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The Paf1 Complex Is Required for Histone H3 Methylation by COMPASS and Dot1p: Linking Transcriptional Elongation to Histone Methylation

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Summary

Methylation of histone proteins is one of their many modifications that affect chromatin structure and regulate gene expression. Methylation of histone H3 on lysines 4 and 79, catalyzed by the Set1-containing complex COMPASS and Dot1p, respectively, is required for silencing of expression of genes located near chromosome telomeres in yeast. We report that the Paf1 protein complex, which is associated with the elongating RNA polymerase II, is required for methylation of lysines 4 and 79 of histone H3 and for silencing of expression of a telomere-associated gene. We show that the Paf1 complex is required for recruitment of the COMPASS methyltransferase to RNA polymerase II and that the subunits of these complexes interact physically and genetically. Collectively, our results suggest that the Paf1 complex is required for histone H3 methylation, therefore linking transcriptional elongation to chromatin methylation.

Introduction

The elongation stage of transcription by RNA polymerase II is a highly regulated process that requires the concerted action of many proteins (Conaway and Conaway 1993; Shilatifard et al., 1997a, 1997b; Shilatifard 1998; Reines et al., 1996; Uptain et al., 1997). RNA polymerase II elongation factors fall into several functional classes. One class, involved in drug-induced arrest or sequence-dependent arrest of transcription, includes P-TEFb (Zhu et al., 1997), DSIF (Spt4, Spt5) (Wada et al., 1998), and SII (Gu and Reines, 1995). A second class, which regulates the rate of transcriptional elongation through a nucleosomal template, includes FACT (facilitates chromatin transcription) (Orphanides et al., 1999). A third class operates to increase the catalytic rate of transcription elongation by altering the K_m and/or the V_{max} of the polymerase and includes TFIIF (Conaway and Conaway, 1993), Elongin (Bradsher et al., 1993; Aso et al., 1995), and the ELL protein family (Shilatifard et al., 1996, 1997a, 1997b; Miller et al., 2000).

Although many of the elongation factors mentioned above have been implicated in the elongation stage of transcription in vitro, very little is known about their role in transcriptional elongation in vivo. We recently demonstrated that the *Drosophila* elongation factor dELL, whose mammalian homolog is required for the development of acute myeloid leukemia (Shilatifard, 1998; DiMartino et al., 2000), behaves as an elongation factor in vivo both cell biologically and genetically (Shilatifard et al., 1996; Gerber et al., 2001; Eissenberg et al., 2002). Our data suggest that the regulation of transcriptional elongation is an essential step in regulation of gene expression and that the various elongation factors play a pivotal role in piloting RNA polymerase II on its voyage down the double helix.

The Paf1 protein complex, which consists of Rtf1, Paf1, Cdc73, Leo1, and Ctr9, associates with the elongating RNA polymerase II (Stolinski et al., 1997; Costa and Arndt, 2000; Mueller and Jaehning, 2002; Squazzo et al., 2002; Pokholok et al., 2002; Krogan et al., 2002a). The sensitivity of strains deleted for some of the Paf1 complex subunits to the drug 6-azauracil (6-AU), which is believed to impede RNA polymerase II elongation by reducing intracellular GTP or UTP levels (by inhibiting enzymes that catalyze their biosynthesis), suggests that the Paf1 complex may also function during the elongation stage of transcription. Indeed, the Paf1 complex associates with the elongating RNA polymerase II. Although the subunits of the Paf1 complex are not essential for viability in yeast, some yeast strains lacking one or more subunits of the complex exhibit transcriptional defects when grown in the presence of 6-AU.

Methylation of lysines 4 and 79 of histone H3 is required for silencing of expression of genes located near chromosome telomeres. COMPASS is a histone methyltransferase that catalyzes methylation of lysine 4; Dot1 catalyzes methylation of lysine 79 (Krogan et al., 2002b; Miller et al., 2001; Roguev et al., 2001; Nagy et al., 2002; Briggs et al., 2001; Ng et al., 2002a; van Leeuwen et al., 2002). By screening extracts of ~4800 nonessential yeast gene deletion mutants, we identified several other proteins required for methylation of lysine 4 and lysine 79 of histone H3 (Dover et al., 2002; Wood et al., 2003). These include the subunits of the Paf1 complex, which have been demonstrated to be associated with the elongating form of RNA polymerase II. We describe results that suggest that Rtf1 and Paf1 genetically and physically interact with COMPASS and Dot1, and are required for: (1) methylation of histone H3 on lysines 4 and 79, (2) telomeric associated gene silencing, and (3) recruitment of COMPASS to the transcribing polymerase. Our results suggest that transcription elongation is linked to histone methylation.

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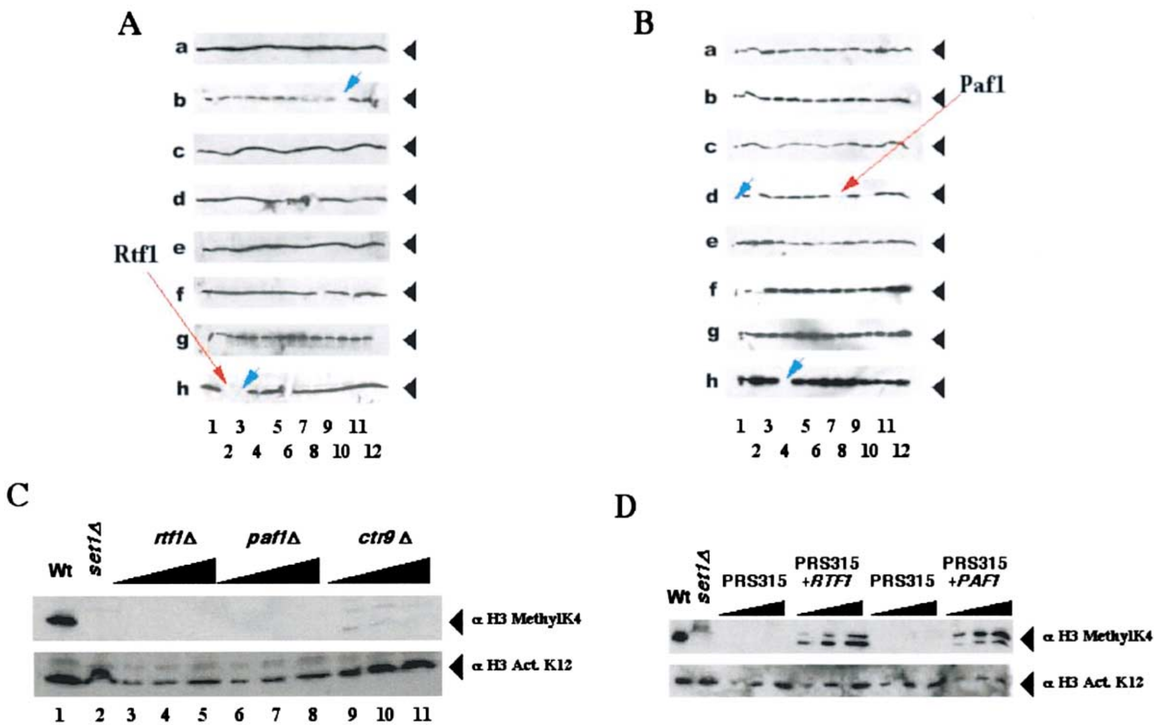


Figure 1. Surveying the *S. cerevisiae* Genome with Global Proteomic Analysis of *S. cerevisiae* Identified the RNA Polymerase II Elongation Complex Paf1 to Be Required for the Methylation of Lysine 4 of Histone H3

(A and B) Extracts of *S. cerevisiae* mutants missing one of the approximately 4800 nonessential genes were tested for the presence of Lys4-methylated histone H3 employing GPS (Dover et al., 2002). Strains lacking either (A) Rtf1 or (B) Paf1 are defective for this histone modification. Blue arrows at position b10 in (A), d1 in (B), and h3 in both indicate empty wells as plate markers.

(C) Extracts of wild-type strains or strains deleted for *rtf1*, *paf1*, and *ctr9* were tested for the presence of Lys4-methylated histone H3. The presence of acetylated histone H4 was used as an internal loading control.

(D) The K4 methylation-deficient phenotype of the *rtf1*Δ and *paf1*Δ cells was complemented by either an empty episomal vector or an episomal vector containing full-length DNA sequence coding for either Rtf1 or Paf1. The presence of acetylated histone H4 was used as an internal loading control.

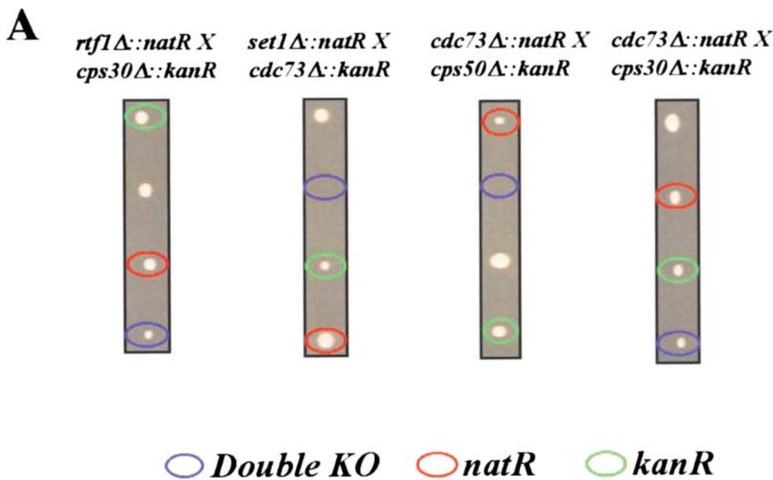
Results

The Paf1 Complex Is Required for Methylation of Lysine 4 of Histone H3

To identify proteins involved in the methylation of histone H3 by COMPASS, we employed Global Proteome analysis of *S. cerevisiae* (GPS) (Dover et al., 2002) to test by Western blotting extracts of each of the ~4800 nonessential yeast gene deletion mutants for defects in methylation of lysine 4 of histone H3, using anti-Lys4-methylated histone H3 as probe. A representative result from this mutant screen is shown in Figures 1A and 1B. This revealed that components of the RNA polymerase II elongation factor Paf1 complex are required for methylation of lysine 4 of histone H3: mutants missing Rtf1, Paf1, and Ctr9 are defective in methylation of K4 of histone H3 (Figure 1C). The histone H3 lysine 4 methylation defect in these mutants is complemented by introducing into them plasmids containing either the *RTF1* or *PAF1* genes (Figure 1D). The Paf1 complex is localized near promoters and with the elongating polymerase (Stolinski et al., 1997; Costa and Arndt, 2000; Mueller and Jaehning, 2002; Squazzo et al., 2002; Pokholok et al., 2002; Krogan et al., 2002b) and is required for expression of several genes, particularly those that respond to signals from the Pkc1/MAP kinase cascade (Betz et al., 2002). In order to determine whether the Paf1 complex may be required for the expression of Set1, we tested the level of expression of Set1 in both wild-type and strains deleted for either Paf1 or Rtf1 employing Set1-specific antibodies. We did not detect any difference in expression level of Set1 in the strains tested, indicating that the Paf1 complex is not required for the expression of Set1 (data not shown).

The Components of COMPASS and the Paf1 Complex Interact

To evaluate the function of COMPASS and the Paf1 complex in gene regulation, we systematically surveyed yeast genes for their genetic interactions with *rtf1* and *paf1* mutations by synthetic genetic array (SGA) analysis (Tong et al., 2001). The *set1, cps50, cps40, cps30*, and *cps25* mutants were crossed to an array of yeast mutant strains missing genes involved in transcriptional regulation (Tong et al., 2001). Nonviable or slow-growing double mutant meiotic progeny identify potential functionally related genes. A representative tetrad analysis of COMPASS and Paf1 complex crosses is shown in Figure



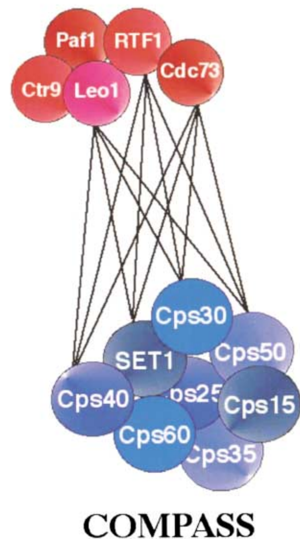


Figure 2. Subunits of COMPASS and the Paf1 Complex Interact Genetically

Genetic interaction network representing synthetic lethality and synthetic growth defects determined by SGA analysis.

(A) A representative tetrad analysis of COMPASS and Paf1 complex crosses shown here demonstrate synthetic growth phenotype.

(B) Genes are represented by circles, and experimentally observed genetic interactions via SGA are represented as lines that connect the circles. All of the interactions were confirmed by both tetrad analysis and random sporulation (data not shown).

2A. We identified approximately 45 genes that result in a growth defect when combined with a mutation in a gene encoding a component of COMPASS. These include the components of the Paf1 complex, Rtf1, Cdc73, and Leo1 (Figure 2B). (*CTR9* and *PAF1* were not identified in the screen because mutants missing these genes are not present in the standard available set of deletion mutants).

Physical Interaction between Components of the Paf1 Complex, RNA Polymerase II, and COMPASS

To test whether COMPASS and the Paf1 protein complex physically interact with each other, we purified the Paf1 complex by immunoprecipitating its Ctr9 subunit fused to the TAP affinity tag (Figure 3A). Employing Set1-specific polyclonal antibodies (Miller et al., 2001), we demonstrated that there is a substoichiometric amount of Set1 associated with the purified Paf1complex (Figure 3B), indicating the presence of physical interaction between COMPASS and the Paf1 complex.

Different phosphorylated forms of the C-terminal domain (CTD) of RNA polymerase II predominate at different stages of transcription (Komarnitsky et al. 2000; Cho et al., 2001). For example, Ser5 phosphorylation of CTD is detected primarily at promoter regions and the early elongation complexes, Ser2 phosphorylation of the CTD is mostly found to be associated with polymerases in productive elongation complexes. Because the Paf1 complex is associated with RNA polymerase II (Stolinski et al., 1997; Costa and Arndt, 2000; Mueller and Jaehning, 2002; Squazzo et al., 2002; Pokholok et al., 2002; Krogan et al., 2002b), we tested whether COMPASS can also associate with RNA polymerase II and found this indeed to be the case (Figure 3C). To determine which phosphorylated form of RNA polymerase II (Ser2 or Ser5 phosphorylated) COMPASS associates with, we purified COMPASS via TAP-tagged Cps30 or Cps60. The pres-

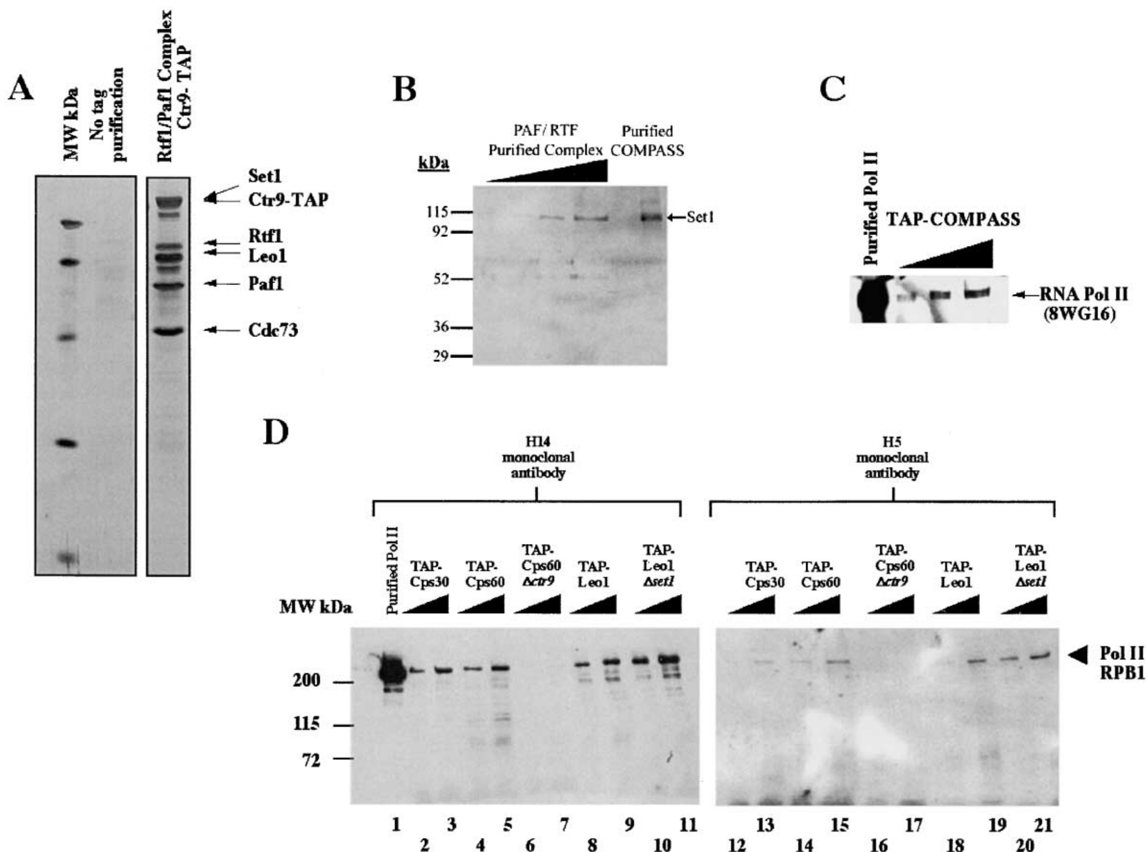


Figure 3. Subunits of COMPASS, the Paf1 Complex, and RNA Polymerase II Are Biochemically Associated within a Macromolecular Complex

(A) The Paf1 complex was purified to homogeneity by TAP tagging the Ctr9 subunit of the complex. The complex was analyzed by SDS-PAGE and silver staining.

(B) The presence of the Set1 subunit of COMPASS in the purified Paf1 complex in (A) was tested by Western analysis using a polyclonal antibody specific to Set1 (antibody to Set1 was generously provided by Dr. Pillus).

(C) The presence of RNA polymerase II in the purified COMPASS (purified by tagging the Cps30 subunit of the complex) was tested by Western analysis using monoclonal antibody 8WG16 specific to the C-terminal domain of the large subunit of RNA polymerase II.

(D) The interaction of COMPASS with RNA polymerase II in the presence or absence of the Paf1 complex and the interaction of the Paf1 complex with RNA polymerase II in the presence and absence of COMPASS were tested. COMPASS was purified from wild-type strains after tagging the Cps30 or Cps60 subunit of COMPASS. The Paf1 complex was purified by tagging the Leo1 subunit of the complex. The presence of RNA polymerase II was tested by Western analysis using either as a phospho-serine 2 (H5) or a phospho-serine 5 (H14) monoclonal antibodies to the C-terminal domain of the large subunit of RNA polymerase II. To determine whether the interaction of COMPASS with RNA polymerase II is dependent on the presence of the Paf1 complex, interaction of RNA polymerase II with COMPASS was tested in strains lacking the Ctr9 subunit of the Paf1 complex (lanes 6, 7, 16, and 17). To further analyze the interaction of the Paf1 complex with RNA polymerase II and to determine whether this interaction is dependent on the presence of COMPASS, purified Paf1 complexes (via tagging the Leo1 subunit of the complex) from wild-type cell or a strain defective for Set1 were tested for the presence of RNA polymerase II (lanes 10, 11, 20, and 21).

ence of RNA polymerase II was detected by Western blotting with anti-phospho-Ser2 or anti-phospho-Ser5 monoclonal antibodies. Our results demonstrate that COMPASS associates mostly with RNA polymerase II phosphorylated in its CTD on Ser5. This interaction requires the Paf1 complex because RNA polymerase II does not interact with COMPASS in strains missing the Ctr9 subunit of the Paf1 complex (Figure 3D, lanes 6, 7, 16, and 17). The converse, however, is not true: interaction of the Paf1 complex with RNA polymerase II does not require the presence of COMPASS (Figure 3D, lanes 10, 11, 20, and 21). These results suggests that COMPASS is primarily associated with RNA polymerase II located at promoters and with the early elongating polymerase and that the presence of stable Paf1 complex is required for such interactions.

The Paf1 Complex Is Required for the Recruitment of COMPASS to Chromatin

If COMPASS is mostly associated with RNA polymerase that is phosphorylated on Ser5 on its CTD, we expect to observe association of COMPASS at the promoter and with the early elongation complexes. Indeed, our chromatin immunoprecipitation experiments indicate that COMPASS is preferentially localized to the promoter and the 5′ end of coding sequence of the *PMA1* gene, suggesting that it is associated with the initiating and early elongating complexes of RNA polymerase

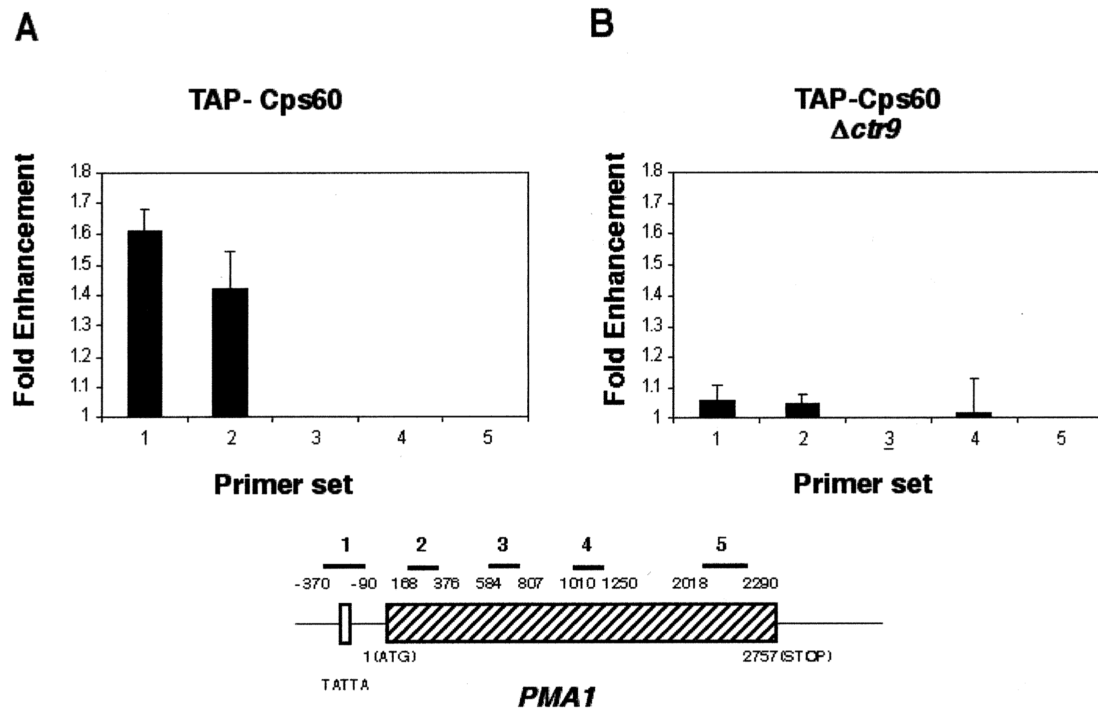


Figure 4. The Paf1 Complex Is Required for the Recruitment of COMPASS to RNA Polymerase II

Chromatin immunoprecipitation (ChIP) assays with Cps60-TAP and Cps60-TAP *ctr9*Δ strains were performed as previously described (Wood et al., 2003).

(A) To monitor the presence of COMPASS along the *PMA1* gene, chromatin was immunoprecipitated in triplicate with rabbit IgG-agarose. PCR amplifications were carried out using primer pairs recognizing promoter (1) or coding (2, 3, 4, 5) regions for *PMA1*. Primer pairs are as follows: $PMA1_{-370}$ and $PMA1_{-90}$ (1), $PMA1_{168}$ and $PMA1_{376}$ (2), $PMA1_{584}$ and $PMA1_{807}$ (3), $PMA1_{1010}$ and $PMA1_{1250}$ (4), $PMA1_{2018}$ and $PMA1_{2290}$ (5). Each PCR contained a second primer pair that amplified a region of chromosome V devoid of ORFs, thus providing an internal control for background. The ratio of the experimental to the control signal for the precipitated DNA was divided by the ratio of the experimental to the control signal for the input DNA.

(B) The presence of COMPASS on the *PMA1* gene was also monitored in strains deleted for *ctr9* as described in (A).

(Figure 4A). Most notably, the Paf1 complex is required for recruitment of COMPASS to chromatin (Figure 4B), suggesting that the interaction of COMPASS with RNA polymerase on chromatin requires the presence of the Paf1 complex. This observation further supports our biochemical data regarding physical interaction between COMPASS with RNA polymerase II and the requirement for the Paf1 complex as presented in Figure 3.

The Paf1 Complex Is Required for Methylation of Lysine 79 of Histone H3

The lysine 79 of histone H3 is also methylated, and Dot1 is the enzyme responsible for the modification of histone H3 (Ng et al., 2002a; van Leeuwen et al., 2002). We set out to determine whether the Paf1 complex is also required for histone methylation on lysine 79 of histone H3. We discovered that components of the Paf1 complex are also required for this histone modification (Figures 5A and 5B). Western blot analysis with antibodies specific to K79-methylated histone H3 results in the appearance of a doublet band with the approximate size of histone H3. Loss of Dot1, the enzyme that catalyzes methylation of histone H3 on K79, results in the total loss of the doublet (data not shown), as does deletion of either *RAD6* or *BRE1*, which encodes the ubiquitination enzymes required for K79 methylation (data not shown). We are not certain why this antibody detects a doublet. It could be due to two different isoforms of H3, one perhaps methylated and phosphorylated (or other modifications) and the other one only methylated.

The requirement of the Paf1 complex for methylation of lysine 79 of histone H3 was confirmed by testing histone H3 K79 methylation in independently generated *rtf1* and *paf1* mutants (Figures 5A–5C) However, in contrast to methylation of K4 of histone H3, we detect residual methylation of K79 in strains lacking *CTR9*. Also, Leo1 (another component of the Paf1 complex) is not required for K79 methylation (data not shown). We also demonstrated that the loss of H3 K79 methylation phenotype in strains deleted for either *rtf1*Δ or *paf1*Δ can be complemented by the wild-type genes as shown in Figure 5D, lanes 3–14. To evaluate the genetic interactions between Dot1p and the Paf1 complex, we systematically surveyed yeast genes for their genetic interactions with *DOT1* by synthetic genetic array analysis (Tong et al., 2001). We found that Dot1p genetically interacts with the subunits of the Rtf/Paf1 complex (data not shown).

The Paf1 Complex Is Required for Silencing of Gene Expression at Chromosome Telomeres

Methylation of histone H3 on K4 (catalyzed by COMPASS) and on K79 (catalyzed by Dot1) is required for

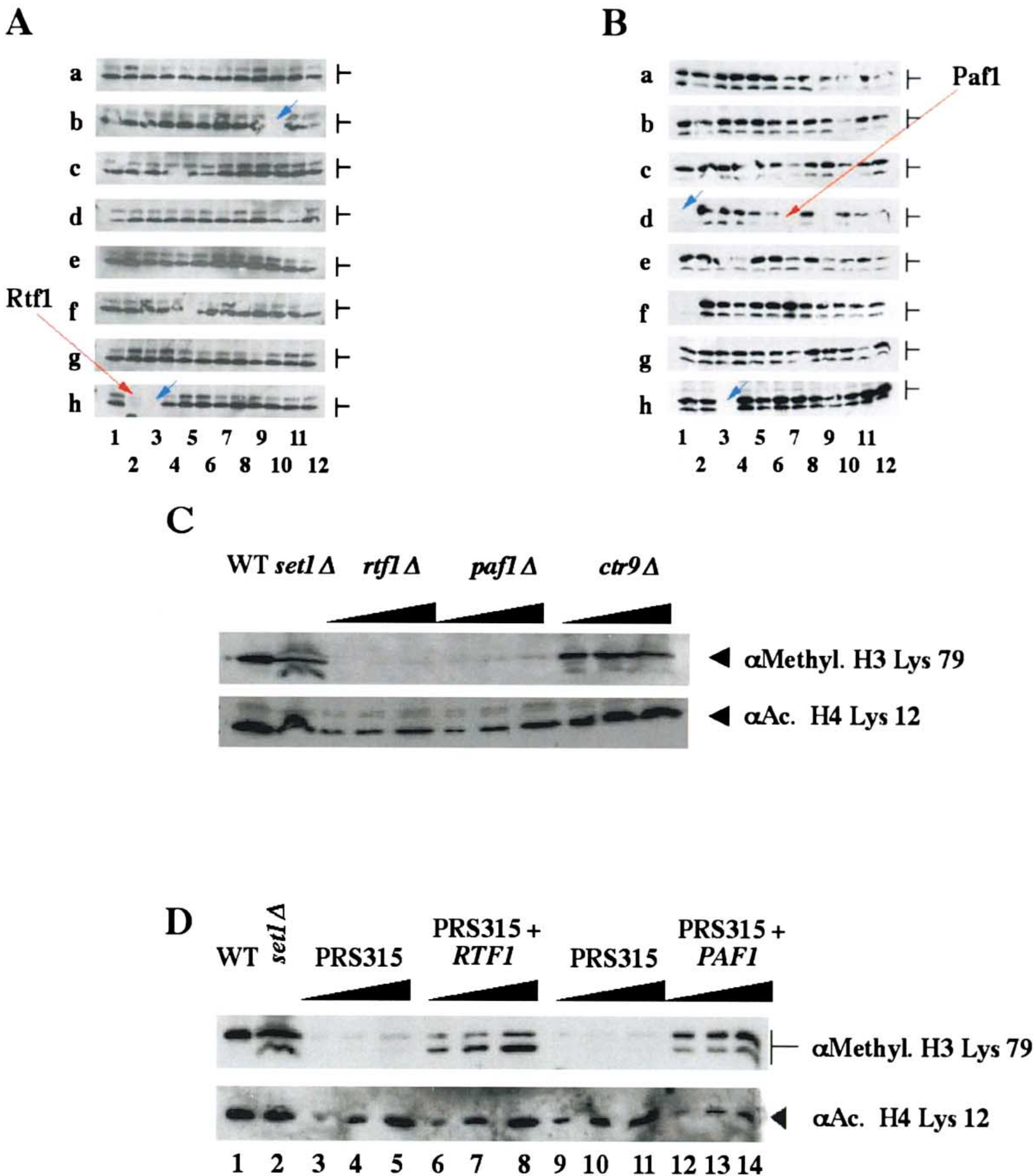


Figure 5. The Paf1 Complex Is Also Required for the Methylation of Lysine 79 of Histone H3

(A and B) Employing GPS (Dover et al., 2002), extracts of *S. cerevisiae* mutants missing one of the approximately 4800 nonessential genes were tested for the presence of Lys79-methylated histone H3. Strains lacking either (A) Rtf1 or (B) Paf1 are defective for this histone modification. Blue arrows at position b10 in (A), d1 in (B), and h3 in both indicate empty wells as plate markers.

(C) Same extracts as in Figure 1. Wild-type strains or strains deleted for *rtf1*, *paf1*, and *ctr9* were tested for the presence of Lys79-methylated histone H3. The presence of acetylated histone H4 was used as an internal loading control.

(D) The K79 methylation-deficient phenotype of the *rtf1*Δ and *paf1*Δ cells was complemented by either an empty episomal vector or an episomal vector containing full-length DNA for Rtf1 or Paf1. The presence of acetylated histone H4 was used as an internal loading control.

silencing of expression of genes located near chromosomal telomere (Nislow et al., 1997; Miller et al., 2001; Krogan et al., 2002a; Ng et al., 2002a; van Leeuwen et al., 2002). Since the Paf1 complex is required for histone methylation by COMPASS and Dot1p, we tested the role of the Paf1 complex in the regulation of silencing of expression of a *URA3* gene located near the telomere of chromosome VII. The Paf1 complex is required for silencing of expression of a *URA3* gene located near the telomere of chromosome VII (Figure 6), an observa-

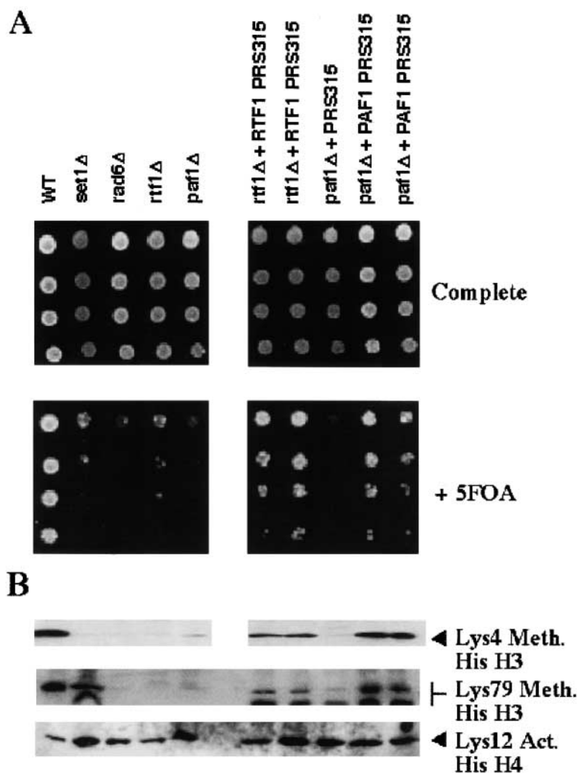


Figure 6. Strains Lacking either Rtf1 or Paf1 Are Defective for the Silencing of Genes Located Near a Chromosomal Telomere

(A) Deletion of either *rtf1* or *paf1* results in a defect in silencing of gene expression at telomeres. The wild-type parental strain (UCC1001, harboring *URA3* near the left telomere of chromosome 7 as a reporter of telomeric gene silencing [Nislow et al., 1997]) and UCC1001 strains deleted for *set1, rad6, rft1*, or *paf1* were tested for defects in silencing of gene expression at telomeres. Wild-type cells silence expression of the telomere-associated *URA3* gene and are therefore resistant to 5FOA. Cells defective for telomeric gene silencing have increased expression of *URA3* and hence are sensitive to 5FOA (14, 20, 25, and 33). A 2-fold serial dilution of cultures (from about 5×10^4 cells) was spotted on minimal glucose plates containing (lower panel) or lacking (upper panel) 5FOA. These plates were incubated at 30°C for 48 (±4) hr.

(B) The ability of each strain to methylate its histone H3 on K4 and 79 was tested by Western analysis. In the same experiment, the addition of two independently cloned plasmids containing wild-type Rft1 or Paf1 complemented both the silencing and methylation-defective phenotypes. The presence of acetylated histone H4 was used as an internal loading control.

tion that is consistent with our previously reported linkage between gene silencing and histone H3 lysine 4 methylation (Krogan et al., 2002a).

Discussion

Methylation of lysine 4 and 79 of histone H3, catalyzed by COMPASS and Dot1p, respectively, requires ubiquitination of histone H2B on lysine 123 (Dover et al., 2002; Sun and Allis, 2002). Ubiquitination of histone H2B is catalyzed by Rad6 (E2) (Robzyk et al., 2000) and its E3 ligase, Bre1, which seems to guide the recruitment of Rad6 to promoters (Wood et al., 2003). Our analyses reported in this manuscript indicate that the components of the Paf1 complex are also required for methylation of histone H3 on K4 and K79. The requirement of the Paf1 complex for histone H3 methylation is supported by the following observations. (1) Loss of the components of the Paf1 complex results in defect in histone methylation on K4 and K79 of histone H3. (2) COMPASS interacts with RNA polymerase II and the Paf1 complex, and the interaction of COMPASS with RNA polymerase II requires the presence of the Paf1 complex. (3) The components of COMPASS genetically interact with the components of the Paf1 complex. (4) Dot1p requires the Paf1 complex for its histone methyltransferase function and genetically interacts with the components of the Paf1 complex. (5) Deletion of genes encoding components of the Paf1 complex result in a defect in telomeric-associated gene silencing. (6) Recruitment of COMPASS to the promoters and the early transcribing polymerase requires the Paf1 complex. Together, our data suggest a link between histone methylation and transcription elongation.

Our systematic survey of yeast genes that genetically interact with *rtf1* or *paf1* by synthetic genetic array analysis (Tong et al., 2001) indicated that deletions of genes encoding components of COMPASS (*set1, cps50, cps30*, and *cps25*) are synthetic lethal with *rtf1* and *paf1* mutations. We were surprised by this result because the Paf1 complex and COMPASS are involved in the same process. This observation may suggest that the Paf1 complex and COMPASS also play other roles in addition to their role in catalyzing histone methylation.

We have observed a substoichiometric amount of COMPASS associated with the purified Paf1 complex. It is possible that these complexes associate stoichiometrically in vivo; however, they may fall apart during the biochemical purification. Alternatively, it is possible that only a small portion of the total COMPASS and the Paf1 complex are found in cocomplex with each other in vivo. We will test this possibility by analyzing the localization of the components of COMPASS and the Paf1 complex on polytene chromosome in vivo to test this hypothesis.

Most recently, results from the Gottschling and Struhl laboratories have suggested that methylation of histone H3 on lysine 79 by Dot1 is also required for regulation of telomeric silencing in yeast (Ng et al., 2002a; van Leeuwen et al., 2002). They have suggested that methylation of histone H3 limits silencing to discrete chromosomal regions by preventing the binding of Sir and other related proteins elsewhere along the genome. It is intriguing that methylation of both lysines 4 and 79 requires ubiquitination of histone H2B (Dover et al., 2002; Sun and Allis, 2002; Ng et al., 2002b; Wood et al., 2003) and that methylation of histone H3 on lysine 4 is also involved in the regulation of telomeric silencing. Together, this may suggest that telomeric silencing through lysine 4 and lysine 79 methylation may function by a similar mechanism.

It was recently suggested that histone H3 methylation and histone H2B ubiquitination may occur on the same nucleosome. However, it appears that there is a large difference in the amount of histone H3 methylation and histone H2B ubiquitination; we find that over 50% of H3 molecules are methylated on K4, but less than 5% of H2B molecules are ubiquitinated on K123. This observa-

tion, in conjunction with our recent finding that both Rad6 and its E3 ligase, Bre1, are recruited to the promoter of a gene (Wood et al., 2003) suggests the possibility that ubiquitination of H2B on a single nucleosome at the promoter may act as a signal for histone methylation on many other nucleosomes. It is also possible that H3 lysine 4 methylation is triggered by ubiquitination of H2B and persists after H2B is deubiquitinated. Identification of lysine 4 methylated histone H3 at the promoter and body of genes (Bernstein et al., 2002; Santos-Rosa et al., 2002) and the association of COMPASS and Dot1 and the elongation complex Paf1 is consistent with this model. We have shown that COMPASS associates with the promoter and the 5′ portion of the *PMA1* gene. It has also recently been demonstrated that this histone modification can be found at promoters and in the body of genes (Bernstein et al., 2002; Santos-Rosa et al., 2002). Indeed, based on these chromatin immunoprecipitation studies it has been proposed that histone methylation may play a role in transcription elongation (Bernstein et al., 2002). Since the Paf1 complex has been implicated to be associated with the elongating RNA polymerase II, demonstration of its requirement for histone methylation by COMPASS and Dot1 at the promoter and body of genes is consistent with this idea.

Another well-characterized histone modification—acetylation—is a dynamic process, being influenced by the ongoing activities of histone acetyltransferases (HATs) and deacetylases (HDACs) (Workman and Kingston, 1998). Because of this, acetylation perhaps would not appear to be an efficient provider of transcriptional memory. Histone methylation, on the other hand, is a more stable modification than ubiquitination, acetylation, or phosphorylation (also no histone demethylating enzymes have yet been identified). Once polymerase has departed the promoter, there presumably is no further communication between the elongating RNA polymerase II and the promoter of a gene. Due to its stability and its presence on the body of genes, histone methylation could play an important role as a mark for transcriptional memory.

Experimental Procedures

Functional Genomic Analyses of Histone Modification by Methylation

Using a 96-well pinning device, the entire collection of 4800 yeast nonessential gene deletion mutants were inoculated from −80°C stocks onto agar plates containing YPD + 200 μg/ml Geneticin (GIBCO) and allowed to grow 48 hr, and used to inoculate 96-tube PCR-plates filled with 100 μl of YPD. After 48 hr of growth at 30°C the plates were centrifuged at 2000 g for 10 min. Medium was removed by wrist-snap inversion and draining into absorbent towels. Plates were then covered and frozen at −80°C for up to 1 week. Cells were thawed at room temperature, resuspended in 30 μl lysis buffer (20 mM Tris [pH 7.5], 50 mM KCl, 1 mM EDTA, 1 mM DTT, 0.1% NP40, 1 mg/ml Zymolyase 100T), and incubated at 37°C for 15 min. Ten microliters of 4× Laemmli loading buffer was added, and the samples were vortexed briefly before heating at 100°C for 5 min. The lysates were subjected to SDS-PAGE, transferred to nitrocellulose membrane, and probed with anti-modified histone antiseras (obtained from Upstate) at 1:1000 dilution, followed by detection of the bound antibody with Horseradish peroxidase-conjugated to anti-rabbit IgG secondary antibodies (1:10,000 dilution).

Isolation of Yeast Extracts at Larger Quantities

To obtain yeast cell extracts in larger quantities, yeast cells were grown to mid-log phase in YPD medium, pelleted, washed with distilled water, pelleted, and resuspended in lysis buffer (20 mM Tris [pH 7.5], 50 mM KCl, 1 mM EDTA, 0.1% NP40, 1 mM DTT) and fresh protease and phosphatase inhibitors (1 μg/ml aprotinin, leupeptin, and pepstatin A; 1 mM PMSF; 1 μM microcystin-LR; 2 mM p-chloromercuriphenylsulfonic acid). Cells were then disrupted by vortexing with glass beads (0.5 mm; Biospec Products) for 15 min at 4°C. The bottoms of the microcentrifuge tubes were punctured, and cell extracts were recovered into a larger tube by brief centrifugation in a microfuge. The lysate was clarified by centrifugation at 20,000 g for 30 min, subjected to SDS-PAGE, transferred to nitrocellulose membrane, and probed with antibodies as indicated in each figure followed by detection of the bound antibody with Horseradish peroxidase conjugated to anti-rabbit IgG secondary antibodies (1:10,000 dilution).

Biochemical Purification of via TAP-Tag

All purifications were carried out at 4°C. All purification steps were performed several times to assure the identification of correct polypeptides. Fractions were tested for RNA polymerase II by Western analysis with anti-Pol II polyclonal antibodies H14, H5, and 8WG16 (Covance). For the affinity purification of complexes, proteins were tagged by chromosomal integration via the C-terminal domain following a previously published method (Miller et al., 2001). Tagged complexes were purified essentially as described on IgG and calmodulin columns from extracts obtained from 1 liter yeast cultures grown in YPD medium to an OD_{600} of 1.0–1.5 as before (Miller et al., 2001). The bound fractions were tested for the presence of either Set1 or phosphorylated RNA polymerase II with the appropriate antibodies as described before (Miller et al., 2001).

Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) assays, in which proteins were crosslinked to DNA in vivo using formaldehyde, were employed to analyze the in vivo distribution of COMPASS and Dot1 along various regions of the *PMA1* gene. Following isolation and shearing of chromatin, TAP-tagged protein were immunoprecipitated with IgG agarose (directed against protein A on the TAP tag). After reversal of the crosslinks, PCR analyses were performed on the coprecipitated DNA. Primer pairs directed against promoter regions, coding regions, and 3′ untranslated regions of the *PMA1* gene were used. Each PCR also contained a second primer pair that amplified a region of chromosome V devoid of ORFs, thus providing an internal control for background. The ratio of the experimental to the control signal for the precipitated DNA was divided by the ratio of the experimental to the control signal for the input DNA.

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References

Aso, T., Lane, W.S., Conaway, J.W., and Conaway, R.C. (1995). Elongin (SIII): a multisubunit regulator of elongation by RNA polymerase II. Science *269*, 1439–1443.

Bernstein, B.E., Humphrey, E.L., Erlich, R.L., Schneider, R., Bouman, P., Liu, J.S., Kouzarides, T., and Schreiber, S.L. (2002). Methylation of histone H3 Lys 4 in coding regions of active genes. Proc. Natl. Acad. Sci. USA *25*, 8695–8700.

Betz, J.L., Chang, M., Washburn, T.M., Porter, S.E., Mueller, C.L., and Jaehning, J.A. (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.

Bradsher, J.N., Jackson, K.W., Conaway, R.C., and Conaway, J.W. (1993). RNA polymerase II transcription factor SIII. I. Identification, purification, and properties. J. Biol. Chem. *268*, 25587–25593.

Briggs, S.D., Bryk, M., Strahl, B.D., Cheung, W.L., Davie, J.K., Dent, S.Y., Winston, F., and Allis, C.D. (2001). Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in Saccharomyces cerevisiae. Genes Dev. *15*, 3286–3295.

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

Conaway, R.C., and Conaway, J.W. (1993). General initiation factors for RNA polymerase II. Annu. Rev. Biochem. *62*, 161–190.

Costa, P.J., and Arndt, K.M. (2000). Synthetic lethal interactions suggest a role for the Saccharomyces cerevisiae Rtf1 protein in transcription elongation. Genetics *156*, 535–547.

DiMartino, J.F., Miller, T., Ayton, P.M., Landewe, T., Hess, J.L., Cleary, M.L., and Shilatifard, A. (2000). A carboxy-terminal domain of ELL is required and sufficient for immortalization of myeloid progenitors by MLL-ELL. Blood *96*, 3887–3893.

Dover, J., Schneider, J., Boateng, M.A., Wood, A., Dean, K., Johnston, M., and Shilatifard, A. (2002). Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. J. Biol. Chem. *277*, 28368–28371.

Eissenberg J.C., Ma, J., Gerber, M.A., Christensen, A., Kennison, J.A., and Shilatifard A. (2002). dELL is an essential RNA polymerase II elongation factor with a general role in development. Proc. Natl. Acad. Sci. USA *99,* 9894–9899.

Gerber, M.A., Jiyan, M.A., Dean, K., Eissenberg, J.C., and Shilatifard, A. (2001). Drosophila ELL is associated with actively elongating RNA polymerase II on transcriptionally active sites in vivo. EMBO J. *20*, 6104–6114.

Gu, W., and Reines, D.J. (1995). Identification of a decay in transcription potential that results in elongation factor dependence of RNA polymerase II. J. Biol. Chem. *270*, 11238–11244.

Komarnitsky, P., Cho, E.J., and Buratowski, S. (2000). Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes Dev. *14*, 2452–2460.

Krogan, N.J., Kim, M., Ahn, S.H., Zhong, G., Kobor, M.S., Cagney, G., Emili, A., Shilatifard, A., Buratowski, S., and Greenblatt, J.F. (2002a). RNA polymerase II elongation factors of Saccharomyces cerevisiae: a targeted proteomics approach. Mol. Cell. Biol. *22*, 6979–6992.

Krogan, N., Dover, J., Khorrami, S., Greenblatt, J.F., Schneider, J., Johnston, M., and Shilatifard, A. (2002b). COMPASS, a histone H3 (lysine 4) methyltransferase required for telomeric silencing of gene expression. J. Biol. Chem. *277*, 10753–10755.

Miller, T., Williams, K., Johnstone, R.W., and Shilatifard, A. (2000). COMPASS: a complex of proteins associated with a trithorax-related SET domain protein. J. Biol. Chem. *275*, 32052–32056.

Miller, T., Krogan, N.J., Dover, J., Bromage, H., Tempst, P., Johnstone, M., Greenblatt, J.F., and Shilatifard, A. (2001b). COMPASS: a complex of proteins associated with a trithorax-related SET domain protein. Proc. Natl. Acad. Sci. USA *98*, 12902–12907.

Mueller, C.L., and Jaehning, J.A. (2002). Ctr9, Rtf1, and Leo1 are components of the Paf1/RNA polymerase II complex. Mol. Cell. Biol. *22*, 1971–1980.

Nagy, P.L., Griesenbeck, J., Kornberg, R.D., and Cleary, M.L. (2002). A trithorax-group complex purified from Saccharomyces cerevisiae is required for methylation of histone H3. Proc. Natl. Acad. Sci. USA *99*, 90–94.

Ng, H.H., Feng, Q., Wang, H., Erdjument-Bromage, H., Tempst, P., Zhang, Y., and Struhl, K. (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., Xu, R.M., Zhang, Y., and Struhl, K. (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.

Nislow, C., Ray, E., and Pillus, L. (1997). SET1, a yeast member of the trithorax family, functions in transcriptional silencing and diverse cellular processes. Mol. Biol. Cell *8*, 2421–2436.

Orphanides, G., Wu, W.H., Lane, W.S., Hampsey, M., and Reinberg, D. (1999). The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature *400*, 284–288.

Pokholok, D.K., Hannett, N.M., and Young, R.A. (2002). Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. Mol. Cell *9*, 799–809.

Reines, D., Conaway, J.W., and Conaway, R.C. (1996). The RNA polymerase II general elongation factors. Trends Biochem. Sci. *21*, 351–355.

Robzyk, K., Recht, J., and Osley, M.A. (2000). Rad6-dependent ubiquitination of histone H2B in yeast. Science *287*, 501–504.

Roguev, A., Schaft, D., Shevchenko, A., Pijnappel, W.W., Wilm, M., Aasland, R., and Stewart, A.F. (2001). The Saccharomyces cerevisiae Set1 complex includes an Ash2 homologue and methylates histone 3 lysine 4. EMBO J. *20*, 7137–7148.

Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C., Schreiber, S.L., Mellor, J., and Kouzarides, T. (2002). Active genes are tri-methylated at K4 of histone H3. Nature *419*, 407–411.

Shilatifard, A. (1998). Factors regulating the transcriptional elongation activity of RNA polymerase II. FASEB J. *12*, 1437–1446.

Shilatifard, A., Lane, W.S., Jackson, K.W., Conaway, R.C., and Conaway, J.W. (1996). An RNA polymerase II elongation factor encoded by the human ELL gene. Science *271,* 1873–1876.

Shilatifard, A., Conaway, J.W., and Conaway, R.C. (1997a). Mechanism and regulation of transcriptional elongation and termination by RNA polymerase II. Curr. Opin. Genet. Dev. *7*, 199–204.

Shilatifard, A., Duan, R.D., Haque, D., Florence, C., Schubach, W.H., Conaway, J.W., and Conaway, R.C. (1997b). ELL2, a new member of an ELL family of RNA polymerase II elongation factors. Proc. Natl. Acad. Sci. USA *94*, 3639–3643.

Squazzo, S.L., Costa, P.J., Lindstrom, D.L., Kumer, K.E., Simic, R., Jennings, J.L., Link, A.J., Arndt, K.M., and Hartzog, G.A. (2002). The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. EMBO J. *21*, 1764–1774.

Stolinski, L.A., Eisenmann, D.M., and Arndt, K.M. (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.

Sun, Z.W., and Allis, C.D. (2002). Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. Nature *418*, 104–108.

Tong, A.H., Evangelista, M., Parsons, A.B., Xu, H., Bader, G.D., Page, N., Robinson, M., Raghibizadeh, S., Hogue, C.W., Bussey, H., et al. (2001). Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science *294*, 2364–2368.

Uptain, S., Kane, C.M., and Chamberlin, M.J. (1997). Basic mechanisms of transcript elongation and its regulation. Annu. Rev. Biochem. *66*, 117–172.

van Leeuwen, F., Gafken, P.R., and Gottschling, D.E. (2002). Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell *109*, 745–756.

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

Wood, A., Krogran, N.J., Dover, J., Schneider, J., Heidt, J., Boateng, M.A., Dean, K., Golshani, A., Zhang, Y., Greenblatt, J.F., et al. (2003). Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. Mol. Cell *11*, 267–274.

Workman, J.L., and Kingston, R.E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu. Rev. Biochem. *67*, 545–579.

Zhu, Y., Peery, T., Peng, J., Ramanathan, Y., Marshall, N., Marshall, T., Amendt, B., Mathews, M.B., and Price, D.H. (1997). Transcription elongation factor P-TEFb is required for HIV-1 tat transactivation in vitro. Genes Dev. *11*, 2622–2632.