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Cotranscriptional Set2 Methylation of Histone H3 Lysine 36 Recruits a Repressive Rpd3 Complex

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Summary

The yeast histone deacetylase Rpd3 can be recruited to promoters to repress transcription initiation. Biochemical, genetic, and gene-expression analyses show that Rpd3 exists in two distinct complexes. The smaller complex, Rpd3C(S), shares Sin3 and Ume1 with Rpd3C(L) but contains the unique subunits Rco1 and Eaf3. Rpd3C(S) mutants exhibit phenotypes remarkably similar to those of Set2, a histone methyltransferase associated with elongating RNA polymerase II. Chromatin immunoprecipitation and biochemical experiments indicate that the chromodomain of Eaf3 recruits Rpd3C(S) to nucleosomes methylated by Set2 on histone H3 lysine 36, leading to deacetylation of transcribed regions. This pathway apparently acts to negatively regulate transcription because deleting the genes for Set2 or Rpd3C(S) bypasses the requirement for the positive elongation factor Bur1/Bur2.

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Introduction

Eukaryotic transcription occurs in the context of chromatin. Accessibility of genes within chromatin is regulated in at least two ways. First, ATP-dependent nucleosome-remodeling complexes can reposition nucleosomes. Second, posttranslational modification of histones can alter chromatin compaction or recruit additional factors. Covalent modifications of nucleosomes include serine phosphorylation and lysine acetylation, methylation, or ubiquitination (Strahl and Allis, 2000). The addition and removal of chemical moieties on histone tails is a dynamic process that both influences and is influenced by transcription. Numerous histone acetylases (HATs) and deacetylases (HDACs) acting at specific residues have been linked to transcriptional activation or repression, as well as DNA replication and repair (Kurdistani and Grunstein, 2003). While there is a general correlation between histone acetylation and transcriptional activity, there are exceptions at some residues (Wang et al., 2002).

Rpd3, the prototypical yeast HDAC, functions as a transcriptional repressor when targeted to promoters such as *INO1*, *IME2*, and *SPO13* by the DNA binding factor Ume6 (Kadosh and Struhl, 1997, 1998; Rundlett et al., 1998; Suka et al., 2002). Rpd3 is known to function as part of a protein complex that contains Ume1, Sin3, Sap30, Sds3, Pho23, and Cti6/Rxt1 (Kurdistani et al., 2002; Lechner et al., 2000; Loewith et al., 2001; Puig et al., 2004; Zhang et al., 1998). Surprisingly, Rpd3 antagonizes Sir2-mediated repression at telomeres, ribosomal loci, and HMR (Sun and Hampsey, 1999). Here we describe a second Rpd3 complex, Rpd3C(S), that functions in the body of genes.

While histone acetylation has been actively investigated for a number of years, the effects of histone methylation have only been appreciated more recently (Kouzarides, 2002; Lachner and Jenuwein, 2002). Methylation occurs at multiple residues on all four histones. Methylations are not uniform throughout the genome, and specific modifications have been correlated with transcription activation or repression (Briggs et al., 2001; Krogan et al., 2002a, 2003c; Ng et al., 2002, 2003; van Leeuwen et al., 2002). At least two yeast histone methyltransferases (HMTs), Set1 and Set2, are recruited to RNA polymerase II (RNApII) transcription complexes at distinct points of the transcription cycle by binding phosphorylated forms of the Rpb1 C-terminal domain (CTD) (Hampsey and Reinberg, 2003). Set1 HMT activity and histone H3-K4 methylation is localized to the 5′

ends of genes through binding serine 5-phosphorylated CTD. In a complementary pattern, Set2 is recruited by CTD serine 2 phosphorylation, which localizes H3-K36 methylation to the body of genes (Krogan et al., 2003c; Li et al., 2003; Schaft et al., 2003; Xiao et al., 2003). Surprisingly, *set1*Δ or *set2*Δ cells have no growth defect, and the functions of these methylations have been difficult to discern.

The Bur1 kinase promotes RNApII transcription elongation (Keogh et al., 2003). Bur1 can phosphorylate RNApII in vitro (Murray et al., 2001), but it is unclear whether this is the physiological substrate (Keogh et al., 2003). The *BUR1* gene is classified as essential for viability because *bur1*Δ cells grow exceedingly slowly. We carried out a screen for gene deletions that could rescue *bur1*Δ cells, predicting that suppressors would encode negative transcription factors. Surprisingly, all isolated suppressors have a role in chromatin modification and include Set2 and RPD3C(S).

Based on biochemical and genetic analyses, we propose that Set2 methylates histone H3 lysine 36 to recruit Rpd3C(S) via its chromodomain subunit Eaf3. This pathway negatively impacts RNApII transcription elongation, an effect that is opposed by the positive elongation factor Bur1.

Results

Identification of New Rpd3-Interacting Proteins

As part of an ongoing effort to systematically purify and characterize transcription factors in *S. cerevisiae*, the HDAC Rpd3/Sin3 was purified using tandem affinity purification (TAP), and proteins were identified by mass spectroscopy. Five previously known Rpd3-interacting proteins were identified: Ume1, Sds3, Pho23, Sap30, and Cti6/Rxt1 (Kurdistani et al., 2002; Lechner et al., 2000; Loewith et al., 2001; Puig et al., 2004; Zhang et al., 1998) (Figure 1A). The purifications contained four additional factors: Rxt2/Ybr095c, Rco1/Ymr075w, Dep1/Yal013w, and Eaf3 (*E*sa1-*a*ssociated *f*actor *3*). Eaf3 was previously identified as a component of the HAT complex NuA4 (Eisen et al., 2001; Krogan et al., 2004a).

To determine whether these proteins were in a single complex, TAP was performed with tagged Ume1, Rco1, Eaf3, Rxt1, Pho23, or Sap30 strains. Two distinct Rpd3/Sin3/Ume1-containing complexes were apparent (Figure 1B; see also Figure S1 and Table S1 in the Supplemental Data available with this article online). Ume1-TAP copurified with both complexes. However, Rco1-TAP purified a five-protein Rpd3-containing complex (Rpd3, Sin3, Ume1, Eaf3, and Rco1), which we designated Rpd3C(S) (Figure 1B). The Eaf3 purification isolated both Rpd3C(S) and NuA4. When Rxt1, Pho23, or Sap30 was purified, we isolated a larger complex, designated Rpd3C(L), that contains Rpd3, Sin3, Ume1, Rxt1, Rxt2, Dep1, Sds3, Pho23, and Sap30 but not Rco1 or Eaf3 (Figure 1B).

Rpd3C(S) and Rpd3C(L) Are Functionally Distinct

To further characterize the Rpd3/Sin3 complexes, synthetic genetic array (SGA) analysis (Tong et al., 2001) was used to systematically identify genetic interactions. The *rpd3*Δ and *sin3*Δ deletion strains were crossed with a miniarray of 384 strains individually deleted of nonessential genes implicated in transcription or chromatin modification. The genetic interactions observed were very similar (Figure 2A and Table S2). Synthetic phenotypes were seen with deletions of subunits of the Set3 HDAC (*SET3*, *SNT1*, *SIF2*, *HOS2*) (Pijnappel et al., 2001) and two HAT complexes, SAGA (*SPT3* and *SPT8*) (Grant et al., 1998) and Elongator (*ELP3*, *ELP4*, and *ELP6*) (Krogan and Greenblatt, 2001; Li et al., 2001; Winkler et al., 2001; Wittschieben et al., 1999). Synthetic growth defects were also observed in combination with deletions of *HTZ1* (histone variant H2A.Z) or genes encoding the SWR complex (*SWR1*, *ARP6*, *SWC2*, *SWC3*, *SWC5*, *SWC6*), which incorporates

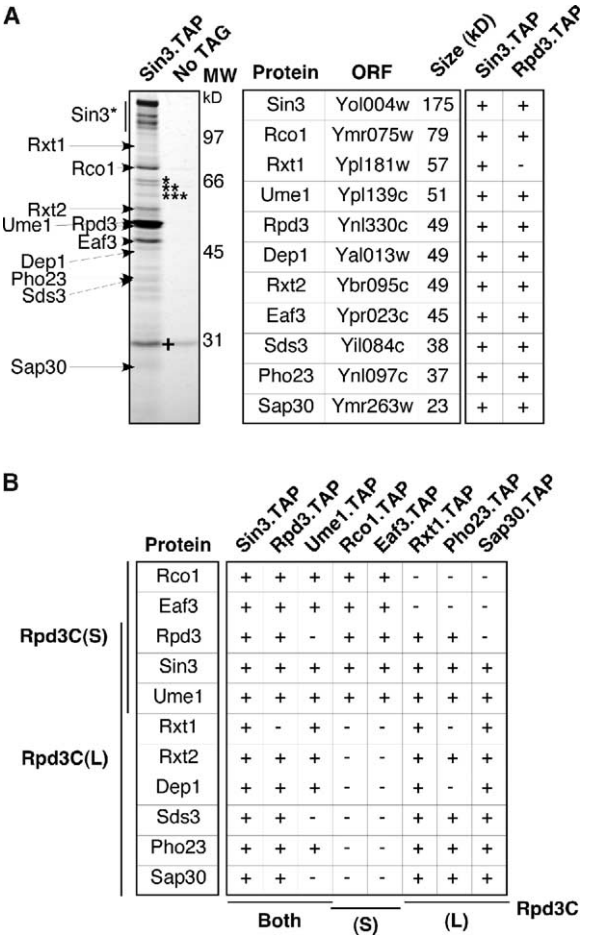
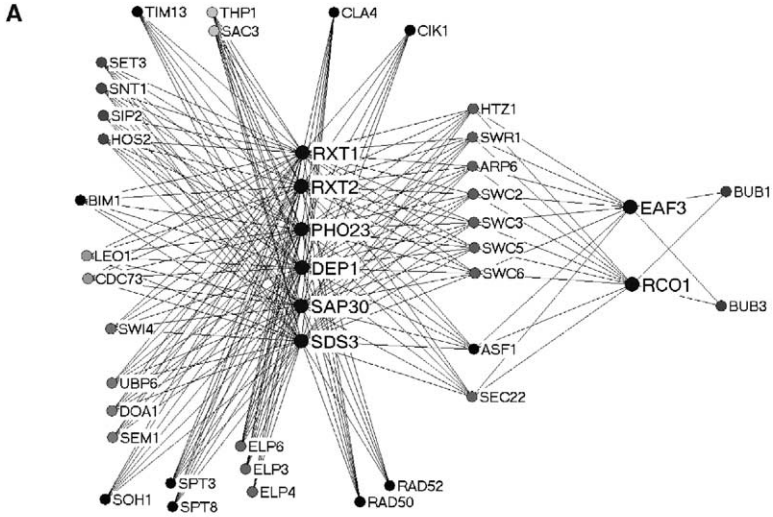


Figure 1. Identification of Two Distinct Rpd3/Sin3 Complexes

(A) Tandem affinity purification from a TAP-tagged Sin3 or an untagged strain. Proteins were resolved by SDS-PAGE, silver stained, and identified by trypsin digestion and mass spectrometry (left panel). Contaminating bands are indicated: ***, Sse1; **, Ssa1; *, Ssb1; +, TEV protease. The right panel summarizes proteins associated with Sin3-TAP and Rpd3-TAP.

(B) Purifications were performed with TAP-tagged Sin3, Rpd3, Ume1, Rco1, Eaf3, Rxt1, Pho23, or Sap30. A summary of the associated proteins identified by mass spectrometry is shown. See Figure S1 for representative purifications of individual complexes and Table S1 for the percent peptide coverage.



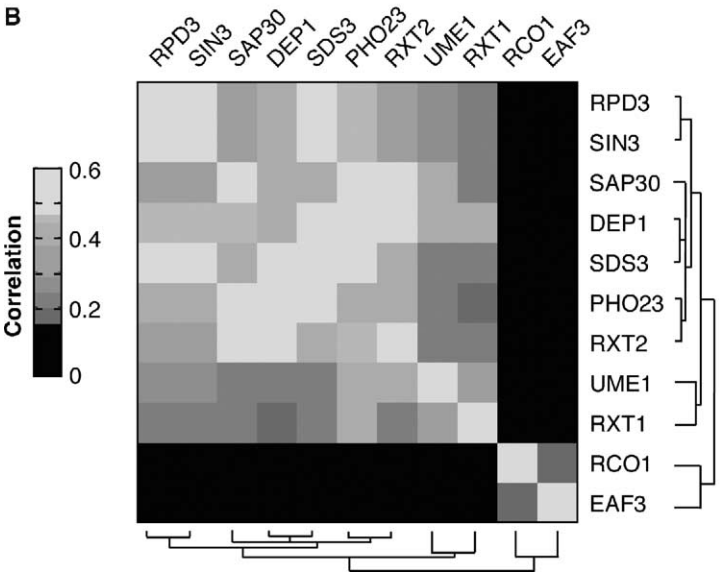


Figure 2. Rpd3C(S) and Rpd3C(L) Are Functionally Distinct

(A) Synthetic genetic interactions for Rpd3C(L) and Rpd3C(S). Lines represent either synthetic lethality or growth significantly slower than the individual mutants. Clusters indicate components of the same protein complex. The proximity of genes in this diagram is unrelated to the strength of the indicated interactions. Similar analyses of *rpd3*Δ and *sin3*Δ produced interactions with all the genes shown except those encoding components of Rpd3C(S) and Rpd3C(L). A more complete listing of the data is found in Table S2.

(B) Microarray analysis of gene expression was performed for the indicated deletion strains. Pearson correlation coefficients were calculated for each deletion pair and organized by 2D hierarchical clustering.

H2A.Z into chromatin (Kobor et al., 2004; Krogan et al., 2003b; Mizuguchi et al., 2004). Interactions were also seen with the chromatin-related factors *ASF1*, *ISW1*, and *CHD1* (Emili et al., 2001) and the PAF1 complex (*LEO1* and *CDC73*) (Krogan et al., 2002b; Mueller and Jaehning, 2002; Squazzo et al., 2002) that functionally links histone methylation to transcriptional elongation by RNApII (Krogan et al., 2003a, 2003c; Ng et al., 2003). Other interacting genes included the *SOH1* component of Mediator (Guglielmi et al., 2004; Linder and Gustafsson, 2004); two components of a complex involved in mRNA export, *THP1* and *SAC3* (Fischer et al., 2002); factors linked to the 19S proteasome (*UBP6*, *SEM1*, and *DOA1*) (Hochstrasser et al., 1999; Krogan et al., 2004b); and the *RAD50* and *RAD52* genes involved in DNA repair.

To evaluate the interaction patterns of genes encoding Rpd3-associated proteins, the corresponding deletion strains were also analyzed with SGA (Figure 2A). Two clear patterns emerged that correspond to the identified protein complexes (Figure 1B). Interaction patterns of *rxt1*Δ, *rxt2*Δ, *pho23*Δ, *dep1*Δ, *sap30*Δ, and *sds3*Δ were very similar to each other and shared the majority of the interactions observed with *rpd3*Δ and *sin3*Δ. However, these differed considerably from the *eaf3*Δ or *rco1*Δ patterns, which were very similar to each other (Figure 2A and Table S1).

The 384 deletion strains in our transcription/chromatin miniarray were crossed against a similar miniarray carrying a different marker, thereby creating a 384 × 384 E-MAP matrix of double-mutant haploid strains (Schuldiner et al., 2005). Growth rates were evaluated by automated image analysis and the results analyzed by two-dimensional hierarchical clustering. Genes with similar functions should produce similar sets of genetic interactions and cluster together. As expected, patterns obtained with *RXT1*, *RXT2*, *SAP30*, *SDS3*, *DEP1*, and *PHO23* deletions were more similar to each other

than to those of the other 378 gene deletions on the array (data not shown). *EAF3* and *RCO1* also clustered next to each other but away from the genes encoding the other Rpd3/Sin3-interacting factors. The *RPD3* and *SIN3* patterns were closest to those of the *RXT1/2* cluster, suggesting that the major role of Rpd3/Sin3 in vivo is in conjunction with this group.

The existence of two functionally distinct Rpd3/Sin3 complexes was further confirmed by microarray gene expression analysis of deletion strains (raw data at http://www.utoronto.ca/greenblattlab/rpd3C.xls). Hierarchical clustering of the gene-expression profiles showed that the effects of deletions of *RXT1*, *RXT2*, *PHO23*, *SDS3*, *SAP30*, and *DEP1* were most similar to each other and to deletions of *RPD3*, *SIN3*, and *UME1* (Figure 2B). Deletions of *EAF3* and *RCO1* had similar effects on gene expression that differed considerably from the effects of the other deletions in these analyses.

Rpd3C(L) Affects Sir2-Mediated Silencing

Deletion of *RPD3* or *SIN3* results in enhanced gene silencing at HMR, ribosomal loci, and telomeres (Bernstein et al., 2000; Sun and Hampsey, 1999). Microarray analysis showed the preferential downregulation of many telomere-proximal genes in *rpd3*Δ and *sin3*Δ strains (Figure 3A, first row). While deletion of *EAF3* or *RCO1* does not specifically affect telomere-proximal gene expression (Figure 3A, second row), *rxt1*Δ, *rxt2*Δ, *pho23*Δ, *dep1*Δ, *sap30*Δ, or *sds3*Δ all mimic the effects of *sin3*Δ and *rpd3*Δ (Figure 3A, third row and data not shown).

The efficiency of silencing of reporter genes integrated near a telomere, in the ribosomal RNA locus, and at HMR were assayed (Figures 3B–3D). Deletion of Rpd3C(L) subunit genes enhanced repression at all three locations. In contrast, deletion strains of Rpd3(S)-specific subunits had normal repression. Together with the microarray data, these experiments suggest that the majority of Rpd3-dependent effects on gene expression are due to Rpd3C(L).

Set2 Methylation of Histone H3-K36 Recruits Rpd3C(S) via the Eaf3 Chromodomain

The genetic-interaction and gene-expression profiles for the Rpd3C(S)-specific factors Eaf3 and Rco1 were compared with other strains in the 384-strain transcription/chromatin miniarray. For both types of analyses, the *EAF3* and *RCO1* patterns were most similar to those of *SET2* (data not shown), the HMT for histone H3 lysine 36. Correlation plots of gene-expression data indicated a high degree of similarity between *set2*Δ and *eaf3*Δ but not two other HMTs, Set1 and Set3 (Figure 4A). These results strongly suggest that the Rpd3C(S) HDAC and the Set2 HMT may function in the same pathway. Chromatin immunoprecipitation (ChIP) of Set2 to transcribed regions was independent of Eaf3 (data not shown), so Rpd3C(S) is not required for recruitment of Set2. Eaf3 contains a chromodomain, a protein motif shown to recognize methylated histones in other proteins (Bannister et al., 2001; Jacobs et al., 2001; Lachner et al., 2001), suggesting that Set2 methylation might recruit Rpd3C(S) to nucleosomes.

We used the structure of the HP1 chromodomain bound to a histone H3-K9-methylated peptide (Jacobs and Khorasanizadeh, 2002; Nielsen et al., 2002) to predict which side chains of the Eaf3 chromodomain would be important for methyl-lysine binding (Figure 4B). Two aromatic residues in the binding pocket (Y81 and W84) were mutated singly or in combination, as were two nearby residues (R96A and I97A). The mutant proteins were all expressed at levels comparable to wild-type Eaf3 (Figure 4C). Mononucleosomes TAP-tagged on histone H4 can precipitate wild-type and R96A/I97A Eaf3 but not the Y81 and W84 mutants (Figure 4D). Therefore, the chromodomain is essential for association of Rpd3C(S) with nucleosomes. Furthermore, this association was no longer seen in nucleosomes from a *set2*Δ strain (Figure 4D). A similar experiment was performed using immunopurified nucleosomes washed in high salt to remove associated proteins. These were incubated with TAP-purified Rpd3C(S). Strong binding was seen to nucleosomes from *SET2* but not *set2*Δ cells (Figure 4E). Therefore, recruitment of Rpd3C(S) to chromatin is dependent on Set2 activity.

If Rpd3C(S) is recruited through binding of Eaf3 to histone H3 methylated at K36, deletion of *SET2* or mutation of K36 should result in increased histone acetylation in transcribed regions. To test this, we monitored histone H4 acetylation levels at several loci by ChIP (Figure 5). Deletion of *RPD3* increased acetylation at all loci tested except for a telomeric region used as a normalizing control. A strain lacking the Rpd3C(L)-specific subunit Dep1 showed increases at a subset of locations, including the *PDR5*, *DYN1*, and *INO1* promoters. Strains lacking Rpd3C(S)-specific subunits Eaf3 or Rco1 had increased acetylation levels, but only at locations where the *DEP1* deletion had no effect (Figures 5B and 5C). These locations included the *PDR5*, *DYN1*, and *PMA1* coding regions. These effects were not due to changes in overall histone levels (Figure 5A and data not shown). We note that Reid et al. (2004) also saw increased acetylation in transcribed regions of genes when *EAF3* was deleted. The nonoverlapping effects of Dep1 and Rco1/Eaf3 on acetylation further support the hypothesis that Rpd3C(L) and Rpd3C(S) have distinct functions. Deletion of *SET2* increased acetylation with exactly the same pattern as deletions of Rpd3C(S) (Figures 5B and 5C), further supporting a model in which Set2 methylation recruits Rpd3C(S) to coding regions. Increased acetylation of coding regions was also seen in a histone H3-K36A mutant (Figure 5E) but not a H3-K4 mutant (data not shown). Finally, the Eaf3 Y81A chromodomain mutant behaved similarly to a complete deletion of Eaf3 (Figure 5D).

Bypass Suppression of a *BUR1* Deletion by Mutations in the Set2/Rpd3C(S) Pathway

In parallel with the characterization of Rpd3C(S), we performed a genetic screen to isolate suppressor mutations that bypass the requirement for the Bur1 kinase. A *BUR1* plasmid shuffling strain was mutagenized with a transposon-based insertion library and transformants selected on 5-fluoro-orotic acid (5-FOA) for improved growth in the absence of Bur1. Insertions into (as well as complete deletions of) the *RTF1* and *CHD1* genes partially bypassed the requirement for Bur1 (Figure 6A

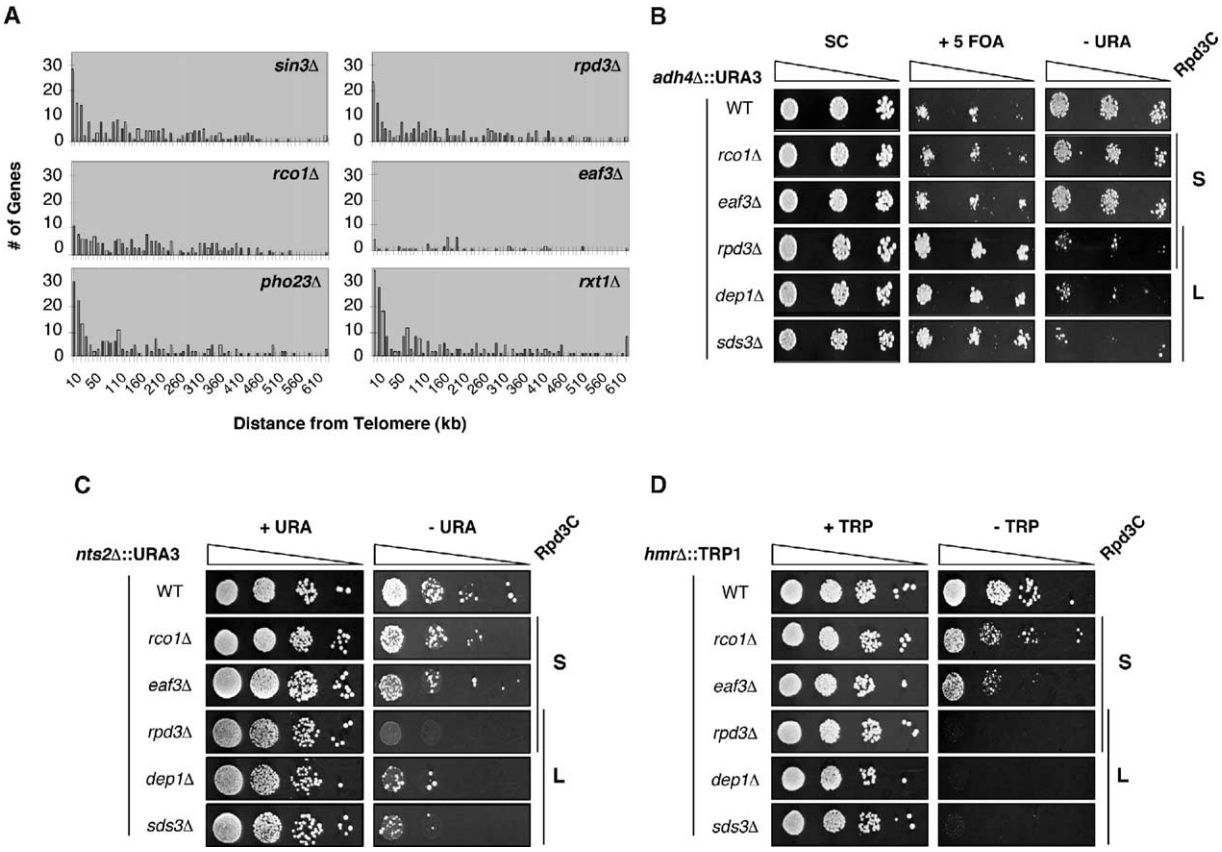


Figure 3. Rpd3C(L) Mutants Enhance Sir2-Mediated Silencing

(A) Microarray analysis of mRNA expression was performed in strains lacking the indicated genes. The number of genes downregulated 1.7-fold or greater was plotted against the distance of the affected gene from the telomere.

(B) To assay telomeric silencing, genes encoding members of the Rpd3-containing complexes were deleted in strain UCC1001, which carries a *URA3* gene near the left telomere of chromosome VII (Nislow et al., 1997). Dilutions of yeast strains were plated on SC medium with 5-FOA or uracil as indicated. SC plates were photographed after 48 hr, plates containing 5-FOA or lacking uracil after 72 hr.

(C) To assay silencing at the ribosomal RNA locus, the indicated genes were deleted in strain ADR3544 containing a *URA3* reporter gene inserted at the nontranscribed spacer (*NTS2*). Growth was assayed as in (B).

(D) To assay expression at a silenced mating-type gene, the indicated genes were deleted in strain ADR1442 containing a *TRP1* reporter gene inserted at *HMR*. Growth was for 48 hr on plates lacking tryptophan.

and data not shown). The *rtf1*Δ and *chd1*Δ suppressors also improved growth of a *bur2*Δ strain (Figure 6B).

The identity of the *bur1*Δ bypass suppressors suggested a connection to chromatin. Rtf1, a subunit of the PAF complex, contributes to recruitment of the HMTs Set1, Set2, and Dot1 (Krogan et al., 2003a; Ng et al., 2003). The chromatin remodeler Chd1 contains a chromodomain and interacts indirectly with the PAF complex via the elongation factor Spt16/Pob3 (FACT) (Krogan et al., 2002b). To further probe a chromatin connection, deletions of known HMTs and other chromatin-related factors were tested (Figure S2). Remarkably, deletion of *SET2*, but none of the other characterized HMTs, efficiently rescued growth of *bur1*Δ and *bur2*Δ strains (Figure 6). Deletions of other PAF-complex subunits and most other chromatin-related factors failed to suppress lethality of *bur1*Δ, and a few showed synthetic-lethal or synthetic-sick phenotypes (Figure S2). However, deletion of the gene for Rad6, a ubiquitin ligase for histone H2B, gave a partial suppression. Furthermore, bypass of *bur1*Δ was observed upon deletion of *SIN3* or *RPD3* (Figure 6A).

The ability of *sin3*Δ and *rpd3*Δ to bypass the requirement for Bur1 could be mediated by the large or small Rpd3 complex. Deletions of genes encoding Rpd3C(L)-specific subunits failed to improve growth of the *bur1*Δ strain (Figure S2). In contrast, deletion of genes for the Rpd3C(S)-specific factors, Eaf3 or Rco1, resulted in vastly improved growth for *bur1*Δ or *bur2*Δ strains (Figure 6). Because Eaf3 is a member of the NuA4 HAT complex as well as the Rpd3C(S) complex, we tested whether deletion of NuA4 subunits could suppress *bur1*Δ. Deletions of *YAF9* or *VID21* or a temperature-sensitive allele of the NuA4 catalytic subunit *ESA1* showed no suppressive effect on a *bur1*Δ strain (Figure S2). Therefore, the Eaf3 function that confers a requirement for Bur1 is mediated by the Rpd3C(S) complex. To test whether the Eaf3 chromodomain is important for this requirement, strains containing the point mutants characterized in Figure 4 were tested for suppression. In the presence of wild-type Eaf3 or the R96A/I97A mutant, *bur1*Δ cells were inviable. In contrast, the Y81 and W84 mutants suppressed the growth defect of a *bur1*Δ strain to the same extent as a complete deletion of

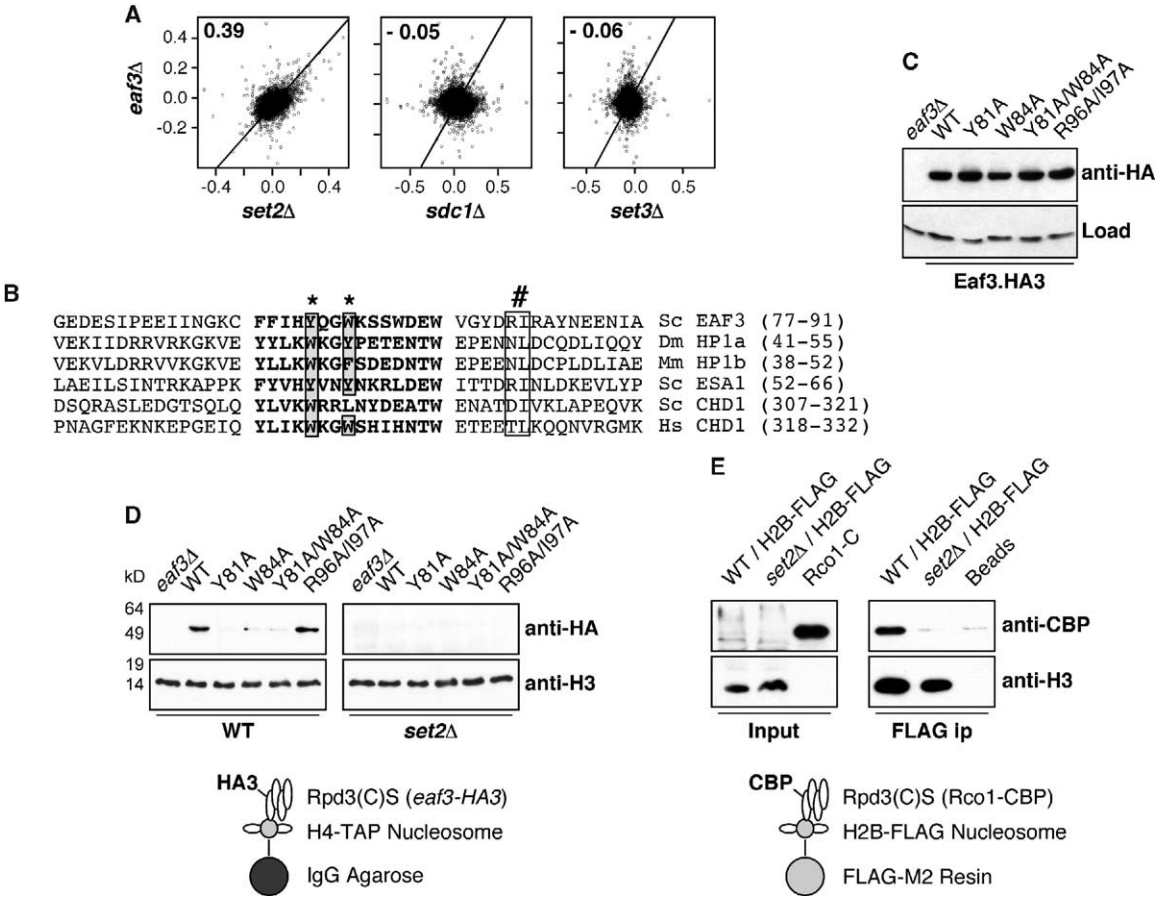


Figure 4. The Eaf3 Chromodomain and Methylated Lysine 36 Recruit Rpd3C(S) to Nucleosomes

(A) The gene-expression profiles of *set2*Δ are similar to *eaf3*Δ. Scatter-plot analysis of the gene-expression profiles of *eaf3*Δ compared with *sdc1*Δ (component of the Set1C, COMPASS), *set2*Δ, or *set3*Δ (component of the Set3C) is shown. x and y axes show the change in expression level in the indicated deletion strain relative to wild-type. Each gene is represented as a dot. Genes that score similarly in both deletions appear along the diagonal line. The number in the upper left corner is the correlation coefficient. While 1.0 represents a perfect correlation, in practice, repeating the same microarray rarely gives a correlation > 0.6.

(B) Sequence alignment of Eaf3 with chromodomains from fly (Dm) and mouse (Mm) HP1, yeast (Sc) Esa1 and Chd1, and human (Hs) Chd1. * indicates conserved aromatic residues in the methyl binding pocket. # indicates a region that may contact the residue next to the methylated lysine.

(C) Immunoblot analysis of HA-tagged Eaf3 mutants shows that the mutant proteins were expressed at levels equivalent to wild-type. The load control shows a nonspecific protein band that crossreacts with the anti-HA antibody.

(D) Chromodomain mutants disrupt the association of Eaf3 with nucleosomes in a Set2-dependent manner. Histone H4-TAP-purified nucleosomes from *SET2* or *set2*Δ strains were incubated with extracts from cells containing the HA-tagged Eaf3 alleles indicated above each lane.

(E) Nucleosomes were purified via a Flag-tagged H2B from *SET2* or *set2*Δ strains and washed at high stringency (400 mM NaCl). Pellets were then incubated with TAP-purified Rpd3C(S) and reprecipitated.

EAF3 (Figure 6C). Therefore, suppression correlated perfectly with the ability of the Eaf3 mutants to bind nucleosomes (Figure 4). If the Eaf3 chromodomain binds Set2-methylated H3, mutating the K36 residue should improve growth in a *bur1*Δ strain. As predicted, the histone H3-K36A substitution (as well as Q and R mutants) significantly improved growth of a *bur1*Δ strain compared to wt or K4A H3 (Figure 6D and data not shown).

The Set2/Rpd3C(S) Pathway Negatively Regulates Transcription

Since Bur1/2 positively regulates RNApII elongation, the suppressing deletions could be negative regulators. This was tested in two ways. First, chromatin immunoprecipitation of RNApII was carried out in mutant and suppressor strains. In the absence of Bur2, very little RNApII was observed to crosslink to transcribed regions despite nearly normal levels of TATA binding protein (TBP) at promoters (Figure 7A). When *SET2*, *EAF3*, or *RTF1* was deleted in the *bur2*Δ background, RNApII crosslinking was restored to relatively normal levels. This agrees with a previous report (Kizer et al., 2005) wherein *SET2* deletion increased levels of RNApII crosslinking at downstream transcribed regions.

A second test for whether *bur1*Δ bypass suppressors act as negative factors was to test their growth on media containing 6-azauracil (6AU) or mycophenolic acid

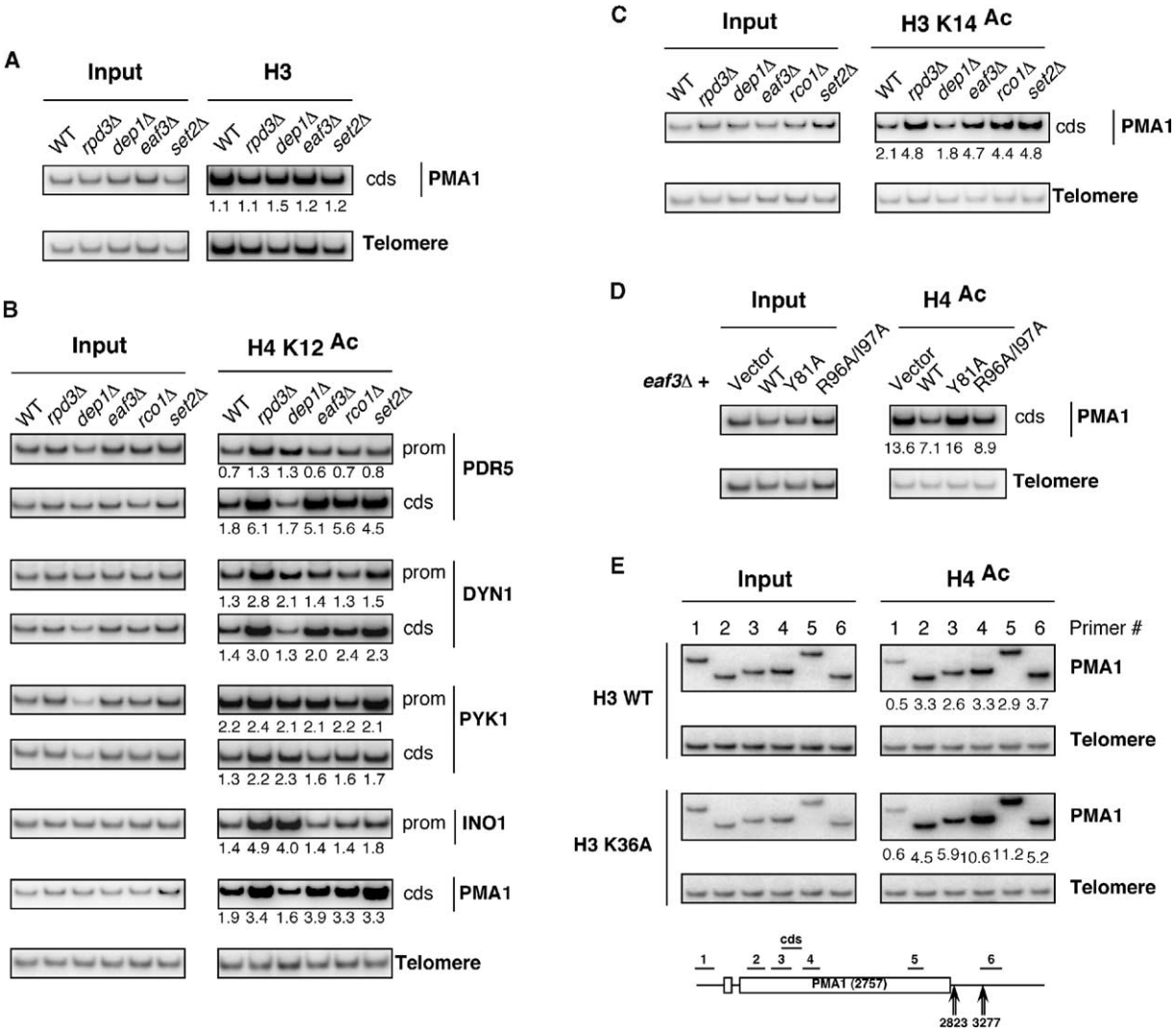


Figure 5. Cells Lacking Set2 or Rpd3C(S) Have Increased Acetylation in Transcribed Regions

(A) Histone H3 levels were unaffected in the indicated deletion strains as assayed by ChIP. The enrichment values (below each lane) are normalized to a region ~500 bp from the end of chromosome VI-R (Telomere) as an internal control and are averaged across three independent experiments. A representative gel is shown.

(B) The indicated deletion strains were tested for acetylation of histone H4-K12 at various locations by ChIP. Deletion of Rpd3C(L) subunit *DEP1* increased acetylation at several promoters (prom), including *INO1*, *PDR5*, and *DYN1*. In contrast, deletion of *EAF3*, *RCO1*, or *SET2* increased acetylation levels in the *PDR5*, *DYN1*, and *PMA1* coding regions (cds).

(C) As in (B), except histone H3-K14 acetylation was analyzed.

(D) Deletion or inactivation of the Eaf3 chromodomain increases acetylation in the *PMA1* coding sequence. In this experiment, an antiserum recognizing multiple H4 acetylation sites was used.

(E) Increased acetylation throughout the PMA1 coding region is observed in a histone H3-K36A mutant. Locations of primers used are shown schematically at bottom.

(MPA). These chemicals reduce NTP pools (Desmoucelles et al., 2002; Exinger and Lacroute, 1992), exacerbating the slow growth phenotypes of cells lacking positive elongation factors such as TFIIS, Bur1, or Spt5 (Archambault et al., 1992; Keogh et al., 2003; Lennon et al., 1998; Squazzo et al., 2002). Mutations in positive elongation factors cause sensitivity to these agents, so mutations in negative elongation factors might give resistance. Deletions of Rpd3C(S) subunit genes *EAF3* or *RCO1* conferred resistance to 6AU and MPA (Figure 7B; note the increased colony size relative to wild-type cells), while deletions of Rpd3C(L)-specific subunits were generally more sensitive. In agreement with earlier results (Kizer et al., 2005), deletion of *SET2* provided some resistance to 6AU and MPA (Figure 7B), as do histone H3-K36 point mutations (Kizer et al., 2005). There have been other reports of weak 6AU sensitivity in *set2*Δ strains (Krogan et al., 2003c; Li et al., 2002, 2003; Schaft et al., 2003), but whether this discrepancy is due to strain differences or other factors remains unclear. Deletion of one other *bur1*Δ bypass suppressor, *CHD1*, also conferred 6AU and MPA resistance (Des-

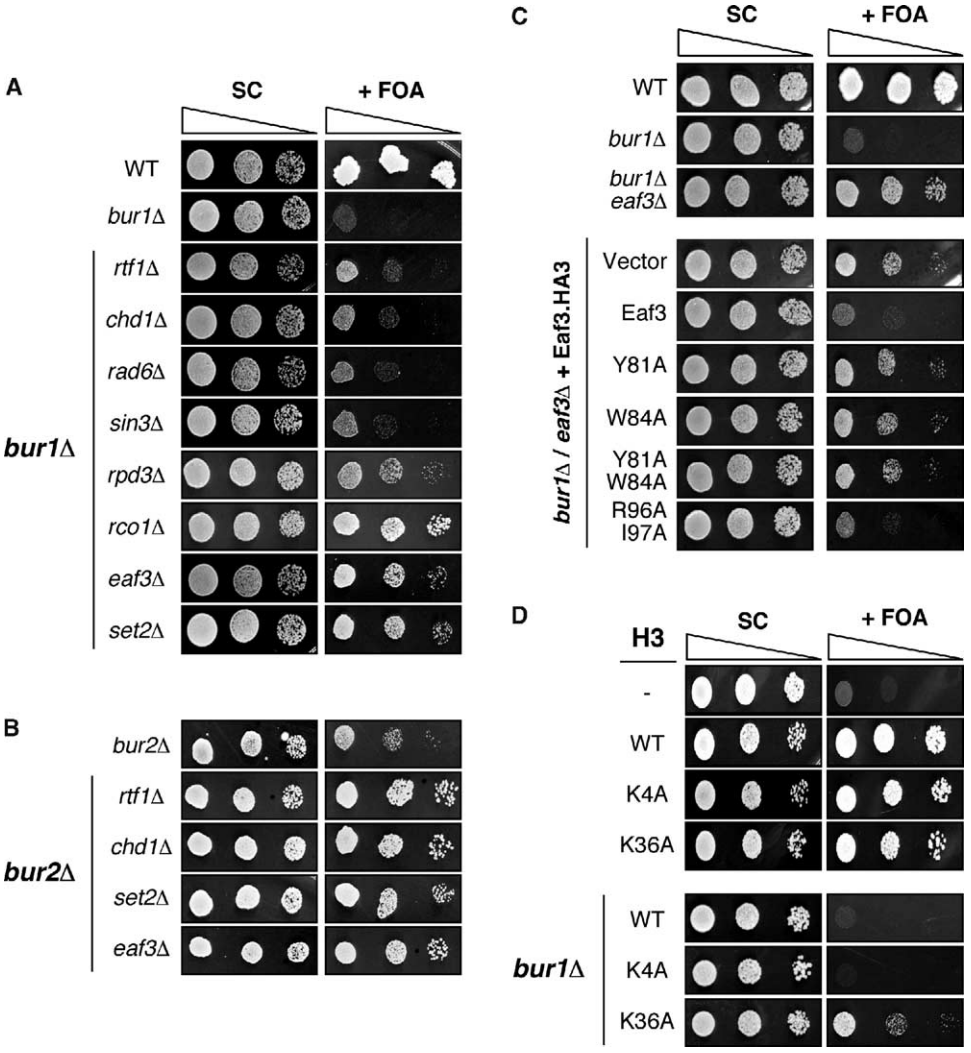


Figure 6. Bypass of the Requirement for Bur1/Bur2 Kinase by Deletion of Chromatin-Related Factors

(A) The indicated gene deletions were introduced into a *BUR1* plasmid shuffling strain. Cells were spotted onto synthetic complete (SC) media or media containing 5-FOA to select against the *BUR1/URA3* plasmid. SC spots were after 48 hr; FOA spots were after 72 hr.

(B) As in (A), except the indicated deletions were tested for improvement of growth in a *bur2*Δ deletion strain.

(C) Strains for simultaneous shuffling of *BUR1* and *EAF3* were transformed with the indicated Eaf3 mutant genes and tested for the ability to grow on 5-FOA, i.e., in the absence of *BUR1*. SC spots were after 48 hr; FOA spots were after 72 hr.

(D) The ability of *HHT1* mutants to support viability in the presence or absence of *BUR1* was tested. BUR1-shuffling cells containing either *HHT1* wild-type (WT), a lysine 4-to-alanine mutation (K4A), or a lysine 36-to-alanine mutation (K36A) were spotted on either SC media or media containing 5-FOA to select against the *BUR1/URA3* plasmid.

moucelles et al., 2002). In contrast, cells lacking Rtf1 or Rad6 were actually more sensitive to these drugs than wild-type cells. Therefore, there may be a distinct mechanism for bypassing the Bur1 requirement at work in these strains.

Discussion

Through a combination of protein purification, high-throughput genetic and gene-expression analyses, and a classical suppressor screen, we identified a transcription regulatory pathway that balances positive and negative effects. The Set2 HMT binds to elongating RNApII and cotranscriptionally methylates histone H3 at lysine 36 (Kizer et al., 2005; Krogan et al., 2003c; Li et al., 2003; Schaft et al., 2003; Xiao et al., 2003). We show that this mark is recognized by the Eaf3 chromodomain, thereby recruiting the Rpd3C(S) complex, which then deacetylates histones. Two lines of evidence argue that this pathway serves to negatively regulate transcription. First, deletions of genes encoding these factors result in some resistance to elongation-inhibiting drugs. Second, the same deletions bypass the requirement for the positive elongation factor Bur1 by improving RNApII recruitment. These findings not only strongly implicate Bur1 activity in transcription through chromatin, they provide the first clear function for the Set2 methylation of histone H3.

The genetically antagonistic relationship between Bur1/Bur2 and Set2/Rpd3C(S) is unlikely to be indirect.

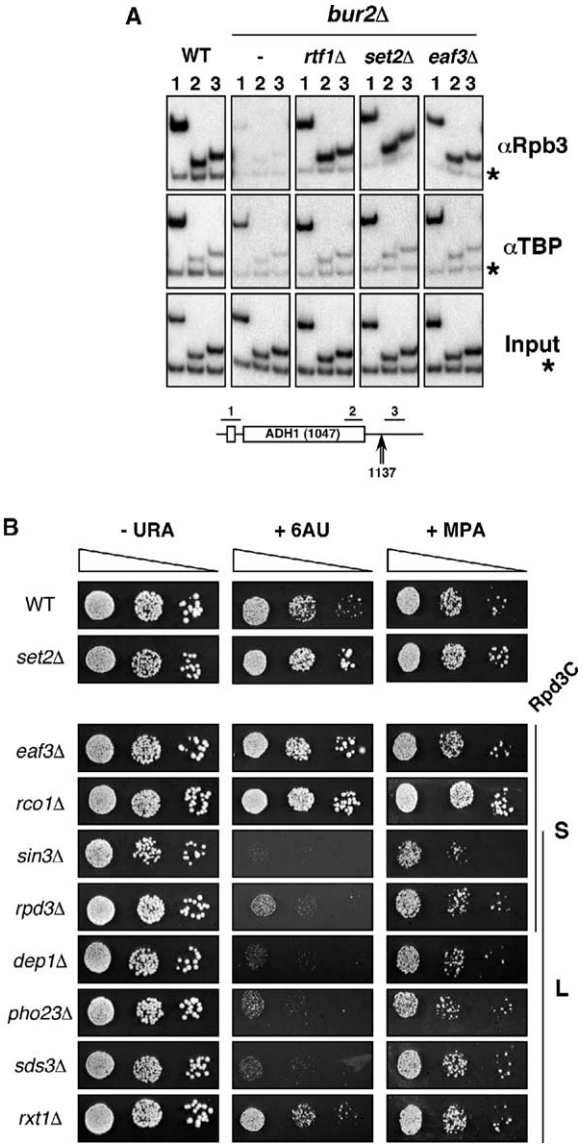


Figure 7. Suppressors of *bur1*Δ Negatively Affect Transcription

(A) Chromatin immunoprecipitation analysis of RNApII (Rpb3 subunit) and TBP was carried out in the indicated strains on the *ADH1* gene. Relative locations of PCR products are shown schematically under gel panels.

(B) Cells lacking Set2 or Rpd3C(S), but not Rpd3C(L), exhibit resistance to 6AU and MPA. The indicated deletion strains were tested for the ability to grow on SC medium with or without 6AU (75 μg/ml) or MPA (15 μg/ml). SC spots were after 48 hr; +MPA and +6AU spots were after 72 hr.

Growing cells in the presence of the general HDAC inhibitor trichostatin A (TSA) does not rescue *bur1*Δ, nor does it improve the suppression efficiency of strains lacking Set2 or Rpd3C(S) (data not shown). Furthermore, disrupting the Set2/Rpd3C(S) pathway does not suppress deletions of the positive elongation factors Fcp1 and Spt5 or the point mutant *spt16-197* (data not shown), arguing that the Set2/Rpd3C(S) pathway does not nonspecifically antagonize all positive elongation factors.

We find that there are two physically distinct Rpd3/Sin3/Ume1-containing complexes. The larger Rpd3C(L) contains Rxt1, Rxt2, Pho23, Sap30, Sds3, and Dep1, while the smaller, more abundant Rpd3C(S) contains Eaf3 and Rco1. The functional differences between these two complexes were shown by clustering of genetic interactions (Tong et al., 2001, 2004) and gene-expression profiles. Interestingly, the profiles generated by *rpd3*Δ, *sin3*Δ, and *ume1*Δ encompassed both groups but were most similar to those of Rpd3C(L) subunits. Therefore, Rpd3C(L) is probably responsible for the majority of Rpd3 effects on gene expression. However, only mutations in Rpd3C(S) could bypass the requirement for Bur1 kinase.

In addition to the Set2/Rpd3C(S) pathway, the genes for Rad6, Chd1, and Rtf1 were isolated in the *bur1*Δ bypass screen. These were the weakest suppressors, so they may operate by a distinct, nonspecific mechanism. Deletion of *RTF1* suppresses mutant phenotypes of certain alleles of the TBP (Stolinski et al., 1997), and *CHD1* deletion restores growth in a mutant strain of the positive elongation factor Spt5 (Simic et al., 2003). Alternatively, an indirect contribution to the Set2/Rpd3C(S) pathway is also possible: deletion of *RTF1* reduces cotranscriptional recruitment of Set2 (Krogan et al., 2003a). Both Rtf1 and Rad6 are necessary for methylation by Set1 of histone H3 lysine 4 near 5′ ends of genes (Ng et al., 2003; Sun and Allis, 2002), and they have more recently been implicated in elongation (Xiao et al., 2005). A double deletion of Set1 and Set2 is synthetically sick (Krogan et al., 2003c), suggesting that loss of Rad6 could indirectly attenuate Set2 activity via Set1. Interestingly, Set1 can also function as a negative transcription regulator, at least at some genes (Carvin and Kladde, 2004). Chd1 interacts with Rtf1 and other elongation factors (Simic et al., 2003), suggesting it may act through Rtf1. However, Chd1 also contains a chromodomain and may have a more direct connection to histone methylation (Pray-Grant et al., 2005).

Presumably, Bur1 counteracts the Set2/Rpd3C(S) pathway through its kinase activity, but the relevant substrate(s) remains unidentified. Deletion of Bur2 results in loss of Rad6-dependent H2B ubiquitylation and methylation of H3-K4 (Laribee et al., 2005), but it is unclear whether this is related to the antagonism between Bur1/2 and the Set2 and Rpd3(S) factors. The deletion phenotypes of *BUR1* and *BUR2* are much more severe than those of *RAD6*, *SET1*, *SET2*, or Rpd3C(S) complex subunits, and the *bur1*Δ bypass with these genes is only partial, so Bur1 is likely to have additional functions besides antagonism of the Set2/Rpd3C(S) pathway.

The recognition of methylated K36 on histone H3 by Eaf3 provides the first clear role for this modification. Direct binding assays, acetylation patterns, and genetic interactions all argue that a major function of Eaf3 is to recruit Rpd3C(S) to Set2-methylated chromatin, i.e., to transcribed regions of genes. We previously showed that K36 methylation increases with distance from the promoter (Krogan et al., 2003c). Similarly, the change in acetylation seen in the absence of H3-K36 methylation increases in 3′ regions of the gene (Figure 5E).

What is the role of Eaf3 in the NuA4 HAT complex? One simple possibility is that Eaf3 also recruits NuA4 to transcribed regions of genes, where it acetylates nu-

cleosomes. However, this model would be difficult to reconcile with the increase in H4 acetylation upon deletion of Set2. Alternatively, Eaf3 might recognize other methylated residues (such as H3-K4) in the context of NuA4 or recognize methylated K36 in a context other than elongation. Along these lines, it has recently been suggested that Set2 may transiently methylate promoter regions and that this modification is necessary for NuA4 recruitment (Morillon et al., 2005).

What is the biological rationale for the repressive Set2/Rpd3C(S) pathway? It may seem paradoxical to link a negative regulator of transcription to the act of transcription itself. However, this type of negative feedback may function to attenuate regulation of genes, which must be not only activated but also repressed with the proper kinetics. The passage of transcribing RNApII results in significant rearrangements of nucleosomes, and a transcription-coupled methylation/deacetylation pathway may allow induced genes to be more rapidly repressed when their expression is no longer required. A second possible function could be to reassemble a repressive chromatin configuration that prevents cryptic promoters within transcribed regions from supporting transcription initiation complexes. Such a function has been shown for Spt6 and Spt16 (Kaplan et al., 2003). Supporting this idea, Carrozza et al. (2005) (this issue of *Cell*) have found that strains lacking Set2 or Rpd3C(S) display internal initiations in several genes. Kaplan et al. (2003) also observed this phenotype in *bur1* and *bur2* mutants. Therefore, a proper balance of the positive Bur1 effects and the negative Set2/Rpd3C(S) effects may be required to keep transcribed chromatin in a state suitable for elongation but not initiation.

Experimental Procedures

Antibodies

Anti-Rpb3 was from Neoclone. Antibodies against specific histone acetylations (H3-K14Ac and H4-K12Ac) are described in Suka et al. (2001). Antibodies against acetylated histone H4 (H4Ac), the calmodulin binding protein (CBP) epitope tag, and the C terminus of H3 were from Upstate Biotechnology, Inc. Protein A and Protein G Sepharose-4 Fastflow were from Amersham. IgG-agarose and Flag-M2-agarose were from Sigma.

Plasmids and Yeast Strains

Histone H3/H4 shuffle strains and the wild-type H3/H4 (HHT2-HHF2) expression plasmid (pWZ414-F12) were from S. Roth-Dent (Zhang et al., 1998). *HHT2* and *EAF3* point mutants were created by the megaprimer PCR method (Keogh et al., 2002, 2003) and confirmed by sequencing. Yeast strains are listed in Table S3.

Phenotypic Analyses

Strains were transformed with a *URA3* plasmid before testing for sensitivity to 6-azauracil (6AU; 10, 25, 50, 75, or 200 μg/ml) or mycophenolic acid (MPA; 15 μg/ml). TSA plates contained 1.3 or 13 μM trichostatin A (Arevalo-Rodriguez et al., 2000). For spotting analyses, cells were resuspended at 10^7/ml and subjected to 10-fold serial dilutions, and 10 μl of each dilution was spotted. Growth was assayed at 48 or 72 hr as indicated. For silencing assays in Figure 3, the indicated genes were deleted in strains containing reporter genes at *TEL VII* (Nislow et al., 1997), the ribosomal RNA locus nontranscribed region NTS2, or the *HMR* silent mating-type gene (Table S3).

Transposon Mutagenesis

The *bur1*Δ bypass screen used a yeast genomic library containing random insertions of a mini-Tn3 (LEU2/LacZ) transposon (Burns et al., 1994). Nineteen thousand Leu$^+$ transformants (estimated >90% genome coverage) in the Bur1 shuffle strain YSB787 (Table S3) were replica plated onto media containing 5-FOA to select against the pRS316-Bur1 plasmid. Ninety positive colonies were tested by PCR for the presence of the *BUR1* gene. For 55 clones lacking *BUR1* sequences, the transposon insertion site was identified by vectorette PCR (Burns et al., 1994). Candidate suppressor genes were confirmed with complete gene deletions introduced into the Bur1 shuffle strain and retesting for 5-FOA resistance.

Chromatin Immunoprecipitations

Chromatin immunoprecipitations were performed as described (Keogh and Buratowski, 2004). Primers used are described in Table S4. Relative signal values were calculated as described (Keogh and Buratowski, 2004; Krogan et al., 2003b).

TAP Purification of Protein Complexes

Proteins with a tandem affinity purification (TAP) tag (Rigaut et al., 1999) were purified as described (Krogan et al., 2002b). Proteins were identified by (1) trypsin digestion of entire fractions followed by sequencing with high-performance capillary-scale liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Cagney and Emili, 2002) and (2) SDS-PAGE, excision of silver-stained gel bands, trypsin digestion, and MALDI-TOF mass spectrometry (Krogan et al., 2002b). Details of peptide identities and protein coverage are in Table S1.

RNA Isolation and Microarray Analyses

Isogenic strains were grown in parallel in synthetic complete (SC) medium at 30°C, and RNA was prepared and extracted for microarray analyses as previously described (Hughes et al., 2000). Pearson correlation coefficients were calculated for each pair of deletions, and the strains were organized by two-dimensional hierarchical clustering according to the degree of similarity of their effects on gene expression.

Epistatic Miniarray Profile Analysis

E-MAP analysis was carried out as described (Schuldiner et al., 2005; Tong et al., 2001). Growth rates were assessed by automated image analysis of colony size (S.R.C. and J.S.W., unpublished data). The results of all screens were analyzed by two-dimensional hierarchical clustering that groups genes according to the degree of similarity of their genetic interactions. >50% of the synthetic genetic interactions shown have been confirmed by tetrad dissection or random sporulation.

Nucleosome Association with the Rpd3C(S)

For Figure 4D, chromatin was fractionated from wild-type Hhf1-TAP or *set2*Δ Hhf1-TAP cells as described (Keogh et al., 2005). Nucleosomes were released by sonication and bound to Protein A-agarose beads. These were used to precipitate HA-tagged Eaf3 mutants from the indicated WCEs prepared as reported (Keogh et al., 2003). After overnight incubation at 4°C, bound complexes were extensively washed with lysis buffer (20 mM Tris-Cl [pH 7.6], 10% glycerol, 200 mM KoAc, 1 mM EDTA, 1 mM DTT + protease inhibitors). Samples were resolved by SDS-PAGE followed by immunoblotting with the indicated antibodies.

For Figure 4E, whole-cell extracts (WCEs) from 100 ml cultures of wild-type H2B-Flag and *set2*Δ H2B-Flag strains were prepared as previously described (Briggs et al., 2001). For chromatin fragments, WCEs were pulse sonicated (30% output and 90% duty cycle; six cycles) and clarified by microfuge centrifugation (13,000 rpm, 15 min). For each pull-down, 2 mg of WCE was mixed with 12.5 μl of Flag-M2 agarose beads for 2 hr at 4°C. After three washes in IP buffer (50 mM Tris [pH 8.0]; 400 mM NaCl; 0.5 mM EDTA; 2 mM PMSF; phosphatase inhibitor cocktail I [Sigma]; and 2 μg/ml pepstatin, aprotinin, and leupeptin), bead bound chromatin was resuspended in 300 μl IP buffer, to which 200 μl of Rco1-TAP-purified Rpd3C(S) was added. Reactions were incubated overnight at 4°C and then washed three times in IP buffer. Bound proteins

were analyzed by SDS-PAGE followed by immunoblotting with the indicated antibodies. Five percent of the yeast input extracts and ten percent of the purified Rpd3C(S) were used for input lanes.

Supplemental Data

Supplemental Data include Supplemental References, four tables, and three figures and can be found with this article online at http://www.cell.com/cgi/content/full/123/4/593/DC1/.

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Supplemental Data

Cotranscriptional Set2 Methylation of Histone H3

Lysine 36 Recruits a Repressive Rpd3 Complex

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Table S1. Coverage of Proteins Identified by MS Analysis of Rpd3C(S) and Rpd3C(L)

	LC-MS			Maldi	
	# Peptides	**% Coverage**	**% Confidence**	**# Peptides**	**% Coverage**
Sap30					
Dep1	2	6.4	99.6	14	35
Rxt1	3	7.9	99.6	ND	
Rxt2	6	18.1	99.6	15	36
Sin3	12	8.7	99.6	32	24
Sds3	8	21.4	99.6	13	44
Sap30	1	8	99.6	10	38
Pho23	3	10	99.6	16	50
Ume1	6	13.9	99.6	ND	
Rpd3	1	3.7	97.9	13	36
Sin3					
Rxt2	5	13.7	99.6	15	33
Pho23	4	17	99.6	8	37
Rco1	1	3.5	99.6	32	46
Rxt1	3	9.5	99.6	10	19
Sap30	5	23.4	99.6	8	40
Sds3	3	9.2	99.6	15	41
Sin3	17	13.2	99.6	16	12
Ume1	11	21.7	99.6	18	58
Eaf3	6	15.7	99.6	16	42
Rpd3	1	3.5	99.4	17	58
Dep1	2	3.3	94.3	13	31
Rco1					
Ume1	9	16.5	99.6	8	28
Sin3	15	15.2	99.6	40	26
Eaf3	10	20.7	99.6	18	46
Rpd3	4	9.7	99.6	19	53
Rco1	4	11.8	99.6	31	46

Table S2. Synthetic Genetic Interactions

	RXT1	RXT2	PHO23	DEP1	SAP30	SDS3	EAF3	RCO1	RPD3	SIN3
SET3	YES	YES	**YES**	**YES**	**YES**	YES	NO	NO	YES	YES
SNT1	YES	YES	**YES**	**YES**	YES	YES	NO	NO	YES	YES
SIF2	**YES**	LG	**YES**	**YES**	**YES**	YES	NO	NO	YES	YES
HOS2	**YES**	YES	YES	**YES**	**YES**	YES	NO	NO	YES	YES
LEO1	**YES**	YES	**YES**	**YES**	**YES**	YES	NO	NO	YES	YES
CDC73	**YES**	**YES**	**YES**	**YES**	**YES**	YES	NO	NO	YES	**YES**
SWI4	YES	YES	YES	**YES**	**YES**	YES	NO	NO	YES	YES
UBP6	YES	YES	YES	**YES**	YES	YES	NO	NO	YES	YES
DOA1	YES	YES	YES	YES	YES	YES	NO	NO	YES	YES
SEM1	YES	YES	YES	**YES**	YES	NO	NO	NO	NO	YES
CIK1	YES	YES	YES	YES	YES	YES	NO	NO	YES	YES
THP1	YES	YES	YES	YES	NO	YES	NO	NO	YES	YES
SAC3	YES	YES	YES	YES	YES	YES	NO	NO	YES	YES
HTZ1	**YES**	YES	**YES**	**YES**	**YES**	YES	YES	YES	NO	LG
SWR1	**YES**	YES	**YES**	**YES**	**YES**	YES	YES	YES	YES	YES
SWC5	**YES**	YES	**YES**	YES	**YES**	YES	YES	YES	YES	YES
ARP6	**YES**	YES	**YES**	**YES**	**YES**	YES	NO	YES	YES	YES
SWC3	**YES**	YES	**YES**	YES	**YES**	YES	YES	YES	YES	YES
SWC6	**YES**	YES	**YES**	**YES**	**YES**	YES	YES	YES	YES	**YES**
SWC2	**YES**	YES	**YES**	**YES**	**YES**	YES	YES	YES	YES	YES
SEC22	YES	YES	**YES**	**YES**	**YES**	YES	YES	YES	YES	YES
ASF1	NO	YES	YES	**YES**	**YES**	YES	YES	YES	YES	YES
ELP2	YES	YES	YES	**YES**	YES	YES	YES	YES	YES	YES
ELP3	YES	YES	YES	**YES**	**YES**	YES	NO	NO	YES	YES
ELP4	NO	NO	YES	**YES**	YES	YES	NO	NO	YES	YES
ELP6	NO	YES	YES	**YES**	YES	YES	NO	NO	YES	YES
ISW1	NO	NO	NO	NO	NO	NO	YES	YES	YES	YES
CHD1	NO	NO	NO	NO	NO	NO	YES	YES	YES	YES
BUB1	NO	NO	NO	NO	NO	NO	YES	YES	YES	YES
TIM13	**YES**	YES	**YES**	**YES**	**YES**	YES	NO	NO	YES	YES
CLA4	YES	**YES**	**YES**	**YES**	**YES**	YES	NO	NO	YES	YES
BIM1	YES	**YES**	**YES**	**YES**	**YES**	YES	YES	YES	YES	YES
RAD52	NO	YES	YES	YES	YES	YES	NO	NO	YES	YES
RAD50	NO	YES	**YES**	**YES**	YES	YES	NO	NO	YES	YES
SPT3	YES	YES	YES	**YES**	**YES**	YES	NO	NO	YES	YES
SPT8	YES	YES	YES	**YES**	**YES**	YES	NO	NO	YES	YES
SOH1	YES	YES	**YES**	**YES**	**YES**	YES	NO	NO	YES	YES

"Yes" represents a genetic interaction between the two genes indicated as determined by SGA analysis, and is bold if confirmed by tetrad dissection or random sporulation (Tong et al., 2001 and data not shown). "No" indicates that the double deletion does not result in a genetic interaction. "LG" indicates that the two genes are in the same linkage group and therefore cannot be tested for a synthetic growth defect using SGA.

Table S3. Yeast Strains Used in This Study

Strain	Genotype	Source/Ref
YSB725	MATα, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2Δ202	(Keogh et al., 2003)
YSB726	MATa, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2Δ202	(Keogh et al., 2003)
YSB787	MATa, bur1Δ::HIS3, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2Δ202 *(pRS316-Bur1)*	(Keogh et al., 2003)
YSB788	MATα, bur1Δ::HIS3, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2Δ202 *(pRS316-Bur1)*	(Keogh et al., 2003)
YSB869	MATα, bur1Δ::HIS3, rtf1Δ::KanMX, ura3-52 or 3Δ0, leu2Δ1 or 2Δ0, trp1Δ63, his3Δ200 or 3Δ1, lys2Δ202 *(pRS316-Bur1)*	This work
YSB992	As YSB869 except chd1Δ::KanMX	This work
YSB1003	As YSB869 except set2Δ::KanMX	This work
YSB1129	As YSB869 except rad6Δ::KanMX	This work
YSB1227	As YSB869 except eaf3Δ::KanMX	This work
YSB1323	As YSB869 except sin3Δ::KanMX	This work
YSB1849	MATa, bur1Δ::HIS3, rco1Δ::KanMX, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2Δ202 *(pRS316-Bur1)*	This work
YSB1267	MATα, bur2Δ::TRP1, ura3-52, leu2Δ1, trp1Δ63, his4-912δ, lys2-128δ, suc2ΔUAS *(pRS316-Bur2)*	This work
YSB1330	MATa, bur2Δ::TRP1, rtf1Δ::KanMX, ura3-52 or 3Δ0, leu2Δ1 or 2Δ0, his3Δ1 or HIS3, trp1Δ63 or TRP1, his4-912δ or HIS4, lys2-128δ or LYS2, suc2ΔUAS or SUC2 *(pRS316-Bur2)*	This work
YSB1331	MATα, bur2Δ::TRP1, chd1Δ::KanMX, ura3-52 or 3Δ0, leu2Δ1 or 2Δ0, his3Δ1 or HIS3, trp1Δ63 or TRP1, his4-912δ or HIS4, lys2-128δ or LYS2, suc2ΔUAS or SUC2 *(pRS316-Bur2)*	This work
YSB1332	MATα, bur2Δ::TRP1, set2Δ::KanMX, ura3-52 or 3Δ0, leu2Δ1 or 2Δ0, his3Δ1 or HIS3, trp1Δ63 or TRP1, his4-912δ or HIS4, lys2-128δ or LYS2, suc2ΔUAS or SUC2 *(pRS316-Bur2)*	This work
YSB1334	MATa, bur2Δ::TRP1, eaf3Δ::KanMX, ura3-52 or 3Δ0, leu2Δ1 or 2Δ0, his3Δ1 or HIS3, trp1Δ63 or TRP1, his4-912δ or HIS4, lys2-128δ or LYS2, suc2ΔUAS or SUC2 *(pRS316-Bur2)*	This work

Strain	Genotype	Source/Ref
WZY43 (YF221)	MATα, hht1-hhf1::pWZ405-F2F9-LEU2, hht2-hhf2::pWZ403-F4F10-HIS3, ura3-52, leu2Δ1, his3Δ200, trp1Δ63, lys2-801, ade2-101 *(YCp50-Copy II (HHT2-HHF2))*	(Zhang et al., 1998)
YSB1269	MATα, hht1-hhf1::pWZ405-F2F9-LEU2, hht2-hhf2::pWZ403-F4F10-his3Δ::TRP1, ura3-52, leu2Δ1, his3Δ200, trp1Δ63, lys2-801, ade2-101 *(YCp50-Copy II (HHT2-HHF2))*	This work
YSB1360	MATα, hht1-hhf1::pWZ405-F2F9-LEU2, hht2-hhf2::pWZ403-F4F10-HIS3, bur1ΔTRP1, ura3-52, leu2Δ1, his3Δ200, trp1Δ63, lys2-801 or 2Δ202, ade2-101 *(pRS317-hht2(K4A)-HHF2, pRS316-Bur1)*	This work
YSB1362	As YSB1360 except: *(pRS317-hht2(K4Q)-HHF2, pRS316-Bur1)*	This work
YSB1364	As YSB1360 except: *(pRS317-hht2(K36A)-HHF2, pRS316-Bur1)*	This work
YSB1365	As YSB1360 except: *(pRS317-hht2(K36Q)-HHF2, pRS316-Bur1)*	This work
BY4741 (YF336)	[a]MATa, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0	(Winzeler et al., 1999)
YF721	[a]As YF336 + SIN3-TAP::HIS3	
YF871	[a]As YF336 + RPD3-TAP::HIS3	
YF660	[a]As YF336 + EAF3-TAP::HIS3	
YF797	[a]As YF336 + RCO1-TAP::HIS3	
YF837	[a]As YF336 + RXT1-TAP::HIS3	
YF858	[a]As YF336 + RXT2-TAP::HIS3	
YF937	[a]As YF336 + RXT3-TAP::HIS3	
YF853	[a]As YF336 + UME1-TAP::HIS3	
YF902	[a]As YF336 + DEP1-TAP::HIS3	
YF919	[a]As YF336 + SDS3-TAP::HIS3	
YF661	[a]As YF336 + ESA1-TAP::HIS3	
YF359	[a]As YF336 + SET2-TAP::HIS3	
YZS276	MATa, hta1-htb1Δ::LEU2, hta2-htb2Δ, ura3̃ı, leu2-3-112, his3-11-15, trp1-1, ade2-1, can1-100 *(pZS145 HTA1-FLAG:HHF2 CEN3 HIS3)*	(Sun and Allis, 2002)

Strain	Genotype	Source/Ref
YTX203	MATa, *set2*Δ::KanMX, hta1-htb1Δ::LEU2, hta2-htb2Δ, ura3̃ı, leu2-3-112, his3-11-15, trp1-1, ade2-1, can1-100 *(pZS145 HTA1-FLAG:HHF2 CEN3 HIS3)*	(Xiao et al., 2005)
UCC1001	MATa, TEL VIIL-adh4::URA3, ura3-52, leu2Δ1, his3Δ200, trp1Δ1, lys2-810^{am}	D. Gottschling
YF1441 (ADR410)	[b]MATa, TRP+, ura3-52, leu2-3-112, his3−11−15, trp1̃1, ade2-1, can1-100	D. Moazed
YF1442 (ADR3275)	[b]MATa, *hmr*ΔE::TRP1, ura3-52, leu2-3-112, his3−11−15, trp1̃1, ade2-1, can1-100	D. Moazed
YF1443 (ADR3543)	[b]MATa, *NTS1*[c]::mURA3-Leu2, ura3-52, leu2-3-112, his3−11−15, trp1̃1, ade2-1, can1-100	D. Moazed
YF1444 (ADR3544)	[b]MATa, *NTS2*[c]::mURA3-Leu2, ura3-52, leu2-3-112, his3−11−15, trp1̃1, ade2-1, can1-100	D. Moazed
YF1445 (ADR3542)	[b]MATa, mURA3-Leu2, ura3-52, leu2-3-112, his3−11−15, trp1̃1, ade2-1, can1-100	D. Moazed

[a]*Saccharomyces* Genome Deletion Project.

[b]W303-1a.

[c]*N*on*t*ranscribed *s*pacer region of the rDNA repeat. Located between the 3'ETS and RDN5.

Table S4. **Oligonucleotides Used in This Study**

Primer or Pair	Chr[a]	Figure	Location	Primer Sequence
TEL-V (*)	V	6F, 7A	F, 9716[b]	GGCTGTCAGAATATGGGGCCGTAGTA
			R, 9823	CACCCCGAAGCTGCTTTCACAATAC
ADH1^{prom} (#1)	XV	7A	F, -235[c]	TTCCTTCCTTCATTCACGCACACT
(ORF = 1047)			R, -18	GTTGATTGTATGCTTGGTATAGCTTG
ADH1^{cds1} (#2)	XV	7A	F, +146[c]	ACGCTTGGCACGGTGACTG
			R, +372	ACCGTCGTGGGTGTAACCAGA
ADH1^{cds2} (#3)	XV	7A	F, +844[c]	TTCAACCAAGTCGTCAAGTCCATCTCTA
			R, +1018	ATTTGACCCTTTTCCATCTTTTCGTAA
ADH1$^{3'UTR}$ (#4)	XV	7A	F, +1231[c]	ACCGGCATGCCGAGCAAATGCCTG
			R, +1400	CCCAACTGAAGGCTAGGCTGTGG
PDR5^{prom}	XV	6	F, -294[c]	CCGTGGTACGATATCTGTTGAACG
(ORF = 4536)			R, -58	CTTTTTCGAAAGTTCCTAGTTGCC
PDR5^{cds}	XV	6	F, +3501[c]	AAAGGTTCGATAACTGCAGCTGAG
			R, +3800	CTCATACAAATCTCTTTGCTGGAC
DYN1^{prom}	XI	6	F, -334[c]	TTCCCACAATTGCCACCTTTTCGC
(ORF = 12279)			R, -54	TTATTGCCCTTCTATGTTCTATGC
DYN1^{cds}	XI	6	F, +10523[c]	CCCAGTGGAGATATTCCAATATTC
			R, +10740	CATTTTCCAACATATTCCCCTGTG
PYK1^{prom}	I	6	F, -294[c]	TCCTTCCCATATGATGCTAGGTAC
(ORF = 1503)			R, -117	AGAGAATAACTTTGAAAGGGGACC
PYK1^{cds}	I	6	F, +750[c]	GACGAAATCTTGAAGGTCACTGAC
			R, +940	TCAGCTCTGGTTGGTCTTGGGTTG

Primer or Pair	Chr[a]	Figure	Location	Primer Sequence
$INO1^{prom}$	X	6	F, -233[c]	TAGGAACCCGACAACAGAACAAGC
(ORF = 1602)			R, -64	CGGCTAAATGCGGCATGTGAAAAG
$PMA1^{cds}$	VII	6	F, +629[c]	GTTACTTGGTTGCTATGACTGGTG
(ORF = 2757)			R, +857	TCCAAAATAGCAATCCATAGACCC
TEL VI	VI	6	F, 269,487[d]	GCGTAACAAAGCCATAATGCCTCC
			R, 269,624	CTCGTTAGGATCACGTTCGAATCC
$PMA1^{UAS}$ (#1)	VII	6F	F, -927[c]	GAAACGGAGAAACATAAACAGG
(ORF = 2757)			R, -678	GTCTCGAGGCCTGGAAGTGC
$PMA1^{cds1}$ (#2)	VII	6F	F, +168[c]	CGACGACGAAGACAGTGATAACG
			R, +376	ATTGAATTGGACCGACGAAAAACATAAC
$PMA1^{cds2}$ (#3)	VII	6F	F, +584[c]	AAGTCGTCCCAGGTGATATTTTGCA
			R, +807	AACGAAAGTGTTGTCACCGGTAGC
$PMA1^{cds3}$ (#4)	VII	6F	F, +1010[c]	GTTTGCCAGCTGTCGTTACCACCAC
			R, +1285	GCAGCCAAACAAGCAGTCAACATCAAG
$PMA1^{cds4}$ (#5)	VII	6F	F, +2018[c]	CTATTATTGATGCTTTGAAGACCTCCAG
			R, +2290	TGCCCAAAATAATAGACATACCCCATAA
$PMA1^{3'UTR}$ (#6)	VII	6F	F, +3287[c]	GAAAATATTTGGTATCTTTGCAAGATG
			R, +3500	GTAAATTTGTATACGTTCATGTAAGTG

[a] Yeast chromosome #.

[b] Position on chromosome.

[c] Relative to ATG (+1).

[d] Chr VI = 270,148 bp.

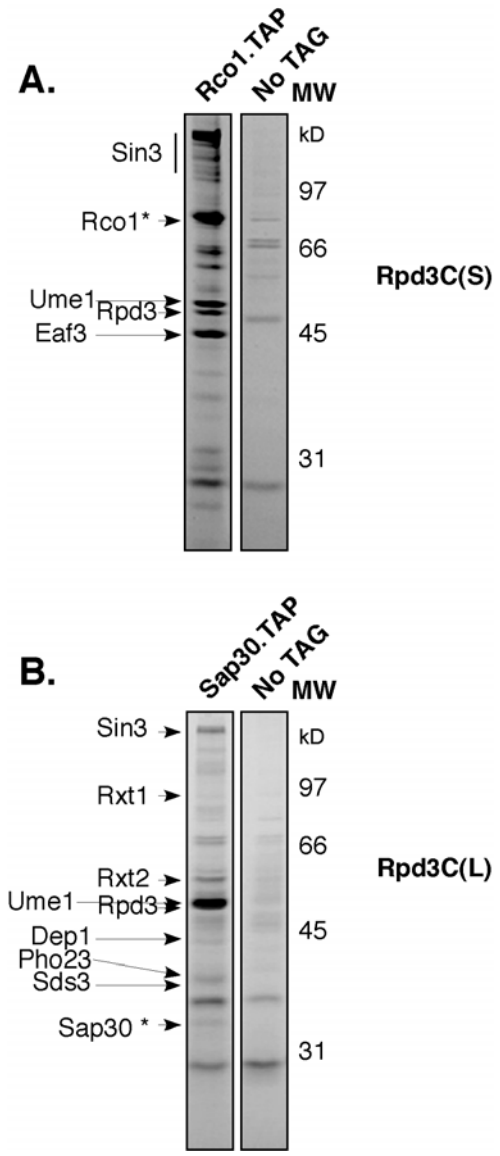

Figure S1. **Purification of Rpd3C(S) and Rpd3C(L)**

(A) Purification of TAP-tagged Rco1.

(B) Purification of TAP-tagged Sap30.

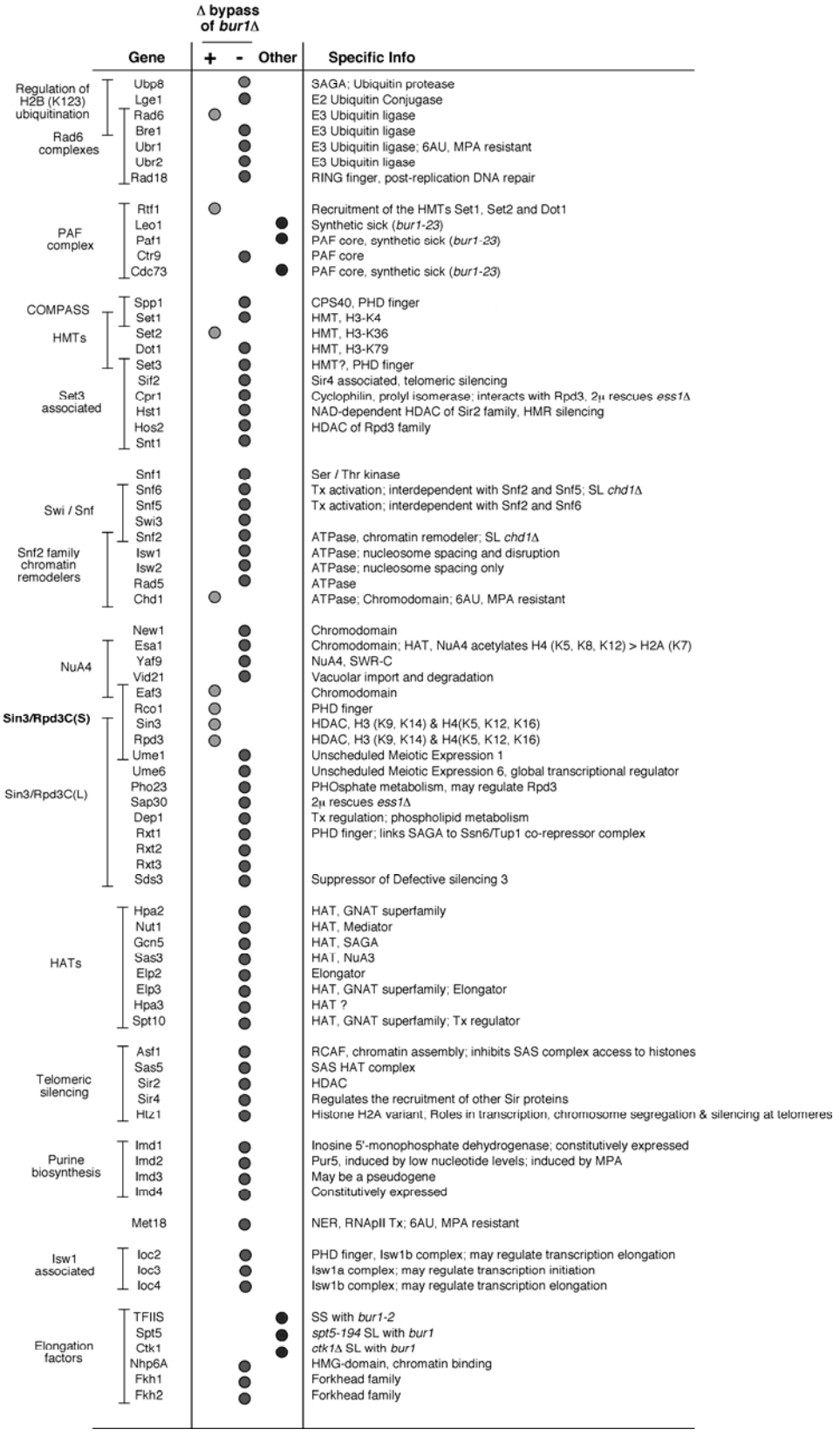

Figure S2. **Summary of Deletions Tested for Ability to Bypass the Requirement for *BUR1***

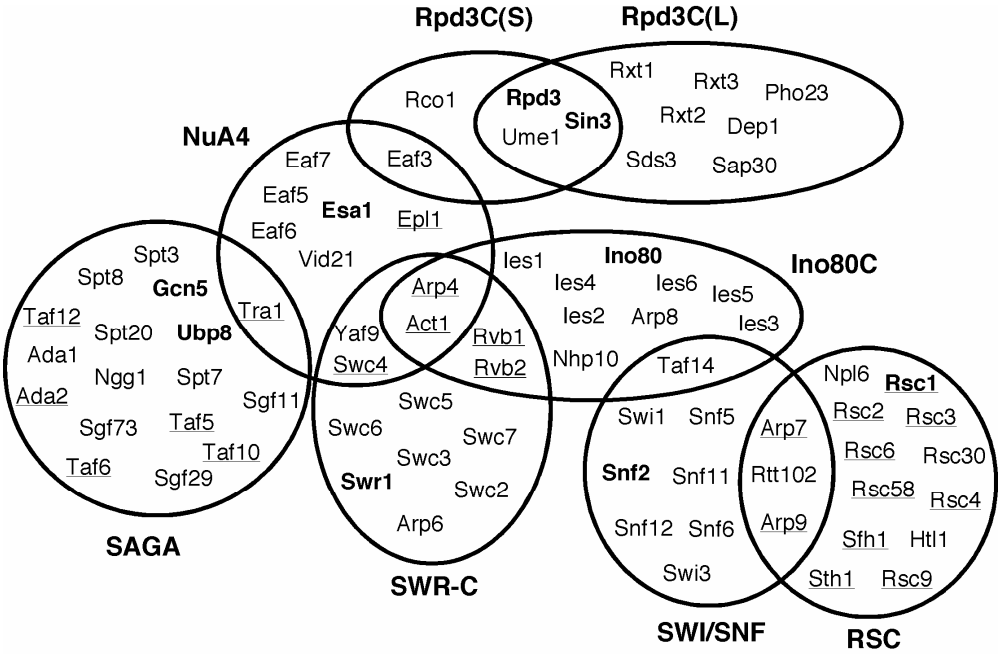

Figure S3. **The Overlapping Subunit Composition of a Number of Yeast Complexes Involved in Chromatin Function**

Rpd3C(L), Rpd3C(S), NuA4, SAGA, SWR-C, SWI/SNF, Ino80C and RSC. The catalytic subunits of these complexes are in bold. Proteins essential for viability are underlined.