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Role for *ADA*/*GCN5* Products in Antagonizing Chromatin-Mediated Transcriptional Repression

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The *Saccharomyces cerevisiae* SWI/SNF complex is a 2-MDa multimeric assembly that facilitates transcriptional enhancement by antagonizing chromatin-mediated transcriptional repression. We show here that mutations in *ADA2*, *ADA3*, and *GCN5*, which are believed to encode subunits of a nuclear histone acetyltransferase complex, cause phenotypes strikingly similar to that of *swi*/*snf* mutants. *ADA2*, *ADA3*, and *GCN5* are required for full expression of all *SWI*/*SNF*-dependent genes tested, including *HO*, *SUC2*, *INO1*, and Ty elements. Furthermore, mutations in the *SIN1* gene, which encodes a nonhistone chromatin component, or mutations in histone H3 or H4 partially alleviate the transcriptional defects caused by *ada*/*gcn5* or *swi*/*snf* mutations. We also find that *ada2 swi1*, *ada3 swi1*, and *gcn5 swi1* double mutants are inviable and that mutations in *SIN1* allow viability of these double mutants. We have partially purified three chromatographically distinct *GCN5*-dependent acetyltransferase activities, and we show that these enzymes can acetylate both histones and Sin1p. We propose a model in which the ADA/GCN5 and SWI/SNF complexes facilitate activator function by acting in concert to disrupt or modify chromatin structure.

The regulation of eukaryotic transcription requires that transcription factors gain access to DNA that is assembled into chromatin. Both in vivo and in vitro studies indicate that the nucleosome core particle, the basic building block of chromatin, represses transcription by competing with transcription factors for DNA binding sites (reviewed in reference 45). How transcription factors contend with the inhibitory effects of chromatin structure remains a central question in understanding gene regulation. Transcriptional activators require both DNA binding and activation domains for their function, and it has been suggested that activation domains might interact with histones to initiate the process of nucleosome disruption (66). Consistent with this view, the strength of an activation domain determines not only the amount of transcriptional activity but also the degree of chromatin disruption (50). A likely target for activator interaction with histone proteins is the highly charged N-terminal domains. These domains play only a minor role in nucleosome structure and stability (reviewed in reference 18); however, they are required for assembly of higher-order chromatin structures (1, 3, 19) and for interactions of nucleosomes with nonhistone proteins (17, 28). The N-terminal domains are also sites for a variety of posttranslational modifications (acetylation, methylation, phosphorylation, and ADP-ribosylation), and the extent of modification often correlates with transitions from transcriptionally inactive to active chromatin (reviewed in reference 22).

Gene-specific activators may also recruit accessory factors that facilitate transcriptional enhancement specifically on chromatin templates. One candidate for a chromatin-disrupting factor is the highly conserved SWI/SNF complex (reviewed in reference 49). The *Saccharomyces cerevisiae* SWI/SNF complex is comprised of 11 different subunits and has an apparent molecular mass of 2 MDa. An intact SWI/SNF complex is required for the function of a variety of gene-specific transcriptional activators and for transcriptional induction of at least 10 genes in yeast. Several observations have led to the suggestion that the SWI/SNF complex facilitates activator function by antagonizing chromatin-mediated repression. For instance, inactivation of the *SIN1* gene, which encodes an HMG1-like protein (37), or mutations in genes that encode histones (30, 36, 52) partially alleviate the transcriptional defects associated with *swi*/*snf* mutations. The SWI/SNF complex is also required for changes in chromatin structure that accompany induction of *SUC2* transcription in vivo (30). Biochemical analyses of purified yeast and human SWI/SNF complexes have shown that these complexes use the energy of ATP hydrolysis to disrupt nucleosome structure and facilitate transcription factor binding to nucleosomal sites (15, 32, 39).

An additional accessory factor that has been implicated in activator function is the putative Ada3p/Ada2p/Gcn5p complex. The *GCN5* gene was initially identified in a genetic screen designed to isolate mutants unable to grow under conditions of amino acid limitation (21). *GCN5*, *ADA2*, and *ADA3* were also identified from yeast mutations that alleviated the toxic effects due to overexpression of the GAL4-VP16 activator (5, 40, 51). These three gene products are required for the function of the GCN4 activator as well as several synthetic LexA-activator fusions (5, 21, 40, 51). In vivo and in vitro studies have indicated that Ada2p, Ada3p, and Gcn5p are subunits of a protein complex that directly interacts with transcriptional activation domains (4, 14, 29, 31, 40, 41, 57). These studies led to the hypothesis that *ADA2*, *ADA3*, and *GCN5* function as transcriptional coactivators that might bridge transcriptional activation domains with components of the general transcription machinery. Recently, Allis and colleagues have shown that the *GCN5* gene encodes a histone acetyltransferase that acetylates several lysine residues in the flexible N-terminal domains of histone H3 and H4 in vitro (10, 38). These results have led to the hypothesis that the ADA/GCN5 complex facilitates transcription by a targeted disruption of chromatin structure.

We present here genetic and biochemical evidence that further links ADA/GCN5 activity to chromatin structure and

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function. We have found that *ADA3*, *ADA2*, and *GCN5* are identical to *SWI7*, *SWI8*, and *SWI9*, three genes which were originally identified as positive regulators of the *HO* gene (8). These subunits of the ADA/GCN5 complex are also required for expression of other *SWI/SNF*-dependent genes, i.e., *SUC2*, *INO1*, and Ty elements. Similarly, genes that do not require SWI/SNF action, such as *LYS2*, *ADH1*, *PHO5*, and *CLN3*, also do not require the ADA/GCN5 complex. *ADA2*, *ADA3*, and *GCN5* do not encode stoichiometric subunits of the SWI/SNF complex, but the *ADA*/*GCN5* and *SWI*/*SNF* genes show similar genetic interactions with chromatin components. We have partially purified three chromatographically distinct *GCN5*-dependent acetyltransferase complexes and show that these activities can acetylate histones as well as the HMG1-like protein Sin1p. We propose that the ADA/GCN5 and SWI/SNF complexes may function in concert to antagonize histone- and non-histone-mediated transcriptional repression.

MATERIALS AND METHODS

Media, strains, and plasmids. Cultures were grown at 30°C in YEPD (2% yeast extract, 1% Bacto Peptone, 2% glucose) unless otherwise specified. S (minimal) medium (Difco Laboratories) contains 6.7 g of yeast nitrogen base per liter without amino acids and is supplemented with required amino acids. The strains used are described in Table 1.

Yeast transformations were performed by the lithium acetate method described previously (20). Other general yeast manipulations were performed as described previously (23). Plasmids expressing GAL4-VP16 (pGAL4VP16), ADA3 (pADA3), ADA2 (pNS3.8), and GCN5 (pRS316GCN5) and knockout plasmids pADA3KO, pADA2KO, and pGCN5KO have been previously described (6, 40, 51). Strains CY561, CY562, CY563, CY564, CY565, CY566, CY569, and CY578 were constructed with the corresponding knockout plasmids as previously described (5, 40).

Preparation of extracts, gel filtration, and Western blot analysis. Crude whole-cell extracts were prepared and fractionated on a fast protein liquid chromatography (FPLC) Superose 6 gel filtration column, and fractions were analyzed by immunoblotting as described previously (47). Immunoblots were probed with affinity-purified rabbit polyclonal anti-Swi3p antibody, followed by goat anti-rabbit secondary antibody coupled to horseradish peroxidase (Boehringer), and developed with a chemiluminescent substrate (Amersham).

Cloning and sequencing of *SWI7*. The *SWI7* gene was isolated on a YCp50-based plasmid by complementation of the defect in *HO-lacZ* expression in strain CY435. The *swi7* mutant strain was transformed with a genomic library in a single-copy ARS-CEN vector which contained a *URA3* marker (54). Ten transformants were blue in plate β-galactosidase assays (2) and thus express the *HO-lacZ* fusion gene. One plasmid that contained an 8-kb genomic insert which complemented the defect in *HO-lacZ* expression upon retransformation was isolated. The insert was subcloned into smaller fragments to yield a 4-kb *Hin*dIII fragment as a minimally complementing clone. This 4-kb fragment was cloned into the integration vector, Yip5, and integrated at the *swi7* locus by cleavage within the *Hin*dIII fragment and transformation into the *swi7* mutant strain, CY435, with selection for Ura^+ cells. Ura^+ integrants were backcrossed to CY432 (*SWI7*$^+$), and tetrad analysis indicated linkage to *SWI7*. Of 16 tetrads analyzed, *URA3* segregated 2:2, and none of the segregants demonstrated a defect for *HO-lacZ* expression. The *Hin*dIII fragment was subcloned into M13mp18 and sequenced by the Sanger method with Sequenase (U.S. Biochemicals). The sequence obtained was analyzed by a BLAST search, which demonstrated the sequence to be identical to that of the *ADA3* gene.

Purification of SWI/SNF and GCN5-dependent acetyltransferase activities. Whole-cell extracts were prepared from derivatives of strains CY448 (wild type), CY449 (*ada2*Δ), CY450 (*ada3*Δ), and CY451 (*gcn5*Δ), which contained a *SWI2-HA-6HIS* fusion gene integrated at the *URA3* locus. The SWI/SNF complex was partially purified from whole-cell extracts essentially as described previously (15). Following the FPLC Mono Q step, fractions were pooled and the SWI/SNF complex was immunoprecipitated with monoclonal antibody 12CA5. Precipitates were then resuspended in 20 μl of sodium dodecyl sulfate (SDS) loading buffer and separated on 10% Laemmli gels. SWI/SNF polypeptides were detected by silver staining.

For purification of GCN5-dependent acetyltransferase activities, whole-cell extracts were prepared from 20-liter cultures of strain CY661 (wild type) or CY663 (*gcn5*Δ). Extracts were fractionated on Ni^{2+}-nitrilotriacetic acid (Ni^{2+}-NTA) agarose, native DNA-cellulose, and FPLC Mono Q as described previously for purification of the SWI/SNF complex (53).

β-Galactosidase assays and invertase assays. Liquid β-galactosidase assays were performed with cells grown to mid-log phase as previously described (59). For invertase assays, cultures were grown to mid-log phase in YEPD and then derepressed for *SUC2* transcription by washing the cells in water and growing them for 2 to 3 h in YEP containing 0.05% glucose. Invertase assays were performed as described previously (13). Invertase units were calculated as micromoles of glucose per minute per milligram (dry weight) of cells. Assays were done in triplicate; results were averaged, and error values were within 20% of the mean.

TABLE 1. Strains

Strain	Relevant genotype	Comment and/or source
CY150	*SWI*$^+$ *SIN*$^+$ *ho::HO-lacZ*	Isogenic to YPH274 (P. Hieter)
CY231	*swi1*Δ*::LEU2 sin1*Δ*::TRP1 ho::HO-lacZ*	Congenic to CY232
CY232	*SWI*$^+$ *SIN*$^+$ *ho::HO-lacZ*	I. Herskowitz
CY237	*sin1*Δ*::TRP1 ho::HO-lacZ*	Congenic to CY232
CY240	*swi1*Δ*::LEU2 ho::HO-lacZ*	Congenic to CY232
CY373	*swi1*Δ*::LEU2 sin1-2 ho::HO-lacZ*	W. Kruger
CY432	*SWI*$^+$ *ho::HO-lacZ*	K. Nasmyth
CY433	*swi10-28(snf5) ho::HO-lacZ*	K. Nasmyth
CY434	*swi9-289 ho::HO-lacZ*	K. Nasmyth
CY435	*swi7-288 ho::HO-lacZ*	K. Nasmyth
CY436	*swi8-299 ho::HO-lacZ*	K. Nasmyth
CY448	*ADA*$^+$ *GCN*$^+$	L. Guarente
CY449	*ada2*Δ	L. Guarente
CY450	*ada3*Δ	L. Guarente
CY451	*gcn5*Δ	L. Guarente
CY561	*ada2*Δ*::URA3-hisG*	Isogenic to CY432
CY562	*ada3*Δ*::URA3-hisG*	Isogenic to CY432
CY563	*gcn5*Δ*::URA3-hisG*	Isogenic to CY432
CY564	*ada2*Δ*::URA3-hisG*	Isogenic to CY232
CY565	*ada3*Δ*::URA3-hisG*	Isogenic to CY232
CY566	*gcn5*Δ*::URA3-hisG*	Isogenic to CY232
CY569	*gcn5*Δ*::URA3-hisG*	Isogenic to CY150
CY571	*SWI*$^+$ *SIN*$^+$ *ADA*$^+$ *ho::HO-lacZ*	Segregant from CY231 × CY565
CY572	*swi1*Δ*::LEU2 ho::HO-lacZ*	Segregant from CY231 × CY565
CY573	*swi1*Δ*::LEU2 sin1*Δ*::TRP1 ho::HO-lacZ*	Segregant from CY231 × CY565
CY574	*ada3*Δ*::URA3 ho::HO-lacZ*	Segregant from CY231 × CY565
CY575	*ada3*Δ*::URA3 sin1*Δ*::TRP1 ho::HO-lacZ*	Segregant from CY231 × CY565
CY576	*swi1*Δ*::LEU2 ada3*Δ*::URA3 sin1*Δ*::TRP1 ho::HO-lacZ*	Segregant from CY231 × CY565
CY578	CY373 × CY232 *ada3*Δ*::URA3-hisG*	Diploid parent
CY579	*SWI*$^+$ *SIN*$^+$ *ADA*$^+$ *ho::HO-lacZ*	Segregant from CY578
CY580	*swi1*Δ*::LEU2 ho::HO-lacZ*	Segregant from CY578
CY581	*swi1*Δ*::LEU2 sin1-2 ho::HO-lacZ*	Segregant from CY578
CY582	*ada3*Δ*::URA3 ho::HO-lacZ*	Segregant from CY578
CY583	*ada3*Δ*::URA3 sin1-2 ho::HO-lacZ*	Segregant from CY578
CY584	*swi1*Δ*::LEU2 ada3*Δ*::URA3 sin1-2 ho::HO-lacZ*	Segregant from CY578
CY661	*ura3::SWI2-HA-6HIS-URA3*	Isogenic to CY150
CY663	*ura3::SWI2-HA-6HIS-URA3*	Isogenic to CY569

RNA analysis. Strains were grown to mid-log phase in the media indicated in the legend to Fig. 2. Total yeast RNA was isolated (48), and 10 to 20 μg of RNA was analyzed by primer extension (37). The DNA primers used were for *INO1* (36), *LYS2* (5′-GCTCGACTCCATCGACATTGC-3′), *ADH1* (46), *CLN3* (46), *PHO5* (5′-CCTAAGGGAATGGTACCTGC-3′), and Ty elements 1 and 2 (5′-TCCTTAGAAGTAACCGAAGC-3′).

Acetylation assays. Liquid acetylation assays were performed with 30-μl reaction mixtures in buffer containing 1 μg of chicken histones (free or assembled into arrays) or purified recombinant glutathione *S*-transferase (GST) protein, 50 mM Tris, 5% glycerol, 0.1 mM EDTA, 50 mM KCl, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride), 10 mM sodium butyrate, and 0.25 μCi of [^{3}H]acetyl coenzyme A. Histones were purified to apparent homogeneity from chicken erythrocytes as described previously (26). Reconstitutions of histone octamers onto 208-12 array templates were performed by salt gradient dialysis with a ratio of histone octamer to 5S rRNA repeat of 1.0 (26). Reactions were initiated with addition of enzyme sample, the mixtures were incubated at 30°C, and then the reactions were stopped by spotting the mixtures onto Whatman P81 cellulose membranes. Membranes were washed in 50 mM carbonate buffer (pH

9.2) three times, washed briefly in acetone, and then dried before counting of radioactivity on the filters in scintillation fluid.

In autoradiographed gels, the reaction in half of an acetylation assay mixture was stopped with addition of SDS sample buffer, and the mixture was then loaded onto 18% polyacrylamide gels. The gels were then fixed in 25% isopropanol–10% acetic acid, followed by incubation in AMPLIFY (Amersham) or ENHANCE (NEN) for 30 min before drying and exposure to film.

Determination of specific activities. Time course acetylation assays were performed to evaluate linearity of [^{3}H]acetate incorporation (data not shown). It was found that at 5 min each of the pools had linear incorporation, and by 30 min incorporation of [^{3}H]acetate was nonlinear. When GST-SIN1 was used as a substrate, incorporation remained linear even after 30 min. Bradford analyses were performed to determine protein concentrations in peak fractions. Five-minute assays were performed in triplicate with two concentrations of fractions to determine specific activities.

Purification of fusion proteins. Recombinant GST and GST-SIN1 proteins were purified from *Escherichia coli* as previously described (48). Briefly, 4 to 6 liters of *E. coli* containing either pGEX or pGEX-SIN1 was grown to an optical density at 600 nm of 0.6, and then expression of protein was induced with 0.5 mM (final concentration) IPTG (isopropyl-β-D-thiogalactopyranoside) for 4 h. Cells were harvested, lysed in phosphate-buffered saline containing 1% Triton X-100 and protease inhibitors, and then clarified. GST fusion proteins were purified with 1-ml glutathione-agarose columns.

RESULTS

SWI7 is required for stability or assembly of the SWI/SNF complex. The purified SWI/SNF complex contains a total of 11 polypeptides (12, 15, 62). Genetic studies have yielded the genes that encode six of these subunits (*SWI1*, *SWI2*/*SNF2*, *SWI3*, *SNF5*, *SNF6*, and *SWP73*), and a two-hybrid screen identified the gene for a seventh subunit, *SNF11* (11, 42, 44, 59, 62). In an attempt to identify the genes for the remaining SWI/SNF subunits, we searched the literature for mutants that had phenotypes consistent with a defect in the SWI/SNF complex. The *SWI7*, *SWI8*, and *SWI9* genes were previously identified from mutations that decreased expression of the *SWI*/*SNF*-dependent gene *HO* (8). These mutants also show growth defects that would be consistent with mutations that inactivate the SWI/SNF complex (43a, 51a); thus, we initiated a further characterization of these mutants.

We showed previously that *swi*/*snf* deletion mutations alter the elution of the remaining subunits from a gel filtration column (47). We used this SWI/SNF assembly assay to determine if *swi7*, *swi8*, or *swi9* mutations alter assembly or stability of the SWI/SNF complex (Fig. 1). Whole-cell extracts were prepared from congenic *SWI*$^+$, *swi7*, *swi8*, and *swi9* strains, and these extracts were fractionated on an FPLC Superose 6 gel filtration column. In extracts prepared from *SWI*$^+$ cells, the Swi3p subunit elutes in a peak centered on fraction 19, which corresponds to an intact SWI/SNF complex with an approximate native molecular mass of 2 MDa (Fig. 1, top panel) (30). Similar elution profiles were observed when extracts were prepared from *swi8* and *swi9* mutants (Fig. 1, lower two panels). In contrast, when extracts were prepared from the *swi7* mutant, the peak of Swi3p shifted from fraction 19 to fraction 21 (Fig. 1, second panel from top). In addition, we found that much less Swi3p was recovered during the analysis, and degradation appeared to be enhanced. Similar results were obtained in four independent experiments. These results are consistent with the possibility that *SWI7* encodes a subunit of the SWI/SNF complex.

***SWI7*, *SWI8*, and *SWI9* are identical to *ADA3*, *ADA2*, and *GCN5*, respectively.** The *SWI7* gene was cloned by complementation of the defect in transcription of an *HO-lacZ* fusion gene. A plasmid which contains a minimal complementing region was integrated into the genome of a *swi7* mutant strain, and tetrad analysis of this integrant confirmed that we had cloned *SWI7* (see Materials and Methods for details). We sequenced 200 bp of *SWI7* and found that the sequence was identical to

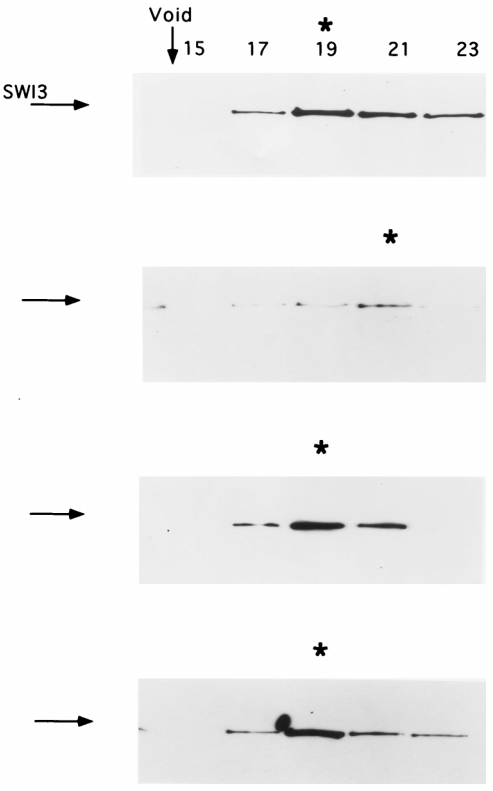

FIG. 1. *SWI7* is required for stability or assembly of the SWI/SNF complex. Immunoblot analysis of fractions obtained from gel filtration of extracts prepared from the wild-type strain (top panel) and from *swi7* (second panel), *swi8* (third panel), and *swi9* (lower panel) mutants is shown. Peak elution of SWI3 is marked with an asterisk above the corresponding fraction. Similar results were found in four separate experiments. Extracts prepared from strains that carried a deletion of *ADA3* showed a similar shift in elution of Swi3p.

that of the previously identified *ADA3* gene. Three additional results are also consistent with the identity of *SWI7* and *ADA3*: (i) the *swi7* mutant resists toxicity due to GAL4-VP16 overexpression (the Ada phenotype); (ii) a *swi7*/*ada3*Δ diploid remains resistant to GAL4-VP16 toxicity, indicating that these two mutants are in the same complementation group; and (iii) a plasmid which expresses only the *ADA3* open reading frame fully complements the *swi7* mutant for the defect in *HO-lacZ* expression (data not shown).

Previous studies have indicated that the *ADA3* gene product functions in concert with the *ADA2* and *GCN5* products (31). Due to the similar phenotypes of *swi7*, *swi8*, and *swi9* mutants (8), we tested whether *SWI8* and *SWI9* are identical to *ADA2* and *GCN5*, respectively. Consistent with this possibility, *swi8* and *swi9* mutants are resistant to toxicity due to overexpression of GAL4-VP16 (data not shown). In addition, *swi8*/*SWI8 ADA2*/*ada2* and *swi9*/*SWI9 GCN5*/*gcn5* diploids remained resistant to toxicity, whereas other mutant combinations were sensitive. These putative homozygous diploids are also sporulation defective, which is a phenotype of both *swi*/*snf* and *ada*/*gcn5* mutants (7, 59). Furthermore, plasmids that express only the *ADA2* or *GCN5* open reading frame restored expression of the *HO-lacZ* fusion gene in a *swi8* or *swi9* strain, respectively (data not shown). These results confirm that *SWI8* is identical to *ADA2* and that *SWI9* is identical to *GCN5*.

The predicted molecular weights of the Ada2p, Ada3p, and Gcn5p proteins are consistent with the sizes of three of the unidentified subunits of SWI/SNF complex (p47, p78, and p50)

(12, 15). Four results, however, show conclusively that these three *ADA*/*GCN5* products are not stoichiometric subunits of SWI/SNF. First, *swi*/*snf* mutations do not confer resistance to GAL4-VP16 overexpression (data not shown). In addition, antibodies directed against Ada2p or Ada3p do not cross-react with polypeptides in preparations of purified SWI/SNF complex (data not shown). Furthermore, SWI/SNF complexes purified from wild-type, *ada2*Δ, *ada3*Δ, and *gcn5*Δ strains contain the same set of 10 stoichiometric subunits that can be visualized by silver staining (53a) (see Materials and Methods). Finally, DNA sequence information derived from microsequencing of SWI/SNF subunits indicates that the unknown subunits are not encoded by *ADA2*, *ADA3*, or *GCN5* (10a).

***ADA3*, *ADA2*, and *GCN5* are required for expression of *SWI*/*SNF*-dependent genes.** To investigate whether the ADA/GCN5 complex might play a role in the function of SWI/SNF, we tested whether *ADA*/*GCN5* products are required for expression of *SWI*/*SNF*-dependent genes. Previous studies have shown that the induced levels of *HIS3*, *TRP3*, and *ILV1* mRNAs are reduced about two- to fourfold by mutations in *GCN5* (21). Other studies of *ADA*/*GCN5* gene products have focused almost exclusively on synthetic LexA fusion activators and artificial reporter genes (5, 21, 40, 51). Breeden and Nasmyth (8) showed that *ada3* (*swi7*), *ada2* (*swi8*), and *gcn5* (*swi9*) alleles decreased expression of an *HO-lacZ* fusion gene. Figure 2A shows that deletion of the *ADA2*, *ADA3*, or *GCN5* gene also leads to a 10-fold decrease in expression of *HO-lacZ*. Furthermore, mutations in *ADA2*, *ADA3*, or *GCN5* lead to a fourfold decrease in expression of the *SUC2* gene (Fig. 2A). A similar decrease in *SUC2* expression is observed in a *snf5* mutant in this genetic background (Fig. 2A). We also analyzed expression of the *INO1* gene and of total Ty elements (Ty1 and Ty2) by a primer extension assay (Fig. 2B). A *swi1* mutation leads to a 4-fold decrease in Ty transcripts detected with this primer (data not shown) and a 30-fold decrease in *INO1* expression (48). Likewise, *ada2*, *ada3*, or *gcn5* mutations each lead to a fourfold decrease in Ty expression (Fig. 2B). Furthermore, *ada2*, *ada3*, or *gcn5* mutations lead to a 6- to 14-fold decrease in *INO1* expression. Thus, it appears that expression of *SWI*/*SNF*-dependent genes also requires ADA/GCN5 products.

The ADA/GCN5 complex, like SWI/SNF, is required for expression of only a subset of yeast genes. *SWI*/*SNF* and *ADA*/*GCN5* products are not required for expression of *HIS4* (21, 27) or for expression of *LYS2*, *ADH1*, or *CLN3* (Fig. 2B). We also investigated expression of the inducible *PHO5* gene. Early studies indicated that *PHO5* induction required *SWI*/*SNF* products; however, subsequent studies have shown that *PHO5* induction is SWI/SNF independent (31a) (Fig. 2B). The steady-state induced level of PHO5 is also insensitive to mutations in *ADA2*, *ADA3*, and *GCN5* (Fig. 2B). Thus, these expression studies suggest that *SWI*/*SNF* and *ADA*/*GCN5* products are required for expression of similar or perhaps identical sets of genes.

Genetic interactions between the ADA/GCN5 complex and genes that encode chromatin components. The *SIN1* gene encodes a distant relative of mammalian HMG1 and is likely to be a nonhistone component of chromatin (37). The semidominant *sin1-2* allele or a *sin1* deletion partially alleviates many of the transcriptional defects caused by inactivation of the SWI/SNF complex (37). Mutations in *SIN1* are more effective suppressors of *swi*/*snf* phenotypes than mutations in histone genes, and furthermore, *sin1* mutants are less pleiotropic (36, 37, 63). The ability of a *sin1* mutation to alleviate the transcriptional defects caused by *ada2* or *ada3* mutations was tested. We constructed *ada2 sin1* and *ada3 sin1* double mutants and then analyzed expression of the *HO-lacZ* and *SUC2* genes. For each assay at least four segregants of each genotype were analyzed, and the results were averaged. In the case of the *HO-lacZ* fusion gene, an *ada3* mutant shows 9% of the expression of a wild-type strain, whereas expression in the *ada3 sin1-2* double mutant is doubled (18% of the wild-type level) (Fig. 3). Likewise, *SUC2* expression is 20% of the wild-type level in the *ada3* mutant, and in the *ada3 sin1*Δ mutant *SUC2* expression is restored to 140% of the wild-type level. Identical results were obtained with *ada2 sin1* double mutants (data not shown). Mutations in the *SIN1* gene also alleviate the slow-growth phenotype of *ada2*, *ada3*, and *gcn5* mutants on synthetic minimal medium (data not shown). One interpretation of these functional interactions is that the ADA/GCN5 complex functions by antagonizing the repressive effects of Sin1p.

In the process of constructing the *ada sin1* double mutants, we were unable to recover *ada swi* double mutants. In a total of 71 tetrads dissected, we recovered most of the *swi1* and *ada* single mutants (38 of 62 and 55 of 60 predicted *swi1* and *ada* segregants, respectively). However, we recovered 0 of 50 predicted *ada swi1* double mutants. This analysis included *ada2* mutants as well as *ada3* mutants, indicating that both *ada2 swi1* and *ada3 swi1* double mutants are nonviable. Likewise we have been unable to obtain *gcn5 swi1* double mutants from dissection of a doubly heterozygous diploid. This synthetic lethality is not due to an additive effect of *swi1* and *ada*/*gcn5* mutations on cell growth, as *ada*/*gcn5* mutants show only a minor growth defect on rich medium. To confirm this apparent synthetic lethality, we constructed a *swi1*/*SWI1 gcn5*/*gcn5* diploid that contained a *URA3*-marked plasmid carrying *GCN5*. Dissection of this diploid yielded *swi1 gcn5* double mutants, although all double mutants harbored the *GCN5*-containing plasmid. These *swi1 gcn5* double mutants, however, were inviable without the *GCN5*-containing plasmid, as these segregants were unable to grow on medium containing 5-fluoro-orotic acid, which selects against cells that retain the *URA3*-marked *GCN5*-containing plasmid. This synthetic lethality between *swi1* and *ada*/*gcn5* mutants is consistent with a role for both the ADA/GCN5 and SWI/SNF complexes in antagonizing chromatin-mediated transcriptional repression. Each complex may perform independent, partially redundant functions, or these genetic interactions may indicate a functional interaction between ADA/GCN5 and SWI/SNF complexes.

In crosses in which *ada*/*gcn5*, *swi1*, and *sin1* mutations were all segregating, we were able to obtain *ada*/*gcn5 swi1 sin1* triple mutants. Although these triple mutants grow poorly on rich medium, expression of the *HO-lacZ* fusion gene is restored to nearly wild-type levels (Fig. 3). Thus, in the absence of *SIN1*, both SWI/SNF and ADA/GCN5 complexes are dispensable for cell viability and *HO-lacZ* expression.

Semidominant mutations (*sin* alleles) in the genes encoding histones H3 and H4 that partially alleviate the transcriptional defects due to inactivation of SWI/SNF have also been identified (36, 52, 63). To investigate whether these Sin^- versions of histones H3 and H4 might also alleviate the transcriptional defects due to loss of the *GCN5* product, low-copy-number plasmids carrying either a wild-type copy of *HHT2* (histone H3), a wild-type copy of *HHF2* (histone H4), a semidominant *sin* allele of *HHT2* (*hht2-2*), or a semidominant *sin* allele of *HHF2* (*hhf2-7*) were introduced into wild-type, *gcn5*, or *snf5* strains. Liquid β-galactosidase assays were then used to measure expression of a chromosomal *HO-lacZ* fusion gene, and the results are shown in Table 2. In the absence of *SNF5*, expression of the *HO-lacZ* fusion gene was reduced to 0.6% of the wild-type level. In the presence of the *hht2-2* or *hhf2-7 sin* allele, transcription was increased two- and eightfold, respectively. These levels of *HO-lacZ* expression in the presence of

plasmid-borne *sin* alleles is similar to those reported previously (36). In the case of the *gcn5* strain, *HO-lacZ* expression is reduced to 2% of the wild-type level. Introduction of the *hht2-2* or the *hhf2-7 sin* allele increased transcription threefold. Thus, Sin^- versions of histones are able to partially alleviate a transcriptional defect due to inactivation of *GCN5*.

In vitro acetylation of Sin1p by distinct *GCN5*-dependent acetyltransferase activities. One interpretation of the genetic interaction between *ADA*/*GCN5* and *SIN1* is that the ADA/GCN5 complex may function by antagonizing the function of Sin1p. One possibility is that this may be due to an indirect mechanism; for instance, acetylation of histones may influence the ability of Sin1p to interact with chromatin. Alternatively, this antagonism may be more direct, perhaps involving the acetylation of Sin1p, disrupting its function. Since nuclear histone acetyltransferases are known to acetylate some nonhistone proteins (primarily HMG1 and HMG2) (65), we investigated whether Gcn5p might acetylate Sin1p. Since the substrate specificity of recombinant Gcn5p appears to be restricted to histones that are not assembled into nucleosomes (38, 67) and Gcn5p is thus unlikely to recognize physiological substrates, we partially purified native *GCN5*-dependent histone acetyltransferase complexes from yeast cells and tested whether these enzymes could acetylate recombinant Sin1p in addition to purified histones.

Whole-cell extracts were prepared from 20-liter cultures of isogenic wild-type and *gcn5*Δ strains. These extracts were then fractionated through three successive chromatography steps: Ni^{2+}-NTA agarose, native DNA-cellulose, and FPLC Mono Q. This purification scheme takes advantage of the observation that the majority of the Gcn5p binds tightly to Ni^{2+}-NTA agarose even though it lacks a hexahistidine tag (15a). The second step of purification utilized a native DNA-cellulose column in order to enrich for chromatin-bound acetyltransferases. Figure 4 shows the profiles of elution from the FPLC Mono Q column for Gcn5p, Ada3p, and histone acetyltransferase activities. When $GCN5^+$ extracts were fractionated, three distinct, reproducible peaks of histone acetyltransferase activity eluted in the Mono Q fractions (peak fractions at 320 [pool 1], 415 [pool 2], and 450 [pool 3] mM NaCl). When free histones were used as substrates, the activity in pool 1 preferentially acetylated histone H4 and weakly acetylated histone H3 (Fig. 5A to C). In contrast, the activity in pools 2 and 3 preferentially acetylated histone H3. The activity in pool 2 also showed weak acetylation of histone H4. All three peaks of acetyltransferase activity cofractionate with Gcn5p and Ada3p detected by Western blotting (Fig. 4, bottom), which is consistent with the presence of three chromatographically distinct ADA/GCN5 acetyltransferase complexes. When extract from the *gcn5*Δ strain was fractionated, only one peak of acetyltransferase activity was detectable in the Mono Q fractions (Fig. 4). The specific activity of this acetyltransferase is consistently much lower than that of the corresponding activity purified from the wild-type strain (Table 3), indicating that the majority of histone acetyltransferase activity in the three peaks found in wild-type Mono Q fractions is *GCN5* dependent.

We also tested whether the *GCN5*-dependent acetyltransferase activities were able to acetylate histones that had been reconstituted into nucleosome arrays. Purified chicken histones were mixed with a DNA template that contains 12 head-to-tail repeats of a 208-bp rRNA nucleosome-positioning sequence (208-12), and an array of 12 positioned nucleosomes was reconstituted by salt gradient dialysis. When nucleosome arrays were used as the substrate, only pool 1 could efficiently acetylate these histones (Fig. 5C and D). As was the case with free histones, the activity in pool 1 preferentially acetylated

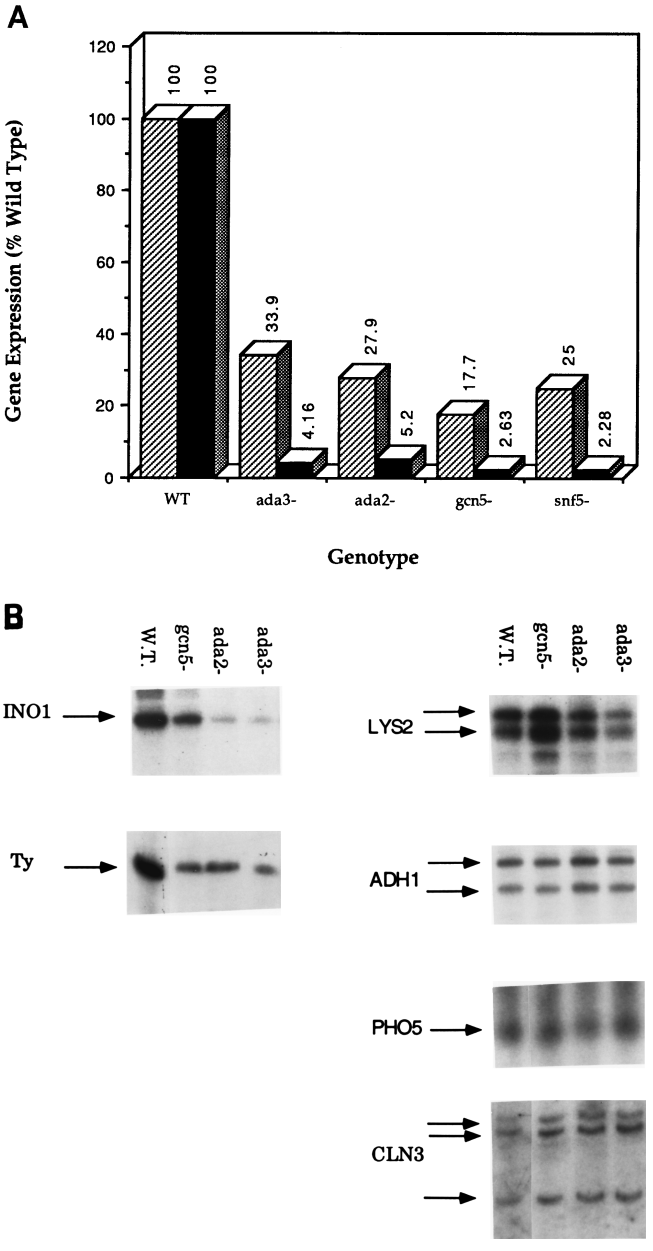

FIG. 2. *ADA2*, *ADA3*, and *GCN5* are required for expression of *SWI*/*SNF*-dependent genes. (A) Expression of *HO-lacZ* and *SUC2*. Strains were grown to mid-log phase in YEPD and then either analyzed for β-galactosidase activity (measurement of *HO-lacZ* transcription) (black bars) or washed in water and then grown for 2 h in YEP with 0.5% glucose before harvesting for invertase assays (measurement of *SUC2* transcription) (hatched bars). The strains used were CY432 (ADA^+) (WT), CY435 and CY562 (*ada3*), CY436 and CY561 (*ada2*), CY434 and CY563 (*gcn5*), and CY433 (*snf5*). Miller units (β-galactosidase) and invertase units were normalized to percentages of wild-type levels. Analyses were done in triplicate, and values were averaged. Values varied by <20%. No differences were seen between *swi7*, *swi8*, *swi9*, and deletion alleles. (B) Primer extension analysis of *INO1*, Ty elements, *LYS2*, *ADH1*, *PHO5*, and *CLN3*. Strains were grown to mid-log phase in S medium for *INO1* and *LYS2* RNA analysis or in YEPD for Ty elements and *CLN3* RNA analysis. For analysis of *ADH1* RNA, cultures were grown to late log phase in YEP containing 2% glycerol, 2% ethanol, and 0.5% sucrose and then washed in water and diluted 1:10 in YEP containing 2% glycerol and 2% ethanol without sucrose and allowed to grow for 6 to 8 h. For analysis of *PHO5* RNA, cultures were grown to late log phase in YEPD and then washed in water and diluted 1:10 in YEPD containing low phosphate (24) and allowed to grow for 8 to 10 h. Total RNA was isolated from cultures and analyzed by primer extension. The strains used were CY432 (ADA^+) (W.T.), CY434 (*gcn5*), CY436 (*ada2*), and CY435 (*ada3*). Similar results were seen in three separate experiments.

histone H4. In contrast, the activities in pools 2 and 3 were able to weakly acetylate histones that were assembled into nucleosome arrays (Fig. 5C and D). Only after very long exposure of the gel shown in Fig. 5D could we detect slight labeling of histone H4 by pool 2.

To determine if Sin1p can serve as an in vitro substrate for the *GCN5*-dependent acetyltransferases, we monitored acetylation of recombinant GST and a GST-SIN1 fusion protein (Fig. 6). Each of the three acetyltransferase activities was able to incorporate [^{3}H]acetate into the GST-SIN1 fusion protein (Fig. 6B). This incorporation was eliminated when pools were prepared from the *gcn5*Δ strain (Fig. 6D). This result suggests that GST-SIN1 acetylation is *GCN5* dependent and furthermore that GST-SIN1 does not have the ability to autoacetylate. Labeling of GST-SIN1 is not due to acetylation of GST residues or to acetylation of the N-terminal residue, since no incorporation is detected when free GST is used as a substrate (Fig. 6B). We also observe several proteins in addition to GST-SIN1 that are labeled with [^{3}H]acetate by the acetyltransferase in pool 1. These proteins are also labeled when no recombinant substrate is added to the reaction (Fig. 6C), and they thus reflect substrates present in the Mono Q fractions.

GST-SIN1 preparations routinely contained several different C-terminal truncation products (Fig. 6D) (which was confirmed by Western blot analysis [Fig. 6A]) that are labeled with [^{3}H]acetate by the acetyltransferase pools. In one preparation of GST-SIN1, a small fusion protein which contained only 10 to 15 kDa of Sin1p N-terminal residues fused to GST predominated. This smaller fusion protein was also a substrate for each of the *GCN5*-dependent acetyltransferase activities (Fig. 6C). This result suggests that at least one site of modification is located in the N-terminal DNA binding domain of Sin1p.

DISCUSSION

We have presented several results that indicate that the *ADA2*, *ADA3*, and *GCN5* products function by antagonizing chromatin and that they may function in concert with the SWI/SNF complex. *ADA*/*GCN5* products are required for expression of several *SWI*/*SNF*-dependent genes, including *HO*, *SUC2*, *TY*, and *INO1*. SWI/SNF and GCN5 are also required for expression of ADH2 in some strain backgrounds (14, 46, 51a). Both *ADA*/*GCN5* and *SWI*/*SNF* products are also required for the function of the GCN4 activator (5, 21, 33, 40, 51) and the mammalian glucocorticoid receptor (29, 68). Inactivation of the HMG1-like protein Sin1p or mutations in genes that encode histones H3 and H4 alleviate the defects in transcription due to inactivation of the SWI/SNF or ADA/GCN5 complex. In addition, *sin1* mutations rescue the lethality of *swi1 ada2*, *swi1 ada3*, and *swi1 gcn5* double mutants. In the case of the *HO-lacZ* gene, expression is restored to wild-type levels in the *ada2 swi1 sin1* triple mutant, indicating that both protein complexes are not required if Sin1p is inactivated. Furthermore, we have found that the substrate specificity of native *GCN5*-dependent acetyltransferases is not restricted to histones but that these enzymes can also acetylate the HMG1-like protein Sin1p.

Role of the ADA/GCN5 complex in contending with chromatin. Early studies on *ADA*/*GCN5* products led to the hypothesis that these proteins functioned as transcriptional adaptors that might bridge transcriptional activation domains to components of the general transcription machinery. This model was supported by biochemical studies in which Ada2p was shown to associate with acidic activation domains in vitro (4, 14, 41, 57) and by genetic studies in which mutations in *ADA*/*GCN5* genes were isolated as suppressors of the toxicity due to overexpression of the potent activator GAL4-VP16 (5, 40, 51). Recent studies have shown, however, that inactivation of the SWI/SNF complex, which is believed to function primarily as a chromatin-remodeling enzyme, also associates with an activator in vitro (68), and *swi*/*snf* mutations alleviate the toxicity due to overexpression of the E1A activator (42). Further-

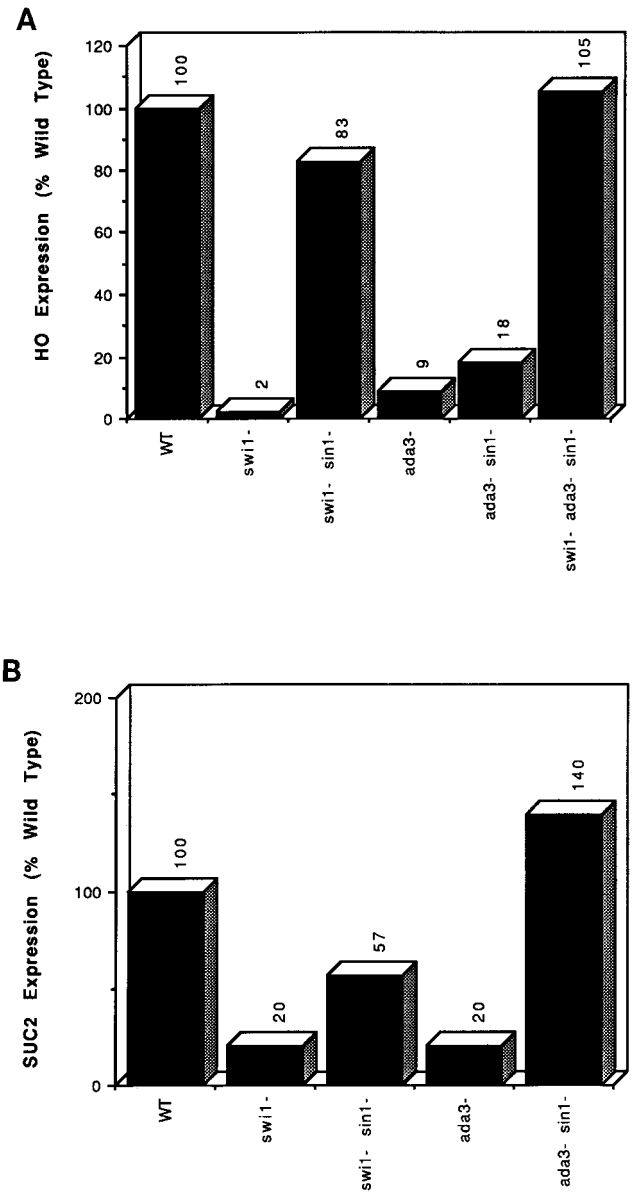

FIG. 3. Mutations in *SIN1* alleviate *ada*/*gcn5* and *swi*/*snf* transcriptional defects. (A) Expression of *HO-lacZ*. Strains were grown to mid-log phase in YEPD and then analyzed for β-galactosidase activity. Strains used for analysis were segregants obtained from tetrad analysis of the diploid parent CY578. The *sin1* mutation is a *sin1-2* allele. Results shown are the averages for three individual segregants of the same genotype, each tested in duplicate. β-Galactosidase levels were measured in Miller units and then converted to percentages of the wild-type (WT) level. Values varied by <20%. (B) Expression of *SUC2*. Strains were grown to mid-log phase in YEPD and then washed in water and grown for 2 h in YEP with 0.05% glucose before harvesting for invertase assays. Strains used were segregants obtained from tetrad analysis of the diploid parent resulting from CY231 × CY565. The *sin1* mutation is a deletion of *sin1*. Results shown are the averages for three individual segregants of the same genotype, each tested in duplicate. Values varied by <20%. Invertase units (nanomoles of glucose per minute per milligram of cells) were converted to percentages of the wild-type value.

TABLE 2. *sin* histone alleles relieve requirement of *GCN5* for transcription

Strain	*HO-lacZ* expression (Miller units) with:			
	HHT2 (WT[a] H3)	*hht2-3* (H3 E-K mutant)	*HHF2* (WT H4)	*hhf2-7* (H4 R-C mutant)
CY432 (WT)	49	36	46	44
CY433 (*snf5*)	0.3	0.6	0.3	2.5
CY434 (*gcn5*)	1.0	2.6	1.6	4.6

[a] WT, wild type.

more, we find that mutations in chromatin components partially alleviate transcriptional defects due to inactivation of ADA/GCN5 or SWI/SNF, which suggests that the primary role of ADA/GCN5 may also be to antagonize chromatin-mediated transcriptional repression. Consistent with this view, it appears that for most *ADA*/*GCN5*-dependent genes chromatin plays an important role in their regulation, since their expression is also dependent on the SWI/SNF complex.

Biochemical studies are also consistent with the ADA/GCN5 complex functioning as a chromatin-remodeling enzyme. Allis and colleagues have shown that recombinant yeast Gcn5p can acetylate lysine residues in the flexible N-terminal domains of histone H3 and H4 (10, 38). However, recombinant Gcn5p does not acetylate histones that are assembled into nucleosomes (67), which led to the suggestion that acetylation of physiological substrates might require an intact ADA/GCN5 complex. Consistent with this view, the *Tetrahymena* homolog of Gcn5p cofractionates with a partially purified protein complex which is able to acetylate nucleosomal histones in vitro (9). We have partially purified from yeast whole-cell extracts three chromatographically distinct acetyltransferase complexes that are dependent on *GCN5* function. One of these activities is able to efficiently modify free histones as well as histones that are assembled into nucleosomal arrays. Both Gcn5p and Ada3p cofractionate with all three pools of acetylase activity, which is consistent with the presence of three distinct ADA/GCN5 acetyltransferase complexes. Furthermore, the acetylase activity in pool 1 sediments in glycerol gradients as a protein of 700 kDa, while the activities in pools 2 and 3 sediment as 2-Mda and 150-kDa proteins, respectively (51a). The sizes of these three acetyltransferase complexes are similar to those of three complexes that contain NGG1/ADA3 and ADA2 (56). Our data does not exclude the possibility of additional acetyltransferase activities, since our purification strategy relies on the fortuitous binding of acetyltransferase activities on Ni^{2+}-NTA agarose. Although the three activities found in the Mono Q pools have been fractionated through three successive chromatography steps, the protein distributions are still complex, and purification to homogeneity will require further fractionation.

The *GCN5*-dependent acetyltransferase in pool 1 appears to

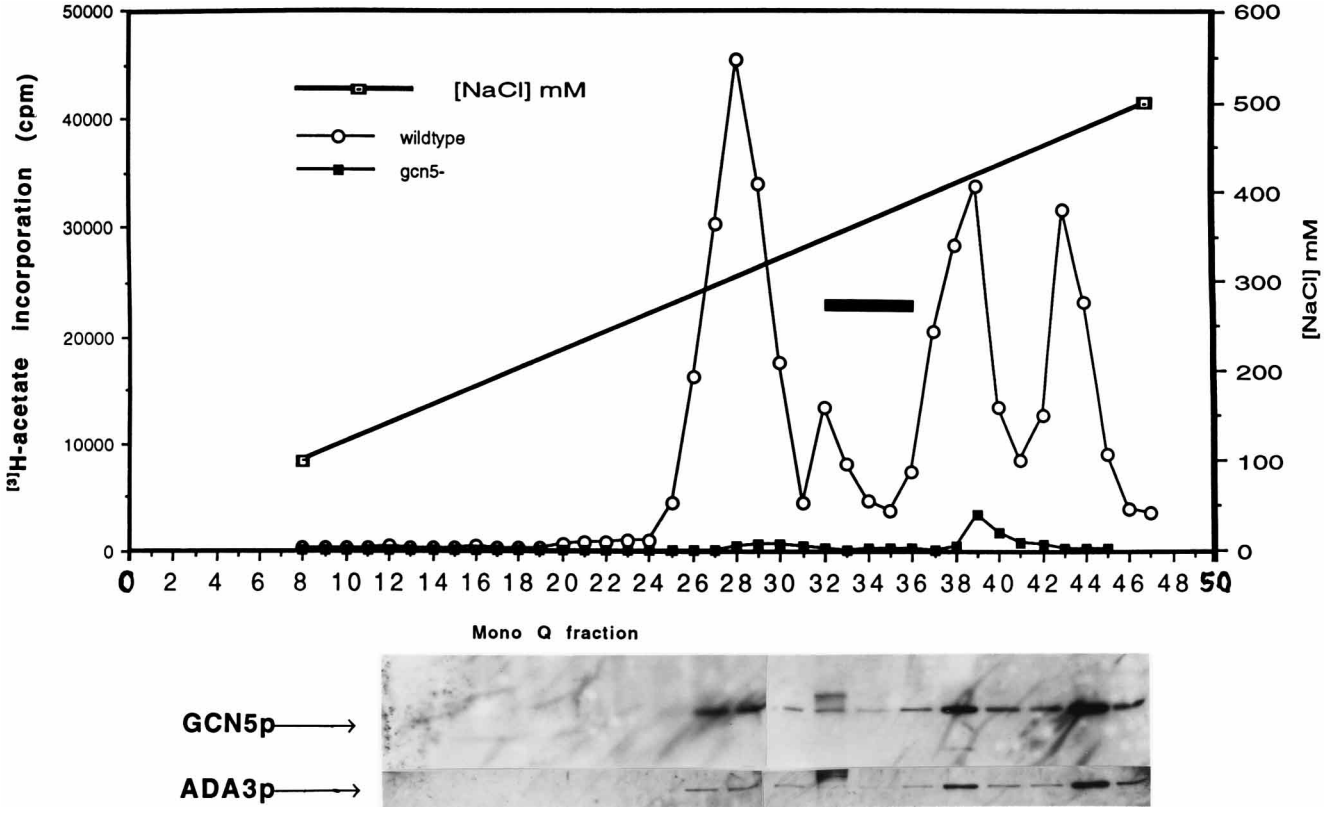

FIG. 4. Isolation of three distinct *GCN5*-dependent histone acetyltransferase activities. Fractions from the FPLC Mono Q column (see Materials and Methods) were analyzed by liquid histone acetylase assays and Western blot analysis. (Top) Acetylase assays. Two micrograms of purified histones was used as a substrate, and 5 μl of each fraction was used as the enzyme in 30-min liquid acetylase assays. The entire reaction mixture was spotted onto filters, and radioactivity was counted. (Bottom) Results from Western blot analysis of wild-type fractions probed with purified anti-GCN5 antibodies or crude anti-ADA3 antibodies, each marked by an arrow. The heavy black bar in the top panel marks fractions containing the SWI/SNF complex. Similar results were found in three separate experiments, with the exception that the apparent small peak of acetyltransferase activity that elutes after pool 1 and overlaps SWI/SNF was not reproducible.

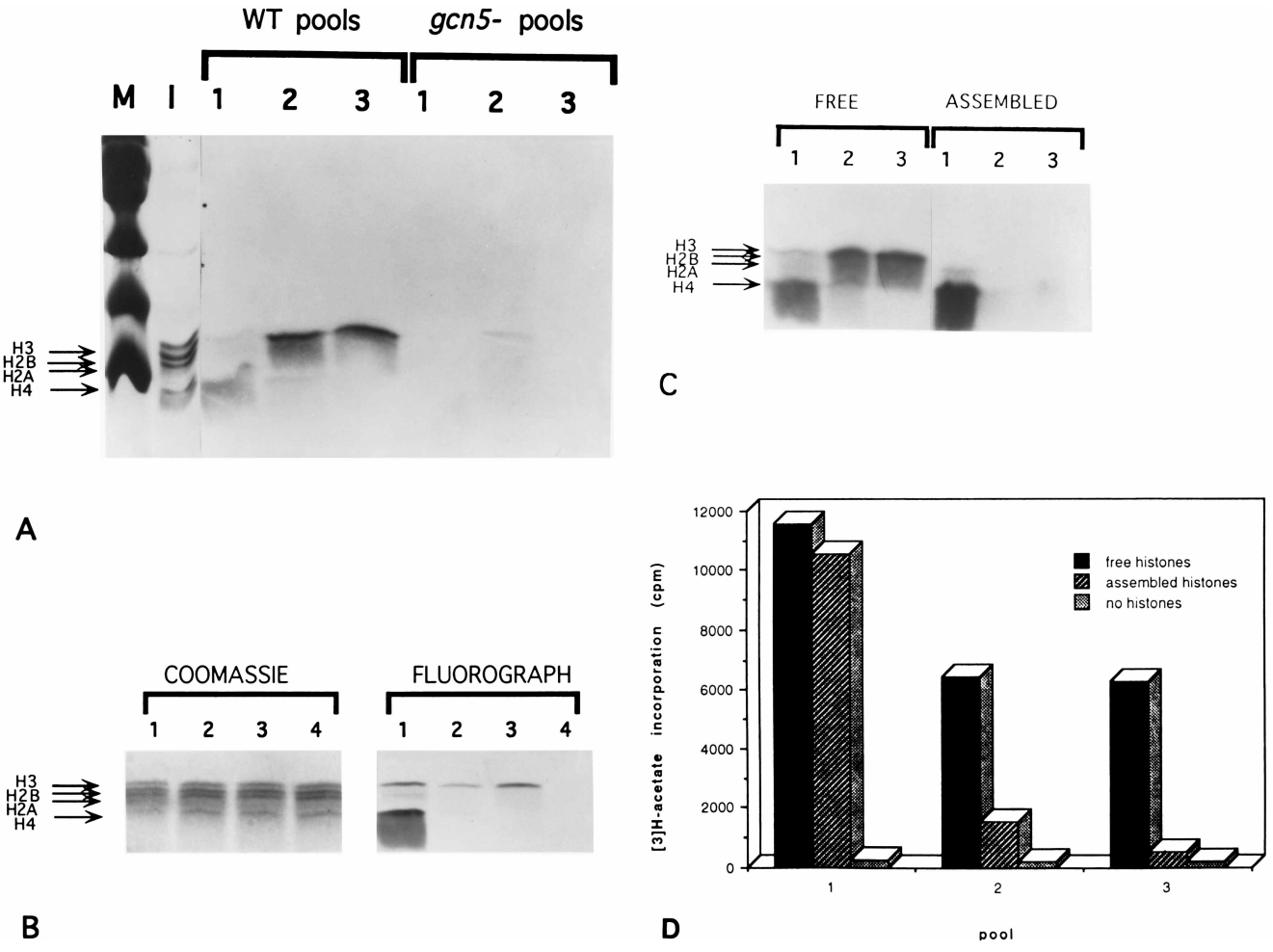

FIG. 5. *GCN5*-dependent acetylation of histones. (A) Acetylation of free histones. One unit of enzyme activity (calculated from specific activities [Table 3]) from each wild-type pool was used in 30-min acetylation assays with 1 μg of free histones as the substrate. Half of the reaction mixture was loaded onto 18% polyacrylamide gels and then fixed and treated for autoradiography. The left two lanes are Coomassie blue-stained marker proteins (M) and 1 μg of histones (I) run on the same gel and then cut off and prepared separately. The right six lanes show results from assays of the three pools of activity from wild-type and *gcn5*Δ strains. The amount of enzyme used from deletion pools was calculated to be the same as the amount of protein used in the corresponding wild-type pool. (B) Histones remain intact following acetylation. Halves of 30-minute acetylation reaction mixtures with 1 μg of free histones as substrate for pool 1, 2, or 3 or no enzyme pool (lanes 1 to 4, respectively) were loaded onto 18% polyacrylamide gels and then fixed and stained with Coomassie brilliant blue before treatment for autoradiography. Staining and fluorography of the same gel are shown and are representative of three separate experiments. (C and D) [^{3}H]acetate incorporation into free and assembled histones. Acetylation assays were performed with 5 μl of each peak fraction and 1 μg of either free chicken histones or histones assembled into nucleosomal arrays. After 30 min, half of the reaction was stopped in SDS sample buffer, and the mixture was loaded onto 18% polyacrylamide gels and then fixed and treated for autoradiography (C). The radioactivity in the other half of the reaction mixture was counted (D). The results shown are representative of three separate experiments.

be distinct from the activities in pools 2 and 3. When free histones or nucleosomal histones are used as a substrate, the activity in pool 1 preferentially acetylates histone H4. In contrast, recombinant Gcn5p (10) and the activities in pools 2 and 3 preferentially acetylate histone H3. In addition to the difference in substrate specificity, the three pools differ greatly in their ability to acetylate histones that are assembled into 12-mer nucleosome arrays. The activity in pool 1 uses both substrates equally well (Fig. 5B and C), while the activities in pools 2 and 3 prefer free histones: nucleosomal histones are acetylated by pool 2 at less than 20% of the levels of free histones, and the activity in pool 3 acetylates nucleosomal histones only very poorly. These results suggest that the physiological substrates for the activities in pools 2 and 3 may be nonhistone proteins, whereas the *GCN5*-dependent activity in pool 1 may represent a true nucleosomal histone-modifying enzyme.

One goal of our biochemical studies was to test if Sin1p could serve as a substrate for an ADA/GCN5 acetyltransferase complex. *SIN1* encodes a distant relative of mammalian HMG1 which behaves genetically as a negative regulator of transcription (37). A recombinant GST-SIN1 fusion protein was acety-

TABLE 3. Enzyme activities

Strain	Peak (fraction)	Salt elution (mM)	Sp act (U/μg)[a]
Wild type	1 (28)	320	0.24
	2 (38)	415	1.34
	3 (43)	450	0.49
*gcn5*Δ	1 (29)	320	0.02
	2 (39)	415	0.08
	3 (44)	450	0.03

[a] 1 U = 1 pmol of [^{3}H]acetate incorporated per 5-min assay.

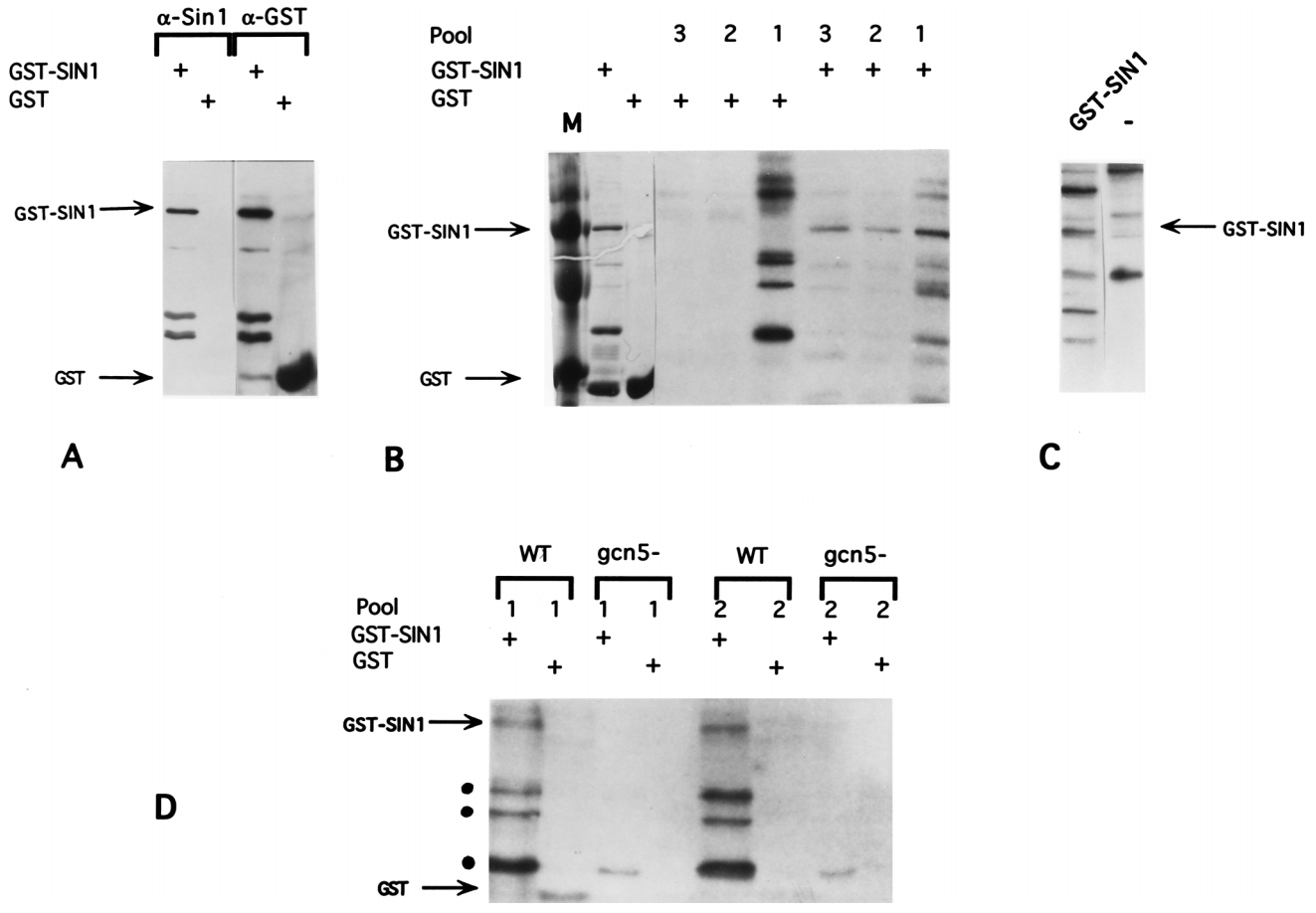

FIG. 6. *GCN5*-dependent acetylation of Sin1p. (A) Western blot analysis of recombinant GST-Sin1p and GST preparations. The same blot was probed with affinity-purified antibodies directed against Sin1 protein (α-Sin1) or monoclonal antibodies directed against GST (α-GST) (Santa Cruz Biotechnology, Inc.). (B to D) Liquid acetylation assays were performed with 5 μl of the peak fraction of each wild-type pool for enzyme and 5 μg of total protein from GST-SIN1 and GST preparations as substrates. (B) The left three lanes show Coomassie blue-stained protein markers (M) and 5 μg of GST-SIN1 or GST. The right six lanes show an autoradiograph of halves of 30-min GST-SIN1 or GST reaction mixtures, which were loaded onto polyacrylamide gels. (C) Pool 1 contains endogenous substrates. Five microliters of pool 1 was used in acetylase assays with addition of either GST-SIN1 (5 μg) or no recombinant substrate. (D) Acetylation of Sin1p is diminished in *gcn5*Δ pools. Halves of acetylation assay mixtures were loaded onto polyacrylamide gels after the reaction. *gcn5*Δ reactions were performed with the same amount of protein as used in the corresponding wild-type (WT) pool. This protein preparation of SIN1-GST was significantly degraded, as the degradation products (circles) were the predominant species in these reactions.

lated by each of the *GCN5*-dependent acetyltransferase activities in vitro, although the extent of acetylation was less than that of free histones. We have not yet mapped the acetylation sites on Sin1p, nor do we know if Sin1p is acetylated by a *GCN5*-dependent acetyltransferase in vivo. Likewise, it is not known if *GCN5*-dependent acetyltransferases acetylate histones in vivo. The in vitro acetylation of proteolytic products of the GST-SIN1 fusion protein indicates that at least one site of modification is within the N-terminal 10 to 15 kDa of Sin1p. This domain contains an HMG box which harbors conserved residues that are present within mammalian HMG1; these residues are known to be sites for acetylation by mammalian nuclear acetyltransferases (60). One possibility is that acetylation of the HMG box of Sin1p disrupts its DNA binding properties, resulting in a chromatin environment that is more permissive for transcription.

Functional interaction between the ADA/GCN5 and SWI/SNF complexes. Mutations in the *ADA*/*GCN5* and *SWI*/*SNF* genes result in many common phenotypes and show similar genetic interactions with mutations in genes that encode chromatin components. One trivial explanation that might explain these similarities is that ADA/GCN5 is required for the expression of one or more SWI/SNF subunits. Several results show that this is not the case. *ada2* and *gcn5* mutations do not affect the integrity or levels of intact SWI/SNF complex detected in whole-cell extracts (Fig. 1). Yet, these mutations yield transcriptional defects that are nearly equivalent to those of *swi*/*snf* mutants. By comparison, expression of Swi3p at <10% of the wild-type level fully complements the transcriptional defects due to a *swi3*Δ (2a). Furthermore, if the phenotype due to *ada*/*gcn5* mutations were due solely to lack of SWI/SNF, then *ada*/*gcn5 swi*/*snf* double mutants should not be inviable.

In contrast to those in *ADA2* and *GCN5*, mutations in *ADA3* have a dramatic effect on the stability or assembly of SWI/SNF (Fig. 1). The reason for this is not known; one possibility is that the ADA/GCN5 complex is associated with SWI/SNF in crude extracts and this interaction is required for SWI/SNF stability. For instance, the absence of Ada3p may lead to increased degradation of SWI/SNF subunits by proteases. Proteins that are components of a complex often show decreased stability when they are expressed individually or discoordinately with their partners (34, 35, 58). This putative interaction, however,

is apparently not stable during our fractionation procedures, since ADA/GCN5 polypeptides do not cofractionate with the SWI/SNF complex in the FPLC Mono Q step (Fig. 4), and they are not detected as stoichiometric subunits of SWI/SNF.

Our genetic analyses also suggest a functional interaction between the ADA/GCN5 and SWI/SNF complexes. Although *ada/gcn5* and *swi* single mutants are viable, we find that *ada2 swi1*, *ada3 swi1*, and *gcn5 swi1* double mutants are inviable. This is not a general effect of combining *swi/snf* mutations with mutations in other chromatin-modifying components, as *swi/snf* mutations are not lethal in combination with *sin3* or *rpd3* (61), which are thought to encode components of histone deacetylase complexes (55; reviewed in reference 64). One possibility is that the SWI/SNF and ADA/GCN5 complexes function together to facilitate an essential step in transcription. In this model, partial function would remain in the absence of one complex, but with the inactivation of both complexes, this essential step would not be completed. Alternatively, synthetic lethality might also be observed if SWI/SNF and ADA/GCN5 function in independent steps that are partially redundant. Two observations suggest that ADA/GCN5 and SWI/SNF may function together. First, the effect of an *ada3* mutation on stability of the SWI/SNF complex is most consistent with a functional interaction between both complexes. Second, our genetic analyses suggest that the ADA/GCN5 complex might regulate the activity of SWI/SNF. In an *ada3 sin1* double mutant, *HO-lacZ* expression is only partially restored (20% of the wild-type level [Fig. 3A]). However, when the SWI/SNF complex is inactivated, expression in the *ada3 swi1 sin1* triple mutant is restored to 100% of the wild-type level. Therefore, the SWI/SNF complex behaves as a repressor of *HO-lacZ* transcription in the absence of a functional ADA/GCN5 complex. In the case of *SUC2*, however, a *sin1* deletion completely alleviates the defect in *SUC2* expression due to an *ada3* mutation (140% of wild type [Fig. 3B]). Thus, ADA/GCN5 function may regulate SWI/SNF activity at only a subset of genes. These genetic and biochemical interactions suggest that the ADA/GCN5 and SWI/SNF complexes may function together to facilitate transcription.

What might be the functional relationship between the ADA/GCN5 and SWI/SNF complexes? Since the ADA/GCN5 complex appears to bind to acidic activation domains, one possibility is that the ADA/GCN5 complex is involved in targeting the SWI/SNF complex to specific activator proteins. Alternatively, the ability of the SWI/SNF complex to disrupt chromatin structure may require the activity of the ADA/GCN5 complex. In vitro, purified SWI/SNF complex can disrupt the rotational positioning of nucleosomal DNA in the absence of the ADA/GCN5 complex (15, 39), but both complexes may be required for the complete spectrum of *SWI/SNF*-dependent changes in chromatin structure that have been observed in vivo (30). For instance, if a major role of histone or nonhistone acetylation is to destabilize compaction of nucleosomal arrays (25), then perhaps the prior action of the ADA/GCN5 acetyltransferase complex is required for SWI/SNF to gain access to the nucleosomal template. This would be consistent with the observation that mammalian SWI/SNF complexes appear to be excluded from heterochromatic regions of nuclear chromatin in vivo (43). In this model the regulation of genes whose expression requires both ADA/GCN5 and SWI/SNF might be strongly influenced by local compaction of chromatin. A functional interaction among the SWI/SNF complex and histone acetyltransferases is unlikely to be restricted to yeast, as a mammalian SWI/SNF subunit may be associated with the histone acetyltransferase, p300/CBP (16).

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