

Activation of the *ADE* Genes Requires the Chromatin Remodeling Complexes SAGA and SWI/SNF[∇]

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The activation of the *ADE* regulon genes requires the pair of transcription factors Bas1 and Pho2. In a genome-wide screen for additional regulators of the pathway, strains with mutations in multiple subunits of the chromatin remodeling complexes SAGA and SWI/SNF were uncovered. These mutants exhibited decreased expression of an *ADE5,7-lacZ* reporter and native *ADE* compared to the wild-type strains, but the expression of the *BAS1* and *PHO2* genes was not substantially decreased. An unregulated Bas1-Pho2 fusion protein depended upon SAGA and SWI/SNF activity to promote transcription of a reporter. A significant but low-level association of Gcn5-myc and Snf2-myc with the *ADE5,7* promoter was independent of adenine growth conditions and independent of the presence of the activator proteins Bas1 and Pho2. However, the increase in occupancy of Bas1 and Pho2 at *ADE5,7* depended on both SAGA and SWI/SNF. The loss of catalytic activity of both SAGA and SWI/SNF complexes in the *gcn5Δ snf2Δ* double mutant was severely detrimental to *ADE-lacZ* reporter expression and native *ADE* gene expression, indicating complementary roles for these complexes. We conclude that Bas1 and Pho2 do not recruit the SAGA and SWI/SNF complexes to the *ADE5,7* promoter but that the remodeling complexes are necessary to increase the binding of Bas1 and Pho2 in response to the adenine regulatory signal. Our data support the model that the SAGA and SWI/SNF complexes engage in global surveillance that is necessary for the specific response by Bas1 and Pho2.

The *ADE* genes of *Saccharomyces cerevisiae* encode the enzymes of the purine nucleotide biosynthetic pathway responsible for the conversion of 5'-phospho-D-ribosyl-1(β)-pyrophosphate into purine nucleotides. Synthesis of purine nucleotides is metabolically regulated by feedback inhibition of the first step of the purine biosynthetic pathway by the end products of the pathway ATP and ADP (39). At the genetic level, *ADE* gene expression is transcriptionally repressed when cells are cultured in the presence of purine bases such as adenine (3, 4, 7, 8, 23, 54). Linking the genetic and metabolic regulation are the intermediates of the biosynthetic pathway SAICAR [1-(5'-phosphoribosyl)-4-(*N*-succinocarboxamide)-5-aminoimidazole] and AICAR [1-(5'-phosphoribosyl)-4-carboxamide-5-aminoimidazole]. Cells with a mutation in any step of the pathway prior to SAICAR synthesis exhibit low, non-derepressible expression of *ADE* genes, whereas cells with a mutation in the steps after SAICAR or AICAR synthesis have high, constitutive *ADE* gene expression (39). Therefore, the regulatory signals for low levels of purine nucleotides are the pathway intermediates SAICAR and AICAR (39, 40).

Nine *ADE* genes are up-regulated by the transcription factors Bas1 and Pho2 under derepressing conditions (3, 58, 65). These two factors also regulate the *HIS1*, *HIS4*, and *HIS7* genes of the histidine biosynthesis pathway and *GLN1*, *SHM2*,

and *MTD1* genes involved in the synthesis of glutamine, glycine, and 10-formyl tetrahydrofolate, respectively (1, 4, 5). Together these 15 genes constitute the *ADE* regulon, although two recent studies have identified up to 56 genes that Bas1 may bind and regulate (21, 24). Both factors are necessary to stimulate expression, but their DNA binding is differentially regulated. Bas1 binds the *ADE* upstream activation sequence (UAS_{*ADE*}) under both repressing and derepressing conditions (51), but Pho2 binds only under derepressing conditions and this binding depends upon interaction with Bas1 (12, 36, 51). The current model for adenine regulation is that the interaction between Bas1 and Pho2 is promoted by the pathway intermediates SAICAR and AICAR (39, 40, 65); this interaction stabilizes Pho2, with its own activation domain, at the UAS_{*ADE*} and unmasks the latent activation domain in Bas1 (12, 36, 65), both of which are necessary to fully activate transcription.

Although Bas1 and Pho2 are required to stimulate *ADE* regulon expression, how they interact with the transcriptional machinery or chromatin remodeling complexes is not known. At *HIS7*, Bas1 and Pho2 are thought to promote transcription through Gcn5-dependent histone acetylation (59). *PHO5* expression, which is regulated by Pho2 in conjunction with Pho4, is dependent on the Snf2 and Gcn5 subunits of the SWI/SNF and SAGA complexes (27, 52). It has also been shown that Pho2 contacts the chromatin remodeling complex NuA4 and that this interaction is necessary for the expression of *PHO5* (29). Thus, several chromatin remodeling complexes may be able to interact with Pho2 and Bas1 in different transcriptional contexts and therefore be relevant as additional regulators of the *ADE* regulon.

In this study, we follow up on an observation that strains with mutations in several subunits of the SAGA and SWI/SNF chromatin remodeling complexes are sensitive to the adenine

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analog 4-aminopyrazolo-(3,4*d*)-pyrimidine (4-APP) (R. J. Rolfes et al., unpublished data). Previous studies of yeast demonstrated that resistance to 4-APP occurs in two different ways. Cells acquired a mutation in the purine-cytosine permease that blocked uptake of the analog (33, 35). Alternatively, cells acquired a specific mutation in the *ADE4* gene that produced a feedback-insensitive form of the first enzyme in the purine nucleotide pathway (33–35). These results indicate that a critical balance between de novo synthesis of purine nucleotides and the intracellular concentration of analog-containing nucleotides is necessary for cells to grow on a medium containing 4-APP. Perturbing this balance can lead to 4-APP resistance or sensitivity (Rolfes et al., unpublished). We found that strains that carry mutations in SAGA and SWI/SNF are more sensitive to 4-APP, suggesting a role for these chromatin remodeling complexes in nucleotide metabolism.

SAGA is a multiprotein complex whose Gcn5 subunit encodes a histone acetyltransferase (HAT). Gcn5 acetylates several lysine residues on the histone tails, including K14 on H3 and K8 and K16 on H4 (19). SAGA is also composed of adaptor proteins of the Ada family, TATA binding protein-associated factors, and Spt proteins involved in the structural integrity of the complex (53, 62). The SWI/SNF complex is a highly conserved remodeling complex that utilizes the energy from its ATPase component Snf2 to remodel the local chromatin structure (26, 31, 50).

The recruitment of the HAT complex SAGA has been associated with recruitment of the SWI/SNF complex in many instances (10, 13, 14, 57). In some cases, acetylation by the SAGA component Gcn5 has been shown to increase retention of the SWI/SNF complex on promoters (13). Thus, it was significant that these two complexes were both identified in our screen, as they may carry out their actions in concert at *ADE* gene promoters. In this report we find that SAGA and SWI/SNF complexes associate with the *ADE5,7* promoter, independently of adenine regulation and independently of Bas1 and Pho2. On the other hand, the Gcn5 subunit of SAGA and the Snf2 component of SWI/SNF are required for efficient binding of Bas1 and Pho2 to the UAS_{*ADE5,7*} under derepressing conditions but not under the basal (repressing) conditions. These results demonstrate a role for SAGA and SWI/SNF in increasing the occupancy of the transcriptional activators in response to the adenine regulatory signal.

MATERIALS AND METHODS

Yeast strains. All strains used in this study are described in Table 1. The homozygous diploid knockout collection was obtained from Research Genetics. To ensure that the strains carried the indicated deletion mutation, each strain selected from the knockout collection for study was analyzed by PCR amplification of the locus (data not shown) using the primers pairs listed on the *Saccharomyces* Genome Deletion Project website (http://sequence-www.stanford.edu/group/yeast_deletion_project/deletions3.html). We obtained strains FY1352, FY1353, FY1354, and FY1360 from Fred Winston (41) and strains HQY367 and HQY453 from Alan Hinnebusch (38, 64). Strain RR439 is a derivative of FY1360 that was constructed to convert the *snf2Δ::LEU2* allele to *snf2Δ::leu2::kanMX* by transformation with BamHI-restricted plasmid m3926 (61). Transformants were selected for G418 resistance and screened for leucine auxotrophy.

Strains RR433, RR434, and RR435 are derivatives of FY1353, FY1354, and FY1360, respectively, that carry the *BAS1-MYC13* allele, and strains RR430, RR431, and RR432 are derivatives of FY1353, FY1354, and FY1360, respectively, that carry the *PHO2-HA3* allele (51). These strains were created by

homologous recombination to insert the epitope tag into the chromosomal genes (51). The *BAS1-MYC13* module was amplified from genomic DNA of strain RR376 using oligonucleotides JO-205 and JO-206, and the *PHO2-HA3* module was amplified from genomic DNA of strain RR409 using oligonucleotides IO-611 and IO-612, as described previously (51). Transformants were selected on yeast extract-peptone-dextrose plates containing 200 μg/ml G418. PCR amplification of the loci from genomic DNA was used to confirm the presence of the tag (data not shown). Expression of the epitope-tagged protein was assessed by immunoblot analysis using anti-myc and antihemagglutinin (anti-HA) antibodies (data not shown).

Strains RR436, RR437, RR440, RR441, and RR442 are derivatives of FY1353, FY1354, RR439, HQY367, and HQY453, respectively, that carry the *bas1-10Δ::hisG* allele. These strains were generated by replacement of the *BAS1* locus with *bas1-10Δ::hisG-URA3-hisG* using the EcoRI-SphI fragment obtained from pR166 and selection on SC medium lacking uracil (51). The *URA3* gene was popped out by culturing the strain on 5-fluoroorotic acid (5-FOA) medium to generate *bas1-10Δ::hisG*. The presence of the *bas1* deletion allele was confirmed by PCR analysis of the genomic locus and by assaying adenine bradytrophy (data not shown).

Strains RR443 and RR444 are derivatives of HQY367 and HQY453, respectively, that carry the *pho2-10Δ::hisG* allele. These strains were generated by integrative transformation with the EcoRI-SphI fragment obtained from pR167 and selection on SC medium lacking uracil (65). The *URA3* gene was popped out by culturing these strains on 5-FOA medium to generate *pho2-10Δ::hisG*. The presence of the *pho2* deletion allele was confirmed by the inability to grow on medium lacking inorganic phosphate and by PCR analysis of the genomic locus (data not shown).

Plasmids. Plasmid pR109 is a fusion of the *lacZ* gene to *ADE4* at codon 71 and was created by inserting *lacZ* into the *ADE4* gene (65) and subcloning it into a *LEU2 CEN6* vector (48) (plasmid construction details available upon request). Plasmid pR115 carries the *ADE5,7-lacZ* reporter, was constructed by inserting the *lacZ* gene into the *ADE5,7* open reading frame (ORF) and then moving the fusion gene into a *LEU2 CEN6* vector, and was described previously (65). Plasmids pR109 and pR115 are similar in that they have large genomic fragment inserts that carry the entire coding region and the entire promoter and regulatory region, extending into the flanking genes. Plasmid pR133, also described previously (65), carries only a small portion (139 bp) of *ADE5,7* that carries the regulatory region (UAS_{*ADE5,7*}) that was inserted into a heterologous system—the *CYC1* core promoter driving the *lacZ* reporter—developed by L. Guarente and colleagues to characterize UAS elements (11). To distinguish these two types of *lacZ* reporters, we refer to this latter type as a UAS_{*ADE5,7-lacZ*} reporter and the former type as an *ADE-lacZ* reporter. Plasmids pR166 (51) and pR167 (65) carry the *bas1-10Δ* and *pho2-10Δ* alleles, respectively. Plasmid m3926 converts *LEU2* to *leu2::kanMX* through a simple insertion (61). Plasmid pCB286 (1) expresses full-length Bas1, and plasmid B273, obtained from Odd Stokke Gabrielsen, expresses the Bas1-Pho2 fusion protein (36).

Media. Strains were cultured at 30°C on YPAD solid medium or SD and SC liquid or solid medium containing 2% glucose, 0.17% yeast nitrogen base (Difco), and 0.5% ammonium sulfate supplemented with amino acids as previously described (47). Adenine was added to a final concentration of 0.15 mM where indicated, and 4-APP was added to a final concentration of 75 μM. Medium lacking inorganic phosphate was prepared by precipitation of magnesium phosphate under alkaline conditions, as described previously (45). Medium containing 5-FOA was made by supplementing SC medium with 0.1% 5-FOA. G418 was added to YPAD medium at 200 μg/ml.

β-Galactosidase assays. β-Galactosidase enzyme assays were performed as previously described (22). Briefly, cells were grown overnight in SC medium containing adenine. Cultures were diluted 1:50 into 50 ml of SC medium with adenine or SC medium lacking adenine and grown for 5 h with rotary agitation at 30°C. Extracts were prepared from pelleted cells by using glass beads, and β-galactosidase activities were measured from clarified whole-cell extracts (22).

4-APP spot test. Cultures were grown overnight at 30°C overnight in 5 ml of YPAD medium. Cultures were diluted to an optical density at 600 nm (OD₆₀₀) of 0.2 and allowed to grow for approximately 3 hours. Approximately 1 ml of cells at an OD₆₀₀ of 0.5 was pelleted and resuspended in 500 μl of sterile water. Serial 10-fold dilutions were prepared, and 5 μl of each dilution was spotted onto the surface of solid medium. Growth was assessed after 48 h of incubation at 30°C.

RNA isolation and Northern blot assays. Cell cultures were grown overnight in SC medium with adenine. Cultures were diluted to an OD₆₀₀ of 0.1 in 50 ml and were allowed to grow to an OD₆₀₀ of 0.5. For derepression, cultures were pelleted, washed twice with SD medium, and resuspended in 50 ml of medium lacking adenine. After 30 min, the cells were pelleted and RNA was isolated using the RNA Isolation Kit (Epicenter); preliminary experiments showed that

TABLE 1. Yeast strains

Name	Genotype	Source or reference
4741/4742	MAT α <i>his3Δ/his3Δ leu2Δ/leu2Δ lys2Δ/LYS2 MET15/met15Δ ura3Δ/ura3Δ</i>	Research Genetics
<i>bas1Δ</i> strain	Isogenic to 4741/4742 with <i>bas1Δ::kanMX/bas1Δ::kanMX</i>	Research Genetics
<i>gcn5Δ</i> strain	Isogenic to 4741/4742 with <i>gcn5Δ::kanMX/gcn5Δ::kanMX</i>	Research Genetics
<i>snf2Δ</i> strain	Isogenic to 4741/4742 with <i>snf2Δ::kanMX/snf2Δ::kanMX</i>	Research Genetics
<i>spt3Δ</i> strain	Isogenic to 4741/4742 with <i>spt3Δ::kanMX/spt3Δ::kanMX</i>	Research Genetics
<i>spt8Δ</i> strain	Isogenic to 4741/4742 with <i>spt8Δ::kanMX/spt8Δ::kanMX</i>	Research Genetics
<i>spt20Δ</i> strain	Isogenic to 4741/4742 with <i>spt20Δ::kanMX/spt20Δ::kanMX</i>	Research Genetics
<i>ada1Δ</i> strain	Isogenic to 4741/4742 with <i>ada1Δ::kanMX/ada1Δ::kanMX</i>	Research Genetics
<i>ada2Δ</i> strain	Isogenic to 4741/4742 with <i>ada2Δ::kanMX/ada2Δ::kanMX</i>	Research Genetics
<i>ada3Δ</i> strain	Isogenic to 4741/4742 with <i>ada3Δ::kanMX/ada3Δ::kanMX</i>	Research Genetics
<i>ubp8Δ</i> strain	Isogenic to 4741/4742 with <i>ubp8Δ::kanMX/ubp8Δ::kanMX</i>	Research Genetics
<i>sgf73Δ</i> strain	Isogenic to 4741/4742 with <i>sgf73Δ::kanMX/sgf73Δ::kanMX</i>	Research Genetics
<i>snf5Δ</i> strain	Isogenic to 4741/4742 with <i>snf5Δ::kanMX/snf5Δ::kanMX</i>	Research Genetics
<i>snf11Δ</i> strain	Isogenic to 4741/4742 with <i>snf11Δ::kanMX/snf11Δ::kanMX</i>	Research Genetics
<i>taf14Δ</i> strain	Isogenic to 4741/4742 with <i>taf14Δ::kanMX/taf14Δ::kanMX</i>	Research Genetics
<i>snf6Δ</i> strain	Isogenic to 4741/4742 with <i>snf6Δ::kanMX/snf6Δ::kanMX</i>	Research Genetics
<i>swi3Δ</i> strain	Isogenic to 4741/4742 with <i>swi3Δ::kanMX/swi3Δ::kanMX</i>	Research Genetics
FY1352	MAT α <i>his3-Δ200 ura3-52 leu2-Δ1 lys2-173R2 gcn5Δ::HIS3 snf2Δ::LEU2</i>	41
FY1353	MAT α <i>his3-Δ200 ura3-52 leu2-Δ1 lys2-173R2</i>	41
FY1354	MAT α <i>his3-Δ200 ura3-52 leu2-Δ1 lys2-173R2 gcn5Δ::HIS3</i>	41
FY1360	MAT α <i>his3-Δ200 ura3-52 leu2-Δ1 lys2-173R2 snf2Δ::LEU2</i>	41
HQY367	MAT α <i>his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0 SNF2-myc₁₃::HIS3</i>	64
HQY453	MAT α <i>his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0 SPT7-myc₁₃::HIS3</i>	38
RR376	MAT α <i>BAS1-MYC13::kanMX6 PHO2 ura3-52</i>	51
RR409	MAT α <i>BAS1 PHO2-HA3::kanMX6 ura3-52</i>	51
RR430	Isogenic to FY1353 with <i>PHO2-HA3::kanMX</i>	This study
RR431	Isogenic to FY1354 with <i>PHO2-HA3::kanMX</i>	This study
RR432	Isogenic to FY1360 with <i>PHO2-HA3::kanMX</i>	This study
RR433	Isogenic to FY1353 with <i>BAS1-MYC13::kanMX</i>	This study
RR434	Isogenic to FY1354 with <i>BAS1-MYC13::kanMX</i>	This study
RR435	Isogenic to FY1360 with <i>BAS1-MYC13::kanMX</i>	This study
RR436	Isogenic to FY1353 with <i>bas1-10Δ::hisG</i>	This study
RR437	Isogenic to FY1354 with <i>bas1-10Δ::hisG</i>	This study
RR439	Isogenic to FY1360 with <i>snf2Δ::kanMX</i>	This study
RR440	Isogenic to RR439 with <i>bas1-10Δ::hisG</i>	This study
RR441	Isogenic to HQY367 with <i>bas1-10Δ::hisG</i>	This study
RR442	Isogenic to HQY453 with <i>bas1-10Δ::hisG</i>	This study
RR443	Isogenic to HQY367 with <i>pho2-10Δ::hisG</i>	This study
RR444	Isogenic to HQY453 with <i>pho2-10Δ::hisG</i>	This study

maximal expression of the *ADE5,7* and *ADE17* loci occurred at 30 min in the wild-type strain (data not shown). RNA samples were separated on 1.2% formaldehyde gels, blotted to GeneScreen Plus filters, and hybridized to probes (46). The *ADE5,7* probe was prepared as described previously (43). Fragments for probes for *BAS1*, *ACT1*, and *ADE17* detection were derived from PCR amplification of genomic DNA using the following oligonucleotides: *BAS1*-up (5'-C ATGGGCCTCACTGGCTGAG) and *BAS1*-down (5'-ATCGCTATCCATGTT GTCC), *ACT*-up (5'-CTTTCTGGAGGAGCAATGATC-3') and *ACT*-down (5'-GGATTCCGGTGATGGTGTAC-3'), and *ADE17*-up (5'-GAGCAGCTG CTAAGAACCATG-3') and *ADE17*-down (5'-ATGGCCAACGCCTCAGGCT C-3'). Radiolabeled probes were prepared using the Random Primed DNA Labeling Kit (Roche) incorporating [α -³²P]dATP (43). RNA transcript levels were quantified by phosphorimaging analysis.

ChIP. Chromatin immunoprecipitation (ChIP) assays were performed as described in reference 9, with the following changes. We added an additional wash of the lysed pellet using fresh FA lysis buffer. DNA in the extracts was fragmented by sonication using a Misonex 3000 sonicator for 30 s on and 30 s off for a total time of 14 min to produce chromatin fragments with an average size of 500 bp, as detected by gel electrophoresis and ethidium bromide staining. Protein G-Dynabeads (Dyna), approximately 2×10^7 in 50 μ l, were preincubated with 2 μ g of the anti-myc (Santa Cruz) or the anti-HA (Roche) antibody for 3 h at 4°C.

Quantitative, multiplex PCR amplification mixtures contained 1 \times Platinum *Taq* polymerase buffer (Invitrogen), 1.5 mM MgCl₂, 0.2 mM of each deoxynucleoside triphosphate, 1.6 μ Ci of [α -³²P]dATP (Amersham), 0.2 μ M *POL1* primer pair, 0.2 μ M *ADE5,7* primer pair, 1.5 U of Platinum *Taq* polymerase

(Invitrogen), and 2 μ l of the immunoprecipitated chromatin sample or diluted input DNA (previously determined to be in the linear range for amplification) in a 15- μ l reaction mixture volume. PCR parameters were 94°C for 4 min; 94°C for 30 s, 52°C for 30 s, and 65°C for 1 min for 26 cycles; and then 65°C for 5 min. PCR products were resolved on 6% polyacrylamide gels and quantified by phosphorimaging analysis. The sequences of the primer pairs were previously described for *POL1* (56) and *ADE5,7* (51).

RESULTS

SAGA and SWI/SNF components are important for resistance to adenine analog 4-APP. In a screen of the yeast deletion collection with the adenine analog 4-APP, we identified ~50 analog-sensitive mutant strains (Rolfes et al., unpublished). The mutants were divided into functional classes. One class contained genes involved in transcription, including *BAS1* and *PHO2*, which are necessary for expression of the adenylate biosynthetic genes (reviewed in reference 42). This class of mutants also included the genes *ADA1*, *ADA3*, *GCN5*, *SPT20*, and *SPT8*, which encode subunits of the SAGA complex, and *SNF2*, *SNF5*, and *SWI3*, which encode subunits of the SWI/SNF complex. Because we identified multiple subunits from

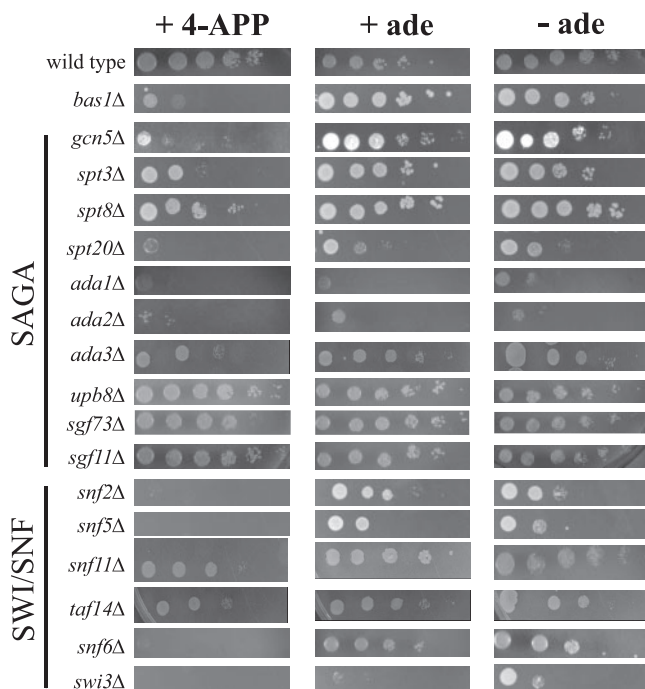


FIG. 1. SAGA and SWI/SNF mutant strains are sensitive to the adenine analog 4-APP. Strains with deletion mutations in the SAGA and SWI/SNF complexes were grown to equivalent cell densities, serially diluted (1:10), and spotted on complete medium containing 4-APP, containing adenine, or lacking adenine. Strains were allowed to grow for approximately 48 h and were photographed.

both complexes, we hypothesized that these complexes are regulators of *ADE* gene expression. To confirm and extend this initial observation, we assessed the 4-APP sensitivity of strains carrying mutations in all available identified subunits of the SAGA and SWI/SNF complexes.

As shown in Fig. 1, we found that these eight originally identified strains were sensitive to 4-APP, as indicated by decreased growth of the mutants on SC medium containing 4-APP compared to the growth of the wild-type strain. We also observed sensitivity to 4-APP in five additional strains that carried deletion alleles of the genes *SPT3*, *ADA2*, *SNF11*, *TAF14*, and *SNF6*. These 13 mutations encode the majority of the nonessential subunits of the SAGA and SWI/SNF complexes (49, 62).

We observed that the sensitivity to 4-APP varied in the different mutant strains. Some of the strains with mutations in the SAGA components exhibited strong sensitivity to 4-APP, including the *spt3Δ* and *spt8Δ* mutants, whereas others, like the *ada3Δ* strain, exhibited only weak sensitivity to 4-APP. Some of the strains had a secondary slow-growth phenotype and an additional 4-APP sensitivity that was very strong for the *gcn5Δ* mutant and weak for the *spt20Δ*, *ada1Δ*, and *ada2Δ* strains. Strains carrying mutations in the other identified SAGA subunit genes, *UBP8*, *SGF73*, and *SGF11* (17, 37), were also tested for a 4-APP sensitivity phenotype; however, these strains showed no appreciable sensitivity to 4-APP (Fig. 1).

Likewise, we observed a range of sensitivities to 4-APP in strains with mutations in different components of the SWI/SNF complex. Strong 4-APP sensitivity was detected in the *snf2Δ*,

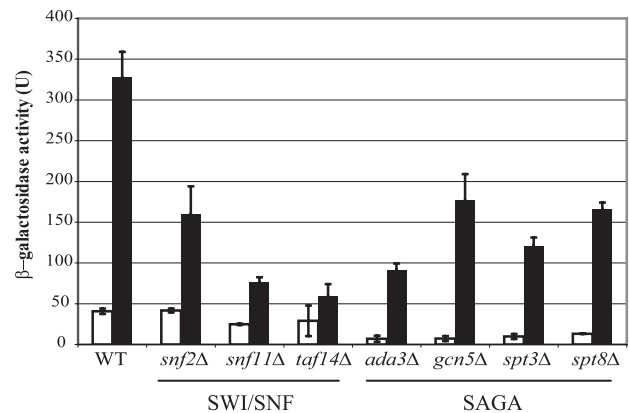


FIG. 2. Expression of a $UAS_{ADE5,7}lacZ$ reporter is compromised in SAGA and SWI/SNF mutants. The wild-type strain (BY4741/BY4742) and isogenic strains with mutations in subunits of SWI/SNF (*snf2Δ*, *snf11Δ*, and *taf14Δ* strains) and SAGA (*gcn5Δ*, *spt3Δ*, *spt8Δ*, and *ada3Δ* strains) were transformed with plasmid pR133 (44), which carries the $UAS_{ADE5,7}lacZ$ reporter. Strains were grown in SC medium containing adenine (open bars) or lacking adenine (filled bars). Extracts were prepared from cell pellets, and β -galactosidase activities were measured in duplicate assays on at least three individual isolates. Error bars indicate standard deviations.

snf5Δ, and *snf6Δ* mutant strains, whereas the *snf11Δ* and *taf14Δ* mutant strains exhibited only a weak sensitivity to 4-APP. A strain with the *swi3Δ* mutation was also extremely sensitive to 4-APP, although it was severely growth compromised even on SC medium containing adenine.

Together these results indicated that the absence of catalytic activity found in Gcn5 and Snf2, as well as the loss of key structural subunits provided by Ada1 and Spt20, led to the greatest degree of 4-APP sensitivity. Interestingly, we did not identify subunits from other remodeling complexes such as NuA3, ISWI, or Ino80. These findings indicate that the SAGA and SWI/SNF complexes play a critical role in *ADE* gene expression.

SAGA and SWI/SNF mutants have decreased *ADE* gene reporter expression. To determine if mutations in SAGA and SWI/SNF affect *ADE* gene expression, we assayed expression of a *lacZ* reporter in strains carrying deletion mutations for subunits of the SAGA and SWI/SNF complexes. This *lacZ* reporter contains binding sites for Bas1 and Pho2 from the *ADE5,7* promoter ($UAS_{ADE5,7}$) in a heterologous context (*CYC1-lacZ*). This plasmid was chosen because the insert was extensively characterized by mutational analysis as the minimum necessary for regulation and because it is strongly derepressed by adenine limitation (44). Strains with mutations in subunits of SWI/SNF (*snf2Δ*, *snf11Δ*, and *taf14Δ*) and SAGA (*gcn5Δ*, *spt3Δ*, *spt8Δ*, and *ada3Δ*) were transformed with this reporter and assayed for effects on expression. We were unable to obtain stable transformants for several of the mutant strains after multiple attempts (*swi3Δ*, *snf5Δ*, *snf6Δ*, *ada2Δ*, *ada1Δ*, and *spt20Δ* strains).

As shown in Fig. 2, we found that each mutant strain was unable to stimulate expression of the $UAS_{ADE5,7}lacZ$ reporter to the level of the wild type under derepressing conditions (SC medium lacking adenine). The greatest effect on β -galactosidase activity was observed in the *ada3Δ*, *snf11Δ*, *spt3Δ*, and

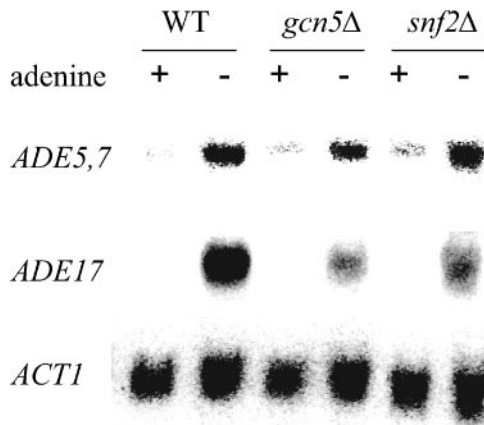


FIG. 3. Expression of *ADE5,7* and *ADE17* is reduced in *gcn5Δ* and *snf2Δ* mutants. RNA was prepared from wild-type (WT) (FY1353) and *gcn5Δ* (FY1354) and *snf2Δ* (FY1360) mutant strains that were harvested after 30 min of growth under repressing conditions (SC medium with adenine) and derepressing conditions (SC medium without adenine). RNA was blotted onto nylon membranes and hybridized to probes for *ADE5,7*, *ADE17*, and *ACT1*. Results were quantified by phosphorimaging analysis, and the levels of *ADE5,7* and *ADE17* were normalized to the reference *ACT1* level.

taf14Δ strains, with expression at approximately 20 to 41% of the wild-type activity. Expression of the reporter was also significantly affected in the *snf2Δ*, *gcn5Δ*, and *spt8Δ* mutant strains, in which β -galactosidase levels decreased to about 56 to 65% of the wild-type level. All of the mutants, with the possible exception of the *taf14Δ* mutant, responded to adenine limitation by increasing expression of the reporter gene; however, the extent of the derepression in each mutant strain was smaller than that found in the wild type. This observation indicates that the mutant strains were able to generate and receive the intracellular derepression signal to stimulate transcription of the *ADE* genes but were unable to fully initiate transcription.

Expression of *ADE* genes is dependent on SAGA and SWI/SNF. We have shown that SAGA and SWI/SNF components are necessary for efficient *lacZ* reporter expression driven by the *ADE5,7* promoter and regulatory region. To investigate the involvement of SAGA and SWI/SNF in *ADE* gene expression, we assayed steady-state RNA levels expressed from the native loci in mutant and wild-type strains using Northern analysis. We chose to assay transcript levels from two well-characterized *ADE* genes, *ADE5,7* and *ADE17* (3, 39, 44), to represent the *ADE* regulon and to use the *gcn5Δ* and *snf2Δ* mutants defective in the catalytic activities found in each of the complexes. We found that the *gcn5Δ* and *snf2Δ* mutant strains were unable to derepress *ADE5,7* and *ADE17* to the wild-type levels (Fig. 3), and no effect was observed on the repressed levels. In the *gcn5Δ* mutant, expression of the *ADE17* and *ADE5,7* loci was reduced to 62 to 63% of the wild-type level, whereas in the *snf2Δ* mutant, expression was modestly reduced to 71 to 82% of the wild-type level. Thus, the SAGA and SWI/SNF chromatin remodeling complexes are required to achieve full expression of at least these members of the *ADE* regulon.

These results show that efficient transcription depends upon the SAGA and SWI/SNF remodeling complexes in addition to

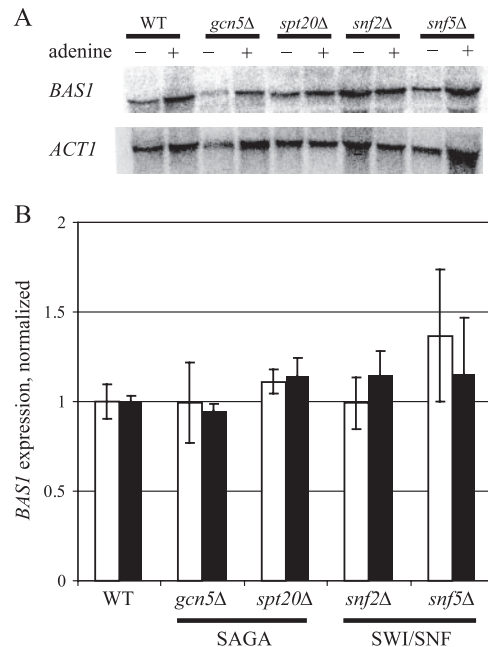


FIG. 4. Expression of *BAS1* is not affected in strains with mutations in SAGA and SWI/SNF. (A) The isogenic wild-type (WT) strain and strains carrying mutations in the *gcn5* and *spt20* genes of SAGA and the *snf2* and *snf5* genes of SWI/SNF were grown in SC medium containing adenine and SC medium lacking adenine. Total RNA was purified from harvested cells, blotted onto nylon membranes, and hybridized to radiolabeled probes for *BAS1* and *ACT1*. This experiment was performed twice, and results from a representative Northern analysis are shown. (B) The results were quantified by phosphorimaging analysis, and the *BAS1/ACT1* ratio was normalized to the wild-type *ACT1* level. Samples were analyzed in duplicate, and the error bars indicate the ranges. Open bars, SC medium containing adenine; filled bars, SC medium lacking adenine.

the Bas1 and Pho2 transcription factors. Several mechanisms could account for the decrease in the *lacZ* reporter and native gene expression that we detected in the mutants. The most likely mechanisms are that the Bas1 and Pho2 factors recruit the remodeling complexes that are necessary for the subsequent engagement of RNA polymerase II or that the remodeling complexes are necessary for the binding of Bas1, Pho2, or both to the *UAS_{ADE}*. Alternatively, an indirect mechanism might be used if the expression of the activator genes *BAS1* and *PHO2* or the synthesis of the signaling molecules SAICAR and AICAR depends on the remodeling complexes. We experimentally address these possibilities below.

SAGA and SWI/SNF mutations do not affect *BAS1* or *PHO2* expression. If expression of the *BAS1* or *PHO2* genes strongly depended on the SAGA and SWI/SNF complexes, then the levels of the activator proteins might be lower in the mutants, decreasing *ADE* gene expression. To determine if the expression of *BAS1* was decreased in SAGA and SWI/SNF mutants, we performed Northern analysis on RNA isolated from strains carrying mutations in the genes encoding structural subunits (*spt20Δ* and *snf5Δ*) and catalytic subunits (*gcn5Δ* and *snf2Δ*) of both complexes. As shown in Fig. 4, we found that strains carrying the deletion mutations were not significantly affected in the transcription of *BAS1*. We also observed that the expression of *BAS1* was not dependent on exogenous adenine in

both the mutant strains and the wild-type strain, consistent with a previous report (65).

Previous reports demonstrated that *PHO2* expression is not affected by mutations in the *SPT7* and *GCN5* genes of SAGA or in the *SNF2* and *SWI1* subunits of SWI/SNF (28, 30, 55). We performed Northern analysis on RNA prepared from our strains and observed no difference in expression of *PHO2* in *gcn5Δ*, *spt20Δ*, *snf2Δ*, and *snf5Δ* mutants (data not shown), in agreement with the previously published reports. Together, these results indicate that mutations in catalytic and structural subunits of SAGA and SWI/SNF do not substantially alter the expression of the genes encoding the transcription factors Bas1 and Pho2.

SAGA and SWI/SNF mutants are defective in *ADE* expression with a constitutively active Bas1-Pho2 fusion protein. A second explanation for the decrease in *ADE* gene expression is that the derepression signals SAICAR and AICAR (39) are produced at lower levels in the mutants. Basal expression of the *ADE* genes is high in *Saccharomyces* (3, 39); thus, when feedback inhibition is initially relieved, basal levels of the pathway enzymes allow for immediate synthesis of downstream products, including the intracellular signals SAICAR and AICAR. If mutations in subunits of the SAGA and SWI/SNF decreased the basal expression of enzymes prior to formation of SAICAR and AICAR, then the ability to generate the derepression signal might be attenuated, leading to decreased reporter and native gene expression.

The role of the regulatory signal is to promote interaction between DNA-bound Bas1 and Pho2 (36, 65). This interaction is critical for unmasking latent activation domains in Bas1. To circumvent the need for the signal, we used a Bas1-Pho2 fusion protein (36). This fusion protein constitutively stimulates expression of target genes and is independent of exogenous adenine levels and the intracellular signals (36). To prevent competition between native Bas1 and the Bas1-Pho2 fusion protein for binding sites, we deleted the *BAS1* gene in the wild-type and remodeling mutant strains prior to transformation with plasmids that express Bas1 or the Bas1-Pho2 fusion protein and the *lacZ* reporters. Because the gene encoding the Bas1-Pho2 fusion protein was marked with *URA3*, we could not use plasmid pR133 as we did above but instead used two different *lacZ* reporters. The *ADE4-lacZ* and *ADE5,7-lacZ* reporters carry the core promoters and regulatory regions from the native *ADE4* and *ADE5,7* genes, and both reporters are carried on plasmids marked by *LEU2*. We then asked whether expression of the *ADE-lacZ* reporter genes was altered in the SAGA and SWI/SNF mutants.

As shown in Fig. 5, in the absence of any Bas1 protein, we found only basal, nonderepressible expression of both the *ADE5,7-lacZ* and *ADE4-lacZ* reporters in all strains, whereas strains expressing Bas1 stimulated adenine-regulated expression in the wild-type strain and decreased expression in the remodeling mutants. As expected, the Bas1-Pho2 fusion was not repressible by adenine and it promoted higher reporter gene expression than the Bas1 and Pho2 proteins in the strain with wild-type remodeling complexes (36). Interestingly in the *gcn5Δ* and *snf2Δ* mutants, the Bas1-Pho2 fusion protein was able to express the *ADE5,7-lacZ* reporter to only 20 to 30% of the wild-type level and the *ADE4-lacZ* reporter to only about 10 to 20% of the wild-type level (Fig. 5A and B). Thus, engi-

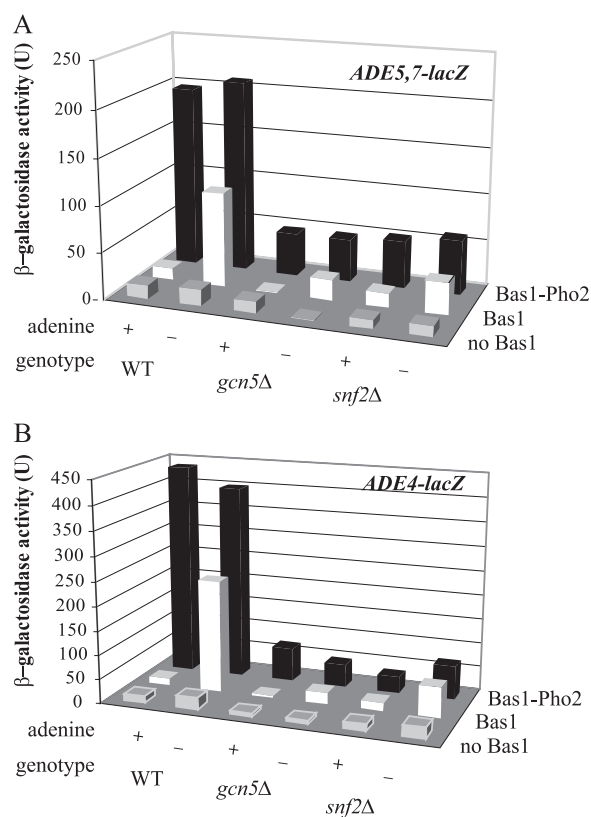


FIG. 5. A constitutively active Bas1-Pho2 fusion protein promotes only low-level expression of *ADE-lacZ* reporters in *gcn5Δ* and *snf2Δ* mutant strains. The wild type (RR436) and strains carrying mutations in *gcn5* (RR437) and *snf2* (RR439) were transformed with empty vector (YCp50), Bas1 (pR201), or the Bas1-Pho2 fusion (B273), as well as reporter plasmids for *ADE5,7-lacZ* (pR115 in panel A) or *ADE4-lacZ* (pR109 in panel B). Strains were grown in SC medium containing or lacking adenine. Extracts were prepared from cell pellets, and β -galactosidase activities were measured in duplicate assays for at least three individual isolates.

neering the transcriptional activators to be independent of the depression signal did not overcome the requirement for the remodeling complexes. These data are consistent with a model in which SAGA and SWI/SNF are involved in promoting transcription after reception of the regulatory signal by the activators, by making the chromatin template more accessible to the transcriptional machinery. However, these data are also consistent with the model that SAGA and SWI/SNF are involved in the binding of the activators to the UAS_{ADE} , affecting Bas1-Pho2 fusion protein occupancy. Since these roles are not mutually exclusive, the remodelers may have both activities.

SAGA and SWI/SNF are present at the *ADE5,7* promoter in both repressing and derepressing conditions. The data presented above suggest that Bas1 and Pho2 are capable of recruiting the SAGA and SWI/SNF complexes. We wanted to determine whether we could detect the presence of these complexes at an *ADE* gene promoter. We performed ChIP assays, using a tagged subunit found in each remodeling complex, Spt7-myc for SAGA and Snf2-myc for SWI/SNF. These tagged strains were used by other researchers to investigate the association of SAGA and SWI/SNF with the *ARG1* and *SNZ1*

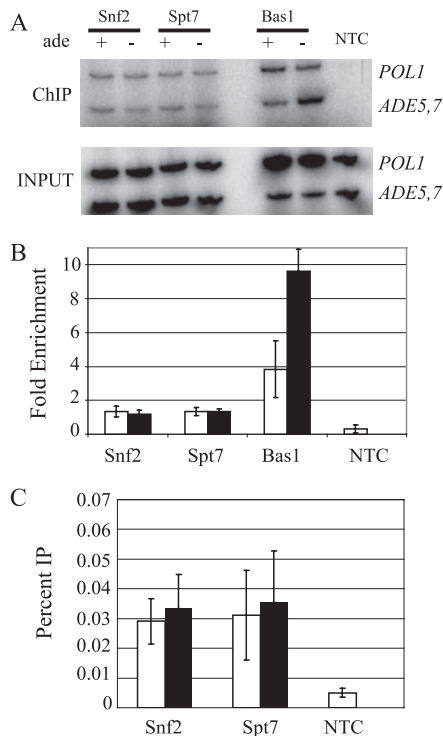


FIG. 6. SAGA and SWI/SNF occupy the *ADE5,7* promoter independently of adenine. (A) ChIP assays were performed using antibodies to the myc tag on proteins Snf2, Spt7, and Bas1 along with an NTC strain, FY1353. A 500-fold dilution of the input control and the undiluted IP DNA were amplified by PCR by using primers specific for *ADE5,7* and *POL1* in the presence of [α - 32 P]dATP. Results from a representative ChIP are shown. The PCR products were resolved on 6% polyacrylamide gels, visualized, and quantified by phosphorimaging analysis. Enrichment (n -fold) was calculated as the ratios of the *ADE5,7* signals in the IP-to-input samples and normalized for the corresponding ratios calculated for *POL1* in the IP-to-input samples. (B) The resulting values from three ChIP experiments were plotted as the averages, with standard deviations, of the results from PCR amplifications of chromatin-immunoprecipitated DNA from three independent experiments. Strains grown with adenine are represented by open bars, and strains grown in medium lacking adenine are represented by filled bars. (C) Calculation of the percent IP was carried out by dividing the IP signal for *ADE5,7* by the corresponding input signal. For all strains, values were significant, $P \leq 0.05$, compared to the NTC. Bars are as defined for panel B.

genes (38, 64). We assayed binding of SAGA and SWI/SNF at the promoter of the *ADE5,7* gene relative to their binding at the *POL1* ORF and asked if their binding increased under the derepressing conditions of SC medium without adenine.

We found that Spt7-myc and Snf2-myc are present at the promoter of the *ADE5,7* gene independently of the presence or absence of adenine (Fig. 6A). Relative to the occupancy of Bas1-myc, we found lower association of both Spt7-myc and Snf2-myc, although in both cases we were able to chromatin immunoprecipitate the promoter fragment significantly more highly than in the no-tag control (NTC) (Fig. 6B). We also calculated the percent IP and observed a seven- to ninefold increase in Snf2-myc and Spt7-myc relative to the NTC strains, indicating their presence at the *ADE5,7* promoter (Fig. 6C). Somewhat surprisingly, we found that both Spt7-myc and Snf2-myc were able to chromatin immunoprecipitate to *POL1*, in-

dicating that SAGA and SWI/SNF are almost as equally abundant in the *POL1* ORF as they are at *ADE5,7* (Fig. 6A). Thus, in comparing the ratio of ChIP at *ADE5,7* to that at *POL1*, we observed a ratio slightly above 1, at about 1.3 for each sample (Fig. 6B), indicating that SAGA and SWI/SNF were only slightly more likely to be found at the *ADE5,7* promoter than at the *POL1* ORF. A similar observation was made by Kim et al. (18), who showed that the ratio of the *HIS3/POL1* ChIP signal was only slightly above 1. We conclude that both SAGA and SWI/SNF have low-level association with the *ADE5,7* promoter that is not increased under derepressing conditions.

SAGA and SWI/SNF association with the *ADE5,7* promoter is independent of Bas1 and Pho2. The association of SAGA and SWI/SNF at *ADE5,7* might depend upon the transcriptional activator Bas1 or Pho2. We performed ChIP assays using an antibody to the myc tag on Spt7 and Snf2 in strains that carried null alleles of *bas1* or *pho2* (Fig. 7). Occupancy of SAGA and SWI/SNF was unchanged by the absence of either transcription factor.

SAGA and SWI/SNF affect factor promoter occupancy of Bas1 and Pho2. We explored the possibility that SAGA and SWI/SNF affect the binding of Bas1 or Pho2 to UAS_{ADE} , altering their occupancy. Previous work from our laboratory, using ChIP assays, showed that Bas1 binds several *ADE* promoters under repressing conditions and its occupancy increases by approximately 2.2-fold under derepressing conditions (51). We used ChIP analysis to look for the presence of the Bas1 protein at the promoter of the *ADE5,7* gene when strains harbored the *gcn5* Δ and *snf2* Δ deletion alleles. Interestingly, we found that the absence of Gcn5 or Snf2 affects the binding of Bas1-myc to *ADE5,7* (Fig. 8A). Under the repressing conditions of SC medium containing adenine, Bas1 is present at the promoter in the mutants at the same level as that found in the wild type. However, under derepressing conditions of SC medium lacking adenine, binding of Bas1 to the promoter is clearly reduced in the mutants (Fig. 8A). These results indicate that the absence of SAGA and SWI/SNF activity decreases Bas1 occupancy at *ADE5,7*.

Similar results were observed with the binding of Pho2 to the *ADE5,7* promoter. We used ChIP analysis to look for the presence of the Pho2 protein at the promoter of the *ADE5,7* gene when strains harbored the *gcn5* Δ and *snf2* Δ deletion alleles. We found that the absence of Gcn5 or Snf2 decreases the binding of Pho2-HA to *ADE5,7* under derepressing conditions but not under repressing conditions (Fig. 8B). The reduction in Pho2 binding could be direct or indirect through the decreased binding of Bas1, as we have shown elsewhere that Pho2 is unable to efficiently bind to the promoter in the absence of Bas1 (51). However, clearly the occupancy of this protein is affected by the loss of Gcn5 and Snf2.

Together, these results demonstrate a role for the SAGA and SWI/SNF complexes in facilitating wild-type levels of occupancy of the Bas1 and Pho2 transcription factors at the promoters under derepressing conditions but with little to no effect under repressing conditions. In the absence of the remodeling complexes, formation of the transcriptionally active Bas1-Pho2 heterodimer is reduced, with effects on the expression of the target *ADE* genes. However, SAGA and SWI/SNF are not apparently recruited by these activators.

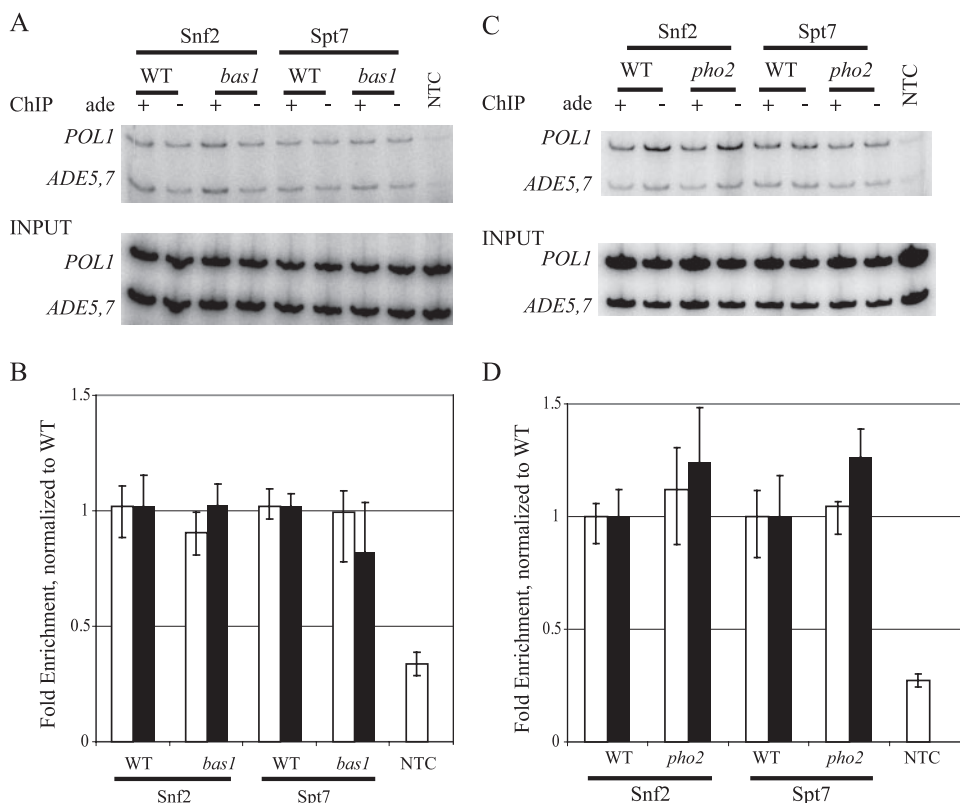


FIG. 7. SAGA and SWI/SNF associate with the *ADE5,7* promoter independently of the Bas1 and Pho2 activators. (A) ChIPs of Spt7-myc and Snf2-myc were performed in wild-type and *bas1*Δ mutant strains (RR441 and RR443) along with an NTC strain, FY1353. (B) Graphical representation of the ChIP results. The ChIP data were plotted, showing the averages and standard deviations of results from PCR amplifications of chromatin immunoprecipitated from three independent experiments and normalized to wild-type levels. (C) ChIPs of Spt7-myc and Snf2-myc were performed in wild-type and *pho2*Δ mutant strains (RR442 and RR444). (D) Graphical representation of the averages and standard deviations of data from PCR amplifications of chromatin immunoprecipitated from three independent experiments and normalized to wild-type levels. WT, wild type. For panels B and D, open and filled bars represent strains grown in medium with and without adenine, respectively.

ADE gene regulation requires SAGA and SWI/SNF catalytic activity. Our results indicate that both SAGA and SWI/SNF are necessary for *ADE* gene transcription; however, disruption of either complex alone lowered but did not completely eliminate *ADE* gene expression. Therefore, we hypothesized that the loss of both Gcn5 and Snf2 would exacerbate the reduction of *ADE* gene expression below that observed in either of the single mutants. We obtained a strain carrying mutations in both *gcn5* and *snf2* from Fred Winston (41); the mutant showed that the combined loss of the two genes resulted in very poor growth even on rich medium. We examined the sensitivity of the *gcn5*Δ *snf2*Δ strain to the adenine analog 4-APP to determine if sensitivity was increased relative to the single mutant strains. As shown in Fig. 9, each of the single *gcn5*Δ and *snf2*Δ mutants in this new strain background showed significant sensitivity to 4-APP compared to the wild type. Interestingly, the double mutant strain was extremely sensitive to 4-APP, much more so than either of the single mutants and about as sensitive as the *bas1*Δ strain (Fig. 9A). This finding suggests additive roles of the SAGA and SWI/SNF remodeling activities at the *ADE* genes.

We confirmed that this sensitivity to 4-APP was correlated with a loss of expression of the $UAS_{ADE5,7-lacZ}$ reporter (65). We found that both single mutant strains in this background

were unable to fully derepress transcription (Fig. 9B). The *snf2*Δ mutation reduced transcription to approximately 35% of that in the isogenic wild type, whereas the *gcn5*Δ mutation had a stronger effect, reducing transcription to 10% of the wild-type level. The effect on gene expression in this strain background was more severe than what we had detected in the Research Genetics diploid knockout strain (Fig. 2). Interestingly, transcription in the *gcn5*Δ *snf2*Δ double mutant was barely detectable and virtually the same as that found under the repressing condition. Thus, the loss of catalytic activity from both complexes eliminated the ability to derepress the *ADE5,7* gene. We obtained the same result using alternate *ADE5,7-lacZ* and *ADE4-lacZ* reporters (pR109 and pR115; data not shown): loss of either catalytic subunit alone decreased expression under derepressing conditions, but the loss of both activities resulted in only basal expression and the elimination of derepression.

To confirm the result from the *lacZ* reporter assays, we assessed expression from the chromosomal *ADE5,7* and *ADE17* loci in wild-type and mutant strains using Northern analysis. For each gene assayed, transcripts were barely detectable in double mutant strains (Fig. 9C). This result was consistent with the *lacZ* reporter expression. These results indicate

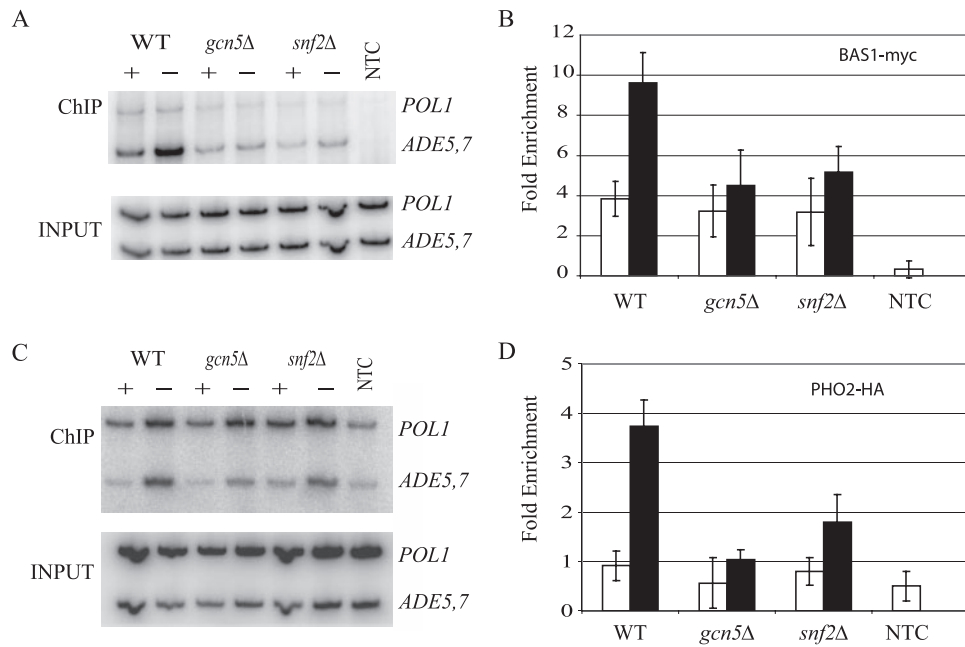


FIG. 8. Promoter occupancy of Bas1 and Pho2 is decreased in SAGA and SWI/SNF mutants. (A) ChIPs of Bas1-myc were performed with wild-type (RR433), *gcn5Δ* (RR434), and *snf2Δ* (RR435) strains along with an NTC strain (FY1353). (B) Enrichment (*n*-fold) of Bas1 binding. ChIP assays were performed on strains grown with adenine (open bars) and without adenine (filled bars). PCR amplification from the ChIP experiments was quantified as the ratio of signal in the IP relative to input DNA, normalized to the *POL1* signals. The average values from three independent ChIP experiments are plotted, with the standard deviations; mutants were significantly different from the wild type under derepressing conditions ($P \leq 0.05$). (C) ChIPs of Pho2-HA were performed with wild-type (RR430), *gcn5Δ* (RR431), and *snf2Δ* (RR432) strains along with the NTC strain (FY1353). (D) Enrichment (*n*-fold) of Pho2 binding. ChIP analyses were prepared from strains grown with adenine (open bars) and without adenine (filled bars). PCR amplification from the ChIP experiments was quantified as the ratio of signal in the IP relative to input DNA, normalized to the *POL1* signals. The average values from three independent ChIP experiments are plotted, with the standard deviations; mutants were significantly different from the wild type under derepressing conditions ($P \leq 0.05$).

that both the SAGA and SWI/SNF complexes are required for wild-type levels of *ADE* gene expression.

DISCUSSION

Genome-wide studies using DNA microarrays have investigated the loss of activity of the SAGA and SWI/SNF complexes. In one study, Holstege and colleagues (15) investigated the loss of both complexes from cells grown in rich (yeast extract-peptone-dextrose) medium, a condition in which the *ADE* regulon is repressed. Not surprisingly, their data showed that there was little to no difference in expression of the *ADE* regulon genes in the *gcn5* and *snf2* mutants relative to the wild type under these repressing conditions. Sudarsanam and colleagues (55) compared gene expression from cells grown in minimal medium with that from cells grown in rich medium to identify additional genes dependent upon SWI/SNF. About 1% of the genome showed an effect of threefold or greater. A great many genes, including several *ADE* genes, had small differences of less than threefold in gene expression in the *snf2* and *swi1* mutants. While these expression effects are small, they might nonetheless be significant under certain growth conditions.

In this report we demonstrate that the SAGA and SWI/SNF complexes are required for full expression of the *ADE4*, *ADE5,7*, and *ADE17* genes, members of the *ADE* regulon. These remodeling complexes were identified by a genome-

wide screen for 4-APP sensitivity, a phenotype exhibited by *bas1Δ* and *pho2Δ* mutants (Fig. 1) (Rolfes et al., unpublished), suggesting a role for the remodeling complexes in the expression of *ADE* genes. In some instances, the recruitment of the SAGA complex and its Gcn5 bromodomain have been shown to stabilize the SWI/SNF complex at promoter nucleosomes (13); thus, it was striking that the screen identified both the SAGA and SWI/SNF complexes. Indeed, the SAGA and SWI/SNF mutant strains exhibited decreased gene expression from several *ADE* genes in *lacZ* reporter and Northern assays (Fig. 2 and 3). Our data are consistent with the data from the Winston laboratory (55) in terms of the magnitude of the effect detected on transcription. Notably, however, we found that this difference in expression due to the loss of SAGA and SWI/SNF imposed a biological effect, namely, a lower tolerance for nucleotide pool imbalances.

Our data also indicate that the SAGA and SWI/SNF complexes are performing nonredundant roles in *ADE* gene expression. The double deletion strain was unable to derepress expression to any significant level above basal expression at *ADE5,7-lacZ* (Fig. 9B) or at native *ADE5,7* and *ADE17* loci (Fig. 9C). Thus, activity from either SAGA or SWI/SNF is necessary for a minimum derepression but full expression required each complex. We conclude that these complexes are performing complementary roles in modifying and remodeling chromatin necessary for *ADE* gene expression.

However, it is interesting that we observed virtually no

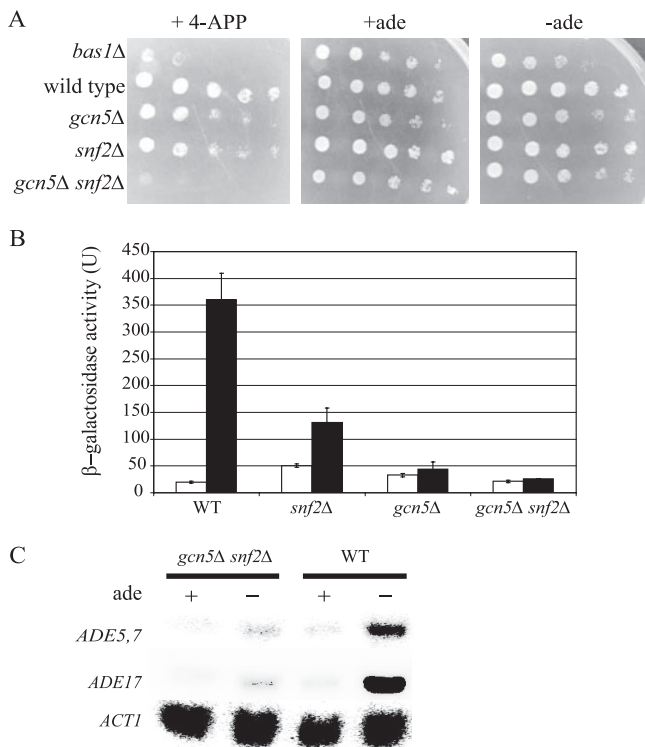


FIG. 9. *gcn5* and *snf2* are nonredundant for *ADE* transcription. (A) Wild-type, single mutant (*gcn5Δ* or *snf2Δ*), and double mutant (*gcn5Δ snf2Δ*) strains were grown to equivalent cell densities; serially diluted (1:10); spotted onto SC medium, SC medium containing 4-APP, and SC medium lacking adenine; and allowed to grow for approximately 48 h at 30°C. (B) The parental wild-type and the single and double mutant strains were transformed with a $UAS_{ADE5,7}lacZ$ reporter (pR133). Strains were grown overnight in SC medium, inoculated into SC medium containing (open bars) or lacking (filled bars) adenine, and assayed for β -galactosidase activity. β -Galactosidase activities were measured in duplicate assays on at least three individual isolates. (C) Expression of *ADE5,7* and *ADE17* in parental wild-type, *gcn5Δ* and *snf2Δ* single mutant, and *gcn5Δ snf2Δ* double mutant strains was measured by Northern analysis. RNA was prepared from the four strains that had been grown in SC medium and transferred to SC medium lacking adenine. Expression was normalized to *ACT1* transcript levels, and the experiment was repeated to confirm results.

4-APP sensitivity in the *ubp8*, *sgf11*, and *sgf73* mutant strains. This finding suggests that the activities performed by these components, such as deubiquitination, may not be critical for *ADE* gene expression. Alternatively, perhaps these subunits are not required for global surveillance (discussed below).

Association of the Bas1 and Pho2 activators with $UAS_{ADE5,7}$ requires chromatin remodelers. Many well-studied activators, such as Gcn4, have been shown to recruit the SAGA and SWI/SNF remodeling complexes during transcriptional activation (9). Less studied is the role of these complexes in the binding of transcription factors to their binding sites (32). We found that the SAGA complex (via Spt7-myc) and the SWI/SNF complex (via Snf2-myc) associate with the *ADE5,7* promoter at a low but significant level that was surprisingly not affected by the SAICAR/AICAR regulatory signal or the presence of the activators Bas1 and Pho2. However, we found that an increase in Bas1 and Pho2 binding that occurs under derepressing conditions was dependent on SAGA and SWI/SNF.

This result was strengthened by the observation that a constitutively active Bas1-Pho2 fusion protein also required the remodeling complexes.

Our data show that the SAGA and SWI/SNF remodeling complexes are not recruited by the Bas1 or Pho2 activator, yet these remodelers associate with the *ADE5,7* gene and their activity is important for the increased binding of the activators and for transcription. This finding agrees with models for the action of the remodeling complexes described by other investigators. Cosma et al. (2) demonstrated that binding of SBF (Swi4/Swi6) required the activity of both SAGA and SWI/SNF whereas the binding of Swi5 did not. The association of Pho4 with nucleosomal UAS2 at *PHO5* also required both Snf2 and Gcn5, although Pho4 binding at UAS1 did not require these remodelers (6, 52). Interestingly, both Swi5 and Pho4 cooperate with Pho2, but neither of these previous studies examined effects on Pho2 binding. We found a dependence on both SAGA and SWI/SNF for the binding of Pho2. While Bas1, Pho4, and Swi5 can all bind to their UAS elements in the absence of the remodeling complexes (presumably once Pho4 and Swi5 become nucleus localized), only Bas1 requires the remodelers to respond to the regulatory signal.

Both SAGA and SWI/SNF catalytic functions are important for *ADE* gene derepression. Our data point to a second role for the chromatin remodeling complexes in transcription. We found that transcription driven by the Bas1-Pho2 fusion protein depended on both Gcn5 and Snf2. Normally, Bas1 and Pho2 do not interact until the regulatory signal is generated; combining the genes to produce the fusion protein makes reception of the signal moot. The Bas1-Pho2 fusion protein promotes transcription constitutively at the *ADE1* (36) and *ADE5,7* and *ADE4* (Fig. 5) genes, indicating that it is constitutively bound to DNA, and yet it still requires the remodeling activities from both SAGA and SWI/SNF. Since a change in promoter occupancy under these conditions is unlikely, our data suggest that SAGA and SWI/SNF are involved at events downstream of factor binding, possibly remodeling chromatin for the binding of the general transcription factors or for transcriptional elongation. Further study will be necessary to define any additional roles.

Global surveillance. How might the SAGA and SWI/SNF complexes associate with the *ADE5,7* promoter since they are not recruited by Bas1 and Pho2? One possibility is that the complexes are recruited by another factor. Dhasarathy and Klade (6) showed that myc-tagged Snf2 and Gcn5 are recruited to *RPS22B* by Abf1, even though the remodelers are not required for expression of this locus. It is possible that Abf1, which binds to the *ADE5,7* promoter (44), recruits SAGA and SWI/SNF. However, since Abf1 is found only in the *ADE5,7* promoter and not in the promoters for other *ADE* genes such as *ADE4* and *ADE17* (25, 44, 63), this putative mechanism would not explain the transcriptional requirement for SAGA and SWI/SNF at these other *ADE* genes. Perhaps other factors not studied in this report facilitate or enhance SWI/SNF and SAGA association, as was observed for Mediator at the *ARG1* promoter (64).

Another explanation for the presence of SAGA and SWI/SNF at promoters is global surveillance. Through the global acetylation mechanism, HATs enzymatically modify histone proteins within large chromatin domains, including both cod-

ing sequences and intergenic regions, without any apparent DNA sequence specificity or specific recruitment. Kuo et al. (20) demonstrated a global role for the activity of the Gcn5 HAT. They showed that the level of H3 acetylation at many genes, including *ADE5,7*, was decreased in a *gcn5* mutant, relative to the wild type, when the Gcn5-containing complexes were not specifically targeted to these promoters. Other researchers have also described a similar phenomenon for the Gcn5 HAT (16, 20, 60).

Our data are consistent with this global role, not only for Gcn5-containing complexes but also for the first time for the SWI/SNF complex. Thus, it is possible that a global surveillance role, while not fully understood, could explain the dependence on these chromatin remodeling complexes for *ADE* gene transcription.

SAGA and SWI/SNF function as coactivators in *ADE* gene derepression. Our current model is that the presence of SAGA and SWI/SNF at the promoter facilitates efficient binding of the activators, Bas1 and Pho2, and thereby they function as coactivators in the derepression of *ADE* genes. SAGA and SWI/SNF are associated with *ADE* gene promoters as a part of their global surveillance role; we propose that they are present to efficiently and quickly turn on the *ADE* genes upon reception of the regulatory signal. There are other remodeling complexes previously shown to be poised under repressive conditions. NuA4, necessary for *PHO5* activation and chromatin remodeling, is critical for chromatin remodeling even prior to activation, demonstrating that NuA4 readies or poises the *PHO5* for activation (29). NuA4 is also present under repressing conditions, and, surprisingly, no increase in its association with promoters was detected upon induction. Interestingly, NuA4 was not actually required once the *PHO5* gene was activated; Nourani et al. proposed that targeted hyperacetylation of nucleosomal histones under repressive conditions prepared the promoter and potentiated a rapid remodeling step, followed by transcriptional induction in response to signals (29). Other researchers have proposed that chromatin remodelers are continuously required because there is rapid, dynamic equilibrium between active and repressive chromatin structures (6). This is a possibility for SAGA and SWI/SNF involvement in *ADE* gene activation as well, where *ADE* genes need to be turned on quickly and efficiently. This “poising” and the continuous presence of the complexes at low levels of binding, as suggested by other researchers (29), are more efficient for stimulating gene expression quickly and thus rapidly producing biosynthetic products.

SAGA and SWI/SNF may therefore associate and be poised at low levels to facilitate the first round of transcription and the increase in factor occupancy. We also cannot rule out the possibility that SAGA and SWI/SNF association will not increase at later time points after induction, not detected at the initial rounds of transcription as well. Interestingly, Dhasarathy and Kladdde observed that as late as 12 h after induction the occupancy of the activator Pho4 and the coactivators SAGA and SWI/SNF at the *PHO5* promoters reached the maximum (6). How this increased binding at later times would correlate with gene expression is not inherently clear. Remodelers might be required not only for initial opening of chromatin but also for the long-term stabilization of the preinitiation complex, allowing for repeated RNA polymerase II loading.

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