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Equivalent Mutations in the Two Repeats of Yeast TATA-Binding Protein Confer Distinct TATA Recognition Specificities

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To investigate the process of TATA box recognition by the TATA box-binding protein (TBP), we have performed a detailed genetic and biochemical analysis of two *Saccharomyces cerevisiae* TBP mutants with altered DNA-binding specificity. The mutant proteins have amino acid substitutions (Leu-205 to Phe and Leu-114 to Phe) at equivalent positions within the two repeats of TBP that are involved in TATA element binding. In an in vivo assay that employs a nearly complete set of single point mutations of the consensus TATAAA sequence, one of the TBP mutants (TBP-L114F) recognizes the sequence TATAAG, while the other TBP mutant (TBP-L205F) recognizes one substitution at the first position of the TATA element, CATAAA, and three substitutions at the 3′ end of the TATA box. Specificity patterns determined from in vitro transcription experiments with purified recombinant wild-type TBP and TBP-L205F agree closely with those observed in vivo, indicating that altered TATA utilization in the mutant strains is a direct consequence of altered TATA recognition by the mutant TBPs. The distinct TATA recognition patterns exhibited by TBP-L114F and TBP-L205F strongly suggest that in vivo, TBP binds to the TATA element in a specific orientation. The orientation predicted from these studies is further supported by the identification of intragenic suppressors that correct the defect of TBP-L205F. This orientation is consistent with that observed in vitro by crystallographic analyses of TBP-TATA box complexes. Finally, the importance of altered DNA-binding specificity in transcriptional regulation at the *S. cerevisiae his4-912δ* promoter was addressed for TBP-L205F. A mutational analysis of this promoter region demonstrates that the nonconsensus TATA element CATAAA is required for a transcriptional effect of TBP-L205F in vivo. This finding suggests that the interaction of TBP with nonconsensus TATA elements may play an important regulatory role in transcription initiation.

The TATA box-binding protein (TBP) plays an essential role in transcription initiation by all three eukaryotic nuclear RNA polymerases (see reference 9 for a review). At promoters transcribed by RNA polymerase II, TBP, as the central component of the general transcription factor TFIID, binds to the TATA box and initiates the assembly of a transcriptionally active preinitiation complex, which consists of a number of different general transcription factors and RNA polymerase II (reviewed in references 6, 23, 25, and 36). As the first step in preinitiation complex formation, binding of TFIID to the TATA box is an important target for the action of regulatory factors (for examples, see references 3, 18, 20, and 34).

The interaction between TBP and the TATA box (consensus, TATAa/tA) has been well characterized in vitro. Crystallographic analyses of TBP-TATA box complexes containing *Arabidopsis thaliana* TBP2 or *Saccharomyces cerevisiae* TBP have demonstrated that the antiparallel β sheets on a concave surface of TBP form specific contacts with bases in the minor groove of an 8-bp TATA element (14, 15). No contacts with bases in the major groove were observed. Binding of the TATA sequence along the underside of the TBP "saddle" results in two sharp kinks in the DNA and a dramatic bend of the duplex toward the major groove. These findings confirmed results from earlier methylation interference (16, 29) and DNA-bending studies with *S. cerevisiae* TBP (12).

Mutational analyses of TBP and TATA elements have provided important insights into their interaction. Systematic analysis of point mutations in the TATA sequence has demonstrated that TBP recognizes the consensus sequences TATAAA and TATATA with a high degree of specificity in vivo and in vitro (5, 8, 33). Deletion analysis of *SPT15*, the gene encoding yeast TBP, has localized the DNA-binding portion of the protein to a highly conserved carboxy-terminal domain of approximately 180 amino acids (13, 24). This region of TBP is dominated by two imperfect direct repeats, each of approximately 60 amino acids, which fold into highly symmetric domains (4, 22). A number of specific amino acid changes within these repeats, obtained either by yeast genetics or through site-directed mutagenesis, disrupt the TBP-TATA interaction (reviewed in reference 9). Crystallographic studies have confirmed that nearly all of the amino acids implicated in DNA binding by mutational analyses form direct contacts with the TATA region (14, 15).

In addition to identifying amino acid changes in TBP that generally impair DNA binding, genetic selections in *S. cerevisiae* have identified at least two TBP mutants with altered DNA-binding specificity (1, 30). One such mutant protein was identified in a genetic selection for TBP mutants that recognize the sequence TGTAAA (30). This mutant contains two amino acid changes, Ile-194 to Phe and Leu-205 to Val, in a highly conserved region of the second direct repeat in the carboxy-terminal domain of TBP. Both substitutions are necessary for the mutant phenotype. In an unrelated genetic selection, a different TBP altered-specificity mutant was identified as a

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suppressor of promoter insertion mutations caused by the yeast retrotransposon Ty (1). Interestingly, this mutant protein, which is encoded by the *spt15-122* mutation, contains a single amino acid change which also occurs at position 205 (Leu-205 to Phe; hereafter L205F). The importance of Leu-205 in TATA box recognition is further supported by recent crystallographic studies, which have concluded that this amino acid directly contacts the TATA element (14, 15).

In a study of the functional similarity of the direct repeats of yeast TBP, we previously reported the construction and characterization of the *spt15-301* mutation which encodes the amino acid change Leu-114 to Phe (hereafter L114F) (1). Leucine 114 lies within the first direct repeat of TBP in a position equivalent to that occupied by leucine 205 in the second repeat. The L114F and L205F substitutions cause similar mutant phenotypes and effects on transcription in vivo, suggesting that TBP-L114F, like TBP-L205F, may have altered DNA-binding specificity. Although these genetic findings argued that both direct repeats of TBP were involved in TATA box binding, they did not identify the specificity patterns for TBP-L114F and TBP-L205F and could not distinguish between models in which the repeats played identical or distinct roles in TATA recognition.

To define the specificity of the *spt15-122* (L205F) and *spt15-301* (L114F) mutant proteins, we have now tested the ability of these TBPs to utilize point mutations of the sequence TATAAA in vivo and in vitro. Our results demonstrate that both proteins recognize specific TATA point mutations. Furthermore, the altered recognition pattern of each mutant protein is distinct, demonstrating that Leu-205 and Leu-114 play distinct roles in TATA box binding. These results strongly suggest that the orientation of TBP binding in vivo is the same as that shown by the crystallographic studies. Lastly, the identification of a TATA variant preferentially recognized by TBP-L205F has led to a demonstration of the importance of altered DNA-binding specificity by TBP in transcriptional regulation.

MATERIALS AND METHODS

Plasmids. The construction of yeast integrating plasmids containing point mutations of the *HIS3* regulatory TATA element (T_R) was described previously (8). The *his3-Δ93* series of plasmids was used in this study. In these plasmids, derivatives of the sequence TATAAA have been inserted at position −83 (relative to the site of transcription initiation at +1) of the *HIS3* promoter at a distance of 23 bp downstream from the GCN4 binding site. The constitutive *HIS3* TATA element (T_C) has been deleted in these constructs; however, the poly(dA-dT) element and sequences further upstream have been retained (31). Presence of the correct *HIS3* T_R point mutations was verified by DNA sequencing for all plasmids used in this study.

Plasmid templates used for in vitro transcription assays with wild-type TBP and TBP-L205F were described previously (33). These plasmids contain the same TATA elements as the *his3-Δ93* plasmids. The TATA elements are inserted upstream (at position −31) of the adenovirus major late initiator element and the G-less cassette from $pML(C_2AT)_{19}\Delta$-50 (27).

Plasmids for the study of *his4-912δ* TATA elements in vivo were constructed as follows. First, plasmid pKA40 was constructed to replace the promoter region of the wild-type *his4-912δ* allele (sequences −403 to +156, relative to the *HIS4* transcription start site) with the yeast *URA3* gene. The vector for pKA40 was derived from pKA1 (1) by restriction with *Xho*I, followed by filling in of the *Xho*I site with Klenow enzyme and then restriction with *Spe*I. The *URA3*-containing insert for pKA40 was excised from plasmid pGP44, a pUC18 derivative which contains the 1.1-kb *Sma*I-*Hin*dIII *URA3* fragment from YEp24 inserted at the *Sma*I site of the polylinker. Plasmid pGP44 was subjected to restriction by *Eco*RI, followed by filling in of the *Eco*RI site with Klenow enzyme and then restriction by *Xba*I. In the final plasmid, the *URA3* gene replaces sequences −403 to +156 of *his4-912δ* such that the direction of *URA3* transcription is opposite to the direction of *HIS4* transcription. Plasmids containing point mutations of the *his4-912δ* TATA region IV and the nonconsensus TATA regions II and III were derived from plasmid pKA41. Plasmid pKA41 is a derivative of pRS306 (28) in which a 1.9-kb *Sal*I fragment containing wild-type *his4-912δ* has been inserted at the *Sal*I site of the polylinker. Mutations were introduced into the *his4-912δ* promoter region of pKA41 by oligonucleotide-directed mutagenesis, using a procedure outlined by Stratagene (La Jolla, Calif.) for the mutagenesis of pBluescript II vectors. At site II, the mutagenic oligonucleotide replaced the nonconsensus TATA sequence TATCAA with the sequence CTGCAG (a *Pst*I restriction site). At site III, the mutagenic oligonucleotide replaced the nonconsensus TATA sequence CATAAA with the sequence GAGCTC (a *Sac*I restriction site). At site IV, the oligonucleotide used for mutagenesis mutated both of the tandem TATAAA sequences of the δ TATA region. The 5′ TATAAA element was mutated to TGTAGA, and the 3′ element was changed to CTGCAG (a *Pst*I restriction site). Introduction of the mutations was confirmed by restriction analysis and, for sites II and III, by DNA sequence analysis of the entire *his4-912δ* promoter region.

Yeast strains and media. Rich (YPD), minimal (SD), synthetic complete (SC), presporulation, and sporulation media were prepared as described previously (26). Nutrient auxotrophies and suppression of δ insertion mutations at *HIS4* and *LYS2* were scored on SD medium supplemented with the appropriate amino acids and other nutrients. Yeast transformants were selected on SC medium lacking the appropriate supplement. Selection of *ura3* strains was carried out on SC medium containing 5-fluoro-orotic acid (26). Medium containing 3-aminotriazole (AT) was SD medium lacking histidine but containing other appropriate supplements and the indicated concentration of AT. The *S. cerevisiae* strains used in this study (Table 1) are isogenic to FY2, a *GAL2*$^+$ *ura3-52* derivative of S288C, and were constructed by standard methods (26).

To replace the genomic *HIS3* gene with the *his3-Δ93* T_R mutations, two-step gene replacements of the *his3Δ200* allele were carried out in *SPT15*$^+$, *spt15-122*, and *spt15-301* strains. Since haploid *spt15-122* strains spontaneously convert to *MAT***a**/*MAT***a** or *MAT*α/*MAT*α diploids with high frequency, we constructed *MAT***a**/*MAT*α diploid strains homozygous for the *SPT15* allele of interest and heterozygous for the *his3-Δ93* mutation of interest. In this way, we attempted to avoid any phenotypic variability due to differences in ploidy. To obtain *SPT15*$^+$ strains with mutations in the *HIS3* T_R element, all of the *his3-Δ93* plasmids, except that containing the double mutation *his3-215,238*, were integrated at the *his3Δ200* allele of the haploid FY661. After 5-fluoro-orotic acid selection, recombinants that retained the T_R mutation were identified (see below) and crossed by FY739 to generate the desired diploid strains. The *his3-215,238* mutation was integrated directly into the diploid strain FY741. To obtain *spt15-301* strains with *HIS3* T_R mutations, all *his3-Δ93* constructs, except *his3-203*, *-208*, *-212*, and *-218*, were first introduced into FY679 to replace *his3Δ200*. The resulting strains were then crossed by FY766. The *his3-203*, *-208*, *-212*, and *-218* alleles were integrated directly into the diploid strain FY780. To generate *spt15-122* strains with the *HIS3* T_R mutations, all *his3-Δ93*

TABLE 1. Yeast strains used in this study

Strain	Genotype
FY546	*MAT***a** *his4-912δ lys2-173R2 ura3-52 trp1Δ63*
FY661	*MAT***a** *his3Δ200 lys2-173R2 ura3-52 ade8*
FY679	*MAT***a** *spt15-301 his3Δ200 lys2-173R2 ura3-52 leu2Δ1 ade8*
FY739	*MAT*α *his3Δ200 lys2-173R2 ura3-52 trp1Δ1*
FY740	*MAT***a**/*MAT*α *his3Δ200/HIS3 lys2-173R2/lys2-173R2 ura3-52/ura3-52 trp1Δ1/TRP1*
FY741	*MAT***a**/*MAT*α *his3Δ200/his3Δ200 lys2-173R2/lys2-173R2 ura3-52/ura3-52 ade8/ADE8 trp1Δ1/TRP1*
FY766	*MAT*α *spt15-301 his3Δ200 lys2-173R2 ura3-52 leu2Δ1 trp1Δ1*
FY777	*MAT***a**/*MAT*α *spt15-122/spt15-122 his3Δ200/his3Δ200 lys2-173R2/lys2-173R2 ura3-52/ura3-52 leu2Δ1/LEU2 ade8/ADE8*
FY778	*MAT***a**/*MAT*α *spt15-301/spt15-301 his3Δ200/HIS3 lys2-173R2/lys2-173R2 ura3-52/ura3-52 ade8/ADE8 leu2Δ1/LEU2 trp1Δ1/TRP1*
FY779	*MAT***a**/*MAT*α *spt15-122/spt15-122 his3Δ200/HIS3 lys2-173R2/lys2-173R2 ura3-52/ura3-52 ade8/ADE8*
FY780	*MAT***a**/*MAT*α *spt15-301/spt15-301 his3Δ200/his3Δ200 lys2-173R2/lys2-173R2 ura3-52/ura3-52 leu2Δ1/leu2Δ1 ade8/ADE8 trp1Δ1/TRP1*
YKA110	*MAT***a** *spt15-122 his4-917δ lys2-173R2 trp1Δ1 ade8*
YKA113	*MAT***a** *his4-912δΔURA3 lys2-173R2 ura3-52 trp1Δ63*
YKA145	*MAT*α *spt15-122 his4-912δΔURA3 lys2-173R2 leu2Δ1 ura3-52*

constructs were introduced directly into the diploid strain FY777. For all strain constructions, *his3-Δ93* plasmids, linearized with either *Xba*I or *Hpa*I to target integration to *his3Δ200*, were transformed into the appropriate strain, and Ura$^+$ transformants were selected. After selection on media containing 5-fluoro-orotic acid, recombinants that had retained *HIS3* T_R mutations and functional *HIS3* genes were identified by scoring the His phenotype of the strains. In *SPT15*$^+$ and *spt15-301* backgrounds, even very deleterious TATA mutations will give some detectable growth on media lacking histidine. In an *spt15-122* background, however, some T_R mutations do not drive enough *HIS3* expression to support growth on media lacking histidine. In these cases, desired recombinants were identified by PCR using primers that flank the *his3Δ200* deletion. All final strains were assayed by Southern analysis and for growth on media containing AT.

Phenotypic analysis of T_R function was carried out by testing growth of strains on media containing AT, a competitive inhibitor of the *HIS3* gene product. For *SPT15*$^+$ and *spt15-301* strains, growth was assayed on AT concentrations of 2, 5, 10, 20, and 40 mM. For reasons unrelated to the level of *HIS3* transcription (see Results), *spt15-122* strains are approximately 40-fold more sensitive than *SPT15*$^+$ or *spt15-301* strains to the concentration of AT in the media. Thus, analysis of the growth of *spt15-122* strains on AT-containing media required the use of lower AT concentrations (0.1, 0.25, 0.5, and 1.0 mM).

Introduction of *his4-912δ* TATA site mutations into an *SPT15*$^+$ strain was carried out in two steps. First, the wild-type *his4-912δ* promoter region (sequences −403 to +156, relative to the *HIS4* transcription initiation site) was replaced with the *URA3* gene by transformation of strain FY546 with the 2.4-kb *Sal*I fragment from pKA40. Elimination of *his4-912δ* sequences and insertion of *URA3* in the integrant strain, YKA113, was confirmed by Southern analysis and by a genetic cross with a *HIS4*$^+$ strain. Second, integration of a particular *his4-912δ* TATA site mutation was accomplished by transforming YKA113 with the 1,459-bp *Pvu*II-*Sal*I fragment from a pKA41 derivative that contained a site-directed change at TATA region II, III, or IV. Transformation mixes were diluted 1:25 in YPD and grown overnight at 30°C prior to plating on medium containing 5-fluoro-orotic acid to identify transformants that lost *URA3* function. Replacement of the *his4-912δΔURA3* allele in YKA113 was confirmed by Southern analyses and appropriate genetic crosses. To analyze suppression of the *his4-912δ* mutations by *spt15-122*, *SPT15*$^+$ strains carrying the mutations (YKA113 derivatives) were crossed by strain YKA145, and the appropriate spores were identified and analyzed.

Isolation of intragenic revertants of *spt15-122*. Plasmid pFW270, a derivative of plasmid pDE59-1 that contains *spt15-122* (1), was mutagenized in vitro by using hydroxylamine (26). The mutagenized plasmid preparation was used to transform yeast strain YKA110, an *spt15-122* mutant, to Trp$^+$, and transformants were screened for those that had become phenotypically Spt$^+$ (based on reversing the Spt$^-$ [Lys$^-$] phenotype of the insertion mutation *lys2-173R2* to Lys$^+$ [1]). Four candidates were identified, and the plasmids were isolated from yeast cells (26) and used to transform *Escherichia coli*. Plasmid DNA prepared from the *E. coli* transformants was then used to retransform YKA110 to confirm that the Spt$^+$ phenotype was conferred by the plasmid. For all four candidates, the entire *spt15* coding region was sequenced as previously described (1). Since control experiments demonstrated that the hydroxylamine mutagenesis raised the frequency of mutations in the plasmid-borne *TRP1* gene to 0.2%, we have assumed that the *spt15* intragenic revertants are of independent origin.

S1 hybridization analysis. For analysis of *HIS3* transcription, strains were grown at 30°C in SD medium lacking histidine to a concentration of 10^7 cells per ml. AT was then added to a final concentration of 10 mM for all strains, and cultures were incubated for an additional 4 h at 30°C. Control experiments showed that *spt15-122* strains do not lose viability during this incubation in 10 mM AT. Preparation of total RNA and S1 hybridization analysis using oligonucleotide probes for *HIS3* and *DED1* transcripts were performed as previously described (5). Reaction products were analyzed on 8% polyacrylamide–8.3 M urea gels. Levels of protected probe were quantitated on a Molecular Dynamics PhosphorImager with ImageQuant software. The data presented in Fig. 2 and Table 3 are representative results from one of five independent experiments.

In vitro transcription analysis. The purification of wild-type TBP and TBP-L205F from *E. coli* strains was described previously (1). The wild-type TBP and TBP-L205F preparations were approximately 50 and 20% pure, respectively. The isolation of general transcription factors from HeLa cells and in vitro transcription assays using these factors and recombinant TBP were performed as described previously (33) except that each reaction contained 200 ng of the test template of interest (described above) and 200 ng of a control template (35). This control template contained a consensus TATA

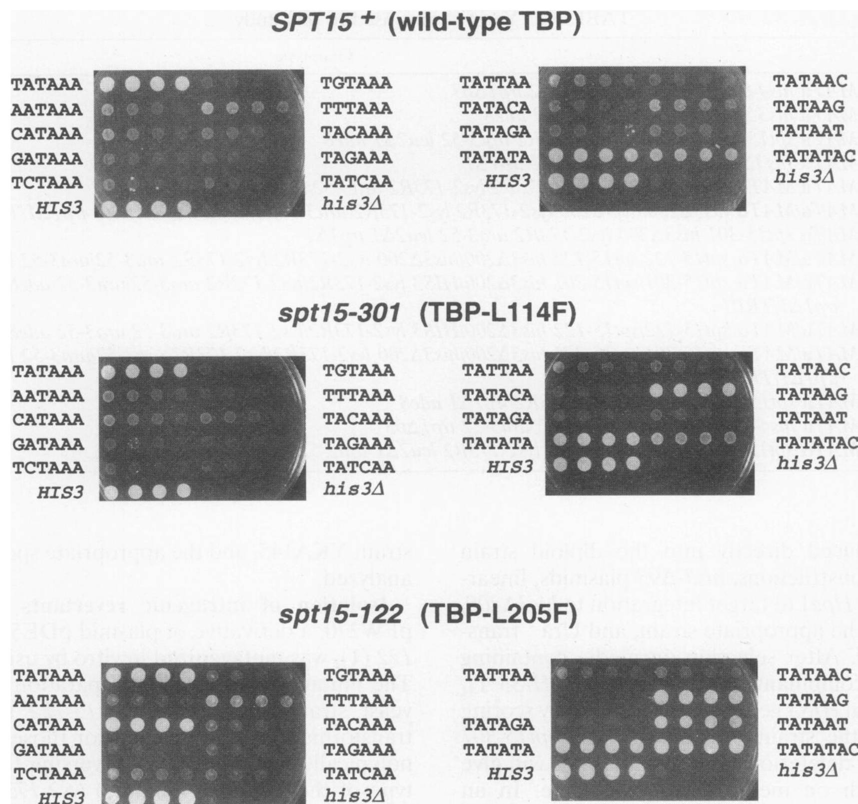

FIG. 1. AT sensitivity of *spt15* mutants containing *HIS3* promoter derivatives. *SPT15*$^+$, *spt15-301*, and *spt15-122* strains containing the indicated TATA elements upstream of the *HIS3* gene were grown to saturation in liquid YPD, washed twice with H_2O, and then spotted onto plates with a multipronged inoculating device. The first spot in the series of four contains an aliquot of a saturated culture (approximately 4×10^8 cells per ml), and the next three spots contain aliquots of twofold serial dilutions of the starting culture. The plates shown contain either 2 mM AT (*SPT15*$^+$ and *spt15-301*) or 0.1 mM AT (*spt15-122*) and were incubated at 30°C for 3 days prior to photography. "*HIS3*" denotes a heterozygous diploid strain that contains the normal *HIS3* promoter and gene and the *his3Δ200* allele (strains FY740, FY778, and FY779). "*his3Δ*" denotes a diploid strain homozygous for *his3Δ200* (strains FY741, FY777, and FY780). The TATACA promoter derivative was not obtained in an *spt15-122* background.

element (TATAAA) upstream of a shortened G-less cassette and was included for normalization purposes. The RNA polymerase II used in these experiments was purified from calf thymus as described previously (11) and was the gift of Rebecca Sundseth and Ulla Hansen. In vitro transcription products were analyzed on 5% denaturing polyacrylamide gels and quantitated on a Molecular Dynamics PhosphorImager with ImageQuant software. In vitro transcription reactions were performed and analyzed twice.

RESULTS

Two yeast TBP mutants recognize specific mutant TATA elements in vivo. In our previous analysis of *spt15* mutations that suppress the transcriptional defects caused by Ty element insertions, we found that the *spt15-122* mutation encoded a mutant TBP (TBP-L205F) with altered DNA-binding specificity (1). In particular, TBP-L205F exhibited increased affinity, relative to wild-type TBP, for two regions within the *his4-912δ* promoter that contain nonconsensus TATA elements (see Fig. 4). In the current study, we sought to identify systematically which nonconsensus TATA elements could be utilized by this mutant TBP. In addition, to determine whether similarities in mutant phenotypes conferred by *spt15-122* (L205F) and *spt15-301* (L114F) were caused by similarities in the patterns of TATA recognition by TBP-L205F and TBP-L114F, we also investigated the specificity of TATA utilization in *spt15-301* mutants.

To determine the preferred recognition sequences of TBP-L205F (*spt15-122*) and TBP-L114F (*spt15-301*) in vivo, we used the methodology originally developed by Chen and Struhl (5) and the plasmids developed by Harbury and Struhl (8) for the analysis of mutant TATA function. The plasmids used in this study contain the promoter of the *HIS3* gene altered so that the regulatory (T_R) and constitutive (T_C) TATA elements (31) are replaced by an oligonucleotide containing the sequence TATAAAGT or variants of that sequence. The GCN4 binding site of the *HIS3* promoter has been retained and lies 23 bp upstream of the TATA element.

HIS3 promoter derivatives bearing 16 of the 18 possible point mutations of the sequence TATAAA and one double mutation, TATATAC, were introduced into *SPT15*$^+$, *spt15-122*, and *spt15-301* diploid strains by integrative transformations and genetic crosses as described in Materials and Methods. The resulting diploid strains were homozygous for the desired *SPT15* allele and contained one copy of the *his3Δ200* allele and one copy of the *HIS3* promoter derivative of interest driving the expression of an intact *HIS3* gene. Relative activities of the TATA elements in promoting *HIS3* expression were determined by testing growth of strains on media containing AT, a competitive inhibitor of the *HIS3* gene product (Fig. 1). For *SPT15*$^+$ and *spt15-301* strains, growth was assayed on AT

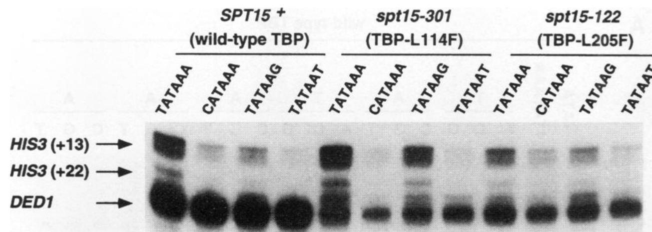

FIG. 2. S1 nuclease protection analysis of *HIS3* transcript levels in strains containing TATA element mutations. Total RNA was prepared from *SPT15*$^+$, *spt15-301*, and *spt15-122* strains containing the indicated *HIS3* promoter derivatives. For each reaction, 10 μg of total RNA was hybridized to an excess of ^{32}P-end-labeled oligonucleotide probe for *HIS3* mRNA and *DED1* mRNA before treatment with S1 nuclease. The amount of protected *DED1* probe serves as a normalization standard for each set of lanes (i.e., for each *SPT15* allele). The positions of migration of probes protected from digestion by *HIS3* mRNA (+13 and +22 initiation sites) and *DED1* mRNA are indicated. The *HIS3* +1-initiated transcript is absent in these strains, because the TATA element responsible for this transcript (T_C) (31) has been deleted in the construction of the *HIS3* promoter derivatives (8). We reproducibly observe heterogeneity at the *HIS3* +13 initiation site in our strain background.

concentrations ranging from 2 to 40 mM. For *spt15-122* strains, it was necessary to measure growth on much lower AT concentrations (0.1 to 1 mM), since higher concentrations of the compound prevented growth of all *spt15-122* strains, including those containing optimal TATA elements. The approximately 40-fold increase in sensitivity to AT exhibited by *spt15-122* strains is not due to a proportional reduction in *HIS3* transcription (Fig. 2) or to a defect in GCN4-mediated activation of *HIS3* (2). Moreover, *spt15-122* strains exposed to 10 mM AT for 4 h in liquid culture do not lose their viability (2). Thus, we conclude that the sensitivity of *spt15-122* strains to AT is most likely due to an indirect effect on cell growth unrelated to *HIS3* expression and that valid conclusions concerning relative TATA utilization can be made for each set of strains individually.

In agreement with previous studies (5, 8), strains that contain wild-type TBP exhibit a strong preference for the TATA sequences TATAAA and TATATA (*his3-215*), although other sequences, including TATAAG (*his3-217*) and TTTAAA (*his3-206*), are used to a measurable extent (Fig. 1 and Table 2). Like *SPT15*$^+$, the two *spt15* mutants also strongly recognize TATAAA and TATATA; however, these mutants have also gained the ability to efficiently utilize certain nonconsensus TATA elements. In this report, we will use the term "altered specificity" to reflect a gain in specificity for a mutant TATA sequence that is not necessarily accompanied by a loss in TATAAA utilization.

The most striking distinction between *SPT15*$^+$ and *spt15-301* strains in their patterns of TATA utilization occurs with the sequence TATAAG (Fig. 1 and Table 2). Whereas *SPT15*$^+$ strains containing this mutant TATA sequence grow weakly on 5 mM AT, *spt15-301* strains containing TATAAG grow on 40 mM AT as well as strains containing TATAAA do. The only other significant difference between the *SPT15*$^+$ and *spt15-301* recognition patterns is a decrease in TTTAAA activity in *spt15-301* strains. This high degree of specificity for TATAAG exhibited by *spt15-301* strains strongly suggests that TBP-L114F has altered DNA-binding specificity for TATA elements.

In contrast to the high degree of specificity exhibited by *SPT15*$^+$ and *spt15-301* strains, *spt15-122* strains containing four

TABLE 2. Growth of *spt15* mutant strains on media containing AT

Relevant sequence[a]	*HIS3* allele(s)	Growth[b]		
		SPT15	*spt15-301*	*spt15-122*
TATAAA	Wild type	++	++	++
AATAAA	*201*	−/+	−/+	−
CATAAA	*202*	−/+	−/+	+/−
GATAAA	*203*	−	−	−
TCTAAA	*204*	−	−	−
TGTAAA	*205*	−	−	−
TTTAAA	*206*	+/−	−	−/+
TACAAA	*208*	−	−/+	−
TAGAAA	*209*	−	−	−
TATCAA	*210*	−	−	−
TATTAA	*212*	−/+	−/+	−
TATACA	*213*	−	−	ND
TATAGA	*214*	−	−	−
TATATA	*215*	++	++	+
TATAAC	*216*	−	−/+	−
TATAAG	*217*	+/−	++	+
TATAAT	*218*	−	−/+	+/−
TATATAC	*215, 238*	+/−	+/−	++

[a] The underlined base indicates the TATA mutation.
[b] Growth of *SPT15*$^+$ and *spt15-301* strains on media containing AT was scored as follows: ++, slow growth on 40 mM AT and normal growth on 20 mM AT; +, slow growth on 20 mM AT and normal growth on 10 mM AT; +/−, slow growth on 5 and 10 mM AT; −/+, very slow growth on 5 mM AT; −, no growth on 5 mM AT. For reasons described in the text, growth of *spt15-122* strains was scored on lower concentrations of AT as follows: ++, slow growth on 1 mM AT and normal growth on 0.5 mM AT; +, slow growth on 0.5 mM AT and normal growth on 0.25 mM AT; +/−, slow growth on 0.25 mM AT and normal growth on 0.1 mM AT; −/+, slow growth on 0.1 mM AT; −, no growth on 0.1 mM AT. ND, not determined.

different mutant TATA derivatives show significant levels of growth on AT media compared with growth of the *spt15-122* TATAAA strain (Fig. 1 and Table 2). One of the four changes occurs at position 1 of the TATA sequence, where the substitution of a C for a T leads to significant levels of *HIS3* expression (*his3-202*). Importantly, the two other possible substitutions at position 1, A (*his3-201*) and G (*his-203*), are not tolerated. The three other mutations utilized by *spt15-122* localize to the 3′ end of the TATA box. Two TATA mutants with substitutions at position 6, TATAAG (*his3-217*) and TATAAT (*his3-218*), are recognized strongly, and the substitution of a C for a G at position 7 in the context of TATATA results in elevated levels of *HIS3* expression (compare *his3-215* and *his3-215, 238*). In agreement with our previous studies, these data indicate that the protein encoded by *spt15-122*, TBP-L205F, has altered specificity for TATA elements. Since only one substitution at position 1 is recognized, whereas three different changes at the 3′ end of the TATA element are tolerated, the primary change in specificity, most likely due to a new amino acid-base contact, appears to occur at position 1; the additional specificity changes may reflect indirect effects of this new contact. Isolation of intragenic suppressors (described below) support this hypothesis. Alternative interpretations of these results are presented in Discussion.

To confirm that the different phenotypes on AT plates were due to changes in *HIS3* transcription, we analyzed GCN4-activated *HIS3* transcript levels in *SPT15*$^+$, *spt15-122*, and *spt15-301* strains (Fig. 2). The activities of four different TATA elements, TATAAA, CATAAA, TATAAG, and TATAAT, were assayed by S1 nuclease protection analysis of RNA produced in vivo. As shown in Fig. 1 and 2, there is good agreement between relative *HIS3* transcript levels and relative growth on AT-containing media. When normalized to TAT

TABLE 3. Transcriptional activities of TATA mutants in vivo

Relevant sequence	HIS3 allele	Relative level of HIS3 transcription[a]		
		SPT15	spt15-301	spt15-122
TATAAA	Wild type	100	100	100
CATAAA	202	17	14	64
TATAAG	217	30	66	77
TATAAT	218	10	9	49

[a] *HIS3* (+13 initiation site) transcript levels were quantitated from the S1 hybridization analysis shown in Fig. 2 and were normalized to *DED1* transcript levels. For each allele of *SPT15*, the normalized *HIS3* transcript levels achieved by the mutant TATA elements are given as percentages of the normalized *HIS3* transcript level produced by TATAAA.

AAA activity, *HIS3* transcription from the mutant TATA elements is affected in the *spt15* mutants in a manner consistent with the phenotypic results. In particular, relative to *SPT15*$^+$ strains, the activity of TATAAG is elevated more than twofold in both mutants, and the activity of CATAAA is increased nearly fourfold in *spt15-122* strains (Table 3).

In vitro analysis of mutant TATA utilization by TBP-L205F. One explanation for the alterations in TATA recognition exhibited by *spt15-301* and *spt15-122* strains is that these mutations cause some indirect effect on *HIS3* transcription. Although such an effect must occur in a TATA-specific manner and therefore seemed rather unlikely, we addressed this possibility for *spt15-122* by testing the transcriptional activities of recombinant wild-type TBP and TBP-L205F on mutant TATA elements in vitro. (A similar analysis of recombinant TBP-L114F was not performed because of the instability of this protein in vitro.) Transcription templates consisting of a TATA element (TATAAA or variants), the adenovirus major late promoter initiator element, and a G-less cassette were transcribed in vitro in a reaction containing partially purified general transcription factors from HeLa cells, calf thymus RNA polymerase II, and recombinant yeast TBP. The TATA sequences used in the construction of these templates were identical to those used in the in vivo analysis of *HIS3* expression described above. As shown in Fig. 3, templates containing the consensus TATA sequence, TATAAA, or the derivative TATATA are highly transcribed by both wild-type TBP and TBP-L205F. However, since the absolute level of transcription by TBP-L205F on TATAAA is approximately 30% of the wild-type TBP level (Fig. 3A) (32), comparisons between the two proteins are based on data that have been normalized for each protein independently according to the transcriptional activity of TATAAA (Fig. 3B). This was done to avoid any bias in our analysis arising from differences in overall activities or stabilities of the two TBP preparations.

The patterns of TATA recognition by wild-type TBP and TBP-L205F in vitro are very similar to those observed in vivo. Wild-type TBP exhibits high specificity for only TATAAA and TATATA in this assay, since none of the other TATA elements exceeds 25% activity relative to TATAAA (Fig. 3). In contrast, TBP-L205F, while having relaxed specificity for a number of TATA sequences, reveals clear preferences for certain mutant TATA elements in vitro. As shown in Fig. 3B, TBP-L205F exhibits transcriptional activities of 40% or higher, relative to TATAAA, for five different TATA sequences: CATAAA, TTTAAA, TATATA, TATAAG, and TATAAT. In addition, TCTAAA is used more than 30% as well as TATAAA in this in vitro assay. Of the TATA elements that support more than 40% of the TATAAA level of transcription with TBP-L205F, CATAAA, TATAAG, and TATAAT discriminate by a factor of 4 or greater between wild-type TBP

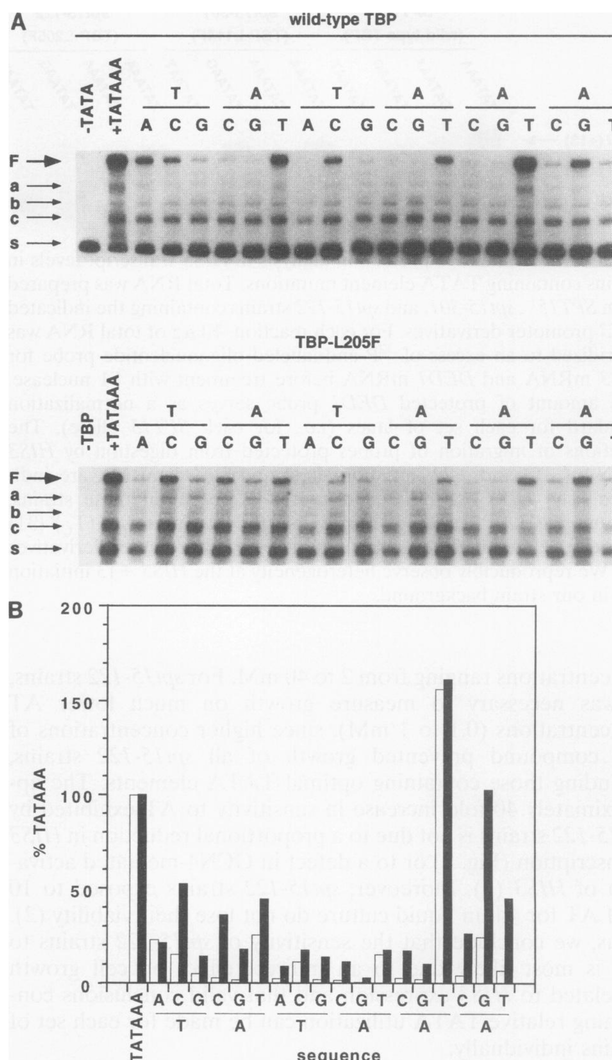

FIG. 3. Transcriptional activity of mutant TATA elements in vitro with wild-type TBP or TBP-L205F. (A) Products of in vitro transcription reactions containing saturating amounts of either wild-type TBP (170 ng; upper panel) or TBP-L205F (50 ng; lower panel) are shown. Reactions contained G-less cassette transcription templates with the indicated derivative of the consensus TATA sequence, TATAAA. The letter directly above each lane indicates the mutant base. The lanes labeled −TATA and −TBP show the products of reactions lacking a test template and recombinant TBP, respectively. F, migration of the full-length, +1-initiated transcription product; s, migration of the transcript produced from a control template that contains a consensus TATAAA element upstream of a shortened G-less cassette (35); a, b, and c, migrations of transcripts internally initiated within the G-less cassette. These RNA polymerase II transcripts are not affected by the TATA element upstream of +1 and serve as useful internal controls for normalization of +1-initiated transcript levels (33). (B) Relative activities of TATA elements in in vitro transcription reactions containing either wild-type TBP (open bars) or TBP-L205F (filled bars). The letter directly below each pair of open and filled bars indicates the mutant base. The +1-initiated transcript levels from two experiments, including that shown in panel A, have been quantitated and normalized to the internally initiated transcript c and to the control template transcript s. For TBP-L205F, the doublet of transcripts at position c were quantitated as a single transcript. The transcriptional activity of each TATA element is presented as the percent activity relative to TATAAA. Because of differences in the overall activity of each TBP preparation, this calculation has been made for each protein independently.

and TBP-L205F. Therefore, the same mutant TATA elements that were identified as targets for TBP-L205F in vivo are preferentially recognized by recombinant TBP-L205F in vitro, confirming that alterations in TATA utilization in vivo are a direct result of alterations in TATA box recognition by the mutant TBP.

Intragenic suppressors of *spt15-122* alter a second amino acid that directly contacts the TATAAA box. The in vivo and in vitro analysis of TBP-L205F, the product of *spt15-122*, has shown that this protein can recognize mutant TATA sequences with a specific base change at position 1 of the TATA sequence, CATAAA, or with three different changes at the 3′ end of the TATA sequence. To analyze this altered specificity in greater detail, we isolated intragenic suppressors of *spt15-122*. Such intragenic suppressors might identify other amino acids in TBP that are important for altered recognition by TBP-L205F. Since the TBP-DNA crystal structure has been determined (14, 15), such amino acid changes might indicate if the primary site of altered recognition for TBP-L205F is at position 1 of the TATA element or at the 3′ end of the TATA element.

We mutagenized a plasmid that contains the *spt15-122* mutant gene and screened yeast transformants for those that now conferred a wild-type phenotype (described in Materials and Methods). By using hydroxylamine, which primarily causes GC-to-AT transitions, as the mutagen, we hoped to favor against isolating true revertants of *spt15-122*, which would require an AT-to-TA transversion. Of 9,132 yeast transformants, we identified four plasmid-borne mutations. When the *spt15* genes on these four plasmids were sequenced, we found that they all still contained the *spt15-122* mutation. In addition, they all contained an identical second mutation, CCT to TCT at triplet 191, which is predicted to change proline 191 to serine (P191S). The TBP-DNA crystal structures (14, 15) suggest that Pro-191 contacts DNA, interacting with the A of the first base pair of the TATA box. This result supports the hypothesis that the primary specificity change of TBP-L205F is at the first base pair of the TATA box and that this altered specificity is corrected in the double mutant, TBP-L205F, P191S.

A sequence containing CATAAA is essential for a transcriptional effect of TBP-L205F at the *his4-912δ* promoter in vivo. Our previous biochemical and genetic analysis of the *spt15-122* mutation suggested a correlation between altered DNA-binding specificity by TBP-L205F and suppression of the Ty δ-element insertion mutation *his4-912δ* (1). In vitro DNase I footprinting analysis revealed that TBP-L205F binds with increased affinity, relative to wild-type TBP, to two regions within the *his4-912δ* promoter that contain a number of near-consensus TATA sequences (sites II and III in Fig. 4A). Interestingly, each of these regions contains a mutant TATA element that we have identified independently in this study as a preferred recognition site for TBP-L205F: region II contains TATAAT and region III contains CATAAA. In addition to binding at regions II and III, TBP-L205F binds with approximately wild-type affinity to the two known, functional TATA elements in the *his4-912δ* promoter region (sites I and IV in Fig. 4A).

To investigate further the mechanism of suppression of *his4-912δ* by *spt15-122*, we made specific mutations in TATA regions II, III, and IV of *his4-912δ* and introduced those mutations into *SPT15*+ and *spt15-122* strains, replacing the endogenous *his4-912δ* alleles. The effect of each of these mutations on suppression of *his4-912δ* was determined by assaying growth on medium lacking histidine. As summarized in Fig. 4B, *SPT15*+ strains containing a wild-type *his4-912δ*

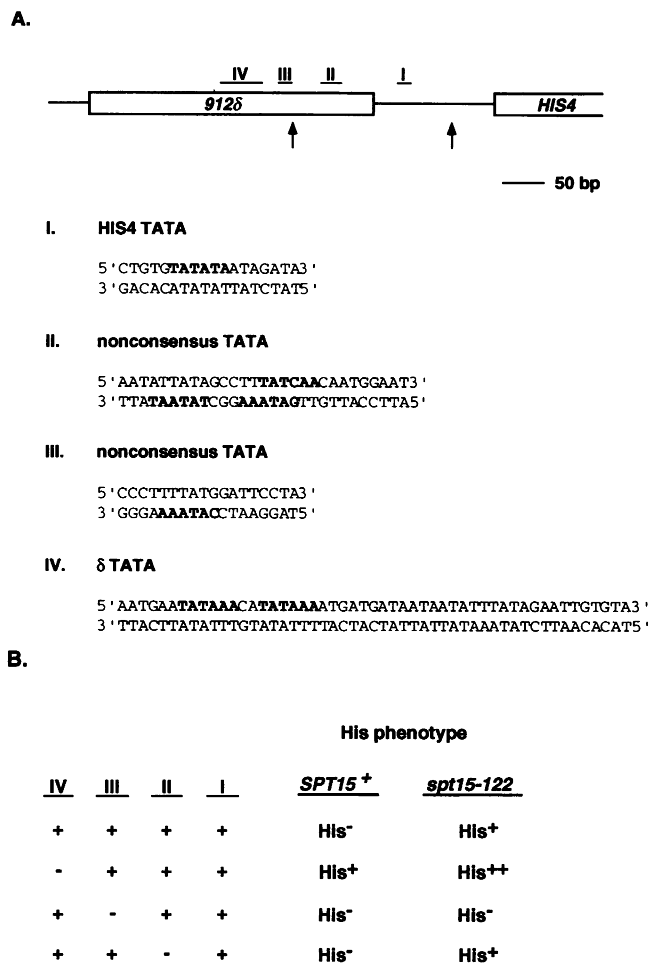

FIG. 4. Mutational analysis of the *his4-912δ* promoter region. (A) Schematic diagram of the *his4-912δ* promoter region, indicating the positions of previously described TBP binding sites I, II, III, and IV. Arrows mark the δ and *HIS4* transcription initiation sites. Given below the diagram are the sequences (sites I to IV) previously shown to be protected from cleavage in DNase I footprinting studies with TBP and TBP-L205F (1). Consensus and nonconsensus TATA elements within these regions of protection are in boldface. For site I, the *HIS4* TATA region, the highlighted TATA sequence has been shown to function in vivo (21). For site IV, the δ TATA region, two consensus TATA elements, both highlighted, are within the region shown to contain TATA function in a related δ element (17). Relative to wild-type TBP, TBP-L205F has increased affinity for sites II and III (1). This diagram was taken from Arndt et al. (1) and is presented here for clarity. (B) Results from a mutational analysis of the TATA elements within sites II, III, and IV. Site-directed mutations were constructed in vitro and integrated into yeast strains as described in Materials and Methods. For site IV, both TATAAA sequences were mutated simultaneously. For site III, the sequence CATAAA was mutated. For site II, the sequences TATCAA and GATAAA were mutated simultaneously. A + under a given site indicates that no mutation has been made in that site; a − indicates that a mutation in the TATA element has been introduced. On the right, the phenotypes conferred by these *his4-912δ* mutations in an *SPT15*+ or an *spt15-122* background are indicated as the growth of strains on plates lacking histidine. A His+ phenotype indicates strong growth on this medium after 3 days at 30°C and represents strong suppression of the *his4-912δ* insertion mutation.

allele are phenotypically His$^-$. In these strains, transcription initiates within the δ element to produce a longer than normal *HIS4* transcript, which is presumed to be translationally nonfunctional. *spt15-122* strains suppress *his4-912δ* by restoring transcription initiation to the normal *HIS4* transcription start site and are therefore phenotypically His$^+$ (1). A site-directed mutation that eliminates both of the consensus TATA elements in region IV, the δ TATA region, enhances suppression by *spt15-122* and causes *SPT15*$^+$ strains to be His$^+$ (Fig. 4B). This result is in agreement with the findings of Hirschman et al. (10), who previously provided genetic evidence for promoter competition between the δ TATA region and the *HIS4* TATA region. Interestingly, a mutation that eliminates the sequence CATAAA in region III, a region preferentially bound by TBP-L205F in vitro, blocks suppression of *his4-912δ* by *spt15-122* (Fig. 4B). Moreover, this mutation suppresses the cold sensitivity and the slight leakiness of *his4-912δ* observed in *SPT15*$^+$ strains (2). Thus, these data strongly suggest that binding of TBP to the nonconsensus TATA element CATAAA of region III in vivo is required for suppression of *his4-912δ* and argue for the importance of DNA-binding specificity in regulating transcriptional initiation. Elimination of two of the three nonconsensus TATA sequences in region II, GATAAA and TATCAA, does not affect the phenotype of either strain. However, since a mutation of the remaining TATA element in region II, TATAAT, has not been analyzed, we cannot rule out the involvement of region II in suppression.

DISCUSSION

The two repeats of TBP are functionally distinct with respect to TATA recognition. To explore the process of TATA box recognition by TBP in vivo, we have performed a detailed genetic analysis of the DNA-binding specificity of two yeast TBP mutants. Using a nearly complete set of TATA element mutations fused to the yeast *HIS3* gene, we have determined that the proteins encoded by the *spt15-122* (TBP-L205F) and *spt15-301* (TBP-L114F) mutations can utilize specific mutant TATA elements more efficiently than wild-type TBP. These findings demonstrate that leucines 114 and 205 play important and distinct roles in TATA box recognition in vivo. Results from in vitro transcription studies that used the same set of TATA mutations and purified recombinant TBPs are in good agreement with the *HIS3* expression results, suggesting that the patterns of TATA utilization observed for TBP-L114F and TBP-L205F in vivo are a direct consequence of altered DNA-binding specificity.

Although we did not directly measure binding of the mutant proteins to the TATA boxes used in these experiments, our results are in very strong agreement with our previous in vitro binding studies of TBP-L205F (1). In those studies, TBP-L205F was shown to preferentially bind to two regions in the *his4-912δ* promoter, one of which contains the sequence CATAAA and the other of which contains the sequence TATAAT. Both of these TATA variants were shown to be preferred both in vivo and in vitro by TBP-L205F in our current studies. In addition, in the previous binding studies, we did not detect binding of TBP-L205F to a different nonconsensus TATA, TATAAC, near the *HIS4* TATA region. This sequence was also shown to be nonfunctional with TBP-L205F in our current studies. Since the in vivo specificity of TBP-L205F fits extremely well with both in vitro transcription and in vitro DNA-binding assays, these results strongly suggest that the in vivo specificity of TBP-L114F (which was too unstable to study in vitro) also reflects altered TATA binding.

The distinct TATA recognition properties of TBP-L114F and TBP-L205F strongly argue that the two direct repeats of yeast TBP are not functionally equivalent and that TBP binds to the TATA element with a specific orientation in vivo. Therefore, the two conserved and symmetrically related domains of TBP, although they may have arisen by an ancestral duplication event, cannot be viewed as identical subunits of a dimeric protein. In contrast, monomeric components of dimeric DNA-binding proteins, such as GCN4 and GAL4, have identical DNA-binding specificities that cannot be distinguished from one another (7, 19).

Relationship of TATA recognition specificity to the crystal structure. Recent crystallographic analyses of two TBP-TATA box complexes have demonstrated that leucines 205 and 114 form direct hydrophobic contacts with bases in the TATA element (14, 15). However, the two studies differ in their assignments of the bases actually contacted by these leucines, possibly reflecting DNA sequence-dependent variations in the TBP-TATA interaction. Crystallographic analysis of a complex composed of *A. thaliana* TBP2 and the adenovirus major late TATA box (TATAAAAG) demonstrated that leucines 205 and 114 (leucines 163 and 72 in the *A. thaliana* sequence) interact directly with bases at positions 2 and 7, respectively, of the TATA sequence (numbering begins with the 5′-most T) (14). In contrast, a second crystallographic study, which was performed on a complex composed of the carboxy-terminal domain of *S. cerevisiae* TBP and a TATA sequence form the *S. cerevisiae CYC1* promoter (TATATAAA), demonstrated that Leu-205 contacts a base at position 3 and Leu-114 contacts a base at position 6 (15). Since our results do not favor one of these assignments over the other, we will interpret our findings with respect to each of these structures.

In comparison with the *A. thaliana* TBP2-TATA complex (14), our results demonstrate that the substitution of either Leu-205 or Leu-114 with a phenylalanine alters the specificity of TATA interaction at a position 1 bp 5′ to the normal site of contact. The most likely explanation for these data is that the bulky side chain of phenylalanine is incompatible with the normal base contact and instead makes a new contact with the base immediately 5′ in the TATA sequence. An alternative explanation is based on the finding that pairs of phenylalanine residues positioned at the 5′ and 3′ ends of the TATA element produce dramatic and critical kinks in the DNA between bp 1 and 2 and between bp 7 and 8 (14, 15). Perhaps the introduction of a new phenylalanine residue 1 bp removed from one of these phenylalanine pairs interferes with the normal formation or location of a kink. With respect to the *S. cerevisiae* TBP-TATA crystal structure (15), the efficient recognition of TATAAG by TBP-L114F can be explained by a direct change in specificity at the base normally contacted by Leu-114. The recognition properties of TBP-L205F are not as easily interpreted from these structural data; however, one possible explanation is that the elimination of an unfavorable contact at position 3 is accompanied by the formation of a new contact 2 bp removed at position 1. Interestingly, in agreement with the interpretations based on either crystal structure, TBP-L205F exhibits altered binding to the adenovirus major late TATA element (1). At the level of resolution achieved by DNase I footprinting analyses, the altered binding by TBP-L205F, relative to the binding by wild-type TBP, corresponds to an approximately 3-bp shift of the region protected from DNase I cleavage; the shift occurs in the 5′ direction.

Our genetic studies also allow us to make predictions concerning the orientation of TBP binding to the TATA box in vivo. TBP-L114F exhibits a high degree of specificity for the sequence TATAAG, placing the DNA contact made by Leu-114 near the 3′ end of the TATA box. A similar placement of

the contact made by Leu-205 is not completely unambiguous, since TBP-L205F recognizes one base substitution at position 1, two substitutions at position 6, and at least one substitution at position 7 of the TATA box. Recognition of the substitution at position 1, CATAAA, appears to occur with greater specificity than recognition of the changes at the 3′ end of the TATA box. In making this observation, we note that all of the substitutions tolerated at positions 6 and 7 support significant levels of transcription by wild-type TBP in vivo and in vitro (5, 8, 33) and that one substitution at position 1, AATAAA, which is comparably transcribed by wild-type TBP, is not preferentially recognized by TBP-L205F. Therefore, one interpretation of these data is that the primary and specific contact made by Leu-205 lies at the 5′ end of the TATA box and that substitution of a phenylalanine for Leu-205 disrupts the TBP-DNA interface such that specificity is relaxed at the 3′ end of the TATA box. This interpretation is strongly supported by the identification of intragenic suppressors of *spt15-122*, which encodes TBP-L205F. All four suppressors encode second amino acid changes at a position that interacts at bp 1 of the TATA box.

An alternative explanation for the recognition by TBP-L205F of both 5′ and 3′ base substitutions is that TBP-L205F can bind bidirectionally to the TATA box. It has long been proposed that the orientation of TBP binding to the TATA box confers directionality on transcription initiation. Indeed, neither crystallographic study, each using a different TBP-TATA box complex, obtained evidence for bidirectional binding by wild-type TBP (14, 15). However, in their analysis of the structural basis for oriented TBP binding, Kim et al. (14) concluded that Leu-205 is one of several amino acids involved in dictating directionality. Thus, it remains an intriguing, albeit unlikely, possibility that the L205F substitution permits bidirectional binding by TBP and that both binding orientations support transcription initiation in the same direction. The structural analysis of TBP-TATA box complexes composed of nonconsensus TATA elements and/or TBP altered-specificity mutants would be particularly interesting in light of this possibility.

Importance of DNA-binding specificity to transcriptional regulation. The altered DNA-binding specificity of TBP-L205F plays an important role in the regulation of transcription initiation at the *his4-912*δ promoter in vivo. Mutagenesis of a nonconsensus TATA element, CATAAA, in a region of the *his4-912*δ promoter (site III) eliminates suppression of *his4-912*δ by the *spt15-122* mutation in vivo. This result provides a very strong link between the altered binding of TBP-L205F to the *his4-912*δ promoter in vitro (1), the effect of TBP-L205F on transcription initiation at *his4-912*δ in vivo (1), and a preferred recognition sequence (CATAAA) for TBP-L205F identified in this study. Surprisingly, this same site III mutation also eliminates suppression of *his4-912*δ by *spt15-301* (2), although this *spt15* mutant showed no clear preference for the sequence CATAAA in our *HIS3* expression studies. Suppression of *his4-912*δ by *spt15-301* is considerably weaker than suppression by *spt15-122*, perhaps as a result of a weaker interaction between TBP-L114F and site III. A potential binding site for TBP-L114F within site III is the sequence TAAAAG, which overlaps CATAAA and differs at only one position from a preferred recognition sequence (TATAAG) for TBP-L114F. Thus, TBP-L205F and TBP-L114F, which cause similar mutant phenotypes yet have distinct TATA recognition specificities, very likely alter transcription initiation at *his4-912*δ by binding to different nonconsensus TATA elements in the same region of the promoter.

The importance of site III in suppression of *his4-912*δ by TBP-L205F may be highly dependent on its location within the promoter. Site III lies within the δ element, just 8 bp 5′ to the δ transcription initiation site (as measured from the 3′ boundary of the DNase I footprint at site III), in the reverse orientation relative to *HIS4*. The close proximity of this sequence to the δ transcription start site as well as its orientation suggest that TBP-L205F may accomplish suppression of *his4-912*δ by binding to this site and sterically blocking transcription initiation from within the δ, thereby favoring initiation at the downstream *HIS4* transcription start site. However, alternative effects of site III occupation by TBP, such as alterations in chromatin structure, cannot be ruled out without further study. Importantly, independent of the actual mechanism of suppression, our data strongly suggest that binding of TBP to transcriptionally nonfunctional TATA elements within the promoter region of a gene can have profound regulatory effects on transcription initiation. This may be a particularly common form of regulation within the AT-rich promoter elements characteristic of genes in *S. cerevisiae*.

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