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Mutations in Chromatin Components Suppress a Defect of Gcn5 Protein in *Saccharomyces cerevisiae*

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The yeast *GCN5* gene encodes the catalytic subunit of a nuclear histone acetyltransferase and is part of a high-molecular-weight complex involved in transcriptional regulation. In this paper we show that full activation of the *HO* promoter in vivo requires the Gcn5 protein and that defects in this protein can be suppressed by deletion of the *RPD3* gene, which encodes a histone deacetylase. These results suggest an interplay between acetylation and deacetylation of histones in the regulation of the *HO* gene. We also show that mutations in either the H4 or the H3 histone gene, as well as mutations in the *SIN1* gene, which encodes an HMG1-like protein, strongly suppress the defects produced by the *gcn5* mutant. These results suggest a hierarchy of action in the process of chromatin remodeling.

Nuclear processes, including transcription, require that enzymes gain access to the eukaryotic DNA template despite the fact that it is complexed with histone and nonhistone proteins to form chromatin. Genetic studies with *Saccharomyces cerevisiae* have identified two groups of genes that appear to link transcriptional regulation to chromatin structure (40). The first group encodes components of the SWI/SNF complex, which has been proposed to antagonize the repressive effects of chromatin on transcription (24). *SWI*/*SNF* genes were identified in genetic screens for mutants defective in the expression of various genes, including the *HO* and *SUC2* genes (2, 18, 22, 32). The second group of genes includes various *SPT* and *SIN* genes, which were defined as suppressors of various types of transcriptional defects (40). The *sin2-1* mutation was found to lie in the *HHT1* gene, which encodes histone H3. Five additional different point mutations, two in histone H3 and three in histone H4, also displayed a Sin^-/Spt^- phenotype (12, 25). These mutations affect residues that are believed either to contact DNA or to be involved in histone-histone contacts within the histone octamer (39). The *SIN1* gene was found to encode a protein with similarities to mammalian HMG1, a structural component of chromatin (11). Although the precise role of yeast SIN1 is not known, the similarity of *sin1* and *sin2-1* mutant phenotypes has led to the inference that these two genes have related physiological functions.

Recently, a group of genes involved in acetylation and deacetylation of histones has been recognized. Histone acetylation has long been correlated with the modulation of gene activity (37). Acetylation of lysines in histone amino-terminal tail domains reduces the positive charge, thereby weakening histone-DNA interactions, destabilizing higher-order structure, and rendering nucleosomal DNA more accessible to transcriptional factors (4, 14). Yeast Gcn5 was originally identified as a regulatory factor required for function of the yeast activator Gcn4 (5), and recently it has been shown that Gcn5 is a histone acetyltransferase (3, 13, 28) that is part of at least two high-molecular-weight complexes called ADA (8) and SAGA (7). The recruitment of these complexes to DNA is thought to direct the local destabilization of nucleosomes, producing more efficient transcriptional activation on a promoter. Aside from transcriptional regulators that function as histone acetyltransferases, there are also regulators that deacetylate the histones (29, 36). These deacetylases comprise part of a transcriptional repression pathway conserved from yeast to vertebrates and provide a molecular mechanism whereby transcription can be continually controlled (19, 38).

In this paper we show that the expression of the *HO* gene is affected by defects in histone acetylation and deacetylation. Previous work has shown that the SWI/SNF complex and structural components of chromatin also affect *HO* expression (11, 12, 16, 22). We present an analysis of single and double mutations in the genes encoding several of these components, and the results suggest a hierarchy in the chromatin remodeling process.

MATERIALS AND METHODS

Strains and media. All strains of *S. cerevisiae* used in this study are described in Table 1. Complete medium (yeast extract-peptone-dextrose [YEPD]) and minimal medium supplemented with the required amino acids were used for yeast growth and transformations (26). Histidine limitation was accomplished by supplementing minimal media with 10 mM 3-amino-1,2,4-triazole (3-AT) (5).

Strain constructions. Single mutants were obtained either by gene disruptions performed by using the one-step replacement method (27) or by gene conversions carried out by a two-step gene replacement procedure (31). Double and triple mutants were obtained by crossing single mutants of opposite mating types and selecting segregants carrying the desired mutations.

A strain carrying a *swi5::LEU2* null allele was generated as described in reference 34. The *gcn5::hisG* strain was generated as described in reference 15. The *HO-lacZ* fusion allele is described in reference 30. The histone mutations were introduced in the chromosome by a two-step replacement procedure (31) using integrating plasmids marked with the *URA3* gene (obtained from R. K. Tabtiang and I. Herskowitz); as these mutations are partially dominant, it is possible to observe their effects, even in the presence of another histone gene copy (12). The *rpd3Δ::LEU2* strain was generated by transforming yeast with pDM176 digested with *Bam*HI. This plasmid carries the *RPD3* locus with a replacement of the entire *RPD3* open reading frame (ORF) with the *LEU2* gene (15a). Correct integration was tested by PCR analysis using oligonucleotides flanking the *RPD3* locus. The *sin1Δ::TRP1* deletion strains were generated by transforming yeast with pUC-SIN1::TRP linearized with *Eco*RI-*Sph*I. This plasmid carries a replacement of the *SIN1* ORF with the *TRP1* gene (11). Correct integration was tested by PCR analysis using oligonucleotides flanking the *SIN1* locus.

RNA analysis. Strains were grown to mid-log phase in YEPD medium. Total-yeast RNA was isolated and fractionated on formaldehyde gels, transferred to nylon membranes (Genescreen; DuPont), and hybridized with random-primed ^{32}P-labeled fragments. The DNA probes used were obtained as PCR fragments by amplification of the desired ORF with specific primers (MapPairs; Research

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TABLE 1. Yeast strains used in this study

Strain	Genotype
FY120	*MAT***a** *ura3-52 leu2Δ1 his4-912δ Lys2-128δ*
RT238	*MATα ura3-52 leu2Δ1 his3 trp1 HO-lacZ*
JJY12	*MATα ura3-52 leu2 Δ1 trp1 lys2-128δ HO-lacZ*
JJY13	Same as JJY12, plus *swi5::hisG*
JJY28	Same as JJY12, plus *gcn5::hisG*
JJY36	Same as JJY12, plus *sin1Δ::TRP1*
JJY42	Same as JJY28, plus *hhf2-8*
JJY43	Same as JJY28, plus *hhf2-13*
JJY44	Same as JJY28, plus *sin2-1*
JJY45	Same as JJY28, plus *sin1Δ::TRP1*
JJY54	Same as FY120, plus *gcn5::hisG*
JJY60	Same as JJY28, plus *swi5::hisG*
JJY64	Same as JJY12, plus *rpd3Δ::LEU2*
JJY65	Same as JJY28, plus *rpd3Δ::LEU2*
JJY72	Same as JJY41, plus *rpd3Δ::LEU2*
JJY73	Same as JJY42, plus *rpd3Δ::LEU2*
JJY74	Same as JJY43, plus *rpd3Δ::LEU2*
JJY75	Same as JJY44, plus *rpd3Δ::LEU2*

Genetics Inc.), with the exception of the *HO* probe, which was obtained as a 2.6-kb *Hin*dIII fragment from the plasmid pGAL-HO (9).

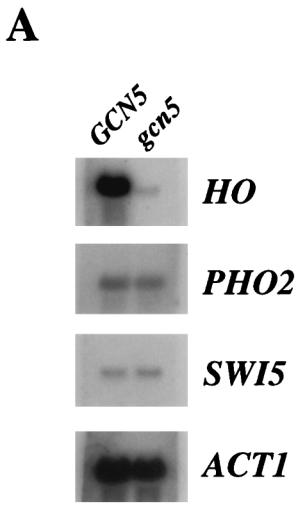
Other methods. Yeast cells were transformed by the LiOAc method (6). β-Galactosidase assays were performed as described elsewhere (26).

RESULTS

The Gcn5 protein is required for *HO* expression. *HO* gene expression is dependent on *SWI5*. This gene encodes a zinc finger DNA-binding protein which binds specifically, along with the PHO2 protein, to the upstream region of the *HO* promoter (1, 34). Genetic studies have described a series of extragenic suppressor mutations that permit expression of *HO* in the absence of the *SWI5* gene product (17, 33). Two of the genes identified in this screen, *RPD3* and *SIN3*, encode, respectively, a histone deacetylase and a protein tightly associated with it (10, 29, 35). The fact that mutations in the gene pair *SIN3*/*RPD3* are able to suppress the absence of the Swi5 protein suggests that one of the roles of the Swi5-Pho2 heterodimer is the recruitment, either directly or indirectly, of a histone acetyltransferase activity. A likely candidate is the *GCN5* gene, which encodes a protein with histone acetyltransferase activity (13). To test this idea, we examined the levels of *HO* mRNA produced in wild-type and isogenic *gcn5* mutant strains (obtained by disruption of the *GCN5* gene; see Materials and Methods). We found that a *gcn5* mutant strain produced significantly less *HO* mRNA (Fig. 1A). By contrast, the absence of the Gcn5 protein did not impair the normal levels of *PHO2* and *SWI5* mRNA.

In principle, *SWI5* and *GCN5* gene products could act in the same pathway or through different pathways to activate *HO* expression. If two genes act in the same pathway, then the phenotype of the double mutant should be the same as that of one of the single mutants. On the other hand, if two genes act through different pathways, then the phenotype of the double mutant should be more severe than that of either single mutant. To distinguish between these two possibilities, we measured the β-galactosidase activity produced by a chromosomal *HO-lacZ* gene fusion in a *swi5 gcn5* double mutant and compared it to those in the single mutants (Fig. 1B). *HO-lacZ* expression in the *gcn5* and *swi5* mutants was reduced 50- and 200-fold, respectively. In the double mutant, *HO-lacZ* expression was reduced 200-fold. The β-galactosidase values of the *swi5* mutant are so low (0.5 Miller units) that we cannot make a conclusive argument about the relationship of *SWI5* and *GCN5*. However, since both defects are suppressed by the same mutations (i.e., by *rpd3*, *sin1*, and *sin2* mutations; see below) and since the levels of mRNA for *SWI5* and *PHO2* genes are not affected by *gcn5* mutations (Fig. 1A), these facts support the idea that *SWI5* and *GCN5* act in the same pathway to stimulate *HO* expression.

Deletion of the *RPD3* gene suppresses the *gcn5* mutation. The results described above are compatible with the idea that histone acetylation is required for maximal *HO* transcriptional activation. According to this hypothesis, a mutation in a gene encoding a deacetylase should be able to suppress a *gcn5* mutation. A likely candidate is the *RPD3* gene, since mutations in this gene suppress the Swi5 requirement in the *HO* gene (35). We therefore measured *HO-lacZ* activity in single and double mutants carrying null alleles of the *GCN5* and *RPD3* genes.



B

Relevant genotype	*HO-lacZ* activity (Miller Units)
wt	101.3
swi5	0.5
gcn5	2.1
swi5, gcn5	0.62

FIG. 1. Gcn5 is required for *HO* expression. (A) Effects of *gcn5* disruption on the mRNA levels of the *HO*, *PHO2*, and *SWI5* genes. Total RNA was extracted from FY120 (*GCN5*) and JJY54 (*gcn5::hisG*) grown in YEPD medium to mid-log phase. *ACT1* mRNA was used as a control. (B) Genetic relationships between *SWI5* and *GCN5*. β-Galactosidase activity was measured in strains carrying an *HO-lacZ* reporter gene integrated in the chromosome at the *HO* locus. The strains used were JJY12 (wild type [wt]), JJY28 (*gcn5::hisG*), JJY13 (*swi5::hisG*), and JJY60 (*gcn5::hisG swi5::hisG*). Values are averages of three independent measurements with less than 10% deviation.

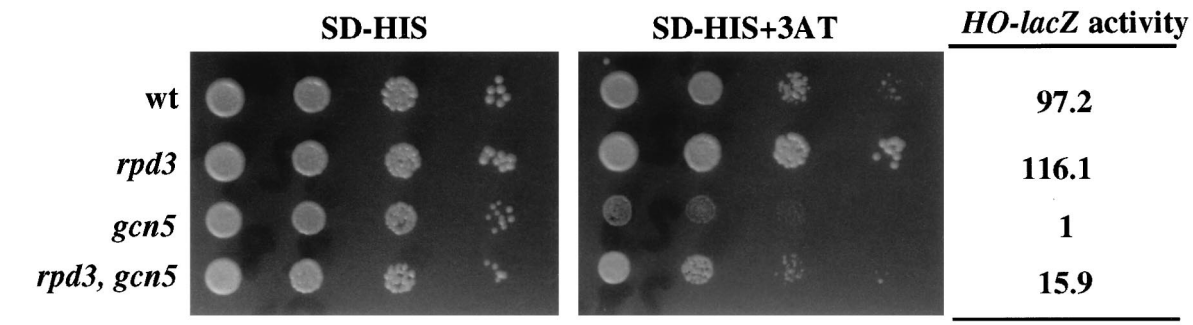


FIG. 2. A deletion of the *RPD3* gene partially suppresses the defects caused by a disruption of the *GCN5* gene. Cultures of JJY1 (wild type [wt]), JJY64 (*rpd3Δ::LEU2*), JJY28 (*gcn5::hisG*), and JJY65 (*rpd3Δ::LEU2 gcn5::hisG*) cells (approximately 5×10^6/ml) were spotted in 10-fold serial dilutions on medium lacking histidine (SD-HIS) and on medium lacking histidine and containing 10 mM 3-AT. Plates were incubated at 30°C for 3 days. The same cultures were used to measure β-galactosidase activity (in Miller units). Values are averages of three independent measurements with less than 10% deviation.

The results (Fig. 2) show that a deletion of the deacetylase gene *RPD3* alleviates the requirement for the histone acetyltransferase gene *GCN5* in *HO* gene expression. The level of suppression of a *gcn5* mutation by the deletion of *RPD3* is similar to that observed in the case of *swi5* mutations (35) and is also similar to the suppression observed in a triple *swi5 gcn5 rpd3* mutant, again supporting the view that *SWI5* and *GCN5* function in the same pathway.

One of the defects originally observed in *gcn5* mutant strains was their inability to grow in media imposing amino acid limitation (20). Thus, a strain carrying a deletion of the *GCN5* gene is defective in growth in media containing 3-AT, a condition that mimics histidine starvation (5). To address whether a deletion in the *RPD3* gene suppresses other defects in *gcn5* strains, we also tested the ability of the *RPD3* deletion to allow growth of a *gcn5* strain in the presence of 3-AT. As shown in Fig. 2, the *gcn5* strain exhibited a growth defect under such conditions compared with an isogenic wild-type strain. Deletion of the *RPD3* gene indeed alleviates this defect, allowing growth of the *gcn5* strain under these conditions.

Disruption of *SIN1*, a gene encoding an HMG1-like protein, also suppresses *gcn5* defects. In addition to *sin3* and *rpd3* mutations, defects in other genes are well-known suppressors of transcriptional deficiencies in *HO*. One of these genes is *SIN1*. This gene encodes a protein with similarities to the mammalian HMG1 protein, and it is believed to be a component of chromatin (11). We have monitored both *HO-lacZ* expression and the ability to grow in the presence of 3-AT of a double mutant defective in both *GCN5* and *SIN1*. The results shown in Fig. 3 indicate that the absence of Sin1 protein relieves the requirement of Gcn5 both for *HO* expression and for growth on 3-AT.

Histone mutations also suppress *gcn5* defects. An explanation for the results obtained with the *sin1* mutant is that the suppression we observed is caused by a defect in chromatin structure, such that this defective chromatin bypasses the requirement for histone acetylation. If this is the case, then other mutations which produce defective chromatin might also be expected to suppress the *gcn5* defects. Certain amino acid changes (*sin* mutations) in either histone H3 or histone H4 alleviate the same set of transcriptional defects as does the *sin1* mutation (12, 23). These *sin* mutations lie in the histone fold domain of histones H3 and H4, and they are in close proximity to one another on the surface of the histone octamer. It has been proposed that residues altered by these mutations may define a functional domain (the SIN domain) that behaves formally as a negative regulator of transcription (12).

To address if defective histones also suppress *gcn5* mutations, the following histone mutant alleles were tested for their ability to suppress a deletion of the *GCN5* gene: *sin2-1* (R116H in *HHT1*), *hhf2-7* (R45C in *HHF4*), *hhf2-8* (V43I in *HHF4*), and *hhf2-13* (R45H in *HHF4*). In spite of the fact that the targets for GCN5 protein are the histone tails, mutations in the histone fold are able to efficiently suppress the defects caused by the absence of the *GCN5* gene product (Fig. 4A).

We also determined the effects of combining a deletion of the *RPD3* gene with the histone *sin* mutations. Levels of *HO-lacZ* activity were determined in single and double mutants, and we found in the double mutants a strong synergistic effect; that is, the activity displayed by the double mutant is higher than the sum of the activities displayed by the single mutants (Fig. 4B). The same synergistic effect is also seen in combinations of *rpd3* and *sin1* mutations (data not shown).

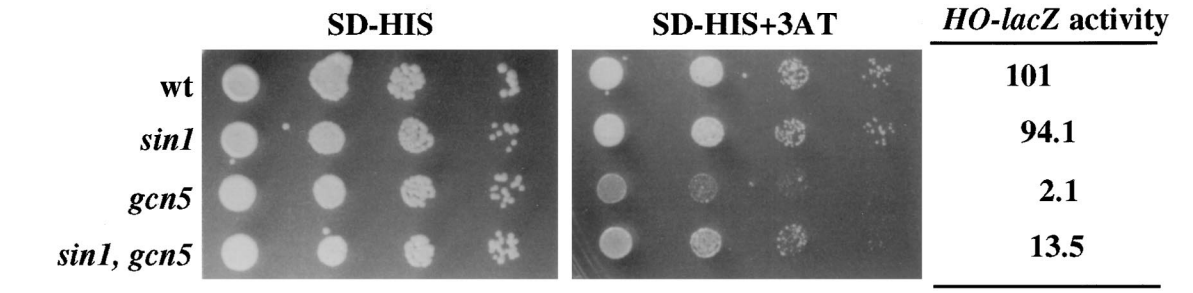
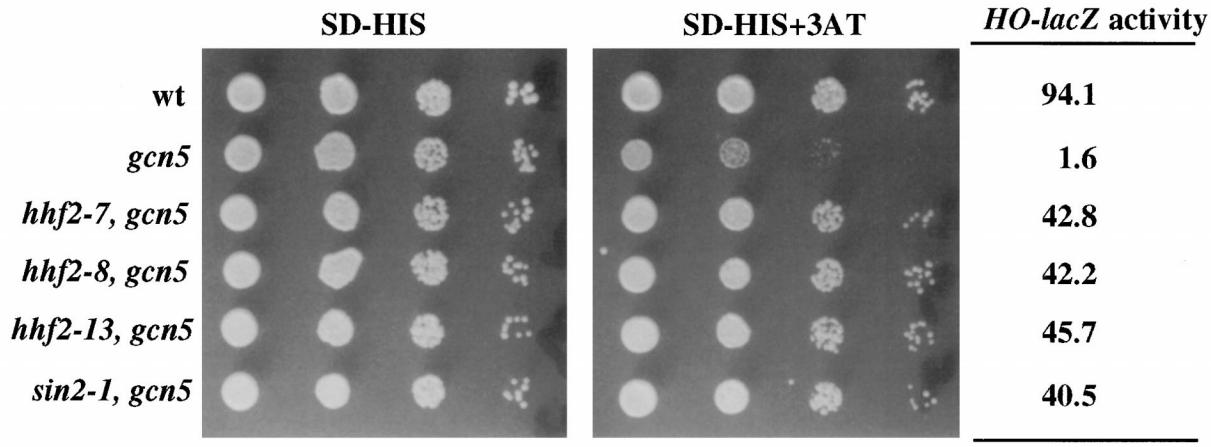


FIG. 3. Deletion of the *SIN1* gene alleviates the defects associated with disruption of the *GCN5* gene. Cultures of JJY12 (wild type [wt]), JJY36 (*sin1Δ::TRP1*), JJY28 (*gcn5::hisG*), and JJY45 (*sin1Δ::TRP1 gcn5::hisG*) cells (approximately 5×10^6/ml) were spotted in 10-fold serial dilutions on medium lacking histidine (SD-HIS) and on medium lacking histidine and containing 10 mM 3-AT. Plates were incubated at 30°C for 3 days. The same cultures were used to measure β-galactosidase activity (in Miller units). Values are averages of three independent measurements with less than 10% deviation.

A



B

Relevant genotype	*HO-lacZ* activity (Miller Units)
wt	106
gcn5	1.6
gcn5, hhf2-7	48.5
gcn5, hhf2-8	41.3
gcn5, hhf2-13	39.5
gcn5, sin2-1	48.4
gcn5, rpd3	20.4
gcn5,hhf2-7,rpd3	163
gcn5, hhf2-8, rpd3	171.4
gcn5,hhf2-13, rpd3	208.4
gcn5,sin2-1, rpd3	162.3

FIG. 4. Histone *sin* mutations suppress *gcn5* defects. (A) Cultures of JJY12 (wild type [wt]), JJY28 (*gcn5::hisG*), JJY41 (*hhf2-7 gcn5::hisG*), JJY42 (*hhf2-8 gcn5::hisG*), JJY43 (*hhf2-13 gcn5::hisG*), and JJY44 (*sin2-1 gcn5::hisG*) cells (approximately 5×10^6/ml) were spotted in 10-fold serial dilutions on medium lacking histidine (SD-HIS) and on medium lacking histidine and containing 10 mM 3-AT. Plates were incubated at 30°C for 3 days. The same cultures were used to measure β-galactosidase activity (in Miller units). Values are averages of three independent measurements with less than 10% deviation. (B) Effects of *rpd3* deletion on the suppression of *gcn5* defects by histone *sin* mutations and *sin1* mutations. The strains used were JJY12, JJY28, and JJY41 through JJY44 (all as described for panel A), as well as JJY65 (*rpd3Δ::LEU2 gcn5::hisG*), JJY72 (*hhf2-7 rpd3Δ::LEU2 gcn5::hisG*), JJY73 (*hhf2-8 rpd3Δ::LEU2 gcn5::hisG*), JJY74 (*hhf2-13 rpd3Δ::LEU2 gcn5::hisG*), and JJY75 (*sin2-1 rpd3Δ::LEU2 gcn5::hisG*). Values are averages of three independent measurements with less than 10% deviation.

DISCUSSION

Regulation of the yeast *HO* gene is complex, and many genes that regulate *HO* have been identified (16). These include genes encoding the SWI/SNF complex (22, 32); *SIN1*, which encodes an HMG1-like protein (11); *SIN2*, which encodes histone H3; *HHF4*, which encodes histone H4 (12); and *SIN3*, which, along with *RPD3*, is involved in the deacetylation of histones (35). In this paper, we show a requirement for the *GCN5* gene, which encodes a histone acetyltransferase (3), for optimal transcription of the *HO* gene.

The identification of histone acetyltransferases and histone deacetylases as transcriptional regulators provides molecular

mechanisms whereby transcription might be turned up or down (38), but so far no such interplay between acetylase and deacetylase activities at a single gene has been reported. The suppression of the *gcn5* defects by deletion of one of the genes encoding a deacetylase activity provides clear support for such interplay at the *HO* promoter. The suppression we observed is only partial, suggesting a functional redundancy in the deacetylase activity. Another protein with deacetylase activity is encoded by the gene *HDA1*, and three additional ORFs with high levels of homology with *RPD3* and *HDA1* have also been described (29). However, we observed that deletion of *HDA1* or of one of these additional ORFs (*HOS1*) does not suppress the *GCN5* requirement in *HO* expression (data not shown). Another explanation for the fact that suppression is only partial is that the *rpd3* deletion may destabilize additional proteins with which it is complexed (7), and these additional proteins may contribute to the activation of *HO*.

The pattern of genetic interactions described in this work suggests a hierarchy of gene function that pertains to chromatin components, histone acetylation, and the SWI/SNF complex. Loss of Swi5 (the major activator protein for the *HO* gene [16]) can be partially suppressed by *sin1*, *sin2* (histone H3), *sin3*, and *rpd3* mutations (33, 35). Loss of GCN5 (a histone acetyltransferase, also required for *HO* transcription) can be suppressed by these same mutations (Fig. 2, 3, and 4A). However, while defects in the SWI/SNF complex can be suppressed by *sin1* (which is thought to be a target of the SWI/SNF complex [21] and *sin2* mutations (11, 12), they cannot be suppressed efficiently by *sin3* or *rpd3* mutations (33, 35). These results indicate that histone acetylation at the *HO* promoter functions upstream of the SWI/SNF complex. Consistent with this view is the strong synergy seen between *rpd3* mutations (which affect the acetylation of histone tails) and *sin1* and *sin2* mutations (which circumvent the need for the SWI/SNF complex) (Fig. 4B). One hypothesis consistent with this genetic hierarchy is that, at the *HO* promoter, histone acetylation precedes and enables the action of the SWI/SNF complex. A similar view has recently been developed independently by Pollard and Peterson (24a).

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