MOLECULAR AND CELLULAR BIOLOGY, Aug. 1991, p. 4121–4127
0270-7306/91/084121-07$02.00/0

Vol. 11, No. 8

SHI, a New Yeast Gene Affecting the Spacing between TATA and Transcription Initiation Sites

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Received 14 November 1990/Accepted 29 May 1991

In a genetic selection for *Saccharomyces cerevisiae* genes involved in transcription start site specification, two mutant genes which restore alcohol dehydrogenase activity to a functionally defective *S. pombe ADH* gene were recovered. Examination of *S. pombe ADH* initiation sites showed that mutations in the *SHI* gene shift the location of the transcription initiation window closer to TATA. The *shi* mutant also affected initiation site selection for two *S. cerevisiae* genes that were tested. For *H2B* mRNA, initiation occurred in the *shi* mutant at a series of initiation sites located 43 to 80 bp 3′ of the histone *H2B* TATA sequence and at the usual initiation sites 102 and 103 bp downstream of the TATA sequence. Weakly used initiation sites ranging from 51 to 80 bp downstream of the TATA sequence were observed for the *S. cerevisiae ADH1* gene in *shi* strains, in addition to the normal *ADH1* initiation sites 89 and 99 bp from the TATA sequence. Restoration of function to the defective *S. pombe ADH* gene occurs only when this gene contains a TATA sequence; a single-base-pair TATA-to-TAGA change is sufficient to prevent this restoration of function. Genetic mapping placed the *SHI* locus on the left arm of chromosome VII, 22.3 centimorgans from *cyh2*; it does not correspond to any previously mapped gene.

Molecular approaches have defined the DNA sequence elements necessary for transcription initiation by *Saccharomyces cerevisiae* RNA polymerase II (PolII). In contrast to higher eucaryotes, mRNA initiation sites in this yeast are chosen within a broad region, termed a window, located approximately 50 to 125 bp downstream of the TATA sequence (reviewed in references 14 and 25). While the TATA sequence is necessary to specify the location of the initiation window (13, 16, 20), the precise mechanism by which yeast initiation sites are selected within the window is not known. The DNA sequence at yeast initiation sites is important (reviewed in reference 25). Several types of consensus initiation sequences have been proposed (13, 16, 17), and it has not been established that all *S. cerevisiae* initiation sites belong to a single category. The identification and characterization of yeast proteins involved in transcription initiation site selection will aid in understanding this process.

The identification in HeLa cells of the general transcription factors TFIIA, TFIIB, TFIID, and TFIIE/F, necessary in addition to RNA PolII for correct in vitro transcription initiation (21; reviewed in reference 23), has made possible the purification of their yeast counterparts. When HeLa cell extracts depleted for specific fractions were complemented with yeast components, the yeast versions of the TATA-binding protein, TFIID (6, 8), and the TFIIA protein (5) were isolated and characterized. The gene that encodes the TFIID protein has been cloned and analyzed (7, 15, 18, 24). This approach has not yielded a protein responsible for yeast start site selection. It has been demonstrated (6) that yeast TFIID does not confer on the hybrid system the typical yeastlike pattern of transcription initiation. Some component other than TFIID must be required to specify the distance from the TATA sequence to the RNA start site.

In this report, we describe a genetic approach used to identify proteins that determine yeast PolII transcription initiation sites. Two previous genetic approaches have identified components of the yeast general PolII transcription machinery by requiring suppression of different transcriptionally inactivated *HIS4* genes. In one approach, Arndt et al. (1) obtained mutations in the largest and second-largest subunits of RNA PolII; in another, Winston and his coworkers (12, 26) obtained 16 *SPT* complementation groups. *SPT15* has been shown to encode the yeast general transcription factor TFIID (11).

Our genetic selection for transcription alterations differs from those cited by requiring the mutant to have a qualitative difference in transcription; that is, the desired altered protein would change the position of transcription initiation for a given promoter. We used a modified *Schizosaccharomyces pombe ADH* test gene that initiates its transcripts downstream of the translation-initiating ATG in *S. cerevisiae*, resulting in nonfunctional messages. By selecting for the function of this gene in *S. cerevisiae*, we obtained mutations in two complementation groups. One group was shown to affect dramatically the position of transcription initiation sites for the test gene and to affect the pattern of initiation sites chosen for two other *S. cerevisiae* genes. Here we describe the isolation and characterization of these mutations, called *shi* for shifted initiation.

MATERIALS AND METHODS

Strains. *S. cerevisiae* WV36-201 (*MATa adh1Δ adh2Δ adh3 ade2 ade4 trp1 ura3*), hereafter referred to as Adh null, was kindly provided by Wolfgang Vogel. This strain contains deletions of the *S. cerevisiae ADH1* and *ADH2* loci and has no alcohol dehydrogenase (ADH) activity. Two *S. pombe ADH* alleles were integrated into the *SUP4* locus of the Adh null strain, along with a linked *URA3* marker gene, as described previously (13). The wild-type *S. pombe ADH* gene was integrated to produce strain Δ0. Adh$^+$ mutants

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with the *MAT***a** mating type were selected from starting strain Δ50, which contains a copy of the *adhΔ50* allele of the *ADH* gene integrated within strain Adh null. A second ADH-negative strain, called Adh0 (*MATα trp1 ura3 can1 cyh2 adh1 adh2 adh3 SUP4::adhΔ50-URA3*), contains point mutation alleles in the *ADH1* and *ADH2* loci and an integrated *adhΔ50* allele. For segregation analysis, heterozygous diploids between six different *shi* strains (110, 232, 233, 285, 326, and 374) and strain Adh0 were made, as well as diploids between dominant mutant 303 and strain Adh0. To carry out a complementation test of allelism between different mutations, 11 of the recessive alleles were crossed into a *MATα* background. Diploids of strain Adh0 crossed to seven mutant strains were plated for random spores to obtain part of the *MATα* mutant-containing strains, and four others were obtained by dissection of Adh0 × mutant diploids.

Media. YEPD medium and antimycin plates have been previously described (13). Cycloheximide resistance was scored on YEPD plates containing 10 μg of filter-sterilized cycloheximide per ml. Dropout medium, complete medium, and potassium acetate plates, as described by Kurjan and Hall (19), were used. 5′-Fluoroorotic acid complete plates for selecting loss of the *adhΔ50-URA3* integration contained 800 mg of 5′-fluoroorotic acid per liter (4).

Plasmids. As described previously (13), plasmid pEG2Δ0 contains a wild-type *S. pombe ADH* gene and pEG2Δ50 contains the *adhΔ50* allele of this gene, which has 50 bp removed in the region between the TATA sequence and the translation-initiating ATG. Plasmid pEG2Δ50-TAGA contains a TATAAA-to-TAGAAA base pair change but is otherwise identical to pEG2Δ50. The mutation was constructed by using the in vitro oligonucleotide-directed mutagenesis kit from Amersham as recommended by the supplier.

Yeast matings, sporulation, transformation, and genetic techniques. Yeast matings were carried out at 30°C, and diploids were selected on appropriate medium and sporulated on potassium acetate plates for 5 days at 30°C. Tetrads were dissected by micromanipulation of glusulase-treated asci on YEPD plates. Yeast transformations were done by the spheroplasting protocol described by Beggs (2). When phenotypes were tested on antimycin plates, double replicas were used; YEPD plates spread with patches of cells were routinely replicated first to another YEPD plate, which was immediately replica plated to an antimycin plate, hereafter called a double replica. The frequency of spontaneous Adh^+ cells arising from strain Δ50 was determined by plating a high density of cells directly onto antimycin plates. Dilutions of the same cultures were plated onto nonselective plates to determine the true number of cells plated; approximately 2×10^{-6} spontaneous Adh^+ cells occurred per viable cell.

Poly(A)$^+$ RNA isolation and primer extension mapping. Preparation of total RNA, poly(A)$^+$ purification, labeling of oligonucleotides, and primer extension reactions were carried out as described previously (13). Three oligonucleotides were used for primer extension experiments. One is an *S. pombe ADH*-specific oligonucleotide (5′-AACCAAGACCT CGTCTTG-3′) complementary to bases +97 to +114. The second is complementary to the *S. cerevisiae ADH1* gene between nucleotides +67 and +84 (5′-CTTTGGAACTG GAATATC-3′). The third oligonucleotide is complementary to the *S. cerevisiae* histone *H2B* gene in the region between +29 and +46 (5′-CAGCTGGGGCTTTGGAGG-3′).

RESULTS

Isolation of *S. cerevisiae* mutations which restore ADH activity to the *S. pombe adhΔ50* allele. Transcription of the *S. pombe ADH* gene in *S. cerevisiae* resembles that of many *S. cerevisiae* genes, in that initiation sites are selected from a range extending between 55 and 125 bp downstream of the TATA sequence (13). The *adhΔ50* allele of the *S. pombe ADH* gene provided the basis for our genetic selection. In this allele, a 50-bp deletion positions the ATG at the start of the ADH reading frame 58 rather than 108 bp downstream of TATA. In the parental *S. cerevisiae* strain, transcription of the *adhΔ50* allele initiates at multiple sites both at the ATG itself and downstream of it. Since these transcripts lack the initiating ATG, they provide no ADH activity; translation beginning at the first available ATG would result in a protein lacking 66 amino acids (13). Mutations that alter the transcription machinery by moving mRNA initiation sites closer to the TATA element should act as suppressors of the Adh^- phenotype of *adhΔ50* by including the initiating AUG codon in the mRNA. To screen for such mutations, a single copy of the *S. pombe adhΔ50* allele was integrated into an Adh null strain to produce strain Δ50. The Adh null strain is completely sensitive to the respiratory inhibitor antimycin, which allows only growth of cells with a functional ADH enzyme (10). Nonselectively grown colonies from strain Δ50 were replica plated to antimycin plates to select spontaneously occurring Adh^+ mutations. The resulting papillations were recovered and single colony purified on antimycin plates. To distinguish between true suppressors of *adhΔ50* and other antimycin-resistant mutations that act independently of *adhΔ50*, the integrated *adhΔ50* gene was evicted by selecting for concomitant loss of the linked *URA3* marker gene on plates containing 5′-fluoroorotic acid, as described by Boeke et al. (4). Only 9% of the candidate mutant clones lost their antimycin resistance as a consequence of *adhΔ50* loss; the other 91% of colonies must therefore be mutants that confer antimycin resistance independently of the resident *adhΔ50* allele.

Extragenic suppressors were distinguished from events at the *adhΔ50* locus by retransformation of mutant strains with plasmid pEG2Δ50, containing the *adhΔ50* allele. We recovered a total of 26 extragenic suppressor mutants, each with the ability to restore ADH activity either to the original integrated *adhΔ50* allele or to a plasmid-encoded *adhΔ50* gene. Additionally, three strains containing mutations at the integrated *adhΔ50* sequences were recovered; analysis of these mutants is described elsewhere (13). The 26 extragenic mutants represent about 4% of the spontaneous Adh^+ mutations recovered from strain Δ50. Since the rate of spontaneous Adh^+ mutations for this strain was determined to be around 2×10^{-6}, the approximate overall frequency of recovery of these mutants was 8×10^{-8}. The 26 mutant strains showed a range of phenotypes with respect to the ability to grow on antimycin plates. The most strongly Adh^+ mutant strains grew almost as well as strain Δ0, which contains an integrated copy of the wild-type *S. pombe ADH* gene, whereas other mutant strains grew slowly on antimycin plates. All of the mutant strains, however, were able to grow on double antimycin replicas.

Complementation testing of the mutations; chromosomal location of the *shi* mutation. Twenty-five of the 26 suppressor mutations were found to be recessive; heterozygous diploids formed by crossing the mutant strains to strain Adh0, an Adh^- strain containing the integrated *adhΔ50* allele, failed to grow on antimycin plates. One strain, mutant 303, proved to

TABLE 1. Segregation of *shi* and mutant 303 with respect to *cyh2*

Type of tetrad	No. of tetrads[a]	
	shi	303
Parental ditype	36	5
Nonparental ditype	0	5
Tetratype	29	35

[a] The *shi* recombination frequency was 22.3%; mutant 303 was unlinked.

harbor a dominant mutation, since the resulting diploid was able to grow on antimycin plates. For complementation testing, diploids from the 25 recessive *MAT***a** strains crossed to a set of 11 *MAT*α strains, each containing a different recessive mutant (derived as described in Materials and Methods), were tested by the double replica plating technique for the ability to grow on antimycin plates. All of these diploids failed to complement; that is, they were able to grow on antimycin plates. This complementation group was given the name *shi*.

To determine whether the Adh$^+$ phenotype in this complementation group was due to a mutation in a single nuclear gene, the segregation of the antimycin resistance phenotype was monitored in crosses between mutant strains and strain Adh0. From diploids heterozygous for the *shi* mutation, 65 four-spored tetrads obtained from six *shi* alleles were scored for their segregating markers. In these tetrads, two spores showed the mutant antimycin resistance phenotype when double replica plated on antimycin plates. The colony size and growth rate on antimycin plates often varied between the two mutant spores. For most of the tetrads, these growth differences were not pronounced; however, under some culture conditions, some of the Adh$^+$ spores grew only weakly or papillated on double antimycin replicas. Since the behavior of these tetrads was consistent with the presence of a single unlinked modifier locus in the *shi* strains which affected the antimycin resistance level, the following experiments were done. Both strongly and weakly antimycin-resistant segregants were crossed back to the Δ50 strain, the diploids were sporulated, and tetrads were dissected. In 12 tetrads analyzed from the backcross of the strongly Ant^r haploid, the two antimycin-resistant spores still showed variation in growth. Eight tetrads from the backcross of the weakly Ant^r haploid showed 2:2 segregation on double antimycin replicas; the antimycin-resistant colonies did not show variation in growth. These results indicate that the Adh$^+$ phenotype in the *shi* complementation group is due to a single gene mutation. However, a second mutation seems also to have arisen in these strains, which, in combination with the *shi* mutation, enables the mutant spore to grow better on antimycin plates. Linkage analysis for segregants in tetrads dissected from crosses between *shi* mutant strains and strain Adh0 (Table 1) placed the *SHI* gene on the left arm of chromosome VII, 22.3 centimorgans from the *cyh2* locus.

From diploids containing the dominant 303 mutant crossed to strain Adh0, 45 tetrads were dissected. The two Adh$^+$ spores from these tetrads often showed marked growth differences on double antimycin replicas, suggesting that a modifying mutation is also present in this strain. The dominant mutation did not display linkage with *cyh2* or any of the other segregating markers; hence, mutant 303 cannot be an allele of the *SHI* gene.

Effects of the *shi* mutation on two *S. pombe ADH* alleles. Both mutant 303 and *shi* restore function to the *adhΔ50* allele. To determine whether this restoration occurred by alteration of the sites of transcription initiation, the 5′ termini of *ADH* mRNA molecules were mapped for the mutant strains. The *S. pombe ADH*-specific oligonucleotide was used to map by primer extension the initiation sites selected for the integrated *adhΔ50* template within mutant 303 and five of the *shi* strains as originally isolated (Fig. 1A). Dominant mutant 303 does not change the pattern of initiation sites selected for the *adhΔ50* gene compared with those selected in a wild-type strain. Other experiments, using the level of *H2B*-specific RNA as an internal standard (data not shown), showed that the amount of RNA produced by mutant 303 does not differ from that of the wild type. One can therefore rule out both dramatic increases in the level of *ADH*-specific RNA and changes in initiation site selection in strain 303.

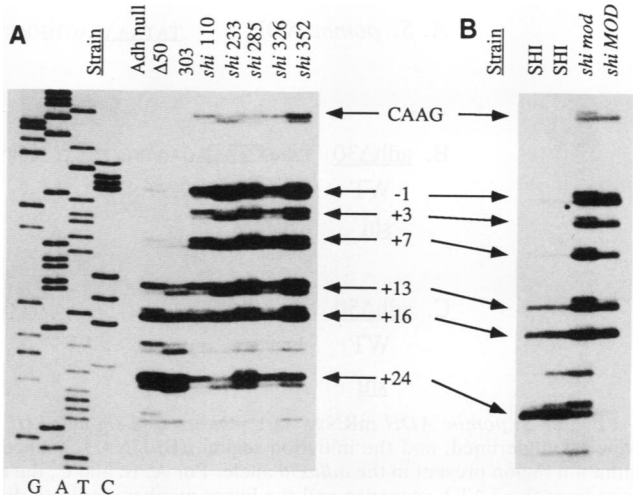

FIG. 1. Primer extension analysis of *S. pombe ADH* RNAs in mutant strains. (A) Total RNA samples prepared from the following strains were extended by using the *S. pombe ADH*-specific oligonucleotide primer: strain Adh null, containing no *S. pombe ADH* gene; strain Δ50, from which the mutant strains were isolated; dominant mutant 303; and *shi* strains 110, 233, 285, 326, and 352. The adjoining sequence ladder is shown for reference. Major initiation sites are designated, and the numbers indicate distances from the ATG. The *shi*-specific initiation site CAAG is also indicated. (B) Total RNAs produced from members of a tetrad in which the *shi* mutation was separated from an unlinked modifier gene were primer extended by using the *S. pombe ADH*-specific oligonucleotide.

In contrast, in all of the *shi* alleles examined, the window permissive for *S. pombe adhΔ50* initiation sites is shifted upstream. In the wild-type strain, the most upstream initiation site used for the *adhΔ50* allele is the $TAAT_{(-1)}$ sequence (measured with respect to the ATG) located 58 bp downstream of the TATA sequence (Fig. 2). A new initiation site, mapping to a CAAG sequence located 46 bp downstream of TATA, is used only in the *shi* mutant. Use of this site has been observed neither for the wild-type *S. pombe ADH* gene nor for any of a series of internally deleted *ADH* alleles transcribed in Shi$^+$ *S. cerevisiae* strains (13). In addition to use of a novel initiation site closer to TATA, the use of all of the other initiation sites is dramatically different in the *shi* strain. The $TAAT_{(-1)}$, $GACT_{(+3)}$, and $TATT_{(+7)}$ initiation sites for the *adhΔ50* allele are used to a much greater extent in the mutant strain than in the Shi$^+$ strain. The downstream initiation site $TGGC_{(+24)}$, used strongly for the *adhΔ50* allele

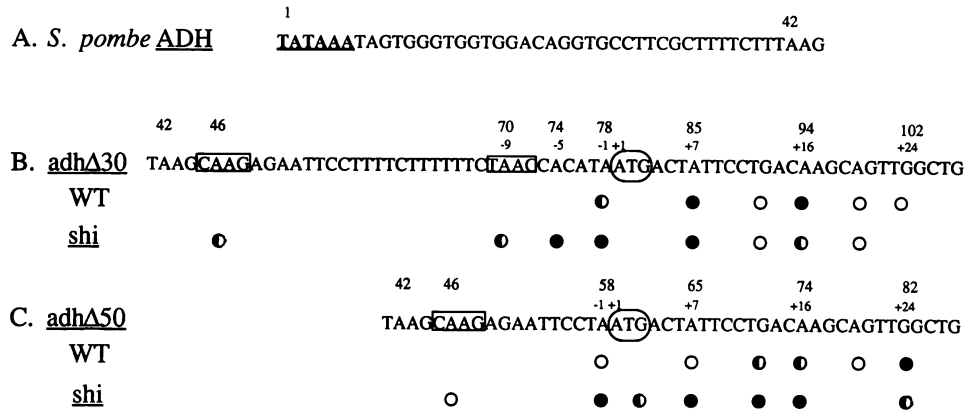

FIG. 2. *S. pombe ADH* mRNA start sites used in *shi* and *SHI* strains. (A) DNA sequence between the *S. pombe ADH* TATAAA box, which is underlined, and the initiation region. (B) DNA sequence of the initiation region for the *adhΔ30 S. pombe ADH* allele. (C) *ADH* initiation region present in the *adhΔ50* allele. For A, B, and C, the upper numbers above the sequences indicate the distances of the initiation sites from the TATA sequence and the lower numbers indicate distances from the ATG, which is circled. Initiation site symbols: ○, weak level of usage; ◐, medium level of usage; ●, strong level of usage. Initiation sites used only in *shi* strains are boxed. WT, wild type.

in a Shi^+ strain, is used at a weaker level in the mutant strain. This suggests that the altered transcription initiation pattern observed in the *shi* mutant strain is caused by a shift in the permissible spacing range rather than by increased recognition of a single new (CAAG) starting site. For this reason, the mutant was termed *shi*, for shifted initiation.

Since the growth of the *shi* strain on antimycin plates appeared to be influenced by an unlinked modifier gene, we wished to demonstrate that the *shi* mutant gene itself was responsible for the observed changes in transcription starts. Total RNA was prepared from all four members of a tetrad which showed a marked difference in growth between the two Adh^+ spores. The segregant growing well on double antimycin replicas contains a modifier gene (*shi mod*), and the other Adh^+ segregant (*shi MOD*) does not. Only one of the remaining Adh^- (Shi^+) spores can contain the second copy of the modifier gene. All four RNAs were primer extended by using the *S. pombe ADH* primer, as shown in Fig. 1B. RNA prepared from strains containing *shi* showed the characteristic shift in initiation pattern, regardless of whether the modifying gene was present. No shift in initiation pattern was seen in strains containing the modifier in the absence of *shi*. Hence, the *shi* allele itself causes the changes in initiation site selection and the modifier gene affects the growth of mutant strains on antimycin-containing medium without affecting the positions of initiation sites.

The *shi* mutant effect of shifting initiation sites is not limited to the *adhΔ50* allele for which suppression was selected. Initiation sites used in *shi* mutant and Shi^+ strains for *adhΔ30*, a 30-bp deletion allele of the *S. pombe ADH* gene, were mapped by primer extension (Fig. 3). The locations of initiation sites are shown schematically in Fig. 2. The CAAG site 46 bp downstream of TATA, the new initiation site for the *adhΔ50* template, is also used for the *adhΔ30* template. As was also seen for the *adhΔ50* template, the more upstream initiation sites for *adhΔ30* are used preferentially in the mutant strain. In the *shi* mutant strain, the $CACA_{(-5)}$ site is used efficiently, as is the previously unused $TAAC_{(-9)}$ site (Fig. 3). The adherence of the new initiation sites for the *ADH* gene to the Py A A/T (Pu) consensus (13) supports the view that the *shi* mutation changes the location of the initiation window but does not affect initiation site preference.

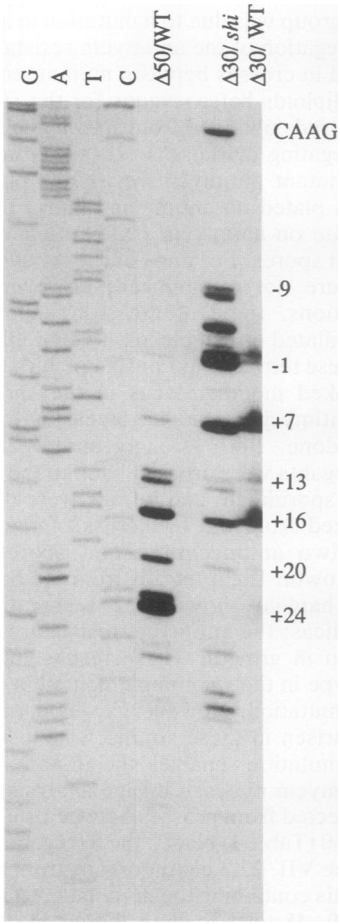

FIG. 3. Primer extension mapping of *adhΔ30* initiation sites in *shi* and *SHI* strains. The *S. pombe ADH*-specific primer was extended by using 25-μg poly(A)$^+$ RNA samples prepared from a *SHI* and a *shi* strain (as originally isolated) containing a plasmid-encoded copy of *adhΔ30* (Δ30). The extension of RNAs made in *SHI* strains containing the *adhΔ50* allele (Δ50) is included for reference. The *shi*-specific CAAG initiation site is indicated. The adjoining sequence was derived from the *adhΔ50* gene by using the *ADH*-specific primer. The numbers indicate positions relative to the ATG.

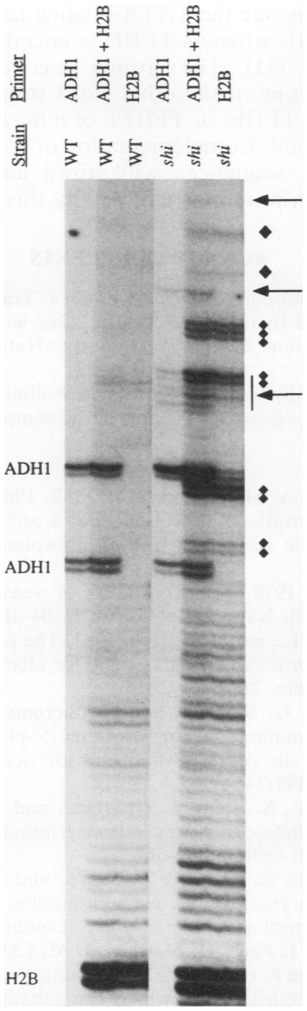

FIG. 4. Primer extension of *S. cerevisiae ADH1* and *H2B* initiation sites used in *shi* and *SHI* strains. Poly(A)$^+$ RNA was prepared from transformants containing a plasmid-encoded copy of the *S. cerevisiae ADH1* gene in a *SHI* and a *shi* strain (as originally isolated), as indicated. A 25-μg sample of RNA was extended with the *S. cerevisiae ADH1*-specific primer and the *H2B*-specific primers, as indicated. The wild-type (WT) *ADH1* and *H2B* initiation sites are labeled on the left. On the right, arrows indicate *shi*-specific *ADH1* initiation sites and diamonds indicate *shi*-specific *H2B* initiation sites.

Effects of *shi* on *S. cerevisiae ADH1* and histone *H2B* initiation sites. A rather general effect of the *shi* mutation upon RNA PolII start site selection is indicated by changes observed in the mRNA initiation sites selected for two other *S. cerevisiae* genes. Since the 10 bp of DNA including and surrounding the TATA box are identical for the *S. pombe ADH* gene and the *S. cerevisiae ADH1* gene, the transcription initiation sites used for the *ADH1* gene were examined in *shi* and *SHI* strains. Poly(A)$^+$ RNA was prepared from *shi* and *SHI* strains transformed with a plasmid-encoded copy of *ADH1* and was primer extended as shown in Fig. 4. The normal initiation sites for this gene are two CAAG sequences 10 bp apart, at positions 89 and 99 bp downstream of TATA (3). In the *shi* strain, these sites are the most strongly used initiation sites; however, a series of sites 66 to 80 bp downstream of TATA is also used at lower levels and an initiation site 51 bp downstream is weakly used.

A stronger effect of the *shi* mutation is seen for initiation sites chosen for the *S. cerevisiae H2B* gene. The poly(A)$^+$ RNAs used to examine *ADH1* initiation sites were also primer extended by using the histone *H2B* oligonucleotide. In wild-type strains, initiation for the *H2B* gene takes place at a CAAG sequence 102 bp downstream of TATA (Fig. 4). In the *shi* mutant, a series of upstream initiation sites is used in addition to the wild-type initiation sites. These new *shi*-specific *H2B* initiation sites, ranging in distance from 43 to 84 bp downstream of TATA, were detected in a total of four independent poly(A)$^+$ RNA preparations from three different *shi* mutant strains (data not shown). (In contrast to animal histone mRNA, yeast histone mRNA is polyadenylated.) *shi*-specific initiation sites mapping to identical locations were also detected by using a different *H2B*-specific primer (data not shown).

The TATA sequence is necessary for the mutant phenotype. We showed previously that deletion of the TATA sequence from the wild-type *S. pombe ADH* gene caused the location of the initiation window in *S. cerevisiae* to shift around 30 bp downstream (13). Since the *shi* mutant also affects the location of the initiation window for this gene, it was interesting to determine whether the *shi* mutant could exert its effects in the absence of a TATA sequence. To avoid affecting spacing relationships, the TATAAATA sequence in the *adhΔ50* allele was changed by a single-base-pair mutation to TAGAAATA. A plasmid containing this change, pEG2Δ50-TAGA, and a plasmid containing the unaltered *adhΔ50* allele were transformed into the *shi* mutant strain. Whereas the TATA-containing *adhΔ50* allele provided ADH function in *shi* mutant strains, transformants of pEG2Δ50-TAGA in the *shi* strain showed no growth on antimycin plates. This indicates that the *shi* mutation cannot restore function to the *adhΔ50* allele without a functional TATA sequence. It also argues strongly that the *shi* mutant protein acts through the normal TATA sequence for the *ADH* gene, rather than causing its effects on initiation sites by allowing a cryptic TATA or alternative promoter sequence to be used in the *shi* strains.

DISCUSSION

For the six yeast genes analyzed, transcription initiation sites are selected within windows spanning similar distance ranges from TATA [*CYC1*, 60 to 100 bp (16); *CYC7*, 45 to 100 bp (17); *HIS3*, 40 to 90 bp (9); *HIS4*, 60 to 110 bp (20); *PHO5*, 55 to 110 bp (22); *S. pombe ADH*, 55 to 125 bp downstream of TATA (13)]. The similar locations of the initiation windows can be explained if, for all six genes, a single mechanism determines the window position; perhaps this mechanism involves a protein that measures the distance from TATA. The criteria by which initiation sites are selected within the window have been harder to define. Many yeast initiation sites adhere to a Py A A/T (Pu) consensus, in which initiation takes place at the first A (13). To identify proteins involved in specifying the transcription initiation window, we undertook a genetic selection requiring restoration of function to a template designed so that its initiation sites fell within the coding region of the gene. Two nonallelic mutations restored function to the *S. pombe adhΔ50* test gene; examination of initiation sites chosen for this and other templates in the mutant strains showed that the *shi* mutation has two related effects upon the selection of transcription initiation sites. One effect is to permit usage of initiation sites

closer to TATA than those used in wild-type strains. For the *S. pombe ADH* gene, a new initiation site at 46 bp downstream of TATA is used in *shi* strains, although in wild-type strains no sites closer than 55 bp downstream of TATA are used for this gene (13). New upstream initiation sites, beginning 40 to 50 bases downstream of TATA, were also observed in *shi* strains for the *S. cerevisiae* histone *H2B* gene and *ADH1* gene. In addition to activating new initiation sites, for the *S. pombe ADH* gene, the *shi* mutation also changes the frequency of usage of initiation sites which are also used in the wild-type strain. In the *shi* mutant, the most frequently used sites for the *ADH* gene are those in the distance range from 58 to 74 bases downstream of TATA. This window of maximum usage of *ADH* initiation sites contrasts with that found in wild-type strains, in which *ADH* initiation sites could be used most efficiently when located between 75 and 115 bases downstream of TATA (13). Both of these effects are consistent with a shift of the position of the transcription initiation window closer to TATA. An alternative explanation of the *shi* mutant effects, namely, that the *shi* mutation allows use of a cryptic TATA sequence closer to the initiation region, is ruled out by the fact that a single-base-pair mutation in the normal TATA sequence abolishes the *shi* Adh^+ phenotype.

Initiation site preferences are not altered in the *shi* mutant. The *shi* mutant-specific initiation sites TAAC, used for the *adhΔ30* allele, and CAAG, used for both that allele and the *adhΔ50* allele, fit the Py A A/T (Pu) consensus which describes the normal *ADH* initiation sites (13). For the *S. cerevisiae* histone *H2B* and *ADH1* genes, most of the new mutant-specific initiation takes place at purines flanked 5′ by pyrimidines, at sites more loosely fitting the Py A A/T (Pu) consensus. The use of consensus initiation sites within the mutant strains suggests that establishment of the initiation window and selection of sites within the window are separable and that *shi* is involved only in the former task.

In addition to its effects on the *S. pombe ADH* gene, the *shi* mutation also affects both of the *S. cerevisiae* genes tested. It was surprising that the *shi* mutation could have such a general effect upon transcription initiation, since it might be assumed that a widespread pattern of upstream initiation for many genes would be disruptive to the cell. Strains in which the *shi* mutation arose also contain a modifying gene, which does not itself affect the transcription initiation sites. The modifying gene may serve to mitigate the adverse effects of the *shi* mutation on cell growth, since strains containing the *shi* mutation in the absence of the modifier gene grow more slowly and are more sensitive to antimycin. However, because the *shi* mutation does not change the initiation site specificity but rather alters the permissive spacing range, wild-type initiation sites continue to be used. For the *ADH* test gene, the use of wild-type initiation sites farther than 75 bp 3′ of TATA is sharply diminished in *shi* strains; however, for the *H2B* and *ADH1* genes, the normal initiation sites at around 100 bp 3′ of TATA continue to be used in mutant strains. Consequently, while the *shi* mutation gives rise to some new mRNAs which may have diminished translatability or stability, for most genes affected by *shi*, mRNA molecules identical to those in wild-type strains should still be present.

Because the *shi* mutation affects the spacing relationship between the TATA element and the transcription start sites and because the phenotypic effect of *shi* upon the *adhΔ50* allele is abolished by a single-base-pair TATAAA-to-TAGAAA mutation, a role for the SHI protein in the transcription initiation complex (23) seems likely. The *SHI* gene does not encode the TATA-binding factor; *shi* maps to chromosome VII, whereas TFIID is encoded by a locus on chromosome V (11). The protein encoded by *SHI* may conceivably be one of the other PolII transcription factors (TFIIA, TFIIB, TFIIE, or TFIIF), or it may be a component of RNA PolII itself. Complementation of the *shi* mutation by wild-type yeast sequences will afford an opportunity to determine which protein is affected by this mutation.

ACKNOWLEDGMENTS

E.F.-G. was supported by a Genetics Training Grant from NIGMS, National Institutes of Health. This work was funded by Public Service grant GM11895 from the National Institutes of Health.

We thank Hans Eppenberger and Theo Wallimann of the Institute for Cell Biology for generous support of the completion of this work.

REFERENCES

1. **Arndt, K., C. A. Styles, and G. R. Fink.** 1989. A suppressor of a *HIS4* transcriptional defect encodes a protein with homology to the catalytic subunit of protein phosphatases. Cell **56**:527–537.
2. **Beggs, J. D.** 1978. Transformation of yeast by a replicating hybrid plasmid. Nature (London) **275**:104–109.
3. **Bennetzen, J. L., and B. D. Hall.** 1982. The primary structure of the *Saccharomyces cerevisiae* gene for alcohol dehydrogenase I. J. Biol. Chem. **257**:3018–3025.
4. **Boeke, J. D., G. R. Fink, and F. Lacroute.** 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast—5′-fluoro-orotic acid resistance. Mol. Gen. Genet. **197**:345–346.
5. **Buratowski, S., S. Hahn, L. Guarente, and P. A. Sharp.** 1989. Five intermediate complexes in transcription initiation by RNA polymerase II. Cell **56**:549–561.
6. **Buratowski, S., S. Hahn, P. A. Sharp, and L. Guarente.** 1988. Function of a yeast TATA element-binding protein in a mammalian transcription system. Nature (London) **334**:37–42.
7. **Cavallini, B., I. Faus, H. Matthes, J. M. Chipoulet, B. Winsor, J. M. Egly, and P. Chambon.** 1989. Cloning of the gene encoding the yeast protein BTF1Y, which can substitute for the human TATA box-binding factor. Proc. Natl. Acad. Sci. USA **86**:9803–9807.
8. **Cavallini, B., J. Huet, J.-L. Plassat, A. Sentenac, J.-M. Egly, and P. Chambon.** 1988. A yeast activity can substitute for the HeLa cell TATA box factor. Nature (London) **334**:77–80.
9. **Chen, W., and K. Struhl.** 1985. Yeast mRNA initiation sites are determined primarily by specific sequences, not by the distance from the TATA element. EMBO J. **4**:3273–3280.
10. **Ciriacy, M.** 1979. Isolation and characterization of further cis- and trans-acting regulatory elements involved in the synthesis of glucose-repressible alcohol dehydrogenase (ADHII) in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **176**:427–431.
11. **Eisenmann, D. M., C. Dollard, and F. Winston.** 1989. *SPT15*, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation in vivo. Cell **58**:1183–1191.
12. **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.
13. **Furter-Graves, E. M., and B. D. Hall.** 1990. DNA sequence elements required for transcription initiation of the *Schizosaccharomyces pombe ADH* gene in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **223**:407–416.
14. **Guarente, L.** 1987. Regulatory proteins in yeast. Annu. Rev. Genet. **21**:425–452.
15. **Hahn, S., S. Buratowski, P. A. Sharp, and L. Guarente.** 1989. Isolation of the gene encoding the yeast TATA binding protein TFIID: a gene identical to the *SPT15* suppressor of Ty element insertions. Cell **58**:1173–1181.
16. **Hahn, S., E. T. Hoar, and L. Guarente.** 1985. Each of three "TATA elements" specifies a subset of the transcription initi-

ation sites at the *CYC1* promoter of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **82**:8562–8566.
17. **Healey, A. M., T. L. Helser, and R. S. Zitomer.** 1987. Sequences required for transcriptional initiation of the *Saccharomyces cerevisiae CYC7* gene. Mol. Cell. Biol. **7**:3785–3791.
18. **Horikoshi, M., C. K. Wang, H. Fujii, J. A. Cromlich, B. A. Weil, and R. G. Roeder.** 1989. Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box. Nature (London) **341**:299–303.
19. **Kurjan, J., and B. D. Hall.** 1982. Mutations at the *Saccharomyces cerevisiae SUP4* $tRNA^{Tyr}$ locus: isolation, genetic fine-structure mapping, and correlation with physical structure. Mol. Cell. Biol. **2**:1501–1513.
20. **Nagawa, F., and G. R. Fink.** 1985. The relationship between the "TATA" sequence and transcription initiation sites at the *HIS4* gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **82**:8557–8561.
21. **Reinberg, D., M. Horikoshi, and R. G. Roeder.** 1987. Factors involved in specific transcription in mammalian RNA polymerase II. J. Biol. Chem. **262**:3322–3330.
22. **Rudolph, H., and A. Hinnen.** 1987. The yeast *PHO5* promoter; phosphate-control elements and sequences mediating mRNA start-site selection. Proc. Natl. Acad. Sci. USA **84**:1340–1344.
23. **Saltzman, A. G., and R. Weinmann.** 1989. Promoter specificity and modulation of RNA polymerase II transcription. FASEB J. **3**:1723–1733.
24. **Schmidt, M. C., C. C. Kao, R. Pei, and A. J. Berk.** 1989. Yeast TATA-box transcription factor gene. Proc. Natl. Acad. Sci. USA **86**:7785–7789.
25. **Struhl, K.** 1989. Molecular mechanisms of transcriptional regulation in yeast. Annu. Rev. Biochem. **58**:1051–1077.
26. **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.