TFII Is Required for Transcription of the Naturally TATA-less but Initiator-containing V\(\beta\) Promoter*

(Received for publication, December 1, 1995, and in revised form, March 5, 1996)

Brenda Manzano-Winkler† Carl D. Novina‡, and Ananda L. Roy§

From the Division of Immunology and the Department of Pathology, Sackler School of Graduate Studies, Tufts University School of Medicine, Boston, Massachusetts 02111

The proximal or core promoter of a typical eukaryotic protein coding gene comprises distinct elements, TATA and/or initiator (Inr). The existence of TATA or Inr at the core promoter suggests that the mechanism of transcription initiation mediated by these two genetic elements may be different. Accordingly, it has been demonstrated that the transcriptional requirements for the TATA-containing, Inr-less (TATA\(^{+}\)Inr\(^{-}\)) promoters are different from the transcriptional requirements for the TATA-less, Inr-containing (TATA\(^{+}\)Inr\(^{+}\)) promoters. Although both types of promoters require the transcription initiation factor (TFIID) in addition to other common initiation factors, a TATA\(^{+}\)Inr\(^{-}\) promoter requires accessory component(s). Here we have employed in vitro analyses to address the transcription factor requirements for a TATA\(^{+}\)Inr\(^{-}\) promoter. We demonstrate that in addition to TFIID, a naturally occurring TATA\(^{+}\)Inr\(^{-}\) promoter requires TFII-I, an Inr element-dependent transcription factor. Consistent with its Inr element-dependent activities, TFII-I is dispensable for a TATA\(^{+}\)Inr\(^{-}\) promoter. Furthermore, we demonstrate that both TFII-I and TFIID activities in nuclear extracts are temperature-sensitive. However, TFII-I is heat-inactivated at temperatures lower than that required to inactivate TFIID. Therefore, differential heat treatment of nuclear extracts provides an assay to discriminate between transcriptional requirements at TATA\(^{+}\)Inr\(^{-}\) and TATA\(^{+}\)Inr\(^{+}\) promoters.

Transcription initiation of protein coding genes is brought about by RNA polymerase II and a set of general transcription factors (1–3). For our analyses, we have used the T cell receptor variable region-derived (V\(\beta\)) promoter (22) as a model TATA\(^{+}\)Inr\(^{-}\) promoter and subsequently restored its transcriptional activity by exogenous addition of purified TFII-I. We demonstrate that: 1) Immunodepletion of nuclear extracts with an anti-TFII-I antibody completely abrogates transcription of the TATA\(^{+}\)Inr\(^{-}\) V\(\beta\) promoter, which is restored by addition of purified TFII-I. Importantly, these antibodies have no effect on the TATA\(^{+}\)Inr\(^{-}\) IgH promoter. 2) TFII-I binds specifically to the V\(\beta\) Inr element. Thus, an oligonucleotide containing the wild type V\(\beta\) Inr element sequence efficiently inhibits V\(\beta\) transcription; exogenously added TFII-I restores V\(\beta\) transcription. A control oligonucleotide containing the mutant V\(\beta\) Inr sequence does not inhibit V\(\beta\) transcription. 3) In addition to TFII-D, TFII-I is temperature-sensitive. Thus, heat...

* 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 U.S.C. Section 1734 solely to indicate this fact.
† These authors contributed equally to this work.
‡ These authors contributed equally to this work.
§ To whom correspondence should be addressed: Division of Immunology/Dept. of Pathology, Sackler School of Graduate Studies, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111.

The abbreviations used are: Inr, initiator; V\(\beta\), T cell receptor variable chain \(\beta\); IgH, immunoglobulin heavy chain; TFII-I, transcription initiation factor-I; TFII-D, transcription initiation factor-D; EMSA, electrophoretic mobility shift analysis; AdML, adenovirus major late; wt, wild type; mut, mutant; TBP, TATA binding protein.
treatment of nuclear extracts impairs both TFII-I and TFIIID activities. However, the two activities are affected at different temperatures. Heat treatment of nuclear extracts at 42 °C ablates TFII-I but not TFIIID activity, whereas heat treatment at 49 °C destroys TFIIID activity as well. Transcriptional complementation assays using heat-treated nuclear extracts demonstrate that although TFIIID is both necessary and sufficient for a TATA “Inr” promoter, TFII-I is additionally required for the TATA “Inr”/β promoter.

**MATERIALS AND METHODS**

**Nuclear Extracts**

Jurkat and HeLa cells were grown in culture, and 3 liters (3 × 10^10 cells) were harvested to prepare nuclear extracts as described (25). Protein concentrations for each nuclear extract were determined spectrophotometrically via Bio-Rad protein assay.

**Heat Inactivation of Nuclear Extracts**

Nuclear extracts (50 μl) were aliquoted and incubated at 42 °C for 6 or 15 min as specified in Fig. 4. Following heat treatment, the extracts were centrifuged for 1 min, and the supernatants were placed in fresh tubes for immediate use in transcription.

**Immunodepletion of Nuclear Extracts**

The anti-TFII-I antibody was raised in rabbits against a synthetic peptide corresponding to the putative DNA binding domain of TFII-I.2 The polyclonal serum was obtained from a 10-week bleed. Immuno-depletion of nuclear extracts was achieved in two ways. One method involved incubation of an extract with either the preimmune serum or with the immune serum (anti-TFII-I antibody) at 30 °C for 10 min prior to starting the transcription reaction. The other method included the additional steps of applying the mixture of nuclear extract and serum to protein A-Sepharose beads and incubating at 0 °C for 30 min; the mixture was centrifuged at low speed for 1 min, and the supernatants were removed for immediate use in transcription.

**Immunodepletion of Nuclear Extracts**

Heat treatment of nuclear extracts impairs both TFII-I and TFIID activities. Differences were not observed between TFII-I and TFIID activity when the TFII-I antibody was preincubated with the preimmune serum (anti-TFII-I antibody) or mock-depleted with the preimmune serum, and, as indicated, also incubated with 100 ng of partially purified TFII-I antibody, which yielded identical results (not shown). All of the above described treatments were done immediately preceding the transcription reactions.

**SDS-PAGE, Western Blot Analyses, and Peptide Block**

A purified preparation of TFII-I (100 ng) and either undepleted, TFII-I-depleted or mock-depleted) urkat nuclear extract (10 μg in each case) was subjected to SDS-PAGE (7.5%) and subsequently transferred to nitrocellulose by Western blot technique. The blotted proteins were probed with the anti-TFII-I antibody (1:2500 dilution) and visualized using ECL technique (Amersham Corp.). Similar methods were employed for visualization of immune precipitates.

**Peptide Block experiments, the anti-TFII-I antibody was preincubated for 30 min at 0 °C prior to probing with 2.5, 0.25, or 0.025 mg/ml of the synthetic peptide derived from the putative DNA binding region of TFII-I.**

**Purification of Transcription Factors**

TFII-D—All procedures were performed at 4 °C. HeLa nuclear extract was chromatographed over a heparin-Sepharose (Pharmacia Biotech Inc.) column equilibrated in buffer A containing 20 mM Tris, pH 7.9, 0.2 mM EDTA, 5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 10% (v/v) glycerol, and 100 mM KCl; the TFII-D activity was eluted in buffer A containing 500 mM KCl. These fractions were pooled and dialyzed against 80 mM Tris, pH 7.9, 0.2 mM EDTA, 0.5% SDS, 100 mM LiCl, 100 μM/μl RNA, 300 mM NaOAc. The transcriptions were then extracted with phenol-chloroform, precipitated with 2-propanol, and washed with 70% (v/v) ethanol.

In the reactions utilizing the IgH G-less template, 500 μM concentrations of each nucleotide ATP, GTP, TTP, and 30 μM [α-32P]CTP were added, supplemented with 30 units of RNase inhibitor (Pharmacia). In the reactions utilizing the IgH G-less cassette, ATP and TTP were added to a final concentration of 500 μM each, along with 25 μM CTP, 100 μM O-methyl-GTP, 30 μM [α-32P]CTP, and 30 units of RNase T1 (Pharmacia). All reactions were incubated for 60 min, including the specified preincubation times, at 30 °C and then stopped by the addition of 400 μl of Stop Mix (8 M urea, 10 mM Tris, pH 7.8, 0.5% SDS, 100 mM LiCl, 100 μg/ml tRNA, 500 mM EDTA) for 3 h at 75°C. The gels were then extracted with formamide and radioactivity were determined.

**RESULTS**

**Vjβ Transcription Requires TFII-I: Antibody-mediated Block of TFII-I Activity**—To test the effects of anti-TFII-I antibody, we employed the antibody in an EMSA. Two different nuclear extracts (HeLa and J urkat) were tested for their ability to bind an Inr element (Fig. 1a). Both nuclear extracts gave a predominant band (lanes 1 and 2) that comigrated with a purified preparation of TFII-I (data not shown). Importantly, the HeLa

---

2 A. L. Roy, P. D. Gregor, C. Carruthers, E. Martinez, and R. G. Roeder, unpublished data.
and J urkat nuclear extract-derived TFII-I band was abrogated by the anti-TFII-I antibody (c, lanes 3 and 5). But a control antibody (anti-TBP antibody, αT; lanes 4 and 6) had no effect on TFII-I binding.

Sequence comparison between different promoters reveals that the Vβ promoter contains a consensus Inr element (Fig. 1b). The Vβ promoter (kind gift from Dr. D. Loh) is typically expressed in T cells (22). Thus, we employed a T cell-derived nuclear extract (J urkat) for all of the in vitro transcriptional assays. We used the anti-TFII-I antibody to deplete TFII-I from a transcriptionally competent J urkat nuclear extract (Fig. 1c). Employing an undepleted nuclear extract (lane 1), the run-off assay from the linearized Vβ promoter (containing wild type sequences from -480 to +260) produced an accurately initiated 260-nucleotide major transcript. Mock depletion of J urkat nuclear extract with a control antibody (preimmune serum) had no appreciable effects on transcription (lane 2). However, immunodepletion with an anti-TFII-I antibody severely impaired the Vβ transcription (lane 3).

To demonstrate that the antibody treatment causes depletion of only TFII-I, we added back TFII-I, exogenously, to an immunodepleted J urkat nuclear extract (Fig. 1d). Antibody-mediated inhibition of transcription (lane 2) was completely restored by exogenous addition of TFII-I (lane 3). Mock depletion (with preimmune serum) had very little effect (lane 4). Therefore, an anti-TFII-I antibody inhibits transcription from a TATA Inr promoter, which can be restored by exogenous addition of TFII-I.

As a control for promoter specificity, we employed the TATA Inr IgH (23, 24) promoter (Fig. 1e). The minimal (–47 to +1) IgH promoter does not exhibit any tissue type specificity in vitro and therefore can be transcribed by a T cell (Jurkat) nuclear extract. Most importantly, TFII-I depletion of a Jurkat nuclear extract did not affect the TATA Inr IgH promoter. Thus, the level of the 400-nucleotide transcript produced from the IgH promoter remains unaltered in undepleted (lane 1), mock-depleted (lane 2), and immunodepleted (lane 3) J urkat nuclear extracts. These data clearly demonstrate that TFII-I is required for a TATA Inr promoter but not for a TATA Inr promoter.

Specificity of the Anti-TFII-I Antibody: Peptide-mediated Block of the Antibody—We further established the specificity of the anti-TFII-I antibody. Western blot analysis of J urkat nuclear extract was carried out and probed with the antibody (Fig. 2a). A purified preparation of TFII-I (120 kDa) was used as a control. This experiment revealed that the antibody, under the assay conditions, recognizes only TFII-I in nuclear extracts. The other cross-reactive species (125 kDa) is a modified (and inactive) form of the 120-kDa form. Most significantly, the antibody reactivity was completely abolished upon treatment with the antigenic peptide, either 2.5 (lanes 3 and 4) or 0.25 mg/ml (lanes 5 and 6) but not with 0.025 mg/ml (lanes 7 and 8). b, antibody depletion (TFII-I dep.) leads to removal of TFII-I from a Jurkat nuclear extract but mock depletion (mock dep.) has no appreciable effect compared with undepleted extract (undep.). c, the immune precipitate (αi ppt) obtained from antibody depletion shows the presence of substantial amount of TFII-I, whereas the precipitate from mock depletion (pl ppt) has no significant amount of TFII-I.

Fig. 1. Immunodepletion of TFII-I affects a TATA Inr+ (Vβ) but not a TATA Inr− (IgH) promoter. a, EMSA of HeLa and J urkat nuclear extracts (lanes 1 and 2) demonstrates a major complex that is blocked by an anti-TFII-I antibody (αI; lanes 3 and 5) but not by an anti-TBP antibody (αT; lanes 4 and 6). A preimmune serum had no effect on TFII-I mobility shift (not shown). For this EMSA we used an AdML promoter-derived Inr element. Identical results were obtained with all Inr elements tested under our conditions. b, sequence comparison of different Inr elements. The initiating nucleotide is indicated by the arrow. c, in vitro transcription using a linearized Vβ template. The run-off transcript from the Vβ template was not affected by mock depletion of J urkat nuclear extract with a preimmune (pl) serum (compare lanes 1 and 2). The transcription was abolished completely upon immune depletion with the anti-TFII-I antibody (I, lane 3). d, the anti-TFII-I antibody (I) severely decreased the Vβ transcription (compare lanes 1 and 2). However, the transcription was restored completely upon exogenous addition of a purified preparation of TFII-I (lane 3). As before, a preimmune (pl) serum had negligible effects on the Vβ transcription (lane 4). e, immunodepletion of TFII-I from J urkat nuclear extract had no significant effect on IgH transcription (compare lanes 1 and 3). Similarly, the control antibody (preimmune serum, pl) had no effects on IgH transcription (lane 2).

Fig. 2. Specificity of the anti-TFII-I antibody. a, the anti-TFII-I antibody specifically recognized p120/TFII-I in J urkat nuclear extract (lane 1) and in purified TFII-I (lane 2). The cross-reactive 125-kDa band in J urkat nuclear extract is a modified form of TFII-I (not shown). Importantly, the antibody reactivity can be blocked by pretreatment with the antigenic peptide, either 2.5 (lanes 3 and 4) or 0.25 mg/ml (lanes 5 and 6) but not with 0.025 mg/ml (lanes 7 and 8). b, antibody depletion (TFII-I dep.) leads to removal of TFII-I from a Jurkat nuclear extract but mock depletion (mock dep.) has no appreciable effect compared with undepleted extract (undep.). c, the immune precipitate (αi ppt) obtained from antibody depletion shows the presence of substantial amount of TFII-I, whereas the precipitate from mock depletion (pl ppt) has no significant amount of TFII-I.

3 C. D. Novina and A. L. Roy, submitted for publication.
Immunodepletion specifically leads to removal of TFII-I from the extract.

**Vβ Transcription Requires Binding of TFII-I to the Inr Element:** Oligonucleotide-mediated Block of TFII-I Activity—In order to correlate the DNA binding and transcriptional activities of TFII-I at the Vβ Inr element, transcription-coupled competitor challenge assays were done. First we demonstrated that a purified preparation of TFII-I specifically binds to the Vβ Inr element (Fig. 3a, lane 2). A wt Vβ Inr sequence containing oligonucleotide could compete for TFII-I binding (lane 3). A mutant oligonucleotide did not block transcription (lane 4). These oligonucleotides were then employed in transcriptional assays.

Consistent with the DNA binding analyses, the wild type Inr oligonucleotide competitively inhibited transcription from the Vβ promoter in nuclear extracts (Fig. 3b, lanes 3 and 8), whereas the mutant oligonucleotide failed to inhibit transcription (lane 4). Most importantly, the Inr oligonucleotide-mediated inhibition of transcription was restored upon the addition of TFII-I (lane 9). An oligonucleotide containing the wild type TATA box sequences from the AdML promoter blocked the Vβ transcription (lane 5); a mutant TATA oligonucleotide did not block transcription (lane 6).

TFII-D has been shown to be required for transcriptional activity of TATA-containing as well as TATA-less promoters (7, 9). Consistent with this notion, an oligonucleotide containing only a wild type (lane 5) but not a mutant TATA box (lane 6) competitively inhibited the Vβ transcriptional activity. It is important to note that TFII-D is necessary but not sufficient to direct Inr-dependent Vβ transcription (see below).

Inr Element-dependent Activity of TFII-I Is Heat-labile Temperature-mediated Block of TFII-I Activity—Heat treatment of nuclear extracts interferes with TFII-I activity leading to transcriptional inhibition of both TATA-containing and Inr-containing promoters (6). The inhibition of a TATA*Inr* promoter can be rescued by exogenous addition of TFII-D (6). However, inhibition of a TATA*Inr* promoter cannot be rescued by the addition of TFII-D (6), suggesting that these promoters require additional heat-sensitive component(s). Because TFII-I is required specifically for a TATA*Inr* promoter, we tested whether TFII-I is heat-sensitive. We heated treated nuclear extracts at various temperatures and for varying periods of time to monitor the heat sensitivity of TFII-I (data not shown). Our analyses demonstrated that the TFII-I binding activity (confirmed by an anti TFII-I antibody, Fig. 4a, compare lanes 1 and 2) in a nuclear extract was abrogated by heat treatment of the nuclear extract minimally at 42 °C for 6 min (lane 3). Similarly heat treatment at 42 °C for 15 min also abolished TFII-I binding (lane 4).

Next, we employed the heat-treated nuclear extracts in vitro transcriptional assays with the Vβ (Fig. 4b) and IgH (Fig. 4c) promoters. Heat treatment of a Jurkat nuclear extract at 42 °C for 6 min led to abrogation of the Vβ transcription (Fig. 4a, compare lanes 1 and 2). Surprisingly, the addition of TFII-I did not alleviate the transcriptional block (lane 3), suggesting that this heat treatment interfered with additional component(s). The addition of a partially purified TFII-D fraction in the absence of exogenous TFII-I did not rescue the transcriptional block (lane 4). However, the addition of both TFII-I and TFII-D simultaneously rescued completely the heat-induced block of Vβ (TATA*Inr*).

**Differential heat treatment of nuclear extracts discriminates between a TATAInr* and a TATA*Inr* promoters:** a, TFII-I activity is temperature-sensitive because heat treatment of nuclear extracts interferes with TFII-I binding. A predominant mobility shift was observed in HeLa nuclear extract (lane 1). The binding was due to TFII-I because the mobility shift was inhibited by an anti-TFII-I antibody (lane 2). Heat treatment of the nuclear extract either at 42 °C for 6 min (lane 3) or 42 °C for 15 min (lane 4) blocked TFII-I binding, b, heat treatment of nuclear extract at 42 °C for 6 min abolished Vβ transcription (compare lanes 1 and 2). Neither TFII-I (lane 3) nor TFII-D (lane 4) completely rescued the heat induced block of Vβ (TATA*Inr*).

**FIG. 4.** Thermal stability of transcription factor II-I (TFII-I) and transcription factor II-D (TFII-D). a, TFII-I activity is temperature-sensitive. TFII-I was competitively inhibited by a wt Inr oligonucleotide (lanes 1 and 2) or a mutant oligonucleotide (lanes 3 and 4). Neither an E-box containing oligonucleotide (lane 2) nor a mutant Inr oligonucleotide (lane 4) inhibited TFII-I transcription. The wt Inr oligonucleotide-containing block of transcription (lane 8) was restored upon the addition of the purified preparation of TFII-I (lane 9). An oligonucleotide containing the wild type TATA box sequences from the AdML promoter blocked the Vβ transcription (lane 5); a mutant TATA oligonucleotide did not block transcription (lane 6).

**FIG. 3.** Specificity of TFII-I binding to the Inr element is essential for TATAInr* transcription. a, a purified preparation of TFII-I (HeLa-derived) binds to the Vβ Inr element (lane 2). The binding was specific because it was competitively inhibited by a wt Vβ Inr element containing oligonucleotide (lane 3) but not by a mutant oligonucleotide (mut, lane 4). b, Vβ transcription by J urkat nuclear extract (lanes 1 and 7) was competitively inhibited by a wt Inr oligonucleotide (lanes 3 and 8). Neither an E-box containing oligonucleotide (lane 2) nor a mutant Inr oligonucleotide (lane 4) inhibited Vβ transcription. The wt Inr oligonucleotide-containing block of transcription (lane 8) was restored upon the addition of the purified preparation of TFII-I (lane 9). An oligonucleotide containing the wild type TATA box sequences from the AdML promoter blocked the Vβ transcription (lane 5); a mutant TATA oligonucleotide did not block transcription (lane 6).

**InrmediatedTranscription**

12079
known, the existence of such a component (activity) has been described before (6). Similar results were obtained when nuclear extracts were heat treated at 42 °C for 15 min (not shown). Furthermore, only TFIIID (and not TBP) was effective in these complementation assays (not shown).

TFII-I is an Inr element-dependent factor and therefore is not required for an Inr-less promoter. Accordingly, mild heat treatment (42 °C for 6 min) of nuclear extracts did not abolish the TATA "Inr" IgH promoter activity (Fig. 4c). In fact, we observed a reproducible increase in the IgH promoter activity upon mild heat treatment (compare lanes 1 and 2). Because TFIIID activity was not affected at 42 °C, the addition of TFIIID had no effect on IgH transcription at this temperature (lane 3). Similarly, heat treatment of nuclear extracts at 42 °C for 15 min had no negative effect on IgH transcription (the background was reduced under these conditions), and subsequently added TFIIID had no appreciable effect on transcription (lanes 4 and 5). Under similar conditions, nuclear extracts heat treated at 42 °C for 15 min failed to transcribe the Vβ promoter (data not shown). Taken together, our data demonstrate that transcription factor requirements between the TATA "Inr" and TATA "Inr" promoters are different. TATA "Inr" promoters require TFII-I, whereas the TATA "Inr" promoters do not.

DISCUSSION

The control region of typical eukaryotic messenger RNA coding genes is comprised of proximal (core) and distal (enhancer) promoter regions (1). The core promoter region consists predominantly of two elements: the TATA box and/or the Inr element, which can be present either alternately (TATA "Inr" or TATA "Inr") or in limited cases simultaneously (TATA "Inr") (26). To understand the various transcriptional strategies that exist in nature, it is important to elucidate why different genes have adopted different core promoter elements and how these elements mediate transcription.

Transcription initiation in eukaryotes is mediated by a set of general transcription factors that assemble at the core promoter to form the preinitiation complex (27-29). The core promoter structures are different for different genes. Consequently, the preinitiation complexes (containing general transcription initiation factors), which assemble at different core promoter elements (TATA or Inr) are different (10, 11). These experiments, however, employed a composite TATA "Inr" core promoter and were reconstituted with purified and/or recombinant proteins (10, 11). To distinguish between the mechanisms of transcription initiation mediated by TATA and Inr, we employed nuclear extracts to transcribe the naturally occurring TATA "Inr" (Vβ) and TATA "Inr" (IgH) promoters. Our analyses demonstrate that the mechanisms of promoter utilization and the requirement of transcription factors are distinct for the two classes of promoters.

We present multiple approaches that were undertaken to demonstrate differences in promoter utilization. First, we depleted various nuclear extracts for the transcription factor TFII-I, which is important for Inr element-containing promoters (10-12). Depletion of TFII-I by an anti-TFII-I antibody led to complete inhibition of transcription of a TATA "Inr" promoter, whereas TFII-I depletion did not have a negative effect on TATA "Inr" transcription. Furthermore, the addition of TFII-I relieved the antibody-mediated inhibition of the Vβ promoter, suggesting that the active component was indeed TFII-I. This conclusion is supported by the fact that the antibody predominantly recognizes TFII-I in nuclear extracts (as evidenced by Western blot analysis) and can be effectively blocked by the antigenic peptide derived from TFII-I (Fig. 2a). However, because the preparation of TFII-I used to reconstitute the transcriptional activity is partially pure, involvement of additional components cannot be ruled out completely.

Second, we demonstrate that an Inr element-containing oligonucleotide, which was competent in TFII-I binding, competitively inhibited transcription of a TATA "Inr" promoter. This observation suggests that interactions of TFII-I to the Inr element is necessary for TATA "Inr" transcription. Consistent with this suggestion, the Inr oligonucleotide-mediated inhibition of transcription was relieved by exogenous addition of TFII-I. Other factors (13-20) have been implicated in Inr element binding. However, under the conditions tested, TFII-I is the predominant factor present in various nuclear extracts that is responsible for Inr-dependent binding and transcriptional activities via the Vβ promoter. This is consistent with our preliminary data, which indicate that TFII-I is also required for the Vβ promoter function in vivo.

TFIIID is required for both TATA "Inr" and TATA "Inr" promoters (6, 7). However, it is questionable whether or not TATA binding activity of TFIIID is required for the TATA "Inr" promoters (6). Accordingly, it has been shown that TATA binding activity is required for some TATA "Inr" promoters but not for others ("true" TATA-less promoters) (6). The definition of a true TATA-less promoter is confusing and thus a TATA-less promoter should be defined by the lack of a consensus TATA sequence and not on the basis of mechanisms of TFIIID binding. The promoter employed here (Vβ) is a naturally occurring TATA-less promoter (lacking a consensus TATA box), a notion further supported by model building studies. However, Vβ transcription requires the TATA binding activity of TFIIID. Although we do not know the exact mechanism of TFIIID recruitment to the Vβ promoter, it is possible that the binding of TFIIID to the promoter may be mediated by TFII-I interactions because TFII-I interacts with the TATA binding subunit (TBP) of TFIIID (11).

Finally, we demonstrate that heat treatment of nuclear extracts affects TATA "Inr" and TATA "Inr" promoters differently. It has been shown that heat treatment of a nuclear extract, normally competent for transcription, rendered the extract inactive for transcription of both types of promoters (6). The transcriptional activity of the extract for a TATA "Inr" promoter could be restored upon exogenous addition of TFIIID (6). However, for a TATA "Inr" promoter, the addition of TFIIID was insufficient, suggesting that an additional heat-labile component...
component(s) was necessary for TATA−Inr+ promoters (6). Here we demonstrate that TFII-I is heat-labile and is required in addition to TFIID for TATA−Inr+ promoter function. Our analyses also suggest the existence of a third component that is required for TATA−Inr+ promoters. This component is present in a partially purified TFIID fraction and is heat-labile (Fig. 5). It is unclear at present whether this component is directly associated or merely copurifies with TFIID.

Our data reveal that different transcription factor activities can be targeted by heat treating nuclear extracts at different temperatures. Thus, although heat treatment of nuclear extracts at 42 °C for 6 min completely ablates TATA−Inr+ transcription, similar treatment does not ablate TATA−Inr− transcription. This mild heat treatment does not affect TFIID activity but destroys other activities (including TFII-I) that are required for TATA−Inr+ transcription. Therefore, differential heat treatment of nuclear extracts at different temperatures can be used as an assay to distinguish between TATA−Inr− and TATA−Inr+–dependent transcriptional activities.

In conclusion, we clearly demonstrate the Inr–dependent function of TFII-I via the TATA−Inr+ Vβ promoter. It is possible that TFII-I may be necessary for transcription of other TATA−Inr+ promoters as well (30). Finally, although it appears at present that different Inr–dependent factors may function through different promoters or under different conditions, it is likely that multiple Inr–dependent factors may work in concert for some TATA−Inr+ promoters.

Acknowledgments—We are grateful to Robert Roeder for providing all gifts of HeLa nuclear extract, Dennis Loh for providing the Vβ5.2 promoter, and Jeff Parvin for providing the IgH promoter construct. We are especially grateful to Ranjan Sen for advice and helpful suggestions for this manuscript. We also thank Danny Reinberg, Robert Roeder, Henry Wortis, and Monica Gaupp for critically reading the manuscript. Finally, we thank Danny Reinberg, Robert Roeder, Stephen Burley, David Baltimore, and Steve Buratowski for insightful discussions.