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 gen5Δ 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-ribose-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-repressible 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 (UASADE) 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 UASADE and unmasks the latent activation domain in Bas1 (12, 36, 51), 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...
analog 4-aminopyrazolo-(3,4d)-pyrimidine (4-APP) (R. J. Rolfe 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 (Rolfs 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 Gen5 subunit encodes a histone acetyltransferase (HAT). Gen5 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 Sp t 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 Gen5 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 Gen5 subunit of SAGA and the Snf2 component of SWI/SNF are required for efficient binding of Bas1 and Pho2 to the UASADE5,7 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 homogenous 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 Yeast Deletions Project website (http://sequence-www.stanford.edu/group/yeast_deletion_project/deletions.html). We obtained strains FY1352, FY1353, FY1354, and FY1360 from Fred Winston (41) and strains HQY367 and HQY453, respectively, that carry the bas1-10a::hisG allele. These strains were generated by replacement of the bas1-10a::hisG locus using the EcoRI-Sphl 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 bas1-10a::hisG. The presence of the bas1 deletion allele was confirmed by PCR analysis of the genomic locus and by assaying adenine bradytrophic phenotype (data not shown).

Strains RR436, RR437, RR440, RR441, and RR442 are derivatives of FY1353, FY1354, FY1355, and FY1360, respectively, that carry the pho2-10a::hisG allele. These strains were generated by integrative transformation with the EcoRI-Sphl 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 pho2-10a::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).

Strains RR434 and RR444 are derivatives of HQY367 and HQY453, respectively, that carry the pho2::hisG allele. These strains were generated by transformation with a modified plasmid R166 (plasmid construction details available upon request). Plasmid pR115 carries the ADE5,7-lacZ reporter, was constructed by inserting the lacZ gene into the ADE4 gene (51) and subcloning it into a LEU2 CEN6 vector (48) (plasmid construction details available upon request). Plasmid pR390 is a fusion of the lacZ gene to ADE5 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 (UASADE5,7) that was inserted into a heterologous system—the CYCl1 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 UASADE5,7-lacZ reporter and the former type as an ADE-lacZ reporter. Plasmids pR166 (51) and pR167 (65) carry the bas1-10a and pho2-10a alleles, respectively. Plasmid m3926 converts LEU2 to leu2::kanMX through a simple insertion (61). Plasmid pBC286 (1) expresses full-length Bas1, and plasmid B273, obtained from Odd Stokke Gabrielsen, expresses the Bas1-Pho2 fusion protein (36).
maximal expression of the $ADE5$ 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).

TABLE 1. Yeast strains

| Name      | Genotype                                      | Source or reference |
|-----------|-----------------------------------------------|---------------------|
| 4741/4742 | MATa/α his3Δ::his3Δ leu2Δ::leu2Δ lys2Δ::LYS2 MET15/met15Δ ura3Δ::ura3Δ | Research Genetics   |
| bas1Δ strain | Isogenic to 4741/4742 with $bas1Δ::kanMX/bas1Δ::kanMX$ | Research Genetics   |
| gcn5Δ strain | Isogenic to 4741/4742 with $gcn5Δ::kanMX/gcn5Δ::kanMX$ | Research Genetics   |
| snf2Δ strain | Isogenic to 4741/4742 with $snf2Δ::kanMX/snf2Δ::kanMX$ | Research Genetics   |
| spt3Δ strain | Isogenic to 4741/4742 with $spt3Δ::kanMX/spt3Δ::kanMX$ | Research Genetics   |
| snf6Δ strain | Isogenic to 4741/4742 with $snf6Δ::kanMX/snf6Δ::kanMX$ | Research Genetics   |
| snf11Δ strain | Isogenic to 4741/4742 with $snf11Δ::kanMX/snf11Δ::kanMX$ | Research Genetics   |
| fob4Δ strain | Isogenic to 4741/4742 with $fob4Δ::kanMX$ | Research Genetics   |
| spt3Δ strain | Isogenic to 4741/4742 with $spt3Δ::kanMX$ | Research Genetics   |
| snf2Δ strain | Isogenic to 4741/4742 with $snf2Δ::kanMX$ | Research Genetics   |
| snf6Δ strain | Isogenic to 4741/4742 with $snf6Δ::kanMX$ | Research Genetics   |
| swi3Δ strain | Isogenic to 4741/4742 with $swi3Δ::kanMX$ | Research Genetics   |
| FY1352 | MATa his3Δ::200 ura3Δ::52 leu2Δ:: lys2Δ::LYS2 gen5Δ::HIS3 snf2Δ::LEU2 | 41 |
| FY1353 | MATa his3Δ::200 ura3Δ::52 leu2Δ:: lys2Δ::LYS2 | 41 |
| FY1354 | MATa his3Δ::200 ura3Δ::52 leu2Δ:: lys2Δ::LYS2 gen5Δ::HIS3 | 41 |
| FY1360 | MATa his3Δ::200 ura3Δ::52 leu2Δ:: lys2Δ::LYS2 snf2Δ::LEU2 | 41 |
| HQY367 | MATa his3Δ::1 leu2Δ::200 met15Δ:: ura3Δ:: SNF2-14;::HIS3 | 64 |
| HQY453 | MATa his3Δ::1 leu2Δ::200 met15Δ:: ura3Δ:: SPT7-myc13;::HIS3 | 38 |
| RR376 | MATa BAS1-MYC13::kanMX6 PHO2 ura3Δ::52 | 51 |
| RR409 | MATa BAS1 PHO2-HA3:kanMX6 ura3Δ::52 | 51 |
| RR430 | MATa to FY1353 with PHO2-HA3:kanMX | This study |
| RR431 | MATa to FY1354 with PHO2-HA3:kanMX | This study |
| RR432 | MATa to FY1360 with PHO2-HA3:kanMX | This study |
| RR433 | MATa to FY1353 with BAS1-MYC13::kanMX | This study |
| RR434 | MATa to FY1354 with BAS1-MYC13::kanMX | This study |
| RR435 | MATa to FY1360 with BAS1-MYC13::kanMX | This study |
| RR436 | MATa to FY1360 with bas1-10Δ::hisG | This study |
| RR437 | MATa to FY1354 with bas1-10Δ::hisG | This study |
| RR439 | MATa to FY1360 with snf2Δ::leu2::kanMX | This study |
| RR440 | MATa to RR439 with bas1-10Δ::hisG | This study |
| RR441 | MATa to HQY367 with bas1-10Δ::hisG | This study |
| RR442 | MATa to HQY453 with bas1-10Δ::hisG | This study |
| RR443 | MATa to HQY367 with pho2-10Δ::hisG | This study |
| RR444 | MATa to HQY453 with pho2-10Δ::hisG | This study |

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 (Rolffes 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
Strains carrying mutations in the other identified SAGA subunits have been shown to be sensitive to 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 adenine, or lacking adenine. Strains were allowed to grow for approximately 48 h and were photographed. As shown in Fig. 1, we found that each mutant strain was sensitive to 4-APP, as indicated by decreased growth of the mutants on SC medium containing adenine. 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 adenine 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 spt20A, 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, 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 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 (UASADE5,7-lacZ) 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 repressed by adenine limitation (44). Strains with mutations in subunits of SWI/SNF (snf2Δ, snf11Δ, and taf14Δ) and SAGA (gcn5Δ, spt3Δ, 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 UASADE5,7-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, and spt3Δ, and
to derepress ADE5,7, and ADE17 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 gen5Δ and snf2Δ mutants defective in the catalytic activities found in each of the complexes. We found that the gen5Δ 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 gen5Δ 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 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, 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, 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 (snf20Δ and snf5Δ) and catalytic subunits (gen5Δ 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 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, gen5Δ, 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 gen5Δ and snf2Δ mutants defective in the catalytic activities found in each of the complexes. We found that the gen5Δ 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 gen5Δ 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
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 \( \text{gen}5\Delta, \text{spt}20\Delta, \text{snf}2\Delta, \) and \( \text{snf}5\Delta \) 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 \( \text{URA3} \), we could not use plasmid \( \text{pR113} \) as we did above but instead used two different lacZ reporters. The \( \text{ADE4-lacZ} \) and \( \text{ADE5,7-lacZ} \) reporters carry the core promoters and regulatory regions from the native \( \text{ADE4} \) and \( \text{ADE5,7} \) genes, and both reporters are carried on plasmids marked by \( \text{LEU2} \). We then asked whether expression of the \( \text{ADE-lacZ} \) 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 \( \text{ADE5,7-lacZ} \) and \( \text{ADE4-lacZ} \) 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 \( \text{gen}5\Delta \) and \( \text{snf}2\Delta \) mutants, the Bas1-Pho2 fusion protein was able to express the \( \text{ADE5,7-lacZ} \) reporter to only 20 to 30% of the wild-type level and the \( \text{ADE4-lacZ} \) reporter to only about 10% of the wild-type level (Fig. 5A and B). Thus, engineering the transcriptional activators to be independent of the derepression 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 \( \text{ARG1} \) and \( \text{SNZ1} \) promoters.
We found that Spt7-myc and Snf2-myc are present at the ADE5,7 gene when strains harbored the *gcn5Δ* and *snf2Δ* deletion alleles. We found that the absence of Gcn5 or Snf2 decreases the association of SAGA and SWI/SNF at the ADE5,7 promoter than in the wild type. However, under derepressing conditions of SC medium lacking adenine, binding of Bas1 to the promoter in the mutants at the same level as that found in the wild type.}

Similarly, we found 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 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-32P]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* < 0.05, compared to the NTC. Bars are as defined for panel B.

**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-32P]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* < 0.05, compared to the NTC. Bars are as defined for panel B.

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, indicating 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 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 *gcn5Δ* and *snf2Δ* 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 *gcn5Δ* and *snf2Δ* 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.
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/H9004 snf2/H9004 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 gcn5Δ and snf2Δ mutant strains 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 bas1Δ 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 snf2Δ mutation reduced transcription to approximately 35% of that in the isogenic wild type, whereas the gcn5Δ 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/H9004 snf2/H9004 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. 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 bas1Δ 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 pho2Δ 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.
that both the SAGA and SWI/SNF complexes are required for wild-type levels of \textit{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 \textit{ADE} regulon is repressed. Not surprisingly, their data showed that there was little to no difference in expression of the \textit{ADE} regulon genes in the \textit{gcn5}\textsuperscript{Δ} and \textit{snf2}\textsuperscript{Δ} 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 \textit{ADE} genes, had small differences of less than threefold in gene expression in the \textit{snf2}\textsuperscript{Δ} and \textit{swi1}\textsuperscript{Δ} 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 \textit{ADE4, ADE5,7, and ADE17} genes, members of the \textit{ADE} regulon. These remodeling complexes were identified by a genome-wide screen for 4-APP sensitivity, a phenotype exhibited by \textit{bas1}\textsuperscript{Δ} and \textit{pho2}\textsuperscript{Δ} mutants (Fig. 1) (Rolles et al., unpublished), suggesting a role for the remodeling complexes in the expression of \textit{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 \textit{ADE} genes in \textit{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 \textit{ADE} gene expression. The double deletion strain was unable to derepress expression to any significant level above basal expression at \textit{ADE5,7-lacZ} (Fig. 9B) or at native \textit{ADE5,7} and \textit{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 \textit{ADE} gene expression.

However, it is interesting that we observed virtually no
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 Gen5, 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 readily 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 Kladde 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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