In Aspergillus nidulans, proline can be used as a carbon and nitrogen source, and its metabolism requires the integration of three signals, including proline induction and nitrogen and carbon metabolite derepression. We have previously shown that the bidirectional promoter in the prnD-prnB intergenic region undergoes drastic chromatin rearrangements such that proline induction leads to the loss of positioned nucleosomes, whereas simultaneous carbon and nitrogen metabolite repression results in the partial repositioning of these nucleosomes. In the proline cluster, the inhibition of deacetylases by trichostatin A leads to partial derepression and is associated with a lack of nucleosome positioning. Here, we investigate the effect of histone acetylation in the proline cluster using strains deleted of essential components of putative A. nidulans histone acetyltransferase complexes, namely, gcnE and adaB, the orthologues of the Saccharomyces cerevisiae GCN5 and ADA2 genes, respectively. Surprisingly, GcnE and AdaB are not required for transcriptional activation and chromatin remodeling but are required for the repression of prnD and prnB and for the repositioning of nucleosomes in the divergent promoter region. Chromatin immunoprecipitation directed against histone H3 lysines K9 and K14 revealed that GcnE and AdaB participate in increasing the acetylation level of at least one nucleosome in the prnD-prnB intergenic region during activation, but these activities do not determine nucleosome positioning. Our results are consistent with a function of GcnE and AdaB in gene repression of the proline cluster, probably an indirect effect related to the function of CreA, the DNA-binding protein mediating carbon catabolite repression in A. nidulans.

SAGA (17). SAGA is a multiprotein complex whose Gcn5p subunit possesses histone acetyltransferase (HAT) activity. Gcn5p acetylates several lysine residues on the N termini of histones, including K9 and K14 on histone H3 and K8 and K16 on histone H4 (21). The Gcn5-containing complexes share several subunits, such as Ada2p, Ada3p, Spt3p, and Tra1p (24), and it has been estimated that the yeast SAGA complex regulates the expression of ~10% of S. cerevisiae genes, with approximately one-third of them being negatively regulated (23). Gcn5p forms a ternary complex with Ada2p and Ada3p, a complex which is conserved in SAGA and ADA (2). The proteins of the complex modulate the acetylating activity of Gcn5p (8, 17, 35) in patterns that are not identical for different promoters or different transcriptional activators (9, 33). In the context of transcriptional regulation, the SAGA complex can have different functions. The role of SAGA has been extensively studied in the S. cerevisiae GAL1 promoter and has been shown to be essential for GAL1 transcription. In the case of GAL1, the first step in transcriptional activation is the binding of the specific Gal4p activator which, in turn, recruits SAGA to the upstream activating sequence, and the upstream activating sequence-bound SAGA then promotes the binding of TATA-binding protein and assembly of the preinitiation complex (5, 22). In contrast to the GAL1 system, in the yeast ADE regulon SAGA is not recruited by the specific activators Bas1p and Pho2p (nor is SWI/SNF, another chromatin remodeling complex recruited in this promoter). Instead, in promoters of this regulon, SAGA function is required for the recruitment and
efficient binding of the specific activators (19). In the yeast nitrogen-carbon utilization regulatory interface, different components of SAGA have been shown to have different functions. The expression of both paralogous glutamate dehydrogenase genes, \textit{GDH1} and \textit{GDH3}, requires different SAGA components, depending on whether glucose or ethanol is the carbon source. \textit{GDH1} expression requires \textit{Ada2p} and \textit{Ada3p} on ethanol as a carbon source but only \textit{Ada3p} on glucose. In both cases, \textit{GDH1} expression is \textit{GCN5} independent (32). In contrast, \textit{GDH3} expression and chromatin remodeling activities in its cognate promoter, which are only seen under carbon derepressing conditions (ethanol), are dependent on the SAGA components \textit{GCN5}, \textit{ADA2}, \textit{ADA3}, and \textit{SPT3}.

There is little information on SAGA function in filamentous fungi. Recent work in \textit{Neurospora crassa} established that blue light-induced transcription of the early light-inducible genes \textit{al-3} and \textit{vvd} depends on increased acetylation of histone H3 at lysine K14 (H3K14) in the promoters of these genes (18). The authors showed that acetylation and transient gene activation require the \textit{N. crassa} \textit{GCN5} homologue \textit{ngf-1}, and it was suggested that the specific activator, White Collar-1, is required for the recruitment of \textit{NGF-1} to \textit{al-3} and \textit{vvd} promoters.

We have recently described the relationship between the transcriptional activation of a number of \textit{Aspergillus nidulans} promoters driving genes of primary metabolism and their chromatin architecture (3, 12, 25, 27–29). The \textit{prn} cluster of \textit{A. nidulans} comprises all the genes involved in proline utilization as a sole nitrogen and/or carbon source. We have studied in detail the bidirectional \textit{prnD-prnB} promoter, driving, respectively, the genes encoding proline oxidase and the specific proline transporter (10, 11, 13–15, 34). The proline metabolic genes are induced by proline and repressed when preferred carbon (glucose) or nitrogen (ammonium or glutamine) sources are available in the medium. A graphical representation of regulatory proteins and their cis-acting recognition motifs involved in the regulation of the bidirectional promoter is shown in Fig. 1A. In this promoter, eight nucleosomes lose their positioning upon induction while simultaneous carbon and nitrogen metabolite repression results in partial nucleosome repositioning. Chromatin restructuring is strictly dependent on the PrnA pathway-specific activator but not on the wide-domain nitrogen regulator AreA, which was proven to be essential for chromatin opening in the \textit{A. nidulans} nitrate regulon (3, 27). AreA in the proline cluster is required only in the presence of glucose, which is consistent with its proposed function (12).

To investigate in more detail how acetylation affects nucleosome positioning in this system, we deleted two genes coding for members of the putative SAGA/ADA acetylation complexes in \textit{A. nidulans}. In this article we describe the effect of \textit{gcnE} and \textit{adaB} deletions, homologues of the \textit{S. cerevisiae} \textit{GCN5} and \textit{ADA2} genes, a putative histone acetylase and adaptor protein, respectively. We studied the effects of these deletions on the transcriptional competence, the chromatin structure, and the acetylation status of H3K9 and H3K14 in the \textit{prnD-prnB} bidirectional promoter. Unexpectedly, low levels of histone H3 acetylation in the deletion strains were found not to affect transcriptional activation but were paradoxically associated with partial derepression of \textit{prnB} and \textit{prnD}.

**MATERIALS AND METHODS**

**Strains and growth conditions.** \textit{A. nidulans} strains used throughout the study are listed in Table 1. A total of 10^6 spores of each strain per ml were inoculated into liquid minimal medium (30) with the appropriate supplements plus 0.1% fructose as the carbon source and 5 mM urea as the nitrogen source. Mycelia were grown for 12 h at 37°C on a rotary shaker with continuous shaking at 180 rpm and then harvested for filtration. For condition-specific growth, aliquots of these precultures were then further incubated for 2 h at 37°C under the following conditions: noninducing, 0.1% fructose–5 mM urea; inducing, 20 mM proline; and inducing-repressing, 20 mM proline plus 1% glucose and 20 mM ammonium (-> +)-tartrate. Finally, mycelia were harvested by filtration for RNA isolation, microcoulcal nucleic (MNase) analysis, and chromatin immunoprecipitation (ChIP). For microscopic observations, cultures were grown on solid complete medium (30) until sporulation occurred (roughly 7 to 10 days for the \textit{adaB} and \textit{gcnE} deletion strains and around 3 days for the wild-type control strains), and surface samples were prepared for microscopy.

**Cloning of \textit{gcnE} and \textit{adaB} and construction of disruption vector.** Using the \textit{S. cerevisiae} \textit{Gcn5p} protein in a BLAST search of the \textit{A. nidulans} genome database (www.broad.mit.edu), we identified gene AN3621.3 as encoding \textit{GcnE}, a putative orthologue of yeast \textit{Gcn5p}, with an overall identity of 66% between the \textit{A. nidulans} and the \textit{S. cerevisiae} proteins (for predicted domain organization and BLAST results, see http://www.broad.mit.edu/annotation/genome/aspergillus_nidulans/). A deletion cassette was constructed by double-joint PCR. Deletion of the \textit{gcnE} open reading frame (ORF) was achieved by replacing it with the \textit{A. fumigatus prg} gene. In this cassette, the \textit{prg} gene is flanked by upstream and downstream \textit{GOF} sequences. The 3’ flanking fragment of \textit{gcnE} was amplified with primers G1F (5’-TACGGACCTCTGATCACAAC-3’) and G2632R (5’-TAAAGACTCTCTGAGATGAAAGTAATGATACCTGG-3’) and the 5’ flanking fragment of \textit{gcnE} was amplified with primers G3OF (5’-GAAAATCCCGCTGTTGCTGATGTTGTT-3’) and G59590R (5’-CTCTCAATACTAAGGCAATGTTGATACCAAG-3’) from \textit{A. nidulans} wild-type genomic DNA. A 1.5-kb genomic DNA fragment from \textit{A. nidulans} \textit{gcnE} gene was amplified from \textit{A. fumigatus} genomic DNA with primers GCNPC1F (5’-GTGCTGTCATCCCTGGGATGATCAT-3’) and GCNPCVR (5’-GCGTGCTACTGGGGATGATCAT-3’), which were used to amplify the corresponding molecule containing 5’-\textit{gcnE}-\textit{prg}-3’-\textit{gcnE}. This \textit{gcnE} deletion cassette was used to transform a \textit{pyg}89 argB26 pantoB100 riboB2 v42 strain. Transformants were selected on minimal medium with appropriate supplements lacking uracil and uridine.

To identify the strains with deletions of \textit{gcnE}, a Southern blot with genomic DNA digested with KpnI was hybridized with a 32P-labeled probe derived from a 2.6-kb PCR product amplified with primers G330OF and G59590R. A single 3-kb band confirmed \textit{gcnE} deletion and single integration of the deletion cassette. The same membrane was stripped and hybridized with a 32P-labeled probe derived from an 800-bp PCR product amplified by GCNORFF (5’-TCGTTGGAGGTATAACCTACG-3’) and GCNORFR (5’-GATGGCTTTGTCCTGATCT-3’) corresponding to the \textit{gcnE} ORF. No signal was detected for the strain with \textit{gcnE} deleted (not shown).

Using the \textit{S. cerevisiae} \textit{Ada2p} protein in a BLAST search of the \textit{A. nidulans} genome database (www.broad.mit.edu), we identified the product of gene AN5974.2 (changed to gene number AN10763.3 in the latest annotation) as a putative orthologue of yeast \textit{Ada2p} with a similarity score of 3 x 10^-7 (for predicted domain organization and BLAST results, see http://www.broad.mit.edu/annotation/genome/aspergillus_nidulans/). The transcription start and ter-
mination points were determined empirically by 5′ and 3′ rapid amplification of cDNA ends, respectively, and were found to correspond to the sites shown in the A. nidulans database. Plasmid pMS12 (Fungal Genetics Stock Center, http://www.fgsc.net) containing the argB gene was used to insert PCR fragments derived from the adaB5 region upstream of argB and from the adaB3 region downstream of argB. The adaB5 and 3′ sequences were obtained by PCR amplification using the sequence information from the A. nidulans sequence database (www.broad.mit.edu). Roughly 700 bp of the adaB5 region was amplified with primers 5′-GATGCAAGACCGCGGCCACAGCAGTCAGC-3′; the NotI site introduced by mutating three bases is underlined) and 5′ reverse (5′-CATCGATCCCCAGAGCCTTCTCACCCGCCG-3′). The fragment was cloned as a NotI-ClaI fragment into the vector. Around 1.4 kb of the adaB3′ region was amplified with primers 3′-CATCGATCCCCAGAGCCTTCTCACCCGCCG-3′; the SalI site introduced by mutating the HindIII restriction site, positioned 50 bp downstream of the empirically determined polyadenylation site, is underlined) and 3′ reverse (5′-GCGAGTTGACTGAGCTCGAGAAGGATCACCCTC-3′). The fragment was cloned as a SalI-XhoI fragment into the vector. The resulting 7.2-kb vector was called p5′-3′adaB::argB. Strain MH 9233 (26) (gift from Michael Hynes, The University of Melbourne) was used as a recipient strain for the knockout construct. To

FIG. 1. Regulation of proline utilization in A. nidulans. (A) Overview of proline cluster regulation. The prnD-B intergenic region is shown. prnD encodes the proline oxidase, and prnB encodes the specific proline transporter (12, 14). The pathway-specific transcription factor PrnA is essential for proline induction of both genes. In the absence of preferential carbon (glucose) and nitrogen (ammonium) sources and the presence of proline, PrnA and the GATA factor AreA bind to their cognate sites in the intergenic region, resulting in the expression of prnD and prnB. Repression requires both glucose activation of the negative regulator CreA and ammonium inactivation of AreA. Full repression occurs only in the simultaneous presence of glucose and ammonium. Repression acts directly on prnB expression; prnD repression is indirect and results from inducer exclusion. (B) Effect of adaB and gcnE deletion on prnB and prnD transcription. Strains (Table 1) were pregrown in liquid minimal medium under noninducing conditions (5 mM urea-0.1% fructose) with the appropriate supplements, harvested, divided into aliquots, and further incubated for 2 h under the conditions indicated. Noninducing (NI), 5 mM urea and 0.1% fructose; inducing (I), induced by 20 mM proline; inducing-repressing (IR), 20 mM proline and repression by 1% glucose and 20 mM ammonium-L(-)-tartrate. Expression levels (bottom) were quantified using phosphorimaging and ImageQuant software analysis. Normalized signals were obtained by comparison of specific signals with actin gene (acnA) expression signals. The induced levels in the adaB5′ and gcnE5′ strains are given in every case the arbitrary value of 100; filled columns represent prnB, and open columns represent prnD expression.
Two microliters of antibody solution was used to incubate 200 ng of chromosomal DNA from a wild-type strain (3.3 μg) and for further studies, an adaB Δ strain was used as a reference strain. An adaB Δ strain with only one additional marker for further studies. An adaB Δ strain was selected from the progeny, and for further studies, a biA1 Δ adaB Δ strain was used as a reference strain.

RNA preparation and Northern blots. Total RNA was isolated with RNA Plus extraction solution (Biogen) following the manufacturer's instructions. RNA electrophoresis and Northern blot hybridizations were carried out as described previously (13). prnB, prnD, and acnA probes were prepared as described by Gomez et al. (14).

Nucleosome positioning. MNase I digestions were performed by the method adapted by Gonzalez and Scazzocchio (16). MNase was used at concentrations ranging from 0.5 to 2.5 U/g of mycelium. DNA was digested with an appropriate restriction enzyme. Probes SC1 and SC2 for the prnB-D intergenic region were prepared as described previously (12).

The position of each MNase cut was calculated by running in each gel a scale of molecular size markers (100 Base-Pair Ladder; Amersham Pharmacia Bio-Tech., Piscataway, NJ). The values reported in the scale adjacent to each gel represent the position of the MNase cut from the ATG of the relevant gene. For each mutant and growth condition, the experiments were performed in triplicate.

ChIP assays. ChIP assays were carried out following our published protocol (4). Antibodies for ChIP analysis of acetylated histone H3 were purchased from Upstate Biotechnology (Charlottesville, VA) and recognized acetylated K9 and K14 of histone H3 (06-599). Rabbit polyclonal antibody recognizing the C-terminal region of the MNase digestion pattern is obtained from the ATG of the relevant gene. For each mutant and growth condition, the experiments were performed in triplicate.

ChIP assays. ChIP assays were carried out following our published protocol (4). Antibodies for ChIP analysis of acetylated histone H3 were purchased from Upstate Biotechnology (Charlottesville, VA) and recognized acetylated K9 and K14 of histone H3 (06-599). Rabbit polyclonal antibody recognizing the C-terminal region of the MNase digestion pattern is obtained from the ATG of the relevant gene. For each mutant and growth condition, the experiments were performed in triplicate.

Quantitative real-time PCR was performed using a Bio-Rad (Hercules, CA) MyIQ Cycler with the Platinum SYBR green qPCR SuperMix-UDG from Invitrogen (Karlsruhe, Germany) for amplification. Chromosomal DNA from a wild-type strain was used as an external standard for setting up the calibration curve. Primers that amplified fragment a (see Fig. 4B) had the following sequences: for prnB+2tor, 5′-TGAGGGATCCCCATTAGTCAAGG-3′; and for prnB+2rev, 5′-GGATCAGGTTCCCTAAAGTCAG-3′. The PCR was calibrated by a dilution series of total DNA extracted from a wild-type strain (3.3 μg μl−1). Each PCR was replicated (technical repetition). For each sample the absolute amount of the specific DNA fragment in the immunoprecipitated sample was divided by the PCR product of the same fragment in the sample before precipitation (normalizing to input DNA). To obtain the relative K9/K14 acetylation level of histone H3, the normalized values obtained from acetylated H3 ChIPs (H3-acetyl) were divided by the normalized values obtained from the H3 C-terminal ChIPs (H3 C-term). The H3-acetyl/H3 C-term ratio obtained in the adaB Δ wild-type strain grown under noninducing conditions was set to one. Two biological repetitions were performed for each condition, and standard deviations were calculated.

RESULTS

Phenotypes of the gcnE and adaB deletions. Both the gcnE and adaB deletion strains showed a strongly reduced growth rate and condensation on solid medium. Both deletions resulted in similar morphological alterations of the conidiophore. Supplemental Fig. S1 shows a selection of pictures obtained from microscopic observations of the adaB deletion strain (see the supplemental material). The strain produces stalks and vesicles, but metulae are missing. Short stalks with vesicle heads and a few phialides repeatedly emerge directly from vegetative mycelia. None of the known conidiation mutants of A. nidulans displays this phenotype.

We tested for specific effects of the gcnEΔ and adaBΔ strains on the utilization of various carbon and nitrogen sources. Compared to growth of the isogenic wild-type strains, both deletion strains displayed reduced growth on all the nutrients tested, including complete medium and proline as a sole carbon or nitrogen source (data not shown).

**TABLE 1. A. nidulans strains used throughout this work**

| Strain | Genotype | Reference or source |
|--------|----------|---------------------|
| gcnEΔ | pyrG89 argB2 pantob100 ribo2 yA2 | This work |
| gcnEΔ | pyrG89 argB2 pantob100 ribo2 yA2 | This work |
| gcnEΔ::pyrGΔ | | |
| adaBΔ | biA1 | FGSC strain A26 |
| adaBΔ | biA1 argB::trpCΔ adaBΔ::argBΔ | This work |
| MH 9233 | wa3 biA1 argB::trpCΔ pyrO4 ribo2 26 | |

*a* FGSC, Fungal Genetics Stock Center (http://www.fgsc.net).
under inducing-repressing conditions and a derepressed phenotype for prnB and prnD expression closely resemble the results obtained when the deacetylase inhibitor TSA is used in the wild type or when MNase patterns are analyzed in a creA d1 strain (10).

We thus used ChIP to determine the acetylation status of histone H3 in the region of nucleosome +2, the only nucleosome that remains positioned under these conditions in the adaBΔ and gcnEΔ strains.
The acetylation status of histone H3 in the region of nucleosome +2 depends on physiological conditions and on the function of GcnE and AdaB. We chose to look at the region of nucleosome +2, as MNase digests indicate that a nucleosome is present in this region under all conditions, whether completely positioned (noninducing conditions) or partially positioned (inducing and inducing-repressing conditions) (12). The acetylation status of H3K9 and H3K14 is a critical mark for gene activation. To investigate the acetylation status of H3K9 and H3K14 in nucleosome +2, we employed ChIP assays using an antibody recognizing both acetyl-H3K9 and acetyl-H3K14, followed by the amplification of a fragment encompassing the region covered by nucleosome +2 (Fig. 2B, fragment a). The ratio between acetylated and total H3 (precipitated with an antibody directed against an H3 C-terminal epitope) is shown in Fig. 3. The acetylation patterns in the adaBΔ and gcnEΔ strains are very similar, but, in contrast to the MNase digests, they are not identical and are therefore presented here for both strains.

In the adaBΔ (wild type) strain, isogenic to the adaBΔ strain, the acetylation of H3K9 and H3K14 increases with induction, indicating a correlation between H3 acetylation, chromatin rearrangements (loss of positioning), and transcriptional activation. Upon repression, the acetylation status of H3K9 and H3K14 does not change significantly, which indicates that the deacetylation of these residues in H3 is not required to trigger gene repression and repositioning of nucleosome +2. In contrast to adaBΔ, in the adaBΔ strain the acetylation of H3K9 and H3K14 is strongly reduced under all conditions; and despite considerable induction of prnB and prnD transcription, an increase in the acetylation of H3 is not seen in these strains. A qualitatively very similar pattern is observed when the gcnEΔ (wild type) control strain is compared to the isogenic gcnEΔ strain under the different growth conditions. As in the adaBΔ strain, the acetylation of H3K9 and H3K14 is strongly reduced in the gcnE deletion strain under all conditions.

**DISCUSSION**

Often, enhanced gene expression correlates with loss of positioning and increased acetylation of nucleosomes (33). In the prnD-prnB bidirectional promoter, we see increased acetylation of H3K9 and H3K14 under inducing conditions in the region corresponding to partially positioned nucleosome +2. However, this increased acetylation is not required for efficient transcription or chromatin remodeling. In the strains lacking essential components of the putative SAGA/ADA complexes, i.e., GenE or AdaB, we detect only low levels of H3 lysine acetylation, but the induction and remodeling of chromatin are not affected. This is not without precedence, as in *S. cerevisiae*, SAGA/ADA-dependent and -independent activators are known. The activation of transcription requires SAGA or ADA complexes in the GAL1 promoter where GAL4 recruits the SAGA complex, and this in turn is necessary for the recruitment of the mediator complex (20). Our results indicate that, while a putative SAGA or ADA complex might be involved in determining the acetylation levels in this region, the PnR-mediated activation and chromatin remodeling of the prnD-prnB promoter do not depend on AdaB and GenE, putative members of *A. nidulans* SAGA/ADA complexes.

It should be noted that in our ChIP experiments, we have tested only histone H3 acetylation at lysines 9 and 14, residues known to be acetylated by SAGA and ADA complexes (9). Other covalent modifications not detected by the antibody used here, such as acetylations of H2A, H2B, and H4 at diverse lysines in their N termini, could also lead to the hyperacetylation of nucleosomes in the prnD-prnB promoter, thus providing the signal for effective chromatin remodeling. In filamentous fungi, it is completely unknown which mechanisms direct the cross talk of histone modification. In a study focusing on the different roles of H3 and H4 acetylation in the yeast *S. cerevisiae*, it was shown that H4 hyperacetylation in promoters of Adr1p-dependent genes does not compensate for low H3 acetylation in a *gen5* mutant strain (1).

The observed partial derepression of prnB and prnD expression in strains with deletions of adaB and genE was unexpected. However, in *N. crassa*, mutation of the GCN5 homologue *nfg-1* results in loss of repression of the blue-light-responsive *al-3* gene in the dark (18). An *nfg-1*Δ*pop* mutant was found to express *al-3* constitutively, suggesting that the HAT is involved not only in blue-light
induction but also in the repression of al-3 in the absence of light. Another example of HAT activities involved in both gene activation and repression is provided by the yeast GAL system. Glucose repression requires Ada2p and Ada3p/Ngg1p, an ADA complex protein functionally associated with Gcn5p and Ada2p (6), and it has been suggested that Ada2p/Ada3p directly inhibits the activation domain of Gal4p. Similarly, in the yeast arginine metabolic system, Gcn5p function and increased histone acetylation are associated with transcriptional repression. In this case, a Gcn5p-mediated increase of H3 acetylation correlates with binding of the arginine repressor complex ARG1/ARGM (31).

However, our nucleosome-positioning and ChIP studies lead to a different picture. Although histone H3 acetylation increases during transcriptional activation, it is not required for transcriptional activation (compare results of inducing conditions in gcnE+/adaBΔ+ strains with those for gcnEΔ or adaBΔ strain in Fig. 1 and 3) or loss of nucleosome positioning (Fig. 2). Additionally, transcriptional repression and repositioning of nucleosomes are not accompanied by deacetylation of H3K9 or H3K14 (Fig. 3, IR lanes). These results suggest that differences in histone acetylation are associated with, but not necessary for, transcriptional activation and nucleosome-positioning processes in the prnD-prnB intergenic region. It was therefore surprising to see that both hyperacetylation by TSA inhibition of deacetylases and hypoacetylation by deletion of adaB or gcnE lead to partial derepression of the proline catabolic genes and a simultaneous lack of nucleosome repositioning.

Transcriptional repression and nucleosome repositioning upon simultaneous glucose and ammonium repression require a functional CreA protein. This has been shown by the results of mutations in both the CreA protein itself (creAΔ1) and in its cognate binding sites (prnDΔ20 or prnDΔ22) in the prnD-prnB promoter (12). The simplest hypothesis integrating our experimental results would therefore propose that both hyperacetylation by TSA treatment and hypoacetylation by adaB or gcnE deletion impair CreA function. Several observations support this view. First, the nucleosomal patterns obtained in the prnD-prnB region by TSA treatment and in adaBΔ and gcnEΔ under inducing-repressing conditions are identical to those found in creAΔ1, prnDΔ20, or prnDΔ22 mutant strains (13). Second, derepression under TSA treatment or in adaBΔ and gcnEΔ strains is only partial, whereas derepression is complete in creA loss-of-function strains. This also implies that while nucleosome repositioning may be necessary for full repression, CreA can still partially repress on completely open chromatin, probably by directly interfering with the activating function of the pathway-specific activator PrnA.

Third, the function of CreA, but not the acetylation status of histones, defines whether nucleosomes are positioned or not. In summary, nucleosomal rearrangements, transcriptional activity, and histone acetylation are clearly distinct processes in the prnD-prnB region, and therefore a direct influence of the acetylation status on nucleosomal positioning and transcription is highly unlikely. Further studies will reveal at which level, transcriptional and/or posttranscriptional, CreA function is impaired by TSA treatment or the lack of HAT activities.

ACKNOWLEDGMENTS

We are grateful for receiving strain MH 9233 from Michael Hynes (The University of Melbourne).

This work was supported by grant P17018 of the Austrian Science Fund FWF to J.S. Work at Orsay was supported by the CNRS, the Université Paris-Sud, and the Institut Universitaire de France. Y.R.-D. was supported by a predocotural studentship of the Ministère de l’Education Supéérieure et de la Recherche and the CONACYT (México). R.F.-M. was supported by a Marie Curie Fellowship (MCFI-2001- 01054). L.G. was supported by postdoctoral fellowships from, successively, the Spanish Ministry of Education and the EU Marie Curie Programme.

REFERENCES

1. Agricola, E., L. Verdone, E. Di Mauro, and M. Caserta. 2006. H4 acetylation does not replace H3 acetylation in chromatin remodelling and transcription activation of Adr1-dependent genes. Mol. Microbiol. 62:1433–1446.
2. Balasubramanian, R., M. G. Pray-Grant, W. Selbeck, P. A. Grant, and S. Tan. 2002. Role of the Ada2 and Ada3 transcriptional coactivators in histone acetylation. J. Biol. Chem. 277:7989–7995.
3. Berger, H., R. Pachinger, I. Morozov, S. Goller, F. Narendran, M. Caddick, and J. Strauss. 2006. The GATA factor AreA regulates localization and in vivo binding site occupancy of the nitrate activator NirA. Mol. Microbiol. 59:433–446.
4. Bernreiter, A., A. Ramon, J. Fernandez-Martinez, H. Berger, L. Araujo-Bazan, E. A. Espeso, R. Pachinger, A. Gallinetzter, I. Ancler, C. Scacciozchico, and J. Strauss. 2007. Nuclear export of the transcription factor NirA is a regulatory checkpoint for nitrate induction in Aspergillus nidulans. Mol. Cell. Biol. 27:791–802.
5. Bhakumik, S. R., and M. R. Green. 2001. SAGA is an essential in vivo target of the yeast acetyl activator Gal4. Genes Dev. 15:1935–1945.
6. Brandi, C. J., J. A. Martens, A. Margaliot, D. Stenning, A. M. Furlanetto, A. Salek, K. S. Hamilton, and J. Geneereaux. 1996. Structure/functional properties of the yeast dual regulator protein Ngg1 that are required for glucose repression. J. Biol. Chem. 271:9296–9306.
7. Brownell, J. E., J. Zhou, T. Ranalli, R. Kobayaschi, D. G. Edmondson, S. Y. Roth, and C. D. Allis. 1996. Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell 84:843–851.
8. Candau, R., J. X. Zhou, C. D. Allis, and S. L. Berger. 1997. Histone acetyltransferase activity and interaction with ADA2 are critical for GCN5 function in vivo. EMBO J. 16:555–565.
9. Carpenter, M. J., R. T. Tran, J. L. Workman, and J. Cote. 2003. The diverse functions of histone acetyltransferase complexes. Trends Genet. 19:321–329.
10. Cubero, B., D. Gomez, and C. Scacciozchico. 2000. Metabolite repression and inducer exclusion in the proline utilization gene cluster of Aspergillus nidulans. J. Bacteriol. 182:233–235.
11. Cubero, B., and C. Scacciozchico. 1994. Two different, adjacent and divergent zinc finger binding sites are necessary for CREA-mediated carbon catabolite repression in the proline gene cluster of Aspergillus nidulans. EMBO J. 13:4107–4115.
12. Garcia, I., R. Gonzalez, D. Gomez, and C. Scacciozchico. 2004. Chromatin rearrangements in the prnD-prnB bidirectional promoter: dependence on transcription factors. Eukaryot. Cell 3:144–156.
13. Gomez, D., B. Cubero, G. Cecchetto, and C. Scacciozchico. 2002. PrmA, a Zn166 activator with a unique DNA recognition mode, requires inducer for in vivo binding. Mol. Microbiol. 44:585–597.
14. Gomez, D., I. Garcia, C. Scacciozchico, and B. Cubero. 2003. Multiple GATA sites: protein binding and physiological relevance for the regulation of the proline transporter gene of Aspergillus nidulans. Mol. Microbiol. 50:277–289.
15. Gonzalez, R., V. Gavrias, D. Gomez, C. Scacciozchico, and B. Cubero. 1997. The integration of nitrogen and carbon catabolite repression in Aspergillus nidulans requires the GATA factor AreA and an additional positive-acting element, ADA. EMBO J. 16:2977–2984.
16. Gonzalez, R., and C. Scacciozchico. 1997. A rapid method for chromatin structure analysis in the filamentous fungus Aspergillus nidulans. Nucleic Acids Res. 25:3955–3956.
17. Grat, P. A., L. Duggan, J. Cote, S. M. Roberts, J. E. Brownell, R. Candau, R. Ohba, T. Owen-Hughes, C. D. Allis, F. Winston, S. L. Berger, and J. L. Workman. 1997. Yeast Gcn5p functions in two multienzyme complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes Dev. 11:1640–1650.
18. Grimdal, B., P. Coiro, P. Filetici, E. Berge, J. R. Dobosy, M. Freitag, E. U. Selker, and P. Ballarino. 2006. The Neurospora crassa White Collar-1 dependent blue light response requires acetylation of histone H3 lysine 14 by Gcn5. Mol. Biol. Cell 17:4576–4583.
19. Koehler, R. N., N. Rachfall, and R. J. Rolfe. 2007. Activation of the ADE2 genes requires the chromatin remodeling complexes SAGA and SWI/SNF, Eukaryot. Cell 6:1474–1485.
20. Koh, S. S., A. Z. Ansari, M. Ptashe, and R. A. Young. 1998. An activator target in the RNA polymerase II holoenzyme. Mol. Cell 2:895–904.
21. Kuo, M. H., J. E. Brownell, R. E. Sobel, T. A. Ranalli, R. G. Cook, D. G. Edmondson, S. Y. Roth, and C. D. Allis. 1996. Transcription-linked acety-
lation by Gcn5p of histones H3 and H4 at specific lysines. Nature 383:269–272.

22. Larschan, E., and F. Winston. 2001. The S. cerevisiae SAGA complex functions in vivo as a coactivator for transcriptional activation by Gal4. Genes Dev. 15:1946–1956.

23. Lee, T. L., H. C. Causton, F. C. Holstege, W. C. Shen, N. Hannett, E. G. Jennings, F. Winston, M. R. Green, and R. A. Young. 2000. Redundant roles for the TFIID and SAGA complexes in global transcription. Nature 405:701–704.

24. Lee, T. I., and R. A. Young. 2000. Transcription of eukaryotic protein-coding genes. Annu. Rev. Genet. 34:77–137.

25. Mathieu, M., I. Nikolaev, C. Scanzocchio, and B. Felenbok. 2005. Patterns of nucleosomal organization in the alc regulon of Aspergillus nidulans: roles of the AlcR transcriptional activator and the CreA global repressor. Mol. Microbiol. 56:535–548.

26. Monahan, B. J., J. A. Fraser, M. J. Hynes, and M. A. Davis. 2002. Isolation and characterization of two ammonium permease genes, meaA and mepA, from Aspergillus nidulans. Eukaryot. Cell 1:85–94.

27. Muro-Pastor, M. I., R. Gonzalez, J. Strauss, F. Narendja, and C. Scanzocchio. 1999. The GATA factor AreA is essential for chromatin remodelling in a eukaryotic bidirectional promoter. EMBO J. 18:1584–1597. (Erratum, 18:2670.)

28. Muro-Pastor, M. I., J. Strauss, A. Ramon, and C. Scanzocchio. 2004. A paradoxical mutant GATA factor. Eukaryot. Cell 3:393–405.

29. Narendja, F., S. P. Goller, M. Wolschek, and J. Strauss. 2002. Nitrate and the GATA factor AreA are necessary for in vivo binding of NirA, the pathway-specific transcriptional activator of Aspergillus nidulans. Mol. Microbiol. 44:573–583.

30. Pontecorvo, G., J. A. Roper, L. M. Hemmons, K. D. MacDonald, and A. W. J. Bilton. 1953. The genetics of Aspergillus nidulans. Adv. Genet. 5:141–238.

31. Ricci, A. R., J. Genereaux, and C. J. Brandl. 2002. Components of the SAGA histone acetyltransferase complex are required for repressed transcription of ARG1 in rich medium. Mol. Cell. Biol. 22:4033–4042.

32. Riego, L., A. Avendano, A. Deluna, E. Rodriguez, and A. Gonzalez, 2002. GDH1 expression is regulated by GLN3, GCN4, and HAP4 under respiratory growth. Biochem. Biophys. Res. Commun. 293:79–85.

33. Roth, S. Y., J. M. Denu, and C. D. Allis. 2001. Histone acetyltransferases. Annu. Rev. Biochem. 70:81–120.

34. Sophianopoulou, V., and C. Scanzocchio. 1989. The proline transport protein of Aspergillus nidulans is very similar to amino acid transporters of Saccharomyces cerevisiae. Mol. Microbiol. 3:705–714.

35. Wang, L., C. Mizzen, C. Ying, R. Candau, N. Barlev, J. Brownell, C. D. Allis, and S. L. Berger. 1997. Histone acetyltransferase activity is conserved between yeast and human GCN5 and is required for complementation of growth and transcriptional activation. Mol. Cell. Biol. 17:519–527.