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SPT5, an Essential Gene Important for Normal Transcription in *Saccharomyces cerevisiae*, Encodes an Acidic Nuclear Protein with a Carboxy-Terminal Repeat

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Mutations in the *SPT5* gene of *Saccharomyces cerevisiae* were isolated previously as suppressors of δ insertion mutations at *HIS4* and *LYS2*. In this study we have shown that *spt5* mutations suppress the *his4-912*δ and *lys2-128*δ alleles by altering transcription. We cloned the *SPT5* gene and found that either an increase or a decrease in the copy number of the wild-type *SPT5* gene caused an Spt⁻ phenotype. Construction and analysis of an *spt5* null mutation demonstrated that *SPT5* is essential for growth, suggesting that *SPT5* may be required for normal transcription of a large number of genes. The *SPT5* DNA sequence was determined; it predicted a 116-kDa protein with an extremely acidic amino terminus and a novel six-amino-acid repeat at the carboxy terminus (consensus = S-T/A-W-G-G-A/Q). By indirect immunofluorescence microscopy we showed that a bifunctional SPT5–β-galactosidase protein was located in the yeast nucleus. This molecular analysis of the *SPT5* gene revealed a number of interesting similarities to the previously characterized *SPT6* gene of *S. cerevisiae*. These results suggest that *SPT5* and *SPT6* act in a related fashion to influence essential transcriptional processes in *S. cerevisiae*.

A large number of mutations that alter transcription in the yeast *Saccharomyces cerevisiae* were isolated by selection for suppressors of Ty or solo δ insertion mutations at *HIS4* and *LYS2* (19, 64, 65). These mutations have identified 16 unlinked genes, named *SPT* genes (for suppressor of Ty). Molecular analysis has revealed that three of the *SPT* genes encode proteins that are believed to play central roles in gene expression: *SPT15* encodes the TATA-binding protein TFIID (16), and *SPT11* and *SPT12* are the same as the genes *HTA1* and *HTB1*, which encode the histone proteins H2A and H2B, respectively (12). Genetic analysis of the remaining *spt* mutants allowed classification of most of the *SPT* genes into three groups, based on distinct mutant phenotypes: the first group includes *SPT11* and *SPT12*, the second includes *SPT15*, and the third includes *SPT13/GAL11* (19, 20).

The *SPT5* gene, characterized in this study, and the *SPT6* gene, analyzed previously (13, 45, 61), belong to the phenotypic class represented by the histone genes *HTA1/SPT11* and *HTB1/SPT12* (12, 19). Mutations in each of these genes suppress the same set of Ty and δ insertion mutations at the *HIS4* and *LYS2* genes (19, 64) as well as some *cis*- and *trans*-acting mutations that severely reduce the expression of the *SUC2* gene and Ty elements (11, 26, 45, 46, 62). Previous studies also demonstrated that altered dosage of the *SPT6* gene or the *HTA1-HTB1* locus causes similar mutant phenotypes (12, 13, 45). Molecular analysis demonstrated that *SPT6* encodes an essential nuclear protein of 170 kDa with an extremely acidic amino terminus (13, 45, 61). These studies suggested that *SPT6* plays an essential role in transcription, perhaps by affecting chromatin structure.

On the basis of the observation that *spt5* mutants are phenotypically similar to *spt6* and histone mutants, we decided to analyze the *SPT5* gene and its product. In this study, we have demonstrated that mutations in the *SPT5* gene suppress δ insertion mutations by altering transcription. We cloned the *SPT5* gene and used the cloned gene for additional molecular and genetic studies of *SPT5*. The nucleotide sequence of the *SPT5* gene predicts a 1,063-amino-acid protein with a highly acidic amino terminus and 15 copies of a six-amino-acid repeat at the carboxy terminus. Analysis of the SPT5 repeat domain indicated that this region is required for SPT5 function. Like *SPT6*, *SPT5* is essential for growth, proper *SPT5* gene dosage is critical for function, and the SPT5 protein appears to be located in the yeast nucleus. This molecular analysis supports the idea that *SPT5* and *SPT6* are functionally related and are required for normal transcription in *S. cerevisiae*.

MATERIALS AND METHODS

Strains and genetic methods. All *S. cerevisiae* strains used in these studies (Table 1) were constructed in this laboratory and are derivatives of strain S288C (*MAT*α *gal2*) except for strains YPH149 (24), SF402-4D (Yeast Genetic Stock Center, Berkeley, Calif.), and K396-11A and K396-22B (36). Parentheses indicate autonomous plasmids, brackets indicate integrated plasmids, and double brackets indicate autonomous linear chromosomal fragments. Standard methods for mating, sporulation, and tetrad analysis were used (43, 56). The *lys2-128*δ allele consists of a solo δ element inserted 153 bp downstream of the *LYS2* translation initiation codon (13, 59). The *his4-912*δ allele is an insertion of a solo δ element at bp -97 relative to the *HIS4* transcription initiation site (8, 18). Strains MS194 and MS195 contain *SPT5-lacZ* hybrid genes integrated at the *spt5*Δ*202*::*LEU2* allele. These strains were constructed by transforming the diploid strain BM80 with pMS50 DNA (MS194) or pBM68 DNA (MS195) that had been linearized by restriction digestion with *Pst*I. The site of integration was determined by sporulating transformants, dissecting tetrads, and scoring the Ura and Leu phenotypes of the spore clones.

Media. Rich medium (YPD), minimal medium (SD), sup-

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TABLE 1. Yeast strains

Strain	Genotype
FY98	*MAT***a** *ura3-52 leu2Δ1*
FY360	*MAT***a** *spt5-194 leu2Δ1*
FY382	*MAT***a** *spt5-8 ura3-52 leu2Δ1*
FY120	*MAT***a** *his4-912δ lys2-128δ ura3-52 leu2Δ1*
FY300	*MAT***a** *spt5-194 ura3-52 his4-912δ lys2-128δ leu2Δ1*
FY276	*MAT***a** *spt5-8 his4-912δ lys2-128δ ura3-52 leu2Δ1*
MS11	*MATα spt5-8 lys2-128δ ura3-52 his4-917 leu2*
MS63	*MAT***a** *spt5-24 his4-912δ lys2-128δ ura3-52 ade2-1 trp5 can1-100*
FW1237	*MAT***a** *his4-912δ lys2-128δ ura3-52*
MS98	*MAT***a** *his4-912δ lys2-128δ ura3-52* [*SPT5*-pMS15-*URA3*]
MS17	*MATα spt5-24 his4-912δ lys2-128δ ura3-52 ade2-1 can1-100*
FY298	*MAT***a** *spt5-194 his4-912δ lys2-128δ ura3-52 leu2Δ1 ade8*
BM60	*MAT***a**/*MATα ura3-52/ura3-52 his4-912δ/his4-912δ lys2-128δ/lys2-128δ leu2-3,112/leu2-3,112 trp1Δ1/trp1Δ1*
BM80	*MAT***a**/*MATα spt5Δ202::LEU2/SPT5 ura3-52/ura3-52 his4-912δ/his4-912δ lys2-128δ/lys2-128δ leu2-3,112/leu2-3,112 trp1Δ1/trp1Δ1*
MS189	*MAT***a** *ura3-52 his4-912δ lys2-128δ leu2Δ1* (pCGS42)
MS190	*MAT***a** *ura3-52 his4-912δ lys2-128δ leu2Δ1* (pMS4)
MS191	*MAT***a** *ura3-52 his4-912δ lys2-128δ leu2Δ1* (pMS24)
YPH149	*MATα ade his7 lys2 trp1Δ1 ura3-52* [[CF/*URA3*/*RAD2* distal]] [[CF/*TRP1*/*RAD2* proximal]]
K396-11A	*MAT***a** *lys7 spo11 ura3 ade1 his1 leu2 met3 trp5*
K396-22B	*MATα lys7 spo11 ura3 ade1 his1 leu2 met3 trp5*
SF402-4D	*MAT***a** *sec59*
MS113	*MAT***a** *spt5-24 sec59 his4-912δ lys2-128δ trp1Δ1*
MS192	*MATα lys7 his4-912δ ura3-52*
MS193	*MATα lys7 his4-912δ ura3-52 ade1*
MS194	*MAT***a**/*MATα* [*spt5Δ202::LEU2-SPT5*(917)-*lacZ-URA3*]/*SPT5 ura3-52/ura3-52 his4-912δ/his4-912δ lys2-128δ/lys2-128δ leu2Δ1/leu2Δ1 trp1Δ1/trp1Δ1*
MS195	*MAT***a**/*MATα* [*spt5Δ202::LEU2-SPT5*(1004)-*lacZ-URA3*]/*SPT5 ura3-52/ura3-52 his4-912δ/his4-912δ lys2-128δ/lys2-128δ leu2Δ1/leu2Δ1 trp1Δ1/trp1Δ1*
BM418	*MAT***a** *spt5-194 his4-912δ lys2-128δ ura3-52 ade8*
BM437	*MATα spt5-194 his4-912δ lys2-128δ ade8* [*ura3-52-SPT5*(1004)-*lacZ-URA3*]
BM443	*MAT***a** *his4-912δ lys2-128δ leu2Δ1* [*ura3-52-SPT5*(1004)-*lacZ-URA3*]
BM444	*MATα his4-912δ lys2-128δ leu2Δ1 ade8* [*ura3-52-SPT5*(1004)-*lacZ-URA3*]
BM448	*MAT***a**/*MATα his4-912δ/his4-912δ lys2-128δ/lys2-128δ leu2Δ1/leu2Δ1 ade8/ADE8* [*ura3-52-SPT5*(1004)-*lacZ-URA3*]/[*ura3-52-SPT5*(1004)-*lacZ-URA3*]
BM454	*MAT***a**/*MAT***a** *his4-912δ/his4-912δ lys2-128δ/lys2-128δ leu2Δ1/leu2Δ1 ade8/ADE8* [*ura3-52-SPT5*(1004)-*lacZ-URA3*]/[*ura3-52-SPT5*(1004)-*lacZ-URA3*]
BM455	*MATα/MATα his4-912δ/his4-912δ lys2-128δ/lys2-128δ leu2Δ1/leu2Δ1 ade8/ADE8* [*ura3-52-SPT5*(1004)-*lacZ-URA3*]/[*ura3-52-SPT5*(1004)-*lacZ-URA3*]
BM467	BM454 × BM455
BM330	*MATα/MATα his4-912δ/his4-912δ lys2-128δ/lys2-128δ ura3-52/ura3-52 trp1Δ63/trp1Δ63*
BM331	*MAT***a**/*MAT***a** *his4-912δ/his4-912δ lys2-128δ/lys2-128δ ura3-52/ura3-52 trp1Δ63/trp1Δ63*
BM339	BM331 × BM330

plemented SD (SD to which amino acids were added from liquid stock solutions), synthetic complete medium lacking a single amino acid, and sporulation medium were prepared as described previously by Sherman et al. (56). GNA presporulation medium contained 10 g of yeast extract, 30 g of nutrient broth, 50 g of dextrose, and 20 g of agar per liter.

Transformations. Yeast cells were transformed by the lithium acetate method (32). *Escherichia coli* HB101 (3) and TB1 (Bethesda Research Laboratories, Gaithersburg, Md.) were transformed as described previously (41).

Enzymes. Restriction enzymes were purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and New England BioLabs (Beverly, Mass.). T4 DNA ligase was purchased from New England BioLabs. All enzymes were used according to the instructions of the supplier.

DNA preparation and analysis. Yeast genomic and plasmid DNAs were prepared as described previously (28). *E. coli* plasmid DNA was prepared by the method of Holmes and Quigley (29) or Birnboim and Doly (2). DNA restriction fragments were separated by electrophoresis through Seakem agarose (FMC Bioproducts, Rockland, Maine) and purified by electroelution, using a device designed by Larry Peck (49). Southern blot hybridization analysis was performed as described previously (51). Radiolabeled DNA probes were prepared by nick translation (50) or random priming (21, 22), using [α-^{32}P]dATP (Amersham) and reagent kits from Boehringer Mannheim.

RNA preparation and analysis. Yeast RNA was prepared as described previously (7) from cells grown in supplemented SD medium at 25°C to a density of 1×10^7 to 2×10^7 cells per ml. RNA was separated in formaldehyde-agarose gels. After denaturation in 0.05 M NaOH and renaturation in 0.1 M Tris (pH 7.5), the RNA was transferred to GeneScreen (New England Nuclear, Boston, Mass.) by capillary blotting in 1× SSC (0.15 M NaCl, 0.015M sodium citrate). RNA was cross-linked to the membrane by UV irradiation (10). Probes were hybridized by the dextran sulfate method as described in the GeneScreen instruction manual. Additional probes were hybridized after the membrane was stripped in 1× SSC–50% formamide at 80°C for 2 h. The amount of RNA in

each lane was normalized by hybridization to *TUB2* DNA (plasmid pYST138; 60).

Plasmids. Vectors used for *SPT5* subcloning were YCp50 (34) for pMS37 and pRS316 (57) for pMS18 and pMS23. Subclones were tested for *SPT5* function after transformation into the *spt5* mutant strain MS63(pMS18) or FY298 (pMS23, pMS37). For integration of the cloned yeast DNA, we constructed plasmid pMS15 by cloning the 4.0-kb *Hin*dIII-*Kpn*I fragment of pMS4 into the *Hin*dIII-*Kpn*I sites of the integrating plasmid pRS306 (57). To study the effects of multiple copies of the *SPT5* gene, plasmid pMS24 was constructed by cloning the 4.8-kb *Hin*dIII-*Eag*I fragment of pMS4 into the *Hin*dIII-*Eag*I sites of the vector pCGS42 (Collaborative Research), which contains part of 2μm circle. Plasmids used as probes were as follows: for *HIS4*, pFW45, a *Bgl*II-*Sal*I restriction fragment internal to *HIS4* cloned in pBR322 (66); for *LYS2*, pFW47, a *Bgl*II-*Xho*I restriction fragment internal to *LYS2* cloned in pBR322 (13), and pFW112, an *Eco*RI-*Bgl*II restriction fragment from the 5′ region of *LYS2* cloned in pBR322 (13); for *TUB2*, pYST138, a 0.24-kb *Bgl*II-*Kpn*I restriction fragment internal to *TUB2* cloned in a pGEM vector (60); and for *SPT5*, pMS22, a 4.0-kb *Hin*dIII-*Kpn*I fragment from pMS4 cloned in pBR322.

We constructed fusions of *SPT5* to the *E. coli lacZ* gene by using integrating plasmids containing a polylinker followed by the *lacZ* gene and the *URA3* gene as a selectable marker (42). Plasmid pBM68 was constructed by cloning the 4.0-kb *Hin*dIII-*Kpn*I restriction fragment of pMS4 into the *Hin*dIII and *Kpn*I sites of YIp356R; the *SPT5-lacZ* fusion gene contained on pBM68, designated *SPT5*(1004)-*lacZ*, encodes the first 1,004 amino acids of SPT5. Plasmid pMS50 was constructed by cloning the 3.7-kb *Hin*dIII-*Pvu*II restriction fragment of pMS4 into the *Hin*dIII-*Sma*I sites of YIp357R; the *SPT5-lacZ* fusion gene contained on pMS50, designated *SPT5*(917)-*lacZ*, encodes the first 917 amino acids of SPT5. Plasmids pBM68 and pMS50 each have a unique *Stu*I restriction site in the *URA3* gene and a unique *Pst*I site in the *SPT5* gene that are useful for directing integration of the plasmids to the *URA3* and *SPT5* genes, respectively, in transformation experiments.

We observed that *E. coli* strains carrying *SPT5* DNA on high-copy-number plasmids formed unusually small colonies.

Construction of an *spt5* null allele. To construct the *spt5* null allele, *spt5Δ202*::*LEU2*, we replaced the 0.8-kb *Sal*I-*Sal*I restriction fragment of pMS23 with a 2.2-kb *Sal*I-*Xho*I restriction fragment containing the *LEU2* gene (1) to generate plasmid pBM19. Next, the 3.1-kb *Pvu*II-*Pst*I restriction fragment from pBM19, which contains the *LEU2* gene flanked by *SPT5* sequences, was used to transform the diploid BM60 to leucine prototrophy. Strain BM80 is a stable Leu^+ transformant resulting from recombination between the fragment and the genome such that one copy of the *SPT5* gene was replaced by the null allele (53). The structure of the *SPT5* loci of the diploid BM80 was verified by Southern hybridization analysis (data not shown). The phenotype of the *spt5* null allele in haploids was analyzed following sporulation of BM80 and dissection of tetrads.

Mapping *SPT5*. The method of Carle and Olson (6) was used to prepare chromosome-size DNA from strain YPH149. Chromosome VII of YPH149 has been fragmented at *RAD2* to allow all of the yeast chromosomes to be resolved as discrete bands by alternating field gel electrophoresis (24). DNA was separated by clamped homogeneous electric field (CHEF) gel electrophoresis (9) through 0.9% agarose in 0.5× TBE buffer (45 mM Tris base, 45 mM boric acid, 1.25 mM EDTA). After electrophoresis, the gel was soaked sequentially for 20 min each in 0.25 M HCl, 0.5 M NaOH–1.0 M NaCl, and 1.5 M NaCl–1.0 M Tris (pH 7.4). The DNA was transferred to nitrocellulose by capillary blotting in 4× SSC. Radiolabeled pMS4 DNA hybridized to several chromosomes as a result of its composition: chromosome IV (*CEN4*), chromosome V (*URA3*), both fragments of chromosome VII (pBR322, *URA3*), and chromosome XIII (*SPT5*). Linkage of *SPT5* to its centromere and to markers on chromosome XIII was determined by tetrad analysis. *spt5-24* was scored by its ability to suppress the His^- phenotype conferred by the δ insertion mutation *his4-912δ*. The *sec59* allele of MS113 was derived from strain SF402-4D.

DNA sequence analysis. Restriction fragments to be sequenced were cloned into the vector M13mp18 or M13mp19 (48). The nucleotide sequence was determined by the method of Sanger et al. (54), using [α-^{35}S]dATP (Amersham) and the Sequenase version 2.0 kit (U.S. Biochemical Corp., Cleveland, Ohio). In 12 cases, where there were no convenient restriction sites, 20-base oligomers were synthesized (Mark Fleming, Biopolymers Laboratory, Department of Genetics, Harvard Medical School) and used as primers. The complete DNA sequence was determined on both strands.

Indirect immunofluorescence. For indirect immunofluorescence experiments, we used tetraploid cells because they are larger than haploid cells. A tetraploid strain containing an *SPT5-lacZ* fusion was constructed in several steps. First, we constructed haploid strains containing the *SPT5*(1004)-*lacZ* fusion by transforming strain BM418 to uracil prototrophy with pBM68 DNA that had been linearized by digestion with *Stu*I. Next, a stable integrant from this transformation, BM437, was crossed with strain FY120 to yield *SPT5* strains carrying the *SPT5*(1004)-*lacZ* fusion. Two of these strains (BM443 and BM444) were then mated by each other to form the diploid BM448. We isolated diploids homozygous at *MAT* by UV irradiating BM448 (300 ergs/mm^2) and screening for diploids that could mate as either **a** or α cells. Mating of the *MAT***a**/*MAT***a** diploid BM454 by the *MAT*α/*MAT*α diploid BM455 yielded the tetraploid strain BM467, which carries four copies of the *SPT5*(1004)-*lacZ* fusion. Strain BM339, similar to BM467 except that it does not contain an *SPT5-lacZ* fusion, was constructed by the same method.

Cells were prepared for immunofluorescence by the method of Kilmartin and Adams (35) with the modifications described previously (61). The SPT5–β-galactosidase fusion protein was identified by incubating the prepared cells with a monoclonal rabbit anti-β-galactosidase antibody (Cappel, Malvern, Pa.) diluted 1:10,000 and a fluorescein-conjugated goat anti-rabbit antibody (Sigma, St. Louis, Mo.) diluted 1:250. DNA was stained with the fluorescent dye 4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI). Fluorescent staining was visualized by using a Zeiss Photomicroscope III equipped for epi-illumination fluorescence with a Zeiss Neofluar 63× lens, numerical aperture 1.25, and photographed with Kodak TMAX-400 film. The exposure time for micrographs of fluorescein fluorescence in the experimental and control strains was 30 s.

Nucleotide sequence accession number. The GenBank accession number for the *SPT5* sequence is M62882.

RESULTS

***spt5* mutations alter transcription.** Recessive mutations in *SPT5* had been identified as suppressors of the insertion

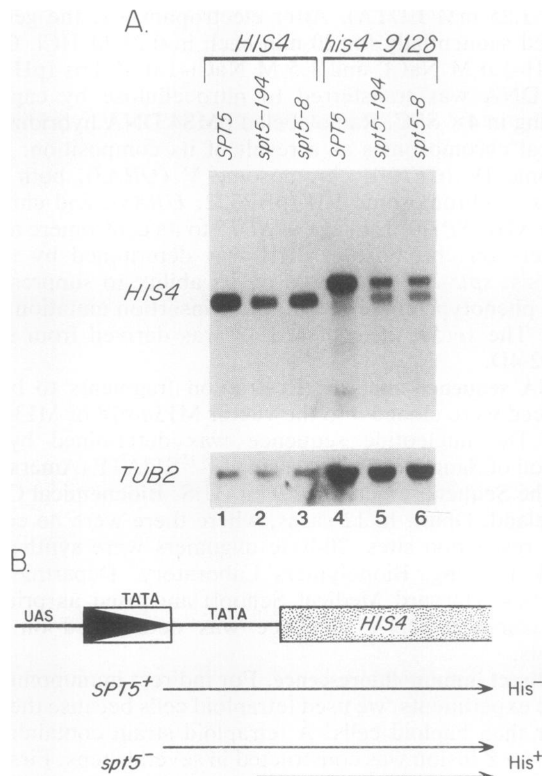

FIG. 1. Effects of *spt5* mutations on transcription of *his4-912δ*. (A) Total yeast RNA was subjected to Northern analysis. The same membrane was hybridized with the *HIS4* probe pFW45 and then rehybridized with *TUB2* probe pYST138. Approximately 2.5 μg of RNA was loaded in lanes 1 to 3, and approximately 10 μg was loaded in lanes 4 to 6. The strains used were (left to right) FY98, FY360, FY382, FY120, FY300, and FY276. (B) The top line depicts the structure of *his4-912δ*. The gray box represents the *HIS4* open reading frame. The thin lines represent flanking DNA. The box with the solid triangle represents a solo δ element. Labels above indicate the relative positions of known TATA boxes (TATA) and upstream activating sequences (UAS). The lower lines depict the probable origins of transcripts in *SPT5*+ and *spt5* strains.

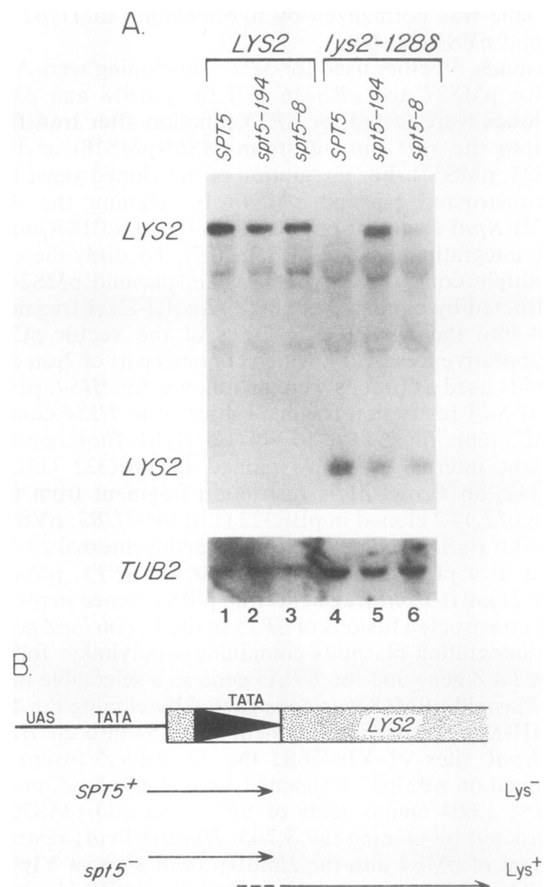

FIG. 2. Effects of *spt5* mutations on transcription of *lys2-128δ*. (A) Total yeast RNA was subjected to Northern analysis. The same membrane was hybridized with a mixture of the *LYS2* probes pFW47 and pFW112 and then rehybridized with the *TUB2* probe pYST138. Since the smaller *LYS2* RNA is much more abundant, the pFW47 probe contained approximately 10-fold more counts per minute than the pFW112 probe. Approximately 2.5 μg of RNA was loaded in lanes 1 to 3, and approximately 10 μg was loaded in lanes 4 to 6. The strains used were the same as those given for Fig. 1A. (B) The top line depicts the structure of *lys2-128δ*. The gray box represents the *LYS2* open reading frame. The thin lines represent flanking DNA. The box with the solid triangle represents a solo δ element. Labels above indicate the relative positions of known TATA boxes (TATA) and upstream activating sequences (UAS). The lower lines depict the probable origins of transcripts in *SPT5*+ and *spt5* strains.

mutations *his4-912δ* and *lys2-128δ* (19, 64). To determine whether the phenotypic changes in *spt5* mutants result from alterations in transcription, we performed Northern (RNA) hybridization analysis. Our results (Fig. 1 and 2) demonstrate that *spt5* mutations cause altered transcription at both *his4-912δ* and *lys2-128δ*.

In an *SPT*+ background, transcription of *his4-912δ* initiates at the δ initiation site and results in an mRNA that is longer than the wild-type *HIS4* mRNA (27, 58; Fig. 1). The 5′ portion of this transcript contains in-frame translation initiation and termination codons which presumably prevent normal translation of the *HIS4* coding region (58). In both *spt5-194* and *spt5-8* mutants, a shorter transcript that comigrated with the wild-type *HIS4* mRNA was produced in addition to the δ-initiated transcript (Fig. 1A, lanes 5 and 6). The levels of the two transcripts were roughly equal. The size of the new transcript indicates that *spt5* mutations allow transcription initiation at the normal *HIS4* initiation site (Fig. 1B). This same alteration in *his4-912δ* transcription is seen in *spt6* and *hta1-htb1* (histone) mutants (12, 13).

Mutations in *SPT5* also affected transcription of *lys2-128δ*. In *SPT*+ strains, transcription of *lys2-128δ* resulted in a short transcript of approximately 580 bases (Fig. 2A, lane 4), probably due to initiation at the *LYS2* initiation site and termination at the δ transcription termination site (13; Fig. 2B). In *spt5* mutant strains, a second transcript was also produced (Fig. 2A, lanes 5 and 6). For *spt5-194* mutants, this second transcript was easily seen; for *spt5-8* mutants, the new transcript was found at a low level and could be seen more clearly with longer exposures. (The two strains were equally Lys+.) This mRNA is slightly shorter than the wild-type *LYS2* transcript and is likely to result from transcription initiation near the δ initiation site and elongation through the *LYS2* gene (Fig. 2B). Mutations in *SPT6* and *HTA1-HTB1* cause similar changes in *lys2-128δ* transcription (12, 13).

Transcription of other sequences was also examined. The

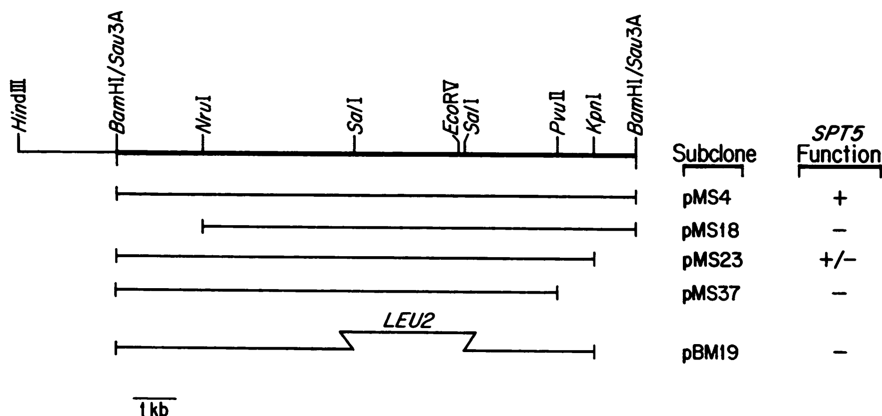

FIG. 3. Restriction map of the *SPT5* locus. The top line represents the *SPT5* locus and its restriction sites. The thinner portion of the line represents vector sequences. The lines below represent DNA fragments that were subcloned to test for *SPT5* function. Plasmid pBM19 was used to construct an *spt5* null mutant.

level of the wild-type *HIS4* and *LYS2* transcripts was slightly reduced by *spt5* mutations (Fig. 1A, lanes 1 to 3; Fig. 2A, lanes 1 to 3). There was no reduction in the level of full-length Ty transcripts in *spt5* mutants (data not shown). Similarily, *spt6* and *hta1-htb1* (histone) mutations do not affect the level of full-length Ty transcripts (4, 13). In contrast, *spt* mutants in a different phenotypic class (*spt3*, *spt7*, *spt8*, and *spt15*) abolish or greatly reduce the level of full-length Ty transcripts (17, 65, 66).

Isolation of the *SPT5* gene. An *SPT5* gene clone was isolated from a library of yeast DNA (52) by screening for plasmids that complemented an *spt5* mutant phenotype. The recipient strain, MS11 (*spt5-8 lys2-128δ ura3-52*), is Lys$^+$ due to suppression of *lys2-128δ* by *spt5-8*. Since *spt5-8* is recessive, transformants of MS11 that carry the wild-type *SPT5* gene on a plasmid should be Lys$^-$. Two Lys$^-$ candidates were identified in a screen of approximately 15,000 Ura$^+$ transformants; both contained the same DNA insert, and one of these plasmids was designated pMS4. This plasmid conferred an Spt$^+$ phenotype when retransformed into strain MS11 or when transformed into strain MS63, which contains a different *spt5* allele.

We confirmed that pMS4 contained the *SPT5* gene by demonstrating that the cloned DNA directed integration of a plasmid to the *SPT5* locus. Plasmid pMS15, an integrating plasmid that contains a restriction fragment from pMS4, was linearized at a unique *Nru*I site within the cloned DNA and used to transform FW1237 (*SPT5$^+$ ura3-52 lys2-128δ*) to uracil prototrophy. A Ura$^+$ transformant (MS98) was crossed to strain MS17 (*spt5-24 ura3-52 lys2-128δ*), and tetrads were dissected. In 17 four-spored and nine three-spored tetrads, the Spt$^+$ and Ura$^+$ phenotypes cosegregated in every tetrad, demonstrating that pMS15 DNA was tightly linked to the *SPT5* locus.

To characterize the *SPT5* gene in greater detail, several subclones were constructed and tested for *SPT5* function (Fig. 3). Although the *Hin*dIII-*Kpn*I fragment (pMS23) partially complemented the *spt5-194* allele, none of the subclones fully complemented the mutant phenotype, suggesting that the *SPT5* gene occupies most of the cloned 3.9-kb DNA fragment.

Construction and analysis of an *spt5* null mutant. We constructed an *spt5* null allele marked by the *LEU2* gene, *spt5Δ202::LEU2*, by deleting the *Sal*I-*Sal*I restriction fragment internal to *SPT5* (Fig. 3) and replacing it with the *LEU2* gene (see Materials and Methods). Integration of this allele into the diploid strain BM60 resulted in a strain heterozygous for the null allele. After sporulation and tetrad dissection of this strain (BM80), viability segregated 2:2 (9 of 10 tetrads) or 1:3 (1 of 10 tetrads). In all cases, the viable spores were Leu$^-$, indicating that they carried the wild-type *SPT5* gene. Therefore, the *SPT5* gene is essential for growth.

Altered *SPT5* gene dosage causes mutant phenotypes. Altered dosage of the *SPT6* or the *HTA1-HTB1* locus confers an Spt$^-$ mutant phenotype (12, 13, 45). To test whether altered *SPT5* gene dosage causes similar phenotypes, we constructed a set of isogenic strains that had either a reduced or increased copy number of the *SPT5* gene. These strains were then tested for suppression of the insertion mutations *his4-912δ* and *lys2-128δ*. A diploid strain (BM80) that contained only one copy of the *SPT5* gene (*SPT5$^+$/spt5Δ202::LEU2*) had a strong Spt$^-$ phenotype (His$^+$ Lys$^+$) (Fig. 4). A haploid *SPT5$^+$* strain (MS191) transformed with a multicopy *SPT5* plasmid (pMS24) had a weak Spt$^-$ phenotype (His$^{+/-}$ Lys$^{+/-}$) (Fig. 4). Therefore, an increase or a decrease in the *SPT5* gene copy number causes an Spt$^-$ phenotype.

Genetic mapping of *SPT5*. To determine whether *SPT5* was a previously identified gene, it was mapped by a combination of physical and genetic methods. To determine the chromosome on which the *SPT5* gene resides, DNA was prepared from strain YPH149, and chromosomes were separated by CHEF gel electrophoresis (9) as described in Materials and

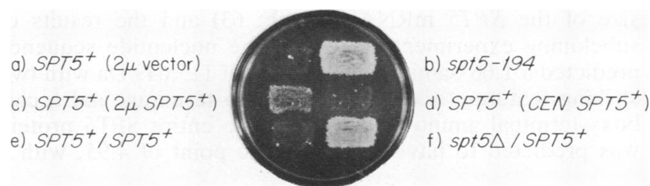

FIG. 4. Suppression of the insertion mutation *lys2-128δ* by altered *SPT5* gene dosage. Patches of the strains of the genotypes indicated were grown on a YPD plate and then replica plated to the plate shown, which contains SC-lys medium. All strains grew on a synthetic complete plate (not shown). (a) MS189(pCGS42); (b) FY300; (c) MS191(pMS24); (d) MS190(pMS4); (e) BM60; (f) BM80.

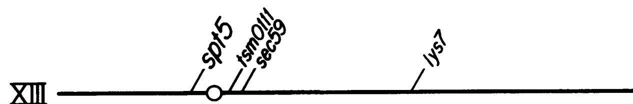

FIG. 5. Genetic map position of *SPT5*. The *SPT5-CEN13* distance was strain dependent (see text).

Methods. Southern hybridization analysis demonstrated that *SPT5* was located on chromosome XIII (63).

Tetrad analysis showed that *SPT5* was located on the left arm of chromosome XIII, tightly centromere linked (Table 2; Fig. 5). The order *SPT5-CEN13-SEC59* was determined by examining tetrads in which *SPT5* and *CEN13* had recombined; in every case (seven tetrads), *SEC59* and *CEN13* did not recombine. This order was also consistent with the segregation of *SPT5* in tetrads in which *CEN13* and *SEC59* had recombined. Similarly, the order *SPT5-SEC59-LYS7* was established by scoring *LYS7* segregation in tetrads in which *SPT5* and *SEC59* had recombined. In those 13 tetrads, *SEC59* and *LYS7* had recombined once, whereas *SPT5* and *LYS7* had recombined 12 times. These results, in conjunction with previous work that showed that *LYS7* maps physically to the right arm of chromosome XIII (23), indicated that *SPT5* resides on the left arm of chromosome XIII. Additional meiotic linkage analysis demonstrated that *SPT5* was not an allele of the centromere-linked *TSM0111* locus, since in one cross (82 tetrads), *SPT5* mapped 4.9 centimorgans (cM) from *TSM0111* (63). Therefore, based on its unique map position, *SPT5* is a previously uncharacterized gene in *S. cerevisiae*.

The map distance between *SPT5* and *CEN13* was strain dependent; two representative crosses are shown in Table 2. In a cross of strain MS113 by strain MS192, there were no *CEN13-SPT5* recombinants in 80 tetrads (<0.6 cM; Table 2). In contrast, when strain MS113 was crossed by strain MS193, there were seven *CEN13-SPT5* recombinants in 51 tetrads (7 cM; Table 2). Analysis of *SPT5* segregation with respect to different centromere-linked markers, *TRP1* (chromosome IV) and *URA3* (chromosome V), showed that the difference between strains MS192 and MS193 lies in the *CEN13-SPT5* interval (Table 2). Similar results were obtained in other *SPT5* crosses: the *CEN13-SPT5* map distance was calculated as either <1 cM or approximately 5 cM (63). The observation that the genetic map position of *SPT5* was strain dependent indicates that there are polymorphisms (possibly insertions or inversions) in the *CEN13* region of some strains.

SPT5 encodes an acidic protein with a carboxy-terminal repeat. The nucleotide sequence of the *SPT5* gene was determined by the method of Sanger et al. (54). The open reading frame of 3,189 bp (Fig. 6) was consistent with the size of the *SPT5* mRNA (3.4 kb; 63) and the results of subcloning experiments (Fig. 3). The nucleotide sequence predicted a 1,063-amino-acid protein of 115,649 Da with two striking features: a very acidic amino terminus and a carboxy-terminal amino acid repeat. The entire SPT5 protein was predicted to have an isoelectric point of 4.95, with a concentration of acidic residues at the amino terminus: residues 1 to 220 had a net charge of -63. One region of 81 amino acids (residues 137 to 217) was 60% glutamic and aspartic acid and included stretches of 11 and 20 consecutive acidic residues. The amino terminus also included four consensus sites for phosphorylation by casein kinase II (37) at positions 122, 188, 241, and 377. The carboxy terminus of the predicted SPT5 protein (residues 887 to 1063) was 31% glycine and contained 15 copies of the six-amino-acid sequence S-T/A-W-G-G-A/Q (Table 3).

TABLE 2. Mapping *SPT5* by tetrad analysis[a]

Cross[b]	Segregating markers	PD	NPD	TT	Linkage (cM)	
					Gene-gene	Gene-*CEN*
1	*spt5, sec59*	76	0	4	3	
	spt5, lys7	41	0	39	24	
	sec59, lys7	43	0	37	23	
	spt5, trp1	42	38	0		<0.6
	sec59, trp1	39	36	5		3
	lys7, trp1	20	20	40		25
	trp1, ura3	36	32	12		8
	spt5, ura3	35	34	11		7
2	*spt5, sec59*	42	0	9	9	
	spt5, lys7	19	0	32	31	
	sec59, lys7	28	0	23	23	
	spt5, trp1	23	21	7		7
	sec59, trp1	23	26	2		2
	lys7, trp1	11	15	25		25
	trp1, ura3	23	20	8		8
	spt5, ura3	19	20	12		12

[a] PD, parental ditype; NPD, nonparental ditype; TT, tetratype. The *spt5-24* allele was scored by its ability to suppress *his4-912δ*; *sec59* was scored by its temperature-sensitive lethality at 37°C; *trp1Δ1* and *ura3-52* were scored by their tryptophan and uracil auxotrophies, respectively; *lys7* was scored by mating spore clones to *lys7* strains (K396-11A and K396-22B) and scoring diploids for complementation of the lysine auxotrophy. The *TRP1* allele, which is very tightly linked to *CEN4* (<1 cM; 44), and the *URA3* allele, which is tightly linked to *CEN5* (8 cM; 44), were used to calculate the map distance from *SPT5* to *CEN13*.

[b] Cross 1 was MS113 × MS192; cross 2 was MS113 × MS193.

A computer search of the National Biomedical Research Foundation Protein Sequence data base (version 26; November 1990) did not reveal proteins with significant sequence similarity to SPT5, with the exception of a number of generally acidic or glycine-rich proteins, including nucleolins (38). No other proteins in the data base contained the six-amino-acid repeat S-T/A-W-G-G-A/Q.

The carboxy-terminal repeat region is critical for SPT5 function. Results of our subcloning experiments suggested that the SPT5 carboxy-terminal repeats were required for SPT5 function. A deletion that removed 6 of the 15 repeats (pMS23) impaired complementation of *spt5-194* (Fig. 3) and eliminated complementation of the *spt5* null mutation. A deletion that removed all 15 repeats (pMS37) eliminated complementation of both the *spt5-194* (Fig. 3) and the *spt5* null alleles.

To examine further the importance of the SPT5 peptide repeats, we constructed two *SPT5-lacZ* fusions: *SPT5*(1004)-*lacZ* encodes residues 1 to 1004 of SPT5 and includes nine

FIG. 6. Nucleotide sequence of the *SPT5* gene and predicted amino acid sequence of its gene product. Nucleotides are numbered on the left; amino acids are numbered on the right. The termination codon is represented by an asterisk. The runs of 11 and 20 consecutive acidic residues and the 15 copies of the six-amino-acid repeat are underlined. The *Pvu*II and the *Kpn*I sites used to construct *SPT5-lacZ* fusions and the *Sal*I sites used to construct the *spt5* null allele are indicated.

```
1                         CTCTTAAGGCCACTTGCGGGAAATCCACGTCTCTGCCACAGTTTCTAATAAGCATCGACCATAAATCATCTATTACTGTTAAATTAT

86    TCATCAGTATCAGTAACTGGCATGTGCATCGTACCAGTTTCGTGAAATCTATTACGTCAGTTCCTTCTATAATGACGTCTTCATTTCTCACCAGTTTCCAAATTG

191   TGGGCTCTAGTTGCAAATCCTTGAAGAACCTTGGTAGTTCATTAACGGTCATATCATGGTCCAGACTGGATTCCGCATATTTTTCCAGAATTTCATCTTCAACTT

296   CGATAGGTACTTTCGGGTTCTCGAATAGCTCTACTCTTTCATAACTAACGTTAGTGGATTTGCTCATTGAAAAAAAACTCGGAAGGTATTTAGGTGACCTTGCGG

401   CTTCTTAATGCTGACACCTGCATGCGTAATTGTTATTATATATATATATGATAGTATATTTCATACTTGTTCCTTCTAAAGGTGACCCGCCTGATATGCGACATA

506   GAAAAAAATTTGGATGAACATGTTCAAAGATTGGTAGCATTGACAAGAACCATTCATACAAGTAGGTAACAGCAGAAGGAAAGAGGAACGGGTGTTGTGATAATT

611   ATGAGTGACAACTCGGACACAAACGTGAGCATGCAGGACCATGATCAACAATTTGCTGATCCCGTAGTGGTTCCGCAGTCAACTGACACTAAAGATGAAAATACT
      M  S  D  N  S  D  T  N  V  S  M  Q  D  H  D  Q  Q  F  A  D  P  V  V  V  P  Q  S  T  D  T  K  D  E  N  T        35

716   AGTGACAAAGATACTGTTGATAGTGGCAATGTGACCACAACGGAAAGTACAGAACGTGCAGAAAGTACAAGCAATATTCCCCCTTTAGATGGGGAAGAAAAAGAA
      S  D  K  D  T  V  D  S  G  N  V  T  T  T  E  S  T  E  R  A  E  S  T  S  N  I  P  P  L  D  G  E  E  K  E        70

821   GCAAAATCTGAGCCACAGCAACCTGAGGATAATGCAGAAACGGCGGCCACAGAGCAGGTTTCATCTAGTAACGGGCCTGCTACAGATGATGCCCAAGCAACTTTG
      A  K  S  E  P  Q  Q  P  E  D  N  A  E  T  A  A  T  E  Q  V  S  S  S  N  G  P  A  T  D  D  A  Q  A  T  L       105

926   AATACGGATTCATCCGAAGCAAATGAAATTGTCAAGAAGGAAGAGGGCTCTGATGAAAGGAAGAGACCTCGCGAAGAGGACACCAAAAACAGTGATGGTGATACC
      N  T  D  S  S  E  A  N  E  I  V  K  K  E  E  G  S  D  E  R  K  R  P  R  E  E  D  T  K  N  S  D  G  D  T       140

1031  AAAGATGAGGGCGATAACAAAGATGAAGACGATGATGAAGACGACGATGACGATGATGATGATGAGGACGATGACGATGAAGCCCCAACTAAAAGGCGTCGTCAG
      K  D  E  G  D  N  K  D  E  D  D  D  E  D  D  D  D  D  D  D  E  D  D  D  D  E  A  P  T  K  R  R  R  Q       175

1136  GAGAGGAACAGATTCTTGGATATTGAAGCTGAGGTTAGTGATGATGAAGATGAAGATGAAGATGAAGAGGATTCAGAGTTGGTTCGTGAAGGTTTCATTACCCAT
      E  R  N  R  F  L  D  I  E  A  E  V  S  D  D  E  D  E  D  E  D  E  E  D  S  E  L  V  R  E  G  F  I  T  H       210

1241  GGTGATGATGAAGATGACGAAGCAAGTGCTCCAGGCGCAAGAAGAGACGATAGATTACATAGACAACTGGACCAAGATTTGAACAAGACTTCAGAAGAAGACGCT
      G  D  D  E  D  D  E  A  S  A  P  G  A  R  R  D  D  R  L  H  R  Q  L  D  Q  D  L  N  K  T  S  E  E  D  A       245

1346  CAAAGGTTAGCGAAAGAATTAAGGGAGCGTTACGGTAGAAGCAGCTCCAAGCAATACCGTGCTGCTGCTCAAGATGGTTACGTGCCCCAGAGGTTTCTCCTACCA
      Q  R  L  A  K  E  L  R  E  R  Y  G  R  S  S  S  K  Q  Y  R  A  A  A  Q  D  G  Y  V  P  Q  R  F  L  L  P       280

1451  AGTGTTGATACAGCTACCATTTGGGGTGTGCGCTGCAGACCAGGTAAAGAAAAGGAATTGATTCGTAAGTTATTAAAAAAAAAATTCAATTTGGATAGGGCGATG
      S  V  D  T  A  T  I  W  G  V  R  C  R  P  G  K  E  K  E  L  I  R  K  L  L  K  K  K  F  N  L  D  R  A  M       315

1556  GGTAAGAAAAAACTGAAAATTTTATCCATTTTCCAAAGGGATAATTACACAGGAAGAATCTATATCGAAGCCCCTAAGCAATCCGTTATTGAAAAATTTTGTAAT
      G  K  K  K  L  K  I  L  S  I  F  Q  R  D  N  Y  T  G  R  I  Y  I  E  A  P  K  Q  S  V  I  E  K  F  C  N       350

1661  GGTGTTCCAGATATTTATATTTCTCAAAAATTGCTAATTCCTGTCCAAGAATTACCTCTATTACTAAAACCAAACAAATCTGATGATGTTGCTTTGGAAGAAGGT
      G  V  P  D  I  Y  I  S  Q  K  L  L  I  P  V  Q  E  L  P  L  L  L  K  P  N  K  S  D  D  V  A  L  E  E  G       385
                                                         SalI
1766  AGCTACGTTCGTATTAAGAGAGGGATCTATAAGGGTGACTTAGCTATGGTCGACCAAATTAGTGAGAATAATTTAGAAGTTATGCTGAAAATTGTTCCTCGTCTG
      S  Y  V  R  I  K  R  G  I  Y  K  G  D  L  A  M  V  D  Q  I  S  E  N  N  L  E  V  M  L  K  I  V  P  R  L       420

1871  GATTATGGTAAATTCGACGAAATTGATCCAACAACACAGCAACGTAAATCCAGAAGACCAACTTTTGCTCATAGAGCACCACCGCAATTATTTAATCCAACAATG
      D  Y  G  K  F  D  E  I  D  P  T  T  Q  Q  R  K  S  R  R  P  T  F  A  H  R  A  P  P  Q  L  F  N  P  T  M       455

1976  GCTCTAAGATTAGACCAAGCTAACCTGTACAAAAGGGATGATCGCCACTTTACTTATAAGAATGAAGATTATATCGATGGTTATCTGTATAAGTCCTTCAGAATT
      A  L  R  L  D  Q  A  N  L  Y  K  R  D  D  R  H  F  T  Y  K  N  E  D  Y  I  D  G  Y  L  Y  K  S  F  R  I       490

2081  CAACATGTGGAAACCAAAAATATTCAGCCAACTGTGGAGGAATTGGCAAGATTCGGTTCCAAAGAGGGCGCGGTAGATCTAACATCAGTTTCACAATCAATCAAG
      Q  H  V  E  T  K  N  I  Q  P  T  V  E  E  L  A  R  F  G  S  K  E  G  A  V  D  L  T  S  V  S  Q  S  I  K       525

2186  AAGGCTCAAGCTGCAAAGGTCACTTTCCAGCCAGGTGATCGTATCGAAGTTCTAAATGGTGAACAACGTGGTTCCAAGGGTATTGTAACAAGAACCACGAAAGAT
      K  A  Q  A  A  K  V  T  F  Q  P  G  D  R  I  E  V  L  N  G  E  Q  R  G  S  K  G  I  V  T  R  T  T  K  D       560

2291  ATTGCTACTATCAAACTAAATGGCTTTACAACGCCTCTAGAATTTCCTATCTCTACTCTGAGGAAAATTTTCGAACCTGGTGATCACGTTACTGTCATCAATGGT
      I  A  T  I  K  L  N  G  F  T  T  P  L  E  F  P  I  S  T  L  R  K  I  F  E  P  G  D  H  V  T  V  I  N  G       595

2396  GAACATCAAGGTGATGCTGGTTTAGTTCTTATGGTAGAGCAAGGTCAAGTGACATTTATGTCAACTCAAACAAGCAGAGAAGTTACCATTACAGCAAATAATCTG
      E  H  Q  G  D  A  G  L  V  L  M  V  E  Q  G  Q  V  T  F  M  S  T  Q  T  S  R  E  V  T  I  T  A  N  N  L       630

2501  TCCAAATCCATCGACACTACAGCTACATCAAGTGAATATGCGCTACATGACATAGTCGAATTGAGTGCTAAAAATGTTGCTTGTATTATTCAAGCTGGCCACGAT
      S  K  S  I  D  T  T  A  T  S  S  E  Y  A  L  H  D  I  V  E  L  S  A  K  N  V  A  C  I  I  Q  A  G  H  D       665
                                   SalI
2606  ATCTTCAAGGTTATTGATGAAACTGGTAAAGTGTCGACAATTACGAAGGGGTCTATCTTGAGTAAAATAAATACTGCTCGTGCACGCGTTTCTAGTGTCGATGCA
      I  F  K  V  I  D  E  T  G  K  V  S  T  I  T  K  G  S  I  L  S  K  I  N  T  A  R  A  R  V  S  S  V  D  A       700

2711  AATGGTAATGAAATCAAAATTGGTGATACCATAGTAGAGAAAGTAGGTTCACGCAGAGAAGGTCAGGTCCTGTACATACAAACACAACAAATTTTTGTTGTTTCA
      N  G  N  E  I  K  I  G  D  T  I  V  E  K  V  G  S  R  R  E  G  Q  V  L  Y  I  Q  T  Q  Q  I  F  V  V  S       735

2816  AAGAAGATTGTTGAAAATGCAGGGGTTTTTGTTGTTAACCCTAGTAACGTTGAGGCCGTGGCATCCAAGGACAATATGTTAAGTAACAAAATGGATCTAAGTAAA
      K  K  I  V  E  N  A  G  V  F  V  V  N  P  S  N  V  E  A  V  A  S  K  D  N  M  L  S  N  K  M  D  L  S  K       770

2921  ATGAATCCAGAAATCATTTCTAAAATGGGACCTCCATCATCTAAGACATTCCAACAACCCATCCAGTCCAGAGGAGGTCGTGAAGTTGCACTCGGCAAAACAGTA
      M  N  P  E  I  I  S  K  M  G  P  P  S  S  K  T  F  Q  Q  P  I  Q  S  R  G  G  R  E  V  A  L  G  K  T  V       805

3026  AGAATTCGTTCTGCTGGTTACAAGGGTCAATTAGGTATTGTGAAAGATGTGAATGGTGATAAAGCTACTGTCGAATTACACTCGAAGAACAAACACATTACAATT
      R  I  R  S  A  G  Y  K  G  Q  L  G  I  V  K  D  V  N  G  D  K  A  T  V  E  L  H  S  K  N  K  H  I  T  I       840

3131  GACAAGCATAAGTTAACATATTACAACCGTGAGGGAGGTGAAGGTATCACGTATGATGAATTGGTTAATAGACGTGGTAGAGTTCCACAGGCCAGAATGGGCCCA
      D  K  H  K  L  T  Y  Y  N  R  E  G  G  E  G  I  T  Y  D  E  L  V  N  R  R  G  R  V  P  Q  A  R  M  G  P       875

3236  AGTTACGTCAGTGCCCCAAGAAACATGGCCACTGGCGGTATTGCAGCAGGTGCTGCGGCTACCTCTTCTGGTCTTAGCGGTGGTATGACACCAGGATGGAGCTCC
      S  Y  V  S  A  P  R  N  M  A  T  G  G  I  A  A  G  A  A  A  T  S  S  G  L  S  G  G  M  T  P  G  W  S  S       910
                 PvuII
3341  TTCGATGGTGGCAAAACACCAGCTGTAAATGCGCATGGAGGCTCAGGTGGTGGCGGTGTCTCCTCATGGGGTGGTGCTTCCACTTGGGGTGGCCAAGGTAATGGA
      F  D  G  G  K  T  P  A  V  N  A  H  G  G  S  G  G  G  G  V  S  S  W  G  G  A  S  T  W  G  G  Q  G  N  G       945

3446  GGTGCATCCGCTTGGGGCGGTGCTGGCGGCGGTGCCTCAGCTTGGGGCGGCCAAGGTACTGGTGCTACTTCTACTTGGGGTGGTGCTTCAGCCTGGGGTAACAAA
      G  A  S  A  W  G  G  A  G  G  G  A  S  A  W  G  G  Q  G  T  G  A  T  S  T  W  G  G  A  S  A  W  G  N  K       980
                                                                             KpnI
3551  TCAAGTTGGGGCGGTGCATCCACTTGGGCGTCGGGTGGTGAATCTAATGGTGCCATGTCTACTTGGGGTGGTACCGGTGATAGGTCAGCCTACGGCGGGGCTTCC
      S  S  W  G  G  A  S  T  W  A  S  G  G  E  S  N  G  A  M  S  T  W  G  G  T  G  D  R  S  A  Y  G  G  A  S      1015

3656  ACCTGGGGAGGAAATAACAATAATAAAAGTACAAGAGATGGCGGAGCTTCTGCATGGGGTAACCAAGACGATGGAAATAGGTCTGCTTGGAACAACCAAGGAAAT
      T  W  G  G  N  N  N  N  K  S  T  R  D  G  G  A  S  A  W  G  N  Q  D  D  G  N  R  S  A  W  N  N  Q  G  N      1050

3761  AAGTCAAACTATGGTGGTAACAGTACATGGGGAGGTCATTAATCACCAAAAGGAGAACCAATATCACCCAAGAAGAAATCAATAAAAAAGACTTTAATATTACCA
      K  S  N  Y  G  G  N  S  T  W  G  G  H  *                                                                        1063

3866  CGTTAATAAGAATAGTTATGAAGATTTCGATGTTTCGATGAAATACTGAATCTCGATCCTCGACGATCG
```

TABLE 3. SPT5 six-amino-acid repeat[a]

Position	Sequence					
931	S	S	W	G	G	A
937	S	T	W	G	G	Q
948	S	A	W	G	G	A
958	S	A	W	G	G	Q
969	S	T	W	G	G	A
975	S	A	W	G	N	K
981	S	S	W	G	G	A
987	S	T	W	A	S	G
1000	S	T	W	G	G	T
1009	S	A	Y	G	G	A
1015	S	T	W	G	G	N
1032	S	A	W	G	N	Q
1043	S	A	W	N	N	Q
1052	S	N	Y	G	G	N
1058	S	T	W	G	G	H
		T				A
Consensus	S	A	W	G	G	Q
No. of matches	15	12	13	13	11	9

[a] The predicted *SPT5* amino acid sequence includes 15 copies of a six-amino-acid repeat at the carboxy terminus. The first serine of each repeat is numbered as in Fig. 6. Residues in boldface type match the consensus, which was derived empirically.

copies of the repeat; *SPT5*(917)-*lacZ* encodes residues 1 to 917 of SPT5 and contains no copies of the repeat (Fig. 6). To determine whether either of these *SPT5-lacZ* fusions complemented the lethal phenotype conferred by an *spt5* null mutation, we constructed diploid strains containing each of the *SPT5-lacZ* fusions integrated at the *spt5Δ202::LEU2* allele (see Materials and Methods). Tetrad dissection of strains that contained either the *SPT5*(1004)-*lacZ* fusion (MS195) or the *SPT5*(917)-*lacZ* fusion (MS194) resulted in 4:0 segregation for viability. However, the spore clones that contained the *SPT5*(917)-*lacZ* fusion gene were extremely sick, taking 5 to 7 days to form colonies at 23°C [as opposed to 2 days for the wild type and 3 days for *SPT5*(1004)-*lacZ* strains]. Spore clones that carried either *SPT5-lacZ* fusion could grow at 30 and 37°C and were phenotypically Spt$^-$. Western immunoblot analysis indicated that strains carrying the two fusions (MS194 and MS195) contained approximately the same amount of SPT5–β-galactosidase protein (63). Therefore, the repeat domain appears to be critical for SPT5 activity.

***SPT5* encodes a nuclear protein.** To characterize further the SPT5 protein, we used indirect immunofluorescence, as described in Materials and Methods, to determine the cellular location of an SPT5–β-galactosidase hybrid protein. The results (Fig. 7A to C) showed that immunofluorescence was coincident with DAPI staining of the nuclear DNA. When treated similarly, an isogenic strain without the fusion was not stained (Fig. 7D to F), demonstrating that the immunofluorescence represented the SPT5–β-galactosidase hybrid protein. Omission of the anti-β-galactosidase antibody from the staining procedure also eliminated fluorescein fluorescence. Previous work has demonstrated that β-galactosidase expressed in yeast cells is located throughout the cytoplasm and nucleus (25, 61). Therefore, the observation that an SPT5–β-galactosidase hybrid protein that contained partial *SPT5* function was localized to the nucleus strongly suggests that the wild-type SPT5 protein is located in the nucleus.

DISCUSSION

Mutations in *SPT5* were initially isolated by genetic selections designed to identify genes that are required for normal transcription in *S. cerevisiae* (19, 64). Previous genetic results had suggested that the *SPT5* gene was related functionally to *SPT6*. These results include the observations that *spt5* and *spt6* mutations suppress the same set of mutant alleles, cause double-mutant lethality in haploid cells, and fail to complement in diploid cells (26, 63, 64). The similarity of the *spt5* mutant phenotypes to those of *spt6* and also *hta1-htb1* (histone) mutants (4, 12, 13, 19, 26, 45, 46, 62) led us to analyze the *SPT5* gene and its product as a step toward understanding the roles of these genes in transcription.

Our current work extends the genetic similarities between the *SPT5* and *SPT6* genes and has also revealed interesting features common to the *SPT5* and *SPT6* gene products. First, *spt5* and *spt6* mutations cause similar alterations in transcription of the *his4-912δ* and *lys2-128δ* alleles (Fig. 1 and 2; 13). Second, *spt5* and *spt6* null mutations each cause lethality (13, 45). Third, increased or decreased gene dosage of either *SPT5* or *SPT6* confers similar mutant phenotypes (Fig. 4; 13). Finally, as judged from immunofluorescence microscopy of fusion proteins, both SPT5 and SPT6 appear to be nuclear proteins (Fig. 7; 61).

The nucleotide sequence of the *SPT5* gene also revealed similarities to the *SPT6* gene. Both predicted proteins have a high concentration of acidic residues at the amino terminus. The sequence of *SPT5* predicted a region of 81 amino acids that was 60% glutamic acid and aspartic acid. Likewise, the *SPT6* nucleotide sequence revealed that 50% of the first 70 amino acids were glutamic acid and aspartic acid (61). The acidic domains of both proteins contain a number of potential sites for phosphorylation by casein kinase II (37).

One class of proteins that have extremely acidic domains are proteins thought to interact with chromatin (see reference 15 for a review). One of these proteins, nucleolin, has both an acidic amino terminus and a glycine-rich carboxy terminus (38), similar to SPT5. Several transcription factors, including the yeast proteins GAL4 (39) and GCN4 (30, 31), have acidic domains that are required for activation of transcription; however, these acidic domains are generally less extensive and less acidic than those predicted for the SPT5 and SPT6 proteins. The human nucleolar transcription factor hUBF does contain a highly acidic carboxy-terminal region (33).

The carboxy-terminal 132 amino acids of the predicted SPT5 protein include 15 copies of the six-amino-acid repeat S-T/A-W-G-G-A/Q. Deletion of the *SPT5* sequences encoding six of the 15 repeats impaired *SPT5* function, while deletion of all 15 repeats virtually eliminated *SPT5* function, as judged by complementation of *spt5* mutations. Analysis of two *SPT5-lacZ* fusion genes that encoded either zero or nine copies of the six-amino-acid repeat also indicated that the repeat domain was critical for SPT5 function. In our analysis of the requirement for the repeat domain, fusions to β-galactosidase appeared to increase slightly the activity of SPT5, possibly by stabilizing the SPT5 portion of the hybrid protein. However, this result does not necessarily mean that the function of the repeats is to stabilize the SPT5 protein. Other possible roles for the SPT5 repeats are to promote protein-protein interactions or to regulate SPT5 activity.

Although no other gene sequences in a large data base predicted proteins with significantly similar repeat sequences, the SPT5 repeat is somewhat reminiscent of the heptapeptide carboxy-terminal repeat of the largest subunit

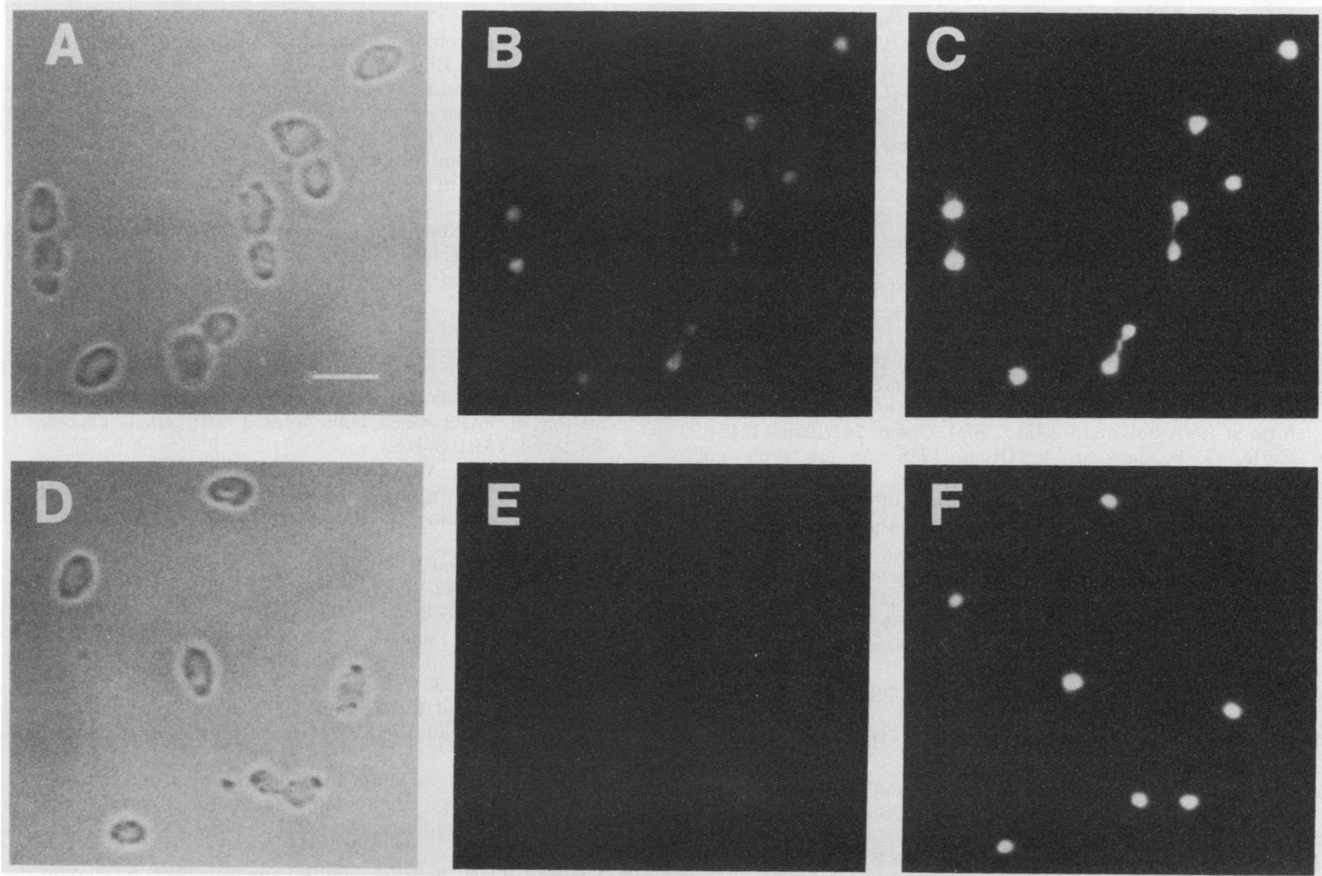

FIG. 7. Nuclear localization of SPT5–β-galactosidase. Strain BM467, containing the SPT5(1004)–β-galactosidase hybrid protein (A to C), and strain BM339, a control strain lacking the hybrid protein (D to F), were stained with anti-β-galactosidase antibody and a fluorescein-conjugated secondary antibody as described in Materials and Methods. Micrographs show phase-contrast (A and D), fluorescein fluorescence (B and E), and DAPI fluorescence, which stains the DNA (C and F). Bar = 10 μm.

of RNA polymerase II (consensus = P-T-S-P-S-Y-S). Both the SPT5 and the RNA polymerase repeats contain residues that could be phosphorylated (S, T), residues known to disrupt secondary structure of proteins (G, P), and an aromatic residue (W, Y). The RNA polymerase repeat is present in eukaryotic RNA polymerases in 26 (yeast) to 52 (mouse) copies (see reference 14 for a review), is phosphorylated (5; reviewed in reference 55), and is required in vivo for yeast RNA polymerase function (47), but its function is not yet known.

One model consistent with our gene dosage results is that the SPT5 and SPT6 proteins function as part of a complex that is sensitive to the stoichiometry of its components. However, strains that contain both *SPT5* and *SPT6* on high-copy-number plasmids also have an Spt$^-$ phenotype (data not shown), suggesting that these are not the only components of the putative complex. Genetic analysis indicates that at least one more gene product, the SPT4 protein, may be required for the function of this putative complex. Mutations in *SPT4* cause phenotypes similar to those of *spt5* and *spt6* mutations, cause double-mutant lethality with *spt5* and *spt6* mutations, and fail to complement *spt5* and *spt6* mutations (63, 64). *SPT4*, however, is distinct in several respects: the gene is not essential for growth, altered dosage does not cause a mutant phenotype, and the SPT4 protein is not acidic (40).

Previous genetic analyses of *spt* mutants revealed a number of similarities between *spt5*, *spt6*, and histone (*hta1/spt11* and *htb1/spt12*) mutant strains. Mutations in these genes suppress the same spectrum of Ty and δ insertion mutations as well as some *cis*- and *trans*-acting mutations that greatly reduce expression of the *SUC2* gene and Ty elements (4, 12, 13, 19, 26, 45, 46, 62). Furthermore, histone gene pairs, like *SPT5* and *SPT6*, exhibit interesting dosage effects: an increase or a decrease in the gene copy number causes an Spt$^-$ phenotype (12). We have now found that *SPT5* and *SPT6* encode essential nuclear proteins with highly acidic amino termini. Given these mutant phenotypes and physical characteristics, we favor a model in which the SPT5 and SPT6 proteins act together to affect chromatin structure, thereby influencing gene expression in yeast cells. Examination of chromatin structure in these *spt* mutants should begin to address this model directly.

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