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Bending DNA Can Repress a Eukaryotic Basal Promoter and Inhibit TFIID Binding

ALYSSA TENHARMSEL AND MARK D. BIGGIN*

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520-8114

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Previous studies indicated that repression by *eve* involves cooperative DNA binding and leads to the formation of a DNA loop which encompasses the DNA sequences normally bound by the RNA polymerase II general transcription factors. To test the general principle of whether bending of a basal promoter sequence can contribute directly to repression of transcription, a minicircle template of 245 bp was used. In a purified transcription system, transcription from the minicircular DNA is greatly reduced compared with that from the identical DNA fragment in linear form. Transcription is also reduced when the minicircle contains a single-stranded nick, indicating that transcription is reduced because of DNA bending, rather than any constraint on supercoiling. We show that the reduced transcription from the minicircle in these experiments is not due to a reduced rate of elongation by RNA polymerase II. Rather, repression occurs, at least in part, because binding of the general transcription factor TFIID to the minicircle is strongly inhibited compared with binding to the linear DNA. We suggest that bending DNA may be a mechanism by which eukaryotic transcription may be regulated, by modulating the activity of the general transcription factors.

The *Drosophila even-skipped* (*eve*) homeodomain protein is a developmental control protein which has been genetically characterized as a repressor of *Ultrabithorax* (*Ubx*) and other *Drosophila* developmental control genes (24, 25). In vitro studies using the *Ubx* and *Adh* distal promoters have provided evidence for two related mechanisms by which *eve* represses transcription (1, 2, 33). On *Ubx*, repression requires the presence of a high-affinity *eve* DNA binding site located 45 bp downstream of the transcription start site. *eve* protein binds cooperatively to this high-affinity site and two other moderate-affinity DNA sites, one of which lies 150 bp upstream of the *Ubx* RNA start site (33). These cooperative interactions cause the intervening DNA (nucleotides −150 to +45) to bend into a loop. Repression occurs, at least in part, because the upstream moderate-affinity site overlaps an element recognized by the *zeste* activator protein, and *eve* competitively inhibits binding of *zeste* protein to prevent activation. A related mechanism also appears to be involved in repression of the *Adh* distal promoter (1). On this promoter, cooperative associations between repressor molecules stabilize binding to low-affinity sites, which may also cause the basal promoter DNA to bend. Repression occurs in this case because binding of TFIID to the promoter is inhibited, apparently through direct competition with the repressor protein for overlapping DNA binding sites.

In both of the examples cited above, competitive inhibition of transcription factor binding to DNA could fully account for repression by *eve* protein. However, work in other systems suggests a second possible mechanism of repression. On several promoters, bending of DNA itself has been implicated in altering the rate of transcription (3, 6, 7, 17, 27, 29). In one of these cases, DNA bending has been specifically shown to either positively or negatively affect the ability of a transcription factor to bind to DNA, depending on the orientation of the bend (17). Given that repression by *eve* leads to bending of promoter DNA (1, 33), it is possible that this could itself be directly preventing transcription factors from binding. Here we have examined whether bending of DNA itself can inhibit transcription and whether this inhibition is due to an effect on the RNA polymerase II general transcription factors.

* Corresponding author. Mailing address: Department of Molecular Biophysics and Biochemistry, Yale University, 260 Whitney Ave., New Haven, CT 06520-8114. Phone: (203) 432-5791. Fax: (203) 432-6178.

MATERIALS AND METHODS

Preparation of transcription templates. To make short templates for transcription containing the *Adh* distal promoter sequences, plasmid pUC7 AdhD was constructed. pUC7 AdhD contains *Adh* distal promoter sequences from −34 to +105 relative to the transcription start site (13). Sequences from pBR322 (approximately 2065 to 2130) make up the rest of the short template, extending to −97 relative to the *Adh* distal transcription start site. The *Adh* distal and pBR322 sequences were inserted into pUC7 at the *Hin*cII sites of the polylinker. The *Adh* distal short template DNA (245 bp) was excised from plasmid pUC7 AdhD by digestion with *Bam*HI, gel purified, and ligated at a concentration of 1 ng/μl to favor formation of primarily monomer minicircles (23). The monomer minicircle was gel purified to separate it from the other ligated species (mainly linear and circular multimers). The linear template was derived from the minicircular template by restriction digestion of the minicircular template with *Bam*HI. The nicked template was constructed by removing the terminal phosphates of the linear fragment by alkaline phosphatase digestion and incompletely replacing the phosphates by phosphorylating the fragment with 0.024 pmol of ATP per ng of DNA fragment. Ligation of the fragment as described above gives mainly linear *Adh* D fragment along with some nicked circle. The nicked circle is separated from the other ligation products by gel purification. All three template forms differed in agarose gel mobility (2% agarose gel, 1.5 μg of ethidium bromide per ml) sufficiently that they could easily be distinguished and separated. The gel purified products were characterized by restriction digestion to confirm that they were monomeric and either circular or linear. Molecular weight markers (1-kb ladder) were from Gibco BRL.

Transcription assays. The purified transcription system contained purified fractions of *Drosophila* general factors TFIID, TFIIF, TFIIH, and RNA polymerase II (1). Recombinant proteins used were human TFIIE, *Drosophila* TFIIB, and *Drosophila* TATA-binding protein (TBP). Amounts of each protein used are described in reference 1. Template DNA (5 ng of pUC7 AdhD per μl or 0.4 ng of minicircle or linear DNA per μl; 2.7 fmol total of either plasmid, linear, or circular DNA) was incubated with either 1.5 μl of TFIID fraction or 30 ng of TBP for 30 min at 21°C; then the rest of the general factors were added, and the reaction mixture was incubated for 30 min at 21°C. Nucleotides were added to the reaction, and transcription was allowed to proceed for 1 min, to give a single round of transcription. S1 nuclease analysis of RNA transcripts was performed as previously described (33) except that the probe used was made up of *Adh* distal promoter sequences from −30 to +49.

DNase I footprinting. DNA probes were prepared as described above except that the DNA was end labeled with ^{32}P before ligation into the circular form. The

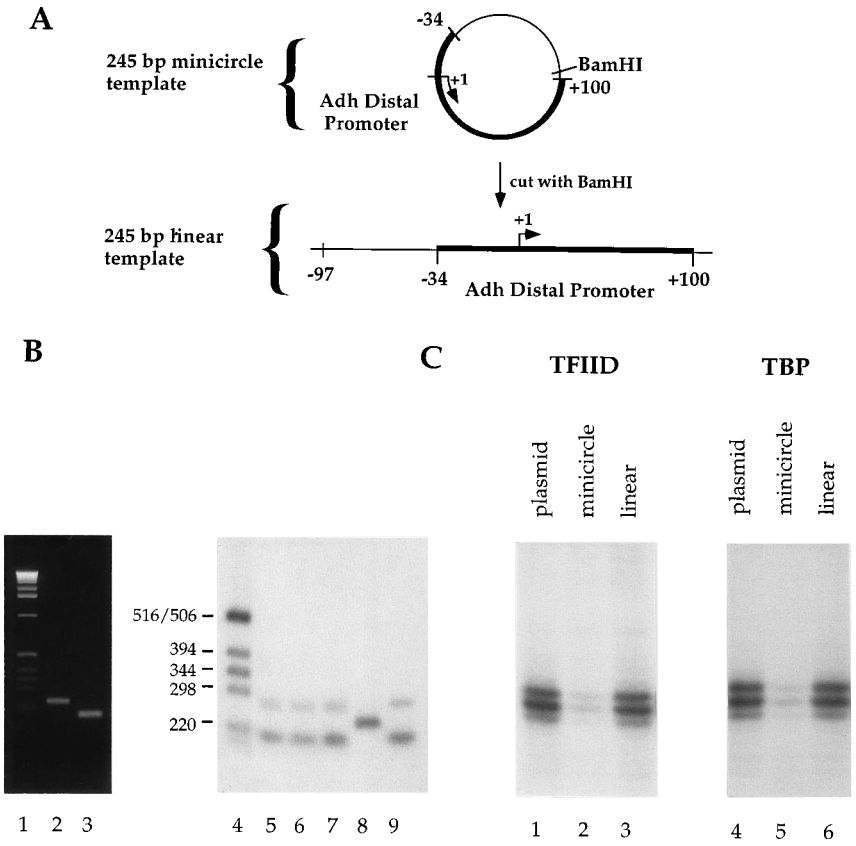

FIG. 1. Transcription is reduced from minicircle DNAs. (A) Diagram of short DNA templates used in transcription. The 245-bp DNA contains *Adh* distal promoter sequences from bp −34 to +100 relative to the transcription start site (thick line) flanked by DNA sequences from pBR322 (thin line; see Materials and Methods). The 245-bp linear DNA template is generated by restriction digestion of the purified minicircle template with the restriction endonuclease *Bam*HI. (B) Characterization of the minicircular and linear DNA templates on 2% agarose gels. Lanes: 1 and 4, molecular weight markers; 2 and 3, 50 ng of either linear (lane 2) or minicircular (lane 3) DNA; 5 to 7, radioactively labeled minicircular templates which have been incubated in transcription reaction mixtures (lane 5, template DNA after one round of transcription; lane 6, template DNA incubated in the absence of nucleotides; lane 7, template DNA after multiple round transcription); 8 and 9, linear and minicircular DNA, respectively. Sizes are indicated in base pairs. (C) S1 nuclease analysis of RNA synthesized in a purified in vitro transcription system. All reaction mixtures contained 2.7 fmol of promoter template. Lanes: 1 and 4, the 3-kb plasmid pUC7 AdhD; 2 and 5, the minicircle DNA; 3 and 6, the linear DNA template. Transcription reaction mixtures included either TFIID (lanes 1 to 3) or TBP (lanes 4 to 6) and the remainder of the general transcription factors (see Materials and Methods).

radioactively labeled minicircular probe contains a small amount of nicked circular probe which results from radiolytic damage of the DNA and which is not efficiently removed by gel purification of the minicircular DNA. Footprinting of TBP was done under previously described conditions (33), and 32 ng of poly(dG-dC) per μl was used as a carrier. Conditions for TFIID binding were as described in reference 9 except that 2.5 ng of *Hae*III-digested *Escherichia coli* DNA per μl was added as a carrier. Proteins were incubated with probe DNA at 21°C for 30 min (TFIID) or 45 min (TBP). After DNase I digestion, the circular probe was restriction digested so that the circular probe would be in linear form. Since the circular and linear probes were labeled at both ends with ^{32}P, a further restriction digestion must be performed, which results in the DNA probes each having a single labeled end (see Fig. 4A). The smaller of these fragments is approximately 30 bp in length, and the DNase I digestion products from this smaller fragment are not observed on a polyacrylamide gel. Therefore, the DNase I digestion pattern seen is only that of the larger fragment.

RESULTS

Bending of DNA inhibits transcription. On the *Ubx* promoter, binding of *eve* protein leads to bending of an approximately 220-bp region of DNA into a looped structure (33). On the *Adh* distal promoter, repressor molecules bind cooperatively to sites separated by 190 bp, and this may bend the DNA between these sites (1). Unfortunately, available methods cannot determine the precise curvature and structure of any of the DNA loops formed by *eve* on these promoters, but it should be possible to mimic the basic features of these loops by circularizing a short DNA fragment 190 to 250 bp in length. By comparing the amount of transcription from such a minicircle with that from a corresponding linear DNA fragment, it should be possible to assess the effect of bending on the activities of the general transcription factors. To do this, we used a reconstituted purified system of the general transcription factors, which removes most sequence specific activators. Transcription from the *Adh* distal promoter was studied, as this promoter gives strong basal transcription. By using this system, these studies seek to establish a general precedent for an effect of bending on basal transcription.

A DNA template was prepared from a linear 245-bp DNA fragment containing the basal promoter sequence of the *Adh* distal promoter (nucleotides −34 to +105) flanked by sequences from pBR322 (13). This promoter fragment lacks activator binding sites and still yields high levels of transcription in the purified system. The 245-bp DNA was then circularized, and the closed circular form of the DNA was isolated from an agarose gel. To control against any change in transcription from this minicircular DNA being due to damage or contamination during the preparation of the template, a portion of this minicircular DNA was restriction digested to produce the linear fragment to be used in transcription assays (Fig. 1A and

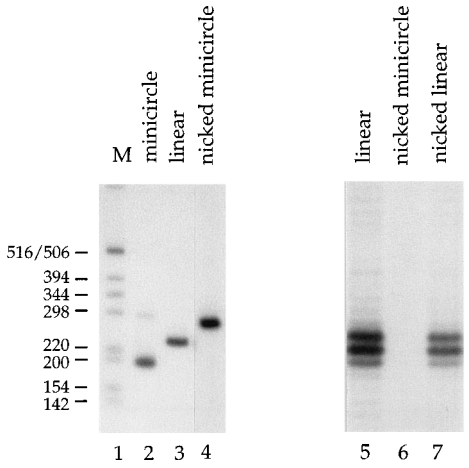

FIG. 2. In vitro transcription from a nicked minicircular template. Agarose gel analysis of radioactively labeled minicircular (lane 2), linear (lane 3), and nicked minicircular (lane 4) templates. The remaining lanes represent an S1 nuclease analysis of RNA synthesized from either the 245-bp linear template (lane 5), a minicircle template which contains a single-stranded nick (lane 6), and a linear template generated from the nicked minicircle DNA by restriction digestion (lane 7). TFIID was present in all reactions, which were performed as described in the legend to Fig. 1. Sizes are indicated in base pairs.

B; Fig. 2). Levels of transcription from the minicircular and linear 245-bp DNA templates were compared. The linear template gave high levels of transcription, and transcription from the minicircle was severely reduced (Fig. 1C; compare lanes 2 and 3), indicating that a bent DNA may be able to repress a eukaryotic basal promoter. Similar results were obtained even when linear DNA was produced by adding *Bam*HI directly to reaction mixtures containing minicircular template prior to addition of transcription factors, compared with minicircular templates that were mock digested prior to the transcription assay (unpublished data). As a control to determine whether the template DNA was changed at all during the course of the reaction, a small amount of radioactively labeled circular template was included in some transcription reaction mixtures. Because of radiolytic damage, the radioactively labeled template contains both closed circular DNA and a small amount of nicked circular DNA, unlike the templates used in the transcription experiments described above, in which all of the minicircle DNA was in closed circular form. After the radiolabeled template-containing transcription reaction mixtures had been incubated with (lanes 2 and 4) or without (lane 3) nucleoside triphosphates (NTPs), the radiolabeled DNA templates were extracted from the reactions and analyzed on an agarose gel (Fig. 1B). No change in the amounts of templates or in the ratio of closed circular to nicked circular templates was observed when the templates were incubated with the general transcription factors in the presence or absence of nucleotides (Fig. 1B; compare lanes 2 to 4 with lane 6). As a further control, the amount of transcription from a molar equivalent of a 3-kb circular plasmid template containing the same basal *Adh* distal promoter sequences was examined. Because the plasmid template is very large compared with the minicircle, the promoter sequences should not be significantly bent, and therefore the level of transcription from the plasmid DNA should be equal to the level of transcription from the linear template. As expected, the levels of transcription from the plasmid and linear templates were essentially equal (Fig. 1C; compare lanes 1 and 3). To provide further evidence that low levels of transcription from the minicircle are not caused by contamination present in the template preparation, an equimolar amount of minicircular template DNA was added to a control reaction mixture containing the plasmid template. No inhibition of transcription from the plasmid was observed (unpublished data).

The experiments described above were performed with the general transcription factors, including TFIID. TFIID is a complex of polypeptides which includes the TBP and eight TBP-associated factors (TAFs) (5; reviewed in reference 8). Since TBP is able to support basal transcription without the TAFs, we were interested in determining if transcription using TBP, rather than TFIID, was also reduced from minicircle templates. Indeed, transcription from the minicircle template is lower than that from the linear template and the plasmid templates in reactions dependent upon TBP activity (Fig. 1C, lanes 4 to 6).

Nicked circular DNA inhibits transcription. From the evidence presented above, it appears that a bend in DNA can inhibit transcription. However, it has been shown that differences in the topological state of DNA (supercoiling) can affect the binding of *E. coli* RNA polymerase (18). To rule out the possibility that supercoiling was reducing transcription from minicircle DNAs, transcription using a nicked minicircle template was carried out. The nicked minicircle template contains a bend but does not have the topological constraints of a closed circle (i.e., it has no torsional stiffness) (18). The linear, closed minicircle and nicked minicircle templates were distinguished by different agarose gel mobilities (Fig. 2, lanes 1 to 4; see also Materials and Methods) and restriction digestions. Note that the radiolabeled minicircular DNA template contains a small amount of nicked circular DNA (also seen in Fig. 1B, lanes 5 to 7 and 9, and Fig. 4D). This is an artifact of radiolytic nicking, and this species is not present in nonradioactive template preparations (Fig. 1B, lane 3). Transcription assays were carried out as described above, using the linear template used for Fig. 1 as well as a nicked minicircle and an additional linear template which was generated by restriction digestion from the nicked minicircle. All of the linear templates give similar levels of transcription (Fig. 2, lanes 1 and 3). There is no observable transcription from the nicked minicircle template (Fig. 2, lane 2). The fact that transcription is low from both closed minicircular and nicked minicircular templates suggests that the reduced transcription is caused by the bend in the DNA rather than by any torsional constraint of the minicircular DNA templates.

Elongation of transcript is not rate limiting. There are several possible ways in which bending DNA could affect transcription. One possibility is that the rate of elongation by RNA polymerase is affected. It has previously been shown that addition of 0.05% the detergent Sarkosyl after incubation of DNA templates with the general transcription factors prevents formation of further preinitiation transcription complexes (11, 12, 16). Addition of nucleotides followed immediately by Sarkosyl allows transcription only from those complexes already formed. By varying the time that elongation of polymerase is allowed to proceed after the addition of nucleotides, it is possible to determine if elongation by RNA polymerase II is rate limiting in this experiment.

Earlier experiments had demonstrated that on large plasmid templates, incubation in the presence of nucleotides for 1 min is sufficient for the RNA polymerase molecules in all preformed complexes to transcribe RNAs at least as long as the S1 probes used (32). In Fig. 3, preinitiation complexes were allowed to form on linear and minicircle templates. Nucleotides were added, Sarkosyl was added immediately thereafter, and elongation was allowed to proceed for either 1 or 30 min (Fig.

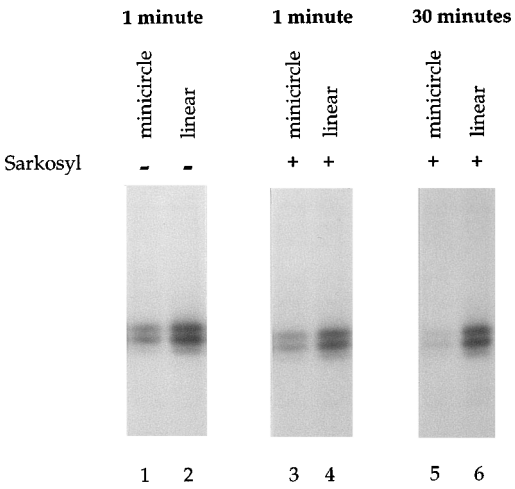

FIG. 3. Elongation is not rate limiting for transcription from the minicircular promoter. S1 nuclease analysis of RNA produced by transcription from minicircular (lanes 1, 3, and 5), and short linear (lanes 2, 4, and 6) DNA templates in the presence (lanes 3 to 6) or absence (lanes 1 and 2) of 0.05% Sarkosyl. All reaction mixtures were first incubated for 30 min in the absence of nucleotides. Nucleotides (NTPs) were added, and in reactions shown in lanes 3 to 6, 0.05% Sarkosyl was added immediately after addition of NTPs. Polymerase elongation was allowed to proceed for either 1 min (lanes 1 to 4) or 30 min (lanes 5 and 6). All reaction mixtures included TFIID; other conditions are as described in the legend to Fig. 1.

3, lanes 3 to 6). As a control, Sarkosyl was omitted from some reactions; for these reactions, elongation was allowed to proceed for 1 min (Fig. 3, lanes 1 and 2). For all three methods of performing the transcription reaction, the amounts of transcription from the minicircular template are significantly lower than those from short linear templates (Fig. 3; compare lanes 1 and 2, lanes 3 and 4, and lanes 5 and 6). Also, the level of transcription from either template does not increase whether elongation proceeds for 1 or 30 min. Taken together, these data suggest that a decrease in elongation rate is not responsible for the reduced transcription from minicircle DNA observed in this experiment. In other words, even if bending DNA can affect elongation, there is no significant effect on our assay. However, we cannot rule out the possibility of inhibition occurring at the earlier step of promoter clearance by RNA polymerase II (10).

Binding of TFIID is affected by bending of DNA. Another possible explanation for the reduced transcription observed in these experiments is that one or more of the general transcription factors is unable to bind to the promoter because of the bend in the minicircle DNA. The general transcription factors bind to a promoter in a defined order (reviewed in reference 38). TFIID (or TBP) binds to the promoter first and is the only factor that can bind in the absence of other general factors. Consequently, we have initially focused on whether TFIID and TBP bind differently to the minicircle and short linear templates.

The DNA probes used were prepared in the same manner as the transcription templates except that the probes were labeled at both ends with ^{32}P before ligation into the circular form. The linear probe was generated from the minicircular probe by restriction digestion. After protein binding and DNase I digestion, both probes were restriction digested to give a unique labeled end for polyacrylamide gel analysis, and in the case of the circular probe, to linearize the DNA (Fig. 4A).

First, it is important to note that there are differences in DNase I digestion patterns between linear and circular DNA probes when no TFIID has been added to the reaction (Fig. 4B or C; compare lanes 5 and 6, regions indicated by − and ●). Compared with the linear DNA, the minicircle DNA shows an alternating pattern of protection (●) and hypersensitive (−) cutting, with an approximately 5-bp periodicity. This type of digestion pattern is observed only when DNA is bent and when most or all of the DNA molecules are bent in the same orientation (4, 26). Thus, this result suggests that the minicircular DNAs adopt either one or a few similar rotational conformations, with most of the molecules presenting the same face of the DNA helix on the outer edge of the circle. Incubation of radiolabeled minicircular DNase I probes with either TBP or the TFIID fraction under the binding conditions described did not damage the minicircular template (Fig. 4D).

Figure 4B shows binding of the TFIID fraction to the *Adh* distal promoter. On the linear DNA probe, TFIID protects the region between nucleotides −40 and +37, which includes both the TATA box and the transcription start site (Fig. 4B, lanes 2 to 4). In contrast, there is no detectable binding of TFIID to the minicircle probe when the same amounts of protein are added (Fig. 4B; compare lanes 3 and 4 with lanes 8 and 9). TFIID is also unable to bind to a nicked, minicircle DNA (data not shown), suggesting that it is the bend in the DNA, rather than any torsional constraint, which is inhibiting binding. Thus, it seems reasonable that transcription may be reduced from a bent template because TFIID cannot bind to the promoter under these circumstances.

Two polypeptides in the TFIID complex, TBP and TAF 150 (35), contact DNA, and interestingly, TBP creates a 90° bend in the TATA box which widens the minor groove in the center of this sequence (15, 20, 21). The pattern of hypersensitive and protected DNase I digestion near the TATA box suggests that the minor groove in the center of the TATA box is compressed in the minicircle. However, in the structure of the TBP-DNA complex, the same region of DNA has a widened minor groove (Fig. 4B) (20, 21). This finding suggests that most of the minicircle DNAs may have adopted a conformation which bends the TATA box in a direction different from that induced by TBP binding. Given results of earlier experiments using catabolite activator protein (CAP) (17) (see Discussion), bending the TATA box in this direction might reduce binding solely through an effect on the TBP polypeptide within the TFIID complex. However, studies using isolated TBP protein suggest that this is not the full explanation.

Binding of TBP to both minicircular and short linear DNAs was examined. TBP protects a region only slightly larger than the TATA box (−35 to −24) (Fig. 4B) (14). Importantly, there is no apparent difference in the ability of TBP to bind either circular or linear DNA probes (Fig. 4C; compare lanes 2 to 4 with lanes 7 to 9). However, binding of TBP to the minicircular probe does induce a change in the DNase I digestion pattern over much of the probe, including regions well away from the TATA box. The alternating pattern of increased and reduced DNase I cleavage is no longer present, suggesting that binding of TBP rotates the minicircles around the longitudinal axis of the DNA, giving the minicircle new preferred conformations. This finding supports the view that in the unbound minicircles, the TATA box is bent in a direction different from that caused by binding of TBP. Presumably the energetic constraint imposed by having to rotate the circular DNA is not sufficient to significantly affect TBP binding. Binding of TFIID to the minicircle may be inhibited because it binds a longer length of DNA (28) and also because it makes specific DNA contacts via other polypeptides (9, 35). For example, the TFIID complex may create further bends in the DNA which cannot be easily

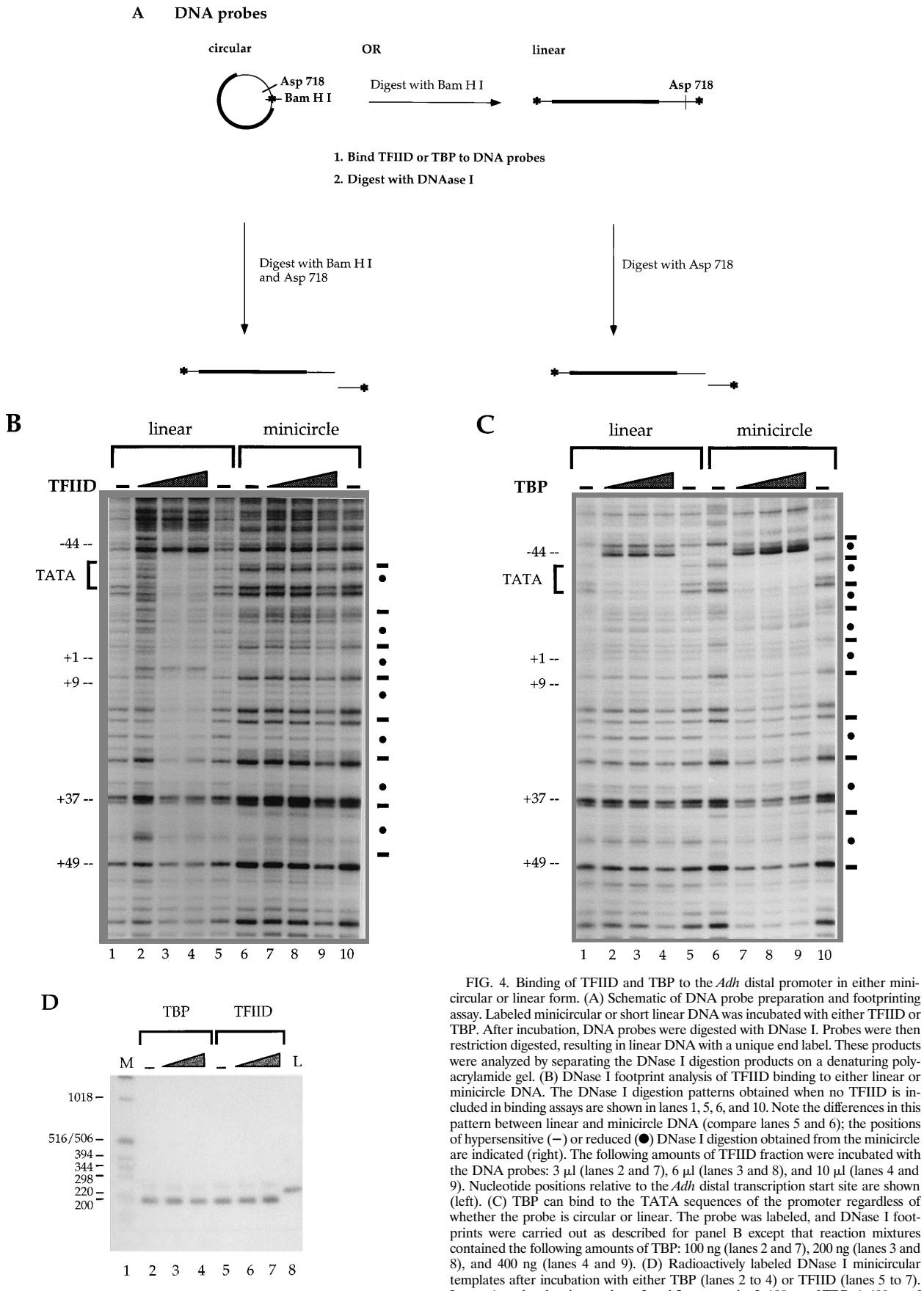

FIG. 4. Binding of TFIID and TBP to the *Adh* distal promoter in either minicircular or linear form. (A) Schematic of DNA probe preparation and footprinting assay. Labeled minicircular or short linear DNA was incubated with either TFIID or TBP. After incubation, DNA probes were digested with DNase I. Probes were then restriction digested, resulting in linear DNA with a unique end label. These products were analyzed by separating the DNase I digestion products on a denaturing polyacrylamide gel. (B) DNase I footprint analysis of TFIID binding to either linear or minicircle DNA. The DNase I digestion patterns obtained when no TFIID is included in binding assays are shown in lanes 1, 5, 6, and 10. Note the differences in this pattern between linear and minicircle DNA (compare lanes 5 and 6); the positions of hypersensitive (−) or reduced (●) DNase I digestion obtained from the minicircle are indicated (right). The following amounts of TFIID fraction were incubated with the DNA probes: 3 μl (lanes 2 and 7), 6 μl (lanes 3 and 8), and 10 μl (lanes 4 and 9). Nucleotide positions relative to the *Adh* distal transcription start site are shown (left). (C) TBP can bind to the TATA sequences of the promoter regardless of whether the probe is circular or linear. The probe was labeled, and DNase I footprints were carried out as described for panel B except that reaction mixtures contained the following amounts of TBP: 100 ng (lanes 2 and 7), 200 ng (lanes 3 and 8), and 400 ng (lanes 4 and 9). (D) Radioactively labeled DNase I minicircular templates after incubation with either TBP (lanes 2 to 4) or TFIID (lanes 5 to 7). Lanes: 1, molecular size markers; 2 and 5, no protein; 3, 100 ng of TBP; 4, 400 ng of TBP; 6, 3 μl of TFIID fraction; 7, 10 μl of TFIID fraction; 8, linear DNA. Sizes are indicated in base pairs.

accommodated in a short minicircle and would therefore inhibit binding.

Since TBP-dependent transcription is reduced on the minicircle DNA, yet TBP binding is not affected, it may be that bending of DNA can inhibit further steps in preinitiation complex formation. We emphasize, however, that TFIID is the more physiologically relevant species, as *Drosophila* and human TBPs are never isolated in the absence of TAFs (reviewed in reference 8). Thus, it is almost certain that the inability of TFIID to bind to the bent promoter is contributing significantly to the transcriptional repression observed in these experiments.

DISCUSSION

Our experiments indicate that bending of DNA can inhibit transcription in a eukaryotic system by affecting the binding of at least one of the general transcription factors. We have shown that the tight bend present in a 245-bp minicircular DNA reduces transcription of the *Adh* distal promoter. Further experiments suggest that this repression occurs, at least in part, because the general factor TFIID is unable to bind to this bent DNA. When transcription reactions are performed with TBP rather than TFIID, transcription from minicircular DNA is also repressed. However, unlike TFIID, TBP can still bind efficiently to the minicircle DNA. Thus, it may be that bending DNA also inhibits the action of one or more of the other general transcription factors. This is a question that we hope to examine in future experiments.

The experiments presented here were undertaken because repression of two different promoters appears to involve cooperative binding of repressor molecules to DNA sites on either side of a basal promoter (1, 33). On the *Ubx* promoter, binding by *eve* protein has been shown to lead to a tight bend in the promoter DNA (33), and it is possible this may also occur on the *Adh* distal promoter (1). The data presented here suggest that in principle, bending of DNA could contribute to repression of both of these promoters. Further experiments suggest that inducing bends in promoter DNA may be a mechanism by which *eve* protein also regulates other promoters. In vivo UV cross-linking studies show that in the embryo *eve* protein binds to many sites throughout various promoters, and it has been suggested that this may lead to the formation of many short loops of DNA between *eve* molecules (36). By bending DNA, *eve* may affect not only the binding of the general transcription factors but also the action of specific regulatory transcription factors on many of these genes.

By virtue of their tertiary structures, a number of prokaryotic transcription factors bend the DNA binding sites that they directly contact, including p4 protein of phage ø29 (29) and CAP (31, 37). Several lines of evidence suggest that this DNA bending may play a direct role in both the activation and repression of transcription (3, 6, 29). For example, it has been shown that DNA which is bent can affect binding of CAP to DNA (17). Minicircle DNAs containing the CAP binding site were generated in which the binding site was bent in different directions by altering its orientation relative to a DNA sequence with a strong intrinsic bend. The binding affinity of CAP was found to vary by more than 200-fold, depending on whether the CAP binding site was bent in a direction which structural studies indicated would be favorable or unfavorable to binding. Given this result, we suggest that binding of TFIID to minicircle DNA is inhibited because the majority of minicircle DNA molecules may have adopted a conformation which is unfavorable to TFIID binding.

As with prokaryotes, many eukaryotic regulatory factors either bend the short section of DNA that they directly contact or, via cooperative interactions between molecules bound at distant sites, cause longer regions of DNA loops to form (7, 15, 19–22, 27; reviewed in references 30 and 34). Although many transcription factors bend DNA, in most cases there is no evidence that this DNA bending itself directly affects transcription. However, two proteins, LEF-1 and YY-1, may affect transcription solely by inducing bends in their DNA binding sites (7, 27). The bends induced by these proteins are thought to act by altering the relative configurations of proteins bound at promoter elements on either side of the bend. Our results differ, in that we suggest that a bend in DNA itself can contribute to repression by inhibiting binding by transcription factors, a result which has been supported by work in prokaryotic systems (6, 17, 29).

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