Mutations in *SPT16/CDC68* Suppress *cis*- and *trans*-Acting Mutations That Affect Promoter Function in *Saccharomyces cerevisiae*

ELIZABETH A. MALONE,[†] CHRIS D. CLARK,[‡] ANNE CHIANG, AND FRED WINSTON*

Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115

Received 3 July 1991/Accepted 26 August 1991

***SPT16* was previously identified as a high-copy-number suppressor of δ insertion mutations in the 5' regions of the *HIS4* and *LYS2* genes of *Saccharomyces cerevisiae*. We have constructed null mutations in the *SPT16* gene and have demonstrated that it is essential for growth. Temperature-sensitive-lethality *spt16* alleles have been isolated and shown to be pleiotropic; at a temperature permissive for growth, *spt16* mutations suppress δ insertion mutations, a deletion of the *SUC2* upstream activating sequence, and mutations in *trans*-acting genes required for both *SUC2* and Ty expression. In addition, *SPT16* is identical to *CDC68*, a gene previously shown to be required for passage through the cell cycle control point START. However, at least some transcriptional effects caused by *spt16* mutations are independent of arrest at START. These results and those in the accompanying paper (A. Rowley, R. A. Singer, and G. C. Johnston, Mol. Cell. Biol. 11:5718–5726, 1991) indicate that *SPT16/CDC68* is required for normal transcription of many loci in *S. cerevisiae*.**

A large number of genes that are required for normal transcription in *Saccharomyces cerevisiae* have been isolated as suppressors of Ty and δ insertion mutations in the 5' regions of the *HIS4* and *LYS2* genes (21, 65, 66). The insertion mutations alter *HIS4* or *LYS2* transcription and result in His[−] or Lys[−] phenotypes. In all cases that have been studied, the suppressor mutations (designated *spt*; suppressor of Ty) restore some level of functional transcription (for examples, see references 15, 18, 21, and 67). Most *spt* mutations have additional effects on gene expression, and many *SPT* genes are essential for growth, suggesting that they play general roles in transcription (for examples, see references 16, 18, 29, and 41).

Genetic and molecular analysis has shown that three *SPT* genes encode proteins that have been analyzed biochemically. *SPT15* encodes the TATA-binding factor TFIID (18), and *SPT11* and *SPT12* are the same as the genes *HTA1* and *HTB1* that encode the histone proteins H2A and H2B (15). The phenotypes of *spt15* mutants and *htal-htb1* mutants differ with respect to their suppression of different Ty and δ insertion mutations and their effects on mating, sporulation, and expression of other genes (15, 18, 28). These phenotypes are representative of two distinct classes of *spt* mutations that may alter transcription in different ways.

Two different approaches have identified alterations in the *SPT11* (*HTA1*)-*SPT12* (*HTB1*) locus as suppressors of insertion mutations. First, *spt11* (*htal*) and *spt12* (*htb1*) mutations were isolated as suppressors of the insertion mutations *his4-912 δ* and *lys2-128 δ* (21). Second, the wild-type *HTA1-HTB1* locus was cloned as a high-copy-number suppressor of *his4-912 δ* and *lys2-128 δ* (15). These results led to the model that changes in the stoichiometry of histone proteins result in altered transcription in *S. cerevisiae*.

In addition to the *HTA1-HTB1* locus, three other genes

that cause an Spt[−] phenotype when their dosage is elevated have been identified. Two of these genes, *SPT5* and *SPT6*, have been extensively characterized elsewhere (16, 62, 63). Mutations in these genes confer many phenotypes similar to those conferred by *htal-htb1* mutations (15, 26, 28, 41, 61). The third gene, *SPT16*, was initially identified as a high-copy-number suppressor of δ insertion mutations (referred to as class 1 in reference 15) and was not characterized further.

Since *SPT16* shares the high-copy-number suppression phenotype with *SPT5*, *SPT6*, and *HTA1-HTB1*, we have analyzed *SPT16* in greater detail. By isolation and analysis of *spt16* null mutations and temperature-sensitive mutations, we have shown that *SPT16* is essential for growth. At the permissive temperature, the temperature-sensitive *spt16* mutations cause suppression of *his4-912 δ* and *lys2-128 δ* and partially suppress *cis*- and *trans*-acting defects in *SUC2* gene expression, similar to *htal-htb1* mutations. Genetic mapping and complementation analysis demonstrate that *SPT16* is the same as the previously identified *CDC68* gene (44, 49). Mutations in *SPT16/CDC68* have previously been shown to cause cell cycle arrest at the control point START (27, 44). However, our results demonstrate that at least some transcriptional alterations caused by *spt16* mutations are independent of cell cycle arrest at START. These results and those in the accompanying manuscript (49) suggest that *SPT16/CDC68* plays a critical role in the transcription of several genes in *S. cerevisiae* and that its function may be related to those of other *SPT* genes.

MATERIALS AND METHODS

Strains and genetic methods. The *S. cerevisiae* strains used are listed in Table 1. With the exceptions of CG379 (obtained from C. Giroux), YPH148 (25), and ART68-1 (obtained from A. Rowley and G. C. Johnston), all strains were constructed in this laboratory and were derived from S288C (*MAT α gal2*). In designating the genotypes, parentheses indicate autonomous plasmids and brackets indicate chromosomal fragments. The *his4-912 δ* mutation and the *lys2-128 δ* muta-

* Corresponding author.

[†] Present address: Department of Genetics, University of Washington, Seattle, WA 98195.

[‡] Present address: Department of Genetics, Stanford University, Stanford, CA 94305.

TABLE 1. Yeast strains

Strain	Genotype
FW1237	<i>MATa his4-9128 lys2-1288 ura3-52</i>
CC131	<i>MATa/MATa his4-9128/his4-9128 lys2-1288/lys2-1288 ura3-52/ura3-52</i>
BM60	<i>MATa/MATa his4-9128/his4-9128 lys2-1288/lys2-1288 ura3-52/ura3-52 leu2-3 leu2-112/leu2-3 leu2-112 trp1Δ1/trp1Δ1</i>
BM72	<i>MATa spt16-101::LEU2 his4-9128 lys2-1288 ura3-52 leu2-3 leu2-112 trp1Δ1</i> (pCC58)
FY56	<i>MATa his4-9128 lys2-1288 ura3-52</i>
LS77	<i>MATa spt16-197 his4-9128 lys2-1288 ura3-52</i>
FY120	<i>MATa his4-9128 lys2-1288 ura3-52 leu2Δ1</i>
FY352	<i>MATa his4-9128 lys2-1288 spt16-197 leu2Δ1</i>
FY98	<i>MATa ura3-52 leu2Δ1</i>
BM361	<i>MATa spt16-197 his4-9128 ura3-52 leu2Δ1 ade8</i>
BM390	<i>MATa spt16-197 his4-9128 lys2-1288 ura3-52 leu2Δ1</i>
BM470	<i>MATa his4-9128 lys2-1288 snf2Δ1::HIS3 his3Δ200 ura3-52</i>
BM493	<i>MATa his4-9128 lys2-1288 spt16-197 snf2Δ1::HIS3 ura3-52 leu2Δ1</i>
YP148	<i>MATa ura3-52 lys2 ade his7 trp1Δ</i> [CF/URA3/RAD2 distal] [CF/TRP1/RAD2 proximal]
CC236	<i>MATa SPT16-43::Tn10-LUK his4-9128 lys2-1288 ura3-52</i>
CG379	<i>MATa ade5 his7-2 leu2-3 leu2-112 trp1-289 ura3-52</i>
CC235	<i>MATa SPT16-43::Tn10-LUK his4-9128 lys2-1288 ura3-52</i>
CC258	<i>MATa lys5 ura3-52</i>
ART68-1	<i>MATa cdc68-1 ade1 ade2 ura3-52 leu2-3 leu2-112</i>

tion have been previously described (16, 20). Null mutations in *SNF* genes have been previously described (1, 12, 13, 19). The mutation *suc2ΔUAS*(-1900/-390) contains a deletion of the *SUC2* 5' sequence from -1900 to -390 with respect to the *SUC2* translation initiation site (50). Standard methods for crosses, sporulation, and tetrad analysis were used (38, 53). Yeast cells were transformed by the lithium acetate method (33).

Escherichia coli HB101 and TB1 were used as hosts for plasmids. These strains were transformed by the CaCl_2 method (37).

Media. The media used include rich medium (YPD), synthetic complete medium (SC), minimal medium (SD), sporulation medium and presporulation medium (47). SC lacking amino acids or other nutrients (e.g., SC-ura lacks uracil) was used to score auxotrophies and to select transformants. Medium containing 1 mg of 5-fluoro-orotic acid (PCR, Gainesville, Fla.) per ml was used to select cells that had lost the *URA3* gene (4). YPsuc and YPrf are media containing 2% sucrose or raffinose and 1 μg of antimycin A (Sigma, St. Louis, Mo.) per ml and were used to determine the ability of mutants to use sucrose and raffinose as carbon sources (11).

DNA preparation and analysis. Plasmid DNA was isolated from yeast cells as described previously (30). *E. coli* plasmid DNA was prepared by the alkaline lysis method (3). DNA restriction fragments were separated in SeaKem agarose (FMC Bioproducts, Rockland, Maine) and purified by electroelution. Southern hybridization analysis was performed as previously described (46, 58). Plasmids used as probes were radiolabelled with [α - ^{32}P]dATP (Amersham, Arlington Heights, Ill.) by nick translation (45) or random priming (23,

FIG. 1. Map of the *SPT16* gene. Each triangle represents a single *Tn10*-LUK insertion. Arrows indicate the relative orientations of the insertion elements. Triangles above the line indicate insertions that abolish or reduce high-copy-number suppression, and triangles below the line represent those that had no effect. *Tn10*-LUK insertions 14, 44, and 45 totally eliminated the high-copy-number suppression phenotype of pCC48. Insertion 37 moderately reduced suppression, and insertion 16 weakly reduced suppression. The extent and orientation of the *SPT16* open reading frame as determined by Rowley et al. (49) is indicated by the long arrow. Shown are the *Bam*HI sites used in subcloning, the *Cla*I site used in mapping the *Tn10*-LUK insertions, the *Sal*I site used in *Tn10*-LUK mapping and for construction of *spt16-101::LEU2*, and the *Sma*BI site used to integrate *spt16-197*.

24) with kits from Boehringer Mannheim (Indianapolis, Ind.).

Enzymes. Restriction enzymes were purchased from New England Biolabs (Beverly, Mass.) or Boehringer Mannheim. DNA ligase was purchased from New England Biolabs, and calf intestinal alkaline phosphatase was from Boehringer Mannheim.

Plasmids. A 4.6-kb *Bam*HI restriction fragment that contains *SPT16* (Fig. 1) was subcloned into a number of vectors: for pCC48, the high-copy-number vector YEp24 (6); for pCC58 and pCC59, the *URA3*-containing centromere vector YCp50 (34); for pBM8, a YIp5 derivative lacking *Sal*I sites, pFW4 (59, 64); for pBM13, the *TRP1*-containing centromere vector pRS314 (56); and for pCC62, the vector pBR322. pSC2-1 contains the *CDC68* gene in the vector YEp24 (44). pBM46 is a derivative of pBM13 that contains *spt16-197*.

Plasmids used as hybridization probes were as follows: for *LYS2*, pFW47 (a *Bgl*III-*Xho*I restriction fragment internal to *LYS2* cloned in pBR322) and pFW112 (an *Eco*RI-*Bgl*III restriction fragment spanning the 5' end of *LYS2* cloned in pBR322) (16); for Ty, B161 (a *Bgl*III fragment internal to Ty cloned in pBR322) (66); for *TUB2* (encoding β -tubulin), pYST138 (57); and for *SPT16*, pCC62 (described above).

Isolation and analysis of *Tn10*-LUK insertion mutations. *Tn10*-LUK mutagenesis (32) was used to generate transposon insertions in pCC48. Restriction digests with *Bam*HI, *Sal*I, *Sma*I, and *Cla*I were used to localize the insertions (Fig. 1). To determine which *Tn10*-LUK insertions affected *SPT16* function, plasmids containing *Tn10*-LUK insertion mutations were transformed into FW1237, and *Ura*⁺ transformants were screened for high-copy-number suppression of δ insertion mutations.

Four of the *Tn10*-LUK insertions that affect the high-copy-number suppression phenotype (insertions 37, 16, 14, and 45; Fig. 1) were recombined into the yeast genome, replacing *SPT16*⁺ (48). To do this, *Bam*HI restriction fragments containing the *SPT16* gene disrupted by a *Tn10*-LUK insertion were purified and used to transform the diploid strain CC131 to *Ura*⁺.

Construction and analysis of *spt16-101::LEU2*. An *spt16*

null allele marked by the *LEU2* gene, *spt16-101::LEU2*, was constructed by inserting a 2.2-kb *SalI-XhoI* restriction fragment containing the *LEU2* gene at the *SalI* site of pBM8, creating pBM10. To determine the phenotype caused by this *LEU2* disruption allele, the *BamHI* fragment containing *spt16-101::LEU2* was used to replace one copy of the wild-type gene in diploid strain BM60 by homologous recombination (48), and the effect of *spt16-101::LEU2* was determined after sporulation and tetrad dissection.

Identification of conditional *spt16* mutations. The plasmid shuffle method (5) was used to identify *spt16* conditional-lethal mutations. pBM13, a low-copy-number plasmid containing *SPT16* and *TRP1*, was mutagenized for 24 h in 7% hydroxylamine (47). *S. cerevisiae* BM72, which contains *spt16-101::LEU2* and the autonomous *SPT16 URA3* plasmid pCC58, was transformed with the mutagenized pBM13. Trp⁺ transformants were replica plated to 5-fluoro-orotic acid plates that were incubated at 15, 30, and 37°C to screen for mutants that could survive loss of the *URA3 SPT16* plasmid at 30°C but not at 15 or 37°C.

Gene replacement of *SPT16*⁺ with *spt16-197(ts)*. The *spt16-197* conditional-lethal allele was integrated into the chromosome by the two-step gene replacement method (51). The *XhoI* restriction fragment of pBM46, which contains the *spt16-197* allele, was subcloned into the integrating vector YIp5, creating pAC4. This plasmid was linearized with *SnaBI* to direct integration to the *SPT16* locus and used to transform strain FY56. Ura⁺ transformants were purified on YPD, streaked on 5-fluoro-orotic acid plates to select for cells that had lost the *URA3* gene because of recombination between the flanking *SPT16* sequences, and screened for the temperature-sensitive and Spt⁻ phenotypes of *spt16-197*. The correct integration of *spt16-197* in strain L577 was confirmed by Southern analysis, tetrad analysis, and complementation by plasmid pCC59.

RNA preparation and analysis. For analysis of *LYS2* RNA, strains were grown in supplemented SD at 30°C to early log phase (approximately 5×10^6 cells per ml). For analysis of Ty transcripts, cells were grown at 30°C in YPD to 1×10^7 to 2×10^7 cells per ml. When necessary, cells were arrested at G₁ by the addition of 5 μ M α -factor (Sigma) or at mitosis by the addition of 10 μ M nocodazole (Sigma) per ml and grown at 30°C for an additional division cycle (3 h). The efficiency of arrest was monitored by observing cell morphology by phase-contrast microscopy. Cultures were transferred to 37°C for 30 min to observe the effects of the *spt16-197* temperature-sensitive allele. RNA preparation (10) and Northern (RNA) hybridization analysis (63) were performed as previously described. The amounts of RNA were normalized by hybridization to pYST138 (*TUB2*).

Mapping of *SPT16*. The chromosome on which *SPT16* resides was determined in several steps. Initially, intact yeast chromosomes were prepared (52) and separated by field inversion gel electrophoresis (9). The DNA was transferred to nitrocellulose by capillary blotting, and the filter was probed with ³²P-labelled pCC62 (58). This *SPT16* probe hybridized to a doublet composed of chromosomes VII and XV. To distinguish between these chromosomes, intact chromosomes were prepared from strain YPH148, in which chromosome VII is fragmented at *RAD2* to allow resolution of chromosomes VII and XV (25). After chromosome separation by field inversion gel electrophoresis and transfer to nitrocellulose, pCC62 hybridized to both fragments of chromosome VII, since they contain pBR322 sequences as a result of the fragmentation procedure (25), and not to chromosome XV. Therefore, *SPT16* is on chromosome VII.

Tetrad analysis was used to demonstrate that *SPT16* is genetically linked to markers on chromosome VII. To monitor *SPT16* segregation in these crosses, we used *SPT16-43::Tn10-LUK*, an insertion adjacent to the *SPT16* gene that apparently does not affect its function, and scored segregation of the *URA3* gene in Tn10-LUK. Linkage of *spt16-197* to *cdc68-1* was determined by segregation of the temperature-sensitive lethality conferred by each mutation.

RESULTS

***SPT16* is essential for growth.** *SPT16* was identified as a high-copy-number suppressor of *his4-9128* and *lys2-1288* (15). To study further the role of *SPT16*, we have constructed and characterized two types of *spt16* null mutations. First, to localize the *SPT16* gene in the cloned DNA, we used Tn10-LUK transposon insertion mutagenesis (32) to disrupt *SPT16*. Seven independent insertions in the 4.6-kb *SPT16* insert were obtained. Five reduced or eliminated the high-copy-number suppression phenotype, while two had no effect (Fig. 1). Second, an *spt16* null mutation was constructed by inserting the *LEU2* gene at a *SalI* site that is within the *SPT16* coding region, as determined by DNA sequence analysis (49). This *LEU2* insertion also eliminated high-copy-number suppression.

To study the phenotypes conferred by these *spt16* null mutations, four of the Tn10-LUK insertion mutations that affect the *SPT16* high-copy-number suppression phenotype and the *LEU2* insertion were each recombined into diploid strains homozygous for *his4-9128* and *lys2-1288*, replacing one copy of *SPT16*⁺. All of these *SPT16*⁺/*spt16* null heterozygotes were His⁻ Lys⁻, demonstrating that the null mutations are recessive. To determine the *spt16* null phenotype in haploid strains, we sporulated and dissected these diploids. In most tetrads (109 of 141), viability segregated 2:2, and in the remainder, fewer than two spores germinated. All of the viable spores were *SPT16*⁺, demonstrating that *SPT16* is essential for growth.

Isolation and analysis of temperature-sensitive *spt16* mutations. Since *spt16* null mutants are inviable, we screened for *spt16* conditional-lethal mutations, as described in Materials and Methods. After screening approximately 20,000 transformants, we identified 53 that exhibited temperature-sensitive lethality. All of these candidates were also His⁺ and Lys⁺ at 30°C, indicating that *his4-9128* and *lys2-1288* were suppressed at a temperature permissive for growth. The plasmids were purified from 12 of the candidate strains, and the plasmids were retransformed into yeast cells. The temperature-sensitive and Spt⁻ phenotypes were linked to the plasmids in 10 of the 12 cases tested. One *spt16* temperature-sensitive allele, *spt16-197*, was recombined into the yeast genome, replacing *SPT16*⁺ (see Materials and Methods). The resulting strain was also His⁺ Lys⁺ and temperature sensitive for growth (Fig. 2). Thus, suppression of *his4-9128* and *lys2-1288* can result from either *spt16* mutations or increased copy number of the wild-type *SPT16* gene.

Other δ and Ty insertion mutations were tested for suppression by an *spt16* mutation and by increased copy number of *SPT16*⁺. The results showed that only *his4-9128* and *lys2-1288* are strongly suppressed (Table 2). This pattern is the same as that observed for *spt4*, *spt5*, and *spt6* mutations (21, 65). A high-copy-number *SPT16* plasmid weakly suppresses *his4-917*, a Ty insertion mutation, in addition to *his4-9128* and *lys2-1288* (Table 2). This suppression pattern also results from mutations in *HTA1-HTB1* (Table 2) (15, 21, 28). Mutations in other *SPT* genes, for instance, *SPT13*/

FIG. 2. Suppression of *his4-9128* by *spt16* and high-copy-number *SPT16*⁺. Yeast strains were replica plated from an SC-ura plate to a plate lacking histidine (SC-ura-his) and grown for 2 days. The strains shown are FY120 transformed with the high-copy-number vector YEp24 (A), FY120 transformed with the high-copy-number *SPT16* plasmid pCC48 (B), FY120 transformed with the low-copy-number vector YCp50 (C), FY120 transformed with the low-copy-number *SPT16* plasmid pCC58 (D), and FY352 (E).

GAL11 and *SPT15*, suppress overlapping but distinct sets of Ty and δ insertion mutations (Table 2) (18, 21).

***spt16-197* suppresses δ insertion mutations by altering transcription.** All *spt* mutations that have been examined suppress insertion mutations at the transcriptional level. To determine whether this is true in *spt16* mutants, we used Northern hybridization analysis to compare transcription of *lys2-1288* in *SPT16* and *spt16-197* strains.

Our results (Fig. 3) show that *spt16-197* causes an alteration in transcription of *lys2-1288*. The *lys2-1288* mutation contains a solo δ element in the 5' coding region of the *LYS2* gene (16). In *SPT*⁺ strains, transcription initiates at the *LYS2* initiation site and results in a transcript of about 580 bases, consistent with termination at the δ termination site (14, 16) (Fig. 3A, lane 3). RNA from an *spt16-197 lys2-1288* strain was examined after growth at 30°C and subsequent transfer to 37°C. In the *spt16-197* mutant, there is a second transcript slightly shorter than the wild-type *LYS2* mRNA, in addition to the 580-bp transcript (Fig. 3A, lanes 4 to 10). The size is consistent with initiation in the δ element and elongation through the *LYS2* open reading frame (Fig. 3B). Although the *spt16* mutant is Lys⁺ at the permissive temperature, there is a dramatic increase in the amount of the long *LYS2* transcript after growth at 37°C for 30 min, and the amount of this transcript remains at the same elevated level with incubation at high temperature for up to 6 h (Fig. 3A, lanes 5 to 10). Although *spt16-197* causes cells to arrest in G₁ after

FIG. 3. Effect of an *spt16* mutation on transcription of *lys2-1288*. (A) Total yeast RNA was subjected to Northern analysis. Cells used for RNA preparation were grown at 30°C (lanes 1 to 4) or grown at 30°C and transferred to 37°C for the indicated times (lanes 5 to 10). The same filter was hybridized with the *LYS2* probes pFW47 and pFW112 and with the *TUB2* probe pYST138. Since the smaller *LYS2* RNA is much more abundant, we used a pFW47 probe that contained approximately 10-fold more counts per minute than the pFW112 probe. The two additional bands are due to rRNA. The strains used were FY98 (lane 1), BM361 (lane 2), FY120 (lane 3), and BM390 (lanes 4 to 10). (B) The top line depicts the structure of *lys2-1288*. The gray box represents the *LYS2* coding region. The thin line represents flanking DNA. The solo δ insertion is represented by the box with the solid triangle. Relative positions of the upstream activating sequence (UAS) and TATA boxes are indicated. The arrows below illustrate potential origins of transcripts in *SPT16*⁺ and *spt16* strains.

TABLE 2. Suppression of insertion mutations by alterations in *SPT16*

Insertion mutation	Growth with ^a :					
	Wild type	<i>spt16</i>	2μm <i>SPT16</i> ^b	<i>htal</i> ^c	<i>spt13</i> ^c	<i>spt15</i> ^c
<i>his4-9128</i>	—	+	+	+	—	+
<i>his4-9178</i>	—	—	—	—/+	—	+
<i>lys2-1288</i>	—	+	+	+	—	+
<i>his4-912</i>	—	—	—	ND	—	—
<i>his4-917</i>	—	—	—/+	+	+	+
<i>lys2-61</i>	—	—	—	ND	+	ND
<i>lys2-173R2</i>	+	+	+	+	ND	—

^a +, —/+, and —, strong growth, weak growth, and lack of growth, respectively, on media lacking histidine for *his4* alleles and lacking lysine for *lys2* alleles; ND, not done.

^b An *SPT*⁺ strain was transformed with pCC48.

^c Suppression patterns have been previously characterized for *htal* (*spt11*) (15, 21), *spt13* (21), and *spt15* (18) and are shown here for comparison.

approximately 4 h at 37°C (see below), there was little change in viability of the cells over the course of this experiment. *spt16-197* also alters *his4-9128* transcription such that a functional *HIS4* transcript is produced (36). The transcription patterns of *lys2-1288* and *his4-9128* in *spt16* mutants are similar to those observed previously in *spt4*, *spt5*, *spt6*, and *htal-htb1* mutants (15, 16, 36, 63).

Other transcriptional defects are suppressed by *spt16-197*. Since *spt16-197* strains have phenotypes similar to those of *spt4*, *spt5*, *spt6*, and *htal-htb1* mutants, we tested whether *spt16-197* strains share another phenotype with these mutants: suppression of *snf2*, *snf5*, and *snf6* mutations (16, 28,

TABLE 3. Suppression of *snf* mutations by *spt16*^a

<i>snf</i> mutation	Growth on YPsuc ^b	
	<i>SPT16</i> ⁺	<i>spt16-197</i>
<i>SNF</i> ⁺	+	+
<i>snf1Δ10</i>	—	—
<i>snf4Δ2</i>	—	—
<i>snf2Δ1::HIS3</i>	—	+
<i>snf5-5::URA3</i>	—	+
<i>snf6Δ2</i>	+/-	+
<i>suc2ΔUAS(-1900/-390)</i>	—	+

^a The ability to use sucrose as a carbon source was determined by replica plating colonies to YPsuc plates and scoring growth after 1 day at 30°C. Similar results were seen after 3 days with YPrf plates, but the *snf spt16-197* and *suc2ΔUAS spt16-197* double mutants did not grow quite as well as the wild type.

^b +, +/-, and —, strong growth, moderate growth, and no growth, respectively.

41, 42). Strains that carry *snf* mutations are unable to transcribe *SUC2* and hence cannot use sucrose or raffinose as carbon sources (40). *SNF2*, *SNF5*, and *SNF6* are distinct from *SNF1* and *SNF4* on the basis of both genetic and biochemical criteria (12, 13, 40, 42). Our results show that *spt16-197* suppresses the defect in *SUC2* expression in *snf2*, *snf5*, and *snf6* mutants but not in *snf1* and *snf4* mutants (Table 3). In addition, a deletion of the *SUC2* upstream activating sequence that causes a Suc⁻ phenotype and is suppressed by mutations in *SPT5*, *SPT6*, and *HTA1-HTB1* (28, 41, 61) is also suppressed by *spt16-197* (Table 3).

In addition to its role in *SUC2* expression, *SNF2* is required for transcription of Ty elements (26). To determine whether an *spt16* mutation suppresses this requirement, we used Northern analysis to examine Ty transcripts in *snf2* mutants. A low-level full-length Ty mRNA was detected in a *snf2 spt16-197* strain grown at 30°C, and there was an increased amount of Ty message detected after growth at 37°C for 30 min (Fig. 4, lanes 7 and 8). Thus, an *spt16* mutation, like one previously characterized class of *spt* mutations, partially bypasses the requirement for proteins involved in transcriptional activation. In a *SNF*⁺ background, *spt16-197* has no effect on Ty transcription (Fig. 4, lanes 1 to 4). Again, this is similar to *spt4*, *spt5*, *spt6*, and *hta1-htb1* mutants. In contrast, some other *spt* mutations eliminate Ty transcription (66, 67).

***SPT16* is identical to *CDC68*.** We determined the *SPT16* genetic map position in two steps. First, we determined that *SPT16* is on chromosome VII on the basis of hybridization of an *SPT16* probe to separated yeast chromosomes (see Materials and Methods). Second, tetrad analysis showed that *SPT16* is 19 centimorgans from *ADE5* and 41 centimorgans from *LYS5* on the left arm of chromosome VII (Table 4), a position similar to that of the *CDC68* gene (39, 44). The *cdc68-1* allele was identified as a temperature-sensitive mutation that causes arrest of cell division at START in G₁ (44).

Molecular and genetic data demonstrated that *SPT16* and *CDC68* are the same gene. First, the cloned *SPT16* and *CDC68* genes have identical restriction maps (Fig. 1) (36, 44, 49). Second, plasmids pCC48 (*SPT16*) and pSC2-1 (*CDC68*) complement the temperature-sensitive lethality of both *spt16-197* and *cdc68-1*. Third, *spt16-197* and *cdc68-1* failed to complement in diploids. Finally, these two mutations are very tightly linked (Table 4).

Because *spt16-197* and *cdc68-1* are allelic, we investigated whether these mutations cause the same mutant phenotypes.

FIG. 4. Effect of an *spt16* mutation on Ty transcription. Total yeast RNA was subjected to Northern analysis. Cells used for RNA preparation were grown at 30°C (lanes 1, 3, 5, and 7) or grown at 30°C and transferred to 37°C for 30 min (lanes 2, 4, 6, and 8). The same membrane was hybridized with the Ty probe B161 and with the *TUB2* probe pYST138. A minor Ty transcript that is slightly less than full length is visible. The two lowermost bands are due to rRNA. The strains used were FY120 (lanes 1 and 2), BM390 (lanes 3 and 4), BM470 (lanes 5 and 6), and BM493 (lanes 7 and 8). Because of different levels of Ty transcription, the Ty hybridization was exposed for 12 h in lanes 1 to 4 (*SNF2*⁺) and for 100 h in lanes 5 to 8 (*snf2Δ1::HIS3*). The *TUB2* hybridization was exposed for the same amount of time in all lanes.

Both *spt16-197* and *cdc68-1* cause 80 to 85% of cells to arrest the cell cycle as unbudded cells after growth for 4 h at the restrictive temperature (37°C). In addition, Rowley et al. (49) have shown that *cdc68-1* suppresses *his4-912δ* and *lys2-128δ*, as does *spt16-197*. Therefore, *spt16-197* and *cdc68-1*, alleles identified in very different ways, confer identical phenotypes, suggesting that they have similar effects on *SPT16* function.

***spt16*-mediated transcriptional suppression is independent of cell cycle arrest at START.** The transcriptional changes in *spt16* mutants are most apparent after growth at the restrictive temperature. These changes may be the direct result of inactivation of the *SPT16* gene product or an indirect effect of arrest or delay of the cell cycle at START. To distinguish between these possibilities, we examined transcription of *lys2-128δ* in *SPT16*⁺ and *spt16-197* cells arrested at two different points in the cell cycle. Cells were arrested at START with α-factor or at the time of chromosome segregation with nocodazole and then shifted to the restrictive

TABLE 4. Mapping *SPT16* by tetrad analysis

Segregating markers (cross no.) ^a	No. of tetrads			Genetic map distance (centimorgans) ^b
	Parental ditype	Nonparental ditype	Tetratype	
<i>SPT16</i> , <i>ade5</i> (1)	27	0	16	19
<i>SPT16</i> , <i>lys5</i> (2)	12	1	26	41
<i>spt16</i> , <i>cdc68</i> (3)	26	0	0	<2

^a The tetrads scored come from the crosses CC236 × CG379 (1), CC235 × CC258 (2), and ART68-1 × L577 (3).

^b Calculated using the formula centimorgans = 50(tetratype + 6(nonparental ditypes))/total tetrads.

FIG. 5. Effect of cell cycle arrest on *spt16-197*-mediated suppression. Cells were grown at 30°C to early logarithmic phase and then were left untreated (---), arrested with α -factor (α f), or arrested with nocodazole (noc). Following arrest, cultures were either left at 30°C or transferred to 37°C for 30 min. RNA was prepared and subjected to Northern hybridization analysis. The same filter was probed with the *LYS2* probes pFW47 and pFW112 and with the *TUB2* probe pYST138. Since the smaller *LYS2* RNA is much more abundant, we used a pFW47 probe that contained approximately 10-fold more counts per minute than the pFW112 probe. The two additional bands are due to rRNA. The strains used were FY98 (lane 1), BM361 (lane 2), FY120 (lanes 3 to 8), and BM390 (lanes 9 to 14). An inconsistency in the blotting makes it difficult to see the *TUB2* normalization in lane 14.

temperature for 30 min (Fig. 5). In the *spt16* mutant, the long *LYS2* transcript previously described was present after growth at the restrictive temperature regardless of whether the cells were previously arrested at START, arrested at mitosis, or unarrested (Fig. 5, lanes 10, 12, and 14). Therefore, *spt16* suppression of *lys2-128δ* does not require arrest at START. In an *SPT16*⁺ strain, there was no long *LYS2* transcript present under any conditions, including in α -factor-arrested cells (Fig. 5, lanes 3 to 8); therefore, arrest at START is not sufficient to cause suppression of *lys2-128δ*. These results demonstrate that alteration of *lys2-128δ* transcription in *spt16* mutants is independent of cell cycle arrest in G₁.

DISCUSSION

The *SPT16* gene was previously identified as a high-copy-number suppressor of δ insertion mutations (15). The results in this paper demonstrate that mutations in *SPT16* also suppress δ insertion mutations, *trans*-acting defects in transcription of the *SUC2* gene and Ty elements, and a deletion of the *SUC2* upstream activating sequence. On the basis of genetic and molecular analysis, we have shown that *SPT16* is the same as the *CDC68* gene (44). Furthermore, the temperature-sensitive-lethality alleles *spt16-197* and *cdc68-1* both cause arrest of the cell cycle at START (44, 49).

The importance of *SPT16* in transcription is illustrated by

the numerous effects of *spt16* mutations on gene expression and the fact that *SPT16* is essential for growth. We have observed effects of *spt16* on four loci: *lys2-128δ*, *his4-912δ*, *SUC2*, and Ty. In all of the cases described here, *spt16-197* permits expression from otherwise nonfunctional promoters. *cdc68-1* also causes increased expression of *HSP82* (49). In contrast, *cdc68-1* reduces transcription of *LEU2*, *ACT1*, and the cyclin genes *CLN1*, *CLN2*, and *CLN3* (49). A dramatic decrease in transcription of the cyclin genes upon transfer to the restrictive temperature is likely to be the cause of the arrest at START in *spt16* mutants (49). Therefore, there are both positive and negative effects on transcription in *spt16* mutants. Other proteins with both positive and negative roles in gene expression have been previously described (for example, RAP1/GRF1/TUF1 [7, 31, 54, 55], PRTF/MCM1/GRM [2, 35], GAL11/SPT13 [22, 60], and E1A [reviewed in reference 8]). Perhaps SPT16 binds directly to promoters and has different roles depending on other proteins bound nearby, similarly to PRTF (2, 35). Alternatively, SPT16 might modify the expression or activity of a variety of activating and repressing transcription factors. Further studies are needed to determine the mechanism by which SPT16 affects transcription initiation.

We have demonstrated that at least one transcriptional alteration caused by an *spt16* mutation does not require arrest at START. *spt16* mutant cells arrested at mitosis with nocodazole show the same change in transcription following transfer to the restrictive temperature as do *spt16* mutant cells growing exponentially or arrested at START with α -factor. Therefore, *SPT16* appears to be required throughout the cell cycle for normal transcription. Two additional observations indicate that changes in transcription in *spt16* mutants are not indirect effects of arrest or delay of the cell cycle at START. First, arrest of *SPT16*⁺ cells at START with α -factor does not mimic the transcription defects observed in *spt16* mutants. Second, some other *cdc* mutations that arrest the cell cycle at START do not suppress δ insertion mutations (36).

Increased copy number of the wild-type *SPT16* gene also causes suppression of *his4-912δ* and *lys2-128δ*. This result suggests that the SPT16 protein functions in a complex and that increased levels of SPT16 alter normal stoichiometry, thereby reducing the level or function of the complex, resulting in an Spt⁻ mutant phenotype. Alternatively, the temperature-sensitive alleles and increased dosage of *SPT16* may both result in increased SPT16 activity at certain promoters or at particular times in the cell cycle.

Among previously characterized *SPT* genes, one group is phenotypically related to *SPT16*. Mutations in *SPT4*, *SPT5*, *SPT6*, and the histone-encoding genes *HTA1* and *HTB1* also strongly suppress *his4-912δ* and *lys2-128δ* but not certain other δ and Ty insertion mutations (15, 21, 65). Also, like mutations in *SPT16*, mutations in all of the *SPT* genes of this class suppress mutations in *SNF2* (26, 28, 41, 42, 61). Furthermore, a *SUC2* upstream activating sequence deletion is suppressed by mutations in *SPT16*, *SPT5*, *SPT6*, and *HTA1-HTB1* (28, 41, 61). Finally, *SPT16*, *SPT5*, *SPT6*, and *HTA1-HTB1* all confer suppression of δ insertion mutations when in high copy number (15, 16, 63). The similar phenotypes exhibited by the *SPT* genes in this class suggest that they are all involved in the same fundamental aspect of transcription.

spt16 mutations are also distinct from *spt4*, *spt5*, and *spt6* mutations in certain respects. Genetic evidence has suggested that *SPT4*, *SPT5*, and *SPT6* are functionally related. Recessive mutations in these genes fail to complement each

other in diploids, and double-mutant combinations are lethal (61, 65). The temperature-sensitive *spt16-197* mutation complements *spt4* and *spt5* mutations and almost completely complements an *spt6* mutation (43). *spt16-197* also fully complements *htal-htb1* and *spt15* mutations. In addition, haploid strains containing *spt16-197* in combination with these other *spt* mutations are viable and have an Spt⁻ phenotype (36). These results suggest that SPT16 functions distinctly from SPT4, SPT5, and SPT6. For example, SPT4, SPT5, and SPT6 proteins may function together in a complex, and the SPT16 protein may function in a separate but related way. However, since *SPT16* is essential for growth, we are limited to studying partial-loss-of-function mutants; genetic interactions with *spt4*, *spt5*, and *spt6* mutations may not be detectable under these conditions or with this particular *spt16* allele.

Sequence analysis of *SPT16*, *SPT5*, and *SPT6* indicates some physical similarities among the three predicted proteins. SPT5 and SPT6 are both nuclear proteins with highly acidic amino termini (62, 63). The SPT16 protein is also very acidic; 49% of the last 78 amino acids are aspartic acid or glutamic acid (49). The acidic regions may have similar functions in these three SPT proteins, perhaps in associating with basic histone proteins. Certain other highly acidic proteins have been suggested to interact with chromatin (reviewed in reference 17), and the similar phenotypes conferred by *spt5*, *spt6*, *spt16*, and histone mutations are consistent with this model. Characterization of chromatin structure in *spt16* mutants and biochemical characterization of the SPT16 protein will allow us to test these ideas.

ACKNOWLEDGMENTS

We are extremely grateful to Adele Rowley and Gerry Johnston for generously sharing strains, plasmids, and unpublished results and for helpful discussions. Craig Giroux and Philip Heiter also kindly provided us with strains. We thank Jan Fassler, Anne Happel, and William Strauss for providing chromosome blots and Liz Howell and Connie Holm for advice on nocodazole arrest.

This work was supported by grants from the National Institutes of Health (GM32967) and the Stroh Brewing Company to F.W. E.A.M. was supported by a National Institutes of Health training grant (T32-GM07196), and E.A.M. and C.D.C. received funds from the Lucille P. Markey Charitable Trust.

REFERENCES

- Abrams, E., L. Neigeborn, and M. Carlson. 1986. Molecular analysis of *SNF2* and *SNF5*, genes required for expression of glucose repressible genes in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 6:3643-3651.
- Bender, A., and G. F. Sprague, Jr. 1987. MAT α 1 protein, a yeast transcription activator, binds synergistically with a second protein to a set of cell-type-specific genes. *Cell* 50:681-691.
- Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* 7:1513-1523.
- Boeke, J. D., F. LaCrute, and G. R. Fink. 1984. A positive selection for mutants lacking orotidine-5'-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. *Mol. Genet.* 197:345-346.
- Boeke, J. D., J. Trueheart, G. Natsoulis, and G. R. Fink. 1987. 5-Fluoro-orotic acid as a selective agent in yeast molecular genetics. *Methods Enzymol.* 154:164-175.
- Botstein, D., S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. Stinchcomb, K. Struhl, and R. W. Davis. 1979. Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. *Gene* 8:17-24.
- Buchman, A. R., W. J. Kimmerly, J. Rine, and R. D. Kornberg. 1988. Two DNA-binding factors recognize specific sequences at silencers, upstream activating sequences, autonomously replicating sequences, and telomeres in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 8:210-225.
- Burk, A. J. 1986. Adenovirus promoters and E1A trans-activation. *Annu. Rev. Gen.* 20:45-79.
- Carle, G. F., M. Frank, and M. V. Olson. 1986. Electrophoretic separations of large DNA molecules by periodic inversion of the electric field. *Science* 232:65-68.
- Carlson, M., and D. Botstein. 1982. Two differentially regulated mRNAs with different 5' ends encode secreted and intracellular forms of yeast invertase. *Cell* 28:145-154.
- Carlson, M., B. C. Osmond, and D. Botstein. 1981. Mutants of yeast defective for sucrose utilization. *Genetics* 98:25-40.
- Celenza, J. L., and M. Carlson. 1989. Mutational analysis of the *Saccharomyces cerevisiae* SNF1 protein kinase and evidence for functional interaction with the SNF4 protein. *Mol. Cell. Biol.* 9:5034-5044.
- Celenza, J. L., F. J. Eng, and M. Carlson. 1989. Molecular analysis of the *SNF4* gene of *Saccharomyces cerevisiae*: evidence for a physical association of the SNF4 protein with the SNF1 protein kinase. *Mol. Cell. Biol.* 9:5045-5054.
- Clark-Adams, C. D. 1988. Ph.D. thesis. Harvard University, Cambridge, Mass.
- Clark-Adams, C. D., D. Norris, M. A. Osley, J. S. Fassler, and F. Winston. 1988. Changes in histone gene dosage alter transcription in yeast. *Genes Dev.* 2:150-159.
- Clark-Adams, C. D., and F. Winston. 1987. The *SPT6* gene is essential for growth and is required for δ -mediated transcription in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 7:679-686.
- Earnshaw, W. C. 1987. Anionic regions in nuclear proteins. *J. Cell Biol.* 105:1479-1482.
- Eisenmann, D. M., C. Dollard, and F. Winston. 1989. *SPT15*, the gene encoding the yeast TATA binding protein TFIID, is required for normal transcription initiation *in vivo*. *Cell* 58:1183-1191.
- Estruch, F., and M. Carlson. 1990. *SNF6* encodes a nuclear protein that is required for expression of many genes in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 10:2544-2553.
- Farabaugh, P. J., and G. R. Fink. 1980. Insertion of the eukaryotic transposable element Ty1 creates a 5-base pair duplication. *Nature (London)* 286:352-356.
- Fassler, J. S., and F. Winston. 1988. Isolation and analysis of a novel class of suppressor of Ty insertion mutations in *Saccharomyces cerevisiae*. *Genetics* 118:203-212.
- Fassler, J. S., and F. Winston. 1989. The *Saccharomyces cerevisiae* *SPT13/GAL11* gene has both positive and negative regulatory roles in transcription. *Mol. Cell. Biol.* 9:5602-5609.
- Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6-13.
- Feinberg, A. P., and B. Vogelstein. 1984. A technique for radiolabeling restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 137:266-267.
- Gerring, S. L., C. Connelly, and P. Heiter. 1990. Positional mapping of genes by chromosome blotting and chromosome fragmentation. *Methods Enzymol.* 194:57-77.
- Happel, A. M., M. S. Swanson, and F. Winston. 1991. The *SNF2*, *SNF5*, and *SNF6* genes are required for Ty transcription in *Saccharomyces cerevisiae*. *Genetics* 128:69-77.
- Hartwell, L. H., J. Culotti, J. R. Pringle, and B. J. Reid. 1974. Genetic control of the cell division cycle in yeast. *Science* 183:46-51.
- Hirschhorn, J. N., S. A. Brown, C. D. Clark, and F. Winston. Unpublished data.
- Hirschhorn, J. N., and F. Winston. 1988. *SPT3* is required for normal levels of α -factor and α -factor expression in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 8:822-827.
- Hoffman, C. S., and F. Winston. 1987. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. *Gene* 57:267-272.
- Huet, J., P. Cottrelle, M. Cool, M.-L. Vignais, D. Thiele, C. Merck, J.-M. Buhler, A. Sentenac, and P. Fromageot. 1985. A general upstream binding factor for genes of the yeast translation apparatus. *EMBO J.* 4:3539-3547.

32. Huisman, O., W. Raymond, K. Froelich, P. Errada, N. Kleckner, D. Botstein, and M. A. Hoyt. 1987. A *Tn10-lacZ-kanR-URA3* gene fusion transposon for insertion mutagenesis and fusion analysis of yeast and bacterial genes. *Genetics* 116:191-199.
33. Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* 153:163-168.
34. Johnston, M., and R. W. Davis. 1984. Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 4:1440-1448.
35. Keleher, C. A., C. Goutte, and A. D. Johnson. 1988. The yeast cell-type-specific repressor $\alpha 2$ acts cooperatively with a non-cell-type-specific protein. *Cell* 53:927-936.
36. Malone, E. A., and F. Winston. Unpublished data.
37. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. Mortimer, R. K., and D. C. Hawthorne. 1969. Yeast genetics, p. 385-460. In A. H. Rose and J. S. Harrison (ed.), *The yeasts*, vol. 1. Academic Press, Inc., New York.
39. Mortimer, R. K., D. Schild, C. R. Contopoulou, and J. A. Kans. 1989. Genetic map of *Saccharomyces cerevisiae*, edition 10. *Yeast* 5:321-403.
40. Neigeborn, L., and M. Carlson. 1984. Genes affecting the regulation of *SUC2* gene expression in *Saccharomyces cerevisiae*. *Genetics* 108:845-858.
41. Neigeborn, L., J. L. Celenza, and M. Carlson. 1987. *SSN20* is an essential gene with mutant alleles that suppress defects in *SUC2* transcription in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 7:672-678.
42. Neigeborn, L., K. Rubin, and M. Carlson. 1986. Suppressors of *SNF2* mutations restore invertase derepression and cause temperature-sensitive lethality in yeast. *Genetics* 112:741-753.
43. Prelich, G., E. A. Malone, and F. Winston. Unpublished results.
44. Prendergast, J. A., L. E. Murray, A. Rowley, D. R. Carruthers, R. A. Singer, and G. C. Johnston. 1990. Size selection identifies new genes that regulate *Saccharomyces cerevisiae* cell proliferation. *Genetics* 124:81-90.
45. Rigby, P. W., M. Diekmann, C. Rhodes, and P. Berg. 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* 113:237-251.
46. Roeder, G. S., and G. R. Fink. 1980. DNA rearrangements associated with a transposable element in yeast. *Cell* 21:239-249.
47. Rose, M. D., F. Winston, and P. Heiter. 1990. Methods in yeast genetics, revised edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
48. Rothstein, R. 1983. One-step gene disruption in yeast. *Methods Enzymol.* 101:202-211.
49. Rowley, A., R. A. Singer, and G. C. Johnston. 1991. *CDC68*, a yeast gene that affects regulation of cell proliferation and transcription, encodes a protein with a highly acidic carboxyl terminus. *Mol. Cell. Biol.* 11:5718-5726.
50. Sarokin, L., and M. Carlson. 1984. Upstream region required for regulated expression of the glucose-repressible *SUC2* gene of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 4:2750-2757.
51. Scherer, S., and R. W. Davis. 1979. Replacement of chromosome segments with altered DNA sequences constructed *in vitro*. *Proc. Natl. Acad. Sci. USA* 76:4951-4955.
52. Schwartz, D. C., and C. R. Cantor. 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* 37:67-75.
53. Sherman, F., G. R. Fink, and C. W. Lawrence. 1978. Methods in yeast genetics, revised edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
54. Shore, D., and K. Nasmyth. 1987. Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. *Cell* 51:721-732.
55. Shore, D., D. J. Stillman, A. H. Brand, and K. A. Nasmyth. 1987. Identification of silencer binding proteins from yeast: possible roles in *SIR* control of DNA replication. *EMBO J.* 6:461-467.
56. Sikorski, R. S., and P. Heiter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122:19-27.
57. Som, T., K. A. Armstrong, F. C. Volkert, and J. R. Broach. 1988. Autoregulation of 2 μ m circle gene expression provides a model for maintenance of stable plasmid copy levels. *Cell* 52:27-37.
58. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503-517.
59. Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis. 1979. High frequency transformation of yeast: autonomous replication of hybrid DNA molecules. *Proc. Natl. Acad. Sci. USA* 76:1035-1039.
60. Suzuki, Y., Y. Nogi, A. Abe, and T. Fukasawa. 1988. *GAL11* protein, an auxiliary transcription activator for genes encoding galactose-metabolizing enzymes in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 8:4991-4999.
61. Swanson, M. S. 1991. Ph.D. thesis. Harvard University, Cambridge, Mass.
62. Swanson, M. S., M. Carlson, and F. Winston. 1990. *SPT6*, an essential gene that affects transcription in *Saccharomyces cerevisiae*, encodes a nuclear protein with an extremely acidic amino terminus. *Mol. Cell. Biol.* 10:4935-4941.
63. Swanson, M. S., E. A. Malone, and F. Winston. 1991. *SPT5*, an essential gene important for normal transcription in *Saccharomyces cerevisiae*, encodes an acidic nuclear protein with a carboxy-terminal repeat. *Mol. Cell. Biol.* 11:3009-3019.
64. Winston, F. Unpublished data.
65. Winston, F., D. T. Chaleff, B. Valent, and G. R. Fink. 1984. Mutations affecting Ty-mediated expression of the *HIS4* gene of *Saccharomyces cerevisiae*. *Genetics* 107:179-197.
66. Winston, F., C. Dollard, E. A. Malone, J. Clare, J. G. Kapakos, P. Farabaugh, and P. L. Minehart. 1987. Three genes are required for *trans*-activation of Ty transcription in yeast. *Genetics* 115:649-656.
67. Winston, F., K. J. Durbin, and G. R. Fink. 1984. The *SPT3* gene is required for normal transcription of Ty elements in *S. cerevisiae*. *Cell* 39:675-682.