Supplementary Methods

Yeast strains, plasmids, and spot test assay.

Yeast strains and plasmids used are listed in **Table S5.** All strains are derivatives of BY4705 (Brachmann *et al*, 1998). Plasmid pYH1 is based on the pRS426 vector; all remaining plasmids are based on pRS316 vector (Brachmann *et al*, 1998), both containing the *URA3* selectable marker. All internal deletions, except that in pYH59, contain a GSGSGS linker at the internal deletion junction. To examine growth phenotype, serial dilutions were spotted on glucose minimal medium lacking uracil and grown for 2 days at 30°C and 37°C (**Fig. S4**).

TAP purifications.

Tandem affinity purifications were performed as previously described but with modifications (Wu *et al*, 2004). Briefly, 12 liters of yeast were grown to an optical density at 600nm (OD_{600}) of 2-5 in YPD (3% glucose). Next, cells were harvested by centrifugation and washed with 200 ml of cold TAP extraction buffer (40 mM HEPES pH 7.5, 350 mM NaCl, 10% glycerol, 0.1% Tween 20, 1 mM dithiothreitol [DTT], 1 mM phenylmethylsulfonyl fluoride [PMSF], 0.31 mg/ml benzamidine, 0.3 μg/ml leupeptin, 1.4 μg/ml pepstatin, 2 μg/ml chymostatin). Cells were resuspended in 150 ml cold TAP extraction buffer and lysed in a BeadBeater (Biospec Products). Cell debris was removed by centrifugation at 15,000 ×g at 4°C for 30 min. The whole cell extracts (WCE) were then cleared by ultracentrifugation at 150,000 ×g at 4°C for 90 min. For TAP-tag purifications, 2.5 ml IgG Sepharose beads (GE Healthcare) were incubated with the WCE at 4°C overnight. The beads were next washed and resuspended in 4 ml cold TEV (tobacco etch virus) cleavage buffer (10 mM Tris pH8, 150 mM NaCl, 0.1% NP-40, 0.5 mM EDTA, 10% glycerol). 25 μg of TEV protease (TEVp) was added to the IgG beads, and the cleavage was performed at 4°C overnight. On day three, the TEVp-cleaved products were collected, and the IgG beads were washed with 3 column volumes (~7.5 ml total) cold Calmodulin binding buffer (15 mM HEPES pH8, 1 mM MgOAc, 1 mM imidazole, 2 mM $CaCl_2$, 0.1% NP-40, 10% glycerol, 300 mM NaCl). $CaCl_2$ was added to the combined eluate at a final concentration

of 2 mM and incubated with 1 ml Calmodulin affinity resin (Agilent Technologies) at 4°C for 4 hours. After incubation, the beads were washed with cold Calmodulin binding buffer and cold Calmodulin wash buffer (same as Calmodulin binding buffer, but containing 0.01% NP-40), and bound proteins were eluted in eleven 0.5 ml fractions with Calmodulin elution buffer (15 mM HEPES pH 8, 1 mM MgOAc, 1 mM imidazole, 2 mM EGTA, 10% glycerol, 300 mM NaCl, 0.01% NP-40) at room temperature. TAP purified complexes were analyzed on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (4-12% acrylamide) gradient gel (Invitrogen) in MOPS buffer. For Spt3 E240K SAGA purification, the Calmodulin binding buffer, Calmodulin wash buffer, and Calmodulin elution buffer contained only 150 mM NaCl. For the purifications of complexes bearing mutations in Sgf73 or Ada3, only 4 liters of yeast cells were grown. The amount of buffer and beads used was adjusted accordingly. Protein concentrations of SAGA complexes were determined by absorbance at 280 nm.

TBP binding assay.

EGTA eluted SAGA complexes (~3 μg) were diluted in 500 μl Calmodulin binding buffer. The diluted complexes were then incubated with 20 μl Calmodulin affinity resin (Agilent Technologies) for 3 hr at 4°C. Beads were washed and blocked with 10% bovine serum albumin (BSA) for 2 hr at 4°C. After blocking, the protein-bound beads were incubated with 0.5 μg recombinant yeast TBP for 1.5 hr at 4°C. Proteins were eluted with 40 μl Calmodulin elution buffer. The eluates were analyzed by SDS PAGE, and visualized by silver stain and Western blot using rabbit polyclonal antibodies against yeast TBP and Ada1. TBP and Ada1 were quantified using the Odyssey infrared imaging system (LiCor Biosciences).

His-tag pull-down and IP assays.

Recombinant proteins used in His-tag pull-down assays were purified from E.coli strain BL21(DE3) RIL. Spt8 was GST-tagged in pGEX-4T-3 vector, and was purified using Glutathione Sepharose 4 Fast Flow resin (GE Healthcare). TBP and mutant K133,138L were His-tagged in pET21a vector, and were purified

using Ni Sepharose High Performance resin (GE Healthcare). Recombinant TBP proteins were also further purified using Source 15S resin (Pharmacia Biotech) in batch. For pull-down assays, 4 μg WT or K133,138L TBP proteins were bound with 20 μl Ni Sepharose beads in 400 μl pull-down buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 10% glycerol, 0.001% NP-40, 1 mM PMSF, 1 mM DTT, 100 μg/ml BSA) for 30 min at 4°C, then washed three times. 0.5 μg GST or GST-Spt8 were incubated with TBP-bound resin or beads only in 400 μl pull-down buffer for 1 hr at 4°C, washed three times with Ni wash buffer (20 mM HEPES pH 8, 300 mM NaCl, 10% glycerol, 20 mM imidazole), and eluted in 40 μl Ni elution buffer (20 mM HEPES pH 8, 300 mM NaCl, 10% glycerol, 200 mM imidazole) with agitation for 7 min at room temperature. 4 μl of eluates (10%) were analyzed on SDS-PAGE, and were visualized by Coomassie Blue staining or Western blot using GST antibody (Z-5, Santa Cruz, sc-459). GST or GST-Spt8 were quantified using the Odyssey infrared imaging system (LiCor Biosciences).

For IP assays, small scale whole cell extracts were prepared by bead beating as described (Knutson & Hahn, 2011), except that TAP extraction buffer was used for TAP IP experiments. Protein concentrations of extracts were determined by Bradford assays. For IP experiments, 2-4 mg of whole cell extracts were incubated with 25 μl IgG Sepharose beads (for TAP IP), or with 25 μl Anti-FLAG M2 Affinity Gel (Sigma; for Flag IP) at 4°C overnight with gentle rocking. For TAP IPs, bound proteins were eluted by incubating beads with 20 μl TEV cleavage buffer containing 0.3 μg TEV protease at room temperature for 2.5 hr. The elution was repeated but for 1.5 hr, and eluates were combined. For Flag IPs, bound proteins were eluted by incubating beads with 25 μl 0.5 mg/ml 3×Flag peptide (Sigma) for 25 min at room temperature. The elution was repeated with 15 μl 0.5 mg/ml 3×Flag peptide for 15 min, and eluates were combined. Alternatively, bound proteins were eluted in 40 μl 1× NuPAGE LDS sample buffer (Invitrogen) at 95°C for 5 min. Eluted proteins were run on an SDS-PAGE and analyzed by Western blot using the following antibodies: M2-Flag antibody (Sigma), Ada2 antibody (yC-20; Santa Cruz, sc-6651), Ada3 antibody (yN-19; Santa Cruz, sc-6652), Gcn5 antibody (yV-19; Santa Cruz, sc-6305), HA-probe

antibody (F-7; Santa Cruz, sc-7392X), and rabbit polyclonal antibody against Taf12. Spt7 was visualized by the Protein A epitope at the C-terminus of the TAP-tag in heat eluted samples.

HAT assays.

Histone acetyltransferase (HAT) assays were performed as previously described (Knutson & Hahn, 2011), but with modifications. Briefly, 60-100 ng of wild type or Sgf73 mutant SAGA complexes normalized by Gcn5 content were incubated with 2 μg recombinant histone H3 or mono-nucleosome (a kind gift from Steven Henikoff at FHCRC) in 100 μl HAT buffer (50 mM Tris pH8, 50 mM KCl, 0.2 mM EDTA, 20 mM sodium butyrate, 5% glycerol, 2 mM DTT, 2 mM PMSF, 0.1% NP-40) at 30°C. After removing a sample at the zero time point, acetyl-CoA (Sigma) was added to a final concentration of 10 μM in each reaction. Equal volume of samples were taken out at 5 min, 10 min, 20 min, and 30 min time points, and were mixed with SDS sample buffer to stop acetylation. Samples were split into two parts, and were analyzed by silver stained SDS-PAGE and by Western blot with Gcn5 antibody, H3K9Ac antibody (Millipore, 07-352), H3K14Ac antibody, and H3K23Ac antibody. The latter two H3 acetylation antibodies were gifts from Toshio Tsukiyama at FHCRC. Gcn5 and acetylated H3 were quantified using the Odyssey infrared imaging system (LiCor Biosciences).

Structural modeling.

Domain prediction and structure similarity searches were performed by HHpred (http://toolkit.tuebingen.mpg.de/hhpred) using HMM PDB under default settings and thresholds (Söding *et al*, 2005). HHpred alignments were then used to manually generate PIR-alignments between query and target sequences. Templates were selected based on the highest probability score and/or orthologous proteins. PIR-alignment files were used as input to generate structural homology models using Modeller 9.10 with default parameters (Sali & Blundell, 1993). Twenty models of each alignment were generated, and the best scoring model was chosen. Sequences and templates used for generating homology models: Taf5 WD40 residues 460-798 were aligned with human

platelet-activating factor acetylhydrolase IB alpha subunit residues 102-402 (PDB ID 1VYH_C); Taf6 HEAT residues 216-429 were aligned with *Antonospora locustae* Taf6 residues 161-349 (PDB ID 4ATG_A); Taf9/Taf6 histone fold residues 30-102 (Taf9) and 5-75 (Taf6) were aligned with *Drosophila melanogaster* Taf9 19-86 (PDB ID 1TAF_A) and Taf6 1-70 (PDB ID 1TAF_B); Ada1/Taf12 histone fold residues 266-315 (Ada1) and 413-490 (Taf12) were aligned with human Taf4 870-918 (PDB ID 1H3O_A) and Taf12 55-128 (PDB ID 1H3O_B); Spt7/Taf10 histone fold residues 984-1030+GSGSGS linker+1065-1081 (Spt7) and 87-135+GSGSGS linker+179-194 (Taf10) were aligned with *Methanothermus fervidus* histone HMfA 6-69 (PDB ID 1B67_A) and 3-67 (PDB ID 1B67_B); Spt8 WD40 residues 142-590 were aligned with yeast Rpn14 residues 20-416 (PDB ID 3VL1_A); Spt3 histone fold residues 6-50 and 188-313 were aligned with human Taf13 31-75 (PDB ID 1BH9_A) and Taf11 113-195 (PDB ID 1BH9_B).

Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, Hieter P & Boeke JD (1998) Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* **14:** 115–132

Knutson BA & Hahn S (2011) Domains of Tra1 important for activator recruitment and transcription coactivator functions of SAGA and NuA4 complexes. *Mol Cell Biol* **31:** 818–831

Sali A & Blundell TL (1993) Comparative protein modelling by satisfaction of spatial restraints. *J Mol Biol* **234:** 779–815

Söding J, Biegert A & Lupas AN (2005) The HHpred interactive server for protein homology detection and structure prediction. *Nucleic Acids Res* **33:** W244–8

Wu P-YJ, Ruhlmann C, Winston F & Schultz P (2004) Molecular architecture of the S. cerevisiae SAGA complex. *Mol Cell* **15:** 199–208