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A Suppressor of TBP Mutations Encodes an RNA Polymerase III Transcription Factor with Homology to TFIIB

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Summary

The *TDS4* gene of S. cerevisiae was isolated as an allele-specific high copy suppressor of mutations within the basic region of the TATA-binding protein (TBP). The gene is essential for viability and encodes a 596 aa protein. The first 300 aa of the TDS4 protein exhibit significant sequence similarity to the RNA polymerase II transcription factor TFIIB. However, TDS4 is required for RNA polymerase III transcription in vivo and in vitro. Antibodies specific for TDS4 or TBP react with the TFIIIB complex, indicating that both proteins are components of the RNA polymerase III initiation complex. These findings suggest that the RNA polymerase II and III initiation mechanisms are extremely similar, and they explain how the TATA-binding protein can function in both systems.

Introduction

Biochemical fractionation of in vitro transcription extracts, in combination with kinetic and physical analyses, has led to basic models for the assembly of eukaryotic RNA polymerases I, II, and III (pol I, pol II, and pol III) initiation complexes. Recent work has led to the surprising discovery that all three polymerase systems are dependent upon the TATA-binding protein (TBP) (Comai et al., 1992; Cormack and Struhl, 1992; Lobo et al., 1991; Margottin et al., 1990; Schultz et al., 1992; Simmen et al., 1991; White et al., 1992; for reviews, see Pugh and Tjian, 1992; Sharp, 1992; Dahlberg and Lund, 1991). TBP was first identified in the yeast Saccharomyces cerevisiae as the pol II transcription factor TFIID (Buratowski et al., 1988; Cavallini et al., 1988). In higher eukaryotes, TFIID apparently consists of TBP and other associated factors (Dynlacht et al., 1991; Pugh and Tjian, 1991; Tanese et al., 1992). It has been shown that TBP is a component of the pol I transcription factor SL1, which is also a multiprotein complex (Comai et al., 1992). A role for TBP in pol III transcription was first demonstrated for the U6 snRNA gene, which contains a canonical TATA element as part of its pol III promoter (Lobo et al., 1991; Margottin et al., 1990; Simmen et al., 1991). Further investigations have demonstrated that TBP is also essential for pol III initiation at non-TATA pol III promoters (Cormack and Struhl, 1992; Schultz et al., 1992; White et al., 1992).

TBP has several functions in the pol II system. It recognizes and binds to the TATA element and subsequently engages in protein–protein interactions with TFIIA and TFIIB (Buratowski et al., 1989; Maldonado et al., 1990; Peterson et al., 1990). On non-TATA pol II promoters, another factor apparently supplies promoter recognition activity, yet TBP is still required for initiation (Pugh and Tjian, 1991). The role of TBP in the other polymerase systems is unclear. Except for the U6 gene, it seems unlikely that TBP is required for sequence-specific DNA recognition. TBP could be necessary for a nonspecific DNA binding role, or it could be involved in protein–protein interactions with components of the pol I and pol III initiation complexes.

In the last few years, it has become feasible to study not only pol III, but also pol II and pol I transcription in extracts derived from yeast (Klekamp and Weil, 1982; Lue and Kornberg, 1987, 1990; Schultz et al., 1991; Woontner and Jaehning, 1990). This experimental system holds great promise owing to the amenability of yeast to genetic analysis. The combined biochemical and genetic approaches have already been fruitful for assigning functions to particular domains of TBP (Arndt et al., 1992; Buratowski and Zhou, 1992; Reddy and Hahn, 1991; Strubin and Struhl, 1992).

In this report, we describe the isolation of a TFIID (TBP) suppressor, the *TDS4* gene. The TDS4 protein exhibits significant sequence similarity to the pol II transcription factor TFIIB. However, TDS4 is essential for RNA polymerase III transcription. These results suggest that the role of TBP in pol III transcription is to interact with TDS4 in a manner homologous to its interaction with TFIIB. The parallels between the pol I, II, and III systems suggest a common initiation mechanism that was established before the evolutionary divergence of the three eukaryotic RNA polymerases.

Results

Genetic Isolation of *TDS4*

A genetic screen was carried out to identify genes whose overexpression can suppress the temperature-sensitive phenotype conferred by mutations in the basic region of TBP. Strain YSB67 carries a mutant TBP gene in which lysine codons at positions 133 and 138 are changed to leucine codons (Buratowski and Zhou, 1992). A yeast genomic library carried in the high copy plasmid YEp24 (Carlson and Botstein, 1982) was used to transform this temperature-sensitive strain. Approximately 8,000 Ura^+ transformants were replica plated and tested for the ability to grow at 38°C. Plasmids were recovered in bacteria (Hoffman and Winston, 1987) from those colonies able to grow at the nonpermissive temperature, and eight suppressed the temperature-sensitive phenotype when retransformed into YSB67.

Restriction digestion revealed that the plasmids fell into three groups. The three isolates with the strongest suppression phenotype contained the wild-type TBP (*SPT15*) gene. Two weakly suppressing plasmids contained overlapping fragments. They carried a gene designated *TDS3*, which will be described elsewhere. The three remaining plasmids contained the *TDS4* gene. The genomic inserts in the plasmids were approximately 10 kb. To localize the

TDS4 gene, a high copy subclone library with inserts of 2–4 kb was constructed and transformed into YSB67. The smallest clone that still conferred supression was approximately 2.5 kb. The suppression phenotype conferred by *TDS4* overexpression is shown in Figure 1. A centromeric (low copy) plasmid containing *TDS4* conferred some ability to grow at the nonpermissive temperature, but this suppression was much weaker than that observed with a high copy *TDS4* plasmid (data not shown).

High copy expression of the *TDS4* gene suppressed the temperature-sensitive phenotypes of TBP mutants containing the double amino acid changes K133,138L and K133,145L, as well as single point mutants at each of those positions. However, overexpression of *TDS4* did not suppress other temperature-sensitive alleles of TBP with mutations in other parts of the protein (data not shown). Such allele specificity is often indicative of a direct interaction between two proteins.

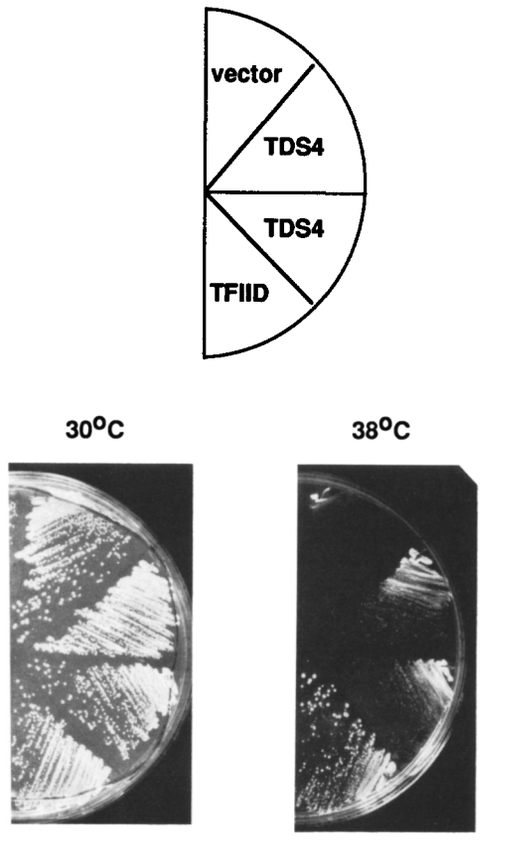


Figure 1. Overexpression of *TDS4* Suppresses the Temperature-Sensitive Phenotype of TFIID Basic Region Mutants

A temperature-sensitive yeast strain (YSB67) containing the TFIID mutation K133, 138L was transformed with either YEp24 (vector), YEpTDS4 (TDS4), or YEpIID (TFIID). The resulting strains were streaked for single colonies at either the permissive (30°C) or nonpermissive (38°C) temperature.

TDS4 Is Essential for Viability

The 2.5 kb insert containing the *TDS4* gene was sequenced (Figure 2). The deduced amino acid sequence predicts that *TDS4* encodes a 596 aa (67 kd) protein with a pI of 6.8. A potential zinc finger (Cys-X_2–Cys-X_{17}–Cys-X_2–Cys) is found within the first 28 aa, and an internally repeated sequence is present (see below). The chromosomal position of *TDS4* was mapped using a chromosome blot and the prime clone grid system (the gift of L. Riles and M. Olsen, Washington State University). *TDS4* maps to chromosome VII, approximately 30 to 40 kb to the centromere distal side of the *pet54* gene.

To test whether *TDS4* is essential for growth, a deletion allele was constructed that replaced the region coding for amino acids 16–430 with the *LEU2* gene. The deletion allele was transformed into a diploid yeast strain, and the resulting Leu^+ strain (YSB106) was sporulated. Of thirty tetrads dissected, all produced two or fewer viable spores (Figure 3). In all cases, the viable spores were Leu^-. Four viable spores were recovered when the same strain was sporulated after transformation with a *TDS4*-containing plasmid (data not shown). Therefore, *TDS4* is essential for viability.

TDS4 Is Related to TFIIB

No exact matches were found when the *TDS4* DNA and protein sequences were compared against all sequences deposited in the GenBank and EMBL databases (using the BLAST program server; Altschul et al., 1990). Therefore, *TDS4* is a previously uncharacterized gene. However, significant similarity was found between the amino acid sequences of TDS4 and the human RNA polymerase II transcription factor TFIIB (Ha et al., 1991; Malik et al., 1991). The recently published amino acid sequence of the yeast TFIIB homolog SUA7 (Pinto et al., 1992) also exhibits significant similarity to TDS4.

An alignment of the TDS4, SUA7, and TFIIB amino acid sequences produced by the BESTFIT program (Devereaux et al., 1984) is shown in Figure 4A. The sequence similarity is confined to the amino-terminal half of the TDS4 protein. The identity between TDS4 and TFIIB is 23%, while conservative changes make the overall similarity 44%. The TDS4 sequence similarity to SUA7 is essentially the same as that with TFIIB. Several small gaps exist in the alignment, but there is only one large gap that might indicate a nonconserved domain.

A second alignment was performed using the DOTPLOT program (Devereaux et al., 1984). Instead of matching individual amino acids, this program averages the match over 15 aa spans. Regions of extended sequence similarity are visualized as diagonal lines (Figure 4B). Using this method, three regions of extended similarity between TDS4 and TFIIB are seen. These are the putative zinc finger region and an internal repeat found in TFIIB (Ha et al., 1991; Malik et al., 1991; Pinto et al., 1992). Additional lines offset from the diagonal represent similarity between the first repeat of one protein and the second repeat of the other. An alignment of the repeats of TDS4, TFIIB, and SUA7 is shown in Figure 4C.

The C-terminal half of TDS4 did not show significant similarity to any proteins within the available databases. However, analysis of the sequence reveals that it is highly charged. Of the last 301 aa, 38% are either acidic (21% Glu or Asp) or basic (17% Lys or Arg). Another 14% of this

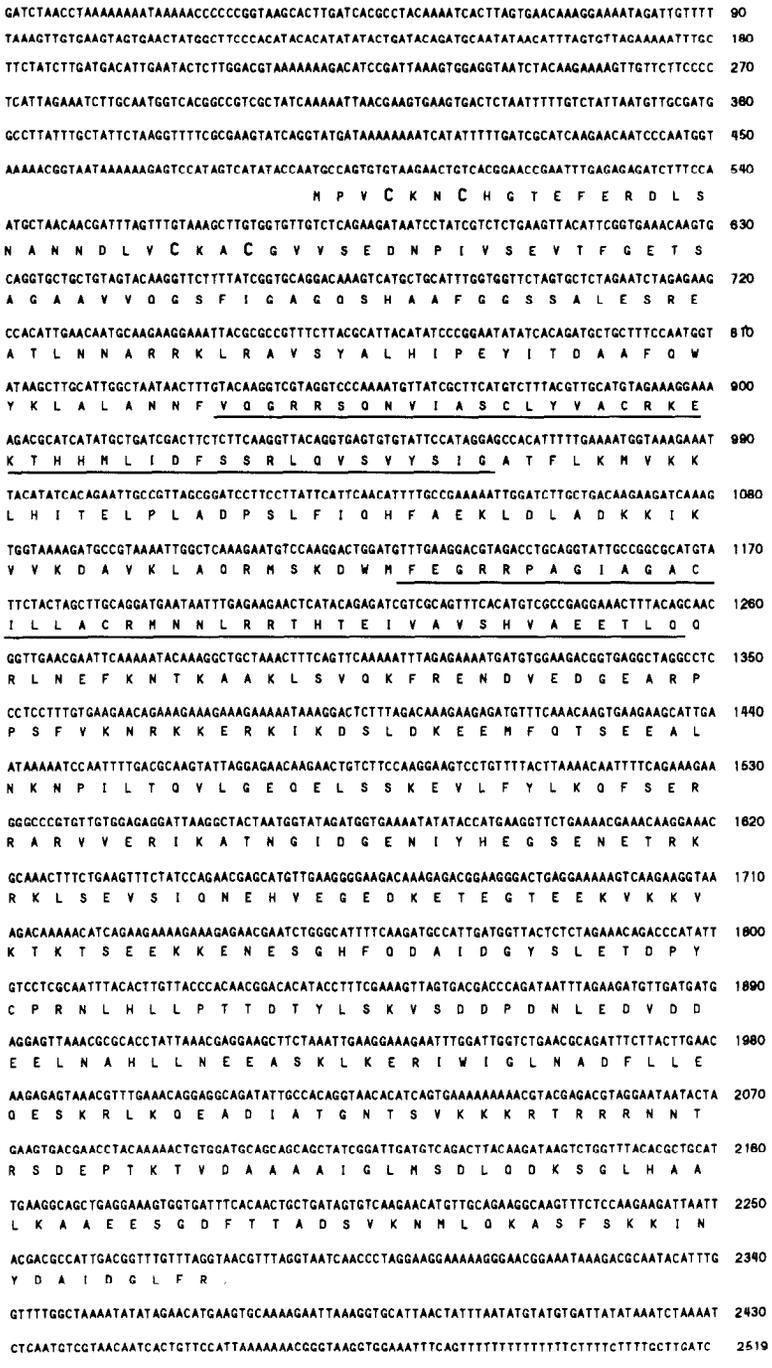


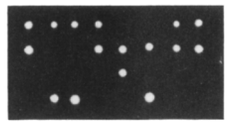
Figure 2. Sequence of the *TDS4* Gene

The deduced amino acid sequence is shown below the DNA sequence. Four cysteines comprising a potential zinc finger motif are shown in large capitals. The direct repeats of TDS4 are underlined.

region consists of Ser or Thr residues. This half of TDS4 is predicted to be highly hydrophilic. It is also essential for TDS4 function, as a deletion of the 35 C-terminal residues destroys the ability to complement a TDS4 deletion (data not shown).

TDS4 Is Essential for RNA Polymerase III Transcription In Vivo

Since TBP has been shown to be involved in transcription initiation by RNA polymerase I (Comai et al., 1992) and RNA polymerase III (Cormack and Struhl, 1992; Lobo et al., 1991; Margottin et al., 1990; Schultz et al., 1992; Simmen et al., 1991; White et al., 1992) as well as RNA polymerase II, an experiment was performed to explore which classes of transcripts required *TDS4* (Figure 5). A yeast strain (YSB112) was constructed that contained a chromosomal deletion of the *TDS4* gene. TDS4 protein was produced by a plasmid containing the *TDS4* coding region fused to a galactose-inducible promoter (pRS316–galTDS4). This strain only grew normally in the presence of galactose. Shifting to glucose-containing media caused a steady decline in the levels of TDS4 protein (data not shown). As a control, a strain (RSY763, the gift of S. Sanders and R. Shekman, University of California, Berkeley)



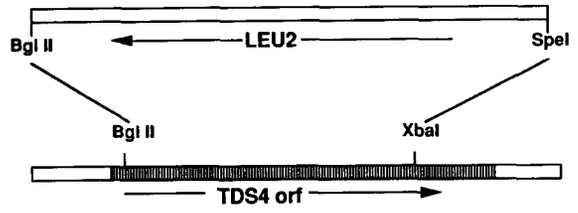


Figure 3. The *TDS4* Gene Is Essential for Viability

A disrupted allele of *TDS4* was constructed by replacing the BglII–XbaI fragment of *TDS4* with a BglII–SpeI fragment containing the *LEU2* gene. A diploid yeast strain was transformed with the resulting fragment and Leu$^+$ transformants were selected. Sporulation of the resulting strain (YSB106) resulted in a 2:2 segregation of viability. Eight tetrads are shown in vertical rows. All viable spores were Leu$^-$, indicating that *TDS4* is essential for viability.

containing the essential gene *SEC61* fused to a galactose-inducible promoter was used. The two strains showed similar growth kinetics upon cessation of galactose induction (Figure 5). Since *SEC61* is not involved in transcription, RSY763 provides an appropriate control for transcription effects attributable only to the change in media or the slowing of the growth rate.

The two strains were grown to mid log-phase in rich media containing galactose (YPGal). The cells were then transferred to the same media containing glucose (YPD), causing repression of the galactose inducible *TDS4* or *SEC61* gene. Both strains continued log-phase growth for about 9 hr (approximately three doublings) before depleting the available TDS4 or SEC61 protein (Figure 5). The cells subsequently grew very slowly, but remained viable (assayed by plating on YPGal) past 20 hr (data not shown). This is probably due to residual TDS4 or SEC61 protein, or perhaps to a small amount of transcription from the GAL promoter under repressing conditions. Total RNA was isolated from the cells at various time points after the shift to glucose, and specific transcripts were quantitated by S1 nuclease-protection assay as described by Cormack and Struhl (1992).

To assay transcription by pol I, a probe against the 3′

A.

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SUA7  24 CPECKVYPPKIVER.FSEG..DVVCALCGLVLSDKLVDTRSEWRTFSNDD 70
TDS4   1 MPVCKNCHGTEFERDLSNANNDLVCKACGVVSEDNPIVSEVTFGETSAGA 50
hIIB  12 RVTCPNHPDAILVEDYRAG..DMICPECGLVVGDRVIDVGSEWRTFSNDK 59

SUA7  71 HNGDDPSRVGEASNPLLDGNNLSTRIGKGETTDMRFTKELNKAQGKNVMDK 121
TDS4  51 A.VVQGSFIGAGQS.....................HAAFGGSSALES 75
hIIB  60 A.TKDPSRVGDSQNPLLSDGDLSTMIGKGTGAASFDEFGNSKYQNRRTMSS 109

SUA7 122 KDNEVQAAFAKITMLCDAAELPKIVKDCADEAYKLCHDEKTLKGKSMESI 171
TDS4  76 REATLNNARRKLRAVSYALHIPEYITDAAFQWYKLALANNFVQGRRSQNV 125
hIIB 110 SDRAMMNAFKEITTHADRINLPRNIVDRTNNLFKQVYEQKSLKGRANDAI 159

SUA7 172 MAASILIGCRRAEVARTFKEIQSLIHVKTKEFGKTLNIMKNILRGKSEDG 221
TDS4 126 IASCLYVACRKEKTHHMLIDFSSRLQVSVYSIGATFLKMV..... 166
hIIB 160 ASACLYIACRQEGVPRTFKEICAVSRISKKEIGRCF.KLI..... 198

SUA7 222 FLKIDTQNMSGAQNLTYIPRFCSHLGLP...MQVTTSAEYTAKKCKEIKE 268
TDS4 167 .KKLHITELPLADPSLFIQHFAEKLDLADKKIKVVKDAVKLAQRMSKDWM 214
hIIB 199 .LKALETSVDLITTGDFMSRFCSNLCLP...KQVQMAATHIARKAVELDL 244

SUA7 269 IAGKSPITIAVVSIYLMILLFQIPITAAKVGQTLQVTEGTIKSGYKILYE 318
TDS4 215 FEGRRPAGIAGACILLACRMNNLRRTHTEIVAVSHVAEETLQQRLNEFKN 264
hIIB 245 VPGRSPISVAAAAIYMASQASAEKRTQKEIGDIAGVADVTIRQSYRLIY. 293

SUA7 319 HR.DKLVDPQLIANGV.....VSLDNLPGVEKK 345
TDS4 265 TKAAKLSVQKFRENDVEDGEARPPSFVKNRKKERK 299
hIIB 294 PRAPDLFPTDFKFD.....TPVDKLPQL 316
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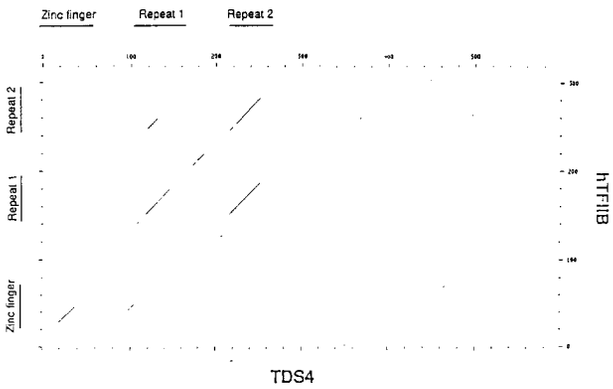
B.



C.

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TDS4 repeat 1   VQGRRSQNVIASCLYVACRKEKTHHMLIDFSSRLQVSVYSIG
TDS4 repeat 2   FEGRRPAGIAGACILLACRMNNLRRTHTEIVAVSHVAEETLQ
TFIIB repeat 1  LKGRANDAIASACLYIACRQEGVPRTFKEICAVSRISKKEIG
TFIIB repeat 2  VPGRSPISVAAAAIYMASQASAEKRTQKEIGDIAGVADVTIR
SUA7 repeat 1   LKGKSMESIMAASILIGCRRAEVARTFKEIQSLIHVKTKEFG
SUA7 repeat 2   IAGKSPITIAVVSIYLMILLFQIPITAAKVGQTLQVTEGTIK
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Figure 4. *TDS4* Is Homologous to Human and Yeast TFIIB

The TDS4 protein sequence was compared to the GenBank and EMBL databases using the BLAST (Altschul et al., 1990) program and found to be significantly similar to the human TFIIB and yeast SUA7 proteins.

(A) Alignment of TDS4 with human TFIIB (hIIB) and yeast TFIIB (SUA7). The BESTFIT program was used to find the optimal alignment between the proteins. Identities are indicated by a solid line, highly conservative changes by a double dot, and partially conservative changes by a single dot.

(B) Graphic alignment of TDS4 and human TFIIB. A second alignment was performed using the DOTPLOT program, which tests for extended sequence similarity over a defined length of amino acids. This method reveals that the highest level of similarity is located in the putative zinc finger region and in the direct repeats of the proteins.

(C) Alignment of the TDS4, human TFIIB, and SUA7 repeats.

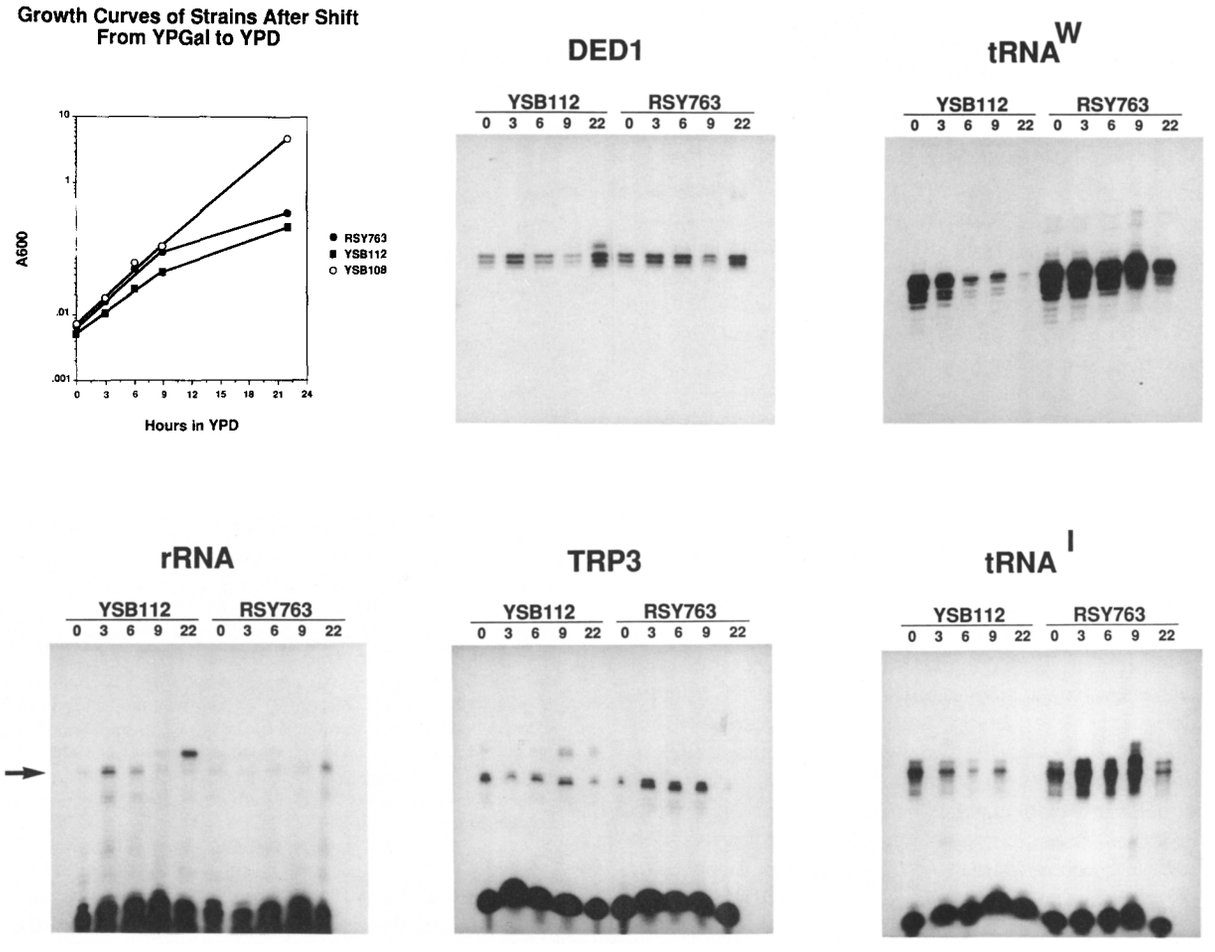

Figure 5. *TDS4* Is Required for Transcription by RNA Polymerase III In Vivo

A yeast strain (YSB112) was constructed in which the *TDS4* gene was transcribed from a galactose-inducible promoter. A second strain (RSY763), in which the essential *SEC61* gene was transcribed from a galactose-inducible promoter, served as a control. Normal growth of these strains is therefore galactose dependent. The growth curve of a wild-type cell is shown by an isogenic strain in which *TDS4* was expressed from its own promoter (YSB108). The yeast strains were grown in the presence of galactose (YPGal) and then shifted to media containing glucose (YPD). Growth of YSB112 and RSY763 continued logarithmically for approximately three doublings (about 9 hr) before slowing. At the indicated times (shown in hours above each lane), samples of cells were taken and total RNA was prepared. The RNA was then subjected to an S1 nuclease protection assay to monitor the production of specific RNAs. Transcription by RNA polymerase I was assayed with a probe specific for the rapidly processed ribosomal RNA precursor (rRNA).The protected fragment is indicated by an arrow. RNA polymerase II transcription was assayed by monitoring the DED1 and TRP3 mRNAs, which have a short half-life in vivo. All three RNAs continued to be produced after *TDS4* and *SEC61* repression. RNA polymerase III transcription was tested by assaying with a probe specific for unstable introns within a family of tryptophan tRNAs ($tRNA^W$) or an isoleucine tRNA ($tRNA^I$). Only expression of the tRNA genes was affected, indicating that transcription by RNA polymerase III is dependent upon *TDS4*. Radioactivity at the bottom of some gels is the remnants of the digested probe.

processing junction of the ribosomal transcript (rRNA) was used. Since the rRNA is quickly processed, the probe signal is directly correlated with the level of transcription (see Cormack and Struhl, 1992). Both YSB112 and RSY763 continued to transcribe ribosomal genes after the shift to glucose. In fact, YSB112 showed a transient increase in rRNA transcription following repression of *TDS4*. Therefore, *TDS4* is apparently not required for transcription by RNA polymerase I.

Pol II transcription was monitored by probing for the mRNAs of the *DED1* and *TRP3* genes, both of which have short half-lives in vivo (see Cormack and Struhl, 1992). Both genes continued to be transcribed after *TDS4* repression. It is therefore unlikely that *TDS4* is required for pol II transcription.

Transcription by RNA polymerase III was assayed using probes against introns within tRNA transcripts. The $tRNA^W$ probe recognizes a family of tryptophan tRNAs, while $tRNA^I$ is specific for an isoleucine tRNA. The tRNA introns are rapidly processed and degraded, making their signal a direct reflection of transcription levels (see Cormack and Struhl, 1992). Both probes indicated a rapid decline in tRNA transcription following repression of *TDS4* expression. The control strain (RSY763) continued to transcribe the tRNA genes after repression of the *SEC61* gene. Therefore, the transcription effects are specific to *TDS4* and not due to the slowdown in growth. Based on these results, we conclude that *TDS4* is required for normal transcription by RNA polymerase III in vivo. Further evidence that *TDS4* is necessary for RNA polymerase III transcrip-

tion in vivo is provided by López-de-León et al. (1992 [this issue of *Cell*]). The authors of that study have found that changes in *PCF4(TDS4)* expression can affect transcription of tandem tRNA genes.

TDS4 and TBP Are Components of TFIIIB

To test whether TDS4 corresponds to previously described RNA polymerase III initiation factors, antibodies were produced against glutathione transferase–TDS4 fusion proteins. The antibodies were found specifically to inhibit transcription of a tRNA gene, consistent with a role for TDS4 in transcription by RNA polymerase III (S. Datta and S. B., unpublished data).

One candidate for TDS4 was a 70 kd protein within the TFIIIB complex that was identified by photo-cross-linking to pol III promoter DNA (Bartholomew et al., 1991; Kassavetis et al., 1991). Fractions containing extensively purified TFIIIB and TFIIIC (Kassavetis et al., 1990) were generously provided by G. Kassavetis and E. P. Geidushek (University of California, San Diego). These fractions were used to assemble RNA polymerase III preinitiation complexes on a tRNA gene. TFIIIC and TFIIIB were incubated with probe to allow complex assembly, and then heparin was added to the binding reaction to strip TFIIIC from the complex. The remaining TFIIIB–promoter complex is stable and can be resolved by native gel electrophoresis (Kassavetis et al., 1990). When anti-TDS4 antibodies were added to the binding reaction (Figure 6, lanes 2 and 4), the TFIIIB complex was supershifted, indicating that the TDS4 protein is a component of the TFIIIB complex. The addition of antibodies produced against the yeast TBP (TFIID) also specifically supershifted the TFIIIB complex (Figure 6, lane 7), consistent with results demonstrating a role for TBP in RNA polymerase III transcription. Preimmune serum had no effect on the TFIIIB complex (Figure 6, lanes 3, 5, and 6). Therefore, both TBP and TDS4 proteins are components of the TFIIIB complex. Based on the similarity in size, it seems likely that *TDS4* encodes the 70 kd TFIIIB protein that cross-links to pol III-transcribed promoters.

Another candidate protein for the *TDS4* gene was a 60 kd protein purified as an essential component of TFIIIB (Klekamp and Weil, 1986). The anti-TDS4 antibodies were used for immunoblotting of partially purified TFIIIB and TFIIIC (generously provided by P. A. Weil, Vanderbilt University; see Klekamp and Weil, 1982). A reacting band of approximately 67 kd was observed in both fractions (data not shown). Antibodies prepared against the 60 kd TFIIIB protein (also provided by P. A. Weil) did not react with recombinant TDS4 protein produced in bacteria (data not shown). These results make it unlikely that TDS4 encodes the 60 kd protein identified by Klekamp and Weil (1986).

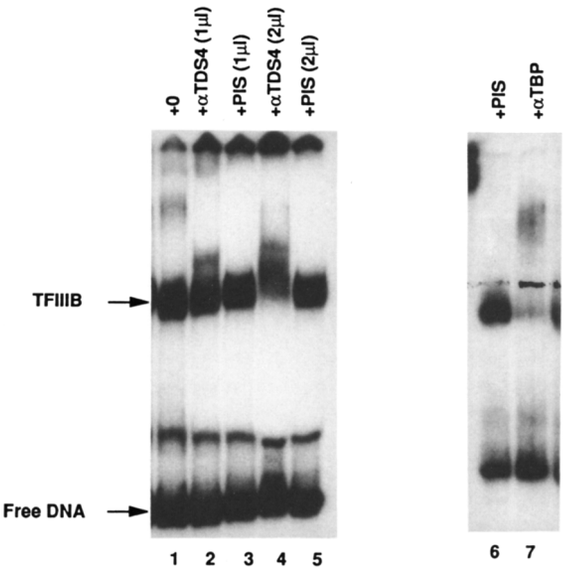

Figure 6. TDS4 and TBP Are Components of the TFIIIB Complex

Partially purified TFIIIB and TFIIIC were incubated with the SUP4 tRNA gene probe. The reactions received no addition (lane 1), preimmune serum (PIS; lanes 3, 5, and 6), anti-TDS4 serum (aTDS4; lanes 2 and 4), or anti-TBP serum (aTBP; lane 7). Heparin was added to strip TFIIIC from the complex, and the reaction was then analyzed by native gel electrophoresis (Braun et al., 1989; Kassavetis et al., 1990). While preimmune serum had no effect, both TDS4 and TBP antibodies caused a supershift of the TFIIIB complex, indicating that both proteins are components of TFIIIB.

Discussion

In this report, we describe the isolation and characterization of an essential TFIIB-like gene that is required for RNA polymerase III transcription initiation. The *TDS4* gene was isolated as an allele-specific high copy suppressor of mutations within the basic region of TBP. The TBP mutations confer a temperature-sensitive phenotype and the mutant proteins are defective in their in vitro interactions with the pol II transcription factor TFIIA (Buratowski and Zhou, 1992). Although we originally suspected that the conditional phenotype was due to defective interactions with the pol II initiation factors, the results presented here suggest that the temperature sensitivity is caused in large part by a defect in pol III transcription. It is likely that the basic region mutations in TBP disrupt protein–protein interactions with both pol II and pol III factors, but that a defect in pol III transcription (which contributes a much larger fraction of the cellular RNA) has a greater effect on the cellular growth rate. It is interesting that overexpression of yeast TFIIB (SUA7), alone or in combination with TDS4 overexpression, did not have an effect on the TBP mutant phenotype (data not shown).

We demonstrate here that TBP and TDS4 interact to form a complex on pol III promoters. Both proteins are components of the TFIIIB complex (Figure 6), although this association could be indirect. The allele-specific suppression of TBP mutants by *TDS4* and the homology between TDS4 and TFIIB suggest that the interaction is probably direct. Mutations in the basic region of TBP may weaken this interaction, causing the temperature-sensitive phenotype. It is likely that overexpression increases the cellular concentration of the TDS4 protein, and this allows a sufficient amount of TBP–TDS4 complex formation to restore viability at the nonpermissive temperature. It should be noted that the suppression by *TDS4* overexpression is only partial, suggesting that defects still exist, possibly in pol I or pol II transcription.

We have recently discovered that *TDS4* has been independently isolated in a genetic selection (Willis et al., 1989) for genes that affect the transcription of tandem tRNA genes. I. Willis and colleagues (Albert Einstein College of Medicine, New York) have shown that a mutation in *TDS4 (PCF4)* can affect pol III transcription of tRNA genes in vivo (López-de-León et al., 1992 [this issue of *Cell*]). These findings complement ours and support the idea that *TDS4* encodes an RNA polymerase III transcription factor.

A TFIIB-like Gene Family

TDS4 is the second TFIIB-like gene isolated from the yeast S. cerevisiae. Several features are shared by members of the TFIIB gene family. All interact with the transcription factor TBP. Human TFIIB binds to a TFIID (TBP)–promoter complex in a native gel electrophoresis assay (Buratowski et al., 1989, 1991; Maldonado et al., 1990; Peterson et al., 1990). The *TDS4* gene is shown here to interact with TBP genetically. Furthermore, both TBP and the TDS4 protein are components of the TFIIIB complex. The *SUA7* gene was isolated in a genetic screen that is specific for RNA polymerase II transcription, and it is very likely that *SUA7* encodes the TFIIB homolog of yeast (Pinto et al., 1992). Bacterially produced SUA7 protein will bind to the TFIID–promoter complex in the gel shift assay (S. B., unpublished data).

Several structural features of the TFIIB-like factors are conserved. At the amino terminus of each protein, there is a potential zinc finger domain of the Cys-X_2–Cys-X_{17}–Cys-X_2–Cys type. Interestingly, the human TFIIB has a histidine residue in the place of the second cysteine and only 15 residues in the central domain. Other residues within this region are also conserved. In addition to the putative zinc finger domain, sequence similarity between the members of this family is highest in the direct repeats. Although the functions of the repeats are currently unknown, there are two obvious possibilities. One is that the TFIIB repeats are responsible for interaction with the direct repeats of TBP. A second plausible theory is that the TFIIB repeats interact with the third largest RNA polymerase subunit, two copies of which are present within a polymerase enzyme complex (Kolodziej et al., 1990). Mutagenesis of conserved amino acids should distinguish between these and other possibilities.

The TDS4 protein is unique in possessing a highly charged 300 aa domain at the carboxyl terminus. This region is essential for TDS4 function, as a deletion of the last 35 aa completely abolishes function in vivo (data not shown). This part of the protein may carry out a function specific to RNA polymerase III initiation. Another interesting possibility is that the RNA polymerase II system contains a corresponding activity that is carried out by a separate protein, perhaps one of the TBP-associated factors.

Parallel Mechanisms of Pol II and Pol III Transcription Initiation

The findings that both TBP and a TFIIB-like protein are essential for RNA polymerase III transcription suggest that the initiation processes of pol II and pol III occur by similar mechanisms. RNA polymerase I is also dependent upon TBP for initiation (Comai et al., 1992). We predict that a TFIIB-like homolog will also be found for the pol I system. It seems likely that the roles of these factors were established in eukaryotes before the evolutionary divergence of RNA polymerases I, II, and III.

It is important to ask how the new findings fit into the preexisting biochemical model of RNA polymerase III initiation. Biochemical fractionation has identified several components besides pol III that are required for reconstitution of transcription in vitro. TFIIIA appears to be specifically required for transcription of 5S RNA. It binds specifically to DNA sequences internal to the 5S gene. TFIIIC also binds to internal promoter elements and appears to be more generally required. TFIIIB binds to the promoter near the site of initiation, in a manner dependent upon TFIIIC (and TFIIIA on 5S genes; Kassavetis et al., 1990). Once bound, TFIIIB appears to be stably associated with the promoter through several rounds of initiation. Based on these results, Kassavetis et al. have concluded that TFIIIA and TFIIIC are "assembly factors" required for association of TFIIIB with the promoter.

TDS4 is a component of the TFIIIB fraction. Yeast TFIIIB has not yet been purified to homogeneity, but photo-cross-linking experiments have identified two components with molecular sizes of approximately 70 and 90 kd (Bartholomew et al., 1991; Kassavetis et al., 1991). The predicted molecular size of TDS4 is 67 kd, which is most consistent with the 70 kd activity. An interesting parallel between yeast TFIIIB and human TFIIB is that both may interact with DNA near the initiation site (Bartholomew et al., 1991; Braun et al., 1989; Buratowski et al., 1989; Kassavetis et al., 1990, 1989; Moncollin et al., 1992). TFIIIB could confer this protection via the TFIIB-like domain of TDS4. In both systems, the TFIIB-like proteins appear to interact with polymerase to recruit it to the promoter.

The similarities between the pol II and pol III systems (and possibly pol I) suggest parallel models of transcription initiation (Figure 7). Upstream binding factor binds upstream of pol I promoters; various regulatory proteins bind both upstream and downstream of pol II promoters; and TFIIIA and TFIIIC bind within the pol III-transcribed 5S and tRNA genes (promoter recognition). These proteins promote the assembly of basal transcription factors, which include TBP and (at least in the case of pol II and III) a TFIIB-like protein (activation of basal complex). Acidic activators of pol II transcription act at the TFIID and/or TFIIB association step (Lewin, 1990; Ptashne and Gann, 1990; for review, see Sharp, 1991). TFIIIA and IIIC promote association of TFIIIB, which contains both the TBP and TDS4 proteins (Kassavetis et al., 1990). Upstream binding factor promotes association of SL1, which contains TBP (see Comai et al., 1992). The very similar components of the committed complexes for pol II and III may explain how some regulatory factors are able to act on both classes of transcription. The committed complexes in turn allow incorporation of the RNA polymerase (and other basal factors) into the initiation complex. TFIIB forms a bridge between TFIID and pol II, and TDS4 probably supplies the analogous activity for RNA polymerase III. It seems likely

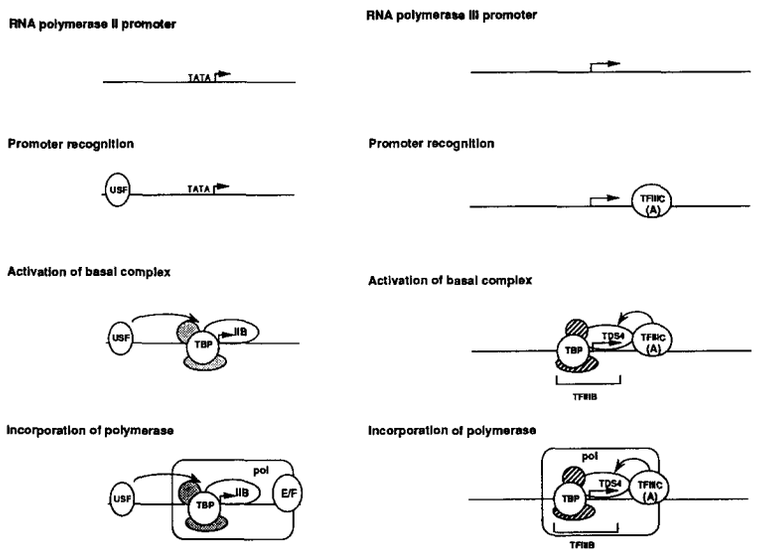

Figure 7. Parallel Mechanisms of Initiation by RNA Polymerases II and III

A schematic representation of the respective initiation models is shown. Promoter recognition by regulatory factors (an upstream binding factor, USF, in the case of pol II; TFIIIC for most pol III genes; TFIIIC and TFIIIA for 5S RNA genes) occurs first. This binding regulates commitment of basal factors to the initiation site. The basal complexes of pol II and pol III both contain TBP and a TFIIB-like factor, as well as other associated factors. Once the basal complex is established, the respective RNA polymerases (and associated factors) recognize and are incorporated into the preinitiation complex and can now carry out initiation. While these models are not meant to be comprehensive, they illustrate the parallels between the two polymerase systems.

that a TFIIB-like protein will be found that bridges the template-associated factors and pol I.

The parallel models of pol I, II, and III transcription initiation should offer new insights to researchers in all three systems. A discovery in one system now becomes a testable prediction for the other two. Just how deeply the parallel features extend remains to be seen, but progress can only be accelerated by considering the similarities and differences between the three polymerase systems.

Experimental Procedures

Yeast Strains and Methods

YSB67.....*MATa, ura3-52, leu2Δ1, his4-917δ, LYS2-173R2, ade8, spt15(K133,138L)*

YSB106...*MATa/MATα, ura3-52/ura3-52, leu2-3,112/leu2-3,112, his3Δ200/his3Δ200, TDS4/tds4Δ::LEU2*

YSB108...*MATa, ura3-52, leu2-3,112, his3Δ200, tds4Δ::LEU2, {pRS316-TDS4}*

YSB112...*MATa, ura3-52, leu2-3,112, his3Δ200, tds4Δ::LEU2, {pRS316-galTDS4}*

RSY763... *MATα, ura3-1, leu2-3,112, ade2-1, trp1-1, his3-11,15, Δsec61::HIS3, {pGAL61}*

Lithium acetate transformation of yeast, preparation of media, tetrad dissection, and other yeast methods were performed by standard methods (Ausubel et al., 1991; Guthrie and Fink, 1991).

Genetic Isolation of *TDS4*

The *TDS4* gene was isolated by transforming yeast strain YSB67 with a YEp24-based yeast genomic library (Carlson and Botstein, 1982). YSB67 is temperature sensitive because of a double mutation (K133, 138L) in the TFIID gene (Buratowski and Zhou, 1992). Approximately 8000 Ura$^+$ transformants were screened for the ability to grow at the nonpermissive temperature (38°C). Plasmids from colonies that grew at 38°C were recovered in bacteria (Hoffman and Winston, 1987) and retested for the ability to suppress the temperature-sensitive phenotype of YSB67. Eight suppressing plasmids were isolated and mapped by restriction enzyme digestion. Three plasmids were determined to contain a copy of the TFIID (*SPT15*) gene. Two others contained an overlapping fragment of DNA that carries the *TDS3* gene. The remaining three plasmids contained overlapping fragments that contain the *TDS4* gene.

The original isolate (YEpTDS4) contained an insert of approximately 10 kb. The *TDS4* gene was localized as follows. DNA from the original isolate was subjected to partial digestion with Sau3AI. Fragments with lengths between 2 and 4 kb were purified after agarose gel electrophoresis. These fragments were cloned into the BamHI site of pRS425 (Sikorski and Heiter, 1989) and the resulting library was transformed into yeast strain YSB67. Plasmids that conferred suppression of the temperature-sensitive phenotype were recovered in bacteria (Hoffman and Winston, 1987). The smallest insert was 2.5 kb (plasmid pRS425-TDS4). This fragment was sequenced using dideoxy-NTPs and Sequenase 2.0 according to the manufacturer's instructions (US Biochemical).

Sequence Analysis

Homology between human TFIIB and TDS4 amino acid sequences was revealed by comparison of the TDS4 sequence to available databases using the BLAST program (Altschul et al., 1990). Further analysis of the homology was performed using the BESTFIT and DOTPLOT routines of the UWGCG package (Devereaux et al., 1984).

Plasmids

A *TDS4* disruption vector (pUCΔH3-tds4Δ::LEU2) was created by replacing the BglII–XbaI fragment of *TDS4* (coding for amino acids 16 to 430) with the 2.7 kb SpeI–BglII fragment containing the *LEU2* gene from YEp13.

For galactose-inducible expression of *TDS4*, an SpeI site was introduced just upstream of the initiation codon by polymerase chain reaction-mediated mutagenesis. The polymerase chain reaction product, which included part of the pBluescript KS$^+$ polylinker sequence downstream of the *TDS4* gene, was digested with SpeI and ligated into the SpeI site of pRS316-GALpro, which contains the GAL10 promoter upstream of the pRS316 polylinker (the gift of D. Miller and G. Fink, Whitehead Institute).

Bacterial expression plasmids were constructed as follows. pGEX–TDS4(BglII–EcoRI) was made by ligating the BglII–EcoRI fragment from TDS4 (encoding amino acids 14 to 262) into the BamHI and EcoRI sites of pGEX1 (Smith and Johnson, 1988). pGEX–TDS4(BamHI–BamHI) was made by ligating the BamHI fragment from pBS–TDS4orf (encoding amino acids 176 to 596) into the BamHI site of pGEX1. Glutathione transferase fusion proteins were produced and purified as described (Smith and Johnson, 1988).

Antibody Production

GST–TDS4 fusion proteins were purified as described (Smith and Johnson, 1988). Two hundred micrograms of each fusion protein was mixed with RIBI adjuvent and injected into rabbits as recommended by the manufacturer (RIBI ImmunoChem Research). The rabbits were

boosted with the same amount of protein one month later, and serum was harvested 12 days after the boost.

S1 Nuclease Protection Assay

Yeast strains YSB112 and RSY763 were grown to OD_{600} = 1.0 in YPGal media. Cells were spun down, washed once in YPD, and then resuspended in YPD to an OD_{600} = 0.1. At the indicated time points, 50 ml of cells was removed. After 9 hr, cells were diluted 1:5 in YPD and allowed to continue growth until 22 hr. Total RNA from the cells was isolated using hot phenol extraction (Ausubel et al., 1991). RNA was quantitated by absorbance at 260 nM and by ethidium bromide staining of 18S and 28S RNAs in agarose gels. The S1 nuclease protection assay was carried out with 15–30 μg of RNA and oligonucleotide probes as described in Cormack and Struhl (1992).

Native Gel Electrophoresis

Binding of TFIIIB and TFIIIC, heparin stripping, and native gel electrophoresis of pol III preinitiation complexes were performed as described (Braun et al., 1989; Kassavetis et al., 1990). The probe used for binding was the SUP4 tRNA gene derived from plasmid pTZ2 (Kassavetis et al., 1989). The TFIIIC fraction (DNA affinity purified, 18 fmol/ml) and TFIIIB fraction (Cibacron Blue purified, 3.7 fmol/ml) were the gift of G. Kassevetis (Kassavetis et al., 1990). Reactions contained 1 fmol of probe, 10 fmol of TFIIIB, and 9 fmol of TFIIIC. The binding reaction was performed at room temperature for 40 min. The indicated amounts of antisera were added, and incubation was continued for an additional 30 min. Heparin was added (300 μg/ml) for 5 min before loading the sample onto the native gel and beginning electrophoresis. Gels were dried and autoradiographed with an intensifying screen overnight.

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