The *Saccharomyces cerevisiae SPT7* Gene Encodes a Very Acidic Protein Important for Transcription *In Vivo*

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ABSTRACT

Mutations in the *SPT7* gene of *Saccharomyces cerevisiae* originally were identified as suppressors of Ty and δ insertion mutations in the 5′ regions of the *HIS4* and *LYS2* genes. Other genes that have been identified in mutant hunts of this type have been shown to play a role in transcription. In this work we show that *SPT7* is also important for proper transcription *in vivo*. We have cloned and sequenced the *SPT7* gene and have shown that it encodes a large, acidic protein that is localized to the nucleus. The SPT7 protein contains a bromodomain sequence; a deletion that removes the bromodomain from the SPT7 protein causes no detectable mutant phenotype. Strains that contain an *spt7* null mutation are viable but grow very slowly and have transcriptional defects at many loci including insertion mutations, Ty elements, the *INO1* gene and the *MFA1* gene. These transcriptional defects and other mutant phenotypes are similar to those caused by certain mutations in *SPT15*, which encodes the TATA binding protein (TBP). The similarity of the phenotypes of *spt7* and *spt15* mutants, including effects of *spt7* mutations on the transcription start site of certain genes, suggests that *SPT7* plays an important role in transcription initiation *in vivo*.

EUKARYOTIC transcription initiation at RNA polymerase II-dependent promoters requires the coordination of many proteins. Analysis of transcription initiation *in vitro* and *in vivo* has shown these proteins to be required for many steps in this process, including overcoming repression by nucleosomes, gene-specific regulation, promoter recognition, recruitment of RNA polymerase II and melting of the DNA strands into an open complex (see recent reviews by CONAWAY and CONAWAY 1993; DRAPKIN *et al.* 1993; BURATOWSKI 1994; PARANJAPE *et al.* 1994; TJIAN and MANIATIS 1994).

Unregulated, or basal transcription can be reconstituted *in vitro* with RNA polymerase II and a set of general transcription factors that are conserved from human to yeast (see recent reviews by CONAWAY and CONAWAY 1993; DRAPKIN *et al.* 1993; MERINO and REINBERG 1993; BURATOWSKI 1994; TJIAN and MANIATIS 1994). One of these factors, TBP, has been shown *in vitro* to play a primary role in recognizing the promoter by binding to the TATA box. This binding initiates an ordered assembly of the other general factors and RNA polymerase II into a preinitiation complex. Regulated transcription can be reconstituted *in vitro* with the further addition of transcriptional activators as well as a group of proteins called TBP-associated factors (TAFs, see recent review by TJIAN and MANIATIS 1994). These *in vitro* studies have concentrated primarily on a small number of TATA-containing promoters. Recent evidence indicates that different types of promoters likely require different auxiliary factors or even different basal factors (PARVIN and SHARP 1993; TYREE *et al.* 1993).

As a complement to the biochemical studies on transcription initiation, genetic studies also have identified components of the basal transcription machinery. For example, mutant hunts in yeast have identified the genes *SPT15*, which encodes yeast TBP (EISENMANN *et al.* 1989), and *SUA7*, which encodes the yeast homologue of another of the general transcription factors, TFIIB (PINTO *et al.* 1992). Mutations in these genes alter transcription *in vivo* and demonstrate the importance of these proteins for transcription *in vivo*.

Several mutant hunts in *S. cerevisiae* also have identified a large number of proteins that, although not present in the most purified reconstituted *in vitro* systems, are nonetheless critical for the transcription of many genes *in vivo*. The characterization of such proteins will help to elucidate the complexities of the mechanism of transcription *in vivo*. Indeed, the genetic identification of one such class of transcription factors, SRB proteins, has led to the identification of an RNA polymerase II-containing complex, the "RNA polymerase II holoenzyme," which can promote both basal and activated transcription (KOLESKE and YOUNG 1994). Another recent study also has identified this complex (KIM *et al.* 1994).

We have taken a genetic approach to identify factors

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important for initiation by RNA polymerase II. This approach involves analysis of suppressors of insertion mutations caused by δ and Ty elements in the 5′ regions of the *HIS4* and *LYS2* genes (reviewed in WINSTON 1992). Currently, these mutant hunts have identified three proteins previously known or suspected to play a general role in transcription: *SPT15*, encoding TBP, and *HTA1/SPT11* and *HTB1/SPT12*, encoding histones H2A and H2B. The other SPT gene products are also important or essential for transcription *in vivo* but are not previously identified transcription factors.

We can divide most of the *SPT* genes into two groups, on the basis of the phenotypes the genes confer when mutant. One group includes the histone genes, *HTA1/SPT11* and *HTB1/SPT12*, and at least five other *SPT* genes (reviewed in WINSTON and CARLSON 1992). The second group includes *SPT15* and three other genes, *SPT3*, *SPT7* and *SPT8* (WINSTON *et al.* 1984, 1987; EISENMANN *et al.* 1994). The pleiotropic nature of the *spt* mutants in each group suggests that these SPT proteins are required for normal transcription of many genes *in vivo*.

The similarity of the phenotypes of *spt3*, *spt7* and *spt8* mutants to those of certain *spt15* mutants and evidence for a physical interaction between SPT3 and TBP (EISENMANN *et al.* 1992) suggest that these SPT proteins may play a role in conjunction with TBP at a step in transcription initiation. In this paper, we present the characterization of the *SPT7* gene and its product. We show that *SPT7* encodes a nuclear protein that is important for growth and transcription. Sequence analysis of *SPT7* reveals that its predicted product is a large, acidic protein that contains a bromodomain motif (HAYNES *et al.* 1992), although the bromodomain is not required for SPT7 function. Finally, although an *spt7* null mutation has very large effects on the expression of certain genes transcribed by RNA polymerase II, it appears to have little or no effect on transcription by RNA polymerase I or on either of the two RNA polymerase III transcripts assayed. These results are all consistent with the idea that *SPT7* is important for transcription initiation by RNA polymerase II. The similarity of the *spt7* mutant phenotypes to those of certain alleles of *spt15* suggests that *SPT7* functions in initiation, perhaps in conjunction with TBP.

MATERIALS AND METHODS

Yeast strains: *S. cerevisiae* strains (Table 1) are all isogenic to S288C, and strains designated FY are isogenic to strain FY2, which is a *GAL2*$^+$ derivative of S288C. (Strain FY2 is described in WINSTON *et al.* 1995). The δ and Ty insertion mutations *his4-917δ* and *lys2-173R2* have been previously described (HAPPEL and WINSTON 1992).

The *spt7Δ402::LEU2* null strains were constructed by a one-step gene replacement of an *Mlu*I-*Sph*I fragment from plasmid pLG59 (described below) into diploids that were made by crossing the following pairs of strains: FY166 × FY168, FY60 × FY61, FY934 × FY935 and FY60 × FY41. The diploids then were sporulated to generate *spt7Δ402::LEU2* haploid progeny. Proper integration was confirmed by genetic analysis of these tetrads and by Southern analysis, using as *SPT7* probes an *Eco*RI-*Sal*I fragment and a *Sal*I-*Cla*I fragment from plasmid pFW127, described below. In comparing *spt7Δ402* progeny constructed in different diploids, we observed two classes with respect to growth rate: one class has a doubling time of ~4 hr in YPD and colonies have a gritty texture on solid media, whereas the second class has a doubling time of ~2 hr and colonies have a normal texture. (Both classes are clumpy in liquid culture; this phenotype is distinct from the colony texture observed on solid media.) One diploid (FY166 × FY168) generated only the slower-growing null progeny, and the other three generated only the other class of null progeny.

TABLE 1

***S. cerevisiae* strains**

Strain	Genotype
FW510	*MAT***a***his4-917δura3-52*
FW1112	*MATα spt7-217 his4-917δlys2-173R2 ura3-52*
FY41	*MAT***a***his4-917δleu2Δ1 trp1Δ63 ura3-52*
FY60	*MATα his4-917δleu2Δ1 ura3-52*
FY61	*MAT***a***his4-917δleu2Δ1 ura3-52*
FY70	*MATα leu2Δ1*
FY149	*MATα spt7-217 his4-917δlys2-173R2 ura3-52*
FY161	*MATα spt7-223 his4-917δlys2-173R2leu2Δ1 ura3-52*
FY162	*MAT***a***spt7-223 his4-917δlys2-173R2 leu2Δ1*
FY166	*MATα his4-917δlys2-173R2 leu2Δ1*
FY168	*MAT***a***his4-917δlys2-173R2 leu2Δ1*
FY293	*MATα spt3-202his4-917δlys2-173R2 ura3-52*
FY294	*MAT***a***spt3-202 his4-917δlys2-173R2 leu2Δ1trp1Δ63 ura3-52*
FY402	*MAT***a***spt15-21 lys2-173R2leu2Δ1 ura3-52*
FY569	*MAT***a***spt7-223 his4-917δlys2-173R2 leu2Δ1 ura3-52*
FY571	*MAT***a***spt7-217 his4-917δlys2-173R2 leu2Δ1trp1Δ63ura3-52*
FY580	*MATα spt7-217 his4-917δlys2-173R2 leu2Δ1*
FY631	*MAT***a***his4-917δlys2-173R2 leu2Δ1 trp1Δ63 ura3-52*
FY773	*MATα spt15-21 lys2-173R2 leu2Δ1 trp1Δ63 ura3-52*
FY920	*MATα spt7Δ402::LEU2his4-917δlys2-173R2 leu2Δ1*
FY922	*MAT***a***spt7Δ402::LEU2his4-917δlys2-173R2leu2Δ1*
FY934	*MAT***a***Ty912Δ44-lacZ lys2-173R2leu2Δ1 ura3-52*
FY935	*MATα Ty912Δ44-lacZ lys2-173R2leu2Δ1 ura3-52*
FY963	*MAT***a***spt7Δ402::LEU2 his4-917δleu2Δ1 ura3-52*
FY1006	*MATα spt7Δ402::LEU2 his4-917δlys2-173R2 leu2Δ1*
FY1007	*MATα spt7Δ402::LEU2 Ty912Δ44-lacZ lys2-173R2 leu2Δ1 ura3-52*
FY1009	*MAT***a***spt7-502 his4-917δleu2Δ1 ura3-52*
FY1010	*MATα spt7-502 Ty912Δ44-lacZlys2-173R2 leu2Δ1 ura3-52*

Thus, all of the *spt7Δ402* progeny of a given *SPT7*$^+$ / *SPT7*$^+$ transformed diploid are of the same class. Crosses between *spt7Δ402* and *SPT7*$^+$ strains demonstrated that this growth difference does not segregate in a Mendelian fashion, but rather is more consistent with either cytoplasmic inheritance or some other epigenetic phenomenon. For example, when a faster-growing *spt7Δ402* strain is crossed by an *SPT7*$^+$ strain from which slow-growing nulls were derived, all of the *spt7Δ402* null progeny have the slow growth rate. Both classes of *spt7Δ402* strains have the same phenotypes with respect to suppression of the *his4-917δ* and *lys2-173R2* insertion mutations, and both classes have the same transcriptional defects on all of the RNA polymerase II transcripts that we have tested. Thus, this difference in growth rate among the *spt7Δ402* strains does not affect our study of RNA polymerase II transcription. We note these preliminary observations, however, simply to document an interesting genetic phenomenon that has come to light in the background of an *spt7* null mutation.

To construct strains in which the bromodomain-deletion derivative allele of *spt7, spt7-502* has replaced *SPT7* in the genome, plasmid pLG79 (described below) was linearized within the *SPT7* coding region with *Bam*HI and used to transform strains FY963 and FY1007 (*spt7Δ402::LEU2*), selecting for Ura$^+$. Transformants were purified, grown in rich media (YPD) and plated on 5FOA plates to select for those that had lost the plasmid. Strains were then screened for concomitant loss of both *URA3* and *LEU2*. Proper integration of the bromodomain-deletion derivative allele was confirmed both by Southern analysis and by tetrad analysis.

Genetic methods and media: Standard methods were used for mating, sporulation and tetrad analysis (ROSE *et al.* 1990). Yeast cells were transformed by the lithium acetate procedure (ITO *et al.* 1983). Media were prepared as described (ROSE *et al.* 1990). Inositolless medium was made as described by SHERMAN *et al.* (1981). To help the growth of cells in liquid inositolless media, all 20 amino acids, adenine and uracil were added. For the experiments to measure *INO1* mRNA levels, inositol was added to the liquid inositolless media to 200 μM for repressing conditions and to 10 μM for derepressing conditions. We chose 10 μM inositol because it has been found to allow partial derepression of the *INO1* gene in wild-type strains while still permitting growth of strains with a mutation in the *INO1* gene (HIRSCH and HENRY 1986). For the solid media in petri plates described in the RESULTS, the inositolless media used was supplemented only with adenine, uracil and four amino acids: histidine, leucine, lysine and tryptophan. No inositol was added to the "−inositol" plate; for the "+inositol" control plate, inositol was spread onto the plate to a final concentration of ~160 μM.

Isolation and analysis of Tn*10*-LUK insertion mutations: Tn*10*-LUK mutagenesis (HUISMAN *et al.* 1987) was used to generate transposon insertions into the *SPT7* gene on plasmid pFW127. Restriction digests with *Bgl*II, *Bam*HI, *Cla*I, *Sal*I, *Eco*RI and *Xho*I were used to determine the positions and orientations of these insertions. To examine the effect of Tn*10*-LUK insertions on *SPT7* function, the plasmids were used to transform strain FW1112. Transformants then were scored for suppression of *his4-917δ*.

DNA sequence analysis: Restriction fragments spanning the *SPT7* gene were subcloned from plasmid pFW127 into the vectors M13mp18 and M13mp19 (NORRANDER *et al.* 1983) and their nucleotide sequence was determined by the method of SANGER *et al.* (1977) using the Sequenase version 2.0 kit from US Biochemical Corp. (Cleveland, OH). Universal M13 primers and synthetic primers were used to determine the DNA sequence on both strands.

DNA preparation and analysis: Yeast genomic and plasmid DNA was prepared for Southern analysis as previously described (HOFFMAN and WINSTON 1987). For Southern analysis, DNA was blotted onto GeneScreen, and hybridization was done according to the instructions of the manufacturer (New England Nuclear, Boston, MA). Manipulation of plasmid DNA using *Escherichia coli* was performed as previously described (AUSUBEL *et al.* 1988).

Enzymes and protease inhibitors: Restriction enzymes, nucleases, DNA polymerases, kinases and other modifying enzymes were purchased from New England BioLabs (Beverly, MA), Boehringer Mannheim Biochemicals (Indianapolis, IN) and Sigma (St. Louis, MO) and used according to instructions of the supplier. Protease inhibitors were purchased from Sigma.

Plasmids: All plasmids were constructed by standard procedures. Plasmid pFW127 contains the *SPT7* gene within a 7-kb *Eco*RI-*Cla*I genomic fragment cloned into YCp50 (JOHNSTON and DAVIS 1984). Plasmid pFW192 was constructed by cloning the *Sal*I-*Cla*I fragment of the *SPT7* gene from plasmid pFW127 into the multicopy (2 μ) vector pCGS42 (Collaborative). This plasmid confers partial complementation of the slow-growth and Spt$^-$ phenotypes of *spt7* mutant strains. There is no defined yeast promoter in this plasmid; we have assumed that cryptic promoter activity must be responsible for the expression of this fragment of *SPT7*. Plasmid pFW124 was constructed by cloning the *Eco*RI-*Sal*I genomic fragment containing *SPT7* sequences into the integrating vector YIp5 (STRUHL *et al.* 1979).

Plasmids pLG17 and pLG20 were created to direct overexpression of the N-terminal quarter and C-terminal half, respectively, of SPT7 in *Escherichia coli*. Plasmid pLG17 encodes a fusion protein consisting of the *E. coli* maltose binding protein (MBP), followed by a factor Xa cleavage site and the N-terminal quarter (351 amino acids) of SPT7, truncated by stop codons where the *SPT7 Nru*I site existed. This fusion is under control of the P_{tac} isopropyl β-D-galactoside (IPTG)-inducible promoter in plasmid vector pMAL-c (New England Biolabs). Introduction of a unique *Kpn*I restriction site for cloning the *SPT7* fragment into pMAL-c and introduction of a factor Xa site immediately upstream of the ATG of *SPT7* was accomplished by oligo-directed mutagenesis (KUNKEL 1985). The stop codons were introduced by cloning a double-stranded oligonucleotide encoding stop codons in all three frames into the *Nru*I site of the *SPT7* coding region. Plasmid pLG20 expresses a fusion protein of MBP and the C-terminal half of SPT7 (617 amino acids) under the control of the P_{tac} promoter. It contains *SPT7* DNA beginning from the *Sal*I site (Figure 1) cloned into the vector pMAL-cRI (New England BioLabs).

Plasmid pLG59 encodes the *spt7Δ402::LEU2* null allele, which was used to construct all of the *spt7Δ402* strains described in this paper. To construct this allele, an *Eco*RI-*Bam*HI fragment from pFW127 (Figure 1) was subcloned into YCp50 to create pFW153. Most of the *SPT7* coding region then was deleted by digesting this plasmid with *Hin*dIII (see Figure 1). The deletion of the *Hin*dIII fragments is entirely internal to the *SPT7* open reading frame. The *LEU2* gene then was inserted in place of the *SPT7 Hin*dIII fragments, after all ends were made blunt ended by using T4 DNA polymerase or Klenow. This allele, hereafter referred to as *spt7Δ402*, lacks over 80% of the *SPT7* coding region.

To integrate an *spt7* deletion that removes the SPT7 bromodomain, we used plasmid pLG79. This plasmid contains the *spt7-502* allele in which codons 465–521 are deleted from *SPT7*. Plasmid pLG79 contains *SPT7* DNA from the *Xba*I site 5′ of the open reading frame to the *Cla*I site 3′ of the open

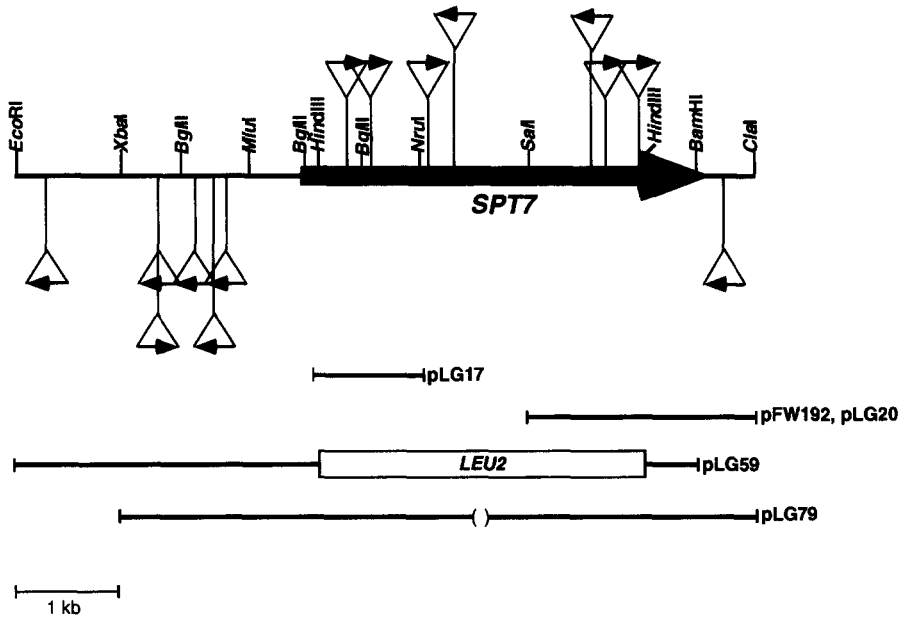

FIGURE 1.—Restriction and functional map of the *SPT7* gene. The *Eco*RI-*Cla*I genomic fragment shown has full *SPT7* complementing activity. The *SPT7* open reading frame is indicated by the large arrow. Tn*10*-LUK insertions into this DNA fragment are indicated as triangles, and the arrowheads show their relative insertion orientation. The locations shown for these insertions are approximate and were determined as described in MATERIALS AND METHODS. The insertions shown above the line disrupted *SPT7* function; the insertions shown below the line did not disrupt function. The *SPT7* fragments used for particular subclones are shown below the restriction map and are described in MATERIALS AND METHODS. For clarity, only the relevant restriction sites for each enzyme used for plasmid constructions and other genetic manipulations are shown in this Figure; there are several additional *Hin*dIII sites and one additional *Xba*I site within the *SPT7* open reading frame that are not shown. The locations of the most 5′ *Bgl*II site and the *Eco*RI, *Xba*I and *Cla*I sites are approximate.

reading frame (Figure 1) cloned into the integrating vector YIp5. In addition, by the nature of its construction, it contains the *Eag*I-*Spe*I region of the polylinker of pRS315 (SIKORSKI and HIETER 1989) 5′ of the *SPT7* sequences. The *spt7-502* deletion was constructed by oligonucleotide-directed mutagenesis (KUNKEL 1985) using a kit from Bio-Rad Laboratories. The mutagenic 40-base oligonucleotide included the 17 nucleotides 5′ to the codon for amino acid residue 465 of *SPT7* and the 23 nucleotides 3′ to the codon for amino acid residue 521. The original plasmid to contain this deletion is pPT4, which was used as the source of *spt7-502* DNA for pLG79.

RNA isolation and Northern analysis: To prepare RNA, cells were grown in liquid media to a concentration of ~1–2 x 10^7 cells/ml. RNA isolation and Northern analysis were performed as previously described (AUSUBEL *et al.* 1988; SWANSON *et al.* 1991). Plasmids B161 (WINSTON *et al.* 1984), pFW45 (WINSTON *et al.* 1984) and pSM39 (MICHAELIS and HERSKOWITZ 1988) were labeled with ^{32}P by nick translation, using a kit from Boehringer Mannheim, and used as hybridization probes for Ty, *HIS4* and *MFA1* mRNAs, respectively. A *Hin*dIII fragment from plasmid pFR2 (kindly provided by Dr. PRATIMA SINHA) and an *Eco*RI-*Hin*dIII fragment of pJH310 (HIRSCH and HENRY 1986) were labeled by random priming to use as hybridization probes for *PYK1* and *INO1* messages, respectively. The amounts of RNA loaded in each lane were determined by A_{260} readings of the RNA samples and visualization of an aliquot of each sample on an ethidium bromide-stained agarose gel. As a loading control, the blots were probed for *PYK1* mRNA. In all of the Northern analyses, we consistently observed a small decrease (generally 1.2- to 2-fold) in the level of *PYK1* mRNA in the *spt7Δ402* strains relative to total RNA, as measured by A_{260} of the RNA loaded. This decrease in *PYK1* levels has been taken into account in our assessment of quantitation of levels of other transcripts relative to *PYK1* levels.

RNA analysis using S1 nuclease: To prepare RNA for S1 analysis, cells were grown to 1–2 × 10^7 cells/ml and total RNA was prepared as described (AUSUBEL *et al.* 1988). For analysis of the *HIS3, DED1, TRP3,* rRNA and isoleucine tRNA transcripts, cells were grown in YPD. The oligonucleotides used as probes for assaying transcript levels of rRNA, an isoleucine tRNA, *TRP3* and U6 were designed by CORMACK and STRUHL (1992). The oligonucleotide probe for the rRNA transcript is complementary to the junction of the 3′ end of the mature 25S transcript and the rapidly processed 3′ sequences of the precursor 25S rRNA (KEMPERS-VEENSTRA *et al.* 1986; SOLLNER-WEBB and TOWER 1986). This probe should measure levels of the precursor rRNA transcript and thus reflect initiation rates of transcription rather than accumulation of stable mature rRNAs. The probe for the tRNA was designed to be complementary to the intron of the isoleucine tRNA (OGDEN *et al.* 1984; O'CONNOR and PEEBLES 1991). Because introns are processed rapidly, RNA levels measured with this probe also should reflect transcription initiation, rather than accumulation of stable mature tRNAs (CORMACK and STRUHL 1992). The oligonucleotide probes used to analyze the *HIS3* and *DED1* transcripts both kindly were provided by the laboratory of K. STRUHL, and both have been described previously (CHEN *et al.* 1987).

To measure RNA levels of the product of the *SNR6* gene, U6, we used CORMACK and STRUHL's experimental design (1992), which took advantage of a derivative of the *SNR6* gene. This derivative is believed to encode an unstable RNA (DAVID BROW, personal communication). Because the wild-

type U6 transcript is so stable, the unstable derivative was used so that measurement of transcript levels would reflect initiation rates of transcription, rather than accumulation of the stable U6. This derivative, generously provided by DAVID BROW, contains all known upstream, downstream and internal promoter elements (BROW and GUTHRIE 1990; BURNOL *et al.* 1993) but has a 14-bp deletion within the gene. The predicted S1 digestion products from hybridization of the U6 probe to the genomic and plasmid-borne *SNR6* transcripts differ in size by ~42 nucleotides and thus are easily distinguishable. For our study, we cloned this *SNR6* derivative on a *Hin*dIII fragment into the polylinker of the yeast centromere vector pRS316 (SIKORSKI and HIETER 1989) in both orientations to avoid influences of particular plasmid flanking sequences (plasmids pLG74 and pLG75). These plasmids were transformed into the *SPT7*$^+$ and *spt7Δ402* strains used for analysis of the other transcripts in this experiment. Two different transformants for each orientation of the *SNR6* derivative in the vector were grown for RNA analysis in synthetic complete media lacking uracil to maintain selection for the plasmids. To control for potential variations in plasmid copy number between *SPT7*$^+$ and *spt7Δ402* strains, we measured the relative plasmid copy number by Southern analysis from an aliquot of each culture grown for the RNA preparations (data not shown). Different transformants with the same plasmid always showed identical results and the same results also were obtained regardless of the orientation of this *SNR6* derivative in the plasmid vector. Therefore, only one representative *SPT7*$^+$ lane and one *spt7Δ402* lane are shown in RESULTS.

The *SNR6* clone contains a solo δ element upstream of all of the known *SNR6* promoter elements (BROW and GUTHRIE 1990). However, deletion analysis has shown that this δ sequence does not appear to affect transcription of *SNR6* (BROW and GUTHRIE 1990; BURNOL *et al.* 1993); we thus can make reasonable conclusions from our experiments looking at this gene.

To perform the S1 nuclease assay, 10 μg of each RNA sample (as measured by A_{260}) was hybridized to an excess of the appropriate ^{32}P-labeled oligonucleotide probes (~3 ng) and treated with S1 nuclease as described previously (CORMACK and STRUHL 1992). Aliquots of the RNA samples also were run on an agarose gel and visualized by staining with ethidium bromide to confirm that equal amounts of each sample were used. The genomic *SNR6* transcript was used as an internal control in the reactions with the *DED1, TRP3,* rRNA, isoleucine tRNA and unstable *SNR6* probes. Because the S1 digestion products of hybrids with the *HIS3* and genomic *SNR6* transcripts are close in size, as an internal control for the *HIS3* reactions, we used the *TRP3* probe and performed these reactions in parallel with reactions containing both the *TRP3* and *SNR6* probes.

Quantitation: For Northern, Southern and S1-nuclease analysis, radioactive signals were quantitated on a Molecular Dynamics PhosphorImager with Image Quant software.

Preparation and affinity-purification of polyclonal antisera against SPT7: Fusion genes encoding the N-terminal quarter (expressed from plasmid pLG17) and the C-terminal half (expressed from plasmid pLG20) of SPT7 fused to the *E. coli* MBP were induced in *E. coli,* and the hybrid proteins were purified over amylose resin affinity columns using the protein fusion and purification system from New England BioLabs (Beverly, MA). The purified MBP-SPT7 (C-terminal half) fusion protein was isolated on an SDS polyacrylamide gel according to a previously described procedure (HARLOW and LANE 1988). The MBP-SPT7 (N-terminal quarter) fusion protein was cleaved with factor Xa protease according to the protocol described by New England Biolabs; and then the factor Xa digestion products were run on an SDS polyacrylamide gel, and the SPT7 fragment band was excised. To confirm that the correct band was excised from the gel, a small portion of the factor Xa digest was run on a separate gel for N-terminal sequencing of the putative SPT7 N-terminal fragment band. The first 20 amino acids were sequenced (by JOHN RUSH, Department of Genetics, Harvard Medical School) and corresponded to the first 20 amino acids encoded by the putative *SPT7* open reading frame (data not shown). Both of the purified fragments of SPT7 were sent to Cocalico Biologicals Inc. (Reamstown, PA) for immunizing rabbits.

The antisera raised against the C-terminal fragment of SPT7 was affinity purified on an immunoblot using a previously described procedure (LILLIE and BROWN 1987; HARLOW and LANE 1988). For use in the indirect immunofluorescence microscopy, antisera raised against the N-terminal fragment was purified over an affinity column of MBP-SPT7 (N-terminal fragment) coupled to activated Affi-Gel 15 gel (BioRad Laboratories). Coupling of the MBP-SPT7 (N-terminal fragment) fusion protein to the Affi-Gel immunoaffinity support was performed according to instructions from Bio-Rad. Preparation by ammonium sulfate precipitation of the crude sera for the immunoaffinity column, passage of the sera over the immunoaffinity column and elution of the affinity-purified antibodies were done according to protocols described by HARLOW and LANE (1988). The affinity-purified antisera was dialyzed against PBS and concentrated by ultrafiltration using Amicon Centriprep and Centricon concentrators according to instructions of the manufacturer.

Preparation of crude yeast protein lysates and Western blotting analysis: Cell cultures were grown to mid to late exponential phase in rich medium (YPD). Crude protein lysates were prepared essentially as described (EISENMANN *et al.* 1992) with some modifications as noted here. First, the buffer used in this work was the Buffer C of the nuclear extract protocol of LUE and KORNBERG with the full complement of protease inhibitors described (LUE *et al.* 1989). In addition, potassium acetate was added to 100 mM. Cells were harvested, washed twice with lysis buffer, transferred to a microcentrifuge tube and pelleted. The cell pellet was frozen in a dry ice/ethanol bath for ≥10 min. The pellet then was thawed and resuspended in 0.2 ml lysis buffer and vortexed with glass beads 12 times for 20 sec each with ~1 min on ice between vortexes to break open the cells. The remainder of the procedure was as previously described (EISENMANN *et al.* 1992) except the lysate was clarified by three centrifugation spins. Seventy-five micrograms of each extract was loaded onto each of two 7.5% polyacrylamide SDS gels, and the gels were run and blotted as described (SWANSON and WINSTON 1992). One blot was probed with affinity-purified antisera against the N-terminal quarter of the SPT7 protein, and the other blot was probed with affinity-purified antisera against the C-terminal half of SPT7. The secondary antibodies were goat anti-rabbit IgG coupled to alkaline phosphatase (Promega) used at a 1:7500 dilution with the alkaline phosphatase detection system from Promega.

Indirect immunofluorescence: Cell cultures were grown to $1-2 \times 10^7$ cells/ml in rich media (YPD) and prepared for indirect immunofluorescence as described (ROSE *et al.* 1990). Immunofluorescence was done on diploid strains. Affinity-purified antisera against the N-terminal portion of the SPT7 protein was used as the primary antibody at a dilution of 1:200. Flourescein-conjugated anti-rabbit secondary antibody raised in goat was obtained from Jackson Immunoresearch Labs (West Grove, PA) and was used at a 1:400 dilution. The fluorescent dye DAPI (4′,6-diamidino-2-phenylindole dihy-

drochloride) was used to stain DNA. Slide preparation, indirect immunofluorescence and DAPI staining were performed as described (ROSE *et al.* 1990). Cells were examined with a Zeiss Axiophot microscope using a ×100 objective, N.A. 1.3, and photographed with Kodak TMAX-400 black-and-white film. The exposure time and degree of enlargement for immunofluorescence micrographs was identical for wild-type and *spt7Δ402* mutant cells.

RESULTS

Cloning *SPT7*: Strains that contain an *spt7* mutation alter the phenotypes of the insertion mutations *his4-917δ* and *lys2-173R2*: *SPT7*$^+$ strains are His$^-$ Lys$^+$, whereas *spt7* mutants are His$^+$ Lys$^-$. To clone the *SPT7* gene, we transformed the *spt7-217* mutant FW1112 with a recombinant yeast plasmid library in the vector YCp50 (ROSE *et al.* 1987), selected for Ura$^+$ transformants and screened for those that had an Spt$^+$ (His$^-$ Lys$^+$) phenotype. Among ~6000 transformants, we identified 3 candidates that contained some common restriction fragments. Subcloning localized the *SPT7* complementing activity to a 7-kb *Cla*I-*Eco*RI restriction fragment (Figure 1). To verify that this fragment contains *SPT7*, a portion of it was subcloned into the integrating vector YIp5 to create plasmid pFW124. Then pFW124 was used to transform the *spt7-217* strain FW1112 to Ura$^+$. When this transformant, which is still Spt$^-$, was crossed to the *SPT7*$^+$ strain FW510, all 19 tetrads were parental ditypes with respect to segregation of *URA3* and *spt7-217*, demonstrating that the insert directed plasmid integration to the *SPT7* locus. We therefore conclude that this fragment contains the *SPT7* gene.

To localize *SPT7* function to a smaller region of DNA, we performed Tn*10*-LUK insertion mutagenesis (HUISMAN *et al.* 1987) on the clone in *E. coli* and screened for insertion mutations that destroyed *SPT7* activity. Restriction mapping of these Tn*10*-LUK insertion mutations further localized the activity to a region of ~5 kb (Figure 1).

***SPT7* DNA sequence:** To analyze the structure of the *SPT7* gene in greater detail, we sequenced 4871 bp of DNA that contains *SPT7* (Genbank accession number L22537). A single open reading frame of 3996 bp was identified. All of the Tn*10*-LUK insertion mutations that eliminate *SPT7* complementing function are within this open reading frame. Additionally, a large deletion within this open reading frame fails to complement the Spt$^-$ phenotypes of the original *spt7-217* mutation. Therefore, we conclude that this open reading frame is the *SPT7* gene. The SPT7 gene product is predicted to be 1332 amino acids long and to have a molecular weight of 154 kD (Figure 2). The predicted gene product is extremely acidic with a net charge of −89.

We compared the *SPT7* DNA sequence and the predicted SPT7 amino acid sequence against Genbank by Blast (ALTSCHUL *et al.* 1990). This analysis revealed that

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   1  MTERIPIKNY QRTNAKALLK LTEKLFNKNF FDLYLTSQQL VVLEYLLSIS
  51  SEEDKLKAWD YFLKGNIALN VEKSFPLTQE EEHHGAVSPA VDTRSDDVSS
 101  QTIKDNNNTN TNTSISNENH VENEIEDKGD NAIANEDNFV NNDESDNVEE
 151  DLFKLDLEDL KQQISGTRFI GNLSLKIRYV LWQCAIDYIY CDRNEFGDEN
 201  DTEYTLLDVE EKEEEEIGKN EKPQNKEGIS KFAEDEDYDD EDENYDEDST
 251  DVKNVDDPPK NLDSISSSNI EIDDERRLVL NISISKETLS KLKTNNVEEI
 301  MGNWNKIYHS FEYDKETMIK RLKLEESDKM IEKGKKKRSR SDLEAATDEQ
 351  DRENTNDEPD TNQKLPTPEG STFSDTGNKR PKQSNLDLTV NLGIENLSLK
 401  HLLSSIQQKK SQLGISDYEL KHLIMDVRKN RSKWTSDERI GQEELYEACE
 451  KVVLELRNYT EHSTPFLNKV SKREAPNYHQ IIKKSMDLNT VLKKLKSFQY
 501  DSKQEFVDDI MLIWKNCLTY NSDPSHFLRG HAIAMQKKSL QLIRMIPNIT
 551  IRNRADLEKE IEDMEKDKDY ELDEEEEVAG SGRKGLNMGA HMLAKENGKV
 601  SEKDSSKTVK DEAPTNDDKL TSVIPEGEKE KDKTASSTVT VHENVNKNEI
 651  KENGKNEEQD MVEESSKTED SSKDADAAKK DTEDGLQDKT AENKEAGENN
 701  EEEEDDDDED EDEDMVDSQS YLLEKDDDRD DLEISVWKTV TAKVRAEICL
 751  KRTEYFKNGK LNSDSEAFLK NPQRMKRFDQ LFLEYKEQKA LESYRQKIEQ
 801  NSIMKNGFGT VLKQEDDDQL QFHNDHSLNG NEAFEKQPND IELDDTRFLQ
 851  EYDISNAIPD IVYEGVNTKT LDKMEDASVD RMLQNGINKQ SRFLANKDLG
 901  LTPKMNQNIT LIQQIRHICH KISLIRMLQS PLSAQNSRSN PNAFLNNHIY
 951  NYTIIDDSLD IDPVSQLPTH DYKNNRELIW KFMHKNISKV AMANGFETAH
1001  PSAINMLTEI AGDYLSNLIK TLKLHHETNS LNRGTNVEML QTTLLENGIN
1051  RPDDLFSYVE SEFGKKTKKL QDIKQKLESF LRALLRPTLQ ELSERNPEDE
1101  SQSFFTGDFA SELTGEDFFG FRELGLEKEF GVLSSSVPLQ LLTTQFQTVD
1151  GETKVQAKKI QPEESDSIVY KKITKGMLDA GSFWNTLLPL LQKDYERSKA
1201  YIAKQSKSSA NDKTSMTSTE DNSFALLEED QFVSKKTATK ARLPPTGKIS
1251  TTYKKKPIAS AFILPEEDLE NDVKADPTTT VNAKVGAEND GDSSLFLRTP
1301  QPLDPLDMDD AFDDTNMGSN SSFSLSLPRL NQ*
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FIGURE 2.—The amino acid sequence of SPT7. Acidic residues are shown in boldface and the bromodomain is underlined. The GenBank accession number for the DNA sequence is L22537.

SPT7 contains a 61 amino acid sequence (amino acids 463–523) that shows significant similarity to sequences in several other predicted protein sequences, including human CCG1 (TAF250), Drosophila FSH and brahma and yeast SNF2 and GCN5 (GEORGAKOPOULOS and THIREOS 1992; HAYNES *et al.* 1992). This sequence, called a bromodomain, is present two times in some protein sequences, although it is present only once in SPT7. The analysis of the SPT7 bromodomain is presented in a later section.

Construction and analysis of an *spt7* null mutant: To determine the phenotypes caused by complete loss of SPT7 function, we constructed and analyzed an *spt7* null mutant, as described in MATERIALS AND METHODS. Briefly, one copy of the wild-type *SPT7* gene in *SPT7*$^+$ /

SPT7$^+$ diploids was replaced with the *spt7Δ402::LEU2* (hereafter called *spt7Δ402*) construct in a one-step gene-replacement integration event. Haploid *spt7Δ402* strains were recovered by sporulating the diploids and choosing Leu$^+$ spores. By Northern analysis using a probe complementary to part of the *SPT7* coding region, we observed a large (~4.5 kb) transcript in *SPT7*$^+$ strains that was absent in *spt7Δ402* strains, consistent with the deletion of the *SPT7* open reading frame (data not shown).

Strains with the *spt7Δ402* mutation are alive at all temperatures tested, from 15 to 37°, but they grow very slowly. They also have several other mutant phenotypes including inositol auxotrophy and poor growth on glycerol or galactose as carbon sources. The cells are clumpy in culture and are altered in morphology relative to wild type, sometimes being enlarged, elongated or otherwise altered depending upon the exact growth conditions. In addition, some heterogeneity was observed in growth rates between *spt7Δ402* mutants derived from different diploids; however this difference caused no detectable effect on RNA polymerase II-dependent transcription (see MATERIALS AND METHODS). Most importantly, the *spt7Δ402* null mutants have an Spt$^-$ phenotype: they show weak to moderate suppression of the *his4-917δ* insertion mutation and strong suppression of the *lys2-173R2* mutation. They also show a strong defect in Ty transcription, as described below, and homozygous *spt7Δ402* diploids are sporulation defective. Thus, the Spt$^-$ phenotype that had been observed previously for the spontaneous *spt7* mutants is because of loss or reduction of SPT7 function.

Analysis of transcriptional defects of Ty elements and of *his4-917δ* in *spt7* mutants: Previous results showed a decreased level of Ty mRNA in *spt7-217* strains (WINSTON *et al.* 1987). To determine whether *spt7* null mutants had the same defect, we analyzed Ty mRNA in an *spt7Δ402* strain as well as in another spontaneous mutant, *spt7-223* (Figure 3A). The effect of the *spt7* mutations on Ty transcription is dramatic: *spt7Δ402* confers an ~50-fold decrease in levels of Ty mRNA, and the other alleles, *spt7-217* and *spt7-223*, cause ~16-fold and 11-fold decreases, respectively. This result fits well with previous results that measured the expression of a Ty-*lacZ* fusion in an *spt7-217* mutant (WINSTON *et al.* 1987). We also observe a qualitative defect previously seen for *spt7-217:* all of the *spt7* mutants show a significant amount of a shorter Ty transcript. Previous work has shown that this shorter Ty RNA has a 5′ end ~800 bp downstream of the normal Ty initiation site (WINSTON *et al.* 1987). These quantitative and qualitative defects in Ty transcription also have been seen in *spt3, spt8* and certain *spt15* mutant strains (reviewed in WINSTON 1992).

To determine whether the suppression of insertion mutations by *spt7* mutations is at the level of transcrip-

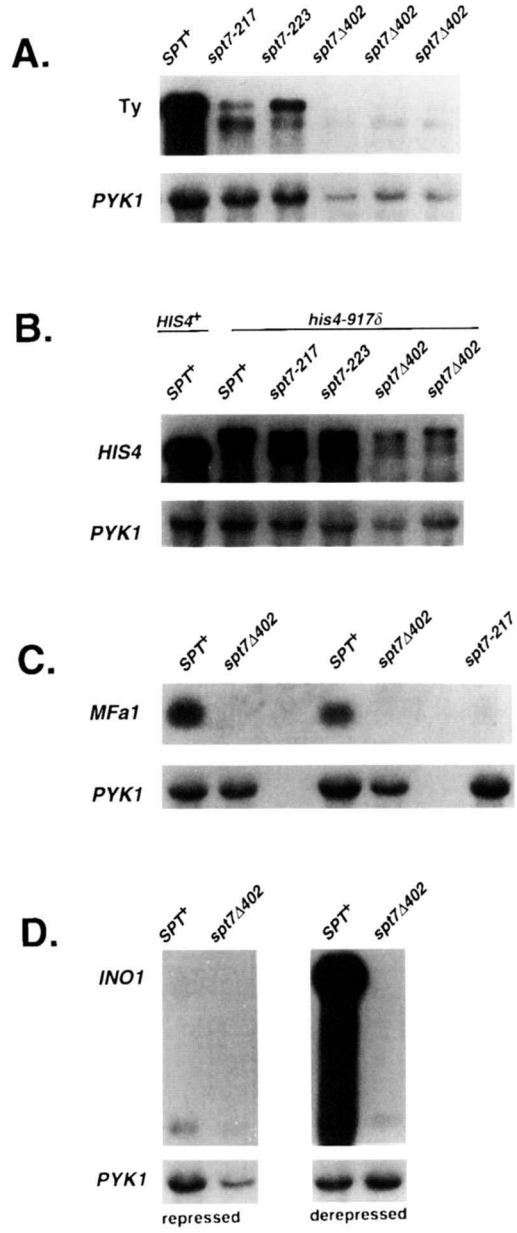

FIGURE 3.—Effects of *spt7* mutations on transcription of various genes. Strains were grown to ~1–2 × 10^7 cells/ml in YPD (A and C), minimal media supplemented with the necessary amino acids (B) or inositolless media made as described in MATERIALS AND METHODS (D). RNA was prepared and subjected to Northern blot analysis as described in MATERIALS AND METHODS. (A) Analysis of Ty transcription in the following strains: *SPT*$^+$ (FY168), *spt7-217* (FY580), *spt7-223* (FY162) and *spt7Δ402* (strains FY920, FY922 and FY1006). (B) Analysis of transcription from *his4-917δ* in the following strains: *SPT7*$^+$ (FY166), *spt7-217* (FY580), *spt7-223* (FY162) and *spt7Δ402* (strains FY920, FY922 and FY1006). The first lane shows the normal *HIS4* transcript in an *SPT7*$^+$, *HIS4*$^+$ strain (FY70). (C) Analysis of *MFA1* transcript levels in *SPT7*$^+$ (strains FY61 and FY168), *spt7Δ402* (strains FY963 and FY922) and *spt7-217* (FY571) strains. (D) Analysis of *INO1* message levels under repressing (200 μM inositol) and derepressing (10 μM inositol) growth conditions in *SPT7*$^+$ (FY61) and *spt7Δ402* (FY963) strains. Strains were grown as described in RESULTS. Each blot also was probed with the *PYK1* gene as an internal control.

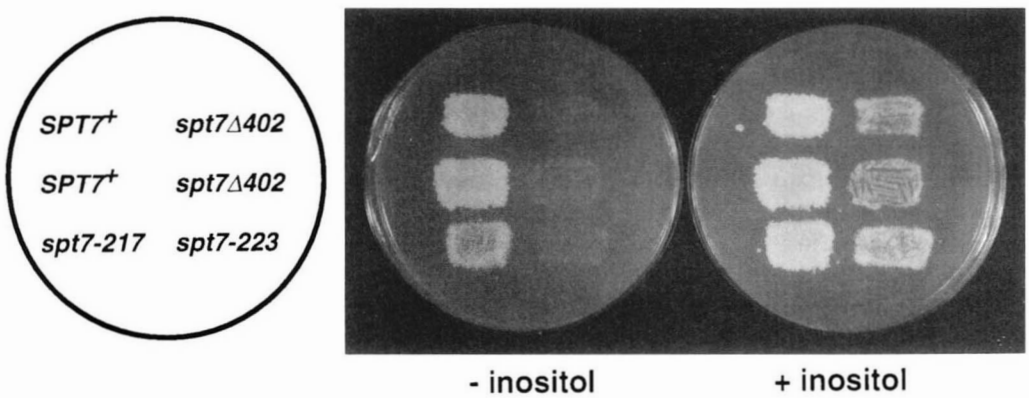

FIGURE 4.—Ino$^-$ phenotypes of *spt7* mutants. The strains used were as follows: top row, *SPT*$^+$ strain FY61 and its *spt7Δ402* derivative FY963; middle row, *SPT*$^+$ strain FY168 and its *spt7Δ402* derivative FY922; bottom row, *spt7-217* strain FY149 and *spt7-223* strain FY161.

tion, we analyzed transcription of *his4-917δ* by Northern analysis. This analysis (Figure 3B) demonstrates that suppression of *his4-917δ* correlates with an alteration of transcription for all three *spt7* alleles tested: *spt7Δ402, spt7-217* and *spt7-223*. In *SPT7*$^+$ *his4-917δ* strains, there is a longer-than-normal *HIS4* mRNA that initiates within the *δ* sequence that is inserted within the *HIS4* promoter upstream of the normal *HIS4* start site (WINSTON *et al.* 1984; K. ARNDT and F. WINSTON, unpublished observations). This transcript is nonfunctional for *HIS4* expression, presumably because of the presence of translational start and stop signals upstream of the normal *HIS4* initiation codon that preclude *HIS4* translation. In the *spt7* mutants there is a decreased level of the long transcript and the appearance of a shorter, wild-type length transcript. This shorter transcript at *his4-917δ* also has been seen in other *spt* mutant strains, including *spt3* and *spt15* (TBP) mutants (WINSTON *et al.* 1984; EISENMANN *et al.* 1992).

***spt7* mutants are defective for transcription of *MFA1*:** Previous studies have shown that *spt7* mutants do not mate as efficiently as wild-type strains (WINSTON *et al.* 1987). A similar result with *spt3* mutants was found to correlate with a decrease in transcription of the *MFA1, MFA2* and *MFα1* mating pheromone genes (HIRSCHHORN and WINSTON 1988). We thus analyzed levels of the *MFA1* transcript in *spt7-217* strains and *spt7Δ402* strains, and we found that the *spt7* mutations caused a large reduction in transcript levels of *MFA1* (Figure 3C).

***spt7Δ402* mutants are defective for transcription of *INO1*:** *spt7* mutants exhibit inositol auxotrophy ranging from very weak Ino$^-$ for *spt7-217* to very strong Ino$^-$ for *spt7Δ402* (Figure 4). This phenotype is of interest because it also is caused by mutations in several genes known to be important for transcription initiation by RNA polymerase II, including certain mutations in *RPB1,* which encodes the largest subunit of RNA polymerase II (SCAFE *et al.* 1990), and certain mutations in *SPT15* (K. ARNDT, S. RICUPERO-HOVASSE and F. WINSTON, unpublished observations). To examine this defect in *spt7* mutants, we examined *INO1* mRNA levels by Northern analysis. To do this, strains were grown first in repressing media (200 μM inositol) and were then shifted to derepressing conditions (10 μM inositol). The results (Figure 3D) show that, consistent with its Ino$^-$ phenotype, the *spt7Δ402* mutant has a greatly reduced level of *INO1* mRNA at 4 hr after derepression, a time at which *SPT7*$^+$ strains have reached their maximal level of *INO1* mRNA. In addition, *spt7Δ402* strains show a three- to sixfold decrease in the uninduced *INO1* levels (detectable in longer exposures; data not shown). In these experiments the *spt7Δ402* strains were not completely defective for *INO1* transcription; rather, they showed a greatly delayed induction. When kept in derepressing media for 10 hr, the *INO1* mRNA levels in *spt7Δ402* strains were similar to the levels reached by *SPT7*$^+$ strains at 4 hr (data not shown). The difference between the very tight *spt7Δ402* Ino$^-$ phenotype observed on plates versus this delayed induction observed in liquid is likely caused by differences in the media used in the two experiments (see MATERIALS AND METHODS).

Analysis of other RNA polymerase II-dependent, TATA-containing promoters: We analyzed transcription of Ty elements, *his4-917δ, MFA1,* and *INO1* because of specific mutant phenotypes observed in *spt7* mutants. We also have analyzed other RNA polymerase II transcripts. First, for *DED1,* we detect a modest and reproducible 1.7-fold decrease in transcript levels relative to total RNA (quantitated by A_{260}) in an *spt7Δ402* strain (Figure 5A). Second, for *PYK1,* we also see a modest and reproducible 1.2- to 2-fold decrease in the *spt7* mutant strains relative to total RNA (quantitated by A_{260}, Figure 3). Thus, *spt7* mutations do not greatly affect all RNA polymerase II-dependent transcripts.

Analysis of other classes of promoters in *spt7* mutant strains: The *SPT15* gene product, TBP, has been shown

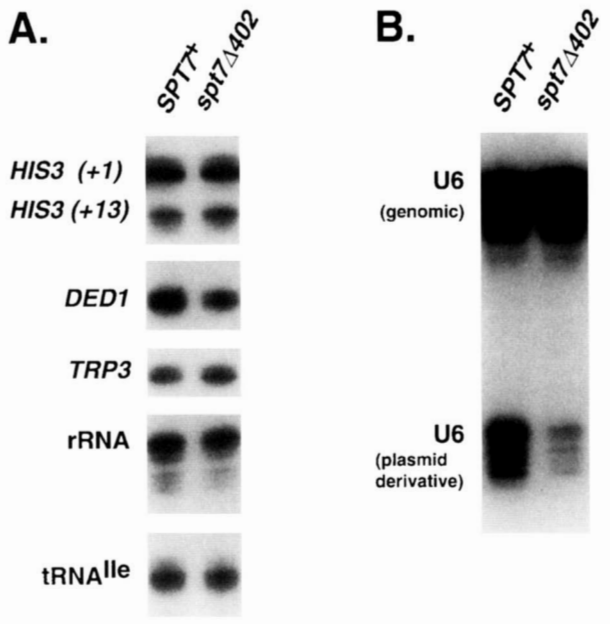

FIGURE 5.—S1 analysis of transcripts from genes transcribed by RNA polymerases I, II and III in *SPT7*$^+$ and *spt7Δ402* strains. (A) Strains FY935 (*SPT7*$^+$) and FY1007 (*spt7Δ402*) were grown in YPD, total RNA was prepared and equal amounts of RNA from each strain were subjected to S1 nuclease analysis with probes for the genes indicated. All reactions were performed at the same time and run on the same gel. For purposes of normalization, an *SNR6* probe was included in each of the reactions containing *DED1*, *TRP3*, rRNA and isoleucine tRNA probes (not shown). The *TRP3* probe additionally was included in the reaction with the *HIS3* probe as an internal control (not shown). (B) Strains FY935 and FY1007 were transformed with a plasmid containing an *SNR6* derivative encoding a putative unstable derivative of the U6 transcript. Total RNA was prepared from the transformants and subjected to S1 nuclease analysis with a probe for the U6 transcript. U6 transcripts derived from the genomic *SNR6* gene and the plasmid derivative are indicated. The difference in levels of the unstable U6-derivative between the wild-type and *spt7Δ402* mutant strains can be attributed to the differences in plasmid copy number observed between the two strains, rather than to transcriptional defects (data not shown).

in vitro and *in vivo* to function at promoters transcribed by RNA polymerases I, II and III, and it is required for transcription at both TATA-containing and TATA-less RNA-polymerase II promoters (reviewed in HERNANDEZ 1993). Because some *spt15* mutations cause the same phenotypes as *spt7* mutations, we investigated whether SPT7 is required at these different classes of promoters. The promoters assayed above, *his4-917δ*, Ty, *MFA1*, *INO1*, *DED1* and *PYK1*, are all RNA polymerase II-dependent and contain TATA box sequences (BURKE *et al.* 1983; ROEDER and FINK 1983; CHEN and STRUHL 1985; MICHAELIS and HERSKOWITZ 1988 and references within; BOEKE 1989; DEAN-JOHNSON and HENRY 1989; DOLAN *et al.* 1989 and references within).

We analyzed transcription from other classes of promoters as was done by CORMACK and STRUHL (1992) in their study of TBP. First, we analyzed two RNA polymerase II-dependent transcripts that are initiated from TATA-less promoters, *TRP3* and the *HIS3*(+1) transcript (AEBI *et al.* 1984; ZALKIN *et al.* 1984; MAHADEVAN and STRUHL 1990). The *HIS3* probe also allowed us to analyze the *HIS3*(+13) TATA-dependent transcript (CHEN and STRUHL 1988). The *spt7Δ402* mutation had no effect on the levels of any of these transcripts (Figure 5A). Second, we analyzed the levels of the RNA polymerase I transcript, rRNA, and found no significant difference in transcript levels between *spt7Δ402* and *SPT7*$^+$ strains (Figure 5A). Finally, we examined two different RNA polymerase III transcripts. One of these was an isoleucine tRNA, and the *spt7Δ402* mutation had no effect on the levels of this transcript (Figure 5A). The other was a derivative of the *SNR6* gene that encodes a U6 transcript believed to be unstable (see MATERIALS AND METHODS). It is interesting to note that, unlike other pol III-dependent promoters, *SNR6* contains a TATA box (BROW and GUTHRIE 1990; BURNOL *et al.* 1993). Plasmids carrying this *SNR6* derivative were transformed into the same strains used for analysis of the other transcripts in this experiment, and the levels of this U6 RNA were assayed (Figure 5B). We found that, although the *spt7Δ402* strain had a lower amount of the putative unstable U6 transcript (Figure 5B), the *spt7Δ402* strain also had a lower plasmid copy number than the *SPT7*$^+$ strains (data not shown); when the transcript levels were normalized to the plasmid copy number in each strain, there was no significant effect of *spt7Δ402* mutations on *SNR6* levels.

The experiments described above were performed on the healthier class of *spt7Δ402* mutants; we also performed the same experiments on the slower-growing class of *spt7Δ402* strains (see MATERIALS AND METHODS). In these strains, we found two differences from the previous experiments. First, the level of the genomic *SNR6* transcript from the *SNR6* gene was increased in the *spt7Δ402* strains by approximately twofold over that in the *SPT7*$^+$ strains, relative to total RNA assayed as measured by A_{260} and visualization on an ethidium bromide-stained gel. Second, the level of the isoleucine tRNA transcript was increased by approximately threefold as compared with *SPT7*$^+$ strains. All other RNA levels were the same as measured for the healthier *spt7Δ402* strains. We did not assay the levels of the unstable *SNR6* derivative in the slower-growing nulls.

We conclude that SPT7 is not required for transcription from either of the two TATA-less RNA-polymerase II promoters assayed, from the RNA polymerase I promoter or from either of the RNA polymerase III promoters assayed. We present some possible explanations for the apparent increases in U6 and tRNA transcript levels in the slower-growing nulls in the DISCUSSION. Taken together, all of these transcriptional analyses

show that SPT7 is required for transcription from particular RNA polymerase II promoters and not from others. We also cannot rule out that it might be required for some untested RNA polymerase III promoters.

Identification of wild-type and mutant SPT7 proteins: To identify directly the SPT7 protein, we raised and affinity-purified polyclonal antisera against the N-terminal quarter and the C-terminal half of the SPT7 protein, as described in MATERIALS AND METHODS. By Western analysis (Figure 6), both antisera recognize one prominent band in *SPT7*$^+$ extracts that migrates at a position corresponding to ~200 kD. This band, recognized by both antisera in *SPT7*$^+$ extracts, is not present in *spt7Δ402* extracts, confirming that it is SPT7. When using the antisera raised against the C-terminal portion of SPT7, we detect an extremely faint band in *spt7Δ402* extracts that migrates at the same position as wild-type SPT7 protein. (This faint band, unlike the other faint background bands in the Westerns in Figure 6, is reproducible.) This result hints at the possibility of a protein antigenically related to SPT7. It is important to note, however, that we do not detect this faint band with the antisera raised against the N-terminus of SPT7. Because the bromodomain of SPT7 was not included in either the N- or C-terminal regions of SPT7 used to raise antisera, the cross-reactivity cannot be caused by recognition of other bromodomains.

We also analyzed the protein products from two spontaneous *spt7* mutants (Figure 6). In *spt7-217* extracts, no full-length SPT7 band was seen; instead, we detected an SPT7 band of slightly lower molecular weight and significantly less intensity than was seen in *SPT7*$^+$ extracts. In *spt7-223* extracts, the N-terminal antisera recognizes a lower molecular weight band of ~140 kD; interestingly, this band is not recognized by the C-terminal antisera. These data suggest that *spt7-217* and *spt7-223* are the result of nonsense mutations at different positions in *SPT7*.

Finally, because SPT7 is related functionally to SPT3 and to TBP, we measured the level of SPT7 in *spt3* and in *spt15* mutants. There is little, if any, change in the levels of SPT7 protein in either *spt3* or *spt15* mutant strains (Figure 6).

The SPT7 protein is nuclear: To determine the intracellular location of the SPT7 protein, we performed indirect immunofluorescence microscopy using affinity-purified antisera against SPT7 (see MATERIALS AND METHODS). In wild-type cells, the SPT7 fluorescent staining colocalizes with the DAPI staining of nuclear DNA, indicating that the SPT7 protein resides in the nucleus (Figure 7, A–C). A number of controls indicate that this immunofluorescence is specific for the SPT7 protein. First, fluorescent staining depends upon the addition of the primary antibody (data not shown). Second, an *spt7Δ402/spt7Δ402* strain gives no signal with the anti-SPT7 antisera (Figure 7, D–F). Finally,

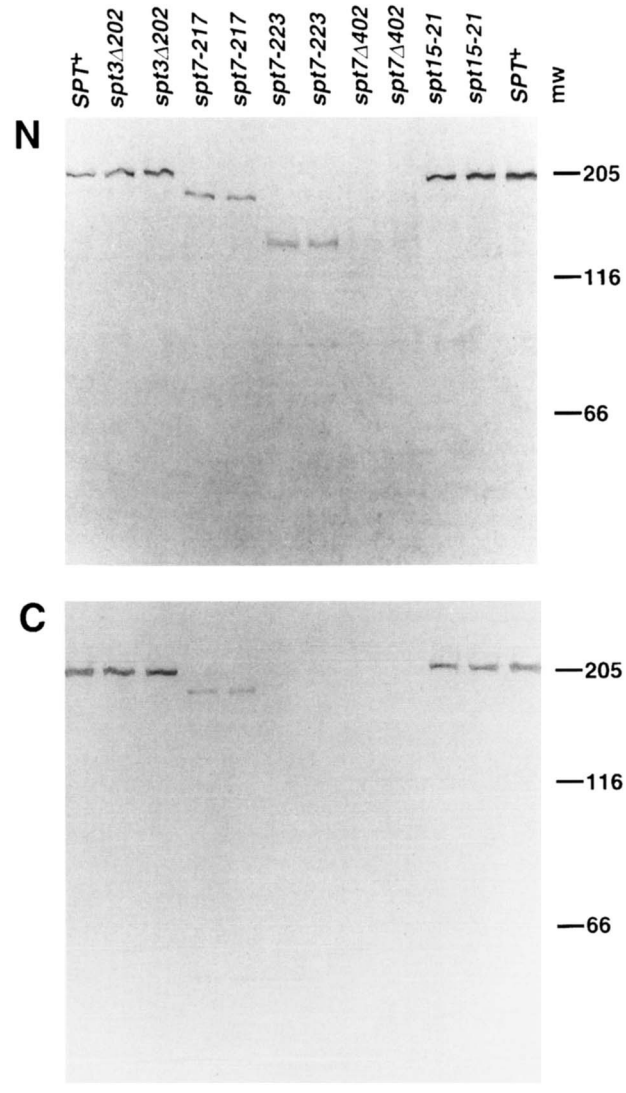

FIGURE 6.—Identification and analysis of the SPT7 protein in *SPT*$^+$, *spt7-217, spt7-223, spt3Δ202* and *spt15-21* strains. Protein extracts were prepared from *SPT*$^+$ and *spt* mutant strains (using two different strains for each *SPT*$^+$ and *spt* genotype). Aliquots (75 μg) of each extract were run on two 7.5% acrylamide gels in parallel, blotted and analyzed by Western using affinity-purified antisera raised against either the N-terminal quarter or C-terminal half of SPT7, as indicated (N, C). Molecular-weight markers are indicated (myosin, 205 kD; β-galactosidase, 116 kD; BSA, 66 kD). The following strains were used: lane 1, FY166 (*SPT*$^+$); lanes 2 and 3, FY294 and FY293 (*spt3Δ202*); lanes 4 and 5, FY571 and FY580 (*spt7-217*); lanes 6 and 7, FY161 and FY569 (*spt7-223*); lanes 8 and 9, FY922 and FY1006 (*spt7Δ402*); lanes 10 and 11, FY402 and FY773 (*spt15-21*); lane 12, FY631 (*SPT*$^+$).

an additional control experiment was performed to confirm that the immunofluorescent staining procedure was adequate for visualization of a nuclear antigen in *spt7Δ402/spt7Δ402* strains: we stained both the wild-type and *spt7Δ402/spt7Δ402* strains with antisera against another nuclear protein, NPL3 (BOSSIE *et al.* 1992), using anti-NPL3 polyclonal sera (generously

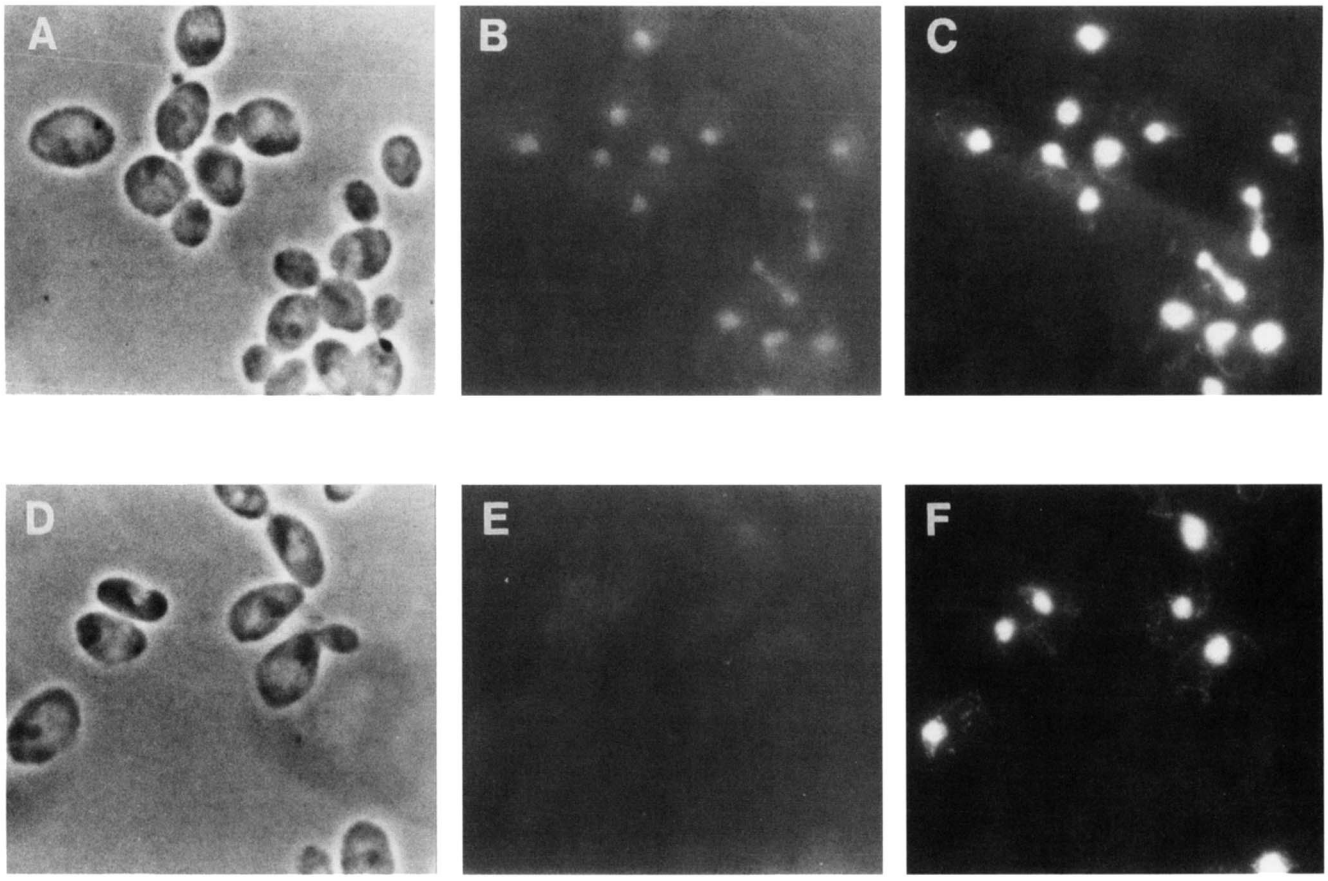

FIGURE 7.—Nuclear localization of SPT7 by indirect immunofluorescence. *SPT7*$^+$ / *SPT7*$^+$ diploid (A–C) and *spt7Δ402*/*spt7Δ402* diploid (D–F) strains were prepared for immunofluorescence as described in MATERIALS AND METHODS. Cells were stained with affinity-purified antisera specific for the N-terminal quarter of the SPT7 protein and anti-rabbit IgG secondary antibodies conjugated to fluorescein. Micrographs shown are phase contrast (A and D), fluorescein fluorescence (B and E) and DAPI fluorescence to stain DNA (C and F). The exposure time and degree of enlargement for the immunofluorescence micrographs was identical for *SPT7*$^+$ and *spt7Δ402* mutant cells. The *SPT7*$^+$ diploid was constructed by crossing strains FY934 × FY60, and the *spt7Δ402* diploid was constructed by crossing strains FY1007 × FY963. Because *spt7* mutants have a mating defect, mating of the *spt7Δ402* haploid strains was facilitated by transforming one of the parents with plasmid pFW192, which encodes the partially complementing C-terminal half of SPT7. This portion of SPT7 does not overlap with the N-terminal fragment of SPT7 used to obtain antisera for the immunofluorescence microscopy. In other experiments performed on *spt7Δ402*/*spt7Δ402* strains after having selected for loss of this plasmid, we obtained the identical result: no fluorescence when using the anti-SPT7 antibody (data not shown).

provided by PAMELA SILVER), and the same fluorescein-conjugated anti-rabbit secondary antibodies used with the anti-SPT7 antisera. The anti-NPL3 antisera yielded a bright nuclear fluorescein staining in both *spt7Δ402*/*spt7Δ402* and *SPT7*$^+$ / *SPT7*$^+$ strains (data not shown). Thus, we conclude that SPT7 is localized to the nucleus. Furthermore, because the staining pattern is nuclear in both budded and unbudded cells, this localization is not cell-cycle dependent.

The bromodomain is not required for SPT7 function: The bromodomain is a loosely conserved sequence of ~60 amino acids that is found throughout eukaryotes in several proteins involved in gene expression, including Drosophila brahma (for which the motif was named), human CCG1/TAF250 and yeast SNF2/SWI2, (HAYNES *et al.* 1992). No functional role for the bromodomain has been identified, and a deletion that removes it from yeast SNF2/SWI2 causes no detectable mutant phenotype (LAURENT *et al.* 1993). To determine whether the SPT7 bromodomain contributes to SPT7 function, we constructed mutants that contain a deletion (*spt7-502*) that removes the bromodomain from the SPT7 protein (described in MATERIALS AND METHODS). We integrated the *spt7-502* allele into the genome at the *SPT7* locus as the sole copy of *SPT7* sequences. The *spt7-502* mutants showed a wild-type phenotype for all phenotypes tested: they grew at the same rate as wild-type cells at all of the temperatures tested, from 15 to 37°; they grew on rich and minimal media without any apparent defect; they were not defective for growth on media containing galactose or glycerol as the carbon source; they were not clumpy; they

were sporulation competent; they were not inositol-auxotrophs; and they did not suppress either *his4-917δ* or *lys2-173R2*. Finally, by Western analysis, there also appeared to be no difference in SPT7 protein levels between *SPT*$^+$ and *spt7-502* strains (data not shown). The lack of a mutant phenotype under any of the conditions tested, and in particular, the lack of an Spt$^-$ phenotype characteristic of every other studied allele of *spt7*, strongly suggests that the bromodomain is not required for any important function of SPT7.

DISCUSSION

In this paper, we have shown that *SPT7* is important for proper transcription of a variety of genes. Mutations in *SPT7* cause a set of pleiotropic phenotypes very similar to those caused by certain mutations in *SPT15*, which encodes the TATA-binding protein (EISENMANN *et al.* 1989, 1992; K. ARNDT, S. RICUPERO-HOVASSE and F. WINSTON unpublished observations). These phenotypes include suppression of a common set of Ty and δ insertion mutations, mating and sporulation defects, inositol auxotrophy and a defect in growth on media containing galactose as carbon source. Many of these phenotypes have been shown to correlate with changes in transcription. By Northern analysis, *spt7* mutant strains show alterations in the transcription initiation sites of the *his4-917δ* and Ty mRNAs, and these start-site alterations are similar to those caused by certain mutations in *spt15* (EISENMANN *et al.* 1989, 1992). These data are consistent with a model in which SPT7 functions in transcription initiation, potentially in conjunction with TBP, to promote proper initiation *in vivo*.

We also investigated the specificity of the role of *SPT7* in transcription with respect to different classes of nuclear promoters. First, we examined transcript levels for several different RNA polymerase II-transcribed genes that contain TATA boxes. For this set of genes, the effects of the *spt7Δ402* mutation ranged from no effect or very small (for *HIS3*(+13), *DED1* and *PYK1*) to drastic (for Ty and *MFA1*). We also examined the transcript levels for two RNA polymerase II-dependent promoters that do not contain TATA boxes, *HIS3*(+1) and *TRP3* (AEBI *et al.* 1984; ZALKIN *et al.* 1984; MAHADEVAN and STRUHL 1990), as well as the levels of the RNA polymerase I-dependent rRNA transcript and the products of two RNA polymerase III-dependent genes. Transcription was not decreased in an *spt7Δ402* strain relative to wild-type strains for any of these genes. These results suggest that *SPT7* may be required specifically for transcription from certain RNA polymerase II-dependent, TATA-containing promoters.

Two issues must be considered in terms of the possible role of SPT7 in RNA polymerase I- and III-dependent transcription. First, because we only examined two RNA polymerase III-dependent genes, the possibility remains that *SPT7* might be required for some, but not all polymerase III transcripts. Second, although the healthier *spt7Δ402* strains showed no change in the levels of rRNA or of the two RNA polymerase III-dependent transcripts examined, the slower-growing *spt7Δ402* strains showed a two- to threefold increase in the levels of the polymerase III transcripts, indicating either an increase in the levels of these RNA polymerase III RNAs or an overall decrease in rRNAs. There are several possible explanations for the differences observed, which are the only transcriptional differences yet observed between the two classes of *spt7Δ402* strains. Although we cannot rule out the possibility that some additional defect in the slower-growing *spt7Δ402* strains plays a direct role in transcription, we think that the difference is likely caused by an indirect effect of the extremely poor growth of these strains.

The sequence of SPT7 contains several interesting features but provides no obvious clues about its role in transcription. SPT7 is very acidic; the N-terminal half of SPT7 (up to the *Sal*I site, Figure 1) has a charge of −69 and a pI of 4.4 and is 23% aspartic acid and glutamic acid residues. The C-terminal half of the protein has a charge of −20 and a pI of 5.02. The extremely acidic nature of SPT7 may account for its slower-than-predicted migration on SDS-PAGE gels (TAKANO *et al.* 1988). The C-terminal half of the protein can complement partially *spt7* mutant phenotypes (data not shown). However, it is not yet clear whether lack of full complementation results from poor expression or stability of this fragment or from lack of a functional component of the N-terminus. SPT7 also contains a bromodomain sequence (HAYNES *et al.* 1992); deletion of this sequence causes no detectable mutant phenotype. The same result has been obtained with the yeast SNF2/SWI2 protein (LAURENT *et al.* 1993). Deletion of the bromodomain from a human SNF2/SWI2 homologue, *hbrm*, resulted in an increased level of this protein as compared to the wild type, hinting at a role for the bromodomain in protein stability (MUCHARDT and YANIV 1993). We have found no difference, however, in SPT7 protein levels between *SPT7*$^+$ strains and strains with the bromodomain deletion allele of *SPT7* (data not shown).

Mutations in two other *SPT* genes, *SPT3* and *SPT8*, cause similar phenotypes to those caused by mutations in *SPT7* and by certain alleles of *SPT15* (WINSTON *et al.* 1984, 1987; EISENMANN *et al.* 1989, 1992, 1994; K. ARNDT, S. RICUPERO-HOVASSE and F. WINSTON, unpublished). The products of these genes may act in a complex together with TBP or they may function at different points in the same pathway toward initiation. The strength of the phenotypes shared among these *SPT* genes varies. For example, all *spt7* null mutants grow more slowly than *spt3* and *spt8* null strains, and both *spt7* and *spt3* null strains exhibit significantly stronger

suppression of the *lys2-173R2* insertion mutation than an *spt8* null strain. These data suggest that SPT7 may play a more direct or more critical role in a step of transcription initiation in which all three of these SPT proteins function, that SPT7 may function in multiple steps in the transcription process, or that it might be required at more promoters than either SPT3 or SPT8. Because *SPT7, SPT3* and *SPT8* are not essential for viability, the relative strengths of their null phenotypes also may reflect the existence of other proteins in the cell with somewhat redundant functions.

The SPT7 protein has not yet been identified biochemically. We were unable, by Western analysis, to detect SPT7 in either the SRB complex of yeast transcription factors (THOMPSON *et al.* 1993; data not shown) or in the yeast TBP-TAF complex (POON and WEIL 1993; data not shown). However, the transcriptional defects caused by mutations in *SPT7* and the poor growth of *spt7* null mutant strains demonstrate that SPT7 function is clearly critical *in vivo*. The fact that it has thus far not been identified among the transcription factors isolated by *in vitro* approaches may suggest that SPT7 plays an as yet uncharacterized role in transcription initiation. Further analysis of *SPT7* should provide interesting insights to the mechanism of transcription initiation *in vivo*.

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