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Histone H3K4 and K36 Methylation, Chd1 and Rpd3S Oppose the Functions of *Saccharomyces cerevisiae* Spt4–Spt5 in Transcription

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ABSTRACT

Spt4–Spt5, a general transcription elongation factor for RNA polymerase II, also has roles in chromatin regulation. However, the relationships between these functions are not clear. Previously, we isolated suppressors of a *Saccharomyces cerevisiae spt5* mutation in genes encoding members of the Paf1 complex, which regulates several cotranscriptional histone modifications, and Chd1, a chromatin remodeling enzyme. Here, we show that this suppression of *spt5* can result from loss of histone H3 lysines 4 or 36 methylation, or reduced recruitment of Chd1 or the Rpd3S complex. These *spt5* suppressors also rescue the synthetic growth defects observed in *spt5* mutants that also lack elongation factor TFIIS. Using a *FLO8* reporter gene, we found that a *chd1* mutation caused cryptic initiation of transcription. We further observed enhancement of cryptic initiation in *chd1 isw1* mutants and increased histone acetylation in a *chd1* mutant. We suggest that, as previously proposed for H3 lysine 36 methylation and the Rpd3S complex, H3 lysine 4 methylation and Chd1 function to maintain normal chromatin structures over transcribed genes, and that one function of Spt4–Spt5 is to help RNA polymerase II overcome the repressive effects of these histone modifications and chromatin regulators on transcription.

EUKARYOTES package their genomes into nucleosomes to form chromatin. Although nucleosomes and higher order chromatin structures permit significant compaction of the genome, they also inhibit transcription by blocking access to underlying DNA and by forming a repeating barrier to elongating RNA polymerases. Strategies used to overcome this inhibition and regulate transcription include: post-translational modification of histone tails; remodeling, eviction, or movement of nucleosomes by both ATP-dependent and -independent mechanisms; and incorporation of histone variants into nucleosomes (Saunders *et al.* 2006; Li *et al.* 2007a; Williams and Tyler 2007).

In contrast to promoters, which are often persistently nucleosome free, the bodies of actively transcribed genes are typically still nucleosome assembled, even though nucleosomes strongly inhibit elongation by purified RNA polymerase II (Studitsky *et al.* 2004; Pokholok *et al.* 2005; Saunders *et al.* 2006; Rando and Ahmad 2007). These observations imply that eukaryotes must possess activities that transiently alter or remove nucleosomes to permit elongation and then restore them to their prior state. Failure to restore chromatin structure after elongation may reveal cryptic promoters, leading to aberrant transcription initiation from internal positions within a gene (Kaplan *et al.* 2003; Mason and Struhl 2003; Carrozza *et al.* 2005). Thus, maintenance of chromatin structure over transcribed sequences presents a unique set of challenges and is critical to appropriate regulation of a cell's transcriptome.

The Spt4–Spt5 complex is an essential, highly conserved regulator of transcription elongation by RNAPII in eukaryotes (Hartzog *et al.* 2002). It joins elongation complexes soon after initiation (Andrulis *et al.* 2000; Ping and Rana 2001) and associates with RNAPII along the entire length of the gene (Kim *et al.* 2004). Although the precise function of Spt4–Spt5 is not known, *in vitro* studies show that it can repress transcription elongation at promoter proximal locations and can promote elongation under nucleotide limiting conditions (Wada *et al.* 1998). Furthermore, a wealth of genetic data implicate it in regulation of elongation and RNA processing *in vivo* (Cui and Denis 2003; Lindstrom *et al.* 2003; Kim *et al.* 2004; Bucheli and Buratowski 2005; Burckin *et al.* 2005; Kaplan *et al.* 2005; Xiao *et al.* 2005). In addition, *spt4* and *spt5* mutations share a number of phenotypes with histone mutations and genetically interact with mutations in genes encoding chromatin remodeling factors, suggesting that the function of Spt4–Spt5 is connected to chromatin (Swanson and Winston 1992; Squazzo *et al.* 2002; Simic *et al.* 2003).

We previously identified a mutation in the *Saccharomyces cerevisiae SPT5* gene, *spt5-242*, which confers a cold-sensitive (Cs^-) growth defect (Hartzog *et al.* 1998). We also identified two classes of suppressors of the Cs^-

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phenotype of *spt5-242* cells. The first class includes mutations in either of the two large, catalytic subunits of RNAPII (Hartzog *et al.* 1998). One of these mutations, *rpb2-10*, displays a decreased elongation rate and lower processivity *in vitro* (Powell and Reines 1996), and *rpb1* suppressors of *spt5-242* alter residues implicated in elongation (Hartzog *et al.* 1998). In addition, *spt5-242* is suppressed by 6-azauracil ((Hartzog *et al.* 1998), which inhibits nucleotide biosynthesis and is believed to impede elongation *in vivo* by starving the polymerase of substrate nucleotides (Exinger and Lacroute 1992). Thus, it appears that the *spt5-242* mutation is suppressed by decreased RNAPII elongation rates. The second class of *spt5-242* suppressors is composed of mutations that likely perturb chromatin structure or dynamics. These include mutations in *CHD1* (Simic *et al.* 2003), which encodes an ATP-dependent chromatin remodeling enzyme (Tran *et al.* 2000; Stockdale *et al.* 2006), with a pair of conserved N-terminal chromodomains, a central Snf/Swi type helicase domain and a C-terminal domain that resembles Myb-type DNA binding domains (Woodage *et al.* 1997). In addition, mutations that perturb the Paf1 complex, which regulates the activity of several histone-modifying enzymes, also suppress *spt5-242* (Squazzo *et al.* 2002).

In this work, we investigate the potential roles of this second class of *spt5-242* suppressors in transcription elongation. We show that these chromatin-based suppressors have effects on the transcription apparatus that are distinct from elongation rate-based suppression. We show that loss of a specific subset of Paf1 complex functions, methylation of histone H3 lysines 4 and 36, are involved in suppression of *spt5-242*. We present evidence that recruitment of Chd1 to transcribed genes may depend in part upon H3K4 and H3K36 methylation; we further show that all three conserved domains of Chd1 are required for its recruitment to chromatin and function. Finally, we find that loss of Chd1 contributes to the appearance of cryptic transcripts, suggesting that Chd1 plays a role in maintaining nucleosomes over transcribed regions. We suggest that the Spt4–Spt5 complex promotes transcription elongation across chromatin templates, acting in opposition to Rpd3S, Chd1, as well as histone H3K4 and H3K36 methylation and downstream effectors of these marks.

MATERIALS AND METHODS

Media and genetic methods: Strain construction and other genetic manipulations were carried out by standard methods (Rose *et al.* 1990). Yeast media was made as described previously (Rose *et al.* 1990). All *S. cerevisiae* strains used in this study (supporting information, Table S1) are isogenic to S288C and are *GAL2*$^+$ (Winston *et al.* 1995). The *GAL1-FLO8-HIS3* reporter construct was a gift of Fred Winston and was integrated as described by Cheung *et al.* (2008). For spot dilutions, strains were grown in rich or synthetic media. Cells were counted with a hemocytometer, 1×10^7 cells were pelleted in a microcentrifuge, and resuspended in 1 ml of water. Fivefold serial dilutions of these cells were prepared and 5–10 μl of each dilution was spotted on rich or synthetic media and incubated at the indicated temperature.

Plasmids: All plasmids used in this study are listed in Table S2. Chromodomain mutations in *CHD1* were constructed in a *HA$_3$-CHD1 URA3 CEN* plasmid, pGH269 (Simic *et al.* 2003). *Age*I sites flanking the chromodomains were generated by PCR mutagenesis. This plasmid was digested with *Age*I, purified, and religated to create the complete chromodomain deletion, pTQ5. PCR products flanked by *Age*I sites containing the first chromodomain and intervening sequence, or the intervening sequence and the second chromodomain, were synthesized and ligated into pTQ5 to create pTQ4 and pTQ3. The plasmid containing a Y316E point mutation in *CHD1* was also derived from pGH269 and was a gift of Patrick Grant (Pray-Grant *et al.* 2005). *rtf1* mutant plasmids in Figure 5A were gifts of Karen Arndt (Warner *et al.* 2007). For use in ChIP assays, *rtf1* mutant plasmids were digested with *Nde*I to remove the HA$_3$ tag and religated.

Chromatin immunoprecipitation: ChIP assays were performed as described previously (Simic *et al.* 2003). Strains containing *hht2* or *rtf1* mutations were grown in SC −Trp media and strains containing plasmid-borne *chd1* mutations were grown in SC −Ura to maintain plasmid selection. All other strains were grown in YPD media. RNAPII was precipitated with a monoclonal antibody, 8WG16 (Covance), and H3K9/14Ac with antihistone H3 K9/14Ac antibody (Upstate). Total histone H3 precipitated with an antibody directed against the C terminus of histone H3 (Abcam). The anti-HA antibody is described in Simic *et al.* (2003).

Quantitative PCR analysis was performed as follows on a Bio-Rad iCycler: 40 cycles of 95° for 30 sec, 55° for 30 sec, and 72° for 45 sec. PCR was carried out in 20-μl reactions in 96-well plates using Eurogentec qPCR MasterMix Plus for SYBR Green I Low ROX. Primers directed against *TEF2* and *PMA1* (Simic *et al.* 2003), YLR454W (Mason and Struhl 2005), and intergenic chromosome V (Komarnitsky *et al.* 2000) were described previously. For ChIP of *STE11*, the promoter primers amplified nucleotides −320 to −563 (relative to the ATG), the 5′ primers amplified nucleotides +1 to +330, and the 3′ primers amplified nucleotides +1641 to +1915. For ChIP of *FLO8*, the promoter primers amplified nucleotides −65 to −287, the 5′ primers amplified nucleotides +60 to +408, and the 3′ primers amplified nucleotides +1969 to +2349.

In Figure 5 and Figure S1, fold enrichment over a nontranscribed sequence on chromosome V was calculated as described previously (Ausubel *et al.* 1991). These values were then normalized to values obtained from control immunoprecipitations from an untagged strain. The RNAPII ChIPs in Figure 1 were analyzed as described in Mason and Struhl (2005), using the chromosome V sequence as a control. The ChIPs of H3AcK9/14 in Figure 9 were normalized to precipitations of total histone H3 to account for any strain-to-strain variation in nucleosome density. The H3Ac ChIPs from the *set2* mutant in Figure 9 are derived from two independent experiments. All other ChIP data were derived from three or more independent experiments.

Northern blots: Northern blotting was performed as described previously (Ausubel *et al.* 1991; Swanson *et al.* 1991; Kaplan *et al.* 2003). Probes for *FLO8*, *STE11*, and *RAD18* were PCR amplified from genomic DNA. The *FLO8* 3′ probe covers nucleotides +1595 to +2349 relative to the ATG. The *STE11* 3′ probe covers nucleotides +1641 to +2153 relative to the ATG. The *RAD18* 3′ probe covers nucleotides +472 to +1472 relative to the ATG. Probes were labeled by random priming as described previously (Ausubel *et al.* 1991).

RESULTS

The *spt5-242* mutation reduces the rate of elongation *in vivo*: To examine the rate of transcription elongation *in vivo*, we used a chromatin immunoprecipitation assay. The *GAL1* promoter, which is induced in galactose media and repressed in the presence of glucose, was integrated upstream of the ∼7.9 kb *YLR454W* gene (Figure 1A) and crossed into strains carrying the *spt5-242* and *chd1*Δ mutations. Cells were grown to log phase in media containing raffinose, which neither represses nor induces the *GAL1* promoter. The *GAL1* promoter was then induced with galactose for 90 min, an aliquot of each culture was formaldehyde crosslinked and frozen, and the remainder of the culture was treated with glucose to repress the *GAL1* promoter. Five minutes after addition of glucose an additional sample of cells was crosslinked and frozen. Both samples were then processed for ChIP using an antibody directed against RNA polymerase II, and a set of five PCR primers was used to monitor the distribution of RNAPII across the hybrid *GAL1-YLR454W* gene.

Previously, Mason and Struhl (2005) used this assay to monitor RNAPII processivity by examining polymerase distribution across the induced *GAL1-YLR454W* gene, and to monitor elongation rate by examining the decay of the RNAPII ChIP signal across *GAL1-YLR454W* following glucose repression. Examining a large collection of mutations, they found that deletion of *CHD1* did not affect processivity or elongation rate, whereas loss of *SPT4* resulted in decreased polymerase processivity, but a normal elongation rate. In addition, they found that *rpb2-10*, a mutation that suppresses *spt5-242* (Hartzog *et al.* 1998) and reduces elongation and processivity *in vitro* (Powell and Reines 1996), also reduced processivity and elongation rate across *GAL1-YLR454W in vivo*.

Consistent with these previous results, we found that RNAPII processivity in a *chd1*Δ strain closely matched that observed in wild type (Figure 1B). In glucose repressed samples, the *chd1* deletion resulted in only a modest increase in the density of RNAPII across *GAL1-YLR454W*. Polymerase density across the induced *GAL1-YLR454W* gene in the *spt5-242* mutant was similar to that observed in wild-type cells. In contrast, following glucose repression, RNAPII levels at *GAL1-YLR45W* in *spt5-242* cells were higher than in wild-type cells (Figure 1C). This effect was most obvious for ChIP probes at the 3′ end of the gene, indicating a decreased rate of clearance of RNAPII from *GAL1-YLR454W*. Thus the *spt5-242* mutation leads to a decreased transcription elongation rate *in vivo*.

Two mechanisms for genetic suppression of *spt5-242*: We were unable to reproducibly measure RNAPII levels at *GAL1-YLR454W* in glucose repressed *chd1*Δ *spt5-242* double mutants. This was likely due to the substantial decrease in RNAPII density in this double mutant observed even under inducing conditions (Figure 1A). As an alternative approach to determining the mechanism by which loss of Chd1 leads to suppression of *spt5-242*, we examined genetic interactions of *spt5-242* and *chd1* with a null allele of *DST1*, which encodes transcription elongation factor TFIIS and functions to overcome transcription arrest by RNAPII (Fish and Kane 2002). At each step in RNA chain elongation, RNAPII may add nucleotides to the 3′ end of the nascent RNA, pause, or arrest. Arrest occurs when the polymerase backtracks, leaving its active site misaligned over the DNA:RNA hybrid of the transcription bubble rather than over the 3′ end of the nascent RNA. TFIIS binds arrested RNAPII elongation complexes, stimulates cleavage of the nascent transcript, creating a new 3′ end that is properly aligned with the active site of the enzyme, allowing elongation to resume.

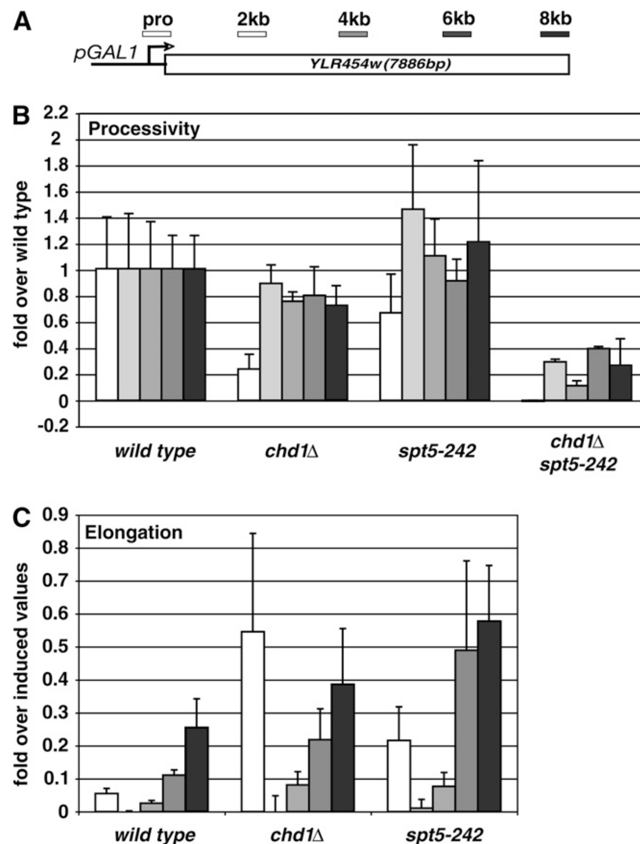

Figure 1.—Reduced rate of RNA polymerase II elongation in *spt5-242* mutant. (A) Location of ChIP probes on hybrid *GAL1-YLR454W* gene. (B) Measurement of RNAPII processivity. ChIP of RNAPII across the hybrid *GAL1-YLR454W* gene in wild-type cells and the indicated mutants was performed under inducing conditions. For each mutant, IP/Input values for each ChIP probe were determined and normalized to the corresponding value measured in the wild-type strain. (C) Measurement of elongation rate. Galactose-induced cells were treated with glucose to repress transcription from the *GAL1-YLR454W* gene and samples were processed for ChIP 5 min later. IP/Input values for each probe are expressed relative to the corresponding value for that probe measure just prior to addition of glucose.

Mutations in RNAPII subunits, including several *rpb1* and *rpb2* alleles that suppress *spt5-242*, cause synthetic growth defects or lethality when combined with TFIIS mutations (Hartzog *et al.* 1998; Lennon *et al.* 1998). These observations were interpreted to indicate that the *rpb* mutations lead to an increased frequency of arrest and dependence on TFIIS. In addition, *spt5 dst1*Δ mutants exhibit strong synthetic growth defects (Figure 2A; Hartzog *et al.* 1998). Thus, it is likely that *spt5* mutations also lead to increased arrest and dependence on TFIIS.

To determine whether the chromatin-based suppressors of *spt5-242* function by a mechanism similar to or distinct from that of the *rpb* suppressors of *spt5-242*, we performed genetic crosses to isolate strains with all possible combinations of *spt5-242*, *dst1*Δ, and *chd1*Δ mutations. Although *spt5-242 dst1*Δ mutants are inviable at temperatures below 37° (Figure 2A; Hartzog *et al.* 1998), a *spt5-242 dst1*Δ *chd1*Δ mutant was viable at temperatures as low as 22° (Figure 2A). In addition, *dst1*Δ *chd1*Δ mutants did not display any other obvious new phenotypes. Thus, loss of Chd1 overcomes the synthetic growth defect of *spt5-242 dst1*Δ mutants.

In contrast to the results with *chd1*Δ, when we crossed strains carrying *dst1*Δ, *spt5-242*, and either the *rpb1-221* or *rpb1-244* mutations, which suppress *spt5-242*, we were unable to obtain viable triple mutants. Thus, although *rpb1-221* and *rpb1-244* suppress *spt5-242*, they cannot overcome the synthetic growth defects observed in *spt5-242 dst1*Δ double mutants. These data support the idea that chromatin defects and elongation defective forms of RNAPII suppress *spt5-242* by distinct mechanisms.

In addition to enhancing the phenotype of *spt5-242*, deletion of *DST1* causes a temperature sensitive (Ts$^-$) growth defect when combined with other transcription elongation factor mutations, including *spt5-194*, *spt4*Δ, and *spt6-14* (Hartzog *et al.* 1998). We therefore crossed strains carrying *chd1*Δ, *dst1*Δ, and these *spt* mutations. Deletion of *CHD1* did not alter growth of the *spt5-194 dst1*Δ strain (data not shown) but did partially suppress the Ts$^-$ phenotype of *spt4*Δ *dst1*Δ and *spt6-14 dst1*Δ (Figure 2, B and C). Thus, Chd1 may have a more general role in elongation since its interactions are not factor specific.

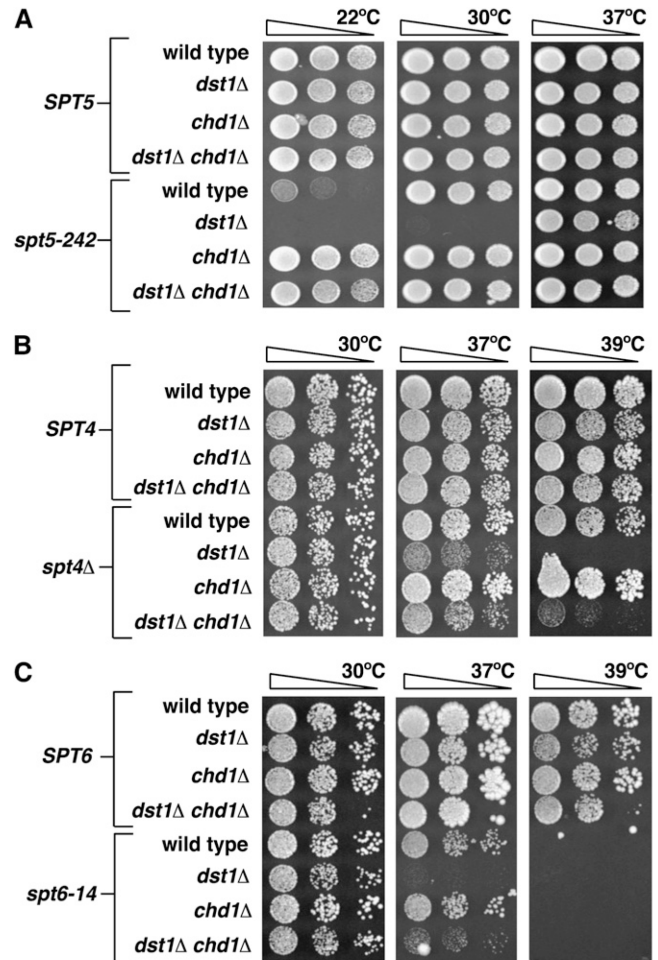

Figure 2.—*chd1* mutations suppress growth defects of *spt dst1* mutants. Genetic crosses were performed to create strains carrying combinations of complete deletions of *DST1* and *CHD1* with the cold-sensitive *spt5-242* mutation, a complete deletion of *SPT4*, and the temperature-sensitive *spt6-14* mutation. Serial dilutions of these strains were spotted to YPD media and grown at the indicated temperature. (A) *chd1*Δ suppresses the growth defect of *spt5-242 dst1*Δ cells at 22° and 30°. (B) *chd1*Δ suppresses the growth defect of *spt4*Δ *dst1*Δ cells at 39°. (C) *chd1*Δ suppresses the growth defect of *spt6-14 dst1*Δ cells at 37°.

Relationships between chromatin-based suppressors of *spt5-242*: The data presented above demonstrate that there are at least two distinct mechanisms for genetic suppression of *spt5-242*, decreasing the elongation rate of RNA polymerase II or disruption of chromatin. We next explored genetic relationships between previously identified chromatin-based suppressor of *spt5-242*, the Paf1 complex and Chd1.

Disruption of an Rtf1–Chd1 interaction suppresses *spt5-242*: Two prior observations suggest that Chd1 interacts with Rtf1, a component of the Paf1 complex. First, a C-terminal fragment of Chd1 interacted with Rtf1 in a two-hybrid experiment. Second, Chd1 did not associate with transcribed chromatin in an *rtf1* mutant (Figure S1; Simic *et al.* 2003). These data suggested the possibility that in addition to its role in Set1 function, the Paf1 complex may play a more direct role in Chd1's association with chromatin via a direct Rtf1–Chd1 interaction.

Several recently characterized Rtf1 mutations support this idea (Warner *et al.* 2007). *rtf1*Δ*1* is an internal deletion that removes amino acids (aa) 3–30 and disrupts the two-hybrid interaction between Chd1 and Rtf1, but does not alter H3K4 methylation. *rtf1*Δ*2*, which deletes aa 30–62, does not alter the two-hybrid interaction with Chd1, histone H3 methylation, or H2B ubiquitylation. In contrast, *rtf1*Δ*3* deletes aa 62–109 and causes defects in histone H2B ubiquitylation and H3K4

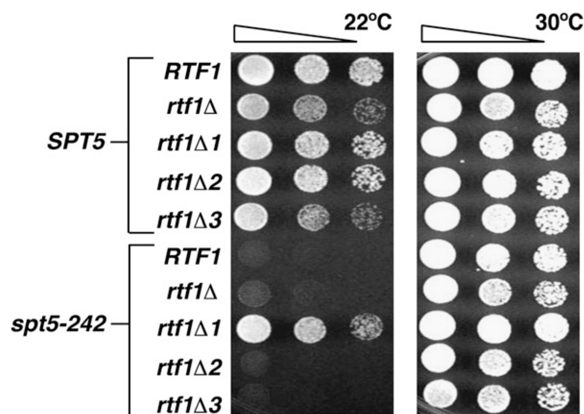

FIGURE 3.—The *rtf1Δ1* mutation suppresses *spt5-242*. Serial dilutions of *SPT5 rtf1Δ* and *spt5-242 rtf1Δ* strains transformed with the indicated *RTF1* plasmids were spotted to SC −Trp media and grown at 22° or 30° for 3 days. Only *rtf1Δ1*, which disrupts Rtf1–Chd1 interactions, suppressed the cold-sensitive phenotype of *spt5-242*.

and H3K79 methylation, but does not alter the Rtf1–Chd1 two-hybrid interaction. Each of these forms of Rtf1 retains the ability to assemble into the Paf1 complex and associate with chromatin. When we combined these *rtf1* mutations with *spt5-242*, we found that *rtf1Δ1* suppressed the Cs⁻ phenotype of *spt5-242* whereas *rtf1Δ2* and *rtf1Δ3* did not (Figure 3A). Consistent with this suppression, in ChIP assays, the *rtf1Δ1* mutation abolishes Chd1's association with transcribed chromatin (WARNER *et al.* 2007; Figure S1). These data suggest that, in addition to its role in regulating histone H3 modification states, Rtf1 plays an important role in Chd1's recruitment to transcribed chromatin during transcription elongation.

Evidence that methylation of histone H3 lysines 4 and 36 impact transcription elongation: The Paf1 complex plays a role in recruiting and regulating the histone H3 methyltransferases Set1, Set2, and Dot1 and also the Rad6/Bre1 histone H2B ubiquitin ligase (KROGAN *et al.* 2003a,b; NG *et al.* 2003; WOOD *et al.* 2003). Suppression of *spt5-242* by perturbation of the Paf1 complex could be due to loss of one or more of these enzymes. We therefore crossed an *spt5-242* mutant to strains carrying deletions of *SET1*, *SET2*, *DOT1*, and *RAD6*. We also created *spt5-242* strains that carried substitutions of histone H3 lysine 4 (H3K4), H3K36, or H3K79 as their sole source of these histones. The progeny of these crosses were monitored for growth at a variety of temperatures (Figure 4 and data not shown). The *set1Δ* mutation suppressed the Cs⁻ phenotype of *spt5-242* weakly at 15° and more strongly at 22°. A similar level of suppression was observed when H3K4 was substituted with either alanine (H3K4A) or arginine (H3K4R). The *set2Δ* and H3K36R mutations both strongly suppressed the growth defect of *spt5-242* at 15° and 22°. We further observed that *spt5-242 set1Δ set2Δ* mutants showed the same level of growth as *spt5-242 set2Δ*

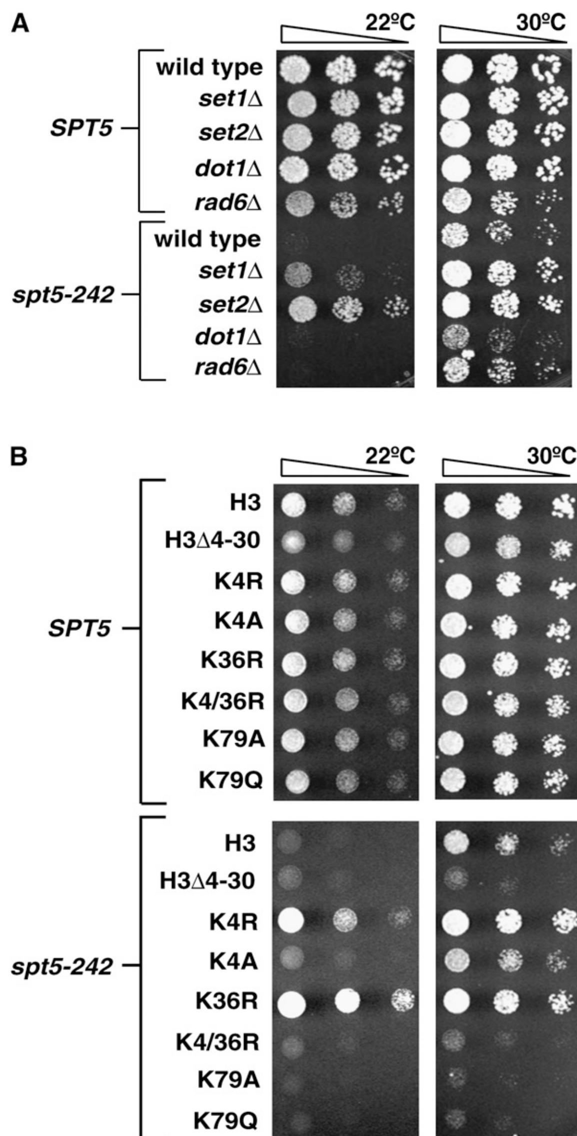

FIGURE 4.—*spt5-242* is suppressed by loss of H3K4 or H3K36 methylation. (A) *Spt5-242* is suppressed by loss of the H3K4 or H3K36 methyltransferases Set1 and Set2 but not by loss of the H3K79 methyltrasferase Dot1 nor the ubiquitin conjugating enzyme Rad6. Strains with the indicated genotypes were spotted to YPD and incubated at 30° for 2 days or 22° for 4 days. (B) Mutations altering H3K4 or H3K36 suppress *spt5-242*. *SPT5* and *spt5-242* strains carrying deletions of both histone H3–H4 loci and a CEN *URA3 HHT1-HHF1* plasmid were transformed with plasmids carrying the indicated histone H3 allele. Trp⁺ transformants were spotted directly to 5FOA and incubated at 30° for 2 days or 22° for 3 days.

mutants. Curiously, *spt5-242* H3K4/K36R mutants did not grow at 15° or 22°. In contrast to the results with *SET1* and *SET2*, deletion of *DOT1* or mutation of its target, H3K79, failed to suppress *spt5-242*. In sum, these data suggest a role for Set1, Set2, and methylation of histone H3 lysines 4 and 36 in Spt5 function.

Normal methylation of H3K4 and H3K79 depends upon Rtf1, Rad6, and H2BK123 ubiquitylation (DOVER *et al.* 2002; NG *et al.* 2002; SUN and ALLIS 2002; KROGAN

et al. 2003a). Curiously, mutations that abolished H2BK123 ubiquitylation, *rtf1*Δ, *rtf1*Δ*3*, and *rad6*Δ, all failed to suppress *spt5-242* (Figures 3 and 4; Squazzo *et al.* 2002). Thus, loss of H2BK123 ubiquitylation appears to impact elongation in a manner that is distinct from and epistatic to the loss of H3K4 or H3K36 methylation. One potential explanation for these observations is that, in addition to its role in histone methylation, H2BK123 ubiquitylation may directly facilitate elongation (Pavri *et al.* 2006; Fleming *et al.* 2008).

Chd1's conserved domains are required for association with transcribed chromatin: The results described above raised the possibility that methylation of H3K4 or K36 might mediate recruitment of Chd1 to chromatin. We therefore sought to identify the domain(s) of Chd1 that mediate its association with chromatin. We focused on Chd1's three conserved domains: its pair of N-terminal chromodomains (CDs), its Swi/Snf-like helicase domain, and its C-terminal domain with homology to Myb-type DNA binding domains (Figure 5A). We previously constructed mutations altering each of these domains in a triple HA epitope-tagged form of Chd1 (HA_3-Chd1), and found that the altered proteins were expressed at normal levels and that the mutations suppressed *spt5-242*, although not always to the same extent as a complete *chd1*Δ (Simic *et al.* 2003).

To examine the contribution of the individual CDs, we generated individual deletions of CDs 1 and 2 (ΔCD1 and ΔCD2) as well as a mutation altering a single residue of CD2, Y316E, which was previously shown to disrupt binding to dimethylated H3K4 peptides *in vitro* (Pray-Grant *et al.* 2005). Western blot analyses indicated that these altered forms of HA_3-Chd1 were expressed at wild-type levels (data not shown). Like the double chromodomain deletion (ΔCD), alterations of individual Chd1 chromodomains suppressed the growth defect of the *spt5-242* mutation moderately at 15° and as well as *chd1*Δ at 22° (Figure 5B and data not shown), implying that deletion of either CD is genetically equivalent to deletion of both and that the CDs play an important role in Chd1 function.

To ask whether these genetic interactions reflect a failure of Chd1 to associate with chromatin, we performed ChIP assays with the chromodomain deletion forms of HA_3-Chd1. Deletion of both CDs, deletion of CD2 alone, and the Y316E mutation all decreased the HA_3-Chd1 ChIP signal over *PMA1* and *TEF2* to background levels (Figure 5, C and D). Deletion of CD1 resulted in a similar decrease at *PMA1*, but only a partial decrease over *TEF2*. These data suggest that CD2 is essential and CD1 is important for the association of Chd1 with actively transcribed chromatin.

We next examined the helicase and C-terminal domains of Chd1. To determine whether Chd1's ATPase activity is required for its association with chromatin, we performed ChIP assays with the HA_3-K407R form of

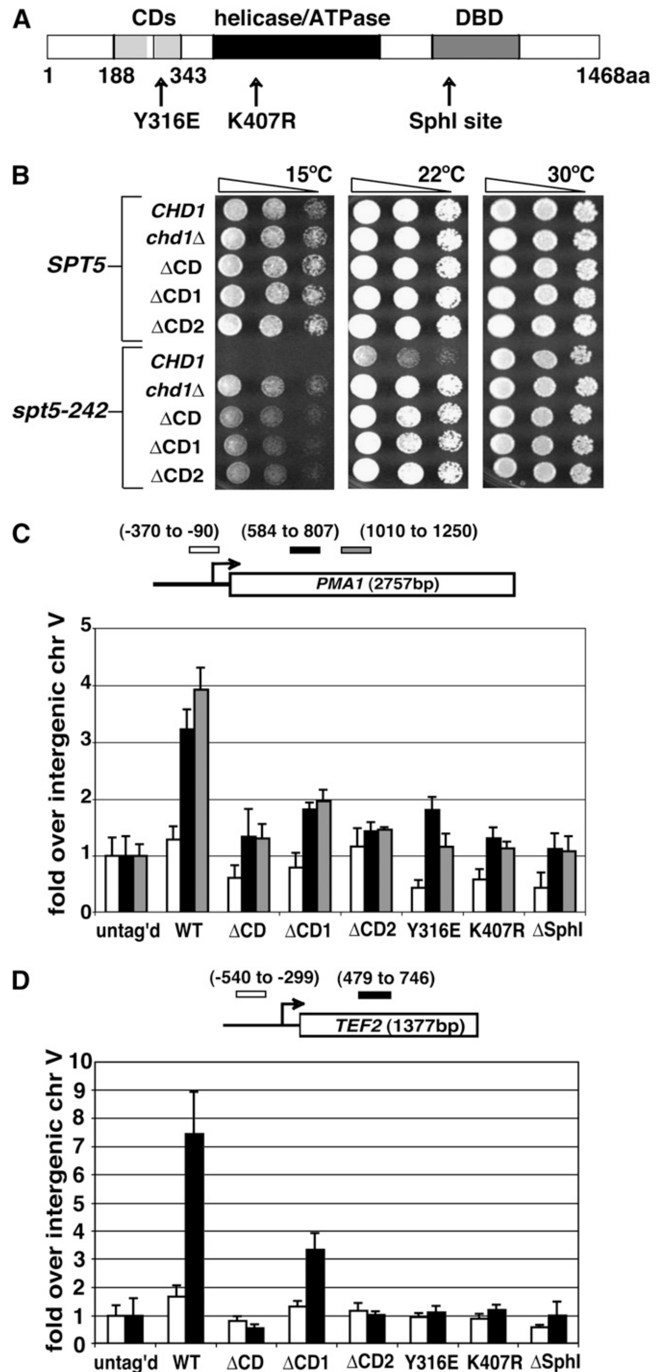

Figure 5.—All three conserved domains of Chd1 are required for its function and localization to chromatin. (A) The location of the conserved sequence motifs of Chd1 as well as sites targeted for mutations. (B) Deletion of either or both chromodomains of Chd1 is sufficient for suppression of *spt5-242*. *SPT5 chd1*Δ and *spt5-242 chd1*Δ strains were transformed with *URA3 CEN* plasmids carrying the indicated *chd1* mutations expressed from the normal *CHD1* promoter. Serial dilutions of cells with the indicated genotypes were spotted to SC −Ura media and grown at the indicated temperature for 4 days. (C and D) All three conserved domains of Chd1 are required for its association with chromatin. Strains expressing HA_3-tagged forms of Chd1 were subjected to anti-HA1 ChIP followed by QPCR analysis using primers directed against the promoters and transcribed regions of *PMA1* (C) and *TEF2* (D).

Chd1, which is expressed at wild-type levels and suppresses *spt5-242*. Lysine 407 falls in the adenine nucleotide-binding motif of Chd1, and similar substitutions in other chromatin remodeling enzymes abolishes their ATP-binding and remodeling activities (Corona *et al.* 1999). Like the CD deletion mutants, we found this mutation to also cause a dramatic decrease in Chd1 levels over the *PMA1* and *TEF2* ORFs (Figure 5, C and D). Consistent with our data, mutations that alter the helicase or chromodomains of mouse Chd1 alter its nuclear distribution in cultured cells (Kelley *et al.* 1999). Finally, we examined a truncated form of Chd1 lacking most of the putative C-terminal DNA-binding domain (HA_3-ΔSphI, Δaa 1083–1468) and found that it also showed a decreased association of Chd1 with *PMA1* and *TEF2* (Figure 5, C and D).

In each of the experiments above, mutations in the chromodomains, ATP-binding site and C terminus of Chd1 gave a similar set of loss-of-function phenotypes. To further examine the effects of these *chd1* mutations on gene expression, we performed DNA microarray analysis of each of them. We compared the expression profiles of these mutants to a set of profiles from 80 mutants defective for a wide variety of functions in gene expression. Hierarchical clustering of the resulting expression data showed no striking differences between these *chd1* mutants in overall gene expression or pre-mRNA splicing (data not shown). In summary, both genetic and DNA microarray analyses suggest that mutation of any one of Chd1's three conserved domains either leads to a nonfunctional protein or prevents Chd1 from reaching its site of action.

Chd1 recruitment does not generally depend upon H3K4 or H3K36 methylation: We next considered the hypothesis that suppression of *spt5-242* by *set1*, *set2*, H3K4, and H3K36 mutations stems from a failure to recruit Chd1 to transcribed chromatin. This hypothesis was suggested by previous observations of chromodomains that bind methylated histone tails (Bannister *et al.* 2001; Lachner *et al.* 2001; Cao *et al.* 2002). Furthermore, one report suggests that yeast Chd1 may bind to H3 tails methylated at lysine 4 (Pray-Grant *et al.* 2005), but others argue that although human Chd1 binds H3K4 methylated tails, yeast Chd1 lacks this activity (Flanagan *et al.* 2005, 2007; Sims *et al.* 2005). We therefore performed ChIP of HA_3-Chd1 from strains carrying the *set1*Δ or *set2*Δ mutations, or from strains that expressed the H3K4R or H3K36R as their only source of histone H3. However, the resulting data did not show consistent changes in the association of HA_3-Chd1 with transcribed chromatin (data not shown).

Genetic evidence that the Rpd3S complex opposes Spt4–Spt5 function: If altered H3K4 or K36 methylation does not result in decreased recruitment of Chd1 to chromatin, then what mechanism might explain suppression of *spt5-242* when H3K4 or K36 methylation is perturbed? In the case of H3K36, a potential explanation is provided by the observation that nucleosomes methylated at H3K36 are targeted by the Rpd3S histone deacetylase complex (Carrozza *et al.* 2005; Keogh *et al.* 2005). This raises the possibility that the genetic interaction between *SET2* and *SPT5* reflects a role for the Rpd3S complex in transcription elongation and Spt5 function. To test this genetically, we crossed the *spt5-242* strain to strains lacking Rpd3, the catalytic subunit of the Rpd3S complex (Figure 6A). The resulting double mutant did not grow at 22° and grew more poorly than *spt5-242* alone at 30°. This enhancement of the *spt5-242* growth defect could be due to Rpd3's participation in multiple histone deacetylase complexes (Carrozza *et al.* 2005; Keogh *et al.* 2005). Thus, we examined mutations of two other genes. One, *EAF3* encodes a chromodomain protein that binds H3K36 methylated nucleosomes and is found in the Rpd3S histone deacetylase and NuA4 histone acetyltransferase complexes (Eisen *et al.* 2001; Carrozza *et al.* 2005; Joshi and Struhl 2005; Keogh *et al.* 2005; Li *et al.* 2007b). The other, *RCO1*, encodes a protein that interacts with Eaf3 and is required for proper Rpd3S complex assembly (Carrozza *et al.* 2005). Both *eaf3*Δ and *rco1*Δ mutations suppressed *spt5-242*'s Cs^- growth defect (Figure 6A). Because Rco1 is found exclusively in the Rpd3S complex (Carrozza *et al.* 2005; Keogh *et al.* 2005), these data suggest a role for the Rpd3S complex in Spt5 function.

We next performed genetic crosses to create *spt5-242 dst1*Δ *set2*Δ and *spt5-242 dst1*Δ *rco1*Δ mutants. As with *chd1*Δ, we found that the *set2*Δ and *rco1*Δ mutations suppressed the growth defects of the *spt5-242 dst1*Δ mutant (Figure 6, B and C). Thus, like *chd1*Δ, suppression of *spt5-242* by loss of Set2 or the Rpd3S complex occurs by a pathway that is distinct from that used by elongation defective forms of RNAPII.

If loss of the Rpd3S complex, which leads to hyperacetylation of histones (Carrozza *et al.* 2005; Joshi and Struhl 2005; Keogh *et al.* 2005), suppresses *spt5-242*, we would expect that decreased histone acetylation should enhance *spt5-242* phenotypes. To test this idea, we crossed *spt5-242* to deletions of *GCN5*, *SPT3*, *SPT7*, and *SPT8*, genes encoding subunits of the SAGA histone acetyltransferase (Grant *et al.* 1997). In each case, the double mutants derived from these crosses failed to grow at 22° and grew very poorly at 30° (Figure 7A and data not shown). In addition, we found that *spt5-242* cells grew poorly when their only source of histone H3 was H3K9/14R or H3K9/14/18/23R (Figure 7B). These observations suggest that histone acetylation plays an important role in transcription elongation, particularly when Spt5 function is compromised.

A role for Chd1 in prevention of cryptic transcription initiation: The Rpd3S complex is implicated in maintaining normal chromatin structure over transcribed regions, and loss of Rpd3S function leads to transcription initiation from cryptic internal promoters

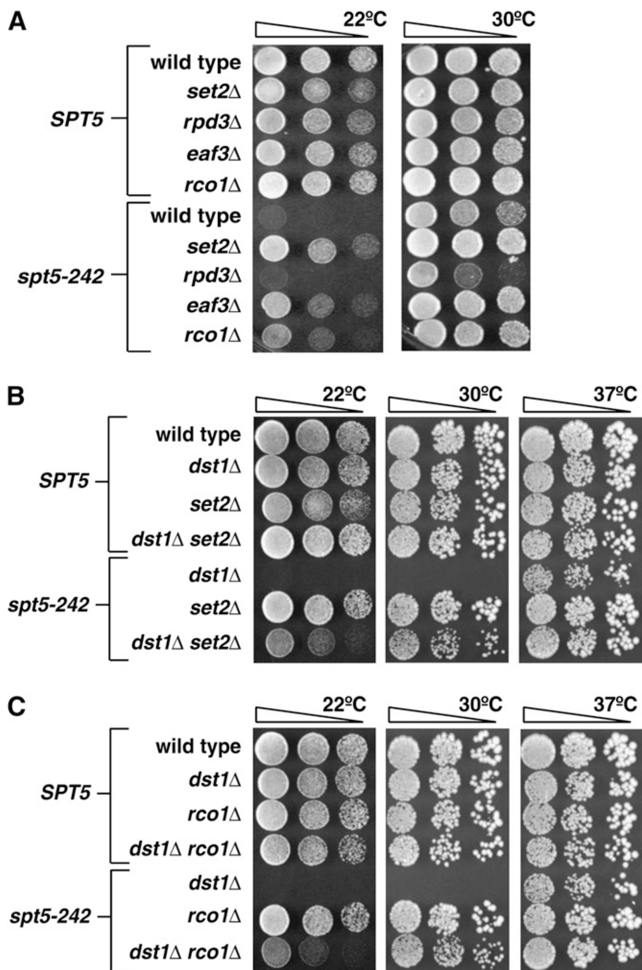

Figure 6.—Mutations that disrupt Rpd3S function suppress *spt5-242*. (A) To determine whether loss or reduced recruitment of the Rpd3S complex leads to suppression of *spt5-242*, strains carrying *set2*Δ, *rpd3*Δ, *eaf3*Δ, or *rco1*Δ mutations in combination with wild-type *SPT5* or *spt5-242* were spotted on YPD and incubated at 22° or 30° for 2 days. (B) Loss of Set2 suppresses the growth defect of *spt5-242 dst1*Δ double mutants. Strains carrying the indicated combinations of *set2*Δ, *dst1*Δ, and *spt5-242* mutations were serially diluted on YPD media and incubated at 22°, 30°, or 37° for 3 days. (C) Loss of Rco1, a subunit of Rpd3S, suppresses the growth defect of *spt5-242 dst1*Δ double mutants. Strains carrying the indicated combinations of *rco1*Δ, *dst1*Δ, and *spt5-242* mutations were serially diluted on YPD media and incubated at 22°, 30°, or 37° for 3 days.

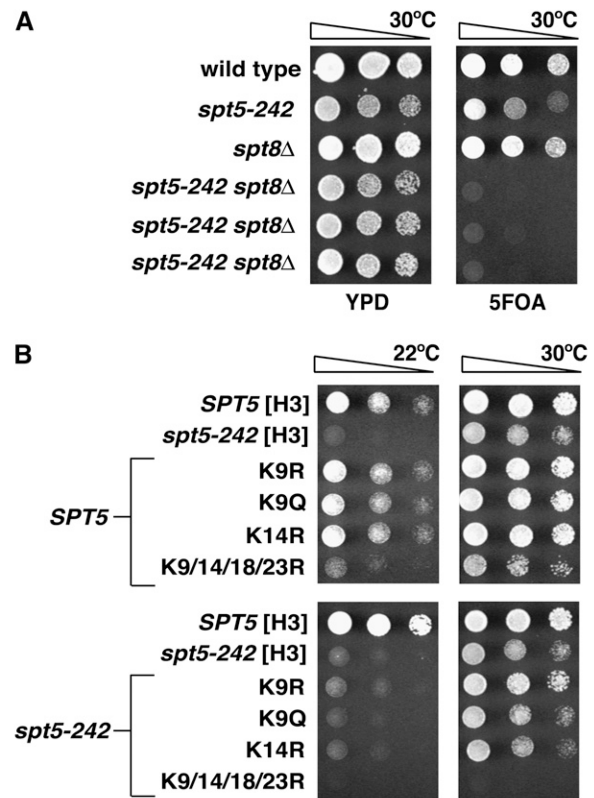

Figure 7.—Mutations that interfere with histone acetylation do not suppress *spt5-242*. (A) Loss of the SAGA subunit Spt8 decreases the viability of *spt5-242* mutants. A strain containing *spt5-242* was crossed to a strain lacking *SPT8*. The resulting diploid was transformed with a *URA3 SPT5* plasmid and then followed through sporulation. The parental strains, a wild-type control, and three representative double mutants were selected and spotted to YPD and 5FOA and incubated at 30° for 2 days. (B) Mutation of H3K9 or H3K14 fails to suppress *spt5-242*, but mutation of H3K9/14/18/23 leads to enhanced *spt5-242* phenotypes. *SPT5* and *spt5-242* strains carrying deletions of both histone H3–H4 loci and a *URA3 HHT1-HHF1* plasmid were transformed with plasmids carrying the indicated histone H3 allele. Trp^+ transformants were spotted directly to 5FOA and incubated at 30° for 2 days or 22° for 3 days.

(Carrozza *et al.* 2005; Li *et al.* 2007b; Cheung *et al.* 2008). To assay for this defect in *chd1*Δ cells, we used a genetic reporter of cryptic initiation over the *FLO8* gene, which has been extensively characterized for cryptic initiation (Kaplan *et al.* 2003; Carrozza *et al.* 2005; Cheung *et al.* 2008). In the reporter, *FLO8* transcription is driven by the strong *GAL1* promoter and the 3′ end of *FLO8* is replaced by the *HIS3* gene (Figure 8A; Cheung *et al.* 2008). Transcription initiation at the normal *FLO8* start site produces a transcript in which the *HIS3* open reading frame is out of frame and not translated. Internal initiation at *FLO8*, however, allows translation of a functional His3 protein, complementing the His^- phenotype of the reporter strain. Consistent with previous reports, we found that *set2* deletion and H3K36R substitution mutations gave strong His^+ phenotypes in the reporter strain. In contrast, a *set1* null mutation gave a His^- phenotype and H3K4 substitutions gave weak His^+ phenotypes that were only apparent after 4–5 days incubation. Interestingly, deletion of the N-terminal tail of histone H3 gave a strong His^+ phenotype. Finally, we observed that a *chd1* null mutation gave a strong His^+ phenotype (Figure 8C).

These observations suggest that the *chd1* mutation causes internal initiation of transcription from the *FLO8-HIS3* reporter. To test for internal initiation defects in the chromosomal *FLO8* gene, we assayed Northern blots of wild-type and mutant strains with

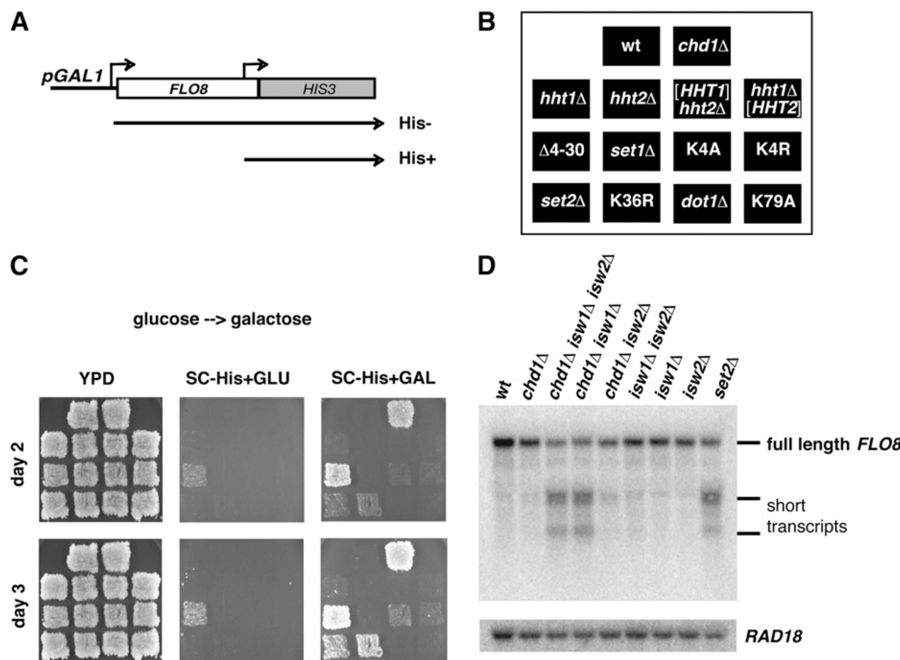

FIGURE 8.—Cryptic, internal initiation of transcription in a *chd1* mutant. Two approaches were used to determine whether loss of Chd1 leads to the appearance of cryptic, internally initiated transcripts over transcribed sequences. (A) Diagram of the p*GAL1*-*FLO8*-*HIS3* reporter gene. Transcription initiation from the normal *FLO8* start site produces a transcript in which *HIS3* is out of frame and not translated. Internal initiation of this transcript produces in frame transcripts and a His^+ phenotype. (B) Diagram showing the pattern of cells carrying the p*GAL1*-*FLO8*-*HIS3* reporter and indicated genotypes that were patched onto YPD media. The strains labeled *hht1*Δ or *hht2*Δ lack one *HHT*-*HHF* locus. [HHT1] and [HHT2] refer to a strain with deletions of both *HHT*–*HHF* loci complemented by a plasmid-borne copy of one of these two loci; in the patch labeled Δ4-30, this strain carries an *hht2*–*HHF2* plasmid, carrying a deletion of codons 4–30 of histone H3. Patches K4A and K4R indicate similar strains with plasmids carrying H3K4 mutations. (C) The YPD plate described in B was replica plated onto SC −His media utilizing either glucose or galactose as the carbon source and incubated at 30° for the indicated number of days. (D) RNA was isolated from the indicated strains and subjected to Northern blot analysis to detect short transcripts from *STE11* (top) and *FLO8* (middle). Hybridization to a *RAD18* probe (bottom) was used as a loading control.

probes derived from the 3′ end of *FLO8*, but did not observe clear evidence of internal initiation (Figure 8D). This observation mirrors recent results of Winston and colleagues, who observed a strong effect of a *chd1* deletion on the *FLO8-HIS3* reporter but very weak effects on internal initiation of normal chromosomal genes as measured by Northern blots (CHEUNG *et al.* 2008). We reasoned that the internal initiation defect caused by a *chd1*Δ mutation might be weak on its own, but enhanced in the presence of other mutations. This phenomenon has been observed for several mutations that activate the *FLO8-HIS3* reporter, but only show clear molecular evidence of internal initiation when combined with other mutations (PRATHER *et al.* 2005; NOURANI *et al.* 2006; CHU *et al.* 2007). Because previous reports have shown synthetic growth defects and chromatin disruption in an *isw1*Δ *isw2*Δ *chd1*Δ mutant (TSUKIYAMA *et al.* 1999; XELLA *et al.* 2006), we analyzed RNA from strains carrying all combinations of *chd1*Δ, *isw1*Δ, and *isw2*Δ mutations (Figure 8D). We observed that the *chd1* mutation showed a strong synthetic cryptic initiation defect when combined with *isw1*Δ. Furthermore, we obtained similar results when we probed Northern blots for *STE11*; neither the *isw1*Δ nor *chd1*Δ single mutants displayed internal initiation, but the *isw1*Δ *chd1*Δ mutant produced a strong internally initiated *STE11* transcript (data not shown).

Increased histone acetylation in *chd1*Δ strains: We next asked whether the cryptic initiation defect of a *chd1*Δ mutation reflected altered histone methylation. Western blot analysis of crude extracts revealed no obvious changes in modifications H3K4me3, H3K36me3, H3K79me2, and H3K9/14Ac (data not shown). We performed ChIP with H3K4me3 and H3K36me3 antibodies and did not observe alterations of these methyl marks in a *chd1*Δ strain. In contrast, when we performed ChIP with antibodies directed against histone H3 acetylated at lysines 9 and 14, we observed increased acetylation in *chd1* and *set2* mutants (Figure 9). Consistent with the relatively weak cryptic initiation phenotype of *chd1*Δ, the increase in acetylation observed at *FLO8* and *STE11* in the *chd1*Δ strain was less than that observed for a *set2*Δ strain.

DISCUSSION

Spt5 is an essential and highly conserved transcription elongation factor that is a core component of transcribing RNAPII. In this work, we have used a unique mutation in the yeast *SPT5* gene to genetically probe the RNAPII transcription apparatus. This mutation, *spt5-242*, causes transcription defects and a cold-sensitive growth defect that can be suppressed by conditions that decrease the rate of transcription elongation. The data presented here show that there is a second mechanism for suppression of *spt5-242* that involves particular perturbations of chromatin. Thus, there appear to be two distinct paths to suppression of *spt5-242*, via decreased elongation rate or alteration of chromatin.

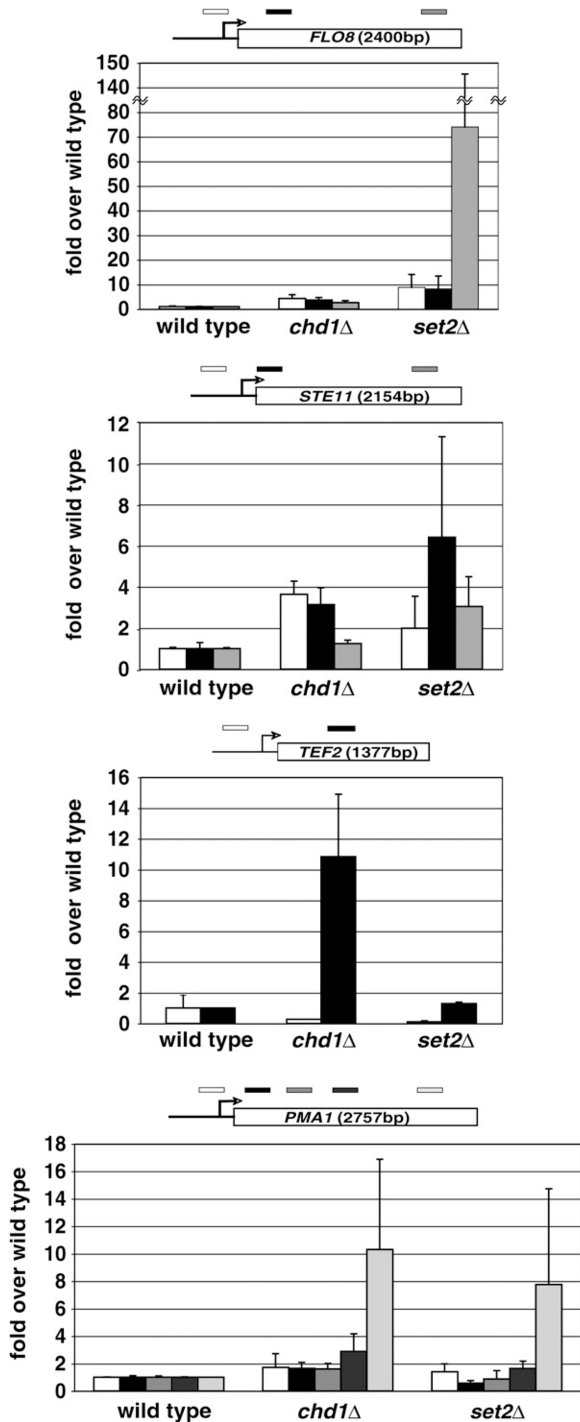

Figure 9.—Loss of Chd1 leads to increased H3 acetylation. Wild-type, *chd1*Δ, and *set2*Δ strains were subjected to ChIP with anti-H3Ac and anti-H3 antisera followed by QPCR analysis using primers directed against the promoters and transcribed regions of *FLO8*, *STE11*, *PMA1*, and *TEF2*. Bar graphs present H3Ac values relative to total histone H3 and are normalized to wild type.

What mechanisms might explain these two pathways to suppression of *spt5-242*? Several groups have previously argued that Set2, Chd1, and Rpd3S oppose transcription (Carrozza *et al.* 2005; Joshi and Struhl 2005; Keogh *et al.* 2005; Kizer *et al.* 2005; Biswas *et al.* 2006, 2007). Evidence for this idea includes the observations that mutations in *SET2*, *CHD1*, and genes encoding subunits of Rpd3S display partial resistance to the elongation inhibitor 6AU (Woodage *et al.* 1997; Keogh *et al.* 2005; Kizer *et al.* 2005; Biswas *et al.* 2006). In addition, similar to the results presented here for suppression of *spt5-242*, *set2*, rpd3S, and *chd1* mutations genetically suppress mutations affecting FACT and the Bur1 kinase (Keogh *et al.* 2005; Biswas *et al.* 2006, 2007; Chu *et al.* 2006). Thus, the genetic relationships between Spt4–Spt5 and chromatin modifiers presented here likely reflect a general set of antagonistic relationships between chromatin and transcription elongation factors (Figure 8).

One clue to a potential biochemical explanation for these relationships is provided by our observations that chromatin-based suppressors of *spt5-242* also alleviated the growth defect of an *spt5-242 dst1*Δ mutant. In contrast, the *rpb1* suppressors of *spt5-242* display synthetic sickness or lethality when combined with *dst1*Δ and do not overcome *spt5-242 dst1*Δ growth defects (Figure 1; Hartzog *et al.* 1998). These observations are consistent with a model in which our chromatin suppressor mutations decrease the probability of transcription arrest and the need for TFIIS *in vivo*. Although TFIIS has been recently implicated in transcription initiation as well as elongation (reviewed in Sikorski and Buratowski 2009), several observations show that Spt4–Spt5's functions are restricted to postinitiation events. First, Spt4–Spt5 does not join transcription complexes until after promoter clearance (Ping and Rana 2001; Bourgeois *et al.* 2002) and only exerts its negative effect on transcription after the nascent transcript has been extended 50–60 nucleotides (Yamaguchi *et al.* 1999; Bourgeois *et al.* 2002). Second, Spt4–Spt5 does not alter initiation *in vitro* (Guo *et al.* 2000; Bourgeois *et al.* 2002; Yamada *et al.* 2006; Zhu *et al.* 2007). Third, although proteomic studies have revealed a myriad of interactions between Spt4–Spt5 and RNAPII, elongation factors and RNA processing factors, Spt4–Spt5 has not been observed to physically associate with initiation-specific factors such as the mediator complex (Krogan *et al.* 2002, 2006; Lindstrom *et al.* 2003; Gavin *et al.* 2006). Finally, using ChIP of RNAPII, two groups have observed decreased processivity of RNAPII in *spt4* mutants (Morillon *et al.* 2003; Mason and Struhl 2005). Thus, the increased dependence of *spt4* and *spt5* mutants on TFIIS function is consistent with the idea that transcription arrest occurs more frequently in the absence of normal Spt4–Spt5 function. Furthermore, barriers to RNAPII elongation, such as nucleosomes, are known to provoke transcription arrest (Fish and Kane 2002; Kireeva *et al.* 2005). Thus we believe that the chromatin-based suppressors disrupt, fail to reassemble, or alter the stability, spacing or location of nucleosomes, which would otherwise lead to increased dependence

upon TFIIS, perhaps due to transcription arrest in *spt5-242* cells.

How is Chd1 recruited to chromatin? Given the shared phenotypes of *chd1*, *set1*, and *set2* mutants, and that Chd1 contains chromodomains, an attractive model is that the H3K4me or H3K36me marks are used to recruit Chd1 to chromatin. Chromatin immunoprecipitation experiments and staining of Drosophila polytene chromosomes indicate that Chd1 associates with actively transcribed genes (Stokes *et al.* 1996; Simic *et al.* 2003; Srinivasan *et al.* 2005; McDaniel *et al.* 2008). However, the mechanism by which Chd1 is recruited to these loci is not clear. In humans, *in vitro* data show that the chromodomains recognize H3K4 methylated nucleosomes (Flanagan *et al.* 2005). However, there are conflicting data regarding the ability of yeast Chd1 to bind H3K4 methylated nucleosomes (Santos-Rosa *et al.* 2003; Pray-Grant *et al.* 2005; Sims *et al.* 2005; Flanagan *et al.* 2007; McDaniel *et al.* 2008). Furthermore, genetic data indicate that H3K4 methylation is unlikely to be the sole determinant of Chd1's localization; in Drosophila, mutations in the Kismet gene prevent association of Chd1 with polytene chromosomes (Srinivasan *et al.* 2005) but have no apparent affect on H3K4me levels (Srinivasan *et al.* 2008), and mutations of the H3K4 demethylase, Lid, result in increased H3K4me without a clear increase in Chd1 levels on polytene chromosomes (Eissenberg *et al.* 2007). Consistent with these observations, Stillman and colleagues have observed that *set1* and H3K4 mutations enhance phenotypes of *spt16* mutations whereas *chd1* mutations suppress *spt16* phenotypes, suggesting distinct functions for Chd1 and H3K4 methylation (Biswas *et al.* 2006, 2007, 2008). Our data suggest that methylation of histone H3 does not have a strong effect on Chd1's localization. In contrast, the *rtf1Δ1* mutation, which abolishes Chd1–Rtf1 interactions, was a strong suppressor of *spt5-242.* These data suggest that Chd1 is initially recruited to transcription complexes via an interaction with Rtf1. It remains possible that H3K4 or H3K36 methylation may affect some other aspect of Chd1 function, such as the persistence of its association with a gene, its ability to maintain contact with histone H3 during remodeling, or its rate of action. The exact relationships between Set1 and H3K4 methylation, Set2 and H3K36 methylation, and Chd1 are difficult to determine. The strong suppression of *spt5-242* by *set2*, *chd1*, and H3K36 mutations prevented clear determination of whether or not *set1* mutations could act additively with these other suppressors of *spt5-242.*

What is the function of Chd1? *Chd1* mutants display few growth defects and only modest defects in gene expression (Tran *et al.* 2000), suggesting that Chd1 does not play a direct or critical role in transcription. Several reports implicate Chd1 in ATP-dependent nucleosome assembly and spacing (Tran *et al.* 2000; Robinson and Schultz 2003; Lusser *et al.* 2005; Stockdale *et al.* 2006), suggesting a role in establishment or maintenance of chromatin structure over transcribed genes. However, *chd1* mutants do not display obvious defects in bulk or transcribed chromatin structure (Xella *et al.* 2006), although it is possible that Chd1 plays a redundant or transient role in establishing or maintaining chromatin structures during transcription.

Another possibility is that Chd1 helps maintain epigenetic information, *i.e.*, histones with specific post-translational marks, over transcribed sequences. We did not observe any clear alterations in levels of H3K4me3 or H3K36me3 in Western blots of *chd1Δ* cell extracts or by ChIP with antisera directed against these modifications, suggesting that Chd1 does not regulate these histone modifications (T. K. Quan and G. A. Hartzog, unpublished data). However, the observation that disruption of H3K36 methylation suppresses *spt5-242* suggested a link to histone acetylation over transcribed genes via the Rpd3S complex. In support of this idea, we found that disruption of Rpd3S is sufficient to suppress *spt5-242.* In contrast, disruption of the SAGA histone acetyltransferase, or H3 residues targeted by SAGA, enhances the phenotypes of an *spt5-242* mutant. Furthermore, just as disruption of the Rpd3S complex leads to inappropriate transcription initiation events from the middle of genes, we found that a *chd1* mutation strongly activates a genetic reporter of cryptic initiation and in Northern blot analysis, causes internal initiation when combined with an *isw1* mutation. Although it is intriguing to note that Isw1 apparently binds to di- and trimethylated H3K4 and depends upon Set1 for its association with chromatin (Santos-Rosa *et al.* 2003), we do not observe suppression of *spt5-242* by an *isw1* mutation (T. K. Quan and G. A. Hartzog, unpublished data). Internal transcription initiation *per se* is unlikely to explain suppression of *spt5-242* by *chd1Δ* as a number of other mutations that give internal initiation phenotypes, *spt6*, H2BK123R, *spt16*, do not suppress *spt5-242* (T. K. Quan and G. A. Hartzog, unpublished data). The observation that H3K9/14Ac is increased over transcribed sequences in a *chd1* mutant may indicate a role for Chd1 in recruitment of Rpd3S to chromatin, or in the dynamics of acetylated histones. Our working model is that Rpd3S, Chd1, and methylation of H3K4 and H3K36 play important roles in maintaining normal chromatin structures over transcribed regions. Loss of these factors or modifications reduces the dependence of elongating RNAPII on Spt4–Spt5 and other positively acting elongation factors at the price of an increased probability of transcription initiation from cryptic promoters.

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Histone H3K4 and K36 Methylation, Chd1 and Rpd3S Oppose the Functions of *Saccharomyces cerevisiae* Spt4–Spt5 in Transcription

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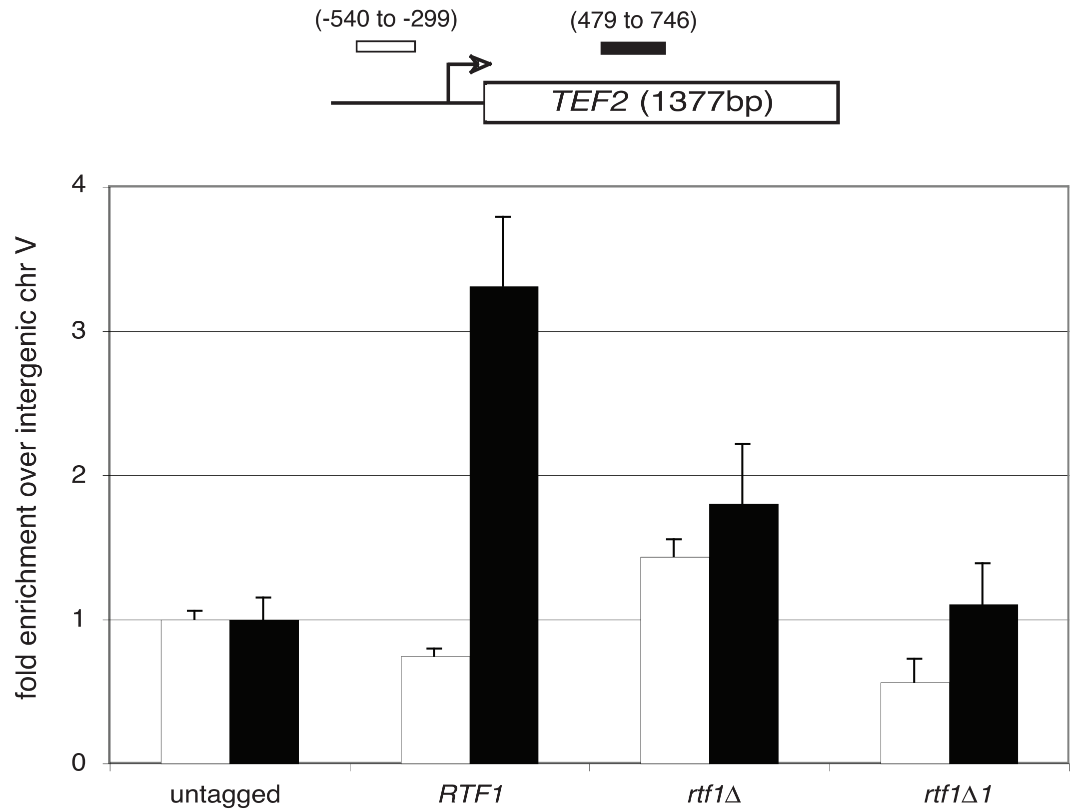

FIGURE S1.—Rtf1 recruits Chd1 to chromatin. To determine if Rtf1-Chd1 binding is required for normal association of Chd1 with chromatin, strains carrying HA1-tagged Chd1 and the indicated *rtf1* mutations were subjected to anti-HA1 ChIP. Association of Chd1 with the promoter or transcribed region of *TEF2* was then determined by QPCR. The data are presented and analyzed as described in Figure 5.

TABLE S1

Yeast strains

Strain	Mat	Genotype	Alias/Source
GHY92	**a**	*his4-912δ lys2-128δ leu2Δ1 ura3-52 spt5-242*	
GHY279	α	*his3Δ200 lys2-128δ ura3-52 leu2Δ1 chd1Δ::HIS3 trp1Δ63*	
GHY305	**a**	*his3Δ200 lys2-128δ ura3-52 leu2Δ1 chd1Δ::HIS3 spt5-242*	
GHY374	**a**	*his4-912δ lys2-128δ leu2Δ1 spt5-242*	
GHY510	**a**	*his3Δ200 lys2-128δ ura3-52 spt5-242*	
GHY513	**a**	*his3Δ200 lys2-128δ leu2Δ1 spt5-242*	
GHY536	α	*his3Δ200 ura3-52 chd1Δ::HIS3*	
GHY766	α	*his4-912δ lys2-128δ leu2(Δ1 or Δ::PET56) ura3-52 gcn5Δ::LEU2*	
GHY773	a	*his3Δ200 lys2-128δ ura3-52 leu2Δ1 HA1₃-CHD1*	
GHY827	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt5-242*	
GHY840	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 (hht1-hhf1)Δ::LEU2 (hht2-hhf2)Δ::HIS3 [pDM9]*	
GHY843	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt5-242 (hht1-hhf1)Δ::LEU2 (hht2-hhf2)Δ::HIS3 [pDM9]*	
GHY985	α	*his3Δ200 lys2-128δ ura3-52 trp1Δ63 spt3-202*	FY1288/Fred Winston
GHY1121	**a**	*his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 rtf1Δ101::LEU2*	KY522/Karen Arndt
GHY1468	α	*his4-912δ lys2-128δ leu2Δ1 trp1Δ63 ura3-52 set1Δ::KANMX*	MB1476/Mary Bryk
GHY1487	α	*his4-912δ lys2-128δ leu2Δ1 ura3-52 suc2Δ(-1900/-390) rad6Δ::URA3*	FY623/Fred Winston
GHY1489	α	*his4-912δ lys2-128δ leu2Δ1 trp1Δ63 ura3-52 set2Δ::KANMX4*	MBY1478/Mary Bryk
GHY1516	α	*his4-912δ lys2-128δ leu2Δ(0 or 1) ura3Δ0 dot1Δ::KANMX*	
GHY1517	**a**	*his3Δ1 leu2Δ(0 or 1) dot1Δ::KANMX spt5-242*	
GHY1521	**a**	*his4-912δ lys2-128δ ura3-52 trp1Δ63 leu2Δ1 spt5-242 set2Δ::KANMX*	
GHY1523	**a**	*his4-912δ lys2-128δ ura3-52 trp1Δ63 leu2Δ1 spt5-242 set1Δ::KANMX*	
GHY1527	α	*his4-912δ lys2-128δ ura3-52 leu2Δ1 spt5-242 rad6Δ::URA3 suc2ΔUAS(-1900/-390)*	
GHY1540	α	*his4-912δ lys2-128δ ura3(Δ0 or -52) leu2Δ(0 or 1) spt5-242 set2Δ::KANMX*	
GHY1578	**a**	*his3Δ1 leu2Δ0 ura3Δ0 isw2Δ::KANMX*	
GHY1661	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 HA1₃-CHD1 trp1Δ63 (hht1-hhf1)Δ::LEU2 (hht2-hhf2)Δ::HIS3 [pDM9]*	
GHY1662	**a**	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 HA1₃-CHD1 set1Δ::NAT*	
GHY1663	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 HA1₃-CHD1 set2Δ::KANMX4*	
GHY1687	α	*his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt5-242 rtf1Δ101::LEU2*	
GHY1696	α	*his4-912δ leu2Δ1 ura3-52 spt7Δ::LEU2*	FY964/Fred Winston

GHY1774	**a**	*his3Δ1 ura3Δ0 leu2Δ0 isw1Δ0::NAT*
GHY1786	**a**	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 rtf1Δ101::LEU2* $HA1_3$*-CHD1*
GHY1855	**a**	*his3Δ1 leu2Δ0 ura3Δ0 isw1Δ::NAT isw2Δ::URA3*
GHY1876	**a**	*his3Δ(1 or 200) leu2Δ(1 or 0) ura3(-52 or Δ0) chd1Δ::HIS3 isw1Δ::NAT isw2Δ::URA3*
GHY1882	α	*his3Δ(1 or 200) leu2Δ(1 or 0) ura3(-52 or Δ0) chd1Δ::HIS3 isw2Δ::URA3*
GHY1883	**a**	*his3Δ(1 or 200) leu2Δ(1 or 0) ura3(-52 or Δ0) chd1Δ::HIS3 isw1Δ::NAT*
GHY1918	**a**	*his3Δ200 lys2-128δ ura3-52 spt5-242*
GHY1919	α	*his3Δ200 lys2-128δ ura3-52 spt5-242 dst1Δ::hisG-URA3*
GHY1920	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 spt5-242 chd1Δ::HIS3*
GHY1921	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 spt5-242 chd1Δ::HIS3 dst1Δ::hisG-URA3*
GHY1923	**a**	*his3Δ200 lys2-128δ ura3-52 dst1Δ::hisG-URA3*
GHY1924	α	*his3Δ200 lys2-128δ ura3-52 chd1Δ::HIS3*
GHY1925	**a**	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 chd1Δ::HIS3 dst1Δ::hisG-URA3*
GHY1959	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63*
GHY1961	**a**	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 chd1Δ::HIS3*
GHY1964	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 chd1Δ::HIS3 dst1Δ::hisG-URA3*
GHY1966	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 dst1Δ::hisG-URA3*
GHY1967	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 spt4Δ2::KAN*
GHY1968	**a**	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt4Δ2::KAN chd1Δ::HIS3*
GHY1969	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 spt4Δ2::KAN dst1Δ::hisG-URA3*
GHY1970	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 spt4Δ2::KAN dst1Δ::hisG-URA3 chd1Δ::HIS3*
GHY1971	**a**	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt6-14*
GHY1972	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 spt6-14 chd1Δ::HIS3*
GHY1973	**a**	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 spt6-14 dst1Δ::hisG-URA3*
GHY1974	**a**	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt6-14 chd1Δ::HIS3 dst1Δ::hisG-URA3*
GHY1975	**a**	*his3Δ(1* or *200) lys2-128δ leu2Δ(0* or *1) ura3(Δ0* or *-52) eaf3Δ0::KANMX6*
GHY1977	**a**	*his3Δ(1* or *200) leu2Δ(0* or *1) ura3(Δ0* or *-52) met15Δ0 spt5-242 eaf3Δ0::KANMX6*
GHY1979	**a**	*his3Δ(1* or *200) lys2-(128δ* or *Δ0) leu2Δ(0* or *1) ura3Δ0 rco1Δ0::KANMX6*
GHY1981	α	*his3Δ(1* or *200) lys2-128δ leu2Δ(0* or *1) spt5-242 rco1Δ0::KANMX6*
GHY1983	**a**	*his3Δ200 lys2-128δ ura3-52 rpd3Δ0::HIS3*

GHY1985	**a**	*his3Δ200 lys2-128δ ura3-52 leu2Δ1 spt5-242 rpd3Δ0::HIS3*	
GHY1987	**a**	*his4-912δ lys2-128δ leu2Δ1 ura3-52 set2Δ::KANMX4 dst1Δ::hisG-URA3*	
GHY1988	α	*his4-912δ lys2-128δ leu2Δ1 ura3-52 spt5-242 dst1Δ::hisG-URA3*	
GHY1989	α	*his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt5-242 set2Δ::KANMX4 dst1Δ::hisG-URA3*	
GHY1990	α	*lys2(-128δ or Δ0) leu2Δ(1 or 0) ura3(-52* or *Δ0) rco1Δ0::KANMX6*	
GHY1991	**a**	*his3Δ200 lys2-128δ leu2Δ(1* or *0) ura3(-52* or *Δ0) spt5-242 rco1Δ0::KANMX6*	
GHY1992	**a**	*his4-912δ lys2(-128δ or Δ0) leu2Δ(1* or *0) ura3(-52* or *Δ0) dst1Δ::hisG-URA3 rco1Δ0::KANMX6*	
GHY1993	**a**	*his3Δ200 lys2(-128δ or Δ0) leu2Δ(1* or *0) ura3(-52* or *Δ0) spt5-242 dst1Δ::hisG-URA3*	
GHY1994	**a**	*his3Δ200 his4-912δ lys2(-128δ or Δ0) leu2Δ(1* or *0) ura3(-52* or *Δ0) spt5-242 dst1Δ::hisG-URA3 rco1Δ0::KANMX6*	
GHY1995	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 pGAL1-FLO8-HIS3::KANMX*	
GHY2001	**a**	*his3Δ200 lys2-128δ leu2Δ(0* or *1) ura3(-52* or *Δ0) trp1Δ63 set1Δ::NAT pGAL1-FLO8-HIS3::KANMX*	
GHY2003	**a**	*his3Δ200 lys2-128δ leu2Δ(0* or *1) ura3(-52* or *Δ0) dot1Δ::caURA3 pGAL1-FLO8-HIS3::KANMX*	
GHY2005	**a**	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 (hht2-hhf2)Δ::NAT pGAL1-FLO8-HIS3::KANMX*	
GHY2006	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63(hht1-hhf1)Δ::LEU2 pGAL1-FLO8-HIS3::KANMX*	
GHY2009	**a**	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 (hht1-hhf1)Δ::LEU2 (hht2-hhf2)Δ::NAT pGAL1-FLO8-HIS3::KANMX [pDM9]*	
GHY2013	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 chd1Δ::URA3 pGAL1-FLO8-HIS3::KANMX*	
GHY2049	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 set2Δ::NAT pGAL1-FLO8-HIS3::KAN*	
GHY2138	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 pGAL1-YLR454w::TRP1*	
GHY2139	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 chd1Δ::HIS3 pGAL1-YLR454w::TRP1*	
GHY2140	α	*his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt5-242 pGAL1-YLR454w::TRP1*	
GHY2141	α	*his3(Δ200 or +) his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 spt5-242 chd1Δ::HIS3 pGAL1-YLR454w::TRP1*	
OY28	α	*his3Δ200 leu2Δ1 ura3-52 gcn5Δ::HIS3*	IPY37/Ines Pinto
OY94	**a**	*his4-912δ lys2-128δ leu2Δ1 ura3-52*	FY120/Fred Winston
OY98	α	*his3Δ200 lys2-128δ leu2Δ1 trp1Δ63 ura3-52*	FY603/Fred Winston
OY382	α	*his3Δ1 lys2Δ0 leu2Δ0 ura3Δ0 spt8Δ::KANMX*	

TABLE S2

Plasmids

Plasmid	Markers	source
pRM430	*hht2Δ4-30 HHF2 TRP1 CEN* AmpR	Michael Grunstein
MBB259	*hht2-K4A HHF2 TRP1 CEN* AmpR	Mary Bryk
MBB257	*hht2-K4R HHF2 TRP1 CEN* AmpR	Mary Bryk
pWZ414-F30	*hht2-K9Q HHF2 TRP1 CEN* AmpR	Sharon Dent
pWZ414-F53	*hht2-K9R HHF2 TRP1 CEN* AmpR	Sharon Dent
pWZ414-F31	*hht2-K14Q HHF2 TRP1 CEN* AmpR	Sharon Dent
pWZ414-F43	*hht2-K14R HHF2 TRP1 CEN* AmpR	Sharon Dent
MBB286	*hht2-K36R HHF2 TRP1 CEN* AmpR	Mary Bryk
pHHT2-K4R,K36R	*hht2-K4R,K36R HHF2 TRP1 CEN* AmpR	LeAnne Howe
pWZ414-F13-K79A	*hht2-K79A HHF2 TRP1 CEN* AmpR	Kevin Struhl
pWZ414-F13-K79Q	*hht2-K79Q HHF2 TRP1 CEN* AmpR	Kevin Struhl
pHHT2-K9,14,18,23R	*hht2-K9R,K14R,K18R,K23R HHF2 TRP1 CEN* AmpR	LeAnne Howe
pRS314-Flag-htb1-K123R	*Flag-htb1-K123R TRP1 CEN* AmpR	Mary Ann Osley
pGH269	*HA_3-CHD1 URA3 CEN* AmpR	Hartzog lab
pTQ5	*HA_3-CHD1-ΔCD URA3 CEN* AmpR	Hartzog lab
pTQ4	*HA_3-CHD1-ΔCD1 URA3 CEN* AmpR	Hartzog lab
pTQ3	*HA_3-CHD1-ΔCD2 URA3 CEN* AmpR	Hartzog lab
pMG316	*HA_3-CHD1-Y316E URA3 CEN* AmpR	Patrick Grant
pKR37	*HA_3-rtf1Δ1 TRP1 CEN* AmpR	Karen Arndt
pTQ10	*rtf1Δ1 TRP1 CEN* AmpR	Hartzog lab
pLS21-5-Δ2	*HA_3-rtf1Δ2 TRP1 CEN* AmpR	Karen Arndt
pKR27	*HA_3-rtf1Δ3 TRP1 CEN* AmpR	Karen Arndt
pDM9	*HHT1-HHF1 URA3 CEN* AmpR	Fred Winston
pJH18	*HHT2-HHF2 TRP1 CEN* AmpR	Mary Bryk
pLS21-5	*HA13-RTF1 TRP1 CEN* AmpR	Karen Arndt