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Mutational and Functional Analysis of Dominant *SPT2* (*SIN1*) Suppressor Alleles in *Saccharomyces cerevisiae*

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The *Saccharomyces cerevisiae SPT2* gene was identified by genetic screens for mutations which are suppressors of Ty and δ insertional mutations at the *HIS4* locus. The ability of *spt2* mutations to suppress the transcriptional interference caused by the δ promoter insertion *his4-912δ* correlates with an increase in wild-type *HIS4* mRNA levels. The *SPT2* gene is identical to *SIN1*, which codes for a factor genetically defined as a negative regulator of *HO* transcription. Mutations in *SPT2/SIN1* suppress the effects of *trans*-acting mutations in *SWI* genes and of partial deletions in the C-terminal domain of the largest subunit of RNA polymerase II. Nuclear localization and protein sequence similarities suggested that the SPT2/SIN1 protein may be related to the nonhistone chromosomal protein HMG1. To assess the significance of this structural similarity and identify domains of SPT2 functionally important in the regulation of *his4-912δ*, we have studied recessive and dominant *spt2* mutations created by in vitro mutagenesis. We show here that several alleles carrying C-terminal deletions as well as point mutations in the C-terminal domain of the SPT2 protein exhibit a dominant suppressor phenotype. C-terminal basic residues necessary for wild-type SPT2 protein function which are absent from HMG1 have been identified. The competence of these mutant SPT2 proteins to interfere with the maintenance of the His$^-$ (Spt$^+$) phenotype of a *his4-912δ SPT2*$^+$ strain is lost by deletion of internal HMG1-like sequences and is sensitive to the wild-type *SPT2*$^+$ gene dosage. Using cross-reacting antipeptide polyclonal antibodies, we demonstrate that the intracellular level of the wild-type SPT2 protein is not affected in presence of dominant mutations and furthermore that the reversion of the dominance by internal deletion of HMG1-like sequences is not mediated by altered production or stability of the mutant polypeptides. Our results suggest that the products of dominant alleles directly compete with the wild-type protein. On the basis of primary sequence similarities, we propose that an HMG-box-like motif is required for SPT2 function in vivo and that this motif also is necessary for the dominant suppressor phenotype exhibited by some mutant *SPT2* alleles.

The *spt2* mutations of *Saccharomyces cerevisiae* were originally characterized by their ability to suppress the effects of transposable element insertions which interfere with gene expression (60, 61, 70). Such Ty or δ insertional mutations in the 5′ noncoding regions of the *HIS4* gene were shown to mediate their effects via inhibition of or interference with the normal transcription of the *HIS4* gene (69). Further insight into possible molecular mechanisms for this type of transcriptional inhibition came from the study of the *his4-912δ* mutation, which confers a cold-sensitive His$^+$ phenotype: yeast strains carrying the *his4-912δ* mutation are phenotypically His$^+$ at 37°C but His$^-$ at 25°C (61). This mutation is caused by the insertion of the transposable element Ty1-912 in the *HIS4* promoter, 97 bp upstream of the transcription initiation site, followed by excision of most of the element by δ-δ recombination, leaving behind a solo δ sequence (or long terminal repeat) inserted between the *HIS4* upstream activating sequences and its TATA box (6, 16, 29, 61). The Ty1-912δ element is inserted in the same transcriptional orientation as the *HIS4* gene and promotes the initiation of a nonfunctional mRNA, probably as a result of the presence of several translational start and stop codons in between the start codon of the *TYA* open reading frame (present within the δ sequence) and that of the *HIS4* gene (6, 16). The δ and *HIS4* promoters were proposed to be in competition for transcriptional initiation such that in *his4-912δ* mutant strains grown at 25°C, the δ-initiated transcript is predominant and causes a His$^-$ phenotype (29). The reversion of this phenotype (by *cis*-acting mutations, growth at 37°C, or *spt* mutations; see below) is always associated with an increase in transcriptional initiation at the *HIS4* promoter (7, 29, 69). Several extragenic suppressor mutations of *his4-912δ* have been characterized and belong to a group of yeast genes known as *SPT* genes (for suppressor of Ty; reviewed in references 6 and 7). Since they can suppress the transcriptional defects caused by the δ insertion, these mutations are thought to define yeast genes whose products are involved directly or indirectly in transcription initiation (17, 82). Consistent with this view, some *SPT* gene products have been implicated in Ty transcription (*SPT3*, *SPT7*, and *SPT8* [83, 84]), chromatin structure (*SPT11* and *SPT12*, coding for histones H2A and H2B [11, 17]), and TATA-box recognition (*SPT15*, coding for the yeast TATA-binding protein yTFIID [15, 17]). Specific point mutations in the genes encoding the two large subunits of yeast RNA polymerase II (*RPO21/RPB1* and *RPO22/RPB2*) were also shown to suppress δ insertions, therefore presenting further evidence for the implication of the transcriptional machinery in the suppression mechanism (23).

Mutations in the *SPT2* gene can suppress Ty and δ insertions at the *HIS4* and *LYS2* loci, suggesting that the wild-type *SPT2* gene product acts independently of the function of the adjacent gene (70). Northern (RNA) blot analysis of the expression of *HIS4* in *his4-912δ SPT2-1* double mutants, which are His$^+$ at 25, 30, and 37°C, showed

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that the suppression correlates with an increase in the levels of both the *HIS4* and δ transcripts (69). In screens for different *SPT* genes, mutations in the *spt2* complementation group represented the major fraction of the mutations recovered, and suppressor *spt2* alleles with recessive or dominant phenotype were characterized (17, 82). Furthermore, a complete deletion of the *SPT2* locus is viable and confers a suppressor phenotype recessive to the wild-type function, demonstrating that the suppression of promoter insertional mutations by *spt2* corresponds to a loss of function (82). Molecular cloning of the *SPT2* gene showed that it has the potential to encode a protein of 333 amino acid residues whose most striking structural features are its high content of charged residues (44.4%), a high degree of predicted α-helical secondary structure (59), and a region of sequence similarity with the HMG1 (HMG for high mobility group) protein (see below). The dominant suppressor allele *SPT2-1* was found to carry a nonsense mutation at position 213 which should produce a truncated polypeptide missing the C-terminal third of the SPT2 protein (59).

SPT2 is identical to *SIN1*, a gene whose product is necessary for negative transcriptional regulation of the *HO* and *INO1* genes in the absence of positive effectors (encoded by the *SWI1*, *SWI2*, and *SWI3* genes [42, 58, 72]). The SPT2/SIN1 protein was shown to be concentrated in the nucleus and to share structural similarities with the chromatin-associated HMG1-like proteins of eukaryotes (42), which constitute an important class of nonhistone chromosomal proteins (35, 77). It was suggested that SWI1/2/3 activates transcription by antagonizing the negative regulatory effects of SPT2/SIN1 (25). A deletion of the *SPT2/SIN1* gene also was shown to partially suppress the phenotypes associated with truncations of the C-terminal domain of the largest subunit of RNA polymerase II (RPO21/RPB1), composed of 26 or 27 heptapeptide repeats (58). On the basis of this functional interaction, it was proposed that SPT2/SIN1 interacts with DNA and mediates negative regulatory effects which must be relieved to allow the function of mutant RNA polymerase II. Further genetic studies reviewed recently (74, 81) suggest that SWI1 (also known as ADR6), SWI2 (also known as SNF2), and SWI3 proteins, along with SNF5 and SNF6 proteins, are required for the transactivation of several regulated yeast genes. Suppressors of *swi/snf* mutations, such as mutations in *SPT2/SIN1*, *SIN2/HHT1* (encoding histone H3), *SPT11/HTA1* and *SPT11/HTB1* (encoding histones H2A and H2B), *SPT6/SSN20*, and other *SPT* genes, were proposed to act as negative regulators of transcription via the establishment of inactive chromatin (81) or by producing the chromatin context necessary for the action of transcriptional regulators (74).

While these genetic studies all suggest that the SPT2 protein is involved in the maintenance of negative transcriptional effects, it is not yet known how this protein mediates this activity or what the functional significance of the sequence similarities with HMG1-like proteins is. On the basis of the available information regarding the structure of the wild-type SPT2 protein and the nature of the dominant *SPT2-1* mutation, we undertook a mutational analysis of the $SPT2^+$ gene product. Our structure-function study was primarily guided by two questions. What are the structural requirements for SPT2-mediated repression and for the dominant suppressor phenotype of some *SPT2* mutants? What is the molecular mechanism of this dominance?

Here we report the construction and analysis of several mutant *SPT2* alleles. We find that truncated gene products composed of the first 179 to 324 amino acids constitute nonfunctional dominant polypeptides interfering with the wild-type SPT2 protein function. This dominant phenotype is not mediated via interference with the transcription or translation of the $SPT2^+$ gene but is sensitive to the levels of wild-type protein. We also show that internal residues overlapping a region of similarity to a DNA-binding motif found in HMG-related proteins (HMG box) are necessary for wild-type negative regulator function and for the dominance of truncated protein. These results provide structural evidence consistent with a molecular mechanism of dominance involving protein-DNA interactions.

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

Name	Genotype
S49	*MATα leu2-1 trp1-1*
S704	*MATa his4-912δ ura3-52 leu2-3 lys1-1 spt2-150*
SLL4	*MATα his4-912δ ura3-52 leu2 lys1-1*
SLL5	*MATa his4-912δ ura3-52 leu2 trp1-1*
SLL7	*MATa his4-912δ ura3-52 leu2 trp1-1 spt2Δ::URA3*
SLL9	*MATa* his4-912δ *ura3-52 leu2 trp1-1 spt2Δ*
SLL10	*MATα his4-912δ ura3-52 leu2 lys1-1 spt2Δ::URA3*
SLL101	*MATa his4-912δ ura3-52 leu2 trp1-1 SPT2-324*
SLL102	*MATa his4-912δ ura3-52 leu2 trp1-1 spt2-324Δ3*

MATERIALS AND METHODS

Strains. *Escherichia coli* JM101 (86) was used for plasmid constructions, yeast shuttle vector propagation, and preparation of single-stranded DNA. *E. coli* RZ1032 (43) was the host for isolation of uracil-containing single-stranded DNA. Growth conditions and transformation of bacteria by the calcium chloride procedure were as described previously (65). Genetic methods and media for yeast growth were as described previously (66, 67). SD is a synthetic minimal medium (67% Bacto Yeast Nitrogen Base [without amino acids], 2% dextrose and 2% Bacto Agar), and SC is a synthetic complete medium (SD supplemented as described previously [66]). Yeast cells were transformed by either the spheroplast (27) or the electroporation (3) procedure. *S. cerevisiae* strains used in this study are listed in Table 1. The diploid strain SLL2 was obtained by mating S49 (laboratory stock, obtained from A. M. Spence) with S704 (obtained from G. S. Roeder) and selecting for mating products on SD+Leu. Strains SLL4 and SLL5 were obtained as meiotic segregants from SLL2; other strains are described below. The His phenotype of the different mutants was monitored as follows. Three independent transformants were grown to saturation in dropout medium selecting for maintenance of the vector(s) (SC-Leu or SC-Leu-Ura for cotransformants); fixed aliquots of the cultures were used to inoculate fresh medium, and the cultures were grown for 12 to 24 h until a cell density of 2.0 to 4.0 units of optical density at 600 nm was reached; equal amounts of cells were spotted on selective dropout plates lacking histidine (SC-Leu-His or SC-Leu-Ura-His) and incubated at 30°C for 2 to 3 days. The β-galactosidase activity of the *SPT2:lacZ* fusion was assayed as described by Ner and Smith (54) on duplicates of three independent transformants.

Genetic nomenclature. We have respected the standard rules of genetic nomenclature for the designation of the in vitro-generated alleles (66). The dominant alleles characterized here are denoted by capital italicized letters (e.g., *SPT2-324*) and are defined as the alleles whose product confers a suppressor (Spt^- [His^+]) phenotype when ex-

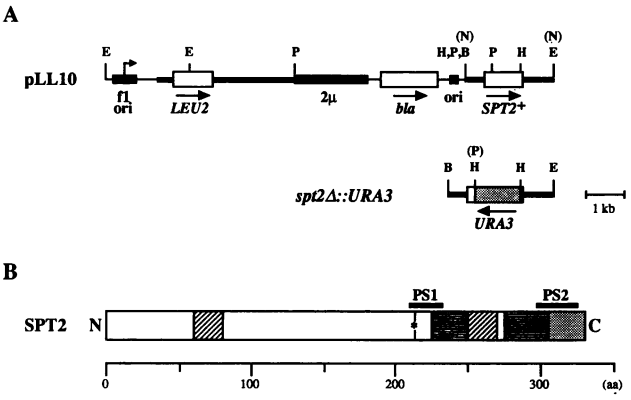

FIG. 1. Plasmid constructions and *SPT2*$^+$ gene product. (A) Map of pLL10 (YEp*SPT2*$^+$), a derivative of pEMBLYe30 (2) which carries the 2.3-kb *Nde*I fragment of the *SPT2*$^+$ locus inserted in the multiple cloning site. In addition to the *E. coli* ampicillin resistance gene and origin of replication (Ori), it contains the yeast *LEU2* gene as a selectable marker, the 2 μm origin of replication, and the filamentous phage f1 origin for single-stranded DNA production. Also shown is the structure of the disruption allele *spt2Δ::URA3* used in strain constructions (see Materials and Methods). Restriction enzymes are abbreviated as follows: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; N, *Nde*I; P, *Pst*I. Parentheses are used to denote sites lost during subcloning steps. (B) Schematic representation of the SPT2 protein. Regions of the protein with significant primary sequence or predicted secondary structures are highlighted. The asterisk indicates the position of the predicted carboxy-terminal residue (Arg-212) in the product of the dominant nonsense mutation *SPT2-1*. Sequences from which the synthetic peptides PS1 and PS2 were designed are shown by solid bars. aa, amino acids; ▨, helix-turn-helix motif (60 to 81 and 250 to 271); ▤, acidic region (226 to 249 and 277 to 303); ▩, polar, helical region (304 to 333).

pressed in a *his4-912* δ*SPT2*$^+$ background. The wild-type allele is designated *SPT2*$^+$; all silent mutations and semidominant and recessive alleles are denoted with lowercase italics. The product of the *SPT2*$^+$ gene will be referred to as the SPT2 protein, or simply SPT2.

Plasmids. Original clones of the wild-type *SPT2* locus (plasmid R559; 4.3-kb *Pst*I-*Pvu*I fragment of *SPT2* inserted at *Pst*I-*Pvu*I site of pBR322) and of the dominant allele *SPT2-1* (plasmid R159; 5.2-kb *Xho*I-*Bam*HI fragment of *SPT2-1* inserted at *Sal*I-*Bam*HI site of pBR325) were kindly provided by G. S. Roeder (59). The *SPT2* coding region, along with approximately 600 bp of 5′ noncoding sequences and 700 bp of 3′-flanking regions, was subcloned as a 2.3-kb *Nde*I fragment (in which the 3′ recessed ends were filled in with the *E. coli* DNA polymerase I Klenow fragment) in the unique *Sma*I site of the multiple cloning site of pEMBLYe30 (2) to obtain plasmid pLL10 (YEp*SPT2*$^+$; Fig. 1A). This yeast shuttle vector is a 2μm-based high-copy-number vector (YEp) carrying the *LEU2* marker for selection in yeast cells and the f1 replication origin, which allows propagation and packaging of single-stranded DNA carrying the coding (mRNA-like) strand of *SPT2*$^+$. The *SPT2-1* allele was similarly subcloned in pEMBLYe30 to form pLL18 (YEp*SPT2-1*). Most nonsense mutations, point substitutions, and internal deletions were obtained as pLL10 derivatives by oligonucleotide-directed mutagenesis (see below). Low-copy-number (YCp) derivatives carrying the *SPT2*$^+$ and *SPT2-1* alleles were constructed by subcloning the 4.2-kb *Bam*HI-*Cla*I fragment of pLL10 and pLL18 in the *Bam*HI-*Cla*I site of YCp50 (37, 62) to form pLL15 (YCp*SPT2*$^+$) and pLL24 (YCp*SPT2-1*), respectively.

For the cotransformation experiments (Fig. 6A), the wild-type *SPT2*$^+$ gene was expressed from the *GAL4* promoter on vectors carrying the *URA3* gene as a selectable marker. Plasmid pPS42B-2, containing a 3.4-kb *Bam*HI-*Eco*RI fragment of *GAL4* (with an additional *Eco*RI site inserted upstream of the ATG, in the *Acc*I site) in pBR322 (obtained from I. Sadowski), was digested with *Bam*HI and *Eco*RI to completion, and the 0.4-kb *GAL4* promoter fragment was subcloned in the *Bam*HI-*Eco*RI site of YCplac33 and YEplac195 (20) to create pLL94 (YCp-*pGAL4*) and pLL95 (YEp-*pGAL4*). The 1.9-kb *Eco*RI fragment from pLL16 (Table 2) carrying the *SPT2* coding region was subcloned in the unique *Eco*RI site of pLL94 and pLL95 to form pLL96 (YCp-*pGAL4:SPT2*$^+$) and pLL97 (YEp-*pGAL4:SPT2*$^+$) in which the insertions are oriented to create a transcriptional fusion.

The *SPT2:lacZ* fusion was constructed by subcloning the 1.7-kb *Sal*I-*Mlu*I fragment of pLL40 (Table 2) in the *Sal*I-*Bam*HI site of pLG-f1 (a derivative of pLG669-Z [21] constructed by S. S. Ner and carrying the f1 replication origin) in the presence of *Mlu*I-*Bam*HI adapters (OLL48a [5′-CGCGGCGGCG-3′] and OLL48b [5′-GATCCGCCGC-3′]), which fuse the two coding regions.

The 2.3-kb *Eco*RI-*Sal*I fragment of pLL10 carrying the *SPT2*$^+$ gene was isolated, treated with *E. coli* DNA polymerase I (Klenow fragment) to fill in the 5′ extensions, and ligated in the 6.1-kb *Sph*I fragment of pVT100-U2 (a derivative of the YEp, *URA3* vector pVT100-U, obtained from T. Vernet [78], in which the unique *Eco*RI site was destroyed) whose 3′ protruding ends were removed by the exonuclease activity of *E. coli* DNA polymerase I. This subcloning strategy regenerates the flanking *Eco*RI and *Sal*I sites and produces a plasmid (pLL77) in which the *Hin*dIII and *Pst*I sites, internal to the *SPT2*$^+$ coding region, are both unique. This plasmid was digested to completion with *Hin*dIII and *Pst*I, treated with *E. coli* DNA polymerase I, and ligated in the presence of excess *Hin*dIII linkers to create a 810-bp deletion in the *SPT2* coding region. The resulting plasmid, pLL80, carries the first 46 codons of *SPT2* fused to codons for the amino acids Lys, Leu, Val, Gly, and Stop (ochre), referred to below as the *spt2-46* allele (or *spt2Δ* in strain constructions). This allele was then subcloned as a *Bam*HI-*Eco*RI fragment in pLL10 to form pLL91 (YEp*spt2-46*). Plasmid pLL81 was obtained by deleting the 1.2-kb *Bgl*II fragment of pLL80 containing the *URA3* gene. The disruption allele *spt2Δ::URA3* was created by inserting the *URA3* gene as a 1.1-kb *Hin*dIII fragment from YEp24 (10) in the *Hin*dIII site of pLL81; in pLL82, the orientation of the *URA3* gene with regard to the deleted *SPT2* locus is such that both transcriptional units are in opposite directions (Fig. 1A).

Mutagenesis and sequence analysis. Oligonucleotide-directed mutagenesis (88) was performed essentially as described previously (44), exploiting the uracil-containing template selection of Kunkel (43) and the single-stranded DNA purification protocol of Dente et al. (14) as described by Inglis et al. (31). The packaging-deficient filamentous helper phage R408 (64) was used for all superinfections. Oligonucleotides were synthesized on an Applied Biosystems 380B or 380A DNA synthesizer and purified on C_{18} SepPak cartridges (Millipore) as described previously (1). Mutagenic primers were phosphorylated chemically during synthesis (30) or enzymatically with T4 polynucleotide kinase (31). Table 2 presents all of the mutations created by oligonucleotide-directed mutagenesis in this study, along with the template and oligonucleotide used in each case. The *SPT2-*

TABLE 2. Oligonucleotide-directed mutagenesis[a]

Template	Mutagenic primer		Plasmid	Mutation(s)	Allele
	Name	Sequence			
pLL10	OLL2	CTTTGAATTCGACTCAAG	pLL16	*Eco*RI site in promoter	*spt2-501*
	OLL18	TCTTGCCTTAATTGAACA	pLL38	R272 ochre	*SPT2-271*
	OLL23	TCAATATTCCTTAAAC GCGTATGCCCTTC	pLL40	V334 *Mlu*I site at 3′ end	*spt2-334*
	OLL26	CTATTTTCTTTTTCTC CAGTTCCTGCCGCCTC	pLL44	Δ60–81	*spt2Δ1*
	OLL30	TTGCCATTTATTATGCCATTTC	pLL47	R304 ochre K305 ochre	*SPT2-303*
	OLL31	CTTCATGCTATTATAACCAAGC	pLL48	K319 ochre K320 amber	*SPT2-318*
	OLL32	TACGGCGTCATTACTCCTCTTC	pLL49	K325 ochre R326 opal	*SPT2-324*
	OLL36	GTTCAGGTTATTACCTTACAAC	pLL63	K117 ochre K118 ochre	*spt2-116*
	OLL37	TAGCGTATGCCTGACTTACGGCGT	pLL57	K330S	*spt2-330*
	OLL38	CGTATGCCCTTTGAACGGCGTCTC	pLL58	K329S	*spt2-329*
	OLL39	ATGCCCTTCTTACTGCGTCTCTT	pLL59	R328S	*spt2-328*
	OLL40	CCCTTCTTACGGCTTCTCTTCTCCT	pLL60	R327S	*spt2-327*
	OLL41	TTCTTACGGCGACTCTTCTCCTCT	pLL61	R326S	*spt2-326*
	OLL42	TTACGGCGTCTTGACTCCTCTTCA	pLL62	K325S	*spt2-325*
	OLL43	TCCAGAAGATTATTAACGCAATGT	pLL68	G180 ochre V181 ochre	*SPT2-179*
	OLL47	TTACGGCGTCTTCTCTCCTCTTCA	pLL73	K325R	*spt2-336*
	OLL49	TGCTATTGCCTCCAGAAGATACTCC CCTTACAACTGGCTTGAGTGGCGCA	pLL75	Δ117–179	*spt2Δ3*
pLL18	OLL26	See above	pLL45	L213 ochre Δ60–81	*SPT2-1Δ1*
pLL49	OLL49	See above	pLL74	K325 ochre R326 opal Δ117–179	*spt2-324Δ3*
pLL62	OLL37	See above	pLL64	K325S K330S	*SPT2-350*
	OLL38	See above	pLL65	K325S K329S	*SPT2-359*
pLL64	OLL49	See above	pLL76	K325S K330S Δ117–179	*spt2-350Δ3*
pLL85	OLL32	See above	pLL86	K325 ochre R326 opal Δ48–179	*spt2-324Δ2*

[a] For each mutation created by site-directed mutagenesis are presented the template and primer (name of oligonucleotide and sequence from 5′ to 3′ ends) used for the mutagenesis reaction as well as the designation of the resulting plasmid, mutation, and allele. Mutant bases are in boldface.

335 allele was obtained by replacing the C-terminal *Hin*dIII-*Mlu*I fragment of pLL40 by a synthetic cassette of two 52-mers introducing mutations K325M, R326I, R327L, R328L, K329M, and K330M to the V-334 silent mutation *spt2-334*. For each mutant allele presented here, the DNA sequence of the entire coding region was confirmed by dideoxy sequencing according to the protocol of Ner et al. (53).

PCR mutagenesis. The deletion of residues 48 to 179 in the SPT2 protein (referred to as Δ2) was constructed by first introducing a *Pst*I site after the codon for residue 179, using polymerase chain reaction (PCR) mutagenesis. The PCR was performed in a Perkin Elmer Cetus DNA Thermal Cycler, using 1 ng of plasmid DNA (pLL10), 50 pmol of each primer (OLL34 [5′-CGCGGATGCCCITCITAIGGIGTITCITCTCC TCTTCATGCTTTTTTAACCA-3′], from the *Hin*dIII-*Mlu*I cassette [I represents inosine residues introduced for random cassette experiments not described here], and OLL51 [5′-CCCCTGGCGCAACATCTGCAGGAGTATCTTCTGG AGGCAATAGC-3′]), 10 nmol of each deoxynucleoside triphosphate (Pharmacia), 1 U of *Taq* DNA polymerase (Pharmacia), and 10 μl of 10× PCR buffer (100 mM Tris-HCl [pH 8.4], 6 mM $MgCl_2$, 0.5% Tween 20, 0.5% Nonidet P-40) in a total volume of 100 μl. The reaction mixture was subjected to a denaturation step of 2 min at 94°C followed by 30 amplification cycles (10 s at 94°C, 30 s at 53°C, and 1 min at 72°C) and a final extension of 5 min at 72°C. The 484-bp product was treated with proteinase K as described previously (13), digested with *Pst*I and *Hin*dIII, and purified by using the Geneclean Kit (Bio 101) after electrophoresis on a 1.5% agarose gel. The PCR product was ligated in the *Pst*I-*Hin*dIII site of pLL77 to obtain pLL85, carrying the *spt2-Δ2* allele. Allele *spt2-324Δ2* was created by oligonucleotide-directed mutagenesis on pLL85 (Table 2), and the *Bam*HI-*Eco*RI fragments of both alleles were subcloned in pLL10 to form pLL89 (YEp*spt2Δ2*) and pLL93 (YEp*spt2-324Δ2*).

Yeast strain constructions. The haploid strain SLL5 (Table 1) was used as a *SPT2*$^+$ parental strain from which was derived a set of four isogenic strains carrying different alleles at the *SPT2* locus. SLL5 was transformed with 3 μg of the 2.6-kb *Eco*RI-*Bam*HI fragment of pLL82 containing the *spt2Δ::URA3* disruption allele, and stable Ura$^+$ transformants were selected by the one-step gene disruption procedure of Rothstein (63). The integration of the *spt2Δ::URA3* disruption allele at the *SPT2* locus of SLL7 was confirmed by genomic DNA analyses and genetic methods (cosegregation of the Ura$^+$ and recessive His$^+$ phenotypes in random spore analysis from the diploid SLL4 × SLL7). We have developed a cotransformation procedure allowing direct allele replacements of the *spt2Δ::URA3* allele in SLL7 and fast screening by PCR analysis (45). Briefly, SLL7 sphero-

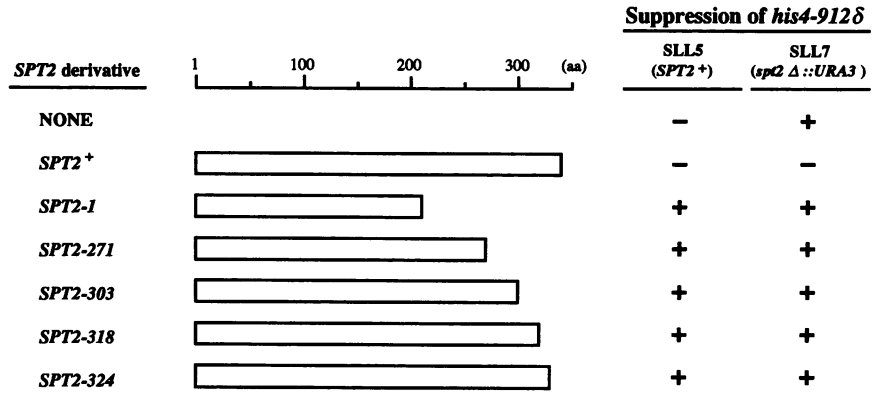

FIG. 2. C-terminal truncations of SPT2 create nonfunctional, dominant polypeptides. Nonsense mutations were introduced in the cloned *SPT2*$^+$ gene to generate alleles encoding truncated proteins of 271, 303, 318, and 324 amino acid (aa) residues. Derivatives of the high-copy-number vector pEMBLYe30 carrying the *LEU2* marker and a *spt2* allele (left) were used to transform the isogenic strains SLL5 (*his4-912*δ *SPT2*$^+$) and SLL7 (*his4-912*δ *spt2*Δ::*URA3*) by selection for leucine prototrophy. Three independent Leu$^+$ transformants were monitored for their ability to grow on SC-Leu-His plates (right). Symbols: +, His$^+$; −, His$^-$ at 30°C. The central panel provides a schematic representation of the expected polypeptide products.

plasts were cotransformed with 1 to 10 μg of digested plasmid DNA carrying the allele of interest and 0.1 to 1 μg of pEMBLYe30 and then plated overnight on SC-Leu plates. The regeneration agar was then transferred on a 5-fluoro-orotic acid–Leu plate, which selects for loss of *URA3* function (8). The transformants were screened by analytical PCR of the *SPT2* locus, and positive integrants were cured of the pEMBLYe30 vector by growth in YPD. Following this procedure, we constructed the isogenic strains SLL9 (*spt2*Δ, from pLL80), SLL101 (*SPT2-324*, from pLL49), and SLL102 (*spt2-324*Δ*3*, from pLL74). All diploid strains presented in Fig. 7 were obtained by mating the appropriate haploids in liquid cultures and selection on SC-Lys-His plates. Strain SLL10 is a meiotic segregant from the diploid SLL4 × SLL7.

Anti-SPT2 antibodies and immunoblotting. We have raised antisera against two synthetic peptides designed from the predicted amino acid sequence of the SPT2 protein: PS1 and PS2, spanning amino acids 209 to 238 and 297 to 325, respectively (Fig. 1B). The peptides were synthesized by using *N*α-*tert*-butyloxycarbonyl-protected amino acids with appropriate side chain protections on an Applied Biosystems 430A synthesizer, using methods described elsewhere (12). The peptides were purified by reverse-phase high-pressure liquid chromatography and coupled to keyhole limpet hemocyanin as described previously (87). The immunization protocol as well as the procedure for the preparation of total yeast extract and their analysis by Western blotting (immunoblotting) were as described previously (49), with the following modifications. All transformants were grown in SC-Leu or SC-Leu-Ura at 30°C. The antisera were assayed for reactivity against the peptides in immunoassays and were immunoaffinity purified on peptide columns (22). For immunoblotting, both antipeptide antisera were combined and used at a 1:800 dilution. Cross-reacting primary antibodies were detected by the enhanced chemiluminescence procedure (Amersham) as described by the manufacturer. The goat anti-rabbit immunoglobulin G-horseradish peroxidase conjugate (Bio-Rad Laboratories) was used at a 1:6,000 dilution. Unless otherwise specified, the blots were exposed 15 to 30 s to Hyperfilm-ECL (Amersham).

Computer analyses. The sequence alignment presented in Fig. 8A is derived from the CLUSTAL program (26), and the statistical significance of the similarities was assessed with the COMPARE program (51). Both programs were from the PCGENE software package (IntelliGenetics Inc.).

RESULTS

C-terminal truncations of the SPT2 protein as dominant suppressors of δ insertional mutations. A yeast strain carrying the *his4-912*δ insertional mutation shows a very weak His$^+$ phenotype at 30°C (61). A complete deletion of the *SPT2*$^+$ gene is a recessive suppressor of the δ promoter insertion, resulting in a His$^+$ phenotype (60, 82). The suppressor phenotype therefore corresponds to a loss of function of the 333-amino-acid SPT2 protein. Several dominant and partially dominant suppressor *spt2* mutations have been characterized genetically, and one such allele, *SPT2-1*, was shown to be caused by a nonsense ochre mutation (TAA) at the Leu-213 codon TTA (59).

To gain a better understanding of the molecular basis of SPT2 function in *S. cerevisiae*, we undertook a mutational analysis of this protein. Isogenic *his4-912*δ *SPT2*$^+$ and *his4-912*δ *spt2*Δ::*URA3* strains (SLL5 and SLL7) were constructed by one-step replacement of the *SPT2*$^+$ gene on chromosome V (see Materials and Methods and Fig. 1A) and were used as reporter strains to monitor the activity of plasmid-borne alleles. That the *spt2*Δ::*URA3* disruption constitutes a null allele deficient in SPT2 protein activity (i.e., an amorphic mutation [50]) is demonstrated by the recessive *his4-912*δ suppressor phenotype of SLL7 and SLL10 (Fig. 2 and 7A) and by the absence of *SPT2*$^+$ product in SLL7 cell extracts (see Fig. 4).

The growth of SLL5 and SLL7 transformants in the absence of histidine provides easily scored dominance and complementation tests for *SPT2* activity, respectively: SLL5 cells transformed with pLL18 (YEp*SPT2-1*) are His$^+$, demonstrating that high-copy-number *SPT2-1* dominates over the chromosomally expressed Spt$^+$ phenotype, and SLL7 cells transformed with pLL10 (YEp*SPT2*$^+$) are His$^-$, confirming the complementation of the *spt2*Δ::*URA3* allele by the cloned *SPT2*$^+$ gene and the recessive phenotype of the chromosomal disruption (Fig. 2). The same results were obtained in all *SPT2*$^+$ and *spt2*$^-$ strains tested, as well as with low-copy-number (YCp) derivatives of pLL10 and

TABLE 3. Dominant C-terminal missense mutations[a]

SPT2 derivative	Protein		Suppression of *his4-912δ*[b]	
	Designation	Sequence[c]	SLL5 (*SPT2*$^+$)	SLL7 (*spt2Δ::URA3*)
None			−	+
SPT2$^+$	wt	EKRRRKKGIR	−	−
SPT2-324	1-324	E	+	+
spt2-334	V334	EKRRRKKGIRV	−	−
SPT2-335	Cassette	EMILLMMGIRV	+	+
spt2-330	K330S	EKRRRKSGIR	−	−
spt2-329	K329S	EKRRRSKGIR	+/−	+/−
spt2-328	R328S	EKRRSKKGIR	−	−
spt2-327	R327S	EKRSRKKGIR	−	−
spt2-326	R326S	EKSRRKKGIR	−	−
spt2-325	K325S	ESRRRKKGIR	+/−	+/−
spt2-336	K325R	ERRRRKKGIR	−	−
SPT2-350	K325S K330S	ESRRRKSGIR	+	+
SPT2-359	K325S K329S	ESRRRSKGIR	+	+

[a] Single and multiple missense mutations were introduced in the wild-type (wt) *SPT2*$^+$ gene and assayed for complementation of *spt2Δ::URA3* and dominance over *SPT2*$^+$ as described in the legend to Fig. 2.

[b] +/−, only partial growth on SC-Leu-His. *spt2-325* (K325S) exhibits a stronger suppressor phenotype than does *spt2-329* (K329S; see text).

[c] Structure of the carboxy terminus from residue E324. Amino acid insertions and substitutions are underlined. The substitutions performed in *SPT2-335* by cassette mutagenesis are K325M, R326I, R327L, R328L, K329M, and K330M, introduced in the V-334 pseudo-wild-type allele *spt2-334*.

pLL18 (see Fig. 7B). No additional phenotype was associated with the increased dosage of the wild-type *SPT2*$^+$ allele.

The predicted translational product of the wild-type *SPT2*$^+$ gene consists of a highly charged protein of 333 amino acids. The truncated polypeptide presumably encoded by the *SPT2-1* mutant allele lacks several distinctive structural motifs present in the C-terminal third of SPT2 protein (Fig. 1B). We first wished to identify elements of this region of the protein, absent in *SPT2-1*, which are necessary for the Spt$^+$ phenotype. Using in vitro mutagenesis, we introduced nonsense mutations in the cloned *SPT2*$^+$ gene to create mutant alleles encoding truncated polypeptides of 271, 303, 318, and 324 amino acids. These mutations were designed to progressively incorporate features of the primary sequence of the C terminus of SPT2 protein missing in the truncated SPT2-1 polypeptide. The mutant alleles were introduced in the isogenic *his4-912δ SPT2*$^+$ and *spt2Δ::URA3* strains, and Leu$^+$ transformants were tested for growth in absence of histidine at 30°C (Fig. 2). None of the nonsense mutations was able to complement *spt2Δ::URA3*, and all of them retained the strong dominant character exhibited by the *SPT2-1* allele when transformed in SLL5. In all cases, Leu$^-$ SLL5 cells which have been cured of the plasmid vector regained the expected His$^-$ phenotype (not shown).

Specific C-terminal basic residues are required for wild-type SPT2 protein function. As seen from the smallest C-terminal deletion (allele *SPT2-324*; Fig. 2), residues at the very end of the SPT2 protein are critical in determining its effects on the phenotype of the δ insertion. Nine amino acid residues of the SPT2 protein are absent form the truncated polypeptide product of the dominant allele *SPT2-324*:Lys-325–Arg–Arg–Arg–Lys–Lys–Gly–Ile–Arg-333. First, the importance of the six consecutive basic residues was demonstrated by a simultaneous replacement of Lys-325 to Lys-330, using a cassette mutagenesis approach in a pseudo-wild-type allele with a phenotypically silent terminal valine insertion (alleles *spt2-334* and *SPT2-335*; Table 3). These multiple substitutions generate a nonfunctional and strongly dominant suppressor allele.

A finer mutational analysis was performed through systematic serine point substitutions at each of the six basic residues. Four of these point mutations could complement the *spt2Δ::URA3* null allele of SLL7 and showed a wild-type Spt$^+$ phenotype (Table 3, alleles *spt2-326* to *spt2-328* and *spt2-330*). The K329S mutant (*spt2-329*) was only partially functional in SLL7 (weak His$^+$ phenotype) and correspondingly was partially dominant when assayed in SLL5. On the other hand, the *spt2-325* allele carrying the K325S substitution showed a stronger mutant phenotype, associated with a more pronounced dominant behavior. The conservative change K325R (allele *spt2-336*) had no apparent effect on SPT2 protein function. The double-point substitutions K325S K330S and K325S K329S showed dominant Spt$^-$ phenotypes indistinguishable from that of the C-terminal deletant *SPT2-324*.

These results demonstrate the importance of specific basic residues in the negative regulation of *his4-912δ* by the SPT2 protein and furthermore establish a correlation between the severity of the effects of C-terminal mutations and their potential to dominate over the Spt$^+$ phenotype.

Amino acids 117 to 179 of the SPT2 protein are required for the dominant suppressor phenotype. The dominant phenotype of mutants at the C terminus of the SPT2 protein suggests that there are at least two regions of the protein required for normal function: (i) the C-terminal basic sequence between residues 325 and 333 and (ii) a positively acting dominance domain which is responsible for the ability of the C-terminal mutants to interfere with the maintenance of the Spt$^+$ phenotype. This domain must lie within the 212-amino-acid sequence of the *SPT2-1* protein. As shown in Fig. 1B, a region of similarity with the DNA-binding helix-turn-helix motif of prokaryotic repressors and homeodomain proteins is present in the N terminus of the SPT2-1 protein (residues 60 to 81 [59]). Since such a functional motif constitutes a potential candidate for the dominance domain, we generated a complete in-frame deletion of these residues (referred to as *Δ1*) in the wild-type *SPT2*$^+$ allele and the dominant suppressor allele *SPT2-1*. The phenotypes of SLL5 and SLL7 transformants (Fig. 3, alleles *spt2Δ1* and *SPT2-1Δ1*), however, disproved this hypothesis and demonstrated that residues 60 to 81 are not required for the

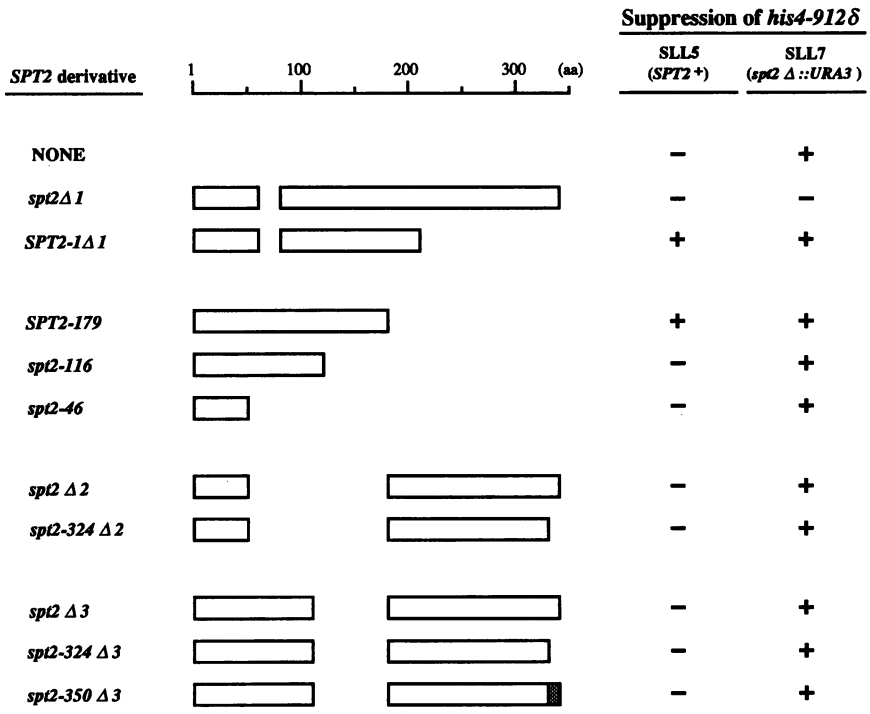

FIG. 3. Residues 117 to 179 of SPT2 are essential for the dominance of truncated products. C-terminal and internal deletion alleles of *SPT2*$^+$ were constructed by in vitro mutagenesis and assayed for complementation and dominance as described for Fig. 2. Symbols: Δ*1*, in-frame deletion of residues 60 to 81; Δ*2*, deletion of residues 48 to 179; Δ*3*, deletion of residues 117 to 179. The double missense mutation of *SPT2-350* (K325S K330S) is represented by the dotted box. aa, amino acids.

dominance of *SPT2-1*, nor are they necessary for the wild-type *SPT2*$^+$ function, at least as far as the Spt phenotype is concerned.

To localize the dominance domain, we produced further truncations of the SPT2 protein and created C-terminal deletion mutants containing 179, 116, and 46 N-terminal amino acids. The 179-amino-acid product still confers a strong dominant suppressor phenotype, but removal of residues 47 to 179 or only 117 to 179 creates recessive *spt2* alleles (Fig. 3, alleles *SPT2-179*, *spt2-116*, and *spt2-46*). We then examined whether a similar loss of dominance could be produced in C-terminal mutants by internal deletions. Our results show that deletions of amino acids 48 to 179 (Δ*2*) and of amino acids 117 to 179 (Δ*3*) have the same effects: they revert the dominance caused by the C-terminal alterations of the SPT2 protein and in themselves create nonfunctional recessive alleles (Fig. 3, alleles *spt2*Δ*2*, *spt2-324*Δ*2*, *spt2*Δ*3*, *spt2-324*Δ*3*, and *spt2-350*Δ*3*). Thus, residues 117 to 179 are necessary for the dominant phenotype of *SPT2* alleles with altered C termini and define a region of the SPT2 protein essential for wild-type function, which we refer to as the dominance domain.

Immunological detection of the SPT2 protein. We have raised antibodies against two synthetic peptides designed from the predicted sequence of the *SPT2*$^+$ gene product (see Materials and Methods and Fig. 1B). The combined immunoaffinity-purified sera cross-react with a protein migrating at an apparent molecular weight of 44,000 in Western blot analysis of total yeast protein extracts (Fig. 4). This protein band is conclusively identified as being SPT2 on the basis of four observations: (i) it is present in total protein extracts from SLL5 (*SPT2*$^+$) but not from SLL7 (*spt2*Δ::*URA3*) (Fig.

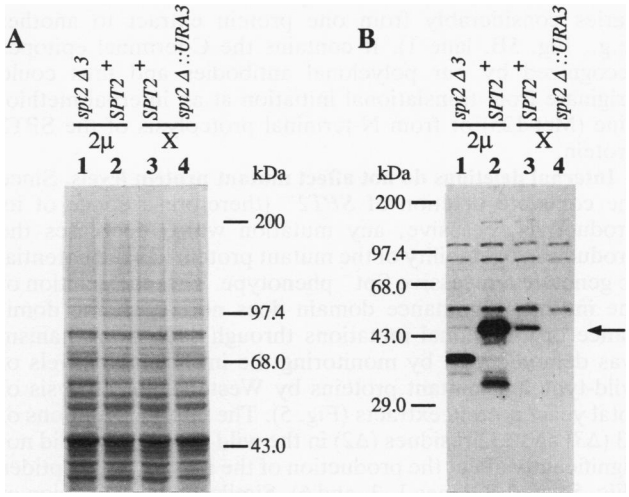

FIG. 4. Immunological detection of the SPT2 protein. Shown are a Coomassie blue-stained gel (A) and Western blot analysis (B) of equal amounts of total protein extracts resolved by electrophoresis on an SDS–10% polyacrylamide gel. Yeast extracts: lane 1, SLL7 (*spt2*Δ::*URA3*)/pLL75 (YEp*spt2*Δ*3*); lane 2, SLL7/pLL10 (YEp-*SPT2*$^+$); lane 3, SLL5 (*SPT2*$^+$); lane 4, SLL7. Designations: 2μ, high-copy-number (YEp) transformants; X, chromosomal expression. For the immunoblot analysis, the electroblotted proteins were reacted with immunoaffinity-purified anti-SPT2 peptide antisera and cross-reacting antibodies were visualized by the enhanced chemiluminescence detection system. The arrow indicates the position of the SPT2 protein.

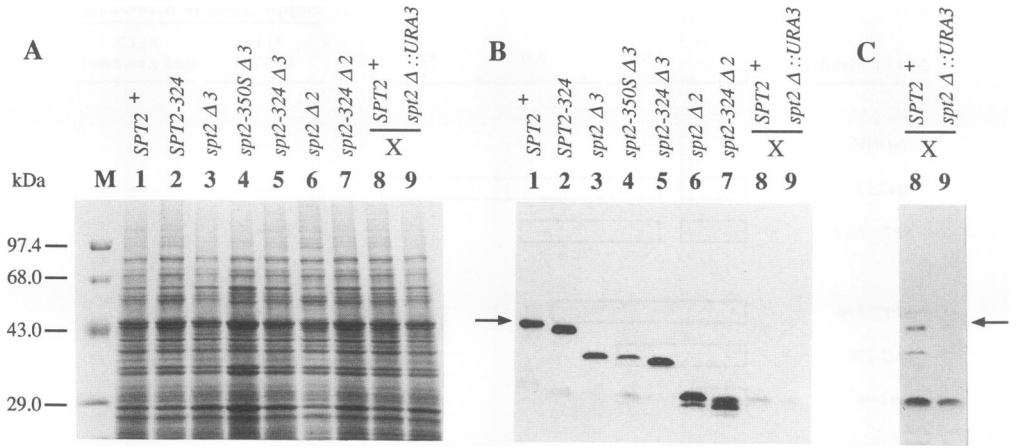

FIG. 5. Production of mutant polypeptides in vivo. (A) Coomassie blue-stained gel; (B and C) Western blot analyses (15-s [B] and 150-s [C] exposure) of total protein extracts, as described for Fig. 4. Yeast extracts: lanes 1 to 7, SLL7 (*spt2*Δ::*URA3*) transformed with the indicated alleles on a high-copy-number vector; lanes 8 and 9, SLL5 (*SPT2*$^+$) and SLL7 respectively (X, chromosomal expression). The arrow indicates the position of the SPT2 protein.

4, lanes 3 and 4); (ii) extracts from SLL7 transformed with the *SPT2*$^+$ high-copy-number plasmid pLL10 (YEp*SPT2*$^+$) reveal an overexpression of the 44-kDa band (Fig. 4, lanes 2); (iii) the migration of the cross-reacting protein is affected by partial deletions of the *SPT2*$^+$ gene (Fig. 4, lanes 1, and Fig. 5); and (iv) the same protein is detected by the antisera raised against the two different peptides when used individually, but not by the preimmune sera (data not shown).

We have noticed an additional cross-reacting band with an apparent molecular weight of 31,000 in YEp*SPT2*$^+$ transformants (Fig. 4B, lane 2). This polypeptide is probably a derivative of SPT2 protein, since its presence seems dependent on overexpression of the protein and its concentration varies considerably from one protein extract to another (e.g., Fig. 5B, lane 1). It contains the C-terminal epitopes recognized by our polyclonal antibodies and thus could originate from translational initiation at an internal methionine (Met-125) or from N-terminal proteolysis of the SPT2 protein.

Internal deletions do not affect mutant protein levels. Since the complete deletion of *SPT2*$^+$ (therefore absence of its product) is recessive, any mutation which decreases the production or stability of the mutant protein has the potential to generate a recessive Spt$^-$ phenotype. That the deletion of the internal dominance domain does not revert the dominance of C-terminal mutations through such a mechanism was demonstrated by monitoring the intracellular levels of wild-type and mutant proteins by Western blot analysis of total yeast protein extracts (Fig. 5). The internal deletions of 63 (Δ*3*) and 132 residues (Δ*2*) in the wild-type protein did not significantly affect the production of the mutant polypeptides (Fig. 5A and B, lanes 1, 3, and 6). Similarly, the reversion of the dominance of the SPT2-324 protein by those deletions is not accompanied by altered protein levels (Fig. 5A and B, lanes 2, 5, and 7). The deletion of residues 117 to 179 (Δ*3*) in the double-point substitution mutant polypeptide SPT2-350 slightly reduced the levels of the product (Fig. 5A and B, lanes 4). However, as for all the other products analyzed here, it is produced in much larger quantities than is the wild-type chromosomal gene product present in SLL5, the strain used for the dominance tests (Fig. 5B and C, lanes 8).

These results clearly show that the reversion of the dominance of *SPT2* alleles by internal deletions cannot be attributed to impaired production or stability of the resulting polypeptides. It therefore can be concluded that the deletion of residues 117 to 179 produces recessive polypeptides which have lost the potential to compete with the maintenance of the Spt$^+$ phenotype, even when overproduced in the wild-type background.

Production of wild-type SPT2 protein in the presence of dominant alleles. A yeast strain carrying a *spt2*Δ null allele and cotransformed with a low-copy-number vector carrying the *SPT2*$^+$ gene and a dominant mutant *SPT2* allele on a high-copy-number plasmid exhibits a His$^+$ phenotype. We have expressed the *SPT2*$^+$ gene from a heterologous promoter, the weak, glucose-repressible *GAL4* promoter, and have observed the same dominance effect in cotransformation experiments (Fig. 6A). The *pGAL4*:*SPT2*$^+$ fusion was subcloned in low- and high-copy-number vectors (YCp and YEp; see Materials and Methods), and three qualitatively different levels of expressions were achieved under repressed (glucose) or derepressed (galactose) conditions. When cotransformed into a *his4-912*δ *spt2*Δ strain along with a control parental vector, only the high-copy-number fusion under derepression produces enough SPT2 protein to fully complement the null allele and generate a His$^-$ phenotype similar to the one observed in cotransformants with a YEp*SPT2*$^+$ vector. At the other extreme, the repressed low-copy-number fusion does not produce enough SPT2 protein to complement *spt2*Δ. The repressed YEp-*pGAL4*:*SPT2*$^+$ and derepressed YCp-*pGAL4*:*SPT2*$^+$ fusions lead to an intermediate His$^+$ phenotype at 30°C, suggesting that in these conditions, partial suppression of the δ insertion is achieved. However, the presence of the dominant allele *SPT2-1* in the cotransformed vector always yields a strong His$^+$ phenotype. We therefore conclude that the dominance occurs even when *SPT2*$^+$ is expressed from an heterologous promoter and that in these experiments, the competence of the high-copy-number *SPT2-1* allele to dominate is not affected by the level of expression of *SPT2*$^+$ (see below). In conjunction with results of our experiments showing that dominant polypeptides interfere with wild-type SPT2 function as antimorphic mutations (see below), these results suggest that the dominance mechanism does not involve a specific transcriptional effect on the *SPT2* promoter.

We also found that the level of the wild-type protein itself

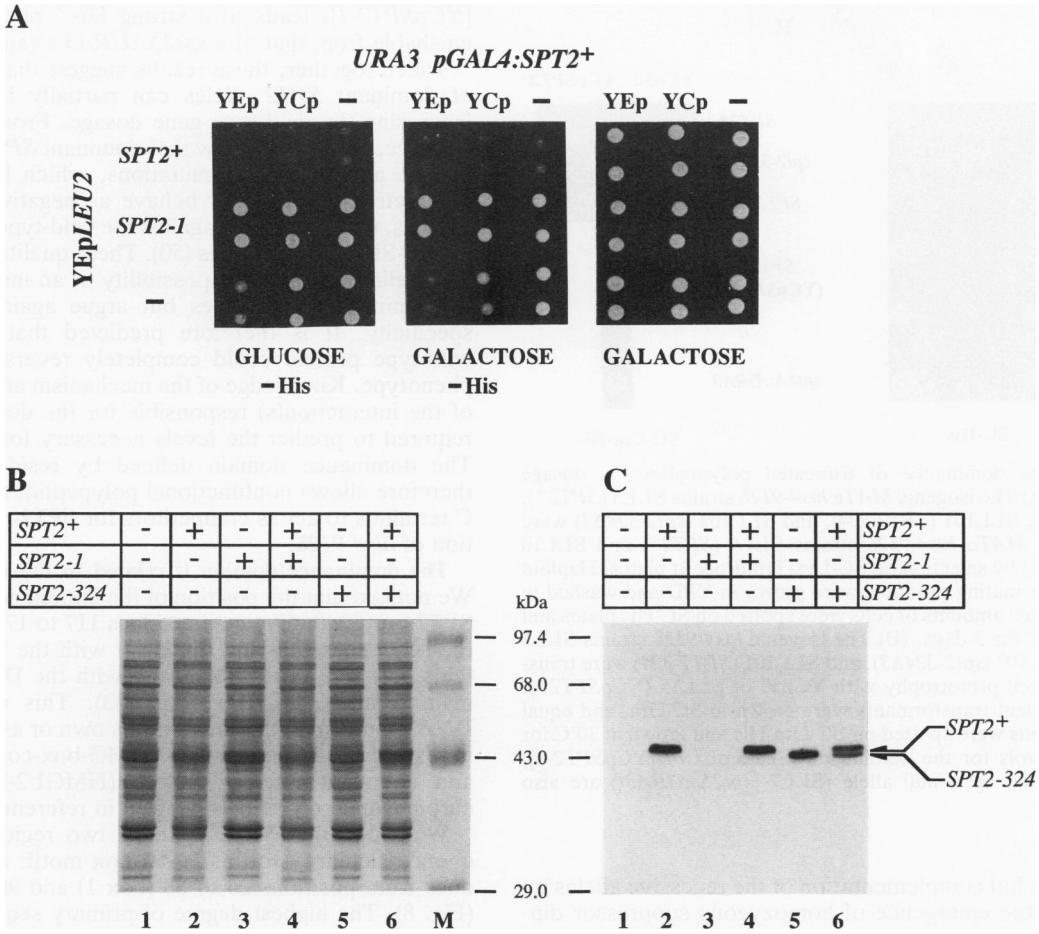

FIG. 6. Truncated polypeptides dominate over a *pGAL4:SPT2*$^+$ fusion and do not alter the production of the wild-type protein. (A) *S. cerevisiae* SLL9 (*his4-912*δ *spt2*Δ) was cotransformed to leucine prototrophy with pLL10 (YEp*SPT2*$^+$), pLL18 (YEp*SPT2-1*), or the parental vector pEMBLYe30 and uracil prototrophy with pLL97 (YEp-*pGAL4:SPT2*$^+$), pLL96 (YCp-*pGAL4:SPT2*$^+$), or pLL95 (YEp-*pGAL4*). Two independent transformants were grown in SC-Ura-Leu dropout medium, and equal amounts of cells were spotted on the indicated media, selecting for both vectors, and grown for 3 days at 30°C. Coomassie blue-stained gel (B) and Western blot analysis (C) of total protein extracts, as described for Fig. 4 but using an SDS–15% polyacrylamide gel. Yeast extracts are from SLL9 cotransformed with pEMBLYe30 (a YEp, *LEU2* vector), pLL18 (YEp*SPT2-1*), or pLL49 (YEp*SPT2-324*) and pVT100-U (a YEp, *URA3* vector) or pLL77 (YEp*SPT2*$^+$). The double arrow points to the wild-type and *SPT2-324* gene products.

is not affected by the presence of a dominant allele (Fig. 6C; compare lane 2 with lanes 4 and 6). These results show that the dominance of the *SPT2-1* or *SPT2-324* allele is not caused by transcriptional or translational inhibition of the wild-type *SPT2*$^+$ gene. This conclusion also was supported by the study of the expression of an in-frame *SPT2:lacZ* fusion in *SPT2*$^+$ and *SPT2-1* backgrounds. When expressed from a high-copy-number vector, the fusion produced 22 to 25 U of β-galactosidase irrespective of the cotransformed allele (not shown). As observed in Fig. 6C, lanes 3 and 4, the dominant negative product of the *SPT2-1* allele is indeed a truncation lacking the C-terminal third of the SPT2 protein, since it does not contain the C-terminal epitopes of the cross-reacting antipeptide sera (see Fig. 1B and Discussion).

The products of dominant alleles interfere with the wild-type SPT2 protein function. Genetic screenings for suppressors of the *his4-912*δ mutation revealed that products of several different *SPT* genes are required to maintain the His$^-$ (Spt$^+$) phenotype. We considered the possibility that dominant proteins mediate their effects by inhibition not of SPT2 protein but of an unrelated *SPT* gene product. Evidence supporting the direct interference of *SPT2*$^+$ function by the products of dominant *SPT2* alleles came from experiments in which the gene dosage of wild-type, null, or dominant alleles was modified.

We constructed a set of four isogenic *MAT***a** *his4-912*δ strains carrying different *spt2* alleles and performed pairwise mating with *MAT*α *his4-912*δ strains with (SLL4) or without (SLL10 *spt2*Δ::*URA3*) the wild-type *SPT2*$^+$ gene (Fig. 7A). The three nonfunctional alleles tested (*spt2*Δ, *spt2-324*Δ*3*, and *SPT2-324*) showed a strong suppressor phenotype both in haploid cells and in combination with the null allele *spt2*Δ::*URA3* in heterozygous diploids. This result suggests that the action of these suppressor alleles is not affected by doubling the dosage of other *SPT* loci. We have also observed that whereas wild-type homozygous diploids show a stable His$^-$ phenotype, heterozygous diploids carrying a noncomplementing recessive allele and a wild-type allele (e.g., *spt2*Δ/*SPT2*$^+$) exhibit a background His$^-$ phenotype with high frequency of His$^+$ reversion. This result is consis-

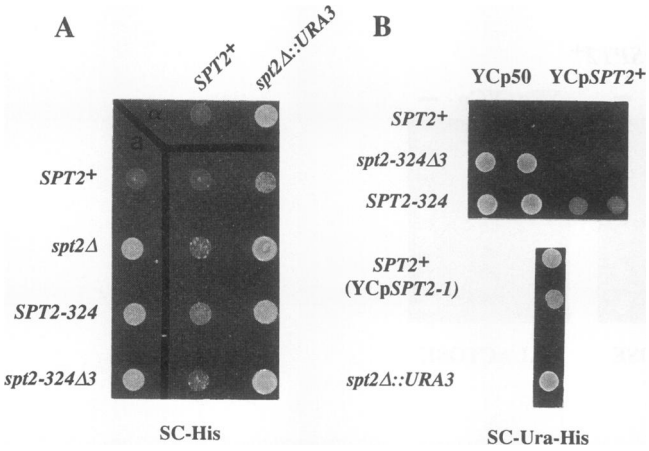

FIG. 7. The dominance of truncated polypeptides is dosage dependent. (A) The isogenic *MAT***a** *his4-912*δ strains SLL5 (*SPT2*$^+$), SLL9 (*spt2*Δ), SLL101 (*SPT2-324*), and SLL102 (*spt2-324Δ3*) were mated to the *MAT*α *his4-912*δ strains SLL4 (*SPT2*$^+$) and SLL10 (*spt2*Δ::*URA3*) by selection on SC-Lys-Trp dropout plates. Haploid cells and their mating products were grown in YPD and washed in water, and equal amounts of cells were spotted on SC-His plates and grown at 30°C for 3 days. (B) The isogenic *his4-912*δ strains SLL5 (*SPT2*$^+$), SLL102 (*spt2-324Δ3*), and SLL101 (*SPT2-324*) were transformed to uracil prototrophy with YCp50 or pLL15 (YCp*SPT2*$^+$). Two independent transformants were grown in SC-Ura, and equal amounts of cells were spotted on SC-Ura-His and grown at 30°C for 3 days. Controls for the dominance (SLL5/pLL24 [YCp*SPT2-1*]) and suppression by a null allele (SLL7 [*spt2*Δ::*URA3*]) are also presented.

tent with the full complementation of the recessive alleles by *SPT2*$^+$ and the emergence of homozygous suppressor diploids by mitotic recombination (e.g., *spt2*Δ/*SPT2*$^+$ becomes *spt2*Δ/*spt2*Δ). The *SPT2-324*/*SPT2*$^+$ diploids clearly showed a stronger His$^+$ phenotype indicative of a semidominant phenotype for the *SPT2-324* allele in diploids. This dominance is abolished by deletion of residues 117 to 179 (*spt2-324Δ3*). Although the background His$^-$ phenotype of *SPT2*$^+$/*spt2* diploids is sometimes masked by the high density of His$^+$ revertants (notably in the case of *SPT2*$^+$/*spt2*Δ::*URA3* diploids; Fig. 7A), spreading of those cells or spotting at lower cell density confirmed the phenotypic distinction between recessive and dominant alleles in heterozygous diploids with the wild-type allele (not shown). The partial dominance of several *spt2* alleles in diploids has previously been observed and in fact was exploited to assign these alleles to the *spt2* complementation group (82).

The phenotypic differences between the heterozygous diploids *SPT2-324*/*SPT2*$^+$ and *SPT2-324*/*spt2*Δ::*URA3* suggest that the extent of suppression is sensitive to the relative levels of dominant and wild-type products and that the two polypeptides are in direct competition in heterozygotes. This conclusion is also supported by experiments in which the gene dosage of the wild-type allele alone was increased by transformation of isogenic strains with a YCp*SPT2*$^+$ construct (Fig. 7B). Increasing the level of the SPT2 protein has no effect on repression of the *his4-912*δ mutation in a *SPT2*$^+$ strain but is sufficient to complement the recessive suppressor mutation *spt2-324Δ3*. When the dominance domain is present, as in the truncated product of *SPT2-324*, the complementation of the Spt$^-$ phenotype by YCp*SPT2*$^+$ is only partial. On the other hand, increasing the dosage of the dominant allele *SPT2-1* in a wild-type background (*SPT2*$^+$ [YCp*SPT2-1*]) leads to a strong His$^+$ phenotype indistinguishable from that of a *spt2*Δ::*URA3* strain (Fig. 7B).

Taken together, these results suggest that the dominance of dominant *SPT2* alleles can partially be abolished by increasing the wild-type gene dosage. From a genetic perspective, our results show that dominant *SPT2* alleles do not behave as neomorphic mutations, which have acquired a new activity, but rather behave as negatively acting antimorphs, which compete against the wild-type protein and act as anti-SPT2 polypeptides (50). These qualitative data do not allow elimination of the possibility of an increase in affinity in dominant polypeptides but argue against a change in specificity. It is therefore predicted that high levels of wild-type protein could completely reverse the dominant phenotype. Knowledge of the mechanism and stoichiometry of the interaction(s) responsible for the dominance will be required to predict the levels necessary for this reversion. The dominance domain defined by residues 117 to 179 therefore allows nonfunctional polypeptides with an altered C terminus to act as competitors for SPT2-mediated regulation of *his4-912*δ.

The dominance domain is related to the HMG-box motif. We noticed that the position of the dominance domain in the SPT2 protein (amino acid residues 117 to 179) overlaps with the sequences showing similarity with the HMG1-like proteins (42) and more specifically with the DNA-binding domain termed the HMG box (33). This motif defines a DNA-binding domain found on its own or as a repeated unit in several regulatory proteins (HMG-box-containing factors) and abundant nuclear factors (HMG1/2-like nonhistone chromosomal proteins [reviewed in reference 52]).

We found that SPT2 contains two regions showing sequence similarity to this HMG-box motif: amino acid residues from positions 26 to 88 (box 1) and 98 to 159 (box 2) (Fig. 8). The highest degree of primary sequence similarity was found with members of the HMG1/2-like protein family (52), although the weak similarity between SPT2 and hUBF has previously been reported (33). We used the Monte Carlo feature of the COMPARE program (51) to assess the statistical significance of the sequence similarities between the putative HMG boxes of SPT2 protein and a group of 15 HMG boxes previously identified (52). Several pairwise alignments were shown to be statistically significant, with actual alignment scores ranging from 3.8 to 4.3 standard deviations (SD) away from the mean score of 150 randomizations. The most significant similarities were seen between SPT2 box 1 and the C-terminal box of HMG-1, box 2 (alignment score of 3.8 SD, with 32% sequence similarity) as well as SPT2 box 2 and the N-terminal box of HMG1, box 1 (3.9 SD, 34% similarity). We also note that at least 50% of the highly conserved residues found in HMG1/2-like proteins are present in each of the two putative HMG boxes of SPT2 (52). Although the alignment presented in Fig. 8 shows that the HMG box 2 of SPT2 covers positions 98 to 159, the similarity between this region of SPT2 and HMG-box-containing transcription factors involved in sex determination extends to amino acid 170 (Fig. 9) (52). The motif PXYK (where X represents any amino acid) is conserved between SPT2 (position 160) and most of these proteins, and the HMG box of the mating-type protein Mc of *Schizosaccharomyces pombe* is 73% similar to SPT2 in this region (8 of 11 conserved residues [38]). It is clear that the HMG boxes of SPT2 are more divergent than in other members of this family, but the structural basis for the DNA-binding activity of this protein domain is still unknown. The fact that the binding characteristics of previously studied HMG-box-

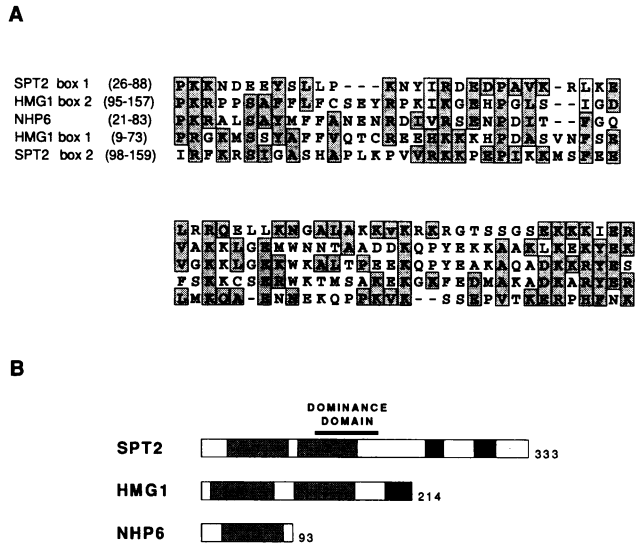

FIG. 8. SPT2 contains two regions of sequence similarity with the HMG-box motif. (A) Alignment between the amino acid sequences of the HMG-box motifs of human HMG1 nonhistone chromosomal protein (80) and of the product of the *S. cerevisiae* NHP6A gene (40) and two regions of the SPT2 protein. Numbers in parentheses give the amino acid positions of the sequences in the respective proteins. Identical or conserved amino acids between SPT2 and the HMG boxes are shaded. Conservative amino acid substitutions are as follows: H = K = R; F = Y; D = E; A = I = L = V; S = T. In SPT2 box 1, v = SGV. This alignment is based on the best match obtained for those six sequences by using the COMPARE program of Needleman and Wunsch (51) from the PCGENE software package (IntelliGenetics Inc.). (B) Schematic representation of the three proteins aligned in panel A. HMG boxes of HMG1, NHP6, and proposed homologs in SPT2 are shaded and numbered as defined for panel A. Hyperacidic sequences are highlighted by black boxes. Also shown are the total number of amino acids in each protein as well as the location of the dominance domain of SPT2 mapped by our mutational analysis.

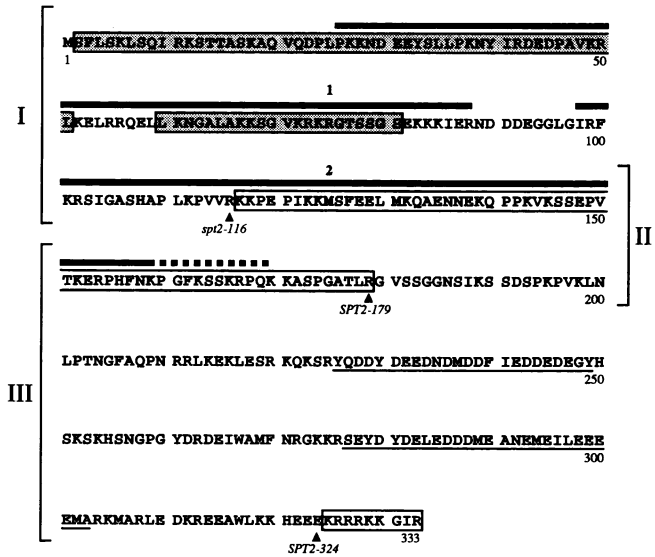

FIG. 9. Primary structure of the SPT2 protein. The predicted amino acid sequence of the *SPT2*$^+$ gene product is divided in three functional regions: region I, residues 1 to 116; region II, residues 117 to 179; and region III, residues 180 to 333. Shaded boxes highlight two regions dispensable for SPT2 activity, and open boxes identify two functional determinants mapped by our analysis: the dominance domain (amino acids 117 to 179) and C-terminal residues (amino acids 324 to 333). Thick lines cover the two HMG-box-like motifs as presented in Fig. 8, and the C-terminal extension of HMG box 2 is shown by the broken line (see text). Thin lines underline the two acidic sequences of region III. Arrows point to the carboxy-terminal residues of three nonsense mutations studied here: the recessive allele *spt2-116* and the dominant alleles *SPT2-179* and *SPT2-324*.

containing proteins range from sequence specific (73, 75) to nonspecific and even structure specific (5, 18) suggests that subtle structural differences could dictate the recognition properties of this domain. In view of our mutational analysis of SPT2, this sequence similarity raises the possibility that the role of amino acids 117 to 179 (dominance domain; Fig. 8B) in the transcriptional repression exerted by the SPT2 protein as well as in the dominance of truncated polypeptides could be mediated by protein-DNA interactions through an intact HMG box 2.

DISCUSSION

Suppression of transcriptional deficiencies by *spt2* mutations. Mutations in the *SPT2* gene of *S. cerevisiae* (also known as *SIN1* [42]) have been associated with the suppression of three types of mutations leading to transcriptional deficiencies: Ty and δ insertional mutations (Spt phenotype), *swi*$^-$*/snf*$^-$ mutations (Sin phenotype), and partial deletions in the C-terminal domain of the largest subunit of RNA polymerase II (Srb phenotype). In each of these systems, discussed in references 6, 81, and 55, respectively, the primary *cis*- or *trans*-acting mutation interferes with the normal transcription of one or several yeast genes and decreases the level of transcriptional initiation from the normal start site(s). Loss-of-function mutations in the *SPT2/SIN1* gene partially suppress these effects, and in each case the reversion phenotype correlates with an increase in the level of transcription at the affected locus or loci (42, 58, 70).

How the SPT2 protein interacts with the transcriptional machinery to repress transcription and what the function of the wild-type protein may be are still unknown, but indirect evidence suggest that SPT2 mediates its effects through alterations in chromatin structure. The *SPT2*$^+$ gene encodes a highly charged protein of 36 kDa (59) with significant sequence similarity with the mammalian HMG1 proteins over more than 50% of its residues (33% similarity [42]). These HMG proteins constitute an important class of nonhistone chromosomal proteins of eukaryotes (reviewed in references 35 and 77). Although their cellular function remains unknown, they have been implicated in nuclear processes such as transcription (71, 76), DNA replication (32), and chromatin assembly (9). Consistent with a role in chromatin structure, it was shown that the SPT2/SIN1 protein is concentrated in the nucleus and that an increase in chromosome instability is observed in *sin1* mutants (42). A similar phenotype has been observed in yeast cells overexpressing the histone H2A and H2B genes (*HTA1-HTB1* locus) or the histone H3 and H4 genes (*HHT1-HHF1* locus [48]). Such alterations in histone gene dosage also affect transcription and can suppress δ insertions and *swi2/snf2* mutations (11, 28). Mutations in the *HTA1* and *HTB1* histone genes were in fact isolated as suppressors of δ insertion mutations (*spt11* and *spt12* [11, 17]), and mutations in one of the histone H3 genes, *HHT1*, were isolated as suppressors of *swi1* mutations (*sin2* [41 and 72]). Several regulatory muta-

tions affecting histone gene expression in *trans*, such as *hcp1/hir3*, *hcp2*, *hir1*, *hir2/spt1*, *spt10*, and *spt21*, were also shown to exhibit a Spt$^-$ phenotype (68, 85). These striking parallels between the phenotypes of *spt2* and histone mutations suggest that the SPT2 protein is part of or regulates a repressor function which mediate its effects through changes in chromatin structure. Mutations in *SPT2/SIN1* do not appear to act indirectly via alterations of histone gene expression (68, 85).

The SPT2 protein of *S. cerevisiae*. That the functional product encoded by the *SPT2*$^+$ gene is a protein was strongly suggested by the finding that the dominant *SPT2-1* mutation is a single-base-pair substitution which introduces a stop codon at residue 213 of a predicted 333-amino-acid protein, presumably resulting in an interfering truncated polypeptide (59). We have raised antisera against two synthetic peptides corresponding to nonoverlapping segments of the C-terminal third of the predicted translational product of *SPT2*$^+$, and these were shown to cross-react with a 44-kDa protein produced by the locus. The predicted molecular weight of the 333-amino-acid SPT2 protein is 36,000. One possible explanation for the discrepancy in molecular weight is that there is read-through of the 56 in-frame codons which follow the stop codon at position 334 of the *SPT2* gene product (59). However, we show here that the introduction of a nonsense mutation at position 325 of the *SPT2*$^+$ coding region in vitro produces a mobility shift in the resulting mutant polypeptide, which suggests that the translation of the 44-kDa protein terminates at codon 334 (Fig. 5B and 6C). Hence, the discrepancy between the apparent molecular weight of the SPT2 protein on SDS-polyacrylamide gels and the molecular weight calculated from the primary sequence of the *SPT2*$^+$ gene is probably a consequence of posttranslational modifications of the nuclear protein or of its high content of charged residues (79). Several proteins which, like SPT2, show a high net positive charge or contain polyacidic sequences have been reported to exhibit abnormal electrophoretic mobilities on sodium dodecyl sulfate (SDS)-polyacrylamide gels (39, 56).

Recessive and dominant suppressors. Genetic analyses of *spt2* suppressor mutations showed that the suppressor phenotype could be recessive or dominant to the wild-type function (70, 82). The observation that a *spt2* null allele confers a recessive suppressor phenotype implies that suppression is caused by the loss of SPT2 activity and that the wild-type SPT2 protein plays an essential role in the transcriptional interference mediated by the δ insertional mutation. A dominant *SPT2* suppressor allele can be described as being able to interfere with the maintenance of the Spt$^+$ phenotype. We find that this dominant phenotype is not associated with a loss of the wild-type SPT2 protein. We furthermore show that increasing the gene dosage of *SPT2*$^+$, but not the dosage of any other *SPT* genes in diploids, attenuates the suppressor phenotype of strains carrying a dominant allele. These results are consistent with a molecular mechanism of dominance based on a direct interference of SPT2 protein function by the dominant polypeptides and confirm that dominant *SPT2* alleles act as true antimorphs (50) or dominant negative alleles (24).

The existence of two different Spt2$^-$ suppressor phenotypes, recessive and dominant, allowed us to address two related questions regarding the transcriptional effects mediated by SPT2. (i) What are the structural elements, missing from the truncated product of the dominant *SPT2-1* allele, which are necessary for the maintenance of the Spt$^+$ phenotype? (ii) What is the structural basis for the dominance of *SPT2* suppressor mutants? Our mutational analysis of the *SPT2*$^+$ gene has identified the two regions of its product containing these structural elements (a carboxy-terminal polar domain and an internal dominance domain) and defined three functionally distinct regions of the protein (Fig. 9).

Region I: dispensable N terminus. The *spt2-116* allele which codes for the first 116 amino acids of the SPT2 protein behaves as a recessive suppressor allele. This result shows that region I of the SPT2 protein, consisting of residues 1 to 116, does not contain structural determinants sufficient for repression or for competition with the wild-type protein. That a substantial part of this region (58%) is furthermore dispensable for SPT2 activity is shown by our observation that the deletion of residues 60 to 81 is silent in both wild-type and dominant products, as well as by previous studies demonstrating that residues 2 to 51 are not required for SIN1/SPT2 activity (42).

Region II: dominance domain. The finding that the dominant *SPT2-1* allele is caused by the introduction of a nonsense mutation at amino acid position 213 and that this allele is transcribed at nearly wild-type levels (59) suggests that the expression of a truncated SPT2 protein interfering with the wild-type SPT2 protein function constitutes the basis for the dominant phenotype. We present three observations confirming that a C-terminal truncation of SPT2 is in itself sufficient for the dominant mutant phenotype: (i) the SPT2-1 polypeptide is not detected by our antisera and therefore does not contain the C-terminal epitopes covered by our peptides (Fig. 6C); (ii) introduction of nonsense mutations at positions 180, 272, 304, 319, and 325 of the wild-type SPT2 protein in vitro produces nonfunctional dominant alleles (Fig. 2 and 3); and (iii) the dominant allele *SPT2-324*, produced by the introduction of a stop codon at position 325, encodes a mutant protein which is detected by our antipeptide antisera and migrates with a slightly higher mobility than does the wild-type protein in SDS-polyacrylamide gel electrophoresis (Fig. 6C).

We show here that the ability of the products of dominant *SPT2* suppressor alleles to compete with the wild-type SPT2 protein and to interfere with the negative regulation of the δ insertion requires a functional domain lost by the deletion of residues 117 to 179. We refer to this region of SPT2 protein as the dominance domain (region II; Fig. 9). Our data do not, however, define the N-terminal boundary of the smallest dominant polypeptide, and we cannot reject the possibility that part of region I is also involved in the structure of a competing polypeptide. The products of recessive alleles carrying a deletion of the dominance domain, although present at high levels in vivo, have lost the ability to interfere with the wild-type function. The observation that the dominance domain overlaps with a HMG-box-like DNA-binding motif provides a model for the molecular basis of the dominance (see below).

Region III: polar C terminus. The *SPT2-179* allele, which codes for the first 179 amino acids of the SPT2 protein, behaves as a dominant suppressor allele. Since the suppression of δ insertional mutations is a consequence of the loss of SPT2 protein function, amino acids 180 to 333 of SPT2 protein (region III; Fig. 9) must contain at least one functional domain which, in conjunction with the dominance domain, is essential for SPT2 activity. The observation that the *SPT2-324* allele, which codes for the first 324 amino acids of the SPT2 protein, also exhibits a dominant suppressor phenotype allowed us to identify such a functional domain in the C terminus of region III; amino acids 325 to 333 and, among these, specific basic residues (Lys-325 and Lys-329)

were shown to be required for SPT2 protein function. At present we have not mapped the N-terminal boundary of this domain, but several features of the C-terminal third of region III suggest that residues 325 to 333 are part of a larger functional structure, mutations in which could also yield dominant suppressor alleles. The last 56 amino acids of SPT2 protein consist of a long polar tail of predicted α-helical structure when analyzed by the secondary structure prediction method of Garnier et al. (19). Composed of 66.5% charged residues, they also contain an acidic subdomain (residues 277 to 303; net negative charge of 15) similar to the one found in several HMG-like proteins (human HMG1 [80], xUBF [47], and hUBF [33]) and previously shown to promote contacts between HMG1 and histone H2A-H2B dimers in vitro (4). A second acidic region (residues 226 to 249; net negative charge of 15) is also present in region III of SPT2 (Fig. 9). It is possible that the mutations studied here act indirectly by affecting the structure of these adjacent acidic domains, but the fact that point mutations at specific residues can mimic the effect of a deletion of residues 325 to 333 suggests that these amino acids may also participate in specific intra- or intermolecular interactions critical for SPT2 protein function. That this positively charged C terminus does not simply act as a nuclear localization signal for SPT2 is supported by the observation that the product of a *SIN1:lacZ* fusion lacking the last 17 amino acids of SIN1/SPT2 is, like the wild-type protein, concentrated in the yeast nucleus (42).

Within the C terminus of the SPT2 protein, we have mutated six consecutive basic residues to serine and found that mutants K325S and K329S exhibit partial loss of SPT2 repression of *his4-912δ*. These results established that point substitutions in the carboxy-terminal region of SPT2 can mimic the effects of carboxy-terminal deletions removing 9 (*SPT2-324* allele) to 154 (*SPT-179* allele) amino acids and suggested that it is not the net positive charge of this domain but rather the presence of positively charged residues at specific positions which is critical for SPT2 function. Thus, mutation K325R conserves the positive charge at position 325 of SPT2 and is phenotypically silent. This strict requirement for positively charged residues at specific terminal positions in repression is reminiscent of the role of N-terminal amino acids of histone H4 in the repression of the silent mating loci *HMLα* and *HMRa* (36, 57). Mutational analysis of histone H4 has shown that, as in the case of SPT2 protein, a small terminal deletion, in this case of amino acids 4 to 19, results in loss of repression and expression of the normally silent mating loci (57). Within this region, four of eight positively charged residues, including Lys-16 and His-18, which are known to undergo reversible modification (acetylation and phosphorylation, respectively), are required for repression. As shown for position 325 of SPT2, elimination of the positive charge at position 16 of histone H4 causes derepression: K16G but not K16R leads to expression of *HMLα* and *HMRa*. Acetylation of K16 would neutralize its positive charge, and this residue was therefore proposed to be deacetylated at *HMLα* and *HMRa* (36). Similarly, the posttranslational modification of K325 and/or K329 of SPT2 could regulate the repressor activity of SPT2 by neutralization of those critical positive charges. Such a mechanism of derepression could play a role in the activation of transcription brought about by SPT2/SIN1 antagonists such as SWI1, SWI2/SNF2, and SWI3 (58).

DNA binding and the mechanism of dominance. The implication of a putative HMG-box DNA-binding domain in the dominance of polypeptides encoded by dominant *SPT2* alleles raises the possibility that the competition between wild-type and dominant products could occur at the level of DNA binding. One possible model consistent with our analysis would imply that wild-type and dominant negative products are both able to bind DNA through the HMG-box motifs (alone or in combination with other specific factors) and that an intact C-terminal domain is necessary to promote the additional interactions mediating the transcriptional negative regulatory effects of SPT2 protein. Since it has previously been shown that a bacterially expressed TrpE:SIN1 fusion binds DNA nonspecifically in vitro (42), it is conceivable that additional nuclear proteins interact with SPT2 protein and restrict its activity by directing the repressor protein to its target genes. The product of a dominant negative allele may also retain the ability to interact with other component(s) of a repressor complex once bound to DNA and disrupt their activity because of a nonfunctional polar tail. This could provide a possible explanation for the stronger Sin^- phenotype of particular dominant negative *SIN1/SPT2* alleles (42) compared with the phenotype of a null deletion allele. According to this model, the HMG boxes of SPT2 protein would not be functionally redundant and HMG box 2, which overlaps with the dominance domain, would be required for high-affinity binding (Fig. 9). Studies on the transcriptional activator hUBF, which contains at least four HMG boxes, have shown that these domains may play different roles not only in DNA binding but also in transcriptional activation (34). This proposed molecular mechanism of dominance therefore bears some resemblance with the mode of action of noninducible LexA and λ repressor proteins (46): both wild-type and mutant repressors can bind to the operator sites, and the dominant mutant phenotype requires an intact DNA-binding domain. An important distinction between the action of these prokaryotic repressors and our model of SPT2 protein negative regulation is that the products of dominant *SPT2* alleles are not functional for repression. Association with other proteins of the transcriptional machinery or chromatin components may be essential for this activity, relieved in presence of activators and an intact RNA polymerase II C-terminal domain (58). Further analysis of the two functional domains of SPT2 protein mapped here and the identification of second-site reversion mutations could clarify the molecular basis of this dual requirement and point to potential partners mediating the transcriptional repression.

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