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Isolation and Characterization of the *SPT2* Gene, a Negative Regulator of Ty-Controlled Yeast Gene Expression

G. SHIRLEEN ROEDER,[1]* CAROLINE BEARD,[2] MICHAEL SMITH,[2] AND SIRKKA KERANEN[3]

Department of Biology, Yale University, New Haven, Connecticut 06511[1]; Department of Biochemistry, University of British Columbia, Vancouver, British Columbia, Canada[2]; and Recombinant DNA Laboratory, University of Helsinki, 00380 Helsinki 38, Finland[3]

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The *his4-917* mutation of *Saccharomyces cerevisiae* results from the insertion of the Ty element Ty917 into the regulatory region of the *HIS4* gene and renders the cell His$^-$. The *his4-912*δ mutant, which carries a solo δ in the 5′-noncoding region of *HIS4*, is His$^+$ at 37°C but His$^-$ at 23°C. Both these mutations interfere with *HIS4* expression at the transcriptional level. The His$^-$ phenotype of both insertion mutations is suppressed by mutations at the *SPT2* locus. The product of the wild-type *SPT2* gene apparently represses *HIS4* transcription in these mutant strains; this repression is relieved when the *SPT2* gene is destroyed by mutation. The repression of transcription by *SPT2* presumably results from an interaction between the *SPT2*$^+$ gene product and Ty or δ sequences. In this paper, we report the cloning and DNA sequence analysis of the wild-type *SPT2* gene and show that the gene is capable of encoding a protein of 333 amino acids in length. In addition, we show that a dominant mutation of the *SPT2* gene results from the generation of an ochre codon which is presumed to lead to a shortened *SPT2* gene product.

Mutations resulting from the insertion of transposable elements have been detected in a variety of eucaryotic organisms. In many cases, the mutant phenotypes caused by these insertions can be suppressed by recessive mutations at unlinked sites in the genome. Thus, for example, mutations resulting from insertion of the *Drosophila* element gypsy can be suppressed by mutations at a locus referred to as suppressor of hairy wing (31). In mice, a gene known as dilute suppressor suppresses the coat color mutation dilute, which results from the insertion of an endogenous retrovirus (9, 25, 50). In maize, a number of controlling elements, including *Ac*, *Spm*, and *Dt*, have a variety of effects on insertion mutations; these include both suppression and destabilization (15). Suppression of these eucaryotic insertion mutations does not appear to be caused by an increase in the rate of excision of the transposable element. Rather, the alteration in phenotype is, at least in some cases, associated with a change in transcription of the transposable element or the affected gene, or both (24).

In *Saccharomyces cerevisiae*, mutations resulting from the insertion of the transposable element Ty (transposon yeast) have been detected at the *HIS4* locus. The *his4-912* and *his4-917* mutations result from the insertion of two different Ty elements, known as Ty912 and Ty917, respectively, into the regulatory region at the 5′ end of the *HIS4* gene. Both these mutations prevent transcription of *HIS4*, thus rendering the cells histidine requiring (8, 14, 37, 38). His$^+$ revertants of these insertion mutations occur by several different mechanisms, including excision of the transposable element (14, 38), chromosomal rearrangements (8, 38), and gene conversion events (39) in which the transposable element at *HIS4* is replaced by a transposable element from elsewhere in the yeast genome. Furthermore, Winston et al. (55) have shown that mutations in any one of seven *trans*-acting genes unlinked to *HIS4* can lead to suppression of the His$^-$ phenotype of Ty insertions at *HIS4*. In addition to their suppressor effects, some of the *spt* (suppressor of Ty) mutations show defects in a variety of cellular functions, including mating, DNA repair, and growth rate (55).

The *spt* mutants described by Winston et al. (55) were selected for suppression of *his4-912*δ, a derivative of the *his4-912* mutation. This derivative is the result of excision of Ty912 by recombination between the directly repeated δ sequences present at the ends of the element; excision leaves behind a solo Ty912 δ in the *HIS4* regulatory region (14). Strains which carry the solo Ty912 δ at *HIS4* are phenotypically His$^+$ at 37°C, weakly His$^+$ at 30°C, and His$^-$ at 23°C (37, 55). In contrast, strains which carry the *his4-912*δ mutation and a recessive mutation in any one of the seven *SPT* genes are His$^+$ at all three temperatures (37, 55).

The subject of this paper is the *SPT* gene referred to by Winston et al. (55) as *SPT2* and previously known as *SPM2* (37). This gene has been genetically mapped to the right arm of chromosome V within 0.25 centimorgan of *RAD4* (55). Mutations at *SPT2* lead to suppression of the His$^-$ phenotype of *his4-912*δ strains grown at 23°C, and they also suppress the His$^-$ phenotype of the *his4-917* mutant (37, 55). The *SPT2* gene is unusual among the *SPT* genes in that both dominant and recessive *his4-912*δ- and *his4-917*-suppressing alleles have been isolated (55). In addition, a recessive deletion mutation which confers suppressor activity has been detected at the *SPT2* locus (55). The product of the wild-type *SPT2* gene, like the products of the other *SPT* genes, is presumed to repress the expression of *HIS4* in the *his4-912*δ and *his4-917* mutant strains. This repression of transcription by *SPT2*$^+$ is apparently specific to Ty- or δ-adjacent genes, because *his4* promoter mutations resulting from the insertion of non-Ty sequences are not suppressed by mutations at *SPT2* (55). Furthermore, mutations at *SPT2* have been shown to suppress Ty and δ insertions at the *LYS2* locus (47). The repression of transcription by *SPT2*$^+$ could occur at the level of a direct interaction between the *SPT2*$^+$ gene product and Ty or δ sequences. Alternatively, *SPT2*$^+$ could act indirectly by controlling the production or activity of a positive regulator of Ty-adjacent gene expression.

In this paper, we describe the cloning and DNA sequence

* Corresponding author.

analysis of the wild-type *SPT2* gene. We show that this gene is capable of encoding a protein of 333 amino acids in length. In addition, we show that a dominant mutation of the *SPT2* gene results from the generation of an ochre codon which presumably leads to a shortened *SPT2* gene product. The nature of the *SPT2* gene product and its possible mechanisms of action are discussed.

MATERIALS AND METHODS

Genetic analysis. Methods of tetrad analysis and media used were as described by Sherman et al. (45).

Cloning of the *SPT2-1* mutant gene. YRp10 plasmid DNA (40 μg) was digested to completion with *Bam*HI. (YRp10 carries the *Eco*RI-*Hin*dIII fragment of *ARS1* inserted between the *Eco*RI and *Hin*dIII sites of pBR322 and the *Hin*dIII fragment of *URA3* inserted at the *Hin*dIII site [5, 6].) DC115 (*his4-Δ29 SPT2-1 ino4-8 HOL1-1*) yeast DNA (200 μg) was partially digested with *Bgl*II to yield fragments of an average size of 10,000 base pairs (bp). The YRp10 and yeast DNA digests were then ligated in a 1-ml volume. The entire ligation was used to transform 10 ml of spheroplasts of yeast strain SR26-12C (*his4-912δ ura3-52 leu2-3-112 SPT2*$^+$). Twenty His$^+$ Ura$^+$ transformants were obtained. In a parallel control experiment selecting for Ura$^+$ transformants, 20,000 transformants were obtained per μg of YRp10 plasmid DNA.

A transformant (SR121-1B) carrying YRp10 and the *SPT2-1* gene integrated at the *SPT2* locus was used to clone the *SPT2-1* gene by excision as described previously (37, 38). SR121-1B DNA (25 μg) was cut to completion with *Bam*HI, and the resulting restriction fragments was circularized by ligation in a 3-ml volume. The ligated DNA was then ethanol precipitated and suspended in 600 μl of TE (10 mM Tris hydrochloride [pH 7], 1 mM EDTA). The DNA solution was used to transform 12 ml of frozen competent *Escherichia coli* HB101 cells, and 24 ampicillin-resistant transformants were obtained.

Cloning of the wild-type *SPT2* gene. Total yeast DNA from a wild-type yeast strain was digested with *Pst*I, and the resulting restriction fragments were separated by electrophoresis on an agarose gel. Fragments in the 2,800-bp size range were eluted from the gel and ligated into the *Pst*I site of pBR322. The ligated DNA was then used to transform *E. coli* to tetracycline resistance. Thirteen percent of the transformants obtained carried *Pst*I inserts as indicated by sensitivity to ampicillin. The ampicillin-sensitive colonies obtained were screened by colony hybridization (21) by using the 2,700-bp *Hin*dIII-*Bgl*II fragment of the cloned *SPT2-1* gene (see Fig. 6d) as a probe. After screening 123 transformants, we found 5 transformants which carried a *Pst*I fragment of the expected size and restriction map.

Elution of fragments from agarose gels. Restriction fragments of DNA were eluted from agarose gels by the protocol of Dretzen et al. (11) with the following modifications. DNA was electrophoresed onto a NA-45 DEAE-cellulose filter from Schleicher & Schuell, Inc. DNA was eluted from the filter by incubation at 60°C in 1.0 M NaCl–0.1 mM EDTA–20 mM Tris hydrochloride (pH 8.0).

Yeast transformations. Yeast transformations were done by the spheroplast method of Hinnen et al. (23). In transformations with YRp10 or its *SPT2*-containing derivatives, 5 μg of plasmid DNA was used to transform 0.2 ml of spheroplasts. Approximately 500 transformants were obtained per transformation.

Co-transformation experiments. To localize the *SPT2-1* mutation within the cloned *SPT2* gene, we used a cotransformation procedure. Restriction fragments of the cloned *SPT2-1* DNA were cloned into pBR322. Before their introduction into yeast cells, the plasmid DNAs were cleaved with restriction enzymes to cut at one or both junctions between the vector DNA and the inserted yeast DNA sequences. Thus, the *SPT2-1* sequences were introduced into yeast cells as linear fragments capable of undergoing substitutive recombination events with yeast genomic DNA (34).

In each cotransformation, 50 μg of cleaved *SPT2-1* plasmid DNA and 2.5 μg of YRp10 DNA were used to transform 0.25 ml of spheroplasts. Restriction fragments carrying the *SPT2-1* mutation generated several hundred to a few thousand His$^+$ Ura$^+$ transformants; those fragments lacking the mutation generated fewer than 10 His$^+$ Ura$^+$ transformants.

Subcloning. Subcloning of *SPT2-1* sequences into pBR322 and YRp10 vectors was performed by standard procedures (26). Frozen competent *E. coli* cells were prepared and transformed by the method of Hanahan (20).

Southern analysis. Methods of DNA preparation, gel electrophoresis, and Southern hybridization have been described previously (38).

Nick translation. DNA fragments used as probes in Southern hybridizations and colony hybridizations were labeled with ^{32}P by the procedure of Maniatis et al. (27).

DNA sequence determination. DNA sequence determination was done by the enzymatic chain termination method of Sanger et al. (41, 42). In some cases, restriction fragments of yeast DNA were inserted into the single-stranded DNA vectors M13mp10 and M13mp11 (30) and then sequenced. In other cases, recombinant plasmids consisting of yeast fragments cloned into the double-stranded DNA vector pUC13 (54) were linearized at one of the insertion boundaries, unidirectionally digested with exonuclease III, and then treated with S1 nuclease and DNA ligase to generate a family of deletions (22). The denatured double-stranded DNAs were sequenced directly by using oligonucleotide primers (48). In some experiments, the universal primer GTAAACGACGGCCAGT or the reverse primer CAGGAAACAGCTATGAC was used to prime DNA synthesis (54). In other experiments, oligodeoxyribonucleotides complementary to yeast DNA were synthesized and used as primers.

Oligodeoxyribonucleotide synthesis. Primers were synthesized by the solid-phase phosphite-triester method with glass bead, solid-phase supports and *N*-acyl,5′dimethoxyltrityl nucleoside-3′-di-isopropyl phosphoramidite intermediates (1, 28).

Northern analysis. Total yeast RNAs from *S. cerevisiae* strains RP123 (*MATα his2 ade1 trp1 met14 ura3*) and GM-3C-2 (*MATα leu2-3 leu2-112 trp1-1 his4-519 cyc1-1 cyp3-1*) were gifts from A. Spence. RNA was fractionated by electrophoresis on a formaldehyde-agarose gel and transferred to a nitrocellulose filter by the procedure of Maniatis et al. (27). Prehybridization, hybridization, and washing of the filters were as described by Thomas (53), except that the hybridization was carried out at 42°C for 48 h.

Preparation of M13 probes used in Northern hybridizations. The universal primer was hybridized with a M13 subclone template, and the primer was extended as described previously (41), except that no dideoxynucleotides were present. The reaction products were treated with restriction endonucleases, purified by electrophoresis on low-melting-temperature agarose gels, and eluted by the procedure of Maniatis et al. (27).

Mapping 5′ ends of *SPT2* mRNA. Total yeast RNAs were isolated from *S. cerevisiae* strains S703 (*MATα his4-912δ spt2-150 ura3-52 lys2-2 can1-100 cry1*), SR106-9B (*MATα his4-912 leu2-3 ura3-52*), and SR106-9D (*MATα his4-912 leu2-3 ura3-52 SPT2-1*) by the method of Broach et al. (7). The primer elongation method (17) was used to map the 5′ ends. The synthetic primer TGCAGTCGTTGATTTTCGTA (nucleotides 425 to 406; see Fig. 5) was labeled with ^{32}P by using T4 polynucleotide kinase and then hybridized to 50 μg of total yeast RNA. The primer extension reaction was carried out at 42°C for 1.5 h with 10 U of avian myeloblastosis virus (AMV) reverse transcriptase. The reaction was then phenol extracted, precipitated with ethanol, and resuspended as for DNA sequencing. Before being loaded on a gel, the sample was made 10 mM in NaOH, incubated at 42°C for 45 min, and then heated at 100°C for 3 min.

An M13mp10 subclone which contained the coding strand of the *SPT2-1* gene was primed with the same synthetic primer and sequenced by the enzymatic chain termination method of Sanger et al. (42). This sequencing ladder was run next to the primer extension reaction on a 6% acrylamide–7 M urea gel.

RESULTS AND DISCUSSION

Cloning of the *SPT2* gene. A mutant allele of *SPT2*, *SPT2-1*, was cloned on the basis of its ability to suppress the His^- phenotype of *his4-912δ* strains grown at 23°C. As described above, strains of the genotype *his4-912δ* $SPT2^+$ are His^+ at 37°C and His^- at 23°C. In contrast, *his4-912δ* strains which carry the *SPT2-1* mutation are strongly His^+ at both 37 and 23°C. Diploid strains which are heterozygous, $SPT2^+/SPT2$-*1*, are phenotypically His^+ at 23°C, indicating that the *SPT2-1* allele is dominant (55). We therefore assumed that a haploid yeast strain carrying the *his4-912δ* mutation, a chromosomal $SPT2^+$ gene, and a plasmid-borne *SPT2-1* gene would also be His^+ at 23°C. Thus, we undertook to clone from an *SPT2-1* strain a fragment of DNA which would allow a *his4-912δ* $SPT2^+$ strain to grow in the absence of histidine at 23°C.

The cloning vector used in these experiments was YRp10 (5, 6), which carries pBR322 vector sequences, the yeast selectable marker *URA3*, and the yeast autonomously replicating sequence *ARS1*. Plasmid YRp10 was linearized by digestion with the restriction enzyme *Bam*HI, and total genomic yeast DNA from an *SPT2-1* strain was partially digested with *Bgl*II. The *Bgl*II fragments of yeast DNA were ligated into the *Bam*HI site of YRp10, and the ligation mixture was used to transform a *his4-912δ* $SPT2^+$ *ura3-52* strain. His^+ Ura^+ transformants were selected at 23°C.

These His^+ Ura^+ transformants were expected to carry an autonomously replicating plasmid consisting of YRp10 sequences and the *SPT2-1* gene. Such transformants should generate His^- Ura^- segregants which have lost the plasmid at high frequency. However, after 20 generations of nonselective growth, all 20 transformants examined generated His^- Ura^- segregants at a frequency of fewer than 1 in 500 cells. These stable His^+ Ura^+ transformants were presumed to result from integration of a plasmid into genomic DNA; some of the transformants were expected to result from integration at the *SPT2* locus. Transformants of this type could result if the plasmid introduced into the cell during transformation carried the *SPT2-1* gene and if a reciprocal crossover took place between the *SPT2* sequences on the chromosome and those on the plasmid (Fig. 1). In such transformants, the Ura^+ and His^+ phenotypes should map to the *SPT2* locus. To examine this possibility, we crossed four Ura^+ His^+ transformants to a *his4-912δ SPT2-1 ura3-52* strain, and tetrads were dissected and analyzed. One transformant (SR121-1B) was found in which Ura^+ segregated 2+:2− and His^+ segregated 4+:0−. The failure to find any His^- segregants indicated that the gene responsible for the His^+ phenotype in the transformants and the original *SPT2-1* mutant gene were at allelic (or at least tightly linked) positions in the genome. This transformant was presumed to carry a duplication of *SPT2* sequences, with YRp10 sequences inserted between the repeats as diagrammed in Fig. 1c. Southern hybridization analysis of this transformant indicated that it carried two or more copies of the YRp10 vector inserted as tandem repeats as indicated in Fig. 1d.

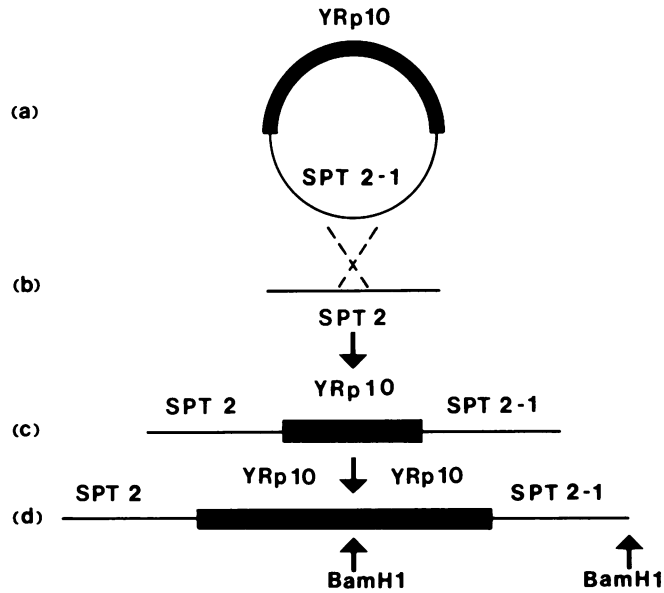


FIG. 1. Cloning of the *SPT2-1* gene. (a) Plasmid YRp10 (black bar) containing the *SPT2-1* mutant gene (solid line). (b) Chromosomal $SPT2^+$ gene. (c) Chromosomal *SPT2* region after integration of the plasmid shown in (a). (d) The chromosomal region shown in (c) after integration of an additional YRp10 plasmid. The *Bam*HI sites define the segment of DNA cloned into *E. coli* and referred to as plasmid pR140.

The *SPT2-1* gene was cloned in *E. coli* by cleavage of total genomic DNA from transformant SR121-1B with *Bam*HI, which left the *SPT2-1* mutant gene and the adjacent YRp10 vector sequences on a single restriction fragment (Fig. 1d). The *Bam*HI fragments of yeast DNA were circularized by treatment with DNA ligase and then used to transform *E. coli* as described above. The *E. coli* transformants obtained carried a plasmid which, when transformed back into a *his4-912δ* $SPT2^+$ *ura3-52* strain, generated transformants which were Ura^+ and His^+ at 23°C. These transformants were unstable and generated His^- Ura^- segregants at high frequency. These transformants carried an autonomously replicating plasmid consisting of YRp10 vector sequences and a segment of yeast DNA carrying the *SPT2-1* mutant gene. This plasmid will be referred to as pR140.

The reason for our inability initially to recover the *SPT2-1* gene on an autonomously replicating plasmid is unclear. Transformants which carry the *SPT2-1* gene integrated into the chromosome are more strongly His^+ than are cells carrying *SPT2-1* on an autonomously replicating plasmid.

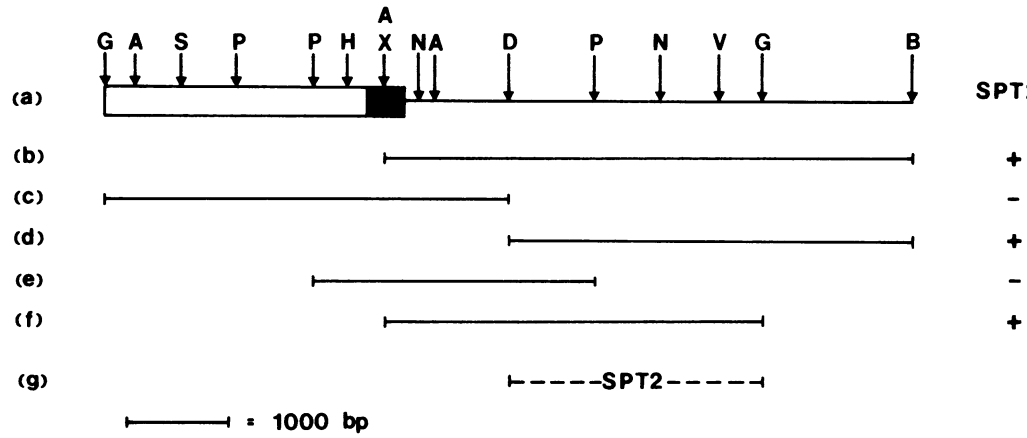


FIG. 2. Restriction mapping of the *SPT2-1* clone and localization of the *SPT2-1* gene. (a) Restriction map of the yeast DNA sequences present in the *SPT2-1* clone pR140. The box represents Ty sequences: the open segment of the box represents the internal region of the transposable element, and the black box represents a δ sequence. The solid line represents unique sequence yeast DNA. The arrows indicate cleavage sites for the restriction endonucleases *Bgl*II (G), *Ava*I (A), *Sal*I (S), *Pst*I (P), *Hpa*I (H), *Nde*I (N), *Xho*I (X), *Hin*dIII (D), *Pvu*I (V), and *Bam*HI (B). (b to f) The solid lines represent fragments of DNA present in various *SPT2-1* subclones. In the column on the right, the ability (+) or inability (−) of the various fragments to suppress the *his4-912*δ mutation when cloned into YRp10 and transformed into yeast cells is indicated. Fragments scored as + cause a *his4-912*δ *SPT2*$^+$ strain to become His$^+$ at 23°C, and fragments scored as − fail to do so. (g) The dotted line defines the segment of DNA to which the *SPT2-1* gene was localized by subcloning experiments.

Thus, transformants in which the plasmid had been integrated into genomic DNA may have been preferentially selected.

Restriction mapping and subcloning. The *SPT2-1*-containing plasmid was analyzed by restriction mapping by using a variety of single- and double-restriction digests. The yeast DNA insert present in plasmid pR140 was approximately 9,000 bp in length and had the restriction map shown in Fig. 2a.

The pR140 plasmid was also used in Southern hybridization analysis to probe total genomic yeast DNA. The plasmid hybridized to multiple fragments of yeast genomic DNA to generate a hybridization pattern characteristic of that obtained when a cloned Ty element is used as a probe. The presence of Ty sequences on the cloned segment was consistent with the restriction mapping data in that the leftmost 3,000 bp of the cloned *SPT2-1* DNA had a restriction map identical to half of a Ty element (40). A subclone of *SPT2-1* DNA which extended from the *Hin*dIII site to the *Bam*HI site hybridized to a unique set of yeast DNA restriction fragments, indicating that these sequences exist as a single copy in the yeast genome. This *Hin*dIII-*Bam*HI fragment was used to probe *SPT2*$^+$ and *SPT2-1* DNAs after digestion with a variety of restriction enzymes. In all digests, identical patterns of hybridization were observed for the wild-type and mutant strains. These observations indicate that the *SPT2-1* mutation is unassociated with any chromosomal rearrangements; the Ty element adjacent to the *SPT2-1* gene must be present at this site in *SPT2*$^+$ strains as well. When the *Hin*dIII-*Bam*HI fragment was hybridized to DNA from a strain which, by genetic criteria, carries a deletion of the *SPT2* gene (55), no hybridization was observed.

Localization of the *SPT2-1* gene. To localize the *SPT2-1* coding sequences within the cloned DNA segment, we constructed and analyzed a variety of subclones. Fragments were subcloned into a YRp10 vector, and the resulting plasmids were used to transform a *his4-912*δ *SPT2*$^+$ *ura3-52* strain. Ura$^+$ transformants were selected and then screened for their ability to grow in the absence of histidine at 23°C. The results of this analysis are indicated in Fig. 2b to 2f. These experiments localized the sequences responsible for the suppression of the His$^-$ phenotype to a 2,700-bp segment of DNA defined by *Hin*dIII and *Bgl*II restriction sites (Fig. 2g).

Localization of the *SPT2-1* mutation. To identify the DNA segment carrying the *SPT2-1* mutation, we cloned a variety of fragments in pBR322 and then analyzed them for their ability to convert the *SPT2*$^+$ gene to *SPT2*. Before introduction into yeast cells, these plasmids were cleaved with restriction enzymes to separate the cloned yeast sequences from the vector sequences so that the *SPT2-1* sequences were introduced into yeast cells as linear fragments. Such linear fragments have been shown to recombine with chromosomal DNA by substitutive events in which the sequences on the chromosome are replaced by the sequences carried by the transforming fragment (34).

The fragments of *SPT2-1* DNA were assayed for their ability to supply the *SPT2-1* mutation in a cotransformation experiment. The fragments of DNA were introduced into a *his4-912*δ *SPT2*$^+$ *ura3-52* yeast strain simultaneously with supercoiled circular YRp10 plasmid DNA, and Ura$^+$ His$^+$ transformants were selected. These Ura$^+$ His$^+$ transformants represent cells which have acquired YRp10 as an autonomously replicating plasmid and in which the wild-type *SPT2* gene on the chromosome has been converted to the *SPT2-1* allele by recombination with the cotransforming linear fragment. A cotransformation procedure was used in these experiments, because transformation with only an *SPT2-1* fragment and then the selection of His$^+$ cells detects primarily cells resulting from mutation of wild-type chromosomal *SPT* genes. Transforming with both *SPT2* DNA and YRp10 DNA and selecting for both His$^+$ and Ura$^+$ phenotypes results in a considerable enrichment for transformants; almost all of the His$^+$ Ura$^+$ colonies obtained were the results of cotransformation. Those fragments of *SPT2-1* DNA which, together with YRp10, generated His$^+$ Ura$^+$ transformants were presumed to carry the *SPT2-1* mutation. Those fragments which failed to generate significant numbers of His$^+$ Ura$^+$ transformants did not include the muta-

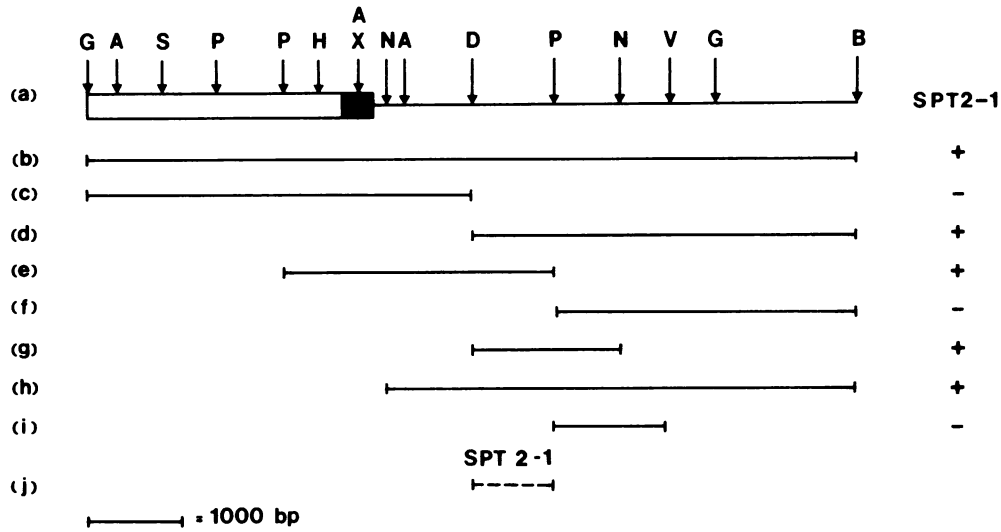


FIG. 3. Localization of the *SPT2-1* mutation. (a) Restriction map of the cloned *SPT2-1* sequences. The box represents Ty sequences: the open segment of the box represents the internal region of the transposable element, and the black box represents a δ sequence. The orientation of the transposable element is such that Ty transcription would begin in the δ not included in this clone and terminate in the δ proximal to *SPT2*. The solid line represents unique sequence yeast DNA. The arrows indicate cleavage sites for the restriction endonucleases *Bgl*II (G), *Ava*I (A), *Sal*I (S), *Pst*I (P), *Hpa*I (H), *Nde*I (N), *Xho*I (X), *Hin*dIII (D), *Pvu*I (V), and *Bam*HI (B). (b to i) The solid lines represent fragments of DNA present in various subclones. In the column on the right, the ability (+) or inability (−) of the various fragments to convert a wild-type *SPT2* gene to the *SPT2-1* mutant allele upon transformation into yeast cells is indicated. Fragments scored as + generated His^+ Ura^+ (at 23°C) transformants when transformed as linear fragments into a *his4-912δ* $SPT2^+$ *ura3-52* strain together with supercoiled YRp10 plasmid DNA. Fragments designated − fail to do so. (j) The dotted line defines the segment of DNA to which the *SPT2-1* mutation was localized by subcloning experiments.

tion. These experiments localized the site of the *SPT2-1* mutation to an 800-bp fragment defined by *Hin*dIII and *Pst*I restriction sites (Fig. 3).

DNA sequence analysis of the *SPT2-1* mutant gene. We also analyzed the cloned *SPT2-1* gene by DNA sequencing (22, 30, 40, 41, 48, 54). The region from the *Ava*I site just to the right of the transposable element extending rightward to the *Bgl*II (see Fig. 6b) site has been sequenced. Analysis of the distribution of nonsense codons (10) in the six possible reading frames indicated three potential protein-coding regions and one incomplete coding region as diagrammed in Fig. 4. Based on the subcloning analysis described above (and assuming that the *SPT2* gene encodes a protein), the leftmost of these coding regions must represent the gene containing the *SPT2-1* mutation. The DNA sequence of this coding region and its flanking DNA is presented in Fig. 5.

The putative *SPT2-1* coding region encodes a protein of 212 amino acids in length which initiates at the methionine (ATG) codon at nucleotide 378 and terminates at an ochre codon, UAA. After this ochre codon, the open reading frame continues for another 120 amino acids. Thus, if translation could proceed through the ochre codon, a protein 333 amino acids in length would be produced. This observation suggested the possibility that the ochre codon present in the middle of the gene is equivalent to the *SPT2-1* mutation and that this termination codon would be absent in the wild-type *SPT2* gene. To examine this possibility, we cloned this region of DNA from an $SPT2^+$ strain and then analyzed it by DNA sequencing.

Cloning and sequencing of the wild-type *SPT2* gene. Fragments of DNA from an $SPT2^+$ strain were cloned into pBR322 and analyzed by colony hybridization by using the *Hin*dIII-*Bgl*II fragment (Fig. 6d) of the cloned *SPT2-1* mutant gene as a probe. The *Pst*I fragment extending from the *Pst*I site in the transposable element to the *Pst*I site in the *SPT2-1* coding sequences (Fig. 6e) was isolated. This fragment includes the site of the ochre codon carried by the *SPT2-1* mutant gene (Fig. 6).

The cloned *Pst*I fragment of $SPT2^+$ DNA was analyzed by DNA sequencing; the sequence of the region from the *Ava*I site just to the right of the transposable element to the *Pst*I site in the *SPT2* coding region was determined. The sequence of this fragment was identical to that of the cloned *SPT2-1* gene, except for the substitution of a T for an A at nucleotide position 1014. This substitution changed the ochre codon (TAA) at amino acid position 213 to a leucine-

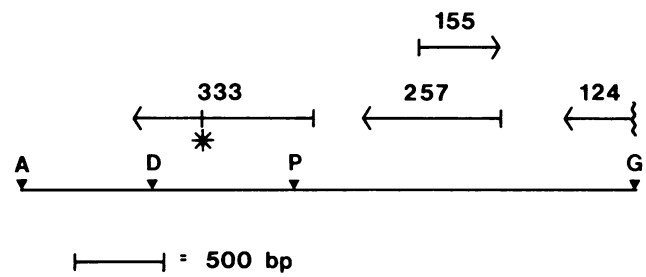
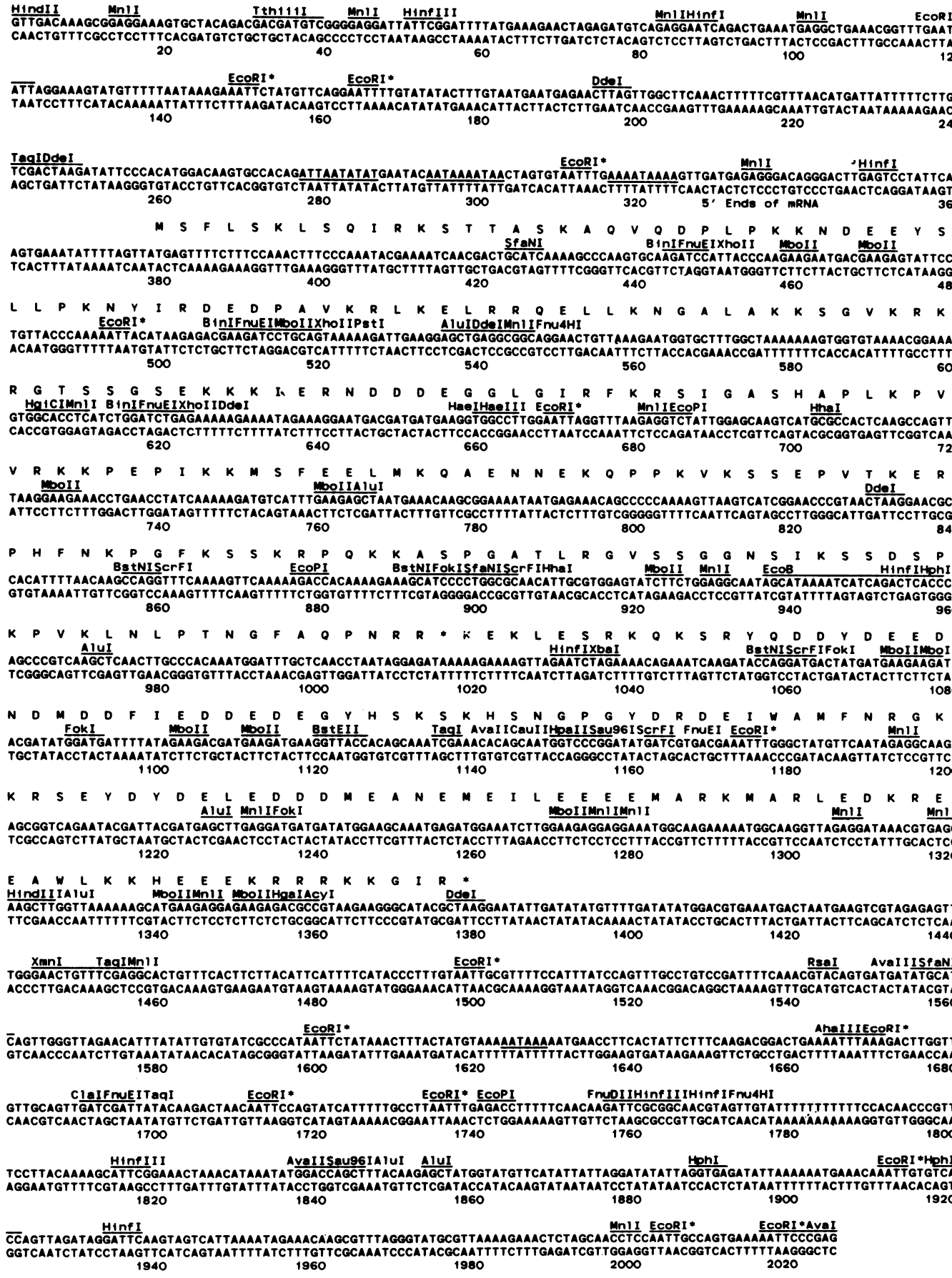


FIG. 4. Open reading frames in the *SPT2-1* clone. A 3,351-bp segment of DNA extending from the *Ava*I site just to the right of the transposable element to the *Bgl*II site near the right end of the cloned fragment (Fig. 6b) is represented. The arrowheads indicate sites for restriction by *Ava*I (A), *Hin*dIII (D), *Pst*I (P), and *Bgl*II (G). The horizontal arrows indicate open reading frames of greater than 50 amino acids in length; arrow direction indicates the direction of translation. The numbers above the arrows indicate the length, in amino acids, of the encoded proteins. The rightmost coding region proceeds through the *Bgl*II site that defines the end of the fragment. The asterisk indicates the position of the ochre codon defined by the *SPT2-1* mutation described in the text.


HindII MnlI Tth111I MnlI HinfIII MnlIHinfI MnlI EcoRI*
EcoRI* EcoRI* DdeI
TaqIDdeI EcoRI* MnlI HinfI
5′ Ends of mRNA
SfaNI BinIFnuEIXhoII MboII MboII
EcoRI* BinIFnuEIMboIIXhoIIPstI AluIDdeIMnlIFnu4HI
HgiCIMnlI BinIFnuEIXhoIIDdeI HaeIHaeIII EcoRI* MnlIEcoPI HhaI
MboII MboIIAluI DdeI
BstNIScrFI EcoPI BstNIFokISfaNIScrFIHhaI MboII MnlI Eco8 HinfIHphI
AluI HinfIXbaI BstNIScrFIFokI MboIIMboII
FokI MboII MboII BstEII TaqI AvaIICauIIHpaIISau96IScrFI FnuEI EcoRI* MnlI
AluI MnlIFokI MboIIMnlIMnlI MnlI MnlI
XmnI TaqIMnlI EcoRI* RsaI AvaIIISfaNI
EcoRI* AhaIIIEcoRI*
ClaIFnuEITaqI EcoRI* EcoRI* EcoPI FnuDIIHinfIIIHinfIFnu4HI
HinfIII AvaIISau96IAluI AluI HphI EcoRI*HphI
HinfI MnlI EcoRI* EcoRI*AvaI

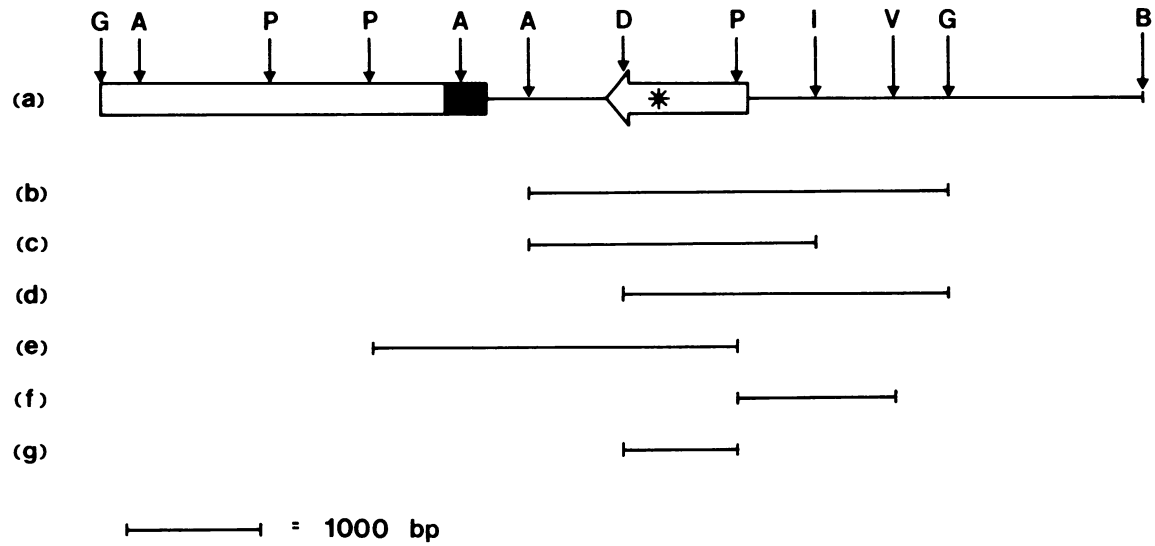


FIG. 6. *SPT2* fragments analyzed by sequencing and used as probes. (a) Restriction map of the cloned *SPT2* DNA. The box represents Ty sequences: the open segment of the box represents the internal region of the transposable element, and the black box represents a δ sequence. The open arrow indicates the *SPT2* coding region, as well as direction of transcription. The arrows above the line indicate cleavage sites for the restriction endonucleases *Bgl*II (G), *Ava*I (A), *Pst*I (P), *Hin*dIII (D), *Hin*cII (I), *Pvu*I (V), and *Bam*HI (B). (b) The *Ava*I-*Bgl*II fragment analyzed by DNA sequencing and represented in Fig. 4. (c) The *Hin*cII-*Ava*I fragment whose sequence is represented in Fig. 5. (d) The *Hin*dIII-*Bgl*II fragment used as a probe for colony hybridizations in cloning the wild-type *SPT2*$^+$ gene. (e) The *Pst*I fragment cloned from a wild-type *SPT2* strain. (f) The *Pst*I-*Pvu*I fragment containing the amino-terminal end and the promoter region of the *SPT2-1* gene. This fragment of the *SPT2-1* mutant gene was combined with the *Pst*I fragment of the cloned wild-type gene to generate an intact *SPT2*$^+$ gene. (g) The *Hin*dIII-*Pst*I fragment used as a probe in Northern hybridization analysis.

encoding triplet (TTA). Thus, the wild-type *SPT2*$^+$ gene encodes a protein which is 333 amino acids in length.

Verification of wild-type *SPT2* activity. To demonstrate that the sequence cloned from the *SPT2*$^+$ strain did indeed specify wild-type *SPT2* function, we constructed and analyzed an intact gene carrying this sequence. The *Pst*I fragment from the wild-type *SPT2* gene (Fig. 6e) was ligated to the *Pst*I-*Pvu*I fragment (Fig. 6f) carrying the amino-terminal end of the *SPT2-1* gene. The reconstructed gene was inserted into the YRp10 autonomously replicating yeast vector to generate plasmid pR561. This plasmid was used to transform a strain (S703) carrying the *his4-912*δ mutation, the *ura3-52* mutation, and a recessive deletion mutation of the *SPT2* gene (*spt2-150*). Ura$^+$ transformants were selected and then analyzed for their ability to grow in the absence of histidine at 23°C. The starting strain, S703, was Ura$^-$ because of the *ura3-52* mutation and His$^+$ at 23°C because of the lack of wild-type *SPT2* repressor activity. When S703 was transformed with only YRp10, the resulting transformants were Ura$^+$ and His$^+$. When the YRp10 plasmid carrying the reconstructed *SPT2* gene was introduced into strain S703, the transformants were Ura$^+$ and His$^-$ at 23°C. Thus, the sequences present on plasmid pR561 conferred the repressor activity characteristic of the wild-type *SPT2* gene and prevented expression of the *his4-912*δ gene at 23°.

Transcription of the *SPT2* gene. To determine whether the *SPT2* gene was transcribed, we carried out Northern hybridization analysis using the cloned *SPT2* gene as a probe. A *Hin*dIII-*Pst*I fragment containing most of the *SPT2* gene (Fig. 6g) detected a single RNA species of 1.6 kilobase pairs in length when RNA from an *SPT2*$^+$ strain was analyzed (Fig. 7).

The length of the *SPT2*$^+$ transcript was consistent with a message which carries the entire *SPT2*$^+$ coding region and a few hundred base pairs of nontranslated RNA. The *SPT2*$^+$ gene sequence did not carry the heptanucleotide TACTAAC, a sequence which is thought to be necessary for the splicing of yeast nuclear gene mRNAs (36). This observation, together with the size of the *SPT2*$^+$ message, suggests that the *SPT2*$^+$ transcript does not undergo splicing.

The initiation point of *SPT2* transcription was mapped by the primer elongation method (17). There were two major and eight minor start sites between nucleotides 328 and 381 (Fig. 8, lanes 1, 3, 4, and 5). These start sites were not seen when the primer elongation experiment was done with RNA isolated from S703, a strain which carries a deletion of the *SPT2* gene (lane 2). All 5′ ends lay downstream of three TATA-like sequences (Fig. 5) and all, except one minor start site, lay upstream of the putative initiation codon at nucleotide 378 (Fig. 5). Approximately equivalent amounts of RNA were produced by both *SPT2*$^+$ strains (Fig. 8, lanes 1, 3, and 5) and the *SPT2-1* strains (Fig. 8, lane 4), indicating that the *SPT2-1* mutation did not significantly alter the stability of *SPT2* mRNA.

FIG. 5. *SPT2-1* DNA sequence. The sequence shown extends from a *Hin*cII site to an *Ava*I site as indicated in Fig. 6c. The *Hin*cII site to the right in Fig. 6c is shown at the top, and the *Ava*I site to the left in Fig. 6c is shown at the bottom. The *SPT2-1* coding sequence begins at nucleotide position 378 (numbering starts at the *Hin*cII site) and ends at the ochre codon at position 1015. The open reading frame continues past this termination codon to position 1376. The protein sequence encoded by the 333-codon reading frame (assuming readthrough of the ochre termination codon) is shown by using the single-letter amino acid code (Fig. 9c). TATA-like sequences between nucleotides 277 and 324 are underlined. A putative 3′ end (position 1623) containing a possible polyadenylation signal is also underlined. The region of mRNA start sites is labeled. Restriction endonuclease cleavage sites are indicated.

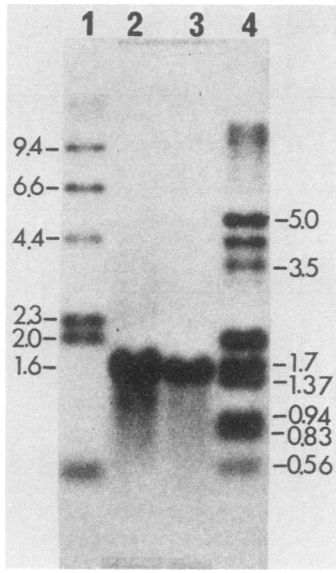


FIG. 7. Northern hybridization analysis of *SPT2* transcript. A 50-μg portion of yeast RNA was fractionated by electrophoresis on a formaldehyde-agarose gel, transferred to a nitrocellulose filter, and then hybridized to a ^{32}P-labeled *Hin*dIII-*Pst*I fragment (Fig. 6g) containing the *SPT2-1* gene. Lane 2, RNA from *S. cerevisiae* RP123. Lane 3, RNA from *S. cerevisiae* GM-3C-2. Both of the yeast strains used in this analysis are wild type at the *SPT2* locus. lanes 1 and 4, Bacteriophage λ DNA was cut with *Hin*dIII or *Hin*dIII and *Eco*RI, respectively, end labeled, and electrophoresed. The numbers indicate the lengths of the RNA transcripts and DNA fragments in nucleotides.

Nature of the *SPT2*$^+$ protein. The wild-type *SPT2* gene encodes a protein 333 amino acids in length. After the termination codon that concludes the wild-type *SPT2* gene, the reading frame continues for another 56 codons. Thus, readthrough of the *SPT2*$^+$ termination codon could lead to a protein product 390 amino acids in length.

The frequency of codon usage in the *SPT2* gene showed almost no codon bias (Table 1). There is more codon bias in yeasts in genes expressed at high levels than in those expressed at low levels (4).

TABLE 1. Codon usage of the *SPT2* 333-codon reading frame

Codon	No.	Codon	No.	Codon	No.	Codon	No.
UUU-Phe	6	UCU-Ser	6	UAU-Tyr	3	UGU-Cys	0
UUC-Phe	2	UCC-Ser	4	UAC-Tyr	5	UGC-Cys	0
UUA-Leu	7	UCA-Ser	11	UAA-		UGA-	
UUG-Leu	6	UCG-Ser	2	UAG-		UGG-Trp	2
CUU-Leu	4	CCU-Pro	5	CAU-His	3	CGU-Arg	5
CUC-Leu	2	CCC-Pro	8	CAC-His	2	CGC-Arg	3
CUA-Leu	1	CCA-Pro	7	CAA-Gln	6	CGA-Arg	1
CUG-Leu	2	CCG-Pro	0	CAG-Gln	4	CGG-Arg	3
AUU-Ile	3	ACU-Thr	2	AAU-Asn	12	AGU-Ser	4
AUC-Ile	2	ACC-Thr	1	AAC-Asn	3	AGC-Ser	3
AUA-Ile	6	ACA-Thr	2	AAA-Lys	27	AGA-Arg	9
AUG-Met	9	ACG-Thr	1	AAG-Lys	23	AGG-Arg	7
GUU-Val	2	GCU-Ala	5	GAU-Asp	19	GGU-Gly	6
GUC-Val	1	GCC-Ala	1	GAC-Asp	7	GGC-Gly	6
GUA-Val	5	GCA-Ala	8	GAA-Glu	25	GGA-Gly	7
GUG-Val	1	GCG-Ala	2	GAG-Glu	14	GGG-Gly	0

The *SPT2*$^+$ gene product contains 39 glutamic acid, 26 aspartic acid, 50 lysine, 28 arginine, and 5 histidine residues. Thus, the protein is basic and polar. The predicted secondary structure (16, 33) of the protein is 46.4% α-helical, 11.4% extended chain, 19.2% reverse turn, and 23.1% random coil. These structural features are dispersed throughout the molecule, with the striking exception of a long, polar helical segment extending from amino acid 276 to amino acid 327. This 52-amino-acid segment contains 62% charged residues, including 12 positively charged and 20 negatively charged amino acids. It is notable that this polar helical region is absent from the abbreviated protein encoded by the *SPT2-1* mutant gene.

Function of the *SPT2*$^+$ protein. Strains which carry the *his4-912*δ mutation and the wild-type *SPT2* gene are phenotypically His$^-$ and fail to produce any *HIS4* transcript when grown at 23°C. In contrast, strains which carry the *SPT2-1*

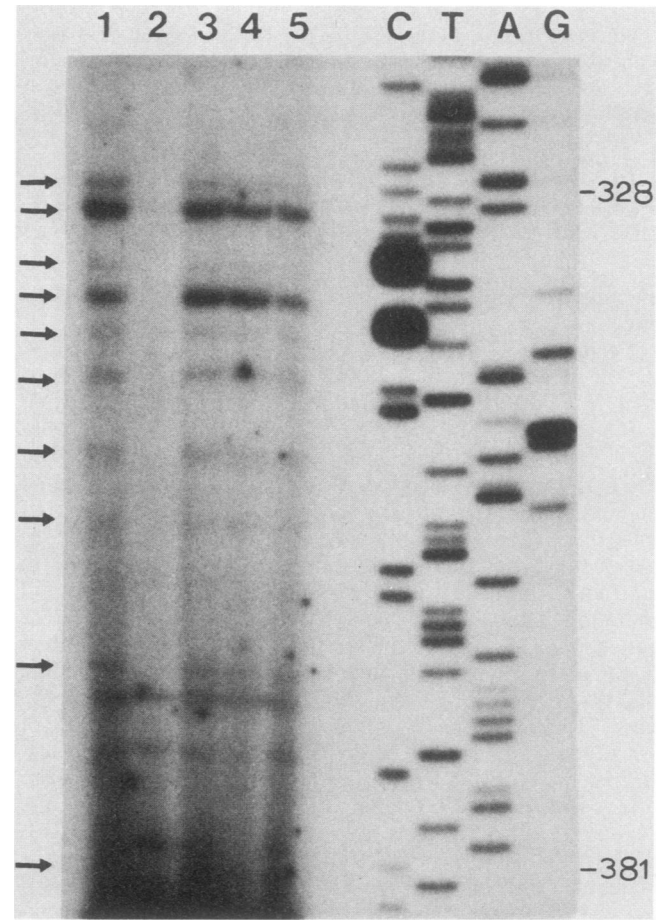


FIG. 8. Mapping of the *SPT2* transcription initiation sites. The 5′ ends of the *SPT2* mRNA were mapped by using the primer elongation method (17). RNA (50 μg) from each of five different *S. cerevisiae* strains was hybridized to a synthetic primer and then extended with reverse transcriptase. Lane 1, strain RP123 (*SPT2*$^+$); lane 2, strain S703 (*spt2-150*); lane 3, SR106-9B (*SPT2*$^+$); lane 4, SR106-9D (*SPT2-1*); lane 5, GM-3C-2 (*SPT2*$^+$). An M13 subclone containing the coding strand of the *SPT2-1* gene was primed with the same primer and then sequenced as shown in the right four lanes of the gel. The arrows to the left indicate the positions of the 5′ ends. The numbers to the right indicate the position in the nucleotide sequence as numbered in Fig. 5.The sequence shown is complementary to the coding sequence shown in Fig. 5.

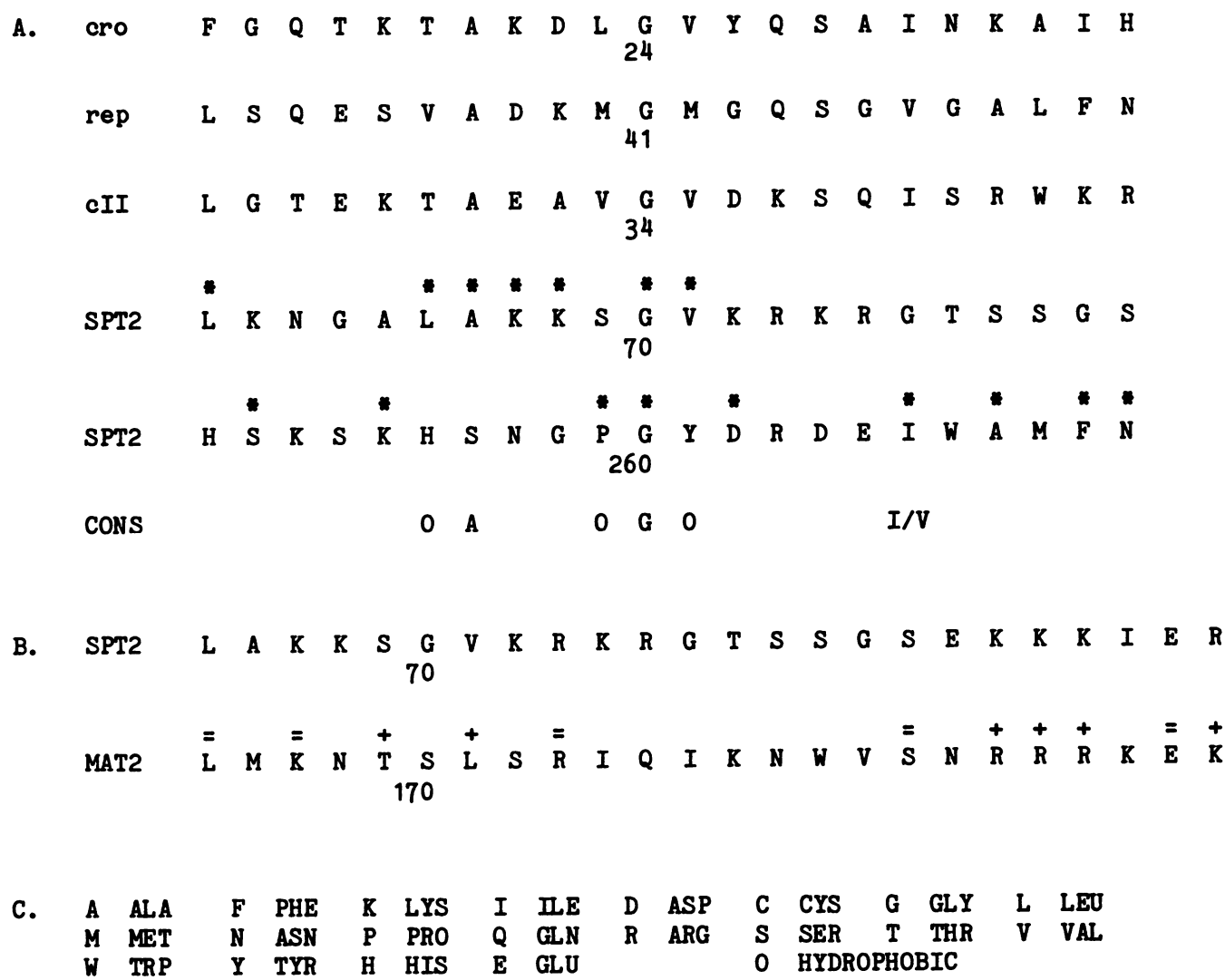


FIG. 9. Amino acid sequence homologies between *SPT2*$^+$ and DNA-binding proteins. In panel A, two different segments of the *SPT2*$^+$ protein are compared with sequences from the bacteriophage λ proteins *cro*, repressor (*rep*), cII. Shown at the bottom of panel A (CONS) are those amino acid residues which are highly conserved in the DNA-binding proteins that have been studied (33, 43, 52). O refers to any hydrophobic amino acid. The asterisk above the *SPT2*$^+$ sequence indicates that the amino acid is identical to that found in one or more of the *cro*, *rep*, or cII proteins or that it is consistent with the consensus sequence, or both. The glycine residues at the center of the two-helix motif are numbered for each protein; numbering starts at the amino-terminal end of each protein. In panel B, homology between *SPT2*$^+$ and the yeast *MATα2* (3) protein is demonstrated. The = refers to identity between the sequences; + refers to amino acids of similar charge, polarity, or hydrophobicity. The one-letter amino acid code is given in panel C. Sequence comparisons were performed by Wayne Anderson by using procedures developed for detecting homologies between potential DNA binding proteins (33). This procedure includes a comparison with random amino acid sequences similar in composition to those of the relevant proteins.

mutation and the *his4-912*δ mutation are His$^+$ at 23°C, and this His$^+$ phenotype is correlated with the production of a *HIS4* transcript of the same size and initiating at the same site in the *HIS4* regulatory region as the transcript produced by the wild-type *HIS4* gene (46). Thus, in *his4-912*δ strains, the wild-type *SPT2* gene product somehow interferes with the transcription of *HIS4*. In the absence of the *SPT2*$^+$ gene product, a normal *HIS4* transcript can be produced even though a δ sequence is still present in the regulatory region. This repression of *HIS4* transcription by *SPT2*$^+$ could be effected by means of a direct interaction between the *SPT2*$^+$ gene product and the δ sequence. Alternatively, *SPT2*$^+$ could affect *HIS4* expression indirectly by controlling the production or activity of a positive regulator of Ty- or δ-adjacent gene expression.

Because the *SPT2*$^+$ gene product acts as a repressor of transcription and may do so by binding to DNA, we looked for similarities between the *SPT2*$^+$ gene product and proteins known to have DNA-binding activity. A variety of procaryotic repressors of transcription have been examined and have been shown to share amino acid sequence homology in a 22-amino-acid segment, usually near their amino termini (33, 43, 52). This region corresponds to two consecutive α-helices in the protein; these structures have been shown to contact the major groove of the DNA helix during binding (2, 29, 35, 49). The *MATa1* protein of *S. cerevisiae*, which is involved in transcription regulation (32), has homology with the procaryotic DNA-binding proteins (33). We searched the *SPT2*$^+$ amino acid sequence for homology with the 22-amino-acid segment from the procaryotic proteins and

found two regions which show limited homology (Fig. 9a). These regions of homology were centered around amino acids 70 and 260 of the wild-type *SPT2* protein. The significance of these homologies is difficult to assess; strict sequence homology is not expected, because the different proteins bind to different DNA sequences.

We also compared the amino acid sequence of the $SPT2^+$ protein with those of many other proteins whose sequences have been determined. Homology was detected between $SPT2^+$ and the *MATα2* gene product of yeast cells, one of the genes which determines mating type (4) and which is known to interact directly with DNA (A. Johnson and I. Herskowitz, personal communication). There are 4 amino acid identities and 6 amino acids of similar charge or polarity within a 24-amino-acid segment (Fig. 9b). The region of $SPT2^+$ which is homologous to *MATα2* is also homologous to the procaryotic DNA binding proteins. Like $SPT2^+$, *MATα2* acts as a negative regulator of gene expression; it represses the expression of **a**-specific mating functions. Thus, the regions of homology between $SPT2^+$ and *MATα2* may represent regions of DNA binding for both proteins. In connection with these homologies, it is interesting that the region of *MATα2* which is homologous to *SPT2* and the region of *MAT***a***1* described above as sharing homology with the procaryotic DNA-binding proteins are both homologous to the homoeo domain of the homoeotic gene products of *Drosophila melanogaster* and *Xenopus laevis* (26, 44).

One of the most intriguing features of the *SPT2* gene is the existence of both dominant and recessive *his4-912*δ-suppressing alleles. The dominant suppressing alleles must encode a gene product which can somehow interefere with the repression effected by wild-type *SPT2* molecules. This interference could be effected if the active form of the wild-type *SPT2* protein is a multimer and if the mutant proteins retain their ability to interact with wild-type subunits. Such interactions could then give rise to hybrid, nonfunctional complexes. An analogous situation exists in the case of the *lac* repressor of *E. coli* which exists in the active form as a tetramer. *trans*-Dominant mutants of the *lac* repressor which lead to constitutive overexpression of the genes in the *lac* operon are thought to be the result of mixing between wild-type and mutant subunits to form hybrid inactive molecules which can no longer bind the operator sequence (18).

Another puzzling feature of Ty-controlled gene expression is the existence of at least seven different *SPT* genes which appear to act as repressors of the expression of Ty- or δ-adjacent genes (55). Why are all seven of these gene products necessary for the repression which occurs in wild-type cells? One possibility is that some or all of the *SPT* gene products interact with each other to form a multimeric complex which is the active DNA-binding form. Alternatively, the *SPT* gene products may bind to the DNA independently, but the binding of all seven proteins may be necessary for the repression of transcription. A third possibility is that most of the *SPT* gene products affect Ty-adjacent gene expression indirectly by controlling the expression of each other or of other regulatory factors which interact directly with Ty sequences. The existence of multiple negative regulators of gene expression is not unprecedented; a similar phenomenon is observed in the regulation of the silent copies of yeast mating type information (*HML* and *HMR*). The products of at least four genes, *SIR1* to *SIR4*, are necessary for repression of the information present in the silent cassettes (19, 32).

Experiments from several labs indicate that the expression of Ty-adjacent genes and the transcription of Ty elements are coordinately regulated. Thus, the transcription of Ty elements and their adjacent genes is repressed in strains which are heterozygous, **a**/α, at the *MAT* locus and in strains which carry mutations at the *STE7*, *ROC1*, or *ROC2* loci (12, 13). Similarly, the transcription of Ty-adjacent genes and of Ty elements is repressed when cells are grown in media containing glycerol as carbon source when compared with cells grown in media containing glucose (51). Because the *SPT* genes apparently control the expression of Ty-adjacent genes, it is possible that these genes also play a role in Ty transcription. The observed coregulation of Ty elements and Ty-adjacent genes leads to the prediction that the wild-type *SPT2* protein represses the transcription of some or all Ty elements. However, it is also possible that *SPT2* activates transcription of Ty or δ sequences and that this activation indirectly leads to the repression of adjacent genes.

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