Analysis of Spt7 Function in the *Saccharomyces cerevisiae* SAGA Coactivator Complex

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The *Saccharomyces cerevisiae* SAGA complex is required for the normal transcription of a large number of genes. Complex integrity depends on three core subunits, Spt7, Spt20, and Ada1. We have investigated the role of Spt7 in the assembly and function of SAGA. Our results show that Spt7 is important in controlling the levels of the other core subunits and therefore of SAGA. In addition, partial SAGA complexes containing Spt7 can be assembled in the absence of both Spt20 and Ada1. Through biochemical and genetic analyses of a series of *spt7* deletion mutants, we have identified a region of Spt7 required for interaction with the SAGA component Spt8. An adjacent Spt7 domain was found to be required for a processed form of Spt7 that is present in a previously identified altered form of SAGA called SLIK, SAGA$_{alt}$, or SALSA. Analysis of an *spt7* mutant with greatly reduced levels of SLIK/SAGA$_{alt}$/SALSA suggests a subtle role for this complex in transcription that may be redundant with a subset of SAGA functions.

The *Saccharomyces cerevisiae* SAGA (Spt-Ada-Gcn5 acetyltransferase) complex is a multisubunit coactivator complex that is important for transcription in vivo (27, 32). Whole-genome mRNA analysis of SAGA mutants has shown that the expression of approximately 10% of *S. cerevisiae* genes are affected by the loss of the SAGA complex (23). Both in vivo and in vitro experiments have shown that SAGA activates transcription after its recruitment by transcriptional activators (5, 22, 42, 43). Other results have suggested that SAGA also represses transcription at particular promoters (4, 23). In addition, several studies have shown that SAGA often acts coordinately with other coactivator complexes at a promoter to achieve normal levels of transcription (27). The SAGA complex is conserved between yeast and humans, strongly suggesting that this type of coactivator is also important in mammalian transcription (6, 25, 26, 28, 46).

The subunits in SAGA can be grouped functionally based on a large body of in vivo and in vitro experiments (27). Three classes of SAGA proteins are involved in distinct aspects of transcriptional control. First, Gcn5 contains the catalytic activity for the histone acetyltransferase (HAT) activity of SAGA (8), and Gcn5's HAT activity is modulated by the Ada2 and Ada3 proteins (2, 16, 39). Second, Spt3 and Spt8 of SAGA have been shown to control the TATA box binding protein (TBP)-TATA interaction at particular promoters (4, 5, 9, 11, 22). Third, Tra1 has been shown to interact with several transcriptional activators in vitro, suggesting that SAGA is recruited to promoters via this subunit and subsequently activates or represses transcription through its different activities (7).

SAGA also contains two additional classes of proteins that are not known to participate directly in regulation and therefore may serve structural roles. In the first class are three proteins, Spt7, Spt20, and Ada1, that function as SAGA core components based on their requirement for integrity of the complex. SAGA is absent from *spt7*Δ, *spt20*Δ, and *ada1*Δ mutants, as assayed by Western analysis and HAT assays (16, 38). Moreover, these mutations cause a broad variety of severe phenotypes, consistent with a complete loss of SAGA function (21, 30, 38). The second class contains a subset of the Tafs (Tafs 5, 6, 9, 10, and 12), proteins initially identified as components of the TFIID complex (17). In referring to the Tafs, we are using the recently revised uniform Taf nomenclature (41). At least one of the Tafs, Taf12, appears to be important for SAGA structure, as its removal via a *taf12* temperature sensitivity mutation affects both SAGA integrity as well as its nucleosomal HAT activity (17).

While the control of transcription by SAGA has been extensively studied, less is known about the control of its assembly and the protein-protein associations within the complex. Previous studies have shown that both Spt7 and Ada1 have histone fold motifs that interact with Taf components of SAGA: Ada1 with Taf12 and Spt7 with Taf10 (13, 14). However, it is not known how the core subunits contribute toward the structural integrity of the complex. To learn more about the roles of one of the SAGA core components, we have chosen Spt7 as the focus of our studies. Spt7 is a 1,332-amino-acid, highly negatively charged protein whose sequence contains two motifs of note. First, as mentioned previously, Spt7 contains a histone fold (amino acids 979 to 1045) required for interaction with the Taf10 subunit of SAGA (13). Second, Spt7 contains a bromodomain (amino acids 463 to 523), a motif found in many transcription factors (20; reviewed in reference 44). A deletion that removes the Spt7 bromodomain does not cause any detectable phenotypes, suggesting that this domain is either redundant or not crucial for Spt7 function (15, 38).

Previous studies suggest that Spt7 may act dynamically to regulate SAGA function. These studies demonstrated that SAGA exists in at least one alternate form that has been named SLIK (SAGA-like), SAGA$_{alt}$, or SALSA (4, 18, 34; D. Sterner and S. Berger, personal communication). This complex

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is referred to hereafter as SLIK/SALSA. The SLIK/SALSA complex has two identified differences from SAGA: a smaller form of Spt7 and the absence of Spt8 (4; Patrick Grant, personal communication). Although little is known about the formation of SLIK/SALSA, evidence suggests involvement of the general amino acid control pathway, because SLIK/SALSA levels increase in cells grown in amino acid starvation conditions (4). However, the requirements for SLIK/SALSA formation, as well as its functions in vivo, remain unknown.

In this study, we examine several aspects of Spt7 protein structure and function. We demonstrate that Spt7 is involved in regulating the levels of Spt20 and Ada1, the two other SAGA core components, suggesting that Spt7 levels control the amount of SAGA present in vivo. Additional experiments reveal that partial Spt7-containing complexes form in the absence of the other core components. To determine regions of function and interaction with other subunits, we generated and analyzed a set of spt7 partial deletion mutants. This analysis allowed us to delineate a region of minimal function in Spt7 as well as a domain for Spt8 interaction. Moreover, we have defined a region of Spt7 that is required for its processing and, hence, for the formation of SLIK/SALSA. Analysis of the spt7 mutant that impairs processing suggests that SLIK/SALSA is not strongly required for transcriptional activation.

MATERIALS AND METHODS

Yeast strains and methods. All *S. cerevisiae* strains used in this study (Table 1) are descended from a $GAL2^+$ derivative of S288C (45). All of the spt3Δ, spt8Δ, gcn5Δ, spt20Δ, and ada1Δ alleles used, as well as spt7Δ402, have been described previously (12, 15, 30, 38). Standard methods for mating, sporulation, transformations, and tetrad analysis were used, and all media were prepared as previously described (31). The Spt phenotype caused by spt7 mutations was scored with respect to two insertion mutations, his4-917δ and lys2-173R2. In an $SPT7^+$ (Spt^+) strain, these insertions confer His^- and Lys^+ phenotypes, respectively; in an spt7 null strain (Spt^-), these insertion mutations cause His^+ and Lys^- phenotypes (15).

The spt7 null mutations spt7Δ403::URA3 and spt7Δ404::HIS3 were constructed by standard methods (3, 24). Deletions that remove specific sequences of SPT7 were constructed using previously described plasmids and procedures (35). We replaced the amino- or carboxy-terminal regions of SPT7 with the hemagglutinin (HA) or Myc epitopes by PCR-mediated integration. To construct strains containing tandem affinity purification (TAP)-tagged SPT7, we used plasmid pBS1479 as previously described (29). Selection was done using the *Kluyveromyces lactis* TRP1 gene. The spt7 internal deletions spt7-200, spt7-300, and spt7-400 were constructed by integrating plasmids pJW10, pJW11, and pJW12, respectively, via a two-step method. First, the plasmid was digested with EcoNI and used to transform the appropriate strain to Ura^+. Second, recombinants that excised the intervening URA3 marker were identified after overnight growth in yeast extract-peptone-dextrose (YEPD) liquid media and plating on 5-fluoroorotic acid (5-FOA) solid media. Verification of the correct integration and recombination events was performed by PCR.

Plasmid DNA construction and analysis. Plasmids were constructed, maintained, and isolated from *Escherichia coli* strains DH5α (1) and MH1 (19) by standard methods (1). Restriction enzymes, T4 DNA ligase, and *Taq* polymerase were purchased from New England Biolabs and Gibco BRL. Plasmid pJW9 contained base pairs 3116 to 3999 of SPT7 subcloned into the integrating URA3 plasmid pRS406 (37). Plasmids pJW10, pJW11, and pJW12, used for construction of spt7-200, spt7-300, and spt7-400, respectively, were generated using the Stratagene QuikChange Site-Directed Mutagenesis Kit.

TAP purifications. TAP purifications were performed as previously described (29) but with several modifications. All steps, except the tobacco etch virus cleavage, were performed at 4°C. Briefly, 2 liters of cells grown to approximately 2×10^7 cells/ml in YEPD were concentrated in 10 ml of extract buffer (40 mM HEPES [pH 7.4], 350 mM NaCl, 10% glycerol, 0.1% Tween, 2 μg of pepstatin A/ml, 2 μg of leupeptin/ml, and 1 mM phenylmethylsulfonyl fluoride [PMSF]), and whole-cell extracts were prepared by bead beating in a mini-beadbeater. Cellular debris was removed by spinning extracts at $10,000 \times g$ for 10 min. The

extract was cleared by ultracentrifugation at $100,000 \times g$ for 1 h. For the TAP purification, 800 μl of 1:1 immunoglobulin G (IgG)-Sepharose (Sigma) slurry was incubated with clarified lysate for 3 h. Beads were washed with 20 ml of extract buffer followed by 10 ml of TEV cleavage buffer (10 mM Tris [pH 8.0], 150 mM NaCl, 0.1% NP-40, 0.5 mM EDTA, 10% glycerol, 1 mM dithiothreitol [DTT]) and then resuspended in 1 ml of the TEV cleavage buffer. TEV cleavage was performed using 10 μl (100 U) of TEV (Gibco BRL) for 2.5 h at 14°C. TEV-cleaved products were added to 3 ml of calmodulin binding buffer (10 mM Tris [pH 8.0], 150 mM NaCl, 1 mM MgOAc, 1 mM imidazole, 2 mM CaCl₂, 0.1% NP-40, 10% glycerol, 1 mM β-mercaptoethanol) along with 3 μl of 1 M CaCl₂ for each milliliter of TEV elution. Calmodulin-Sepharose (Stratagene) purification was performed by binding eluate to 400 μl of bead slurry for 2 h at 4°C. Proteins bound to calmodulin beads were eluted in 0.2-ml fractions using calmodulin elution buffer (10 mM Tris [pH 8], 150 mM NaCl, 1 mM MgOAc, 1 mM imidazole, 2 mM EGTA, 0.1% NP-40, 10% glycerol, 1 mM β-mercaptoethanol). TAP-purified complexes were run on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (5 to 20% acrylamide) gradient gel. Silver staining was performed according to the protocol from the Yeast Resource Center at the University of Washington (http://depts.washington.edu/~yeastrc /ms_silver.htm). Gels were dried under vacuum at 80°C for 1 h.

Western analysis. Whole-cell extracts were prepared as described above, and protein concentrations were determined by the Bradford assay (Bio-Rad). Equal amounts of whole cell extracts, column fractions, or TAP-purified complexes were separated on SDS-PAGE gels. Transfer was performed as previously described (40a). The following antibodies were used at the given concentrations: HA (12CA5 from Boehringer Mannheim, 1:2,500), Myc (A14 from Santa Cruz, 1:1,000), Ada1 (1:2,000), Spt20 (1:1,000), Spt7 (1:1,000), Taf1 (1:2,000), Taf6 (1:2,000), Taf10 (1:2,000), Taf12 (1:1,000), Spt3 (1:1,000), Spt8 (1:1,000), Ada2 (1:1,000), and Gcn5 (1:1,000). Horseradish peroxidase (HRP)-conjugated secondary antibodies (Bio-Rad) were used at a 1:5,000 dilution and were detected by chemiluminescence.

Mono Q fractionations. Extracts were fractionated using a Mono Q column on an AKTA fast-performance liquid chromatograph (FPLC) (Amersham Pharmacia) (10). Cells were grown in 1 liter of YEPD to approximately 2×10^7 cells/ml, and clarified lysate was prepared as described in the previous section. Purification of approximately 10 mg of extract was achieved by fractionation on a 1-ml Mono Q column with a 100 to 500 mM NaCl gradient in the following buffer: 50 mM Tris-HCl (pH 8.0), 10% glycerol, 0.1% Tween 20, and protease inhibitors (2 μg of leupeptin/ml, 2 μg of pepstatin A/ml, 1 mM PMSF). Every other fraction was analyzed by Western blotting for the presence of SAGA subunits.

RNA isolation and Northern analysis. To prepare RNA for Northern analyses, cells were grown in liquid media to a concentration of 1×10^7 to 2×10^7 cells/ml. For induction of HIS3 and TRP3, 40 mM 3-aminotriazole (Sigma) was added to cells grown in SC-His; cultures were induced for 2 h before harvesting. RNA isolation and Northern analysis were performed as previously described (1, 40). All probes were synthesized by PCR amplification of each open reading frame from genomic DNA and labeled with [α-³²P]dATP by random priming (1). RNA levels were quantitated using the Molecular Dynamics PhosphorImager and normalized to the level of ACT1 mRNA.

RESULTS

Levels of Spt20 and Ada1 are dependent on Spt7. Both biochemical and genetic evidence implicate Spt7, Spt20, and Ada1 as core components of the SAGA complex. From these studies, no distinction is apparent between the requirement for Spt7, Spt20, or Ada1 in SAGA integrity or assembly. To test if they might control each other's protein levels, we measured the amounts of these three core components in spt7Δ, spt20Δ, and ada1Δ mutants. Western blottings of whole-cell extracts (Fig. 1A) demonstrated that in an spt7Δ mutant, both Spt20 and Ada1 proteins are barely detectable. Conversely, the level of Spt7 is only mildly decreased in an ada1Δ mutant, although a more significant decrease is observed in an spt20Δ mutant. Finally, Spt20 and Ada1 affect each other's levels, although the effects are not nearly as severe as those caused by spt7Δ. These results suggest that Spt7 plays an important in vivo role in maintaining appropriate levels of these other two SAGA core

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype
FY3	*MAT*a *ura3-52*
FY632	*MAT*a/*MAT*α *his4-917δ his4-917δ lys2-173R2/lys2-173R2 leu2Δ1 leu2Δ1 ura3-52/ ura3-52 trp1Δ63/trp1Δ63*
FY971	*MAT*a *gcn4Δ::LEU2 lys2-173R2 leu2Δ1 ura3-52*
FY1093	*MAT*a *spt7Δ402::LEU2 his4-917δ lys2-173R2 leu2Δ1 ura3-52 trp1Δ63 ade8*
FY1291	*MAT*α *spt20Δ200::ARG4 arg4-12 trp1Δ63 leu2Δ1 lys2-173R2 ura3-52*
FY1977	*MAT*α *HA-SPT20 his4-917δ lys2-173R2 leu2Δ0 ura3Δ0*
FY2025	*MAT*a *HA-SPT7-Myc ura3Δ0 his3Δ200 leu2Δ0 lys2-128δ*
FY2026	*MAT*a *HA-SPT20 his3Δ200 spt7Δ403::URA3 ura3Δ0 leu2Δ0 lys2-128δ*
FY2027	*MAT*a *HA-SPT20 ada1Δ::HIS3 his3Δ200 leu2Δ0 lys2-173R2 his4-917δ ura3Δ0 trp1Δ63*
FY2028	*MAT*a *spt7Δ404::HIS3 ura3Δ0 his3Δ200 leu2Δ0 lys2-128δ*
FY2029	*MAT*a *HA-spt7-100 ura3-52 his3Δ200 leu2Δ1 lys2-128δ trp1Δ63*
FY2030	*MAT*a *HA-SPT7 ura3Δ0 leu2Δ1 trp1Δ63 his4-917δ lys2-173R2*
FY2031	*MAT*a *HA-SPT7-TAP::TRP1 ura3Δ0 leu2Δ1 trp1Δ63 his4-917δ lys2-173R2*
FY2032	*MAT*a *HA-spt7-1125–TAP::TRP1 ura3Δ0 leu2Δ1 trp1Δ63 his4-917δ lys2-173R2*
FY2033	*MAT*a *HA-spt7-1180–TAP::TRP1 ura3Δ0 leu2Δ1 trp1Δ63 his4-917δ lys2-173R2*
FY2034	*MAT*a *HA-SPT7-TAP::TRP1 ura3Δ0 leu2Δ1 his3Δ200 gcn5Δ::HIS3 trp1Δ63 lys2-173R2*
FY2035	*MAT*a *HA-SPT7-TAP::TRP1 ura3Δ0 leu2Δ1 trp1Δ63 his3Δ200 ada1Δ::HIS3 lys2-173R2 his4-917δ*
FY2036	*MAT*a *HA-SPT7-TAP::TRP1 ura3Δ0 leu2Δ1 trp1Δ63 his4-917δ lys2-173R2 spt20Δ100::URA3*
FY2037	*MAT*a *HA-SPT7-TAP::TRP1 trp1Δ63 his4-917δ lys2-173R2 spt8Δ302::LEU2 ura3-52*
FY2038	*MAT*a *HA-SPT7-Myc leu2Δ1 spt8Δ302::LEU2 ura3-52 lys2-128δ his4-917δ trp1Δ63*
FY2039	*MAT*α *HA-SPT7-Myc leu2Δ0 spt8Δ302::LEU2 ura3-52 trp1Δ63*
FY2040	*MAT*a *HA-SPT7-TAP::TRP1 trp1Δ63 spt3Δ203::TRP1 ura3Δ0 his4-917δ lys2-173R2*
FY2041	*MAT*α *HA-SPT7-TAP ada1Δ::HIS3 spt20Δ100::URA3 his4-917δ trp1Δ63 lys2-173R2 his3Δ200 ura3Δ0*
FY2042	*MAT*a *HA-spt7-873–TAP::TRP1 ura3Δ0 leu2Δ1 trp1Δ63 his4-917δ lys2-173R2*
FY2043	*MAT*a *HA-spt7-200–TAP::TRP1 ura3Δ0 leu2Δ1 trp1Δ63 his4-917δ lys2-173R2*
FY2044	*MAT*a *HA-spt7-300–TAP::TRP1 ura3Δ0 leu2Δ1 trp1Δ63 his4-917δ lys2-173R2*
FY2045	*MAT*a *HA-spt7-200–Myc ura3Δ0 his3Δ200 leu2Δ0 lys2-128δ*
FY2046	*MAT*a *HA-spt7-300–Myc ura3Δ0 his3Δ200 leu2Δ0 lys2-128δ*
FY2047	*MAT*α *HA-spt7-200–TAP::TRP1 arg4-12 ura3-52 trp1Δ63 lys2-173R2*
FY2048	*MAT*α *HA-spt7-300–TAP::TRP1 arg4-12 trp1Δ63 lys2-173R2 ura3Δ0*
FY2049	*MAT*a *HA-spt7-400–TAP::TRP1 lys2-173R2 trp1Δ63 ura3Δ0*
FY2050	*MAT*a *HA-spt7-1180–TAP::TRP1 spt8Δ302::LEU2 his4-917δ trp1Δ63 leu2Δ0 ura3-52 lys2-173R2*
FY2051	*MAT*a *HA-spt7-1180–TAP::TRP1 gcn5Δ::HIS3 lys2-173R2 leu2Δ1 ura3Δ0 trp1Δ63*
FY2052	*MAT*a *HA-spt7-1180–TAP::TRP1 spt3-401 his4-917δ lys2-173R2 leu2Δ1 trp1Δ63 ura3-52*
FY2053	*MAT*a *HA-spt7-1125–TAP::TRP1 spt8Δ302::LEU2 his4-917δ trp1Δ63 leu2Δ1 ura3-52 lys2-173R2*
FY2054	*MAT*a *HA-spt7-1125–TAP::TRP1 gcn5Δ::HIS3 ura3Δ0 leu2Δ1 trp1Δ63 lys2-173R2*
FY2055	*MAT*α *HA-spt7-1125–TAP::TRP1 spt3-401 his4-917δ lys2-173R2 ura3Δ0 trp1Δ63 leu2Δ1*
FY2056	*MAT*a *HA-spt7-200–TAP::TRP1 spt8Δ302::LEU2 ura3-52 his4-917δ lys2-173R2 trp1Δ63 leu2Δ1*
FY2057	*MAT*α *HA-spt7-200–TAP::TRP1 gcn5Δ::HIS3 ura3Δ0 his4-917δ lys2-173R2 trp1Δ63 leu2Δ1*
FY2058	*MAT*α *HA-spt7-200–TAP::TRP1 spt3-401 his4-917δ lys2-173R2 leu2Δ1 ura3Δ0 trp1Δ63*
FY2059	*MAT*a *HA-spt7-300–TAP::TRP1 spt8Δ302::LEU2 ura3-52 his4-917δ lys2-173R2 trp1Δ63 leu2Δ1*
FY2060	*MAT*a *HA-spt7-300–TAP::TRP1 gcn5Δ::HIS3 his4-917δ lys2-173R2 leu2Δ1 arg4-12 ura3Δ0 trp1Δ63*
FY2061	*MAT*a *HA-spt7-300–TAP::TRP1 spt3-401 his4-917δ lys2-173R2 leu2Δ1 ura3Δ0*
FY2062	*MAT*a/*MAT*α *HA–spt7-100/ HA–spt7-873–TAP::TRP1 ura3Δ0/ura3Δ0 his3Δ200/HIS3 leu2Δ0/ leu2Δ1 lys2-128δ/lys2-173R2 trp1Δ63/TRP1 his4-917δ/HIS4*
FY2063	*MAT*a/*MAT*α *HA–spt7-100/HA–spt7-100–TAP::TRP ura3Δ0/ura3Δ0 his3Δ200/his3Δ200 leu2Δ1/leu2Δ0 lys2-128δ/lys2-128δ trp1Δ63/TRP1*
FY2064	*MAT*a/*MAT*α *HA–spt7-873–Myc/HA–spt7-873–TAP::TRP1 ura3Δ0/ura3Δ0 leu2Δ1/leu2Δ0 trp1Δ63/TRP1 his4-917δ/HIS4 his3Δ200/HIS3 lys2-173R2/lys2-128δ*
FY2065	*MAT*a/*MAT*α *spt7Δ403::URA3/spt7Δ404::HIS3 ura3Δ0/ura3Δ0 his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2-128δ/lys2-128δ*
L861	*MAT*α *spt3-401 his4-917δ lys2-173R2 leu2Δ1 ura3-52*

components and therefore in determining the level of the entire SAGA complex.

As SAGA is a transcriptional coactivator, we wanted to determine whether the reduction in protein levels observed in *spt7Δ*, *spt20Δ*, and *ada1Δ* mutants occurs transcriptionally or posttranscriptionally. Northern analysis (Fig. 1B) shows that deletion of either *SPT7*, *SPT20*, or *ADA1* does not significantly alter the mRNA levels of the two remaining genes. The only reproducible effect observed was an approximately twofold increase in the level of *SPT7* mRNA in *ada1Δ* and *spt20Δ* mutants; however, this increase does not correlate with the decreased Spt7 protein levels in these strains. Thus, the effect

on core component protein levels in *spt7Δ*, *spt20Δ*, and *ada1Δ* mutants is posttranscriptional, probably at the level of stability.

Partial SAGA complexes form in *spt20Δ* and *ada1Δ* mutants. The results described in the previous section demonstrate the importance of Spt7 in the integrity of the SAGA complex. As a significant level of Spt7 exists in *spt20Δ* and *ada1Δ* mutants, it is possible that partial SAGA complexes assemble in these strains. To gain insight into the protein interactions within SAGA and potential intermediates in complex formation, we used the TAP tag to purify Spt7-containing complexes. First, we constructed a version of *SPT7* that encodes a TAP tag at the carboxy terminus of Spt7 and an HA
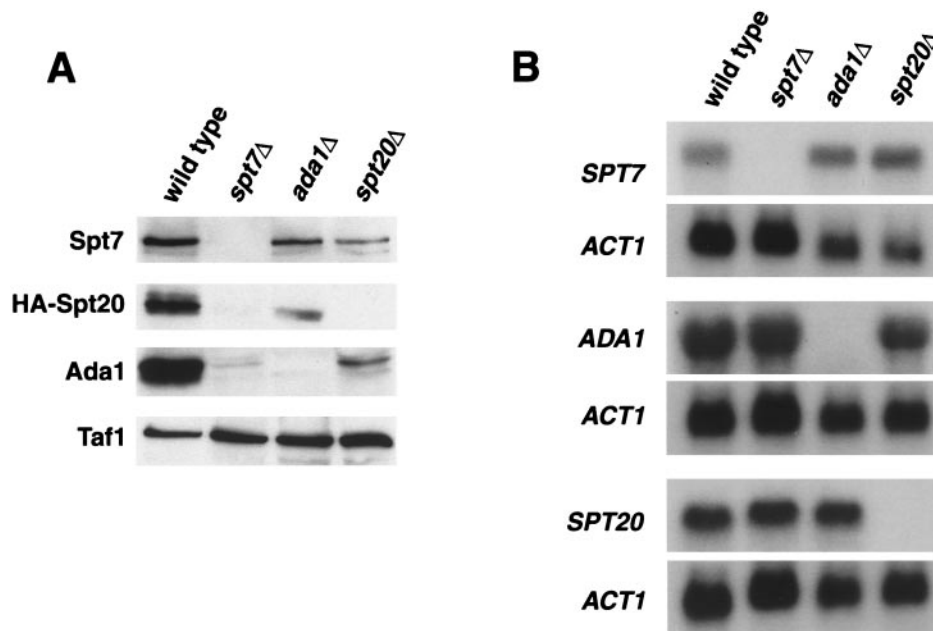
FIG. 1. Levels of Spt20 and Ada1 are dependent on Spt7. (A) Western analysis was performed to measure the levels of Spt7, Spt20, and Ada1. Wild-type (strain FY1977), *spt7*Δ (FY2026), *spt20*Δ (FY1291), and *ada1*Δ (FY2027) strains were grown in YEPD medium, and whole-cell extracts were prepared and subjected to Western blot analysis, using antibodies to Spt7, Ada1, and HA (to detect the HA-Spt20 fusion protein). Taf1 levels were measured as a loading control. (B) Northern analysis of mRNA levels in *spt7*Δ, *spt20*Δ, and *ada1*Δ mutants. The same wild-type, *spt7*Δ, *spt20*Δ, and *ada1*Δ strains were grown in YEPD medium, and RNA was isolated and analyzed by Northern analysis. *ACT1* mRNA levels were measured as a loading control.

epitope tag at the amino terminus. This version of *SPT7*, *HA-SPT7-TAP*, encodes wild-type Spt7 function by all phenotypes tested (data not shown). Second, strains were constructed that contained *HA-SPT7-TAP* in combination with deletions of genes that encode other SAGA components. Then, Spt7 and associated proteins were purified (see Materials and Methods) and were analyzed on silver-stained gradient SDS-PAGE gels (Fig. 2) and by Western analysis (Fig. 3). Silver stains were used as the primary indication for the presence of SAGA subunits, and these data were supplemented with Western analyses for the detection of low levels of associated proteins. In a wild-type background, the form of SAGA purified by Spt7-TAP (Fig. 2, lane 1) appeared to be identical to SAGA as previously purified by conventional chromatography (16).

As expected, both *spt20*Δ and *ada1*Δ mutations caused major disruptions to SAGA; however, partial complexes are present in both mutants. The *ada1*Δ mutant produced a partial complex that contained detectable levels of Spt8, Taf5, Taf6, Taf9, and Taf10 (Fig. 2, lane 7) by silver staining and a small amount of Spt20 visible by Western analysis (Fig. 3, lane 7). The absence of Taf12 agrees with a previously described interaction between Taf12 and Ada1 (14). The *spt20*Δ mutant also produced an altered complex distinct from that produced in either the wild-type or *ada1*Δ mutant (Fig. 2, lane 5). Although the silver stain shows the presence of only a few SAGA subunits, including Spt8, Taf6, Taf9, Taf10, and Ada1 (Fig. 2, lane 5), Western analysis indicates that this complex contained low but detectable levels of all SAGA proteins for which we tested (Fig. 3, lane 5). Finally, we also examined an *ada1*Δ *spt20*Δ double mutant and found that four SAGA proteins were still associated with Spt7, including Taf6, Taf9, Taf10, and Spt8

(Fig. 3, lane 6). The association of Taf10 with Spt7 in the absence of other core components supports previous findings (13).

Several conclusions can be made from these data. First, both Ada1 and Spt20 can associate with Spt7 in the absence of the other core member, albeit at reduced levels. Second, Tra1, the SAGA subunit required for the recruitment of SAGA by transcriptional activators in vitro (7), requires both Ada1 and Spt20 to be present in SAGA. Since SAGA from an *spt20*Δ mutant is still associated with at least most of the other known SAGA components, this result suggests that Tra1 is not absolutely required for normal assembly of other SAGA components. Third, we can tentatively assign four other SAGA proteins, Taf12, Spt3, Gcn5, and Ada2, as requiring Ada1 for association in SAGA. Fourth, the fact that at least four SAGA subunits associate with Spt7 in the *ada1*Δ *spt20*Δ double mutant suggests that they interact directly with Spt7 or with each other. Overall, these results demonstrate that Spt7, Spt20, and Ada1 are each required in distinct ways for the formation of SAGA and that partial SAGA complexes can form in the absence of two of the core subunits.

To determine the dependence of SAGA on three members involved in transcriptional control, we also purified SAGA from *spt3*Δ, *spt8*Δ, and *gcn5*Δ mutants. In contrast to loss of the core subunits, for each of these mutants we detected only the loss of the subunit corresponding to the deleted gene (Fig. 2 and 3, compare lane 1 with lanes 2, 3, and 4). Therefore, each of these proteins associates with SAGA independently of the others. These results correlate with previous data that showed that Spt3 and Spt8 are not dependent upon each other for their
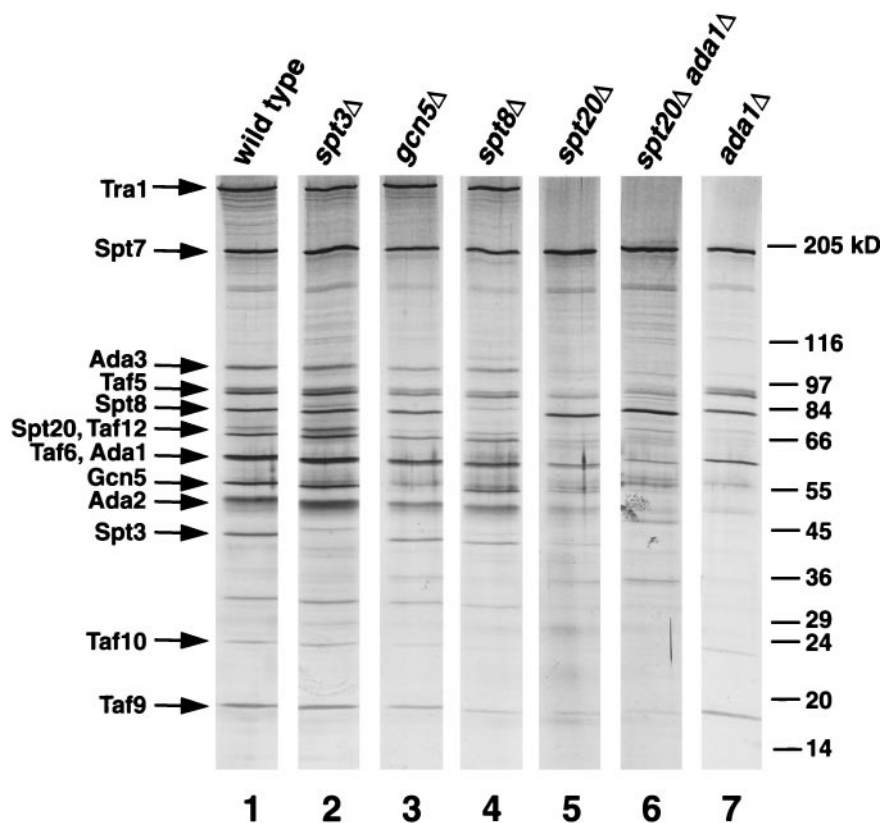
FIG. 2. Characterization of SAGA purified from wild-type and SAGA mutant strains. TAP-purified complexes from wild-type (strain FY2031), *spt3Δ* (FY2040), *gcn5Δ* (FY2034), *spt8Δ* (FY2037), *ada1Δ* (FY2035), *spt20Δ ada1Δ* (FY2041), and *spt20Δ* (FY2036) strains were prepared, run on SDS-PAGE (5 to 20% acrylamide) gels, and silver stained. The amount of each complex loaded was adjusted to have an equal level of Spt7 in each lane. All samples were run on one gel.

association with SAGA and that loss of each protein has only mild effects on the complex (38).

Biochemical and genetic characterization of Spt7 carboxy-terminal deletions. Based on past studies and our new results described in the previous section, Spt7 has an important structural role within SAGA, perhaps as a scaffold for several SAGA components. To determine regions of Spt7 required for interactions with other SAGA components, we constructed a set of *spt7* deletion mutations at the endogenous *SPT7* locus (see Materials and Methods). Since past work suggested that the carboxy-terminal portion of Spt7 was important for Spt7 function (13, 15), the deletions were constructed to remove increasing amounts from this end of the protein (Fig. 4A). These strains were then examined for a set of mutant phenotypes (Fig. 4B) and for the composition of the SAGA complex (Fig. 5). A deletion mutation that removes the carboxy-terminal 459 amino acids, *spt7-873*, confers phenotypes close to those of an *spt7* null mutation (Fig. 4B). Although it produces a stable product (Fig. 5, lane 6), this truncated protein does not associate with any SAGA components stoichiometrically. The region of *SPT7* missing in Spt7-873 contains the previously identified histone fold motif (amino acids 979 to 1045) (13). As we describe in a later section, expression of only these carboxy-terminal 459 amino acids is largely sufficient for Spt7 function.

Two smaller deletion mutations that leave the histone fold motif intact, *spt7-1180* and *spt7-1125*, cause different mutant

phenotypes and were studied further. The larger deletion mutant, *spt7-1125*, causes stronger Gal⁻, Ino⁻, and Spt⁻ phenotypes than does *spt7-1180* (Fig. 4B; Table 2), although the proteins are present at the same level (data not shown). To correlate the phenotypes with biochemical interactions, we purified SAGA from these strains. For both deletions, the only detectable difference from wild-type SAGA was the absence of Spt8, indicating that a region in the carboxy-terminal 152 amino acids of Spt7 is required for the association of Spt8 with SAGA (Fig. 5, compare lane 1 with lanes 4 and 5). This result is further supported by the observation that the carboxy-terminal 459 amino acids of Spt7 are sufficient for interactions with Spt8 (data not shown).

Since *spt7-1125* causes more severe phenotypes than does *spt7-1180*, we analyzed the region between amino acids 1125 and 1180 in greater detail. First, we constructed an internal deletion of this region (*spt7-400*; Fig. 4A) and observed that this mutation caused phenotypes identical to those of *spt7-1125* (data not shown). Next, we divided this region by two smaller deletions: *spt7-200* removes codons 1125 to 1150 and *spt7-300* removes codons 1150 to 1180 (Fig. 4A). The *spt7-300* mutation caused phenotypes very similar to those of *spt7-1180* (Fig. 4B). We also noted that both *spt7-1180* and *spt7-300* caused phenotypes very similar to an *spt8Δ* mutation (Table 2). Consistent with these mutant phenotypes, Spt8 failed to associate with SAGA in the *spt7-300* mutant (Fig. 5, lane 3). These results
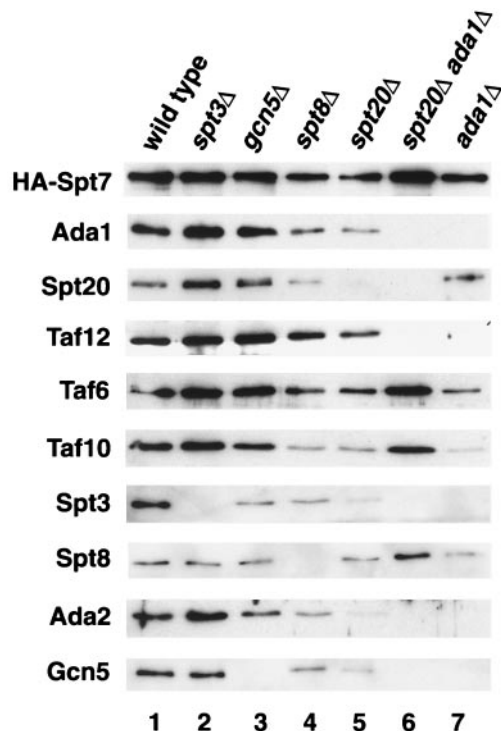
FIG. 3. Western analysis of TAP-purified mutant SAGA complexes. TAP-purified complexes from the same extracts as in Fig. 2 were run on SDS-PAGE gels and Western blotted using the following antibodies: HA (12CA5), Ada1, Spt20, Taf6, Taf10, Taf12, Spt3, Spt8, Ada2, and Gcn5.

strongly suggest that a region of Spt7 that spans amino acid 1180 is required for interaction with Spt8. In contrast, analysis of the *spt7-200* mutant showed that it was phenotypically distinct from *spt7-300*. The *spt7-200* mutation caused no mutant phenotypes among those tested (Fig. 4B), and Spt8 is still associated with SAGA in this mutant (Fig. 5, lane 2). The *spt7-200* mutant is also wild type for several additional phenotypes that are possessed by an *spt7* null mutant, including sensitivities to caffeine, hydroxyurea, formamide, 3-aminotriazole, and mycophenolic acid and the inability to utilize glycerol as the sole carbon source (data not shown). Therefore, while the *spt7-200* deletion strengthens mutant phenotypes when combined with deletion of the adjacent region, it does not cause detectable phenotypes in an otherwise wild-type background. Thus, two regions of Spt7 are defined by these deletions: one required for association with Spt8 and a second shown in a later section to be required for carboxy-terminal processing.

Further genetic characterization of Spt7 carboxy-terminal deletions. To test if the *spt7-1180* and *spt7-300* mutations are truly genetically equivalent to *spt8Δ*, we performed three additional genetic tests. First, double mutants were constructed between *spt8Δ* and each of these *spt7* mutations. If the sole defect in these *spt7* mutants was loss of Spt8, then the double mutants should have no greater phenotype than any of the single mutants. Indeed, this result was observed (Table 2; compare *spt8Δ*, *spt7-1180*, *spt7-1180 spt8Δ*, *spt7-300*, and *spt7-300 spt8Δ*). Second, double mutants were constructed between

each of these *spt7* mutations and *gcn5Δ*. Previous results showed that *spt8Δ gcn5Δ* double mutants have more severe phenotypes than either single mutant. Again, the *spt7* mutations mimicked the *spt8Δ* mutation, as the *spt7-1180 gcn5Δ* and *spt7-300 gcn5Δ* double mutants have significantly more severe phenotypes than any of the single mutants (Table 2, compare *spt8Δ*, *gcn5Δ*, *spt8Δ gcn5Δ*, *spt7-1180*, *spt7-1180 spt8Δ*, *spt7-1180 gcn5Δ*, *spt7-300*, and *spt7-300 gcn5Δ*). Third, we tested the *spt7-300* and the *spt7-1180* mutations with respect to a previously described genetic interaction between *spt8Δ* and a mutation in another SAGA-encoding gene, *SPT3*. In this case, it was demonstrated that *spt8Δ* and the *spt3-401* mutation mutually suppress each other with respect to their Spt⁻ mutant phenotypes (12). Therefore, to test if the *spt7-1180* and *spt7-300* mutations act like *spt8Δ*, we constructed *spt7-1180 spt3-401* and *spt7-300 spt3-401* double mutants. As observed for *spt8Δ*, these *spt7* mutations exhibited mutual suppression when in combination with *spt3-401* (Table 3). Thus, we have shown by several genetic tests that the only role of the region defined by the *spt7-300* and *spt7-1180* deletions is to assemble or maintain Spt8 in the SAGA complex.

We also analyzed *spt7-1125* and *spt7-200* by an analogous set of double mutants to those described above for *spt7-1180* and *spt7-300*. Mutant analysis of *spt7-1125* and *spt7-200* suggested that the region of Spt7 from amino acids 1125 to 1150 is required for a function unrelated to the association with Spt8. The results of the double mutant analysis showed that *spt7-1125* behaves similarly to *spt7-1180* with respect to interactions with *spt8Δ* and *gcn5Δ* (Table 2, *spt7-1125*, *spt7-1125 spt8Δ*, and *spt7-1125 gcn5Δ*). However, unlike *spt7-1180*, the Spt phenotype of *spt7-1125* is not suppressed by *spt3-401* (Table 3), supporting the idea that this deletion impairs Spt7 beyond losing its ability to interact with Spt8. The analysis of double mutants with *spt7-200* was consistent with this hypothesis, as *spt7-200* caused a mutant phenotype when in combination with *spt8Δ* (Table 2, *spt7-200* and *spt7-200 spt8Δ*). The *spt7-200 spt8Δ* double mutant showed synthetic Ino⁻, Gal⁻, and Spt⁻ phenotypes, similar to those of *spt7-1125*. Additionally, in a triple mutant, *spt7-200* negated the suppression of *spt8Δ* by *spt3-401* (data not shown). The phenotypes of the *spt7-200 spt8Δ* double mutant, then, are the same as the more severe phenotypes of *spt7-1125* and are indicative of the loss of two distinct Spt7 functions. Taken together, these data support the existence of a function for the 1125 to 1150 region of Spt7.

Intragenic complementation by Spt7 amino- and carboxy-terminal deletions. The deletion analysis of Spt7 described above demonstrated that the carboxy terminus is necessary for critical functions of the wild-type protein. To determine if this portion of the protein is sufficient for Spt7 function, we constructed a mutant lacking the first 873 codons of *SPT7*, *spt7-100* (Fig. 4A). The design of this mutant was based on a previous observation that a cloned restriction fragment that encodes the carboxy-terminal fragment of Spt7 can partially complement an *spt7Δ* mutation (15). Indeed, the *spt7-100* mutant is only partially defective with respect to several phenotypes caused by *spt7* mutations, including growth, Spt, Gal, and Ino (Fig. 6A and data not shown). As the Spt7 carboxy terminus provides some, but not all, functions of the wild-type protein, we investigated if coexpression of two nonoverlapping portions of Spt7, using the *spt7-100* (encoding amino acids 874 to 1132) and
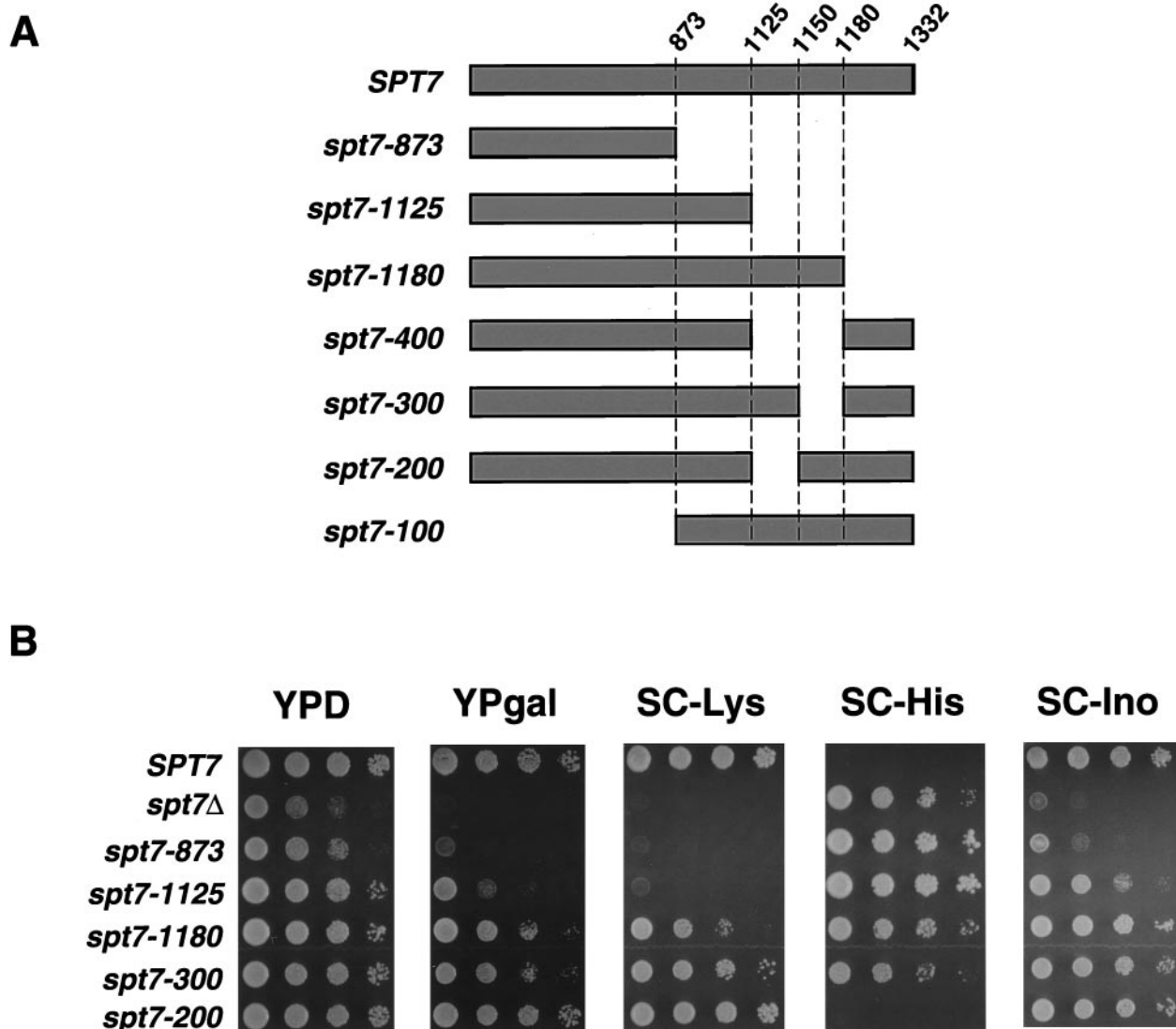
FIG. 4. Spt7 deletion analysis. (A) Diagram of Spt7 deletion mutants described in this study. Deletion mutations were constructed as described in Materials and Methods. (B) Phenotypic and biochemical analyses of *spt7* deletion mutants. The strains used are as follows: wild-type (strain FY2031), *spt7Δ* (FY1093), *spt7-873* (FY2042), *spt7-1125* (FY2032), *spt7-1180* (FY2033), *spt7-300* (FY2044), and *spt7-200* (FY2043). All strains contain the *his4-917δ* and *lys2-173R2* alleles for determining the Spt phenotype (see Materials and Methods). An Spt⁻ phenotype corresponds to growth on media lacking histidine and no growth on media lacking lysine. Cells were grown to saturation overnight in YEPD medium and spotted in a dilution series from 10^8 to 10^4 cells/ml, and the plates were incubated at 30°C. The plates shown were incubated for the following times: YEPD, 2 days; SC-Lys, 3 days; SC-His, 4 days; YPgal, 3 days; SC, 2 days; and SC-Ino, 2 days.

spt7-873 (encoding amino acids 1 to 873) mutations, might be able to complement. To do this test, a diploid strain containing *spt7-100* and *spt7-873* was constructed and its phenotypes were determined (Fig. 6A). The *spt7-100/spt7-873* heterozygote exhibits phenotypes close to those of an *SPT7⁺/SPT7⁺* strain, demonstrating intragenic complementation.

To determine whether the intragenic complementation arose from the partial function of two different mutant SAGA complexes or, alternatively, from the assembly of the two partial Spt7 products into a single SAGA complex, we purified SAGA from an *spt7-100/spt7-873* heterozygote containing TAP-tagged Spt7-873. Silver staining showed that the complex from this strain appears similar to that from an *spt7-873* mutant (data not shown). However, by Western analysis, we de-

tected copurification of Spt7-100 with Spt7-873, suggesting the presence of both Spt7 truncations in one complex (Fig. 6B, lane 3). This evidence, along with the genetic complementation, indicates that the amino- and carboxy-terminal portions of Spt7 can be coassembled to form a largely functional SAGA complex.

Spt7 processing occurs in the carboxy terminus. The SAGA complex has been shown to exist in an alternate form, referred to here as SLIK/SALSA (4, 18, 34; D. Sterner and S. Berger, personal communication). Characterization of SLIK/SALSA has shown that it differs from SAGA in at least two ways: it contains a smaller form of Spt7 and lacks Spt8 (4; Patrick Grant, personal communication). The smaller size of Spt7 in SLIK/SALSA suggests that it is proteolytically processed. To
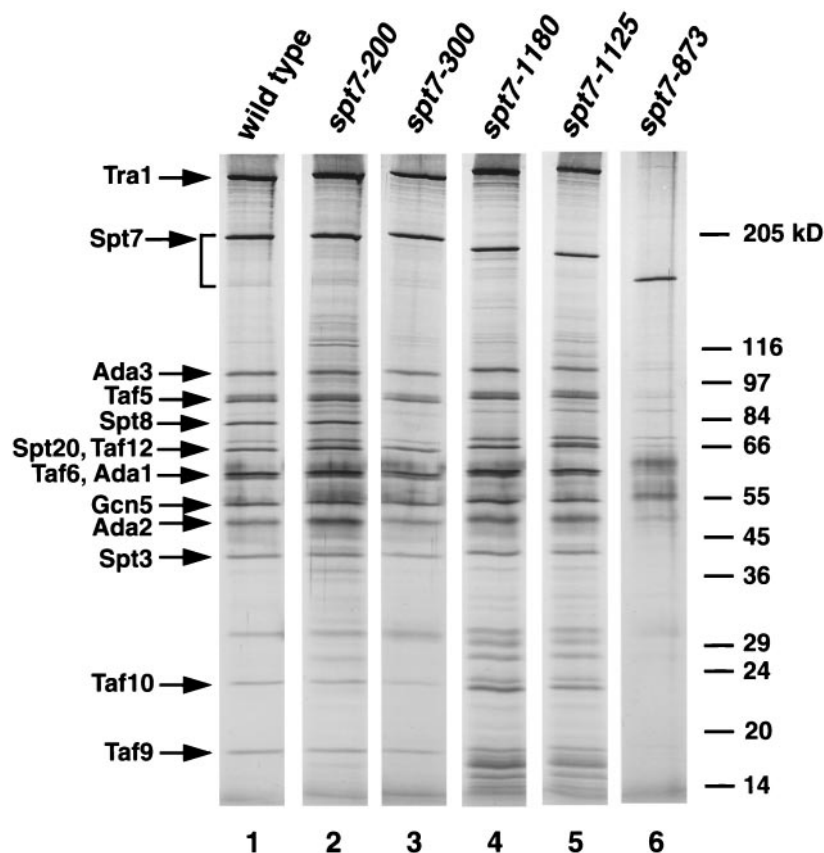
FIG. 5. Silver stain of SAGA purified from wild-type and *spt7* mutant strains. TAP-purified SAGA complex from strains analyzed for phenotypes in Fig. 4B were visualized on an SDS-PAGE (5 to 20% acrylamide) gel by silver stain. The amount of each complex loaded was adjusted to have an equal level of Spt7 in each lane. All samples were run on one gel. The additional faint bands present in lanes 4 and 5 were not observed reproducibly and are not part of SAGA.

test this idea, full-length Spt7 was epitope tagged at both the amino and carboxy termini with HA and Myc tags, respectively. This doubly tagged version of Spt7 had wild-type function as determined by several phenotypic tests (data not shown). To examine the two forms of Spt7, whole-cell extracts were fractionated on a Mono Q column, previously shown to separate SAGA and SLIK/SALSA (4), and fractions were assayed for each Spt7 epitope tag by Western analysis (Fig. 7). Our results demonstrate that the smaller form of Spt7 can be detected with the antibody against the amino-terminal HA epitope tag but not with the antibody against the carboxy-terminal Myc epitope tag (Fig. 7, top, fractions 34 and 36). These results strongly suggest that the smaller form of Spt7 arises from the removal of part of its carboxy terminus. Confirmation of this result was obtained by probing whole-cell extracts with the HA and Myc antibodies (Fig. 8, upper panels). Consistent with results from the Mono Q fractionation, both forms of Spt7 are detectable with the antibody against the amino-terminal HA epitope, while only the longer form is detectable with the antibody against the carboxy-terminal Myc epitope. Moreover, a processed piece of Spt7 with a molecular mass of approximately 40 kDa containing the Myc epitope can be observed by Western blotting (Fig. 8, lower panel). These results indicate that Spt7 processing occurs in the carboxy terminus and is

TABLE 2. Genetic analysis of *spt7* deletion mutants

Relevant genotype	Phenotypes[a]			
	Growth	Spt	Ino	Gal
SPT7[+]	+	+	+	+
spt8Δ	+/−	−/+	+	+/−
gcn5Δ	+/−	+/−	+	+/−
spt8Δ gcn5Δ	−	−	−	−/+
spt7-1180	+/−	−/+	+	+/−
spt7-1180 spt8Δ	+/−	−/+	+	+/−
spt7-1180 gcn5Δ	−	−	−/+	−/+
spt7-300	+/−	+/−	+	+/−
spt7-300 spt8Δ	+/−	−/+	+	+/−
spt7-300 gcn5Δ	−	−	−/+	−/+
spt7-1125	−/+	−	+/−	−/+
spt7-1125 spt8Δ	−/+	−	+/−	−/+
spt7-1125 gcn5Δ	−	−	−	−
spt7-200	+	+	+	+
spt7-200 spt8Δ	−/+	−	+/−	−/+
spt7-200 gcn5Δ	+/−	+/−	+/−	+/−

[a] Representative strains for each genotype tested above are listed in Table 1. Growth was assessed by streaking for single colonies on YEPD plates, and other phenotypes were determined by replica plating. All phenotypes were scored as +, +/−, −/+, or − (+, wild-type phenotype; −, the *spt7* null phenotype). The phenotypes tested were as follows: Ino, inositol auxotrophy; Gal, utilization of galactose as the sole carbon source; Spt, suppression of the *lys2-173R2* and/or *his4-917δ* alleles.

TABLE 3. *spt3-401* suppresses *spt7-1180* and *spt7-300* but not *spt7-1125*

Genotype	Spt phenotype[a]
Wild type	+
spt3-401	−
spt8Δ	−
spt7-1180	−
spt7-300	−/+
spt7-1125	−
spt3-401 spt8Δ	+/−
spt3-401 spt7-1180	+/−
spt3-401 spt7-300	+/−
spt3-401 spt7-1125	−

[a] Phenotypes were determined by growing patches of cells on YEPD plates and then replica plating onto synthetic complete plates lacking histidine (SC-His) to assess suppression of *his4-917δ* (Spt⁻ phenotype). Phenotypes are scored on a scale using +, +/−, −/+, and − (+, wild-type phenotype; −, the *spt8Δ* phenotype).

unlikely to be an artifact of fractionation, because it is observed out of whole-cell extracts.

To map the region of Spt7 involved in this processing event, several *spt7* deletion mutants described in a previous section were analyzed. Extracts from *spt7-1180* and *spt7-1125* strains were fractionated on a Mono Q column and Western blotted. The Spt7-1180 protein was processed while Spt7-1125 protein was not (data not shown), suggesting that amino acids 1125 to 1180 of Spt7 are required for the processing to occur. To delineate further the region involved in processing, we examined the two *spt7* internal deletion mutants that are missing either amino acids 1125 to 1150 (in *spt7-200*) or 1150 to 1180 (in *spt7-300*). The *spt7-200* mutation greatly impairs Spt7 processing, as there are barely detectable levels of the shorter form of Spt7 in this mutant (Fig. 7 and 8). In contrast, the *spt7-300* mutation causes no detectable effect on processing
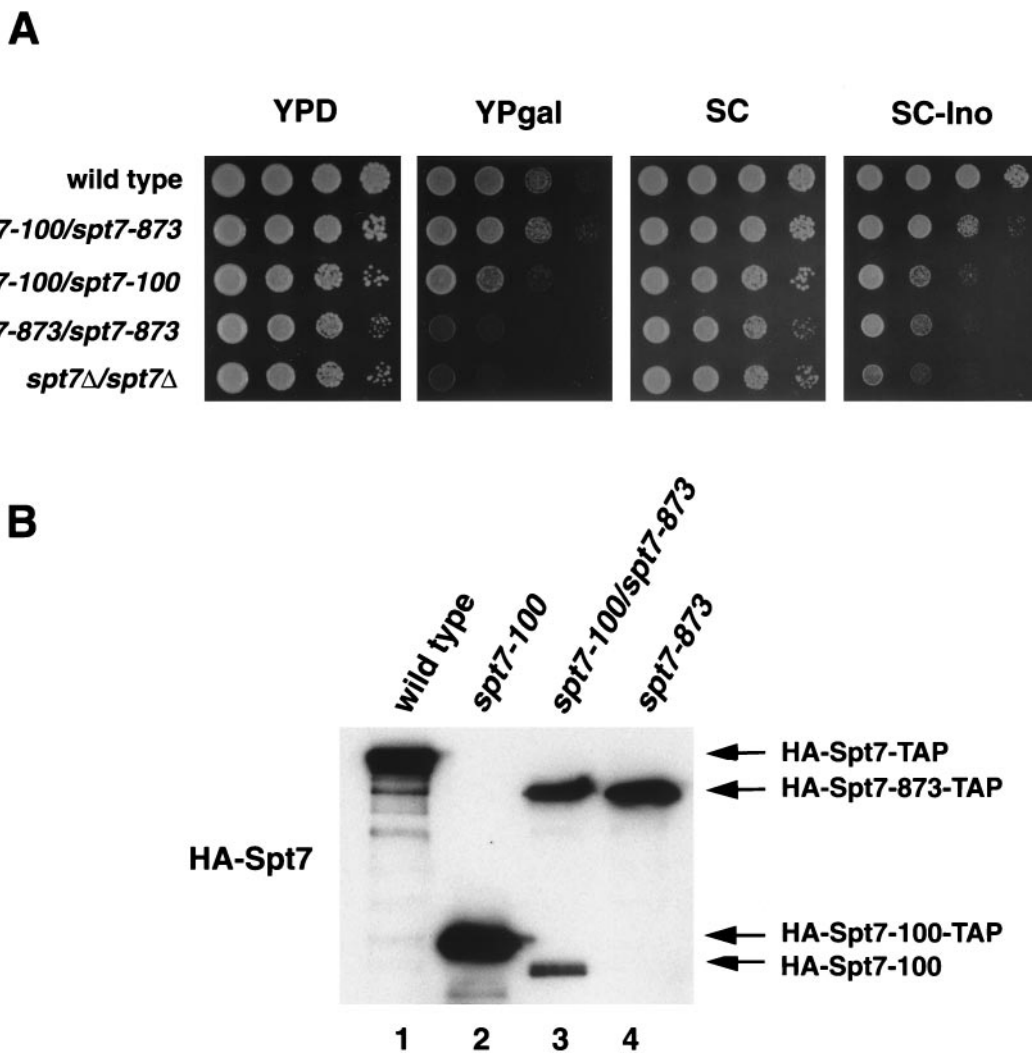

FIG. 6. Analysis of intragenic complementation between *spt7-100* and *spt7-873*. (A) Comparison of the phenotypes of wild-type (strain FY632), *spt7Δ/spt7Δ* (FY2065) *spt7-100/spt7-100* (FY2063), *spt7-873/spt7-873* (FY2064), and *spt7-873/spt7-100* (FY2062) diploids. Cells were grown to saturation overnight in YEPD medium and spotted in a dilution series from 10⁸ to 10⁴ cells/ml, and the plates were incubated at 30°C for 2 days. (B) Western analysis of TAP-purified complexes from wild-type (FY2031), *spt7-100* (FY2029), and *spt7-873* (FY2042) haploids as well as *spt7-873/spt7-100* (FY2062) heterozygous diploids. Purified complexes were run on SDS-PAGE (8% acrylamide) gels and probed using the HA (12CA5) antibody.
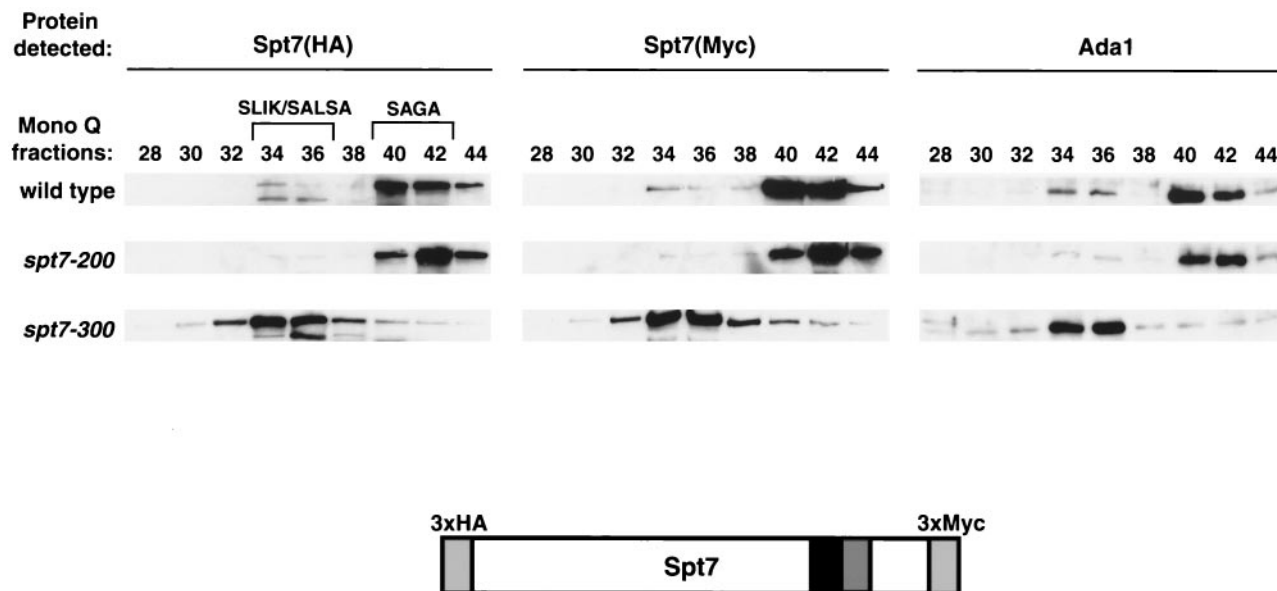
FIG. 7. Spt7 in SLIK/SALSA lacks part of the carboxy terminus. Mono Q fractionation of yeast whole-cell extract from wild-type (strain FY2025), *spt7-200* (FY2045), and *spt7-300* (FY2046) strains expressing HA- and Myc-tagged Spt7. Western analyses of Mono Q fractions were probed with antibodies to HA or Myc (to detect Spt7) as well as antibodies to Ada1. The diagram depicts the doubly-tagged Spt7 protein; Spt7-200 lacks the region in black, and Spt7-300 lacks the region in gray. The faster-migrating band in the panel showing the anti-HA antibody-probed blot of Mono Q fractions from a wild-type strain represents the carboxy-terminally processed form of Spt7. The presence of Ada1 in the fractions containing SLIK/SALSA in the *spt7-200* panel is likely due to the loss of Spt8 from SAGA upon fractionation.

(Fig. 7 and 8). Similar to an *spt8Δ* mutant, the *spt7-300* mutant has an altered elution profile on a Mono Q column. These results demonstrate that adjacent regions of Spt7, as defined by *spt7-200* and *spt7-300*, are required independently for Spt7 processing and for association with Spt8, respectively. Furthermore, they show that Spt8 is not required for Spt7 processing, a conclusion confirmed by the presence of processed Spt7 in an *spt8* deletion mutant (Fig. 8, lane 5). Finally, the results from

FIG. 8. Analysis of yeast whole-cell extract from untagged and tagged wild-type (strain FY2025), *spt7-200* (FY2045), *spt7-300* (Y2046), and *spt8Δ* (FY2038) strains on Western blots probed with HA (12CA5) or Myc (A14) antibodies. Equal amounts of protein were loaded in each lane.

the Mono Q fractionation show that the *spt7-200* mutant has severely reduced levels of SLIK/SALSA, using Ada1 as a marker for the presence of both SAGA and SLIK/SALSA. The low level of Ada1 in the characteristic SLIK/SALSA fractions may actually be due to SAGA that has lost Spt8 during fractionation, since we see the larger form of Spt7 in those fractions both wild-type and *spt7-200* strains as well.

Nonprocessed Spt7 mutant has normal transcriptional activation of *HIS3* and *TRP3*. The identification of a nonprocessed form of Spt7 allowed us to examine the requirement for SLIK/SALSA in vivo. Previous studies showed that, under conditions of amino acid starvation, SLIK/SALSA is a predominant form of SAGA-related complexes (4). This finding raised the possibility that SLIK/SALSA is required for activation of genes under general amino acid control. We tested the requirement for SLIK/SALSA under these conditions by measuring the level of *HIS3* and *TRP3* mRNAs in the *spt7-200* mutant, where SLIK/SALSA levels are severely reduced. Our results (Fig. 9) show that there is no significant effect on activated levels of *HIS3* or *TRP3* mRNAs in the *spt7-200* mutant. In addition, the level of these transcripts is normal in the *spt7* mutants that have lost interaction with Spt8. These results strongly suggest that SLIK/SALSA is not necessary for activation of *HIS3* or *TRP3* and may not be required in general for activation via the general amino acid control pathway.

Previous studies also showed that *spt8Δ* and *spt3Δ* mutations cause increased levels of basal transcription of *TRP3* and *HIS3*, suggesting a possible role for SAGA in controlling the uninduced levels of mRNAs of these genes (4). Northern analysis (Fig. 9) shows that there is an approximately twofold increase in the uninduced levels of *HIS3* and *TRP3* mRNAs in all of the *spt7* mutants, including *spt7-200*, in which Spt7 processing does
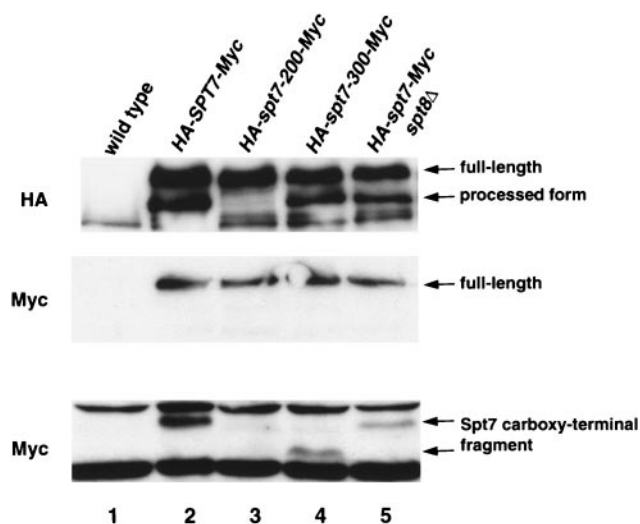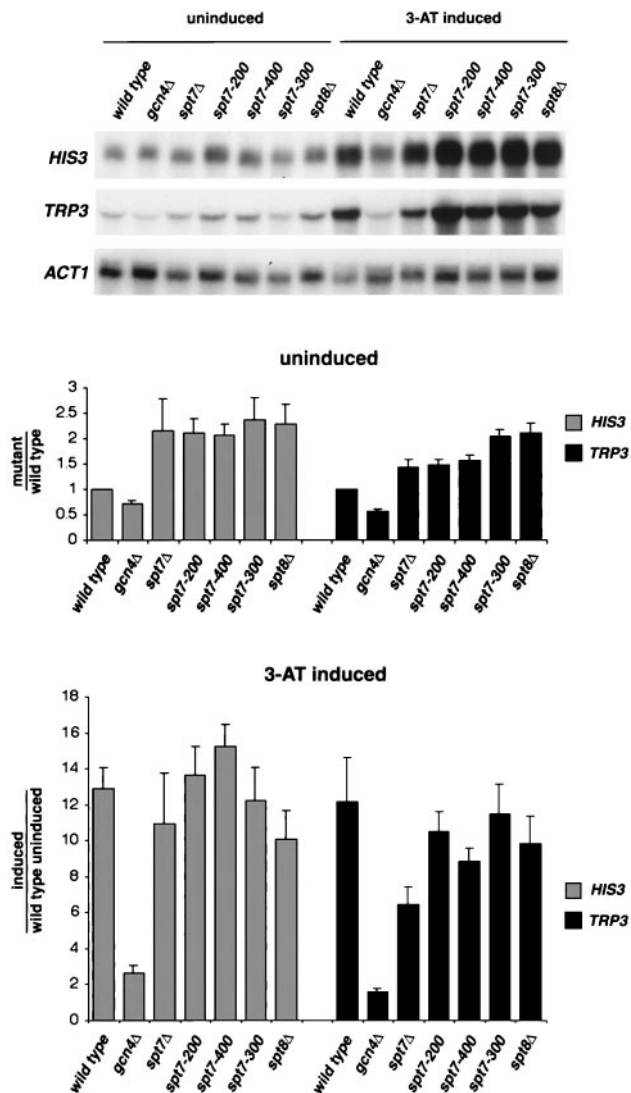
FIG. 9. Northern analysis of *spt7* internal deletion mutants. Wild-type (strain FY3), *gcn4Δ* (FY971), *spt7Δ* (FY2028), *spt7-200* (FY2047), *spt7-300* (FY2048), *spt7-400* (FY2049), and *spt8Δ* (FY2039) mutants were grown in SC-His to approximately 1×10^7 to 2×10^7 cells/ml and induced with 40 mM 3-aminotriazole for 2 h. RNA was then made from uninduced and induced samples. Northern blots were probed separately with *HIS3*, *TRP3*, and *ACT1* DNA probes. Shown is a representative Northern blot. The bar graphs below the Northern blot show the average and standard error measured for each mRNA level, with the wild-type, uninduced level equal to 1.0. Each value was determined three to six times.

not occur and *spt7-300*, which does not associate with Spt8. As previously shown (4), an *spt8Δ* mutation also causes an approximately twofold increase in *HIS3* and *TRP3* mRNA levels. These results suggest that perturbations to the level of SAGA and/or SLIK/SALSA cause mild defects in this aspect of transcriptional control.

DISCUSSION

Our studies have investigated the functions of Spt7, a core component of the *S. cerevisiae* SAGA coactivator complex.

These results have strongly suggested that Spt7 plays an important role in SAGA assembly and have demonstrated that partial SAGA complexes that contain Spt7 can be formed in the absence of the core subunits Ada1 and Spt20. Deletion analysis has delineated a minimal functional Spt7 that encompasses the carboxy-terminal portion of the protein. Further analyses have established that one carboxy-terminal region of Spt7 is required for interaction with Spt8, and an adjacent small region is required for Spt7 carboxy-terminal processing. We have shown that this carboxy-terminally processed Spt7 is the form previously described to be in SLIK/SALSA, an altered version of SAGA (4, 18). An *spt7* mutant that impairs Spt7 processing has only weak mutant phenotypes, suggesting that processed Spt7 and the SLIK/SALSA complex are not strongly required under the conditions tested.

Spt7 and SAGA complex formation. Previous biochemical and genetic studies showed that Spt7, Spt20, and Ada1 are required for integrity of the SAGA complex. Our studies have shown that Spt7 must play an early and central role in SAGA formation as it is necessary for normal levels of Spt20 and Ada1. In addition, Spt7, Spt20, and Ada1 are each required for the presence of other SAGA components in the complex. Thus, directly or indirectly, Spt7 is required for the presence of most SAGA subunits. These results strongly suggest that one of the first steps in SAGA assembly is the association of the three core subunits, Spt7, Spt20, and Ada1. In addition, the key role for Spt7 in modulating the level of other SAGA subunits raises the possibility that the level of SAGA is regulated under some conditions by controlling the level of Spt7. Recent work shows that *SPT7* mRNA levels are decreased in cells treated with rapamycin or shifted to low quality nitrogen or carbon sources, suggesting regulation by the Tor pathway (36). Another study demonstrated that Spt7 is ubiquitinated, indicating a possible mechanism to control Spt7 levels (33).

Several results suggest that different regions of Spt7 play distinct roles in SAGA assembly and function. Our studies have shown that Spt7 associates with several SAGA members in the absence of Spt20 and Ada1, including Spt8, Taf6, Taf9, and Taf10. Previous results demonstrated that the histone fold region of Spt7 interacts with Taf10 (13). Our results, discussed below, show that a different region is required for the association between Spt7 and Spt8. Furthermore, we have observed intragenic complementation between nonoverlapping *spt7* deletions, strongly suggesting that the two different pieces of Spt7 expressed in the heterozygote play independent roles in SAGA assembly and/or function.

Spt7-Spt8 interaction. Our analysis has shown that Spt8 requires a region in the carboxy terminus of Spt7 for assembly into SAGA. Two results suggest that the Spt7-Spt8 interaction is direct. First, two *spt7* deletions, *spt7-300* and *spt7-1180*, cause loss of Spt8 from SAGA without the detectable loss of any other SAGA components. Second, Spt8 is still associated with Spt7 in an *spt20Δ ada1Δ* double mutant. In addition, the two *spt7* deletions that cause the loss of Spt8 from SAGA result in the same set of mutant phenotypes as does an *spt8Δ* mutation, strongly suggesting that the only function of this carboxy-terminal region of Spt7 is for association with Spt8. The extremely similar mutant phenotypes displayed by *spt8Δ*, *spt7-1180*, and *spt7-300* strongly suggest that Spt8 functions in vivo solely through its presence in SAGA.

The SAGA complex has been divided into distinct functional subgroups based on both genetic and biochemical results (27, 32). One subgroup contains Spt8 and Spt3, as several studies have suggested that these factors play related roles in transcription initiation by controlling the binding of TBP to TATA regions (22). However, previous studies showed that their physical association in SAGA is independent: Spt8 is still present in SAGA purified from an *spt3Δ* mutant and Spt3 is still present in SAGA from an *spt8Δ* mutant (38). Our results have confirmed this finding and have provided additional information on the independent association of these two factors within SAGA. While Spt8's association with SAGA is dependent upon Spt7, the presence of Spt3 in SAGA is dependent upon Spt20 and Ada1. In spite of these distinct associations, Spt8 and Spt3 may still interact directly in the assembled complex.

Analysis of in vivo requirement for Spt7 processing. Several studies have provided strong evidence for multiple forms of SAGA (4, 18, 34). One form of SAGA previously described, named SLIK or SALSA, was defined as having an altered form of Spt7 and lacking Spt8 (4; Patrick Grant, personal communication). Our analysis of Spt7 has provided a view of how this form of SAGA arises. First, analysis of wild-type Spt7 showed that this altered form of Spt7 lacks a portion of its carboxy-terminal end. In addition, the *spt7-200* mutant identified a region of Spt7 required for the presence of this altered form, suggesting that it arises by cleavage within or very near amino acids 1125 to 1150. Since this region is immediately amino terminal to the region of Spt7 required for the association of Spt8 with Spt7, the cleavage of Spt7 would result both in generating the shorter form of Spt7 and the loss of Spt8. Analysis of an *spt8Δ* mutant demonstrated that Spt8 is not required for Spt7 processing.

Since the *spt7-200* mutation blocks the carboxy-terminal processing of Spt7, resulting in little or no SLIK/SALSA complex in vivo, we were able to address the requirement for this form of SAGA in vivo. As described in Results, *spt7-200* causes only a few weak mutant phenotypes. While these mild defects are likely caused by the loss of Spt7 processing, we cannot rule out the possibility that the *spt7-200* deletion impairs Spt7 independently of the processing defect. In an *spt7-200* mutant, activation of transcription of the *HIS3* and *TRP3* genes was normal, strongly suggesting that SLIK/SALSA plays no significant role in the activation of genes regulated by general amino acid control. Since a previous study suggests that SLIK/SALSA is the major form of SAGA in cells induced with 3-AT, SLIK/SALSA and SAGA may be functionally equivalent under most conditions. We cannot rule out that there may be defects in gene expression that we did not detect. We also cannot rule out the possibility that SLIK/SALSA function is actually dependent upon something other than the processing of Spt7 and loss of Spt8. Regardless of these uncertainties, a significant role for SLIK/SALSA in vivo remains to be discovered.

Conservation of Spt7. Several SAGA components have been shown to be conserved and to exist in similar mammalian complexes (6, 25, 26, 28, 46). With respect to the three SAGA core components, mammalian homologues of Spt7 and Ada1 have been recently identified (26), while no mammalian homologue of Spt20 has yet been reported. Comparison of the sequences of human and *S. cerevisiae* Spt7 reveals that the

human version, named STAF65γ, is considerably smaller (414 amino acids) than *S. cerevisiae* Spt7 (1332 amino acids). Interestingly, STAF65γ is homologous to the carboxy-terminal 543 amino acids of *S. cerevisiae* Spt7, including the previously identified histone fold motif (13) and the region of Spt7 required for processing. This conservation corresponds extremely well with our finding that expression of the carboxy-terminal 459 amino acids of Spt7 can complement many *spt7Δ* mutant phenotypes. In mammalian cells, the functions provided by the amino terminus of Spt7 in *S. cerevisiae* may not be required or may be encoded by another gene.

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