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# SAGA binds TBP via its Spt8 subunit in competition with DNA: implications for TBP recruitment

**Decha Sermwittayawong and Song Tan***

Center for Gene Regulation, Department of Biochemistry & Molecular Biology, The Pennsylvania State University, University Park, PA, USA

**In yeast, the multisubunit SAGA (Spt-Ada-Gcn5-acetyltransferase) complex acts as a coactivator to recruit the TATA-binding protein (TBP) to the TATA box, a critical step in eukaryotic gene regulation. However, it is unclear which SAGA subunits are responsible for SAGA's direct interactions with TBP and precisely how SAGA recruits TBP to the promoter. We have used chemical crosslinking to identify Spt8 and Ada1 as potential SAGA subunits that interact with TBP, and we find that both Spt8 and SAGA bind directly to TBP monomer in competition with TBP dimer. We further find that Spt8 and SAGA compete with DNA to bind TBP rather than forming a triple complex. Our results suggest a handoff model for SAGA recruitment of TBP: instead of binding together with TBP at the TATA box, activator-recruited SAGA transfers TBP to the TATA box. This simple model can explain SAGA's observed ability to both activate and repress transcription.**

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## Introduction

Eukaryotic transcription requires an intricate interplay between activator proteins, coactivator complexes, general transcription factors and RNA polymerase acting on a chromatin template (Hampsey, 1998). One popular mechanism for transcriptional activation postulates that activator proteins bind directly via sequence-specific interactions or indirectly with DNA to recruit coactivator complexes including SAGA (Spt-Ada-Gcn5-acetyltransferase), TFIID and the Mediator complexes (Naar *et al*, 2001). These coactivator complexes facilitate the binding of general transcription factors such as TBP to promoter sequences that ultimately recruit RNA polymerase II into a preinitiation complex. Several studies have implicated the recruitment of TBP to the promoter as a critical step in preinitiation complex assembly (Chatterjee and Struhl, 1995; Xiao *et al*, 1995; Kuras and Struhl, 1999; Li *et al*, 1999).

SAGA is a megadalton coactivator complex that regulates the activity of approximately 10% of all yeast genes, particularly stress-related genes (Lee *et al*, 2000; Huisinga and Pugh, 2004). The SAGA coactivator complex binds TBP directly (Sterner *et al*, 1999) and multiple studies have demonstrated the importance of this interaction to the recruitment of TBP to the promoter (Dudley *et al*, 1999; Belotserkovskaya *et al*, 2000; Bhaumik and Green, 2001; Larschan and Winston, 2001; Ricci *et al*, 2002; Barbaric *et al*, 2003; Yu *et al*, 2003; Qiu *et al*, 2005).

However, two key issues have not been resolved. Firstly, the precise subunit(s) of SAGA that interacts directly with TBP has been controversial. Substantial evidence supports a close tie between the SAGA Spt3 subunit and TBP. For example, an Spt3 mutation (*spt3–401*) suppresses a mutation (*spt15–21*) in the TBP gene in an allele-specific manner, often an indication of direct interaction between the two gene products (Eisenmann *et al*, 1992). Additionally, chromatin immunoprecipitation assays show that Spt3 is required for efficient recruitment of TBP to several SAGA-dependent promoters *in vivo* (Dudley *et al*, 1999; Larschan and Winston, 2001; Bhaumik and Green, 2001, 2002; Barbaric *et al*, 2003). However, it is not clear that Spt3 is actually required for SAGA to interact with TBP. Sterner *et al* (1999) found that SAGA lacking Spt3 still binds TBP *in vitro*. In contrast, SAGA without its Spt8 subunit, another TBP-interaction candidate identified by genetic studies (Eisenmann *et al*, 1994), binds TBP weakly suggesting that Spt8 is more important than Spt3 for binding of TBP to SAGA (Sterner *et al*, 1999). Spt8 was also determined to be required for efficient binding of TBP to four of five SAGA-dependent promoters *in vivo* (Bhaumik and Green, 2002), and to be in close proximity to both TBP and TFIIA in an assembled preinitiation complex *in vitro* (Warfield *et al*, 2004).

The other unresolved issue is the perplexing ability of SAGA to activate or repress transcription depending on its context. For example, SAGA is required for GAL1 transcription mediated by the activator Gal4, and SAGA's Spt3 subunit is necessary for TBP recruitment by SAGA (Dudley *et al*, 1999; Bhaumik and Green, 2001; Larschan and Winston, 2001). Similarly, Spt3 is required for full activation of the PHO5 promoter by the Pho4 activator, and Spt3 also strongly increases TBP recruitment to this promoter (Barbaric *et al*, 2003). Furthermore, both Spt3 and Spt8 are required for TBP binding at four other SAGA-dependent promoters (Bhaumik and Green, 2002). Thus, SAGA plays a positive role to recruit TBP to these promoters during transcriptional activation. However, at the HIS3 and TRP3 promoters, SAGA apparently inhibits transcription under noninduced conditions (Belotserkovskaya *et al*, 2000), although a smaller inhibitory effect was detected in a separate study (Wu and

*Corresponding author. Center for Gene Regulation, Department of Biochemistry & Molecular Biology, 108 Althouse Laboratory, The Pennsylvania State University, University Park, PA 16802-1014, USA. Tel.: +1 814 865 3355; Fax: +1 814 863 7024; E-mail: sxt30@psu.edu

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Winston, 2002). Instead of helping TBP bind to the core promoter, the Spt3 and Spt8 subunits actually inhibit binding of TBP to the HIS3 TATA element *in vitro* (Belotserkovskaya *et al*, 2000). Furthermore, Spt3 has been shown to inhibit TBP binding to the HO promoter *in vivo* (Yu *et al*, 2003). These observations are difficult to reconcile with a model where SAGA recruits and directly stabilizes TBP–promoter interactions.

To address the question of what SAGA subunit(s) interact with TBP, we have used chemical crosslinking as an unbiased method to identify which SAGA subunits are in close proximity to TBP in the SAGA/TBP complex, and then verified the interaction by direct binding experiments. Our results suggest an important direct role of Spt8 as well as a novel potential role for Ada1 in SAGA complex binding to TBP. We have also examined the mechanism by which Spt8 alone and the SAGA complex binds to TBP, and we find that both Spt8 and SAGA bind the TBP monomer but not TBP dimer and, unexpectedly, that this binding is competitive with DNA. Our findings suggest a simple model for how SAGA recruits TBP to a promoter, which explains the positive and negative roles of SAGA in transcriptional regulation.

## Results

### *The yeast TBP R171E point mutant is defective in its interaction with the SAGA complex*

To study the interactions between SAGA and the general transcription factor TBP, we purified native SAGA complexes from yeast using the tandem affinity purification (TAP) procedure supplemented by anion-exchange chromatography. Silver stained SDS–PAGE gels of SAGA complex isolated from wild-type yeast cells show the presence of all expected SAGA subunits (Figure 1A, lane 1), and Western blotting confirms the identity of individual subunits (Figure 1B, lane 1). Fluorography of histone samples treated with the purified SAGA complex verifies its ability to acetylate histone H3 (Figure 1A, lower panel lane 1).

We analyzed the direct interaction between yeast SAGA complex and TBP using a pull-down assay. As the N-terminus of TBP is dispensable for yeast viability and response to acidic transcriptional activators such as Gal4 (Reddy and Hahn, 1991; Cormack *et al*, 1994), we used the conserved C-terminal core of TBP for our experiments. We purified recombinant TBP with an engineered N-terminal Strep tag (Figure 1C, lane 1) and immobilized this to Strep-Tactin resin for binding studies with SAGA complex. We find that SAGA H3 HAT activity binds to and is eluted from the TBP beads (Figure 1D, lanes 4 and 5), whereas only faint background activity is detected when TBP is not added (Figure 1D, lanes 2 and 3). These results show that highly purified wild-type SAGA complex is sufficient to bind to the conserved C-terminal domain of TBP.

To examine the specificity of the SAGA–TBP interaction, we used the TBP point mutants R171E and T153I. Mutations in TBP R171 were originally identified as an allele-specific suppressor of the *spt3–401* mutation, suggestive that TBP R171 as involved in the SAGA–TBP interaction (Eisenmann *et al*, 1992). We also investigated the TBP T153I mutant because this mutant fails to respond to transcriptional activation unless artificially recruited to promoters (Stargell and Struhl, 1996), possibly due to defects in its interaction with coactivators such as SAGA.

We therefore expressed and purified Strep-tagged TBP R171E and T153I proteins (Figure 1C, lanes 2 and 3) and used these point mutants in binding assays with SAGA. We find that SAGA is preferentially retained on immobilized wild-type TBP and TBP T153I proteins compared to TBP R171E (Figure 1D, lanes 4–9), suggesting that TBP R171E is specifically defective in its interactions with SAGA. In three independent pull-down assays, the R171E mutant possesses only about one-third the binding activity of wild-type TBP (Figure 1E). In contrast, TBP T153I exhibits the same SAGA-binding activity as wild-type TBP within experimental error (Figure 1D, lanes 8 and 9, Figure 1E). Thus, these results identify a specific mutation on TBP that adversely affects its interaction with SAGA.

### *The Spt8 and Ada1 subunits are in close proximity to TBP in the SAGA/TBP complex*

Although previous genetic and biochemical studies have shown that the Spt3 and/or Spt8 subunits are important for SAGA's interaction with TBP, it is not clear which SAGA subunits bind to or are in close proximity to TBP. To address this question, we utilized a photo-crosslinking label transfer procedure. In particular, we used Sulfo-SBED, a photoactivatable lysine-specific reagent that transfers a biotin group from the bait to the target subunit. The same reagent was used to identify Tra1 as the SAGA target of activators and Swi1, Snf4 and Swi2/Snf2 as the Swi/Snf targets of activators (Brown *et al*, 2001; Neely *et al*, 2002). This technique allows the identification of potential SAGA subunits, which interact with TBP in the context of the entire SAGA complex.

When TBP conjugated with Sulfo-SBED is reacted with highly purified Ada1-TAP-tagged SAGA complex, we detect a strong biotin-linked band with apparent molecular weight of 80 kDa, and a weaker band with apparent molecular weight of 70 kDa (Figure 2, lanes 1–4). Both bands required the presence of SAGA and UV photoactivation, indicating that they represent specific SAGA targets of TBP. Using antibodies against protein candidates at these positions on the same membrane, we identified the strong 80 kDa crosslinked band as Spt8 and the weaker 70 kDa band as Ada1-TAP (Figure 2, lanes 5, 6, 9 and 10). The crosslink to Ada1-TAP is not dependent on the TAP tag as untagged Ada1 is also crosslinked by Sulfo-SBED TBP when Spt7-TAP-tagged SAGA is used instead of Ada1-TAP SAGA (data not shown). The 80 and 70 kDa bands were the only two bands to be consistently detected in our crosslinking studies. In particular, we did not detect Spt3 by this procedure. This suggests that Spt3 is not in close proximity to the conjugated TBP in the SAGA/TBP complex, but we cannot exclude the possibility that crosslinks are not formed for other reasons such as lack of appropriate functional groups on either TBP or Spt3 to form the Spt3-TBP crosslink. Thus, crosslinking data suggest that Spt8 and Ada1, but apparently not Spt3, are candidate targets of TBP in the SAGA complex.

### *Spt8 but not Spt3 binds directly to TBP*

Our photo-crosslinking results suggested that Spt8 might be responsible for SAGA's interaction with TBP. To test the hypothesis that Spt8 but not Spt3 is sufficient to bind to TBP, we examined the interaction between TBP and purified,

 

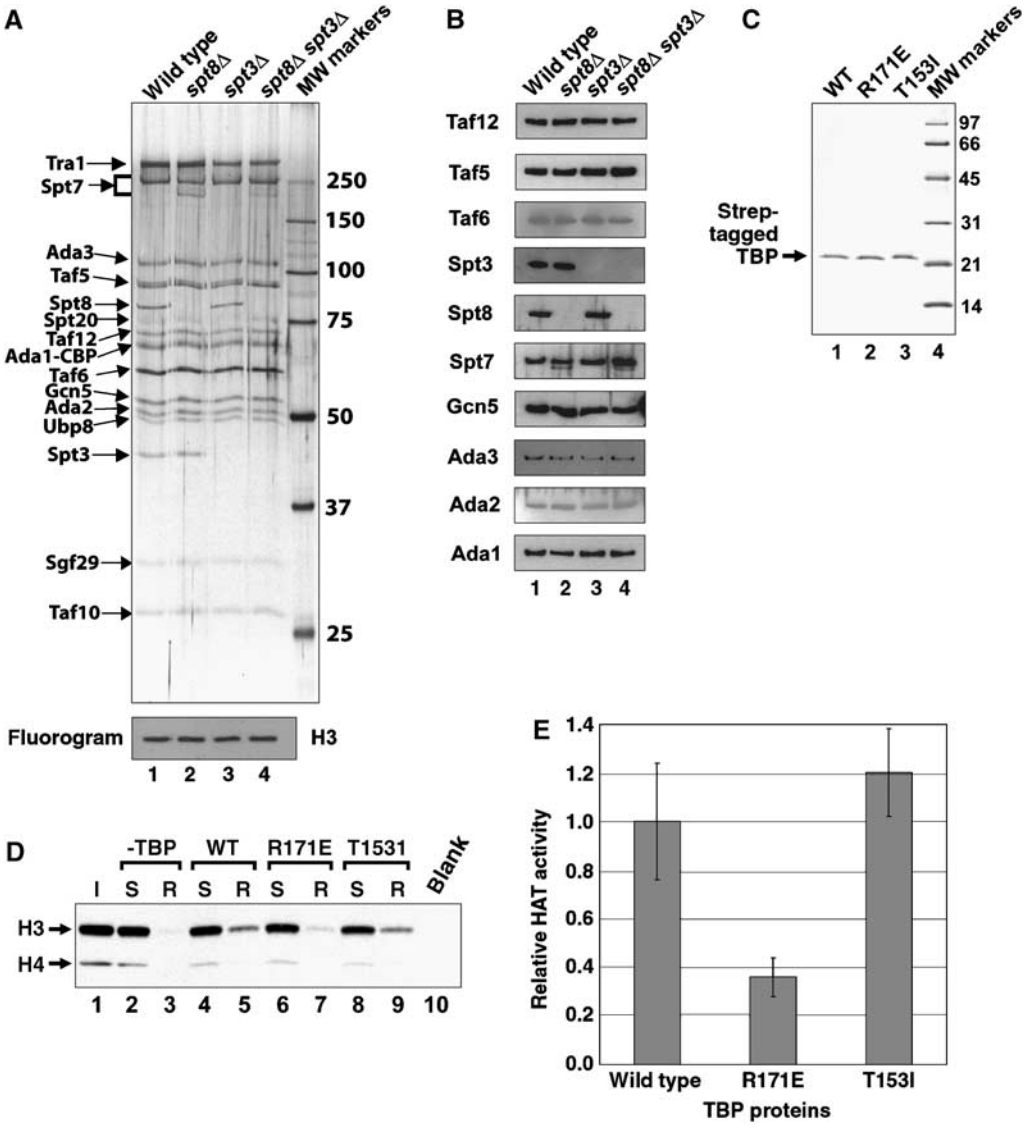


**Figure 1** The yeast TBP R171E mutant is defective in its interaction with the SAGA complex. (**A**) Silver-stained gel of TAP-tagged purified wild-type, *spt8*Δ, *spt3*Δ and *spt8*Δ*spt3*Δ SAGA complexes normalized by HAT activity in lanes 1–4, respectively. Lower panel fluorogram shows equivalent H3 activity on core histone substrates. Three independent HAT assays quantitated by scintillation counting show that the wild-type, *spt8*Δ, *spt3*Δ and *spt8*Δ*spt3*Δ SAGA complexes normalized by Gcn5 content have relative core histone HAT activity of $1.00 \pm 0.04$, $0.98 \pm 0.04$, $0.97 \pm 0.07$ and $1.10 \pm 0.06$, respectively, with wild-type SAGA set to 1.00. (**B**) Western blot of SAGA subunits confirms equivalent amounts of individual subunits in the wild-type and variant SAGA complexes and verifies absence of Spt3 and Spt8 subunits in appropriate samples. (**C**) Coomassie-stained gel of purified Strep-TEV-tagged C-terminal domain of wild-type, R171E and T153I TBP proteins (lanes 1–3, respectively). (**D**) Pull-down assay of SAGA binding to immobilized wild-type and variant TBP. Equivalent amounts of SAGA input (I), unbound supernatant (S) and released after TEV cleavage (R) fractions were assayed on core histone substrates and the acetylated histones H3 and H4 visualized by fluorography. The HAT assay in lane 10 was performed without enzyme to estimate the background counts for the assay. (**E**) Pull-down efficiency between SAGA and variant TBP molecules quantitated by HAT enzyme activity, with wild-type TBP set to 1.0. The bar chart shows data from three separate experiments.

recombinant Spt8 or Spt3 proteins. As with our binding study between TBP and SAGA, we employed a pull-down assay using wild-type TBP or TBP point mutants immobilized to the Strep-Tactin resin.

Our results show that Spt8 alone can bind to wild-type TBP (Figure 3A, lanes 1–5). Furthermore, Spt8 binds more weakly to the TBP R171E mutant (Figure 3A, lanes 6 and 7), consistent with the weaker interaction between the SAGA complex and this TBP mutant. This is a specific effect of the TBP R171E mutation as the TBP T153I mutant shows binding affinity to Spt8 similar to wild-type TBP (Figure 3A, lanes 8 and 9), again consistent with TBP T153I's undiminished interaction with the SAGA complex. These results show that Spt8 binds to TBP directly and specifically, and that the interaction does not require additional factors.

In contrast, Spt3 protein did not interact with wild-type TBP, TBP R171E or TBP T153I (Figure 3B). To eliminate the possibility that the C-terminal HIS-tag on the Spt3 protein might interfere with binding to TBP, we additionally purified recombinant Spt3 lacking the C-terminal tag. This untagged Spt3 was also unable to interact with TBP in the pull-down assay (data not shown). These results are consistent with our photo-crosslinking data where Spt3 in the context of SAGA complex did not photo-crosslink to TBP to any detectable

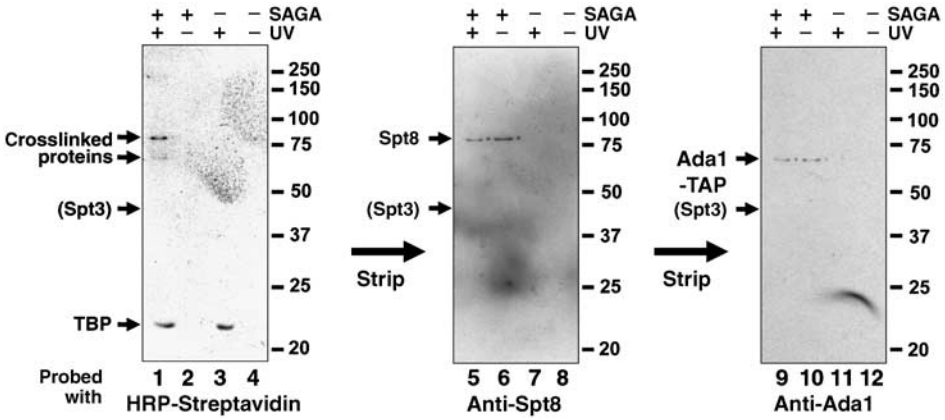


**Figure 2** Label transfer photo-crosslinking identifies Spt8 and Ada1 as proximal SAGA subunit targets of TBP. The biotin label on the crosslinked samples was visualized by Western blotting using HRP-conjugated Streptavidin (lanes 1–4) and using anti-Spt8 (lanes 5–8) and anti-Ada1 antibodies (lanes 9–12) after the same blot was stripped and reprobed.

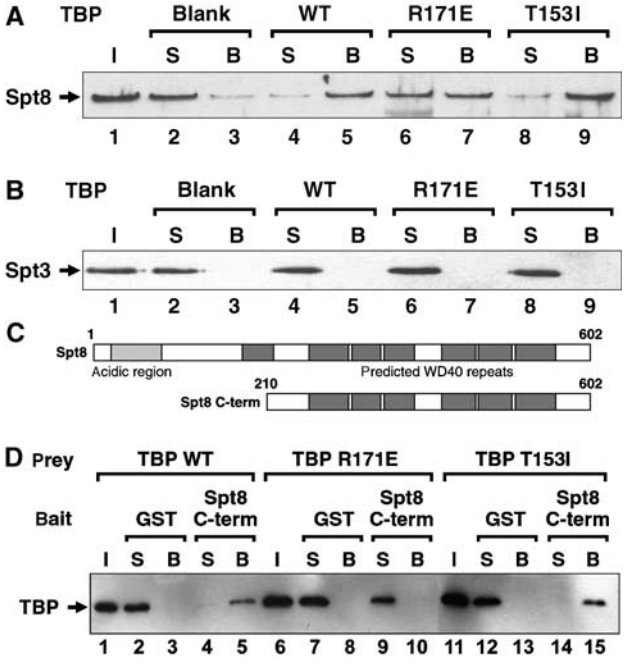


**Figure 3** Recombinant purified Spt8, but not Spt3, protein is sufficient to interact with TBP. (**A**) Spt8 pull-down experiment using Strep-tagged TBP immobilized on Strep-Tactin resin shows that wild-type TBP (lanes 4 and 5) and TBP 153I mutant (lanes 8 and 9) bind to TBP but that TBP R171E is defective in this interaction (lanes 6 and 7). Input, supernatant (unbound) and bound fractions are labeled I, S and B, respectively. Spt8 proteins were detected via anti-Spt8 antibodies. (**B**) Same comments as for (A) except that Spt3 protein was used and detected using anti-HIS antibodies which recognized the C-terminal hexahistidine tag engineered on the recombinant Spt3. (**C**) Schematic of Spt8 and Spt8 (210–602) showing location of the seven WD40 repeats predicted by CD-Search (Marchler-Bauer and Bryant, 2004). The N-terminal acidic region is shown in light gray, and the predicted WD40 repeats in gray. (**D**) GST pull-down experiment shows that Spt8 (210–602) is sufficient for specific, direct interactions with TBP. GST only or GST-Spt8 (210–602) immobilized on glutathione–Sepharose was incubated with Strep-tagged TBP, TBP R171E or TBP T153I proteins, and visualized with anti-Strep antibodies (IBA) by Western blotting.

level. Thus, Spt3 on its own or within the SAGA complex does not appear to physically interact with TBP, although Spt3 could still contribute indirectly to SAGA's binding to TBP.

### *The C-terminal WD40 repeat region of Spt8 is sufficient for its interaction with TBP*

Analysis of the Spt8 amino-acid sequence suggested Spt8 might contain seven WD40 repeats at the C-terminal two-thirds of the protein (Figure 3C), more than the single WD40 repeat previously noted (Eisenmann *et al*, 1994; Warfield *et al*, 2004). As many WD40 repeat proteins participate in protein–protein interactions, we asked if the C-terminal WD-40 repeats mediated Spt8's binding to TBP. For this experiment, we performed a GST pull-down assay using recombinant Spt8 (210–602) as a GST fusion. We find that wild-type TBP binds to GST-Spt8 (210–602) via the Spt8 moiety as binding does not occur with GST alone (Figure 3D, lanes 1–5). To analyze the specificity of the interaction, we employed the TBP R171E and T153I point mutants. Consistent with the results obtained using the SAGA complex or full-length Spt8, we find that GST-Spt8 (210–602) is defective in its interaction with TBP R171E (Figure 3D, lanes 6–10). We note that the TBP R171E mutation appears to affect interaction with Spt8 (210–602) even more severely than with full-length Spt8. The defective interaction of the TBP R171E mutation with Spt8 is specific to that mutation as the TBP T153I has no detectable effect on binding to GST-Spt8 (210–602) (Figure 3D, lanes 11–15). Our observed interaction between the acidic C-terminal WD40 repeat of Spt8 and TBP is specific as we do not observe interaction between the same region of Spt8 and hDot1 (2–416), which shares a similarly high pI of around 10 as the C-terminal domain of TBP (data not shown). We therefore conclude that the Spt8 (210–602) C-terminal WD40 repeat region is sufficient for its direct and specific interaction with TBP.

### *Both Spt3 and Spt8 contribute to TBP–SAGA interactions*

To evaluate the contributions of Spt8 and Spt3 to TBP binding in the context of SAGA, we created yeast mutant strains deleted in the Spt8 gene (*spt8*Δ), Spt3 gene (*spt3*Δ) or both Spt8 and Spt3 (*spt8*Δ*spt3*Δ), and purified SAGA complexes from these strains. Silver staining of samples separated by SDS–PAGE establishes that all expected SAGA subunits are present (Figure 1A, lanes 2–4). Western blotting further verifies the absence of Spt3 and/or Spt8 isolated from the appropriate deletion strains (Figure 1B, lanes 1–4). The four

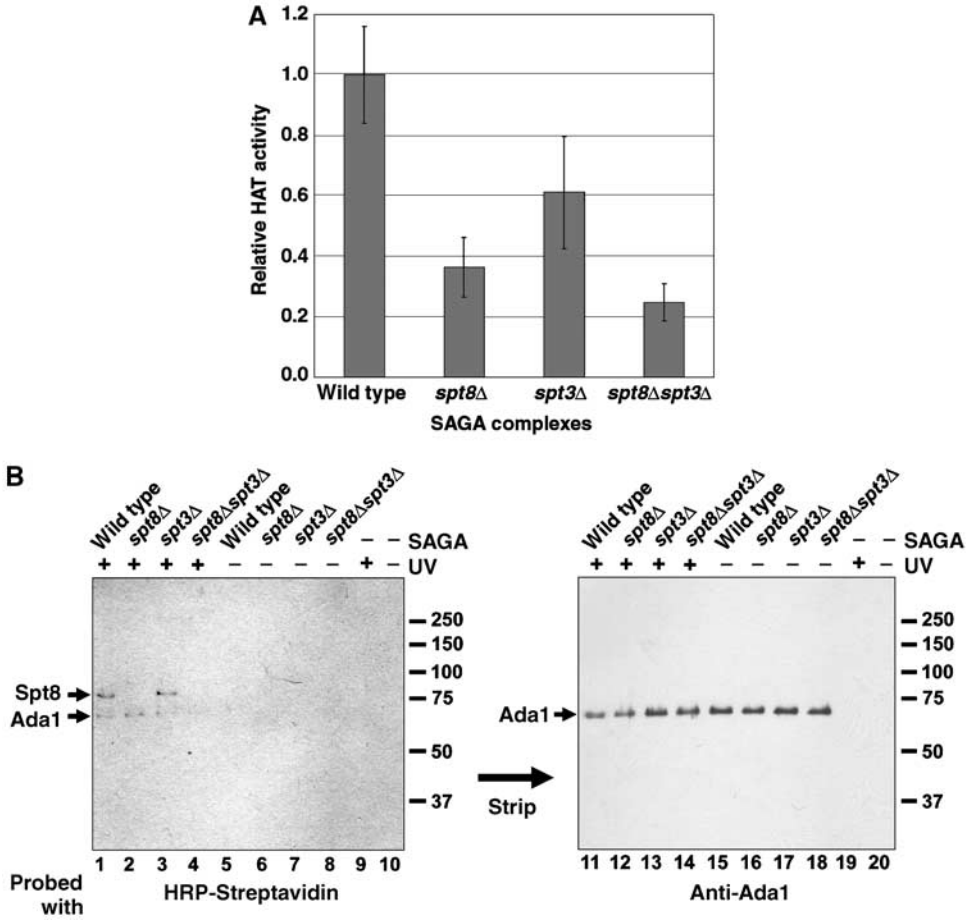


**Figure 4** Role of Spt3 and Spt8 subunits in SAGA binding to TBP. (**A**) Pull-down assay between immobilized TBP and wild-type, *spt8*Δ, *spt3*Δ and *spt8*Δ*spt3*Δ SAGA complexes, quantitated by core histone HAT activity after TEV cleavage of TBP from resin, with wild-type SAGA set to 1.0. Results are from three separate experiments. (**B**) Label transfer photo-crosslinking of SAGA subunits by Sulfo-SBED-conjugated TBP for wild-type, *spt8*Δ, *spt3*Δ and *spt8*Δ*spt3*Δ SAGA complexes with (lanes 1–4, respectively) or without UV illumination (lanes 5–8, respectively). Crosslinked bands were visualized by Western blotting using HRP-Streptavidin. The blot was then stripped and reprobed with anti-Ada1 antibodies to identify the location of the Ada1 subunit on the blot (lanes 11–18).

SAGA complexes (wild type, *spt8*Δ, *spt3*Δ, *spt8*Δ*spt3*Δ) have the same histone acetylase-specific activity (Figure 1A, lower panel, see figure legend for liquid HAT assay results). The equivalent specific activity of the four complexes also allows us to compare the amount of SAGA present by the measured HAT activity.

We find that *spt8*Δ SAGA binds TBP only one-third as well as wild-type SAGA (Figure 4A), showing that Spt8 is important for SAGA to bind TBP. Although we do not detect direct interactions between Spt3 and TBP, SAGA complexes lacking the Spt3 subunit binds less well to TBP, indicating an indirect role for Spt3 in TBP binding by the SAGA complex. Furthermore, deletion of both Spt3 and Spt8 subunits results in lower binding ability compared to the single deletions of either subunit. However, weak binding (~20% after adjusting for background counts) is still observed between this *spt8*Δ*spt3*Δ SAGA complex and TBP, suggesting that other SAGA subunits might also interact with TBP.

To examine what other subunits in SAGA might interact with TBP, we repeated the photo-crosslinking assay using the *spt8*Δ, *spt3*Δ and *spt8*Δ*spt3*Δ SAGA variants. In the absence of Spt8, the crosslinked Spt8 band disappears, providing corroborating evidence that the crosslinked band was, in fact, Spt8 (Figure 4B, lanes 1 and 2). However, the fainter crosslinked Ada1 is still present. When Spt3 is deleted, both Spt8 and Ada1 in SAGA receive the crosslinking biotin label, similar to wild-type SAGA (Figure 4B, lanes 3 and 13). No crosslinking to Spt8 and only a faint crosslinked Ada1 band is detected when both Spt8 and Spt3 are deleted (Figure 4B, lanes 4 and 14), perhaps due to the relatively weak binding between this double deletion SAGA and TBP. These results provide additional data to support a role of Ada1 in the binding of TBP by the SAGA complex.

### *Spt8 and SAGA bind to TBP monomer*

Our pull-down experiments showed that the TBP R171E point mutant is impaired for interactions with Spt8 and SAGA. Although these observations could indicate that TBP R171 residue directly contacts Spt8 and SAGA, the location of the R171 residue in the TBP three-dimensional structure suggests an alternate explanation. The TBP R171 residue is positioned on the alpha-helix along the side of the TBP saddle near its C-terminal stirrup (Figure 5A). This region is part of the dimer interface observed in crystal structures of the TBP protein (Nikolov *et al*, 1992; Chasman *et al*, 1993; DeDecker *et al*, 1996). In particular, R171 is in close proximity

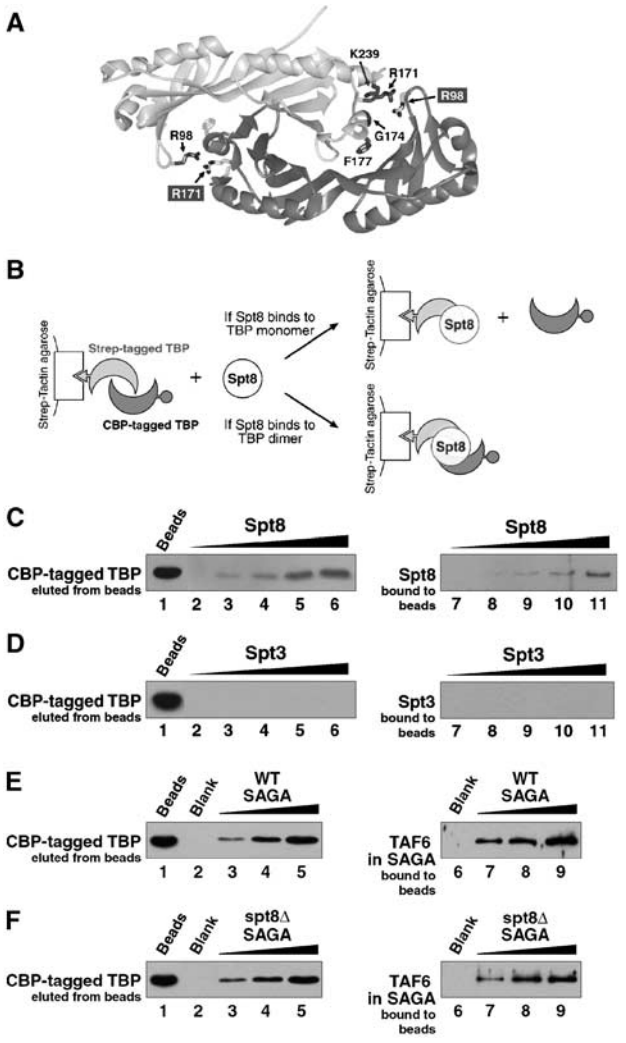


**Figure 5** Spt8 and SAGA bind TBP monomer in competition with the TBP dimer. (**A**) Ribbon representation of yeast TBP dimer showing the location of *spt15* point mutations that suppress the *spt3–401* mutation: R171, G174, F177 and K239. The R98 side chain in close proximity to the likewise positively charged R171 residue of the dimer partner TBP molecule is also shown. This figure was prepared using the MidasPlus software (Ferrin *et al*, 1988). (**B**) Schematic of experimental design for TBP dimer competition experiment. (**C**) Spt8 competes with TBP dimer for binding to TBP monomer. Spt8 protein was incubated with the heterodimeric CBP-tagged TBP/Strep-tagged TBP resin, and supernatant and bound fractions analyzed on Western blots using anti-CBP antibodies to detect the eluted CBP-tagged TBP, and anti-Spt8 antibodies to detect the bound Spt8 protein. (**D**) Spt3 does not interact with TBP. Same comments as for (C) except that Spt3 was used instead of Spt8, and anti-HIS antibodies were used to detect the bound Spt3. (**E**) SAGA competes with TBP dimer for binding to TBP monomer. Same comments as for (C) except that SAGA complex was used instead of Spt8 and bound SAGA complexes were detected on the Western blot using anti-TAF6 antibodies. Blank washes did not contain the SAGA complex. (**F**) SAGA complex lacking Spt8 subunit also competes with TBP dimer for binding to TBP monomer. Same comments as for (E).

to R98 of the partner TBP in the dimer. In fact, the R171E mutation stabilizes the TBP dimer presumably by favorable charge interactions with R98, and suppresses phenotypes *in vivo* associated with an unstable TBP dimer (Jackson-Fisher *et al*, 1999; Kou and Pugh, 2004). The R171E mutation thus favors the TBP dimer in the dimer/monomer equilibrium, resulting in less TBP monomer. Thus, TBP R171E mutation could adversely affect TBP's interaction with SAGA if Spt8 and SAGA bind to TBP monomer and not to TBP dimer.

We therefore designed an experiment to test the hypothesis that Spt8 competes with TBP dimer for the TBP monomer (Figure 5B). Strep-tagged TBP was bound to Strep-Tactin resin and the resulting Strep-tagged TBP resin incubated with CBP (calmodulin-binding peptide)-tagged TBP to create heterodimers of Strep- and CBP-tagged TBP bound to the resin. After washing to remove excess unbound TBP, this heterodimeric TBP resin was incubated with increasing amounts of Spt8 protein. If Spt8 does compete with CBP-tagged TBP, then increasing amounts of CBP-tagged TBP will elute from the resin as increasing amounts of Spt8 are added and bind to the Strep-tagged TBP resin. On the other hand, if Spt8 binds to TBP dimer, then CBP-tagged TBP should not elute upon incubation with Spt8, even though Spt8 would bind to the TBP resin.

Our experiments show that increasing amounts of CBP-tagged TBP do elute off the Strep-tagged TBP resin with increasing amounts of Spt8 added (Figure 5C, lanes 1–6). Concomitantly, Spt8 binds to the TBP resin in increasing quantities (Figure 5C, lanes 7–11). In contrast, Spt3 does not cause CBP-tagged TBP to elute off the TBP resin, and Spt3 itself dos not bind to the TBP resin (Figure 5D). These results indicate that Spt8 competes with TBP dimer for binding to TBP monomer.

We next examined if the entire SAGA complex also competes with TBP dimer for binding to TBP monomer using an analogous experimental design. We observe a similar elution pattern using SAGA complex when only Spt8 protein was incubated with the resin (Figure 5E), suggesting that SAGA also binds to TBP as a monomer and competes with the TBP dimer to do so. Interestingly, the competitive binding to TBP monomer also occurs in the absence of Spt8 (Figure 5F), indicating that SAGA with or without Spt8 binds to TBP monomer.

### *Spt8 and SAGA compete with TATA DNA for binding to TBP*

TBP binds to DNA as a monomer using the concave underside of the TBP saddle-like structure (Kim *et al*, 1993a, b). As this same surface is used to bind a second TBP in the TBP dimer (Nikolov *et al*, 1992; Chasman *et al*, 1993), binding of DNA by TBP monomer is mutually exclusive with binding a second TBP monomer to form a TBP dimer (Coleman *et al*, 1995). Given that Spt8 and SAGA bind to TBP monomer, we wondered if this binding would permit simultaneous binding of TBP to DNA. We employed a variation of the competition pull-down experiment used in the previous section: Spt8 was bound to Strep-tagged TBP attached to the Strep-Tactin resin via the Strep tag and the resulting resin then incubated with TATA-containing DNA (Figure 6A). If Spt8 competes with TATA DNA for binding to TBP, Spt8 should be displaced from the TBP resin as the TATA DNA binds to TBP. Conversely, if Spt8 binds to the TBP/DNA complex to form a ternary Spt8/TBP/DNA complex, TATA DNA should bind to the TBP resin without simultaneous release of Spt8. By monitoring both the elution of Spt8 from the TBP resin and the binding of DNA to TBP resin, we can distinguish between competitive binding and potential elution of Spt8 by DNA.

 

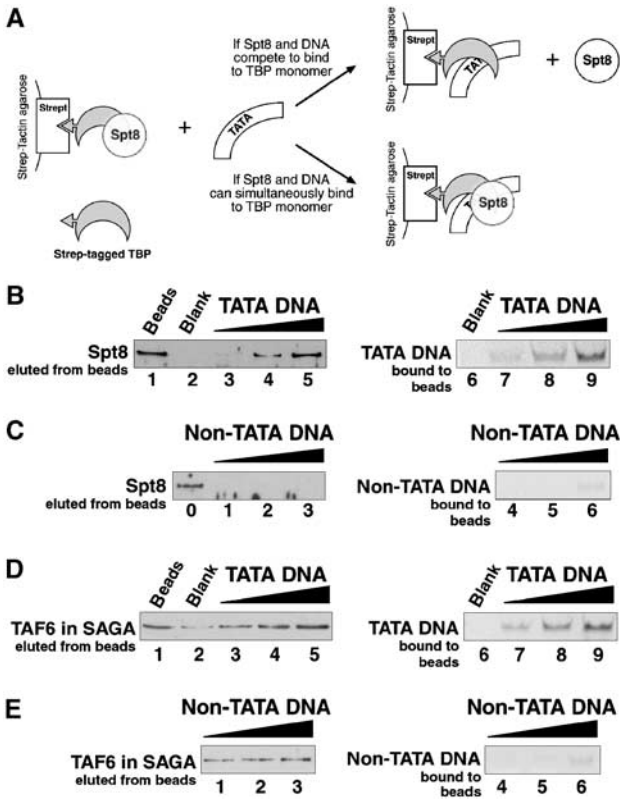


**Figure 6** Spt8 and SAGA bind TBP in competition with DNA. (**A**) Schematic of experimental design for DNA competition experiment. (**B**) Spt8 competes with TATA DNA for binding to TBP. Spt8 protein was bound to Strep-tagged TBP immobilized on Strep-Tactin before incubation with TATA box DNA. Wash fractions were then analyzed on Western blots using anti-Spt8 antibodies (lanes 2–5). The DNA that bound to the resin was visualized by ethidium bromide staining after native polyacrylamide gel electrophoresis (lanes 7–9). Blank washes omitted the DNA fragment (lanes 2 and 6). (**C**) Similar comments as for (B) except that non-TATA DNA was used instead. Lane 0 shows the position of the Spt8 band as reference for lanes 1–3. (**D**) SAGA complex competes with TATA DNA for binding to TBP. Same comments as for (B) except that SAGA complex was used instead of Spt8 protein, and anti-TAF6 antibodies were used in the Western blot. (**E**) Same comments as for (D) except that non-TATA DNA was used instead.

Our results show that Spt8 is eluted from the TBP resin concomitant with binding of TATA DNA to TBP (Figure 6B). This competitive binding requires TATA-containing DNA as the same concentrations of non-TATA sequence DNA do not release Spt8 and the non-TATA sequence binds only weakly to the TBP resin (Figure 6C). Thus, Spt8 binds to TBP monomer apparently to the exclusion of formation of a ternary Spt8/TBP/DNA complex.

We next performed this DNA competition experiment using the SAGA complex instead of its Spt8 subunit. We find that similar to Spt8, the SAGA complex is displaced from the TBP resin by TATA DNA with corresponding binding of the DNA by TBP (Figure 6D). The SAGA complex is washed from the resin in lesser amounts when non-TATA DNA is used, presumably due to SAGA's ability to bind to naked DNA (Lee *et al*, 2005) (Figure 6E). However, this effect is not due to displacement of SAGA from TBP by DNA as the non-TATA DNA does not bind to the TBP resin in appreciable amounts (a faint signal can be seen in Figure 6E, lane 6 only at the highest concentrations of non-TATA DNA used). Thus, we conclude that SAGA binds to TBP in competition with TATA DNA.

## Discussion

We have investigated subunits of SAGA which interact with TBP. We used label-transfer crosslinking to identify SAGA's Spt8 and Ada1 subunits as being within approximately 20 Å of TBP in the SAGA/TBP complex. We have verified that recombinant Spt8 is sufficient to interact with TBP by pull-down experiments, showing that the interaction detected by crosslinking is functional. The highly acidic N-terminus of Spt8 apparently is not necessary to interact with TBP as the C-terminal WD40 repeats of Spt8 alone can bind TBP. Previous observations have shown that Spt8 is required for efficient TBP binding by SAGA (Sterner *et al*, 1999) and our results now establish that Spt8 is a direct target of TBP when TBP binds to SAGA. Our data are consistent with the recent report by Warfield *et al* (2004) that the Spt8 protein alone suffices to bind to TBP.

Our crosslinking experiments also identified, for the first time, Ada1 as a SAGA subunit that may interact with TBP. Ada1 had not been previously identified as a potential target of TBP in the SAGA complex presumably because Ada1 is required for the structural integrity of SAGA (Sterner *et al*, 1999) and many prior experiments examined the effects of deleting individual SAGA subunits. This inability to isolate SAGA from cells where the Ada1 gene was deleted also complicated our attempts to validate the interaction between Ada1 and TBP using Δ*ada1* SAGA. We were also unable to test direct binding of Ada1 to TBP using recombinant proteins, because the full-length Ada1 polypeptide is not soluble on its own or even when complexed with its histone-fold partner TAF12 (Gangloff *et al*, 2000), another component of the SAGA complex (data not shown). However, we still observed the crosslinked interaction between Ada1 within the Δ*spt3* and Δ*spt8* SAGA complexes and TBP. These results suggest a new role for Ada1 in the binding of TBP by the SAGA complex.

Our experiments show that Spt8 binds to TBP monomer suggesting that Spt8 binds to the TBP concave surface, or at least close enough to this surface so that binding of Spt8 to TBP occludes binding of a second TBP monomer. Similar experiments show that the SAGA complex also binds to TBP monomer. SAGA lacking Spt8 likewise binds to TBP competitive with TBP dimer formation, which suggests that Ada1 may also bind to or very close to the concave surface.

We find that SAGA binds TBP in competition with DNA, an unexpected result as many models for SAGA's role in transcriptional regulation assume that SAGA can bind to the TBP/DNA complex (Belotserkovskaya *et al*, 2000; Bhaumik and Green, 2001; Larschan and Winston, 2001, 2005). While unexpected, the result is consistent with available published data and provides a mechanistic explanation for several previous observations. For example, SAGA can be localized to the UAS by chromatin immunoprecipitation experiments, presumably by SAGA's interactions with activator proteins that bind directly or indirectly to the UAS elements. However, it was puzzling that the SAGA coactivator complex does not localize to the TATA box by chromatin immunoprecipitation (Bhaumik and Green, 2001; Larschan and Winston, 2001). Our finding that SAGA competes with DNA to bind to TBP

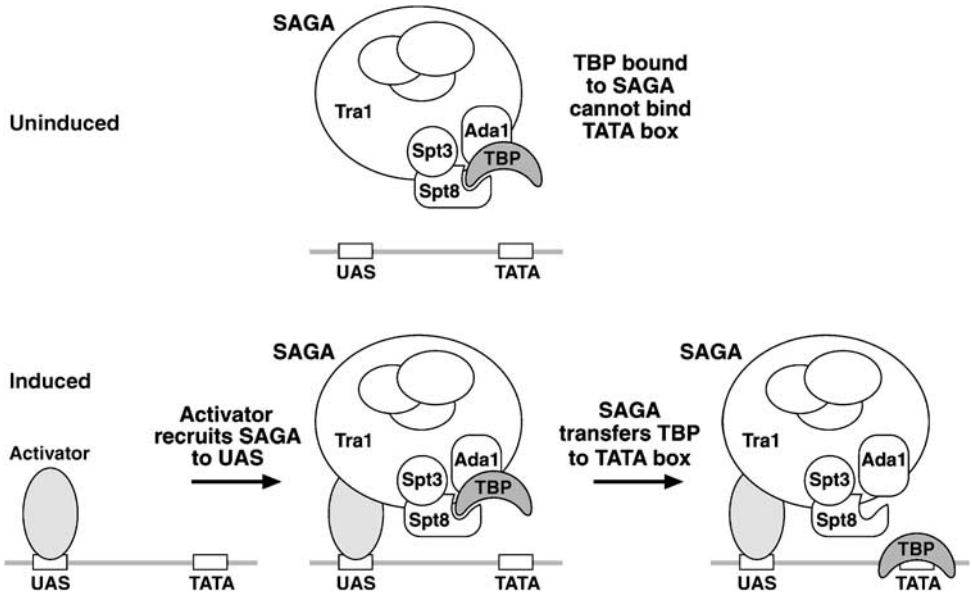


**Figure 7** Handoff model for SAGA recruitment of TBP to TATA box. In the uninduced or repressed state, the SAGA complex binds TBP via Spt8 and perhaps Ada1 subunits. TBP does not bind SAGA and DNA simultaneously, and TBP is not delivered to the promoter. In the induced or activated state, the binding of activator to the UAS recruits SAGA by direct interactions with SAGA subunits such as Tra1. The recruited SAGA/TBP complex does not bind to the TATA box, but instead transfers or hands off TBP to the TATA box. This results in TBP binding to the TATA box while breaking the direct interactions between SAGA subunits and TBP.

indicates that SAGA cannot interact with TBP bound to DNA, and thus provides an explanation for why SAGA does not chromatin immunoprecipitate with the TATA box.

Based on our results, we propose the following 'handoff' model for how SAGA recruits TBP to the core promoter (Figure 7). Under noninducing or repressive conditions, SAGA binds to TBP via its Spt8 and possibly the Ada1 subunits, reducing the pool of available TBP and preventing TBP from binding to the core promoter. Thus under such conditions, SAGA inhibits transcription. Deletion of Spt3 or Spt8 derepresses uninduced or repressed transcription because SAGA lacking Spt3 or Spt8 binds TBP less tightly and therefore releases TBP to bind to TATA box, consistent with the observations of Belotserkovskaya *et al* (2000) and Govind *et al* (2005). In contrast, under activating conditions, the appropriate activator binds to the UAS and recruits SAGA to this element. Once at the promoter, SAGA transfers TBP to the TATA box, permitting assembly of the preinitiation complex. This model can explain the positive role of SAGA at activated promoters such as GAL1 and PHO5, in particular the requirement of Spt8 for TBP recruitment to the core promoter (Dudley *et al*, 1999; Bhaumik and Green, 2001; Larschan and Winston, 2001; Barbaric *et al*, 2003). As SAGA does not associate with TBP after delivering it to the TATA box, the handoff model implicitly provides not only the mechanism for how SAGA delivers TBP to the TATA box but also for how to dissociate SAGA from TBP after that delivery.

What then is the role of Spt3 in TBP recruitment by SAGA? Our results indicate that Spt3 does not bind on its own to TBP, although it does contribute to the binding of TBP to the SAGA complex. It is possible that Spt3 does interact directly with TBP, but only in the context of other SAGA subunits such as Ada1, or Spt3 could alter the conformation of Spt8 and/or Ada1 for interaction with TBP (even though Spt8 on its own is already competent to bind to TBP). Spt3 could also be involved in the handoff of TBP from SAGA to the TATA box, a potentially critical regulatory step. If so, Spt3 might be required for TBP recruitment not because it binds TBP directly, but because it coordinates the release of TBP from SAGA. It is possible, for example, that the Spt3 mutations, which suppress TBP mutation in an allele-specific fashion (Eisenmann *et al*, 1992), reflect the possible involvement of Spt3 in handing TBP off to the TATA box.

If the handoff of TBP from SAGA to the core promoter is an important regulatory step, we might expect that additional determinants might influence the ability of SAGA to recruit TBP to the core promoter. The HO promoter may constitute such an example. At the HO promoter, the Swi5 activator protein binds to upstream sequences and recruits the Mediator coactivator and the Swi/Snf chromatin remodeling complexes, which in turn recruit SAGA to the upstream sequences. Histone acetylation of the HO promoter by SAGA then permits the SBF activator protein to bind to its UAS and to activate HO expression (Cosma *et al*, 1999; Yu *et al*, 2003). In this case, although SAGA is recruited to the promoter, it appears to continue to repress transcription by preventing TBP binding as deletion of Spt3 and Spt8 subunits derepresses the promoter and allows TBP binding to the HO promoter (Yu *et al*, 2003). Thus, it appears that some mechanism downregulates the ability of SAGA to hand TBP off to the HO promoter TATA box. A second example where TBP handoff by SAGA might be downregulated is the ARG1 promoter where SAGA acts to repress transcription in rich medium (repressing conditions) and turns on expression in minimal medium (activating conditions) (Ricci *et al*, 2002). Spt3 and Spt8 are required for repression in rich media, as predicted by the handoff model, but their absence increases transcription in minimal medium. As the HAT activity of SAGA is required for activation at the Arg1 promoter, these

 

results suggest that chromatin modification may be SAGA's primary role in activating the Arg1 promoter and that SAGA's TBP recruitment function is prevented by impeding TBP handoff. In this case, TBP might be recruited by other coactivators such as the Mediator complex (Qiu *et al*, 2005).

The handoff model for the SAGA recruitment of TBP to the core promoter recalls the two-step handoff model for TFIID recruitment by transcriptional activators in several but not all respects. TFIID's largest subunit, TAF1, contains a repressive N-terminal domain (TAND), which binds to the concave underside of TBP in competition with DNA (Liu *et al*, 1998). The two-step handoff model proposes that certain activator proteins can displace the TAND (first step) to permit TBP to bind to DNA (second step) (Nishikawa *et al*, 1997; Burley and Roeder, 1998; Kotani *et al*, 2000). In both the TFIID and SAGA handoff models, TBP must be recruited to the promoter by a coactivator. Both models involve a coactivator subunit which binds to TBP at or near its concave underside competitive with TBP binding to DNA. However in the two-step handoff model, the entire TFIID complex (TBP together with the TAFs) is transferred to the core promoter, whereas in the SAGA handoff model, only TBP is recruited to the TATA box.

This feature, that only TBP is recruited to the TATA box, is also what distinguishes the SAGA handoff model from previous models of TBP recruitment by SAGA (Belotserkovskaya *et al*, 2000; Bhaumik and Green, 2001; Larschan and Winston, 2001, 2005). Those models suggested that the coactivator SAGA could interact with activators bound to UAS as well as TBP bound to the TATA box. The significance of this difference is that the handoff model imposes a directionality to the recruitment process independent of any regulatory effects of chromatin. As SAGA does not stay bound to TBP at the TATA box, the recruitment process is unidirectional: activators recruit SAGA which delivers TBP to the TATA box. However, the reverse scheme whereby TBP could recruit SAGA which then binds to activators would not be possible as if SAGA extracts TBP from the TATA box in the absence of activators, SAGA would no longer be tethered to the promoter. This corollary is consistent but not necessarily the sole explanation for observations that Spt3 is required for TBP recruitment but not for Gal4 recruitment at the GAL1 promoter (Bhaumik and Green, 2001; Larschan and Winston, 2001), and with the finding that deletion of the Arg1 TATA element does not affect recruitment of SAGA to the UAS by Gcn4 (Qiu *et al*, 2005).

In summary, our investigations indicate that SAGA binds to TBP via its Spt8 and perhaps Ada1 subunits in competition with DNA. These results suggest a simple model for how SAGA recruits TBP to the promoter which resolves many formerly puzzling experimental observations. Future investigations will now be needed to study how SAGA transfers TBP to DNA and to more precisely define the role of the Spt3 subunit in this process.

## Materials and methods

### *Yeast strains, purification of SAGA complexes and purification of recombinant proteins*

Yeast wild-type SAGA complex in this study was isolated from wild-type BY4742 *MAT*α, which contains a genomic C-terminally TAP-tagged ADA1 gene. The variant *spt8*Δ SAGA, *spt3*Δ SAGA and *spt8*Δ*spt3*Δ SAGA complexes were isolated from the same strain background but have genotypes of *spt8*Δ::kanMX4, *spt3*Δ::kanMX4, and *spt8*Δ::LEU2; *spt3*Δ::kanMX4, respectively. SAGA complexes were purified by the TAP scheme (Puig *et al*, 2001), except that an additional MonoQ fractionation from 100 to 500 mM NaCl at pH 8.0 was included after TEV protease cleavage from the IgG-Sepharose column. Recombinant Strep-TEV-tagged TBP (61–240), Spt8 and Spt3 were expressed in Escherichia coli and purified by affinity and conventional chromatography. Details of the recombinant protein expression and purification are available in the Supplementary data.

### *Label transfer photo-crosslinking*

The photo-crosslinking label transfer experiment was performed essentially as described (Neely *et al*, 2002), except that the Sulfo-SBED-conjugated TBP was purified from excess crosslinking reagent by Source S chromatography (Sulfo-SBED obtained from Pierce).

### *Pull-down assay*

For pull-down assays, equilibrated Strep-Tactin Superflow (IBA) was incubated with Strep-TEV-tagged TBP, washed with buffer, incubated with recombinant Spt3 or Spt8 protein or with SAGA complex, washed with buffer before fractionation by SDS–PAGE and analysis was by Western blotting. Additional details are available in the Supplementary data.

The amount of SAGA complex in the input, supernatant (unbound) and eluted fractions were quantitated by HAT assays and fluorography or scintillation counting as described previously (Eberharter *et al*, 1998). SAGA complex was eluted from the resin by TEV protease to cleave the fusion tag on TBP and thus to release both TBP and TBP-bound SAGA complex from the resin. Control experiments show essentially complete cleavage of the fusion TBP protein by TEV protease. Separating SAGA complex from the Strep-Tactin resin significantly reduced nonspecific interactions between the HAT assay substrates (histones) and the resin. We found HAT activity measurements to be more sensitive and reliable than Western blotting for quantitative measurements, due in part to the high background associated with detecting small quantities of SAGA by Western blotting.

### *Competition assay*

Competition experiments between TBP dimer and Spt8 or SAGA were performed by binding Strep-TEV-tagged TBP (61–240) to Strep-Tactin Superflow resin, washing, incubating the resin with CBP-tagged TBP (61–240), washing three times , incubating with Spt8 protein or SAGA complex, washing three times, and analyzing bound or unbound fractions by Western blotting. Competition experiments between DNA and Spt8 or SAGA for binding to TBP were performed in an analogous manner: Strep-TEV-tagged TBP (61–240) bound to Strep-Tactin resin was incubated with Spt8 or SAGA, washed three time, incubated with TATA DNA (26 bp double-stranded oligonucleotide CYC1 promoter DNA: 5′-TGCTCTGTATG TATATAAAACTCTTG-3′) or non-TATA DNA (26 bp double-stranded oligonucleotide DNA 5′-TGCTCTGTATGCAGATAAAACTCTTG-3′), and bound or unbound samples were analyzed by Western blotting or by ethidium bromide staining of native PAGE. Details of the competition assays are provided in the Supplementary data.

### *Supplementary data*

Supplementary data are available at *The EMBO Journal* Online.

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