Snf1p-dependent Spt-Ada-Gcn5-acetyltransferase (SAGA) Recruitment and Chromatin Remodeling Activities on the HXT2 and HXT4 Promoters*\[5\]

We previously showed that the Spt-Ada-Gcn5-acetyltransferase (SAGA) complex is recruited to the activated HXT2 and HXT4 genes and plays a role in the association of TBP-associated factors. Using the HXT2 and HXT4 genes, we now present evidence for a functional link between Snf1p-dependent activation, recruitment of the SAGA complex, histone H3 removal, and H3 acetylation. Recruitment of the SAGA complex is dependent on the release of Snf6p-Tup1p repression by Snf1p. In addition, we found that the Gcn5p subunit of the SAGA complex preferentially acetylates histone H3K18 on the HXT2 and HXT4 promoters and that Gcn5p activity is required for removal of histone H3 from the HXT4 promoter TATA region. In contrast, histone H3 removal from the HXT2 promoter does not require Gcn5p. In conclusion, although similar protein complexes are involved, induction of HXT2 and HXT4 displays important mechanistic differences.

The yeast Saccharomyces cerevisiae utilizes glucose fermentation to generate metabolic energy. To optimize this process, glucose concentrations are carefully monitored, and gene expression is tightly linked to glucose availability. When glucose is present, genes involved in the uptake and fermentation of glucose are actively transcribed. However, glucose can also mediate repressive signals for genes involved in processing non-fermentable carbon sources and genes involved in the uptake and processing of alternative carbon sources. This latter process is known as glucose repression (1, 2).

A central component in the glucose repression pathway is the Tup1p-Ssn6p complex. The Snf6p-Tup1p complex has no DNA binding activity and depends on sequence-specific factors such as Mig1p for association to its target promoters (3, 4). The Snf6p-Tup1p repressor complex genetically interacts with components of the Mediator complex and is associated with histone deacetylation (HDAC) enzymes (5–9). Moreover, deletion of either SSN6 or TUP1 results in an altered chromatin organization of RN3, FLO1, SUC2, and a-cell-specific genes (10–14). A direct molecular link between Tup1p and chromatin organization was suggested by the interaction of the Tup1p repression domain with the N-terminal tails of histone H3 and H4 (15). In addition, Tup1p preferentially binds hypoacetylated histones on a subset of genes in vivo (16, 17).

To ensure efficient influx of glucose, yeast cells can express different hexose transporter (HXT) genes. The HXT2 and HXT4 genes encode high affinity glucose transporters, whose expression is repressed by high levels of glucose (18). Repression of the HXT2 and HXT4 genes is mediated by both subunits of the Snf6p-Tup1p complex, because deletion of either SSN6 or TUP1 results in promoter activation of these genes under non-inducing conditions (18, 19). To release Snf6p-Tup1p-mediated repression under inducing conditions (i.e. low glucose), the HXT2 and HXT4 genes require Snf1p function (18). Snf1p encodes a serine/threonine kinase and is required for the expression of glucose-repressed genes (20, 21). Snf1p kinase phosphorylates Mig1p, which results in a functional disruption of the Mig1p-Ssn6p interaction on repressed genes (22–24). Furthermore, Snf1p has also been reported to phosphorylate serine 10 of histone H3 (H3S10), which is important for SAGA recruitment to the INO1 promoter (25).

The SAGA complex is a multisubunit complex with distinct transcriptional activities (26, 27). It is composed of different classes of proteins, which were identified in independent genetic screens. The Spt3p and Spt8p proteins regulate TBP activity, whereas the Gcn5p subunit has histone acetyltransferase (HAT) activity (28–32). Furthermore, subunits, including Spt7p and Spt20p, comprise the structural backbone of the SAGA complex supporting the Spt3p-Spt8p-TBP and Gcn5p-HAT modules (30, 32, 33). The SAGA complex is specifically recruited via its Tra1p subunit by activators like Gal4p and Gcn4p (34). SAGA is essential for the Spt3p-dependent TBP recruitment to the TATA box region of the GAL1 promoter (35–37). In addition, Spt3p and Gcn5p were shown to recruit Mot1p to the GAL1 gene and to play a role in nucleosomal arrangement of the GAL1 promoter after activation (38). However, deletion of GCN5 only has a mild effect on GAL1 transcription (35, 36). Surprisingly, SAGA recruitment to the GAL1 gene involves Ctf6p, a protein that physically bridges Snf6p to the SAGA complex (39).

We have shown recently that SAGA is specifically recruited to the upstream regions of the HXT2 and HXT4 genes (40). Although the SAGA complex is required for the recruitment of both Mot1p and the TFIIID subunit, Taf1p, to the TATA box regions via different modules, SAGA is only essential for HXT4 expression (40). Here, we describe a functional link between release of repression by Snf1p, SAGA recruitment, and its consequences for chromatin regulation of the HXT2 and HXT4 promoters. Acetylation of histone H3 depends on the HAT activity of Gcn5p but does not play a role in transcription activation. Together, these experiments show a novel relationship between Snf6p-dependent activation, release of Snf6p-

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2 The abbreviations used are: HDAC, histone deacetylation; SAGA, Spt-Ada-Gcn5-acetyltransferase; HAT, histone acetyltransferase; HA, hemagglutinin; TBP, TATA box-binding protein; UAS, upstream activating sequence.
Tup1p repression, SAGA recruitment, and chromatin regulation on the HXT2 and HXT4 genes.

MATERIALS AND METHODS

Yeast Strains—The S. cerevisiae strains used in this study are listed in Table 1. All procedures were performed according to standard methods (41). The HA3-MOT1 (DP107) and HA3-TAF1 (YBY838) strains have been described previously (42, 43). Single deletion strains were constructed as described (44). However, the gcn5Δ strain was constructed using a GCN5 disruption cassette kindly provided by Dr. G. Thireos (45). All deletions were verified by PCR analysis with primers corresponding to both wild-type and disruption alleles. The TUP1-HA3 and SPT20-TAP alleles have been described previously (16, 40). Sequences of oligonucleotides used in this study are available upon request.

Growth Conditions—For glucose concentration shift experiments, cells were grown in SC medium (0.67% (w/v) yeast nitrogen base without amino acids (Difco) supplemented with complete supplement mixture (Qiagen)) containing 4% glucose. When cells had reached mid log (A600 = 0.55–0.6), 10 ml of the culture was removed at the indicated time points. Cells were collected by centrifugation, refrozen in liquid N2, and stored at −80 °C upon further processing.

Northern Blotting—RNA isolation and hybridization conditions were described previously (46). Oligonucleotide sequences and the labeling procedure used for HXT genes have been described previously (47). A 1-kb fragment of the ACT1 coding region (spanning the region +324 to +1347) was used to analyze variations in mRNA loading. The ACT1 DNA fragment was labeled with the RediprimeTMII system according to the manufacturer’s protocol (Amersham Biosciences). PhosphorImager quantification of HXT2 and HXT4 mRNA signals has been described previously (46). Data from triplicate experiments were used for quantification.

Chromatin Immunoprecipitation Assay—Chromatin extracts were prepared as previously described (46). Chromatin extract (200 μl) was used for immunoprecipitation, and 10 μl of chromatin extract was used for input control preparation. Specific protein-DNA complexes were recovered as described previously (46) with 25 μl of Protein G-agarose beads (Roche). Specific protein-DNA complexes were recovered as described previously (46). Chromatin extract (200 μl) was used for immunoprecipitation, and 10 μl of chromatin extract was used for input control preparation. Specific protein-DNA complexes were recovered as described previously (46) with 25 μl of Protein G-agarose beads (Roche). Specific protein-DNA complexes were recovered as described previously (46). Chromatin extract (200 μl) was used for immunoprecipitation, and 10 μl of chromatin extract was used for input control preparation. Specific protein-DNA complexes were recovered as described previously (46) with 25 μl of Protein G-agarose beads (Roche).

RESULTS

Activation and Repression of HXT2 and HXT4 mRNA Expression—Transcription of the HXT2 and HXT4 genes is regulated by glucose levels (23). Previously, it was shown that deletion of components of the repression complex (i.e. MIG1, RGT1, SSN6, and TUP1) leads to (partial) derepression of the HXT2 and HXT4 promoters depending on the growth conditions (18, 19). In contrast, deletion of the SNF1 gene completely abolishes HXT2 and HXT4 induction under conditions of low glucose (18). In these experiments HXT2 and HXT4 promoter activity was measured in a plasmid-based system using the LacZ reporter gene. To study the early kinetics of HXT2 and HXT4 mRNA induction we have developed a rapid cell harvesting protocol (40). Using this method, we studied the kinetics of mRNA induction from the endogenous HXT2 and HXT4 genes in repression and derepression mutant strains (Table 1). To analyze variations in mRNA loading, HXT2 and HXT4 mRNA were quantified relative to ACT1 mRNA levels (Fig. 1, C and D), which is invariant during the glucose concentration shift (data not shown). As shown in Fig. 1 and as previously described (40), a 20- to 25-fold reduction of HXT2 and HXT4 mRNA was observed, which occurred already 5 min after a shift to low glucose. Deletion of SNF1 completely abolished induction of HXT2 and HXT4 mRNA, which is in agreement with previous results (18). Mig1p and Rgt1p are repressors recruiting the Sn6p-Tup1p complex to glucose-repressed genes (1). Mutant cells deleted for either MIG1 or RGT1 displayed a 3- to 5-fold increase of basal levels compared with wild-type cells (Fig. 1). Nevertheless, shifting to low glucose still resulted in mRNA induction of HXT2 and HXT4 to levels, which slightly exceeded wild-type levels. In contrast, deletion of TUP1 resulted in (an almost complete) derepression and deletion of SSN6 completely derepressed HXT2 and HXT4 transcription under non-inducing conditions. Thus, the Sn6p-Tup1p complex represses mRNA expression of the HXT2 and HXT4 genes under non-inducing conditions and relief of repression requires Snf1p as has been described previously (18, 19). The results also suggest that both Mig1p and Rgt1p can recruit the Sn6p-Tup1p complex to the HXT2 and HXT4 promoters.

A Decrease in Tup1p Binding Preccedes Decreased Histone H3 Binding and Preinitiation Complex Formation—The Sn6p-Tup1p repressor complex is recruited by Mig1p to several glucose-repressed genes such as CYC1 and GAL1 (3, 24, 48). Furthermore, deletion of either component of the Sn6p-Tup1p complex resulted in a constitutive expression of the HXT2 and HXT4 genes (Fig. 1). Therefore, to investigate the direct association of the Sn6p-Tup1p complex to the HXT2 and HXT4 promoters, we determined the binding kinetics of Tup1p during a shift to low glucose. This was investigated by chromatin immunoprecipitation assays employing yeast cells expressing a hemagglutinin (HA)-tagged version of Tup1p (see Table 1). Cells were grown in SC medium containing 4% glucose and shifted to 0.1% glucose. Subsequently, formaldehyde-cross-linked chromatin was isolated at various times after the glucose shift. Immunopurified DNA was analyzed by real-time PCR using primer sets, which amplify the TATA or upstream regions of the HXT2 and HXT4 genes (Fig. 2A). An intragenic fragment of the POL1 gene was used as a normalization control (49). When cells were grown in 4% glucose we observed strong and specific binding of Tup1p to the putative UAS regions of the HXT2 and HXT4 promoters, which was 35- to 40-fold above the POL1 background signal (Fig. 2B). Directly after cells were shifted to low glucose, Tup1p binding was slightly decreased (less than 2-fold) at the putative UAS of HXT2 and HXT4. Association of Tup1 with the flanking regions was lower, and activation did not reduce this association.
Tup1p has been reported to bind to the N-terminal tails of histone H3 and H4, and Tup1p binding correlates with hypoacetylated histone H3 on the STE2 and STE6 promoters in vivo (15, 16). Therefore we analyzed the binding of histone H3 to the HXT2 and HXT4 promoters by using an antibody directed against the C-terminal 12 residues of H3. Under levels of high glucose, we observed histone H3 binding to the UAS and TATA regions of the HXT2 and HXT4 promoters. Shifting cells to low glucose resulted in a 3- to 8-fold decrease in histone H3 binding to both UAS and TATA regions with minimal binding after 2–5 min (Fig. 2C). Interestingly, H3 binding inversely correlated with TBP and polymerase II binding, which is at maximum levels 2–5 min after the glucose shift (Fig. 2D) as we observed previously (40, 46). Thus, after a shift to low glucose the small decrease in Tup1p association precedes a strong decrease in histone H3 binding and increases in TBP and polymerase II association.

HXT2 and HXT4 Promoters Are Preferentially Acetylated at Histone H3K18 after Activation—A correlation between Tup1p association and histone H3 acetylation was reported for the HXT2 (A) and HXT4 (B) in wild-type, snf1Δ, mig1Δ, rgt1Δ, tup1Δ, and snn6Δ strains upon shifting to low glucose. Cells were grown in 4% glucose and shifted to 0.1% glucose for 0, 2, 5, and 10 min before collection (see "Material and Methods"). RNA samples from wild-type and mutant cells were processed in parallel and analyzed in parallel on a single RNA blot to ensure direct comparison of hybridization signals. HXT mRNA was detected by oligonucleotide probes as described previously (46, 47). ACT1 probes were used as RNA loading control. Data shown are representative of at least two independent experiments. C and D, quantification of HXT2 and HXT4 mRNAs. HXT mRNA hybridization signals were quantified by PhosphorImager and are expressed relative to ACT1 mRNA signals.

### TABLE 1

Yeast strains and genotypes used in this study

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**FIGURE 1.** Induced transcription of the HXT2 and HXT4 genes in different mutant strains. Kinetics of mRNA expression of HXT2 (A) and HXT4 (B) in wild-type, snf1Δ, mig1Δ, rgt1Δ, tup1Δ, and snn6Δ strains upon shifting to low glucose. Cells were grown in 4% glucose and shifted to 0.1% glucose for 0, 2, 5, and 10 min before collection (see "Material and Methods"). RNA samples from wild-type and mutant cells were processed in parallel and analyzed in parallel on a single RNA blots to ensure direct comparison of hybridization signals. HXT mRNA was detected by oligonucleotide probes as described previously (46, 47). ACT1 probes were used as RNA loading control. Data shown are representative of at least two independent experiments. C and D, quantification of HXT2 and HXT4 mRNAs. HXT mRNA hybridization signals were quantified by PhosphorImager and are expressed relative to ACT1 mRNA signals.
FIGURE 2. Tup1p and histone H3 binding decrease after activation of the HXT2 and HXT4 promoters. A, HXT promoter structure and location of primers used to analyze Tup1p, histone H3, TBP, and polymerase II binding. Boxes indicate binding sites for Mig1p (vertical stripes), Rgt1p (horizontal stripes), putative UAS (filled), and TBP (open) (19). Lines represent the DNA fragment, which was amplified by real-time PCR analysis. Numbers represent the location of HXT2 and HXT4 primers relative to the translation start site. Cells expressing a HA-tagged version of Tup1p (Table 1) were subjected to a glucose shift, and cross-linking was initiated by addition of formaldehyde at the indicated time points. Input and immunoprecipitated DNA using anti-HA (B) anti-H3 (C) anti-TBP and polymerase II (D) antibodies was analyzed by real-time PCR using primers spanning the TATA box (TATA), putative UAS (UAS), or further upstream (UPS) regions of the HXT genes as indicated. HXT signals are presented as the ratio of immunoprecipitation to input material relative to the POL1 ratio of immunoprecipitation to input material (40, 46). Signals represent the average of two independent glucose shift experiments, which were analyzed at least twice by real-time PCR.
SAGA Recruitment and Histone H3 Modification Depend on Snf1p—The observed reduction in histone H3 binding and increase in its acetylation suggests a link between the action of the Snf6p-Tup1p repressor and SAGA co-activator complex. Previous experiments showed that Snf1p can phosphorylate Mig1p resulting in a functional disruption of the Mig1p-Snf6p interaction and relief of Snf6p-Tup1p repression (22, 24). To test whether Snf1p activity is essential for the reduction of histone H3 binding and acetylation of histone H3, we analyzed histone H3 and H3K18Ac levels on the activated HXT2 and HXT4 promoters in yeast cells deleted for Sfn1p. This showed that histone H3 binding levels did not decrease in the absence of Sfn1p (Fig. 2, A and B). In contrast, deletion of SSN6 resulted in an almost complete loss of histone H3 binding regardless of the glucose concentration. Under non-inducing conditions histone H3 binding in tup1Δ cell was partially reduced, and levels further decreased after the glucose shift. This correlates with the partial derepression of HXT2 and HXT4 expression in tup1Δ cells (Fig. 1). When H3K18Ac was analyzed (Fig. 4, C and D) we found that H3K18Ac was completely absent in snf1Δ cells. In contrast, deletion of either SSN6 or TUP1 resulted in high H3K18Ac levels under repressing conditions, which were induced to wild-type levels after shifting of cells to low glucose.

Previously, we showed that the SAGA complex was recruited to the presumptive UAS regions of the HXT2 and HXT4 promoters upon their activation (40). Interestingly, Tup1p shows a weak preference for binding to the UAS region (Fig. 2A). To test whether SAGA association is linked to Snf1p activation and release of Snf6p-Tup1p repression, we tested association of the architectural Spt20p subunit of SAGA to the HXT2 and HXT4 promoters in sfn1Δ, tup1Δ, and ssn6Δ cells. Spt20p binding to the UAS regions of the HXT2 and HXT4 promoters is severely reduced in cells deleted for Sfn1p. In contrast, Spt20p associated in ssn6Δ cells and (to a lesser extent) in tup1Δ cells under non-inducing conditions (Fig. 4, E and F). Shifting these strains to low glucose resulted in Spt20p binding levels, which were similar to the wild-type level strain.

Besides Mig1p, Snf1p can mediate phosphorylation of serine 10 of histone H3, and this is important for SAGA recruitment to INO1 (25). To test whether the activated HXT2 and HXT4 genes display a similar dependence on H3S10 phosphorylation we analyzed induction in a strain expressing a S10A mutant form of histone H3. Fig. 5 shows that accumulation of HXT2 and HXT4 mRNAs occurred normally in this H3S10A strain, whereas as expected the sfn1Δ strain was entirely defective for induction.

In conclusion, under repressive conditions the Snf6p-Tup1p complex seems to prevent association of the SAGA complex to the HXT2 and HXT4 promoters. Under activating conditions Snf1p is essential for the recruitment of the SAGA complex, histone H3 removal, and H3K18Ac.

Gcn5p Mediates Removal of Histone H3 from the HXT4 TATA but Not from the HXT2 TATA Region—To test the role of SAGA in chromatin regulation of the HXT2 and HXT4 promoters, we analyzed histone H3 binding and histone H3K18Ac in the TATA box region in several SAGA mutant strains. Deletion of the Spt20p subunit decreased histone H3 removal from the HXT2 TATA region. In contrast, histone H3 binding to the HXT4 TATA box region was not affected during activation and remained at non-inducing levels (Fig. 6A). This shows that Snf1p-dependent histone H3 removal from the HXT2 promoter is partially dependent on SAGA activity, whereas on the HXT4 promoter histone H3 removal is strongly SAGA-dependent.

To test which component of the SAGA complex is crucial for histone H3 removal, gcn5Δ, spt3Δ, or spt8Δ cells were assayed for histone H3 binding. Whereas Gcn5 deletion had little effect on H3 binding to the HXT2 promoter, the spt3Δ and spt8Δ cells resemble the spt20Δ strain.

FIGURE 3. HXT2 and HXT4 genes are preferentially acetylated on histone H3K18Ac. Chromatin immunoprecipitations were performed with the same extracts as used for Fig. 2 using antibodies against histone H3K18Ac (A) and histone H3K14Ac (B). Histone H3K14Ac and H3K18Ac signals on the HXT2 (C) and HXT4 promoters (D) were divided for total histone H3 signals (see Fig. 2) to normalize acetylation levels. Signals are presented as described for Fig. 2.

against individual acetylated lysine residues of histone H3. We observed a strong increase in H3K18Ac relative to a control region of the POL1 open reading frame (46, 50) on both HXT2 and HXT4 promoters after a shift to low glucose (Fig. 3A). In contrast, we did not detect changes in H3K14Ac levels (Fig. 3B) and only a 2-fold increase in H3K9Ac levels (supplementary Fig. S1). However, when compared with the total amount of histone H3 on the HXT2 and HXT4 promoters, H3K14Ac and H3K9Ac increased after the glucose shift (Fig. 3, C and D). In addition, normalization to total histone H3 levels further confirms that the HXT2 and HXT4 promoters are preferentially acetylated on H3K18 (Fig. 3, C and D). Thus, analysis of the modification status of histone H3 reveals a specific histone H3 acetylation pattern on the HXT2 and HXT4 promoters during activation.
In contrast, yeast cells deleted for either SPT3 or SPT8 displayed only a mild defect in histone H3 removal from the HXT4 TATA box region. In this case, deletion of GCN5 prevented removal of H3 from the HXT4 promoter similar to spt20Δ cells (Fig. 7A). In contrast, acetylation was still induced in spt3Δ and spt8Δ cells by a shift to low glucose.

To test whether histone H3K18Ac is specifically dependent on the HAT activity of Gcn5p, we introduced a plasmid-based mutant allele encoding the GCN5 allele harboring the F221A amino acid substitution and the corresponding wild-type allele in gcn5Δ/H9004 cells. The F221A mutation is localized in the HAT domain of Gcn5p and completely abolishes activity (51). Cells containing wild-type Gcn5p showed an increase of histone H3K18Ac on both promoters (Fig. 8A), which is comparable with cells containing endogenous Gcn5p (see Fig. 3). In contrast, in cells expressing the mutant allele histone H3K18Ac induction is completely abolished (Fig. 8A).

To test whether the HAT activity of Gcn5p also plays a role in the removal of histone H3 after activation, we analyzed histone H3 binding. As expected, inactivation of the HAT domain does not affect removal of histone H3 binding from the HXT2 promoter (Figs. 8B and 6B). However, on the HXT4 promoter we observed only a small defect in histone H3 removal, which was less severe as observed in gcn5Δ cells (Figs. 8B and 6C). To test whether GCN5 play a role in the induction of mRNA of HXT2 and HXT4 we performed a Northern blot analysis. Fig. 8C shows that deletion of GCN5 or inactivation of its HAT activity has no effect on HXT2 and HXT4 mRNA induction as found previously (40). In conclusion, these experiments show that the Gcn5p subunit of the SAGA complex is responsible for the observed increase in histone H3K18Ac on the HXT2 and HXT4 promoters and suggest that,
in the case of HXT4, this acetylation is linked to the reduction in histone H3 association. However, GCN5 does not play an essential role in transcription activation of the HXT2 and HXT4 genes.

DISCUSSION

In this study the role of chromatin-related complexes in the transcriptional induction of the HXT2 and HXT4 genes was investigated in response to glucose levels. We show that the HXT2 and HXT4 promoters are subjected to acetylation of histone H3 at Lys-9, Lys-14, and Lys-18 and that histone H3 binding is reduced upon activation. Second, we find that Tup1p remains bound at the HXT2 and HXT4 promoters during activation. Thirdly, we show that Snf1p activation and derepression of the Snf6p-Tup1p complex is essential for binding of the SAGA complex to the HXT2 and HXT4 promoters. Although these chromatin-related complexes are recruited to both the HXT2 and HXT4 promoters upon their activation, the functional consequences of this are distinct.

Repression of the HXT2 and HXT4 Genes Does Not Solely Depend on Either Mig1p or Rgt1p Function—Whereas previous studies employed plasmid-based reporter genes to analyze activity of the HXT2 and HXT4 promoters (18, 19), we directly determined mRNA levels of these genes. As expected we observed a strong defect in mRNA accumulation of the HXT2 and HXT4 genes in a snf1Δ strain and constitutive mRNA expres-

FIGURE 6. Histone H3 binding in SAGA mutant strains. Cells deleted for SPT20 (A) or GCN5, SPT3, or SPT8 (B and C) were subjected to a shift to low glucose. Chromatin extracts were immunoprecipitated with antibodies directed against histone H3. Immunoprecipitated DNA was analyzed by real-time PCR with primers spanning the TATA box regions of the HXT genes as indicated. Signals are presented as described for Fig. 2.

FIGURE 7. Histone H3K18 acetylation in SAGA mutant strains. Cells deleted for GCN5, SPT3, or SPT8 (A and B) were subjected to a shift to low glucose. Chromatin extracts were immunoprecipitated with antibodies directed against histone H3K18Ac. Immunoprecipitated DNA was analyzed by real-time PCR with primers spanning the TATA box regions of the HXT genes as indicated. Signals are presented as described for Fig. 2.

The recruitment of the SAGA complex to the HXT2 and HXT4 genes completely depends on Snf1p. However, the exact mechanism on how
Snf1p recruits SAGA is not clear yet. Snf1p-dependent phosphorylation of histone H3S10 is involved in recruiting the SAGA complex to the INO1 promoter but it is not important for SAGA recruitment to the GAL1 promoter (25). Fig. 5 shows that the H3S10A mutation does not affect induction of HXT2 and HXT4 mRNAs. This suggests that Snf1p-dependent SAGA recruitment to HXT2 and HXT4 does not rely on H3S10 phosphorylation. Possibly, under non-inducing conditions, the Snf6p-Tup1p complex prevents SAGA recruitment by inhibiting binding of a hitherto unknown activator to the HXT2 and HXT4 promoters. For example, on the GAL1 promoter, the acidic activator Gal4p recruits the SAGA complex via Tra1p to the upstream promoter region (36, 37). This hypothesis is supported by the observations that Tup1p and SAGA association predominantly localize to the putative UAS regions of the HXT2 and HXT4 promoters. Furthermore, a small decrease in Tup1p binding precedes SAGA recruitment in time (40 and this report), and removal of the Sn6p-Tup1p repressor complex results in constitutive SAGA binding. We have tried to identify this activator by analysis of HXT2 and HXT4 mRNA expression in yeast cells deleted for several activators, which are known to be involved in stress-response. However the activators tested (e.g. MSN2, MSN4, YAP1, and YAP2) did not have any effect on HXT2 and HXT4 induction (data not shown). Interestingly, SAGA is only required for HXT4 expression (40), and the essential function of Snf1p can be explained by its role in facilitating the binding of the SAGA complex. In contrast, SAGA is not required for HXT2 expression. This suggests that Snf1p has additional roles beyond SAGA recruitment, which are essential for the expression.

**Acetylation of the HXT2 and HXT4 Promoters Might Serve as a Mark for Factor Binding**—To maintain a stable repressed state of promoters, the Snf6p-Tup1p repressor complex directly interacts with the N-terminal tails of histones (15), and Tup1p is associated with HDACs to preserve hypoacetylated histones (8, 9, 54). Deacetylation of histones by HDACs stimulates Tup1p interaction, and this interaction may prevent acetylation of histones in its turn (16, 17). Our observations do not support this view. Whereas we also observed that, under non-inducing conditions, Tup1p binding is associated with hypoacetylated histone H3, shifting cells to activating conditions or deleting either Tup1p or Snf6p resulted in decreased histone H3 binding and a concomitant increase in histone H3 acetylation. However, during activation Tup1p also remained bound at the HXT2 and HXT4 promoters. Interestingly, removal of the HAT activity by deletion of Gcn5p strongly diminished histone H3 acetylation on the HXT2 and HXT4 promoter and histone H3 removal on the HXT4 promoter but has no effect on transcription (40). Thus, Gcn5p-dependent chromatin modification and remodeling is not essential to counteract a possible repressive chromatin environment mediated by the Snf6p-Tup1p complex.

The observation that Gcn5p-dependent acetylation of the HXT2 and HXT4 promoters does not play a role in release from repression raises the question of its contribution to the expression of these genes. Recently, we showed that Gcn5p function is required for the recruitment of TFIID to the HXT2 and HXT4 promoters (40). Recruitment of the SAGA complex leads to an increase in histone H3K18Ac and to lesser extents in H3K9Ac and H3K14Ac on the HXT2 and HXT4 promoters, which depends on the HAT activity of Gcn5p. In the context of the native SAGA complex, Gcn5p has a broad substrate specificity in vitro and can acetylate Lys-9, Lys-14, Lys-18, and Lys-21 of histone H3 (55). The capacity of the SAGA complex to acetylate different histone H3 residues has been shown on the INO1 promoter, which has been reported to be acetylated on H3K14 and H3K18 by Gcn5p in vivo (25, 56). Interestingly, Tup1p interacts with class I HDACs Rpd3p, Hos1p, and Hos2p (8, 9) and has been shown to interact with HDAC1 to deacetylate histone H3 on the ENA1 promoter (54). Because Tup1p remains bound at the HXT2 and HXT4 promoters the opposite activities of Gcn5p and HDACs ultimately determine specific acetylation patterns during activation. This pattern might then serve as a mark for TFIID binding to the HXT2 and HXT4 promoters. Interestingly, Gcn5p also plays a role in the removal of histone H3 on the HXT4 promoter, which is partially dependent on the HAT activity. Whether Gcn5p-dependent histone H3 removal also plays a role in the possible recruitment of the TFIID complex is not known, and further experiments are necessary to elucidate the exact role of histone H3 acetylation and chromatin remodeling in the induction of the HXT2 and HXT4 genes.
REFERENCES
