

# SAGA is an essential *in vivo* target of the yeast acidic activator Gal4p

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Despite major advances in characterizing the eukaryotic transcriptional machinery, the function of promoter-specific transcriptional activators (activators) is still not understood. For example, in no case have the direct *in vivo* targets of a transcriptional activator been unambiguously identified, nor has it been resolved whether activators have a single essential target or multiple redundant targets. Here we address these issues for the prototype acidic activator yeast Gal4p. Gal4p binds to the upstream activating sequence (UAS) of *GAL1* and several other *GAL* genes and stimulates transcription in the presence of galactose. Previous studies have shown that *GAL1* transcription is dependent on the yeast SAGA (Spt/Ada/GCN5/acetyltransferase) complex. Using formaldehyde-based *in vivo* cross-linking, we show that the Gal4p activation domain recruits SAGA to the *GAL1* UAS. If SAGA is not recruited to the UAS, the preinitiation complex (PIC) fails to assemble at the *GAL1* core promoter, and transcription does not occur. SAGA, but not other transcription components, is also recruited by the Gal4p activation domain to a plasmid containing minimal Gal4p-binding sites. Recruitment of SAGA by Gal4p and stimulation of PIC assembly is dependent on several SAGA subunits but not the SAGA histone acetyl-transferase (HAT) GCN5. Based on these and other results, we conclude that SAGA is an essential target of Gal4p that, following recruitment to the UAS, facilitates PIC assembly and transcription.

[Key Words: Transcription; *GAL1*; SAGA; Gal4p; TAF; TBP]

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Transcription of eukaryotic structural genes often involves a *cis*-acting element that functions at a distance from the core promoter, termed an enhancer in mammalian cells (McKnight and Tjian 1986) and a UAS (upstream activating sequences) in yeast (Stargell and Struhl 1996). Enhancers and UASs provide binding sites for transcriptional activator proteins (activators), which contain two functional domains: one that directs DNA binding and another that activates transcription (Ptashne 1988). A central question in the field is how different promoter-specific activators function through a common set of general transcription factors (GTFs) that assemble on the core promoter to form the preinitiation complex (PIC). Although it is clear that activators function, at least in part, by stimulating PIC assembly (Orphanides et al. 1996; Roeder 1996; Ptashne and Gann 1997; Lee and Young 2000), the detailed mechanism by which this occurs has not been clearly elucidated.

Activator-mediated stimulation of PIC assembly is believed to result from a direct interaction between the activation domain and one or more components of the transcription machinery, termed the target. Based prima-

rily on *in vitro* protein-protein interaction experiments, a variety of factors have been proposed to be the direct targets of activators (Orphanides et al. 1996; Ptashne and Gann 1997; Zaman et al. 1998). However, whether any of these are bona fide *in vivo* targets required for stimulation of PIC assembly and transcription remains to be determined. An important prediction is that a true target may be directly recruited to the promoter by the activator *in vivo* and in a manner not dependent on other transcription components.

The well-characterized acidic activator Gal4p is responsible for the transcriptional induction of *GAL* genes, such as *GAL1*, which contain Gal4p-binding sites in their promoters (Johnston 1987; Johnston and Carlson 1992; Dudley et al. 1999). In this study, we use formaldehyde-based *in vivo* cross-linking (Orlando and Paro 1993; Strahl-Bolsinger et al. 1997) in conjunction with transcriptional, mutational, and kinetic analyses to identify the target of Gal4p.

## Results

### Recruitment of SAGA to the *GAL1* UAS

Yeast SAGA is a 1.8-MD complex, which contains  $\geq 14$  subunits: Ada1p, Ada2p, Ada3p, Ada5p/Spt20p, Spt3p, Spt7p, Spt8p, Gcn5p, Tra1p, TAF<sub>II</sub>17, TAF<sub>II</sub>25, TAF<sub>II</sub>60,

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TAF<sub>II</sub>68, and TAF<sub>II</sub>90 (Hampsey 1997; Grant et al. 1998; Brown et al. 2000). Several of these subunits (e.g., Spt3p, Spt7p, Spt8p, and Spt20p) are present exclusively in SAGA and thus can be used to study the intact complex. Because previous studies have shown that transcription of *GAL1* (Roberts and Winston 1997; Dudley et al. 1999; Sterner et al. 1999) as well as other genes (Horiuchi et al. 1997) is dependent on SAGA, we analyzed recruitment of SAGA to the *GAL1* promoter using a formaldehyde-based in vivo cross-linking/immunoprecipitation assay. For these experiments, we used either a c-myc mouse monoclonal antibody and yeast strains expressing a C-terminal epitope-tagged SAGA subunit (Spt3p, Spt20p, Gcn5p, or Ada2p) or polyclonal antibodies to various SAGA TAF<sub>II</sub>s. Two sets of promoter-specific primer-pairs were used that, as shown below, could distinguish binding to the *GAL1* UAS or core promoter.

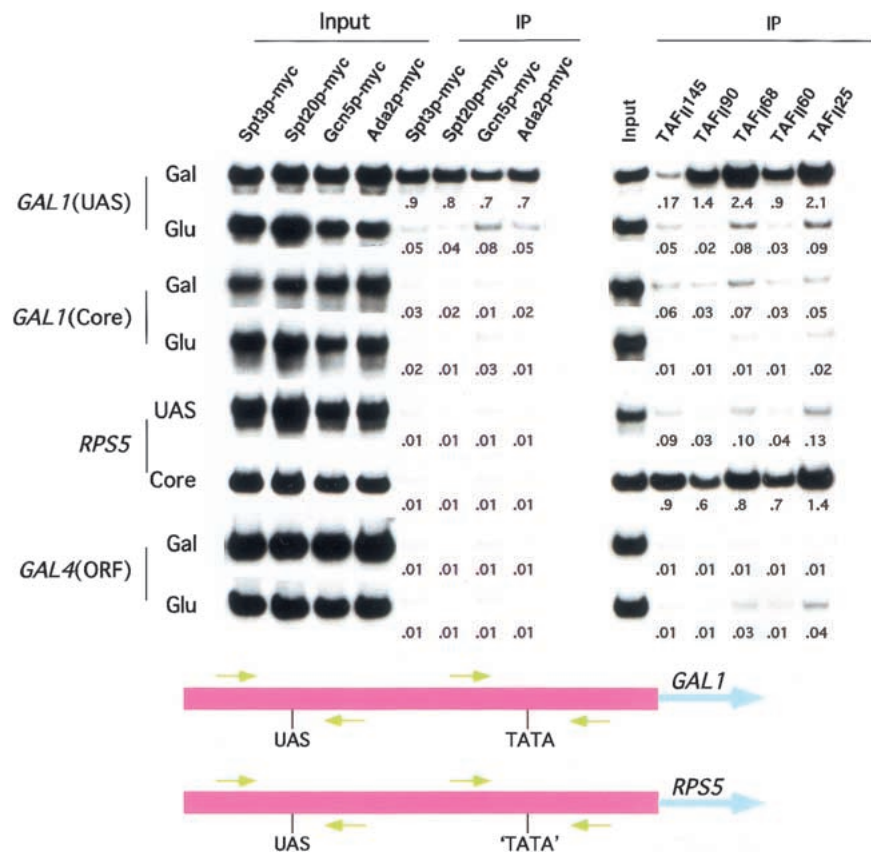
Figure 1 shows that under conditions in which the Gal4p activation domain was active (galactose media), the SAGA subunits Spt3p, Spt20p, Gcn5p, and Ada2p were present at the *GAL1* UAS but not the core promoter. Significantly, these same SAGA subunits were not associated with the *GAL1* UAS when the Gal4p activation domain was inactive (glucose [Fig. 1] or raffinose [see below] media). In addition, SAGA was not associated with an irrelevant DNA sequence (*GAL4* ORF) or with the UAS or core of the *RPS5* promoter, the transcription of which does not require SAGA (Lee et al. 2000; see below).

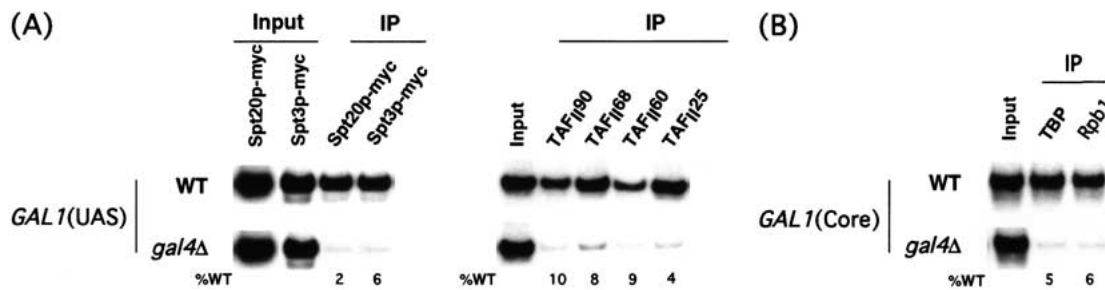
We also analyzed the association of the TAF<sub>II</sub>s with the *GAL1* promoter. Previous studies have shown that TAF<sub>II</sub>s are not required for *GAL1* transcription and that TBP binds the TATA box in a form not associated with TAF<sub>II</sub>s (Li et al. 2000). Figure 1 shows that following growth in galactose, the SAGA TAF<sub>II</sub>s (TAF<sub>II</sub>90, TAF<sub>II</sub>68, TAF<sub>II</sub>60, and TAF<sub>II</sub>25), like the other SAGA components, were associated with the *GAL1* UAS but not the core promoter. TAF<sub>II</sub>145, a component of TFIID but not SAGA, was not associated with the *GAL1* UAS. These results indicate that the TAF<sub>II</sub>s are recruited to the *GAL1* UAS as part of the SAGA complex. In contrast, on the *RPS5* promoter, the transcription of which requires TAF<sub>II</sub>s (Shen and Green, 1997; Li et al. 2000) but not SAGA, the TFIID-specific TAF<sub>II</sub>145 and the other TAF<sub>II</sub>s are associated with the core promoter but not the UAS. These results indicate that TFIID is recruited to the *RPS5* core promoter during PIC assembly.

#### *Gal4p* mediates SAGA recruitment

Figure 1 indicates that in the presence of galactose, SAGA was specifically recruited to the UAS, which harbors the Gal4p-binding sites. These results strongly suggest that recruitment of SAGA to the UAS requires Gal4p. To confirm this supposition, we performed in vivo cross-linking in strains lacking Gal4p. Figure 2A shows that in a *GAL4* deletion strain, SAGA was not recruited to the UAS.

**Figure 1.** Recruitment of SAGA to the *GAL1* upstream activating sequence (UAS). Yeast strains were grown at 30°C in 1% yeast extract containing 2% peptone plus 2% glucose or galactose as indicated. Formaldehyde-based in vivo cross-linking/immunoprecipitation was performed as previously described (Li et al. 2000). Primer-pairs located in the UAS or core promoter of *GAL1* and *RPS5* (see Materials and Methods) were used for PCR analysis of the immunoprecipitated DNA samples. A PCR fragment corresponding to the *GAL4* ORF was used as a control for background binding. Immunoprecipitation was performed using a mouse monoclonal antibody against the c-myc epitope-tag (9E10; Santa Cruz) or a polyclonal antibody against the indicated TAF<sub>II</sub>. The promoter, primer-pair, and media are indicated on the left. The ratio of immunoprecipitate over the input is indicated below each band. Ratios ≤0.01 are designated .01. IP, immunoprecipitate.





**Figure 2.** Gal4p mediates recruitment of SAGA to the *GAL1* upstream activating sequence (UAS). Wild-type and *GAL4* deletion strains were first grown in glucose-containing (YPD) and then shifted to galactose-containing (YPG) media 5 h before treatment with formaldehyde. Primer-pairs located in the (A) *GAL1* UAS or (B) core promoter were used for PCR analysis of the immunoprecipitated DNA samples. Immunoprecipitations were performed using polyclonal antibodies against a TAF<sub>II</sub> or TBP, the mouse monoclonal antibody 9E10 (Santa Cruz) against the c-myc epitope-tag, or the mouse monoclonal antibody 8WG16 (Covance) against the CTD domain of the RNA polymerase II large subunit (RPB1).

We have shown previously that in the presence of galactose, Gal4p stimulates PIC assembly as evidenced by recruitment of GTFs and RNA polymerase II to the core promoter (Li et al. 1999, 2000). Consistent with these previous results, in a *GAL4* deletion strain, TBP and RNA polymerase II were not recruited to the core promoter (Fig. 2B).

#### Role of specific SAGA subunits

We next analyzed various yeast deletion strains to determine the role of individual SAGA subunits in SAGA recruitment, PIC assembly, and transcription. In yeast strains lacking *SPT20*, SAGA was not recruited to the *GAL1* UAS (Fig. 3A), and PIC assembly (Fig. 3B) and transcription (Fig. 3C) were reduced substantially. Thus, Spt20p is required for recruitment of SAGA to the *GAL1* promoter, consistent with previous studies showing that Spt20p is required for integrity of the SAGA complex (Grant et al. 1997; Sterner et al. 1999). Figure 3D shows that strains lacking *SPT20* were also defective for transcription of the Gal4p-dependent *GAL2*, *GAL7*, and *GAL10* genes. Thus, the requirement for SAGA is not specific to *GAL1* but rather is general to Gal4p-dependent genes.

In strains lacking *SPT3*, recruitment of SAGA to the UAS was reduced only modestly (Fig. 3A). Significantly, however, PIC assembly (Fig. 3B) and transcription (Fig. 3C) were reduced substantially. Thus, Spt3p appears to function by facilitating PIC assembly following recruitment of SAGA to the UAS.

Finally, in strains lacking *GCN5*, SAGA recruitment, PIC assembly, and transcription occurred at near wild-type levels (Fig. 3A–C). Collectively, these data reveal that individual SAGA components are differentially required for SAGA recruitment, PIC assembly, and transcription.

#### Requirement of SAGA following *GAL80* deletion

The Gal4p activation domain is controlled through binding of the negative regulator Gal80p. In the presence of

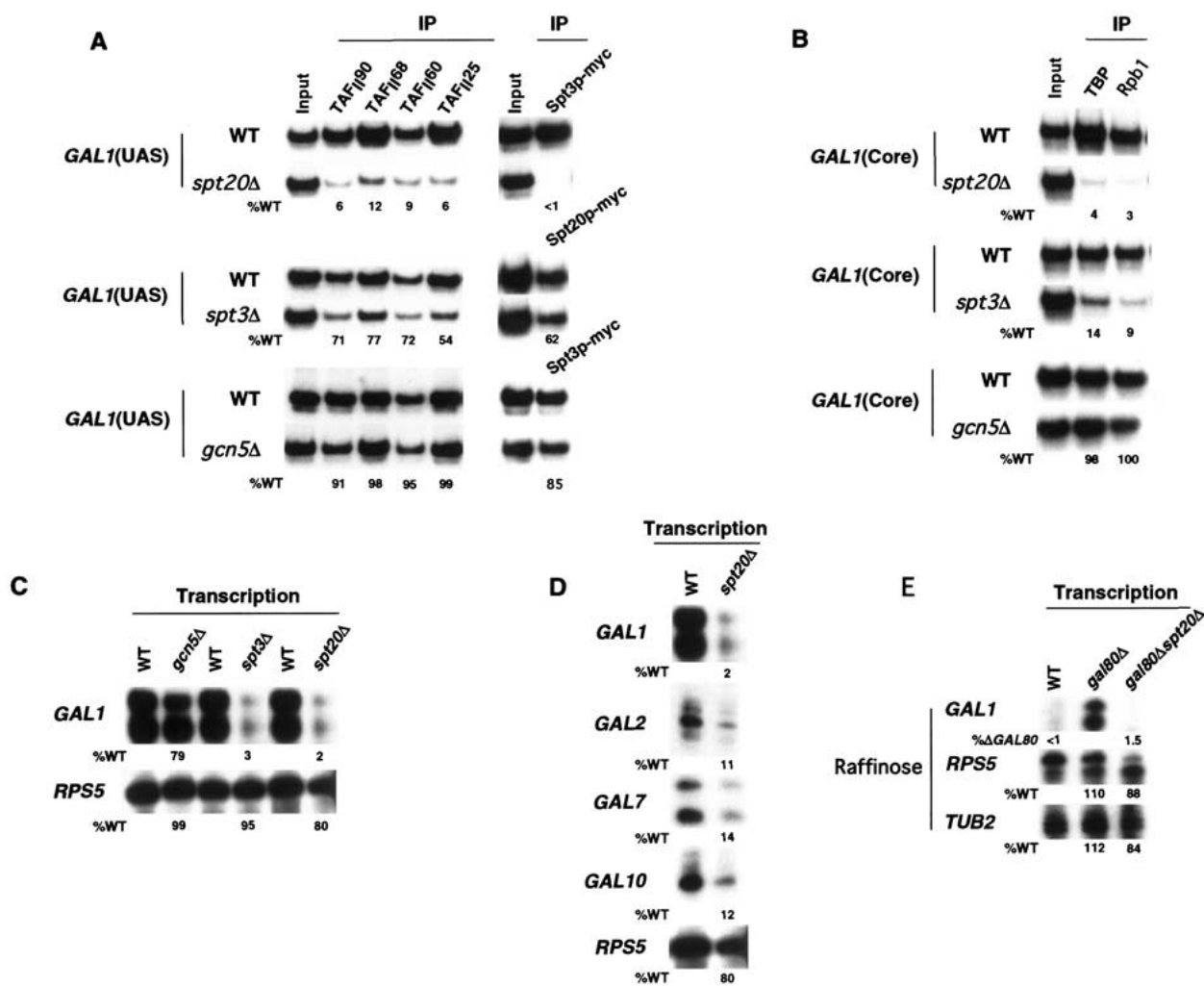
galactose, Gal4p is activated through a complex pathway that ultimately counteracts Gal80p (Johnston 1987; Johnston and Carlson 1992; Ostergaard et al. 2001). It was therefore possible that the inability of Gal4p to activate transcription in SAGA deletion strains was caused by an inability to counteract Gal80p and not by the absence of an essential target of the Gal4p activation domain.

To distinguish between these possibilities, we artificially activated Gal4p by deleting the gene encoding the negative regulator Gal80p and analyzed the requirement for SAGA (Fig. 3E). As expected, in raffinose media *GAL1* was transcribed at high levels in a *GAL80* deletion but not a wild-type strain. Significantly, inactivation of SAGA by deletion of *SPT20* again dramatically reduced *GAL1* transcription, even under these artificial activation conditions. As expected, transcription of *TUB2* and *RPS5*, which do not require SAGA (Dudley et al. 1999; Lee et al. 2000), was unaffected by deletion of *SPT20* (or *GAL80*). Thus, the requirement of SAGA for *GAL1* transcription is at a step subsequent to establishment of a functional Gal4p activation domain.

#### Recruitment of SAGA to minimal Gal4p-binding sites

The above experiments indicate that in the presence of active Gal4p, SAGA is recruited to the UAS, the PIC assembles at the core promoter, and transcription occurs. These observations raise the possibility that SAGA is a direct target of the Gal4p activation domain. However, because these experiments were performed with the intact *GAL1* promoter under conditions permissive for transcription, it remained possible that other transcription components, such as GTFs, played a role in SAGA recruitment to the UAS. To address this issue, we asked whether SAGA could be recruited by Gal4p to a plasmid bearing only Gal4p-binding sites and not other promoter elements. Figure 4A shows that similar to the results with the *GAL1* UAS, SAGA was recruited to a plasmid bearing Gal4p-binding sites in galactose but not glucose media.

The requirement of galactose for SAGA recruitment,



**Figure 3.** Requirement of SAGA subunits for *GAL1* complex assembly and transcription. Wild-type and SAGA subunit deletion mutants were grown as in Fig. 2 before treatment with formaldehyde. Primer-pairs located in the (A) *GAL1* upstream activating sequence (UAS) or (B) core promoter were used for PCR analysis of the immunoprecipitated DNA samples. (C) Transcription. Total cellular RNA was prepared from the wild type or deletion mutants (*top*), and transcription from the indicated gene (*left*) was quantitated by primer-extension. The percentage transcription relative to wild type is indicated below. (D) Other Gal4p-dependent genes (as in panel C). (E) Requirement of SAGA following artificial activation of Gal4p (as in panel C).

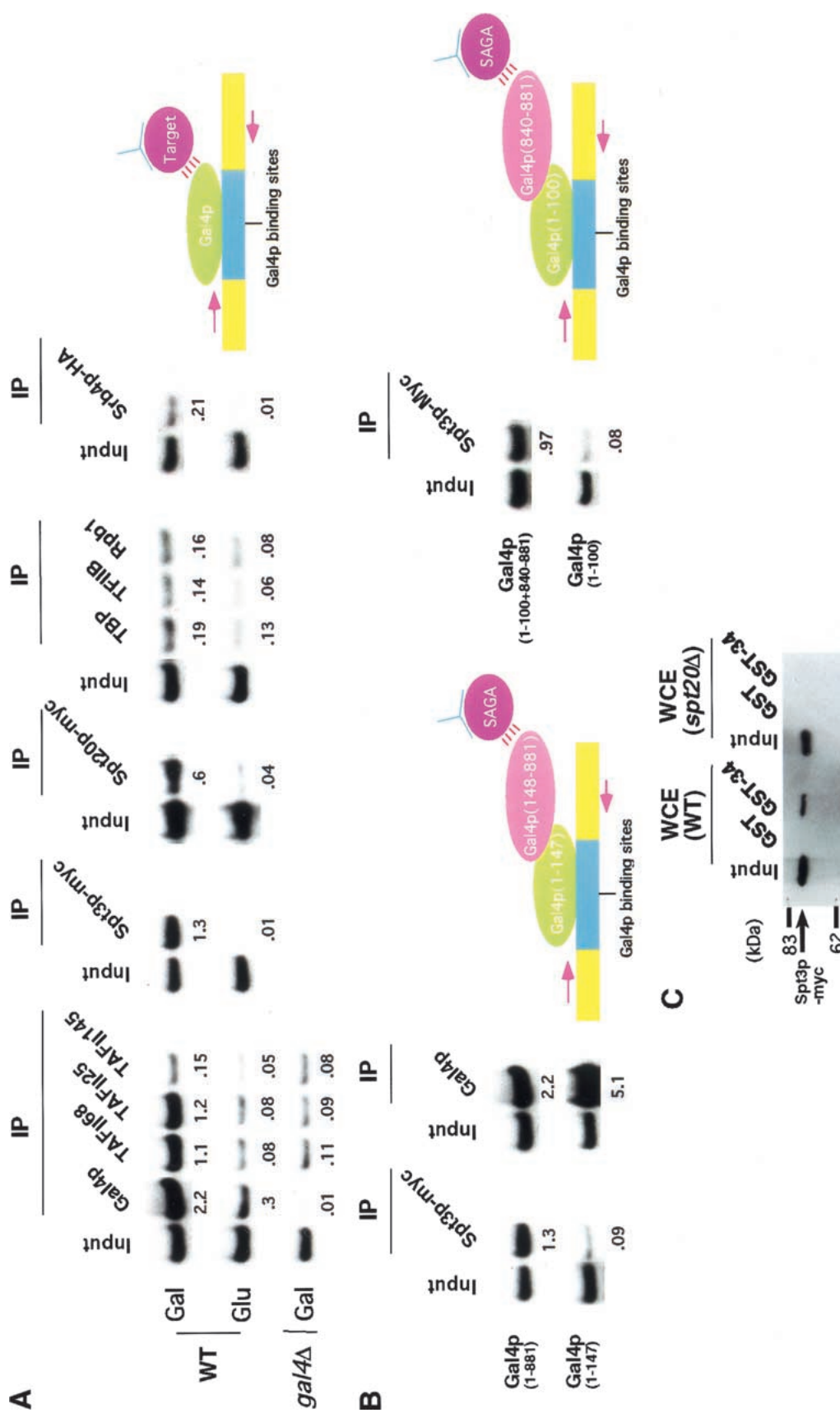
suggested an essential role for the Gal4p activation domain. Consistent with this idea, Figure 4B shows that SAGA was not recruited by Gal4p(1–147), which contains an intact DNA-binding domain but lacks the activation domain. In vivo cross-linking indicated that Gal4p(1–147) bound to the Gal4p-binding sites efficiently; in fact, the level of Gal4p(1–147) binding was actually higher than that of Gal4p. Thus, the inability of Gal4p(1–147) to recruit SAGA was not because of a failure to bind DNA.

Previous studies have shown that residues 840–881 of Gal4p comprise a functional galactose-dependent transcriptional activation domain (Wu et al. 1996). Figure 4B shows that addition of this 41 amino acid activation domain to a minimal Gal4p DNA-binding domain, Gal4p(1–100), also resulted in SAGA recruitment. Thus, SAGA is also targeted by the Gal4p 41 amino acid C-terminal activation domain.

Finally, we performed an in vitro protein affinity-chromatography experiment to detect interactions between the Gal4p activation domain and SAGA. Figure 4C shows that in a yeast whole-cell extract, SAGA bound to a glutathione-S-transferase (GST) fusion-protein containing a 34-residue Gal4p activation domain (841–875) but not to a control GST protein. Consistent with the in vivo cross-linking data of Figure 3A, the Gal4p activation domain–SAGA interaction did not occur in a yeast whole-cell extract prepared from an *SPT20* deletion mutant.

We also used the in vivo cross-linking assay to look for interactions between Gal4p and several other transcription components, some of which are proposed Gal4p targets on the basis of in vitro protein-protein interaction experiments (Melcher and Johnston 1995; Wu et al. 1996; Ansari et al. 1998; Koh et al. 1998; Xie et al. 2000a,b). Figure 4A shows that although SAGA was recruited by Gal4p relatively efficiently (cross-linking signal within





**Figure 4.** Recruitment of SAGA to minimal Gal4p-binding sites. (A) Gal4p recruits SAGA but not other transcription factors to minimal Gal4p-binding sites. Yeast strains were grown, and in vivo cross-linking analysis was performed as described in Fig. 1. Immunoprecipitation was performed using polyclonal antibodies against the indicated TAF<sub>II</sub> or TBP, or a mouse monoclonal antibody against the c-myc epitope-tag, HA epitope-tag, or TFIIB. The primers used for the PCR analysis are adjacent to the Gal4p-binding sites in the plasmid. (B) Dependence of SAGA recruitment on the Gal4p activation domain. As in panel A except strains were used that expressed the Gal4p derivative indicated on the left. (C) In vitro interaction between the Gal4p activation domain and SAGA. Whole-cell extracts were prepared from a wild-type strain or an *SPT20* deletion mutant and incubated with immobilized GST or GST-34 in buffer A for 30 min at 4°C. The eluate was analyzed by immunoblotting using the c-myc mouse monoclonal antibody against Spt3p-myc. The position of Spt3p-myc is indicated.

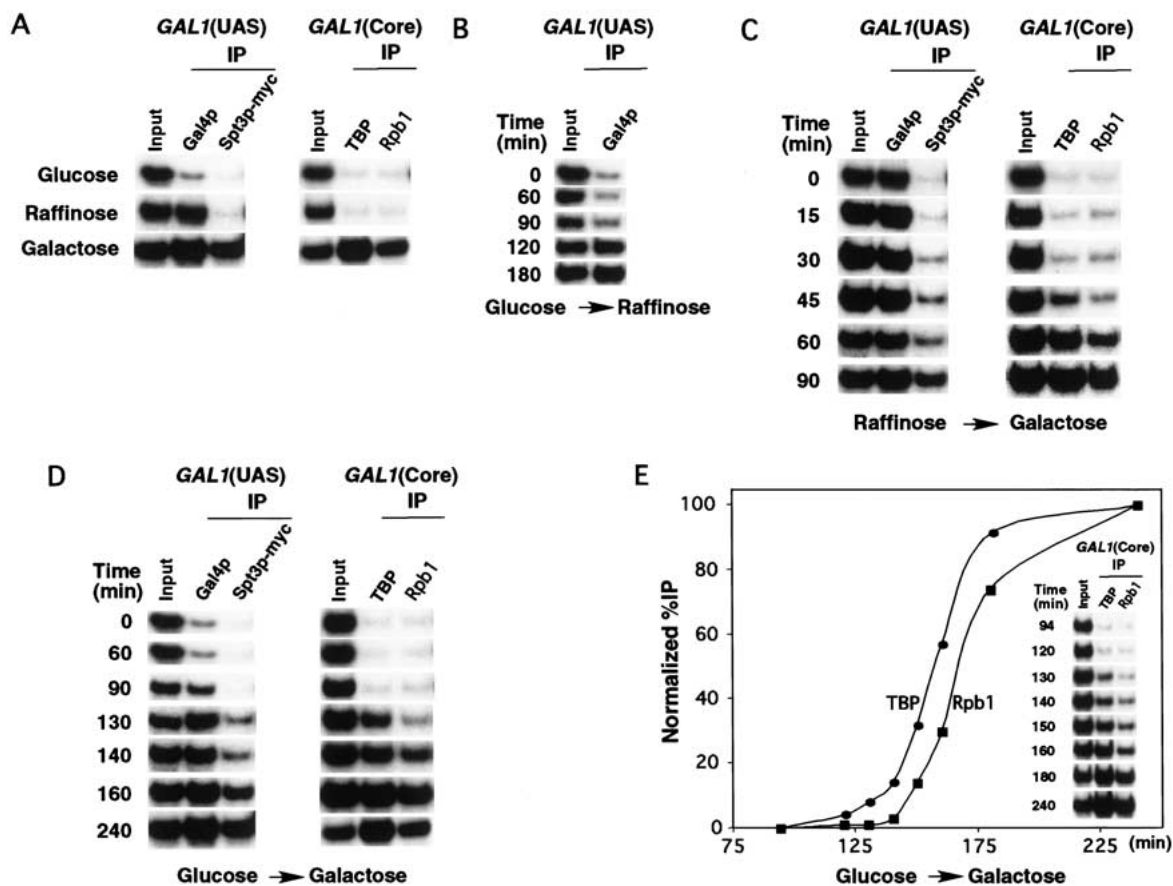
twofold of Gal4p itself), cross-linking of TFIID components (TAF<sub>II</sub>145 and TBP), TFIIB, RNA polymerase II, and Srb4p were substantially lower, which in some cases approximated the background level of the assay. Although these data do not rule out these other factors as possible Gal4p targets, the low level of cross-linking argues that these other factors are not required to mediate (in particular bridge) the Gal4p–SAGA interaction. Collectively, these data indicate that SAGA is a direct target of the Gal4p activation domain.

#### A stepwise pathway of transcription complex assembly on the *GAL1* promoter

Next, we analyzed the kinetics of Gal4p and SAGA recruitment to the UAS and several GTFs to the core pro-

motor. The analysis was performed in three carbon sources: glucose (repressing and noninducing), raffinose (noninducing), and galactose (inducing). Consistent with previous studies (Johnston 1987; Johnston and Carlson 1992; Lohr et al. 1995) and the results presented above, Figure 5A shows that in glucose, Gal4p was bound at a low level, SAGA was not associated with the UAS, and GTFs were not associated with the core promoter. In raffinose, Gal4p was bound to the UAS, but neither SAGA [UAS] nor GTFs (core) were recruited. Finally, in galactose, in which *GAL1* is transcriptionally active, Gal4p and SAGA were associated with the UAS, and the GTFs were associated with the core promoter.

Figure 5B shows that on switch from glucose to raffinose media, Gal4p binding was first detectable in ~2 h, reflecting the time required for relief of glucose repres-



**Figure 5.** Kinetic analysis of transcription complex assembly on the *GAL1* promoter. (A) Association of Gal4p, SAGA, and GTFs with the *GAL1* promoter in different carbon sources. The carbon source is indicated on the left and association of transcription factors (top) is given with the upstream activating sequence (UAS) or core region of the *GAL1* promoter analyzed by formaldehyde-mediated cross-linking/immunoprecipitation. (B) Kinetics of Gal4p binding on switch from glucose to raffinose. Cells were grown in glucose and shifted to raffinose for the times (includes 15 min cross-linking) indicated on the left. Binding of Gal4p to the *GAL1* UAS was analyzed by formaldehyde-mediated cross-linking/immunoprecipitation. (C) Kinetics of complex assembly on switch from raffinose to galactose. Cells were grown in raffinose and shifted to galactose for the times indicated on the left. Association of the indicated transcription factor (top) with the UAS or core region of the *GAL1* promoter was analyzed by formaldehyde-mediated cross-linking/immunoprecipitation. (D) Kinetics of complex assembly on switch from glucose to galactose. As in panel C except that cells were grown in glucose and shifted to galactose. (E) Plot of TBP and RNA polymerase II association with the *GAL1* core promoter upon switch from glucose to galactose. The data from an independent experiment (inset) was quantitated and plotted. Each point was normalized to the maximum cross-linking signal (assigned value of 100%).

sion (Johnston 1987; Johnston and Carlson 1992; Lohr et al. 1995; Ostergaard et al. 2001). Figure 5C shows that on switch from raffinose to galactose media, recruitment of SAGA to the UAS was first detectable between 30 and 45 min, and concomitantly, binding of TBP to the TATA box became evident. Shortly thereafter (60 min), recruitment of RNA polymerase II to the core promoter was first detectable. Figure 5D shows that on switch from glucose to galactose, association of Gal4p was first detectable at 90 min, SAGA and TBP at 130 min, and RNA polymerase II at 140 min. Figure 5E plots the kinetics of TBP and RNA polymerase II association with the *GAL1* core promoter following shift from glucose to galactose and again demonstrates entry of TBP shortly before RNA polymerase II. Collectively, the data of Figure 5 reveal a kinetic pathway of complex assembly on the *GAL1* promoter initiated by binding of Gal4p, followed by recruitment of SAGA, the apparent simultaneous association of TBP, and finally entry of RNA polymerase II.

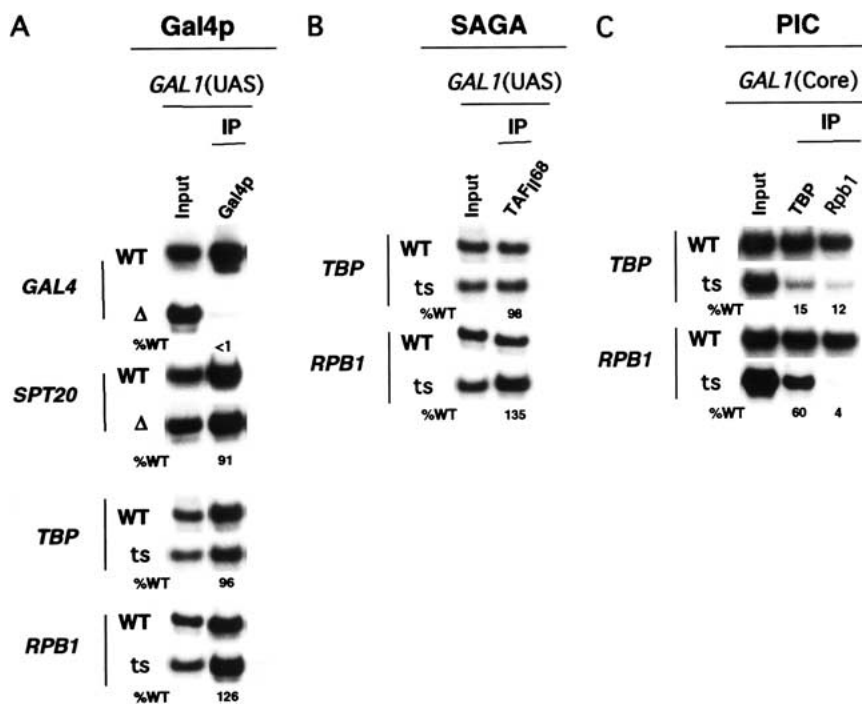
To determine whether this kinetic pathway reflects a series of obligatory steps, we analyzed complex assembly in yeast strains bearing mutations in Gal4p, SAGA, TBP, or RNA polymerase II. As described above, strains deleted of *GAL4* failed to recruit SAGA to the UAS (Fig. 2A) and GTFs to the core promoter (Fig. 2B). In the *SPT20* deletion strain, Gal4p binding was normal (Fig. 6A), but SAGA was not recruited to the UAS (Fig. 3A) and PIC assembly did not occur (Fig. 3B). Temperature-sensitive inactivation of TBP did not interfere with recruitment of Gal4p and SAGA to the UAS (Fig. 6A,B), but the PIC failed to assemble (Fig. 6C). Finally, temperature-sensitive inactivation of RNA polymerase II did not interfere with recruitment of Gal4p or SAGA to the UAS (Fig. 6A,B) and had only a modest effect on recruitment of

TBP to the TATA box (Fig. 6C). Thus, the kinetic assembly pathway reflects a stepwise series of obligatory interactions shown schematically in Figure 7 and discussed below.

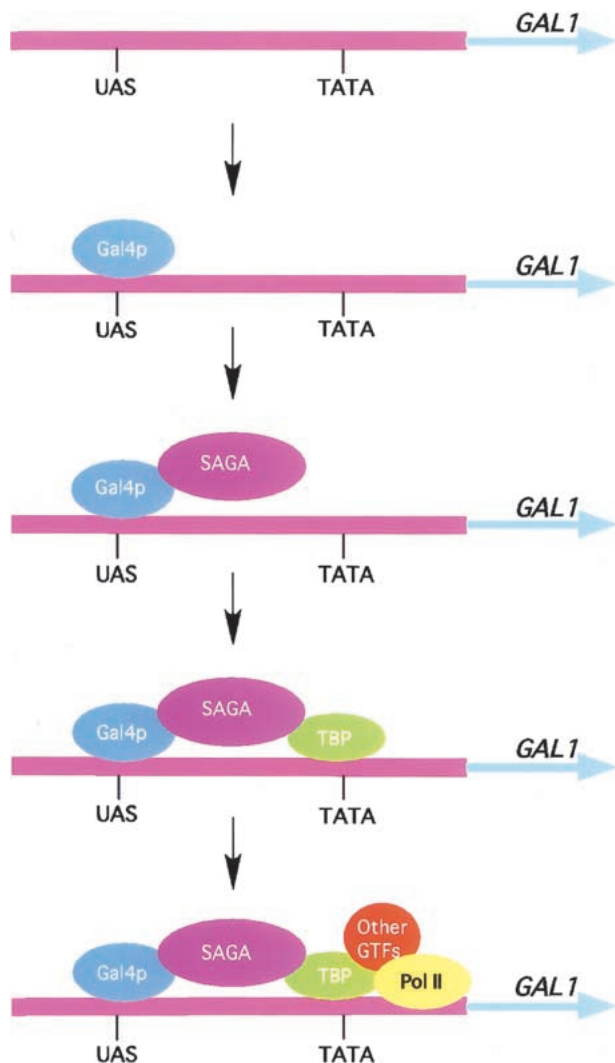
## Discussion

The major conclusion of this present study and the study of Larschan and Winston (2001) is that SAGA is an essential and likely direct target of the prototype acidic activator Gal4p. This conclusion is supported by several lines of evidence. First, SAGA is required for Gal4p to stimulate PIC assembly and transcription, indicating that SAGA performs an essential and nonredundant function. Second, in vivo Gal4p recruits SAGA to the *GAL1* UAS as well as to minimal Gal4p-binding sites. Recruitment of SAGA is dependent on a functional Gal4p activation domain. In this same assay, other transcription components, including some previously proposed Gal4p targets, were not recruited by Gal4p, strongly suggesting that they are not involved in SAGA recruitment. Finally, the proposed pathway of transcription complex assembly on *GAL1* (Fig. 7), based on kinetic and mutational analyses, is also consistent with the direct targeting of SAGA by Gal4p.

Our work suggests a model in which Gal4p first recruits SAGA to the UAS, and then UAS-bound SAGA facilitates PIC assembly and thus transcription (Fig. 7). The most likely mechanism by which UAS-bound SAGA functions is by serving as an adaptor that directly contacts one or more components of the PIC. This model fits in very well with several other studies. For example, it has been previously shown that SAGA is required for *GAL1* transcription (Roberts and Winston 1997; Dudley



**Figure 6.** A stepwise pathway of transcription complex assembly on the *GAL1* promoter. Deletion strains and their isogenic wild-type counterparts were first grown in glucose, switched to galactose for 5 h, and treated with formaldehyde. Temperature-sensitive mutant strains and their isogenic wild-type versions were grown in galactose to an  $OD_{600}$  of 0.85 at 23°C, switched to 37°C for 1 h, and treated with formaldehyde. Association of Gal4p (A) and SAGA (TAF<sub>1168</sub>; B) with the *GAL1* upstream activating sequence (UAS) or TBP and RNA polymerase II (C) with the *GAL1* core promoter was analyzed by formaldehyde-mediated cross-linking/immunoprecipitation.



**Figure 7.** Summary of transcription complex assembly on the *GAL1* promoter. The entry of SAGA and TBP were not kinetically resolvable, but the results of mutational experiments (Fig. 6) indicate that SAGA can associate with *GAL1* in the absence of TBP. Although not analyzed here, it has been shown previously that other GTFs are associated with the transcriptionally active *GAL1* promoter (Li et al. 1999, 2000). The model shows the other general transcription factors (GTFs) entering subsequent to TBP, but it is possible that some GTFs may be recruited to the promoter simultaneously with TBP (see Li et al. 1999, 2000).

et al. 1999; Sterner et al. 1999), acts following Gal4p binding (Dudley et al. 1999), and facilitates the TBP-TATA box interaction (Dudley et al. 1999).

The detailed mechanism by which UAS-bound SAGA interacts with the transcriptional machinery and stimulates PIC assembly remains to be determined. However, our results suggest that the SAGA subunit Spt3p may be involved: Spt3p was dispensable for SAGA recruitment but required for Gal4p-mediated PIC assembly (Fig. 3). Significantly, previous studies have provided genetic and physical evidence for interactions between Spt3p and

TBP (Eisenmann et al. 1992, 1994). Thus, an attractive idea would be that UAS-bound SAGA facilitates PIC assembly by interacting with TBP through Spt3p. The manuscript by Larschan and Winston (2001) also provides strong, independent support for this possibility.

Several experimental observations have suggested that activators, in particular acidic activators, have multiple, functionally redundant targets. For example, in vitro protein-protein interaction experiments have shown that a single activator can interact with multiple transcription components (Struhl 1996; Ptashne and Gann 1997). Moreover, in some instances a single activator can stimulate transcription synergistically on a promoter bearing multiple activator-binding sites (see Carey et al. 1990). The interpretation of this synergistic effect has been that each of the bound activators contacts a different component of the PIC.

An important conclusion of our studies is that SAGA is a nonredundant target of Gal4p. It is possible that Gal4p is atypical and that most activators will, as generally believed, have multiple, redundant targets. However, the previous experimental observations suggesting target redundancy require re-examination. For example, although an activator might interact in vitro with more than one transcription component, these interactions may not be involved in transcriptional activation in vivo. With regard to transcriptional synergy, not all activators can synergize with themselves (see Davidson et al. 1988; Fromental et al. 1988), suggesting distinct classes of activators, which likely function through different targets. Even for activators that can synergize with themselves, the activator could interact with a single target, such as SAGA, which in turn makes multiple contacts with the PIC. According to this idea, it is the adaptor, rather than the activator, that has redundant targets.

In several instances, a HAT-containing component or complex has been shown to be required for transcription of or recruited to a particular gene (e.g., p300; Sterner and Berger 2000; Roth et al. 2001). This result has in general been interpreted as a requirement for the HAT activity. Here we find that a HAT-containing complex, SAGA, is recruited to the *GAL1* promoter and required for transcription. Because SAGA is a multi-subunit complex, we have been able to show that the HAT activity is dispensable for recruitment to the promoter, stimulation of PIC assembly, and transcription, consistent with previous results (Dudley et al. 1999). Our results raise the possibility that in these other instances, the major function of the HAT-containing component or complex may not be to provide a HAT activity.

Several of the critical SAGA subunits (e.g., Spt3p, Spt20p) are not essential for yeast viability (Roberts and Winston 1996), and whole-genome expression analysis indicates that SAGA is required for expression of only a small subset of yeast genes (Lee et al. 2000). Therefore, the activator targets for the vast majority of yeast genes must be components other than SAGA. The experimental approaches described here may be generally useful to identify the targets of other activators.



## Materials and methods

### Plasmids

Plasmids pRJR182 (Gal4p 1–100+840–881; Wu et al. 1996), pRJR217 (Gal4p 1–100; Wu et al. 1996), and pMA241 (Gal4p 1–147; Ma and Ptashne 1987) were obtained from Mark Ptashne (Memorial Sloan Kettering Cancer Center, NY). Plasmids pGEX2T (GST) and pGEXCSNGal4AD (GST-34) (Melcher and Johnston 1995) were obtained from Stephen A. Johnston (University of Texas Southwestern Medical Center, TX). Plasmid SGP4 (three Gal4p-binding sites) was generated by cloning a DNA fragment containing three Gal4p-binding sites into the low copy number plasmid pRS416.

### 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, MA) (Roberts and Winston 1996, 1997; Sterner et al. 1999). Strains FY1992 (*MATa his4–917 $\delta$  lys2–173R2 ura3–52 leu2 $\Delta$ 1*), FY1993 (*MATa his4–917 $\delta$  lys2–173R2 ura3–52 leu2 $\Delta$ 1 gal80 $\Delta$ ::LEU2*), and FY1994 (*MATa his4–917 $\delta$  lys2–173R2 ura3–52 leu2 $\Delta$ 1 gal80 $\Delta$ ::LEU2 spt20 $\Delta$ ::URA3*) were obtained from Erica Larschan and Fred Winston (Harvard Medical School, Boston, MA). The myc-tagged strains, SGY1 (Spt3p-myc; *TRP1*), SGY2 (Spt20p-myc; *TRP1*), SGY3 (Ada2p-myc; *TRP1*), and SGY4 (Gcn5p-myc; *TRP1*) were generated by insertion of multiple myc-epitope tags at the original chromosomal loci of *SPT3*, *SPT20*, *ADA2*, and *GCN5*, respectively, in FY631 (Longtine et al. 1998). SGY96 (Spt20p-myc; *TRP1*) was generated by multiple myc-epitope tags at the original chromosomal locus of *SPT20* in FY294. SGY97 (Spt3p-myc; *HIS3*) and SGY98 (Spt3p-myc; Kan) were generated by multiple myc-epitope tags at the original chromosomal locus of *SPT3* in IPY36 and IPY37, respectively. The strains SGY7 and SGY8 were generated by multiple myc tags at the chromosomal locus of *SPT3* in FY67 and FY1097. The endogenous *GAL4* gene of SGY1 and SGY2 was disrupted using the PCR method (Brachmann et al. 1998) to generate SGY14 (Spt3p-myc; *gal4 $\Delta$ ::URA3*) and SGY15 (Spt20p-myc; *gal4 $\Delta$ ::URA3*), respectively. The plasmid SGP4, carrying three Gal4p-binding sites, was transformed into SGY27 (multiple myc-epitope tags with *TRP1* at the original chromosomal locus of *SPT3* in w303a) to generate SGY30. The *GAL4* gene of SGY27 was disrupted to generate SGY28 (*gal4 $\Delta$ ::URA3*), which was then streaked on a 5FOA plate to pop out *URA3*. *URA3*-popped-out SGY28 was transformed by the plasmids pMA241 (Gal4p 1–147) and SGP4 or pRJR216 (Gal4p 1–100) and SGP4 or pRJR182 (Gal4p 1–100 + 840–881) and SGP4 to generate SGY71, SGY72, and SGY73, respectively. The plasmid SGP4 was also transformed into SGY2 and SGY81 (HA3 epitope tag with *TRP1* at the chromosomal locus of *SRB4* in w303a) to generate SGY99 and SGY82, respectively.

Glucose-repressed strains were grown in YPD (YP + 2% glucose) to an OD<sub>600</sub> of 1. Galactose-induced strains were grown in YPG (YP + 2% galactose) to an OD<sub>600</sub> of 1. Raffinose noninduced strains were grown in YPR (YP + 2% Raffinose). For *gal4 $\Delta$* , *spt3 $\Delta$* , *spt20 $\Delta$* , and *gcn5 $\Delta$*  strains, cells were first grown in YPD to an OD<sub>600</sub> of 0.8 and then transferred to YPG for 5 h. Minimal media containing either 2% glucose or galactose were used for strains with reporter plasmid, protein expression plasmid, or both.

### GST protein affinity chromatography

GST and GST-34 were overexpressed in 100 mL of *Escherichia coli* culture by IPTG induction for 3.5 h. The overexpressed proteins were immobilized on 0.2 mL of a 50% slurry of glutathione beads for 30 min at 4°C with gentle agitation. The beads were washed four times with phosphate-buffered saline with 1% triton X-100. Finally, the beads were washed twice with buffer A (50 mM HEPES at pH 7.5, 150 mM NaCl, 10% glycerol, 0.1% tween 20, 0.5 mM DTT, 1 mM PMSF, 2  $\mu$ g/ $\mu$ L leupeptin, and 2  $\mu$ g/ $\mu$ L pepstatin A). Yeast whole cell extract (WCE) was prepared from *Saccharomyces cerevisiae* SGY7 and SGY8 as described by Woontner et al. (1991).

To 50  $\mu$ L GST or GST-34 immobilized beads, 20  $\mu$ L (20  $\mu$ g/ $\mu$ L) yeast WCE and 180  $\mu$ L buffer A were added and gently agitated at 4°C for 30 min. Beads were washed four times with buffer A and boiled in 1 $\times$  SDS-PAGE buffer for 5 min at 95°C. The eluate was analyzed by immunoblotting using the c-myc monoclonal antibody.

### Primer-extension analysis

Primer-extension analysis was performed as described by Li et al. (2000). The primers used for analysis of *GAL1*, *GAL2*, *GAL7*, *GAL10*, *RPS5*, and *TUB2* mRNA are as follows: *GAL1*, 5'-CCTTGACGTTAAAGTATAGAGG-3'; *GAL2*, 5'-GCTTGGGGTTGCTGTGAAACA-3'; *GAL7*, 5'-CGGTTAGTGGATTGTAACGTCT-3'; *GAL10*, 5'-CAATGTATCCAGCACCTGT-3'; *RPS5*, 5'-GACTGGGGTGAATTCTTCAACAACCTTC-3'; and *TUB2*, 5'-CCAATTTGGTTACCACACTGACCT-3'.

### Formaldehyde-based in vivo cross-linking

Formaldehyde-based in vivo cross-linking and chromatin immunoprecipitation was performed as described by Li et al. (2000). The immunoprecipitated DNA was amplified by PCR. Primer-pairs used for PCR analysis are as follows: *GAL1* (UAS), 5'-CGCTTAACTGCTCATTGCTATATTG-3' and 5'-TTGTT CGGAGCAGTGC GGCGC-3'; *GAL1* (Core), 5'-ATAGGATGATAATGCGATTAGTTTTTTAGCCTT-3' and 5'-GAAAATGTGAAAAGTATTAGTTAAAAGTGGTTATGCA-3'; *RPS5* (UAS), 5'-AGAAACAATGAACAGCCTTGAGTTCTC-3' and 5'-GCA GGGCCATTCTCATCTGA-3'; *RPS5* (Core), 5'-GGCCAACCTCTACGCTCACGTTAG-3' and 5'-CGGTGTCAGACATCTT TGGAATGGTC-3'; and *GAL4* (ORF), 5'-CTTGTTCATGCA GTCCCTAGTACCC-3' and 5'-CACAAGTCTGGATTTTAAAA GTGGCC-3'.

Primer-pairs flanking Gal4p-binding sites in the plasmid SGP4 are 5'-GGTGGCGCCGCTCTAGAACTAGT-3' and 5'-TTGACCGTAATGGGATAGGTCACG-3'.

Autoradiograms were scanned and quantitated by the National Institutes of Health image 1.62 program. Immunoprecipitated (IP) DNAs were quantitated and presented as the ratio of IP to input.

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