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In simple synthetic promoters YY1-induced DNA bending is important in transcription activation and repression

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ABSTRACT

Depending on promoter context, YY1 can activate or repress transcription, or provide a site for transcription initiation. To investigate whether the ability of YY1 to induce DNA bending influenced its ability to activate and repress transcription, simple synthetic promoters were constructed in which the YY1 binding site was inserted between the TATA box and either the NF1 or AP1 recognition sequences. In transient transfections of COS cells, the NF1YY1TATA and NF1RYY1TATA promoters exhibited a dramatic 15–20-fold increase in correctly initiated transcription. These promoters exhibited even larger 60–80-fold increases in transcription in HeLa cells. Neither multiple copies of the YY1 binding site alone, nor placement of a YY1 site upstream of the NF1 site activated transcription. Deletion of 4 bp between the NF1 and YY1 sites, which changes the phase of the DNA bends, abolished the 16-fold activation of transcription by NF1YY1TATA. Insertion of the YY1 site between the AP1 site and the TATA box decreased transcription ~3-fold. Replacing the YY1 binding site with an intrinsic DNA bending sequence mimicked this transcription repression. Sequences of similar length which do not bend DNA fail to repress AP1-mediated transcription. Gel mobility shift assays were used to show that binding of YY1 to its recognition sequence did not repress binding of AP1 to its recognition sequences. Our data indicate that YY1-induced DNA bending may activate and repress transcription by changing the spatial relationships between transcription activators and components of the basal transcription apparatus.

INTRODUCTION

The ability of transcription factors to regulate gene expression is thought to be a function of their affinity for their DNA recognition sequences, their interactions with other transcription factors, and the efficiency with which they make protein–protein contacts with components of the basal transcription apparatus (1–3). Interaction between components of the basal transcription machinery and regulatory proteins bound to non-contiguous sites on DNA may require distortion of the DNA helix by DNA looping (4,5), or by DNA bending (6). In prokaryotes, a role for DNA bending in replication, recombination and regulated transcription has been clearly established (7–13). However, evidence implicating DNA bending in eukaryotic transcription regulation is less direct. Steroid/nuclear receptors, other transcription activators (14–18), and several basal transcription factors, bend DNA upon binding to their recognition sequences (19–22). Prebending of DNA alters the affinity for their recognition sequences of DNA binding proteins such as the TATA binding protein (23), or the estrogen receptor DNA binding domain (J. Kim *et al*., unpublished observations). Recently, we reported that intrinsically bent DNA in a transcription factor binding site potentiates transcription (24,25). These and other eukaryotic bending studies suggest that altering promoter architecture by DNA bending may exert a significant effect on gene transcription. Nevertheless, a functional role for transcription factor-induced DNA bending in the regulation of eukaryotic transcription has not been definitively established.

While many eukaryotic transcription regulatory proteins possess domains which enable them to function as independent activators or repressors of transcription, recent studies demonstrate the existence of a different class of transcription regulatory protein. These proteins act indirectly by bending the DNA and creating a promoter architecture which facilitates or impairs interactions between other transcription factors and components of the basal transcription machinery (26). Among the proteins proposed to work as structural or ‘architectural’ transcription regulators are LEF1 (27,28), HMG I/Y (29), SRY (30,31) and UBF (32). Some regulatory proteins, such as YY1, may have properties in common with both classes of protein.

YY1 is a ubiquitously expressed zinc finger protein whose binding sites have been identified in several different promoters and enhancers (30,33–42). YY1 represses transcription from the c-*fos* (30), adeno-associated virus p5 (33), β-casein (34) and skeletal muscle α-actin promoters (35). YY1 activates transcription of the c-*myc* promoter (36), and the promoters of ribosomal proteins L30 and L32 (37). An unusual characteristic of YY1 is the coincidence of its binding site with the transcription initiation site in the adeno-associated virus p5 promoter (38,39). This functional versatility of YY1 has been explained by several

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models. Interaction with cellular or viral regulatory proteins such as cyclophilin A, c-*myc* and viral EIA appears to alter the activity of YY1 (40,41). The ability of YY1 to activate and repress transcription has also been attributed to the presence of distinct activation and repression domains (42). Another simple model to explain the functional versatility of YY1 is based on the observation of Natesan and Gilman (30) that binding of YY1 to its recognition sequence in the c-*fos* promoter induces a DNA bend of ~80° toward the major groove. Although the role of YY1 in transcription regulation has received considerable study, the contribution of the YY1-induced DNA bend to transcription activation and repression is not clear.

In this work we investigate the role of YY1-induced DNA bending in the modulation of transcription by NF1 or AP1. We studied YY1 action in an *in vivo* context, in which proteins which interact with YY1 are present. We provide evidence that YY1-induced DNA bending, which can bring NF1 closer to the basal transcription apparatus, is important in NF1-mediated transcription activation. We also show that YY1-induced DNA bending is likely to be responsible for repression of AP1-mediated transcription, since we can mimic YY1-induced repression with an intrinsic DNA bend producing the same degree of DNA bending as YY1.

MATERIALS AND METHODS

Plasmid constructions

All the plasmids used in this study were constructed by inserting oligonucleotides or DNA fragments into the polylinker sites of a TATACAT plasmid which contains a consensus TATA box from the efficiently transcribed *Xenopus* vitellogenin B1 gene, and the 5′-flanking region of the vitellogenin B1 promoter up to –42. This sequence contains no known transcription factor binding sites other than the TATA sequence (24,43). The plasmids NF1TATACAT and AP1TATACAT were prepared by cloning the synthetic NF1 consensus sequence 5′-ATTGGCTATGAGCCAAT-3′ (44), or the synthetic AP1 consensus sequence 5′-CATGAGTCAGAT-3′ (45), respectively, into the blunt-ended *Xba*I site of the TATACAT plasmid. The plasmids YY1TATACAT, mYY1TATACAT, 8YY1TATACAT, NF1YY1TATACAT, NF1RYY1TATACAT (reverse orientation of the YY1 binding site) and NF1mYY1TATACAT were constructed by inserting double-stranded oligonucleotides containing the YY1 binding site 5′-GATCTGACCATCTCTAGATC-3′ (30) or the mutated core YY1 binding site 5′-GATCTGCAGATCTCTAGATC-3′ (30) into the blunt-ended *Bgl*II site of the TATACAT or NF1TATACAT plasmids. The plasmids, $NF1_{(-4)}YY1_{(-4)}TATACAT$, $NF1_{(-4)}mYY1_{(-4)}TATACAT$ designed to test helical phasing and the AP1-containing plasmids, AP1YY1TATACAT and AP1mYY1TATACAT were prepared by direct insertion of YY1 or mYY1 oligonucleotides into the *Bgl*II site of NF1TATACAT or AP1TATACAT. Plasmids YY1NF1TATACAT and YY1AP1TATACAT were prepared by insertion of the YY1 oligonucleotide into the blunt-ended *Sal*I site of NF1TATACAT or AP1TATACAT. DNA fragments containing the intrinsic DNA bending sequence, AAAAAAGCGC (which we refer to as A_6GCGC; 46), from the plasmid 4 (A_6GCGC)TATACAT (24) and a control sequence of the same length from the plasmid 4($A_2C_2A_2$GCGC)TATACAT, which was constructed as described for the 4(A_6GCGC)TATACAT plasmid (24,46), and the multiple cloning site (MCS) of the pTZ18U plasmid (47), were blunt-ended by T4 DNA polymerase after *Pst*I/*Xba*I or *Eco*RI/*Hin*dIII digestion. Gel-purified DNA fragments were inserted into the blunt-ended *Bgl*II site of AP1TATA. DNA sequencing was used to confirm the identity of all constructions.

Cell culture, transfection and CAT assays

Transient transfections of HeLa cells were carried out essentially as described by Natesan and Gilman (30). COS-7 cells were grown at 37°C in Dulbecco's modified Eagle's medium/F-12 medium (DME-F12, Sigma) supplemented with 10% fetal bovine serum. A total 8 µg DNA, including 4 µg reporter plasmid, 0.5 µg TK-luciferase as an internal standard, and 3.5 µg pTZ18U plasmid as carrier, was transfected by the calcium phosphate–DNA coprecipitation method, as we have recently described (24). Five hours after adding crystals to the cells, the cells were subjected to a 3 min shock with 10% DMSO in serum free-medium. Forty-eight hours after shock, the cells were harvested for CAT and luciferase assays. The transfected cells were broken by three rounds of freezing and thawing and cell debris was sedimented by centrifugation. The supernatant was assayed by the quantitative mixed phase CAT assay method (48). CAT activity was normalized to luciferase activity assayed in the same amount of crude whole cell extracts.

Gel mobility shift assays

COS cell nuclear extracts were prepared as described (24,49). DNA fragments (*Sal*I/*Xba*I) containing NF1 or AP1 sites and a double stranded YY1 oligonucleotide were labeled with [α-^{32}P]dCTP. The DNA fragments were fractionated on a 5% native acrylamide gel, and eluted by shaking the gel segments overnight in TE buffer. For unlabeled competitor DNAs, DNA fragments were digested with restriction enzymes, fractionated and electroeluted. Gel shift assays were carried out as described (24), with minor modifications. Briefly, the ^{32}P-labeled DNA fragment (10 000 c.p.m.) was incubated for 15 min at room temperature with the following: 5.2 µg COS nuclear extracts, 2 µg poly dI/dC, 0.5 µg pTZ18U plasmid, 10% glycerol, 40 mM KCl, 15 mM Tris–HCl, pH 7.9, 0.2 mM EDTA and 0.4 mM dithiothreitol in a final volume of 20 µl. In the competition experiments, the indicated amounts of unlabeled DNA fragments were preincubated with the reaction mixture lacking the labeled probes for 15 min on ice. The binding reaction was initiated by adding the labeled probe and the samples were incubated for an additional 15 min at room temperature. Radiolabeled bands were visualized by autoradiography and quantitated with a PhosphorImager (Molecular Dynamics Corp.).

RESULTS

COS cell nuclear extracts contain proteins which bind to the YY1, NF1 and AP1 sites

The role of YY1-induced DNA bending in gene expression was investigated using NF1 and AP1 sites in simple synthetic promoters. NF1, AP1 and YY1 are well-studied transcription regulators, which are present in a variety of cells. To determine whether COS cells, which were used in most of these studies, contained proteins able to bind to the NF1, AP1 and YY1 recognition sequences, gel mobility shift assays were carried out (Fig. 1). Labeled oligonucleotides containing YY1, NF1 and AP1 sites showed distinct protein–DNA complexes in the presence of COS cell nuclear extracts. To demonstrate that the gel shifted

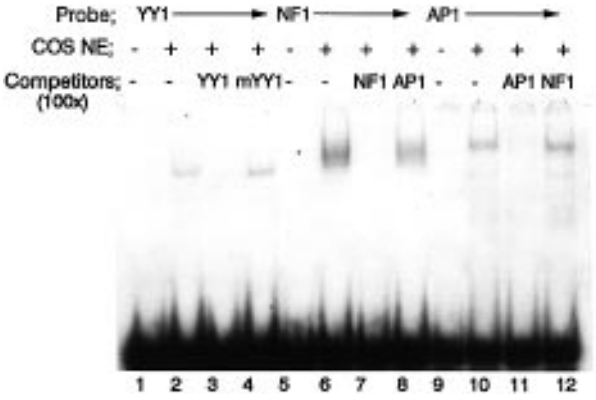

Figure 1. COS cell nuclear extracts contain proteins which bind to the YY1, NF1 and AP1 recognition sequences**.** DNA fragments (*Sal*I–*Xba*I) containing AP1 or NF1 sites and a double stranded oligonucleotide containing YY1 were labeled with [α-^{32}P]dCTP and competition gel mobility shift assays were carried out as described in 'Materials and Methods'. A 100-fold molar excess of the unlabeled oligonucleotides, YY1, mYY1, NF1 or AP1 were added to the indicated COS cell nuclear extracts and the binding reaction was initiated by adding the YY1, NF1 or AP1 radioactive probes. Protein–DNA complexes and free probe were resolved by acrylamide gel electrophoresis at low ionic strength and visualized by autoradiography.

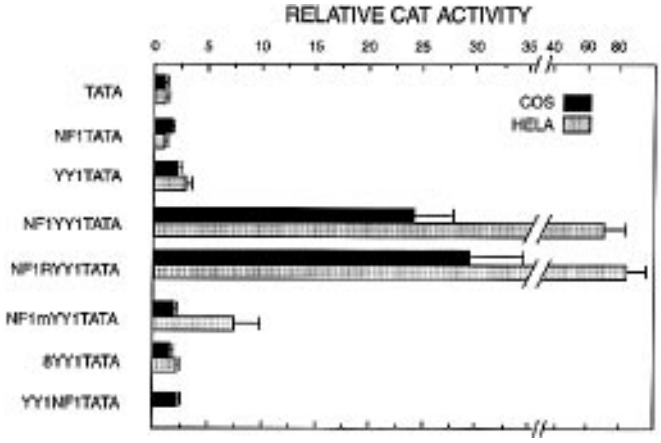

Figure 2. YY1 potentiates transcription activation by NF1. Plasmid constructions, transfections, CAT and luciferase assays were as described in 'Materials and Methods'. The plasmids were cotransfected into COS cells (black bars) or into HeLa cells (shaded bars) with TK luciferase (used as an internal standard). After 48 h, the cells were harvested and assayed for CAT and luciferase activity. CAT activity was normalized to the activity obtained with the TATACAT plasmid, which was set equal to 1. The data for each sample represent the mean of five independent transfections ± s.e.m. The plasmid NF1RYY1TATA contains the YY1 binding site in the same position relative to the NF1 site, but in the reverse orientation. In plasmids without any special notation the YY1 site CCAT is in its original orientation. In the plasmid 8YY1TATA, eight copies of the YY1 binding site were inserted at the same relative position as the YY1 site in the YY1TATA plasmid. In the YY1NF1TATA plasmid, the YY1 site was inserted into the *Sal*I site of the NF1TATA plasmid, which is 35 nucleotides upstream of the NF1 site.

bands represented sequence-specific binding by YY1, NF1 and AP1, we added to the binding reaction a 100-fold excess of either a specific recognition sequence, or a non-specific competitor of similar size. Consistent with previous studies (30), unlabeled YY1 sequence effectively competed for binding, while a closely related mutated YY1 sequence (mYY1) was unable to compete (Fig. 1, lanes 2–4). Binding to NF1 and AP1 was also sequence-specific (Fig. 1, lanes 6–8 and 10–12). Similar results were observed with HeLa cell nuclear extract, indicating that HeLa cells also contain YY1, NF1 and AP1 binding proteins (data not shown).

YY1 potentiates transcription activation by NF1

To investigate the effect of YY1 binding on transcription by NF1, we prepared simple synthetic promoters containing single copies of the NF1 or YY1 recognition sequences linked to a consensus TATA box. Plasmids containing the promoters were transfected into COS and HeLa cells, and CAT activity was determined. CAT activity from the plasmids containing the activator sequences in various combinations was compared with the activity of the TATACAT plasmid, which was set equal to 1. In COS and HeLa cells the plasmids containing single copies of the NF1 or YY1 recognition sequences exhibited a minimal increase in transcription (Fig. 2). Insertion of a YY1 binding site between the NF1 site and the TATA box resulted in a dramatic additional increase in activity of 16-fold in COS cells and by >60-fold in HeLa cells. This increase was largely abolished when the mutated YY1 binding site was present, indicating that the increase in activity was due to binding of YY1 to its recognition sequence (Fig. 2, NF1mYY1TATA). Since primer extension analysis showed one extension product at +1 in COS cells (data not shown), which is the authentic transcription start site of the TATA box we employed (50), the dramatic increase in activity observed with the NF1YY1TATA plasmid was not due to the generation of a new transcription initiation site. In both COS and HeLa cells, reversing the orientation of the YY1 site in NF1RYY1TATA did not abolish the increase in activity and actually led to a slight increase in activity relative to NF1YY1TATA (Fig. 2). The HeLa cell data demonstrates that the increase in activity exhibited by NF1YY1TATA and NF1RYY1TATA is a general phenomenon, and is not specific to COS cells.

The strong activation of transcription seen with the NF1YY1TATA plasmid (Fig. 2) could be due to YY1-induced DNA bending, or to synergistic activation of transcription by YY1 and NF1, or to a combination of these factors. Most weak activators exhibit synergistic activation of transcription when they are multimerized (51). Since a plasmid containing eight tandem copies of the YY1 binding site did not activate transcription in COS cells or HeLa cells (Fig. 2, 8YY1TATA), YY1 is unlikely to be a weak independent activator of transcription in these cell–promoter contexts. One way to examine the question of whether the presence of bound YY1 near the NF1 site activates transcription by either facilitating or stabilizing the binding of NF1 to its recognition sequence is to reverse the order of the NF1 and YY1 binding sites. When the YY1 binding site was placed upstream of the NF1 binding site (YY1NF1TATA), there was no increase in transcription in COS cells (Fig. 2).

One plausible explanation for the transcription activation seen with the NF1YY1TATA construct is that the YY1-induced DNA bend changes the geometry of the DNA so that the bound NF1 is brought into closer proximity to components of the basal transcription apparatus bound to the TATA region. In contrast, when the YY1 binding site is upstream of the NF1 site, the YY1- induced DNA bend does not affect the spatial relationship between bound NF1 and the components of the basal transcription apparatus, and

therefore does not facilitate NF1-mediated transcription. If this model is correct, one critical determinant of transcription activation will be the rotational orientation of the NF1, YY1 and TATA sequences.

Altering the rotational orientation of the YY1-induced DNA bend relative to the TATA box abolishes transcription activation

Because the DNA rotates through 360° approximately every 10 base pairs, the direction of bent DNA in 3-dimensional space also changes when the bend is located at different positions on the DNA helix. This is referred to as the rotational orientation or helical phasing of the DNA. To test whether the rotational orientation of the DNA bend induced by binding of YY1 was important in transcription activation, plasmids were constructed in which the YY1 and mYY1 binding sites were positioned 4 bp closer to the TATA sequence (Fig. 3A). In these constructions the DNA bend introduced by YY1 binding was in nearly the opposite direction relative to the bend induced by binding of TBP and its associated proteins to the TATA box (Fig. 3B). In contrast, in the original NF1YY1TATA construct the DNA bends were in the same direction. Because the face of NF1 exposed to the transcription complex could also influence transcription activation, we also deleted an additional four nucleotides between the YY1 and NF1 sites. This rotates the NF1 site ~300° so that NF1 and the TATA binding proteins have nearly the same rotational orientation as in the original NF1YY1TATA construct (Fig. 3B). In contrast with the NF1YY1TATA construct which activated transcription by 16-fold, the $NF1_{(-4)}YY1_{(-4)}$TATA construct activated transcription by only 2-fold (Fig. 4). These data support the view that a change in promoter architecture produced by YY1-induced DNA bending can facilitate transcription by NF1, when the YY1 and TATA sites are bent in the same direction (Fig. 3B, NF1YY1TATA). However when the YY1 and TATA sites bend in the opposite direction, the YY1-induced DNA bend does not facilitate protein–protein interactions important in transcription activation [Fig. 3B, $NF1_{(-4)}YY1_{(-4)}$TATA].

YY1 represses AP1-mediated transcription and an intrinsic DNA bending sequence mimics transcription repression

A correctly phased jun–fos induced DNA bend is thought to be important in transcription activation by the jun–fos heterodimer bound to the AP1 recognition sequence (14). As a model for the effect of YY1 in a system in which DNA bending plays an important role in transcription activation, we examined the effect of inserting a YY1 binding site (or an mYY1 sequence) between the AP1 site and the TATA box. A single AP1 site stimulated transcription in transfected COS cells by ~50-fold relative to the transcriptional activity of TATACAT (Fig. 5). Insertion of one or two YY1 sites between AP1 and TATA repressed AP1-mediated transcription by 2.5- and 5-fold, respectively (Fig. 5). Since the mYY1 site did not repress transcription, changes in the position of the AP1 site were not responsible for repression. Insertion of a YY1 site upstream of the AP1 sequence did not affect transcription (Fig. 5). These data do not distinguish between the possibilities that YY1-induced DNA bending, impaired protein–protein interaction, or impaired AP1 binding, or a combination of these factors, are involved in YY1-mediated repression of AP1 transcription.

If repression of AP1-mediated transcription is due to YY1-induced DNA bending, it might be possible to mimic YY1 repression by inserting a synthetic DNA bending sequence. In previous work (24) we showed that the intrinsic DNA bending sequence $4(A_6GCGC)$ does not bind cellular proteins and does not activate transcription (Fig. 6). To achieve an overall DNA bending angle similar to the YY1-induced DNA bend, we inserted four copies of the A_6GCGC intrinsic DNA bending sequence between the AP1 and TATA sequences. To account for effects due to increasing the distance between the AP1 and TATA sequences, a control plasmid containing a DNA insert the same length as the $4(A_6GCGC)$ sequence was prepared using a segment of the multiple cloning site (MCS) of pTZ18U (47). This sequence does not exhibit intrinsic DNA bending. The plasmid AP1 1MCSTATA, in which the AP1 site was moved 55 bp away from the TATA box, showed a small 20% reduction in transcription relative to AP1TATA (Fig. 6). The AP1 $4(A_6GCGC)$TATA plasmid exhibited a striking 5-fold reduction in transcription (Fig. 6). Since the multiple cloning site sequence in AP11MCSTATA is not related to the $4(A_6GCGC)$ intrinsic bending sequence, the possibility that a repressor protein not detected in our gel shift assays (24) bound to this sequence *in vivo*, and was responsible for the repression of AP1-mediated transcription could not be formally excluded. We previously showed that the sequence $4(A_2C_2A_2GCGC)$, which is closely related to the $4(A_6GCGC)$ intrinsic bending sequence, does not induce DNA bending. A plasmid containing this control sequence AP14$(A_2C_2A_2GCGC)$TATA did not repress AP1 activity, and actually exhibited a 20% increase in activity [from 34 ± 5 for AP1TATA to 41 ± 2 for AP14$(A_2C_2A_2GCGC)$TATA] sequence. It therefore seems highly unlikely that a repressor protein undetectable in gel shift assays is responsible for the repression of AP1-mediated transcription by the $4(A_6GCGC)$ intrinsic bending sequence. The ability of an intrinsic DNA bending sequence to mimic YY1-mediated repression of AP1-induced transcription provides strong evidence that YY1-induced DNA bending is responsible for repression of transcription in this simple promoter system.

Repression of AP1-induced transcription by YY1 and the $4(A_6GCGC)$ is not due to altered AP1 binding

Competition gel mobility shift assays were carried out to evaluate whether the YY1 or the $4(A_6GCGC)$ intrinsic DNA bending sequences altered jun–fos binding to the AP1 sequence in the synthetic promoters. A DNA fragment containing the AP1 site was used as a probe and 10- and 25-fold excesses of the unlabeled DNA fragments containing YY1 and mYY1 sites were used as competitors. Both AP1YY1 and AP1mYY1 fragments showed a similar ability to compete for AP1 binding in the gel mobility shift assay (Fig. 7A). The unlabeled DNA fragment of AP1 $4(A_6GCGC)$TATA was actually a slightly better competitor than the AP1 1MCSTATA fragment (Fig. 7B). In addition, the AP1YY1 DNA fragment showed a level of co-occupancy by YY1 and AP1 consistent with their ability to occupy fragments containing only a single binding site (YY1 or AP1) (data not shown). These data indicate that, in our synthetic promoters, neither YY1 nor the $4(A_6GCGC)$ intrinsic DNA bending sequence represses transcription by inhibiting binding of the jun-fos proteins to the AP1 element.

A.

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                -90        -80        -70        -60        -50        -40        -30
TATA                                              CCTGCAGGTCGACTCTAGAGGATCCAGATCTTTCTGCTATAAATA
NF1                                         ATTGGCTATGAGCCAATCTAGAGGATCCAGATCTTTCTGCTATAAATA
NF1YY1      ATTGGCTATGAGCCAATCTAGAGGATCCAGATCGATCTGACCATCTCTAGATCGATCTTTCTGCTATAAATA
NF1mYY1     ATTGGCTATGAGCCAATCTAGAGGATCCAGATCGATCTGCAGATCTCTAGATCGATCTTTCTGCTATAAATA
NF1(-4)YY1(-4)      ATTGGCTATGAGCCAATCTAGAGGATCCAGATCTGACCATCTCTAGATCTTTCTGCTATAAATA
NF1(-4)mYY1(-4)     ATTGGCTATGAGCCAATCTAGAGGATCCAGATCTGCAGATCTCTAGATCTTTCTGCTATAAATA
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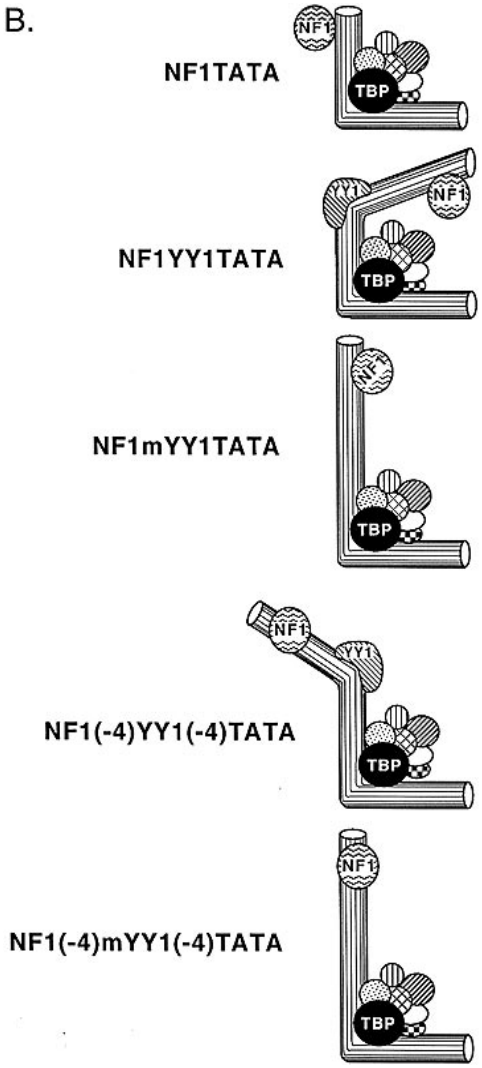

Figure 3. Sequences and cylindrical projections of NF1, YY1 and the basal transcription complex in NF1–YY1 constructions. (**A**) Consensus binding sites for the NF1, YY1 and TBP protein are underlined and bending centers are indicated by a dot. The GATC sequences deleted in the plasmids with different rotational orientations are also indicated. These plasmids (see Materials and Methods) carry the same upstream sequence except for the 4 bp difference in spacing between the NF1 and YY1 sequences and between YY1 and TATA. (**B**) DNA represented schematically as a cylindrical projection. The position of the proteins on the DNA helix is illustrated by placing them in front of or behind the cylindrical projection of the DNA, or in the same plane. In evaluating the role of helical phasing and DNA bending, we calculated the number of nucleotides from one DNA bending center to another. Although our preliminary data indicates that there is a small (~30°) NF1-induced DNA bend, this bend is omitted from the figure for simplicity. In the absence of data on the location of the NF1 bending center, we used the center of the NF1 binding site as the bending center. YY1 bends DNA ~80° toward the major groove, with the center of the bend at the center of the YY1 recognition sequence, CCAT (30). Solution of the crystal structure of the TBP–TATA complex revealed sharp kinks bending DNA ~80° toward the major groove at each end of the sequence TATAAATA (19–22).

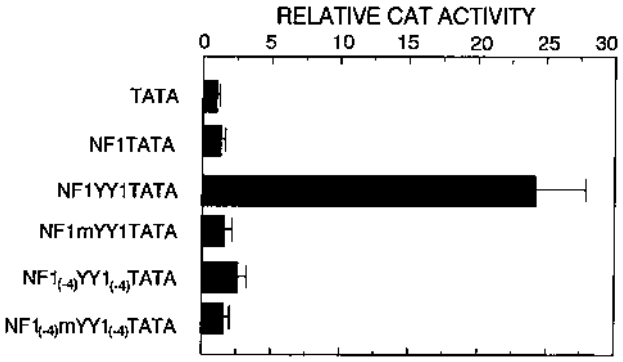

Figure 4. Changing the rotational orientation of the YY1-induced DNA bend abolishes transcription activation. COS cell transfections employed 0.5 μg internal standard TK-luciferase and 4 μg reporter plasmid. The transfections were carried out and the data plotted as described in 'Materials and Methods'. The CAT activity data represent the average ± s.e.m. for five independent transfections.

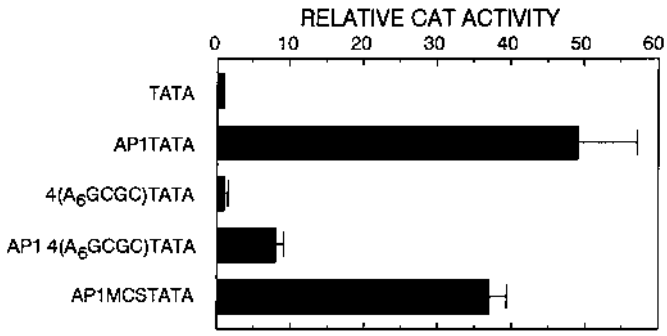

Figure 6. An intrinsic DNA bending sequence mimics YY1 repression of AP1 transcription. Four copies of the intrinsic DNA bending sequence, A_6GCGC (46), or the same length (55 bp) of sequence (which is not an intrinsic DNA bending sequence) from the multiple cloning site of the pTZ18U plasmid (47) were inserted into the *Bgl*II site of the AP1TATACAT plasmid. COS cell transfections, and determinations of CAT and luciferase activity were as described in 'Materials and Methods'. The data represent the mean ± s.e.m. for three separate transfections.

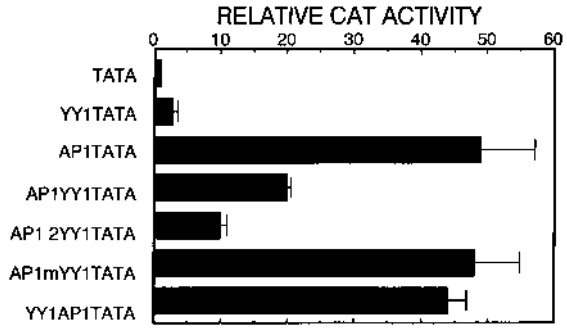

Figure 5. YY1-induced DNA bending represses transcription of AP1 TATA. Oligonucleotides containing the YY1 or mYY1 site were inserted either between AP1 and TATA, or upstream of the AP1 site. COS cell transfections, and determinations of CAT and luciferase activity were as described in 'Materials and Methods'. The data represent the mean ± s.e.m. for three to five separate transfections.

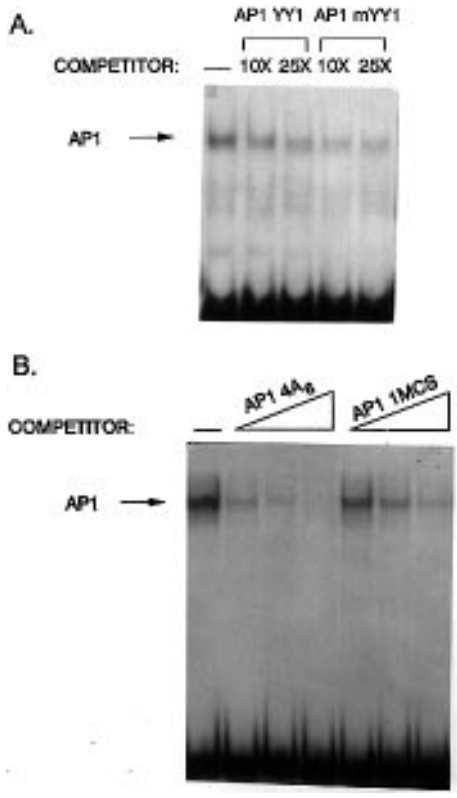

Figure 7. YY1 repression of AP1-mediated transcription is not due to decreased binding to the AP1 site. Increasing amounts of the unlabeled DNA fragments AP1YY1, AP1mYY1 (**A**), AP1 4(A6GCGC) or AP1 1MCS (**B**) were preincubated with the COS nuclear extracts on ice for 15 min and the reaction mixtures were further incubated for 15 min at 22°C after adding the probe containing the AP1 site. After fractionation by polyacrylamide gel electrophoresis, the intensity of the bands was quantitated with a PhosphorImager.

DISCUSSION

Although it was clear that binding of YY1 to its recognition sequence results in DNA bending (30), it was still uncertain whether DNA bending is simply part of the process by which YY1 binds to DNA, or also contributed to activation and repression of transcription by YY1. To address this question, we examined the ability of YY1 to modulate transcription by the NF1 and AP1 upstream activators in simple synthetic promoters.

Several factors were evaluated as potential contributors to YY1 potentiation of NF1 transcription. (i) YY1 could act as a direct activator of transcription and exhibit strong synergy with NF1 activation. The failure of 8YY1TATA to activate transcription and the absence of synergistic activation of transcription by YY1NF1TATA make direct transcriptional synergy unlikely. (ii) In our system, which employs synthetic promoters containing an effective TATA box, YY1 did not affect transcription by generating a new transcription initiation site. (iii) Changing the spacing between upstream activators bound at their recognition sequences and the basal transcription complex bound at the TATA region has often been shown to change the activity of transcription factors (4). By changing the distance between NF1 and the TATA box in the NF1YY1TATA construction, transcription may have

been stimulated by exposing a different face or domain of NF1 to the basal transcription apparatus. To evaluate this possibility we prepared the NF1mYY1TATA construct, in which the spacing between the NF1 and the TATA complex is the same as in NF1YY1TATA (Fig. 3A). No increase in activity was seen with NF1mYY1TATA, indicating that changing the distance between the NF1 and TATA sequences, or the face of NF1 available for interaction with components of the basal transcription apparatus is not responsible for activation. This data demonstrates that binding of YY1 to its recognition sequence is required for the enhanced activity we observe with NF1YY1TATA.

These data indicate that increased transcription may be due to either DNA bending by YY1 or to interaction between NF1 and YY1, or to a combination of these factors. To evaluate the possibility that protein–protein contacts between YY1 and NF1 were critical to activation, we reversed the orientation of the YY1 site. The NF1RYY1TATA promoter strongly activated transcription, and was actually slightly more effective in activating transcription than NF1YY1TATA. Since reversing the orientation of the YY1 binding site should change the regions of YY1 available for direct contact with NF1, the retention of activity in this promoter argues against contacts between the NF1 and YY1 proteins playing a critical role in the activation of transcription we observed. In the absence of detailed structural information on a YY1–DNA interaction, the effect of reversing the orientation of the YY1 binding site on the rotational orientation of the YY1 bend relative to the TATA and NF1 sites cannot be stated with certainty. The 5′-CCAT-3′ sequence is critical for YY1 binding, and is located near the center of the inserted YY1 sequence in both NF1YY1TATA and NF1RYY1TATA. The junction between CC and AT has been reported as the putative bend center (30). Also, by analogy to some other proteins which bend DNA at the junction of an AT and GC monomer, the YY1-induced DNA bend might be expected to be at or very near the junction between CC and AT. If the YY1-induced DNA bend is at or near the CC and AT junction, then reversing the orientation of the YY1 site would not be expected to significantly change the rotational orientation of the YY1 induced DNA bend relative to the NF1 and TATA sequences, and should not abolish the YY1 activation of transcription. The retention of activity in the NF1RYY1TATA promoter is therefore consistent with the view that YY1-induced DNA bending is important for the activity of these promoters.

The possible role of YY1-induced DNA bending in transcription activation was also examined by changing the rotational orientation of the YY1 and NF1 sequences. Plasmids in which four nucleotides were deleted between the YY1 and TATA sequences and the NF1 and YY1 sequence were constructed. This changes the direction of the YY1 induced DNA bend so that it is in the opposite direction relative to the TBP induced DNA bend (Fig. 3B), and should move the bound NF1 away from the basal transcription apparatus bound near the TATA box. Because this four base deletion also rotates the NF1 site relative to the TATA box, an additional four nucleotides were deleted between the YY1 and NF1 sequences which approximately realigns these two sites. This exposes a similar face or domain of NF1 to the basal transcription apparatus (Fig. 3B). The failure of this plasmid to demonstrate the strong 16-fold activation of transcription seen with NF1YY1TATA is most consistent with the view that YY1-induced DNA bending is responsible for transcription activation.

While the possibility of direct protein–protein interaction between YY1 and NF1 can not be completely eliminated by our experiments, the inability of YY1 to activate NF1 when it is upstream of NF1 in YY1NF1TATA, and downstream of NF1 in $NF1_{(-4)}YY1_{(-4)}$TATA and the strong activation of transcription in the NF1RYY1TATA promoter make this possibility much less likely than a role for DNA bending.

In contrast with activation of NF1-mediated transcription, YY1 is also able to repress transcription. In an important study Natesan and Gilman showed that YY1 is a position-dependent repressor of transcription from the CRE in the natural c-*fos* promoter (30). In this work we extend those studies by using synthetic AP1-containing promoters and an intrinsic DNA bending sequence. YY1-mediated repression of AP1-induced transcription in the AP1YY1TATA construct was successfully mimicked by replacing the YY1 site with an intrinsic DNA bending sequence exhibiting a similar degree of DNA bending. The AP1 4(A_6GCGC)TATA plasmid showed >5-fold repression. A closely related control sequence of similar length which does not bend DNA, 4($A_2C_2A_2$GCGC) did not elicit a similar repression of transcription. Since the 4(A_6GCGC) sequence does not bind any cell proteins in gel shift assays (24), and does not impair binding to the AP1 site (Fig. 7B), its ability to repress AP1-induced transcription is almost certainly due to its intrinsic DNA bend. Although theoretically possible, it seems highly improbable that an intracellular repressor protein not detected in our *in vitro* assays, and unable to bind *in vivo* to the closely related 4($A_2C_2A_2$GCGC) sequence, is responsible for repression of AP1-mediated transcription by the 4(A_6GCGC)sequence. It also seems improbable that the repression of AP1-mediated transcription by 4(A_6GCGC) is due to its ability to block *in vivo* binding of AP1, when the quite similar 4($A_2C_2A_2$GCGC) non-bending sequence fails to repress AP1-mediated transcription. These data strongly support the view that the YY1-induced DNA bend is responsible for repression of AP1-mediated transcription, and provide the first demonstration that an intrinsic bending sequence can mimic YY1 repression of transcription. In the absence of detailed structural information on the relevant protein–DNA complexes a detailed model for the role of YY1-induced DNA bending in the activation of NF1 transcription and in the repression of AP1 transcription remains largely speculative.

Taken together, our observations strongly support the view that, at least for simple synthetic TATA box containing promoters, YY1-induced DNA bending is important for transcription activation and repression. YY1-induced DNA bending might influence the architecture of the DNA around the promoter, and thereby facilitate or impair the ability of upstream activators bound to their recognition sequences to physically contact proteins in the basal transcription complex bound to the TATA region. It remains possible that in other promoter contexts [as has been reported for LEF-1 (27,28)], YY1 may also act as a conventional transcription factor, functioning primarily by protein–protein interactions with other transcription regulatory proteins. Interaction with regulatory proteins may alter or modulate the ability of YY1 to interact with its recognition sequence on DNA. For example, interaction of YY1 with adenovirus E1A and E1A-associated protein p300 modulate the ability of YY1 to repress transcription (42). These protein–protein interactions may alter other YY1–protein interactions, or indirectly affect transcriptional activation by altering bending of

DNA by YY1. This idea that YY1 complexes with other protein could alter DNA bending is supported by studies showing that the ability of E2F to activate transcription was based on DNA bending, and that cell cycle-dependent binding of the retinoblastoma gene product, pRB, to E2F reversed DNA bending. This reversal of DNA bending may be responsible for converting E2F from an activator to a repressor (52).

The ability of upstream activators to regulate promoter activity by making stable contacts with proteins in the basal transcription apparatus can be influenced by several factors. These include the identity of the interacting sequences on the proteins, the distance between the two proteins, and their positions relative to each other. The relative rigidity of DNA over short distances influences both the frequency with which the proteins will make contact, and requires an energy input from the protein–protein contacts to deform the linear DNA structure. In the NF1TATA plasmid, transcription activation is relatively weak (<2-fold, Fig. 2). This suggests that the contacts between NF1 and the transcription complex do not provide sufficient free energy to induce and maintain the stable distortion of the DNA helix required for formation of an efficient transcription complex. YY1-induced DNA bending distorts the DNA structure, which may bring NF1 into close proximity to proteins bound at the TATA region, and thereby reduce the free energy requirement for interaction between NF1 and these proteins. In the NF1YY1TATA plasmid, the distance between the NF1 and TATA sequences is approximately six helical turns of the DNA (62 bp). Thus, the YY1 induced DNA bend will both position protein bound at the NF1 site in close physical proximity to the TATA complex, and on the same face of the DNA helix as protein bound to the TATA box (Fig. 3B). This allows efficient interaction between the proteins and results in a large (16-fold) increase in transcription. In the related phasing plasmid $NF1_{(-4)}YY1_{(-4)}$TATA, although bound NF1 is in a similar plane as in NF1YY1TATA, the rotational orientation of the YY1 induced DNA bend is different. The bound NF1, therefore, is not brought into proximity to components of the basal transcription complex and there is no significant activation of transcription. These results indicate that changes in promoter topology resulting from YY1-induced DNA bending can facilitate or impair protein–protein contacts required for efficient transcription.

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