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Targeted Recruitment of Set1 Histone Methylase by Elongating Pol II Provides a Localized Mark and Memory of Recent Transcriptional Activity

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Summary

Set1, the yeast histone H3-lysine 4 (H3-K4) methylase, is recruited by the Pol II elongation machinery to a highly localized domain at the 5′ portion of active mRNA coding regions. Set1 association depends upon the TFIIH-associated kinase that phosphorylates the Pol II C-terminal domain (CTD) and mediates the transition between initiation and elongation, and Set1 interacts with the form of Pol II whose CTD is phosphorylated at serine 5 but not serine 2. The Rtf1 and Paf1 components of the Pol II-associated Paf1 complex are also important for Set1 recruitment. Although the level of dimethylated H3-K4 is fairly uniform throughout the genome, the pattern of trimethylated H3-K4 strongly correlates with Set1 occupancy. Hypermethylated H3-K4 within the mRNA coding region persists for considerable time after transcriptional inactivation and Set1 dissociation from the chromatin, indicating that H3-K4 hypermethylation provides a molecular memory of recent transcriptional activity.

Introduction

Chromatin-modifying activities are often recruited to specific gene-regulatory sequences, whereupon they cause localized changes in chromatin structure and specific transcriptional effects. Histone acetylases (e.g., SAGA and NuA4) are often recruited to promoters by DNA binding activator proteins (Cosma et al., 1999; Agalioti et al., 2000; Reid et al., 2000), whereas histone deacetylases (e.g., the Sin3/Rpd3 complex) are recruited by DNA binding repressors (Pazin and Kadonaga, 1997; Struhl, 1998). Nucleosome-remodeling complexes (e.g., Swi/Snf, Isw2, RSC) can be recruited by specific activators or repressors, depending on the promoter (Cosma et al., 1999; Agalioti et al., 2000; Goldmark et al., 2000; Martens and Winston, 2002; Ng et al., 2002b). In addition, the RSC complex is recruited to all genes transcribed by RNA polymerase (Pol) III, presumably by the Pol III machinery itself (Ng et al., 2002b). Distinct histone H3 methylases that modify different lysine residues are targeted to active promoters (Milne et al., 2002; Nakamura et al., 2002) or to repressor binding sites (Cao et al., 2002; Czermin et al., 2002; Kuzmichev et al., 2002; Muller et al., 2002; Ogawa et al., 2002). In addition to being targeted to specific gene-regulatory sequences, histone-modifying activities also function in a nontargeted, genome-wide fashion (Kuo et al., 2000; Reid et al., 2000; Vogelauer et al., 2000; Katan-Khaykovich and Struhl, 2002; Ng et al., 2003). Global histone acetylases and deacetylases rapidly restore chromatin structure back to the ground state at the end of a transcriptional response (Vogelauer et al., 2000; Katan-Khaykovich and Struhl, 2002).

Methylation of histone H3 at lysine 4 (H3-K4) is associated with active chromatin in a wide range of eukaryotic organisms (Strahl et al., 1999; Litt et al., 2001; Boggs et al., 2002; Gendrel et al., 2002; Noma and Grewel, 2002; Reuben and Lin, 2002). The first H3-K4 methylase to be identified was the *Saccharomyces cerevisiae* Set 1 complex (Briggs et al., 2001; Roguev et al., 2001; Miller et al., 2001; Krogan et al., 2002a; Nagy et al., 2002), and the catalytic subunit Set1 is homologous to human MLL/ALL and *Drosophila* trithorax. MLL/ALL is a component of an H3-K4 methylase that is recruited to active *Hox* promoters (Milne et al., 2002; Nakamura et al., 2002). The trithorax complex maintains homeotic genes in an active state that is antagonistic to the repressed state mediated by the polycomb complex (Cavalli and Paro, 1999; Francis and Kingston, 2001; Petruk et al., 2001; Poux et al., 2002; Simon and Tamkun, 2002). Importantly, both the active and repressed states of homeotic genes are epigenetically inherited through many generations, indicating that H3-K4 methylation is linked to long-term transcriptional memory.

In *Saccharomyces cerevisiae*, Set1 and H3-K4 methylation are important for ribosomal DNA (Briggs et al., 2001; Bryk et al., 2002) and telomeric silencing (Nislow et al., 1997; Krogan et al., 2002a). However, the level of H3-K4 methylation is low at silenced loci (Bernstein et al., 2002; Bryk et al., 2002), and by analogy with H3-K79 methylation (van Leeuwen et al., 2002; Ng et al., 2003), it is likely that H3-K4 methylation inhibits binding of the Sir silencing proteins and hence is associated with active loci as in other organisms. H3-K4 methylation occurs throughout the genome, but it appears slightly higher at the coding regions of active genes (Bernstein et al., 2002). This preferential association occurs with the trimethylated but not the dimethylated form of H3-K4 (Santos-Rosa et al., 2002). Genetic (Nislow et al., 1997) and transcriptional profiling (Miller et al., 2001; Bernstein et al., 2002) experiments have identified genes whose expression appears to be affected by the loss of Set1, but there is no overlap between the genes identified by different groups, and the effects are quantitatively modest.

Transcription of mRNA coding genes by RNA polymerase II (Pol II) is a complex process (Conaway et al., 2000; Lemon and Tjian, 2000; Orphanides and Reinberg, 2000; Naar et al., 2001) that is linked to subsequent events of mRNA production including capping, splicing, polyadenylation, and nuclear export (Bentley, 2002; Ma-

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niatis and Reed, 2002; Orphanides and Reinberg, 2002). In yeast cells, the general initiation factors localize specifically to promoter regions in a mutually interdependent manner that is strongly correlated with transcriptional activity (Kuras and Struhl, 1999; Li et al., 1999, 2000; Kuras et al., 2000; Pokholok et al., 2002). Upon preinitiation complex assembly, TFIIH phosphorylates serine 5 of the C-terminal domain (CTD) of the largest Pol II subunit at or near the promoter (Komarnitsky et al., 2000), thereby marking the transition between initiation and elongation. At a later stage, elongating Pol II is phosphorylated at serine 2 of the CTD by Ctk1, with the level of serine 2 phosphorylation increasing toward the 3′ end of the gene (Komarnitsky et al., 2000; Cho et al., 2001). Putative elongation factors such as TFIIS, Spt4/5, Spt6, TREX, and the Paf complex interact with Pol II (Sopta et al., 1985; Shi et al., 1996; Wada et al., 1998; Krogan et al., 2002b) and associate with coding regions of active genes (Lei et al., 2001; Krogan et al., 2002b; Licatalosi et al., 2002; Pokholok et al., 2002; Strasser et al., 2002). Thus, Pol II-associated factors are exchanged during the transition between initiation and elongation in vivo (Pokholok et al., 2002).

Here, we show that Set1 histone methylase is targeted to a discrete location within the 5′ portion of active mRNA coding regions by the Pol II elongation machinery. Set1 recruitment requires Kin28, the TFIIH-associated kinase that phosphorylates the Pol II CTD, and it is affected by the Rtf1 and Paf1 components of the Paf1 complex. Set1 interacts with the form of Pol II whose CTD is phosphorylated at serine 5 but not at serine 2. Targeted Set1 generates a localized domain of trimethylated H3-K4 that is distinct from the genome-wide dimethylated H3-K4 that arises from the untargeted action of Set1. Hypermethylated H3-K4 within the mRNA coding region persists for considerable time after transcriptional inactivation and Set1 dissociation from the chromatin, indicating that H3-K4 hypermethylation provides a molecular memory of recent transcriptional activity. Potential biological roles for this memory of recent transcriptional activity are discussed.

Results

Set1 Is Targeted to a Discrete Location within the mRNA Coding Regions of Active Pol II Genes

To address whether Set1 histone methylase is recruited to specific chromosomal regions in vivo, we performed genome-wide location analysis, a procedure that combines chromatin immunoprecipitation with hybridization on DNA microarrays representing the entire genome (Ren et al., 2000; Iyer et al., 2001; Ng et al., 2002b). Set1 associates with DNA sequences corresponding to open reading frames (ORFs) and intergenic regions in a manner that correlates with Pol II transcriptional activity (Figure 1A). Set1 occupancy is generally higher at any given ORF than at its flanking intergenic regions (Figure 1B). Set1 association is not observed at silenced loci, Pol III-transcribed genes, and the Pol I-transcribed rDNA locus. Thus, Set1 is generally targeted to active Pol II-driven genes.

We mapped Set1 occupancy at several genes using specific primers in a quantitative real-time PCR assay.

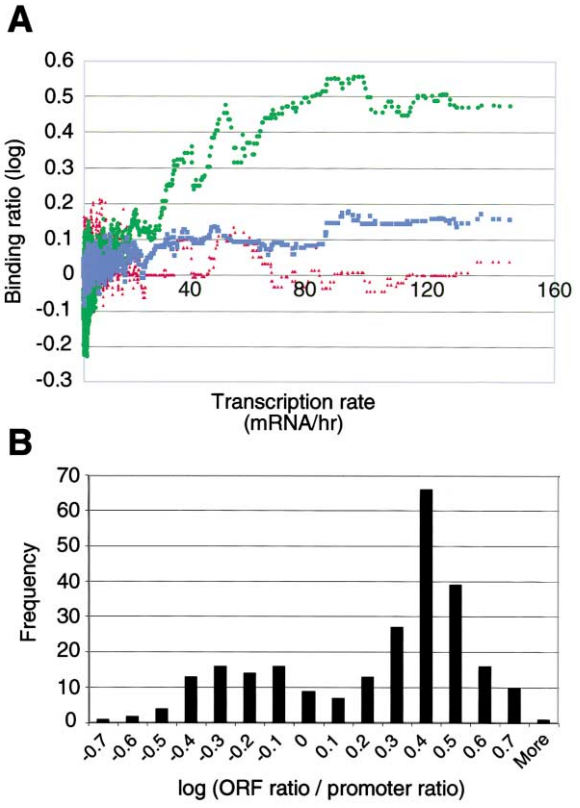

Figure 1. Set1 Associates with Transcriptional Active Genes

(A) Set1 occupancy correlates with Pol II transcription rate. The log binding ratio of the immunoprecipitated (IP) and whole-cell extract (WCE) DNAs for Set1 binding to all yeast ORFs (green circles), Set1 binding to all intergenic regions (blue squares), and Rsc8 binding to all intergenic regions (Ng et al., 2002b; red triangles) was determined, and moving median (window size = 20) is plotted as a function of expression level as determined by microarray analysis (Holstege et al., 1998).

(B) Preferential Set1 binding to ORFs versus promoter regions. The frequency of the log transformation of the binding ratio at the ORF over binding ratio at the promoter is shown for all genes associated with Set1 ($p < 0.01$ for either ORF or promoter). Positive values represent increasingly higher binding at the respective ORFs, whereas negative values represent increasingly higher binding at the respective promoters.

Strikingly, Set1 is localized specifically to the 5′ portion of the coding regions of the divergently transcribed and highly active *RPS16A/RPL13B* genes (Figure 2A). Although fold enrichments do not represent absolute measurements of genome occupancy, Set1 association at these active ribosomal protein genes is remarkably high (60-fold over background) and far above that observed for other chromatin-modifying complexes (typically 5- to 10-fold at best using proteins tagged with the same epitope). Higher resolution mapping of the *TSL1* gene indicates that the region occupied by Set1 is about 300 bp (Figure 2B), which corresponds to 2 nucleosomes and is quite similar to the domain of localized histone deacetylation that occurs upon recruitment by a specific DNA binding repressor (Kadosh and Struhl, 1998; Rundlett et al., 1998). Similar results are observed with Bre2, an integral component of the Set1 complex (Figure 2C). Thus, the Set1 complex associates specifically with a

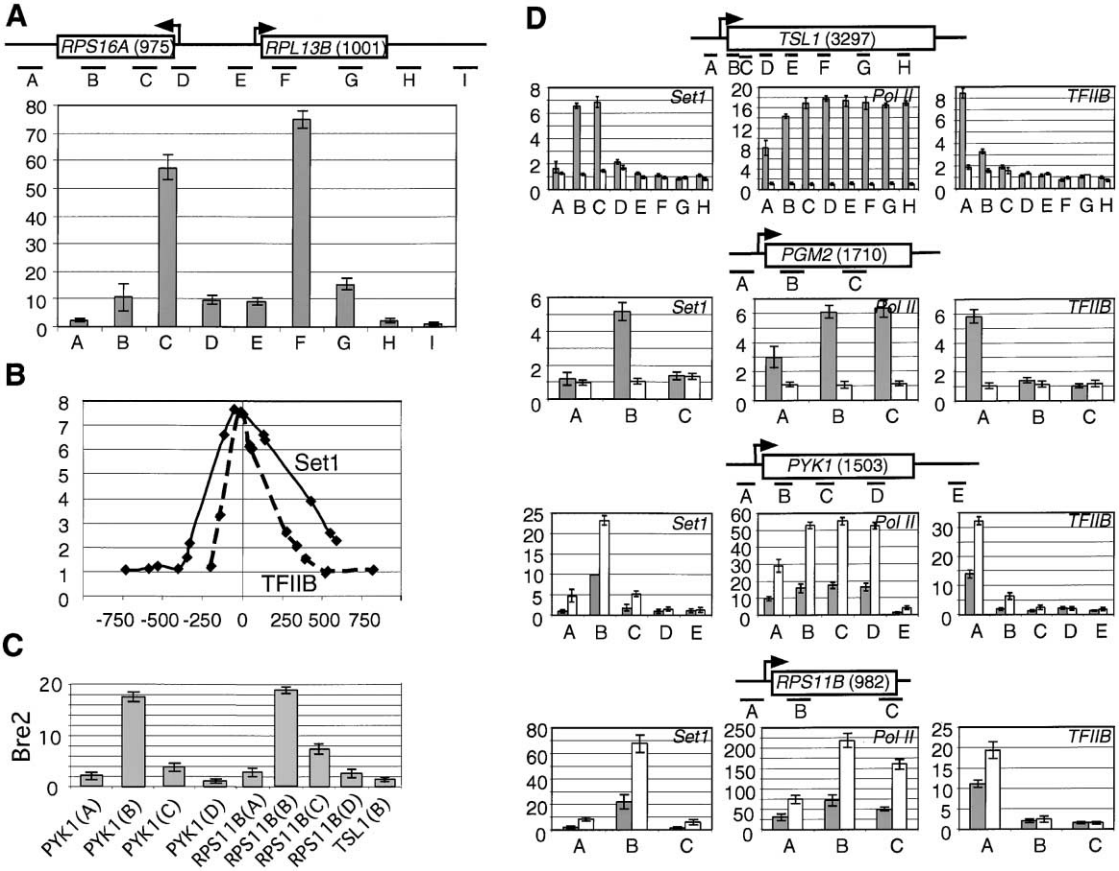

Figure 2. Set1 Associates with a Highly Localized Domain of the mRNA Coding Region

(A) Set1 occupancy (fold enrichment over an intergenic region on chromosome VIII) at the indicated regions (defined by PCR primer pairs) of the *RPS16A/RPL13B* locus (drawn to scale).

(B) Set1 and TFIIB occupancy at various regions of the *TSL1* locus (defined by the centers of the PCR products in bp, with position 0 representing peak occupancy) in cells growing in 3% ethanol as the carbon source. With respect to the ATG initiation codon, peak TFIIB occupancy occurs 200 bp upstream, and peak Set1 occupancy occurs 200 bp downstream. The Set1 occupancy profile is approximately 300 bp broader than the TFIIB occupancy profile, indicating that Set1 is localized over a domain of 2 nucleosomes.

(C) Occupancy of Bre2, a component of the Set1 complex, with the indicated genomic regions.

(D) Set1 occupancy at the 5′ portion of the mRNA coding region correlates with transcriptional activity. Set1, TFIIB, and Pol II occupancies at the indicated regions of *TSL1*, *PGM2*, *PYK1*, and *RPS11B* in ethanol-grown cells (gray bars) that were shifted for 1 hr to medium containing 3% glucose (white bars).

limited 5′ portion of the mRNA coding region in vivo. This striking localization pattern is distinct from all previously described proteins that are involved in transcriptional initiation, elongation, mRNA capping, splicing, and nuclear export (Komarnitsky et al., 2000; Cho et al., 2001; Lei et al., 2001; Krogan et al., 2002b; Licatalosi et al., 2002; Pokholok et al., 2002; Strasser et al., 2002).

Set1 association with 5′ mRNA coding regions is dynamic in accord with changes in gene expression (Figure 2D). When cells are switched from ethanol-containing to glucose-containing medium, *TSL1*, *PGM2*, and *GSY2* are repressed, whereas *RPB11B*, *RPL2B*, and *PYK1* are induced. As expected, TFIIB and Pol II occupancies under these conditions correlate with transcriptional activity, with TFIIB being localized to the promoter and Pol II associated over the entire mRNA coding region (Figure 2D, data not shown). In all cases tested, Set1 is recruited to the 5′ portion of the coding regions of these genes only under conditions when they are active. Thus, Set1 is recruited to specific genomic regions by a mechanism fundamentally different from previously examined chromatin-modifying activities, all of which are recruited to Pol II promoters by specific DNA binding regulators in a manner independent of transcriptional activity (Cosma et al., 1999; Agalioti et al., 2000; Goldmark et al., 2000; Martens and Winston, 2002; Ng et al., 2002b).

Set1 Targeting to mRNA Coding Regions Is Mediated by Elongating Pol II and Requires CTD Phosphorylation at Serine 5

The fact that Set1 associates with a specific location at the beginning of active Pol II coding regions strongly suggests that the Pol II elongation machinery targets Set1. Thus, mutations in the transcription apparatus that affect elongation but not formation of the preinitiation complex should affect Set1 recruitment to the coding region. Indeed, Set1 occupancy is drastically reduced upon thermal inactivation of Kin28 (Figure 3A), the TFIIH-

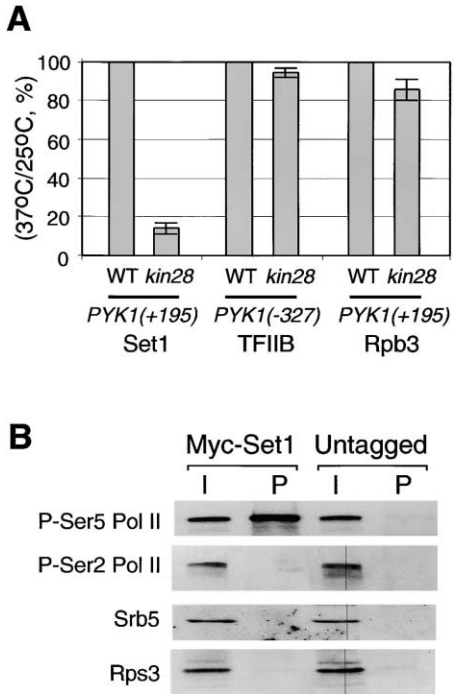

Figure 3. Set1 Recruitment Requires Kin28-Dependent Phosphorylation of Pol II

(A) Set1, TFIIB, and Pol II occupancies at the indicated *PYK1* regions in wild-type and *kin28* ts strains; data is plotted as the percent occupancy after a 1 hr shift to 37°C with respect to occupancy at 25°C. Western blotting indicates that levels of myc-tagged Set1 are unaffected by inactivation of Kin28 (data not shown).

(B) Set1 coimmunoprecipitates with Pol II phosphorylated at serine 5 but not serine 2 on the CTD. Cell-free extracts containing myc-tagged or untagged Set1 were immunoprecipitated with anti-myc antibody. Input (I) and immunoprecipitated (P) samples were examined by Western blotting using the indicated antibodies. Indistinguishable results were observed when immunoprecipitations were performed in the presence of 200 μg/ml ethidium bromide.

associated kinase that phosphorylates the Pol II CTD at serine 5 and mediates the transition between transcriptional initiation and elongation (Komarnitsky et al., 2000). As expected (Kuras and Struhl, 1999; Komarnitsky et al., 2000), inactivation of Kin28 does not affect promoter occupancy of transcriptional initiation factors such as TBP, TFIIB, and Pol II.

Set1 coimmunoprecipitates Pol II whose CTD is phosphorylated at serine 5 (Figure 3B), the residue affected by Kin28 in vivo (Komarnitsky et al., 2000). Importantly, Set1 does not associate with Pol II phosphorylated at serine 2 of the CTD (Figure 3B), the form of Pol II that is generated by Ctk1 kinase and preferentially associates with 3′ portions of the mRNA coding regions (Komarnitsky et al., 2000; Cho et al., 2001). In addition, Set1 does not associate with Srb5, a subunit of the mediator complex that is required for preinitiation complex formation in vivo (Kuras and Struhl, 1999; Li et al., 1999) and is localized at the promoter (Pokholok et al., 2002). Thus, recruitment of Set1 to the coding region occurs after preinitiation complex formation, and it requires Kin28-mediated phosphorylation of the Pol II CTD at serine 5 and association with a specific form of elongating Pol II.

Targeted Recruitment of Set1 Generates a Localized Domain of H3-K4 Trimethylation, Whereas Untargeted Action of Set1 Mediates Genome-Wide H3-K4 Dimethylation

Lysines 4 and 79 of histone H3 are found in different methylation states, ranging from mono-, di-, and trimethyl (Santos-Rosa et al., 2002; van Leeuwen et al., 2002), and Set1 mediates both di- and trimethylation of H3-K4 in vitro (Santos-Rosa et al., 2002). It has been reported that di- and trimethylated H3-K4 are associated with both the intergenic and the coding region of transcriptionally active genes (Bernstein et al., 2002; Santos-Rosa et al., 2002). However, these studies did not map the location of dimethylated and trimethylated H3-K4, an important issue given our finding that Set1 localization is highly localized to the 5′ portion of active coding regions.

There is a striking correlation between trimethylated H3-K4 and Set1 occupancy, with H3-K4 trimethylation being concentrated at the 5′ portion of coding region of all active genes tested and essentially absent at the inactive *TSL1* gene (Figure 4). In contrast, the pattern of dimethylation correlates poorly with Set1 occupancy. As previously reported (Bernstein et al., 2002), the level of dimethylated H3-K4 appears to be higher at coding regions than at promoters. However, as assayed by a myc-tagged version of histone H4, nucleosome occupancy at the promoter regions of *RPS11B*, *PYK1*, and *TSL1* is lower than at the corresponding ORFs (Figure 4). When normalized to the level of myc-H4, the level of dimethylated H3-K4 is constant throughout these genes. This genome-wide histone H3-K4 dimethylation presumably reflects nontargeted and relatively inefficient association of Set1 with bulk nucleosomes, in a manner similar to that of histone acetylases and deacetylases (Kuo et al., 2000; Reid et al., 2000; Vogelauer et al., 2000; Katan-Khaykovich and Struhl, 2002) and Dot1 histone methylase (Ng et al., 2003). Thus, targeted recruitment of Set1 directly generates a localized domain of chromatin with trimethylated H3-K4, presumably due to its high local concentration at discrete sites within active Pol II mRNA coding regions. It is possible that Set1 recruitment might also result in methylation of a component of the elongating Pol II machinery or some other nonhistone substrate.

Rtf1 and Paf1 Components of the Paf1 Complex Are Important for Targeting of Set1 to mRNA Coding Regions

Kin28-mediated phosphorylation of the Pol II CTD at serine 5 is necessary for Set1 recruitment (Figure 3A), but it is not sufficient because phosphorylation of CTD-serine 5 occurs at the promoter, yet Set1 association is restricted to the 5′ portion of the mRNA coding region. Given the direct connection between Set1 and transcriptional elongation, we examined whether Set1 recruitment involves a factor(s) that interacts with Pol II, associates with active mRNA coding regions in vivo, and is implicated in Pol II elongation. We first determined levels of trimethylated H3-K4, a specific marker of targeted Set1 recruitment, in a set of mutant strains lacking such elongation factors. Interestingly, strains lacking Rtf1 or Paf1 display drastically reduced levels of trimethylated

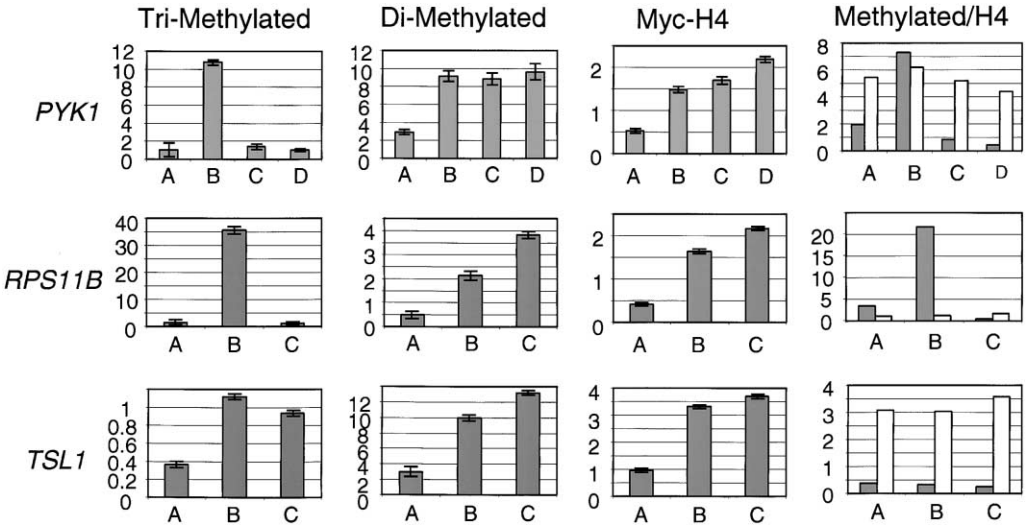

Figure 4. Di- and Trimethylated H3-K4

Levels of dimethylated H3-K4, trimethylated H3-K4, and myc-tagged H4 (fold enrichment over the intergenic region of chromosome VIII) at the indicated regions (see Figure 2B) of *PYK1*, *RPS11B*, and *TSL1*. The normalized (to myc-H4) levels of dimethylated (white bars) and trimethylated (gray bars) are indicated.

H3-K4, whereas all other mutant strains behave indistinguishably from the wild-type strain (Figure 5A). Rtf1 and Paf1 are components of the Pol II-associated Paf complex that plays a role in transcriptional elongation (Costa and Arndt, 2000; Krogan et al., 2002b; Mueller and Jaehning, 2002; Pokholok et al., 2002; Squazzo et al., 2002). Unlike Rtf1 and Paf1, the Cdc73 and Leo1 components of the Paf1 complex do not affect the level of H3-K4 trimethylation. Association of Set1 and Bre2 with the 5′ portions of the *RPS11B* and *PYK1* coding regions is reduced 3- to 5-fold in an *rtf1* deletion strain and 6- to 10-fold in a *paf1* deletion strain. Set1 protein is expressed at

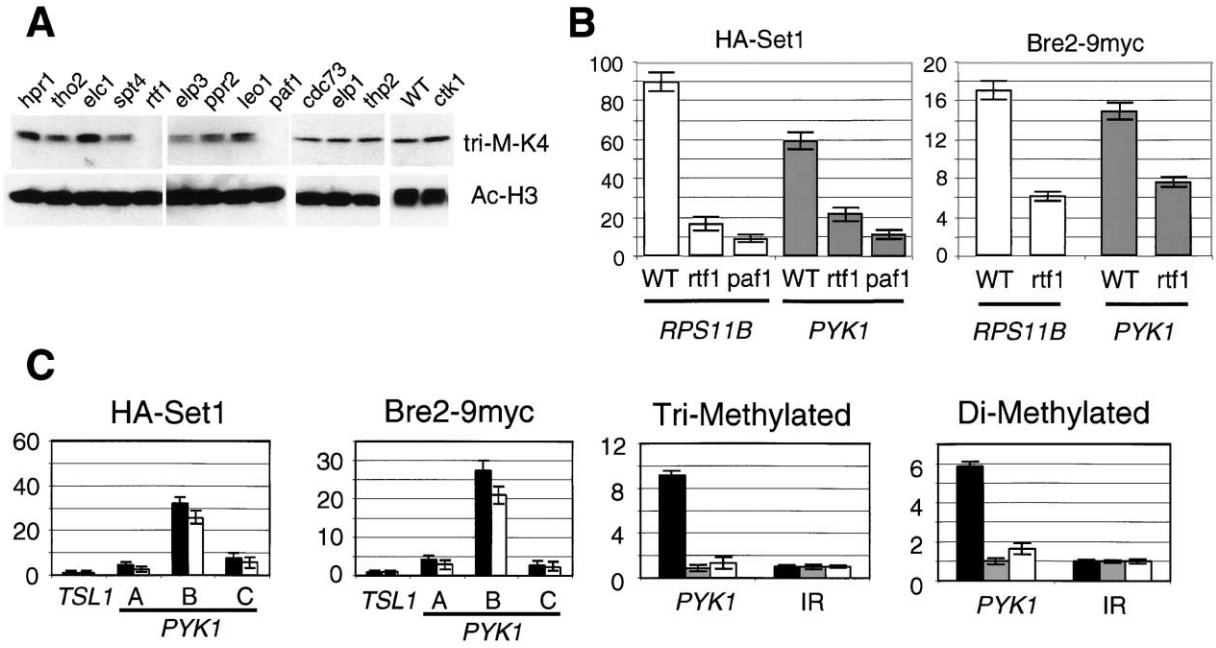

Figure 5. Rtf1 and Paf1 but Not Rad6 Are Important for Set1 Recruitment

(A) Western blots of yeast whole-cell extracts from the wild-type and indicated mutant strains were probed with antibodies against the acetylated H3 tail or trimethylated H3-K4.

(B) Set1 and Bre2 occupancies within the *RPS11B* (white bars) and *PYK1* (gray bars) coding regions in wild-type and *rtf1* and *paf1* deletion strains. Western blotting indicates that levels of HA-tagged Set1 or myc-tagged Bre2 are unaffected by loss of Rtf1 or Paf1 (data not shown).

(C) Set1 and Bre2 occupancies and levels of di- and trimethylated H3-K4 at the indicated regions (see Figure 2B; IR is an intergenic region) in wild-type (black bars), *rad6* mutant (white bars), and *set1* (gray bars) strains.

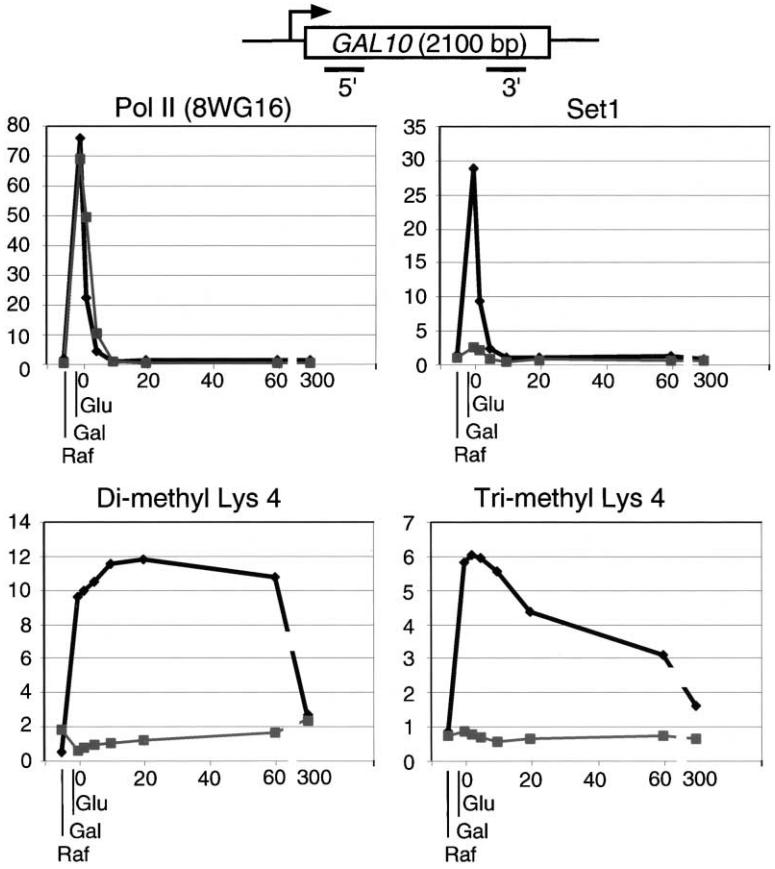

Figure 6. Hypermethylation of H3-K4 Persists after Transcriptional Inactivation and Set1 Dissociation

Set1 and Pol II occupancies and levels of di- and trimethylated H3-K4 at the 5′ (black) and 3′ (gray) regions of *GAL10* in cells grown in 2% raffinose (Raf), induced in 2% galactose for 1.5 hr (Gal), and subjected to 2% glucose shut-off for the indicated times.

wild-type levels in *rtf1* and *paf1* mutant strains (data not shown). Thus, Rtf1 and Paf1 are important (although not absolutely required) for targeting of Set1 to mRNA coding regions.

Set1-dependent methylation of H3-K4 in vivo depends on Rad6-dependent ubiquitination of histone H2B at lysine 123 (Dover et al., 2002; Sun and Allis, 2002). However, recruitment of Set1 or Bre2 to the 5′ proximal region of all coding regions tested occurs at normal levels in strains lacking Rad6, even though H3-K4 methylation at these regions is virtually abolished (Figure 5C). Thus, the lack of H3-K4 methylation in *rad6* mutant cells is not due to a lack of recruitment of Set1, and targeting Set1 to nonubiquitinated nucleosomes does not result in H3-K4 methylation in vivo.

H3-K4 Methylation within mRNA Coding Regions Is Maintained for Considerable Time after Transcriptional Inactivation and Set1 Dissociation

The rapid and regulated recruitment of Set1 to active genes provides an approach for determining the dynamics of histone methylation in vivo. Specifically, we monitored Pol II and Set1 occupancies and the level of dimethylated and trimethylated H3-K4 within the *GAL10* coding region during a time course experiment in which *GAL10* expression was rapidly induced or shut off (Figure 6). For unknown reasons, the level of H3-K4 dimethylation within the *GAL10* coding region under noninducing conditions (raffinose medium) is about 3-fold lower than that observed at other genomic regions. As expected, galactose induction results in the rapid association of Set1 and Pol II with the *GAL10* coding region, with Set1 localized to the 5′ proximal region and Pol II associated throughout the gene. The induced recruitment of Set1 leads to a concomitant increase in both di- and trimethylation of H3-K4, with both forms being localized to the 5′ region of the gene. The atypical increase in dimethylated H3-K4 levels upon induction of *GAL10* is likely due to a combination of unusually low levels in uninduced cells and high Set1 occupancy in induced cells. Upon switching to glucose medium, Pol II and Set1 occupancies are rapidly and dramatically reduced with similar kinetics, again suggesting an intimate link between Pol II and Set1.

Interestingly, despite the rapid dissociation of Set1, both the di- and trimethylated states of H3-K4 persist for considerable time. After 60 min, the trimethylated state is reduced only 2-fold, whereas the dimethylated state is unchanged. Thus, even though the gene is inactive, the persistence of methylated H3-K4 forms at the 5′-proximal region of *GAL10* represents a genomic mark of recent transcriptional activity. This genomic mark does not persist indefinitely; the levels of dimethylated and trimethylated H3-K4 return to near-background levels after 5 hr. These observations suggest that the induced state of H3-K4 methylation is not transmitted

to newly synthesized histones that are assembled into chromatin and hence is not stably inherited through cell divisions.

Discussion

Recruitment of Set1 Histone Methylase to a Highly Localized Portion of Active mRNA Coding Regions

Many chromatin-modifying activities are targeted in a highly localized manner to promoter regions by activators and repressors (see Introduction). Here, we show that, unlike all chromatin-modifying activities examined to date, Set1 histone methylase is not recruited to gene-regulatory sequences but rather is targeted to active mRNA coding regions. Targeting of Set1 within the mRNA coding region is highly localized to a domain that is comparable in size (approximately 2 nucleosomes) to that observed for activator- or repressor-mediated recruitment of chromatin-modifying activities to promoters. Such highly localized targeting within a specific portion of an mRNA coding region has not been observed for any protein involved in processes that generate functional mRNAs (e.g., capping, splicing, polyadenylation, and nuclear export). Thus, Set1 represents a direct and unique connection between transcriptional elongation and a specific chromatin-modifying activity.

Recruitment of Set1 by the Elongating Pol II Machinery

The transition between transcriptional initiation and elongation results in a dramatic change in the factors that associate with Pol II in vivo (Pokholok et al., 2002). During the process of initiation at the promoter, Pol II associates with general transcription factors and mediator proteins. After TFIIH-dependent phosphorylation of Pol II, these initiation factors dissociate from Pol II and are replaced by a variety of so-called elongation factors, such as the Spt4,5, TREX, and Paf complexes. These elongation factors associate with the entire mRNA coding region (Andrulis et al., 2000; Kaplan et al., 2000; Krogan et al., 2002b; Pokholok et al., 2002; Strasser et al., 2002), and in some cases it has been shown that they travel with elongating Pol II (Strasser et al., 2002).

The striking localization of Set1 to the 5′ end of the mRNA coding region is distinct from all previously described proteins involved in elongation and other post-initiation processes (Komarnitsky et al., 2000; Cho et al., 2001; Lei et al., 2001; Krogan et al., 2002b; Licatalosi et al., 2002; Pokholok et al., 2002; Strasser et al., 2002). Thus, Set1 is recruited by, but does not travel with, the elongating Pol II machinery. Set1 recruitment to mRNA coding regions depends on TFIIH-dependent phosphorylation of the Pol II CTD at serine 5, and Set1 interacts with Pol II that is phosphorylated at CTD-serine 5. However, TFIIH-dependent phosphorylation of the Pol II CTD at serine 5 can occur at the promoter (Komarnitsky et al., 2000) and hence is not sufficient for Set1 recruitment, which is restricted to the mRNA coding region. Set1 recruitment to mRNA coding regions also involves the Rtf1 and Paf1 components of the Pol II-associated Paf complex. However, the Paf complex associates with essentially the entire active mRNA coding region (Krogan et al., 2002b; Pokholok et al., 2002), indicating that

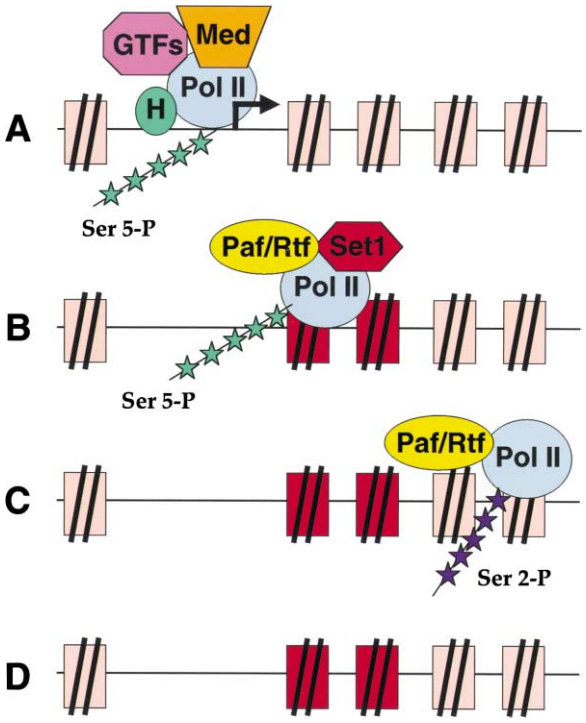

Figure 7. Model for Set1 Recruitment and Memory of Recent Transcriptional Activity

The diagram illustrates a single round of transcription.

(A) Preinitiation complex containing Pol II, mediator (Med), TFIIH (H), and other general transcription factors (GTFs) at the initiation site (arrow). TFIIH phosphorylates the CTD (line) at serine 5 (green stars). Nucleosomes contain dimethylated H3-K4 (pink).

(B) Early stage of elongation in which mediator and general transcription factors have dissociated and the Set1 and Paf/Rtf complexes are recruited to the 5′ portion of the coding region. Set1 generates a localized domain of H3-K4 trimethylation (red nucleosomes).

(C) Late stage of elongation in which CTD becomes phosphorylated at serine 2 (purple stars) and dephosphorylated at serine 5. Set1 dissociates from the elongating Pol II machinery, whereas the Paf/Rtf complex remains associated.

(D) Localized mark of H3-K4 trimethylation persists for considerable time after cessation of transcription.

the presence of the Paf complex on an active gene does not necessarily result in Set1 association. Thus, Set1 is recruited by a specific and heretofore undescribed form of the elongating Pol II complex

The simplest model is that Set1 recruitment occurs specifically with elongating Pol II during the exchange of the associated initiation and elongation factors (Figure 7). We suggest that Set1 association occurs after dissociation of the mediator complex from Pol II and around the time or after association of the Paf complex (and possibly other factors that travel with elongating Pol II). Although TFIIH-dependent phosphorylation of CTD-serine 5 at the promoter initiates this transition, we presume that mediator dissociation and/or Paf complex association occur downstream of the promoter. In this view, Set1 association would depend on a form of Pol II that is phosphorylated at CTD-serine 5 and contains the Paf complex (and perhaps other elongation factors),

and Set1 association with Pol II might be inhibited by the mediator complex. We further suggest that continued Set1 association with the mRNA coding region does not occur because CTD-serine 5 phosphorylation is strongly biased to the 5′ coding region (Komarnitsky et al., 2000), presumably reflecting the activity of a CTD-serine 5 phosphatase. Consistent with this idea, Set1 does not associate with Pol II phosphorylated at serine 2 of the CTD (Figure 3B), and Ctk1-mediated phosphorylation of serine 2 occurs preferentially at downstream portions of mRNA coding regions (Komarnitsky et al., 2000; Cho et al., 2001). It is also possible that Set1 association might be actively inhibited by CTD-serine 2 phosphorylation or by the full complement of associated factors that travel with Pol II throughout the entire gene. Although the molecular details remained to be determined, our results clearly demonstrate that Set1 recruitment defines a distinct, and previously undescribed, stage of the Pol II transcription process that occurs after initiation.

Localized Marking of Active Pol II Genes by Targeted Recruitment of Set1 Provides a Memory of Recent Transcriptional Activity

Targeted H3-K4 trimethylation (and induced dimethylation) constitutes a molecular memory within the mRNA coding region for recent transcriptional activity. Specifically, H3-K4 trimethylation in the mRNA coding region occurs only during the act of transcription, yet this specific and localized chromosomal mark persists for considerable time after transcriptional inactivation and Set1 dissociation (Figure 7). In other words, H3-K4 trimethylation within a given mRNA coding region informs the cell that transcription of that gene occurred in the recent past but is not necessarily happening at the present time. Thus, by the definition of memory, during the time period when a gene contains a localized mark of H3-K4 trimethylation yet is transcriptionally inactive, the cell remembers that this gene was transcribed recently.

This molecular memory clearly lasts for a significant portion of an individual cell cycle, and the induced modifications might be transmitted to new progeny after cell division. However, this memory of recent transcriptional activity is not faithfully transmitted to all daughter cells and hence is mechanistically distinct from long-term epigenetic memory as occurs in transcriptional silencing and position-effect variegation. Of particular interest, the relatively short-term memory described here is different from the long-term memory during *Drosophila* development mediated by the Set1-like trithorax protein (Petruk et al., 2001; Poux et al., 2002) or from epigenetic silencing in yeast heterochromatin which is affected by Set1 itself (Briggs et al., 2001; Bryk et al., 2002). Thus, H3-K4 methylation is involved in biologically and mechanistically distinct short-term and long-term memories of transcriptional activity.

In principle, loss of H3-K4 trimethylation (and induced dimethylation) upon transcriptional inactivation can be due to nontransmission of the modification to both sister chromosomes during DNA replication and/or to nonreplicative exchange of histones (or, less likely, active histone demethylation). The loss of H3-K4 trimethylation (and induced dimethylation) occurs somewhat more rapidly than can be accounted for solely by dilution upon cell division, suggesting that nonreplicative histone exchange contributes to removal of the memory mark. Histone exchange during this time would favor dimethylated H3-K4 over the trimethylated form, and this (and perhaps also incomplete dissociation of Set1 at the early time points) might explain why dimethylated H3-K4 levels decrease more slowly than trimethylated H3-K4 levels upon transcriptional inactivation.

Why Do Cells Have a Memory Mechanism for Recent Transcriptional Activity?

Our results clearly demonstrate that yeast cells have evolved a robust and highly specific memory mechanism for recent transcriptional activity. Set1 targeting is robust (as high as 80-fold over background), highly specific (restricted to a limited domain of active Pol II genes), dynamic (Set1 association and dissociation occurs within 1–2 min), and it generates a specific and diagnostic molecular signal (H3-K4 trimethylation) that is relatively stable and not easily reversed. It is inconceivable that such a robust and highly specific phenomenon occurring in living wild-type cells under normal physiological conditions represents a biological artifact or evolutionary accident.

There are many possible reasons, not mutually exclusive, why cells have evolved such a memory mechanism. First, this mechanism might be important for maintaining, but not initiating, an active transcriptional state, particularly given the distinct genetic requirements for transcriptional induction and steady-state maintenance (Klein and Struhl, 1994; Bhaumik and Green, 2001; Larschan and Winston, 2001; Reinke et al., 2001). Second, this memory mechanism might be important for genes that are rapidly switched on and off by environmental changes. Most stress-inducible transcriptional responses in yeast cells are transient (Gasch et al., 2000; Causton et al., 2001), and genes regulated in such a fashion will retain a memory of their recent activation. Third, H3-K4 trimethylation might be an inhibitory mark to prevent recently active genes from becoming silenced, and in this regard, the Sir silencing proteins preferentially associate with chromatin containing undermethylated H3-K4 (Bernstein et al., 2002; Bryk et al., 2002). Fourth, the memory mechanism might not be relevant for mRNA levels per se but rather some other mRNA-related process (e.g., splicing, polyadenylation, or nuclear export) or for some other chromatin-dependent process (e.g., DNA repair, recombination, mitosis, meiosis, or long-range genomic or nuclear architecture) for which cells wish to distinguish between currently or recently active genes as opposed to inactive genomic regions. Whatever the ultimate purpose(s) of this memory phenomenon, our results demonstrate that yeast cells have evolved a robust, dynamic, and highly specific mechanism for targeting Set1 histone methylase to a localized domain of the mRNA coding region, thereby marking a gene that has undergone recent transcriptional activity.

Experimental Procedures

Yeast Strains

For most experiments, the wild-type strain was a derivative of FT4 (a *ura3*-52 *trp1*-Δ63 *his3*-Δ200 *leu2*::*PET56*) (Tzamarias and Struhl,

1994) that expresses a modified Set1 protein containing three copies of the HA epitope at the N terminus from the natural *SET1* promoter. This strain behaves indistinguishably from an isogenic strain with untagged Set1 as assayed by growth and H3-K4 methylation, and Western blotting indicates that it expresses $(HA)_3$-Set1 of the predicted molecular weight. Bre2 was tagged at its C termini with a 9myc::*TRP1* cassette (Cosma et al., 1999) in a W303 AR5 strain background. Histone H4 occupancy was monitored using a strain expressing Myc-tagged histone H4 (Ng et al., 2003). For the experiment in Figure 3A isogenic wild-type and *kin28*-ts16 strains (Cismowski et al., 1995) were analyzed as described previously (Kuras and Struhl, 1999). To screen for genes affecting H3-K79 trimethylation, we used deletion derivatives of BY4741 (*MAT***a**, *his3*-Δ1, *leu2*-Δ0, *met15*-Δ0, *ura3*-Δ0), which were obtained from Research Genetics. Preparation of cell-free extracts and Western blot analysis were performed as described previously (Ng et al., 2002a).

Genome-Wide Location Analysis

Genome-wide location analysis of $(HA)_3$-Set1 was performed on microarrays containing 6400 intergenic regions and protein coding regions as described previously (Ng et al., 2002b). At least three independent chromatin preparations were immunoprecipitated with monoclonal anti-HA antibodies (F7, Santa Cruz Biotechnology). Searchable and downloadable datasets are available at the author's website at http://web.wi.mit.edu/young/set1.

Chromatin Immunoprecipitation

Chromatin immunoprecipitation was carried out with a modified version of a procedure described previously (Kuras and Struhl, 1999). Cells (200 ml at A_{600} = 0.8) were fixed in 1% formaldehyde for 20 min at room temperature and lysed with zirconia-silica beads (BioSpec Products) in a mini-bead beater (BioSpec Products). Chromatin was first pelleted by high-speed centrifugation and then solubilized by sonication (Branson Sonifier 350, five times, 100% duty, power 5, 40 s for each cycle). The resulting DNA has an average length of 350 bp (100 to 650 bp in range). Crosslinked chromatin was immunoprecipitated with monoclonal antibodies against the HA (F7, Santa Cruz Biotechnology) and myc (9e10, Upstate Biotechnology) epitopes, Rbp1 (8WG16, Covance), Rps3 (1Y26, NeoClone Biotechnology), and polyclonal antibodies against di and trimethylated H3-K4 (AbCam) and TFIIB. Quantitative PCR analyses (primer sequences are available at http://web.wi.mit.edu/young/set1) were performed in real time using an Applied Biosystems 7700 sequence detector. Relative occupancy values were calculated by determining the apparent immunoprecipitation efficiency (amount of PCR product in the immunoprecipitated sample divided by the amount of PCR product in the input sample) and normalized to the level observed at an intergenic region of chromosome VIII, which was defined as 1.0.

Coimmunoprecipitation Experiments

Whole-cell extracts were prepared from yeast strain W303 and a derivative expressing myc-Set1 from the *ADH1* promoter. Four milligrams of each extract was incubated overnight at 4°C with anti-myc-coupled Dynabeads in E buffer (20 mM HEPES [pH 8], 350 mM NaCl, 10% glycerol, 0.1% Tween 20) with or without β-mercaptoethanol (200 μg/ml). Beads were washed five times with E buffer and loaded on 4%–15% SDS page gradient gel together with 50 μg of the input material. Proteins were analyzed by Western blotting using antibodies against CTD-phosphoserine 5 (H14, Covance), CTD-phosphoserine 2 (H5, Covance), Rpb3 (NeoClone Biotechnology), Srb5, and Rps3 (kindly provided by Matthias Seedorf).

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