SPT4, *SPT5* and *SPT6* Interactions: Effects on Transcription and Viability in *Saccharomyces cerevisiae*

Michele S. Swanson[1] and Fred Winston

Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115

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ABSTRACT

The *SPT4*, *SPT5* and *SPT6* genes of *Saccharomyces cerevisiae* were identified originally by mutations that suppress δ insertion mutations at *HIS4* and *LYS2*. Subsequent analysis has demonstrated that *spt4*, *spt5* and *spt6* mutations confer similar pleiotropic phenotypes. They suppress δ insertion mutations by altering transcription and are believed to be required for normal transcription of several other loci. We have now analyzed interactions between *SPT4*, *SPT5* and *SPT6*. First, the combination of mutations in any two of these three genes causes lethality in haploids. Second, some recessive mutations in different members of this set fail to complement each other. Third, mutations in all three genes alter transcription in similar ways. Finally, the results of coimmunoprecipitation experiments demonstate that at least the SPT5 and SPT6 proteins interact physically. Taken together, these genetic and biochemical results indicate that SPT4, SPT5 and SPT6 function together in a transcriptional process that is essential for viability in yeast.

IN *Saccharomyces cerevisiae*, a large number of genes have been identified as being important or essential for transcription. Among these, the *SPT* genes (SPT = suppressor of Ty) were identified by selection for extragenic suppressors of transcriptional defects caused by δ and Ty insertions in the 5′ regions of *HIS4* and *LYS2* (WINSTON *et al.* 1984, 1987; FASSLER and WINSTON 1988). These suppressor mutations, which define 17 *SPT* genes, are pleiotropic; for example, some affect mating and sporulation and others cause temperature-sensitive lethality (WINSTON *et al.* 1984; HIRSCHHORN and WINSTON 1988; EISENMANN, DOLLARD and WINSTON 1989). Genetic and molecular analysis of *SPT* genes and of *spt* mutant strains has led to the formation of three distinct classes of *SPT* genes. One group includes *SPT15*, which encodes the TATA binding protein TFIID (EISENMANN, DOLLARD and WINSTON 1989); another includes *SPT13/GAL11*, important for normal levels of transcription from several promoters (SUZUKI *et al.* 1988; FASSLER and WINSTON 1989; NISHIZAWA *et al.* 1990); and the third group includes *SPT11/HTA1* and *SPT12/HTB1*, which encode histone proteins H2A and H2B, respectively (CLARK-ADAMS *et al.* 1988). Each group of mutants is distinct with respect to the pattern of suppression of Ty and δ insertion mutations and other mutant phenotypes.

In this study, we have characterized the interactions between the *SPT4*, *SPT5* and *SPT6* genes. *SPT4*, *SPT5* and *SPT6* are members of the phenotypic class that includes the histone genes *HTA1* and *HTB1*. Mutations in *SPT4*, *SPT5* and *SPT6* were isolated originally as suppressors of the δ insertion mutations *his4-912δ* and *lys2-128δ* (WINSTON *et al.* 1984; FASSLER and WINSTON 1988). Mutations in all three genes have been shown to suppress both of these insertion mutations by altering the patterns of transcription of *HIS4* and *LYS2*, respectively (CLARK-ADAMS and WINSTON 1987; SWANSON, MALONE and WINSTON 1991; E. A. MALONE, J. FASSLER and F. WINSTON, unpublished data). Mutations in *SPT6* also have been identified in two other genetic studies. First, mutations in *SPT6* (called *SSN20*) were demonstrated to be extragenic suppressors of the defect in transcription of the *SUC2* gene caused by *snf2*, *snf5* or *snf6* mutations (NEIGEBORN, RUBIN and CARLSON 1986). In these studies, mutations in *SPT6* were also shown to suppress *cis*-acting mutations at *SUC2*: they restore expression from *SUC2* genes that lack upstream sequences that are necessary for regulation by glucose repression (NEIGEBORN, CELENZA and CARLSON 1987). In a second set of studies, one *SPT6* allele, called *cre2-1*, was isolated as a mutation that allowed glucose-insensitive expression of *ADH2* and bypassed the requirement for the ADR1 transcriptional activator protein (DENIS 1984; DENIS and MALVAR 1990).

Previous molecular and genetic analysis revealed that the *SPT5* and *SPT6* genes share a number of interesting features. First, both genes are essential for mitotic growth (NEIGEBORN, CELENZA and CARLSON 1987; CLARK-ADAMS and WINSTON 1987; SWANSON, MALONE and WINSTON 1991). Second, *S. cerevisiae* cells are sensitive to altered dosage of either the *SPT5*

[1] Current address: Department of Molecular Biology and Microbiology, Tufts University Medical School, Boston, Massachusetts 02111.

or the *SPT6* gene: increased or decreased gene copy number causes an Spt⁻ phenotype (NEIGEBORN, CELENZA and CARLSON 1987; CLARK-ADAMS and WINSTON 1987; SWANSON, MALONE and WINSTON 1991). Third, both genes encode nuclear proteins with unusually acidic amino termini (SWANSON, CARLSON and WINSTON 1990; SWANSON, MALONE and WINSTON 1991).

The genetic analyses presented here extend the similarities between *SPT5* and *SPT6* to include a third gene, *SPT4*. Interestingly, *spt4* mutations cause similar phenotypes as *spt5* and *spt6* mutations with respect to effects on gene expression, yet SPT4 function is distinct in other ways. For example, the *SPT4* gene is not essential for growth, and altered *SPT4* dosage does not cause an Spt⁻ phenotype (E. A. MALONE, J. FASSLER and F. WINSTON, unpublished data).

In this study, we have used a number of techniques to analyze *SPT4*, *SPT5* and *SPT6* interactions. First, we demonstrate that virtually every double mutant combination among the *SPT4*, *SPT5* and *SPT6* genes causes lethality in haploid strains. Second, we show that certain mutations in *SPT4*, *SPT5* and *SPT6* fail to complement each other in diploid strains. Third, we show that *spt4* and *spt5* mutations that were isolated as suppressors of δ-insertion mutations also suppress the *SUC2* transcriptional defect caused by a *snf2* allele, similar to *spt6* mutations. Finally, by coimmunoprecipitation experiments, we present evidence that the SPT5 protein associates with the SPT6 protein. These results suggest that the *SPT4*, *SPT5* and *SPT6* genetic interactions reflect physical interactions between the SPT4, SPT5 and SPT6 proteins which are essential for some aspect of transcription in yeast.

MATERIALS AND METHODS

Yeast strains: The yeast strains used in this study (Table 1) are derivatives of strain S288C (*MATα gal2*) and are from our laboratory collection. Parentheses indicate episomal plasmids, and brackets indicate integrated plasmid DNA. The δ-insertion mutations *his4-912δ* (CHALEFF and FINK 1980; FARABAUGH and FINK 1980) and *lys2-128δ* (SIMCHEN *et al.* 1984; CLARK-ADAMS and WINSTON 1987) cause histidine and lysine auxotrophies, respectively.

The *spt4Δ1::URA3* allele is a deletion of approximately half of the *SPT4* coding region and includes insertions of Tn*5* sequences and the *URA3* gene; *spt4-289* and *spt4-6* are nonsense mutations; and *spt4-3* is an internal deletion (E. A. MALONE, J. FASSLER and F. WINSTON, unpublished data). The mutations *spt5-24*, *spt5-8*, *spt5-194*, *spt6-140*, *spt6-113* and *spt6-167* are spontaneous, presumed partial loss-of-function mutations (WINSTON *et al.* 1984; FASSLER and WINSTON 1988). Both *spt6-140* and *spt6-113* cause lethality at 37°, and *spt6-167* causes slow growth at 37°. The mutations *spt3Δ203::TRP1* (HAPPEL AND WINSTON 1992) and *snf2Δ1::HIS3* (ABRAMS, NEIGEBORN and CARLSON 1986) are null alleles. The *suc2Δ(−1900/−390)* allele contains the *SUC2* TATA region, but lacks sequences from −1900 to −390 relative to the translational start site for the precursor to secreted invertase (NEIGEBORN, CELENZA and CARLSON 1987). Strains MS222, MS223 and MS224 have plasmid pBM68 integrated at the *URA3* locus, such that the *SPT5-lacZ* gene is expressed from the *SPT5* promoter (SWANSON, MALONE and WINSTON 1991). Both the *cis*-acting mutation *suc2Δ(−1900/−390)* and the *trans*-acting mutation *snf2Δ1::HIS3* were used. In our suppression studies we observed that *spt6-140 suc2Δ(−1900/−390)* strains have a Suc⁺ phenotype with lower levels of invertase than measured for *SPT6⁺ snf2Δ1::HIS3* (Suc⁻) strains; this difference is likely caused by the pleiotropic effects of the *snf2Δ1::HIS3* mutation (NEIGEBORN and CARLSON 1984).

Media: The media used were as described by SHERMAN, FINK and LAWRENCE (1978) and include the rich media YEP + 2% glucose (YPD) and YEP + 0.05% glucose, as well as synthetic complete media lacking a specific amino acid (for example, SC − Lys), minimal media supplemented with a particular amino acid (such as SD + Lys), and sporulation media. YP + sucrose consisted of YEP + 2% sucrose + 1 μg/ml antimycin A. GNA presporulation medium contained 10 g yeast extract, 30 g nutrient broth, 50 g dextrose and 20 g agar per liter.

Genetic methods: Standard methods for mating, sporulation and tetrad analysis were used (MORTIMER and HAWTHORNE 1969; SHERMAN, FINK and LAWRENCE 1978). For analysis of *spt* double mutants, at least 15 tetrads were scored for each cross. The *spt* genotypes of viable spore clones were determined by complementation tests using *spt* tester strains L448, L449, L450, L451, L452 and L453 (Table 1). First, tetrads and parental strains were crossed to *MAT***a** and *MATα spt* lawns on YPD media. Then, diploids were selected on SD + His + Lys plates and suppression of *lys2-128δ* and *his4-912δ* was scored on SD + His and SD + Lys plates, respectively. As described in RESULTS, we observed that some pairs of unlinked mutations failed to complement; however, in no case was the double heterozygote Spt⁻ phenotype as strong as the homozygous Spt⁻ phenotype. Therefore, the genotype of spore clones could be determined unambiguously by scoring their phenotype relative to that of each *spt* parent strain. Yeast cells were transformed by the lithium acetate method (ITO *et al.* 1983).

RNA preparation and analysis: For analysis of *SUC2* RNA under derepressing conditions, yeast cells were grown at 30° in YPD medium to 1–1.5 × 10^7 cells per ml, washed twice with sterile water, then incubated for 2.5 h at 30° in YEP + 0.05% glucose to relieve glucose repression. For analysis of *SPT4*, *SPT5* and *SPT6* RNA, cells were grown at 30° in YPD medium to 1–2 × 10^7 cells per ml. RNA was prepared as described previously (CARLSON and BOTSTEIN 1982) and separated by electrophoresis in formaldehyde-agarose gels as described previously (SWANSON, MALONE and WINSTON 1991). Hybridization probes were radiolabeled by nick-translation (RIGBY *et al.* 1977) of plasmid DNA using [α-^{32}P]dATP (Amersham Corp.) and a reagent kit from Boehringer Mannheim.

Plasmids: Plasmids used to prepare hybridization probes were, for *SUC2*, plasmid pRB59 (CARLSON and BOTSTEIN 1982), and for *TUB2*, plasmid pYST138, a 0.24-kb *Bgl*II-*Kpn*I restriction fragment internal to *TUB2* cloned in a pGEM vector (SOM *et al.* 1988).

Several plasmids were used to express SPT fusion proteins for analysis by coimmunoprecipitation experiments. Plasmid pMS33, which is a derivative of the *CEN4-ARS1-URA3* vector YCp50 (JOHNSTON and DAVIS 1984), encodes the nine amino acid HA1 epitope (NIMAN *et al.* 1983) fused to the entire SPT6 protein; the HA1-SPT6 fusion protein is functional, as judged by its ability to complement the temperature-sensitive lethality caused by the *spt6-140* mutation (SWANSON, CARLSON and WINSTON 1990). Plasmid pMS39

is a derivative of the *CEN6-ARSH4-LEU2* vector pRS315 (SIKORSKI and HEITER 1989) and encodes the HA1-SPT6 hybrid gene; it was constructed by digesting plasmid pMS33 partially with *Hin*dIII and completely with *Eag*I to obtain a 7.0-kb *Hin*dIII-*Eag*I fragment containing the *HA1-SPT6* gene, which was then ligated to the *Eag*I and *Hin*dIII sites of the vector pRS315 (SIKORSKI and HEITER 1989). Plasmid pMS35, which is a derivative of the 2-μm circle-*URA3* vector YEp353 (MYERS *et al.* 1986), encodes a fusion between the HA1 epitope and the β-galactosidase protein of *Escherichia coli* expressed from the yeast *ADH1* promoter (SWANSON, CARLSON and WINSTON 1990). Plasmid pAB1, which is a derivative of the *CEN-ARS-TRP1* plasmid pJK1521 (KAMENS *et al.* 1990), encodes a LexA-SPT5 fusion protein expressed from the *ADH1* promoter; it was constructed by digesting plasmid pMS4 (SWANSON, MALONE and WINSTON 1991) with *Sph*I, treating with Klenow, and then ligating the 3.5-kb fragment containing the *SPT5* gene to the *Sma*I site of plasmid pJK1521 (KAMENS *et al.* 1990). The LexA-SPT5 fusion protein consists of the first 87 amino acids of the *E. coli* LexA protein fused to the SPT5 protein beginning at amino acid 12; this plasmid causes a weak Spt$^-$ mutant phenotype in an *SPT5*$^+$ background (A. BORTVIN and F. WINSTON, unpublished data). Plasmid pBM68 is an integrating plasmid containing the *SPT5(1004)-lacZ* gene; this hybrid gene encodes the first 1004 amino acids of SPT5 fused to β-galactosidase and partially complements an *spt5* null mutation (SWANSON, MALONE and WINSTON 1991). Plasmid pMS50 is an integrating plasmid carrying the *SPT5(917)-lacZ* gene; this hybrid gene, which encodes the first 917 amino acids of SPT5 fused to β-galactosidase, lacks the entire SPT5 repeat domain and provides a very low level of SPT5 activity (SWANSON, MALONE and WINSTON 1991). The vector YEp13 contains part of the yeast 2-μm circle and the *LEU2* gene as a selectable marker (BROACH, STRATHERN and HICKS 1979).

Invertase assays: Yeast cultures were grown to exponential phase (1–3 $\times$ 10^7 cells per ml) in YPD medium, then glucose-repressed and glucose-derepressed cells were prepared as described by CELENZA and CARLSON (1984). Secreted invertase activity was assayed on whole cells using the method of GOLDSTEIN and LAMPEN (1975) as described by CELENZA and CARLSON (1984). For calculation of units of invertase activity, we assumed that 1 ml of cells at a density of 50 Klett units was equivalent to 0.38 mg dry weight of cells. A twofold variation in calculated invertase units occurs for cultures grown to 1 $\times$ 10^7 *vs.* 2 $\times$ 10^7 cells per ml (G. PRELICH, personal communication).

Coimmunoprecipitation assays: Yeast cell lysates were prepared from cultures grown at 30° to 1–2 $\times$ 10^7 cells per ml of SC media lacking the appropriate amino acid(s) for plasmid maintenance. Protein lysates were prepared as described previously (CELENZA and CARLSON 1986), except for the composition of the lysis buffer, which was 20 mM HEPES (*N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]) [pH 7.4], 100 mM potassium acetate, 2 mM magnesium acetate and 2 mM phenylmethyl sulfonate. For cross-linking, 0.5 mg/ml of protein lysate was incubated with 0.2 mg/ml dithiobis(succinimidylpropionate) (DSP, Pierce) for 30 min at 4°. The cross-linking reaction was quenched by the addition of 0.5 M ammonium acetate for 5 min at 4°, and the lysate was clarified by centrifugation at 16,000 $\times$ *g* for 10 min at 4°. For mock-cross-linked lysates, the order of the DSP and ammonium acetate incubations was reversed. The protein concentrations of the cell lysates were determined by the method of BRADFORD (1976) using a dye reagent from Bio-Rad Laboratories and bovine serum albumin (BSA) as the standard.

The immunoprecipitation reactions consisted of 250 μg of freshly prepared protein lysate and either 2.5 μl of ascites fluid containing the HA1 epitope- specific monoclonal antibody 12CA5 (NIMAN *et al.* 1983) or 1 μl of anti-β-galactosidase antisera (Promega) in 0.5 ml of immunoprecipitation (IP) buffer (50 mM Tris(hydroxymethyl)aminomethane-HCl (Tris-HCl) [pH 7.5], 1% Triton X-100, 150 mM NaCl, 500 mM ammonium acetate and 0.5 mg/ml BSA. (During our initial coimmunoprecipitation experiments, we found that addition of 250–750 mM ammonium acetate to the IP buffer virtually eliminated a nonspecific precipitation of both the LexA-SPT5 and SPT5-β-galactosidase fusion proteins without reducing their association with the HA1-SPT6 protein.) After 60 min on ice, aggregates were removed by centrifugation at 16,000 $\times$ *g* (14,000 rpm in an Eppendorf microcentrifuge) for 10 min at 4°. Next, the supernatants were incubated with 50 μl of an 8.3% suspension of Pansorbin (Calbiochem Corp., La Jolla, California) for 30 min at 4°. The protein-Pansorbin complexes were collected by centrifugation for 40 sec at 16,000 $\times$ *g*, washed three times with 0.5 ml of IP buffer, and then resuspended in 0.5 ml of IP buffer lacking BSA. The protein-Pansorbin suspension was transferred to a new tube, washed two times in IP buffer lacking BSA, and then resuspended in 35 μl of protein sample buffer (60 mM Tris-HCl [pH 6.8], 2% sodium dodecyl sulfate (SDS), 8% glycerol, 0.4% bromphenol blue and 2% β-mercaptoethanol). The samples were heated in a boiling water bath for 3 min, cleared by centrifugation at 16,000 $\times$ *g* for 2 min, and then separated by electrophoresis through 7.5% acrylamide gels and analyzed by immunoblotting.

Immunoblotting analysis: Protein samples were analyzed by immunoblotting as described previously (CELENZA and CARLSON 1984) using one of the following primary antibody preparations: the HA1-SPT6 and HA1-β- galactosidase fusion proteins were identified with a 1:500 dilution of 12CA5 ascites fluid (NIMAN *et al.* 1983); the LexA-SPT5 fusion protein was identified with a 1:1500 dilution of rabbit serum containing LexA-specific polyclonal antibodies (kindly provided by R. BRENT); and the SPT5(1004)-β-galactosidase fusion protein was identified with a 1:7500 dilution of a β-galactosidase-specific monoclonal antibody (Promega). The primary antibodies were detected using the Protoblot System from Promega with a 1:7500 dilution of rabbit or mouse IgG-specific antibody affinity-purified from goat serum and conjugated to alkaline phosphatase.

RESULTS

Combinations of mutations in the *SPT4*, *SPT5* and *SPT6* genes cause lethality: Mutations in the *SPT4*, *SPT5* and *SPT6* genes were isolated originally as suppressors of δ-insertion mutations in the 5′ regions of the *HIS4* and *LYS2* genes (WINSTON *et al.* 1984; FASSLER and WINSTON 1988). During the initial characterization of *spt* mutant strains, WINSTON *et al.* (1984) observed that the combination of any two of the mutations *spt4-3*, *spt5-194* and *spt6-140* caused lethality at 30°. The double mutant lethality appeared to be specific to these three genes, since the same mutations in combination with mutations in 13 other *SPT* genes were viable (WINSTON *et al.* 1984; FASSLER and WINSTON 1988; M. SWANSON, E. A. MALONE and F. WINSTON, unpublished data).

We have extended this analysis to determine

TABLE 1

Yeast strains

a.	Double mutant lethality studies	
	MS53	*MAT***a** *spt4-289 his4-912δ lys2-128δ leu2-3 can*^{R}
	MS63	*MAT***a** *spt5-24 ura3-52 his4-912δ lys2-128δ leu1 trp5 ade1 can1-100*
	L242	*MATα spt6-140 ura3-52 his4-912δ lys2-128δ leu1 trp5-1*
	FW147	*MATα spt6-167 ura3-52 his4-912δ lys1-1 cry1*
	MS22	*MAT***a** *spt4Δ1::URA3 ura3-52 his4-912δ lys2-128δ leu1 trp5*
	MS23	*MATα spt6-167 his4-912δ lys2-128δ ade2-1*
	FW146	*MAT***a** *spt6-167 his4-912δ lys1-1 leu2-3 cry1*
	MS17	*MATα spt5-24 ura3-52 his4-912δ lys2-128δ ade2-1 can1-100*
	MS14	*MAT***a** *spt4-289 his4-912δ lys2-128δ ura3-52 leu2-1 ade2-1 trp5*
	L448	*MAT***a** *spt6-140 his4-912δ lys2-128δ ade8*
	L449	*MATα spt6-140 his4-912δ lys2-128δ ade8*
	L450	*MATα spt4Δ1::URA3 his4-912δ lys2-128δ ade8*
	L451	*MAT***a** *spt4Δ1::URA3 his4-912δ lys2-128δ ade8*
	L452	*MAT***a** *spt5-24 his4-912δ lys2-128δ ade8*
	L453	*MATα spt5-24 his4-912δ lys2-128δ ade8*
b.	Complementation studies	
	MS73	*MAT***a** */MATα his4-912δ/his4-912δ lys2-128δ/lys2-128δ trp2/trp2 leu2/leu2 canl-100/can1-100*
	MS70	*MAT***a**/*MATα spt4Δ1::URA3/+ ade8/+ his4-912δ/his4-912δ lys2-128δ/lys2-128δ leu2/LEU2 trp2/TRP2 can1-100/CAN1*
	MS169	*MAT***a**/*MATα spt5-24/SPT5 ade8/ade8 his4-912δ/his4-912δ lys2-128δ/lys2-128δ*
	MS69	*MAT***a**/*MATα spt6-140/SPT6 ade8/ADE8 his4-912δ/his4-912δ lys2-128δ/lys2-128δ leu2/LEU2 trp2/TRP2 can1-100/CAN1*
	MS208	*MAT***a**/*MATα spt6-113/SPT6 his4-917/his4-912δ lys2-128δ/lys2-128δ ura3-52/URA3 leu2/leu2*
	MS72	*MAT***a**/*MATα spt4Δ1::URA3/spt4Δ1::URA3 ade8/ade8 his4-912δ/his4-912δ lys2-128δ/lys2-128δ*
	MS166	*MAT***a**/*MATα spt5-24/spt5-24 ade8/ade8 his4-912δ/his4-912δ lys2-128δ/lys2-128δ*
	MS71	*MAT***a**/*MATα spt6-140/spt6-140 ade8/ade8 his4-912δ/his4-912δ lys2-128δ/lys2-128δ*
	MS209	*MAT***a**/*MATα spt6-113/spt6-113 his4-917/his4-917 lys2-128δ/lys2-128δ ura3-52/ura3-52 leu2/leu2*
	MS167	*MAT***a**/*MATα spt4Δ1::URA3/SPT4 spt5-24/SPT5 ade8/ade8 his4-912δ/his4-912δ lys2-128δ/lys2-128δ*
	MS68	*MAT***a**/*MATα spt4Δ1::URA3/SPT4 spt6-140/SPT6 ade8/ade8 his4-912δ/his4-912δ lys2-128δ/lys2-128δ*
	MS168	*MAT***a**/*MATα spt5-24/SPT5 spt6-140/SPT6 ade8/ade8 his4-912δ/his4-912δ lys2-128δ/lys2-128δ*
	MS93	*MAT***a**/*MATα spt4Δ1::URA3/SPT4 spt5-24/SPT5 spt6-140/SPT6 ura3-52/ura3-52 his4-912δ/his4-912δ lys2-128δ/lys2-128δ ade2-1/ADE2 can1/CAN1*
	MS210	*MAT***a**/*MATα spt4Δ1::URA3/SPT4 spt6-113/SPT6 ade8/ADE8 his4-912δ/his4-917 lys2-128δ/lys2-128δ ura3-52/URA3 leu2/LEU2*
	MS217	*MAT***a**/*MATα spt5-8/SPT5 ade8/ADE8 his4-912δ/his4-912δ lys2-128δ/lys2-128δ ura3-52/URA3 leu2Δ1/leu2 trp2/TRP2 can1-100/CAN1*
	MS218	*MAT***a**/*MATα spt5-8/spt5-8 ade8/ADE8 his4-912δ/his4-912δ lys2-128δ/lys2-128δ ura3-52/URA3 leu2Δ1/LEU2*
	MS219	*MAT***a**/*MATα spt4Δ1::URA3/SPT4 spt5-8/SPT5 ade8/ADE8 his4-912δ/his4-912δ lys2-128δ/lys2-128δ ura3-52/ura3-52*
	MS220	*MAT***a**/*MATα spt5-8/SPT5 spt6-113/SPT6 ade8/ADE8 his4-912δ/his4-917 lys2-128δ/lys2-128δ ura3-52/URA3 leu2Δ1/leu2*
c	Invertase assays	
	FW1238	*MATα ura3-52 his4-912δ lys2-128δ*
	MS1	*MAT***a** *snf2Δ::HIS3 ura3-52 his4-912δ lys2-128δ*
	MS87	*MAT***a** *spt4Δ1::URA3 ura3-52 his4-912δ lys2-128δ*
	L381	*MATα spt4Δ1::URA3 ura3-52 lys2-128δ*
	L331	*MAT***a** *spt4-289 ura3-52 his4-912δ lys2-128δ ade2-1*
	MS3	*MATα spt5-194 his4-912δ ade2-1*
	FW256	*MATα spt5-194 his4-912δ ade2-1*
	MS11	*MATα spt5-8 ura3-52 his4-917 lys2-128δ leu2*
	L807	*MATα spt5-8 ura3-52 his4-917 lys2-128δ leu2*
	MS36	*MAT***a** *spt6-140 ura3-52 his4-912δ lys2-128δ*
	MS155	*MAT***a** *spt6-140 ura3-52 his4-912δ lys2-128δ*
	MS39	*MATα spt6-140 snf2Δ1::HIS3 ura3-52 his4-912δ lys2-128δ*
	MS31	*MATα spt4Δ1::URA3 snf2Δ1::HIS3 ura3-52 lys2-128δ*
	MS56	*MATα spt4-289 snf2Δ1::HIS3 his4-912δ lys2-128δ ade2-1*
	MS58	*MATα spt4-289 snf2Δ1::HIS3 ura3-52 his4-912δ lys2-128δ*
	MS10	*MATα spt5-8 snf2Δ1::HIS3 ura3-52 his4-917 lys2-128δ leu2*
	MS13	*MAT***a** *spt5-8 snf2Δ1::HIS3 ura3-52 his4-912δ lys2-128δ*
	MS4	*MAT***a** *spt5-194 snf2Δ1::HIS3 ura3-52 his4-912δ ade2-1*
	MS7	*MAT***a** *spt5-194 snf2Δ1::HIS3 his4-912δ lys2-128δ*
	MS29	*MATα spt6-167 snf2Δ1::HIS3 ura3-52 his4-912δ lys2-128δ*
	MS30	*MAT***a** *spt6-167 snf2Δ1::HIS3 ura3-52 his4-912δ lys2-128δ leu2-3*
	FY51	*MAT***a** *spt3-203::TRP1 ura3-52 his4-917δ leu2Δ1 trp1Δ63*
	FY416	*MATα spt3-203::TRP1 snf2Δ1::HIS3 ura3-52 his4-917δ*
	A745	*MATα spt3-203::TRP1 snf2Δ1::HIS3 ura3-52 his4-917δ*
	FY121	*MAT***a** *suc2-Δ-1900/-390 ura3-52 his4-912δ lys2-128δ trp1Δ63*
	MS163	*MATα suc2-Δ-1900/-390 ura3-52 his4-912δ lys2-128δ*

	MS161	*MATα spt4Δ1::URA3 suc2-Δ-1900/-390 ura3-52 his4-912δ lys2-128δ*
	MS162	*MATa spt4Δ1:URA3 suc2-Δ-1900/-390 ura3-52 his4-912δ lys2-128δ*
	MS164	*MATα spt5-194 suc2-Δ-1900/-390 his4-912δ*
	MS165	*MATa spt5-194 suc2-Δ-1900/-390 his4-912δ lys2-128δ ade2-1*
	FY129	*MATα spt6-140 suc2-Δ-1900/-390 ura3-52 his4-912δ lys2-128δ ade8*
	FY141	*MATa spt6-140 suc2-Δ-1900/-390 ura3-52 his4-912δ lys2-128δ ade8*
d.	*SUC2* transcription	
	FW1237	*MATa ura3-52 his4-912δ lys2-128δ*
	MS37	*MATα snf2Δ1::HIS3 ura3-52 his4-912δ lys2-128δ*
	MS91	*MATα spt4Δ1::URA3 snf2Δ1::HIS3 ura3-52 his4-912δ lys2-128δ*
	MS4	*MATa spt5-194 snf2Δ1::HIS3 ura3-52 his4-912δ ade2-1*
	MS38	*MATa spt6-140 snf2Δ1::HIS3 ura3-52 his4-912δ lys2-128δ*
e.	Coimmunoprecipitation experiments	
	MS225	*MATa his4-912δ lys2-128δ ura3-52 leu2Δ1 trp1Δ63* (pMS39) (pAB1)
	MS227	*MATa his4-912δ lys2-128δ ura3-52 leu2Δ1 trp1Δ63* (pRS315) (pAB1)
	MS242	*MATa his4-912δ lys2-128δ ura3-52 leu2Δ1 trp1Δ63* (pMS35) (pAB1)
	MS232	*MATa his4-912δ lys2-128δ spt5-194 leu2Δ1* [*ura3-52::URA3-SPT5(1004)-lacZ*] (pMS39)
	MS234	*MATa his4-912δ lys2-128δ spt5-194 leu2Δ1* [*ura3-52::URA3-SPT5(1004)-lacZ*] (YEp13)

TABLE 2

Combinations of *spt* mutations cause lethality

Cross	Relevant genotype	Phenotype of double mutant[a]
FW256 × MS53	*spt4-289 spt5-194*	Dead
L381 × MS63	*spt4Δ1::URA3 spt5-24*	Dead
MS63 × L242	*spt5-24 spt6-140*	Dead
L381 × MS36	*spt4Δ1::URA3 spt6-140*	Dead
WINSTON *et al.* (1984)[b]	*spt4-3 spt6-140*	Dead
WINSTON *et al.* (1984)	*spt4-3 spt5-194*	Dead
WINSTON *et al.* (1984)	*spt5-194 spt6-140*	Dead
MS14 × FW147	*spt4-289 spt6-167*	Spt$^-$, Ts$^-$
MS22 × MS23	*sp4Δ1::URA3 spt6-167*	Spt$^-$, Ts$^-$
FW146 × MS17	*spt5-24 spt6-167*	Spt$^-$, Ts$^-$
FW146 × FW256	*spt5-194 spt6-167*	Spt$^-$, Ts$^-$

[a] Greater than 15 tetrads were scored for each cross. The *SPT* genotypes of viable spore clones were determined by complementation tests as described in MATERIALS AND METHODS. Strains designated Spt$^-$ and Ts$^-$ had an Spt$^-$ phenotype at 30° and failed to grow at 37°.

[b] Crosses designated WINSTON *et al.* (1984) contain data originally presented in that publication.

whether the *spt4*, *spt5* and *spt6* double mutant lethality is allele-specific. Tetrad analysis demonstrated that most double mutant combinations examined caused lethality in haploids at 30° (Table 2). The only exception was the *spt6-167* allele, which unlike the other *spt6* mutation tested, *spt6-140*, does not itself cause temperature-sensitive growth and is thus likely to be a less severe mutation. Even in cases with this allele, the *spt4 spt6-167* and *spt5 spt6-167* double mutants grew poorly at 30° and failed to grow at 37° (Table 2). Therefore, the double mutant lethality caused by *spt4*, *spt5* and *spt6* mutations is not allele-specific. These data suggest that the double mutant lethality occurs by the cumulative reduction of function(s) caused by mutations in *SPT4*, *SPT5* and *SPT6*.

Some *spt4*, *spt5* and *spt6* mutations fail to complement each other: During our analysis of *spt* single and double mutants, we frequently observed incomplete complementation of *spt4*, *spt5* and *spt6* mutations. To investigate this genetic interaction more systematically, we constructed diploid strains heterozygous at two of these three *SPT* loci and compared their phenotypes to strains that were either homozygous mutant or homozygous wild type. The phenotype examined was suppression of the lysine auxotrophy caused by the insertion mutation *lys2-128δ*. This analysis has demonstrated that among mutations in *SPT4*, *SPT5* and *SPT6*, some recessive mutations in any one of these *SPT* genes fail to complement recessive mutations in the other two *SPT* genes (Table 3). For example, the *spt4Δ1::URA3* and *spt6-140* mutations were recessive for suppression of *lys2-128δ* (diploid strains MS70 and MS69 were Lys$^-$), yet a diploid that was heterozygous for both an *spt4Δ1::URA3* and an *spt6-140* mutation had an Spt$^-$ phenotype (MS68 was Lys$^{+/-}$; Table 3, Figure 1). The failure of unlinked *spt* mutations to complement each other was also observed for most other combinations of *spt4*, *spt5* and *spt6* alleles that were tested (Table 3). In general, the double heterozygote mutant phenotypes were not as strong as those of homozygous mutant strains. [Similar results have been observed for suppression of the mutations *his4-912δ* and *suc2Δ(-1900/-390)* (M. SWANSON, G. PRELICH and F. WINSTON, unpublished data).] The results of testing different combinations of mutations suggest that the cumulative severity of the *spt* alleles determines the degree of complementation. First, the *spt5-8* allele, which fully complemented both an *spt4Δ1::URA3* and an *spt6-113* mutation (Table 3), is probably a leaky allele, as judged by its weak effects on *lys2-128δ* transcription (SWANSON,

TABLE 3

Mutations in *SPT4*, *SPT5* and *SPT6* fail to complement each other

Diploid Strain	Relevant genotype	Lys phenotype
MS73	Wild type	−
MS70	*spt4Δ1::URA3/SPT4*	−
MS169	*spt5-24/SPT5*	−
MS217	*spt5-8/SPT5*	−
MS69	*spt6-140/SPT6*	−
MS208	*spt6-113/SPT6*	−
MS72	*spt4Δ1::URA3/spt4Δ1::URA3*	+
MS166	*spt5-24/spt5-24*	+
MS218	*spt5-8/spt5-8*	+
MS71	*spt6-140/spt6-140*	+
MS209	*spt6-113/spt6-113*	+
MS167	*spt4Δ1::URA3/SPT4 spt5-24/SPT5*	+/−
MS68	*spt4Δ1::URA3/SPT4 spt6-140/SPT6*	+/−
MS210	*spt4Δ1::URA3/SPT4 spt6-113/SPT6*	+/−
MS168	*spt5-24/SPT5 spt6-140/SPT6*	+/−
MS219	*spt4Δ1::URA3/SPT4 spt5-8/SPT5*	−
MS220	*spt5-8/SPT5 spt6-113/SPT6*	−
MS93	*spt4Δ1::URA3/SPT4 spt5-24/SPT5 spt6-140/SPT6*	+

Suppression of *lys2-128δ* was scored after replica-plating patches of each strain to SC and SC − Lys plates and incubating at 30° for 1.5 days. + indicates growth; +/− indicates weak growth; − indicates no growth.

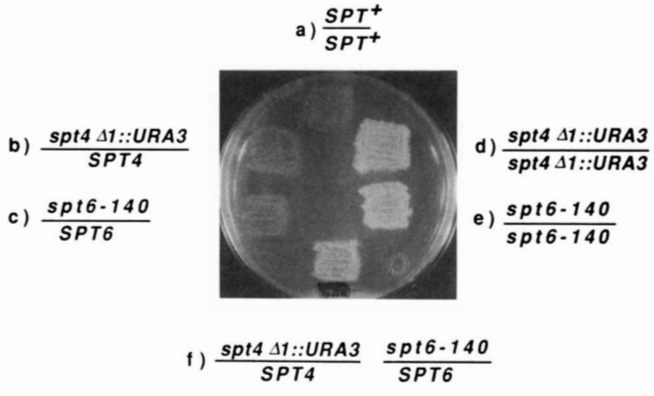

FIGURE 1.—Mutations in *SPT4* and *SPT6* fail to complement for suppression of *lys2-128δ*. Patches of strains of the genotype indicated were grown on a YPD plate, then replica plated to the SC and SC − Lys plates shown here. The plates were photographed after 1.5 days of growth at 30°. The strains shown are (a) MS73, (b) MS70, (c) MS69, (d) MS72, (e) MS71 and (f) MS68.

MALONE and WINSTON 1991) and suppression of *snf2* (described in the next section). Second, the mutant phenotype of a triple heterozygote (Table 3, strain MS93) was more severe than that of isogenic double heterozygotes (Table 3). These effects are also consistent with the previous observations that *spt5* and *spt6* null alleles, which are recessive lethal, are dominant for an Spt⁻ phenotype. Taken together, these results indicate that the SPT4, SPT5 and SPT6 gene products function in a similar process.

Mutations in *SPT4*, *SPT5* and *SPT6* each restore invertase expression to *snf2* mutants: A number of *spt6* mutations were identified as suppressors of mutations in the *SNF2* and *SNF5* genes, which are required for high level expression of *SUC2*, the structural gene for invertase (NEIGEBORN and CARLSON 1984; NEIGEBORN, RUBIN and CARLSON 1986). Several of these *spt6* mutations were shown to restore high levels of invertase activity to *snf2* mutant strains by increasing the level of *SUC2* RNA (NEIGEBORN, RUBIN and CARLSON 1986; NEIGEBORN, CELENZA and CARLSON 1987).

TABLE 4

Effects of *spt4*, *spt5* and *spt6* mutations on invertase activity

Relevant genotype	Secreted invertase activity[a]	
	Repressed	Derepressed
Wild type	3 ± 1	141 ± 7
spt4Δ1::URA3	3 ± <1	149 ± 15
spt4-289	3 ± 1	187 ± 33
spt5-8	3 ± 1	131 ± 13
spt5-194	3 ± 1	224 ± 29
spt6-140	3 ± 1	252 ± 20
spt3Δ203:TRP1	7 ± 1	112 ± 1
snf2Δ1::HIS3	2 ± <1	17 ± 2
snf2Δ1::HIS3 spt4Δ1::URA3	2 ± <1	50 ± 6
snf2Δ1::HIS3 spt4-289	2 ± <1	60 ± 1
snf2Δ1::HIS3 spt5-8	4 ± 1	59 ± 9
snf2Δ1::HIS3 spt5-194	3 ± <1	104 ± 10
snf2Δ1::HIS3 spt6-140	5 ± 1	109 ± 13
snf2Δ1::HIS3 spt6-167	10 ± 3	107 ± 17
snf2Δ1::HIS3 spt3Δ203:TRP1	3 ± <1	6 ± 1
suc2Δ(−1900/−390)	3 ± 1	3 ± 1
suc2Δ(−1900/−390) spt4Δ1::URA3	3 ± 1	5 ± 1
suc2Δ(−1900/−390) spt5-194	4 ± 1	9 ± 1
suc2Δ(−1900/−390) spt6-140	7 ± 1	12 ± 1

[a] Micromoles of glucose released per min per 100 mg (dry weight) of cells as described in MATERIALS AND METHODS. Values are the averages of two strains, each assayed at least twice; ± standard error.

We reasoned that if the SPT4 and SPT5 gene products were related functionally to the SPT6 gene product, *spt4* and *spt5* mutations might also suppress the defect in *SUC2* transcription caused by *snf2* mutations. To test this possibility, we measured the effect of *spt* mutations on *SUC2* expression by assaying invertase activity in *spt4 snf2*, *spt5 snf2* and *spt6 snf2* double mutant strains. As shown in Table 4, mutations in either *SPT4* or *SPT5* partially suppressed the defect in *SUC2* expression in *snf2* mutant strains. The *spt4Δ1::URA3*, *spt4-289* and *spt5-8* alleles had weak effects, while the *spt5-194*, *spt6-140* and *spt6-167* mutations were stronger, restoring nearly wild-type levels of *SUC2* expression (Table 4). In contrast, null mutations in the *SPT3* gene, which belongs to a different phenotypic class of *SPT* genes (WINSTON *et al.* 1987), showed no suppression of *snf2* (Table 4).

To verify that suppression of *snf2* by *spt4*, *spt5* and *spt6* occurs at the level of transcription, we determined *SUC2* mRNA levels by Northern hybridization analysis. This analysis demonstrated that at least *spt5* and *spt6* mutations increased *SUC2* mRNA levels in a

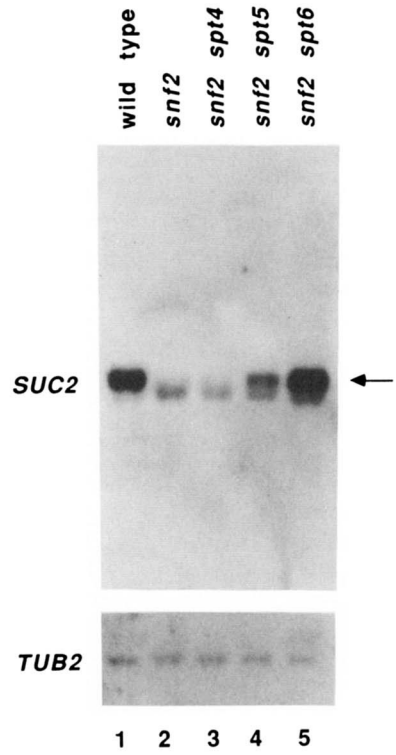

FIGURE 2.—Mutations in *SPT5* and *SPT6* restore expression of *SUC2* RNA in *snf2* mutant strains. Total yeast RNA was prepared from cells grown under derepressing condition and analyzed by Northern hybridization analysis. The filter was hybridized with a *SUC2* probe, then rehybridized with *TUB2* as a standardization probe. Each lane contains approximately 5 μg of RNA from strains (1) FW1237, (2) MS37, (3) MS91, (4) MS4 and (5) MS38. The arrow indicates the position of the 1.9-kb *SUC2* mRNA, which encodes the secreted form of invertase. The 1.8-kb RNA encodes an intracellular form of invertase of unknown function (CARLSON and BOTSTEIN 1982).

snf2 mutant background (Figure 2). The *SUC2* gene encodes two mRNAs that are regulated differently: a glucose-repressible 1.9-kb mRNA encodes the secreted form of invertase, and a constituitive 1.8-kb mRNA encodes an intracellular form of invertase of unknown function (CARLSON and BOTSTEIN 1982). The *spt5-194 snf2* mutant had an intermediate level of the 1.9-kb *SUC2* RNA (Figure 2, lane 4). An *spt6-140 snf2* mutant strain had an approximately wild-type level of *SUC2* RNA (Figure 2, lane 5), similar to that previously described by NEIGEBORN, CELENZA and CARLSON (1987). By this assay, which is less sensitive than measurement of invertase activity, the relatively weak suppression of *snf2* by the *spt4Δ1::URA3* mutation was not detectable (Figure 2, lane 3). We observed that the *spt6-140 snf2* strain had higher levels of *SUC2* RNA than the *spt5-194 snf2* strain (Figure 2), while the average units of invertase activity for two strains of each genotype were approximately equal (Table 4). We believe that this difference is a result of an effect of cell density on the level of invertase measured (see MATERIALS AND METHODS).

Mutations in *SPT6* were also shown previously to restore low levels of invertase activity to strains with upstream activation sequence (UAS) deletions at *SUC2* (NEIGEBORN, CELENZA and CARLSON 1987). To determine whether *spt4* or *spt5* mutations also partially bypassed the requirement for the *SUC2 UAS*, we assayed invertase activity in *spt suc2ΔUAS* strains. As shown in Table 4, both the *spt5-194* and the *spt6-140* mutations restored a low level of invertase activity in a *suc2ΔUAS* background. However, the *spt4Δ1::URA3* mutation had no detectable effect on expression of the *suc2ΔUAS* allele (Table 4).

Since the *spt5* and *spt6* effects on the *suc2ΔUAS* allele were slight, we also used a plate assay to analyze suppression of this *cis*-acting mutation. Invertase is required by yeast to utilize sucrose as a carbon source, and growth on YP + sucrose plates is a sensitive indicator of low levels of invertase. Therefore, we spotted cell suspensions of wild type, *suc2ΔUAS*, *spt4 suc2ΔUAS*, *spt5 suc2ΔUAS*, and *spt6 suc2ΔUAS* strains on YPD and YP + sucrose plates. The ability of these strains to grow was consistent with our invertase assays. After 2 days at 30°, the wild type and the *spt6-140 suc2ΔUAS* strains were Suc^+, while the *spt5-194 suc2ΔUAS* strain was $Suc^{+/-}$. In contrast, both the *spt4Δ1::URA3 suc2ΔUAS* and the *suc2ΔUAS* strain were Suc^-, even after a third day on a YP + sucrose plate. (All of the strains grew well on YPD medium.) The inability of the *spt4Δ1::URA3* mutation to suppress the *suc2ΔUAS* mutation was consistent with its weak suppression of the less severe defect caused by the *snf2Δ1::URA3* mutation.

Our observations that an *spt5-194* mutation was a strong suppressor of the *snf2* transcriptional defect and that some *spt5* mutations failed to complement *spt6* mutations raised the possibility that some temperature-sensitive *ssn20* alleles (NEIGEBORN, RUBIN and CARLSON 1986) might actually be *spt5* mutations. Complementation tests using the cloned *SPT5* and *SPT6* genes indicated that *ssn20-2*, *ssn20-3* and *ssn20-11* were *spt5* alleles; genetic linkage tests confirmed their identity as *spt5* mutations (E. A. MALONE, M. CARLSON and F. WINSTON, unpublished data). These alleles have been renamed *spt5-2*, *spt5-3* and *spt5-11*, respectively.

Physical association of the SPT5 and SPT6 proteins: The genetic interactions among *SPT4*, *SPT5* and *SPT6* are consistent with two classes of models. In the first class, the SPT4, SPT5 and SPT6 proteins function in a regulatory pathway, with one SPT protein regulating the level or the activity of the next SPT protein. In the second class of models, the SPT4, SPT5 and SPT6 proteins function in a protein complex and contribute to a common activity. The results of our analysis of *SPT4*, *SPT5* and *SPT6* mRNA and protein levels in wild type, *spt4*, *spt5* and *spt6* mutant strains did not support models in which *SPT4*, *SPT5* and *SPT6* regulate the levels of mRNA or protein for

other *SPT* genes in this triad (SWANSON 1991). Therefore, we investigated whether the SPT5 and SPT6 proteins interact physically. (Since the SPT4 protein is detectable only when overexpressed, we did not include it in these studies.)

In the first series of experiments, we immunoprecipitated an HA1-SPT6 fusion protein (SWANSON, CARLSON and WINSTON 1990) using a monoclonal antibody specific to the nine amino acid HA1 epitope (NIMAN *et al.* 1983), and then determined whether the immune complexes contained a LexA-SPT5 fusion protein using LexA-specific antibody. In this test, the LexA-SPT5 protein coimmunoprecipitated with the HA1-SPT6 protein (Figure 3A, lane 1). Several control experiments were done to test the specificity of the LexA-SPT5 coimmunoprecipitation. These control experiments demonstrated that the precipitation of the LexA-SPT5 fusion protein required the HA1-SPT6 protein, since the LexA-SPT5 protein was not precipitated when the HA1 antibody was omitted (Figure 3A, lane 4), nor when the antibody was incubated with extracts that either lacked the HA1-epitope (Figure 3A, lane 2) or contained a fusion of the HA1 epitope to β-galactosidase instead of SPT6 (Figure 3A, lane 3).

To confirm that the association between the HA1-SPT6 and the lexA-SPT5 fusion proteins required the SPT5 protein and was independent of the LexA domain, we determined whether an SPT5-β-galactosidase fusion protein associated with HA1-SPT6. Similar to the results obtained with the LexA-SPT5 protein, the SPT5-β-galactosidase protein coimmunoprecipitated with the HA1-SPT6 protein (Figure 3B, lane 2). Precipitation of the SPT5-β-galactosidase protein was dependent on HA1-SPT6, since SPT5-β-galactosidase was not detected in immune complexes that lacked either the HA1 antibody (Figure 3B, lane 3) or the HA1 epitope (Figure 3B, lane 1). In the converse experiment, coimmunoprecipitation of the HA1-SPT6 protein with the SPT5-β-galactosidase protein also occurred, although it required pretreatment with the cross-linking reagent DSP (Figure 3C, lanes 1 and 3). Cross-linking is often required to detect specific coimmunoprecipitation (for example see SANDERS *et al.* 1992).

The predominant species of the SPT5-β-galactosidase fusion protein present in the HA1-SPT6 immune complexes migrated faster than full length SPT5-β-galactosidase and appeared to be approximately 15 kD smaller. This species was also seen in the total protein extract. In the same experiment, the full length SPT5-β-galactosidase fusion protein was precipitated by β-galactosidase-specific antibody (Figure 3C, lanes 1 and 3), indicating that the full length protein was present in the immunoprecipitation reaction. Therefore, we believe that the apparently smaller form of SPT5-β-galactosidase is a degradation product that interacts with HA1-SPT6 more efficiently than does the full length SPT5-β-galactosidase, and thus is enriched by the HA1-SPT6 immunoprecipitation. A simple explanation for this observation is that the addition of β-galactosidase to the carboxy terminus of SPT5 interferes with the SPT5-SPT6 protein interaction, either due to direct interference by β-galactosidase sequences with the SPT6 interaction or to tetramerization of the SPT5-β-galactosidase hybrid protein (ZABIN and FOWLER 1978).

The *SPT5* nucleotide sequence predicts that the carboxy-terminal domain of the protein consists almost exclusively of 15 copies of a 6-amino acid repeat with the consensus S-T/A-W-G-G-A/Q (SWANSON, MALONE and WINSTON 1991). While this repeat domain appears to be critical for SPT5 activity (SWANSON, MALONE and WINSTON 1991), its function is not known. We assessed whether the SPT5 repeat domain was required for SPT5-SPT6 protein interactions by the coimmunoprecipitation assay using SPT5-β-galactosidase hybrid proteins that contain either zero or nine copies of the SPT5 repeat. Equal amounts of each SPT5-β-galactosidase species coimmunoprecipitated with SPT6 (data not shown). Therefore, the SPT5 carboxy-terminal repeat domain does not appear to be required for the physical interaction between the SPT5 and SPT6 proteins. Of course, we cannot rule out the possibility that β-galactosidase substitutes for the SPT5 repeat domain with respect to interaction with SPT6.

DISCUSSION

In this study, we investigated the functional relationship between *SPT4*, *SPT5* and *SPT6*, three genes previously identified as important for transcription in yeast (WINSTON *et al.* 1984; FASSLER and WINSTON 1988; CLARK-ADAMS and WINSTON 1987; SWANSON, MALONE and WINSTON 1991; E. A. MALONE, J. FASSLER and F. WINSTON, unpublished data). On the basis of several genetic and biochemical criteria, the SPT4, SPT5 and SPT6 proteins appear to function together in a transcriptional process that is essential for viability. First, the combination of mutations in any two of these three genes causes lethality. Second, some mutations in any two of these three genes fail to complement each other. Third, *spt4*, *spt5* and *spt6* mutations suppress a common set of *cis*- and *trans*-acting mutations that reduce transcription of the *HIS4*, *LYS2* and *SUC2* genes (WINSTON *et al.* 1984; FASSLER and WINSTON 1988; CLARK-ADAMS and WINSTON 1987; NEIGEBORN, CELENZA and CARLSON 1987; SWANSON, MALONE and WINSTON 1991; E. A. MALONE, J. FASSLER and F. WINSTON, unpublished data). In a related study, we found that mutations in *SPT4*, *SPT5* and *SPT6* suppress the defect in Ty transcription caused

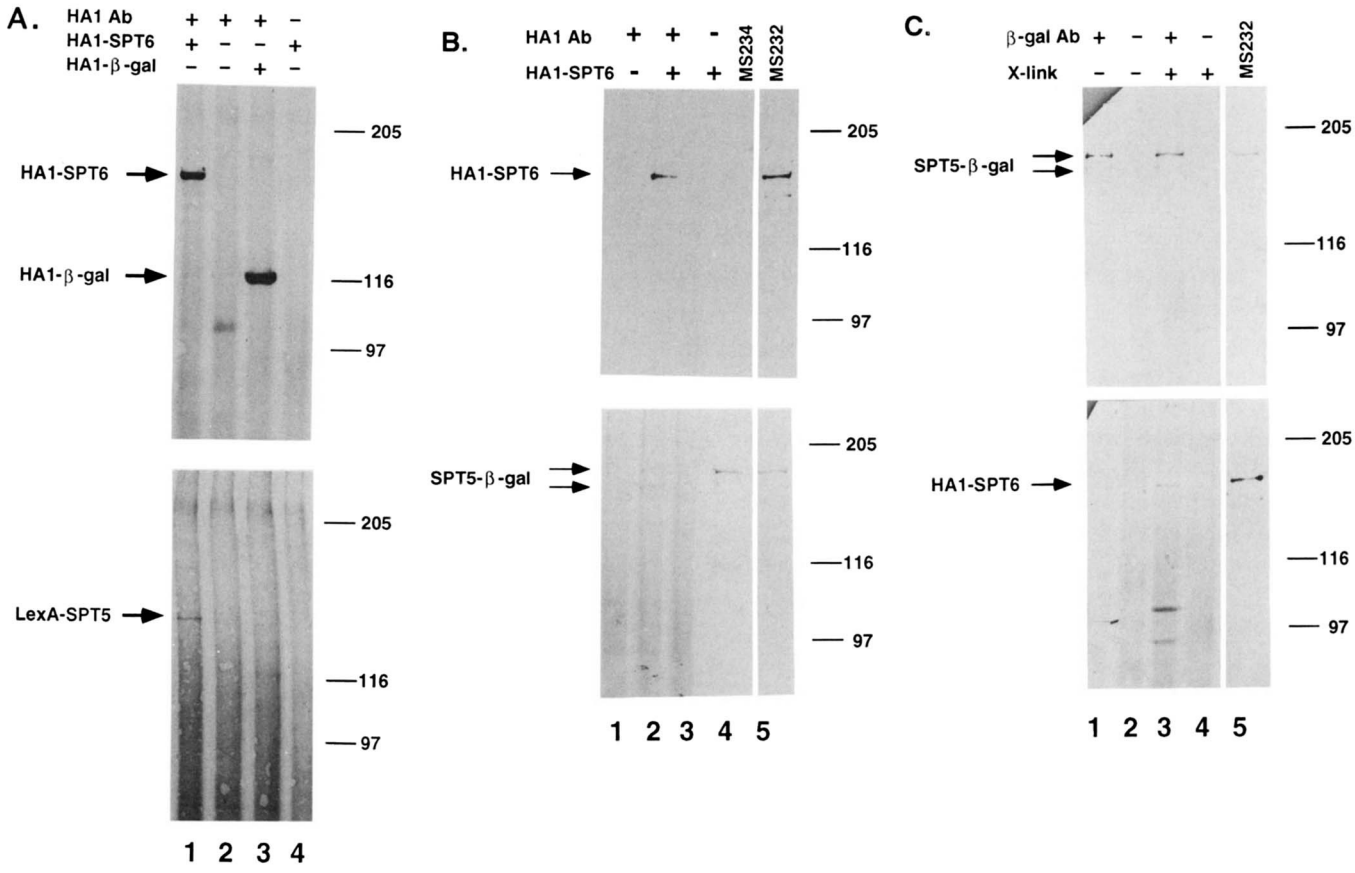

FIGURE 3.—Coimmunoprecipitation of SPT5 fusion proteins with the HA1-SPT6 protein. (A) Coimmunoprecipitation of LexA-SPT5 with HA1-SPT6. Protein lysates prepared from strains MS225 (lanes 1 and 4), MS227 (lane 2) and MS242 (lane 3) were immunoprecipitated with HA1-specific antibody, then the immune complexes were analyzed by immunoblotting with HA1-specific antibody (top panel, 5-μl sample per lane) and LexA-specific antibody (bottom panel, 25-μl sample per lane). Strains MS225, MS227 and MS242 each encode the LexA-SPT5 fusion protein. (B) Coimmunoprecipitation of SPT5-β-galactosidase with HA1-SPT6. Protein lysates prepared from strains MS234 (lane 1) and MS232 (lanes 2 and 3) were immunoprecipitated with HA1-specific antibody, then the immune complexes were analyzed by immunoblotting with HA1-specific antibody (top panel, 5-μl sample per lane) and β-galactosidase-specific antibody (bottom panel, 25-μl sample per lane). Total protein lysates prepared from strain MS234 (lane 4) and MS232 (lane 5) were analyzed by immunoblotting with HA1-specific antibody (top panel, 7.5 μg per lane) and β-galactosidase-specific antibody (bottom panel, 7.5 μg per lane). In this experiment, we estimate that 15–20% of total HA1-SPT6 is immunoprecipitated and that 2–4% of the SPT5-β-galactosidase coimmunoprecipitates. (C) Coimmunoprecipitation of HAI-SPT6 with SPT5-β-galactosidase. Protein lysate prepared from strain MS232 was either cross-linked with DSP (lanes 3 and 4) or mock-cross-linked (lanes 1 and 2) and immunoprecipitated with β-galactosidase-specific antibody. The immune complexes were analyzed by immunoblotting with β-galactosidase-specific antibody (top panel, 5-μl sample per lane) or with HA1-specific antibody (bottom panel, 25-μl sample per lane). In this experiment, we estimate that 20–30% of the total SPT5-β-galactosidase is immunoprecipitated and that 1–2% of the HA1-SPT6 coimmunoprecipitates. For each figure a + indicates the presence and a − indicates the absence during the immunoprecipitation reaction of the indicated HA1-fusion protein, the antibody or the cross-linking reagent DSP. The panels represent identical gels used to analyze two aliquots of each immunoprecipation reaction.

by *snf2* mutations (HAPPEL, SWANSON and WINSTON 1991). Thus, the *spt4*, *spt5* and *spt6* effects on Ty transcription parallel the effects demonstrated for *SUC2* expression. Finally, results of coimmunoprecipitation experiments indicate that the SPT5 and SPT6 proteins interact physically.

Our genetic data are consistent with a model in which the sum of SPT4, SPT5 and SPT6 activities is required to be above a particular threshold to result in a wild-type phenotype. By this model, reduction below this threshold results in an Spt$^-$ phenotype; reduction below a lower threshold results in inviability. This model is supported by results examining both haploid and diploid strains. In haploids, leaky effects (leaky mutations in *SPT5* or *SPT6* or any mutation in *SPT4*), cause an Spt$^-$ phenotype; tight effects (double mutants or null mutations in *SPT5* or *SPT6*) cause inviability. Similarly, in diploids leaky effects (complete loss of one copy of *SPT5* or *SPT6*, or heterozygosity at more than one of these three *SPT* loci) cause an Spt$^-$ phenotype; tight effects (homozygous null mutations in *SPT5* or *SPT6*) cause lethality. Thus, reducing the sum of SPT4, SPT5 and SPT6 activities by either a single severe mutation or a combination of less severe mutations in two of these three genes results in a mutant phenotype. Also consistent with this model is the result that we have not observed

allele-specific *spt4*, *spt5* and *spt6* interactions in our studies of double mutant lethality.

The failure of unlinked recessive mutations to complement has been reported previously for other groups of functionally related genes and is generally thought to indicate a physical interaction between the respective gene products (For examples see BISSON and THORNER 1982; ATKINSON 1985; RINE and HERSKOWITZ 1987; JAMES *et al.* 1988; STEARNS and BOTSTEIN 1988; REGAN and FULLER 1988; HAYS *et al.* 1989; GREEN *et al.* 1990). For example, in both *S. cerevisiae* and *D. melanogaster*, particular mutations in α-tubulin-encoding genes fail to complement particular mutations in β-tubulin-encoding genes (STEARNS and BOTSTEIN 1988; HAYS *et al.* 1989). In both cases, the failure of these mutations to complement was allele specific, indicating that the mutant proteins interact to form either an inactive complex or a "poison" (STEARNS and BOTSTEIN 1988; HAYS *et al.* 1989). STEARNS and BOTSTEIN (1988) also showed that recessive mutations in *TUB1* and *TUB3*, which encode the major and minor forms of α-tubulin, respectively, also fail to complement each other. In this case, however, the failure to complement was not allele-specific and most likely reflects a cumulative reduction of protein below the minimal threshold necessary for function. The unlinked noncomplementation between *spt4*, *spt5* and *spt6* mutations, in conjunction with our other genetic data, is most consistent with a cumulative reduction of function, as opposed to specific interactions between mutant proteins, as the cause for the failure to complement. This model is strongly supported by the result that an *spt4* null allele fails to complement either *spt5* or *spt6* mutations.

Our evidence that the SPT4, SPT5 and SPT6 proteins interact and contribute to a common activity is consistent with results of previous studies of the effects of altered dosage of the SPT5 and SPT6 gene products. For both *SPT5* and *SPT6*, increased or decreased gene copy number causes an Spt$^-$ phenotype (CLARK-ADAMS and WINSTON 1987; NEIGEBORN, CELENZA and CARLSON 1987; SWANSON, MALONE and WINSTON 1991). A simple model that is consistent with these dosage effects is that the SPT5 and SPT6 proteins function in a complex that is sensitive to altered stoichiometry. Altered stoichoimetry of proteins that are components of a complex has been observed to cause mutant phenotypes in several other cases. For example, assembly of the bacteriophages T4 and λ are disrupted by altered levels of some gene products important for head morphogenesis (FLOOR 1970; STERNBERG 1976). In addition, mitotic chromosome transmission and transcription can be affected by altered dosage of some histone genes (MEEKS-WAGNER and HARTWELL 1986; CLARK-ADAMS *et al.* 1988).

SPT4 appears to be distinct from *SPT5* and *SPT6* in several respects. First, both *SPT5* and *SPT6* encode large, nuclear proteins with unusually acidic amino terminal domains (SWANSON, CARLSON and WINSTON 1990; SWANSON, MALONE and WINSTON 1991). In contrast, the SPT4 protein is not large or acidic, and it is present in much lower amounts as compared to either the SPT5 or SPT6 protein. Second, altered *SPT4* gene dosage does not cause an Spt$^-$ phenotype. Finally, the *SPT4* gene is not essential (E. A. MALONE, J. FASSLER and F. WINSTON, unpublished data). Therefore, the function of the SPT4 protein is likely to be distinct from that of the SPT5 and SPT6 proteins. Analysis of the SPT4 interactions with SPT5 and SPT6 must await more sensitive methods of detecting SPT4.

Mutations in the *SPT16/CDC68* gene and the histone genes *SPT11/HTA1* and *SPT12/HTB1* alter transcription of many of the same loci as mutations in *SPT4*, *SPT5* and *SPT6*. For example, the pattern of *his4-912δ* and *lys2-128δ* transcription is similar in *spt16*, *spt11*, *spt12* and *spt6* mutant strains (CLARK-ADAMS *et al.* 1988; CLARK-ADAMS and WINSTON 1987; SWANSON, MALONE and WINSTON 1991; MALONE *et al.* 1991). Also, the defect in transcription of *SUC2* and Ty elements caused by *snf2* mutations is suppressed by *spt4*, *spt5* and *spt6* mutations, as well as mutations in either *SPT16* or the *SPT11/HTA1-SPT12/HTB1* locus (HAPPEL, SWANSON and WINSTON 1991; MALONE *et al.* 1991; J. HIRSCHHORN, S. BROWN, C. D. CLARK and F. WINSTON, unpublished data). These phenotypic similarities suggest that *SPT4*, *SPT5* and *SPT6*, together with *SPT16* affect transcription via an effect on chromatin structure. However, double mutants of *spt16* with either *spt4*, *spt5* or *spt6* are viable as haploids and *spt16* mutations complement these other mutations in diploids (MALONE *et al.* 1991). Therefore, the SPT16 protein does not appear to function in a protein complex with the SPT4, SPT5 and SPT6 proteins.

On the basis of the phenotypic similarities to histone mutant strains, as well as the physical characteristics of the SPT5 and SPT6 proteins, we favor a model in which the SPT4, SPT5 and SPT6 proteins function as a nuclear protein complex that is important for establishment or maintenance of proper chromatin structure. Given that *spt4*, *spt5* and *spt6* mutations suppress the loss of transcriptional activation in *snf2*, *snf5* and *snf6* mutants, we hypothesize that the SPT4-SPT5-SPT6 complex would be required for repression of gene expression. Therefore, *SPT4*, *SPT5* and *SPT6* may serve a role that is somewhat analogous to that postulated for the genes in the *Polycomb* group of *D. melanogaster*–chromatin-associated proteins that are required to maintain chromatin in a repressed state (PARO 1990). Alternatively, *SPT4*, *SPT5* and *SPT6* may encode functions required for establish-

ment of a repressed chromatin state. For example, chromatin assembly on SV40 DNA during *in vitro* replication requires CAF-1, a nuclear, multisubunit protein complex (SMITH and STILLMAN 1989; 1991a,b). Possibly, reduced levels of such an activity would result in altered establishment of chromatin states *in vivo*. Analysis of chromatin structure in the *spt4*, *spt5* and *spt6* mutants, as well as continued genetic and biochemical studies of SPT4, SPT5 and SPT6, should help to elucidate the mechanism by which these proteins affect transcription in yeast.

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