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A comparison of the DNA bending activities of the DNA binding proteins CRP and TFIID

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ABSTRACT

Protein-induced DNA bending is of importance in the formation of complex nucleoprotein assemblies such as those involved in the initiation of DNA replication or transcription initiation. We have compared the DNA bending characteristics of the *Escherichia coli* cyclic AMP receptor protein (CRP or CAP), an archetypal DNA bending protein, to those of TFIID, the eukaryotic TATA-element binding transcription factor. By altering the helical phasing between a CRP binding site and the *E.coli melR* promoter we have mapped a DNA sequence-directed bend in the downstream region of the promoter. This intrinsic DNA bend may be important in the regulation of the *melR* promoter by CRP *in vivo*. Gel retardation assays and DNAse I footprinting show that human TFIID binds to the *melR* promoter −10 region. Taking advantage of this fact, and using the CRP-induced DNA bend as a standard, we have employed phase sensitive detection to show that the DNA bend angle induced by TFIID is far less than that induced by CRP. Further evidence to support this conclusion comes from a comparison of the relative mobilities of CRP-DNA and TFIID-DNA complexes. These results place limits on the role of any DNA bending induced by TFIID alone in the initiation of transcription.

INTRODUCTION

The regulation of gene expression in both prokaryotes and eukaryotes involves sequence-specific DNA binding proteins which bind at a variety of distances from the transcription start point and stimulate transcription initiation. On binding DNA, many of these proteins have also been shown to induce DNA bending, reviewed by Travers (1), however, it is not clear at this time whether these protein-induced DNA bends are important for the mechanism of transcription activation.

The most comprehensively studied DNA bending transcription factor is the cyclic AMP receptor protein (CRP or CAP) of *E.coli* (2). On binding cyclic AMP (cAMP), the CRP-cAMP complex binds to a 22bp sequence found upstream of a number of catabolite sensitive genes and activates transcription (3–5). DNA bending induced by CRP binding has been demonstrated by a number of independent techniques, for example, the anomalous electrophoretic mobility of CRP-cAMP-DNA complexes (6,7), alteration of the intramolecular ligation rate of DNA molecules on binding CRP-cAMP (8), electron microscopy (9), and electrodichroism measurements of rotational relaxation times of CRP-cAMP-DNA complexes (10). Furthermore, DNA bending is also apparent in the crystal structure of the CRP-cAMP-DNA complex (11).

Transcription factor TFIID was first identified as a chromatographic fraction essential for the transcription of eukaryotic genes *in vitro* (12–14). TFIID binds to DNA templates carrying a TATAAA or related sequence (the TATA box), which is usually found around 30bp upstream from the transcription start point (15). Binding of TFIID to the TATA box appears to be a rate-limiting step in the formation of a complex competent for transcription initiation (16–18). TFIID is a multisubunit protein of which one subunit is the TATA box binding protein or TBP (19). The complementary DNA (cDNA) of the TBP has been cloned from a variety of species, reviewed by Greenblatt (20) and the encoded proteins shown to bind as monomers to the TATA box (21).

Gel retardation assays (22,23) can be used to investigate both sequence-specific and protein-induced DNA bending (24,7). DNA fragments which contain either an intrinsic DNA bend or a protein-induced DNA bend migrate slower in polyacrylamide gels if the locus of bending is near the centre of the fragment rather than near one end. When two bending loci are present on one DNA fragment, the mobility of the DNA depends on the phasing of the bend centres; when the bends are in phase, the mobility is lower than when they are out of phase (25). Using a circular permutation assay in which a TFIID binding site is placed at different positions relative to the ends of a series of DNA fragments, Horikoshi et al. (26) have recently shown that TFIID induces a change in the conformation of the DNA to which it binds. However, an important limitation of the circular permutation assay is that it cannot differentiate between a protein-induced DNA bend and an increase in DNA flexibility at the protein binding site, nor can this assay determine the direction of any induced DNA bend (25).

Taking the DNA bend induced by CRP as a reference we have used phase sensitive detection to identify a sequence-dependent bend in the DNA downstream of the *E.coli melR* promoter

(27,28). Using the same technique we then investigated the DNA bending properties of human TBP. We demonstrate that under conditions where CRP induces significant changes in DNA bending human TBP has little effect. These results are discussed with regard to the role TFIID-induced DNA bending may have in the formation of complexes competent for transcription initiation.

MATERIALS AND METHODS

DNA fragments

Plasmid pAA182 (29) and *E. coli* host strain M182 Δ*crp* (30) were used for all recombinant DNA work. Plasmid DNA isolation, restriction endonuclease treatment and isolation and labelling of DNA fragments were performed as described by Maniatis et al. (31). For gel retardation assays and DNAse I footprinting experiments, DNA fragments carrying each promoter were isolated using *Eco*RI and either *Hae*III, *Hha*I, or *Bst*NI. Fragments were labelled at the *Eco*RI end with α^{32}P dATP using Klenow enzyme (the DNA fragments used in this study are shown in Figure 1).

Purified proteins

CRP was purified from *E.coli* strain M182 *crp*$^+$ using cAMP-agarose (Sigma, Cat. No. A6885) according to the method of Ghosaini et al. (32). Purified human TFIID, TATA binding protein (TBP) was obtained from Promega (Cat. No. E3081).

Gel retardation assays

Labelled DNA fragments (less than 1nM) were incubated with either 10nM CRP and 200μM cAMP or various amounts of human TBP in standard buffer (40mM Tris pH 8·0, 10mM $MgCl_2$, 100mM potassium glutamate, 1mM DTT, and 0·1mg/ml bovine serum albumin). After 30 minutes incubation at room temperature, free and bound DNA were separated on 6% polyacrylamide gels run in TBE as previously described (33). The gels were dried onto Whatman 3MM paper and exposed to Kodak X-0MAT film at −70°C with an intensifying screen.

DNAse I footprinting

DNA fragments (2−5nM) labelled at the *Eco*RI ends were incubated with increasing amounts of TFIID in the conditions described above. After 30 minutes incubation the complexes formed were digested with DNAse I (34) and the reaction products separated on 8% sequencing gels and visualised by autoradiography as previously described.

RESULTS

Binding of CRP to *melR* promoter fragments

We have previously described a series of synthetic promoters (shown in Figure 1) based on the *E. coli melR* promoter, but containing a CRP binding site which more closely resembles the consensus (35) positioned at a variety of distances upstream from the *melR* −10 region (28). Promoter CC has a 31bp spacing between the centre of symmetry of the CRP binding site and the −10 region, the same spacing found in the wild-type *melR* promoter (27). The other members of the promoter series contain insertions between the CRP binding site and the −10 region, for example, CC+4 and CC+10 contain 4 and 10bp insertions, respectively. The CRP-cAMP complex activates transcription from these promoters *in vivo* only when the CRP binding site and the *melR* −10 region are separated by integral or near integral turns of the DNA helix (28).

We have investigated the binding of CRP-cAMP to this series of promoters using gel retardation assays. Labelled 341bp *Eco*RI-*Hae*III DNA fragments carrying each of the promoters shown in Figure 1 were incubated with CRP-cAMP as described in the methods. Free and bound DNA were then resolved by electrophoresis on a 6% polyacrylamide gel and visualised by autoradiography. As can be seen from the resulting autoradiograph (Figure 2A) the mobility of the CRP-cAMP-DNA complex depends on the phasing of the CRP binding site and sequences present in the *melR* fragment. The mobilities of the retarded bands show a helical periodicity indicating that the *melR* fragment contains a sequence-induced DNA bend. When the CRP-induced DNA bend and the *melR* sequence-induced DNA bend are in phase (linker lengths 0, 10, 11, 19 and 21bp) the mobility of the complex is low, when the bends are out of phase (linker lengths 4 and 14bp) the mobility of the complex is high. Similar results have been reported by Zinkel and Crothers (25) who showed that phase sensitive detection could be used to identify intrinsic bends in DNA fragments carrying the *lac* promoter.

To map the position of the sequence-directed bend in the *melR* fragment, the restriction enzymes *Hha*I and *Bst*NI were used to sequentially remove DNA from the 3′ end of the *Eco*RI-*Hae*III fragment (see Figure 1). Removal of sequences from +254 to +122 by digestion with *Hha*I has no effect on the relative mobilities of the CRP-DNA complexes (Figure 2B). After removal of sequences between +122 and +5 by digestion with *Bst*NI the relative mobilities of the CRP-DNA complexes no longer show significant helical phase variation (Figure 2C). These

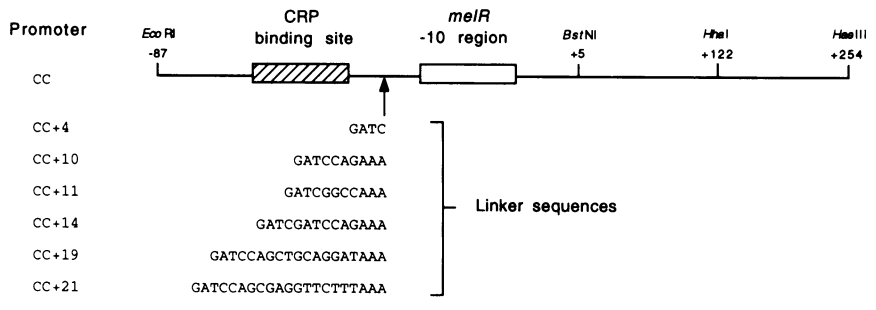

Figure 1. DNA fragments used in this study. The construction of this series of *melR* promoter derivatives has been previously described (28). Each construct contains a CRP binding site (hatched box) separated by a variable length linker from the *melR* −10 region (empty box); for clarity only the sequences of the variable linkers are shown. DNA fragments labelled at the *Eco*RI end were digested with either *Hae*III, *Hha*I, or *Bst*NI to generate the DNA's used in this study.

results show that the *melR* fragment contains a sequence-dependent DNA bend located between the region around base pair +5 and base pair +122. Importantly, Figure 2C also shows that the slight change in the size of the DNA fragments as the linker length is increased has little or no effect on the migration of the CRP-DNA complex.

TFIID binds to the *melR* promoter −10 *in vitro*

In order to investigate TFIID-induced DNA bending, we decided to utilise the *melR* series of constructions. The prokaryotic −10 region or Pribnow box (36) has long been known to be similar in sequence to the TATA box of eukaryotic genes (15). However, the binding of TFIID or TBP to a prokaryotic −10 region has not been demonstrated. On the basis of a comparison of the consensus TATA box with the consensus Pribnow box (shown in Figure 3A), and knowledge of the effects of mutations of the TATA box sequence on TFIID binding (37), we reasoned that TFIID should be able to bind the *melR* −10 region. We incubated labelled *Eco*RI-*Hae*III DNA fragments carrying the CC promoter with increasing amounts of purified TFIID as described in the methods. After 30 minutes incubation at room temperature, free and bound DNA fragments were separated by electrophoresis and visualised by autoradiography. Figure 3B shows that as the concentration of TBP is increased a retarded band becomes apparent (indicated by the thick arrow in Figure 3B). This shows that TBP binds specifically to the *melR* fragment. At higher TBP concentrations a faint band of lower mobility can be seen (indicated by the thin arrow in Figure 3B); this band probably corresponds to the weak binding of a second molecule of TBP to the fragment.

To determine the exact position of the strong TBP binding site on the *melR* promoter fragment, we used DNAse I footprinting. Labelled CC promoter fragment was incubated with TBP exactly as above except that after 30 minutes the DNA was digested with DNAse I as described in the methods. The digestion products were separated on 8% sequencing gels and visualised by autoradiography (in each case both the upper and lower strands were digested with DNAse I although only the results obtained with one of the strands are shown). Probing with DNAse I revealed that TFIID weakly protects sequences in the *melR*

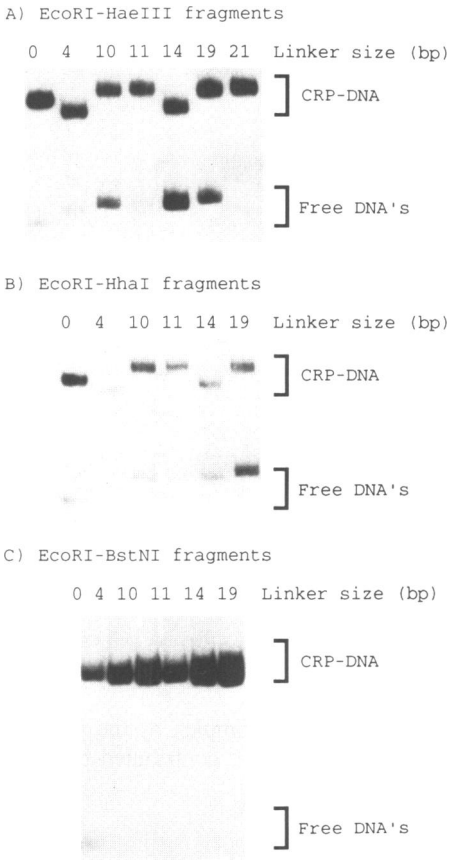

Figure 2. Binding of CRP to the CC promoter series. (A) *Eco*RI-*Hae*III fragments labelled at the *Eco*RI end were incubated with 10nM CRP in the presence of 10μM cAMP for 20 minutes at room temperature. Free and bound DNA were then separated on 6% polyacrylamide gels and visualised by autoradiography. (B) and (C) show the same experiment repeated using *Eco*RI-*Hha*I and *Eco*RI-*Bst*NI fragments respectively.

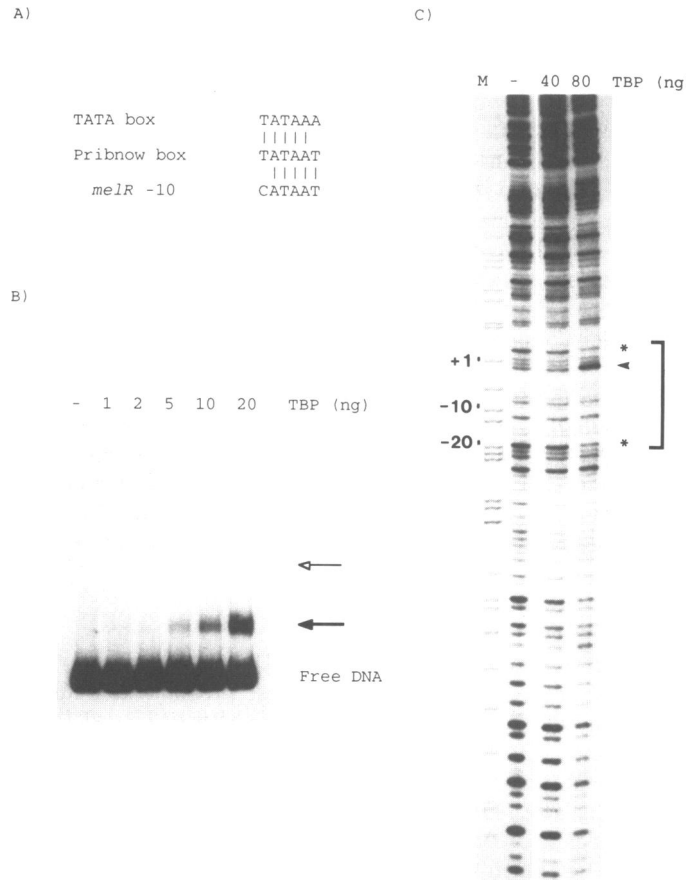

Figure 3. Binding of TFIID to the *melR* −10 region. (A) The consensus eukaryotic TATA box sequence (top line) is shown aligned with the consensus prokaryotic −10 region (middle line) and the *melR* −10 region (bottom line). Bases that are shared between any two sequences are indicated by vertical bars. (B) Labelled *Eco*RI-*Hae*III fragment carrying the CC promoter was incubated with increasing quantities of human TBP as described in the text. Free and bound DNA were then separated by electrophoresis on a 6% polyacrylamide gel. The major TBP-DNA complex is indicated by the thick arrow, whereas, the minor, weaker complex is indicated by the thin arrow. (C) Protein-DNA complexes from part B were attacked with DNAse I as described in the text. The products of digestion were separated on an 8% polyacrylamide sequencing gel and visualised by autoradiography. Lane M contains M13 sequence marker fragments. Protection (stars) and enhancement (arrow head) of DNAse I cleavage and the limits of the DNAse I footprint (bracket) are shown in the figure. The numbers indicated refer to the position of bases relative to the *melR* transcription start point.

promoter −10 region and leads to the enhancement of cleavage at one position (indicated by the arrow head in Figure 3C). These results demonstrate that the *melR* −10 region functions as a binding site for human TBP.

As there is no change in the helical phasing of the TBP binding site (*melR* −10) and the sequence-directed DNA bend present in the *melR* transcribed region, the binding of TBP alone to the *Eco*RI-*Hae*III series of fragments produces TBP-DNA complexes which have identical relative mobilities (data not shown). In order to examine any DNA bending induced by TBP it is necessary to compare the mobility of CRP-TBP-DNA ternary complexes in which the phasing of the CRP and TBP binding sites has been altered.

Non-cooperative binding of CRP and TBP

Although the CRP-cAMP complex binds strongly to the consensus CRP binding site present in the synthetic *melR* promoter derivatives, CRP-cAMP only binds to the wild-type *melR* promoter in the presence of RNA polymerase (27). This cooperative DNA binding is also seen when CRP and RNA polymerase bind to other promoters (34) and when CRP binds in conjunction with other proteins, for example, the CytR repressor (38). In view of this potential for cooperativity, we assayed the effect of CRP-cAMP on the DNA binding activity of TBP. Figure 4 shows the result of a gel retardation experiment in which increasing amounts of TBP were incubated with labelled *Eco*RI-*Hae*III fragment carrying the CC *melR* promoter in either the presence (lanes 3 to 6) or absence (lanes 7 to 10) of CRP-cAMP. As can be seen from the figure, CRP alone binds to the CC fragment and produces a retarded band (Figure 4, lane 2). In the presence of CRP and TBP, a ternary complex consisting of CRP-cAMP and TBP bound to the DNA can be seen migrating slower than the CRP-cAMP-DNA complex (Figure 4, lanes 3 to 6). As the concentration of TBP is increased the intensity of this band rises, however, this increase is similar in the absence of CRP (compare lanes 3 to 6 with lanes 7 to 10). Therefore, the data show that there is no cooperativity in the binding of CRP and TBP.

A comparison of DNA bending by CRP and TFIID

The CRP-DNA complex and the TBP-DNA complex show very different gel mobilities even when these proteins are bound to identical DNA fragments (for example compare Figure 4, lane 2 and lane 9). The relative mobility shift induced by CRP is much larger than that induced by TBP even though the proteins are of similar size (47kD and 38kD respectively). This shows that under identical conditions the extent of DNA bending induced by CRP is much greater than that induced by TBP. Further evidence to support this conclusion comes from a comparison of the gel mobilities of CRP-TBP-DNA complexes in which the helical phasing of the TBP binding site (the *melR* −10 region) and the CRP binding site is varied. Figure 5 shows the results of a gel binding assay in which TBP is bound to either the *Eco*RI-*Hae*III series of fragments (Figure 5A) or the *Eco*RI-*Bst*NI series of fragments (Figure 5B) in the presence of CRP. As can be seen from the figure, the helical phase of the CRP binding site and the TBP binding site (*melR* −10 region) does not affect the mobility of the CRP-TBP-DNA complex to any great extent. The *Eco*RI-*Hae*III series shows the same helical variation of mobility when either CRP alone or CRP and TBP are bound (compare Figure 2A with Figure 5A) as does the *Eco*RI-*Bst*NI series (compare Figure 2C with Figure 5B). Therefore, any DNA bending contributed to the complex by the presence of TBP is insignificant compared to that contributed by CRP.

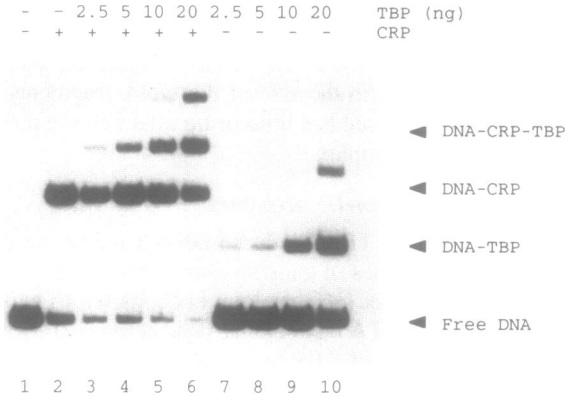

Figure 4. CRP and TFIID bind non-cooperatively to the *melR* promoter. An *Eco*RI-*Hae*III fragment carrying the CC promoter and labelled at the *Eco*RI end was incubated with the indicated amounts of human TBP in the presence (lanes 3 to 6) or absence (lanes 7 to 10) of 10nM CRP. The various protein-DNA complexes were resolved on a 6% polyacrylamide gel and visualised by autoradiography as above.

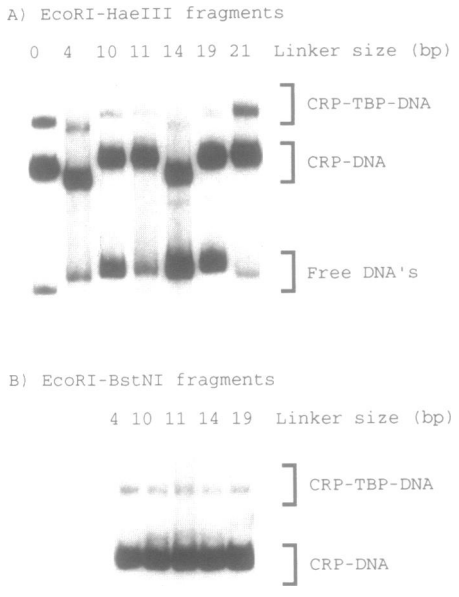

Figure 5. The mobility of TBP-CRP-DNA complexes. (A) *Eco*RI-*Hae*III fragments carrying each promoter construct were incubated with CRP-cAMP as in Figure 2 except that 10ng TBP was included in the reaction mix. Protein-DNA complexes were separated on 6% polyacrlyamide gels exactly as before. (B) This shows the same experiment as in part A performed using *Eco*RI-*Bst*NI fragments.

DISCUSSION

The formation of complex protein-DNA assemblies important for cellular processes such as replication, transcription, and recombination, involves the folding of DNA into ordered three dimensional arrays. Sequence-specific and protein-induced DNA bending is thought to be important in the formation and maintenance of these structures. One situation in which DNA bending has been shown to be important is the regulation of

transcription initiation in prokaryotes (40–42). Sequence-specific or protein-induced DNA bends can activate transcription both *in vitro* and *in vivo* (43–45). Furthermore, the binding of RNA polymerase itself has been shown to induce DNA bending in the region downstream from the transcription start site (46).

Transcription from the *E. coli melR* promoter is totally dependent on the CRP-cAMP complex, an archetypal DNA bending protein (27,47). Taking the DNA bend induced by CRP as a reference we have used phase sensitive detection to map a sequence-directed bend in the DNA between residues +5 and +122 of the *melR* promoter. This region of the *melR* promoter contains a homopolymeric run of five A residues from position +57 to +61 which may be the source of the DNA bend (48,49). It is interesting to note that the distances between the CRP site and the −10 region at which CRP activates transcription from the *melR* promoter *in vivo*, are the same as those at which the CRP-induced DNA bend and the intrinsic *melR* DNA bend are in phase. Thus, at the CRP site to −10 spacings at which the CRP-DNA complex has low mobility (linker lengths 0, 10, 11, 19, and 21bp) the corresponding promoters (CC, CC+10, CC+11, CC+19, and CC+21) are activated by CRP *in vivo* (28). At the CRP site to −10 spacings at which the CRP-DNA complex has high mobility (linker lengths 4 and 14bp) the corresponding promoters (CC+4 and CC+14) are not activated by CRP *in vivo*. The *melR* sequence-specific DNA bend might therefore be important in the activation of transcription from this promoter by CRP. The presence of the sequence-directed DNA bend might facilitate activation by CRP, either by promoting RNA polymerase binding as has been shown for upstream DNA bends in the *galP1* promoter (42) or by aiding the escape of RNA polymerase after open complex formation, a step in transcription initiation at which CRP has previously been shown to act in the *malT* promoter (50).

Using gel retardation assays and DNAse I footprinting we have shown that human TBP, the DNA binding component of TFIID, binds to the *melR* −10 region. This confirms the well known similarity between the TATA box sequence, found around 30bp upstream from the transcription start point in eukaryotic promoters, and the Pribnow box sequence (or −10 region), found around 10bp upstream from the transcription start point in prokaryotic promoters. The ability of TBP to bind to this DNA was predicted from the sequence requirements of TFIID. Mutations which change the consensus TATA box from TATAAA to CATAAA or TATAAT, the two differences between the TATA box and the *melR* −10 (CATAAT), have been shown to reduce but not abolish TFIID-dependent transcription *in vitro* (37). We have exploited the −10 binding activity of TBP to compare the DNA bending properties of human TBP with those of CRP.

Horikoshi et al. (26) have reported that human and yeast TBP induce DNA bending on binding to circular permuted DNA fragments carrying the adenovirus TATA box. Using the phase sensitive assay that successfully mapped the intrinsic DNA bend in the *melR* promoter we failed to detect DNA bending induced by human TBP. This implies that under the conditions used here any DNA bending induced by the binding of human TBP alone is of minor importance compared to that induced by either CRP or the *melR* sequence-directed bend. Further evidence to support this conclusion comes from a comparison of the mobilities of CRP-DNA and TBP-DNA complexes. The binding of CRP to *melR* promoter fragments produces a much greater relative mobility shift than does the binding of TBP (Figure 4). This indicates that the DNA bend angle induced by CRP is far greater than that induced by TBP. A similar result has been reported by Koudelka (39) who showed that differences in the relative mobility shifts induced by CRP and 434 repressor reflect differences in protein-induced DNA bending. Although it is possible that the absence of detectable TBP-induced DNA bending in these assays is due to the fact that we are not using a natural TFIID binding site, this seems unlikely given the fact that TFIID binds to a large number of DNA sequences which deviate widely from the consensus (51,37). In addition, the DNAse I footprint of TBP bound to the *melR* −10 is very similar to that of TBP bound to the TATA box present in the SV40 early promoter (K.G. unpublished observations).

Circular permutation assays cannot distinguish between a protein-induced DNA bend and a protein-induced increase in DNA flexibility (25). Since phase sensitive detection shows little or no TBP-induced DNA bending, one possible explanation for our results is that TBP increases the flexibility of the DNA surrounding the TATA box. However, an increase in DNA flexibity at the TBP binding site would be expected to affect the phase of the CRP-induced and sequence-dependent bends in the CRP-TBP-DNA complex and this is not the case (see Figure 5A). Another possibility is that the bend angle induced by human TBP is too low to be measured by this method, in which case its role *in vivo* must be open to question. Finally, it may be that human TBP-induced DNA bending is augmented by the presence of TBP accessory proteins. This may also explain the reported differences in DNA bending activity of human and yeast TBP (26). The small changes in the mobility of human TBP-DNA complexes as the position of the TBP binding site is varied (26) may simply be due to the intrinsic effects of altering the position of the bound protein, whereas, yeast TBP may have true DNA bending activity.

In some circumstances a protein-induced DNA bend can be replaced by a sequence-specific DNA bend or one DNA bending protein may be able to functionally replace another DNA bending protein. For example, Goodman and Nash (52) have shown that the DNA bend induced by IHF (integration host factor) can be functionally replaced by a CRP-induced DNA bend. Similarly, the DNA bend induced by CRP can, to some extent at least, be replaced by suitably positioned sequence-directed DNA bends (43,45). It will be interesting to determine whether any DNA bending induced by human TBP can be functionally replaced by either of these elements.

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