TFIIA Induces Conformational Changes in TFIID via Interactions with the Basic Repeat

DONG KUN LEE, JEFF DEJONG, SHIGERU HASHIMOTO,
MASAMI HORIKOSHI,[†] AND ROBERT G. ROEDER*

*Laboratory of Biochemistry and Molecular Biology, The Rockefeller University,
1230 York Avenue, New York, New York 10021*

Received 18 June 1992/Returned for modification 28 July 1992/Accepted 4 August 1992

DNA-binding studies with *Saccharomyces cerevisiae* TFIID point mutants indicated that TFIIA interacts with the basic repeat region of TFIID and induces structural changes. The latter was shown by the ability of TFIIA to compensate for TFIID point mutants defective for DNA binding. Interaction with TFIIA also rendered TFIID binding temperature independent, thus mimicking the effect of removing the nonconserved N terminus of TFIID. In addition, N-terminal truncation of the TFIID point mutants defective for DNA binding mimicked the ability of TFIIA to restore DNA binding of those mutants. Taken together, these results suggest that TFIIA enhances TFIID binding to DNA by eliminating an otherwise inhibitory effect of the nonconserved N terminus of TFIID. Furthermore, analyses of TFIID contact points on DNA and binding studies with TATA-containing oligonucleotide probes showed that TFIIA decreases the effect of sequences flanking the adenovirus major late TATA element on TFIID binding to DNA, suggesting a possible role of TFIIA in allowing TFIID to recognize a wider variety of promoters.

Transcription of most eukaryotic class II genes by RNA polymerase II involves common transcription factors that include TFIIA, -B, -D, -E, -F (for a review, see references 42 and 46), -H (5, 10, 12), and -G/J (7, 51). Components of the general transcription machinery are functionally conserved among eukaryotes, as evidenced by the ability of TFIIA and TFIID from *Saccharomyces cerevisiae* to substitute for the corresponding HeLa factors in vitro (2, 4, 14, 21). TFIID binding to the TATA element initiates the assembly of the other factors into a preinitiation complex (1, 34, 54, 55). Yeast TFIID (yTFIID) and the TATA-binding subunit of TFIID (designated TFIID τ or TBP) from higher eukaryotes contain a highly conserved 180-amino-acid C-terminal domain and divergent N-terminal regions (3, 8, 11, 15–18, 22, 25, 33, 35, 47, 52). Biochemical and genetic studies have shown that the conserved C-terminal domain of TFIID τ contains all of the essential regions for DNA binding, transcription initiation, and species specificity (6, 13, 23, 36, 40, 56, 57). TFIID shows unusual binding properties compared with those of the other sequence-specific DNA binding proteins: e.g., a temperature dependency and slow on and off rates (20, 28, 48).

The binding of TFIID to DNA can be stimulated by TFIIA, apparently by direct interactions of TFIIA with TFIID (1, 30). The ability of TFIIA to interact directly with TFIID has allowed efficient purification of this protein from both HeLa cells (7, 7a, 53) and *S. cerevisiae* (38). TFIIA has been shown to function at an early step in the formation of a preinitiation complex (10, 41). In contrast to the absolute requirement for the other transcription factors, a consistent requirement for TFIIA in the initiation of transcription has not been observed (for a review, see reference 42). This may reflect the nature of the TFIID-employed cross-contamination of factors, the presence of alternative factors, or the

particular conditions of transcription (1, 7, 31, 32, 41–45, 51, 55). For example, the transcription factor TFIIG initially was identified as a factor which substitutes for TFIIA in basal transcription and which further stimulates TFIIA-saturated transcription (51). The initiator-binding transcription factor TFII-I (43) has been shown to substitute for TFIIA in transcription of the adenovirus major late (AdML) core promoter, suggesting alternative pathways for the formation of preinitiation complexes, possibly with different rates of formation and/or complex stabilities (for a review, see reference 42; 42a). The function(s) of TFIIA in preinitiation complex formation and transcription initiation has yet to be elucidated.

Interactions of negative cofactors which bind competitively with TFIIA to TFIID on DNA have also been reported elsewhere (31, 32). These negative cofactors dissociate TFIIA or TFIIB from the intermediary preinitiation complexes to produce nonproductive preinitiation complexes (negative cofactor-TFIID-DNA complexes) that result in repressed promoter states (for a review, see reference 42). Preincubation of promoters with TFIIA and native TFIID was shown to increase basal levels of transcription with corresponding decreases in promoter stimulation by activators, suggesting the involvement of TFIIA in the regulation of transcription initiation (32).

We report here that TFIIA interacts with the basic repeat of TFIID and that TFIIA-TFIID complexes exhibit DNA binding properties similar to those observed with N-terminal truncated TFIID. Taken together, these data suggest a hypothetical model for the structure of the TFIIA-TFIID complex and possible functions of TFIIA in the regulation of transcription.

MATERIALS AND METHODS

Preparation of TFIIA. Human TFIIA (hTFIIA) was purified through phosphocellulose, Q-Sepharose, hydroxylapa-

* Corresponding author.

[†] Present address: Institute of Applied Microbiology, University of Tokyo, Bun Kyo-Ku, Tokyo 113, Japan.

tite, mono Q column, hydroxylapatite high-performance liquid chromatography (HPLC), and DEAE-5PW HPLC (7a).

yTFIIA was purified from nuclear extract through DEAE-Sepharose and heparin-Sepharose (38). Fractions containing yTFIIA were applied to a hydroxylapatite column equilibrated with 20 mM Tris-OAc (pH 7.8), 20% glycerol, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM EDTA, 5 mM dithiothreitol (buffer A) containing 0.3 M KOAc, and 10 mM potassium phosphate buffer (pH 8.0). The column was washed first with 3 column volumes of buffer A containing 0.1 M potassium phosphate buffer and then with 3 column volumes of buffer A containing 0.15 M potassium phosphate buffer. Fractions containing TFIIA activity were eluted with buffer A containing 0.3 M potassium phosphate. These fractions were dialyzed and applied to a DEAE-5PW HPLC column equilibrated with 20 mM Tris-OAc (pH 7.8), 10% glycerol, 0.5 mM EDTA, 1 μ g of leupeptin per ml, and 5 mM dithiothreitol (buffer B) containing 0.1 M KOAc. Protein was eluted with a linear gradient from 0.1 to 0.7 M KOAc.

DNA-binding assays. Electrophoretic mobility shift assays were performed as described before (21), except that 5 mM dithiothreitol was substituted for 2-mercaptoethanol. For the analysis of the temperature sensitivities of protein-DNA complexes, DNA and proteins were mixed on ice. The reaction mixtures were divided, with one portion incubated at 30°C and the other at 4°C. Complexes formed in the reaction mixtures incubated at 30 and 4°C were resolved by native gel electrophoresis at room temperature and 4°C, respectively.

N-terminal truncation of yTFIID mutants. yTFIID point mutants were overexpressed in bacteria and purified as described before (19). TFIID was proteolyzed as described before (28). Briefly, sequencing-grade trypsin was incubated with TFIID (weight ratio, 1:20) in a solution containing 20 mM Tris-Cl (pH 7.5), 0.2 M NaCl, 20% glycerol, and 5 mM dithiothreitol at 30°C for 20 min. Trypsin was inactivated by the addition of a molar equivalent of soybean trypsin inhibitor. For the blank samples, TFIID was incubated with inactivated trypsin which was formed by premixing trypsin and trypsin inhibitor.

RESULTS

TFIIA interacts with the basic repeat of TFIID. TFIIA has been thought to enhance the formation of a TFIID-DNA complex through direct interaction with TFIID, although the mechanisms for such an enhancement are not known (1, 30). In order to identify the exact regions of TFIID which interact with TFIIA, point mutants of yTFIID were analyzed. We chose K→L point mutants, a subset of the mutants studied by Yamamoto et al. (56), because some of them do not bind independently to DNA but nonetheless support basal transcription in the presence of the other transcription factors. The capacity of TFIIA to interact with TFIID was analyzed by a gel shift assay in which TFIIA can effect a supershift of the TFIID-DNA complex. This analysis employed hTFIIA that had been highly purified through five chromatographic steps (7a), including an HPLC hydroxylapatite step to remove the TFIID activity reported by Cortes et al. (7). Point mutants of yTFIID were expressed in bacteria and purified as described elsewhere (19). As shown by Yamamoto et al. (56), mutant proteins with changes in residues conserved in each of the direct repeats (K110, K120, K127, K201, K211, and K218) failed to produce TFIID-DNA complexes (Fig. 1, upper panel, lanes 4 to 6 and 14 to 16). In contrast, mutant

FIG. 1. TFIIA interacts with the basic repeat of TFIID and induces structural changes in TFIID. Wild-type yTFIID and derived point mutants were overexpressed and purified. yTFIID (with or without hTFIIA) was incubated with the labeled 140-bp AdML promoter fragment (−86 to +47), and bound probes were separated on 5% native gels. (Top) Mobility shift analysis with yTFIID point mutants. Direct repeats are denoted by arrows. Positions of mutations are designated by numbers. (Bottom) Mobility shift analysis with yTFIID point mutants and hTFIIA. wt and noD, wild-type yTFIID and no addition of yTFIID, respectively.

proteins with K→L changes either in residues not conserved between the direct repeats (K83, K97, K156, K167, K199, and K239) or in residues in the basic repeat region between the direct repeats (K133, K138, K145, and K151) did generate TFIID-DNA complexes (Fig. 1, upper panel). These complexes were similar to the wild-type TFIID-DNA complex with respect to other DNA-binding characteristics, including TFIID-dependent DNA bending and specific base contacts in DNA (data not shown). The DNA-binding properties observed here with purified recombinant proteins were the same as those observed with in vitro-translated proteins (56).

The effects of hTFIIA on the DNA-binding activities of yTFIID are indicated in Fig. 1 (lower panel). The association of hTFIIA regenerated the DNA-binding capacities of five direct-repeat mutants (K110L, K120L, K127L, K201L, and K218L) which lacked intrinsic DNA-binding activities, suggesting the possibility that conformational changes in TFIID are induced by hTFIIA (Fig. 1, lower panel, lanes 4 to 6, 14, and 16). The same results were obtained with yTFIIA partially purified as described in Materials and Methods (data not shown). Interestingly, two of the basic repeat region mutants (K138L and K145L) which showed normal levels of TFIID-DNA complexes nonetheless failed to generate hTFIIA-yTFIID-DNA complexes (Fig. 1, lanes 8 and 9). hTFIIA alone did not give any detectable DNA-protein complexes under the conditions used (Fig. 1, lane 18). These results argue that the basic repeat of TFIID is at least in part important for protein-protein interactions with TFIIA, which appears to induce structural changes in TFIID. Figure 2 shows a schematic summary of the analyses with the K→L point mutations in the DNA-binding and yTFIID-hTFIIA interaction assays.

TFIIA decreases the effect of sequences flanking the TATA element on TFIID binding to promoters. To analyze how TFIIA enhances TFIID binding to DNA, we employed guanine-adenine methylation interference assays to determine whether TFIIA can change the contact points between TFIID and DNA. Since TFIIA regenerates DNA-binding activities of TFIID mutants K110L, K120L, K127L, K201L,

FIG. 2. A schematic of the results with yTFIID point mutants and hTFIIA. The direct repeats and basic repeat regions are denoted by solid and broken arrows, respectively. Positions of mutations are indicated by numbers.

and K218L (Fig. 1, lower panel; summarized in Fig. 2), contact points between DNA and protein were compared for TFIIA-TFIID-DNA complexes formed with wild-type versus mutant TFIIDs.

End-labeled DNA fragments were partially modified with dimethyl sulfate and incubated with yTFIID and hTFIIA. DNA-protein complexes produced with yTFIID, yTFIID-hTFIIA, yTFIID (K110L)-hTFIIA, and yTFIID (K201L)-hTFIIA were isolated. Purified DNAs were treated to cleave the phosphodiester backbone at the modified base positions, and the cleaved products were analyzed on DNA sequencing gels (26). To identify the positions of the DNA sequences at which methylation interferes with formation of the DNA-protein complex, cleaved products of the bound probe were compared with those of input DNA, i.e., modified DNA itself (Fig. 3). As expected from earlier studies (26, 49), methylation of any conserved adenine residue in the TATA element interfered with binding of yTFIID alone. However, the same interference pattern was observed for complexes formed with TFIIA and TFIID or with TFIIA and two mutant yTFIIDs (K110L or K201L) dependent on TFIIA for binding (Fig. 3). This indicates that hTFIIA does not alter the interaction of yTFIID with the minor groove of the TATA element and that the hTFIIA-regenerated DNA-binding activities of the mutant yTFIIDs (K110L or K201L)

retained their sequence specificities. However, whereas methylation of the guanine residue at position -23 in the nontranscribed strand interfered with DNA binding by yTFIID alone, it did not interfere with binding of TFIID in association with hTFIIA (Fig. 3b). Thus, the association of hTFIIA appears to decrease the influence of sequences flanking the TATA element on yTFIID binding. This result suggests that hTFIIA association might relax the yTFIID-binding specificity and facilitate stable interaction with a wider variety of promoters.

This possibility was confirmed by binding studies with synthetic oligonucleotides. A guanine-to-adenine change at position -23 (3' GC cluster) in the nontranscribed strand decreased yTFIID binding to DNA by threefold (Fig. 4, lane 4 versus lane 7). In contrast, a guanine-to-adenine change at position -34 (5' GC cluster) enhanced binding of yTFIID by 2.5-fold, consistent with previous quantitative dimethyl sulfate methylation interference assays (26). However, in the presence of either yTFIIA or hTFIIA, the levels of protein-

FIG. 4. Binding of TFIID to DNA requires DNA sequences flanking the TATA element, while the binding of the TFIIA-TFIID complex does not. Oligonucleotides (-50 to +15 of the AdML promoter) were synthesized to change one nucleotide in the GC cluster flanking the TATA element. The labeled oligonucleotides were incubated with yTFIID alone or together with yTFIIA or hTFIIA. DNA-protein complexes were resolved on native gel electrophoresis. The types of oligonucleotide used are designated at the top of the figure. The mutations in the 5' and 3' GC clusters involved guanine-to-adenine changes at positions -34 and -23, respectively, in the nontranscribed strand.

FIG. 5. Effect of TFIIA on the temperature sensitivity of yTFIID binding to DNA. A labeled 140-bp DNA fragment containing the AdML promoter was incubated with yTFIID alone or together with TFIIA as indicated at the tops of the panels. The amounts of TFIIA were adjusted to effect a 50% shift of the yTFIID-DNA complex. DNA and proteins were mixed on ice and divided into two. One portion (a) was incubated at 30°C and resolved on a native gel at room temperature, while the other portion (b) was incubated at 4°C and resolved on a native gel at 4°C.

DNA complexes formed with the two mutated oligonucleotides were virtually the same as those formed with the wild-type oligonucleotide (1.2-fold increase for the 5' mutation and 0.9-fold for the 3' mutation). The result in Fig. 4 clearly shows that the formation of a yTFIID-DNA complex was greatly affected by the DNA sequences flanking the TATA element, whereas the formation of a TFIID-TFIIA-DNA complex was not.

These methylation interference and mutated-oligonucleotide binding assays indicate that the stimulatory effect of TFIIA on TFIID binding to DNA may be through a mechanism that does not involve extension of specific physical contacts between TFIID and DNA.

TFIID-TFIIA complexes are not temperature dependent for the interaction with DNA. Although TFIIA appears to enhance the formation of TFIID-DNA complexes, earlier studies of association and dissociation constants for yTFIID on the AdML promoter showed almost no changes in the presence or absence of partially purified yTFIIA (14). An alternative explanation for the enhanced binding of TFIID to DNA is that TFIIA may change the relative amounts of inactive and active (DNA binding) forms of TFIID in a fixed population. Deletion of the nonconserved N-terminal domain of yTFIID was also shown to enhance formation of the TFIID-DNA complex (23, 28). Therefore, it seemed possible that the stimulatory effect of TFIIA on TFIID binding to DNA may be related in some way to the effect observed upon removal of the N-terminal domain of yTFIID.

Evidence that TFIID binding to DNA might result from conformational changes in TFIID has been provided by studies of the temperature dependence of the binding reaction. N-terminal truncated TFIID, but not intact yTFIID, can bind to the TATA element at 0°C, suggesting a temperature-dependent conformational change in TFIID that involves the N-terminal domain (28). The possibility of a similar TFIIA-induced conformational change in TFIID was examined by assessing the temperature sensitivity of TFIIA-TFIID complex binding to DNA. Figure 5 compares the DNA-binding properties of intact and N-terminal truncated yTFIID in the presence or absence of TFIIA at 30 versus 4°C. Consistent with previous results (28), both intact and N-terminal truncated yTFIID interacted with DNA at 30°C (Fig. 5a, lanes 1 and 4), whereas only the N-terminal truncated TFIID showed a significant interaction at 4°C (Fig. 5b, lanes 1 and 4). However, the presence of yTFIIA markedly enhanced the binding of intact yTFIID at low

temperature, generating a yTFIIA-yTFIID-DNA complex (Fig. 5b, lane 2 versus lane 1). This contrasts with the lack of a significant effect of yTFIIA on the binding of N-terminal truncated yTFIID, which shows significant independent binding to DNA at low temperature (Fig. 5b, lane 4 versus lane 5). Interestingly, whereas hTFIIA interacted with the N-terminal truncated yTFIID as efficiently as yTFIIA at low temperature (Fig. 5b, lane 6 versus lane 5), hTFIIA apparently failed to interact efficiently with intact yTFIID under the same conditions (Fig. 5b, lane 3).

The effect of hTFIIA versus yTFIIA on the binding of hTFIID τ and yTFIID at 30 versus 4°C is shown in Fig. 6. Both hTFIID τ and yTFIID showed markedly reduced binding to DNA at low temperature relative to the efficient binding observed at 30°C (Fig. 6, lane 7 versus lane 1 and lane 10 versus lane 4). At low temperatures, hTFIIA and yTFIIA markedly enhanced binding of hTFIID τ and yTFIID, respectively (Fig. 6, lane 8 versus lane 7 and lane 12 versus lane 10), whereas heterologous combinations of TFIIA and TFIID showed much less cooperativity under the same conditions (Fig. 6, lane 9 versus lane 7 and lane 11 versus lane 10). The homologous combinations of factors at low temperature generated levels of TFIIA-TFIID-DNA com-

FIG. 6. Homologous but not heterologous TFIIA-TFIID complexes show temperature-independent DNA binding. DNA and proteins were mixed on ice, and DNA-protein complexes were incubated and resolved as described in the legend to Fig. 5. Incubation temperatures are designated at the top of figure.

FIG. 7. N-terminal truncations of TFIID point mutants defective in DNA binding mimic the effect of TFIIA. The DNA-protein complexes obtained with wild-type and N-terminal truncated yTFIID are designated. The numbers above the lanes give the positions of the point mutations. yTFIID mutants were digested with trypsin as described in Materials and Methods and derived products were verified by SDS-polyacrylamide gel electrophoresis. For the control (inactivated trypsin), trypsin was mixed with soybean trypsin inhibitor prior to addition of yTFIID.

plexes comparable to those observed at 30°C (Fig. 6, lane 12 versus lane 6 and lane 8 versus lane 2).

These studies of both yTFIID and hTFIID suggest that the N-terminal domain may hold the core domain mainly in a form inactive for DNA binding. As suggested before (28), it appears that TFIID may contain at least two distinct populations, one that is inactive for DNA binding and normally present in larger relative amounts and another that has undergone a transition to an actively binding species and is usually present in lower relative amounts. The results in Fig. 5 and 6 suggest the hypothesis that the position of the N-terminal domain in TFIID may be changed either by thermal energy or by TFIIA, but probably more efficiently by TFIIA, to render the core domain of TFIID competent for DNA binding.

Truncation of the nonconserved N-terminal domain regenerates the DNA-binding activities of several mutants. The possibility of a common mechanism for enhancement of TFIID binding to DNA by TFIIA and the N-terminal truncation was analyzed further. The previously described mutant proteins which lack intrinsic DNA-binding activities were subjected to N-terminal truncation in order to determine whether DNA-binding activities were regenerated in a fashion which paralleled the effects of TFIIA interactions with the same mutants. Positive results would indicate similarities in structural transitions induced in these mutants by either TFIIA or the N-terminal truncation.

Results of band shift assays with trypsin-treated yTFIID point mutants K110L, K120L, K127L, K201L, K211L, and K218L are presented in Fig. 7. As shown before, trypsin removed an approximately 6-kDa fragment from both wild-type and mutant TFIIDs (data not shown). Deletion of the N-terminal domain enhanced DNA-binding activity of wild-type yTFIID about fivefold (Fig. 7, lanes 1 and 15). Interestingly, deletions of the N-terminal domain regenerated significant DNA-binding activities for mutants K110L, K120L, K127L, K201L, and K218L but not for mutant K211L. The latter result with K211L is consistent with the observed failure of TFIIA to regenerate binding ability with the same mutant (Fig. 1). Since this mutant supports basal transcription in the presence of the other transcription factors (56), the inability to regenerate DNA-binding activity by TFIIA is an intrinsic property of this mutant. Moreover, as revealed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, it does not result from aberrant trypsin digestion relative to that observed for wild-type TFIID or for the other point mutants (data not shown). Thus, the effect of TFIIA on regeneration of the DNA-binding activities of mutants K110L, K120L, K127L, K201L, and K218L is mimicked by the N-terminal truncations of these mutants.

Altogether, these results suggest that TFIIA, by interacting with the basic repeat region of TFIID, may overcome an inhibitory effect of the N-terminal domain.

DISCUSSION

TFIIA interactions with the basic repeat of TFIID. Although supposedly not essential for basal transcription mediated by yTFIID or hTFIID τ under optimal in vitro conditions, TFIIA can enhance the binding of these forms of TFIID and appears to be essential for both basal and activator-dependent transcription in the presence of native hTFIID (for a review, see reference 42) and yTFIIA is required for viability in *S. cerevisiae* (39). To better appreciate the role of TFIIA in the regulation of transcription initiation, we have employed yTFIID point mutants and highly purified hTFIIA to identify regions of TFIID important for interactions with TFIIA. The stability of TFIIA-TFIID-DNA supershifted complexes in native gel electrophoresis allowed a convenient screening for yTFIID point mutants deficient in the ability to interact with hTFIIA. As summarized in Fig. 2, mutants (K138L and K145L) with changes of lysine residues in the basic repeat region of yTFIID were unable to form hTFIIA-yTFIID-DNA complexes. Since these lysine residues are conserved between species (for a review, see reference 18), they are likely to play an important role in the interaction between TFIIA and TFIID from HeLa cells.

The basic lysine repeat region of TFIID was proposed as a potential target for acidic upstream activators (22) on the basis of its potential for forming an amphipathic helix with a high basic charge and in light of previous suggestions of activation mechanisms involving direct interactions between upstream activators and TFIID (20, 37). Indeed, the prototypic acidic upstream activator VP16 was shown to interact directly with both yeast and human TFIID (50), although it also was demonstrated to interact more strongly with the basic residue-rich TFIIB (29). Recently, the activator E1A was demonstrated to interact with the basic repeat region of hTFIID τ (24, 27). These reports, together with the present results, support the hypothesis that there may be a functional interplay between TFIIA and transcriptional activators, as was also proposed from mechanistic studies of activators and cofactors in reconstituted systems (32). Negative cofactors important for physiological levels of induction also have been demonstrated to compete with TFIIA for binding to TFIID (31, 32). However, it is not yet clear whether negative cofactors could bind competitively with TFIIA to the basic repeat region of TFIID or whether putative altered TFIID structures induced by TFIIA (dis-

FIG. 8. Model for the TFIID-TFIIA complex. The schematic structure of TFIID is based on that described by Yamamoto et al. (56). The basic repeat region is shown as a dark line. The N-terminal domain, direct repeats, and C-terminal domain are indicated by open, striped, and stippled areas, respectively. Oval and rectangular shapes are used to distinguish active (DNA binding) and inactive forms of the core domain. Different shadings in the direct repeat and C-terminal domains represent the putative differences in structural motifs within the core domain between TFIIA-associated TFIID and TFIID alone.

cussed below) might prevent association of negative cofactors with TFIID. Identification of TFIID domains required for negative-cofactor interactions will clarify this point.

TFIIA-induced structural changes in TFIID. Two different lines of evidence support the idea that TFIIA induces structural changes in TFIID. First, TFIIA association regenerated the DNA-binding activities of yTFIID mutants (K110L, K120L, K127L, K201L, and K218L) which had little or no DNA-binding activities by themselves (Fig. 1). The TFIIA-induced regeneration of the DNA-binding activities could explain the ability of these mutants to mediate basal-level transcription (56). Second, methylation interference studies showed that yTFIID contacts in the 3' flanking region of the TATA element are lost in the presence of TFIIA (Fig. 3). This alteration of contact points between TFIID and DNA by TFIIA suggests a structural alteration in the DNA-binding motifs within TFIID.

Further support for the relevance of TFIIA-induced structural changes in TFIID to the TFIIA-stimulated binding of TFIID to DNA was provided by the demonstration that TFIIA effected binding of TFIID at low temperature, in a fashion which mimicked the effect of an N-terminal truncation of TFIID (Fig. 5 and 6). This indicates that TFIIA may eliminate an inhibitory effect of the N-terminal domain, probably more efficiently than thermal energy itself. The suggestion from these studies that TFIIA and the N-terminal truncation might enhance TFIID binding to DNA by similar mechanisms received further support from the demonstra-

tion that DNA-binding activities of a specific subset of TFIID point mutants were restored by the N-terminal truncation as well as by TFIIA (Fig. 7). In summary, these results suggest the working model shown in Fig. 8 for the stimulation of TFIID binding to DNA by TFIIA interactions. Since an analysis of contact points between TFIID and DNA indicated changes resulting from TFIIA association, the structure of the TFIIA-independent DNA-binding form of TFIID appears not to be precisely identical to that of the TFIIA-associated form.

Possible functions of TFIIA in the regulation of transcription initiation. Even though TFIIA can be a component of the preinitiation complex, an absolute requirement in transcription initiation has not been rigorously demonstrated (for a review, see reference 42). Such efforts have been impeded so far by the lack of completely purified and/or cloned factors and are further complicated by variable requirements for TFIIA in the presence of recombinant TATA-binding subunit versus native TFIID. However, preincubation of templates with TFIIA and native TFIID was found to raise basal-level transcription and to reduce the stimulatory effects of the upstream activator upstream stimulating factor (USF) (32), suggesting that TFIIA may have an intermediary role in the regulation of transcription initiation. TFIIA has been reported to release an inhibitory factor in the partially fractionated native TFIID (7, 32) but to have no effect on transcription of the AdML promoter with the recombinant TATA-binding subunit (7). However, the effect of TFIIA on transcription with the TATA-binding subunit may be dependent on experimental variables such as the concentration of TFIID and/or template, since TFIIA may increase the proportion of the active binding form of TFIID and/or the DNA-binding affinity of TFIID. In addition, TFIIA may regulate transcription initiation in other ways. First, as suggested by the present study (Fig. 3 and 4), it might decrease the effect of flanking sequences on TFIID binding to the TATA element, thereby allowing TFIID to interact with a wider range of promoters. Second, given the present studies on mutations affecting TFIIA binding, it is likely that TFIIA and at least some upstream activators can compete with each other for binding to the basic repeat region of TFIID (or possibly to the basic rich regions of TFIIB).

ACKNOWLEDGMENTS

We thank J. D. Johnson and members of the Laboratory of Biochemistry and Molecular Biology, especially Ernest Martinez, and Jong-Bok Yoon for valuable discussions; and Richard Bernstein, Stephen Burley, Ernest Martinez, Christopher Min, and Camilo Parada for critical comments on the manuscript.

Deoxyoligonucleotides were synthesized by the Protein Sequence Facility of the Rockefeller University.

J.D. is supported by an American Cancer Society Fellowship, and M.H. was an Alexandrine and Alexander L. Sinsheimer Scholar. This work was supported by National Institutes of Health grants to R.G.R. (CA42567 and AI27397) and M.H. (GM45258) and by funds from the Pew Charitable Trusts to the Rockefeller University.

REFERENCES

1. 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.
2. Buratowski, S., S. Hahn, P. A. Sharp, and L. Guarente. 1988. Function of a yeast TATA element-binding protein in a mammalian transcription system. *Nature (London)* 334:37-42.
3. Cavallini, B., I. Faus, H. Mattes, J. M. Chipoulet, B. Winsor,

- J. M. Egly, and P. Chambon. 1989. Cloning of the gene encoding the yeast protein BTF1Y, which can substitute for the human TATA box-binding factor. *Proc. Natl. Acad. Sci. USA* **86**:9803-9807.
4. Cavallini, B., J. Huet, J.-L. Plassat, A. Sentenac, J. M. Egly, and P. Chambon. 1988. A yeast activity can substitute for the HeLa cell TATA box factor. *Nature (London)* **334**:77-80.
5. Conaway, R. C., and J. W. Conaway. 1989. An RNA polymerase II transcription factor has an associated DNA-dependent ATPase (dATPase) activity strongly stimulated by the TATA region of promoters. *Proc. Natl. Acad. Sci. USA* **86**:7356-7360.
6. Cormack, B. P., M. Strubin, A. S. Ponticelli, and K. Struhl. 1991. Functional differences between yeast and human TFIID are localized to the highly conserved region. *Cell* **65**:341-348.
7. Cortes, P., O. Flores, and D. Reinberg. 1992. Factors involved in specific transcription by mammalian RNA polymerase II: purification and analysis of transcription factor IIA and identification of transcription factor III. *Mol. Cell. Biol.* **12**:413-421.
- 7a. DeJong, J., and R. G. Roeder. Unpublished data.
8. Eisenmann, D. M., C. Dollard, and F. Winston. 1990. SPT15, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation in vivo. *Cell* **58**:1183-1191.
9. Fire, A., M. Samuels, and P. A. Sharp. 1984. Interactions between RNA polymerase II, factors and template leading to accurate transcription. *J. Biol. Chem.* **259**:2509-2516.
10. Flores, O., H. Lu, and D. Reinberg. 1992. Factors involved in specific transcription by mammalian RNA polymerase II. *J. Biol. Chem.* **267**:2786-2793.
11. Gasch, A., A. Hoffmann, M. Horikoshi, R. G. Roeder, and N.-H. Chua. 1990. *Arabidopsis thaliana* contains two genes for TFIID. *Nature (London)* **346**:390-394.
12. Gerard, M., L. Fischer, V. Moncollin, J.-M. Chipoulet, P. Chambon, and J.-M. Egly. 1991. Purification and interaction properties of the human RNA polymerase B(II) general transcription factor BTF2. *J. Biol. Chem.* **266**:20940-20945.
13. Gill, G., and R. Tjian. 1991. A highly conserved domain of TFIID displays species specificity in vivo. *Cell* **65**:333-340.
14. Hahn, S., S. Buratowski, P. A. Sharp, and L. Guarente. 1989. Identification of a yeast protein homologous in function to the mammalian general transcription factor, TFIIA. *EMBO J.* **8**:3379-3382.
15. Hahn, S., S. Buratowski, P. A. Sharp, and L. Guarente. 1989. Isolation of the gene encoding the yeast TATA binding protein TFIID: a gene identical to the SPT15 suppressor of Ty element inactivation. *Cell* **58**:1173-1181.
16. Hoey, T., B. D. Dynlacht, M. G. Peterson, B. F. Pugh, and R. Tjian. 1990. Isolation and characterization of the *Drosophila* gene encoding the TATA box binding protein, TFIID. *Cell* **61**:1179-1186.
17. Hoffmann, A., M. Horikoshi, C. K. Wang, S. Schroeder, P. A. Well, and R. G. Roeder. 1990. Cloning of the *Schizosaccharomyces pombe* TFIID gene reveals a strong conservation of functional domains present in *Saccharomyces cerevisiae* TFIID. *Genes Dev.* **4**:1141-1148.
18. Hoffmann, A., E. Sinn, T. Yamamoto, J. Wang, A. L. Roy, M. Horikoshi, and R. G. Roeder. 1990. Highly conserved core domain and unique N terminus with presumptive regulatory motifs in a human TATA factor (TFIID). *Nature (London)* **346**:387-390.
19. Horikoshi, M., C. Bertuccioli, R. Takada, J. Wang, T. Yamamoto, and R. G. Roeder. 1992. Transcription factor IID induces DNA bending upon binding to the TATA element. *Proc. Natl. Acad. Sci. USA* **89**:1060-1064.
20. 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.
21. Horikoshi, M., C. K. Wang, H. Fujii, J. A. Cromlish, P. A. Well, and R. G. Roeder. 1989. Purification of a yeast TATA box-binding protein that exhibits human transcription factor IID activity. *Proc. Natl. Acad. Sci. USA* **86**:4843-4847.
22. Horikoshi, M., C. K. Wang, H. Fujii, J. A. Cromlish, P. A. Well, and R. G. Roeder. 1989. Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box. *Nature (London)* **341**:299-303.
23. Horikoshi, M., T. Yamamoto, Y. Ohkuma, P. A. Well, and R. G. Roeder. 1990. Analysis of structure-function relationships of yeast TATA box binding factor TFIID. *Cell* **61**:1171-1178.
24. Horikoshi, M., K. Maguire, A. Kralli, E. Maldonado, D. Reinberg, and R. Weinmann. 1991. Direct interaction between adenovirus E1A protein and the TATA box binding transcription factor IID. *Proc. Natl. Acad. Sci. USA* **88**:5124-5128.
25. Kao, C. C., P. M. Lieberman, M. Schmidt, Q. Zhou, R. Pel, and A. J. Berk. 1990. Cloning of a transcriptionally active human TATA binding factor. *Science* **248**:1646-1650.
26. Lee, D. K., M. Horikoshi, and R. G. Roeder. 1991. Interactions of TFIID in the minor groove of the TATA element. *Cell* **67**:1241-1250.
27. Lee, W. S., C. C. Kao, G. O. Bryant, X. Liu, and A. J. Berk. 1991. Adenovirus E1A activation domain binds the basic repeat in the TATA box transcription factor. *Cell* **67**:365-376.
28. Lieberman, P. M., M. Schmidt, C. C. Kao, and A. J. Berk. 1991. Two distinct domains in the yeast transcription factor IID and evidence for a TATA box-induced conformational change. *Mol. Cell. Biol.* **11**:63-74.
29. Lin, Y.-S., and M. R. Green. 1991. Mechanism of action of an acidic transcriptional activator in vitro. *Cell* **61**:1217-1224.
30. Maldonado, E., I. Ha, P. Cortes, L. Weiss, and D. Reinberg. 1990. Factors involved in specific transcription by mammalian RNA polymerase II: role of transcription factors IIA, IID, and IIB during formation of a transcription-competent complex. *Mol. Cell. Biol.* **10**:6335-6347.
31. Meisterernst, M., and R. G. Roeder. 1991. Family of proteins that interact with TFIID and regulate promoter activity. *Cell* **6**:557-567.
32. Meisterernst, M., A. L. Roy, H. M. Lieu, and R. G. Roeder. 1991. Activation of class II gene transcription by regulatory factors is potentiated by a novel activity. *Cell* **66**:981-993.
33. Muhich, M. L., C. T. Iida, M. Horikoshi, R. G. Roeder, and C. S. Parker. 1990. cDNA clone encoding *Drosophila* transcription factor TFIID. *Proc. Natl. Acad. Sci. USA* **87**:9148-9152.
34. 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.
35. Peterson, M. G., N. Tanese, P. F. Pugh, and R. Tjian. 1990. Functional domains and upstream activation properties of cloned human TATA binding protein. *Science* **248**:1625-1630.
36. Poon, D., S. Schroeder, C. K. Wang, T. Yamamoto, M. Horikoshi, R. G. Roeder, and P. A. Well. 1991. The conserved carboxy-terminal domain of *Saccharomyces cerevisiae* TFIID is sufficient to support normal cell growth. *Mol. Cell. Biol.* **11**:4809-4821.
37. Ptashne, M., and A. F. Gann. 1990. Activators and targets. *Nature (London)* **346**:329-331.
38. Ranish, J. A., and S. Hahn. 1991. The yeast general transcription factor TFIIA is composed of two polypeptide subunits. *J. Biol. Chem.* **266**:19320-19327.
39. 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.
40. Reddy, P., and S. Hahn. 1991. Dominant negative mutations in yeast TFIID define a bipartite DNA-binding region. *Cell* **65**:349-357.
41. Reinberg, D., M. Horikoshi, and R. G. Roeder. 1987. Factors involved in specific transcription by mammalian RNA polymerase II. *J. Biol. Chem.* **262**:3322-3330.
42. Roeder, R. G. 1991. The complexities of eukaryotic transcription initiation: regulation of preinitiation complex assembly. *Trends Biochem. Sci.* **16**:402-408.
- 42a. Roy, A. L., S. Malik, M. Meisterernst, and R. G. Roeder. Unpublished data.
43. Roy, A. L., M. Meisterernst, P. Pognonec, and R. G. Roeder. 1991. Cooperative interaction of an initiator-binding transcrip-

- tion initiation factor and the helix-loop-helix activator USF. *Nature (London)* **354**:245–248.
44. Samuels, M., and P. A. Sharp. 1986. Purification and characterization of a specific RNA polymerase II transcription factor. *J. Biol. Chem.* **261**:2003–2013.
  45. Sawadogo, M., and R. G. Roeder. 1985. Interaction of a gene-specific transcription factor with the adenovirus major late promoter upstream of the TATA box region. *Cell* **43**:165–175.
  46. Sawadogo, M., and A. Sentenac. 1990. RNA polymerase B(II) and general transcription factors. *Annu. Rev. Biochem.* **59**:711–754.
  47. Schmidt, M. C., C. C. Kao, R. Pel, and A. J. Berk. 1989. Yeast TATA-box transcription factor gene. *Proc. Natl. Acad. Sci. USA* **86**:7785–7789.
  48. Schmidt, M. C., Q. Zhou, and A. J. Berk. 1989. Sp1 activates transcription without enhancing DNA-binding activity of the TATA box factor. *Mol. Cell. Biol.* **9**:3299–3307.
  49. Starr, D. B., and D. K. Hawley. 1991. TFIID binds in the minor groove of the TATA box. *Cell* **67**:1231–1240.
  50. Stringer, K. F., C. J. Ingles, and J. Greenblatt. 1990. Direct and selective binding of an acidic activation domain to the TATA-box factor TFIID. *Nature (London)* **345**:783–786.
  51. Sumimoto, H., Y. Ohkuma, T. Yamamoto, M. Horikoshi, and R. G. Roeder. 1990. Factors involved in specific transcription by mammalian RNA polymerase II: identification of general transcription factor TFIIG. *Proc. Natl. Acad. Sci. USA* **87**:9158–9162.
  52. Tamura, T., K. Sumita, I. Fujino, A. Aoyama, M. Horikoshi, A. Hoffmann, R. G. Roeder, M. Muramatsu, and K. Mikoshiba. 1991. Striking homology of the variable N-terminal as well as the conserved core domains of the mouse and human TATA-factors (TFIID). *Nucleic Acids Res.* **19**:3861–3865.
  53. Usuda, Y., A. Kubota, A. Berk, and H. Handa. 1991. Affinity purification of transcription factor IIA from HeLa cell nuclear extracts. *EMBO J.* **10**:2305–2310.
  54. Van Dyke, M., R. G. Roeder, and M. Sawadogo. 1988. Physical analysis of transcription preinitiation complex assembly on a class II gene promoter. *Science* **241**:1335–1338.
  55. Van Dyke, M., M. Sawadogo, and R. G. Roeder. 1989. Stability of transcription complexes on class II genes. *Mol. Cell. Biol.* **9**:342–344.
  56. Yamamoto, T., M. Horikoshi, J. Wang, S. Hasegawa, P. A. Weil, and R. G. Roeder. 1992. A novel bipartite DNA binding domain composed of direct repeats in the TATA box-binding factor TFIID. *Proc. Natl. Acad. Sci. USA* **87**:2844–2848.
  57. Zhou, Q., M. C. Schmidt, and A. J. Berk. 1991. Requirement for acidic amino acid residues immediately N-terminal to the conserved domain of *Saccharomyces cerevisiae* TFIID. *EMBO J.* **10**:1843–1852.