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Chd1 and yFACT Act in Opposition in Regulating Transcription▽†

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***CHD1* encodes an ATP-dependent chromatin remodeler with two chromodomains. Deletion of *CHD1* suppresses the temperature-sensitive growth defect caused by mutations in either *SPT16* or *POB3*, which encode subunits of the yFACT chromatin-reorganizing complex. *chd1* also suppresses synthetic defects caused by combining an *spt16* mutation with other transcription factor mutations, including the synthetic lethality caused by combining an *spt16* mutation with TATA binding protein (TBP) or TFIIA defects. Binding of TBP and RNA polymerase II to the *GAL1* promoter is reduced in a *pob3* mutant, resulting in low levels of *GAL1* expression, and all three defects are suppressed by removing Chd1. These results suggest that Chd1 and yFACT have opposing roles in regulating TBP binding at promoters. Additionally, overexpression of Chd1 is tolerated in wild-type cells but is toxic in *spt16* mutants. Further, both the ATPase and chromodomain are required for Chd1 activity in opposing yFACT function. Similar to the suppression by *chd1*, mutations in the *SET2* histone methyltransferase also suppress defects caused by yFACT mutations. *chd1* and *set2* are additive in suppressing *pob3*, suggesting that Chd1 and Set2 act in distinct pathways. Although human Chd1 has been shown to bind to H3-K4-Me, we discuss evidence arguing that yeast Chd1 binds to neither H3-K4-Me nor H3-K36-Me.**

Chromatin structure limits the accessibility of DNA sequences in eukaryotic chromosomes. Accessibility is enhanced through three major processes in vivo. First, posttranslational histone modifications either change the properties of the chromatin structure or create binding sites for other transcription factors (50). These posttranslational modifications include phosphorylation of serine residues and acetylation, methylation, and ubiquitylation of lysine residues (11). Several transcription factors that recognize specific histone modifications for their recruitment have been described. For example, the bromodomain-containing proteins recognize acetylation of histone proteins (61), and chromodomain-containing proteins are involved in recognizing methylation marks on histone proteins (6, 11). Second, ATP-dependent chromatin-remodeling factors promote accessibility by repositioning nucleosomes (9, 21, 55). These factors utilize the energy from ATP hydrolysis to establish or disrupt repressive chromatin structures (21). The third way by which the DNA sequence is made available is through ATP-independent chromatin-reorganizing factors that change the properties of nucleosomes in a localized manner (17). For example, the yFACT complex changes the properties of nucleosomes without requiring ATP hydrolysis (39, 41). The reorganization by yFACT has been shown to alter DNA accessibility both in vivo and in vitro (5, 14, 17, 30, 44, 45).

The FACT complex (*fa*cilitates *c*hromatin *t*ranscription) was first identified as a factor that promoted RNA polymerase II (Pol II) transcription in vitro using assembled chromatin as a template (33). The mammalian FACT complex is composed of two subunits, p140 and SSRP1. The homologs of p140 and SSRP1 in yeast are Spt16 and Pob3, respectively (34). The Spt16 and Pob3 proteins are always present in a heterodimer to form the SP complex in yeast (57). Although the N-terminal DNA binding domain of SSRP1 is absent in Pob3, Nhp6, a high-mobility group protein, is thought to serve as the DNA binding activity of the SP complex to form the yFACT complex (7, 18). Genetic and biochemical evidence suggests that yFACT is involved in regulating both transcription and DNA replication (1a, 3, 5, 18, 19, 27, 30, 34, 42, 56, 57). While the association of yFACT with elongation factors (27, 45) and with transcribed regions of genes (30, 42) supports an elongation role, studies also suggest that the FACT complex has a role in transcription initiation (5, 44). We have shown earlier that yFACT has a role in regulating TATA binding protein (TBP) binding during the transcriptional initiation step (5). The evidence for this included synthetic lethality between certain mutations of TBP and TFIIA and defective alleles of *SPT16*, reduced binding of TBP at some promoters in *spt16* mutants, and enhanced binding of TBP to a TATA box within nucleosomal DNA in presence of TFIIA and yFACT.

The yeast chromodomain protein (Chd1) is a member of the Snf2-like subfamily of nucleic acid-stimulated ATPases (21) and has ATP-dependent chromatin-remodeling activity in vitro (29, 40). Chd1 and other CHD proteins have two chromodomains near the N terminus, a centrally located Snf2-related helicase/ATPase domain, and a Myb-related DNA binding domain near the C terminus (59). Chd1 is thought to promote formation of inhibitory chromatin, as extracts derived from cells lacking Chd1 are unable to produce the same level of DNase I resistance at specific loci that results from similar preparations derived from normal cells (40). Genetic interactions have been reported between mutations of *CHD1* and mutations in transcription elongation factors such as Spt5, Isw1, and Isw2 (45, 54). Chd1 also physically interacts with several transcription elongation factors, such as members of the Paf1 complex, the Spt4-Spt5 complex, and components of

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yFACT (24, 27, 45, 54). Recently Chd1 has been shown to physically associate with the SAGA/SLIK complex in yeast and to bind histone tail peptides methylated at K4 (37). However, binding of yeast Chd1 to methylated H3-K4 has not been observed by others (32, 46). Relatively little is known about the functional role of Chd1 in vivo in regulating transcription, although it was recently reported that a *chd1* mutation affects chromatin structure of the *ADH2* gene and the kinetics of *ADH2* activation (60). In this report, we show that part of the role of Chd1 is to oppose the function of the positive transcription factor yFACT. We present evidence which suggests that Chd1 negates yFACT's ability to enhance TBP binding at promoters. We show that in a strain with a yFACT defect, deletion of *CHD1* results in increased TBP binding and increased Pol II binding at promoters. Finally, we find that deletion of *CHD1* suppresses synthetic lethalities between *spt16* mutations and TBP mutations as well as between *spt16-11* and TFIIA mutations.

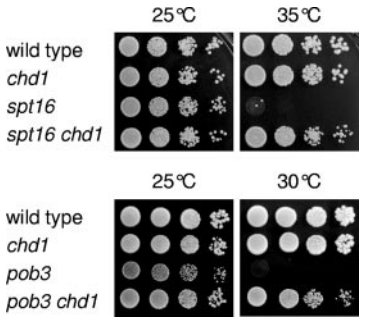

FIG. 1. *chd1* suppresses *spt16* and *pob3* phenotypes. (Top panels) Tenfold dilutions of strains DY150 (wild type), DY6957 (*chd1*), DY8107 (*spt16*), and DY9151 (*spt16 chd1*) were plated on complete medium at the indicted temperature for 2 days. (Bottom panels) Tenfold dilutions of strains DY150 (wild type), DY6957 (*chd1*), DY7379 (*pob3*), and DY9458 (*pob3 chd1*) were plated on complete medium at the indicted temperature for 3 days.

MATERIALS AND METHODS

The yeast strains used are isogenic with W303 (52) and are listed in Table S1 in the supplemental material. Standard genetic methods were used for strain construction (43). Cells were grown in YPD medium (43) at 30°C, except as noted, or in synthetic complete medium (43) with 2% glucose and supplemented with adenine, uracil, and amino acids, as appropriate. For the galactose induction experiments, cells were grown at 25°C in YP medium supplemented with 2% raffinose to mid-log phase and shifted to 30°C for 2 h, and then galactose was added to a final concentration of 2%. Plasmids are listed in Table S2 in the supplemental material. RNA levels were determined with S1 nuclease protection assays as described previously (2, 3).

Chromatin immunoprecipitations (ChIPs) were performed as described previously (1), using the 8WG16 monoclonal antibody against the Pol II C-terminal repeat and a polyclonal anti-TBP serum generously provided by Tony Weil (42a). Real-time PCR was performed as described previously (14a), using the open reading frame (ORF)-free chromosome I region (30a) as a nontranscribed region control.

For all ChIP experiment the value for each ChIP output PCR signal was divided by that for the ChIP output PCR signal for the ORF-free control, and to control for primer pair efficiencies, this ratio was further divided by a similar ratio of target to nontranscribed regions but using input DNA PCR signals, resulting in a ChIP ratio. Each PCR was performed in triplicate, and the normalized mean and standard deviation of the ratio were calculated as described previously (14a).

RESULTS

Deletion of *CHD1* suppresses phenotypes of yFACT mutant strains. It was previously reported that a *chd1* mutation can suppress the growth defects of a *pob3-272* mutant (10). We asked whether a *chd1* deletion could suppress the temperature-sensitive growth defects of *spt16-11* and *pob3*(*L78R*) mutations in our strain background. Figure 1 shows that *spt16 chd1* and *pob3 chd1* strains grow well under conditions where the *spt16* and *pob3* single mutants are inviable. Thus, the *chd1* suppression of yFACT mutant phenotypes suggests that yFACT and Chd1 have opposing roles in regulating transcription.

The yFACT complex, in addition to Spt16 and Pob3, contains the Nhp6 HMGB (18). Nhp6 is encoded by two redundant genes, *NHP6A* and *NHP6B*, and the *nhp6ab* double mutant strain is temperature sensitive for growth. Based on our observation that a *chd1* mutation suppresses the temperature-sensitive growth phenotype of the *spt16* and *pob3* strains, we asked whether *chd1* could also suppress temperature sensitivity of the *nhp6ab* strain. We constructed a *nhp6ab chd1* triple mutant strain, but this strain failed to grow at the restrictive temperature (data not shown). Nhp6 has a role in transcription by Pol III (23) and interacts with other chromatin proteins besides yFACT, including Swi/Snf, RSC, and Ssn6/Tup1 (4, 20, 51). The ability of a *chd1* mutation to suppress *spt16* and *pob3* but not *nhp6ab* may reflect these additional roles of Nhp6.

Mutations in the ATPase domain and in the chromodomain of Chd1 suppress yFACT mutations. The Chd1 protein has ATPase activity (53), and it also contains two chromodomain sequence motifs (59). We investigated the roles of the ATPase and chromodomains of Chd1 in the genetic suppression of *spt16* and *pob3* mutations. Strains were constructed with an integrated *CHD1*(Δ*CD*)-*HA* allele, lacking both Chd1 chromodomains, and an integrated *CHD1*(*K407R*)-*HA* allele, with a mutation within the consensus ATP binding motif (45). The control strains had wild-type *CHD1-HA* integrated and also hemagglutinin (HA) tagged at the 3′ end. In this assay, the strain will not grow if a mutant complements the gene deletion, indicating restoration of the normal ability to oppose yFACT function. Similarly, growth indicates a failure to provide Chd1 function. The *pob3 chd1* strain grows at 30°C, while the *pob3 CHD1* strain is inviable. The ΔCD and K407R mutant versions of Chd1 allow growth of the *pob3* mutant, and thus both the chromodomain and the ATPase are required for the Chd1 activity that, here, is toxic in *pob3* mutants (Fig. 2A). With *spt16*, again both the chromodomain and ATPase mutations in Chd1 allow growth (Fig. 2B), although growth in the *spt16 CHD1*(Δ*CD*)-*HA* strain is less robust, suggesting that the chromodomain plays a less prominent role than the ATPase in generating toxicity in the *spt16* strain. Finally, comparing growth of the *pob3 CHD1* and the *pob3 CHD1-HA* strains suggests that the HA-tagged allele is not fully functional. In summary, these experiments suggest that both the ATPase activity and the chromodomain of Chd1 are required for Chd1 to be toxic in yFACT mutants.

Deletion of *CHD1* suppresses synthetic lethality between *spt16* and other transcription factors. The Isw1 and Isw2 chromatin complexes have been implicated in both transcriptional elongation and repressing transcriptional initiation (31). Ad-

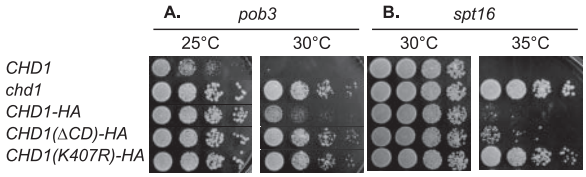

FIG. 2. Both the chromodomain and the ATPase contribute to Chd1 toxicity in yFACT mutants. (A) Tenfold dilutions of strains DY7379 (*pob3*), DY9458 (*pob3 chd1*), DY11724 (*pob3 chd1 CHD1-HA*), DY11736 [*pob3 chd1 CHD1*(Δ*CD*)*-HA*], and DY11770 [*pob3 chd1 CHD1*(*K407R*)*-HA*] were plated on complete medium at 25°C or 30°C for 2 days. (B) Tenfold dilutions of strains DY8107 (*spt16*), DY9152 (*spt16 chd1*), DY11614 (*spt16 chd1 CHD1-HA*), DY11624 (*spt16 chd1 CHD1*(Δ*CD*)*-HA*], and DY11643 [*spt16 chd1 CHD1*(*K407R*)*-HA*] were plated on complete medium at 30°C or 35°C for 2 days.

ditionally, the *isw1 isw2 chd1* triple mutant shows additive growth defects at elevated temperatures (54). Based on these results, we looked for genetic interactions between *spt16*, *chd1*, *isw1*, and *isw2*. The *spt16 isw1* double mutant shows a significant growth defect at 33°C (Fig. 3A), and the *spt16 isw1 isw2* triple mutant is completely dead at 33°C (Fig. 3B). These synthetic growth defects suggest that the yFACT chromatin-reorganizing complex and the Isw remodeling complexes may perform similar functions in vivo. Importantly, a *chd1* mutation suppresses both the *spt16 isw1* and the *spt16 isw1 isw2* growth defects, supporting the idea that Chd1 acts in opposition to yFACT for the function that overlaps Isw-mediated remodeling.

As a *chd1* mutation suppresses a number of *spt16* phenotypes, we asked whether *chd1* can also suppress other synthetic lethal phenotypes seen with *spt16*. *spt16* shows marked growth

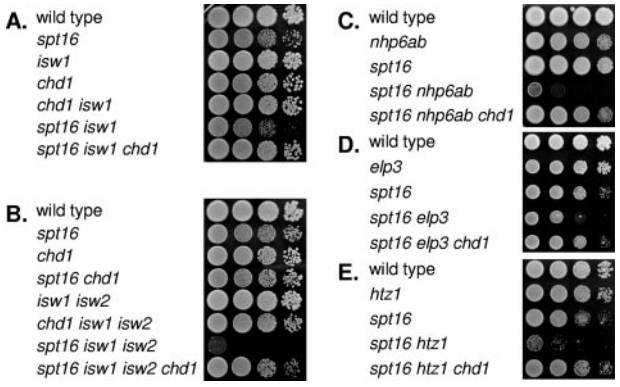

FIG. 3. *chd1* suppresses *spt16* synthetic growth defects. (A) Tenfold dilutions of strains DY150 (wild type), DY8107 (*spt16*), DY9816 (*isw1*), DY9809 (*chd1*), DY9827 (*chd1 isw1*), DY9055 (*spt16 isw1*), and DY9834 (*spt16 isw1 chd1*) were plated on complete medium at 33°C for 2 days. (B) Tenfold dilutions of strains DY150 (wild type), DY8107 (*spt16*), DY9809 (*chd1*), DY9152 (*spt16 chd1*), DY7656 (*isw1 isw2*), DY9823 (*chd1 isw1 isw2*), DY9820 (*spt16 isw1 isw2*), and DY9831 (*spt16 isw1 isw2 chd1*) were plated on complete medium at 33°C for 2 days. (C) Tenfold dilutions of strains DY150 (wild type), DY6612 (*nhp6ab*), DY8107 (*spt16*), DY8808 (*spt16 nhp6ab*), and DY9978 (*spt16 nhp6ab chd1*) were plated on complete medium at 33°C for 2 days. (D) Tenfold dilutions of strains DY150 (wild type), DY8156 (*elp3*), DY8107 (*spt16*), DY8185 (*spt16 elp3*), and DY9965 (*spt16 elp3 chd1*) were plated on complete medium at 33°C for 2 days. (E) Tenfold dilutions of strains DY150 (wild type), DY7836 (*htz1*), DY8107 (*spt16*), DY9808 (*spt16 htz1*), and DY9811 (*spt16 htz1 chd1*) were plated on complete medium at 33°C for 2 days.

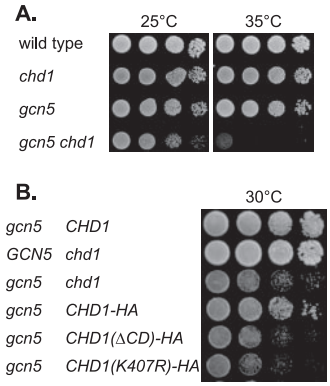

FIG. 4. *chd1* and *gcn5* show a synthetic growth defect. (A) Tenfold dilutions of strains DY150 (wild type), DY9809 (*chd1*), DY5926 (*gcn5*), and DY9873 (*gcn5 chd1*) were plated on complete medium at 25°C for 3 days or at 35°C for 2 days. (B) Tenfold dilutions of strains DY5925 (*gcn5*), DY6957 (*chd1*), DY11500 (*gcn5 chd1*), DY11716 (*gcn5 chd1 CHD1-HA*), DY11694 [*gcn5 chd1 CHD1*(Δ*CD*)*-HA*], and DY11703 [*gcn5 chd1 CHD1*(*K407R*)*-HA*] were plated on complete medium at 30°C for 2 days.

defects when combined with mutations in both *nhp6a* and *nhp6b* (18), and an *spt16 nhp6ab* double mutation is lethal at 33°C. A *chd1* mutation suppresses this synthetic lethality, as seen by growth of the *spt16 nhp6ab chd1* strain (Fig. 3C). *ELP3* encodes a histone acetyltransferase subunit of the elongator complex (58), and *elp3* is synthetic lethal with *spt16* (19). The *spt16 elp3* synthetic lethality is suppressed by a *chd1* mutation (Fig. 3D). *HTZ1* encodes the yeast H2A.Z histone variant of H2A (12), and we recently showed that *htz1* and *spt16* are synthetic lethal (3). A *chd1* mutation also suppresses the *spt16 htz1* synthetic lethality (Fig. 3E). Htz1 is believed to function at promoter regions, as it localizes preferentially at promoter regions of genes (28, 38, 62), and this suppression suggests that Chd1 might influence promoter function.

We recently showed that a *set2* mutation can suppress many *spt16* phenotypes (3). Like *chd1*, *set2* suppresses the *spt16 np6ab*, *spt16 elp3*, and *spt16 htz1* synthetic lethalities. There are also differences in the suppression profiles, however. While *chd1* suppresses the *spt16 isw1 isw2* lethality, a *set2* mutation does not (data not shown). Conversely, *set2* suppresses synthetic lethality of the *spt16 gcn5* double mutant (3), but a *chd1* mutation does not (data not shown). In fact, a *gcn5 chd1* double mutant shows a growth defect at 25°C and is synthetic lethal at 35°C (Fig. 4A). The *CHD1*(Δ*CD*)*-HA* and *CHD1*(*K407R*)*-HA* alleles both also show strong growth defects when combined with the *gcn5* disruption (Fig. 4B). This suggests that both the chromodomain and the ATPase activity are required for the Chd1 activity that is needed when Gcn5 is not active. It was reported that Chd1 is present in the SAGA/SLIK coactivator complexes (37), and the synthetic effects of combining *gcn5* and *chd1* mutations could reflect distinct functions of these two proteins in the same protein complex.

A *chd1* mutation suppresses the synthetic lethality of *spt16* with *set1* or histone H3(K4R) mutations. Human Chd1 binds to methylated K4 of histone H3 (46). If Chd1 function in yeast requires interaction with methylated H3-K4-Me, then either replacement of this residue or a *set1* gene disruption that eliminates the methyltransferase that modifies K4 of histone

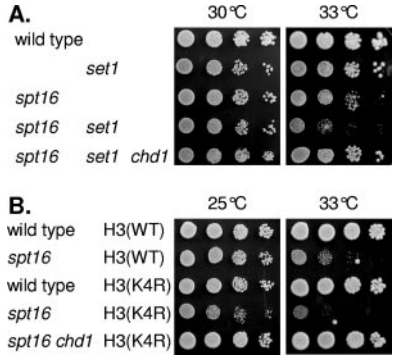

FIG. 5. A *chd1* mutation suppresses the synthetic growth defect of *spt16* with *set1* or histone H3(K4R) mutations. (A) A *chd1* mutation suppresses the *spt16 set1* synthetic growth defect. Tenfold dilutions of strains DY150 (wild type), DY8875 (*set1*), DY8107 (*spt16*), DY9206 (*spt16 set1*), and DY9271 (*spt16 set1 chd1*) were plated on complete medium at 30°C or 33°C for 2 days. (B) A *chd1* mutation suppresses the *spt16* histone H3(K4R) synthetic growth defect. Tenfold dilutions of strains DY8862 [*hht1-hhf1 hht2-hhf2* + YCp-*TRP1*:H3(wild type)-H4(wild type)], DY8865 (*spt16 hht1-hhf1 hht2-hhf2* + YCp-*TRP1*:H3(wild type)-H4(wild type)], DY8863 [*hht1-hhf1 hht2-hhf2* + YCp-*TRP1*:H3(K4R)-H4(wild type)], DY8866 [*spt16 hht1-hhf1 hht2-hhf2* + YCp-*TRP1*:H3(K4R)-H4(wild type)], and DY10472 [*spt16 chd1 hht1-hhf1 hht2-hhf2* + YCp-*TRP1*:H3(K4R)-H4(wild type)] were plated on complete medium at the indicated temperature for 2 days.

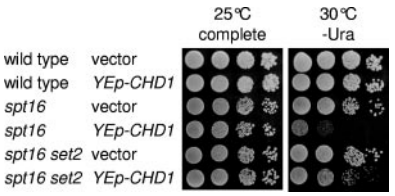

FIG. 6. *CHD1* overexpression is toxic in an *spt16* mutant. Strains DY150 (wild type), DY8117 (*spt16*), and DY8799 (*spt16 set2*) were transformed with either YEp-*CHD1* or the empty YEp-URA3 vector and plated on complete medium at 25°C for 3 days or on medium lacking uracil at 30°C for 2 days.

H3 (8) should lead to suppression of yFACT defects. Instead, combining *spt16 and set1* mutations leads to a synthetic defect, lethality at 33°C (3). Thus, the simple idea of the absence of Chd1 binding via methylated K4 of histone H3 is not sufficient to explain the suppression caused by loss of Chd1 (see Discussion).

As a *chd1* mutation suppresses many *spt16* phenotypes, including some synthetic lethal interactions, we constructed an *spt16 set1 chd1* triple mutant strain. As shown in Fig. 5A, *chd1* suppresses the *spt16 set1* growth defect at 33°C, similar to the suppression of *spt16 set1* by *set2* (3). Similar to *set1*, a K4R substitution in histone H3 is synthetic lethal with *spt16* at 33°C, and this is also suppressed by a *chd1* mutation (Fig. 5B). Similar effects can be seen with *pob3* mutants, where *pob3 set1* and *pob3* H3(K4R) mutations are lethal but can be suppressed by *chd1* (data not shown). The fact that similar genetic effects are seen with either a *set1* or a histone H3(K4R) mutation is consistent with lysine 4 of H3 being the critical target for the Set1 enzyme. These data are also consistent with a recent report showing suppression of the *set1* growth defect by *chd1* (63). Importantly, we find that the effects of Set1 and H3(K4R) mutations in a yFACT mutant are different from those in a *chd1* mutant. This suggests that the mechanism by which Chd1 opposes yFACT does not involve methylation of H3-K4.

***CHD1* overexpression is toxic in yFACT mutant strains.** Our experiments suggest that yFACT and Chd1 act in opposition during regulation of transcription. Thus, the activity of Chd1 is toxic in cells that have a partially defective yFACT chromatin-reorganizing factor, and a *chd1* mutation relieves this toxicity. This model predicts that Chd1 overexpression could be toxic in strains with yFACT mutations. We transformed wild-type and *spt16* mutant strains with a multicopy *CHD1* plasmid and assessed growth on selective medium (Fig. 6). *CHD1* overexpression has no effect in the wild-type stain but is very toxic in the *spt16* strain. Interestingly, a *set2* mutation partially reverses the toxicity of *CHD1* overexpression in the *spt16* mutant. There is no phenotypic consequence of *CHD1* overexpression in *set1* or *set2* single mutant strains (data not shown), and thus the effect appears to be specific to yFACT mutant strains. We conclude that the amount of Chd1 is of critical importance in strains with a defect in the yFACT complex.

A *chd1* mutation suppresses a galactose induction defect in a *pob3* strain. Although genetic and biochemical experiments suggest a role for Chd1 in regulating transcription in eukaryotes, the exact mechanism of Chd1 function is unclear. ChIP experiments showed that Chd1 was bound to the coding regions of the *TEF2* and *GAL10* genes, suggesting an elongation function (45). However, the ChIP studies also showed that Chd1 was recruited to the *GAL10* promoter, consistent with a role in initiation of transcription. We recently showed that a *pob3* mutation reduces expression of a *GAL1*-YLR454w gene fusion and that the *pob3* mutation reduces binding of both Pol II and TBP to the *GAL1* promoter (3). We performed similar experiments examining the effect of *pob3* and *chd1* mutations on expression and factor binding at *GAL1*-YLR454w (Fig. 7). Four isogenic strains were grown first in raffinose medium at 25°C and then shifted for 2 h to 30°C, galactose was added to the medium to induce *GAL1*-YLR454w expression, and samples were taken at timed intervals for mRNA and ChIP analyses. There is a rapid rise in *GAL1*-YLR454w mRNA levels following galactose induction in wild-type and *chd1* cells (Fig. 7B and C). There is a marked defect in *GAL1*-YLR454w induction in the *pob3* mutant, but this defect is completely suppressed in the *pob3 chd1* double mutant. We conclude that Chd1 has a negative role at the *GAL1* promoter, opposing the yFACT-dependent transcriptional activation at this promoter.

To examine the molecular mechanism of suppression by *chd1* of the defect in transcriptional induction in the *pob3* mutant, we used ChIP experiments to measure Pol II occupancy following galactose induction. Samples were harvested at various times after induction with formaldehyde to cross-link and were processed for ChIP. We used PCR probes specific for four different regions of the 8-kb-long YLR454w gene: the *GAL1*-YLR454w promoter, 1 kb downstream of start codon, the middle of the YLR454w ORF (position +3600), and the 3′ end of the gene (position +7800) (Fig. 7A). The ChIP results shown in Fig. 7D show Pol II occupancy at 60 min after galactose induction at different regions of the *GAL1*-YLR454w gene. A mutation with an elongation defect should cause decreased Pol II binding along the gene, but Pol II binding at the promoter should not be affected. In contrast, the

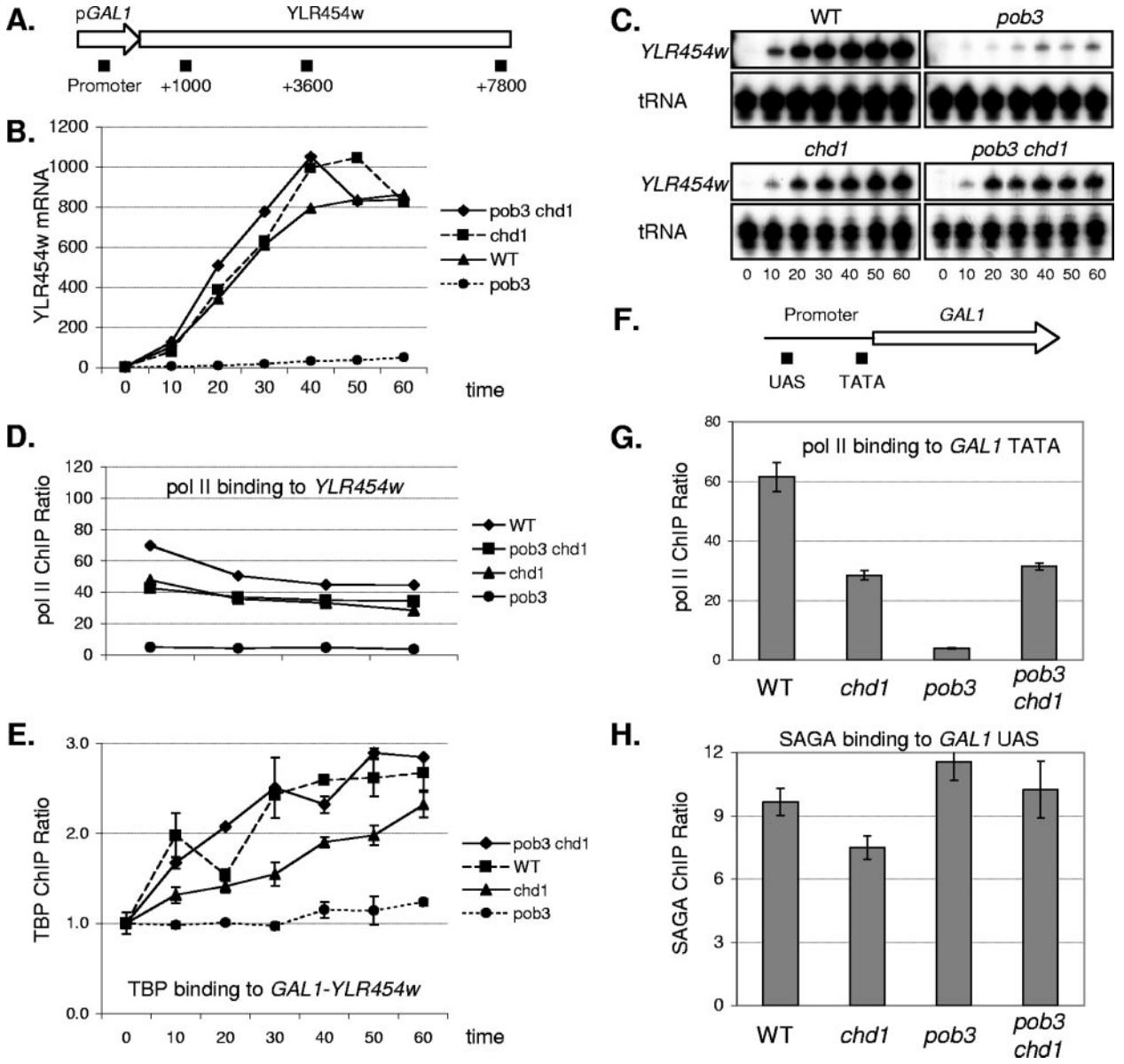

FIG. 7. A *chd1* mutation suppresses defects in *GAL1* induction and Pol II and TBP binding caused by a *pob3* mutation. Strains DY9591 (*GAL1*-YLR454w), DY9959 (*chd1 GAL1*-YLR454w), DY9972 (*pob3 GAL1*-YLR454w), and DY10020 (*pob3 chd1 GAL1*-YLR454w) were grown on YP medium with 2% raffinose. Galactose was added to 2%, and samples were taken at 10-min intervals and processed for ChIP analysis to measure Pol II and TBP binding. (A) Map of the *GAL1*-YLR454w allele showing the positions of regions amplified by at the promoter and within the gene. (B) YLR454w mRNA levels measured from the *GAL1*-YLR454w allele, quantified after phosphorimaging of the gels in panel C. WT, wild type. (C) S1 protection assays to measure YLR454w mRNA from the *GAL1*-YLR454w allele, using probes specific for YLR454w and a tRNA internal control. (D) Distribution of Pol II at 60 min following galactose induction at different *GAL1*-YLR454w regions in four different strains. Error bars show variance (standard deviations) among replicate PCRs. (E) TBP binding to the *GAL1*-YLR454w promoter following galactose induction in four different strains. ChIP values were normalized to binding at time zero. Error bars show variance among replicate PCRs. (F) Map of the native *GAL1* gene showing the positions of regions amplified by at the upstream activation sequence (UAS) and TATA within the promoter. (G) Pol II binding to the TATA region (positions −190 to + 54) of the native *GAL1* promoter at 30 min following galactose induction in four different strains. Error bars show variance among replicate PCRs. (H) SAGA binding to the native UAS region (positions −496 to −316) of the native *GAL1* promoter at 30 min following galactose induction in four different strains. Error bars show variance among replicate PCRs.

pob3 mutation sharply reduces Pol II binding at all regions of the gene, including the promoter, suggesting that the *pob3* mutation affects recruitment of Pol II to the promoter. Importantly, Pol II binding is effectively restored in the *pob3 chd1* double mutant. Similar results were seen at the native *GAL1* promoter, where Pol II binding is reduced in a *pob3* mutant but restored in the *pob3 chd1* double mutant (Fig. 7G). It is not clear why a *chd1* mutation, alone, results in reduced Pol II binding to the promoter, compared to the wild type.

Next, we used ChIP assays to measure binding of TBP to the *GAL1*-YLR454w promoter following galactose induction (Fig. 7H). TBP binding was severely reduced in the *pob3* mutant,

and TBP binding approached wild-type levels in the *pob3 chd1* double mutant strain. These results are consistent with our earlier data suggesting that yFACT has a role in facilitating formation of the TBP-TFIIA complex on DNA. The observation that deletion of *CHD1* overcomes the defect in TBP binding in the *pob3* strain suggests that Chd1 has a negative role regulating TBP binding at the *GAL1* promoter region.

The SAGA complex is required for activation of *GAL1*, and mutations in SAGA prevent binding of TBP (13). We used ChIP assays to examine binding of the Ada2-Myc subunit of SAGA. The results show no defect in SAGA binding to *GAL1* in a *pob3* mutant (Fig. 7F), and thus the defect that we observe in TBP binding at *GAL1* is not due to failure of SAGA to be recruited to the promoter.

Deletion of *CHD1* suppresses the synthetic lethality between *spt16-11* and TBP mutations as well as between *spt16-11* and TFIIA mutations. It has been shown earlier that several transcriptional coactivators regulate transcription initiation by regulating formation of the TBP-TFIIA complex. The Swi/Snf chromatin-remodeling complex uses the energy from ATP hydrolysis to regulate TBP binding both in vivo and in vitro (4, 22). Our genetic and biochemical data also showed that yFACT has a role in regulating TBP-TFIIA complex formation. As the defect in TBP binding to the *GAL1* promoter caused by a yFACT mutation can be suppressed by deletion of *CHD1*, we asked whether a *chd1* mutation can also suppress the synthetic lethalities between TBP mutations and *spt16* mutations that we have described earlier (5). We used a plasmid shuffle assay to address this question. We constructed two isogenic strains containing the wild-type *CHD1* gene and deletion of the *CHD1* gene. In both of these strains the TBP gene and *SPT16* genes were disrupted. (The *SPT15* gene encodes TBP, but we will refer to it as the TBP gene to avoid confusion.) Since these genes are essential for cell viability, the strains were kept alive by providing these genes on YCp-*URA3* plasmids. We transformed these strains with the TBP plasmid and *spt16* plasmid combination that showed synthetic lethality in our earlier genetic assays (5). The transformants were grown on medium containing 5-fluoroorotic acid (5-FOA) so that the strains are required to lose the parental YCp-*URA3* plasmid containing both the wild-type TBP gene and *SPT16* for their growth. The strain transformed with empty vectors could not grow on a 5-FOA plate (5). However, these strains transformed with wild-type copies of TBP and *SPT16* plasmids could grow on medium containing 5-FOA. The introduction of some combinations of TBP mutations and *spt16* mutations resulted in either synthetic lethality or a synthetic growth defect in a *CHD1* strain background. Interestingly, deletion of *CHD1* rescued some of these synthetic lethalities or synthetic growth defects (compare *CHD1* with *chd1* on 5-FOA plates). This in vivo evidence strongly suggests that Chd1 has a negative role in yFACT-mediated TBP binding. A deletion of this negative factor rescues the synthetic lethal or synthetic growth defect phenotypes associated with *spt16* mutations and TBP mutations.

During transcriptional initiation, TBP binding is followed by TFIIA binding to form a stable TBP-TFIIA complex on DNA. Yeast TFIIA is a heterodimer composed of the Toa1 and Toa2 subunits. Some *toa2* mutations that abolished the TFIIA interaction with TBP when assayed in an in vitro binding reaction

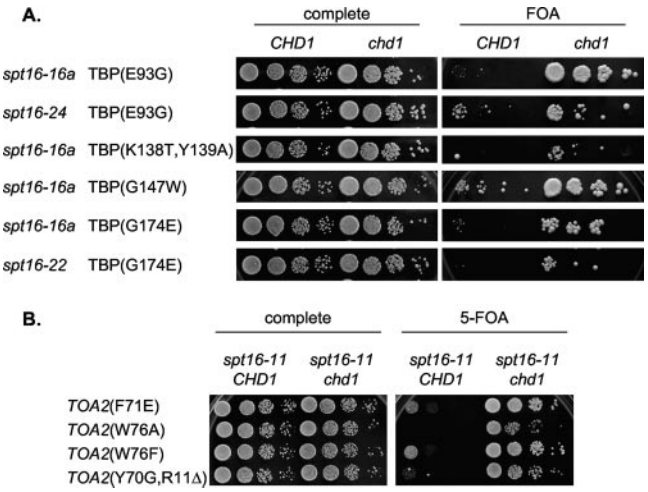

FIG. 8. A *chd1* mutation suppresses the synthetic lethality of an *spt16* mutation with either TBP or TFIIA mutations. (A) Strains DY8552 (*spt15 spt16* + YCp-*URA3*-TBP-Spt16) (indicated as "*CHD1*") and DY10141 (*spt15 spt16 chd1* + YCp-*URA3*-TBP-Spt16) (indicated as "*chd1*") were transformed with two plasmids, a YCp-*TRP1* plasmid encoding a TBP mutant and a YCp-*LEU2* plasmid with either wild-type *SPT16* or *spt16* mutations, and 10-fold dilutions were plated at 33°C either on complete medium for 2 days or on FOA medium for 3 days. (B) Strains DY8700 (*spt16-11 toa2* + YCp-*URA3-TOA2*) (indicated as "*CHD1*") and DY10214 (*spt16-11 chd1 toa2* + YCp-*URA3-TOA2*) (indicated as "*chd1*") were transformed with a YCp-*LEU2* plasmid with the indicated *toa2* mutant, and 10-fold dilutions were plated for 2 days on complete medium at 25°C and on FOA medium at 30°C.

were described previously (35). We have earlier shown that some of these *toa2* mutations are synthetic lethal with the *spt16-11* mutation (5). Since our data presented here strongly suggest that Chd1 has a negative role in regulating TBP binding in vivo, we asked whether a *chd1* mutation would also suppress the synthetic lethal interactions between *spt16-11* and *toa2* mutations. Two isogenic strains, the *spt16-11 toa2* and *spt16-11 toa2 chd1* strains, were constructed. Since *TOA2* is an essential gene for cell viability, the strains were kept alive by providing the *TOA2* gene on a YCp-*URA3* plasmid. Both strains were transformed with plasmids containing *toa2* mutations that showed a synthetic growth defect or synthetic lethal phenotype with *spt16-11*. The transformants were grown on a 5-FOA plate so that the strains are required to lose the parental YCp-*URA3-TOA2* plasmid and depend on the mutant *toa2* plasmid for their growth. Some *toa2* mutations show a synthetic lethal phenotype with *spt16-11* mutation in the presence of wild-type *CHD1*. Importantly, deletion of *CHD1* rescued these synthetic lethalities between *spt16-11* and *toa2* mutations (Fig. 8B). We also have observed a synthetic growth defect with some *toa2* mutations in combination with the *spt16-11* mutation. Deletion of *CHD1* also restored this synthetic growth defect between *spt16-11* and *toa2* mutations (Fig. 8B). Collectively, these data once again strongly suggest a negative role played by Chd1 in yFACT-mediated TBP binding during the transcriptional initiation step.

This work and previous studies suggest a dual role played by yFACT and Chd1 in regulating transcriptional initiation and elongation. Consistent with these observations, other elonga-

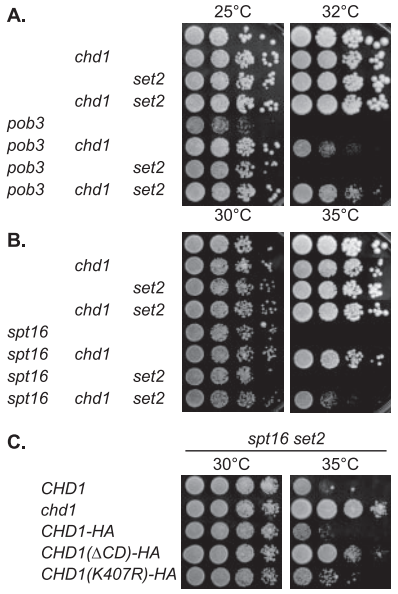

FIG. 9. Additive suppression of *pob3* by *chd1* and *set2* mutations. (A) Tenfold dilutions of strains DY150 (wild type), DY9809 (*chd1*), DY8690 (*set2*), DY9838 (*chd1 set2*), DY7379 (*pob3*), DY9458 (*pob3 chd1*), DY8878 (*pob3 set2*), and DY9547 (*pob3 chd1 set2*) were plated on complete medium at 25°C or 32°C for 3 days. (B) Tenfold dilutions of strains DY150 (wild type), DY9809 (*chd1*), DY8690 (*set2*), DY9838 (*chd1 set2*), DY8107 (*spt16*), DY9152 (*spt16 chd1*), DY8777 (*spt16 set2*), and DY9153 (*spt16 chd1 set2*) were plated on complete medium at either 30°C or 35°C for 2 days. (C) Tenfold dilutions of strains DY8777 (*spt16 set2*), DY9153 (*spt16 set2 chd1*), DY11619 (*spt16 set2 chd1 CHD1-HA*), DY11626 [*spt16 set2 chd1 CHD1*(Δ*CD*)*-HA*], and DY11645 [*spt16 set2 chd1 CHD1*(*K407R*)*-HA*] were plated on complete medium at 30°C for 2 days or at 35°C for 3 days.

tion factors such as the Rtf1 component of the PAF complex (49) and TFIIS (36) have also shown genetic interactions with TBP, thereby suggesting a dual role played by these factors in regulating transcription.

Chd1 and Set2 act in different pathways in vivo. We find that the defects caused by yFACT mutations can be similarly suppressed by *chd1* and *set2* mutations. These similar suppressive effects could mean that Chd1 and Set2 function in a similar pathway, possibly with the Chd1 chromodomain recognizing the H3-K36 residue methylated by Set2 or other modified histone residues. To explore this possibility, we introduced both *chd1* and *set2* disruptions into strains with yFACT mutations. At 32°C a *pob3* strain does not grow at all, while the *pob3 chd1* strain grows weakly (Fig. 9A). The *pob3 set2* double mutant does not grow at 32°C (Fig. 9A), but it does grow at 30°C (5), a temperature at which the *pob3* single mutant does not grow. The *pob3 chd1 set2* triple mutant shows much better growth than either double mutant. This additive defect shows that Chd1 and Set2 act in different pathways. A slightly different result is seen with the *spt16* mutant, where *chd1* suppresses well the 35°C growth defect but *set2* does not suppress at all at this temperature (Fig. 9B). Interestingly, the *spt16 chd1 set2* triple mutant shows an intermediate phenotype. The difference in the *spt16* response to *chd1* and *set2* is consistent with these two regulators functioning in distinct pathways, although we do not see an additive effect here.

Thus, the *pob3*(*L78R*) and *spt16-11* alleles differ in terms of additive suppression by *chd1* and *set2*. This is not completely unexpected, as the *pob3*(*L78R*) and *spt16-11* mutants show opposite effects in response to changes in copy number of histone genes or to an *rpd3* mutation (19).

We also examined the *CHD1* alleles with mutations in the chromodomain and ATPase domains for effects in the *spt16 set2* strain (Fig. 9C). The *CHD1 spt16 set2* strain fails to grow at 35°C, while the *chd1 spt16 set2* strain is alive. If a *CHD1* mutant complements the strain will not grow; failure to complement will result in growth. The ΔCD and K407R mutant versions of Chd1 both allow partial growth of the *spt16 set2* mutant at 35°C, and thus both the chromodomain and the ATPase are required for the Chd1 activity that is toxic in the *spt16 set2* strain.

In summary, the additive effect of *chd1* and *set2* disruptions in suppressing the *pob3* growth defect demonstrates that Chd1 and Set2 function in different pathways. We also show that both the chromodomain and the ATPase contribute to this function of Chd1.

Consistent with our observations that Chd1 and Set2 both play negative roles in regulating yFACT-mediated transcription, a negative role for these factors in transcriptional elongation was suggested by an earlier study. The Bur1 kinase is thought to promote elongation by phosphorylating Pol II (26), and the severe growth defect caused by a *bur1* deletion can be suppressed by disruption of either *CHD1* or *SET2* (25). Since Bur1 promotes elongation, those authors concluded that Chd1 and Set2 act negatively on elongation.

DISCUSSION

The yeast Chd1 protein has ATPase activity and two chromodomains, and the protein functions as an ATP-dependent chromatin remodeler in vitro (29, 48). We describe yFACT as a chromatin-"reorganizing" complex because its activity is ATP independent, to distinguish it from the chromatin remodelers that require ATP. We find that a *chd1* gene disruption suppresses numerous phenotypes caused by mutations in the Spt16 and Pob3 subunits of yFACT, including temperature-sensitive growth, and synthetic growth defects in combination with other transcriptional regulators. This suppression by *chd1* mutations suggests that Chd1 and yFACT act in opposition at the steps involved in causing these phenotypes. Previous studies have suggested that Chd1 and yFACT both function in transcriptional elongation (19, 27, 45, 47). However, our results here show that the defect in *GAL1* transcription caused by a *pob3* mutation can be suppressed by *chd1* and that this suppression includes restoration of binding by the TBP basal transcription factor and Pol II. Additionally, *spt16* mutations show synthetic lethality in combination with mutations in TBP or TFIIA, and this synthetic lethality can be suppressed by deletion of the *CHD1* gene. These results suggest that both Chd1 and yFACT function at promoters, regulating chromatin accessibility for DNA binding by TBP and TFIIA. However, the possibility remains that the effect on TBP recruitment is indirect. Mason and Struhl (30) found that inactivation of a thermosensitive Spt16 results in reduced binding of TBP and TFIIB at promoters, and those authors suggested that this

reduced TBP binding is an indirect result of inappropriate TBP binding to cryptic sites elsewhere in the genome.

Our genetic experiments suggest that Chd1 and yFACT act in opposition, with Chd1 being toxic in *spt16* or *pob3* mutants with a partially defective yFACT chromatin-reorganizing factor. In support of this model, we showed that overexpression of Chd1 is toxic in an *spt16* mutant; importantly, Chd1 overexpression is not detrimental in an *SPT16* strain (Fig. 6).

We have shown that disruption of the *SET2* gene, encoding a histone methyltransferase acting on K36 of histone H3, can also suppress growth defects caused by *spt16* and *pob3* mutations (3). Similar to a *chd1* mutation, *set2* also suppresses defects in *GAL1* transcription, TBP binding, and Pol II binding, as well as the synthetic lethality seen with TBP or TFIIA mutants. Thus, *chd1* and *set2* have very similar effects in suppressing yFACT mutants. A simple model would have Chd1 and Set2 functioning in a similar pathway, possibly with the Chd1 chromodomain recognizing the H3-K36 residue methylated by Set2 or other modified histone residues. However, we find that *chd1* and *set2* show additivity in their ability to suppress the *pob3* growth defect (Fig. 9A). This genetic experiment clearly shows that Chd1 and Set2 function in distinct pathways.

Structural work has shown that the two chromodomains of human Chd1 form a single structural unit and that this double chromodomain binds to the histone H3 tail with methylated K4 (16). The results with yeast Chd1 are controversial, with one group showing yeast Chd1 binding to H3-K4-Me (37) and two labs failing to detect this interaction (32, 46). Our genetic experiments argue strongly against the idea that Chd1 binds H3-K4-Me. While a *chd1* mutation suppresses yFACT defects, either an H3-K4R substitution or disruption of *SET1*, encoding the H3-K4 methyltransferase, shows strong synthetic defects when combined with either *spt16* or *pob3* (3). Thus, *chd1* has opposite effects from those of either H3-K4R or *set1*, and thus it seems unlikely that the ability of Chd1 to oppose yFACT requires binding of Chd1 to H3-K4-Me. Like the suppression by *chd1*, H3-K36R or *set2* mutations also suppress yFACT defects (3). However, the additive effect seen by *chd1* and *set2* in suppressing the *pob3* growth defect also makes it unlikely that Chd1 binds to H3-K36-Me. Consistent with these genetic data, a recent structure of yeast Chd1 shows that it lacks aromatic residues involved in binding methyl-lysine and suggests that it will not bind this modified residue (15).

Chd1 has two chromodomains along with its ATPase domain, and we used Chd1 mutants to test whether these protein functions are required for the toxicity of Chd1 in yFACT mutants. We used two mutants, *CHD1*(Δ*CD*), where the chromodomain has been deleted, and *CHD1*(*K407R*), with a mutation in a lysine residue required for ATPase activity. Our experiments show that the chromodomain and ATPase mutations in Chd1 both partially relieve the growth defect in *pob3* and *spt16* mutants (Fig. 2). These experiments suggest that the ATPase activity and the chromodomain of Chd1 are both required for Chd1 toxicity in yFACT mutants. We found a strong growth defect in the *gcn5 chd1* double mutant, and this growth defect is also seen in *gcn5* mutants with either the *CHD1*(Δ*CD*) or *CHD1*(*K407R*) allele affecting the chromodomain or the ATPase (Fig. 4). Thus, both the ATPase activity and the chromodomain are required for the Chd1 activity that opposes yFACT.

Our genetic experiments show that Chd1 and yFACT act in opposition in regulating transcription, and this may involve regulating TBP binding at promoters. An association between Chd1 and yFACT has been shown by purification of TAP-tagged proteins and by immunoprecipitation (27, 45). Further work is needed to understand how these two chromatin factors function and what is the role of the chromodomain in Chd1.

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