G., S. JIMENO, M. GARCIA-RUBIO and A. AGUILERA, 2003 Molecular evidence that the eukaryotic THO/TREX complex is required for efficient transcription elongation. J Biol Chem **278:** 39037-39043.

ROSE, M., and D. BOTSTEIN, 1983 Construction and use of gene fusions to lacZ (beta-galactosidase) that are expressed in yeast. Methods Enzymol **101:** 167-180.

ROSE, M. D., F. WINSTON, AND P. HIETER, 1990 Methods in Yeast Genetics: a Laboratory Course Manual. Cold Spring Harbor Laboratory Press.

ROSONINA, E., S. KANEKO and J. L. MANLEY, 2006 Terminating the transcript: breaking up is hard to do. Genes Dev **20:** 1050-1056.

ROSSMANN, M. P., W. LUO, O. TSAPONINA, A. CHABES and B. STILLMAN, 2011 A common telomeric gene silencing assay is affected by nucleotide metabolism. Mol Cell **42:** 127-136.

ROTHSTEIN, R., 1991 Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. Methods Enzymol **194:** 281-301.

ROUGVIE, A. E., and J. T. LIS, 1988 The RNA polymerase II molecule at the 5' end of the uninduced hsp70 gene of D. melanogaster is transcriptionally engaged. Cell **54:** 795-804.

ROZENBLATT-ROSEN, O., C. M. HUGHES, S. J. NANNEPAGA, K. S. SHANMUGAM, T. D. COPELAND *et al.*, 2005 The parafibromin tumor suppressor protein is part of a human Paf1 complex. Mol Cell Biol **25:** 612-620.

ROZENBLATT-ROSEN, O., T. NAGAIKE, J. M. FRANCIS, S. KANEKO, K. A. GLATT *et al.*, 2009 The tumor suppressor Cdc73 functionally associates with CPSF and CstF 3' mRNA processing factors. Proc Natl Acad Sci U S A **106:** 755-760.

RUPRECHT, R. M., N. C. GOODMAN and S. SPIEGELMAN, 1973 Conditions for the selective synthesis of DNA complementary to template RNA. Biochim Biophys Acta **294:** 192-203.

RUSCHE, L. N., A. L. KIRCHMAIER and J. RINE, 2003 The establishment, inheritance, and function of silenced chromatin in Saccharomyces cerevisiae. Annu Rev Biochem **72:** 481-516.

SADO, T., Z. WANG, H. SASAKI and E. LI, 2001 Regulation of imprinted X-chromosome inactivation in mice by Tsix. Development **128:** 1275-1286.

SAIARDI, A., J. J. CAFFREY, S. H. SNYDER and S. B. SHEARS, 2000 Inositol polyphosphate multikinase (ArgRIII) determines nuclear mRNA export in Saccharomyces cerevisiae. FEBS Lett **468:** 28-32.

SAIARDI, A., H. ERDJUMENT-BROMAGE, A. M. SNOWMAN, P. TEMPST and S. H. SNYDER, 1999 Synthesis of diphosphoinositol pentakisphosphate by a newly identified family of higher inositol polyphosphate kinases. Curr Biol **9:** 1323-1326.

SALMERON, J. M., JR., K. K. LEUTHER and S. A. JOHNSTON, 1990 GAL4 mutations that separate the transcriptional activation and GAL80-interactive functions of the yeast GAL4 protein. Genetics **125:** 21-27.

SANTOS-ROSA, H., R. SCHNEIDER, A. J. BANNISTER, J. SHERRIFF, B. E. BERNSTEIN *et al.*, 2002 Active genes are tri-methylated at K4 of histone H3. Nature **419:** 407-411.

SARCINELLA, E., P. C. ZUZARTE, P. N. LAU, R. DRAKER and P. CHEUNG, 2007 Monoubiquitylation of H2A.Z distinguishes its association with euchromatin or facultative heterochromatin. Mol Cell Biol **27:** 6457-6468.

SAUER, F., J. D. FONDELL, Y. OHKUMA, R. G. ROEDER and H. JACKLE, 1995 Control of transcription by Kruppel through interactions with TFIIB and TFIIE beta. Nature **375:** 162-164.

SCHAFT, D., A. ROGUEV, K. M. KOTOVIC, A. SHEVCHENKO, M. SAROV *et al.*, 2003 The histone 3 lysine 36 methyltransferase, SET2, is involved in transcriptional elongation. Nucleic Acids Res **31:** 2475-2482.

SCHMITT, M. E., T. A. BROWN and B. L. TRUMPOWER, 1990 A rapid and simple method for preparation of RNA from Saccharomyces cerevisiae. Nucleic Acids Res **18:** 3091-3092.

SCHULTZ, J., L. MARSHALL-CARLSON and M. CARLSON, 1990 The N-terminal TPR region is the functional domain of SSN6, a nuclear phosphoprotein of Saccharomyces cerevisiae. Mol Cell Biol **10:** 4744-4756.

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

SHAHBAZIAN, M. D., and M. GRUNSTEIN, 2007 Functions of site-specific histone acetylation and deacetylation. Annu Rev Biochem **76:** 75-100.

SHELDON, K. E., D. M. MAUGER and K. M. ARNDT, 2005 A Requirement for the Saccharomyces cerevisiae Paf1 complex in snoRNA 3' end formation. Mol Cell **20:** 225-236.

SHEMA, E., I. TIROSH, Y. AYLON, J. HUANG, C. YE *et al.*, 2008 The histone H2B-specific ubiquitin ligase RNF20/hBRE1 acts as a putative tumor suppressor through selective regulation of gene expression. Genes Dev **22:** 2664-2676.

SHI, X., M. CHANG, A. J. WOLF, C. H. CHANG, A. A. FRAZER-ABEL *et al.*, 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.

SHI, X., A. FINKELSTEIN, A. J. WOLF, P. A. WADE, Z. F. BURTON *et al.*, 1996 Paf1p, an RNA polymerase II-associated factor in Saccharomyces cerevisiae, may have both positive and negative roles in transcription. Mol Cell Biol **16:** 669-676.

SHIRRA, M. K., R. R. MCCARTNEY, C. ZHANG, K. M. SHOKAT, M. C. SCHMIDT *et al.*, 2008 A chemical genomics study identifies Snf1 as a repressor of GCN4 translation. J Biol Chem **283:** 35889-35898.

SHIRRA, M. K., S. E. ROGERS, D. E. ALEXANDER and K. M. ARNDT, 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, T. W., and S. BURATOWSKI, 2009 The basal initiation machinery: beyond the general transcription factors. Curr Opin Cell Biol **21:** 344-351.

SIMIC, R., D. L. LINDSTROM, H. G. TRAN, K. L. ROINICK, P. J. COSTA *et al.*, 2003 Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. EMBO J **22:** 1846-1856.

SIMS, R. J., 3RD, C. F. CHEN, H. SANTOS-ROSA, T. KOUZARIDES, S. S. PATEL *et al.*, 2005 Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. J Biol Chem **280:** 41789-41792.

SMITH, E., C. LIN and A. SHILATIFARD, 2011 The super elongation complex (SEC) and MLL in development and disease. Genes Dev **25:** 661-672.

SMITH, J. S., and J. D. BOEKE, 1997 An unusual form of transcriptional silencing in yeast ribosomal DNA. Genes Dev **11:** 241-254.

SMITH, R. L., and A. D. JOHNSON, 2000 Turning genes off by Ssn6-Tup1: a conserved system of transcriptional repression in eukaryotes. Trends Biochem Sci **25:** 325-330.

SPRAGUE, E. R., M. J. REDD, A. D. JOHNSON and C. WOLBERGER, 2000 Structure of the C-terminal domain of Tup1, a corepressor of transcription in yeast. EMBO J **19:** 3016-3027.

SPROUSE, R. O., T. S. KARPOVA, F. MUELLER, A. DASGUPTA, J. G. MCNALLY *et al.*, 2008a Regulation of TATA-binding protein dynamics in living yeast cells. Proc Natl Acad Sci U S A **105:** 13304-13308.

SPROUSE, R. O., I. SHCHERBAKOVA, H. CHENG, E. JAMISON, M. BRENOWITZ *et al.*, 2008b Function and structural organization of Mot1 bound to a natural target promoter. J Biol Chem **283:** 24935-24948.

SQUAZZO, S. L., P. J. COSTA, D. L. LINDSTROM, K. E. KUMER, R. SIMIC *et al.*, 2002 The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. EMBO J **21:** 1764-1774.

STAVROPOULOS, N., R. K. ROWNTREE and J. T. LEE, 2005 Identification of developmentally specific enhancers for Tsix in the regulation of X chromosome inactivation. Mol Cell Biol **25:** 2757-2769.

STEINMETZ, E. J., C. L. WARREN, J. N. KUEHNER, B. PANBEHI, A. Z. ANSARI *et al.*, 2006 Genome-wide distribution of yeast RNA polymerase II and its control by Sen1 helicase. Mol Cell **24:** 735-746.

STOCKDALE, C., A. FLAUS, H. FERREIRA and T. OWEN-HUGHES, 2006 Analysis of nucleosome repositioning by yeast ISWI and Chd1 chromatin remodeling complexes. J Biol Chem **281:** 16279-16288.

STOKES, D. G., K. D. TARTOF and R. P. PERRY, 1996 CHD1 is concentrated in interbands and puffed regions of Drosophila polytene chromosomes. Proc Natl Acad Sci U S A **93:** 7137-7142.

STOLINSKI, L. A., D. M. EISENMANN and K. M. ARNDT, 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, B. D., P. A. GRANT, S. D. BRIGGS, Z. W. SUN, J. R. BONE *et al.*, 2002 Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. Mol Cell Biol **22:** 1298-1306.

SUN, Z. W., and C. D. ALLIS, 2002 Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. Nature **418:** 104-108.

SWANSON, M. J., H. QIU, L. SUMIBCAY, A. KRUEGER, S. J. KIM *et al.*, 2003 A multiplicity of coactivators is required by Gcn4p at individual promoters in vivo. Mol Cell Biol **23:** 2800-2820.

SWANSON, M. S., E. A. MALONE and F. WINSTON, 1991 SPT5, an essential gene important for normal transcription in Saccharomyces cerevisiae, encodes an acidic nuclear protein with a carboxy-terminal repeat. Mol Cell Biol **11:** 3009-3019.

TAKAHASHI, Y. H., J. M. SCHULZE, J. JACKSON, T. HENTRICH, C. SEIDEL *et al.*, 2011 Dot1 and Histone H3K79 Methylation in Natural Telomeric and HM Silencing. Mol Cell **42:** 118-126.

TAVERNA, S. D., S. ILIN, R. S. ROGERS, J. C. TANNY, H. LAVENDER *et al.*, 2006 Yng1 PHD finger binding to H3 trimethylated at K4 promotes NuA3 HAT activity at K14 of H3 and transcription at a subset of targeted ORFs. Mol Cell **24:** 785-796.

TENNEY, K., M. GERBER, A. ILVARSONN, J. SCHNEIDER, M. GAUSE *et al.*, 2006 Drosophila Rtf1 functions in histone methylation, gene expression, and Notch signaling. Proc Natl Acad Sci U S A **103:** 11970-11974.

THIEBAUT, M., E. KISSELEVA-ROMANOVA, M. ROUGEMAILLE, J. BOULAY and D. LIBRI, 2006 Transcription termination and nuclear degradation of cryptic unstable transcripts: a role for the nrd1-nab3 pathway in genome surveillance. Mol Cell **23:** 853-864.

THOMPSON, D. M., and R. PARKER, 2007 Cytoplasmic decay of intergenic transcripts in Saccharomyces cerevisiae. Mol Cell Biol **27:** 92-101.

TOMSON, B. N., C. P. DAVIS, M. H. WARNER and K. M. ARNDT, 2011a Identification of a Role for Histone H2B Ubiquitylation in non-coding RNA 3'-end Formation Through Mutational Analysis of Rtf1 in Saccharomyces cerevisiae. Genetics **188:** 273-289.

TOMSON, B. N., C. P. DAVIS, M. H. WARNER and K. M. ARNDT, 2011b Identification of a Role for Histone H2B Ubiquitylation in non-coding RNA 3'-end Formation Through Mutational Analysis of Rtf1 in Saccharomyces cerevisiae. Genetics.

TOUS, C., and A. AGUILERA, 2007 Impairment of transcription elongation by R-loops in vitro. Biochem Biophys Res Commun **360:** 428-432.

TRAN, H. G., D. J. STEGER, V. R. IYER and A. D. JOHNSON, 2000 The chromo domain protein chd1p from budding yeast is an ATP-dependent chromatin-modifying factor. EMBO J **19:** 2323-2331.

TRIPIC, T., D. G. EDMONDSON, J. K. DAVIE, B. D. STRAHL and S. Y. DENT, 2006 The Set2 methyltransferase associates with Ssn6 yet Tup1-Ssn6 repression is independent of histone methylation. Biochem Biophys Res Commun **339:** 905-914.

TURNER, S. D., A. R. RICCI, H. PETROPOULOS, J. GENEREAUX, I. S. SKERJANC *et al.*, 2002 The E2 ubiquitin conjugase Rad6 is required for the ArgR/Mcm1 repression of ARG1 transcription. Mol Cell Biol **22:** 4011-4019.

TZAMARIAS, D., and K. STRUHL, 1995 Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 corepressor complex to differentially regulated promoters. Genes Dev **9:** 821-831.

UHLER, J. P., C. HERTEL and J. Q. SVEJSTRUP, 2007 A role for noncoding transcription in activation of the yeast PHO5 gene. Proc Natl Acad Sci U S A **104:** 8011-8016.

UJVARI, A., F. K. HSIEH, S. W. LUSE, V. M. STUDITSKY and D. S. LUSE, 2008 Histone N-terminal tails interfere with nucleosome traversal by RNA polymerase II. J Biol Chem **283:** 32236-32243.

VAN LEEUWEN, F., P. R. GAFKEN and D. E. GOTTSCHLING, 2002 Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell **109:** 745-756.

VAN WERVEN, F. J., H. VAN BAKEL, H. A. VAN TEEFFELEN, A. F. ALTELAAR, M. G. KOERKAMP *et al.*, 2008 Cooperative action of NC2 and Mot1p to regulate TATA-binding protein function across the genome. Genes Dev **22:** 2359-2369.

VAN WERVEN, F. J., H. A. VAN TEEFFELEN, F. C. HOLSTEGE and H. T. TIMMERS, 2009 Distinct promoter dynamics of the basal transcription factor TBP across the yeast genome. Nat Struct Mol Biol **16:** 1043-1048.

VANACOVA, S., J. WOLF, G. MARTIN, D. BLANK, S. DETTWILER *et al.*, 2005 A new yeast poly(A) polymerase complex involved in RNA quality control. PLoS Biol **3:** e189.

VANDEMARK, A. P., M. M. KASTEN, E. FERRIS, A. HEROUX, C. P. HILL *et al.*, 2007 Autoregulation of the rsc4 tandem bromodomain by gcn5 acetylation. Mol Cell **27:** 817-828.

VENTERS, B. J., J. D. IRVIN, P. GRAMLICH and B. F. PUGH, 2011 Genome-wide transcriptional dependence on conserved regions of mot1. Mol Cell Biol **31:** 2253-2261.

VOGELAUER, M., J. WU, N. SUKA and M. GRUNSTEIN, 2000 Global histone acetylation and deacetylation in yeast. Nature **408:** 495-498.

WADA, T., T. TAKAGI, Y. YAMAGUCHI, A. FERDOUS, T. IMAI *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.

WADE, P. A., W. WEREL, R. C. FENTZKE, N. E. THOMPSON, J. F. LEYKAM *et al.*, 1996 A novel collection of accessory factors associated with yeast RNA polymerase II. Protein Expr Purif **8:** 85-90.

WAGNER, E., and J. LYKKE-ANDERSEN, 2002 mRNA surveillance: the perfect persist. J Cell Sci **115:** 3033-3038.

WANG, A., S. K. KURDISTANI and M. GRUNSTEIN, 2002 Requirement of Hos2 histone deacetylase for gene activity in yeast. Science **298:** 1412-1414.

WANG, X., C. LEE, D. S. GILMOUR and J. P. GERGEN, 2007 Transcription elongation controls cell fate specification in the Drosophila embryo. Genes Dev **21:** 1031-1036.

WARNER, M. H., K. L. ROINICK and K. M. ARNDT, 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.

WATSON, A. D., D. G. EDMONDSON, J. R. BONE, Y. MUKAI, Y. YU *et al.*, 2000 Ssn6-Tup1 interacts with class I histone deacetylases required for repression. Genes Dev **14:** 2737-2744.

WILSON, M. A., S. MEAUX and A. VAN HOOF, 2007 A genomic screen in yeast reveals novel aspects of nonstop mRNA metabolism. Genetics **177:** 773-784.

WIND, M., and D. REINES, 2000 Transcription elongation factor SII. Bioessays **22:** 327-336.

WINSTON, F., D. T. CHALEFF, B. VALENT and G. R. FINK, 1984 Mutations affecting Ty-mediated expression of the HIS4 gene of Saccharomyces cerevisiae. Genetics **107:** 179-197.

WINSTON, F., C. DOLLARD and S. L. RICUPERO-HOVASSE, 1995 Construction of a set of convenient Saccharomyces cerevisiae strains that are isogenic to S288C. Yeast **11:** 53-55.

WINZELER, E. A., D. D. SHOEMAKER, A. ASTROMOFF, H. LIANG, K. ANDERSON *et al.*, 1999 Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science **285:** 901-906.

WOOD, A., N. J. KROGAN, J. DOVER, J. SCHNEIDER, J. HEIDT *et al.*, 2003a Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. Mol Cell **11:** 267-274.

WOOD, A., J. SCHNEIDER, J. DOVER, M. JOHNSTON and A. SHILATIFARD, 2003b 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.

WU, C. H., C. LEE, R. FAN, M. J. SMITH, Y. YAMAGUCHI *et al.*, 2005 Molecular characterization of Drosophila NELF. Nucleic Acids Res **33:** 1269-1279.

WU, C. H., Y. YAMAGUCHI, L. R. BENJAMIN, M. HORVAT-GORDON, J. WASHINSKY *et al.*, 2003 NELF and DSIF cause promoter proximal pausing on the hsp70 promoter in Drosophila. Genes Dev **17:** 1402-1414.

WYERS, F., M. ROUGEMAILLE, G. BADIS, J. C. ROUSSELLE, M. E. DUFOUR *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.

XIAO, T., H. HALL, K. O. KIZER, Y. SHIBATA, M. C. HALL *et al.*, 2003 Phosphorylation of RNA polymerase II CTD regulates H3 methylation in yeast. Genes Dev **17:** 654-663.

XIAO, T., C. F. KAO, N. J. KROGAN, Z. W. SUN, J. F. GREENBLATT *et al.*, 2005 Histone H2B ubiquitylation is associated with elongating RNA polymerase II. Mol Cell Biol **25:** 637-651.

XIN, H., S. TAKAHATA, M. BLANKSMA, L. MCCULLOUGH, D. J. STILLMAN *et al.*, 2009 yFACT induces global accessibility of nucleosomal DNA without H2A-H2B displacement. Mol Cell **35:** 365-376.

XU, Z., W. WEI, J. GAGNEUR, S. CLAUDER-MUNSTER, M. SMOLIK *et al.*, 2011 Antisense expression increases gene expression variability and locus interdependency. Mol Syst Biol **7:** 468.

XU, Z., W. WEI, J. GAGNEUR, F. PEROCCHI, S. CLAUDER-MUNSTER *et al.*, 2009 Bidirectional promoters generate pervasive transcription in yeast. Nature **457:** 1033-1037.

YAMAGUCHI, Y., J. FILIPOVSKA, K. YANO, A. FURUYA, N. INUKAI *et al.*, 2001 Stimulation of RNA polymerase II elongation by hepatitis delta antigen. Science **293:** 124-127.

YAMAGUCHI, Y., N. INUKAI, T. NARITA, T. WADA and H. HANDA, 2002 Evidence that negative elongation factor represses transcription elongation through binding to a DRB sensitivity-inducing factor/RNA polymerase II complex and RNA. Mol Cell Biol **22:** 2918-2927.

YAMAGUCHI, Y., T. MURA, S. CHANARAT, S. OKAMOTO and H. HANDA, 2007 Hepatitis delta antigen binds to the clamp of RNA polymerase II and affects transcriptional fidelity. Genes Cells **12:** 863-875.

YARRAGUDI, A., T. MIYAKE, R. LI and R. H. MORSE, 2004 Comparison of ABF1 and RAP1 in chromatin opening and transactivator potentiation in the budding yeast Saccharomyces cerevisiae. Mol Cell Biol **24:** 9152-9164.

YART, A., M. GSTAIGER, C. WIRBELAUER, M. PECNIK, D. ANASTASIOU *et al.*, 2005 The HRPT2 tumor suppressor gene product parafibromin associates with human PAF1 and RNA polymerase II. Mol Cell Biol **25:** 5052-5060.

YEUNG, K., S. KIM and D. REINBERG, 1997 Functional dissection of a human Dr1-DRAP1 repressor complex. Mol Cell Biol **17:** 36-45.

YOON, H. G., D. W. CHAN, Z. Q. HUANG, J. LI, J. D. FONDELL *et al.*, 2003a Purification and functional characterization of the human N-CoR complex: the roles of HDAC3, TBL1 and TBLR1. EMBO J **22:** 1336-1346.

YOON, S., C. K. GOVIND, H. QIU, S. J. KIM, J. DONG *et al.*, 2004 Recruitment of the ArgR/Mcm1p repressor is stimulated by the activator Gcn4p: a self-checking activation mechanism. Proc Natl Acad Sci U S A **101:** 11713-11718.

YOON, S., and A. G. HINNEBUSCH, 2009 Mcm1p binding sites in ARG1 positively regulate Gcn4p binding and SWI/SNF recruitment. Biochem Biophys Res Commun **381:** 123-128.

YOON, S., H. QIU, M. J. SWANSON and A. G. HINNEBUSCH, 2003b Recruitment of SWI/SNF by Gcn4p does not require Snf2p or Gcn5p but depends strongly on SWI/SNF integrity, SRB mediator, and SAGA. Mol Cell Biol **23:** 8829-8845.

YOUN, M. Y., H. S. YOO, M. J. KIM, S. Y. HWANG, Y. CHOI *et al.*, 2007 hCTR9, a component of Paf1 complex, participates in the transcription of interleukin 6-responsive genes through regulation of STAT3-DNA interactions. J Biol Chem **282:** 34727-34734.

ZANTON, S. J., and B. F. PUGH, 2006 Full and partial genome-wide assembly and disassembly of the yeast transcription machinery in response to heat shock. Genes Dev **20:** 2250-2265.

ZEITLINGER, J., A. STARK, M. KELLIS, J. W. HONG, S. NECHAEV *et al.*, 2007 RNA polymerase stalling at developmental control genes in the Drosophila melanogaster embryo. Nat Genet **39:** 1512-1516.

ZHANG, H., D. O. RICHARDSON, D. N. ROBERTS, R. UTLEY, H. ERDJUMENT-BROMAGE *et al.*, 2004 The Yaf9 component of the SWR1 and NuA4 complexes is required for proper gene expression, histone H4 acetylation, and Htz1 replacement near telomeres. Mol Cell Biol **24:** 9424-9436.

ZHANG, H., D. N. ROBERTS and B. R. CAIRNS, 2005a Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. Cell **123:** 219-231.

ZHANG, X., A. KOLACZKOWSKA, F. DEVAUX, S. L. PANWAR, T. C. HALLSTROM *et al.*, 2005b Transcriptional regulation by Lge1p requires a function independent of its role in histone H2B ubiquitination. J Biol Chem **280:** 2759-2770.

ZHANG, X. Y., H. K. PFEIFFER, A. W. THORNE and S. B. MCMAHON, 2008 USP22, an hSAGA subunit and potential cancer stem cell marker, reverses the polycomb-catalyzed ubiquitylation of histone H2A. Cell Cycle **7:** 1522-1524.

ZHANG, Z., and J. C. REESE, 2004 Redundant mechanisms are used by Ssn6-Tup1 in repressing chromosomal gene transcription in Saccharomyces cerevisiae. J Biol Chem **279:** 39240-39250.

ZHAO, J., B. K. SUN, J. A. ERWIN, J. J. SONG and J. T. LEE, 2008a Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. Science **322:** 750-756.

ZHAO, Y., G. LANG, S. ITO, J. BONNET, E. METZGER *et al.*, 2008b A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. Mol Cell **29:** 92-101.

ZHOU, K., W. H. KUO, J. FILLINGHAM and J. F. GREENBLATT, 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.

ZHU, B., S. S. MANDAL, A. D. PHAM, Y. ZHENG, H. ERDJUMENT-BROMAGE *et al.*, 2005a The human PAF complex coordinates transcription with events downstream of RNA synthesis. Genes Dev **19:** 1668-1673.

ZHU, B., Y. ZHENG, A. D. PHAM, S. S. MANDAL, H. ERDJUMENT-BROMAGE *et al.*, 2005b Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. Mol Cell **20:** 601-611.