Transcription factor TFIID induces DNA bending upon binding to the TATA element

(TATA box-binding factor/RNA polymerase II/transcription initiation/transcription regulation/DNA structure)

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ABSTRACT  The TATA box-binding factor TFIID plays a primary role in the process of transcription initiation by RNA polymerase II and its regulation by various gene-specific factors. Here we employ a permuted binding site/gel retardation assay with recombinant yeast and human TFIID to show that this factor induces DNA bending around the TATA element. These results are consistent with the presence of G+C-rich sequence elements flanking the consensus TATA element and led to the recently confirmed suggestion that TFIID interacts with the TATA element via the minor groove. They also raise the possibility that TFIID-induced bending might facilitate promoter interactions of other general factors in the preinitiation complex or interactions between general transcription factors and regulatory factors bound at upstream sites.

The general transcription initiation factor TFIID plays a key role in the activation and regulation of eukaryotic promoters. Binding of TFIID to the common TATA element initiates the assembly of a preinitiation complex involving RNA polymerase II and the other general initiation factors (1–3). Although any of the general factors are potential targets for the positive or negative action of regulatory factors, some of these factors have been found to interact directly with TFIID (4–8) or to facilitate TFIID interactions in functional assays (9–13). A unique role for TFIID in promoter activation and regulation was further suggested by the presence of novel structural motifs in the DNA-binding domain (14–18) and by the unusual binding properties of TFIID, requiring high temperature and characterized by slow on and off rates (4, 6, 19–21). This raises the possibility of coupled conformational changes in TFIID and the promoter. Prompted by these observations, we further investigated the TFIID–promoter interactions and report experiments showing that TFIID bends the promoter DNA around the TATA box.

MATERIALS AND METHODS

Purification of Bacterially Expressed Yeast and Human TFIID. The yeast TFIID gene and the human TFIID cDNA were inserted into the T7 bacterial expression vector (22) after changing the DNA sequences around the translation initiation regions to Nde I sites. Transformed Escherichia coli were grown and induced with isopropyl β-d-thiogalactopyranoside when the OD600 of the culture reached 0.8. Three hours after induction, the bacteria were collected and washed with buffer containing 20 mM Tris-HCl (pH 7.9 at 4°C) and 200 mM NaCl. The washed E. coli cells then were suspended in a high-salt buffer containing 20 mM Tris-HCl (pH 7.9 at 4°C), 50 mM 2-mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride, 10% (vol/vol) glycerol, 0.5 M NaCl, and pepstatin/leupeptin (10 μg/ml) and were sonicated until the OD950 was reduced to <10% of the original value. The sonicated lysate was centrifuged and the supernatant was analyzed by Coomassie brilliant blue staining after SDS/PAGE (Fig. 1a). Bacterially expressed yeast TFIID was further purified through two successive chromatographic steps (DEAE-Sepharose, heparin-Sepharose), which yielded a protein of >90% purity (Fig. 1a). Bacterially expressed human TFIID was further purified through four successive chromatographic steps (DEAE-Sepharose, heparin-Sepharose, S-Sepharose, CM-Sepharose). This yielded a protein of about 90% purity, as shown previously (23). In vitro transcription assay mixtures (24) contained general transcription factors and RNA polymerase II from HeLa cells, the adenovirus major late (AdML) promoter as template, and either no exogenous TFIID or about 10 ng of bacterially expressed and purified human or yeast TFIID.

Construction of DNA Fragments for DNA Bending Studies. Polymerase chain reaction (PCR) techniques were used to isolate AdML promoter fragments of identical length but with the TATA box in different positions at regular intervals. Oligonucleotide primers contained restriction enzyme sites (Xba I and BamHI) at the ends to facilitate subcloning of the PCR product into a vector. After digestion of the plasmids with both restriction enzymes, the PCR products were isolated by electrophoresis in an agarose gel and subcloned into a vector for nucleotide sequence determination.

Gel Mobility-Shift Assay. The DNA fragments used for the mobility-shift assay were isolated from clones of confirmed sequence and were end-labeled with [α-32P]dCTP by using the Klenow fragment of DNA polymerase I. End-labeled DNA fragments (~50,000 cpm/ng) were separated from nucleotides by gel filtration on Sephadex G-50.

Gel mobility-shift assays with these fragments employed standard transcription conditions (24), except that nucleotides were omitted, the promoter concentrations were 10-fold lower, the MgCl2 concentration was reduced to 3 mM (Fig. 5) or 5 mM (Fig. 3), and 50 ng (Fig. 3) or 250 ng (Fig. 5) of poly(dG·dC) was added. One femtomole (50,000 cpm/ng) of each end-labeled AdML fragment was incubated with purified recombinant yeast or human TFIID at 30°C for 30 min (Fig. 5) or 40 min (Fig. 3), and TFIID–DNA complexes were resolved from free DNA fragments by electrophoresis in either a 4% polyacrylamide gel with running buffer containing 1× TGE (25 mM Tris base/190 mM glycine/1 mM EDTA, pH 8.3) and 5 mM MgCl2 (Fig. 3) or a 5% polyacrylamide gel with running buffer containing 0.5× TBE (45 mM Tris base/45 mM boric acid/0.1 mM EDTA, pH 8.0) and 2 mM MgCl2 (Fig. 5). The gel was supplemented with buffer containing either 1× TGE, 10% glycerol, 0.5 mM dithiothreitol, and 4 mM MgCl2 (Fig. 3) or 0.5× TBE, 0.5 mM dithiothreitol, and 2 mM MgCl2 (Fig. 5).
RESULTS

The availability of cDNA clones encoding yeast TFIID and the TATA-binding component (TFIIDr) of natural human TFIID (reviewed in ref. 25) enabled us to obtain nearly homogeneous preparations of bacterially expressed proteins (Materials and Methods) that were equivalent to natural human TFIID in stimulating transcription from the AdML promoter. The purified yeast protein (Fig. 1a, lane 4) was identical in size (27 kDa) to the natural yeast TFIID (24) and was active in effecting transcription from the AdML promoter in conjunction with other general factors from human cells (Fig. 1b). The purified human protein (37 kDa; ref. 17) was nearly identical in size to the TATA-binding component in natural human TFIID (R.T., Y. Nakatani, T. Kokubo, M.H., and R.G.R., unpublished data) and showed transcriptional activity on the AdML promoter equivalent to that of yeast TFIID. This equivalence of yeast and human TFIID in effecting basal transcription (24, 26, 27) is consistent with studies indicating that the 180-residue C-terminal domain that is necessary and sufficient for DNA binding (16) is highly conserved from yeast to man (14, 17, 18, 28-37) and that the structural motifs within this domain (direct repeats, basic repeat, Myc homology, and σ homology) are even more highly conserved (reviewed in ref. 17).

DNA Bending by Yeast TFIID in the TATA-Box Region. To investigate the DNA-bending activity of yeast TFIID, we took advantage of the permuted binding site/gel retardation assay developed by Wu and Crothers (38) to show DNA bending by the bacterial catabolite gene activating protein (CAP). This method has also been used to demonstrate DNA bending by other proteins involved in the enzymatic manipulation of DNA (reviewed in refs. 39 and 40). The assay is based on the increasingly anomalous electrophoretic mobility of uniform-length DNA fragments as the bending center is moved from the ends to the center of the DNA fragment in question. In the present study, PCR amplification was employed to generate a series of uniform-length (187-base-pair) DNA fragments with the TATA box at regularly scheduled intervals (summarized in Fig. 2).

The 32P-labeled DNA probes were incubated with bacterially expressed TFIID under normal TFIID binding conditions and the resulting DNA–protein complexes were analyzed by electrophoresis under nondenaturing conditions. As shown in Fig. 3, each free DNA fragment showed the same mobility, indicating the absence of any intrinsic bending. In contrast, the DNA–protein complexes formed showed uniformly sharp bands with distinct electrophoretic mobilities. The mobility of the DNA–protein complex decreased uniformly as the position of the binding site was moved from...
either end to the center of the DNA fragment. This pattern is typical of that observed for other site-specific DNA-binding proteins whose DNA bending properties have been verified by other techniques (including electron microscopic visualization, ligation rate assays, and direct structural studies). We interpret the above data to indicate that TFIID binding to the TATA element induces DNA bending. To determine the bending center the data were plotted as shown in Fig. 4. Extrapolation of the binding curves places the bending center within the TATA box, apparently near the left side.

DNA Bending by Human TFIID in the TATA-Box Region. Given the structural and functional relationships between yeast and human TFIID, DNA-bending activity of the latter protein was also tested with the same DNA fragments by using slightly modified gel shift conditions (Materials and Methods). The mobility of the human TFIID–DNA complex (Fig. 5 Left, lanes 1–5) also varied as a function of the position of the TATA element, although complexes were not clearly detected with the two DNA fragments containing the least 5' flanking sequence (lanes 6 and 7). Control experiments showed that under these electrophoretic conditions (Fig. 5 Right), yeast TFIID–DNA complexes showed essentially the same pattern of mobility as observed earlier. These results show that human TFIID has a DNA-bending activity but further suggest that formation of a stable human TFIID–DNA complex requires a minimal amount of flanking region on the 5' side of the TATA box. Somewhat surprisingly, it appears that the DNA bending center is shifted further 5' from the TATA box, relative to the case for yeast TFIID. This is indicated by the fact that the human TFIID–DNA complex with the slowest mobility was observed with the DNA fragment in which the TATA box is located to the right of the center position. However, the bending center was not determined accurately, since the two DNA fragments with the TATA element in the most 5' position did not show human TFIID–DNA complexes.

**DISCUSSION**

The present data indicate that TFIID can induce DNA bending at the TATA box of the AdML promoter. They extend previous studies of induced bending of DNA by a number of other site-specific DNA-binding proteins to include a commonly used (general) transcription initiation factor. Questions that arise relate to the mechanisms and specific sequences involved in promoter specificity and the possible functional significance of TFIID-dependent bending. Studies of nucleosomes (40) and CAP-promoter complexes (39) have established that, at points of DNA–protein contact, A+T-rich sequences favor minor-groove compression whereas G+C-rich sequences favor major-groove compression. This plus the structure of the AdML promoter, which contains a consensus TATA element (TATTTAAA) flanked by long G+C-rich clusters (Fig. 4 legend), suggested that TFIID might contact the TATA element primarily through the minor groove, with secondary (stabilizing) contacts in flanking regions enhanced by bending. This hypothesis has since been tested and verified for yeast TFIID (54). Other proteins, including integration host factor (IHF) (41, 42) and high-mobility-group protein 1 (HMG-1) (43) have been shown to bind to A+T-rich DNA sequences through minor-groove contacts that may be accompanied by DNA bending and corresponding secondary contacts (IHF). Although the promoter specificity (and sequence dependence) of TFIID-induced bending has not been examined, variations associated with flanking sequences could explain the variable footprints and binding affinities observed with promoters containing similar or identical TATA elements (19, 44).

A surprising finding was an apparent difference in the position of the induced-bending center by yeast and human TFIID. What region of TFIID determines this species difference? Since a detailed mutagenesis analysis (ref. 16; T.Y., M.H., J.W., P.A. Weil, and R.G.R., unpublished data) indicates that conserved residues in both direct repeats contribute to the DNA binding, it is reasonable to consider that the conserved C-terminal 180-residue domain plays a major role in the bending. The observations that the core is sufficient for TATA box binding (16) and determines some species-specific functions in vivo (45–49) support this idea. However, since deletion of the N terminus of yeast TFIID stimulates TATA box-binding activity (16), we cannot exclude the possibility that the divergent N-terminal domains are responsible for differences in the binding properties of yeast and human TFIID. This possibility can be tested with appropriate mutations in various species of TFIID. A related question is whether the differences in DNA-bending ability
might contribute to functional differences between yeast and human TFIID on certain promoters. The availability of functional tests for both basal and activator-dependent transcription in response to TFIID will allow these points, and the significance of the bending phenomenon, to be tested.

Although the functional relevance of the observed bending is not yet established, the key role of TFIID in initiating the assembly of the other initiation factors (and RNA polymerase II) into a functional preinitiation complex makes this an interesting possibility. In particular, the bending may serve to stabilize interactions of the other general factors or, as suggested (40) for the bacterial RNA polymerase complex, energy expended in bending might later be converted to torsional stress that facilitates unwinding of the double helix. Alternatively, the altered DNA conformation could facilitate contact between regulatory factors bound at upstream sites and one or more components of the general transcriptional machinery (Fig. 6). In the extreme case the sole function of bending might be to bring other factors in contact, as indicated for IHF (50) and potentially for CAP (51). Although seemingly unlikely in the case of TFIID, this possibility is raised by the recent demonstration (52) of other core promoter elements (initiators) and associated factors (53) that may play a dominant role in facilitating assembly of preinitiation complexes on some promoters. In any case, the possibility of promoter-specific variations in TFIID-induced bending, which could account for the unusually high basal activity of the AdML promoter, allows for another level of control on the diverse group of class II promoters.

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Fig. 5. DNA-bending activity of human TFIID in the TATA-box region. Gel shift analysis was carried out in the presence of purified recombinant human (Left) or yeast (Right) TFIID with various DNA fragments. DNA probes in lanes 1–7 correspond to the constructs in Fig. 2.

Fig. 6. Model of enhanced interactions between regulatory factors and basic transcription factors resulting from TFIID-induced bending.