Cell, Vol. 68, 721–730, February 21, 1992, Copyright © 1992 by Cell Press

Yeast and Human TFIID with Altered DNA-Binding Specificity for TATA Elements

Michel Strubin and Kevin Struhl
Department of Biological Chemistry
and Molecular Pharmacology
Harvard Medical School
Boston, Massachusetts 02115

Summary

TFIID is the highly conserved, but species-specific, component of the RNA polymerase II transcription machinery that binds specifically to the TATA element (consensus TATAAA). Using a genetic selection, we isolated an altered specificity derivative of yeast TFIID that permits transcription from promoters containing a mutated TATA element (TGTAAA). Biochemical analysis indicates that this TFIID derivative has specifically gained the ability to bind TGTAAA efficiently. The mutant protein contains three substitutions within a 12 amino acid region; two of these are necessary and primarily responsible for the altered specificity. An analogous version of human TFIID, generated by introducing the same amino acid substitutions in the corresponding region of the protein, can support basal and GCN4-activated transcription in yeast cells from a TGTAAA-containing promoter. These results define a surface of TFIID that directly interacts with the TATA element, and they indicate that human TFIID can respond to acidic activator proteins in conjunction with the other components of the yeast transcription machinery.

Introduction

Most RNA polymerase II promoters contain a TATA element (consensus TATAAA) that is recognized by the general transcription factor TFIID (reviewed by Sawadogo and Sentenac, 1990). After binding of TFIID to the TATA element, the other initiation factors and RNA polymerase II assemble into the active transcriptional initiation complex. This basic RNA polymerase II machinery supports TATA element–dependent transcription in vitro, but requires gene-specific activator proteins for efficient transcription in vivo. Interactions between activator proteins and TFIID have been observed both genetically (Struhl, 1986, 1987; Homa et al., 1988; Simon et al., 1988; Harbury and Struhl, 1989) and biochemically (Sawadogo and Roeder, 1985; Horikoshi et al., 1988; Stringer et al., 1990; Ingles et al., 1991), but their role in the transcriptional activation mechanism remains to be elucidated.

TFIID is highly conserved throughout the eukaryotic kingdom. Yeast and human TFIIDs are functionally interchangeable in basal transcription reactions reconstituted with yeast or human components (Buratowski et al., 1988; Cavallini et al., 1988; Flanagan et al., 1990). Yeast and human TFIIDs have nearly identical DNA sequence requirements for TATA-dependent transcription in vitro (Wobbe and Struhl, 1990), and Schizosaccharomyces pombe TFIID can functionally substitute for Saccharomyces cerevisiae TFIID in vivo, even though these yeasts are evolutionarily distant (Fikes et al., 1990). The C-terminal 180 residues of the various TFIIDs are at least 80% identical in amino acid sequence. This C-terminal core domain is necessary and sufficient for TATA element binding and basal transcription in vitro (Hoey et al., 1990; Horikoshi et al., 1990; Peterson et al., 1990; Lieberman et al., 1991) and for the essential functions of TFIID in yeast cells (Cormack et al., 1991; Gill and Tjian, 1991; Reddy and Hahn, 1991; Zhou et al., 1991).

Despite these striking similarities, it appears that TFIID function is species specific. Biochemical experiments suggest that yeast TFIID cannot respond to the Sp1 activator, unlike its human and fly counterparts (Pugh and Tjian, 1990), and that the human-specific N-terminal region is required for activation by Sp1 and GAL4-VP16 (Peterson et al., 1990). More directly, human TFIID cannot carry out the essential functions of yeast TFIID in vivo (Cormack et al., 1991; Gill and Tjian, 1991). However, differences scattered throughout the core domains, not the divergent N-termini, account for this phenotypic distinction, with some regions within the conserved core being more important than others (Cormack et al., 1991; Gill and Tjian, 1991). From these observations, it has been suggested that species specificity might reflect differential interactions between TFIID and putative adaptor proteins or subtle differences in TATA element specificity.

TFIID appears to be an unusual sequence-specific DNA-binding protein. First, TFIID is extremely slow at binding to and dissociating from TATA elements (Schmidt et al., 1989), and it has a surprisingly high affinity for single-stranded DNA (Hahn et al., 1989). Second, unlike most specific DNA-binding proteins, TFIID binds as a monomer (Horikoshi et al., 1990) and it undergoes a conformational change upon binding to the TATA element (Lieberman et al., 1991). Third, optimal binding is not constrained to a simple target DNA site that conforms to the consensus of naturally occurring TATA elements. Although nearly all possible mutations of the canonical TATAAA sequence seriously reduce TFIID function in vivo (Chen and Struhl, 1988) and in vitro (Wobbe and Struhl, 1990), a variety of nonconsensus sequences interact efficiently with TFIID (Hahn et al., 1989; Singer et al., 1990). Fourth, the TFIID DNA-binding domain is not localized to a short (60–100 amino acids) region, as is typical of most transcriptional regulatory proteins. Instead, small deletions throughout the 180 C-terminal residues invariably eliminate DNA binding activity (Horikoshi et al., 1990). This observation and proteolytic cleavage experiments (Lieberman et al., 1991) suggest that the structural integrity of the entire core domain is crucial.

Another unusual feature of the TFIID conserved core is the presence of two direct 67 amino acid repeats that are separated by a highly basic region (see Figure 1). Recently, dominant-negative mutations in either repeat of

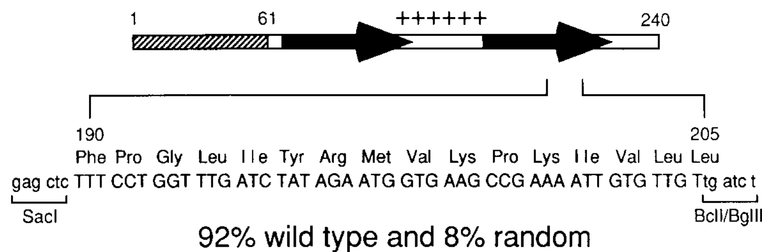

Figure 1. TFIID Mutagenesis

The structural motifs of yeast TFIID (240 amino acids) are depicted as follows: nonconserved N-terminal region (hatched box), direct repeat sequences implicated in DNA binding (solid arrows), basic region (marked with plus signs). Shown below are the nucleotide and amino acid sequences corresponding to the region between residues 190 and 205 of TFIID. This region was mutagenized at a frequency of 8% per base pair by cloning a degenerate oligonucleotide flanked by SacI and BclI sites between the indicated SacI and BglII sites in the TFIID coding region.

yeast TFIID have been isolated that eliminate DNA binding while maintaining at least some aspects of normal structure and function (Reddy and Hahn, 1991). These mutations have led to the suggestion that the TFIID monomer contains a bipartite DNA-binding domain in which each repeat contributes to sequence recognition (Reddy and Hahn, 1991). However, as the mutations eliminate TFIID binding activity, it is unclear whether they disrupt direct protein–DNA contacts or, more indirectly, affect the conformation of the subdomains.

We have been interested in developing a new approach for addressing aspects of TFIID function that involves the isolation of derivatives that are transcriptionally active on mutated TATA elements. Such altered specificity mutants, which have been obtained for prokaryotic σ factors (Gardella et al., 1989; Siegele et al., 1989; Zuber et al., 1989) and a variety of other DNA-binding proteins (Youderian et al., 1983; Ebright et al., 1984; Hochschild et al., 1986; Wharton and Ptashne, 1987; Lehming et al., 1987; Hanes and Brent, 1991; Tzamarias et al., 1992), provide the strongest genetic evidence for a direct protein–DNA contact. Moreover, by "genetically marking" TFIID with the property of altered TATA element specificity, the activities of heterologous or mutated TFIIDs can be specifically assayed in vivo even in the presence of wild-type TFIID, which might be required for viability of the organism.

In this paper, we combine localized mutagenesis and a genetic selection to identify altered specificity mutants of yeast TFIID that can support transcription from a promoter containing a mutated TATA element, T**G**TAAA. Furthermore, we generate an equivalent altered specificity version of human TFIID and show that it responds to acidic activator proteins in yeast cells. These results are discussed with respect to DNA-binding and species specificity of TFIID as well as potential uses of altered specificity TATA factors.

Results

Selection for TFIID Mutants Allowing Transcription from a Nonfunctional TATA Element

Saturation mutagenesis of the yeast *his3* T_R TATA element revealed that almost all single base pair substitutions in the core sequence (TATAAA) severely compromised promoter function in vivo and in vitro (Chen and Struhl, 1988; Wobbe and Struhl, 1990). Consequently, cells carrying *his3* alleles with such defective TATA elements grow poorly on medium lacking histidine and not at all in the presence of aminotriazole (AT), a competitive inhibitor of the *his3* gene product. Thus, we designed a genetic selection in which potential TFIID mutants with altered specificity for TATA elements would be isolated by virtue of their ability to increase transcription from a defective *his3* TATA element and hence to confer AT resistance.

To search for altered specificity mutants, the region of TFIID between amino acids 190 and 205 was heavily mutagenized by replacing it with a degenerate oligonucleotide containing 8% non-wild-type residues per base pair (Figure 1). This small region was targeted because it displays a weak sequence similarity (Horikoshi et al., 1989) with a region of prokaryotic σ factors thought to interact with DNA (Helmann and Chamberlin, 1988). A library (10^6 independent clones) containing the collection of TFIID mutant proteins was introduced into a set of yeast strains with defective *his3* TATA elements (T**G**TAAA, **G**ATAAA, TA**G**AAA, and TATA**G**A) whose transcriptional activities in vitro are at least 10-fold reduced compared with the wild-type TATAAA (Wobbe and Struhl, 1990). All of these strains contain the wild-type TFIID gene on the chromosome to carry out the essential functions for cell growth.

When the resulting *TRP*$^+$ transformants were tested for the ability to grow in the presence of 5 mM AT, colonies were observed only in the yeast strain containing the T**G**TAAA element (approximate frequency of 10^{-5}). Four such transformants lost the ability to grow on AT when their TFIID-containing plasmids were removed (by screening for Trp$^-$ segregants), as expected for altered specificity derivatives. To confirm this more directly, the TFIID-containing plasmids from these four transformants were reintroduced into the parental strain. As shown in Figure 2, cells transformed with a plasmid carrying the wild-type TFIID gene grow slowly in the absence of histidine and not at all when AT is added to the medium. In contrast, the four putative TFIID mutants allow cells to grow at a normal rate in the absence of histidine and also confer AT resistance, with strain 3 being able to grow at higher drug concentrations (strain 4 behaves indistinguishably from strains 1 and 2; data not shown). Thus, four TFIID-containing plasmids have been isolated that allow transcription from a promoter with a defective TATA element.

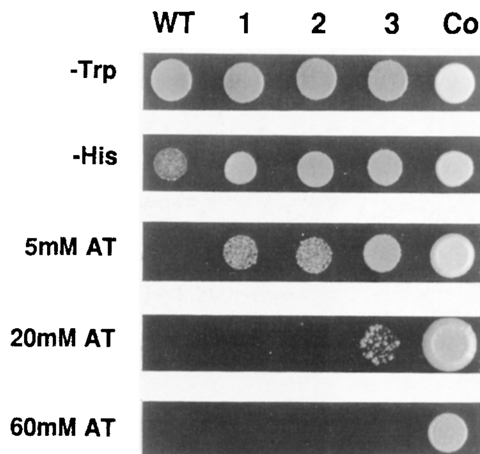

Figure 2. Isolation of Altered Specificity Mutants of Yeast TFIID

Strains containing the *his3*-Δ93 and the **TG**TAAA allele that had been transformed with plasmids containing the wild-type (WT) or indicated mutant TFIIDs were plated on glucose minimal medium lacking either tryptophan (−Trp) or histidine (−His) or containing the indicated concentrations of AT.

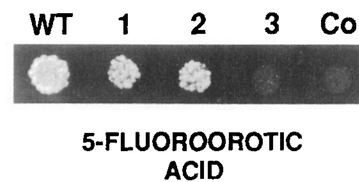

Figure 3. The TFIID Mutants Do Not Support Normal Rates of Cell Growth

Strains containing a *URA3* plasmid encoding wild-type TFIID and a *TRP1* plasmid encoding the indicated TFIID derivative were plated on medium containing 5-fluoroorotic acid. These conditions require loss of the *URA3* plasmid and hence functional activity of the derivative encoded on the *TRP1* plasmid for cell growth. Co, a control *TRP1* plasmid that does not contain any TFIID derivative.

The Mutants Are Less Effective in Carrying Out the Essential Functions of Wild-Type TFIID

By analogy with altered specificity mutants of several DNA-binding proteins, TFIID derivatives with the novel property of functioning at mutated TATA sequences might be expected to be defective in activating transcription from promoters with natural TATA elements. Because the wild-type TFIID gene was present in these cells, the ability of the different mutants to perform this and other essential functions of wild-type TFIID could not be tested. However, as determined by the plasmid shuffle complementation assay (Boeke et al., 1987), yeast cells containing mutants 1 or 2 as the sole source of TATA-binding protein are viable, but they grow significantly less well than wild-type (Figure 3). Overproduction of these mutant TFIID proteins, by introducing the genes on a multicopy 2μ plasmid, partially overcomes this slow growth phenotype (data not shown). For yeast cells containing molecule 3, very slow growing colonies are observed only after prolonged incubation; overexpression of this derivative has no observable effect on cell growth. Thus, TFIID proteins bearing mutations that allow them to induce transcription from a defective TATA element are less efficient in promoting transcription from wild-type promoters. Moreover, the derivative allowing the highest *his3* expression from altered TATA element is also the least effective in carrying out the essential functions of wild-type TFIID.

Allele Specificity of the TFIID Mutants

Three of the mutated TFIID derivatives were tested for their ability to suppress nonfunctional TATA elements other than the **TG**TAAA sequence used in the original screening. Since the wild-type TFIID protein is present in the cell to carry out essential functions, the analysis is restricted to those TATA sequences that show virtually no transcriptional activity. Plasmids expressing the various TFIID mutants were introduced into yeast strains that differ only by the presence of a nonconsensus G:C base pair at position 1, 2, or 3 of the *his3* TATA element (**G**ATAAA, T**G**TAAA, T**C**TAAA, and TA**G**AAA). Figure 4 shows that all three TFIID alleles confer AT resistance only in cells containing the T**G**TAAA allele used for their isolation. Moreover, strains with substitutions at the fifth position (TATA**G**A) show the same sensitivity to AT, regardless of which TFIID version is present (data not shown). Thus, as expected for an altered specificity mutant, these TFIID derivatives suppress mutations of the *his3* TATA element in an allele-specific manner.

The Mutant TFIIDs Contain Two Common Amino Acid Substitutions

DNA sequencing of the region encoded by the degenerate oligonucleotide revealed the surprising result that all four TFIID-altered specificity derivatives bear the same double amino acid substitution, Ile-194 to Phe-194 and Leu-205 to Val-205 (Figure 5). Since each mutant contains specific additional nucleotide changes, the four molecules must represent independent clones. Molecule 3, which supports the highest *his3* expression from the T**G**TAAA-containing promoter, also contains a third amino acid

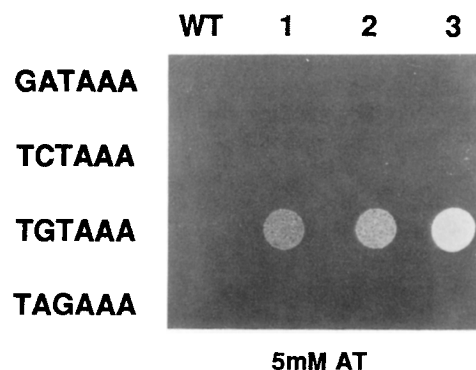

Figure 4. Allele Specificity of the TFIID Derivatives

Strains containing the *his3*-Δ93 and the indicated TATA allele were transformed with plasmids containing the wild-type (WT) or indicated mutant TFIIDs and plated on glucose minimal medium containing 5 mM AT.

```
      190                                                           205
WT    TTT CCT GGT TTG ATC TAT AGA ATG GTG AAG CCG AAA ATT GTG TTG T tg
      Phe Pro Gly Leu Ile Tyr Arg Met Val Lys Pro Lys Ile Val Leu Leu

1     ------------T-------------A------T------------G -
              Phe                                  Val

2     --------C--T----------------C A-----------------G -
              Phe              Gln                 Val

3     ------------T-----------------------------AC ---G -
              Phe                               Thr  Val
                                                203

4     ------------T----------------T------------------G -
              Phe              Asn                 Val
              194                                  205
```

Figure 5. TFIID Mutant Sequences

The deviations in DNA and deduced amino acid sequences between residues 190 and 205 of the four mutant TFIIDs are shown with respect to those of wild-type yeast TFIID.

amino acid 194	203	205		amino acid 194	203	205
m	m	m		m	+	m
m	m	+		m	+	+
+	m	m		+	+	m
+	m	+		+	+	+

Figure 6. Altered TATA Element Specificity Requires Two Amino Acid Substitutions

Strains containing the *his3*-Δ93 and the TGTAAA allele that had been transformed with plasmids representing all possible combinations of mutations at positions 194, 203, and 205 (+ indicates the wild-type residue and m indicates the residue found in mutant 3) were plated on glucose minimal medium containing 0.5 mM AT.

change, Val-203 to Thr-203, that is not present in the three other molecules. The substitutions of either glutamine (molecule 2) or asparagine (molecule 4) for the lysine at position 199 appear to be neutral because these molecules are phenotypically indistinguishable from molecule 1, which contains the wild-type residue.

The presence of the same double mutation in four independent mutants strongly suggests that both changes are required to produce a TFIID protein with altered specificity. To assess this hypothesis, new TFIID alleles carrying all possible combinations of the mutations found in molecule 3 were tested for their ability to induce transcription from the TGTAAA-containing promoter. As expected, none of these TFIID derivatives could support detectable growth, even at a low AT concentration (Figure 6). We conclude that both changes at positions 194 and 205 are required for generating a TFIID protein with altered specificity, and that the efficiency of such mutants to support transcription from the defective TATA element can be enhanced by a third mutation within this region.

Transcriptional Activation by GCN4

GCN4 activator protein binds specifically to the promoter region of the *his3* gene and induces its transcription in response to amino acid starvation (Arndt and Fink, 1986; Hope and Struhl, 1985). The ability of the TFIID mutant 3 to respond to GCN4 and stimulate transcription from defective TATA elements was determined directly by measuring *his3* RNA levels in cells grown in rich medium (no GCN4 protein present; CAA) or in medium containing AT (inducing GCN4 protein synthesis; AT) (Figure 7). In a strain containing the nonsuppressible TATA element TAGTAA, both the basal and GCN4-activated levels of transcription are unaffected by the presence of an additional copy of the wild-type TFIID gene or of mutant 3. In contrast, a yeast strain carrying the TGTAAA element shows substantially increased *his3* RNA level in the presence of TFIID mutant 3 only. Moreover, this basal transcription is stimulated by GCN4, with the fold induction being comparable with that expected for a wild-type promoter. The absolute level of *his3* transcription mediated by TFIID mutant 3 on the TGTAAA promoter is about 20% of that mediated by wild-type TFIID on an equivalent promoter containing TATAAA (Harbury and Struhl, 1989). A related set of experiments indicates that mutant 3 also supports GAL4-activated transcription from the TGTAAA element (data not shown). Thus, the altered TFIID protein not only allows constitutive transcription to occur from a defective TATA element, but is also responsive to an acidic activator protein.

Altered DNA-Binding Specificity

The DNA-binding specificities of wild-type TFIID and mutant 3 were directly examined by synthesizing the proteins in vitro and incubating them with the complete set of TATA sequences that differ at position 2. As expected from in vivo (Chen and Struhl, 1988) and in vitro (Wobbe and Struhl, 1990) transcriptional analyses of the identical

CAA AT : TFIID : CAA AT
– WT 3 3 WT – – WT 3 3 WT –
+1
+13
ded1
TAGAAA TGTAAA

Figure 7. TFIID Mutant 3 Increases *his3* Transcription from the TGTAAA Promoter

Total RNA from *his3*-Δ93 strains containing either the TAGAAA (left panel) or TGTAAA (right panel) allele that had been transformed with plasmids containing wild-type (WT), mutant 3, or no TFIIDs was incubated with *his3* and *ded1* oligonucleotide probes and treated with S1 nuclease. The positions of the *his3* +1 and +13 transcripts as well as the *ded1* control transcript are indicated.

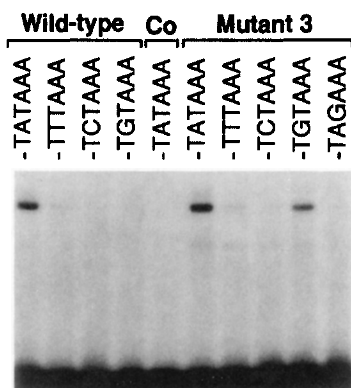

Figure 8. Altered DNA-Binding Specificity of the Mutant TFIID

Equivalent amounts of wild-type TFIID and mutant 3 were incubated with the indicated TATA sequences, and the resulting protein–DNA complexes were electrophoretically separated from the unbound DNA probes. The lane marked Co represents control in vitro translation products generated in the absence of TFIID RNA.

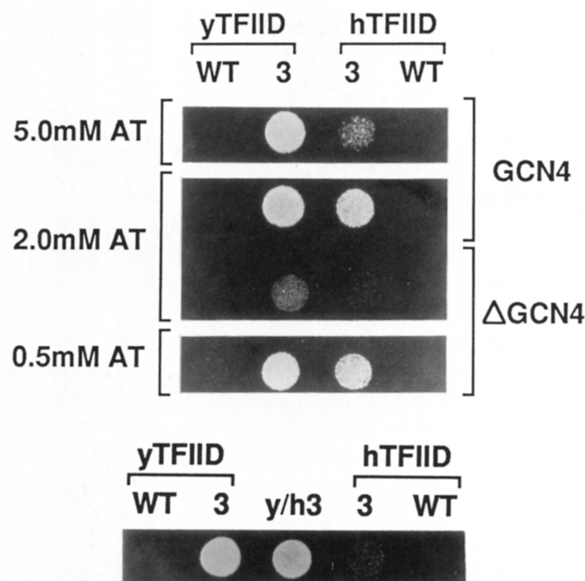

Figure 9. The Altered Specificity Derivative of Human TFIID Activates Transcription in Yeast

(Top) Strains containing the TGTAAA element with (*his3*-Δ93) or without (*his3*-Δ94) the GCN4-binding site upstream were transformed with plasmids representing the wild-type and mutant 3 derivatives of yeast and human TFIID and plated in the presence of the indicated concentration of AT.

(Bottom) Similar to the above experiment except for the addition of a strain carrying the yeast TFIID derivative containing residues 190 to 205 from human TFIID mutant 3 (y/h3) and plating on medium containing 1 mM AT.

TATA sequences, wild-type TFIID binds efficiently to TATAAA, weakly to TTTAAA, and extremely poorly to both TGTAAA and TCTAAA (Figure 8); it also fails to bind detectably to TAGAAA (data not shown). In striking contrast and in accordance with the genetic observations described here, the mutant protein binds much more efficiently than wild-type TFIID to the TGTAAA sequence. Otherwise, TFIID mutant 3 behaves very similarly to the wild-type protein on the four TATA sequences tested. Consistent with the observation that mutant 3 stimulates transcription in vivo from TGTAAA with less than full wild-type efficiency, the mutant protein binds TGTAAA without somewhat lower affinity than TATAAA. It is perhaps surprising that the mutant 3 protein binds efficiently to the TATAAA sequence given that this derivative is unable to support cell growth (see Discussion for potential explanations). Nevertheless, these biochemical experiments directly demonstrate that TFIID mutant 3 displays altered DNA-binding specificity. In particular, the mutant protein specifically gains the ability to bind TGTAAA efficiently while maintaining many of the DNA sequence recognition properties of wild-type TFIID.

Human TFIID Responds to an Acidic Activator in Yeast

Since human TFIID cannot replace yeast TFIID for supporting cell growth (Cormack et al., 1991; Gill and Tjian, 1991), its ability to interact in vivo with acidic activator proteins and other components of the RNA polymerase II transcription machinery cannot be easily assessed. To circumvent this problem, it was necessary to generate a "genetically marked" derivative of human TFIID whose activity could be easily distinguished from that of wild-type yeast TFIID by virtue of altered TATA element specificity. Thus, we introduced mutations analogous to those found in molecule 3 into equivalent positions of the wild-type human TFIID gene in the hope that this derivative would function at the TGTAAA element.

Plasmid DNAs expressing either wild-type or mutant human TFIID were introduced into two yeast strains that differ only by the presence or the absence of a GCN4-binding site upstream of the mutated TGTAAA element in the *his3* promoter. For both strains, the mutant human TFIID confers AT resistance to the cells, whereas the wild-type human TFIID does not (Figure 9, top). This indicates that the mutant human TFIID has altered TATA element specificity and that it can function with the components of the yeast transcription machinery. Moreover, the altered specificity human TFIID responds to the GCN4 activator protein, because the presence of a GCN4 site in the *his3* promoter results in higher drug resistance to the cell.

Although the altered specificity derivative of human TFIID is competent to support basal and GCN4-activated transcription from TGTAAA promoters in yeast cells, it appears to be less effective than the analogous derivative of yeast TFIID. One possibility for this apparent defect is that amino acid differences between yeast and human TFIID in the region between residues 190 and 205 might have nonequivalent effects on the altered TATA element specificity conferred by the Phe-194, Thr-203, and Val-205 mutations. However, this possibility seems unlikely because a derivative of yeast mutant 3 (y/h3) that contains Ile-198 and Arg-201 (human-specific residues) in place of Val-198 and Lys-201 (yeast-specific residues) behaves indistinguishably from yeast mutant 3 (Figure 9, bottom), even

though the region between residues 190 and 205 is equivalent to that found in human mutant 3.

Discussion

Altered Specificity Mutants of TFIID

By employing a genetic selection on a collection of TFIID molecules that had been heavily mutagenized between amino acids 190 and 205, we have isolated derivatives that activate transcription from promoters containing the defective TATA element T**G**TAAA. In the case of mutant 3 (and presumably the other mutants), this novel transcriptional property can be ascribed to increased DNA-binding affinity for the mutant TATA element. Moreover, the biochemical experiments directly demonstrate that this mutant TFIID has altered DNA-binding specificity at position 2, because it strongly increases binding to T**G**TAAA without significantly affecting binding to the other TATA sequences tested. The mutant TFIID differs from a rare subclass of altered specificity mutants in that increased affinity to a mutant sequence is not associated with decreased affinity to the wild-type sequence. Nevertheless, it is clear that the mutant protein has not simply "relaxed" or "lost" DNA-binding affinity or specificity at position 2 (and at other positions tested), but instead has specifically gained the novel ability to recognize a particular sequence with high affinity.

A surprising, perhaps unprecedented, feature of these altered specificity derivatives is that two mutations spaced 11 residues apart (I194F and L205V) are necessary to generate the observed phenotype. Moreover, the presence of a third mutation within this region (V203T) results in increased activity from T**G**TAAA promoters and the inability to carry out the essential functions of TFIID. Given that all four altered specificity mutants share two particular base pair substitutions, it is very likely (roughly 90% probability) that any other pair of nucleotide changes within the mutagenized region could not yield TFIID derivatives that would pass the genetic selection employed here. Thus, increased activity from T**G**TAAA promoters can not be accomplished with at least 80% of the possible single amino acid substitutions. These considerations emphasize the importance of high frequency, localized mutagenesis; indeed, several attempts to isolate TFIID specificity mutants by using the identical genetic selection following classical in vivo mutagenesis procedures were unsuccessful (W. Chen and K. S., unpublished data). Of course, the results here do not address the possibility that mutations outside of residues 190–205 can generate TFIIDs with altered specificities for TATA elements.

The reduced ability of the altered specificity mutants to support cell growth presumably reflects a transcriptional defect at the wild-type promoter(s) of one or more essential yeast genes. It is unlikely that these mutants inhibit normal growth by inappropriately activating genes, because the poor growth phenotype is recessive (i.e., not observed in the presence of wild-type TFIID). Why then does TFIID mutant 3 fail to support cell growth even though it binds with normal affinity to a TATAAA sequence? One explanation is that the mutant protein fails to activate one (or a few) of the 1000 essential genes (Goebl and Petes, 1986), because it binds with lower affinity to a TATA element(s) whose sequence diverges from the TATAAA consensus. This might be due to DNA-binding specificity differences at position 2 that are influenced by the context of adjacent nucleotides, or it might reflect effects at other nucleotide positions. Another possibility is that mutant 3 causes a subtle reduction in DNA binding to many TATA elements, such that inviability results from the combined effect on the 1000 essential genes. In distinguishing between these possibilities, it is important to stress that cell viability is an extraordinarily sensitive assay, and our biochemical experiments are limited by the small number of TATA sequences examined and by the difficulty in measuring subtle differences in DNA-binding affinity. Nevertheless, these considerations do not detract from the primary conclusion that the TFIID mutants described here have altered DNA-binding specificity.

Implications for DNA Binding by TFIID

By analogy with altered specificity mutants of a variety of DNA-binding proteins, our results provide strong genetic evidence that the region of TFIID between residues 190 and 205 directly interacts with the TATA element. This very region had previously been implicated as being important for DNA binding, because single substitutions at positions 196, 203, and 207 abolish TATA element interaction while retaining at least some aspects of normal TFIID structure and function (Reddy and Hahn, 1991). In addition, a different substitution at position 205 is observed in the *spt15-122* allele of TFIID that alters the transcription pattern at the *his4-917δ* locus, possibly by affecting TATA element utilization (Eisenmann et al., 1989) (F. Winston, personal communication).

Altered specificity derivatives of DNA-binding proteins are generally interpreted as disrupting and/or creating interactions between individual amino acids and base pairs (Youderian et al., 1983; Ebright et al., 1984; Hochschild et al., 1986; Wharton and Ptashne, 1987; Lehming et al., 1987; Hanes and Brent, 1991; Tzamarias et al., 1992), and such direct contacts have often been confirmed by high resolution structures of the protein–DNA complexes (Aggarwal et al., 1988; Jordan and Pabo, 1988; Kissinger et al., 1990). Given that mutant 3 specifically increases binding to T**G**TAAA, the most likely explanation for altered specificity is the existence of a new contact between protein and DNA that does not interfere with the normal TATA element interactions mediated by TFIID. However, the standard interpretation cannot be easily applied, because altered TATA element specificity requires two amino acid substitutions spaced 11 residues apart and is increased by a third substitution within the region. It is possible that residues 194, 203, and 205 are in close proximity in the folded structure of TFIID, with one or more of them directly interacting with position 2 of the TATA element. Alternatively, the mutations might subtly disrupt the conformation of a critical surface of TFIID and affect specificity more indirectly through another amino acid residue.

Implications for Species Specificity of TFIID

Human TFIID cannot substitute for its yeast counterpart to support cell growth, indicating that some aspect of TFIID function is species specific (Cormack et al., 1991; Gill and Tjian, 1991). To address the mechanistic basis of this species difference, we analyzed an altered specificity version of human TFIID whose activity can be distinguished from wild-type yeast TFIID whose presence in the cell is necessary for viability. The results indicate that in vivo human TFIID can function with other components of the yeast transcription machinery to support TATA-dependent transcription that is stimulated by the acidic activators GCN4 and GAL4. Consistent with this observation, recent experiments show that yeast and human TFIID are functionally interchangeable for basal and activated transcription in vitro (Kelleher et al., 1992).

Although the altered specificity version of human TFIID is transcriptionally competent and responsive to acidic activation domains in yeast cells, it appears to be less active than the equivalent derivative of yeast TFIID (as determined by *his3* expression levels). This phenotypic distinction might be trivial, reflecting either lower intracellular TFIID protein levels (which is unlikely; see Cormack et al., 1991) or reduced DNA binding activity due to the three amino acid substitutions in the context of the human protein. Alternatively, it is certainly possible that the reduced activity in vivo could be due to a relatively inefficient interaction between human TFIID and some component(s) of the yeast transcription machinery. While an inefficient interaction of human TFIID could, in principle, affect basal and/or activated transcription, our results would be more consistent with a defect in basal transcription because the human protein confers reduced function in the absence of an activator protein, yet shows the expected response to GCN4. Although recent experiments show that yeast and human TFIID are functionally interchangeable for basal and activated transcription in vitro (Kelleher et al., 1992), yeast cell viability is an extremely sensitive assay; hence, a subtle functional defect could be responsible for the observed species specificity.

The altered specificity mutations described here partially overlap the C2a region of TFIID that contains an important determinant for species specificity between yeast and human (Cormack et al., 1991). Extending the connection between DNA-binding and species specificity, several regions throughout the highly conserved C-terminal core are responsible for the functional difference between yeast and human TFIID (Cormack et al., 1991; Gill and Tjian, 1991) and are proposed to constitute the bipartite DNA-binding domain (Reddy and Hahn, 1991). Thus, despite the strikingly similar DNA sequence requirements for yeast and human TFIID in vitro (Wobbe and Struhl, 1990), it remains possible that subtle differences in TATA element specificity account for the inability of human TFIID to support yeast cell growth, presumably by affecting the relative mRNA levels of the numerous yeast genes.

Although human TFIID cannot support yeast cell growth (Cormack et al., 1991; Gill and Tjian, 1991), it is important to stress that viability requires proper expression of thousands of yeast genes, including 1000 essential genes (Goebl and Petes, 1986), and hence represents an extraordinarily sensitive assay. The basic observation in vivo was of interest primarily because of suggestions from indirect biochemical experiments that species specificity of TFIID function reflects fundamental incompatibilities in the transcriptional activation mechanism, particularly regarding proposed interactions between TFIID and putative adaptor proteins (Berger et al., 1990; Kambadur et al., 1990; Kelleher et al., 1990; Lewin, 1990; Meisterernst et al., 1990; Peterson et al., 1990; Pugh and Tjian, 1990). Our results demonstrate conclusively that there are no fundamental incompatibilities between TFIID and adaptor proteins for the response to acidic activator proteins in vivo. Instead, they strongly suggest that yeast and human TFIIDs are functionally conserved at this level, and that the observed species specificity in vivo represents an extremely subtle and mechanistically trivial difference between the proteins.

Potential Applications for Altered Specificity TFIIDs

A major goal of this work was to obtain a "genetically marked" TFIID whose activity could be distinguished from that of the wild-type protein by virtue of its transcriptional competence on a defective TATA element. The main value of this approach is that TFIID derivatives can be functionally analyzed in vivo even if they are unable to support cell growth. In this paper, we have utilized an altered specificity mutant to show that human TFIID can respond to acidic activator proteins in conjunction with the yeast transcription machinery. The reciprocal experiment could be done by introducing the altered specificity derivative of yeast TFIID and a T**G**TAAA-containing promoter into human cells. It should also be relatively straightforward to utilize this approach to define the regions of human TFIID that are necessary for specific functions in human cells. For example, the suggestion from in vitro transcription experiments that the human-specific N-terminal region of TFIID is necessary for responding to acidic or glutamine-rich transcriptional activation domains (Peterson et al., 1990; Pugh and Tjian, 1990) can be addressed directly. Finally, the ability to use simple genetic screens and selections to assay the transcriptional activity of yeast TFIID derivatives independently of the requirement for cell viability could prove to be very useful for identifying mutants with specific functional defects. By using appropriate promoters, it might be possible to isolate mutations that carry out the basic TATA-dependent reaction but fail to respond to acidic activator proteins.

Experimental Procedures

DNA Manipulations

The mutant TFIID library was derived from a molecule containing the 2.4 kb EcoRI-BamHI fragment encoding the yeast TFIID gene cloned into a version of the *TRP1* centromeric vector YCplac22, in which the XbaI site in the polylinker region was deleted (Cormack et al., 1991). Using the polymerase chain reaction, the TFIID coding region was modified by introducing a SacI site at amino acid 188 (using oligo ySacI) and a BglII site at amino acid 205 (using oligo yBglII), such that the sequence of the encoded protein was not altered. The region of

the TFIID gene encoding residues 190 to 205 was mutagenized at an approximate frequency of 8% per base pair by using mutually primed synthesis (Oliphant et al., 1986) to generate the double-stranded form of the degenerate oligonucleotide CGGCGAGCTC**TTTCCTGGTTTGATCTATAGAATGGTGAAGCCGAAAATTGTGTTG**TTGATCAAC-3′ (bold residues indicate positions of degeneracy and are flanked by SacI and BclI recognition sequences; see Figure 1). After cleavage with SacI and BclI, the degenerate oligonucleotide was inserted between newly created SacI and BglII sites in the TFIID gene; this required a three fragment ligation because the BglII site is not unique. The degenerate oligonucleotide was characterized by sequencing 18 nonselected DNA molecules. Sixty-nine non-wild-type nucleotides were observed with G residues being somewhat underrepresented (17 expected and 5 observed); this corresponds to a mutation frequency of 8.3% per base pair or to approximately 2.4 amino acid substitutions per oligonucleotide.

To generate the final library, the ligation mixture was introduced into Escherichia coli strain DH5α by electroporation. After recovery, the cell suspension was inoculated into 700 ml of medium containing ampicillin, grown to saturation, and plasmid DNA representing the complete library was prepared. The complexity of this library, determined by immediately plating a small sample of the recovered cells in the presence of ampicillin, was approximately 2×10^6 independent clones.

Yeast TFIID alleles carrying different combinations of the amino acid substitutions found in mutant 3 were obtained by cloning two degenerate oligonucleotides essentially as described above. Both oligonucleotides were programmed to be 50% A and 50% T at position 580 (first nucleotide of the triplet encoding Ile-194 in wild-type TFIID), 50% T and 50% G at position 613 (first nucleotide of the triplet encoding wild-type Leu-205), and flanked by SacI and BclI recognition sites used for insertion between the SacI and BglII sites created in the yeast TFIID gene. One such oligonucleotide encodes the wild-type valine at position 203, whereas the other one encodes the Thr-203 allele present in mutant 3. The desired single, double, and triple mutations were identified by DNA sequencing.

The three amino acid changes found in yeast altered specificity mutant 3 were introduced in the human TFIID sequence by polymerase chain reaction amplification using human-yeast TFIID hybrid 11 (Cormack et al., 1991) as a DNA template and oligonucleotides hBclI-1 and hBclI-2 as primers. After cleavage with BclI, the two amplified fragments were ligated together and the resulting product was cleaved with XbaI and BamHI before being inserted into TFIID hybrid 12 (Cormack et al., 1991) in place of the yeast sequence, hence generating human TFIID mutant 3. The altered specificity mutant y/h3 (Figure 8B) containing the Ile-198 and Arg-201 residues found in human TFIID in place of Val-198 and Lys-201 present in the yeast sequence was obtained by a similar procedure. All fragments generated by polymerase chain reaction amplification were sequenced in their entirety before being cloned into the final constructs. The oligonucleotides used for the constructs are listed below with restriction sites in bold.

ySacI: CGGC**GAGCTC**TGGCTCATAGGAGGAGAAAG.
yBglII: CGGC**AGATCT**TTGTTTCAGGAAAGATTGTTC.
hBclI-1: CCGCT**GATCA**AACCCAGAATTACTCTCGTTATTTTTG.
hBclI-2: GCGC**TGATCA**TTCTGTAGAATAAACCAGG.

Isolation of TFIID Altered Specificity Mutants

The library of mutant TFIID proteins was introduced into a set of S. cerevisiae strains containing *his3*-Δ93, an allele in which the T_C element has been deleted such that the GCN4-binding site in the native *his3* promoter lies just upstream of the T_R TATA element, a position where GCN4 can activate transcription (Harbury and Struhl, 1989). The various strains differ in that the wild-type T_R (TATAAA) sequence has been replaced by T**G**TAAA, **G**ATAAA, TA**G**AAA, or TATA**G**A (Harbury and Struhl, 1989). The cell suspension, which contained approximately 10^6 independent yeast transformants, was inoculated into 500 ml of glucose minimal medium lacking tryptophan and incubated for 40 hr at 30°C, at which time the culture consisted of approximately 80% Trp^+ cells. The resulting cells were tested for growth in the presence of 5 mM AT, a concentration at which the starting strain does not grow at all. Plasmid DNAs were recovered from slow growing colonies that appeared after 4 days of incubation at 30°C by transformation into E. coli and retested to confirm their phenotype. For the four plasmids that conferred AT resistance when reintroduced into the starting strain, the DNA sequences of the region corresponding to the degenerate oligonucleotide were determined.

Additional Phenotypic Analyses

The TFIID plasmids that permitted growth on the *his3*-Δ93 promoter containing the T**G**TAAA sequence were tested for allele specificity by introducing them into equivalent strains containing the following other TATA sequences: **G**ATAAA, T**C**TAAA, TA**G**AAA, and TATA**G**A (Harbury and Struhl, 1989). To examine whether transcription mediated by the altered specificity derivatives depended on the GCN4 activator protein, the relevant plasmid DNAs were introduced into strains containing the T**G**TAAA element in the context of *his3*-Δ94, which deletes the GCN4-binding site (Harbury and Struhl, 1989). In all cases, transformants were tested for growth on AT by plating about 10^4 cells on appropriate glucose minimal medium containing either casamino acids (CAA) or various concentrations of AT.

The plasmid shuffle complementation assay (Boeke et al., 1987) was employed to determine whether the TFIID mutants could support cell growth. The relevant TFIID derivatives (present on *TRP1* centromeric plasmids) were introduced into yeast strain BYΔ2, whose sole copy of the TFIID gene is carried on a *URA3* centromeric plasmid (Cormack et al., 1991). After growth of the resulting transformants on medium lacking uracil and tryptophan, approximately 10^5 cells were spotted on glucose minimal medium containing 5-fluoroorotic acid.

RNA Analysis

Total RNA was prepared from cells grown in glucose minimal medium containing either casamino acids (normal conditions with low GCN4 levels) or 20 mM AT (conditions of amino acid starvation that result in high levels of GCN4). The *his3* mRNA levels were determined directly by S1 nuclease protection experiments as described previously (Chen et al., 1987), except that the *ded1* radiolabeled oligonucleotide probe was diluted 10-fold prior to hybridization.

DNA-Binding Experiments

Plasmids (pGEM) encoding wild-type TFIID or mutant 3 were transcribed in vitro with SP6 RNA polymerase, and the resulting RNAs were purified after treatment with DNAase I followed by phenol extraction and ethanol precipitation. Approximately 1 μg of each RNA was translated (50 μl reaction) using a rabbit reticulocyte lysate according to the instructions of the supplier (Promega). The amount and integrity of the synthesized TFIIDs were determined by adding 10 μCi of $[^{35}S]$methionine to a 5 μl portion of each reaction and analyzing the translation products on SDS–polyacrylamide gel electrophoresis. Equivalent amounts of in vitro translated TFIIDs were incubated with 6 fmol (20,000 cpm) of internally ^{32}P-labeled DNA fragments containing the various TATA elements. These DNA probes, which were generated by the polymerase chain reaction, extend from positions −47 to −10 of the promoter region present in the pGC plasmids described previously (Wobbe and Struhl, 1990). The DNA-binding reactions were carried out in 15 mM HEPES (pH 7.9), 50 mM KCl, 6 mM $MgCl_2$, 1 mM dithiothreitol, 0.5 mM EDTA, 10% glycerol, and 2 μg of poly(dG–dC) per μl of lysate. After incubation at 30°C for 15 min, the reactions were supplemented with 5 μg of sonicated single-stranded salmon sperm DNA, and then incubated for another 15 min at room temperature. This procedure greatly reduced nonspecific protein–DNA complexes without affecting the amount of the TFIID-containing complex. Protein–DNA complexes were resolved from free DNA by electrophoresis on 5% polyacrylamide gel.

Acknowledgments

This work was supported by a postdoctoral fellowship to M. S. from the Swiss National Science Foundation and by research grants to K. S. from the National Insititutes of Health (GM 30186) and the Lucille Markey Trust.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *“advertisement”* in accordance with 18 USC Section 1734 solely to indicate this fact.

Received August 26, 1991; revised November 5, 1991.

References

Aggarwal, A. K., Rodgers, D. W., Drottar, M., Ptashne, M., and Harrison, S. C. (1988). Recognition of a DNA operator by the repressor of phage 434: a view at high resolution. Science *242*, 899–907.

Arndt, K., and Fink, G. (1986). GCN4 protein, a positive transcription factor in yeast, binds general control promoters at all 5′ TGACTC 3′ sequences. Proc. Natl. Acad. Sci. USA *83*, 8516–8520.

Berger, S. L., Cress, W. D., Cress, A., Triezenberg, S. J., and Guarente, L. (1990). Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell *61*, 1199–1208.

Boeke, J. D., Trueheart, J., Natsoulis, G., and Fink, G. R. (1987). 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Meth. Enzymol. *154*, 164–175.

Buratowski, S., Hahn, S., Sharp, P. A., and Guarente, L. (1988). Function of a yeast TATA element-binding protein in a mammalian transcription system. Nature *334*, 37–42.

Cavallini, B., Huet, J., Plassat, J. L., Sentenac, A., Egly, J.-M., and Chambon, P. (1988). A yeast activity can substitute for the HeLa TATA box factor. Nature *334*, 77–80.

Chen, W., and Struhl, K. (1988). Saturation mutagenesis of a yeast *his3* TATA element: genetic evidence for a specific TATA-binding protein. Proc. Natl. Acad. Sci. USA *85*, 2691–2695.

Chen, W., Tabor, S., and Struhl, K. (1987). Distinguishing between mechanisms of eukaryotic transcriptional activation with bacteriophage T7 RNA polymerase. Cell *50*, 1047–1055.

Cormack, B. P., Strubin, M., Ponticelli, A. S., and Struhl, K. (1991). Functional differences between yeast and human TFIID are localized to the highly conserved region. Cell *65*, 341–348.

Ebright, R. H., Cossart, P., Gicquel-Sanzey, B., and Beckwith, J. (1984). Mutations that alter DNA sequence specificity of the catabolite gene activator protein of *E. coli*. Nature *311*, 232–235.

Eisenmann, D. M., Dollard, C., and Winston, F. (1989). *SPT15*, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation in vivo. Cell *58*, 1183–1191.

Fikes, J. D., Becker, D. M., Winston, F., and Guarente, L. (1990). Striking conservation of TFIID in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. Nature *346*, 291–294.

Flanagan, P. M., Kelleher, R. J. I., Feaver, W. J., Lue, N. F., LaPointe, J. W., and Kornberg, R. D. (1990). Resolution of factors required for the initiation of transcription by yeast RNA polymerase II. J. Biol. Chem. *265*, 11105–11107.

Gardella, T., Moyle, H., and Susskind, M. M. (1989). A mutant *Escherichia coli* s^{70} subunit of RNA polymerase with altered promoter specificity. J. Mol. Biol. *206*, 579–590.

Gill, G., and Tjian, R. (1991). A highly conserved domain of TFIID displays species specificity in vivo. Cell *65*, 333–340.

Goebl, M. G., and Petes, T. D. (1986). Most of the yeast genomic sequences are not essential for cell growth and division. Cell *46*, 983–992.

Hahn, S., Buratowski, S., Sharp, P. A., and Guarente, L. (1989). Yeast TATA-binding protein TFIID binds to TATA elements with both consensus and nonconsensus DNA sequences. Proc. Natl. Acad. Sci. USA *86*, 5718–5722.

Hanes, S. D., and Brent, R. (1991). A genetic model for interaction of the homeodomain recognition helix with DNA. Science *251*, 426–430.

Harbury, P. A. B., and Struhl, K. (1989). Functional distinctions between yeast TATA elements. Mol. Cell. Biol. *9*, 5298–5304.

Helmann, J. D., and Chamberlin, M. J. (1988). Structure and function of bacterial sigma factors. Annu. Rev. Biochem. *57*, 839–872.

Hochschild, A., Douhan, J., III, and Ptashne, M. (1986). How λ repressor and λ Cro distinguish between O_R1 and O_R3. Cell *47*, 807–816.

Hoey, T., Dynlacht, B. D., Peterson, M. G., Pugh, B. F., and Tjian, R. (1990). Isolation and characterization of the Drosophila gene encoding the TATA box binding protein, TFIID. Cell *61*, 1179–1186.

Homa, F. L., Glorioso, J. C., and Levine, M. (1988). A specific 15-bp TATA box promoter element is required for expression of a herpes simplex virus type I late gene. Genes Dev. *2*, 40–53.

Hope, I. A., and Struhl, K. (1985). GCN4 protein, synthesized in vitro, binds to *HIS3* regulatory sequences: implications for general control of amino acid biosynthetic genes in yeast. Cell *43*, 177–188.

Horikoshi, M., Hai, T., Lin, Y.-S., Green, M. R., and Roeder, R. G. (1988). Transcription factor ATF interacts with the TATA factor to facilitate establishment of a preinitiation complex. Cell *54*, 1033–1042.

Horikoshi, M., Wang, C. K., Fujii, H., Cromlish, J. A., Weil, P. A., and Roeder, R. G. (1989). Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box. Nature *341*, 299–303.

Horikoshi, M., Yamamoto, T., Ohkuma, Y., Weil, P. A., and Roeder, R. G. (1990). Analysis of structure–function relationships of yeast TATA box binding factor TFIID. Cell *61*, 1171–1178.

Ingles, C. J., Shales, M., Cress, W. D., Triezenberg, S. J., and Greenblatt, J. (1991). Reduced binding of TFIID to transcriptionally compromised mutants of VP16. Nature *351*, 588–590.

Jordan, S. R., and Pabo, C. O. (1988). Structure of the lambda complex at 2.5 Å resolution: details of the repressor-operator interactions. Science *242*, 893–899.

Kambadur, R., Culotta, V., and Hamer, D. (1990). Cloned yeast and mammalian transcription factor TFIID gene products support basal but not activated metallothionein gene transcription. Proc. Natl. Acad. Sci. USA *87*, 9168–9172.

Kelleher, R. J., III, Flanagan, P. M., and Kornberg, R. D. (1990). A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. Cell *61*, 1209–1215.

Kelleher, R. J., III, Flanagan, P. M., Chasman, D. I., Ponticelli, A. S., Struhl, K., and Kornberg, R. D. (1992). Yeast and human TFIIDs are interchangeable for the response to acidic transcriptional activators in vitro. Genes Dev. *6*, 296–303.

Kissinger, C. R., Liu, B., Martin-Blanco, E., Kornberg, T. B., and Pabo, C. O. (1990). Crystal structure of an engrailed homeodomain–DNA complex at 2.8 Å resolution: a framework for understanding homeodomain–DNA interactions. Cell *63*, 579–590.

Lehming, N., Sartorius, J., Niemoller, M., Genenger, G., von Wilcken-Bergmann, B., and Muller-Hill, B. (1987). The interaction of the recognition helix of *lac* repressor with *lac* operator. EMBO J. *6*, 3145–3153.

Lewin, B. (1990). Commitment and activation at Pol II promoters: a tail of protein–protein interactions. Cell *61*, 1161–1164.

Lieberman, P. M., Schmidt, M. C., Kao, C. C., and Berk, A. J. (1991). Two distinct domains in the yeast transcription factor IID and evidence for a TATA box-induced conformational change. Mol. Cell. Biol. *11*, 63–74.

Meisterernst, M., Horikoshi, M., and Roeder, R. G. (1990). Recombinant yeast TFIID, a general transcription factor, mediates activation by the gene-specific factor USF in a chromatin assembly assay. Proc. Natl. Acad. Sci. USA *87*, 9153–9157.

Oliphant, A. R., Nussbaum, A. L., and Struhl, K. (1986). Cloning of random-sequence oligodeoxynucleotides. Gene *44*, 177–183.

Peterson, M. G., Tanese, N., Pugh, F., and Tjian, R. (1990). Functional domains and upstream activation properties of cloned human TATA binding proteins. Science *248*, 1625–1630.

Pugh, B. F., and Tjian, R. (1990). Mechanism of transcriptional activation by Sp1: evidence for coactivators. Cell *61*, 1187–1197.

Reddy, P., and Hahn, S. (1991). Dominant negative mutations in yeast TFIID define a bipartite DNA-binding region. Cell *65*, 349–357.

Sawadogo, M., and Roeder, R. G. (1985). Interaction of a gene-specific transcription factor with the adenovirus major late promoter upstream of the TATA box region. Cell *43*, 165–175.

Sawadogo, M., and Sentenac, A. (1990). RNA polymerase B (II) and general transcription factors. Annu. Rev. Biochem. *59*, 711–754.

Schmidt, M. C., Zhou, Q., and Berk, A. J. (1989). Sp1 activates transcription without enhancing DNA-binding activity of the TATA box factor. Mol. Cell. Biol. *9*, 3299–3307.

Siegele, D. A., Hu, J. C., Walter, W. A., and Gross, C. A. (1989). Altered

promoter recognition by mutant forms of the s^{70} subunit of *Escherichia coli* RNA polymerase. J. Mol. Biol. *206*, 591–604.

Simon, M. C., Fisch, T. M., Benecke, B. J., Nevins, J. R., and Heintz, N. (1988). Definition of multiple, functionally distinct TATA elements, one of which is a target in the *hsp70* promoter for E1A regulation. Cell *52*, 723–729.

Singer, V. L., Wobbe, C. R., and Struhl, K. (1990). A wide variety of DNA sequences can functionally replace a yeast TATA element for transcriptional activation. Genes Dev. *4*, 636–645.

Stringer, K. F., Ingles, C. J., and Greenblatt, J. (1990). Direct and selective binding of an acidic transcriptional activation domain to the TATA-box factor TFIID. Nature *345*, 783–786.

Struhl, K. (1986). Constitutive and inducible *Saccharomyces cerevisiae* promoters: evidence for two distinct molecular mechanisms. Mol. Cell. Biol. *6*, 3847–3853.

Struhl, K. (1987). Promoters, activator proteins, and the mechanism of transcriptional initiation in yeast. Cell *49*, 295–297.

Tzamarias, D., Pu, W. T., and Struhl, K. (1992). Mutations in the bZIP domain of yeast GCN4 that alter DNA-binding specificity. Proc. Natl. Acad. Sci. USA *89*, in press.

Wharton, R. P., and Ptashne, M. (1987). A new-specificity mutant of 434 repressor that defines an amino acid-base pair contact. Nature *326*, 888–891.

Wobbe, C. R., and Struhl, K. (1990). Yeast and human TATA-binding proteins have nearly identical DNA sequence requirements for transcription in vitro. Mol. Cell. Biol. *10*, 3859–3867.

Youderian, P., Vershon, A., Bouvier, S., Sauer, R. T., and Susskind, M. M. (1983). Changing the DNA-binding specificity of a repressor. Cell *35*, 777–783.

Zhou, Q., Schmidt, M. C., and Berk, A. J. (1991). Requirement for acidic amino acid residues immediately N-terminal to the conserved domain of *Saccharomyces cerevisiae* TFIID. EMBO J. *10*, 1843–1852.

Zuber, P., Healy, J., Carter, H. L. I., Cutting, S., Moran, C. P. J., and Losick, R. (1989). Mutations changing the specificity of an RNA polymerase sigma factor. J. Mol. Biol. *206*, 605–614.