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Architectural Transcription Factors and the SAGA Complex Function in Parallel Pathways To Activate Transcription

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Recent work has shown that transcription of the yeast *HO* gene involves the sequential recruitment of a series of transcription factors. We have performed a functional analysis of *HO* regulation by determining the ability of mutations in *SIN1*, *SIN3*, *RPD3*, and *SIN4* negative regulators to permit *HO* expression in the absence of certain activators. Mutations in the *SIN1* (=*SPT2*) gene do not affect *HO* regulation, in contrast to results of other studies using an *HO:lacZ* reporter, and our data show that the regulatory properties of an *HO:lacZ* reporter differ from that of the native *HO* gene. Mutations in *SIN3* and *RPD3*, which encode components of a histone deacetylase complex, show the same pattern of genetic suppression, and this suppression pattern differs from that seen in a *sin4* mutant. The Sin4 protein is present in two transcriptional regulatory complexes, the RNA polymerase II holoenzyme/mediator and the SAGA histone acetylase complex. Our genetic analysis allows us to conclude that Swi/Snf chromatin remodeling complex has multiple roles in *HO* activation, and the data suggest that the ability of the SBF transcription factor to bind to the *HO* promoter may be affected by the acetylation state of the *HO* promoter. We also demonstrate that the Nhp6 architectural transcription factor, encoded by the redundant *NHP6A* and *NHP6B* genes, is required for *HO* expression. Suppression analysis with *sin3*, *rpd3*, and *sin4* mutations suggests that Nhp6 and Gcn5 have similar functions. A *gcn5 nhp6a nhp6b* triple mutant is extremely sick, suggesting that the SAGA complex and the Nhp6 architectural transcription factors function in parallel pathways to activate transcription. We find that disruption of *SIN4* allows this strain to grow at a reasonable rate, indicating a critical role for Sin4 in detecting structural changes in chromatin mediated by Gcn5 and Nhp6. These studies underscore the critical role of chromatin structure in regulating *HO* gene expression.

The *Saccharomyces cerevisiae HO* gene encodes an endonuclease that is responsible for initiating mating type switching in yeast. The transcriptional regulation of *HO* is complex and has been the subject of intensive study (for reviews, see references 22 and 37). Recent studies have shown that transcription of specific genes can be affected by chromatin structure at the promoter (for reviews, see references 25, 28, 54, and 59). Chromatin structure plays an important role in regulation of *HO* transcription, as *HO* expression is altered by mutations in a number of important transcriptional regulators, including components of the Swi/Snf chromatin remodeling complex, the SAGA histone acetyltransferase complex, and the Sin3/Rpd3 histone deacetylase complex. *GCN5*, *ADA2*, and *ADA3*, which encode members of the SAGA histone acetyltransferase complex (18), are required for *HO:lacZ* expression (43), and native *HO* expression is also reduced in a *gcn5* mutant (41). The yeast *RPD3* gene encodes a histone deacetylase that is associated with Sin3 (26, 27). *SIN3* and *RPD3* are negative regulators of transcription, and mutations in *SIN3* or *RPD3* allow an *HO:lacZ* reporter to be expressed in the absence of specific activators (41, 53).

HO is cell cycle regulated and is expressed in late G_1 (36). Recent work using chromatin immunoprecipitation provides new insights as to changes at the *HO* promoter during the cell cycle. Cosma et al. (12) showed that activation of *HO* transcription involves ordered recruitment of transcription factors. Swi5 enters the nucleus at the end of anaphase, binds to the promoter, and then recruits Swi/Snf. Swi/Snf, in turn, recruits SAGA, and Swi/Snf and SAGA are both required for SBF binding. It is believed that SBF, composed of the Swi4 and Swi6 factors, is then directly responsible for *HO* activation. Krebs et al. (30) showed that a 1-kb region of the *HO* promoter undergoes histone acetylation in mid-G_1 phase of the cell cycle, and these promoter changes require the activity of the Swi5, Swi/Snf, and SAGA transcription factors. Mutations in *SIN3* or *RPD3* result in acetylation of the *HO* promoter throughout the cell cycle.

The *SIN4* gene was identified as regulator of *HO* expression (24). A *sin4* mutation causes decreased expression of some genes, including *HIS4*, *CTS1*, and *MAT*α. However, expression of other genes, including *HO:lacZ*, *IME1*, *GAL1*, *SUC2*, *DIT1*, *DIT2*, and **a**-specific genes, is increased in a *sin4* mutant (11, 14, 16, 24, 50, 56). A *sin4* mutation has effects similar to those seen in strains with histone mutations, including changes in linking number of plasmid DNA and sensitivity of chromatin to nucleases, and it has been suggested that these effects on transcription are caused by changes in chromatin structure (23, 24, 33). The Sin4 protein is part of the RNA polymerase II holoenzyme/mediator, in a subcomplex with Rgr1, Gal11, Med2, and Pgd1 (32, 35). Importantly, mutations in other components of the RNA polymerase II mediator complex also have diverse effects on transcriptional regulation (for reviews, see references 5, 9, and 20). It has been recently demonstrated that Sin4 is also part of the SAGA complex (P. Grant and J. Workman, personal communication).

The *HO* gene promoter is quite large, by yeast standards, with regulatory sites identified nearly 2 kb from the transcription start site. The *SWI5* gene encodes a zinc finger DNA binding protein that is required for *HO* expression. There are

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two Swi5 binding sites in the *HO* promoter, at −1800 and at −1300. Genetic analysis demonstrates that both Swi5 binding sites are required for *HO* expression, suggesting that there is a physical interaction between these two sites separated by 500 bp (34). The term "architectural transcription factor" has been applied to proteins that bend DNA and promote assembly of distantly bound factors into a productive complex (58). It is possible that architectural transcription factors, by promoting DNA bending, could facilitate this proposed interaction between Swi5 molecules bound at these two sites. We decided to examine whether architectural transcription factors contribute to Swi5-dependent activation of *HO* by determining whether mutations in these factors affect *HO* expression.

Architectural transcription factors often contain the DNA-binding domain first identified in mammalian high-mobility-group 1 and 2 (HMG1/2) proteins (8). There are a number of yeast genes encoding proteins with homology to the HMG domain, including *ABF2*, *ROX1*, *SIN1*, and the duplicated *NHP6A* and *NHP6B* genes. Some HMG proteins, such as Rox1 (15), bind DNA in a sequence-specific manner; other HMG proteins have little specificity in DNA sequence recognition but may recognize structural elements in DNA or chromatin, such as cruciform structures (4). We directed our attention to *SIN1* and *NHP6A*/*B* because mutations in these genes have been reported to affect transcriptional regulation.

The *SIN1* gene was originally identified as *SPT2*, as *sin1*/*spt2* mutations suppress the transcriptional defects due to insertions of the Ty1 transposable element into the *HIS4* and *LYS2* promoters (49). *SIN1* mutations were also identified as bypass suppressors allowing expression of an *HO:lacZ* reporter in strains lacking either the Swi1 or Swi5 transcriptional activator (51). As we show below, *sin1* mutations do not restore expression of the native *HO* gene; the original observation appears to be an artifact of the bacterial sequences present in the *HO:lacZ* reporter. A *sin1* mutation can suppress the transcriptional defects at the *SUC2* and *HIS3* loci caused by mutations in *SWI1* and *GCN5*, respectively (41, 43). Additionally, a *sin1* mutation increases expression of the *SSA3* gene (1).

The 11-kDa Nhp6 protein of yeast shows 40% identity to the HMG domain of mammalian HMG1/2 proteins (29). There are two highly related genes, *NHP6A* and *NHP6B*, that express the Nhp6 protein. These two genes appear to be functionally redundant, as deletion of both genes is required for any observable phenotype (13). The *nhp6a nhp6b* double mutant is temperature sensitive for growth and shows defects in transcriptional activation of a number of LacZ reporter constructs (13, 39). Finally, in vitro experiments show that Nhp6 protein can promote the assembly of multicomponent protein-DNA complexes (40).

In this report we show that the Gcn5 and Nhp6 proteins are required for expression of *HO*. Suppressor analysis shows that mutations in the *SIN3*, *RPD3*, or *SIN4* gene can allow *HO* expression in the absence of these activators. We also find that *gcn5 nhp6a nhp6b* triple mutants are very sick, suggesting that Gcn5 and Nhp6 are both required for transcription of important genes. A *sin4* mutation suppresses this growth defect, suggesting that Sin4 has a unique role in regulating chromatin structure. The genetic analysis shows differences in the ability of *sin3* and *sin4* mutations to suppress *swi5* and *swi6* defects, and these results provide new insights as to regulation of *HO* expression.

MATERIALS AND METHODS

The yeast strains used in this study are listed in Table 1. Standard genetic methods were used for strain construction (45, 46). W303 strains with *SWI5*, *SIN3*, and *SIN4* disruptions have been previously described (24, 57). W303 strains with gene disruptions in *GCN5*, *HDA1*, and *HPR1* were provided by Sharon Roth, Michael Grunstein, and Hannah Klein, respectively. The *SIN1* gene was disrupted with plasmid WB39 (31), provided by Ira Herskowitz, and the *NHP6A* and *NHP6B* genes were disrupted with plasmids pDK201 and pDK262, respectively, provided by David Kolodrubetz. All gene disruptions were confirmed by Southern analysis. The *swi6::TRP1* allele from the closely related K1107 strain background was backcrossed four times into W303. Plasmid M4195 was constructed by inserting a 2.2-kb *Eco*RI-*Hin*dIII fragment with NHP6B from plasmid pDK227 (from David Kolodrubetz) into YEplac195 (17).

Cells were grown at 30°C in standard media (46). YEPD medium was used, except where use of YEP-galactose medium is indicated or when strains had plasmids. In the latter case, cells were grown in synthetic complete medium with 2% glucose supplemented with adenine, uracil, and amino acids, as appropriate, but lacking essential components to select for plasmids.

RNA levels were determined with S1 nuclease protection assays using *HO* and *CMD1* probes as described elsewhere (3). Protein extracts were prepared for quantitative measurement of β-galactosidase activity as described previously (7).

RESULTS

Role of architectural transcription factors in *HO* transcription. Genetic studies demonstrated that Swi5 binding at two sites, separated by 500 bp, was required for transcription of the *HO* gene (34). An architectural transcription factor might promote interaction between Swi5 molecules bound at these sites, and we determined whether mutations in the *SIN1* (=*SPT2*) gene, which encodes an HMG protein (31), affect *HO* expression. RNA was isolated from isogenic *SIN1* and *sin1* strains, and *HO* mRNA levels were measured with an S1 nuclease protection assay. As shown in Fig. 1A, a *sin1* mutation does not affect expression of *HO* (compare lanes 1 and 3). Two groups recently reported that a *gcn5* mutation reduced expression of an *HO:lacZ* reporter (41, 43). It was also reported that a *sin1* mutation suppresses the *gcn5* mutation, as the *HO:lacZ* reporter is expressed in the *gcn5 sin1* double mutant (41). However, we measured *HO* mRNA and found that while the *gcn5* mutation does reduce *HO* expression (Fig. 1A, lane 2), this reduction is not reversed in the *gcn5 sin1* mutant (Fig. 1A, lane 4). We attribute this difference in results in the *gcn5 sin1* double mutant to the use of an *HO:lacZ* reporter rather than native *HO*. (The differences between regulation of the native *HO* gene and the *HO:lacZ* reporter are considered in Discussion.) As *GCN5* encodes a histone acetyltransferase, it seemed possible that a mutation in a histone deacetylase would suppress the *gcn5* defect in *HO* expression. *HO* is expressed in a *gcn5 rpd3* double mutant (Fig. 1B, lane 4), consistent with results with an *HO:lacZ* reporter (41). In contrast, a mutation in a different histone deacetylase, *HDA1*, does not suppress the *gcn5* mutation (Fig. 1B, lane 6). The S1 protection assay in Fig. 1C shows that *HO* is not expressed in a *swi5 sin1* or *swi2 sin1* double mutant. Thus, a *sin1* mutation does not suppress defects in *HO* transcription caused by mutations in *GCN5*, *SWI5*, or *SWI2*. These results suggest that *SIN1*/*SPT2* is not a true negative regulator of native *HO* expression.

We next evaluated the role of the *NHP6A* and *NHP6B* genes, which encode HMG proteins, in activation of *HO*. The two genes encode nearly identical proteins, and a temperature-sensitive phenotype is seen in the *nhp6a nhp6b* double mutant but not in either single mutant (13). Expression of a number of *lacZ* reporters is reduced in a *nhp6a nhp6b* mutant strain (39). We find that expression of *HO* is reduced nearly 20-fold in the *nhp6a nhp6b* double mutant (Fig. 2, lane 2). Sidorova and Breeden (47) recently showed that *NHP6A* acts as a multicopy suppressor allowing expression of an *HO:lacZ* reporter at the nonpermissive temperature in a *swi6* temperature-sensitive mutant. The YEp-*NHP6A* plasmid does not suppress a *swi6* null mutation, however. They also observed reduced *HO* expression in a *nhp6a nhp6b* double mutant; however, the strains

TABLE 1. Yeast strains used

DY150[a]	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*
DY151[a]	*MATα ade2 can1 his3 leu2 trp1 ura3*
DY161[a]	*MAT***a** *swi5::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY411[a]	*MAT***a** *swi5::hisG ade2 can1 his3 leu2 trp1 ura3*
DY773[a]	*MAT***a** *sin3::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY775[a]	*MAT***a** *swi5::hisG sin3::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY984[a]	*MAT***a** *sin3::ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY1699[a]	*MAT***a** *sin4::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY1702[a]	*MAT***a** *sin4::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY2133[a]	*MAT***a** *swi5::LEU2 sin4::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY2348[a]	*MAT***a** *swi2::HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY2378[a]	*MAT***a** *nhp6a::URA3 ade2 can1 his3 leu2 trp1 ura3*
DY2380[a]	*MAT***a** *nhp6b::HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY2381[a]	*MATα nhp6a::URA3 nhp6b::HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY2382[a]	*MAT***a** *nhp6a::URA3 nhp6b::HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY2389[a]	*MATα ade2 can1 his3 leu2 lys2 trp1 ura3*
DY2395[a]	*MAT***a** *ade2 can1 his3 leu2 lys2 trp1 ura3*
DY2499[a]	*MATα swi2::ADE2 sin4::TRP1 ade2 can1 leu2 trp1 ura3*
DY2763[a]	*MAT***a** *sin4::TRP1 ade2 can1 leu2 lys2 trp1 ura3*
DY2870[a]	*MAT***a** *swi2::ADE2 sin3::LEU2 ade2 can1 leu2 trp1 ura3*
DY3944[a]	*MATα swi2::ADE2 ade2 can1 leu2 lys2 trp1 ura3*
DY3658[a]	*MATα sin1::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY4548[a]	*MATα rpd3::LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5068[a]	*MATα hda1::URA3 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5116[a]	*MAT***a** *gcn5::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY5153[a]	*MAT***a** *nhp6a::URA3 nhp6b::HIS3 sin1::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY5155[a]	*MAT***a** *nhp6a::URA3 nhp6b::HIS3 sin4::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY5157[a]	*MAT***a** *nhp6a::URA3 nhp6b::HIS3 sin3::ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY5168[a]	*MATα gcn5::TRP1 hda1::URA3 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5170[a]	*MATα gcn5::TRP1 rpd3::LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5199[a]	*MATα gcn5::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5265[a]	*MAT***a** *gcn5::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5285[a]	*MAT***a** *sin3::ADE2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5289[a]	*MAT***a** *gcn5::TRP1 sin4::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY5294[a]	*MAT***a** *sin3::ADE2 sin4::LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5297[a]	*MAT***a** *gcn5::TRP1 sin3::ADE2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5299[a]	*MAT***a** *swi5::hisG-URA3-hisG sin3::ADE2 sin4::LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5306[a]	*MAT***a** *gcn5::TRP1 nhp6a::URA3 nhp6b::HIS3 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5315[a]	*MAT***a** *gcn5::TRP1 sin3::ADE2 sin4::LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5323[a]	*MAT***a** *sin1::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5326[a]	*MAT***a** *gcn5::hisG-URA3-hisG sin1::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5410[a]	*MAT***a** *swi5::LEU2 sin1::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5420[a]	*MAT***a** *swi2::HIS3 sin1::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5780[a]	*MAT***a** *swi6::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY5781[a]	*MATα swi6::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY5820[a]	*MATα sin4::LEU2 gcn5::TRP1 nhp6a::URA3 nhp6b::HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY5825[a]	*MAT***a** *gcn5::TRP1 nhp6a::URA3 nhp6b::HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY5907[a]	*MAT***a** *swi6::TRP1 sin4::URA3 ade2 can1 his3 leu2 trp1 ura3*
DY5908[a]	*MATα swi6::TRP1 sin4::URA3 ade2 can1 his3 leu2 trp1 ura3*
DY5909[a]	*MAT***a** *swi5::LEU2 swi6::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY5910[a]	*MATα swi5::LEU2 swi6::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY5911[a]	*MAT***a** *sin4::URA3 swi5::LEU2 swi6::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY5912[a]	*MATα sin4::URA3 swi5::LEU2 swi6::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY6103[a]	*MATα sin3::LEU2 swi6::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY881[b]	*MAT***a** *ade2 his3 leu2 lys2 trp1 ura3*
DY1712[b]	*MAT***a** *sin4::URA3 ade2 his3 leu2 lys2 trp1 ura3*
DY2532[b]	*MATα nhp6A::URA3 nhp6B::HIS3 ade2 his3 leu2 lys2 trp1 ura3*
DY2533[b]	*MATα nhp6A::URA3 nhp6B::HIS3 sin4::TRP1 ade2 his3 leu2 lys2 trp1 ura3*

[a] Isogenic in the W303 background (55).
[b] Isogenic in the S288C (YPH500) background (48).

were not isogenic and only a modest reduction in *HO* expression was seen in this study (47).

Mutations in *sin3* and *sin4* suppress *nhp6* and *gcn5* transcription defects. Isogenic yeast strains were constructed to test the ability of mutations in regulatory genes to suppress the *nhp6* defect in *HO* transcription. A *sin1* mutation does not suppress the defect in *HO* expression due to the absence of the Nhp6 protein (Fig. 2, lane 4). However, mutations in the *SIN3* or *SIN4* genes do permit *HO* expression in the *nhp6a nhp6b* mutant (Fig. 2, lanes 6 and 8).

As *sin3* and *sin4* mutations were effective in suppressing defects in *nhp6* mutants, we sought to determine whether *sin3* or *sin4* could suppress defects in other activators of *HO* expression, such as *GCN5* and *SWI5*. A *sin3* mutation suppresses the defects in *HO* expression caused by a *gcn5* mutation (Fig. 3, lane 6) or a *swi5* mutation (Fig. 3, lane 10) to 64 or 45%,

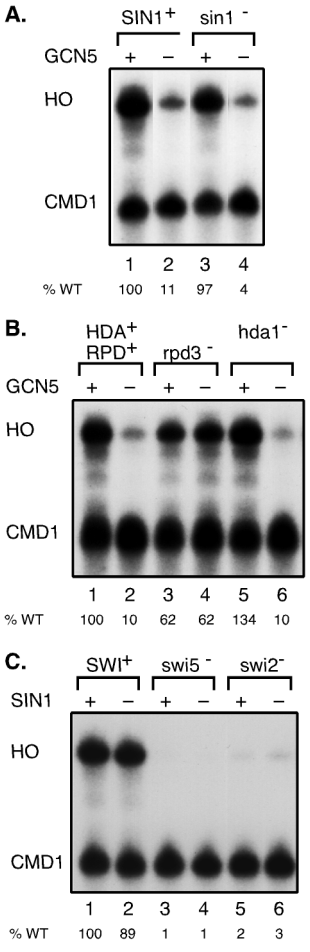

FIG. 1. *HO* expression is not altered by a *sin1* mutation. S1 nuclease protection assays were performed using probes specific for *HO* and *CMD1* (internal control). *HO* RNA levels were quantitated by phosphorimager, normalized by dividing by the value for *CMD1*, and expressed as a percentage of the wild-type (WT) value in lane 1 in each panel. RNAs were prepared from strains DY2395, DY5116, DY5323, and DY5326 (A), DY2389, DY5199, DY4548, DY5170, DY5068, and DY5168 (B), and DY150, DY5323, DY161, DY5410, DY2348, and DY5420 (C).

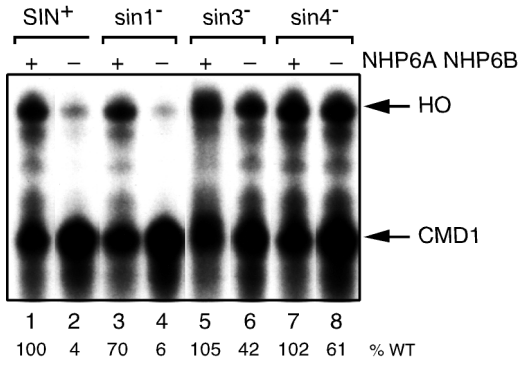

FIG. 2. The *nhp6* defect in *HO* transcription can be suppressed by *sin3* or *sin4* mutations. S1 nuclease protection assays were performed using probes specific for *HO* and *CMD1* (internal control). *HO* RNA levels were quantitated by phosphorimager, normalized by dividing by the value for *CMD1*, and expressed as a percentage of the wild-type (WT) value in lane 1. RNAs were prepared from strains DY150, DY2381, DY3658, DY5153, DY984, DY5157, DY1699, and DY5155.

respectively, of the wild-type level. Similar levels of suppression are seen in an *rpd3* mutant (data not shown). This last result is not surprising as mutations in *SIN3* and *RPD3* cause similar phenotypes (53) and the Sin3 protein physically interacts with the Rpd3 histone deacetylase (26, 27). A *sin4* mutation shows striking differences in the ability to suppress *gcn5* or *swi5* mutations for expression of *HO. HO* is not expressed in a *swi5 sin4* strain (Fig. 3, lane 11), despite the fact a *sin4* mutation does suppress the *swi5* defect when an *HO:lacZ* reporter is used (24) (see Discussion). In contrast, *HO* is expressed in a *gcn5 sin4* mutant at 104% of wild-type levels (Fig. 3, lane 7), and thus *sin4* is an effective *gcn5* suppressor. The combination of the *sin3* and *sin4* mutations is able to suppress either a *gcn5* mutation (Fig. 3, lane 8) or a *swi5* mutation (Fig. 3, lane 12). In summary, a *sin3* mutation is able to suppress both *swi5* and *gcn5* defects in *HO* expression, but a *sin4* mutation can suppress only *gcn5*. Thus, *sin3* and *sin4* suppress transcriptional defects by different mechanisms.

Analysis of suppression of *swi6* and *swi2* transcription defects by *sin3* and *sin4*. Based on the difference in suppression of a *swi5* mutation by *sin3* and *sin4*, we decided to examine suppression of mutations affecting other types of *HO* transcriptional activators. *SWI2* encodes part of the Swi/Snf chromatin remodeling complex, and *HO* is not expressed in a *swi2* mutant. We first examined *HO* expression in *swi2 sin3* and *swi2 sin4* mutants to look for suppression of the *swi2* transcriptional defect. The results in Fig. 4A show that neither *sin3* nor *sin4* can suppress the reduced *HO* expression caused by the *swi2* mutation. Thus, the requirement for the Swi/Snf chromatin remodeling complex cannot be suppressed by mutations in either *SIN3* or *SIN4*.

The SBF DNA binding factor binds to the *HO* promoter only after Swi/Snf and SAGA are recruited to the *HO* promoter (12). The *SWI6* gene encodes a subunit of SBF, and *HO* is not expressed in a *swi6* mutant (Fig. 4B, lanes 1 to 4). However, in the *swi6 sin4* double mutant, *HO* is expressed at nearly wild-type levels (Fig. 4B, lanes 5 and 6). Thus, *HO* can be expressed in a *sin4* mutant in the absence of SBF. Importantly, *HO* is not expressed in the *swi5 swi6 sin4* mutant (Fig. 4B, lanes 9 and 10). This suggests that Swi5, or a factor recruited in a *SWI5*-dependent manner such as Swi/Snf or SAGA, is still required for *HO* expression in the *sin4* mutant. Finally, *HO* is not expressed in a *swi6 sin3* double mutant, and

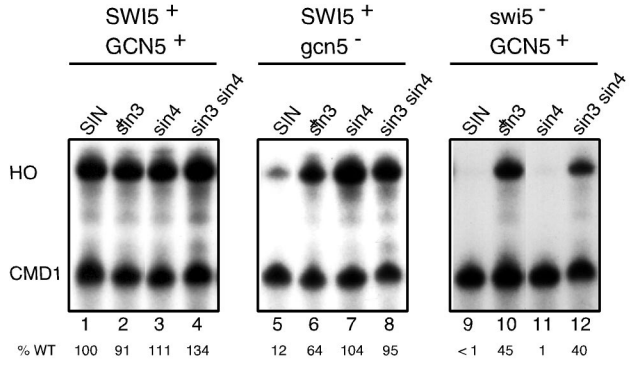

FIG. 3. Both *sin3* and *sin4* mutations suppress the *gcn5* defect. S1 nuclease protection assays were performed using probes specific for *HO* and *CMD1* (internal control). *HO* RNA levels were quantitated by phosphorimager, normalized by dividing by the value for *CMD1*, and expressed as a percentage of the wild-type (WT) value in lane 1. RNAs were prepared from strains DY150, DY5285, DY2763, DY5294, DY5265, DY5297, DY5289, DY5315, DY411, DY775, DY2133, and DY5299.

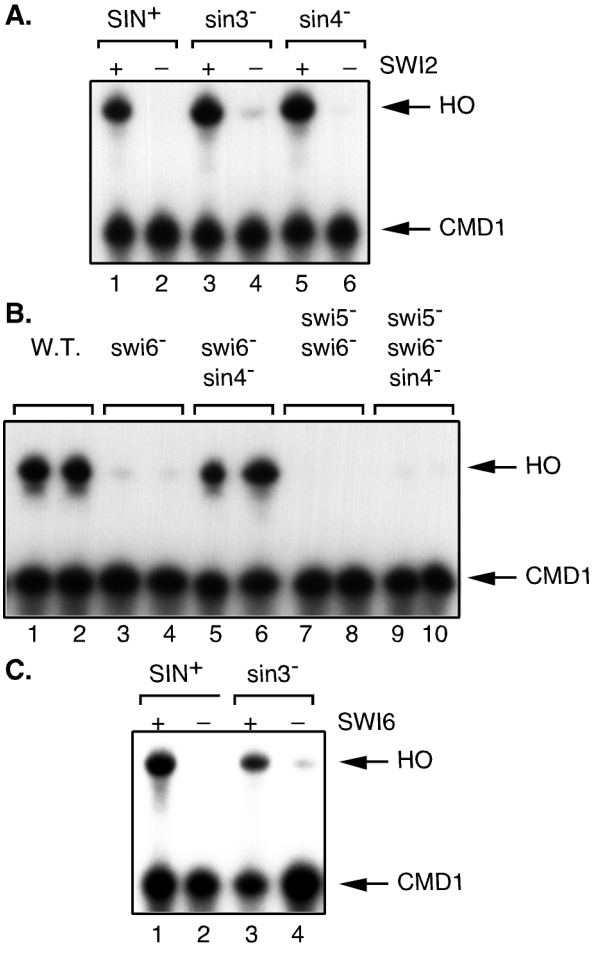

FIG. 4. A *sin4* mutation suppresses *swi6* but not *swi2*. S1 nuclease protection assays were performed using probes specific for *HO* and *CMD1* (internal control). (A) *HO* is not expressed in *swi2 sin3* or *swi2 sin4* strains. RNAs were prepared from strains DY150, DY3944, DY773, DY2870, DY1702, and DY2499. (B) *HO* is expressed in *swi6 sin4* strains. RNAs were prepared from strains DY150, DY151, DY5780, DY5781, DY5907, DY5908, DY5909, DY5910, DY5911, and DY5912. (C) *HO* is not expressed in a *swi6 sin3* strains. RNAs were prepared from strains DY150, DY5780, DY773, and DY6103.

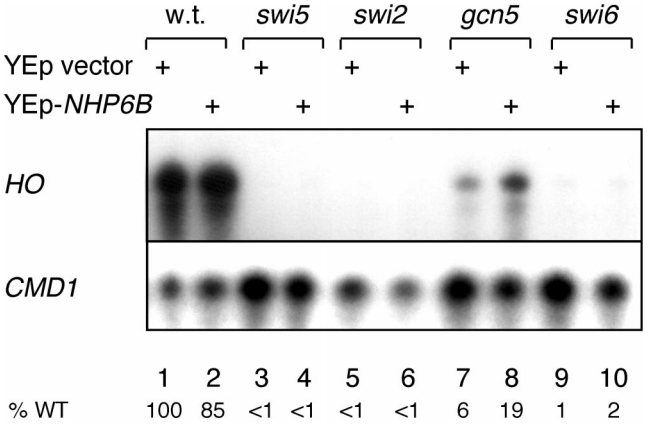

FIG. 5. Nhp6b overexpression partially suppresses the *gcn5* defect. Strains DY150 (wild type [w.t.]), DY161 (*swi5*), DY2348 (*swi2*), DY5116 (*gcn5*), and DY5780 (*swi6*) were transformed with either the YEplac195 vector or M1195, a YEp-NHP6B plasmid. S1 nuclease protection assays were performed using probes specific for *HO* and *CMD1* (internal control), using RNA prepared from strains grown under selective conditions to maintain the plasmid. The upper panel was exposed to film for 8 h; the lower panel was exposed for 24 h.

thus a *sin3* mutation does not permit *HO* transcription without SBF (Fig. 4C). Thus there is a striking difference in the ability of *sin3* and *sin4* mutations to suppress activator mutations. A *sin4* mutation allows *HO* to be expressed in the absence of the SBF factor, while a *sin3* mutation does not suppress. The pattern of suppression of a *swi5* mutation (Fig. 3) is just the opposite, with a *sin3* mutation suppressing but not *sin4*.

Suppression of *gcn5* by Nhp6b overexpression. We determined whether overexpression of Nhp6b could suppress *HO* transcriptional defects caused by mutations in transcriptional activators. A YEp multicopy plasmid with the *NHP6B* gene was transformed into various strains. An S1 nuclease protection assay shows that overexpression of Nhp6b does not suppress *swi5*, *swi2*, or *swi6* null mutations (Fig. 5). However, Nhp6b overexpression partially suppresses the reduced *HO* expression caused by a *gcn5* mutation (lanes 7 and 8). In the *gcn5* mutant, *HO* is expressed at 6% of the wild-type level, and YEp-*NHP6B* causes a threefold increase in *HO* expression.

Genetic interactions based on growth phenotypes. As the *sin3* and *sin4* mutations suppress the defect in *HO* transcription caused by the lack of the Nhp6 protein, we investigated whether these mutations would also suppress other *nhp6a nhp6b* phenotypes. The *nhp6a nhp6b* double mutant displays a number of phenotypes, including temperature-sensitive growth (13) and inability to grow on galactose medium (Fig. 6). Interestingly, we observed this galactose growth defect for *nhp6a nhp6b* double mutants only in the S288C background, not in W303 strains. The *sin3* mutation was unable to suppress any of the *nhp6* defects; in fact, the *nhp6a nhp6b sin3* triple mutant grows much more slowly than either the *nhp6a nhp6b* or *sin3* mutant strains. We were unable to demonstrate suppression of the 37°C growth defect, as the *sin4* single mutant is also temperature sensitive for growth (24). However, a *sin4* mutation is able to suppress one of the *nhp6* phenotypes. The *nhp6a nhp6b sin4* triple mutant can grow on galactose, whereas the *nhp6a nhp6b* double mutant cannot (Fig. 6). This suggests that the suppression of *nhp6* by *sin4* may be more general and not limited to *HO* transcription.

Combining two mutations can sometimes cause a severe additive phenotype, suggesting that these two genes affect the same function but from different pathways (19). For example, Roberts and Winston (44) found that combining a *gcn5* muta-

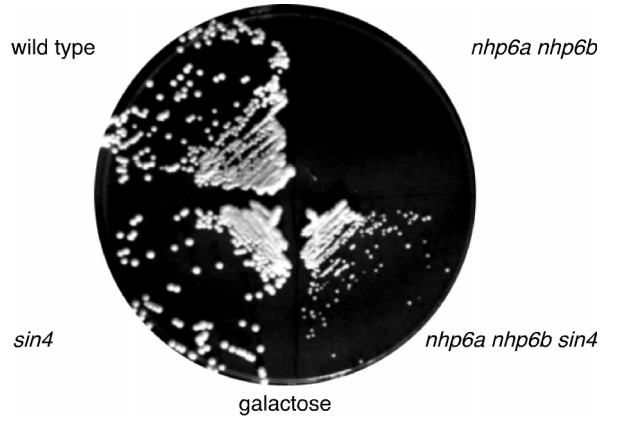

FIG. 6. A *sin4* mutation suppresses the *nhp6* growth defect on galactose. Strains DY881, DY1712, DY2532, and DY2533 were plated on YEP-galactose medium and grown for 4 days at 30°C.

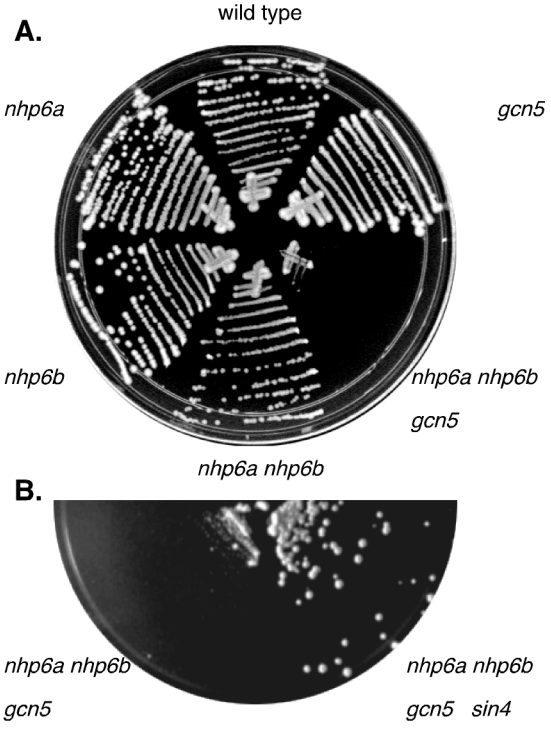

FIG. 7. The severe growth defect of a *gcn5 nhp6a nhp6b* triple mutant is suppressed by a *sin4* mutation. (A) Strains DY150, DY2378, DY2380, DY2382, DY5116, and DY5306 were plated on YEPD medium and grown for 3 days at 30°C. (B) Strains DY5825 and DY5820 were plated on YEPD medium and grown for 5 days at 30°C.

tion with a mutation in the Swi/Snf chromatin remodeling complex causes a severe growth defect. They suggested that either the SAGA histone acetyltransferase complex (which contains Gcn5) or the Swi/Snf chromatin remodeling factor can supply certain critical functions for gene activation, but that the absence of both activities is manifested as the growth defect. We constructed *gcn5 nhp6a nhp6b* triple mutant strains and found that these strains grew extremely poorly (Fig. 7A). To test whether a *sin4* mutation could suppress this growth defect, we crossed a *gcn5 nhp6a* strain to a *nhp6a nhp6b sin4* strain and examined the phenotype of haploid progeny. The experiment in Fig. 7B show that the *gcn5 nhp6a nhp6b sin4* quadruple mutant strain grows much better than the *gcn5 nhp6a nhp6b* triple mutant. Thus, the effect of a *sin4* mutation is not limited to allowing *HO* expression in *gcn5* or *nhp6* mutants. *SIN4* has global effects on transcription, as a *sin4* mutation overcomes the marked growth defects in a *gcn5 nhp6a nhp6b* strain.

DISCUSSION

The promoter of the yeast *HO* gene is large and complex, and genetic analysis has shown that chromatin structure plays an important role in transcriptional regulation of this gene. Through studies of *HO* regulation, we have identified common features between the *NHP6A* and *NHP6B* genes, which encode architectural transcription factors, and *GCN5*, which encodes a hitone acetyltransferase subunit of the SAGA complex. *HO* expression is reduced in either an *nhp6a nhp6b* double mutant or a *gcn5* mutant. Moreover, these mutants show similar suppression patterns, with either a *sin3* or a *sin4* mutation restoring *HO* expression despite mutations in transcriptional activators. We found that a *nhp6a nhp6b gcn5* triple mutant grows extremely slowly. One interpretation of this result is that Nhp6 and Gcn5 may provide two distinct mechanisms for transcriptional activation of certain important genes. Disruption of the *SIN4* gene suppresses this defect, and thus the *nhp6a nhp6b gcn5 sin4* quadruple mutant grows reasonably well. A *sin4* mutation also suppresses galactose growth defects of a *nhp6a nhp6b* mutant.

How do *sin3* and *sin4* mutations suppress transcriptional defects caused by the absence of Gcn5 or Nhp6? To investigate this further, we determined whether *sin3* or *sin4* can suppress other mutations in other activators required for *HO* expression (Table 2). Cosma et al. (12) used chromatin immunoprecipitation to examine transcription factor binding to the *HO* promoter, and they showed that factors bind sequentially. Their model is that Swi5 enters the nucleus in late anaphase, binds to the promoter, and recruits Swi/Snf. Swi/Snf, in turn, recruits SAGA, and SAGA is required for SBF binding. It is suggested that SBF is responsible for recruiting general transcription factors to the promoter (12). (The term "recruit" means brings to the promoter and does not necessarily imply a direct physical interaction.) Krebs et al. (30) showed that histone acetylation of a 1-kb region of the *HO* promoter occurs in late G_1 phase, and this histone acetylation is dependent on Swi5, Swi/Snf, and the Gcn5 component of SAGA. Importantly, inactivation of the Sin3/Rpd3 histone deacetylase complex causes the promoter to be constitutively acetylated. In light of these findings, we explain our results on suppression by *sin3* mutations by suggesting that SBF binds poorly to *HO* when it is deacetylated, and that either the *sin3* mutation or activity of the Gcn5 histone acetyltransferase results in histone acetylation that permits SBF binding. This model explains why a *sin3* mutation is able to suppress *swi5* and *gcn5* mutations (Table 2). The failure of a *sin3* mutation to suppress the *swi6* defect in *HO* transcription is also consistent with this model. Why then is *HO* not expressed in a *swi2 sin3* double mutant? We suggest that Swi/Snf has multiple roles in activation of *HO* expression, with only one being to recruit SAGA. By this model, the second role of Swi/Snf, revealed in the *swi5 sin3* mutant, is to assist the TATA-binding protein (TBP), or possibly SBF, to bind the *HO* promoter. We suggest that Swi/Snf need not be stably bound to the *HO* promoter to assist TBP to bind. Thus, Swi/Snf is still required for *HO* activation in a *swi5* mutant although it may not be stably bound to the promoter.

Suppression of *HO* activation defects by a *sin4* mutation is quite different (Table 2). The Sin4 protein is a component of the RNA polymerase II mediator complex (32), and thus it is possible that the *sin4* mutation relaxes the RNA polymerase holoenzyme's specificity, allowing it to activate in the absence of certain factors such as SBF. RNA polymerase binding and transcriptional initiation at *HO* normally require both SBF and Swi/Snf, and a *sin4* mutation could allow polymerase to start in the absence of SBF. According to this scenario, the mediator

TABLE 2. Suppression of mutations in activators of *HO* transcription by *sin3* and *sin4*

Genotype	Function	*SIN*$^+$	*sin3* (deacetylase)	*sin4* (mediator, SAGA)
SWI$^+$		+	+	+
swi5	DNA-binding factor	−	+	−
swi2	Swi/Snf complex	−	−	−
gcn5	SAGA	−	+	+
nhp6ab	Architectural transcription factor	−	+	+
swi6	SBF DNA-binding factor	−	−	+
swi5 swi6		−	ND	−

[a] ND, not determined.

TABLE 3. Differences in expression of an integrated *HO:lacZ* reporter and the native *HO* gene

Genotype	% Expression	
	HO:lacZ reporter	Native *HO* gene
SWI5	100	100
swi5	1	1
swi5 sin3	30	45
swi5 sin4	150	1

[a] Values for the integrated *HO:lacZ* reporter are taken from Stillman et al. (53); values for the native *HO* gene are taken from Fig. 3.

part of RNA polymerase functions as an "activator checkpoint," verifying that an activator is at the promoter before RNA polymerase can initiate transcription.

An alternative model for Sin4 function is possible based upon the recent observation that Sin4 is also present in the SAGA complex (Grant and Workman, personal communication). Genetic analysis clearly shows that the SAGA complex has additional roles besides the Gcn5 histone acetyltransferase complex; one of these functions, mediated by the Spt3 and Spt8 proteins, may be to inhibit DNA binding by TBP (2, 52). The model most consistent with the data suggests that in a *sin4* mutant the activity of SAGA is altered, with the *sin4*-mutant SAGA not inhibiting, and thus stimulating, TBP binding. In the wild type, TBP binding requires SBF and Swi/Snf; in the *sin4* mutant, TBP binding occurs in the absence of SBF. This model fits the data nicely as *HO* can be activated in the absence of SBF in a *sin4* mutant. Similarly, a *sin4* mutation allows *HO* to be expressed in the absence of Gcn5, normally required for SBF binding. *HO* is not expressed in a *swi2 sin4* double mutant because Swi/Snf is still required, probably to promote binding by TBP.

We first examined the role of Nhp6 in *HO* expression based on the hypothesis that architectural transcription factors might be involved in bridging the two Swi5 molecules bound at distant sites (34). While we have shown that the Nhp6 protein is required for *HO* activation, at present we have no evidence that it mediates this long-range interaction in vivo. Instead, our data suggest that Nhp6 functions with the Gcn5 histone acetyltransferase. The *nhp6a nhp6b* mutant shows the same suppression pattern as the *gcn5* strain (Table 2), and thus Nhp6 may work through SAGA. The Nhp6 protein could assist in the recruitment of SAGA to the *HO* promoter, possibly by stabilizing binding by SAGA. Alternatively, Nhp6 could act downstream of SAGA, by establishing a chromatin structure that facilitates activities of SAGA, or by assisting in DNA binding by SBF. Overexpression of Nhp6 allows *HO* expression in the absence of Gcn5 (Fig. 5) and suppresses the reduced *HO:lacZ* expression caused by mutations within the ankyrin repeat region of Swi6 (47). Increased levels of Nhp6 do not suppress *swi6* null mutations, however. In contrast, the fact that the *nhp6a nhp6b gcn5* mutant grows very slowly suggests that Nhp6 and Gcn5 have independent functions. How does a *sin4* mutation suppress the growth defect in the *nhp6a nhp6b gcn5* mutant? Further work will be needed to determine whether the absence of Sin4 from the holoenzyme or from SAGA is responsible for suppression of this growth defect. Finally, while we believe that the Nhp6 and Sin4 have direct effects on *HO* expression, it remains possible that there are indirect effects caused by these mutations altering expression of other genes.

Differences between native *HO* and an *HO:lacZ* reporter. The *sin3* and *sin4* mutations were identified as suppressor mutations that allow an *HO:lacZ* reporter to be expressed in the absence of the *SWI5* transcriptional activator. As shown in Table 3, a *swi5* mutation reduces expression of the *HO:lacZ* reporter 100-fold. A mutation in either *SIN3* or *SIN4* restores expression of *HO:lacZ*, although to different extents. We have found that the regulation of the *HO:lacZ* reporter can be strikingly different from that of the native *HO* gene. This *HO:lacZ* reporter is integrated at the *HO* locus on chromosome IV, with the entire flanking *HO* promoter sequences present. *HO* is expressed in a *swi5 sin3* double mutant strain, and thus *SIN3* is a bona fide *swi5* suppressor. However, a *sin4* mutation does not overcome the defect in *HO* expression due to the mutation in the *SWI5* transcription factor. This inability to allow *HO* expression in a *swi5* mutant was described before for the *sdi3-1* allele of *sin4* (38).

Are there other differences between *HO:lacZ* and *HO* in terms of regulatory properties? Although *sin1* mutations do suppress the defect in *HO:lacZ* expression due to the absence of the Swi5 or Swi2 transcriptional activators (data not shown) (31, 41, 43), the same result is not observed when native *HO* mRNA is measured. The difference between the effects of a *sin1* mutation on *HO* versus *HO:lacZ* regulation may reflect unique properties of *HO*, as a *sin1* mutation has marked effects on regulation of *SUC2*, *INO1* and *SSA3* (1, 42, 43).

A *pho2* mutation reduces expression of an *HO:lacZ* reporter (6) but has no effect on expression of the native *HO* gene (34). Zhu et al. (60) reported that an *hpr1* mutation reduced expression of an *HO:lacZ* reporter. However, we have determined that expression of the native *HO* gene is not affected by an *hpr1* mutation (data not shown). Chávez and Aguilera (10) have shown that an *hpr1* mutation has different effects on *lacZ* reporters and native genes, and that these effects are transcriptional and not translational.

It is possible that there are sequences present within the bacterial *lacZ* gene that act in *cis* to affect regulation of the *HO* promoter, and that these effects become apparent in a *sin4* mutant. Supporting this idea of *cis* effects from within *lacZ*, W. Hörz (personal communication) has shown that a *sin4* mutation affects expression of a *PHO5-lacZ* reporter, but a *sin4* mutation does not cause derepression of the native *PHO5* gene. Additionally, the fact that a *sin4* mutation derepresses *PHO5* transplaced into the *URA3* locus, but not the native *PHO5* locus, suggests that effects of a *sin4* mutation can be influenced by the chromosomal context (21). Finally, the concept of *cis*-acting effects of *lacZ* sequences affecting transcriptional regulation is supported by the work of Chávez and Aguilera (10) showing that an *hpr1* mutation affects native genes and *lacZ* reporters differently.

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