Direct recognition of initiator elements by a component of the transcription factor IID complex

Jörg Kaufmann and Stephen T. Smale

Howard Hughes Medical Institute, Molecular Biology Institute, and Department of Microbiology and Immunology, University of California, Los Angeles School of Medicine, Los Angeles, California 90024-1662 USA

A core promoter element called an initiator (Inr) overlaps the transcription start site of numerous mammalian protein-coding genes. In promoters that lack a TATA box, the Inr is functionally analogous to TATA, in that it is capable of directing basal transcription by RNA polymerase II and of determining the precise site of transcription initiation. In promoters that contain a TATA box, the Inr can greatly enhance promoter strength. Mammalian Inr consensus sequences have been defined through functional studies and sequence comparisons of the start site regions of protein-coding genes. Here, we show that, in a DNase I footprinting assay with synthetic promoters, the purified TATA-binding protein complex TFIID specifically contacted the Inr. The TFIID–Inr interaction relies on the precise nucleotides needed for Inr function. Detection of the interaction was dependent either on a TATA box or on Spl bound to upstream sites. Furthermore, recombinant TFIIB appeared to influence the TFIID–Inr interaction, whereas TFIIA stabilized the TFIID–TATA interaction. These results demonstrate that distinct components of TFIID interact with the TATA boxes and Inr elements of core promoters for RNA polymerase II.

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Accurate transcription initiation from mammalian protein-coding genes depends on specific recognition of core promoter elements. For promoters that contain a TATA box, this recognition event is carried out by TFIID, a multiprotein complex containing a TATA-binding subunit called TBP and several additional factors known as TAFs (Zawel and Reinberg 1992; Hernandez 1993). For promoters that lack a TATA box, the proteins that functionally recognize the core control elements have not been clearly established. One class of TATA-lacking promoters includes those that contain an Inr element (Weis and Reinberg 1992; Kollmar and Farnham 1993; Smale 1994). An Inr can be defined as a basal control element that overlaps a transcription start site and is capable of determining the location of the start site in a promoter that lacks a TATA box (Smale and Baltimore 1989). Furthermore, an Inr can enhance the strength of a promoter that contains a TATA box, if the Inr is located ∼25 bp downstream of TATA (Smale and Baltimore 1989; Smale et al. 1990).

Recently, through a functional analysis of 80 random and mutant Inr elements, we defined a loose consensus sequence for Inr activity in mammalian cells (Javahery et al. 1994). This consensus sequence, Py Py A+1N T/A Py Py, is similar to a start site consensus that had been defined solely through a sequence comparison of 500 genes transcribed by RNA polymerase II (Bucher 1990). Moreover, virtually every mammalian Inr that has been described matches this consensus (see Weis and Reinberg 1992; Kollmar and Farnham 1993; Smale 1994). Although a variety of proteins have been reported to bind to Inr elements in specific genes (Means and Farnham 1990; Roy et al. 1991; Seto et al. 1991; Du et al. 1993), binding of these proteins does not rely on the same nucleotides that are required for Inr activity (Kollmar and Farnham 1993; Javahery et al. 1994).

Two primary lines of evidence suggest that a component of the general transcription machinery, and specifically a component of the TFIID complex, may be responsible for Inr recognition. First, during in vitro transcription experiments, Inr activity typically has been observed in the presence of TFIID, but not in the presence of recombinant TBP (Smale et al. 1990; Conaway et al. 1991; Pugh and Tjian 1991; Wang and Van Dyke 1993; see Fig. 1A, below). Second, with some promoters that contain both TATA and Inr elements, DNase I footprinting experiments have revealed that TFIID contacts the DNA at the TATA box and also at sequences downstream of TATA (Sawadogo and Roeder 1985; Zhou et al. 1992; Purnell and Gilmour 1993). In two studies, mutations in an Inr element reduced the interaction between partially purified TFIID and the transcription start site.
region (Purnell and Gilmour 1993; Wang and Van Dyke 1993).

Despite the above evidence in support of a TFIID–Inr interaction, specific mutations in the functional Inr element of the adenovirus major late (AdML) promoter appear to have no effect on the binding of extensively purified TFIID (Chiang et al. 1993; J. Kaufmann and S.T. Smale, unpubl.). Instead, binding of purified TFIID to the AdML promoter appears to depend primarily on the TATA box and on sequences located upstream and/or downstream of the Inr (Chiang et al. 1993). This finding suggests that either (1) TFIID does not functionally interact with the Inr, or (2) in the AdML promoter, a specific interaction with the Inr is masked by the strong binding of TFIID to surrounding sequences.

To analyze in more detail the role of TFIID in Inr activity, we studied the binding of highly purified TFIID preparations to synthetic core promoters that contain a strong Inr but that lack the surrounding sequences found in the AdML promoter. Our results demonstrate that a component of TFIID specifically interacts with the Inr, with the interaction depending on the precise sequences required for Inr function.

Results

The purified TFIID complex is required for Inr function

To investigate the possibility of a direct interaction between TFIID and Inr elements, we wished to analyze the binding of the purified TFIID complex to a core promoter containing a TATA box and an Inr but lacking the surrounding sequences that apparently contact TFIID in the AdML promoter. To this end, we studied a synthetic promoter containing only the AdML TATA box fused to the strong Inr from the terminal transferase (TdT) gene (plasmid J1634; see plasmid IV in Smale et al. 1990). For all of our experiments, TFIID was isolated from HeLa cells containing a retroviral insert expressing an epitope-tagged TBP. Markers are shown in lane 2. The TAFs that are smaller than TBP (see Zhou et al. 1992) were not visible on the stained gel.

Efficient transcription from the synthetic J1634 promoter was dependent on both the Inr element and on the intact TFIID complex (Fig. 1A). In a crude nuclear extract, the J1634 promoter was considerably stronger than a promoter containing a single base pair mutation in the Inr (J3102, +3G; Fig. 1A, lanes 1,2). Transcription from both promoters was eliminated when the extract was depleted of TFIID by heat treatment (Nakajima et al. 1988) (lanes 3-8). Reactions were supplemented with 5 µl of human TBP expressed in E. coli or 5 µl of purified TFIID. Templates (300 ng) were either J1634 (TATA/Inr) or J3102 (lnr mutant, +3G, see Fig. 3). (B) Purified TFIID (30 µl) (lane 1) was visualized on an SDS–polyacrylamide gel by silver staining. TFIID was purified as described (Zhou et al. 1992) from HeLa cells expressing an epitope-tagged TBP. Markers are shown in lane 1. The TAFs that are smaller than TBP (see Zhou et al. 1992) were not visible on the stained gel.

Figure 1. The intact TFIID complex is required for Inr activity. [A] In vitro transcription reactions were performed with 50 µg of crude HeLa nuclear extract (lanes 1,2) or nuclear extract that had been depleted of TFIID activity by heat treatment [Nakajima et al. 1988, lanes 3–8]. Reactions were supplemented with 5 µl of human TBP expressed in E. coli or 5 µl of purified TFIID. Templates (300 ng) were either J1634 [TATA/Inr] or J3102 [lnr mutant, +3G, see Fig. 3]. (B) Purified TFIID (30 µl) (lane 1) was visualized on an SDS–polyacrylamide gel by silver staining. TFIID was purified as described (Zhou et al. 1992) from HeLa cells expressing an epitope-tagged TBP. Markers are shown in lane 2. The TAFs that are smaller than TBP (see Zhou et al. 1992) were not visible on the stained gel.

The purified TFIID complex is required for Inr function

To test for binding of TFIID to the strong TdT Inr, we
performed DNase I footprinting experiments with the J1634 promoter. With the purified TFIID, we detected binding to the TATA box and weak protection of sequences downstream of TATA [Fig. 2B]. Interestingly, a strong hypersensitive band was detected mapping to nucleotide +5 relative to the start site [Fig. 2B]. This band is at a location that borders the 3' end of the Inr. With recombinant TBP expressed in E. coli, strong protection of the TATA box was observed, but the hypersensitive site at +5 was absent [Fig. 2A]. Furthermore, the hypersensitive site was strongly reduced when we tested a promoter containing a mutant TATA box (with the sequence GATATC) that was reported previously to reduce TATA activity by ~100-fold [Zenzie-Gregory et al. 1993] [Fig. 2C, lanes 10,11, see also Fig. 7, lane 12, below]. A 2-bp mutation that abolished Inr activity (−1G, +1G; Javahery et al. 1994) also eliminated the hypersensitivity [Fig. 2C, lane 15]. Thus, the hypersensitive site, but not the weak protection, appears to depend on the Inr element. [It should be noted that, in a given panel, all of the DNase footprinting probes were prepared by PCR with the same 32P-labeled primer, allowing all probes to possess the same specific activity.]

Although the transcribed strand in Figure 2B primarily exhibited a hypersensitive site at the Inr, protection could be observed in DNase I footprinting experiments with a probe labeled on the nontranscribed strand. In Figure 2D, lane 18, weak protection was observed at the TATA box and at multiple locations downstream of TATA. Notably, two bands contained within the Inr were consistently protected relative to the bands both above and below the Inr. To achieve this result, it was necessary to concentrate the purified TFIID preparation threefold by centrifugation through a Centricon 30 concentrator [Amicon]. The protection was reduced when a probe containing a 2-bp mutation in the Inr was tested [Fig. 2D, lane 22]. Because this same mutation abolished the hypersensitive site observed on the transcribed strand [Fig. 2C, lane 15], the protection and the hypersensitive site most likely result from the same TFIID--Inr interaction. Taken together, the results in Figure 2 reveal an interaction between TFIID and the start site region that relies on sequence-specific contacts at both the −30 region and the Inr.

The TFIID--Inr interaction depends on the precise nucleotides needed for Inr function

The data presented above reveal a TFIID interaction at the Inr that may be required for Inr activity. An important criterion that must be met, however, is that the TFIID--Inr interaction and Inr activity must be depen-
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dent on the same nucleotides. To address this issue, we analyzed nine mutant Inrs that had been tested previously for activity in the context of a promoter lacking a TATA box (Javahery et al. 1994). These mutants (Fig. 3, bottom) were inserted into a vector containing the AdML TATA box, but no upstream activator. In vitro transcription analyses with these plasmids (Fig. 3, lanes 1–10) revealed Inr activities similar to those found in the context of the upstream Sp1 sites (Javahery et al. 1994), with only minor quantitative differences.

DNase I footprinting experiments with each of these mutant promoters revealed that the presence of the hypersensitive site correlates with Inr activity (Fig. 4). Mutants that eliminate activity (J3102, J1123, and J1116) did not exhibit hypersensitivity nor did random Inr elements that are largely inactive (J444 and J2549). (With these mutants, the weak TATA protection was also somewhat reduced, suggesting that one role of the TFIID–Inr interaction may be to stabilize binding of TBP to the TATA box.) In contrast, mutants with considerable Inr activity (J3521, J3121, and J2219) retained the hypersensitive site. [As noted by the question mark at the bottom of Figure 1-10) revealed Inr activities similar to those found in the context of the upstream Sp1 sites (Javahery et al. 1994), with only minor quantitative differences.

A gel mobility shift analysis was employed to confirm the correlation between Inr activity and TFIID binding. The gel shift experiments used agarose gel electrophoresis (see Materials and methods) and probes containing both the TATA and Inr elements. With the wild-type TdT Inr, a strong band was detected migrating at the expected location (Fig. 5, lane 6; Zhou et al. 1992). Mutation of the TATA box strongly reduced the TFIID–DNA complex [lane 3]. A 2-bp mutation at −1 and +1 (J1116, −1G, +1G) or a single base pair mutation at +3 (J3102, +3G) reduced the intensity of the complex by two- to threefold (Fig. 5, lanes 9,12). Similar results were found in three independent experiments. These two Inr mutations strongly reduced Inr activity (see Fig. 3). Conversely, the mutation at +3 that retained Inr activity (J3121, +3A) resulted in no significant effect on the TFIID–DNA complex [Fig. 5, lane 15]. This result suggests that the binding of TFIID to the Inr increases the affinity of TFIID for a TATA-containing promoter. The mutant analysis using both DNase I footprinting and mobility retardation assays implicates TFIID as the only factor that has been identified whose interaction with the Inr depends on the precise sequences required for Inr activity.

**Figure 3.** Functional activity of Inr mutants in the context of an upstream TATA box. In vitro transcription reactions were performed with 50 μg of HeLa nuclear extract and 300 ng of template DNA. Templates contained the AdML TATA box and either the TdT Inr [J1634, lane 1] or a mutant Inr [lanes 2–10]. Inr sequences are shown at the bottom, with the nucleotides underlined that differ from the TdT Inr. The relative activities of the promoters (the mean of two independent experiments; J1634 = 100%) are shown at the bottom of Fig. 4.

**Figure 4.** DNase I footprinting of Inr mutants in the context of the upstream Spl sites (Javahery et al. 1994; see Materials and methods). The relative activities of the promoters (the mean of two independent experiments; J1634 = 100%) are shown at the bottom of Fig. 4.

**Figure 5.** A gel mobility retardation assay was employed to confirm the correlation between Inr activity and TFIID binding. Two general transcription factors known to interact with TFIID are TFIIA and TFIIIB. In some assays, TFIIA stabilizes the binding of TBP to TATA boxes (Ranish et al. 1992; Zawel and Reinberg 1992). TFIIA has been shown to interact both with TBP and with *Drosophila* TAF40 (Buratowski et al. 1989; Zawel and Reinberg 1992; Goodrich et al. 1993). Moreover, TFIIA induced an interaction with the start site region of the AdML promoter when added to a binding reaction that contained TFIID (Buratowski et al. 1989; Maldonado et al. 1990; Moncollin et al. 1992). To analyze the effect of TFIIA and TFIIIB on the specific interaction between TFIID and the Inr element, we performed the experiments shown in Figure 6. For these experiments we began with a lower concentration of TFIID that when added alone is not sufficient to reveal interactions with the core promoter (Fig. 6, lanes 2,7,11). These conditions were found to be
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Figure 4. The TFIID–Inr interaction and Inr activity depend on the same sequences. DNase I footprinting experiments were performed with 5000 cpm of 32P-labeled probes (see Fig. 2) containing wild-type and mutant promoters. All probes were prepared with the same labeled primer, resulting in similar specific activities. Reactions were performed in the absence (odd-numbered lanes) or presence (even-numbered lanes) of 7.5 µl of purified TFIID. The location of the +5 hypersensitive site is indicated.

Figure 5. The TFIID–Inr interaction enhances TFIID binding in a mobility retardation assay. This assay was performed using agarose gel electrophoresis as described (see Materials and methods). Probes were derived from plasmid I (lanes 1–3, see Smale et al. 1990), J1634 (lanes 4–6), J116 (lanes 7–9), J3102 (lanes 10–12), and J3121 (lanes 13–15). Binding conditions were the same as for DNase I footprinting experiments and lacked lanes 1,2,4,5,7,8,10,11,13,14 or contained 7.5 µl of purified TFIID. Quantitation was performed by Phosphorlmager analysis (Molecular Dynamics). The results are representative of three independent experiments. The arrow indicates the specific protein–DNA complex.

Figure 6 (lanes 3, 4) shows that recombinant yeast TFIIA (Ranish et al. 1992) strongly induces binding of TFIID to the TATA box. However, the TFIIA did not induce the hypersensitive site at the Inr. Thus, increasing the affinity of TFIID for the TATA box does not appear to enhance the binding of TFIID to the Inr. (In Fig. 6, lanes 3–5, the TFIIA appears to result in nonspecific protection of the probe; this protection most likely results from contaminating proteins in the partially purified TFIIA preparation.) Interestingly, recombinant human TFIIB (Ha et al. 1991) influenced the interactions taking place near the Inr but had little influence on TFIID binding to the TATA box (Fig. 6, lanes 8, 9). As expected, TFIIB did not induce the hypersensitive site in the presence of recombinant TBP (data not shown). In Figure 6, TFIIB resulted in a modest hypersensitivity of the +5 band relative to that observed with TFIID alone (cf. lanes 7 and 9). However, the most striking effect of TFIIB was to induce protection of sequences downstream of the Inr (lane 9). Thus, although TFIIB may stabilize the binding of TFIID to the Inr and downstream sequences, an alternative explanation is that TFIIB itself may interact with sequences near the Inr in a TFIID-dependent manner.

With both TFIIA and TFIIB added to the binding reaction, clear interactions were detected at the TATA box and at the Inr (Fig. 6, lane 13). However, despite the efficient protection of the TATA box and the strong hypersensitive site at the Inr, the Inr remained only weakly protected on the transcribed strand. Analysis of the Inr mutants again revealed that the TFIID–Inr interaction correlates with the sequence requirements for activity. Most strikingly, the J3121 (+3A) mutant retained strong hypersensitivity, but only a weak interaction was de-
Figure 6. TFIIA and TFIIB differentially influence the interaction between TFIID and synthetic promoters. DNase I footprinting experiments were performed with the J1634 promoter (lanes 1–13) or the Inr mutants J3102 (lanes 14,15), J3521 (lanes 16,17), or J3121 (lanes 18,19). Reactions contained no protein (lanes 1,6,12,14,16,18), 2.5 µl of purified TFIID (lanes 2–4,7–9,11,13,15,17,19), 0.25 µl (lane 3) or 2.5 µl (lanes 4,5,13,15,17,19) of partially purified recombinant yeast TFIIA expressed in E. coli [Ranish et al. 1992], and 0.25 µl (lane 8) or 2.5 µl (lanes 9,10,13,15,17,19) of partially purified recombinant TFIIB [Ha et al. 1991]. The locations of the TATA box, Inr, and +5 hypersensitive site are indicated.

In the presence of Sp1, TFIID interacts with the Inr in the absence of a TATA box

The experiments described above demonstrate that a component of TFIID specifically interacts with the Inr in a promoter that contains a TATA box, but little evidence of the interaction was detected in the absence of a TATA box (see Fig. 2C, lanes 10,11). Figure 7, lane 12, shows the best interaction between TFIID and the isolated TdT Inr that we detected after numerous attempts. In this reaction, a weak hypersensitive site is visible at the +5 position and protection is visible downstream of the Inr. However, the ratio between the bands at +5 and +8 is not altered dramatically, as it is in the presence of a TATA box (cf. Fig. 7, lane 12, with Fig. 2B, lane 8, and Fig. 4, lane 2). In functional assays, transcription directed...
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by the isolated Inr is extremely weak but can be greatly enhanced by the binding of Sp1 to upstream sites [Smale and Baltimore 1989, Smale et al. 1990]. Therefore, to determine whether TFIID is capable of interacting with the Inr in the absence of a TATA box, we performed DNase I footprinting experiments in the presence of TFIID and purified Sp1.

With a synthetic promoter containing six Sp1-binding sites upstream of both TATA and Inr elements, TFIID bound to the TATA box and to sequences downstream of the Inr in the absence of Sp1 [Fig. 7, lane 17]. In this experiment [lanes 17 and 18], the hypersensitive site at +5 was very weak because a smaller amount of TFIID was added relative to the amounts used in Figures 2, 4, and 7 [lanes 1–15]. With both Sp1 and TFIID added to the reaction, the hypersensitive site was enhanced [lane 18]. Similar effects of activator proteins on the binding of TFIID to TATA-containing promoters have been reported [e.g., Horikoshi et al. 1988].

In another promoter, the TATA box was mutated to a sequence that reduces TATA activity by ~100-fold (TATAAA to GATATC; Zenzie-Gregory et al. 1993). With this promoter, no specific interactions were observed at the core promoter with TFIID in the absence of Sp1 [Fig. 7, lane 2]. However, in the presence of Sp1, a clear interaction was detected at the Inr and protection was observed downstream of the Inr [lane 3]. Sp1 did not induce the binding of TFIID to a promoter containing a point mutant at the Inr [lane 8] or to the promoter containing the isolated Inr [cf. lanes 12 and 13]. These results demonstrate that the specific interaction between TFIID and an Inr can occur in the absence of a functional TATA box.

Discussion

The data presented in this manuscript demonstrate that a component of the TFIID complex is involved in Inr activity. In vitro transcription experiments with promoters containing only a TATA box and an Inr element confirmed that Inr activity requires the intact TFIID complex. DNase I footprinting studies then revealed that purified TFIID directly contacts the Inr. The TFIID–Inr interaction depended on the same nucleotides that were found in an extensive mutant analysis to be required for Inr function. The relevance of the interaction was further supported by experiments suggesting that the general transcription factor TFIIB specifically influences the interaction at the Inr. Finally, we found that TFIID can contact the Inr in a promoter that lacks a functional TATA box, primarily if Sp1 is bound to upstream sites.

In our experiments the TFIID–Inr interaction was revealed most clearly by the presence of a strong hypersensitive site in the DNase I footprinting experiments with a probe labeled on the transcribed strand. The absence of strong protection of the Inr partially results from the fact that the TFIID purified by immunoaffinity chromatography is not highly concentrated. However, the same protein preparations are concentrated enough to give strong protection of the TATA box [Figs. 6 and 7] and of sequences downstream of the start site in the AdML promoter [data not shown]. Therefore, it appears that a low affinity of the TFIID–Inr interaction also contributes to the absence of protection. The loose consensus sequence for Inr activity [Javahery et al. 1994] previously suggested to us that the functional Inr-binding protein would interact with a relatively low affinity. If the functional protein bound independently and with high affinity, this protein would be capable of tightly interacting with the genome at an enormous number of sequences that match the consensus but are not associated with promoter regions. Another reason why we might expect a low-affinity interaction concerns the events that must occur in the vicinity of the Inr after the initial recognition event. Subsequent to this initial recognition, RNA polymerase II must become associated with the start site region and the DNA must unwind to allow the initiation of RNA synthesis. If a high-affinity interaction occurs, it would most likely block transcription initiation.

The possible stabilization of the TFIID–Inr interaction by TFIIB is consistent with results published previously [Buratowski et al. 1989, Maldonado et al. 1990, Moncolli et al. 1992]. In particular, one study with purified components revealed protection of the AdML start site region in a DNase I footprinting reaction containing both TFIIB and the TFIID complex [Maldonado et al. 1990]. In those experiments, it was not clear whether TFIID or TFIIB interacted directly with the start site or whether the interaction was dependent on an Inr. Recently, TFIIB has been shown to interact with Drosophila TAF40 [Goodrich et al. 1993], suggesting that this protein–protein interaction may enhance the binding of TFIID to the Inr and, furthermore, that Drosophila TAF40 and the human homolog of this TAF may be the Inr-binding subunit of TFIID.

In the absence of a TATA box, TFIID bound to the Inr primarily when Sp1 was bound to upstream sites. Sp1 interacts directly with Drosophila TAF110 [Hoey et al. 1993], but we have not established that Sp1 induces the TFIID–Inr interaction by directly binding to this TAF. For the purposes of this study, the use of Sp1 allowed us to confirm that TFIID is capable of interacting with an Inr in the absence of a TATA box.

It may be noteworthy that with the AdML promoter and with the synthetic promoter shown in Figure 7, TFIID appears to bind more tightly to the sequences surrounding the Inr than to the Inr itself [Fig. 7, lanes 15–19; Chiang et al. 1993; J. Kaufmann and S.T. Smale, unpubl.]. In contrast, the Inr enhances promoter strength much more strongly than do the surrounding sequences [data not shown]. This observation suggests that the strength of a core promoter does not depend simply on the overall affinity of the TFIID complex. Instead, the interaction between TFIID and the Inr is likely to play an important mechanistic role during the initiation reaction. Possibly, the TFIID–Inr interaction aids in the recruitment of TFIIB to the transcription start site, as suggested by the data in Figure 6. Alternatively, because the TFIID–Inr interaction occurs at the actual site of initia-
tion, it may play a direct role in template unwinding or in the recruitment and positioning of RNA polymerase II.

Although the human TFIID used in our experiments appears to bind very weakly to the Inr and more strongly to surrounding sequences in promoters like AdML (Chiang et al. 1993; J. Kaufmann and S.T. Smale, unpubl.), Drosophila TFIID appears to contact the consensus Inr in the Drosophila hsp70 promoter much more efficiently (Purnell and Gilmour 1993; D. Gilmour, pers. comm.). Moreover, the interaction of TFIID with the hsp70 Inr does not appear to be obscured by interactions between TFIID and surrounding sequences as in the AdML promoter (Purnell and Gilmour 1993). The stronger TFIID–Inr interaction observed in the Drosophila system may reflect the fact that the Drosophila Inr consensus sequence, although similar to the mammalian consensus, appears to be more restrictive (Cherbas and Cherbas 1993). This restrictive consensus sequence may be needed for TFIID to bind with higher affinity and with specificity within the Drosophila genome.

Several other proteins have been reported as specific Inr-binding proteins, including E2F(HIP1), TFII-I, USF, and YY1 (Means and Farnham 1990; Roy et al. 1991; Seto et al. 1991, Du et al. 1993). However, the binding of these proteins does not depend on the precise sequences required for Inr activity (Javahery et al. 1994), making it highly unlikely that the binding detected in our experiments results from these proteins contaminating our TFIID preparations. Instead, we suggest that these proteins act as auxiliary activators that stimulate transcription through gene-specific Inr elements. It remains to be determined whether the elements recognized by any of these other proteins can carry out Inr activity in a core promoter that lacks a TFIID recognition site at either the –30 region or the start site.

A previous study by Carcamo et al. (1991) demonstrated that purified RNA polymerase II activates transcription from CA dinucleotides, in the absence of TFIID and other general transcription factors. Our results do not exclude the possibility that in addition to recognition of the Inr by TFIID, RNA polymerase II may also prefer to begin RNA synthesis from CA dinucleotides. In support of this idea, a comparison of 500 promoters (Bucher 1990) found a strong preference for a C at the –1 position, yet we found no strong preference for a C (vs. a T) when we defined the functional Inr consensus sequence (Javahery et al. 1994). Possibly, the prevalence of a C at that position reflects a preference by RNA polymerase II during the actual initiation of RNA synthesis. Thus, the consensus Inr derived through sequence comparisons (Bucher 1990) may contain recognition sites for both TFIID and RNA polymerase II.

Materials and methods

Protein preparation

The epitope-tagged TFIID complex was isolated from LTRa3 cells by affinity purification as described (Zhou et al. 1992).

Preparation of the partially purified epitope-tagged TBP protein from E. coli was as described previously (Zhou et al. 1992). For the experiment in Figure 2D, the TFIID was concentrated threefold by centrifugation through a Centricon 30 concentrator (Amicon). Partially purified recombinant yeast TFIIA and human TFIIB expressed in E. coli were kindly provided by Dr. Michael Carey (University of California, Los Angeles). The human Spl was affinity purified (Promega).

In vitro transcription assays

In vitro transcription reactions were performed as described (Smale and Baltimore 1989) with 300 ng of template DNA and 50 μg of crude HeLa nuclear extract or nuclear extract that had been depleted of TFIID activity by heat treatment (Nakajima et al. 1988). RNA products were purified and analyzed by primer extension analysis using a 32P-labeled SP6 promoter primer (Promega, see Smale et al. 1990). CDNA products were visualized by electrophoresis on an 8% denaturing polyacrylamide gel, followed by autoradiography. Quantitation of signals was determined by PhosphorImager analysis (Molecular Dynamics).

DNA binding assays

DNA probes were prepared by PCR using a 5’ end-labeled SP6 primer (Promega), complementary to sequences downstream of the AdML promoter, and an unlabeled primer (5’–GGCCGATTCATTAAATGCAGG-3’), complementary to sequences upstream of the promoter. For Figure 2D, the opposite primer was labeled and PCR was performed with an unlabeled SP6 primer. Binding reactions for DNase I footprinting experiments were performed for 30 min at 30°C in a total volume of 15 μl, containing 5000 cpm of 32P-labeled probe, 10 mM MgCl2, 2 μg of BSA, 1 mM DTT, 10 mM HEPES (pH 7.9), 10% glycerol, 0.1 mM EDTA, and 50 mM KCl. Nonspecific competitor DNA was not added to the reactions. The labeled probes were then partially digested with DNase I, and cleaved DNA molecules were purified and analyzed by electrophoresis on an 8% denaturing polyacrylamide gel (see Lo et al. 1991).

Mobility retardation experiments were performed using agarose gel electrophoresis by the method described (Zhou et al. 1992), with a modified running buffer described by P. Lieberman and A. Berk (in prep.). Labeled probes for mobility retardation experiments were prepared by the same method as those used for DNase I footprinting experiments. Binding conditions were the same as above, except 10,000 cpm of each 32P-labeled probe was used. Signals were quantitated by PhosphorImager analysis.

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References

Bucher, P. 1990. Weight matrix descriptions of four eukaryotic RNA polymerase II promoter elements derived from 502 unrelated promoter sequences. J. Mol. Biol. 212: 563–578.
Buratowski, S., S. Hahn, L. Guarente, and P.A. Sharp. 1989. Five intermediate complexes in transcription initiation by RNA polymerase II. *Cell* 56:549–561.

Carcamo, J., L. Buckbinder, and D. Reinberg. 1991. The initiator directs the assembly of a TFIID-dependent transcription complex. *Proc. Natl. Acad. Sci.* 88:8052–8056.

Cherbas, L. and P. Cherbas. 1993. The arthropod initiator: The capsite consensus plays an important role in transcription. *Insect Biochem. Mol. Biol.* 23:81–90.

Chiang, C.-M., H. Ge, Z. Wang, A. Hoffmann, and R.G. Roeder. 1993. Unique TATA-binding protein containing complexes and cofactors involved in transcription by RNA polymerases II and III. *EMBO J.* 12:2749–2762.

Conaway, J.W., J.P. Hanley, K.P. Garrett, and R.C. Conaway. 1991. Transcription initiated by RNA polymerase II and transcription factors from liver: Structure and action of transcription factors $e$ and $\tau$. *J. Biol. Chem.* 266:7804–7811.

Du, H., A.L. Roy, and R.G. Roeder. 1993. Human transcription factor USF stimulates transcription through initiator elements of the HIV-1 and the Ad-ML promoters. *EMBO J.* 12:501–511.

Goodrich, J.A., T. Hoey, C.J. Thut, A. Admon, and R. Tjian. 1993. Drosophila TAF40 interacts with both a VP16 activation domain and the basal transcription factor TFIIB. *Cell* 75:519–530.

Ha, I., W.S. Lane, and D. Reinberg. 1991. Cloning of a human gene encoding the general transcription initiation factor IIB. *Nature* 352:689–695.

Hernandez, N. 1993. TBP, a universal eukaryotic transcription factor? *Genes & Dev.* 7:1291–1308.

Hoey, T., R.O. Weinzierl, G. Gill, J.L. Chen, B.D. Dynlacht, and R. Tjian. 1993. Molecular cloning and functional analysis of Drosophila TAF110 reveal properties expected of a coactivator. *Cell* 72:247–260.

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

Javahery, R., A. Khachi, K. Lo, B. Zennie-Gregory, and S.T. Smale. 1994. DNA sequence requirements for transcriptional initiator activity in mammalian cells. *Mol. Cell. Biol.* 14:in press.

Kollmar, R. and P.J. Farnham. 1993. Site-specific initiation of transcription by RNA polymerase II. *Proc. Soc. Exp. Biol. Med.* 203:127–139.

Lo, K., N.R. Landau, and S.T. Smale. 1991. LyF-1, a transcriptional regulator that interacts with a novel class of promoters for lymphocyte-specific genes. *Mol. Cell. Biol.* 11:5229–5243.

Maldonado, E., I. Ha, P. Cortes, L. Weis, and D. Reinberg. 1990. Factors involved in specific transcription by mammalian RNA polymerase II: Role of transcription factors IIA, IIB, and IIB during formation of a transcription-competent complex. *Mol. Cell. Biol.* 10:6335–6347.

Means, A.L. and P.J. Farnham. 1990. Transcription initiation from the dihydrofolate reductase promoter is positioned by HIP1 binding at the initiation site. *Mol. Cell. Biol.* 10:653–651.

Moncollin, V., L. Fischer, B. Cavallini, J.-M. Egly, and P. Chambron. 1992. Class II [B] general transcription factor (TFII B) that binds to the template-committed preinitiation complex is different from general transcription factor BTF3. *Proc. Natl. Acad. Sci.* 89:397–401.

Nakajima, N., M. Horikoshi, and R.G. Roeder. 1988. Factors involved in specific transcription by mammalian RNA polymerase II: Purification, genetic specificity, and TATA box-promoter interactions of TFIID. *Mol. Cell. Biol.* 8:4028–4040.

Pugh, B.F. and R. Tjian. 1991. Transcription from a TATA-less promoter requires a multisubunit TFIID complex. *Genes & Dev.* 5:1935–1945.

Purnell, B.A. and D.S. Gilmour. 1993. Contribution of sequences downstream of the TATA element to a protein-DNA complex containing the TATA-binding protein. *Mol. Cell. Biol.* 13:2593–2603.

Ranish, J.A., W.S. Lane, and S. Hahn. 1992. Isolation of two genes that encode subunits of the yeast transcription factor IIA. *Science* 255:1127–1129.

Roy, A.L., M. Meisterernst, P. Pognonec, and R.G. Roeder. 1991. Cooperative interaction of an initiator-binding transcription initiation factor and the helix-loop-helix activator USF. *Nature* 354:245–248.

Sawadogo, M. and R.G. Roeder. 1985. Interaction of a genespecific transcription factor with the adenovirus major late promoter of the TATA box region. *Cell* 43:165–175.

Seto, E., Y. Shi, and T. Shenk. 1991. YY1 is an initiator sequence-binding protein that directs and activates transcription in vitro. *Nature* 354:241–245.

Smale, S.T. 1994. Architecture of core promoters for eukaryotic protein-coding genes. In *Transcription: Mechanisms and regulation* (ed. R.C. Conaway and J.W. Conaway), pp. 63–81. Raven Press, New York.

Smale, S.T. and D. Baltimore. 1989. The "initiator" as a transcription control element. *Cell* 57:103–113.

Smale, S.T., M.C. Schmidt, A.J. Berk, and D. Baltimore. 1990. Transcriptional activation by Sp1 as directed through TATA or initiator: Specific requirement for mammalian transcription factor IID. *Proc. Natl. Acad. Sci.* 87:4509–4513.

Wang, J.C. and M.W. Van Dyke. 1993. Initiator sequences direct downstream promoter binding by human transcription factor IID. *Biochim. Biophys. Acta* 1216:73–80.

Weis, L. and D. Reinberg. 1992. Transcription by RNA polymerase II: Initiator-directed formation of transcription-competent complexes. *FASEB J.* 6:3300–3309.

Zawel, L. and D. Reinberg. 1992. Advances in RNA polymerase II transcription. *Curr. Opin. Cell Biol.* 4:488–495.

Zennie-Gregory, B., A. Khachi, I.P. Garraway, and S.T. Smale. 1993. Mechanism of initiator-mediated transcription: Evidence for a functional interaction between the TATA-binding protein and DNA in the absence of a specific recognition sequence. *Mol. Cell. Biol.* 13:3841–3849.

Zhou, Q., P.M. Lieberman, T.G. Boyer, and A.J. Berk. 1992. Holo-TFIID supports transcriptional stimulation by diverse activators and from a TATA-less promoter. *Genes & Dev.* 6:1964–1974.
Direct recognition of initiator elements by a component of the transcription factor IID complex.

J Kaufmann and S T Smale

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References

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