2002-10-09

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MOLECULAR AND CELLULAR BIOLOGY, Nov. 2002, p. 7365–7371 Vol. 22, No. 21
0270-7306/02/$04.00+0 DOI: 10.1128/MCB.22.21.7365–7371.2002

Differential Requirement of SAGA Components for Recruitment of TATA-Box-Binding Protein to Promoters In Vivo

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Received 24 May 2002/Returned for modification 11 July 2002/Accepted 6 August 2002

The multisubunit *Saccharomyces cerevisiae* SAGA (Spt-Ada-Gcn5-acetyltransferase) complex is required to activate transcription of a subset of RNA polymerase II-dependent genes. However, the contribution of each SAGA component to transcription activation is relatively unknown. Here, using a formaldehyde-based in vivo cross-linking and chromatin immunoprecipitation assay, we have systematically analyzed the role of SAGA components in the recruitment of TATA-box binding protein (TBP) to SAGA-dependent promoters. We show that recruitment of TBP is diminished at a number of SAGA-dependent promoters in *ada1*Δ, *spt7*Δ, and *spt20*Δ null mutants, consistent with previous biochemical data suggesting that these components maintain the integrity of the SAGA complex. We also find that Spt3p is generally required for TBP binding to SAGA-dependent promoters, consistent with biochemical and genetic experiments, suggesting that Spt3p interacts with and recruits TBP to the core promoter. By contrast, Spt8p, which has been proposed to be required for the interaction between Spt3p and TBP, is required for TBP binding at only a subset of SAGA-dependent promoters. Ada2p and Ada3p are both required for TBP recruitment to Gcn5p-dependent promoters, supporting previous biochemical data that Ada2p and Ada3p are required for the histone acetyltransferase activity of Gcn5p. Finally, our results suggest that TBP-associated-factor components of SAGA are differentially required for TBP binding to SAGA-dependent promoters. In summary, we show that SAGA-dependent promoters require different combinations of SAGA components for TBP recruitment, revealing a complex combinatorial network for transcription activation in vivo.

The expression of most eukaryotic genes is controlled at the level of transcription initiation. Transcription by RNA polymerase II requires binding of the TATA-box binding protein (TBP) and the assembly of multiple basal transcription factors into a preinitiation complex (PIC) at the core promoter. The primary promoter recognition factor is TFIID, a complex that consists of TBP and at least 14 TBP-associated factors (TAFs). Transcription of eukaryotic protein-coding genes also often involves the action of transcriptional activator proteins (activators), which bind to specific *cis*-acting promoter elements. Activators function by stimulating PIC assembly via a mechanism that is thought to involve a direct interaction with one or more components of the transcription machinery.

Recent studies in *Saccharomyces cerevisiae* have demonstrated the presence of two distinct classes of promoters: those that depend on multiple TAFs for transcription (TAF dependent) and those that have no apparent TAF requirement (TAF independent) (24, 28). At TAF-dependent promoters, TAFs are present at levels comparable to that of TBP and are required for the delivery of TBP to the core promoter. The recruitment of TAFs to these promoters is activator dependent. At TAF-independent promoters, TAFs are not required for transcriptional activity or TBP recruitment (28).

The mechanism by which TBP is recruited to TAF-independent promoters is an intriguing problem that researchers are only now beginning to understand. One pathway for activating TAF-independent promoters involves the SAGA (Spt-Ada-Gcn5-acetyltransferase) complex (6, 25). SAGA is a large multiprotein complex that is required for the normal transcription of approximately 10% of yeast genes (26). The role of SAGA in transcription activation has been studied extensively at *GAL1*, a TAF-independent promoter that requires SAGA (13, 34, 37). At the *GAL1* promoter, SAGA is first recruited by the Gal4p activator to the upstream activating sequence (UAS), and the UAS-bound SAGA then facilitates the binding of TBP to the core promoter, thereby stimulating PIC assembly and transcription (6, 25). SAGA is essential for *GAL1* transcription: if SAGA is not recruited to the UAS, the PIC is not assembled and transcription does not occur (6, 25). SAGA is thought to function by serving as an adaptor that directly contacts one or more components of the PIC. However, the detailed molecular mechanism by which SAGA interacts with the transcriptional machinery and stimulates PIC assembly remains to be elucidated.

In yeast, the nonessential components of SAGA can be classified into three groups on the basis of their distinct mutant phenotypes: (i) Ada1p, Spt7p, and Spt20p; (ii) Spt3p and Spt8p; and (iii) Gcn5p, Ada2p, and Ada3p (8, 18, 20, 22, 34, 37). Gcn5p, the most extensively characterized component of SAGA, possesses a histone acetyltransferase (HAT) activity (36). The SAGA complex also includes a subset of TAFs: TAF5, TAF6, TAF9, TAF10, and TAF12 (formerly known as TAF90, TAF60, TAF17, TAF25, and TAF61/68, respectively [38]). Several of these TAFs have demonstrable roles in SAGA function and hence transcriptional stimulation (17, 32). Finally, SAGA also contains the ATM/PI-3-kinase-related pro-

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tein Tra1p, which has been reported to be a direct target of certain activators (7). Collectively, these observations suggest that SAGA components make distinct contributions to the function of the complete complex.

To understand in greater detail the roles of individual SAGA components in transcription activation, we have analyzed their contribution in TBP recruitment to several SAGA-dependent promoters by a formaldehyde-based in vivo cross-linking and chromatin immunoprecipitation (ChIP) assay. Our results reveal differences in the roles of various subunits of the SAGA complex.

MATERIALS AND METHODS

Yeast strains and media. Yeast strains harboring null mutations in *SPT3* (FY294), *SPT20* (FY1097), and *GCN5* (FY1370) and their isogenic wild-type equivalents, FY631, FY67, and FY1369, respectively, were obtained from Fred Winston (Harvard Medical School, Boston, Mass.) (34, 35, 37). Yeast strains carrying deletion mutations in *ADA1* (FY1559), *ADA3* (FY1542), *SPT7* (FY963), and *SPT8* (FY462) and their wild-type equivalents (FY602 for *ada1*Δ and *ada3*Δ, FY61 for *spt7*Δ, FY631 for *spt8*Δ) were also obtained from Fred Winston (35, 37). The *ada2*Δ yeast strain (PSY316Δ*ada2*) and its wild-type counterpart (PSY316) were obtained from Shelly Berger (Wister Institute, Philadelphia, Pa.) (5, 10, 39). Temperature-sensitive *taf6* (YSB555) and *taf12* (YSB547) strains and their isogenic wild-type counterparts (YSB553 and YSB590, respectively) were obtained from Stephen Buratowski (Harvard Medical School) (31). The temperature-sensitive *taf9* strain (LY761) and its wild-type counterpart (LY740) were generated previously in our laboratory (2).

For the studies at the *GAL1* promoter, cells were first grown in YPD (yeast extract-peptone plus 2% dextrose) to an optical density at 600 nm (OD_{600}) of 0.8 and then transferred to YPG (yeast extract-peptone plus 2% galactose) for 5 h at 30°C prior to formaldehyde cross-linking. Yeast strains harboring temperature-sensitive mutations in *TAF9*, *TAF6*, and *TAF12* were grown in YPG at 23°C to an OD_{600} of 0.8 and then transferred to 37°C for 1 h. For the studies at the *ADH1*, *BDF2*, *VTC3*, and *PHO84* promoters, yeast strains were grown in YPD to an OD_{600} of 1.0.

Primer extension analysis. Primer extension analysis was carried out as described previously (28). Primers used for the analysis of *GAL1*, *ADH1*, *SED1*, *RPS5*, *PHO84*, *VTC3*, and *BDF2* mRNA are as follows: *GAL1*, 5′-CCTTGACGTTAAAGTATAGAGG-3′; *ADH1*, 5′-TATCCTTGTGTTCCAATTTACCGTGG-3′; *SED1*, 5′-AGTAGTCGAGGCTAAACCGG-3′; *RPS5*, 5′-GACTGGGGTGAATTCTTCAACAACTTC-3′; *PHO84*, 5′-GAAGACTTCTTTCAGCAACATG-3′; *VTC3*, 5′-TGAATCCTTCCAGGGAGGATATAC-3′; and *BDF2*, 5′-TGCTGCCAGTAAAGCAGAATGTGC-3′.

Formaldehyde-based in vivo cross-linking and ChIP. Formaldehyde-based in vivo cross-linking and chromatin immunoprecipitation were performed as described previously (28). For quantitative analysis, 5 and 100 μl of whole-cell extract (from 400 μl of the total obtained from 50 ml of yeast culture) were used for input and ChIP, respectively. Total input DNA was dissolved in 100 μl of TE, pH 8.0 (10 mM Tris-HCl [pH 8.0], 1 mM EDTA), and 1/100 of input DNA was used for PCR analysis in a 25-μl volume. Total immunoprecipitated (IP) DNA was dissolved in 20 μl of TE, pH 8.0, and 1/20 of IP DNA was used for PCR analysis in a 25-μl volume. Serial dilutions of input and IP DNA were used to assess the linear range of DNA amplification. Autoradiograms were scanned and quantitated by the National Institutes of Health Image version 1.62 program. IP DNA was quantitated and presented as the ratio of IP to input relative to that of the wild type. The following sets of primer pairs were used for PCR analysis: *GAL1* (UAS), 5′-CGCTTAACTGCTCATTGCTATATTG-3′ and 5′-TTGTTCGGAGCAGTGCGGCGC-3′; *GAL1* (Core), 5′-ATAGGATGATAATGCGATTAGTTTTTTAGCCTT-3′ and 5′-GAAAATGTTGAAAGTATTAGTTAAAGTGGTTATGCA-3′; *GAL4* (open reading frame), 5′-CTTGTTCAATGCAGTCCTAGTACCC-3′ and 5′-CACAAGTCTGGATTTTAAAAGTGGCC-3′; *PHO84* (Core), 5′-GATCCACTTACTATTGTGGCTCGT-3′ and 5′-GTTTGTTGTGTGCCCTGGTGATCT-3′; *VTC3* (Core), 5′-GAGAGCGGCTTACATCAGACATCT-3′ and 5′-CGCTCTAATAGCCAAATGACCTATAGTG-3′; *BDF2* (Core), 5′-ATCGCCGCGGCAGAGAATGACTCAAATAAATGCGC-3′ and 5′-ATTAGGATCCGTATCCATGTTAGTACGAGACATAGC-3′; *ADH1* (Core), 5′-GGTATACGGCCTTCCTTCCAGTTAC-3′ and 5′-GAACGAGAACAATGACGAGGAAACAAAAG-3′.

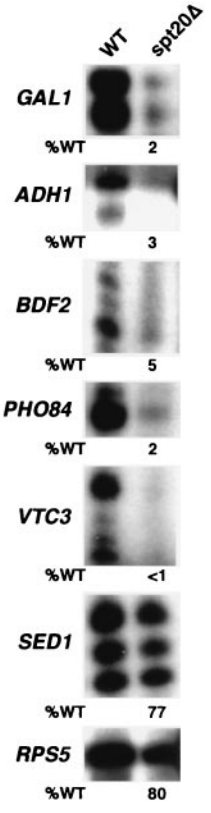

FIG. 1. Identification of a set of promoters that require SAGA for transcription. Total cellular RNA was prepared from either a wild-type (WT) or *spt20*Δ strain, and transcription from the indicated promoter was monitored by primer extension. The level of transcription in the *spt20*Δ strain relative to that of the wild type is indicated (%WT).

RESULTS

Identification of a set of promoters that require SAGA for transcription. To obtain a set of SAGA-dependent promoters for further analysis, we analyzed the promoters of *ADH1*, *BDF2*, *PHO84*, *VTC3*, and *SED1* (also known as *PHM2*), which were predicted to require SAGA based on genome-wide expression data (26). Transcription of each gene was measured in a strain harboring a null mutation in *SPT20*, a gene encoding a SAGA component required for the integrity and function of the complex (16, 37). Consistent with previous results, transcription of *GAL1* was significantly reduced in the absence of Spt20p (Fig. 1) (6). Similarly, *ADH1*, *BDF2*, *PHO84*, and *VTC3* also required Spt20p, indicating that these promoters were also SAGA-dependent. In contrast, *SPT20* was dispensable for the transcription of *SED1*. As expected, transcription from a promoter that does not require SAGA, namely, *RPS5*, was not affected by the *spt20*Δ mutation.

General requirement of Ada1p, Spt7p, and Spt20p for recruitment of TBP to SAGA-dependent promoters. To understand in greater detail the roles of SAGA components in transcription activation, we analyzed their contribution in the recruitment of TBP to the SAGA-dependent promoters identified in Fig. 1 by using a ChIP assay. We first investigated the role of Ada1p, Spt7p, and Spt20p. We have previously shown

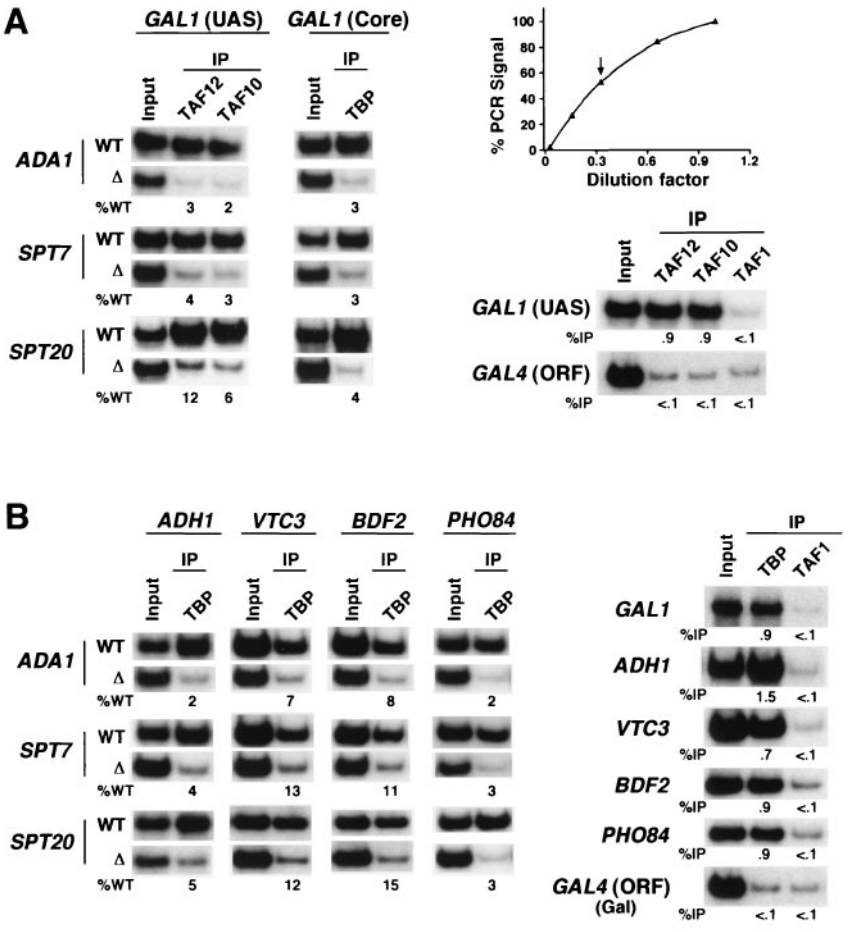

FIG. 2. General requirement of Ada1p, Spt7p, and Spt20p for recruitment of TBP to SAGA-dependent promoters. (A) Wild-type (WT), *ada1*Δ, *spt7*Δ, and *spt20*Δ strains were first grown in glucose-containing medium (YPD) and then shifted to galactose-containing medium (YPG) 5 h prior to treatment with formaldehyde. Formaldehyde-based in vivo cross-linking and ChIP were carried out as previously described (28). Immunoprecipitation assays were performed with polyclonal antibodies against TAF10, TAF12, or TBP. TAF10 and TAF12 are representative SAGA components used to monitor recruitment of the SAGA complex. Primer pairs located in the *GAL1* UAS or core promoter were used for PCR analysis of the input and immunoprecipitated (IP) DNA samples. All PCRs were carried out in the linear range of DNA amplification, as indicated by the arrow in the curve shown in the top right panel. The percentage of DNA immunoprecipitated relative to that of the wild type (%WT) is indicated. Background levels in the immunoprecipitation assay are shown by using an irrelevant DNA sequence (*GAL4* ORF [open reading frame]) and an irrelevant antibody control (TAF1 is a specific component of the TFIID complex, which is not associated with the core promoters of the genes analyzed) which immunoprecipitated less than 0.1% of DNA. (B) All strains were grown in YPD to an OD_{600} of 1.0 prior to formaldehyde treatment. Analysis of TBP binding to the core promoters of *ADH1*, *VTC3*, *BDF2*, and *PHO84* was performed as described for panel A. The background signal obtained with the irrelevant anti-TAF1 antibody control is shown on the right.

that Spt20p is required for the recruitment of SAGA and TBP to the *GAL1* promoter, whereas SAGA is not recruited to an irrelevant DNA sequence (6). Analysis of Ada1p and Spt7p revealed a similar requirement for SAGA recruitment and TBP binding: in the absence of either Ada1p or Spt7p, the association of TBP and SAGA with the *GAL1* promoter was at a background level comparable to that obtained with an irrelevant antibody control (Fig. 2A). These results indicate that Ada1p, Spt7p, and Spt20p are essential for the recruitment of SAGA to the *GAL1* promoter, which is consistent with previous in vitro biochemical data that show that these three components maintain the integrity of the SAGA complex (16, 37). These observations suggest that there might be a general requirement for these three components at other SAGA-dependent promoters. Consistent with this prediction, deletion of *ADA1*, *SPT7*, or *SPT20* also compromised the recruitment of TBP to the core promoters of *ADH1*, *VTC3*, *BDF2*, and *PHO84* (Fig. 2B).

General requirement of Spt3p but not Spt8p in recruitment of TBP to SAGA-dependent promoters. We next analyzed the roles of Spt3p and Spt8p in the recruitment of TBP to SAGA-dependent promoters. Spt3p is a functionally conserved eukaryotic transcriptional regulator (29, 41) believed to play roles in both the activation and repression of transcription (4, 26, 40). Genetic and biochemical studies have shown that Spt3p interacts with TBP (13, 14, 27). In addition, recent studies have shown that the deletion of *SPT3* interferes with the recruitment of TBP but not SAGA to the *GAL1* promoter (6, 25). These results have been interpreted to indicate that Spt3p interacts with TBP and facilitates its binding to the *GAL1*

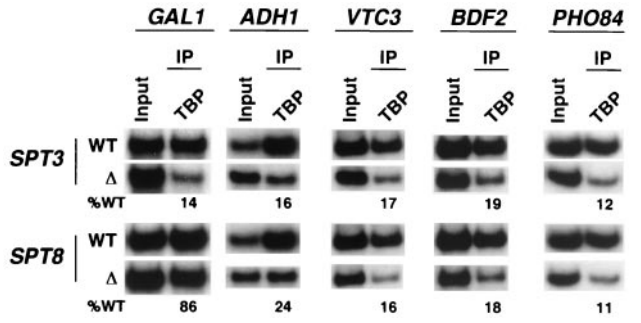

FIG. 3. General requirement of Spt3p but not Spt8p in recruitment of TBP to SAGA-dependent promoters. Wild-type (WT), *spt3*Δ, and *spt8*Δ deletion mutants were grown as described in the legend to Fig. 2 prior to formaldehyde treatment. Primer pairs located in the core promoters of the *GAL1*, *ADH1*, *VTC3*, *BDF2*, and *PHO84* genes were used for PCR analysis of the input and immunoprecipitated (IP) DNA samples.

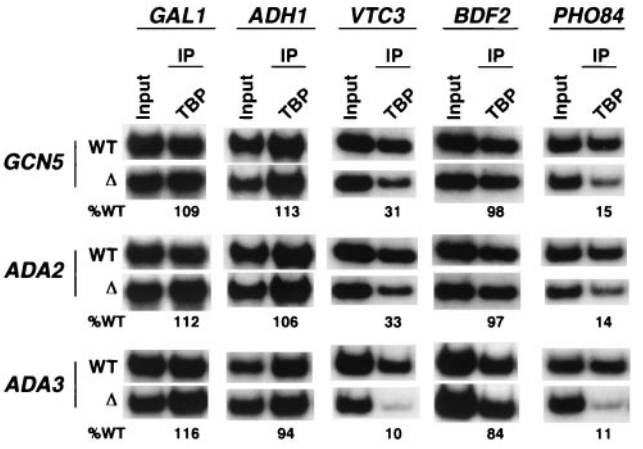

FIG. 4. Gcn5p, Ada2p, and Ada3p are required for TBP recruitment to a common set of SAGA-dependent promoters. Wild-type (WT), *gcn5*Δ, *ada2*Δ, and *ada3*Δ strains were grown as described in the legend to Fig. 2 prior to formaldehyde treatment. Primer pairs located in the core promoters of the *GAL1*, *ADH1*, *VTC3*, *BDF2*, and *PHO84* genes were used for PCR analysis of the input and immunoprecipitated (IP) DNA samples.

promoter. To test whether other SAGA-dependent promoters also required Spt3p for TBP recruitment, we analyzed TBP binding to the *ADH1*, *VTC3*, *BDF2*, and *PHO84* promoters in an *spt3*Δ mutant background. Figure 3 shows that, similar to the *GAL1* promoter, Spt3p was required for the efficient recruitment of TBP to these promoters.

Genetic evidence has suggested that *SPT8* is required for the functional interaction between Spt3p and TBP (15). It has also been demonstrated that Spt8p interacts with TBP in vitro (37). Therefore, we anticipated that Spt8p, like Spt3p, might also be generally required for the recruitment of TBP to SAGA-dependent promoters. Figure 3 shows that Spt8p was required for efficient TBP recruitment to the *ADH1*, *VTC3*, *BDF2*, and *PHO84* core promoters. In contrast, Spt8p was dispensable for the recruitment of TBP to the *GAL1* core promoter, consistent with previous genetic studies showing that *spt8*Δ mutants are Gal^+ (37). Thus, Spt8p is required for TBP binding at a subset of SAGA-dependent promoters.

Gcn5p, Ada2p, and Ada3p are required for TBP recruitment to a common set of SAGA-dependent promoters. Next, we analyzed the roles of Gcn5p, Ada2p, and Ada3p in the recruitment of TBP to SAGA-dependent promoters. It has been proposed that the HAT activity of Gcn5p, which is important for transcriptional activation both in vivo and in vitro (36), remodels local chromatin structure and subsequently facilitates TBP binding (37). However, the transcription of a number of SAGA-dependent genes does not require Gcn5p (26), indicating that the HAT activity of SAGA may be dispensable for PIC assembly. For example, Gcn5p is not required for transcription of *GAL1* (13) and, accordingly, it is dispensable for the recruitment of TBP to the *GAL1* promoter (6, 25). These considerations raise the question of whether Gcn5p is required at Gcn5p-dependent promoters for TBP recruitment or some other step. To test this, we assessed TBP recruitment in *gcn5*Δ mutant strains at promoters that are either Gcn5p dependent (*VTC3* and *PHO84*) or Gcn5p independent (*ADH1* and *BDF2*) (26). Figure 4 shows that, like the *GAL1* promoter, Gcn5p was dispensable for TBP recruitment to the core promoters of *ADH1* and *BDF2*. In contrast, the deletion of *GCN5* decreased TBP binding to the Gcn5p-dependent *VTC3* and *PHO84* promoters. Thus, Gcn5p is required for facilitating TBP recruitment at Gcn5p-dependent promoters.

Ada2p and Ada3p interact genetically and biochemically with Gcn5p and with each other (10, 11, 21, 30). Although recombinant Gcn5p can acetylate only free histones (9, 16), a Gcn5p-Ada2p-Ada3p complex possesses in vitro nucleosomal HAT activity with the lysine specificity of the intact SAGA complex (3). These observations suggest that promoters that require the HAT activity of Gcn5p for efficient TBP recruitment should also require Ada2p and Ada3p. To test this hypothesis, we analyzed the recruitment of TBP to several promoters in *ada2*Δ and *ada3*Δ mutant backgrounds. Figure 4 shows that the requirement for Ada2p and Ada3p mirrored that for Gcn5p: *VTC3* and *PHO84* required Ada2p and Ada3p for TBP binding, whereas *GAL1*, *BDF2*, and *ADH1* did not. Thus, Ada2p and Ada3p are required in vivo for efficient TBP recruitment to Gcn5p-dependent promoters.

Differential requirement of SAGA TAFs for recruitment of TBP to SAGA-dependent promoters. TAF5, TAF6, TAF9, TAF10, and TAF12 are integral components of the SAGA complex, and several studies have indicated that they have important roles in SAGA function (17, 32). However, their precise roles in transcriptional activation have not been clearly defined. We therefore analyzed the requirement of a subset of these TAFs for TBP recruitment to SAGA-dependent promoters. Figure 5 shows that TAF6, TAF9, and TAF12 are dispensable for TBP binding to the *ADH1* and *GAL1* promoters, which is consistent with previous reports that TAF6 and TAF9 are not required for transcription of these genes (28). In contrast, all three TAFs were required for efficient TBP recruitment to the *PHO84* promoter. The *BDF2* and *VTC3* promoters displayed a differential requirement for the TAFs: *BDF2* required TAF6 but not TAF12, whereas *VTC3* required TAF12 but not TAF6. Thus, our data clearly demonstrate that differ-

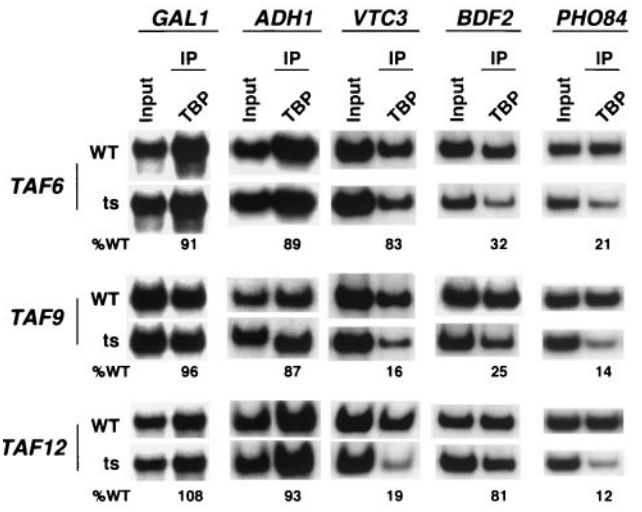

FIG. 5. Differential requirement of SAGA TAFs for recruitment of TBP to SAGA-dependent promoters. Yeast strains harboring temperature-sensitive mutations in *TAF9*, *TAF6*, and *TAF12* were first grown at 23°C to an OD_{600} of 0.8 and then transferred to 37°C for 1 h prior to treatment with formaldehyde. Primer pairs located in the core promoters of the *GAL1*, *ADH1*, *VTC3*, *BDF2*, and *PHO84* genes were used for PCR analysis of the input and immunoprecipitated (IP) DNA samples.

ent SAGA-dependent promoters show differing degrees of dependence on SAGA TAFs for TBP recruitment.

DISCUSSION

In this report, we have analyzed the in vivo role of SAGA components for TBP recruitment to SAGA-dependent promoters. Our major findings are summarized in Table 1 and are discussed below. All of the SAGA-dependent promoters examined required Ada1p, Spt3p, Spt7p, and Spt20p for TBP recruitment. Previous biochemical experiments have indicated that Ada1p, Spt7p, and Spt20p are required for the integrity of the SAGA complex (16, 37), which is consistent with the general requirement for these components that we observed. However, our results do not rule out that, in addition to complex integrity, Ada1p, Spt7p, and Spt20p also have a more direct role in TBP recruitment. In addition, genetic and biochemical experiments have suggested that Spt3p interacts with TBP (13, 14, 27). Our finding that Spt3p is generally required for TBP recruitment is again consistent with this possibility.

In contrast to the general requirement of Ada1p, Spt3p, Spt7p, and Spt20p, we found that Spt8p, Ada2p, Ada3p, Gcn5p, and the TAF components of SAGA were differentially required for TBP recruitment to SAGA-dependent promoters. Significantly, we found that the promoter requirements for Gcn5p, Ada2p, and Ada3p were identical, which is consistent with previous work showing that Ada2p and Ada3p are required for Gcn5p's HAT activity on nucleosomal histones (3). TAF12 was also required at the same subset of promoters as Gcn5p, Ada2p, and Ada3p, consistent with the finding that TAF12 is required for SAGA's in vitro HAT activity on nucleosomal substrates (17). Finally, genetic evidence has suggested that Spt8p is required for the functional interaction between Spt3p and TBP (15), which may be mediated through an Spt8p-TBP interaction (37). Our results suggest that if this interaction occurs in vivo, it is required only at certain promoters.

The SAGA-dependent promoters displayed differential sensitivities to temperature-sensitive mutations in *TAF6*, *TAF9*, and *TAF12*. The differential requirement for TAF6, TAF9, and TAF12 at SAGA-dependent promoters is consistent with the results of genome-wide expression analysis (26). However, several studies have found that different temperature-sensitive mutant alleles within a single TAF can result in distinct phenotypes and gene expression profiles (for an example, see reference 23). This finding is most likely explained by the fact that TAFs can have multiple domains, can be present in more than one complex, can mediate different functions, and can also be required for complex integrity (1, 19). Thus, although our results suggest that SAGA-dependent promoters will have differential TAF requirements, it will be important to verify this supposition by analysis of additional TAF mutants.

SAGA is a complex, multisubunit transcription factor that has at least two distinct activities: it can serve as the direct target (adaptor) for transcriptional activation domains, and it has a HAT activity that can modify chromatin structure. These two activities are carried out by distinct subunits of the SAGA complex. We speculate that the multiplicity of SAGA functions is related to our finding that various SAGA-dependent promoters differentially require specific SAGA subunits. For example, the differential requirement for Gcn5p's HAT activity suggests that the chromatin structure differs at various SAGA-dependent promoters. Likewise, the activators at some SAGA-dependent promoters, such as Gal4p, may require the SAGA adaptor function; however, at other SAGA-dependent promoters, SAGA's adaptor function may not be essential because, for example, SAGA is redundant with other targets. Recognizing and understanding the specific features of SAGA-dependent promoters that determine their requirement for specific SAGA subunits will require further research. It is

TABLE 1. Summary of the requirement for SAGA components for TBP recruitment to SAGA-dependent promoters[a]

Promoter	*SPT20*	*SPT7*	*ADA1*	*SPT3*	*SPT8*	*GCN5*	*ADA2*	*ADA3*	*TAF12*	*TAF9*	*TAF6*
GAL1	+	+	+	+	−	−	−	−	−	−	−
ADH1	+	+	+	+	+	−	−	−	−	−	−
BDF2	+	+	+	+	+	−	−	−	−	+	+
PHO84	+	+	+	+	+	+	+	+	+	+	+
VTC3	+	+	+	+	+	+	+	+	+	+	−

[a] +, required; −, not required. A SAGA component was defined as required if, when deleted or mutated, TBP recruitment was reduced to less than one-third of the level of the wild type.

intriguing, however, that, like SAGA, several other multisubunit transcription complexes, including TFIIA (12), TFIIE (33), and TAFs (26), are also differentially required.

Previous studies have shown that transcription of the TAF-independent *GAL1* promoter is dependent on SAGA (13, 34, 37). On the basis of genome-wide transcription profiling, we predicted that other TAF-independent promoters would, like *GAL1*, also require SAGA for transcription (26). Consistent with this prediction, we found that the TAF-independent *ADH1* promoter required SAGA for transcription as well as TBP recruitment. In contrast, SAGA was dispensable for transcription from the TAF-independent promoters *SED1* (Fig. 1) and *PGK1* (data not shown) (28). These results indicate that only a subset of TAF-independent promoters are SAGA dependent. The mechanism by which promoters that are both TAF and SAGA independent are transcriptionally activated remains to be determined. Our results raise the possibility that another complex in addition to TFIID and SAGA is involved in TBP recruitment and transcription activation.

In summary, we have shown here that SAGA components are differentially required for TBP binding to SAGA-dependent promoters in vivo. Our in vivo analysis is remarkably consistent with previous genetic, biochemical, and genome-wide expression data that SAGA components are differentially required for the gene activity (16, 17, 22, 26, 32, 34, 37). However, the molecular basis of the distinct but selective effects of individual SAGA components on TBP recruitment and hence transcription remains to be elucidated. The present functional analysis of SAGA reinforces an important concept: the individual subunits of transcription complexes may have distinct and selective functions.

ACKNOWLEDGMENTS

We thank Fred Winston, Shelly Berger, and Stephen Buratowski for generously providing yeast strains and Sara Evans for editorial assistance.

This work was supported in part by a grant from the NIH to M.R.G. M.R.G. is an investigator and S.R.B. is an associate of the Howard Hughes Medical Institute.

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