The SPT3 Gene is Required for Normal Transcription of Ty Elements in S. cerevisiae

Fred Winston,* Karen J. Durbin,† and Gerald R. Fink*†

* Department of Genetics
Harvard Medical School
Boston, Massachusetts 02115
† Section of Biochemistry, Molecular and Cell Biology
Cornell University
Ithaca, New York 14850
‡ Whitehead Institute of Biomedical Sciences
and Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Summary

The transposable Ty elements consist of a central core, ε, flanked by direct repeats called 8s. In wild-type strains Ty transcripts initiate in one 8 and terminate in the other. Insertion mutations caused by Ty elements have a wide variety of phenotypes, ranging from inhibition of gene expression to constitutive gene expression. Mutations in the SPT3 gene suppress these effects of Ty and 8 insertion mutations on adjacent genes. In spt3 null mutants the Ty transcription pattern for the entire ensemble of Ty elements is changed. The 8-ε transcripts are absent and initiation begins at a position 800 bp into the ε region. In these spt3 strains, transcription that initiates in solo 8s and proceeds into adjacent structural genes is also abolished. The requirement of SPT3 for normal transcription from 8 can explain the ability of spt3 mutations to suppress the mutants caused by Ty and 8 insertions. In SPT3 strains transcription from the δ into adjacent sequences interferes with normal expression of those sequences, whereas in spt3 strains the aberrant transcript is not made. spt3 mutations also lead to defects in diploid formation and sporulation, suggesting that SPT3 is important for the expression of genes in addition to Ty elements.

Introduction

The Ty elements of yeast are a dispersed, repetitive set of elements, present in 30–35 copies per haploid yeast genome (Cameron et al., 1979). They are 5.9 kb long and consist of 330 bp direct terminal repeats (δ sequences) flanking the internal ε sequence. The role, if any, of Ty elements in normal yeast growth has not yet been elucidated.

Ty insertion mutations in the 5’ noncoding regions of genes have been identified at several loci (Errede et al., 1980; Roeder et al., 1980; Williamson et al., 1981; Bach, 1984; Eibel and Philippson, 1984; Rose and Winston, 1984; Simchen et al., 1984). These insertions cause inhibition or otherwise altered regulation of adjacent genes, often placing these genes under the control of unlinked genes. For example, many Ty mutations that activate adjacent gene expression are controlled by the yeast mating-type locus, MAT (Errede et al., 1980; Williamson et al., 1983). In these cases, constitutive gene expression is observed in haploid strains, but is greatly reduced in a/a diploids. The MAT control of genes adjacent to Ty insertions is likely to be a consequence of the fact that Ty transcription is itself under mating-type control (Elder et al., 1980) in a fashion that parallels the behavior of Ty insertion mutations; Ty transcription is high in haploid cells, but reduced in a/a diploids.

We have reported previously that mutations in seven SPT genes (SPT = suppressor of Ty) can suppress a variety of Ty and 8 insertion mutations at the HIS4 and LYS2 loci (Simchen et al., 1984; Winston et al., 1984). The effects of these trans-acting genes suggests that some might act by affecting Ty or 8 transcription; hence, an spt mutation could affect the expression of a gene by its effect on expression of the adjacent Ty or δ element. Suppressors of insertion mutations have also been found in Drosophila (Modolell et al., 1983) and mice (Sweet, 1983).

In this paper, we present an analysis of the molecular basis for suppression of Ty and 8 insertion mutations by spt3 mutations. The results show that spt3 mutations alter the start site of transcription of Ty elements from the δ sequence to a new position 800 bp downstream, spt3 suppression of δ and Ty insertion mutations can be explained by a similar transcriptional effect. In addition to these suppression properties, spt3 mutations affect fundamental cellular properties including diploid formation and sporulation. These results suggest that the SPT3 gene product is important for Ty transcription, Ty-mediated gene expression, and other cellular functions.

Results

Construction of Null Alleles of SPT3

We constructed an spt3 null mutant to ensure the complete loss of SPT3 function since the spontaneous mutations we isolated previously (Winston et al., 1984) might be leaky and have residual SPT3 function. To make null alleles in vitro, the SPT3 gene was cloned by screening a yeast recombinant plasmid library in the autonomous plasmid YEp24 (which contains the yeast URA3 gene) (Carlson and Botstein, 1982). The recipient strain for cloning SPT3 was 8582-2B (MATa spt3-1 his4-177 his3-112 ura3-52). The his4-177 mutation is a Hisδ insertion that is suppressed by spt3 mutations; therefore, this strain has a His phenotype. Since spt3-1 is recessive to SPT3, 8582-2B transformants with the wild-type SPT3 gene on a plasmid have a His phenotype. The results of subcloning experiments localized the SPT3 gene to a 2.5 kb Eco RI-Bgl II fragment (Figure 1). The identity of the cloned gene was verified to be SPT3 by showing that it directs integration of one of the originally isolated plasmids, pFW18, to the SPT3 locus.

To study SPT3 null alleles, we constructed a frameshift and a deletion in the cloned SPT3 gene and recombined each of these mutations into the genome, replacing the
wild-type SPT3 gene (see Experimental Procedures). Since haploids with these mutations are viable, SPT3 is not an essential gene for mitotic growth. However, strains with the spt3 frameshift (spt3-101) or the spt3 deletion (spt3-201) have impaired growth; they have doubling times 1.3–1.5 times greater than wild-type strains. These null mutations, like the spontaneous spt3 mutations, suppress the His" phenotype of the insertion mutations his4-9126, his4-9176, and his4-917 (Winston et al., 1984). Therefore, suppression is due to loss of function of the SPT3 gene product.

spt3 Alters the 5' End of Ty Transcripts

The spt3 mutation has a qualitative effect on the transcription of all Tys in the cell. In an SPT3 background, Ty transcription originates predominantly in the δ terminal repeat, runs through the 5.3 kb internal sequence, and terminates in the downstream δ (Elder et al., 1983). The spt3 mutation greatly diminishes production of the full-length RNA and makes an RNA shortened by 800 bp instead of the transcript of normal size (Figure 2a).

We studied the structure of the new transcript in a strain that carried a Ty-HIS4 fusion at the HIS4 locus. This allele, his4-Δ5, was isolated as a spontaneous His4C revertant of his4-912, a Ty insertion mutation (Chaleff and Fink, 1980). Expression of the carboxy-terminal portion of the HIS4 gene product was restored by a deletion that fuses 1.5 kb of upstream Ty sequences to a position early in the coding sequence of HIS4 (P. Farabaugh, unpublished data). A single transcript originates in the δ of the Ty and terminates at the wild-type HIS4 position (Silverman and Fink, 1984). The his4-Δ5 fusion permits the analysis of a uniquely identifiable Ty transcript.

The spt3 mutation has a qualitative effect on transcription of his4-Δ5; in an spt3 his4-Δ5 strain, the HIS4-specific RNA was restored by a deletion that fuses 1.5 kb of upstream Ty sequences to a position early in the coding sequence of HIS4 (P. Farabaugh, unpublished data). A single transcript originates in the δ of the Ty and terminates at the wild-type HIS4 position (Silverman and Fink, 1984). The his4-Δ5 fusion permits the analysis of a uniquely identifiable Ty transcript.

The 5' end of the total Ty RNA from spt3 strains maps to a site downstream of the δ, in the internal sequence of the Ty (Figure 4b, lanes 2 and 4). We conclude that the SPT3 gene product is required for initiation of transcription in a δ sequence. In addition to regulation by the SPT3 gene, Ty transcription is under control of the MAT locus; the level of transcription is 20 times lower in a/a diploids than in haploids and a/a and α/α diploids (Elder et al., 1980). This regulation is quantitative rather than qualitative; full-length transcripts

Figure 1. Restriction Map of the SPT3 Locus

The top line represents SPT3 DNA and its restriction sites. The three lines below represent the subcloned fragments. These were tested for SPT3 function after transformation into the spt3 mutant strain 8582-28.

Figure 2. Northern Analysis of Ty Transcripts in SPT3 and spt3 Strains

(a) Northern analysis of Ty RNA in SPT3 and spt3 strains. Poly(A) RNA was isolated from SPT3 (SS248-1A) and spt3 (KD1581C) strains and Northern analysis was performed as described in Experimental Procedures. The probe was SPT3 plasmid, a Ty clone in pBR322.

(b) Northern analysis of HIS4-specific RNA in SPT3 and spt3 strains. Total RNA was isolated from the strains indicated in the figure and subjected to Northern analysis. The probe was pFW45, an internal fragment of HIS4 cloned in pBR322. HIS4 mRNA is 2.6 kb in length, his4-Δ5 mRNA is 2.6 kb in an SP73 strain and 2.8 kb in an spt3 mutant. The transcript in the SPT3 background appears as a diffuse band because it runs just above the SS5 ribosomal RNA. The lower band visible in lanes 1-4 is caused by the presence of 18S ribosomal RNA. The strains represented are (left to right): FW452, FW451, KD88-12C, KD88-2C, KD195-19C, and KD195-19C.
are still seen in the a/α cells. his4-Δ5 is under both SPT3 and MAT control; the spt3 mutation causes a change in size of the his4-Δ5 mRNA (Figure 2b), while the a/α genotype causes a reduction in the level of the transcript but no change in its size (not shown).

Another distinction between SPT3 and MAT control is that regulation by SPT3 does not require expression of α and α information. If the spt3 mutation acted on Ty indirectly, by allowing expression of the silent mating type cassettes at HML and HMR, expression of the spt3 mutant phenotype would require the presence of α and α alleles at these loci in a haploid. To test this possibility, we constructed strains of genotype HMLα MATα HMRα spt3. Since these strains are devoid of functional α information, they could not express αα control. In such strains, SPT3 is still required for the transcription initiation at 5α; these strains show suppression of his4-917β and produce the short form of the his4-Δ5 mRNA (Figure 2b, lanes 5 and 6). Thus SPT3 control of Ty transcription is distinct from MAT control.

Effects of spt3 on Adjacent Gene Transcription

The effect of spt3 on transcription of HIS4 and LYS2, when these genes are adjacent to Ty and δ sequences, was analyzed by Northern hybridization experiments. We examined two constructions where an spt3 mutation suppresses an insertion from − to +, his4-917δ and his4-917β, and a construction where spt3 causes a + to + change in phenotype, lys2-1/73R2 (Table 1).

For his4-917, a Ty insertion, there is no visible HIS4 transcript made in an SPT3 background (Figure 5). This result was expected since Ty917 is inserted just 5 bp upstream of the normal HIS4 transcriptional start site. In an spt3 mutant, an apparently normal size of HIS4 mRNA is made.

In his4-917β strains, HIS4 transcription occurs in both SPT3 and spt3 bands, but the transcripts differ in size. In the SPT3 strain, which is His+, the predominant HIS4 transcript is longer than the wild-type HIS4 transcript (Figure 6, lane 3). A transcript that initiated in the 9175 sequence and terminated at the normal HIS4 termination site would have the length observed. Such a transcript, based on the known DNA sequence (Roeder et al., 1980;
Donahue et al., 1982) would have both an AUG and a UAA codon in frame upstream of the HIS4 AUG. If the normal HIS4 AUG codon could not be used, then this transcript would be nonfunctional for HIS4 activity. In the spt3 his4-9176 mutant, which is His+, the predominant HIS4 transcript comigrates with the HIS4 wild-type transcript (Figure 6, lane 4). Therefore, the spt3 mutation allows an apparently normal HIS4 transcript to be made, even in the presence of the 9176 sequence.

Examination of an insertion mutation that fails to inhibit gene expression reveals that spt3 causes the same transcriptional effect, but results in a different phenotypic effect. lys2-173R2 is a Lys+ revertant of lys2-173, a Ty insertion in the 5' end of the LYS2 (Simchen et al., 1984). However, spt3 lys2-173R2 double mutants are Lys-.

Northern analysis of the spt3 effect on lys2-173R2 shows that a transcriptional change occurs that is qualitatively similar to the other cases studied (not shown).

**spt3 Mutants Are Defective in Diploid Formation and in Sporulation**

Other phenotypes not obviously related to Ty insertion mutations are also observed in spt3 mutants. spt3 strains form diploids at a greatly reduced frequency when mated by each other (Table 2). The frequency of diploid formation is reduced 5 to 10-fold when one parent is wild type and the other is spt3, and it is reduced nearly 100-fold when both parents are spt3. This has been tested for two different sets of parents carrying the spt3-101 frameshift mutation, with the same results obtained each time.

Table 2. Diploid Formation Frequency

<table>
<thead>
<tr>
<th>Cross</th>
<th>Strains</th>
<th>MAT</th>
<th>spt3</th>
<th>% Diploids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FW209</td>
<td>a</td>
<td>+</td>
<td>57.5</td>
</tr>
<tr>
<td>2</td>
<td>FW283</td>
<td>a</td>
<td>+</td>
<td>0.4</td>
</tr>
<tr>
<td>3</td>
<td>FW284</td>
<td>a</td>
<td>101</td>
<td>5.2</td>
</tr>
<tr>
<td>4</td>
<td>FW282</td>
<td>a</td>
<td>101</td>
<td>9.7</td>
</tr>
</tbody>
</table>

The frequency of diploid formation in spt3 mutants. The frequency of diploids formed was measured as described in Experimental Procedures. The percent diploids indicates the amount measured after 6 hr of mating.

Another phenotype of spt3 mutants is failure of spt3 homozygous diploids to sporulate. Heterozygous SPT3/spt3 diploids sporulate at a normal frequency, but spt3/spt3 homozygotes do not sporulate (Table 3). The cells in the sporulation culture appear not to have arrested in G1 as most of them are budded.

**Discussion**

Analysis of transcription of all Ty elements, of a specific Ty, and of solo 6s shows that the SPT3 gene is required for transcription initiation in 6 sequences (Figure 7). In the absence of SPT3 function, transcription fails to initiate in 6, and instead, initiates at a site downstream. This alteration of transcription can explain the suppression of Ty and 6 insertions in strains carrying spt3 mutations. It
Table 3. Sporulation Frequencies

<table>
<thead>
<tr>
<th>SPT3 Alleles</th>
<th>% Tetrads</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>20.3</td>
</tr>
<tr>
<td>+</td>
<td>32.1</td>
</tr>
<tr>
<td>101</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>101</td>
<td>32.1</td>
</tr>
<tr>
<td>+</td>
<td>22.8</td>
</tr>
<tr>
<td>+</td>
<td>32.1</td>
</tr>
</tbody>
</table>

Sporulation in spt3 mutants. Sporulation was measured as described in Experimental Procedures. All diploids had the same genotype with the exception of the SPT3 alleles as indicated. The four diploids came from the parents FW282, FW283, FW284, and FW299.

Figure 7. Model for SPT3 Function

SPT3 function is necessary for initiation of transcription in δ sequences of (a) Ty elements, (b) a Ty-HIS4 fusion, hisΔ5 and (c) solo δ insertions.

also suggests an explanation for the other phenotypic effects of these mutations.

The effect of spt3 mutations on Ty-specific transcription is due to loss of SPT3 function as shown by studies of null mutations. Spontaneous spt3 mutations were isolated based on their ability to suppress insertion mutations in the 5' noncoding region of HIS4 (Winston et al., 1984). In vitro-constructed spt3 frameshift and deletion mutations result in the same suppressor phenotypes as the spontaneous mutations.

Suppression of Ty and δ Insertions

We have examined the effect of spt3 mutations on total Ty transcription, as well as on the transcription of a number of insertion mutations involving Ty or δ elements (his4-917, his4-917δ, his4-912δ (Silverman and Fink, 1984), his4-Δ5, and lys2-17382). In all cases (except for his4-917, which is discussed below), the same qualitative transcriptional effect is observed. In SPT3 strains, transcription initiates predominantly in a δ sequence; in spt3 mutants, transcription fails to initiate in the δ and initiates further downstream. For Ty elements and his4-Δ5, we have identified the new site of initiation by S1 mapping. For the other insertions, the size of the transcripts determined by Northern analysis is consistent with this interpretation. Elder et al. (1983) observed that a minor fraction of Ty RNA made in wild-type strains is shorter at the 3' end. This 3' heterogeneity is unrelated to the spt3 effects we have studied.

The absence of a δ transcript can explain the suppression of solo δ insertions by spt3. In SPT3 strains, the transcript initiating in the δ is most likely nonfunctional because there are translational starts and stops prior to the normal initiation codon of the HIS4 coding sequence. In spt3 strains, the transcript fails to initiate in the δ and instead begins closer to the site of translation initiation. This shorter transcript is functional because it contains the normal AUG for translation initiation and lacks the upstream starts and stops found in the larger transcript.

The mechanism of spt3 suppression of a complete Ty insertion may be different from that of a solo δ. The HIS4 mRNA, which is missing in a his4-917 SPT3 strain, is made in the spt3 mutant (Figure 5). This change in transcription of HIS4 may be caused indirectly as the result of alteration of Ty917 transcription.

Ty and δ insertions are structurally similar to avian leukemia virus (ALV) insertions upstream of the c-myc gene. ALV-induced bursal lymphomas are thought to be caused by transcriptional activation of the c-myc gene. In some cases activation occurs by promoter insertion, utilizing the ALV LTR sequences (Hayward et al., 1981; Payne et al., 1982). This transcription from inserted retroviral sequences is analogous to transcription from the δ insertions in front of HIS4 and LYS2. In other cases, activation is less direct, as when an ALV provirus is inserted upstream of c-myc in the transcriptionally opposite orientation (Payne et al., 1982; Corcoran et al., 1984). This activation by complete retroviral proviruses is analogous to activation by Ty insertions. Perhaps some ALV insertions are under regulation of cellular genes, analogous to SPT3 regulation of δ and Ty insertion mutations.

The Activity of the SPT3 Gene Product

The SPT3 gene product is not necessary for transcription of its target gene. Rather, it determines the site of initiation of transcription. In this way it resembles the α subunit of procaryotic RNA polymerases that determine specificity for promoters, as in E. coli (Burgess et al., 1969) or for specific classes of promoters, as in B. subtilis (reviewed by Neidig and Porin, 1981).

The SPT3 gene product could involve direct interaction with the δ sequence to make its promoter or initiation site functional. Regulation by binding to DNA has been shown for Sp1 factor for transcription from the early promoter of SV40 (Dyman and Tjian, 1993) and I IS17 for transcription of the Drosophila hsp70 gene (Parker and Topol, 1984). Another mechanism by which a cellular gene regulates transcription of an autonomous element is via methylation; the Dam methylase of E. coli inhibits transcription of the IS10R transposase gene by methylation of its promoter (Kleckner et al., 1984).

Alternatively, the SPT3 gene product could act indirectly, by regulating the expression of other genes whose prod-
ucts are required for Ty transcription. This indirect control does not involve expression of a and α information because Ty transcription is still altered by the spt3 mutation in cells that contain only α information at MAT, HML, and HMR.

Other spt3 Phenotypes
Other phenotypes for spt3 mutants that are not obviously related to Ty insertions include failure to form diploids when both parents are spt3 and failure of spt3 homozygous diploids to sporulate. It is possible that all of the genes responsible for these other phenotypes are next to Ty or 6 sequences and are therefore under SPT3 control. Errede et al. (1980) proposed that Ty elements control several genes involved with mating. Alternatively, SPT3 may recognize transcription signals that are present in both α and α genes. However, the test of these undefined genes could be the major role of SPT3. Finally, the observed phenotypes could be indirect effects of a major alteration of the cell’s transcription pattern.

Experimental Procedures

Yeast Strains and General Genetic Methods
All yeast strains are listed in Table 1 and were constructed for these studies. We have used standard genetic nomenclature: capital letters indicate the wild-type allele for a gene and lowercase letters indicate a mutant allele for the same gene. Standard yeast genetic procedures of crossing, sporulation, and tetrad analysis were followed as described by Mortimer and Hawthorne (1969) and Sherman et al. (1978). We have used standard genetic nomenclature: capital letters indicate the wild-type allele (for example, SPT3) and lowercase letters indicate a mutant allele.

Table 1. Yeast Strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>BY522-9</td>
<td>MATα his4-9176 spo3-1 ade2-1 ura3-52 isol1 iso4</td>
</tr>
<tr>
<td>S224-1A</td>
<td>MATα cry1 his4-Δ5 arg11</td>
</tr>
<tr>
<td>KD158-1C</td>
<td>MATα cry1 his4-Δ5 spo5-101 lys2 arg11</td>
</tr>
<tr>
<td>FW452</td>
<td>MATα leu2-1</td>
</tr>
<tr>
<td>FW451</td>
<td>MATα spo3-101 leu2-1</td>
</tr>
<tr>
<td>KD88-12C</td>
<td>MATα his4-Δ5 arg11</td>
</tr>
<tr>
<td>KD88-1C</td>
<td>MATα his4-Δ5 arg11</td>
</tr>
<tr>
<td>KD195-18C</td>
<td>MATα his4-Δ5 HMLα HMRα ura3-52 met4</td>
</tr>
<tr>
<td>KD195-19C</td>
<td>MATα his4-Δ5 HMLα HMRα his4-9176 spo3-101 his1</td>
</tr>
<tr>
<td>FW712</td>
<td>MATα his4-917 lys2-173R2 ura3-52 leu2-1</td>
</tr>
<tr>
<td>FW711</td>
<td>MATα his4-917 spo3-101 lys2-173R2 ura3-52 leu2-1</td>
</tr>
<tr>
<td>FW278</td>
<td>MATα his4-917 leu2-1</td>
</tr>
<tr>
<td>FW292</td>
<td>MATα his4-917 spo3-101 leu2-1</td>
</tr>
<tr>
<td>FW282</td>
<td>MATα his4-917 spo3-101 ura3-52</td>
</tr>
<tr>
<td>FW283</td>
<td>MATα his4-917 leu2-1 ade2-1</td>
</tr>
<tr>
<td>FW294</td>
<td>MATα his4-917 spo3-101 leu2-1 ade2-1</td>
</tr>
<tr>
<td>FW299</td>
<td>MATα his4-917 ura3-52 iso4</td>
</tr>
</tbody>
</table>

Enzymes
All restriction enzymes, T4 DNA ligase, and DNA polymerase I were purchased from New England Biolabs and used as suggested by the supplier. DNAase I was purchased from Sigma. S1 and Exo VII were purchased from Bethesda Research Labs.

Plasmids
Plasmids used as probes were as follows: for SPT3, pFW24, a Hind III-Bgl II restriction fragment containing SPT3 cloned into plasmid pBR322; for HIS4, pFW45, a HIS4 internal Bgl II-Sal I restriction fragment in pBR322 and B66; a Sal I restriction fragment covering the 5' end of HIS4; for LYS2, pFW47, a LYS2 internal Bgl II-Xho I restriction fragment in pBR322; for Ty, S13 (Cameron et al., 1979) and B163, a Ty Bgl II restriction fragment in pBR322.(R. Surosky, B. K. Tye, and G. R. Fink, unpublished). Plasmids used for subcloning were YEp24 (Botstein et al., 1979) and pCGS42 (provided by Collaborative Research). The 11 cloning vectors were MGM11 and MGM2 (kindly provided by G. Voss, Collaborative Research).

DNA Preparations
Yeast DNA mini-preps for recovery of plasmids were done as described by Winston et al. (1983) using either 10 ml or 40 ml overnight cultures. E. coli plasmid mini-preps were done by the boiling method as described by Holmes and Gugli (1981). Large scale E. coli plasmid preparations were prepared and purified on ethidium bromide–cesium chloride gradients as described by Davis et al. (1980).

Restriction fragments were purified from agarose gels by the glass powder method of Vogelstein and Gillespie (1979).

Construction of spt3 Frameshift and Deletion Mutants
A Hind III-Bgl II restriction fragment containing SPT3 was subcloned into plasmid pFW44, which is identical with plasmid YEp6 (Struhl et al., 1979), except that it has no Sal I site in the pBR322 region. (F. W. G. and G. R. Fink, unpublished). The resulting plasmid, pFW30, contains a single Sal I site in the SPT3 gene. The plasmid was digested with Sal I, made completely double-stranded by using polyadenylated virus reverse transcriptase (Smith and Calvo, 1980), ligated, and transformed into E. coli. Plasmids that became fully double-stranded would have lost the Sal I site, gained a Pvu II site, and will have a 4 base insertion (+1 frameshift) in SPT3. Such plasmids were recovered and the spt3 mutation was designated spt3-101.

Other events also occurred, one of which was a deletion from near the Sal I site in SPT3 to near an Eco RI site outside the gene. This deletion was designated spt3-201. The frameshift and deletion mutations were recombinated into the yeast genome as described by Scherer and Davis (1979). The Uni+ segregants were identified after no inositol death enrichment by the method of Winston et al. (1983) adapted from Henry et al. (1975).

Agarose Gels
DNA restriction fragments were analyzed by fractionation on a 0.6% agarose gels. Gels were run in 0.5×TE buffer (Roeder and Fink, 1980).

Northern Hybridization Analysis
Cells for RNA prep were grown in supplemented SD medium to 1 x 107 cells/ml. Yeast RNA was prepared essentially as described by Carlson and Botstein (1982). To purify poly(A) RNA, RNA was loaded onto oligo(dT)–cellulose columns (Collaborative Research) in loading buffer (0.5 M LiCl, 0.01 M Tris, pH 7.4, 0.1% lithium dodecyl sulfate). The columns were washed with 2 x 10 ml of loading buffer. poly(A) RNA was then eluted with 4 ml of elution buffer (0.1 M Tris, pH 7.4, 0.05% SDS, 0.001 M EDTA) and concentrated by ethanol precipitation. Northern analysis was performed as described in the instructions for Gene Screen (New England Nuclear) by the method that uses deoxalin sulfate.

S1 Mapping
The procedure used was a variant of that described by Favaro et al. (1980). Restriction fragments from the 5' end of HIS4, his4-Δ5, and Ty12 were subcloned into T phage (Zinder and Boeke, 1982). Single-stranded phage DNA was mixed with total yeast RNA in hybridization buffer (20% formamide, 0.4 M NaCl, 0.04 M Pipes, pH 7.4, 0.001 M EDTA) and
hybridized overnight at 52°C. Hybridizations were digested 20-fold in ice-cold S1 buffer (0.25 M NaCl, 0.03 M NaAC, pH 4.6, 0.004 M ZnSO4, 20 μg/ml sheared, denatured calf thymus DNA) containing 60 U BRL S1 nuclease and incubated 30 min at 37°C. The hybrids were electrophoresed in 1.5% alkaline or neutral agarose gels, transferred to nitrocellulose, and probed using the Southern hybridization procedure described by Roeder and Fink (1980). For Exo VI analysis, plasmid DNA was nicked with DNAase I at 1 ng/ml for 15 min at 37°C in 0.125 M NaCl, 0.02 M MgCl2, 0.004 M Tris, pH 8.0, 0.03% BSA, and was phenol-extracted before being mixed with RNA. Exo VI digestion were performed at 37°C for 30 min in 0.03 M KCl, 0.01 M Tris, pH 8.0, 0.01 M EDTA containing 6 U BRL enzyme.

Measurement of Diploid Formation

The frequency of diploid formation was adapted from the filter-mating procedure of Duttcher and Hartwell (1983). The filters with the two parents were placed on YPD plates and incubated at 30°C for 6 hr. Dipsoids were measured by plating on selective media.

Measurement of Sporulation Frequency

Cultures were sporulated for 1 day at 23°C and 2 days at 30°C on solid sporulation medium. Sporulated cultures were examined by light microscopy in a hemacytometer. Sporulation frequency was determined by the sum of tetrads and unsporulated cells.

Acknowledgments

We thank Dave Garfinkel and Jet Boeke for critical reading of the manuscript, and Tam Brodick for its preparation. K. J. D. was supported by a National Research Service Award 5 T32 GM072773-10 granted to the Section of Biochemistry, Molecular and Cell Biology of Cornell University. This work was supported by National Institutes of Health grants GM32967 to F. W. and CA34429 and GM18012 to G. R. F., an American Cancer Society Research Professor of Genetics.

The costs of publication of this article were defrayed in part by the advertisement in accordance with 18 U.S.C. Section 1734 solely to support the costs of publication of this article. This work was supported by National Institutes of Health grants GM32967 to F. W. and CA34429 and GM18012 to G. R. F., an American Cancer Society Research Professor of Genetics.

Received September 11, 1984; revised October 9, 1984

References


251 pp.


