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Near-Zero Linking Difference upon Transcription Factor IID Binding to Promoter DNA

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Binding of yeast transcription factor IID (TFIID) to the adenoviral major late promoter in circular DNA molecules caused a linking number change of less than 0.1. TFIID on its own therefore fails to unwind DNA appreciably, or else it causes both unwinding and compensatory writhing. Highly purified, recombinant yeast TFIID relaxed supercoiled DNA, because of a contaminant of bacterial topoisomerase I. Relaxing activity of topoisomerase I was enhanced by the adenoviral major late promoter, suggesting an instability of the TATA sequence or a destabilizing effect on flanking DNA.

Transcription factor IID (TFIID), which binds the TATA element of RNA polymerase II promoters, is involved in the initiation of transcription by all eukaryotic polymerases (6, 8, 16, 20, 24). TFIID appears to be important for promoter recognition and for the recruitment of other general initiation factors to polymerase II promoters (2, 19) and may play additional roles. Genes for TFIID have been cloned from many organisms, and exhibit a remarkable degree of sequence conservation of a 180-amino-acid region that suffices for activity in vitro and in vivo (7, 13, 14, 17). A pair of directly repeated sequences within the conserved region appear to be involved in DNA binding (18). Interaction with DNA involves contacts in the minor groove and bends the double helix (12, 15, 21). The possibility that TFIID not only bends but also unwinds DNA (1) has been raised, which is of particular interest because of the requirement for unwinding some 10 to 20 bp for the initiation of transcription (5).

A role of the TATA element in unwinding was earlier suggested on the basis of preferential cleavage of this sequence in supercoiled plasmids by single strand-specific nucleases (9). Transient unwinding of the TATA sequence could be understood in terms of the instability of the TpA dinucleotide step, attributed to a comparatively small degree of stacking overlap (22). It might therefore be imagined that TFIID exploits an inherent property of the TATA sequence to bring about the unwinding of DNA for transcription. We report here on measurements done to reveal any unwinding upon TFIID binding to promoters in circular DNA molecules.

MATERIALS AND METHODS

The following oligonucleotide, containing residues −53 to +1 of the adenoviral major late promoter (MLP), was ligated with *Bam*HI-digested pUC19:

5′-GATCCCCTGAAGGGGGGCTATAAAAGGGGGTGGGGGCGCGTTCGTCCTCACTCTCTTCCA-3′
3′-GGGACTTCCCCCCGATATTTTCCCCCACCCCCGCGCAAGCAGGAGTGAGAGAAGGTCTAG-5′

Plasmids containing one (pUC1×MLP) or two (pUC2×MLP) copies of the oligonucleotide were obtained. Isolation of the tandemly repeated promoters from pUC2×MLP on a *Sma*I-*Hin*cII fragment and ligation with *Sma*I-digested pUC19 gave a plasmid with four copies of the starting oligonucleotide (pUC4×MLP). Gel electrophoretic analysis of purified pUC1×, 2×, and 4×MLP DNAs showed the superhelix densities of the three plasmids were essentially the same. Recombinant yeast TFIID was as previously described (14).

RESULTS

Linking number change upon TFIID binding to promoter DNA. For measurement of the linking number change associated with TFIID binding, a synthetic oligonucleotide with the sequence of residues −53 to +1 of the adenoviral MLP was inserted in a circular DNA molecule, pUC19. To enhance the sensitivity of the measurements, as many as four copies of the oligonucleotide were incorporated. DNase I footprint analysis showed that all four TATA sequences could be simultaneously bound by TFIID (Fig. 1).

The linking difference upon binding was assessed by treatment of naked DNA or TFIID-DNA complexes with wheat germ topoisomerase I and then by deproteinization and electrophoresis in a chloroquine-containing agarose gel to reveal the topoisomer distribution. The center of mass of the distribution was essentially unchanged upon TFIID binding (Fig. 2). A shift of the center of mass by half the distance from one topoisomer band to the next would have been readily detectable, so the linking difference associated with binding was less than 0.1 (taking into account the presence of four promoters in the DNA molecule; if the occurrence of one TATATA and two TATAAA sequences [residues 1598 to 1604, 944 to 949, and 2653 to 2658, respectively, of pUC19] and of other related TFIID binding sequences in the vector DNA is considered, the upper limit on the linking difference associated with TFIID binding is

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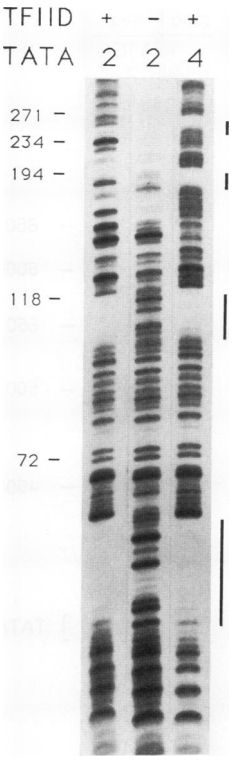

FIG. 1. TFIID binding to tandemly repeated promoters. Plasmids containing two or four copies of the adenoviral MLP (pUC2×MLP or pUC4×MLP; number of TATA sequences indicated above the lanes) were cleaved with *Eco*RI, treated with calf intestinal alkaline phosphatase, and labelled with T4 polynucleotide kinase and [γ-^{32}P]ATP. Labelled DNA (28 ng, 10^5 cpm) was combined with 2 μg of TFIID (+) or not (−) in 20 μl of 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.5)–50 mM KCl–5 mM $MgCl_2$–0.1 mM EDTA containing 0.75 μg of poly(dG-dC) and 2.5 μg of bovine serum albumin, and the mixture was incubated for 20 min at 30°C. Following treatment with DNase I (3 ng in 5 μl of the same buffer, containing 3 mM $CaCl_2$) for 30 s at 20°C, 100 μl of proteinase K (0.5 mg/ml in 100 mM NaCl–10 mM EDTA–0.5% SDS) was added, and the mixture was incubated for 15 min at 37°C. DNA was extracted with phenol, precipitated with ethanol, dissolved in 80% formamide containing 8.9 mM Tris-borate–8.9 mM boric acid–0.2 mM EDTA, and subjected to electrophoresis in a 7 M urea–8% polyacrylamide gel and autoradiography. Lengths (in nucleotides) of DNA fragments are indicated at the left, and regions protected by TFIID from DNase I digestion are indicated by vertical lines on the right.

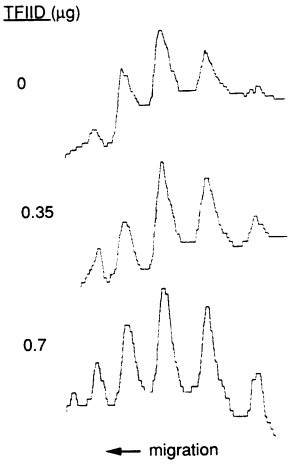

FIG. 2. Near-zero linking number change upon TFIID binding to promoter DNA. Plasmid DNA containing four copies of the adenoviral MLP (pUC4×MLP, 600 ng) was treated with 10 U of wheat germ topoisomerase I in 15 μl of 25 mM HEPES (pH 7.5)–50 mM KCl–0.1 mM EDTA–0.1 mg of bovine serum albumin per ml for 30 min at 37°C. TFIID was added in the amounts indicated (multiples of 350 ng), followed by incubation for 5 min at 37°C, addition of 10 U of wheat germ topoisomerase I, incubation for another 30 min at 37°C, addition of 0.5 μl of 5% SDS, and electrophoresis at 50 V for 18 h in a 0.9% agarose (SeaKem GTG, FMC BioProducts) gel (23 by 14.5 by 0.3 cm) containing 1.3 μM chloroquine. The gel was washed extensively with water and stained with ethidium bromide. Densitometer tracings of a photographic negative are shown. The direction of electrophoresis was from left to right.

even lower). This result was the same regardless of whether TFIID interacted initially with negatively supercoiled or relaxed DNA.

Relaxation of supercoiled DNA by bacterially expressed TFIID due to topoisomerase I. A control experiment in which TFIID-DNA complexes were formed but wheat germ topoisomerase I was omitted gave the surprising result that the supercoiled DNA was extensively relaxed (Fig. 3, compare lanes 1 and 2). This effect was dependent on the number of promoters in the supercoiled DNA molecule (Fig. 4A), as would be expected for an activity of TFIID. It further seemed that the relaxing activity must be due to TFIID since the preparation was apparently homogeneous, as judged from sodium dodecyl sulfate (SDS) gel electrophoresis (14). We nonetheless considered the possibility that the activity was due to a contaminant in the bacterially expressed TFIID preparation, for example, bacterial single-strand binding protein or topoisomerase I. Pure *Escherichia coli* single-strand binding protein had no effect (not shown). However, when antibodies against *E. coli* topoisomerase I were added together with the TFIID preparation, they abolished the relaxing activity (Fig. 3, lane 3). Topoisomerase I alone was sufficient for all the observations made with the TFIID preparation (Fig. 3, lanes 4 and 5; Fig. 4B), including the dependence of relaxing activity on the number of promoters

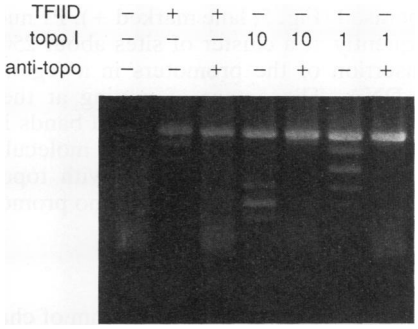

FIG. 3. Relaxation of supercoiled DNA due to *E. coli* topoisomerase I (topo I) contaminating bacterially expressed TFIID. Plasmid DNA containing four copies of the adenoviral MLP (pUC4×MLP, 450 ng) was combined with TFIID (280 ng), *E. coli* topoisomerase I (19 ng), and anti-*E. coli* topoisomerase antiserum (anti-topo; 1 μl) (as indicated above the lanes) in 15 μl of the same solution as for Fig. 2. Following incubation for 30 min at 30°C, 35 μl of water was added, and DNA was extracted twice with phenol, precipitated with ethanol, subjected to electrophoresis and stained as for Fig. 2.

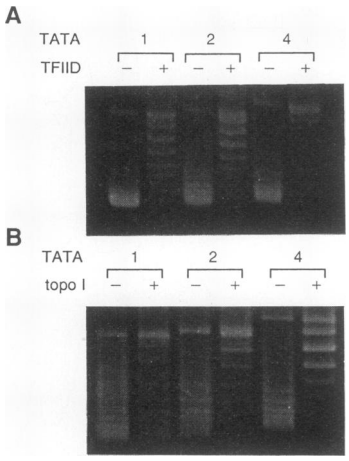

FIG. 4. Promoter sequences enhance the relaxing activity of *E. coli* topoisomerase I (topo I). Plasmids pUC1×MLP, pUC2×MLP, and pUC4×MLP, containing one (1×), two (2×), or four (4×) copies of the adenoviral MLP, respectively, were treated with TFIID (+) or not (−) (A) or with *E. coli* topoisomerase I (+) or not (−) (B) as indicated. Conditions and procedure were as for Fig. 3, except that reactions were terminated with SDS and gel electrophoresis was performed directly without extraction of DNA, as for Fig. 2.

in the DNA. Addition of TFIID did not augment or stimulate topoisomerase I action but rather was inhibitory (not shown; the inhibition may have been due either to TFIID binding to the TATA element or to nonspecific DNA binding, revealed in DNase I footprints by spreading of protection in the presence of increasing amounts of TFIID from the TATA element to flanking regions).

Inasmuch as topoisomerase I acts on single-stranded DNA (23), we thought the enhancement of its activity by promoters might be due to the single-stranded character of the TATA sequence mentioned above (see Introduction). We investigated this possibility with the use of the single-strand-specific P1 nuclease, which is active under the same conditions as topoisomerase I, in contrast with S1 nuclease used previously (9), which requires a low pH. P1 nuclease caused no detectable cleavage of the TATA sequence in supercoiled molecules (Fig. 5, lane marked −), but in the presence of topoisomerase I (Fig. 5, lane marked +), P1 nuclease did cut more frequently at a cluster of sites about 250 bp from the site of insertion of the promoters in a region of bacterial (pUC19) DNA. The extent of cutting at these sites (the intensity integrated over the cluster of bands in Fig. 5) was as much as 5.8- or 11-fold greater for molecules containing one or two promoters and treated with topoisomerase I, respectively, than for molecules with no promoter (pUC19).

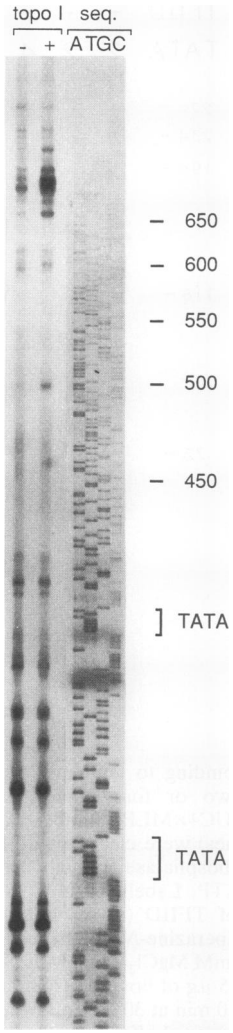

FIG. 5. Effects of *E. coli* topoisomerase I (topo I) on P1 nuclease digestion of promoter sequences in a supercoiled plasmid. Plasmid containing two copies of the adenoviral MLP (pUC2×ADMLP) was treated with *E. coli* topoisomerase I (+) or not (−) as for Fig. 3. After the incubation at 30°C, P1 nuclease (1 U; Pharmacia) was added and was incubated for 30 s at 37°C. P1 digestion was stopped by treatment with 8.3 μl of 0.4 M NaOH–25 mM EDTA for 10 min at room temperature, and DNA was extracted with phenol, precipitated with ethanol, dissolved in 15 μl of 50 mM Tris-HCl (pH 7.3)–10 mM $MgCl_2$–1 mM dithiothreitol containing 280 ng of 5′-^{32}P-labelled universal primer (Bio-Rad). The mixture was heated for 2 min at 90°C, allowed to cool to 37°C, adjusted to 20 μl with the same buffer, made 0.125 mM in all four deoxynucleotide triphosphates, and treated with 3 U of DNA polymerase I, large fragment (GIBCO), for 10 min at 37°C, and then 10 μl of 0.9 M sodium acetate–60 mM EDTA was added. DNA was precipitated with ethanol and analyzed in a 5% LONG RANGER (AT Biochemicals) sequencing gel.

DISCUSSION

A change in linking number is the sum of changes in twist and writhing (11). So the near-zero linking difference associated with TFIID binding to promoter DNA may signify either a lack of unwinding by TFIID or an amount of unwinding that is balanced by writhing in the opposite sense. When the path of DNA in the complex with TFIID is known, the writhing and thus the unwinding can be calculated. It is also possible that TFIID unwinds DNA in the presence of one or more of the other general initiation factors and RNA polymerase II. We have thus far investigated TFIIA in this regard, since it potentiates TFIID binding to DNA and interacts directly with TFIID (3), and have found no effect on unwinding by TFIID (unpublished data). Poorly efficient assembly of the entire set of general factors and RNA polymerase into functional initiation complexes in systems currently available may preclude measurements of the sort reported here to assess unwinding in the complete complex.

The contamination of purified, bacterially expressed

TFIID by topoisomerase I should be borne in mind when supercoiled templates are transcribed in systems reconstituted from purified components. Such contamination led, in the present work, to the surprising finding that *E. coli* topoisomerase I action on a supercoiled plasmid is enhanced by a mammalian promoter in the plasmid. This enhancement may reveal a significant property of TATA sequences. Topoisomerase I is believed to act at transiently single-stranded regions, and the enhancement of its activity may reflect a greater tendency for unwinding within the promoter, for example at the TATA sequence, or an effect of the promoter on unwinding elsewhere in the circular DNA molecule. Digestion with P1 nuclease revealed no single-stranded character of the TATA sequence, but there was an increase in cutting in the presence of topoisomerase at sites about 250 bp away in a region of the bacterial plasmid. Lack of P1 cutting does not rule out the possibility of transient unwinding of the TATA element or of unwinding facilitated by topoisomerase I. The occurrence of P1 cutting at remote sites in the presence of topoisomerase I might reflect a tendency of the nuclease to cleave there in relaxed molecules or interaction of topoisomerase with the remote sites. Such interaction might, in turn, be enhanced by the TATA element through a variety of mechanisms, including the following: (i) Initial binding of topoisomerase to the TATA element, followed by "oozing" due to interaction of further topoisomerase molecules with a series of adjacent sites along the DNA; (ii) Initial interaction of topoisomerase with the TATA element, followed by wrapping around the enzyme to bring about the closer proximity of distant sites in the DNA; (iii) Transient unwinding at the TATA element, followed by propagation of the single-stranded region to distant sites (10); and (iv) a direct influence of the TATA element on the properties of adjacent regions through cooperative interactions (4). Further work is needed to distinguish among these possibilities and assess the implications for transcription.

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