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

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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, TFIIIB, TFIID, and TFIIIE/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, TFIIID (6, 8), and the TFIIA protein (5) were isolated and characterized. The gene that encodes the TFIIID 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 TFIIID does not confer on the hybrid system the typical yeastlike pattern of transcription initiation. Some component other than TFIIID 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 TFIIID (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 W316-201 (MATa adh1Δ adh2Δ adh3 ade2 ade4 trpl1 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 MATa 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 pEG2A50 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 pEG2A50-TAGA contains a TATAAA-to-TAGAAA base pair change but is otherwise identical to pEG2A50. 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 ascii on YEPD plates. Yeast transformations were done by the spheroplasting protocol described by Beggs (2). When phenotypes were tested on antimycin plates, double replicates 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 on nonselective plates to determine the true number of cells plated; approximately 2 x 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'-CTTGGAAACTG GAATATC-3'). The third oligonucleotide is complementary to the S. cerevisiae histone H2B gene in the region between +29 and +46 (5'-CAGCTGGGTCCTGGAGG-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 AUG 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 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 x 10^-6, the approximate overall frequency of recovery of these mutants was 8 x 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 or not at all 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

<table>
<thead>
<tr>
<th>Type of tetrad</th>
<th>No. of tetrads*</th>
</tr>
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<tbody>
<tr>
<td>Parental diatype</td>
<td>shi</td>
</tr>
<tr>
<td></td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>303</td>
</tr>
<tr>
<td>Nonparental diatype</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Tetratype</td>
<td>29</td>
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<td>35</td>
</tr>
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* The shi recombination frequency was 22.3%; mutant 303 was unlinked.

harbor a dominant mutation, since the resulting diploid was able to grow on antimonycin plates. For complementation testing, diploids from the 25 recessive MATa strains crossed to a set of 11 MATa 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 antimonycin plates. All of these diploids failed to complement; that is, they were able to grow on antimonycin 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 antimonycin 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 antimonycin resistance phenotype when double replica plated on antimonycin plates. The colony size and growth rate on antimonycin 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 antimonycin 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 antimonycin resistance level, the following experiments were done. Both strongly and weakly antimonycin-resistant segregants were crossed back to the Adh0 strain, the diploids were sporulated, and tetrads were dissected. In 12 tetrads analyzed from the backcross of the strongly Adh* diploid, the two antimonycin-resistant spores still showed variation in growth. Eight tetrads from the backcross of the weakly Ant* haploid showed 2:2 segregation on double antimonycin replicas; the antimonycin-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 antimonycin 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 shi mutant 303 crossed to strain Adh0, 45 tetrads were dissected. The two Adh* spores from these tetrads often showed marked growth differences on double antimonycin 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.

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 down-stream 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 ShiΔ 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↓1, and TATT↓1 initiation sites for the adhΔ50 allele are used to a much greater extent in the mutant strain than in the ShiΔ strain. The downstream initiation site TGCC↓24 used strongly for the adhΔ50 allele...
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. 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.

**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.
ADHJ gene and ADHJ initiation from transformants. A
in the observed sites.

ers, the isolated), (3). In the normal shi and SHI strains.

 initiation sites. 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 TATAATA sequence in the adhA50 allele was changed by a single-base-pair mutation to TAGAAATA. A plasmid containing this change, pEG2A50-TAGA, and a plasmid containing the unaltered adhA50 allele were transformed into the shi mutant strain. Whereas the TATA-containing adhA50 allele provided ADH function in shi mutant strains, transformants of pEG2A50-TAGA in the shi strain showed no growth on ampicillin plates. This indicates that the shi mutation cannot restore function to the adhA50 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 (CYC7, 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 adhA50 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 downstream of TATA is also used at lower levels and an initiation site 51 bp downstream is weakly used.

**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
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 *ADH1* initiation sites contrasts with that found in wild-type strains, in which *ADH1* 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 allele and the *adhΔ50* allele, fit the Py A/T (Pu) consensus which describes the normal *ADH1* 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/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 TFIIID 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, TFIIIB, TFIIIE, or TFIIIF), 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.

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shi AFFECTS SPACING BETWEEN TATA AND INITIATION SITES


