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# Direct binding of yeast transcription factor (TFIID) to the ribosomal protein L32 (rpL32) TATA-less promoter sequence

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The ribosomal protein L32 (rpL32) gene transcribed by RNA polymerase II lacks a canonical TATA element, that binds the transcription factor TFIID $\tau$ or TBP (TATA binding protein). Instead this promoter contains an element, termed $\gamma$, located at −30 relative to the transcription initiation site. We previously reported that, despite the lack of a canonical TATA element the rpL32 gene utilizes yeast TFIID $\tau$ for its transcriptional initiation. Whether TFIID $\tau$ participates in rpL32 gene transcription by binding directly to a promoter element or through another protein has not been resolved. These studies reveal that proteins ranging in size from 20–40 kDa binds to the $\gamma$-element. The 40 kDa protein(s) displays strong affinity for the canonical TATA element and may be related or equivalent to TFIID $\tau$. Furthermore, cloned and purified yeast TFIID (TBP) binds directly to the $\gamma$-element implying that the $\gamma$-element directs RNA polymerase II-dependent transcription of the rpL32 gene.

Transcription factor IID; RNA polymerase II; Ribosomal protein L32

## 1. INTRODUCTION

Regulation of gene expression is a complex process involving the interaction of both sequence-specific DNA binding and general initiation factors [1]. One of the general transcription factors, TFIID, plays a pivotal role in the decoding of most genes, by interacting with sequence-specific factors to achieve transcriptional enhancement [2]. Hence, the study of TFIID involvement in transcription of a particular gene is of considerable interest. Recent reports have indicated that native TFIID in higher organism contains, in addition to the TATA binding subunit (TFIID $\tau$ or TBP), a number of other polypeptides required specifically for activator-dependent transcription [3–6]. Identification of a yeast protein capable of substituting for mammalian TFIID in basal transcription led to the cloning of genes encoding the yeast protein and, ultimately, the corresponding TATA binding component from higher eukaryotes (see [6]). These proteins, like native TFIID [7,8], bind to the TATA element to initiate preinitiation complex assembly (reviewed in [2]). Subsequently, other general initiation factors and RNA polymerase II interact in a sequential manner with the functional preinitiation complex ([9,10]; reviewed in [2]).

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Transcription factor TFIID is essential for formation of RNA polymerase II initiation complexes and is thought to act by recognizing and binding to the asymmetric TATA element (consensus TATAAAA) that is present in most class II promoters. Recent studies suggest, however, that TFIID $\tau$ is also involved in the transcription of genes catalysed by RNA polymerase I and RNA polymerase III (reviewed in [11,12]), most of which do not contain a TATA element. Moreover, many promoters transcribed by RNA polymerase II also lack a canonical TATA element. Among these TATA-less promoters are the simian virus (SV40), interferon regulatory factor and the mouse ribosomal protein gene rpL32 [13–16]. Known elements in the rpL32 gene promoter are shown in Fig. 1.

In spite of the absence of a canonical TATA element, the rpL32 gene initiates transcription at one site [17–21]. Our previous studies have indicated that the rpL32 promoter utilizes yeast TFIID $\tau$ despite the absence of the canonical TATA element [20]. Whether the yeast TFIID $\tau$ participates in rpL32 gene transcription, by binding directly to the $\gamma$-element of the rpL32 promoter segment or indirectly to a protein which binds to the promoter segment, is unclear. A sequence comparison of the rpL32 $\gamma$-element and adenovirus major late promoter (Ad ML) TATA element is shown in Fig. 2.

In this communication we demonstrate that the rpL32 $\gamma$-element binds proteins ranging in size from 20–40 kDa. Of these, the 40 kDa protein(s) displays a strong affinity for the canonical TATA element, whereas the 20 kDa protein does not. Furthermore, our results reveal that yeast TFIID $\tau$ binds to the rpL32

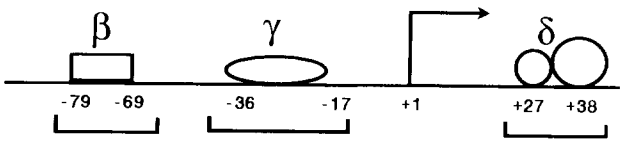


Fig. 1. Schematic organization of the 5′ regulatory region of the rpL32 gene promoter. Segments containing specific binding sites are indicated. Nuclear proteins that bind to the particular region of the rpL32 promoter were localized by transfection experiments [26], cell-free transcription [18] and gel-shift assay ([21,26] and this study).

γ-element in a sequence-dependent manner, implying that TFIID τ binds directly to a non-canonical TATA element in the rpL32 promoter.

## 2. MATERIALS AND METHODS

### 2.1. *Cell culture and nuclear extract preparation*

Preparation and partial purification of mouse L1210 nuclear extracts were performed as previously described [18–21].

### 2.2. *UV cross-linking of factors to the γ-element*

All the procedures employed were essentially as outlined by Gronostajski [22]. The specific γ-labelled DNA was prepared as follows: oligonucleotides (5′-GCGCGCAAGGTATGATGGGAC-3′) were made duplex by hybridization of a primer (5′-GTCCCATCATAC-3′) followed by extension using the large fragment of DNA polymerase I as previously described [22]. Labelled oligonucleotide, 0.25–0.50 ng, was incubated with a partially purified (DEAE fraction) nuclear extract. After incubation at 30°C for 30 min, the samples were UV-irradiated for 30 min. Following irradiation, $CaCl_2$ was added to a final concentration of 10 mM, with subsequent addition of DNAse I and micrococcal nuclease. The mixture was then digested at 37°C and electrophoresed in a 12.5% SDS-polyacrylamide gel, dried and the labelled peptides identified by autoradiography.

### 2.3. *Gel mobility shift assay*

An oligonucleotide (5′-CTCCGTCCCATCATACCTTGCGC-3′) was $^{32}P$ labelled and annealed to the opposite strand. Gel mobility shift assays were performed with 0.1 ng of the labelled oligonucleotide fragment in the presence of 100 ng poly (d[G–C]) incubated with purified yeast TFIID at 30°C for 30 min. The protein–DNA complexes were resolved from free DNA by electrophoresis on 4% polyacrylamide gels.

### 2.4. *Expression and purification of yeast TFIID*

The procedures employed were described by Horikoshi et al. [23].

## 3. RESULTS

### 3.1. *UV cross-linking identifies proteins interacting with the γ-element*

In an attempt to characterize interacting proteins, oligonucleotides (20 bp) containing the γ-element were synthesized with $^{32}P$-labelled dCTP and incubated with nuclear extracts from mouse L1210 cells. After irradiation with UV light the cross-linked material was subjected to nuclease digestion and the resulting products were analyzed on denaturing 12.5% SDS-polyacrylamide gels. These UV cross-linking experiments identified labelled polypeptides migrating with an apparent molecular mass of 40 kDa and two or three additional species at 20 kDa (Fig. 3, lane 2). To verify the specificity of these proteins, competition studies were carried out with an unlabelled oligonucleotide containing the γ-element sequence and an unrelated oligonucleotide (Fig. 3, lanes 3 and 4, respectively). The presence of the specific unlabelled competitor eliminated the labelled polypeptides, while inclusion of an unrelated competitor did not (lane 4), implying that these polypeptides bind specifically to the γ-element.

Ad-ML. TATA element - CTATAAAAGGGGGTGG

rpL32 gamma element - CCATCATACCCTGCGCG

Fig. 2. The nucleotide sequence of a canonical TATA element (a typical TFIID binding site) and rpL32 γ-element.

### 3.2. *The γ-factor binds to the TATA element*

The above studies suggest that the 40 kDa and 20 kDa proteins specifically interact with the γ-element. The mouse TATA binding subunit (TFIID τ) is approximately 40 kDa in size [24]. Consequently a series of UV cross-linking competition analyses were performed to determine whether similar proteins interact with both the γ- and canonical TATA elements. In these experiments a labelled oligonucleotide (20 bp) containing the

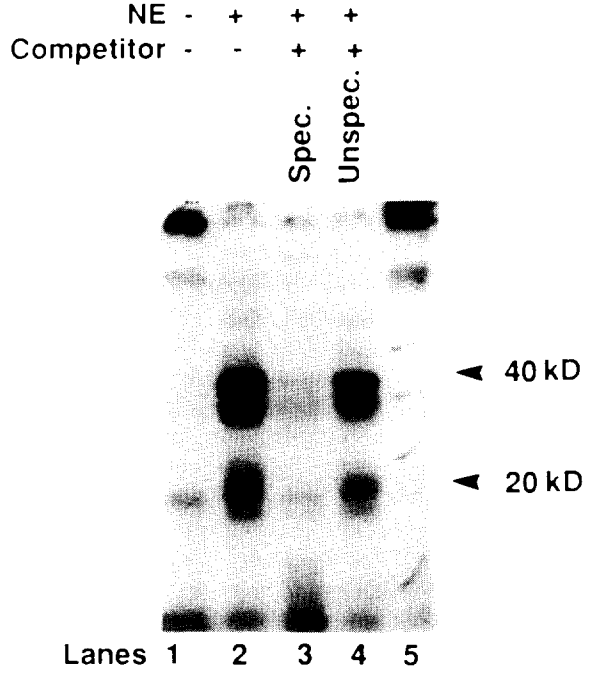


Fig. 3. Identification of the protein(s) binding to the γ-element. The labelled γ-element was incubated with nuclear extracts in the presence of an unlabelled oligonucleotide containing the γ site or an unrelated oligonucleotide lacking the γ site followed by UV irradiation and separation by SDS-polyacrylamide gel electrophoresis. Lanes: 1, no nuclear extract; 2, nuclear extract; 3, nuclear extract plus 100-fold molar excess of specific oligonucleotide; 4, nuclear extract plus 100-fold molar excess of unrelated oligonucleotide; 5, standard molecular weight protein markers.

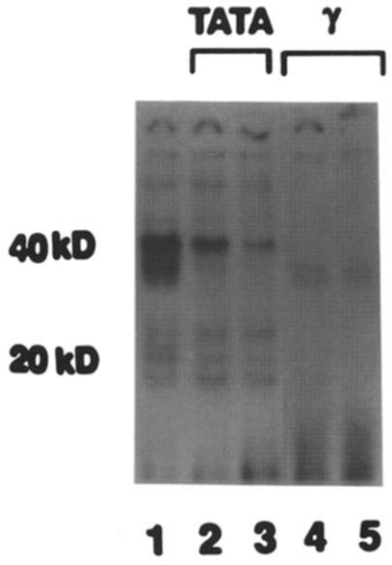


Fig. 4. One of the γ proteins interacts with the TATA element. UV cross-linking studies were performed as described in section 2. The labelled oligonucleotide containing the γ-element was incubated with nuclear extract. Lanes: 1, nuclear extract with no competitor; 2,3, nuclear extract with 50-, and 100-fold molar excess, respectively, TATA oligonucleotide; 4,5, nuclear extract with 50- and 100-fold molar excess of oligonucleotide containing the γ-element.

γ-element was incubated with a partially purified (DEAE cellulose) nuclear extract fraction. Competition with an unlabelled oligonucleotide containing a canonical TATA element (from Ad-ML promoter) specifically eliminated binding of the 40 kDa protein(s) to the γ-element (Fig. 4, lanes 2 and 3). On the other hand, an unlabelled nucleotide containing the γ-element eliminated binding of both proteins (Fig. 4, lanes 4 and 5). These findings suggest that the 40 kDa protein(s) may reflect interaction of the mouse TFIID τ with the γ-element. Whether this interaction results from direct association or through the lower molecular weight proteins is currently unknown.

### 3.2. *Yeast TFIID forms a complex with the γ-element*

Our UV cross-linking analyses revealed that a number of proteins (20–40 kDa) bind specifically to the γ-element. Further, the 40 kDa protein(s) interacts not only with the γ-element but also with a canonical TATA element. It is tempting to speculate that the 40 kDa protein which interacts with the γ-element is related to or is the mouse TFIID. Recent studies have indicated that TFIID τ from various species exhibits significant homology [25]. In view of these reports and the functional data presented in our previous study [20], gel mobility shift assays were performed with cloned yeast TFIID τ to determine whether it binds directly to the γ-element. Experiments were performed with purified yeast TFIID τ and labelled γ-element. Gel mobility shift analysis revealed a specific complex migrating at a slower rate than the free DNA probe (Fig. 5, lanes 1 and 2). Adding increasing amounts of recombinant yeast TFIID τ to the reaction mixture produced a more intensely labelled complex (Fig. 5, lanes 2–4). Moreover, addition of a specific unlabelled competitor abolished the appearance of the labelled complex (Fig. 5, lane 6), whereas addition of an unrelated oligonucleotide did not (Fig. 5, lane 5). These observations suggest that yeast TFIID τ, and presumably murine TFIID τ, binds directly to the γ-element without the interaction of another protein.

## 4. DISCUSSION

The rpL32 promoter lacks a canonical TATA element [15]. Despite its absence the rpL32 gene can utilize yeast TFIID τ for transcription [20]. This was shown by specifically inactivating TFIID τ in a transcriptionally active nuclear extract and then supplementing the extract with cloned and purified yeast TFIID τ [20]. Al-

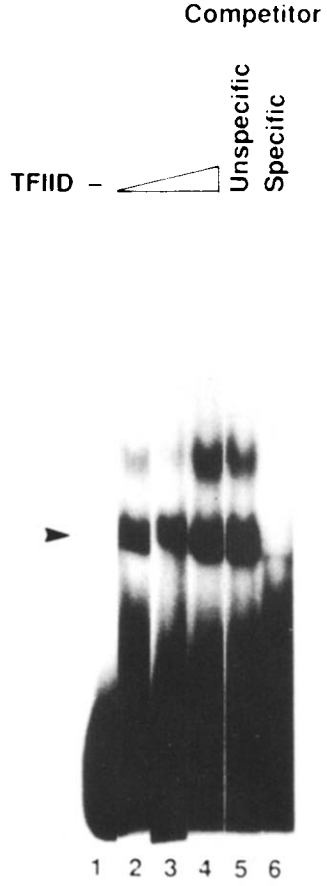


Fig. 5. Cloned and purified yeast TFIID binds directly to the γ-element. To determine whether TFIID by itself binds to the γ-element the labelled γ oligonucleotide and yeast TFIID were incubated as indicated in section 2. Protein–DNA complexes were resolved by native polyacrylamide gel electrophoresis as described by Horikoshi et al. [23] Lanes: 1, no yeast TFIID; 2–4, increasing amount of yeast TFIID; 5, 50 ng of yeast TFIID and 100-fold molar excess of unrelated oligonucleotide; 6, 50 ng of yeast TFIID and 100-fold molar excess of γ oligonucleotide.

though our previous studies provided strong biochemical evidence for the involvement of yeast TFIID $\tau$ in transcription of the rpL32 gene, it did not address its mode of interaction with the promoter. In this communication we established that the −30 region of rpL32 binds purified yeast TFIID $\tau$, and based on the extreme sequence conservation we expect that the corresponding mouse protein (TFIID $\tau$) will interact in the same manner. The −30 region, designated the $\gamma$-element (Fig. 1), has been shown previously to be important for transcription in vivo [26]. We conclude, therefore, that binding of TFIID $\tau$ to the $\gamma$-element plays an important role in the transcriptional initiation of this gene. In a recent report, Mertz and colleagues also demonstrated that TFIID $\tau$ binds to −30 of several promoters lacking a canonical TATA element [27]. It is well established that binding of TFIID $\tau$ (TBP) allows RNA polymerase II and other general initiation factors to interact with the promoter [2]. The studies presented here suggest that the $\gamma$-element can substitute functionally for the canonical TATA element and that it directly recognises TFIID $\tau$. Along with the position of the $\gamma$-element (−30 bp upstream of the start site) these observations imply that this element acts in transcriptional initiation in a manner similar to that of the canonical TATA element. Furthermore, these data indicate that TFIID $\tau$ can bind to a sequence distinct from that found in the canonical TATA element.

The UV cross-linking data also reveal the potential for proteins distinct from TFIID $\tau$ (or TBP) to bind specifically to the $\gamma$-element. The lower molecular mass (20 kDa) proteins present are clearly distinct from the already established basic transcription factors, but are similar in size to polypeptides found associated with TBP in natural TFIID purified from mammalian cells by epitope tagging and antibody affinity purification (C.M. Cheng, H. Ge, A. Hoffmann and R.G. Roeder, unpublished observations). Except for TFIID $\tau$ none of the required basic transcription factors has been shown to be sequence-specific. The sequence-specific 20 kDa DNA binding proteins observed in this study may play a role in stabilizing the interaction of TFIID $\tau$ to the $\gamma$-element of the rpL32 promoter. In the in vivo situation, the affinity of TFIID $\tau$ for the $\gamma$ sequence of the rpL32 promoter may be less than its affinity for canonical TATA elements. Consequently the lower molecular weight proteins may function by recruiting TFIID to the $\gamma$-element or increasing its affinity for the $\gamma$-element. A clear understanding of these protein–protein interactions requires further purification and characterization of these proteins. In summary, we have shown that yeast TFIID $\tau$ can bind directly to the rpL32 gene through a unique promoter element, and that other cellular factors, perhaps associated with the natural murine TFIID $\tau$, can also interact with this element.

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