

# Specific Components of the SAGA Complex Are Required for Gcn4- and Gcr1-Mediated Activation of the *his4-912δ* Promoter in *Saccharomyces cerevisiae*

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## ABSTRACT

Mutations selected as suppressors of Ty or solo  $\delta$  insertion mutations in *Saccharomyces cerevisiae* have identified several genes, *SPT3*, *SPT7*, *SPT8*, and *SPT20*, that encode components of the SAGA complex. However, the mechanism by which SAGA activates transcription of specific RNA polymerase II-dependent genes is unknown. We have conducted a fine-structure mutagenesis of one widely used SAGA-dependent promoter, the  $\delta$  element of *his4-912δ*, to identify sequence elements important for its promoter activity. Our analysis has characterized three  $\delta$  regions necessary for full promoter activity and accurate start site selection: an upstream activating sequence, a TATA region, and an initiator region. In addition, we have shown that factors present at the adjacent UAS<sub>HIS4</sub> (Gcn4, Bas1, and Pho2) also activate the  $\delta$  promoter in *his4-912δ*. Our results suggest a model in which the  $\delta$  promoter in *his4-912δ* is primarily activated by two factors: Gcr1 acting at the UAS <sub>$\delta$</sub>  and Gcn4 acting at the UAS<sub>HIS4</sub>. Finally, we tested whether activation by either of these factors is dependent on components of the SAGA complex. Our results demonstrate that Spt3 and Spt20 are required for full  $\delta$  promoter activity, but that Gcn5, another member of SAGA, is not required. Spt3 appears to be partially required for activation of *his4-912δ* by both Gcr1 and Gcn4. Thus, our work suggests that SAGA exerts a large effect on  $\delta$  promoter activity through a combination of smaller effects on multiple factors.

**T**RANSSCRIPTION initiation in eukaryotes is a complex process requiring the coordination of multiple DNA-protein and protein-protein interactions. RNA polymerase II-dependent promoters in *Saccharomyces cerevisiae* contain combinations of four elements: upstream activating sequences (UAS), upstream repressing sequences (URS), TATA elements, and sites of transcriptional initiation (for review, see Struhl 1995). UAS and URS elements are binding sites for gene-specific activators and repressors, respectively, and usually determine a promoter's regulation in response to environmental stimuli. TATA elements are the site of binding for the TATA-binding protein (TBP) and for the assembly of the general transcription initiation factors. In *S. cerevisiae*, transcription often initiates at multiple sites within a window of 30–120 nucleotides 3' of the TATA element, although the sequence determinants of start site selection are poorly understood. The binding of transcription factors to the appropriate promoter elements facilitates the assembly of RNA polymerase II into a preinitiation complex.

In eukaryotes, transcription by RNA polymerase II is often regulated by multiple activators, repressors, TATA

elements, and transcription initiation sites within the same promoter. Promoters in larger eukaryotes are often complex, spanning up to several thousand nucleotides and containing many promoter elements (for example, see Thanos and Maniatis 1995). In contrast, RNA polymerase II-dependent promoters in *S. cerevisiae* are usually compact, consisting of only a few hundred nucleotides and containing only a few promoter elements, facts that have made yeast an excellent system for the study of transcription initiation *in vivo*. However, one class of complex promoters that exists in *S. cerevisiae* are the composite promoters generated by insertions of Ty1 and Ty2 retrotransposons into the 5' regions of other genes (for review, see Winston 1992). Because the Ty promoter resides in its 5' long terminal repeat ( $\delta$ ), the presence of solo  $\delta$  elements often alters transcription from promoters into which they insert due to the introduction of additional promoter elements. One such insertion mutation is the well-characterized allele *his4-912δ*, in which a Ty1  $\delta$  element lies between the UAS and TATA elements of *HIS4* (Farabaugh and Fink 1980). Strains containing *his4-912δ* are His<sup>-</sup> because transcription initiates at the Ty mRNA start site within the  $\delta$ , instead of at the *HIS4* start site, resulting in a nonfunctional  $\delta$ -*HIS4* mRNA transcript (Silverman and Fink 1984; Hirschman *et al.* 1988). The presence of multiple promoter elements makes *his4-912δ* a good model for complex promoters such as those found in larger eukaryotes.

The UAS<sub>HIS4</sub> region, located ~25 bp upstream of the

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$\delta$  in *his4-912 $\delta$* , has been extensively characterized. This region, which contains binding sites for the activators Rap1, Bas1, Pho2 (Bas2/Grf10), and Gcn4 (Arndt and Fink 1986; Arndt *et al.* 1987; Tice-Baldwin *et al.* 1989; Devlin *et al.* 1991), regulates *HIS4* via two different pathways (Arndt *et al.* 1987). The basal pathway is constitutively active and requires the binding of both Bas1 and Pho2 to their adjacent sites within the UAS<sub>*HIS4*</sub> (Arndt *et al.* 1987; Tice-Baldwin *et al.* 1989). The general amino acid control pathway is regulated by amino acid levels and is mediated by the binding of the strong transcriptional activator Gcn4 (Hinnebusch *et al.* 1985; Hope and Struhl 1985; Arndt and Fink 1986). *HIS4* transcription via both pathways is dependent on the transcriptional activator/repressor protein Rap1, which is believed to allow binding of the other factors by maintaining an accessible chromatin structure (Devlin *et al.* 1991). Only combinations of mutations in genes encoding components of both the constitutive and general amino acid control pathways, for example, both *bas1 $\Delta$*  and *gcn4 $\Delta$* , abolish promoter activity and confer a His<sup>-</sup> phenotype (Arndt *et al.* 1987). In addition to activating the *HIS4* promoter, there is some evidence that Gcn4 at the UAS<sub>*HIS4*</sub> can activate the  $\delta$  promoter of *his4-912 $\delta$*  (Silverman and Fink 1984), although  $\delta$  activation by UAS<sub>*HIS4*</sub> factors has not been extensively characterized.

In contrast to the *HIS4* promoter, relatively few sequence elements important for  $\delta$  promoter activity have been identified. Two previous deletion analyses demonstrated that the first 100 nucleotides of the  $\delta$  are dispensable for full promoter activity (Liao *et al.* 1987; Fulton *et al.* 1988). However, the two studies reached different conclusions regarding the importance of the  $\delta$  UAS element. One study found no sequences upstream of the TATA region to be necessary for expression of a Ty1 element (Fulton *et al.* 1988), while a second study identified a UAS region important for  $\delta$  promoter activity in a Ty2 element (Liao *et al.* 1987). The activity of the Ty2 UAS is dependent on the transcriptional activator Gcr1, which can bind the UAS <sub>$\delta$</sub>  *in vitro* (Türkel *et al.* 1997). The  $\delta$  promoter of *his4-912 $\delta$*  also contains two consensus TATA elements that bind TBP with equal affinity *in vitro* (Arndt *et al.* 1992). Although several studies have shown that mutations in the  $\delta$  TATA region significantly decrease promoter activity (Liao *et al.* 1987; Coney and Roeder 1988; Fulton *et al.* 1988; Hirschman *et al.* 1988; Arndt *et al.* 1994), the relative contributions of the two consensus TATA elements present in many  $\delta$  sequences have not been analyzed. Finally, the transcription start sites of both Ty1 and *his4-912 $\delta$*  have been mapped to the same single nucleotide (Elder *et al.* 1983; Silverman and Fink 1984), although no sequence determinants for this start site selection have been identified. Thus, previous studies have identified a region containing two consensus TATA elements and possibly a UAS element as being required for full  $\delta$

promoter activity, although other regions of the  $\delta$  have not been examined by a fine-structure analysis.

Despite the fact that sequence elements within the  $\delta$  have not been thoroughly characterized, genetic analysis has identified a large number of *trans*-acting factors required for  $\delta$  promoter function (for review, see Winston 1992). Many of these factors were originally identified by mutations that cause an Spt<sup>-</sup> (Suppressor of Ty) phenotype, that is, the ability to suppress transcription of full-length Ty elements or solo  $\delta$  insertion alleles such as *his4-912 $\delta$* . One class of these suppressors includes mutations in *SPT3*, *SPT7*, *SPT8*, and *SPT20*. Mutations in these genes share a set of mutant phenotypes with certain mutations in *SPT15*, which encodes the TATA-binding protein, and cause transcriptional defects in a subset of RNA polymerase II-dependent promoters (Winston 1992). Recently, these Spt proteins were shown to be components of the 1.8-MD SAGA (Spt/Ada/Gcn5/acetylase) complex (Grant *et al.* 1997). Although SAGA was originally purified on the basis of its ability to acetylate histones in a nucleosomal template (Grant *et al.* 1997), genetic evidence suggests that this large complex contains a variety of additional functions (Horiuchi *et al.* 1997; Roberts and Winston 1997). However, the mechanism by which large transcription complexes such as SAGA are targeted to, and function at, specific subclasses of RNA polymerase II-dependent promoters is not yet understood.

In addition to components of the SAGA complex, genetic analysis has demonstrated that several other important classes of transcription factors are required for normal expression of *his4-912 $\delta$* . Components of RNA polymerase II (Hekmatpanah and Young 1991); the Srb/mediator complex, Gal11/Spt13 (Fassler and Winston 1988, 1989), Rgr1 (Jiang and Stillman 1992), and Sin4 (Jiang and Stillman 1992); and the general transcription apparatus, TBP, (Eisenmann *et al.* 1989), TFIIA (Madison and Winston 1997), and Mot1 (Madison and Winston 1997) are all required for expression from the  $\delta$  promoter of *his4-912 $\delta$* . Expression of *his4-912 $\delta$*  also depends on many factors that influence chromatin structure, including histones (Clark-Adams *et al.* 1988; Prelich and Winston 1993; Smith *et al.* 1996; Santisteban *et al.* 1997); factors that regulate histone gene expression (Sherwood and Osley 1991; Xu *et al.* 1992); Spt4, Spt5, and Spt6 (Winston *et al.* 1984a); and the Snf/Swi complex (Happel *et al.* 1991). The  $\delta$  promoter of *his4-912 $\delta$*  also appears to be sensitive to epigenetic regulation (Jiang and Stillman 1996). Thus, further characterization of *his4-912 $\delta$*  may provide new information on the function of these factors and complexes at other promoters.

In this study, we have characterized the *cis*-acting elements necessary for the  $\delta$  promoter activity of *his4-912 $\delta$*  by fine-structure mutagenesis of a *Ty-lacZ* fusion. We have analyzed the roles of several sequences in the  $\delta$ : a UAS, a weak URS, two consensus TATA elements, and

a region surrounding the transcription start site. We have also analyzed the requirement for transcriptional activators that act at the UAS<sub>HIS4</sub> (Bas1, Pho2, and Gcn4) and the UAS<sub>δ</sub> (Gcr1 and a currently unidentified factor) and the requirement for the SAGA complex. Our analysis suggests that the *his4-912δ* promoter is activated primarily by two factors: Gcr1 at the UAS<sub>δ</sub> and Gcn4 at the UAS<sub>HIS4</sub>. In addition, our results demonstrate that this activation is dependent on some but not all components of the SAGA complex. Surprisingly, one SAGA component, Spt3, appears to be partially required for the activity of a number of *his4-912δ* promoter factors, including Gcr1 and Gcn4. Our work suggests a model in which specific SAGA components exert large effects on the  $\delta$  promoter as a sum of smaller effects on multiple factors.

## MATERIALS AND METHODS

**Yeast strains, genetic methods, and media:** All yeast strains used in this study are isogenic and were originally derived from a *GAL2*S288C derivative (Winston *et al.* 1995).  $\beta$ -Galactosidase assays and primer extension analyses were performed on two to six independent strains of each relevant genotype, with minor variations in mating type and auxotrophic markers. A representative strain of each genotype is presented in Table 1. A complete strain list is available upon request. The *spt3-202* (Winston and Minehart 1986), *spt20Δ100::URA3* (Roberts and Winston 1996), *gcn5Δ::HIS3* (Roberts and Winston 1997), *gcn4Δ::LEU2* (Drysdale *et al.* 1995), *bas1-2* (Arndt *et al.* 1987), *gcr1Δ::LEU2* (Baker 1986), and *pho2Δ::LEU2* (Brazas and Stillman 1993) mutations have all been described previously. Construction of the *Ty912Δ44-lacZ* alleles is described below. Yeast strains were transformed by the lithium acetate procedure (Elble 1992). Standard methods of mating, sporulation, and tetrad analysis were used (Rose *et al.* 1990). Rich (YPD), minimal (SD), synthetic complete (SC), 5-fluoroorotic acid (5-FOA), and sporulation media were prepared as described previously (Rose *et al.* 1990).

**Construction of  $\delta$  promoter mutations:** To facilitate the analysis of a large number of promoter mutations in a variety of mutant backgrounds,  $\delta$  promoter mutations were constructed in a *Ty-lacZ* fusion. The structure of this reporter gene, *Ty912Δ44-lacZ*, has been described previously (Winston *et al.* 1987) and contains the entire 334 bp of *Ty1-912δ* and 54 bp of the epsilon region fused in-frame to the *Escherichia coli lacZ* gene. The *Ty912Δ44-lacZ* fusion will be referred to as *his4-912δ-lacZ* and the  $\delta$  sequence itself as *912δ*. The promoter fusion does not contain the *HIS4*TATA region or transcription start site, and thus all *his4-912δ-lacZ* expression is a result of  $\delta$  promoter function. In the present study, each of the *his4-912δ-lacZ* alleles is integrated at the *HIS4* locus, such that the position of the  $\delta$  relative to the UAS<sub>HIS4</sub> is the same as the  $\delta$  in *his4-912δ* (Farabaugh and Fink 1980).

To identify sequences important for *912δ* promoter activity, a series of small, clustered base pair substitutions was constructed between *912δ* nucleotides 90 and 250 (Figure 1 and Table 2). Each mutation alters the sequence of 6–11 consecutive base pairs and creates a restriction site that is unique in *912δ*. In addition to the mutations listed in Table 2, three double mutants were constructed. The double UAS mutant contains both mutations 12 and 13 (Table 2). The double 13/18 mutant contains both mutations 13 and 18 (Table 2). The double TATA mutant was constructed in a previous study and alters the sequence of the TATA region from

(5' TATAAACATATAAAA 3') to (5' TGTAGACACTGCAG 3'), where the 3' TATA is replaced by a *PstI* site (Arndt *et al.* 1994).

Mutation 23 and the double TATA (17/18) mutations were constructed in a previous study (Arndt *et al.* 1994) and subcloned from pKA41 derivatives that contain the region III and IV TATA mutations, respectively (Arndt *et al.* 1994). The remaining mutations were constructed by site-directed mutagenesis using methods of Kunkel (1985) and Ho *et al.* (1989) or the QuickChange mutagenesis kit (Stratagene, La Jolla, CA). The presence of the correct base-pair changes as well as the absence of secondary mutations was confirmed by DNA sequence analysis. To reduce the amount of DNA sequencing, a 320-bp *PacI-BseRI* fragment containing each of the *912δ* mutations created in pLG39 was subcloned into the same sites of an unmutagenized *his4-912δ-lacZ* in pAD1. Since mutation 25 abolishes the *BseRI* site, a larger region of the original pLG39 derivative was sequenced and used for one-step gene replacement technique. Each *his4-912δ-lacZ* allele was stably integrated into the genome of FY1022 at the *HIS4* locus by one- or two-step gene replacement technique (Rose *et al.* 1990). The structure of the reporter gene and integration at the *HIS4* locus were confirmed by Southern blot analysis (Southern 1975). The presence of each promoter mutation was confirmed by restriction digest of PCR products generated from each integrated *his4-912δ-lacZ* allele.

**Plasmids:** The plasmid pLG40 contains a mutant allele of *his4-912δ*, *his4-912δ::URA3-lacZ*, which was integrated at the *HIS4* locus to facilitate the selection of one- and two-step integrants of *his4-912δ-lacZ* mutant alleles. pLG40 was derived from pKA40 (Arndt *et al.* 1994) in which the promoter region of *his4-912δ* (sequences –403 to +156 relative to the *HIS4* transcription start site) is replaced by the yeast *URA3* gene. To create pLG40, pKA40 was linearized with *NheI* and filled with Klenow enzyme. A 3-kb *BamHI* fragment containing the *E. coli lacZ* gene was isolated from pMC1871 (generously provided by Dr. Malcolm Casadaban), filled with Klenow enzyme, and subcloned into the filled *NheI* site of pKA40.

Unless noted, each of the *912δ* promoter mutations was constructed in pLG39, which contains a fragment of the *his4-912δ-lacZ* fusion sufficient for site-directed mutagenesis and integration at the *HIS4* locus via *his4-912δ::URA3-lacZ*. To create pLG39, pRS306 (Sikorski and Hieter 1989) was linearized with *BamHI*, filled with Klenow enzyme, and digested with *SacI*. pLG39 was constructed by subcloning a *SacI-HpaI* fragment from pFW82 (Winston *et al.* 1987), which contains a portion of the *his4-912δ-lacZ* fusion, into this pRS306 vector. To provide an additional marker, *TRP1*, for screening two-step integration candidates, the plasmid pAD1 was constructed by subcloning the 1.5-kb *SacI-ClaI* fragment from pLG39 into pRS304 (Sikorski and Hieter 1989).

The plasmids pAD11 through pAD24 contain the *his4-912δ-lacZ* alleles 11–24 in pAD1, respectively. pAD25 contains the *his4-912δ-lacZ* allele 25 in pLG39. The plasmids pAD28, pAD29, and pAD30 contain the *his4-912δ-lacZ* double UAS, double TATA, and double 13/18 alleles in pAD1, respectively.

The plasmid pBM947 is a *URA3* derivative of pHR307a (Liu *et al.* 1993) that lacks the 0.8-kb *NcoI* fragment containing *TRP1*. Briefly, this plasmid contains *HIS3* under the transcriptional control of a *GAL1* promoter from which the UAS has been deleted and replaced by a multiple cloning site. The plasmid pAD9 was created by subcloning annealed oligonucleotides that contained the UAS<sub>δ</sub> sequence, sites 12–14 (Table 2), flanked by *BamHI*-compatible ends into the pBM947 *BamHI* site. pAD9 contains three tandem insertions of the UAS<sub>δ</sub> sequence, with the first two copies inserted in the correct orientation and the third inserted in the reverse orientation, with respect to the orientation of the  $\delta$  promoter.

TABLE 1

*S. cerevisiae* strains

Strain	Genotype
FY114	<i>MATa lys2-173R2 ura3-52</i>
FY251	<i>MATa ura3-52 his3Δ200 leu2Δ1 trp1Δ63</i>
FY1022	<i>MATα his4-9128::URA3-lacZ lys2-173R2 ura3-52 trp1Δ63</i>
FY1472	<i>MATα Ty912Δ44-lacZ (wild type) lys2-173R2 ura3-52 trp1Δ63</i>
FY1765	<i>MATα Ty912Δ44-lacZ (Mutant 1) lys2-173R2 ura3-52 trp1Δ63</i>
FY1766	<i>MATα Ty912Δ44-lacZ (Mutant 12) lys2-173R2 ura3-52 trp1Δ63</i>
FY1767	<i>MATα Ty912Δ44-lacZ (Mutant 13) lys2-173R2 ura3-52 trp1Δ63</i>
FY1768	<i>MATα Ty912Δ44-lacZ (Mutant 14) lys2-173R2 ura3-52 trp1Δ63</i>
FY1473	<i>MATα Ty912Δ44-lacZ (Mutant 15) lys2-173R2 ura3-52 trp1Δ63</i>
FY1475	<i>MATα Ty912Δ44-lacZ (Mutant 16) lys2-173R2 ura3-52 trp1Δ63</i>
FY1477	<i>MATα Ty912Δ44-lacZ (Mutant 17) lys2-173R2 ura3-52</i>
FY1479	<i>MATα Ty912Δ44-lacZ (Mutant 18) lys2-173R2 ura3-52 trp1Δ63</i>
FY1769	<i>MATα Ty912Δ44-lacZ (Mutant 19) lys2-173R2 ura3-52 trp1Δ63</i>
FY1770	<i>MATα Ty912Δ44-lacZ (Mutant 20) lys2-173R2 ura3-52 trp1Δ63</i>
FY1771	<i>MATa Ty912Δ44-lacZ (Mutant 21) lys2-173R2 ura3-52</i>
FY1772	<i>MATα Ty912Δ44-lacZ (Mutant 22) lys2-173R2 ura3-52 trp1Δ63</i>
FY1773	<i>MATα Ty912Δ44-lacZ (Mutant 23) lys2-173R2 ura3-52 trp1Δ63</i>
FY1774	<i>MATα Ty912Δ44-lacZ (Mutant 24) lys2-173R2 ura3-52 trp1Δ63</i>
FY1775	<i>MATα Ty912Δ44-lacZ (Mutant 25) lys2-173R2 ura3-52 trp1Δ63</i>
FY1776	<i>MATα Ty912Δ44-lacZ (Mutant 12/13) lys2-173R2 ura3-52 trp1Δ63</i>
FY1481	<i>MATα Ty912Δ44-lacZ (Mutant 17/18) lys2-173R2 ura3-52 trp1Δ63</i>
FY1777	<i>MATα Ty912Δ44-lacZ (Mutant 13/18) lys2-173R2 ura3-52 trp1Δ63</i>
FY1778	<i>MATa Ty912Δ44-lacZ (wild type) lys2-173R2 ura3-52 leu2Δ1 trp1Δ63 pho2Δ::LEU2</i>
FY1779	<i>MATa Ty912Δ44-lacZ (Mutant 12) lys2-173R2 ura3-52 leu2Δ1 pho2Δ::LEU2</i>
FY1780	<i>MATa Ty912Δ44-lacZ (Mutant 13) ura3-52 leu2Δ1 pho2Δ::LEU2</i>
FY1781	<i>MATα Ty912Δ44-lacZ (Mutant 17) ura3-52 leu2Δ1 trp1Δ63 pho2Δ::LEU2</i>
FY1782	<i>MATα Ty912Δ44-lacZ (Mutant 18) lys2-1286 ura3-52 leu2Δ1 trp1Δ63 pho2Δ::LEU2</i>
FY1783	<i>MATa Ty912Δ44-lacZ (Mutant 17/18) lys2-173R2 ura3-52 leu2Δ1 trp1Δ63 pho2Δ::LEU2</i>
FY1784	<i>MATa Ty912Δ44-lacZ (wild type) lys2-173R2 ura3-52 leu2Δ1 trp1Δ63 bas1-2</i>
FY1785	<i>MATα Ty912Δ44-lacZ (Mutant 12) lys2-173R2 ura3-52 leu2Δ1 bas1-2</i>
FY1786	<i>MATα Ty912Δ44-lacZ (Mutant 13) ura3-52 leu2Δ1 trp1Δ63 bas1-2</i>
FY1787	<i>MATα Ty912Δ44-lacZ (Mutant 17) lys2-173R2 ura3-52 leu2Δ1 trp1Δ63 bas1-2</i>
FY1788	<i>MATa Ty912Δ44-lacZ (Mutant 18) lys2-173R2 ura3-52 leu2Δ1 trp1Δ63 bas1-2</i>
FY1789	<i>MATα Ty912Δ44-lacZ (Mutant 17/18) lys2-173R2 ura3-52 leu2Δ1 bas1-2 trp1Δ63</i>
FY1790	<i>MATa Ty912Δ44-lacZ (wild type) lys2-173R2 ura3-52 trp1Δ63 leu2Δ1 gcn4Δ::LEU2</i>
FY1791	<i>MATa Ty912Δ44-lacZ (Mutant 12) lys2-173R2 ura3-52 leu2Δ1 trp1Δ63 gcn4Δ::LEU2</i>
FY1792	<i>MATa Ty912Δ44-lacZ (Mutant 13) ura3-52 leu2Δ1 gcn4Δ::LEU2</i>
FY1793	<i>MATa Ty912Δ44-lacZ (Mutant 17) lys2-173R2 ura3-52 leu2Δ1 gcn4Δ::LEU2</i>
FY1794	<i>MATα Ty912Δ44-lacZ (Mutant 18) lys2-173R2 ura3-52 leu2Δ1 gcn4Δ::LEU2</i>
FY1795	<i>MATα Ty912Δ44-lacZ (Mutant 17/18) leu2Δ1 lys2-173R2 ura3-52 trp1Δ63 gcn4Δ::LEU2</i>
FY1796	<i>MATα Ty912Δ44-lacZ (wild type) lys2-173R2 ura3-52 trp1Δ63 his3Δ200 gcn5Δ::HIS3</i>
FY1797	<i>MATα Ty912Δ44-lacZ (wild type) lys2-173R2 ura3-52 trp1Δ63 spt20Δ100::URA3</i>
FY1798	<i>MATa Ty912Δ44-lacZ (wild type) lys2-173R2 ura3-52 spt3-202</i>
FY1799	<i>MATα Ty912Δ44-lacZ (Mutant 12) lys2-173R2 ura3-52 spt3-202</i>
FY1800	<i>MATa Ty912Δ44-lacZ (Mutant 13) lys2-173R2 ura3-52 spt3-202</i>
FY1801	<i>MATa Ty912Δ44-lacZ (Mutant 17) lys2-173R2 ura3-52 spt3-202</i>
FY1802	<i>MATα Ty912Δ44-lacZ (Mutant 18) lys2-173R2 ura3-52 spt3-202</i>
FY1803	<i>MATα Ty912Δ44-lacZ (Mutant 13/18) lys2-173R2 ura3-52 spt3-202</i>
FY1804	<i>MATα Ty912Δ44-lacZ (wild type) lys2-173R2 ura3-52 trp1Δ63 leu2Δ1 gcn4Δ::LEU2 spt3-202</i>
FY1814	<i>MATa his3Δ200 lys2-1286 leu2Δ ura3-52 gcr1Δ::LEU2</i>
FY1815	<i>MATa his3Δ200 lys2-173R2 leu2Δ ura3-52 gcn4Δ::LEU2</i>

The plasmid B238 is derived from plasmid p164 (Hinnebusch 1985) and contains a constitutively expressed *GCN4* allele in which the four upstream regulatory ORFs have been abolished by point mutations (Mueller and Hinnebusch 1986).

**β-Galactosidase assays:** Cells were grown to  $1-2 \times 10^7$  cells/

ml in SD media supplemented with the appropriate amino acids. Crude extracts were prepared and assayed as described previously (Rose *et al.* 1990). β-Galactosidase units, normalized to total protein concentration, are calculated as described in Rose *et al.* (1990). Protein concentrations were determined by Bradford protein assay (Bio-Rad, Richmond, CA). Because

TABLE 2  
 $\delta$  sequence changes introduced by the promoter mutations used in this study

Mutation	Nucleotides <sup>a</sup>	Wild-type sequence	Mutant sequence
11	90–99	ATGAGAAATA	GGTACCATCA
12	100–110	GTCATCTAAAT	CTCGGTACCAA
13	111–120	TAGTGGGAAGC	GAGAATTCGT
14	121–131	TGAAACGCAAG	TGAGAATTCTG
15	132–143	GATTGATAATGT	GAGGTACCTCGT
16	144–154	AATAGGATCAA	CTCCAGTCCAA
17	155–164	TGAATATAAA	AGTGGTACCA
18	165–175	CATATAAAATG	CTGGTACCATG
19	176–186	ATGATAATAAT	GACATCTCGAG
20	187–192	ATTTATAGAATT	CTTCTCGAGATG
21	193–201	GTGTAGAATTG	GTGCTCGAGTG
22	202–211	CAGATTCCCT	CTGAGAATTC
23	212–217	TTTATG	GAGCTC
24	222–231	CCTAAATCCT	GGTAAGCTTT
25	232–241	TGAGGAGAAC	AAGCTTGTC

<sup>a</sup> The nucleotide position refers to the 334 bp of the  $\delta$  sequence, where the first nucleotide of the  $\delta$  = 1.

of the large number of promoter mutations examined in this study,  $\beta$ -galactosidase assays were performed on groups of strains as indicated in figure and table legends. Each group consisted of two to six strains per genotype and was assayed three to six times. The average  $\beta$ -galactosidase units for each genotype are presented plus and minus the standard error, which was <10% in most cases. For reasons not understood, the absolute  $\beta$ -galactosidase values sometimes varied between experiments, although the relative differences between mutants were highly reproducible. For this reason, two controls were included in every experiment. First, a negative control strain containing the disrupted *his4-912δ::URA3-lacZ* allele produced 0–3 units of  $\beta$ -galactosidase activity (A. M. Dudley and F. Winston, unpublished data). Also, the same set of wild-type strains was included in each group. Thus, the differences between the values of these wild-type strains represent the experiment-to-experiment variability of the absolute  $\beta$ -galactosidase values. All relevant comparisons were performed within the same experiments.

**RNA isolation and primer extension analysis:** Cells were grown to  $1-2 \times 10^7$  cells/ml in SD media supplemented with the appropriate amino acids. Total RNA was isolated by the hot-phenol method (Ausubel *et al.* 1988). Two oligonucleotides, *lacZ* (5'GGGTAACGCCAGGGTTTTCCAGTCACGACGTTGTA 3') and U6.48-72 (5'GCAGGGGAAGTCTGATCATCTCT 3'), were 5' end labeled with [ $\gamma$ -<sup>32</sup>P]ATP using T4 polynucleotide kinase (New England Biolabs, Beverly, MA) as described previously (Ausubel *et al.* 1988). Primer extension reactions were performed on 20  $\mu$ g of total RNA combined with 1.2 ng labeled *lacZ* oligo, 1 ng labeled U6 oligo, and 10 ng unlabeled U6 oligo as described previously (Prelich and Winston 1993). The *lacZ* oligo is used to detect *Ty-lacZ* mRNA and produces a 212-nucleotide product that corresponds to the *Ty* transcription initiation site (Elder *et al.* 1983). Extension of U6.48-72 produces a 72-nucleotide U6-specific product (Brow and Guthrie 1988) that serves as a normalization control.

**Electrophoretic mobility shift assays:** Yeast nuclear extracts were prepared from FY114 as described previously (Hull *et al.* 1995). The following oligonucleotides were annealed to generate probe and competitor DNA: short wild type (AD14 5' GATCCGTCATCTAAATTAGTG 3'; AD15 5' GATCCAC TAATTTAGATGACG 3'), long wild type (AD6 5'GATCCG

TCATCTAAATTAGTGGGAAGCTGAAACGCAAGGG 3'; AD7 5' GATCCCTTGCGTTTCAGCTTCCACTAATTTAGATGACG3'), mutant 12 (AD10 5'GATCCCTCGGTACCAATAGTGGAAGCTGAAACGCAAGGG 3'; AD11 5'GATCCCTTGCGTTTCAGCTTCCACTATTGGTACCGAGG 3'), and mutant 13 (AD12 5'GATCCGTCATCTAAATGAGAATTCGTTGAAACGCAAGGG 3'; AD13 5'GATCCCTTGCGTTTCAACGAATTCATATTTAGATGACG 3'). Pairs of oligonucleotides were designed to recreate a double-stranded DNA molecule containing the appropriate wild-type or mutant UAS<sub>8</sub> sequence flanked by *Bam*HI-compatible ends when annealed. Underlined regions indicate the presence of the appropriate mutations. The double-stranded probe was labeled by filling the *Bam*HI-compatible ends of the annealed oligonucleotides with Klenow enzyme in the presence of [ $\alpha$ -<sup>32</sup>P]dGTP. Cold competitor substrates were generated in the same manner, except in the presence of cold nucleotides.

Mobility shift assays were carried out in the following DNA-binding buffer: 20 mM Hepes, 10 mM NaCl, 1 mM MgCl<sub>2</sub>, 10% glycerol, and 0.1% Nonidet P-40. A total of 20  $\mu$ l of binding reactions contained 1 $\times$  binding buffer, 5 mM dithiothreitol, 0.5  $\mu$ g poly[dG-dC], 100  $\mu$ g bovine serum albumin,  $\sim$ 1 ng <sup>32</sup>P-labeled probe DNA, 10  $\mu$ g yeast nuclear extract, and unlabeled competitor DNA, where indicated. Reaction mixtures containing all components, except the <sup>32</sup>P-labeled probe and unlabeled competitor DNA, were assembled on ice and incubated at room temperature for 10 min. Labeled probe DNA and unlabeled competitor DNA were added, and the reaction proceeded at room temperature for an additional 20 min. Reactions were loaded onto a 2.5% glycerol/6% polyacrylamide gel (60:1 cross-linking) containing 1 $\times$  Tris-glycine (50 mM Tris, 380 mM glycine, 2 mM EDTA, pH 8.5) and electrophoresed against 1 $\times$  Tris-glycine buffer at 100 V. Following electrophoresis, gels were vacuum dried onto Whatman filter paper and exposed to Kodak XAR X-OMAT autoradiographic film.

## RESULTS

**Mutant analysis defines regions of the  $\delta$  necessary for full promoter activity and accurate initiation:** To study

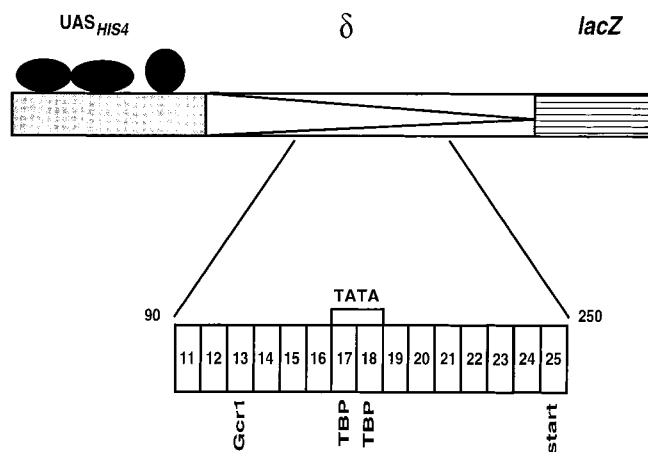


Figure 1.—Relative positions of the mutations created in this study. The structure of the *his4-912δ-lacZ* reporter gene is shown, including the positions of the *UAS<sub>HIS4</sub>*, which contains Rap1-, Pho2-, Bas1-, and Gcn4-binding sites, the  $\delta$  consensus Gcr1 site, the  $\delta$  consensus TATA elements, and the  $\delta$  site of transcription initiation. The mutagenized region corresponds to  $\delta$  nucleotides 90 through 250.

promoter elements in  $\delta$  sequences, we chose to study the  $\delta$  of a widely used and well-characterized insertion mutation, *his4-912δ*. For our studies, the  $\delta$  sequence of *his4-912δ* was fused in frame to the *E. coli lacZ* gene (materials and methods; Winston *et al.* 1987). Because *lacZ* is fused to the  $\delta$ , not to the *HIS4* TATA or transcription initiation site, *his4-912δ-lacZ* expression is a result of  $\delta$  promoter function. This fusion produces moderately high levels of *lacZ* mRNA in an *SPT*-dependent manner (Winston *et al.* 1987) and thus serves as an accurate reporter of *his4-912δ* promoter activity. To identify sequences important for *912δ* promoter function, we constructed a series of small, clustered base pair substitution mutations (Figure 1) in a region of *912δ* previously shown to be important in other  $\delta$  elements (Liao *et al.* 1987; Fulton *et al.* 1988).

The effect of each *912δ* mutation on promoter function was measured by  $\beta$ -galactosidase assay (Figure 2). This analysis revealed that mutations in two regions of *912δ* decreased  $\beta$ -galactosidase activity. The first region contains a UAS element, defined by mutations 12, 13, and (to a lesser extent) 14. Mutations 12 and 13 both decreased expression to  $\sim 35\%$  of wild type, while the 12/13 double UAS mutant decreased expression to 25% of wild type. While this work was in progress, an identical UAS sequence of a different  $\delta$  was shown to bind the transcription factor Gcr1 (Türkel *et al.* 1997). The Gcr1-binding site corresponds to site 13 in our studies (Figure 1). The second region of decreased activity contains two consensus TATA elements that are abolished by mutations 17 and 18. Interestingly, these elements do not contribute equally to *912δ* promoter activity. Mutation 17 in the 5' TATA only decreased promoter activity to 87% of the wild-type level, while mutation 18

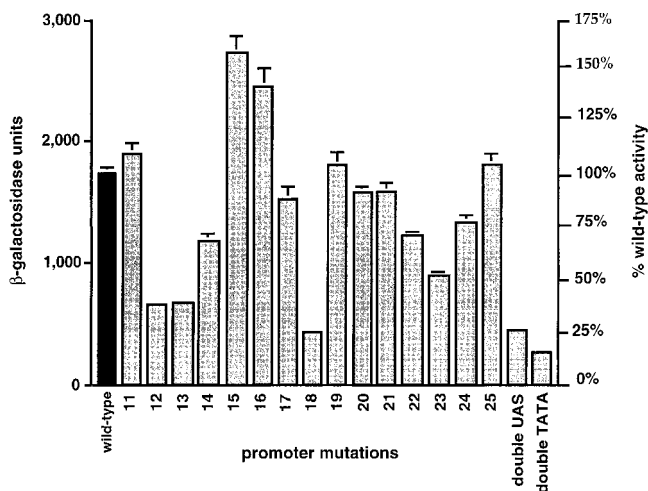


Figure 2.— $\beta$ -Galactosidase activity of the  $\delta$  promoter mutants. The  $\beta$ -galactosidase activity of  $\delta$  mutations 11–25, double UAS, and double TATA are graphed  $\pm$  standard error. The left *y*-axis presents the scale in  $\beta$ -galactosidase units and the right *y*-axis presents the scale as percent activity relative to the wild-type *his4-912δ-lacZ* promoter (black bar).

in the 3' TATA decreased promoter activity to 24% of the wild-type level. The double TATA mutant decreased expression to 14% of the wild-type level. Thus, as was shown for other Ty elements (Liao *et al.* 1987; Coney and Roeder 1988), the UAS and TATA regions of *912δ* are both necessary for full promoter activity. Our results suggest that the *912δ* UAS may contain two elements, defined by sites 12 and 13, and that the 3' TATA element is required for most of the *912δ* TATA activity.

Mutations in two other regions of *912δ* caused weak effects on expression. One region, defined by mutations 15 and 16, may contain a weak negative site. Mutations 15 and 16 caused modest but reproducible increases in  $\beta$ -galactosidase activity (Figure 2). This region contains an *in vitro* binding site for the Mot3 protein (Madison *et al.* 1998), and genetic evidence suggests that Mot3 weakly represses the *912δ* promoter via this site (Madison *et al.* 1998). A second region, defined by mutations 22 and 23, may contain a weak positive site.

To determine whether  $\beta$ -galactosidase values correlated with *his4-912δ-lacZ* mRNA levels and to examine the effects of the promoter mutations on the position of transcription initiation, we analyzed *his4-912δ-lacZ* mRNA in each *912δ* promoter mutant by primer extension analysis (Figure 3). Results from the measurement of mRNA levels agreed with the  $\beta$ -galactosidase assays, showing the same relative effects across the promoter region. Mutations within the UAS $_{\delta}$ , 12 and 13, decreased *lacZ* mRNA expression to 50% of wild type, while the 12/13 double mutant decreased expression to 30% of wild type. Mutations 15 and 16, which contain a Mot3-binding site, produced a 150–200% increase in *lacZ* mRNA levels. Mutations that abolish the strong TATA element, site 18, decreased *lacZ* mRNA levels to 40% of

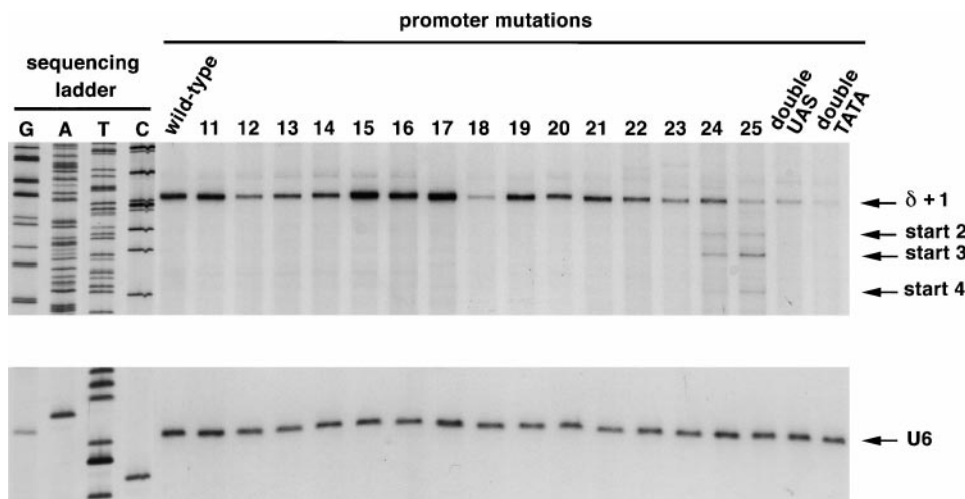


Figure 3.—Primer extension analysis of the  $\delta$  promoter mutants. Primer extension reactions were performed on RNA isolated from strains containing *his4-912δ-lacZ* promoter mutations. A *lacZ* primer detects *his4-912δ-lacZ* transcripts, and the U6 primer detects the U6 snRNA as a normalization control. A *his4-912δ-lacZ* sequencing ladder, generated using the *lacZ* oligo, was run in parallel with the primer extension products to map transcription start sites. The positions of the correct Ty mRNA ( $\delta + 1$ ), the three novel transcripts seen in mutants 24 and 25 (start 2–4), and the U6 snRNA are indicated by arrows. This figure is a composite of different exposures of the same gel and was produced with Adobe Photoshop and a Fujix Pictography 3000 printer.

wild type, while the double TATA mutation decreased expression to 20% of wild type. Thus, the relative effects of the *912δ* promoter mutations detected by  $\beta$ -galactosidase assay correlate well with *lacZ* mRNA levels, although the degree of the effects is slightly less.

Two mutations, 24 and 25, altered transcriptional start site selection (Figure 3). Mutation 25 spans the  $\delta$  nucleotide at which transcription initiates (Elder *et al.* 1983) and changes the initiation base from a G to an A. Mutation 24 spans a region 5' of the initiation site (Table 2). Both mutations cause initiation to occur at three new sites (at or near positions +16, +25, and +35) in addition to the normal initiation site (+1). The correct transcription initiation site and the three downstream sites of initiation are utilized with relatively equal frequency in both promoter mutants, although mutation 25 shows a greater effect. Mutations in this region do not significantly decrease  $\beta$ -galactosidase activity (Figure 2), presumably because all four mRNA species initiate upstream of the Ty translation initiation codon and encode functional  $\beta$ -galactosidase. These two mutations define a 20-bp region surrounding the transcription initiation site that is necessary for accurate start-site selection.

**Evidence for the activity of a second activator binding to the *912δ* UAS:** Our analysis of the *912δ* UAS indicated that both sites 12 and 13 contain UAS activity. Recent work has demonstrated that Gcr1 binds to the  $\delta$  UAS over the region defined by site 13 (Türkel *et al.* 1997). We have confirmed Gcr1-binding to site 13 using a recombinant Gcr1-binding domain peptide (generous gift of Dr. Henry Baker) in electrophoretic mobility shift assays (A. M. Dudley and F. Winston, unpublished results).

Since mutations in site 12 affect UAS $_{\delta}$  activity but this site is distinct from the Gcr1-binding site, we also assayed

binding to site 12. As shown in Figure 4, a DNA-binding activity in yeast nuclear extracts was able to gel shift a short DNA probe that contains site 12 and only 3 bp of site 13 (Figure 4B, lane 2). The binding was competed by unlabeled, double-stranded oligonucleotides containing site 12 sequence (Figure 4B, lanes 3–5 and 9–14) but not by oligonucleotides in which site 12 was mutated (Figure 4B, lanes 6–8), demonstrating that the DNA-binding activity is specific for site 12. Two pieces of evidence strongly suggest that this mobility-shifted complex is not a result of binding by Gcr1. First, the short, wild-type probe used in this assay does not contain the consensus Gcr1-binding site, and a recombinant Gcr1-binding domain peptide (generous gift of Dr. Henry Baker) is unable to bind this probe (A. M. Dudley and F. Winston, unpublished results). Second, the gel shift was completed by a double-stranded, unlabeled oligonucleotide containing a site 13 mutation, which destroys the Gcr1-binding site (Figure 4B, lanes 9–11). Thus, genetic and biochemical evidence support the hypothesis that a factor in addition to Gcr1 is able to bind and activate the UAS $_{\delta}$ .

**Upstream activators at both the UAS $_{HIS4}$  and the UAS $_{\delta}$  contribute to *912δ* promoter activity:** Previous results suggested that Gcn4, acting at the adjacent UAS $_{HIS4}$ , exerts significant control over transcription from the  $\delta$  initiation site at *his4-912δ* (Silverman and Fink 1984). To confirm this aspect of *his4-912δ* regulation and to test whether other elements of the UAS $_{HIS4}$  controlled *his4-912δ-lacZ*, we measured the effects of *bas1Δ*, *pho2Δ*, and *gcn4Δ* mutations on its expression. Expression from the *his4-912δ-lacZ* fusion decreased to 23% of wild type in a *gcn4Δ* mutant and to ~50% of wild type in both *bas1Δ* and *pho2Δ* mutants (Table 3). These results are similar to those obtained previously for a *HIS4-lacZ* fusion tested under similar growth conditions (Arndt *et*

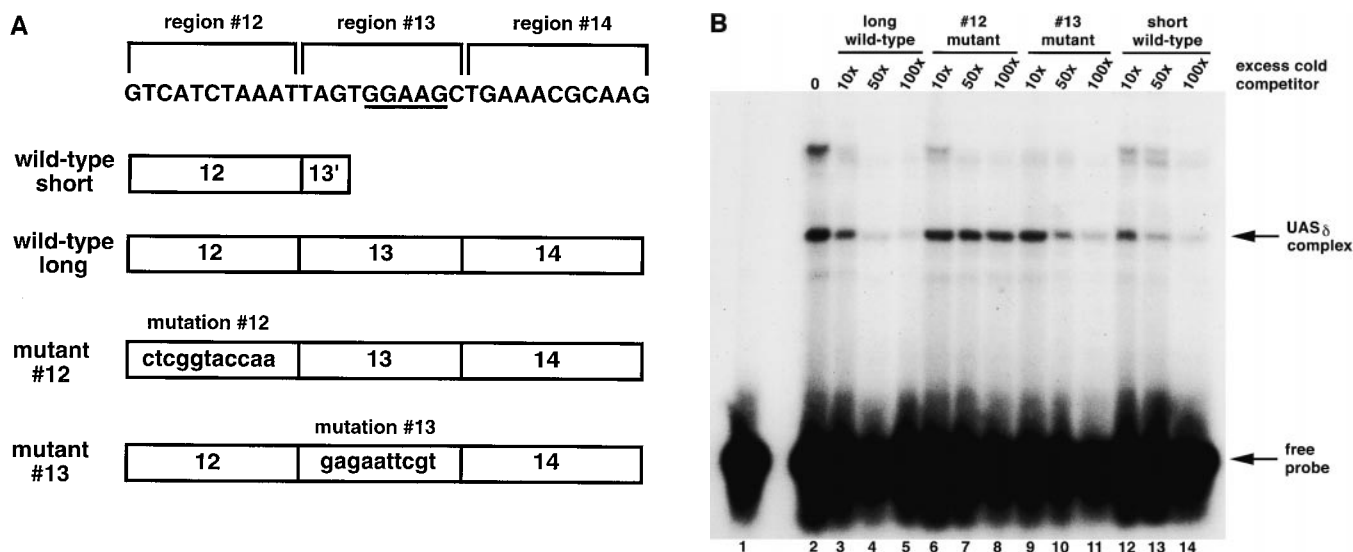


Figure 4.—A factor in yeast nuclear extracts is able to bind  $\delta$  site 12. (A) Schematic diagram of probes and competitors used for electrophoretic mobility shift analysis. The short, wild-type probe contains the entire sequence of site 12 and 3 bp of site 13. The long, wild-type competitor contains the entire sequence of sites 12, 13, and 14. Mutant 12 and mutant 13 competitors are identical to the long, wild-type competitor except that they contain mutant 12 and 13 base pair substitutions, respectively. The Gcr1-consensus-binding site is underlined. All probes and competitors are double-stranded and were created by annealing single-stranded oligonucleotides (materials and methods). (B) Electrophoretic mobility shift analysis was performed using 10 ng of yeast nuclear extract and 0.8 ng of the short wild-type probe. All lanes contain the labeled probe. Lanes 2–14 contain extract; lanes 3–5 contain 10-, 50-, and 100-fold excess cold wild-type long competitor; lanes 6–8 contain 10-, 50-, and 100-fold excess cold 12 mutant competitor; lanes 9–11 contain 10-, 50-, and 100-fold excess cold 13 mutant competitor; lanes 12–14 contain 10-, 50-, and 100-fold excess cold wild-type short competitor. The positions of free probe and the specific UAS <sub>$\delta$</sub>  complex are indicated by arrows. This figure was produced with Adobe Photoshop and a Fujix Pictography 3000 printer.

*al.* 1987). Thus, factors bound at the UAS<sub>HIS4</sub> activate the  $\delta$  promoter in a manner similar to their regulation of *HIS4*.

To compare the relative contributions of transcriptional activators at the UAS<sub>HIS4</sub> and the UAS <sub>$\delta$</sub> , we measured *his4-912 $\delta$ -lacZ* expression in double mutants that affect both UAS elements (Table 3). In combination with either a *bas1 $\Delta$*  or *pho2 $\Delta$*  mutation, the 912 $\delta$  site 12 or 13 mutations caused reductions in  $\beta$ -galactosidase activity equal to those predicted for the double mutant combinations,  $\sim$ 18% of the wild-type activity. Similarly, a *gcn4 $\Delta$*  site 12 double mutant exhibited close to the expected reduction in promoter activity. In contrast, the combination of *gcn4 $\Delta$*  with a mutation in the Gcr1-binding site (site 13) produced a greater-than-expected decrease in expression, to only 2% of the wild-type activity. The distinct behaviors of site 12 and site 13 mutations in a *gcn4 $\Delta$*  mutant support the hypothesis that the two elements are bound by different activators. Taken together, these results suggest that, among the various activators known to bind either the UAS<sub>HIS4</sub> or the UAS <sub>$\delta$</sub> , significant 912 $\delta$  promoter function requires either Gcr1 or Gcn4. Only in the presence of at least one of these factors can other activators at either the UAS<sub>HIS4</sub> or the UAS <sub>$\delta$</sub>  contribute additional activity.

To test if Gcn4 activates via the UAS <sub>$\delta$</sub>  as well as the UAS<sub>HIS4</sub>, we examined the effect of a *gcn4 $\Delta$*  on the activity of the UAS <sub>$\delta$</sub>  in a heterologous context. Multiple copies

of the UAS <sub>$\delta$</sub>  sequence were cloned 5' to a heterologous reporter gene as described in materials and methods. As expected, the UAS <sub>$\delta$</sub>  was able to activate expression of the *HIS3* reporter in a wild-type strain; however, this activation was abolished by a *gcr1 $\Delta$*  mutation (Figure 5), consistent with previous studies (Türkel *et al.* 1997). Moreover, the UAS <sub>$\delta$</sub>  was still able to activate in a *gcn4 $\Delta$*  strain (Figure 5). The Gcn4 independence of the UAS <sub>$\delta$</sub>  strongly suggests that the effects observed in double mutant combinations between *gcn4 $\Delta$*  and UAS <sub>$\delta$</sub>  mutants (Table 3) are not solely caused by the loss of Gcn4 activation, but rather by the combined loss of multiple activators.

To test the relative contribution of Gcn4 activation under conditions that mimic amino acid starvation, Gcn4 was constitutively expressed. To do this, we transformed cells containing either the wild-type *his4-912 $\delta$ -lacZ* promoter or the double UAS (12/13) mutant with a plasmid that carries a *GCN4* constitutive mutation (Table 4). Because this allele of *GCN4* is no longer under its normal translational repression, it is highly expressed regardless of amino acid abundance (Müller and Hinnebusch 1986). Constitutive expression of *GCN4* increases *his4-912 $\delta$ -lacZ* activity approximately twofold in both the presence and absence of UAS <sub>$\delta$</sub>  activity (Table 4). Similarly, constitutive *GCN4* expression increases *his4-912 $\delta$ -lacZ* activity approximately twofold in an *spt3 $\Delta$*  mutant (Table 4). These results demonstrate

**TABLE 3**  
Factors at the UAS<sub>HIS4</sub> and the UAS<sub>δ</sub> contribute to  
*his4-912δ-lacZ* activity

Relevant genotype <sup>a</sup>		β-Galactosidase units <sup>b</sup>	Percentage <sup>c</sup>
<i>HIS4</i> UAS	δ UAS		
+	+	1729 ± 60	100
+	12	637 ± 16	37
+	13	646 ± 32	37
+	+	2396 ± 109	100
<i>bas1Δ</i>	+	1243 ± 41	53
<i>bas1Δ</i>	12	312 ± 16	13
<i>bas1Δ</i>	13	387 ± 20	16
+	+	2620 ± 145	100
<i>pho2Δ</i>	+	1440 ± 115	56
<i>pho2Δ</i>	12	485 ± 32	19
<i>pho2Δ</i>	13	539 ± 39	20
+	+	1950 ± 129	100
<i>gcn4Δ</i>	+	449 ± 39	23
<i>gcn4Δ</i>	12	215 ± 11	11
<i>gcn4Δ</i>	13	48 ± 1	2

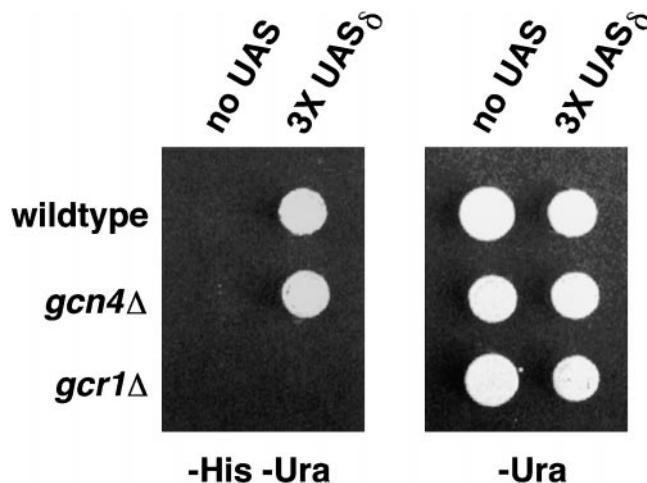
<sup>a</sup> The activities of factors at the UAS<sub>HIS4</sub> were abolished by *trans*-acting mutations (*bas1Δ*, *pho2Δ*, and *gcn4Δ*). The activities of factors at the UAS<sub>δ</sub> were abolished by *cis*-acting mutations (12 and 13).

<sup>b</sup> β-Galactosidase activities are shown plus and minus the standard error. Assays were done in groups as indicated. β-Galactosidase values for the first group (wild-type, 12, and 13 alleles in a wild-type background) are taken from the data in Figure 2.

<sup>c</sup> The percentage is based on the wild-type strain in each group as 100%.

that high levels of *GCN4* expression are not sufficient to overcome the defects caused by a UAS<sub>δ</sub> or *spt3Δ* mutation.

**Interactions of mutations in the two 912δ TATA regions with mutations that affect HIS4 and 912δ UAS activity:** To assess the roles of the two 912δ TATA elements with respect to activation by Gcn4 and Gcr1, we examined a set of double mutants that affect both UAS and TATA function. First, we examined the effect of mutations in the 912δ TATA elements in combination with a *gcn4Δ* mutation (Table 5). Similar to the severe defect seen when both Gcn4 and Gcr1 activation were abolished, expression decreased to 2% of wild type in a 3' TATA (18) *gcn4Δ* double mutant. This result suggests a model in which virtually all the activation by Gcr1 occurs via the 3' TATA; that is, the *gcn4Δ* site 18 double mutant mimics loss of both Gcn4 and Gcr1. The fact that the weak defect observed for the 5' TATA mutation (17) is the same in *GCN4* and *gcn4Δ* backgrounds suggests that this TATA element may be responsive to activation by Gcn4. However, the weak activity of this TATA in the presence of a functional 3' TATA element makes this analysis difficult. These results sug-



gest that 912δ requires the activity of either Gcn4 or TATA 18 to have any significant activity. Figure 5.—The UAS<sub>δ</sub> is *GCR1* dependent and *GCN4* independent. Wild-type, *gcr1Δ*, and *gcn4Δ* strains were transformed with plasmids containing a *HIS3* reporter gene, either without a UAS (no UAS) or with three copies of the UAS<sub>δ</sub> sequence. Approximately  $2 \times 10^5$  cells were spotted onto solid media containing histidine (SC-Ura) and lacking histidine (SC-Ura-His) and the plates were incubated at 30°. The media contained 2% glycerol/2% ethanol as carbon sources to allow the growth of the *gcr1Δ* strains, which grow poorly on glucose.

gest that 912δ requires the activity of either Gcn4 or TATA 18 to have any significant activity.

To test the requirement of the 3' TATA (18) in Gcn4 activation, we constructed a *his4-912δ-lacZ* derivative containing mutations in the Gcr1-binding site (mutation 13) and in the 3' TATA (mutation 18). The decrease in the site 13/18 double mutant was less severe than in the *gcn4Δ* site 18 double mutant, 9% vs. 2% of wild-type activity (Table 5). These results suggest that in the absence of Gcr1 and the 3' TATA, Gcn4 is able to weakly activate the 912δ promoter, presumably through the 5' TATA. Taken together, these results suggest that Gcr1 activates via TATA 18 and Gcn4 activates via both TATA sequences.

**Expression of *his4-912δ-lacZ* is dependent on specific classes of SAGA components:** Transcription of full-length Ty elements as well as many solo δ insertion alleles is strongly dependent on certain Spt proteins (Winston *et al.* 1987) that are components of the SAGA complex (Grant *et al.* 1997). SAGA contains a histone acetyltransferase activity that is dependent upon one SAGA component, Gcn5 (Brownell *et al.* 1996; Grant *et al.* 1997). To learn more about SAGA control of the δ promoter, we have analyzed *his4-912δ-lacZ* expression in three different classes of SAGA mutants, *spt20Δ*, *spt3Δ*, and *gcn5Δ* (Table 6). Recent work has shown that *spt20Δ* likely abolishes all SAGA activities, while *spt3Δ* and *gcn5Δ* each affect distinct subsets of SAGA activities (Grant *et al.* 1997; Horiuchi *et al.* 1997; Roberts and Winston 1997). As expected (Winston *et al.* 1984b; Roberts and Winston 1996), *spt3Δ* and *spt20Δ* mutations caused decreased levels of *his4-912δ-lacZ* expres-

TABLE 4

Analysis of the contribution of Spt3 and the UAS<sub>δ</sub> under conditions of constitutive Gcn4 expression

Relevant genotype <sup>a</sup>				
<i>SPT3</i>	<i>HIS4</i> UAS	δ UAS	β-Galactosidase units <sup>b</sup>	Percentage <sup>c</sup>
+	+	+	2217 ± 173	100
+	<i>GCN4</i> <sup>c</sup>	+	4721 ± 273	213
+	+	12/13	346 ± 31	16
+	<i>GCN4</i> <sup>c</sup>	12/13	835 ± 78	38
<i>spt3Δ</i>	+	+	166 ± 11	7
<i>spt3Δ</i>	<i>GCN4</i> <sup>c</sup>	+	445 ± 34	20

<sup>a</sup> The activity of Gcn4 at the UAS<sub>HIS4</sub> was altered by transforming the strain with either B238, which expresses Gcn4 constitutively (*GCN4*<sup>c</sup>), or with YCp50 (+). The activity of factors at the UAS<sub>δ</sub> was abolished by the double UAS mutation (12/13).

<sup>b</sup> β-Galactosidase activities are shown plus and minus the standard error. Assays were performed in the same experiment.

<sup>c</sup> The percentage is based on the wild-type strain as 100%.

sion. However, *gcn5Δ* caused no defect in *his4-912δ-lacZ* expression. These results support the hypothesis that SAGA contains multiple functions that may be required at different promoters to modulate their expression. For example, the Spt3-dependent SAGA activity is required for *his4-912δ-lacZ* expression, whereas the Gcn5 histone acetyltransferase activity is not required.

**Several elements of the *his4-912δ* promoter are partially Spt3 dependent:** The Spt3-dependent function of SAGA is strongly required at some but not all RNA polymerase II-dependent promoters in *S. cerevisiae*. However, the factors or promoter elements that confer Spt3-dependence on these promoters are unknown. Unexpectedly, our analysis of promoter elements within *912δ* did not identify any single element that could account

for the large decrease in expression observed in an *spt3Δ* mutant. On the basis of this analysis, we reasoned that Spt3 activity might be exerted over a number of promoter elements, or even the entire promoter region. If the function of a promoter element was completely dependent upon Spt3, we would expect that in an *spt3Δ* mutant, a mutation in that promoter element would not further reduce expression. To determine whether the activities of important *912δ* promoter elements were dependent on Spt3, we measured the activity of a set of *912δ* promoter mutants in an *spt3Δ* background (Table 7). This analysis shows that, while none of the promoter elements tested were completely Spt3 dependent, several promoter elements appeared partially dependent. The strongest example of this partial dependence

TABLE 5

Analysis of the contribution of Gcn4 at the UAS<sub>HIS4</sub> and Gcr1 at the UAS<sub>δ</sub> in δ TATA mutants

Relevant genotype <sup>a</sup>				
<i>HIS4</i> UAS	δ UAS	δ TATA	β-Galactosidase units <sup>b</sup>	Percentage <sup>c</sup>
+	+	+	1729 ± 60	100
+	+	17	1507 ± 125	87
+	+	18	415 ± 16	24
+	+	+	1950 ± 129	100
<i>gcn4Δ</i>	+	+	449 ± 39	23
<i>gcn4Δ</i>	+	17	420 ± 30	22
<i>gcn4Δ</i>	+	18	38 ± 2	2
+	+	+	1161 ± 10	100
+	13	18	101 ± 7	9

<sup>a</sup> The activity of Gcn4 at the UAS<sub>HIS4</sub> was abolished by the *trans*-acting mutation (*gcn4Δ*). The activity of factors at the UAS<sub>δ</sub> was abolished by *cis*-acting mutations (13, 17 and 18).

<sup>b</sup> β-Galactosidase activities are shown plus and minus the standard error. Assays were done in groups as indicated. β-Galactosidase values for the first group (wild-type, 17, and 18 alleles in a wild-type background) are taken from the data in Figure 2.

<sup>c</sup> The percentage is based on the wild-type strain in each group as 100%.

TABLE 6

Expression of *his4-912δ-lacZ* in three classes of SAGA mutations

Relevant genotype	β-Galactosidase units <sup>a</sup>	Percentage <sup>b</sup>
Wild type	1649 ± 124	100
<i>spt3Δ</i>	123 ± 5	7
Wild type	2705 ± 297	100
<i>spt20Δ</i>	395 ± 7	15
Wild type	2582 ± 115	100
<i>gcn5Δ</i>	2670 ± 81	103

<sup>a</sup> β-Galactosidase activities are shown plus and minus the standard error. Assays were done in pairs as indicated.

<sup>b</sup> The percentage is based on the wild-type strain for each pair as 100%.

occurred with the Gcr1-binding site. In an *spt3Δ* background, a mutation in the Gcr1-binding site (13) in the *912δ* promoter caused a significantly weaker defect than in an *SPT3<sup>+</sup>* background. Similar effects were seen for several other elements, including the strong 3' TATA box (18), Gcn4, and the unidentified protein that binds to site 12 of the *912δ* UAS (Table 7). We also assayed the 13/18 mutation in an *spt3Δ* background and demonstrated a strong but still partial dependence upon Spt3. Taken together, these results support a model in which Spt3 activity at the *912δ* promoter occurs via partial effects at a number of important promoter elements.

## DISCUSSION

In this study we have conducted a genetic analysis of a Ty  $\delta$  insertion mutation, *his4-912δ*, to identify sites and factors important for  $\delta$  promoter function *in vivo*. Past studies and this work have shown that several activators act at the two UAS elements present in this complex promoter. However, our analysis has demonstrated that two of these activators, Gcr1 acting at the UAS $\delta$ , and Gcn4 acting at the UAS<sub>HIS $\delta$</sub> , are the primary activators required for *912δ* expression. In addition, we found that multiple *his4-912δ* promoter elements are partially dependent upon Spt3, a component of the SAGA complex. Thus, the Spt3-dependent SAGA activity appears to exert large effects on the  $\delta$  promoter through a combination of partial effects on several promoter elements.

**Identification of *912δ* sequences important for promoter function:** The region identified as a UAS in *912δ* is identical to the UAS identified previously in a Ty2 element in sequence and position (Liao *et al.* 1987). Our results have provided evidence for two distinct sites within this UAS, which we have called sites 12 and 13. Our analysis of site 13 is consistent with a previous study that demonstrated binding of Gcr1 to this region of the UAS $\delta$  (Türkel *et al.* 1997). Although we have not yet

TABLE 7

Analysis of Spt3-dependence of expression of *his4-912δ*

Relevant genotype	Relative expression <sup>a</sup>	
	<i>SPT3<sup>+</sup></i>	<i>spt3Δ</i>
Wild type	100	100
12	37	51
13	37	79
17	87	79
18	24	38
13/18	9	42
<i>gcn4Δ</i>	19	57

<sup>a</sup> The relative expression is calculated as the percentage of β-galactosidase units in the strains with mutant promoters compared to the strains with a wild-type promoter. For *SPT3<sup>+</sup>* strains, mutants 12, 13, 17, and 18 were assayed in one group, in which the wild-type β-galactosidase value was 1729 ± 60, and another group (13/18 and *gcn4Δ*), in which the wild-type β-galactosidase value was 1171 ± 10. For the *spt3Δ* strains, mutants 12, 13, 17, and 18 were assayed in one group, in which the wild-type β-galactosidase value was 123 ± 5, and another group (13/18 and *gcn4Δ*), in which the wild-type β-galactosidase value was 142 ± 13.

identified the factor that binds to site 12, the site 12 sequence hints that this factor might be a homeodomain protein. The site 12 sequence TAAATTA contains AATT and TAAT sequences often seen in sites bound by homeodomain proteins (Wolberger 1996). We have tested null mutations in several genes encoding homeodomain proteins for an effect on *his4-912δ-lacZ* expression. There was no decrease in *his4-912δ-lacZ* expression for any of the mutations tested in the genes *MAT $\alpha$ 1*, *MAT $\alpha$ 2*, *PHO2*, *CUP9*, and *YGL096* (A. M. Dudley and F. Winston, unpublished results). In addition, gel shift experiments have suggested that the factor binding to site 12 does not bind cooperatively with Gcr1 (A. M. Dudley and F. Winston, unpublished data). More analysis is required to understand fully the role of site 12 and the factor that binds to it.

Mutations in the two *912δ* consensus TATA elements also decreased expression from *his4-912δ-lacZ*. Although many studies have demonstrated the importance of this TATA region for  $\delta$  activity (Liao *et al.* 1987; Coney and Roeder 1988; Fulton *et al.* 1988; Hirschman *et al.* 1988; Arndt *et al.* 1994), the relative contribution of each TATA element was unknown prior to this study. Surprisingly, the activity of these identical TATA elements is not equivalent; mutation of the 5' TATA produces a very modest decrease in expression while mutation of the 3' TATA causes a decrease of severalfold. Two previous studies have examined the utilization of identical TATA elements introduced into the *S. cerevisiae* genes *HIS4* (Nagawa and Fink 1985) and *CYC1* (Li and Sherman 1991). In both cases, only the 5' TATA element was functional. In contrast, the 3' TATA is the most active in the *912δ* promoter. Several possible

mechanisms could explain this differential TATA selection. The function of specific activators could increase the activity of a particular TATA element. Certain activators may prefer specific TATA elements based on position or some other mechanism. Alternatively, the flanking sequence surrounding a consensus TATA element may affect its utilization *in vivo* (for example, see Harbury and Struhl 1989). There is evidence that sequences flanking the TATA in *9126* are important for promoter function (Hirschman *et al.* 1988). Although the mechanism for *9126* TATA preference is still unknown, our results suggest that at some promoters the mechanism may be more complex than merely choosing the best consensus TATA located closest to the UAS.

Our analysis identified a region of the  $\delta$  required for accurate transcription initiation. Mutations in this  $\delta$  initiator element alter the position of initiation without affecting the overall level of expression. The role of initiator elements is not well understood in *S. cerevisiae*. Surveys of the transcription start site of a number of genes and analysis of the effects of distance from the TATA element have led to a model in which transcription usually initiates from a site resembling a loose consensus sequence around  $\sim 30$  to 120 nucleotides from the TATA element (Chen and Struhl 1985; Hahn *et al.* 1985; Nagawa and Fink 1985; Healy *et al.* 1987; Rudolph and Hinnen 1987; Maicas and Friesen 1990; Li and Sherman 1991). Start site selection appears to be intimately linked to the general transcription apparatus since mutations in the genes encoding TFIIB (Pinto *et al.* 1992), Rbp1 (Berroteran *et al.* 1994), and Rpb9 (Hull *et al.* 1995) alter the transcriptional initiation patterns of several genes. We speculate that Ty1 may have evolved a strong initiator element because a single initiation site is important for some aspect of its life cycle, such as translation, mRNA packaging, or reverse transcription.

**Transcription from *his4-9126* is controlled by promoter elements of both the  $\delta$  and the UAS<sub>HIS4</sub>:** Ty  $\delta$  promoter function in *his4-9126* is determined by the activity of a variety of UAS and TATA promoter elements and is therefore a good model for the complex promoters found in larger eukaryotes. Our results have led to a model in which the *9126* promoter is primarily activated by Gcr1 and Gcn4 (Figure 6). In this model, Gcn4 at the UAS<sub>HIS4</sub>, Gcr1 at the UAS $\delta$ , and TBP at the 3'  $\delta$  TATA are all crucial for promoter function. Other factors at the UAS<sub>HIS4</sub> (Bas1 and Pho2), the UAS $\delta$  (an unidentified factor at site 12), and the 5' TATA (TBP) increase expression from *9126* as long as Gcr1 or Gcn4 is present. Thus, the ability of the  $\delta$  promoter to respond to the activity of transcription factors positioned near it as a result of transposition allows activation of the promoter via  $\delta$ -specific and heterologous factors.

**How does SAGA function at a promoter?** Our results have demonstrated that the Spt3-dependent activity of SAGA is required for *9126* promoter function and that

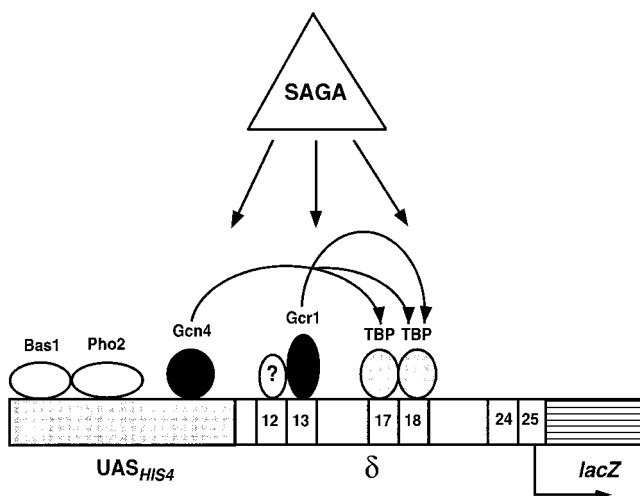


Figure 6.—A model for *his4-9126* promoter function. The drawing shows the relative positions of several factors known to bind to the *his4-9126* promoter region: Bas1, Pho2, and Gcn4 binding at the UAS<sub>HIS4</sub>; Gcr1 and a currently unidentified factor binding at the UAS $\delta$ ; and TBP binding at the two consensus TATA elements. The transcription start site is indicated by the arrow. In this model, Gcr1 activates via the 3' TATA element and Gcn4 activates via both TATA sequences. Many of these important promoter elements are partially dependent on the Spt3-dependent activity of the SAGA complex, resulting in a large Spt3-dependent effect on the  $\delta$  promoter as the sum of partial effects at a number of promoter elements.

the Gcn5-dependent histone acetyltransferase activity of SAGA is not required. These results are consistent with previously observed Spt<sup>-</sup> phenotypes of *spt3Δ* and *spt20Δ* mutants and the Spt<sup>+</sup> phenotype of a *gcn5Δ* mutant (Winston *et al.* 1984b; Roberts and Winston 1996, 1997). The results are also consistent with previous models that suggest that functions encoded by different components of SAGA may be required at different promoters (Horiuchi *et al.* 1997; Roberts and Winston 1997).

Our analysis of the effect of an *spt3Δ* mutation in combination with mutations in important promoter elements suggests that activation by both Gcn4 at the UAS<sub>HIS4</sub> and Gcr1 at the UAS $\delta$  is partially Spt3 dependent. Our results provide the first evidence that Spt3 may exert large effects on the transcriptional activity of a promoter by a combination of smaller effects at multiple promoter elements. The relatively weak SPT3 dependence of the *9126* TATA elements was surprising as previous results suggested that Spt3 interacts with TBP (Eisenmann *et al.* 1992). However, Spt3 may affect the activity of specific TATA elements either by direct interaction with TBP or by controlling communication between TBP and activators.

Our results are consistent with two obvious models for SAGA function at *9126*: a transcription-factor-binding model and a transcription-factor-activation model. In the factor-binding model, activators such as Gcr1 and Gcn4 would partially require the Spt3-dependent SAGA

activity to bind their cognate sites. If this model is correct, these activators must be able to bind their sites, albeit to a lesser degree, in an *spt3Δ*, because the activities of Gcr1 and Gcn4 are not completely Spt3 dependent. One parameter that could affect the binding of multiple proteins over a region of this length is the chromatin structure (for review, see Kingston *et al.* 1996; Steger and Workman 1996). However, it is unlikely that the nucleosome acetylation activity of SAGA facilitates the binding of these factors because a *gcn5Δ*, which completely abolishes the histone acetylase activity of SAGA (26), has no effect on *his4-9128* transcription. In the factor-activity model, activators such as Gcr1 and Gcn4 would not require SAGA to bind their sites, but would at least partially require the Spt3-dependent SAGA activity for a subsequent step in transcriptional activation. This model is consistent with previous models proposed for the function of several classes of SAGA components, including Spt proteins (Eisenmann *et al.* 1992), Ada proteins (Berger *et al.* 1992), and Gcn5 (Georgakopoulos and Thireos 1992). In the context of this model, our results suggest that Spt3 and other components of the SAGA complex would mediate the interaction of multiple activators with the transcription machinery. Experiments designed to distinguish between these two models will greatly increase our understanding of SAGA function, and the detailed analysis of SAGA-dependent promoters will provide important tools for such studies.

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