Retinoblastoma Protein Reverses DNA Bending by Transcription Factor E2F*

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E2F is a mammalian transcription factor involved in cell cycle regulation. The retinoblastoma gene product, pRB, binds to E2F in a cell cycle-dependent manner and appears to turn E2F from a transcriptional activator into a repressor. We show here that in vitro binding of pRB has three major effects on the DNA binding properties of E2F affinity-purified from HeLa cells; pRB binding increases the half-life of E2F-DNA complexes 10–15-fold, it reduces E2F’s specific DNA binding in the presence of nonspecific DNA by sequestering E2F, and it partially reverses the DNA bending induced by E2F. Upon specific DNA binding, E2F induces a DNA bend with a flexure angle of 125°. Both full-length pRB105 and the N-terminally truncated pRB60 bind to the E2F-DNA complex with a K_d of 150 ps and reduce the apparent DNA bending to less than 80°. DNA footprinting analysis indicates that the nonspecific DNA binding activity of pRB is not involved in this effect. Our biochemical data suggest that transcriptional activation by E2F may involve DNA bending and that the reversal of bending upon binding of pRB may turn E2F into a repressor.

The mammalian transcription factor E2F, originally identified as a cellular factor recruited by adenosviruses for transformation of the viral E2 promoter, appears to play an important role in cell cycle control (reviewed in Ref. 1). E2F participates in the regulation of genes expressed coordinately with cell cycle progression. E2F DNA binding sites have been identified in the promoters of genes such as c-myc, cdc2, polA, and DHFR (2–5), and transcriptional activation by E2F has been demonstrated for the promoters of c-myc, cdc2, and DHFR (2, 3, 5, 6).

E2F is found in multiple complexes with cellular proteins, such as the product of the retinoblastoma tumor suppressor gene (pRB)† (7–9), the pRB-related protein p107 (10, 11), and additional unidentified proteins (12), probably including p130. The composition of the complexes varies with progression through the cell cycle and appears to involve different forms of E2F (12). The viral oncoproteins E1a, large T, and HPV E7 disrupt the E2F-pRB complex by sequestering pRB, suggesting that complexed E2F is transcriptionally silent and that the release of free E2F is required to enter or complete the cell cycle (reviewed in Ref. 13). Experimental support for this hypothesis came from transfection experiments in which overexpression of pRB arrested cells in G1 (14, 15), and reduced the transcription from promoters containing E2F sites (16–18). Furthermore, pRB binding to E2F suppressed transcriptional activation by other promoter elements (19), indicating a pRB-mediated transcriptional switch from activator to repressor.

E2F from HeLa cells fractionates into at least five components that can be separated into two complementation groups. Reconstitution of E2F with optimal DNA and pRB binding activity requires dimerization of two proteins, one from each group (20). Several cDNAs encoding proteins with properties similar to HeLa E2F have been cloned recently (21–26). E2F-1 and E2F-2 belong to the same complementation group (25), while DP-1 is a member of the second group and heterodimerizes with E2F-1 to form high affinity E2F (24). The E2F-1/DP-1 heterodimer transactivates from E2F promoter sites and transcription is repressed by pRB coexpression (24) as is the case for native E2F. E2F-1, E2F-2, and DP-1 all contain the basic region-helix-loop-helix DNA binding motif, as well as a possible leucine zipper dimerization domain (21–26). E2F thus belongs to the growing family of heterodimeric transcription factors that includes the Jun/Fos, Myc/Max, and NFκB families (27). The existence of several different complementing polypeptides suggests the potential for cell type- or cell cycle-specific E2F complexes with different affinities for DNA and pRB and with different functions.

pRB is a 105-kDa nuclear phosphoprotein with a central domain ("pocket") that is necessary for the binding of the viral oncoproteins Ela, large T, and HPV E7, and the cellular D cyclins (28–30), as well as other proteins of unknown function (31). The C terminus and the pocket of pRB are involved in binding of E2F (15), DNA (32), and c-Myc (33). Binding of the viral oncoproteins prevents pRB not only from binding to E2F (34), but also from binding to DNA (35) and Myc (33). No function has been assigned to the N-terminal 390 amino acids of pRB, and an N-terminally truncated 60-kDa form, pRB60 (36), retains all known binding activities.

While the pRB-E2F interaction involves large domains on pRB (15), the binding site on E2F is apparently rather small. On E2F-1 a pRB binding site of 18 amino acids in length has been identified within the C-terminal transactivation domain (23), suggesting that pRB may inhibit E2F transactivation by blocking the interaction between transactivation domain and other elements of the transcription complex. Little biochemical characterization of the pRB-E2F complex and its interaction with DNA has been carried out, although it has been shown that the DNA sequence recognized by E2F is not significantly altered by binding of pRB (37). In the current contribution, we report the effect of pRB binding to E2F in vitro and show that the interaction affects DNA binding affinity and DNA bending, providing an alternate model for the transcriptional switch induced by pRB.

EXPERIMENTAL PROCEDURES

Materials

Proteins—E2F from HeLa cells was purified as described (30). E2F-1 was cloned with a C-terminal anti-tubulin epitope and affinity-purified

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1 The abbreviations used are: pRB, retinoblastoma protein; bp, base pair(s).
E2F DNA binding domains were expressed as glutathione S-transferase fusion proteins (25) and affinity-purified on glutathione resin. Expression and affinity purification of pRB60 have been described (36). pRB105 was affinity-purified from Sf9 cell lysate (a gift from Carol Rives, Columbia University) as described for pRB60. Anti-pRB60 monoclonal antibody Z6S was a gift from Ed Harlow (Massachusetts General Hospital, Boston).

DNA—Plasmid pUC-E2F was constructed by inserting a 40-bp DNA oligomer (5'-TAGTTTTCCGATATTAAATTTGAGAGGGCCGCAAACGTAG-3') containing a single E2F binding site (underlined) into the Smal restriction site of pUC19 in the orientation that placed the HindIII site between the HindIII and SalI (filled in with Klenow fragment) sites of pUC-E2F and reinserting it into the site found in the L' virus E2 promoter, but containing a single E2F site, served as a template for the formation of E2F DNA complexes. The resulting 194-bp duplication allows the isolation of circularly permuted 194-bp fragments containing a single E2F site.

**Methods**

**Gel Mobility Shift Assay**—Assays were performed essentially as described (20). Standard protein concentrations were as follows: 0.8 ng/ml HeLa E2F, 6.7 nM pRB60, 3.8 nM pRB105. Unless otherwise indicated, nonspecific DNA was not included. Three 40-bp DNA probes derived from the sequence of the adenovirus type 5 E2 promoter were synthesized by Midland Certified Reagent Co. (Midland, TX). The first two probes have a mutated distal E2F site, the remaining E2F site is wild-type in the first oligomer and palindromic in the second one, similar to the site found in the L' virus promoter: (5'-TAGTTTTCCGATATTAAATTTGAGAGGGCCGCAAACGTAG-3') and (5'-TAGTTTTCCGATATTAAATTTGAGAGGGCCGCAAACGTAG-3'). E2F binding sites are underlined. A control oligomer with both E2F sites mutated was used to test specificity of binding (5'-TAGTTTTCCGATATTAAATTTGAGAGGGCCGCAAACGTAG-3').

**Dissociation Assay**—To measure dissociation rates of E2F-DNA complexes, E2F and 32P-labeled DNA probe were preincubated at room temperature in gel shift buffer in the presence or absence of pRB. At time zero an approximately 200-fold excess of unlabeled DNA probe was added to trap dissociating E2F. Re-equilibration of E2F was terminated by addition of 5 mM EDTA and transfer onto ice. Samples were then assayed within a few minutes.

**DNA Footprinting**—The DNA binding of HeLa E2F in the absence or presence of pRB was compared using the DNase I footprinting technique (39). A 210-bp DNA probe with a single E2F site was obtained by cutting plasmid pUC-E2F with restriction enzyme BamHI, SaI, PstI, FokI, BglII, BclII, EcoRI, or Asp718, respectively. Fragments were labeled with T4 DNA polymerase and [α-32P]dATP. Mobilities of pRB-E2F-DNA complexes were determined in standard gel shift assays and plotted as a function of the position of the E2F site within the DNA probe. The equation \( \alpha = 2 \cdot \cos^{-1}(1 - A/k) \) (41) and the KaleidaGraph program (Synergy Software, Reading, PA) were used to curve-fit the relative mobilities and estimate the DNA flexure angles induced by E2F and pRB-E2F binding. The amplitude, \( A \), was determined from the cosine curve fit of the normalized mobilities (1 - (density/density of DNA probe)). The coefficient \( k \) was determined to equal 1.07 with a set of DNA standards containing 3-7 phased (dA)n tracts corresponding to intrinsic DNA bends of 54° to 126° (41, 42) (provided by T. Kerppola, Roche Institute of Molecular Biology, Nutley, NJ).

**RESULTS**

Transcription factor E2F was purified from HeLa cells (20), and its specific DNA binding was verified in a DNA gel shift assay (Fig. 1A). 40-bp DNA oligomers derived from the adenovirus E2 promoter, but containing a single E2F site, served as specific probes. Preincubation of E2F and DNA probe with either full-length pRB105 or the "pocket" protein pRB60 (36) supershifted the E2F-DNA complex as expected. The binding of all complexes was specific as shown by the competition with wild-type and mutant DNA oligomers (Fig. 1A). No binding of pRB to DNA probe was detectable in the absence of E2F (results not shown). Titration of pRB provided an estimate for the apparent binding constant of the interaction of pRB with the E2F-DNA complex. Both pRB60 and pRB105 supershifted half of the E2F-DNA complex at 150 pm (Fig. 1B), a concentration approximating \( K_d \) since the E2F dimer concentration was kept below 10 pm. At the higher Mg2+ concentration of 10 mM, routinely used in earlier reports (6, 34, 43), the binding constant increased approximately 3-fold (data not shown). The apparent DNA binding affinity of E2F and E2F-pRB was also reduced at 10 mM Mg2+ (Fig. 1A). Unless otherwise indicated, subsequent experiments were performed at 1 mM Mg2+, a concentration closer to physiological levels of free Mg2+.

pRB has intrinsic nonspecific DNA binding activity (35, 44), and the inclusion of nonspecific DNA in the gel shift assay affected the formation of pRB-E2F-DNA complexes (Fig. 2).
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A

\[ \text{SSDNA} = 0, 10, 20, 40, 80 \mu\text{g/ml} \]

B

\[ \text{SSDNA} = 0.4, 80 \mu\text{g/ml} \]

Fig. 2. Effect of nonspecific DNA on E2F-pRB-DNA complex formation in the gel mobility shift assay. A, pRB60 supershifting of the E2F-DNA complex in the presence of increasing concentrations of salmon sperm DNA (SS DNA). pRB60 concentration was 6.7 nM. The position of the "free E2F"-DNA complex is indicated by an arrowhead. B, titration of E2F-DNA complexes with pRB of increasing concentration in the presence of 0.4 or 80 \mu\text{g/ml} salmon sperm DNA.

Salmon sperm DNA competed poorly with specific probe for E2F binding; however, in the presence of pRB, the competition became much more effective, as demonstrated by the more rapid disappearance of the pRB-E2F versus E2F band (Fig. 2A). Nonspecific DNA also increased the apparent binding constant of pRB for the E2F-DNA complex, to 1 nM in the presence of 0.4 \mu\text{g/ml} DNA and to 5 nM at 80 \mu\text{g/ml} DNA (Fig. 2B). At very high concentrations of nonspecific DNA, as commonly used in gel mobility assays, the pRB-supershifted complex was no longer visible. Western blotting with anti-pRB and anti-E2F antibodies detected most protein close to the wells of the gel (data not shown). Apparently, the DNA binding activity of pRB localized the pRB-E2F complex to the nonspecific DNA. The resulting low mobility complexes contained no \textsuperscript{32}P label, suggesting that simultaneous binding of labeled DNA probe and nonspecific DNA by the pRB-E2F complex is not possible. To avoid the complication introduced by pRB's DNA binding, nonspecific DNA was omitted from subsequent experiments.

To determine the effect of pRB on the stability of the E2F-DNA complex, the half-life of the complex was determined in the presence or absence of pRB. Dissociation from the radiolabeled probe was monitored by trapping dissociated E2F with excess unlabeled DNA probe. The concentration of Mg\textsuperscript{2+} had a dramatic effect on the stability of the complexes. The half-lives of both complexes dropped from greater than 10 min in the absence of Mg\textsuperscript{2+} to less than 0.1 min in the presence of 10 mM Mg\textsuperscript{2+} (Fig. 3A and results not shown). At 1 mM Mg\textsuperscript{2+} significant stabilization of the E2F-DNA complex by pRB was apparent (Fig. 3A and B). pRB60 and pRB105 increased the half-life of the E2F-DNA complex from approximately 3 min to 30 and 45 min, respectively (Fig. 3, B and C).

Association of E2F and DNA was too fast to be measured by gel shift analysis, even at 0 °C and in the absence of Mg\textsuperscript{2+} (data not shown). Analysis of equilibrium binding, though, suggested
that the equilibrium dissociation constant for the E2F-DNA interaction was not significantly changed by pRB in the absence of nonspecific DNA (Fig. 4). The pRB105 concentration was adjusted so that approximately half of the E2F-DNA complex was supershifted. The concentration of the labeled DNA probe was then lowered to become limiting. Densitometric scanning of the autoradiograph showed that the relative intensities of the bands of free and pRB-bound E2F did not change significantly, indicating similar affinities for the specific DNA probe (Fig. 4 and data not shown).

The finding that pRB, with its nonspecific DNA binding activity, increased the half-life of E2F-DNA complexes, was suggestive of additional protein-DNA interactions in the presence of pRB. DNase I footprinting was used to detect protein binding sites on a 210-bp DNA fragment containing one E2F binding site. DNase I-treated complexes of free or pRB-supershifted E2F and DNA were isolated from DNA retardation gels and rerun on a sequencing gel (Fig. 5). E2F protected the consensus binding site 5'-TTCGCGC as well as several bases on either side as has been described (43). However, no footprints due to pRB60 or pRB105 were detected within 140 bp 5' and 60 bp 3' of the E2F site, even in the absence of KCl and MgCl₂, conditions that favor both specific E2F-DNA binding and nonspecific pRB-DNA binding. pRB-DNA contacts more distal to the E2F site may exist but would not contribute to the interactions analyzed on our standard 40-bp probe with its short flanking sequences. These results, while not ruling out weak pRB-DNA interactions too short-lived to inhibit DNase I cleavage, suggest that pRB stabilizes the E2F-DNA complex by allosteric interaction rather than by direct DNA contacts.

Several recent reports have indicated that binding of pRB to E2F, as measured by supershifting of the E2F-DNA complex in a gel mobility shift assay, may not occur with purified components, suggesting a requirement for additional factors (45, 46). Although the inclusion of nonspecific DNA in the assay can be responsible for the absence of pRB supershifting (Fig. 2B), and supershifting can be observed with highly purified (20) and cloned E2F components (24), we attempted to reproduce published procedures to identify other potential explanations. Using DNA probes longer than our standard 40-bp oligomer, we observed that the presence of a pRB supershift, or more generally, the direction of the mobility shift, depended on the length of the DNA probe. Specifically, on DNA fragments of greater than 120 bp the addition of pRB60 resulted in faster, rather than slower, migrating E2F-DNA complexes (Fig. 6A). To confirm that pRB60 was actually part of complexes with increased mobility, we treated preformed complexes with various concentrations of deoxycholate (Fig. 6B). Deoxycholate preferentially disrupts protein-protein over protein-DNA interactions. It regenerated a slower moving complex which comigrated with free E2F, indicating that binding of pRB60 caused the original faster migrating complex. Supershifting of the preformed complex by anti-pRB antibody XZ55 also demonstrated that pRB60 was part of the complex (Fig. 6B, rightmost lane). Antibody XZ55 had no effect on the E2F-DNA complex in the absence of pRB (data not shown). These results demonstrate that the degree and the direction of supershifting of E2F by pRB in a gel shift assay depend on the length of the DNA probe.
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FIG. 6. pRB60 increases the mobility of complexes of E2F and large DNA fragments in the gel shift assay. A, direction of pRB supershift depends on DNA length. E2F, pRB60, and \(^{32}\)P-labeled DNA fragments of the indicated length were preincubated and run in a standard gel shift assay. DNA fragments containing one E2F binding site were prepared by digesting plasmid pUC-E2F with HindIII and either KpnI, EcoRI, HaeIII, MaeIII, FokI, PvuII, or PvuI. The E2F site is located 67 bp from the HindIII site. B, effect of deoxycholate and anti-pRB antibody X255 on pRB60-E2F-DNA complex formation. Deoxycholate of the indicated concentration and X255 at 70 pg/ml (lane labeled +) were present during the preincubation of E2F, pRB, and DNA. Addition of pRB60 results in a faster migrating complex on the 213-bp HindIII-PvuI fragment.

and that with certain combinations of DNA and pRB super-shifting may not be detectable at all.

The mobility of protein-DNA complexes in non-denaturing gels is determined by both the size and the conformation of the complexes (47). The unexpected effect of pRB binding on the mobility of E2F-DNA complexes, as well as the fact that the effect was dependent on DNA length, suggested again that pRB induced a conformational change in the E2F-DNA complex. To test for DNA bending at the E2F site, circular permutation analysis was performed (40). Linear 194-bp DNA fragments of identical but circularly permuted sequence containing one E2F site were created as described under "Experimental Procedures." The effect of E2F binding to the circularly permuted probes in the absence or presence of pRB was analyzed in a standard gel shift assay (Fig. 7). The DNA probes in the absence of any protein showed virtually identical mobility, demonstrating that the E2F binding site has no intrinsic bend (data not shown). Binding of E2F alone caused a dramatic variation in mobility depending on the position of the E2F site within the DNA probe (Fig. 7A, left panel). The lowest mobility was observed when the E2F site was located near the middle of the DNA (Fig. 7A, lanes D) as predicted for protein-induced DNA bends (40). The addition of pRB60 or pRB105 alleviated much of the mobility variation, suggesting that binding of pRB relaxed some of the E2F induced DNA bending (Fig. 7A, middle and right panels). The mobilities of the different complexes were plotted as a function of the distance between the E2F site and center of the DNA fragment and normalized with respect to the maximal mobility of the "E2F only" complex. Symbols used are as follows: circle, E2F; square, E2F + pRB60; triangle, E2F + pRB105. C, schematic representation of the 194-bp DNA fragments used for circular permutation analysis. The boxes represent the E2F binding sites. Distances between the center of the E2F site and the center of the respective DNA fragments (in bp) are as follows: fragment A, -62; B, -50; C, -19; D, 9; E, 29; F, 47; G, 71; H, 83.
the calculated values will be referred to as DNA flexure angles, as has been suggested (41). Cosine amplitudes and derived DNA flexure angles are summarized in Table I. pRB60 and pRB105 reduce the flexure angle of the E2F-DNA complex from 125° to 67° and 79°, respectively. Homodimeric E2F-1, a protein with properties similar to those of HeLa E2F, induces DNA bending with a flexure angle similar to that of HeLa E2F. Analysis of E2F-1 fragments showed that the smallest polypeptide that retains DNA binding activity (amino acids 130–191) (25) also bent DNA, although to a lesser extent than full-length protein (Table I).

The cosine curve fit for free HeLa E2F is centered around the predicted symmetry axis of a palindromic E2F binding site, indicating symmetrical bending by the E2F heterodimer. In the presence of pRB, however, the center of bending is shifted by 10 to 15 bp toward the 3′ end of the TTTCGCGC binding site (Fig. 7B), suggesting formation of an asymmetric complex.

**DISCUSSION**

The interaction between pRB and transcription factor E2F was originally identified from pRB specific supershifting of E2F-DNA complexes in mobility shift assays (9, 48). Subsequently, pRB has been shown to suppress transcriptional activity of E2F (16, 19, 49), as well as of a recently cloned protein with E2F-like activity, E2F-1, in *vivo* (50). The pRB binding site on E2F-1 overlaps with the transactivation domain (21–23) and this finding provides a simple explanation for the transcriptional repression of E2F (37), the occupation of the transactivation domain by pRB may turn E2F into a repressor. In the current study, we have shown that pRB has several effects on E2F-DNA binding in *vivo* that may affect E2F's ability to transactivate and may also explain the observed transcriptional switch upon pRB binding.

pRB bound the E2F-DNA complex very tightly, with an apparent *Kₐ* of 150 pm. Binding of pRB in turn stabilized the E2F-DNA interaction and increased the half-life of the complex more than 10-fold, making it a more powerful effector: pRB itself has intrinsic nonspecific DNA binding activity (32, 44, 51), suggesting that pRB may stabilize E2F-DNA complexes through additional DNA contacts. DNA footprint analysis, however, provided no support for this model. The functional consequences of the nonspecific DNA binding activity of pRB in the context of the E2F interaction in *vivo* are not clear at this point.

In the presence of excess uncoated DNA pRB could cause translocation of the E2F-pRB complex from specific to nonspecific binding sites. While this effect could provide an alternate route to E2F inactivation, its biological significance is uncertain since most of the DNA in a living cell is contained in nucleosomes and may not be accessible to pRB. Furthermore, in *vivo* experiments (19) rather conclusively demonstrate that, in the presence of pRB, E2F acts as a transcriptional repressor dependent on the presence of an E2F binding site. Separation of the two activities of pRB, i.e. DNA and E2F binding, would help to elucidate their respective functions. However, earlier work had indicated that DNA and E2F binding involves overlapping sequences in the C terminus of pRB (15, 22, 33) and we were unable to create pRB mutants that retained E2F binding but were deficient in DNA binding. Analysis of a series of C-terminal deletions of pRB suggested that these amino acids are more important for E2F binding than for DNA binding (results not shown).

Analysis of pRB/E2F supershifting on DNA probes with a circularly permuted E2F binding site showed that E2F induced a DNA bend upon specific binding, an effect partially reversed by subsequent pRB binding. DNA flexure angles were calculated as described (41), but the degree of static DNA bending and the absolute orientation of the bend have not been determined. DNA flexure, as determined in permutation analysis, is thought to result from static DNA bending and increased dynamic flexibility of DNA adjacent to protein binding sites (41). Results obtained with other large DNA binding proteins such as Jun/Fos and Myc/Max suggest that the actual bending angle will be smaller (42, 52). The DNA flexure angle (125°) induced by E2F is among the largest determined for eukaryotic proteins and is similar to those induced by LEF (130°) (53), NFκB (75°–110°) (54), the Jun-Fos heterodimer (94°) (41), and the thyroid hormone receptor (75°) (55). Recombinant E2F-1 caused DNA flexure similar to E2F from HeLa cells. The minimal DNA bending domain of E2F-1 (25) still exhibited DNA bending, although the flexure angle was considerably smaller, suggesting a role for flanking sequences in DNA interaction.

Protein-induced DNA bending is involved in processes as diverse as DNA packaging, replication, recombination, and transcription. In prokaryotes DNA bending has been shown to be the essential function of several transcription stimulators. Some transcription factors can be functionally replaced by intrinsically bent DNA to bring distal promoter elements into proximity of the transcription machinery (56, 57), and DNA bending can be sufficient to cause a switch in usage of alternate promoters (58). The situation in eukaryotes may be more complex. Some factors, such as "high mobility group" (HMG) domain containing LEF, appear to work primarily through DNA bending (53). However, most transcription factors with DNA bending activity have additional essential interaction domains, such as transactivation domains, and proper juxtaposition of elements of the transcription machinery is just one of many potential mechanisms for transcriptional stimulation by DNA bending (reviewed in Ref. 59).

Many transcription factors, such as Jun/Fos, Myc/Max, and NFκB consist of families of proteins that bind DNA as homo- or heterodimers (27), allowing for multiple combinations with different transcriptional activities. E2F has been shown to bind DNA as a heterodimer (20, 24), and the recently cloned genes E2F-1 (21–23), E2F-2 (25), and DP-1 (24, 26) are likely the first members of a larger family whose existence is also suggested by the multiple E2F complexes observed in *vivo* (12). Interestingly, in the Jun/Fos transcription factor family, the orientation of DNA bending (60) and the effect on transcriptional activation by the glucocorticoid receptor (61) are opposite for Jun-Jun homodimers and Jun-Fos heterodimers. Similarly, c-Myc and Max dimers bend DNA in opposite orientation (52). The same potential for differential regulation through variable DNA bending exists for different E2F dimers. It should be noted that, while the extent of bending by the E2F-1 homodimer and HeLa E2F are similar, the direction of the bend, and the effect on transactivation, may be opposite. Interestingly, the E2F-1 homodimer is a comparatively poor transactivator in *vivo* (50). The availability of E2F clones from both complementation groups should now allow the dissection of the respective contributions to DNA bending and transactivation.
DNA binding by E2F was partially reversed by binding of pRB. The significance of this effect is not yet apparent, but if DNA binding is essential to transcriptional activation this response to pRB may well turn E2F from an activator into a repressor as observed in vivo (19). E2F by itself bent DNA at the center of the asymmetric E2F binding site derived from the E2 promoter (with the center being defined by the center of the palindromic E2F site in the DHFR promoter). Upon binding of pRB the center of bending shifted downstream by 10–15 base pairs. This asymmetry indicates that the E2F heterodimer binds to the asymmetric DNA recognition site in a directional manner. Subsequent pRB binding to the E2F-DNA complex must be asymmetric as well. This conclusion is consistent with the finding that DP-1, one possible partner of E2F-1 in the E2F heterodimer, has no sequence homology to the pRB binding domain of E2F-1 (24, 26) and will likely not contribute a pRB binding site. This result opens up the interesting possibility that the orientation of an asymmetric E2F site may determine the extent of transactivation by E2F heterodimers, as well as the response to binding of pRB, p107, and other E2F-binding proteins.

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