RESEARCH COMMUNICATION

Yeast transcription factor IID participates in cell-free transcription of a mammalian ribosomal protein TATA-less promoter

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We analysed transcription of the gene for the ribosomal protein (rp) L32 of the mouse, which is transcribed in mouse L1210 nuclear extracts *in vitro*. The rpL32 gene lacks a canonical TATA box. Hence it has been suggested that this gene has an alternative transcription pathway not requiring transcription factor IID (TFIID). Selective inactivation of TFIID in nuclear extract completely abolished the transcription of *rpL32 in vitro*. Selective inactivation was restored by the addition of cloned and purified yeast TFIID (yTFIID), indicating that this TATA-less *rpL32* promoter utilizes TFIID for its transcription initiation. Furthermore, addition of an oligonucleotide-containing TATA sequence interfered with the *rpL32* transcription and this was overcome by the addition of yTFIID. To further examine the stage of involvement of TFIID in *rpL32* transcription, TATA oligonucleotide was added to nuclear extract before and after the formation of the transcription complex. The results reveal that TFIID associates with the pre-initiation complex and that this complex is largely resistant to added TATA oligonucleotide. Our results show, for the first time, that the TATA-less *rpL32* gene utilizes TFIID for transcription initiation.

INTRODUCTION

Most eukaryotic RNA polymerase II promoters contain a TATA-element consensus sequence TATAAA, required for the precise initiation of transcription *in vivo* and *in vitro* [1,2]. The pivotal factor in the basal apparatus is termed transcription factor IID (TFIID), which binds to a specific TATA sequence just upstream of the start site, and stimulates transcriptional initiation [3–5]. Following the binding of TFIID, other transcriptional factors and polymerase II bind to the DNA. TFIID is regarded as a commitment factor the binding of which to the TATA box is a prerequisite for the assembly of the basal transcription apparatus.

The factor which interacts with the TATA sequence has only been partially purified. A protein with similar activities has been identified in yeast, plant and human cells. The genes encoding the TATA binding protein have also been isolated from several species including yeast, *Drosophila*, plant and human [6–9].

Studies have revealed that a number of eukaryotic promoters including mouse ribosomal proteins (rps) L32, L30, S16, small nuclear RNAs (Sn RNAs) and the mouse terminal deoxynucleotidyltransferase (*TdT*) gene lack a TATA element [10–13]. Despite the absence of a TATA box the mouse *rpL32* gene, which is transcribed by polymerase II and contains a γ element [10,13] instead of the usual TATA motif, initiates transcription at a precise start site [14–17]. While cell-free transcription has been achieved for the *rpL32* gene, the exact mechanism controlling transcriptional initiation is still unclear. Whether the γ element utilizes TFIID for correct positioning of polymerase II is unknown.

Our studies are designed to examine the involvement of TFIID in the transcription of the *rpL32* gene promoter. To assist our

understanding of this phenomenon we have employed a cell-free transcription system using the *rpL32* gene and purified TFIID. Our results suggest that, despite the lack of a canonical TATA box, TFIID is involved in transcription of the mouse *rpL32* gene.

MATERIAL AND METHODS

Cell culture and nuclear extract preparation

Mouse L1210 cells were maintained in culture (with shaking) in Dulbecco's modified Eagle's medium supplemented with 5% (v/v) fetal calf serum. Nuclear extracts were obtained using the procedure of Dignam *et al.* [18]. Nuclear extracts were fractionated on DEAE-cellulose (Whatman DE52) according to the protocol of Zahradka & Sells [15].

Cloning and purification of yeast TFIID (yTFIID)

The procedures employed were essentially as described by Horikoshi *et al.* [6].

Cell-free transcription

Individual reaction mixtures contained 12 mM-Hepes, pH 7.9, 5 mM-MgCl₂, 70 mM-KCl, 10% (w/v) glycerol, 500 μ M (each) of ATP, CTP and GTP, 25 μ M-[³²P]UTP, 0.4 mM-EDTA, 0.5 mM-dithiothreitol, 0.25 μ g of *Eco*RI-digested plasmid DNA containing the *rpL32* gene and 3 μ l of DEAE fraction in a total volume of 25 μ l. After a 60 min incubation period at 30 °C the reaction was stopped by the addition of 175 μ l of stop buffer [15]. The transcript was isolated by extraction with phenol/chloroform and then precipitated with ethanol and resolved by urea/PAGE. Gels were dried and autoradiographed. A plasmid containing the promoter segment and coding region of *rpL32* was digested with *Eco*RI and gave rise to a 1500 bp-specific transcript.

Abbreviations used: TFIID, transcription factor IID; rp, ribosomal protein; Sn RNAs, small nuclear RNAs; yTFIID, yeast TFIID; NTPs, nucleotide triphosphates.

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Depletion of endogenous TFIID from L1210 nuclear extract

TFIID inactivation was achieved by heat treatment (15 min at 47 °C) of the DEAE fraction of L1210 essentially as described in Nakajima *et al.* [2].

RESULTS AND DISCUSSION

The following experiments were designed to determine the requirement for TFIID in transcription involving the *rpL32* promoter. This was accomplished by selectively inactivating TFIID by mild heat treatment of nuclear extracts followed by supplementation with purified cloned yTFIID. Mild heat treatment of nuclear extracts (47 °C for 15 min) completely abolished transcription of the *rpL32* gene (Fig. 1, compare lane 1 with lane 2). When these heat-treated extracts were supplemented with purified yTFIID, faithful transcription of the *rpL32* gene occurred (Fig. 1, lanes 3–5). This observation demonstrates that yTFIID can function in the transcription of the *rpL32* gene. Addition of increasing amounts of yTFIID resulted in the formation of more transcription product, again indicating direct involvement of TFIID in *rpL32* gene transcription. The participation of yTFIID in the transcription of the TATA-less mouse

Fig. 1. Analysis of cell-free transcriptional activity of the *rpL32* gene using yTFIID

Transcription using the plasmid containing the *rpL32* gene was measured in a heat-treated (TFIID-deficient) nuclear extract supplemented with buffer or yTFIID. Lane 1, untreated nuclear extract as control; lane 2, heat-treated nuclear extract supplemented with buffer; lanes 3–5, heat-treated nuclear extract supplemented with indicated amounts of purified yTFIID. The position of the correctly initiated transcript is indicated by the arrow.

Fig. 2. Involvement of the TATA binding factor in cell-free transcription of the *rpL32* gene

Lane 1, control extract without oligonucleotide (oligo.); lane 2, extract containing 100 ng of non-specific oligonucleotide; lanes 3–7, extract containing 100 ng of TATA oligonucleotide; supplemented with buffer (lanes 3 and 4); or 10, 25, 50 ng respectively of yTFIID (lanes 5–7).

rpL32 gene suggests that this TATA-less promoter utilizes TFIID in its transcriptional initiation.

To assess whether TFIID, or a different protein with similar activity in the L1210 nuclear extract, contributes to the transcription of the *rpL32* gene the following experiment was conducted. An oligonucleotide containing the TATA element was added to the nuclear extract. The TATA element specifically inhibited transcription (Fig. 2, lanes 3 and 4) and added yTFIID restored transcription (Fig. 2, lanes 5–7). A control oligonucleotide had no significant effect upon transcription (Fig. 2, lane 2). These results clearly demonstrate that the TATA binding factor, TFIID, is involved in initiating *rpL32* gene transcription. The transcript product appeared as a doublet in some lanes; however, the nature of this doublet is unknown.

To examine further this premise a competition experiment was performed. An oligonucleotide containing the TATA sequence was included in the transcription reaction mixture before or after formation of the pre-initiation complex. Pre-initiation complexes were formed, by pre-incubation in the absence of nucleotide triphosphates (NTPs). Transcription was then initiated by the addition of NTPs, and the transcripts generated over 60 min were analysed. Addition of the TATA oligonucleotide before formation of the pre-initiation complex abolished transcription (Fig. 3, lanes 2 and 3), whereas addition of the TATA oligonucleotide following formation of the pre-initiation complex slightly decreased transcription (Fig. 3, lanes 4 and 5). Addition of control oligonucleotide before (lane 1) or after (results not shown) complex formation had no significant effect upon transcription. These observations imply that the TATA-binding factor in extracts from L1210 is involved in initiation of transcription of the *rpL32* gene. The fact that addition of the TATA element after pre-initiation complex formation reduced transcription suggests that TFIID within the *rpL32* pre-initiation complex is still partially susceptible to competition by the TATA element.

These results reveal that the TATA-binding protein, TFIID, plays a role in transcription of the *rpL32* gene. Participation of this factor in the transcription of the *rpL32* gene was shown using the TFIID protein produced from a cloned yeast gene. It is well established that binding of TFIID allows RNA polymerase II and other general initiation factors to interact with the promoter sequence [4,5,19,20]. Our results also reveal that yTFIID can substitute for the mouse TFIID in a cell-free transcription system. Previous reports have also suggested that yTFIID can replace human TFIID in cell-free transcription [6,21,22]. The results of this investigation indicate that the requirement of the TATA consensus sequence for TFIID binding may not be as essential as previously thought. These results are consistent with other reports [23,24].

To date, six functional mouse *rp* genes have been cloned, but none of these six contains a canonical TATA box [25–30]. Therefore it is believed that these *rp* genes employ an alternative transcription initiation mechanism which is different from the typical TFIID pathway. Five of these gene promoters contain A+T-rich sequences at the usual TATA element site, while *rpS16* contains a completely different sequence in this region [27]. Perry and co-workers have also shown that a factor binding to this sequence is different from TFIID [31]. The studies reported here suggest that one of the *rp* genes, *rpL32*, utilizes TFIID for transcriptional initiation *in vitro*. It is conceivable that another factor may play a role *in vivo*. Further experiments *in vivo* are needed to verify this phenomenon. To the best of our knowledge this is the first report of cell-free transcription which shows that TFIID protein is directly associated with the transcription of a *rp* gene. It will be of interest to determine whether other *rp* genes also utilize TFIID for transcriptional initiation.

Fig. 3. TFIID forms pre-initiation complexes with rpl32 promoter

Various amounts of the TATA element or a non-specific oligonucleotide (oligo.) were included in the transcription reaction mixture as indicated. Lane 1, non-specific oligonucleotide (100 ng); lane 2, 50 ng of TATA element and lane 3, 100 ng of TATA element added before the formation of pre-initiation complexes; lane 4, 50 ng of TATA element and lane 5, 100 ng of TATA element added after the formation of pre-initiation complexes. The TATA element used in the current studies contained 31 bp of duplex DNA spanning the sequence of Ad2 MLP from -45 to -15.

This research was supported by the MRC (Canada) and the NSERC (Canada) (to B.H.S.) by the NIH (to M.H. and R.G.R.). M.H. is an Alexandrine and Alexander Sinsheimer Scholar.

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Received 27 April 1992; 20 May 1992; accepted 26 May 1992