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Differential Requirement of SAGA Subunits for Mot1p and Taf1p Recruitment in Gene Activation†

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Transcription activation in yeast (*Saccharomyces cerevisiae*) involves ordered recruitment of transcription factor complexes, such as TFIID, SAGA, and Mot1p. Previously, we showed that both Mot1p and Taf1p are recruited to the *HXT2* and *HXT4* genes, which encode hexose transporter proteins. Here, we show that SAGA also binds to the *HXT2* and *HXT4* promoters and plays a pivotal role in the recruitment of Mot1p and Taf1p. The deletion of either *SPT3* or *SPT8* reduces Mot1p binding to *HXT2* and *HXT4*. Surprisingly, the deletion of *GCN5* reduces Taf1p binding to both promoters. When *GCN5* is deleted in *spt3*Δ or *spt8*Δ strains, neither Mot1p nor Taf1p binds, and this results in a diminished recruitment of TATA binding protein and polymerase II to the *HXT4* but not the *HXT2* promoter. This is reflected by the SAGA-dependent expression of *HXT4*. In contrast, SAGA-independent induction of *HXT2* suggests a functional redundancy with other factors. A functional interplay of different SAGA subunits with Mot1p and Taf1p was supported by phenotypic analysis of *MOT1* SAGA or *TAF1*/SAGA double mutant strains, which revealed novel genetic interactions between *MOT1* and *SPT8* and between *TAF1* and *GCN5*. In conclusion, our data demonstrate functional links between SAGA, Mot1p, and TFIID in *HXT* gene regulation.

Yeast cells respond to environmental stress by changing their transcription profiles. Changes in the environment are relayed via a cascade of signal transduction pathways to transcriptional activators. These activators recruit distinct cofactors which can modify the chromatin organization of promoter regions, enabling association of the mediator complex to the core promoter and subsequent recruitment of the TATA binding protein (TBP) and the basal transcription machinery (36). The recruitment of different activities at a given time during transcription activation is a gene-specific event (14).

Recent analysis has indicated that yeast can have different requirements for the TFIID or SAGA coactivator complexes. They interact with activators, possess histone acetyltransferase (HAT) activity, and facilitate TBP binding to promoter sites (24). SAGA and TFIID share a common set of TBP-associated factors (TAFs) (25), which are required for the integrity of both complexes (33, 57). In contrast, TBP is an integral part of the TFIID complex but not of SAGA. However, TBP shows multiple genetic interactions with the SAGA components *SPT3* and *SPT8* (20, 21) and physically interacts with Spt8p (50, 55). An alternative SAGA-like complex, SALSA/SLIK, contains a truncated form of Spt7p and lacks Spt8p (48). Moreover, SAGA shares the Ada1p/Gcn5p HAT module with the ADA complex (19).

In vivo protein-DNA cross-linking studies classified yeast promoters as either TAF dependent or TAF independent based on the ratio of TBP/TAF binding to promoter sites (29, 34). Genome-wide expression analysis of various strains carrying mutations in genes encoding subunits of TFIID and SAGA also allowed classification into SAGA-dependent genes and TFIID-dependent genes (28, 32). Interestingly, consensus TATA elements which constitute the optimal binding site for TBP are overrepresented in SAGA-dependent genes (5). This suggests that TFIID and SAGA complexes are recruited to different sets of target promoters via different molecular mechanisms. SAGA is specifically recruited to upstream activator sequences (UAS) (8, 9, 31). In contrast, TFIID is specifically recruited to core promoter regions which require TAF subunits that either interact with activators or have specificity for promoter sequences (11).

Mot1p has initially been characterized as a global negative regulator of transcription (16, 42, 44). Mot1p has been found in a stable complex with TBP (43), and the Mot1p/TBP complex binds TATA DNA with high affinity (26). The C-terminal ATPase domain of Mot1p can disrupt Mot1p-TBP-TATA ternary complexes in vitro upon addition of ATP (2). Thus, Mot1p can exert its negative role in transcription by disruption of TBP-TATA complexes in an ATP-dependent manner (41). Genome-wide expression analysis of two different *mot1* mutant strains shows that Mot1p has both negative and positive effects on transcription regulation (1, 15, 22). In addition, in vivo protein-DNA cross-linking studies show that Mot1p binds to heat shock, copper-regulated, or salt-inducible promoter regions after their activation, which correlates with TBP and RNA polymerase II (Pol II) binding to these promoters (22, 23). Moreover, the promoters of the heat shock genes *SSA3*, *SSA4*, *HSP104*, and *CTT1* and the copper-inducible *CUP1* gene have been described as *TAF* independent (29). This suggests a positive role of Mot1p in the activation of these stress-regulated genes. Moreover, it was proposed that stress conditions activate the Mot1p/TBP complex (23). In agreement with

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TABLE 1. List of yeast strains used in this study

Strain	Genotype	Reference or source
COy001	*MAT*α *leu2-3,112 his3-11,15 trp1-1 can1-100 ade2-1 ura3-1*	This paper
COy015	Isogenic to COy001 except *spt3*Δ::*URA3*	This paper
COy017	Isogenic to COy001 except *ada2*Δ::*URA3*	This paper
COy019	Isogenic to COy001 except *spt7*Δ::*URA3*	This paper
COy021	Isogenic to COy001 except *spt8*Δ::*URA3*	This paper
COy023	Isogenic to COy001 except *spt20*Δ::*URA3*	This paper
COy026	Isogenic to COy001 except *gcn5*Δ::*KANA*	This paper
COy054	Isogenic to COy001 except *spt3*Δ::*URA3 gcn5*Δ::*KANA*	This paper
COy055	Isogenic to COy001 except *spt8*Δ::*URA3 gcn5*Δ::*KANA*	This paper
COy056	Isogenic to COy001 except *spt3*Δ *spt8*Δ::*URA3*	This paper
COy059	Isogenic to COy001 except *mot1-1*::*URA3*	This paper
DPY107	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-*Δ*1 his3-*Δ*200 leu2-*Δ*1 mot1*::*TRP1*::*flu3*::*MOT1*	43
COy089	Isogenic to DPY107 except *spt3*Δ::*URA3*	This paper
COy091	Isogenic to DPY107 except *spt8*Δ::*URA3*	This paper
JCA001	Isogenic to DPY107 except *gcn5*Δ::*KANA*	This paper
COy095	Isogenic to DPY107 except *spt3*Δ::*URA3 gcn5*Δ::*KANA*	This paper
COy096	Isogenic to DPY107 except *spt8*Δ::*URA3 gcn5*Δ::*KANA*	This paper
COy097	Isogenic to DPY107 except *spt3*Δ *spt8*Δ::*URA3*	This paper
YBY838	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-*Δ*1 his3-*Δ*200 leu2-*Δ*taf11*Δ::*TRP1* pRS313-3Flu-TAF130-His6-FLAG (HIS CEN)	3
COy098	Isogenic to YBY838 except *spt3*Δ::*URA3*	This paper
COy100	Isogenic to YBY838 except *spt8*Δ::*URA3*	This paper
JCA002	Isogenic to YBY838 except *gcn5*Δ::*KANA*	This paper
COy104	Isogenic to YBY838 except *spt3*Δ::*URA3 gcn5*Δ::*KANA*	This paper
COy105	Isogenic to YBY838 except *spt8*Δ::*URA3 gcn5*Δ::*KANA*	This paper
COy106	Isogenic to YBY838 except *spt3*Δ *spt8*Δ::*URA3*	This paper
COy142	Isogenic to COy001 except *SPT20*::*TAP*::*TRP1*	This paper
COy140	Isogenic to COy001 except *SPT3*::*TAP*::*TRP1*	This paper
COy036	Isogenic to COy001 except *mot1-1*::*URA3 SPT20*::*TAP*::*TRP1*	This paper
COy182	Isogenic to FHY58 except *spt3*Δ::*URA3*	This paper
COy184	Isogenic to FHY58 except *spt8*Δ::*URA3*	This paper
COy180	Isogenic to FHY58 except *gcn5*Δ::*KANA*	This paper
COy181	Isogenic to FHY59 except *spt3*Δ::*URA3*	This paper
COy183	Isogenic to FHY59 except *spt8*Δ::*URA3*	This paper
COy179	Isogenic to FHY59 except *gcn5*Δ::*KANA*	This paper
COy185	Isogenic to COy001 except *mot1-1 spt3*Δ::*URA3*	This paper
COy187	Isogenic to COy001 except *mot1-1 gcn5*Δ::*KANA*	This paper

these results, we found that Mot1p is involved in recruitment of TBP to the *HXT2* and *HXT4* genes. TBP recruitment was severely compromised in two different *mot1* mutant strains but only weakly in a *taf1* mutant (1).

The *HXT2* and *HXT4* genes are part of a family of hexose transporters comprising 18 different genes in yeast. *HXT2* and *HXT4* are high-affinity transporters which are transcribed under conditions of low glucose (37, 38, 56). Under conditions of high glucose, the *HXT2* and *HXT4* genes are repressed by Mig1p and Rgt1p, which recruit the general repressor complex Tup1p/Ssn6p. Under low-glucose conditions, the Snf1p kinase phosphorylates Mig1p, which leads to derepression of the *HXT2* and *HXT4* genes (37) and subsequent recruitment of Taf1p, Mot1p, TBP, and Pol II (1). How these factors are recruited to the *HXT2* and *HXT4* promoters remains an open question. Possibly, the SAGA complex is involved in this process. Shifting cells to low glucose concentrations could be regarded as a stress condition, and SAGA has been proposed to regulate stress-dependent genes (28). Moreover, *MOT1* displays genetic interactions with *SPT3* (12, 35), a component of SAGA. In addition, it has been shown that Spt3p and Mot1p are recruited to the *GAL1* promoter in an interdependent manner (53).

Here, we show that SAGA is recruited to the *HXT2* and *HXT4* promoters upon activation by low glucose. In addition, deletion of the *SPT3* or *SPT8* subunit affects recruitment of Mot1p. In contrast, Taf1p recruitment is dependent on the Gcn5p module of SAGA. Our results provide novel insight into the interplay of SAGA, TFIID, and Mot1p on activated promoters.

MATERIALS AND METHODS

Yeast strains. The *Saccharomyces cerevisiae* strains used in this study are listed in Table 1, except for FHY59, a *taf1ts2* strain, and the congenic wild-type strain (54). The COy001 strain represents the W303-derived DY150 strain purchased from the American Type Culture Collection (Manassas, VA). All procedures were performed according to standard methods (10). The HA_3-*MOT1* (DP107) and HA_3-*TAF1* (YBY838) tagged strains have been described previously (3, 43). Single or double SAGA mutant strains were constructed as described previously (47). However, the *gcn5*Δ strain was constructed using a *GCN5* disruption cassette (51). To construct an *spt3*Δ *spt8*Δ strain, COy015 (*spt3*Δ::URA3) was grown on 5-fluoroorotic acid plates to select for colonies which lost the URA3 cassette. Subsequently, the obtained strain was retransformed to replace the *SPT8* coding region (47). All deletions were verified by PCR analysis with primers corresponding to both wild-type and disruption alleles. SPT20-TAP and SPT3-TAP strains were constructed as described previously (46). Proper integration was verified by Western blot analysis and by PCR analysis with primers corresponding to both the wild-type and the tagged allele. To introduce the *mot1-1* allele in the W303 background, a 292-bp fragment of the *mot1-1* allele, harboring a Trp1730Stop mutation, was amplified by PCR (47) and transformed into the COy001 strain. Transformants carrying the *mot1-1* allele were identified based on a tempera-

ture-sensitive (TS) phenotype by growth at 37°C. From these clones, the 3′ end of *MOT1* was PCR amplified and sequenced to verify the *mot1-1* mutation. To test for genetic interactions between SAGA and *MOT1*, single mutant strains were crossed and sporulated, followed by tetrad analysis to check for proper marker segregation. Sequences of oligonucleotides used in this study are available upon request.

Growth conditions. For glucose concentration shift experiments, cells were grown in SC medium containing 4% glucose. When cells had reached mid-log phase (optical density at 600 nm = 0.55 to 0.6), 250 ml of culture was cross-linked for 20 min at room temperature by addition of 1% formaldehyde (corresponding to time $t = 0$). The remaining cells were harvested by centrifugation for 6 min at 1,700 × *g* in a Sorvall SLA3000 rotor, resuspended in a small volume of SC with 4% glucose, and diluted in SC without glucose to adjust the glucose concentration to 0.1%. Cells were incubated at 30°C and subjected to cross-linking at the indicated time points.

For RNA analysis, cells were grown in SC medium containing 4% glucose. When cells had reached mid-log phase (optical density at 600 nm = 0.55 to 0.6), 10 ml of the culture was directly added to 25 ml liquid N_2 ($t = 0$). After shifting to low glucose, 10 ml of the culture was removed at the indicated time points and was frozen directly in liquid N_2. After thawing, cells were collected by centrifugation, refrozen in liquid N_2, and stored at −80°C upon further processing.

Northern blotting. RNA isolation and hybridization conditions were described previously (1). Oligonucleotide sequences and the labeling procedure used for *HXT* genes have been described previously (17). 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 RediprimeII system according to the manufacturer's protocol (Amersham). PhosphorImager quantification of *HXT2* and *HXT4* mRNA signals has been described previously (1). Data from duplicate experiments were used for this quantification.

Chromatin immunoprecipitation (ChIP) assay. Chromatin extracts (CE) were prepared as previously described (1). CE (200 μl) were used for immunoprecipitation (IP), and 10 μl CE was used for input control preparation. IPs were performed with 25 μl of Prot G-agarose beads (Roche) prebound to 10 μg anti-yTBP, 30 μg antihemagglutinin (anti-HA) antibodies (12CA5), or 10 μl anti-Pol II CTD monoclonal antibody (8WG16) or with 30 μl immunoglobulin G Sepharose 6 fast flow beads (Amersham). IPs were performed as described previously (1). After elution, cross-links were reversed by incubating the eluates with 200 μg proteinase K for 2 h at 37°C and overnight at 65°C. DNA from input samples was prepared similarly. After DNA purification from the eluates using Qiaquick columns (QIAGEN), DNA was analyzed by multiplex PCR (1).

RESULTS

Transcriptional induction of *HXT4*, but not *HXT2*, depends on the SAGA complex. The SAGA complex consists of multiple subunits involved in different aspects of its coactivator and histone acetyltransferase functions (49). Biochemical and structural analysis showed that the Spt7p and Spt20p subunits are essential for the structural integrity of the complex (50, 57). In order to test whether SAGA is involved in transcription activation of the *HXT2* and *HXT4* genes, we analyzed their expression in yeast strains with deletions of specific SAGA subunits after shifting to low glucose concentrations. To allow the analysis of early time points, we devised a rapid cell harvesting protocol, which involves the instantaneous freezing of cells (see Materials and Methods). In wild-type yeast cells, both *HXT2* and *HXT4* mRNAs accumulated, peaking at 10 min after the shift to 0.1% glucose (Fig. 1A and D). In contrast, *spt7*Δ or *spt20*Δ mutant cells were severely compromised in *HXT4* induction. Deletion of these SAGA subunits only mildly affected *HXT2* activation. Interestingly, *HXT2* induction seemed to be somewhat delayed in the *spt20*Δ and *spt7*Δ strains (Fig. 1A and D; see also the supplemental material).

To investigate contributions of the different SAGA modules, we constructed additional yeast deletion strains to compare transcriptional induction of the *HXT2* and *HXT4* genes (Fig. 1B, C, and D and the supplemental material). As expected, *HXT2* mRNA induction was not affected by the deletion of individual SAGA modules, but induction shows a delay similar to *spt20*Δ cells (Fig. 1B and D; see also the supplemental material). Removal of the HAT activity by deletion of either *GCN5* or *ADA2* did not affect the induction of *HXT4* mRNA. In contrast, the deletion of either *SPT3* or *SPT8*, which comprise the TBP module of SAGA (20, 21), resulted in decreased *HXT4* transcription. When *spt3*Δ *spt8*Δ cells were analyzed, we found that this double deletion did not further reduce *HXT4* induction. The observed reduction in *HXT4* transcription in *spt8*Δ cells shows that SAGA is involved and that alternative complexes, such as the SALSA/SLIK or ADA complexes, do not play a role (19, 48). In contrast, in *gcn5*Δ *spt3*Δ or *gcn5*Δ *spt8*Δ cells, expression was further reduced to a level comparable to that seen in *spt7*Δ or *spt20*Δ cells.

Taken together, these observations indicate that SAGA plays an important role in *HXT4* gene induction after shifting to low glucose conditions. The Spt3p/Spt8p TBP module of SAGA seems to be most important in this. The contribution of its HAT module (Gcn5p-Ada2p-Ada3p) is apparent only in strains already compromised in this TBP module.

Transcriptional activation of *HXT2* and *HXT4* coincides with SAGA recruitment. It has been shown that the SAGA coactivator complex can bind to UAS regions of target promoters upon their activation (4, 8, 13, 31). To investigate whether SAGA is directly involved in the activation of the *HXT* genes, we tested the association of the architectural Spt20p and TBP-interacting Spt3p subunits of SAGA during activation of the *HXT2* and *HXT4* promoters. This was investigated by ChIP experiments employing yeast strains expressing tandem affinity purification (TAP)-tagged versions of Spt20p or Spt3p (Table 1). The TAP-tagged strains were grown in SC medium containing 4% glucose and shifted to SC medium containing 0.1% glucose to induce the *HXT2* and *HXT4* genes. Subsequently, formaldehyde-cross-linked chromatin was isolated at various times after the shift to low glucose. Immunopurified chromatin was analyzed by multiplex PCR using primer sets that amplify the TATA and upstream region of the *HXT* genes (Fig. 2A) or an intragenic fragment of the *POL1* gene as a normalization control (30). Directly after shifting to 0.1% glucose, binding of Spt20p and Spt3p to the upstream regions of both *HXT2* and *HXT4* is specifically increased and localized to −441/−222 and −523/−238 of *HXT2* and *HXT4*, respectively (Fig. 2B). A putative activator colocalizes the *HXT2* region (39), but this is not known yet for the *HXT4* fragment. Spt20p and Spt3p association is transient and can already be detected 2 minutes after a shift to low glucose. This precedes the induction of *HXT* mRNA expression (compare Fig. 1A). After 5 minutes, Spt20p and Spt3p start to decrease to background levels. The rapid recruitment of SAGA subunits coincides with Mot1p binding kinetics (1). Mot1p becomes associated to the TATA region of the *HXT2* and *HXT4* promoters 2 minutes after the shift to low glucose and decreases after 5 to 10 minutes.

In conclusion, these experiments show that SAGA is recruited to both *HXT2* and *HXT4* promoters under conditions of activation.

Mot1p and Taf1p recruitment to *HXT* genes depends on different modules of SAGA. We reported previously that Mot1p and the Taf1p subunit of TFIID are recruited to the

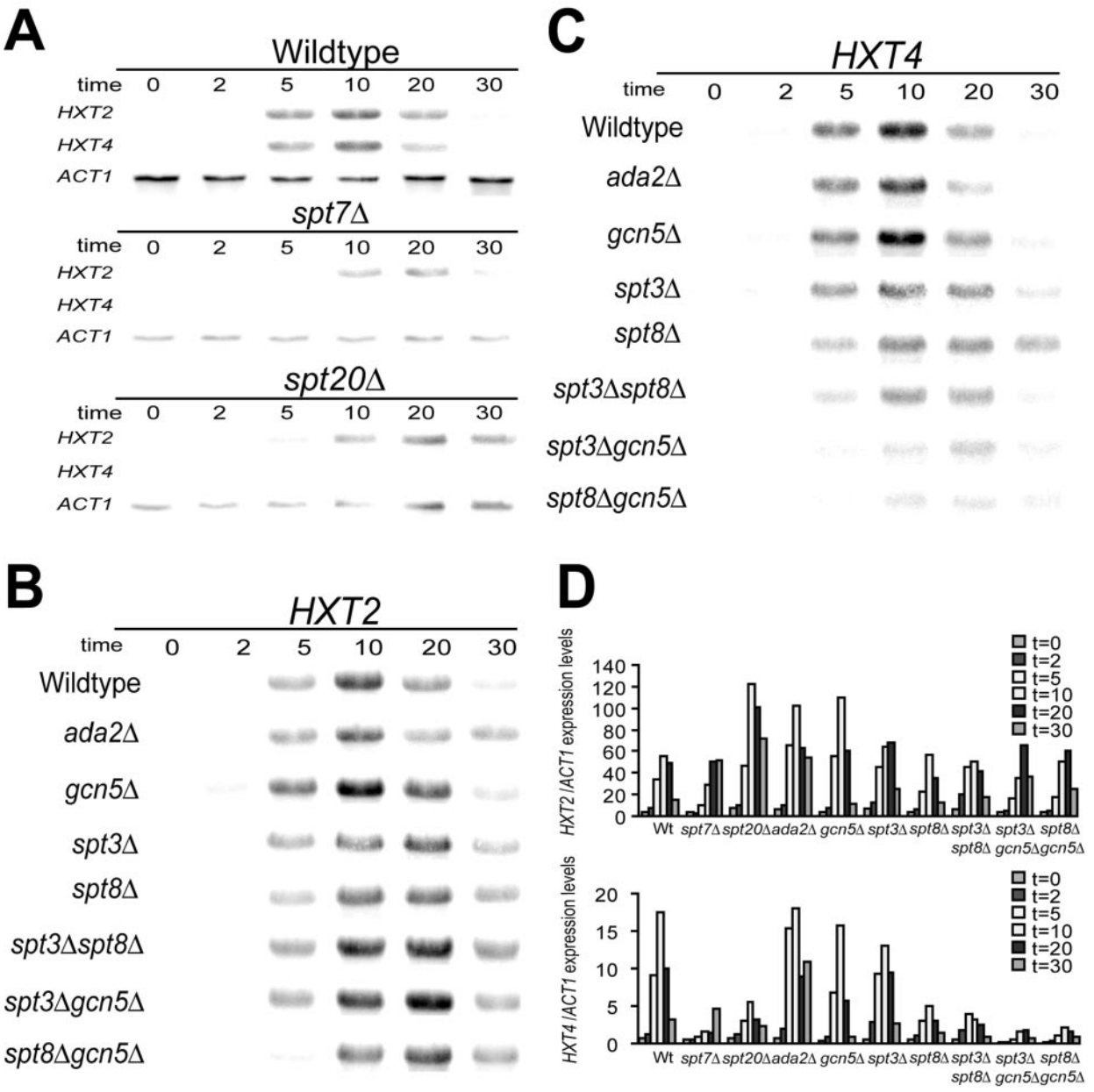

FIG. 1. Transcription of *HXT4* but not *HXT2* is affected in SAGA mutant strains. (A) Kinetics of mRNA expression of *HXT* genes in wild-type, *spt7*Δ, and *spt20*Δ strains upon a shift to low glucose. Cells were grown in 4% glucose and shifted to 0.1% glucose for 0, 2, 5, 10, 20, and 30 min before collection (see Materials and Methods). RNA samples from wild-type, *spt7*Δ, and *spt20*Δ strains were processed in parallel and analyzed on a single Northern blot to ensure direct comparison of hybridization signals. *HXT* mRNAs were detected using oligonucleotide probes described previously (1, 17). *ACT1* probes were used us an internal loading control. Data shown are representative of at least two independent experiments. (B and C) Kinetics of mRNA expression of the *HXT2* gene (B) or the *HXT4* gene (C) in various SAGA mutant strains. RNA samples from wild-type, *ada2*Δ, *spt3*Δ, *gcn5*Δ, *spt8*Δ, *spt3*Δ *gcn5*Δ, *spt8*Δ *gcn5*Δ, and *spt3*Δ *spt8*Δ strains were analyzed on single Northern blots as in panel A. Reprobing of the blots with the *ACT1* probe verified equal mRNA loading (data not shown). (D) Quantification of *HXT2* and *HXT4* mRNAs. *HXT* hybridization signals were quantified by a PhosphorImager and are expressed relative to *ACT1* mRNA signals.

HXT2 and *HXT4* promoters upon a change to low glucose concentrations (1). Mutations in *MOT1* affect TBP recruitment, whereas mutations in *TAF1* have little effect (1). These findings and those described above suggest that SAGA could be involved in Mot1p and/or Taf1p recruitment. In addition, observations made in other experimental systems showed that Spt3p and Spt8p can be involved in the recruitment of TBP (7, 8, 18, 31). Spt3p and Gcn5p were also implicated in the recruitment of Mot1p to the activated *GAL1* promoter (53). To test SAGA involvement in Mot1p and or Taf1p recruitment, we constructed several SAGA mutant strains expressing HA-tagged versions of Mot1p (43) or of Taf1p (3). Subsequently,

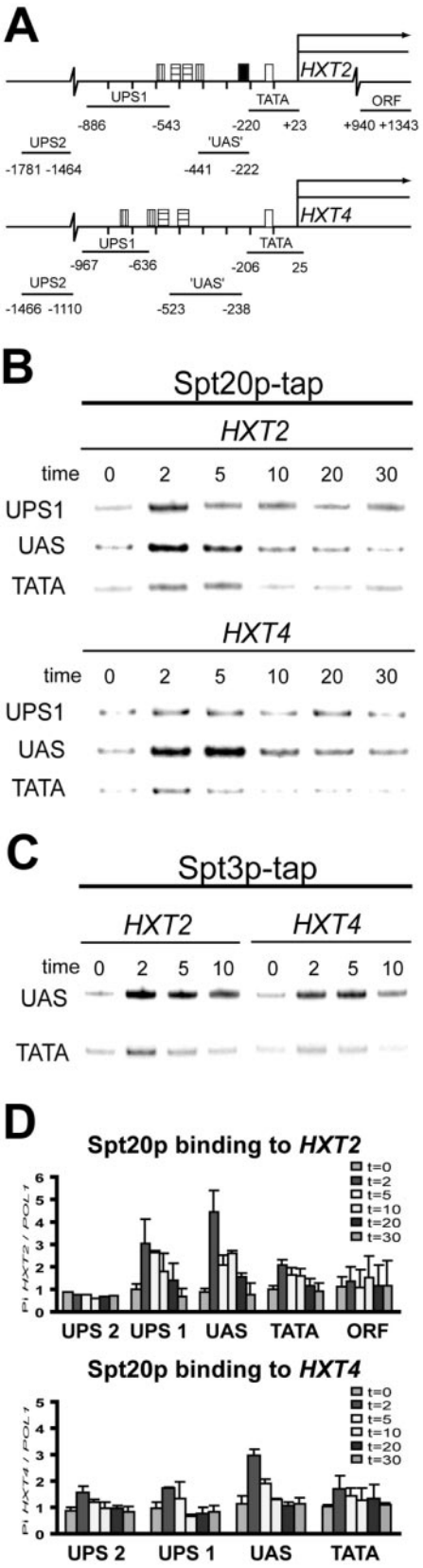

FIG. 2. SAGA specifically binds to the upstream region of both *HXT2* and *HXT4* genes. (A) *HXT* promoter structure and location of primers used to analyze Spt20p and Spt3 binding. Boxes indicate binding sites for Mig1p (vertical stripes), Rgt1p (horizontal stripes), putative UAS (filled), and TBP (open) (39). Lines represent the DNA fragment, which was amplified in a multiplex PCR analysis. Numbers represent the exact location of *HXT2* and *HXT4* primers relative to the start site of the coding region. (B and C) Cells expressing a TAP-tagged version of Spt20p (B) or Spt3p (C) were subjected to a glucose shift, and cross-linking was initiated by addition of formaldehyde at the indicated time points. Input and immunoprecipitated DNA was analyzed by multiplex PCR with primers spanning the TATA box (TATA), putative UAS, or further upstream (UPS1) regions of the *HXT* genes as indicated. (D) Quantification of ChIP signals over the *HXT2* and *HXT4* genes. Radioactive signals of the indicated PCR fragments were quantified with a PhosphorImager. The signals are expressed relative to the *POL1* signal, which was included as a negative control in the multiplex PCR analysis.

recruitment of these factors, TBP, and Pol II binding to the activated *HXT* promoters was determined by using ChIP assays (see Materials and Methods).

Mot1p was recruited within 2 minutes to the *HXT2* and *HXT4* promoters in a wild-type strain, in agreement with our previous findings (Fig. 3A) (1). In contrast, Mot1p recruitment to both promoters was severely impaired in the *spt3*Δ and *spt8*Δ cells and reduced only slightly in the *gcn5*Δ strain (Fig. 3A). To test whether recruitment of SAGA also depends on Mot1p, we analyzed Spt20p binding in the *mot1-1* strain (Fig. 3B), which is severely impaired in TBP recruitment (1). We found that Spt20p was recruited normally to the *HXT2* and *HXT4* promoters in the *mot1-1* mutant. Thus, in the case of the *HXT* promoters, the Spt3p-Spt8p module of SAGA is essential for efficient Mot1p recruitment.

In contrast to Mot1p, the deletion of *GCN5* severely impaired Taf1p recruitment (Fig. 3C). Taf1p recruitment was also reduced in *spt8*Δ and to a lesser extent *spt3*Δ cells. To test whether the impaired recruitment of Mot1p and Taf1p correlates with reduced preinitiation complex formation on *HXT* start site regions, we analyzed TBP and Pol II recruitment to these promoters. As expected (1), the kinetics of TBP recruitment to the activated *HXT* promoters is very rapid and coincides with Mot1p and Taf1p binding (Fig. 3D). Surprisingly, TBP and Pol II association with both the *HXT2* and *HXT4* promoters is only mildly affected by the deletion of the SAGA subunits (Fig. 3D). The strongest reduction, however, is observed in the *spt8*Δ strain, which also displays the strongest effect on mRNA accumulation. The weakest effects on TBP recruitment are observed in the *gcn5*Δ strain, which displays a severe reduction in Taf1p recruitment. This is in agreement with observations that Taf1p plays a minor role in TBP recruitment to activated *HXT* promoters (1).

Different modules of SAGA cooperate in Mot1p and Taf1p recruitment to *HXT* genes. The observations that different SAGA subunits are involved in the recruitment of Mot1p and Taf1p prompted us to test whether different modules of SAGA cooperate in the recruitment of Mot1p and Taf1p. To this end, we constructed *gcn5*Δ *spt3*Δ and *gcn5*Δ *spt8*Δ strains expressing either HA-Mot1p or HA-Taf1p. Subsequent ChIP analysis showed that Mot1p and Taf1p recruitment was absent or strongly reduced to both *HXT* promoters in *gcn5*Δ *spt3*Δ and *gcn5*Δ *spt8*Δ cells (Fig. 4). These findings indicate that Gcn5p can work in concert with Spt8p and Spt3p to recruit Mot1p to the *HXT2* and *HXT4* promoters. In addition, the deletion of

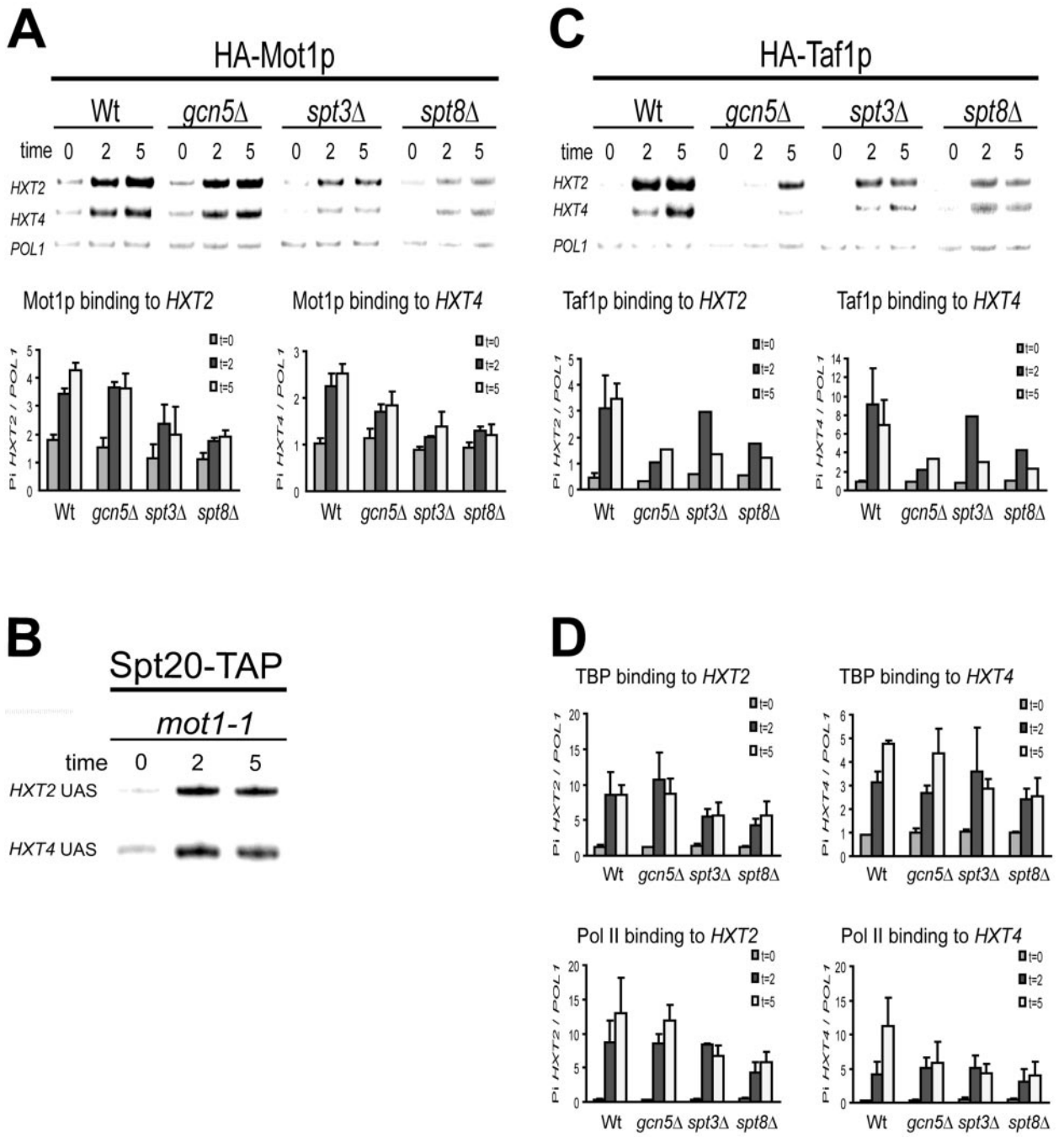

FIG. 3. Binding kinetics of SAGA, Mot1p, Taf1p, TBP, and Pol II to *HXT* genes in SAGA and *MOT1* mutant strains after a shift to low glucose. (A) Representative PCR and PhosphorImager quantification of Mot1p binding to *HXT* genes in wild-type (Wt), *gcn5*Δ, *spt3*Δ, and *spt8*Δ cells. The top panel shows multiplex PCR analysis in which *POL1* primers have been included as a normalization control. Primers amplifying *HXT2* and *HXT4* are specific for the core promoter region as indicated for Fig. 2A. Each sample was analyzed at least in duplo. The lower panel displays the quantification. Factor binding is expressed as *HXT*/*POL1* ratios, as described previously (1). (B) Analysis of Spt20p binding to *HXT* genes in a *mot1-1* mutant strain after a shift to low glucose. (C) Taf1p binding to *HXT* in Wt, *gcn5*Δ, *spt3*Δ, and *spt8*Δ cells. (D) TBP and Pol II recruitment to *HXT* genes in Wt, *gcn5*Δ, *spt3*Δ, and *spt8*Δ strains.

SPT3 but not *SPT8* further reduces Gcn5p-dependent Taf1p recruitment (Fig. 4). Next we analyzed TBP and Pol II recruitment in these double mutant strains. Surprisingly, TBP recruitment to *HXT2* was not affected, and the efficiency of TBP recruitment to *HXT4* was only mildly reduced (Fig. 4B). Therefore, in the absence of a functional SAGA complex, TBP does not absolutely require Mot1p or the TFIID complex to associate with the *HXT2* and *HXT4* promoters during their activa-

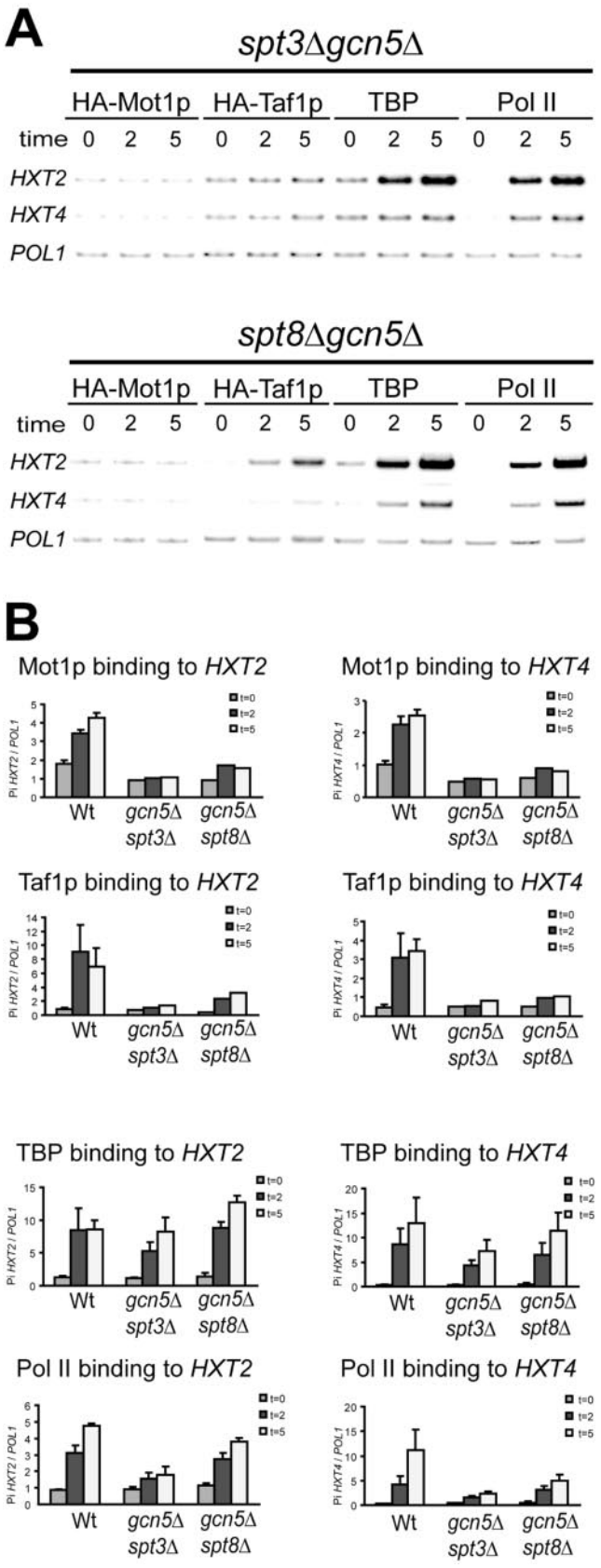

FIG. 4. Binding kinetics of Mot1p, Taf1p, TBP, and Pol II to *HXT* genes in SAGA double deletion strains. (A) Recruitment of Mot1p, Taf1p, TBP, and Pol II in *gcn5*Δ *spt3*Δ and *gcn5*Δ *spt8*Δ cells after a shift to low glucose. Multiplex PCR signals of immunoprecipitated DNA as indicated above the lanes are displayed for the TATA-containing fragments of *HXT2* and *HXT4* as described for Fig. 2A. (B) PhosphorImager quantification of Mot1p, Taf1p, TBP, and Pol II binding to the indicated *HXT* promoters.

tion by a shift to low glucose. In contrast to this, Pol II recruitment to *HXT4* is severely reduced, but little effect is observed on Pol II binding to *HXT2* (Fig. 4B). This parallels mRNA accumulation of these genes upon a shift to low glucose (Fig. 1B). These findings also suggest that TBP complexes formed on the *HXT4* promoter are not functional. In conclusion, disruption of both the Gcn5p HAT and Spt3p/Spt8p TBP modules of SAGA prevent Mot1p recruitment to the *HXT* promoters.

Genetic interactions of *MOT1* or *TAF1* with SAGA subunits. Differential SAGA subunit requirement as observed for the *HXT* promoters suggests separate pathways for Mot1p and Taf1p recruitment. We decided to investigate whether this could be a general phenomenon by testing for genetic interactions of *MOT1* and *TAF1* with different SAGA modules. The deletion of *GCN5* in *taf1ts2* cells elicited a severe growth defect on SC plates containing 2% glucose (Fig. 5A, row 6). When these *gcn5*Δ/*taf1ts2* mutant cells were spotted on SC plates containing 0.5% glucose, the growth defect was even more pronounced. In contrast, *spt8*Δ/*taf1ts2* and *spt3*Δ/*taf1ts* cells grew like the wild type and displayed a very mild defect, respectively (Fig. 5A, rows 7 and 8). In order to cross the *mot1-1* mutant with SAGA mutant strains in the W303 background, we first created the *mot1-1* allele in this background (see Materials and Methods). In contrast to *taf1ts2* cells, we noted that *mot1-1* cells already displayed a growth defect at a lower glucose concentration (Fig. 5, compare panels A and B). The *mot1-1* strain was crossed with the *spt3*Δ, *spt8*Δ, and *gcn5*Δ mutant strains. Resulting diploids were subjected to sporulation to obtain double disruptant strains. In the case of *mot1-1*/*spt8*Δ, analysis of 21 tetrads revealed synthetic lethality between *mot1-1* and *spt8*Δ. The other combinations were viable and were analyzed for growth at different temperatures and glucose concentrations in synthetic media. The deletion of *SPT3* in *mot1-1* cells created a strong growth defect, which was most apparent at lower glucose concentrations (Fig. 5B). This agrees with earlier reports of *spt3*Δ/*mot1-1* cells as severely growth defective (12, 35). In contrast, the deletion of *GCN5* had no effect on *mot1-1* mutant cells.

The observed growth defect of *gcn5*Δ/*taf1ts2* cells suggests a Gcn5p-Taf1p pathway. In contrast, *mot1-1* cells do not depend on Gcn5p but rather on Spt3p and Spt8p, suggesting the existence of a separate Spt3p/Spt8p-Mot1p pathway.

DISCUSSION

In this paper we showed that SAGA is recruited to the activated *HXT2* and *HXT4* promoters and that SAGA is essential for the recruitment of Mot1p and the Taf1p subunit of TFIID. The deletion of the Spt3p/Spt8p TBP module of SAGA affected predominantly the recruitment of Mot1p, whereas the deletion of *GCN5* selectively impaired Taf1p recruitment.

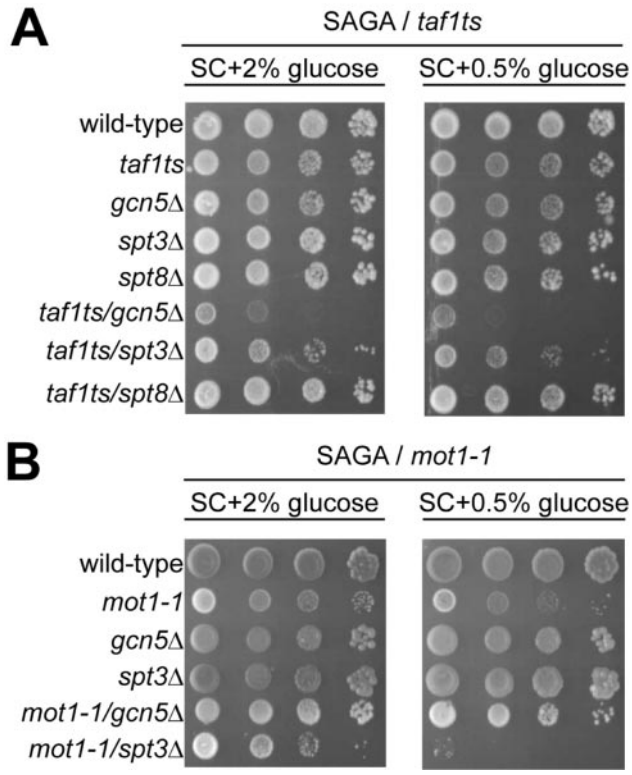

FIG. 5. Genetic interactions between SAGA, *MOT1*, and *TAF1* during conditions of stress. (A) Wild-type (FHY58) and SAGA/*TAF1* or (B) wild-type (W303) and SAGA/*MOT1* mutant strains (as indicated) were serially diluted (10-fold steps) and grown on SC plus 2% glucose or SC plus 0.5% glucose. Cells were grown for 4 days at 33°C.

When both the Spt3p/Spt8p module and the Gcn5p HAT module were absent, Mot1p and Taf1p were not recruited. Surprisingly, TBP recruitment is relatively unaffected in such SAGA mutant strains. In contrast, Pol II association to the *HXT4* but not the *HXT2* promoter is reduced, which is reflected by differences in *HXT2* and *HXT4* mRNA accumulation. Functional interactions of SAGA, Mot1p, and Taf1p were supported by phenotypic analyses of double mutant strains grown under normal and glucose-restricted conditions. A *mot1-1* strain shows phenotypes with *spt8*Δ and *spt3*Δ alleles but not with *gcn5*Δ. Conversely, a *taf1ts2* TS strain displays a synthetic growth phenotype with *gcn5*Δ.

Recruitment of the SAGA complex to *HXT* genes. *HXT2* and *HXT4* encode high-affinity hexose transporters and are transcribed under low glucose concentrations (38). We used activation of these genes as a model system to investigate transcriptional regulation by Mot1p and Taf1p. Our previous work showed that both of these TBP-interacting proteins can be recruited to the *HXT2* and *HXT4* promoters and that Mot1p is essential for TBP recruitment (1). Relatively little is known about positively acting transcription factors involved in activation of these *HXT* genes. While SAGA is clearly recruited to both *HXT* promoters, it remains unclear which gene-specific activators are responsible for this. Deletion analysis of the *HXT2* promoter identified a UAS element located between −291 and −218 relative to the start of the gene (39). Thus, one possible mechanism for SAGA recruitment is through the activator(s) binding to this region. Alternatively, activation of the Tup1p/Ssn6p complex mediates SAGA binding to the *HXT* promoters. It has been shown that under stress conditions, the Tup1p/Ssn6p corepressor complex transforms into a coactivator, which facilitated SAGA binding to *GAL1* and several osmotic stress-induced genes (40, 45). In this case, SAGA recruitment required the Cti6p protein (40). Possibly, Cti6p plays a similar role for the *HXT* genes studied here.

It is also important to note that although recruited to the *HXT2* promoter, the SAGA complex is not required for transcriptional activation of this gene. This indicates a functional redundancy of SAGA, which is not apparent in *HXT4* transcription. This could either be due to other coactivators present at the *HXT2* promoter that substitute for SAGA function or be due to a different chromatin organization of this gene. In either case, a different requirement for SAGA could be beneficial for yeast cells, as it allows differential regulation of *HXT2* and *HXT4* genes.

Recruitment of Mot1p and Taf1p by distinct SAGA modules. The described molecular function of SAGA includes recruitment of TBP to the transcription start sites of SAGA-dependent promoters via Spt3p and Spt8p (4, 7, 8, 31) and chromatin modification via the histone acetyltransferase activity of Gcn5p (4). A recently determined low-resolution structure of the SAGA complex (57) shows that Spt3p/Spt8p is organized in a separate module, termed domain V. In contrast, Gcn5p localizes to a central position in SAGA and faces a cleft, which could accommodate a nucleosome-like particle (52, 57).

Our results demonstrate that Gcn5p is essential for Taf1p recruitment, whereas the Spt3p/Spt8p module is most important for Mot1p. This suggests that these factors are differentially recruited. Taf1p recruitment via the Gcn5p module may involve its HAT activity, which could result in recruitment of other bromodomain-containing factors, such as the Swi/Snf complex, or in stabilization of SAGA binding (27). However, Gcn5p-dependent Taf1p recruitment seems less important for *HXT4* transcription, as induction of this gene is not affected in *gcn5*Δ or *ada2*Δ cells (Fig. 1B). In contrast, the Spt3p/Spt8p module, which is primarily responsible for Mot1p recruitment, is essential for efficient induction of *HXT4* transcription. This agrees with earlier findings that Mot1p is primarily responsible for TBP recruitment to *HXT* promoters (1).

A connection between Mot1p and SAGA components was also observed during induction of *GAL1* transcription. In this case, Mot1p recruitment depends on Spt3p and Gcn5p (53). For *GAL1*, this is interdependent, because SAGA was not recruited in the *mot1-1* strain. In contrast, for the *HXT* promoters Gcn5p is not essential for Mot1p recruitment. In addition, the recruitment of SAGA was not affected in *mot1-1* cells. Thus, although Mot1p recruitment to *GAL1* and the *HXT* genes depends on SAGA, different mechanisms which may reflect differences in activator and/or promoter context are involved.

In light of our earlier findings of Mot1p-dependent TBP recruitment (1), it was unexpected that recruitment of TBP is only mildly affected in strains lacking both Gcn5p and the Spt3p/Spt8p module (Fig. 4B). How can it be explained that in the absence of detectable Taf1p or Mot1p, TBP is still recruited to the *HXT* promoters? It was suggested that Spt3p and

Spt8p play a dual role in transcription and can inhibit TBP binding (6). Possibly, in wild-type cells recruitment of TBP requires TBP-associated factors, like Mot1p and Taf1p, to overcome inhibition by Spt3p/Spt8p. In *gcn5*Δ *spt3*Δ and *gcn5*Δ *spt8*Δ strains, the recruitment of Mot1p and Taf1p is abolished but inhibition of TBP association would also be relieved. These observations also strengthen the notion that yeast cells contain pools of TBP, which are not in a TFIID or Mot1p complex (23), and argue against the model that SAGA and TFIID together account for all transcription (28). How TBP becomes recruited in the absence of functional SAGA, Taf1p, or Mot1p remains an open question. But the results with the *HXT4* promoter suggest that SAGA can be required at a post-TBP recruitment step as Pol II recruitment is abolished (Fig. 4B). This view is supported by recent findings that Spt8p can contact TFIIA and Taf4p of TFIID (55). Possibly, these Spt8p interactions may play a role in the activation of *HXT4*.

Interplay between SAGA, Mot1p, and Taf1p in transcriptional regulation. The finding that the recruitment of Taf1p (and presumably TFIID) to the *HXT* genes is dependent on the Gcn5p HAT module of SAGA is rather unexpected in light of recent reports (5, 28), which grouped promoters into TATA-less and TFIID dominated or TATA containing and SAGA dominated. The latter (and smaller) group consists for the most part of stress-induced genes (28). In contrast, previous genome-wide mRNA expression studies indicated that SAGA and TFIID are functionally redundant, suggesting that they serve overlapping sets of promoters (32). This is in better agreement for the *HXT* genes, as we found that both SAGA and TFIID are recruited to the *HXT2* and *HXT4* promoters but that only SAGA is functionally required for *HXT4*. Strikingly, both promoters contain TATA consensus sequences (5) and both would be classified as SAGA dominated. Also, the genetic interaction between *gcn5*Δ and the *taf1* TS allele (Fig. 5A) suggests cooperation between TFIID and SAGA complexes rather than completely separated transcriptional pathways.

The connection between Mot1p and SAGA could extend beyond the *GAL1* and *HXT* genes and have general implications for transcriptional regulation. Several lines of evidence support this suggestion. First, Mot1p- and SAGA-dependent genes are involved in stress responses (23, 28). Second, *MOT1* genetically interacts with *SPT3* (12, 35) and *SPT8* (this study). Third, a recent genome-wide localization study suggested direct involvement of Mot1p in regulation of the SAGA pathway (58).

While our analysis of the *HXT4* gene indicates that SAGA and Mot1p functionally cooperate, it was suggested that Mot1p rather acts as a negative factor for SAGA-dominated stress-induced genes (58). This is also at odds with results showing that Mot1p and TFIIB can cooccupy stress-induced promoters (23). Clearly, more detailed studies involving more promoters are required to clarify whether the cooperation between Mot1p and SAGA on the *HXT4* promoter is a general phenomenon.

Altogether, our study revealed mechanistic insight into the regulation of hexose transporters in yeast. In addition, we demonstrated functional interplay between SAGA, Mot1p, and TFIID in glucose-regulated transcription, and our conclusions provide a framework to study the interaction of these pivotal transcription regulators at other stress-regulated genes.

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