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# Opposite orientations of DNA bending by c-Myc and Max

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**ABSTRACT** **The control of gene transcription requires specific protein–protein and protein–DNA interactions. c-Myc, the protein product of the c-*myc* protooncogene, is a member of the basic helix–loop–helix leucine-zipper class of transcription factors. Although c-Myc is able to bind to a specific core hexanucleotide DNA sequence (CACGTG), its precise function in modulating transcription remains unclear. The recent discovery of Max, a basic helix–loop–helix leucine-zipper partner protein for c-Myc, suggests that the ability of c-Myc to regulate transcription is modulated by the presence of Max. By taking advantage of the altered mobility of protein-bound DNA in the mobility-shift assay, we demonstrate that homo- and heterodimeric complexes of c-Myc and Max are able to cause increased DNA flexure as measured by the circular permutation assay. Based on phasing analysis, c-Myc and Max homodimers bend DNA in opposite orientations, whereas c-Myc–Max heterodimers cause a smaller bend, in an orientation similar to that induced by Max homodimers. To address the possibility that the apparent opposite orientation of bending was the result of DNA unwinding by one of the proteins, we measured the ability of c-Myc and Max homodimers to affect DNA unwinding; we were unable to show any specific unwinding caused by c-Myc or Max. In addition to demonstrating that members of the basic helix–loop–helix leucine-zipper class of transcription factors are able to induce DNA bending, these results suggest that different transcription factor dimers are able to bind to identical DNA sequences and yet have distinct structural effects.**

Control of gene transcription requires protein–protein and protein–DNA interaction. Many transcription factors mediate the regulation of DNA transcription by virtue of their ability to specifically interact with particular DNA sequences and proteins of the basal transcription apparatus (1–5). The c-*myc* protooncogene is important in the regulation of normal cell growth and differentiation (6–8). Although c-Myc, its protein product, shares homology with a number of transcription factors, the specific function of c-Myc remains unclear. The recent discovery of Max (9, 10), a partner protein for c-Myc with a basic-region helix–loop–helix leucine-zipper motif, has led to speculation that the ability of c-Myc to regulate transcription is modulated by the presence of Max.

c-Myc and Max have been shown to bind to a specific core nucleotide sequence, CACGTG (9–12). Previous work from this laboratory (13) using bacterially produced proteins has shown that full-length c-Myc alone is unable to bind to this sequence. A truncated form of c-Myc, amino acids 342–439, however, is able to bind to this sequence, as are Max and a combination of full-length c-Myc and Max. Binding to this core nucleotide sequence is mediated by formation of homo- or heterodimers. Dimerization is believed to occur by interaction at the helix–loop–helix leucine-zipper region, thereby aligning the basic regions into the proper spatial orientation to enable specific DNA recognition. A specific arginine residue ($Arg^{367}$) within the basic region is essential for recognition of the central CG dinucleotide pair in the core hexanucleotide sequence (14). To understand the potential significance of specific DNA recognition by c-Myc and Max, we sought to determine whether c-Myc–Max heterodimers and Max homodimers bind to DNA in a physically symmetrical fashion and whether a higher order of specificity is dictated by the manner in which these proteins recognize DNA. Kerppola and Curran (15, 16) had demonstrated that binding of Fos and Jun induces a change in the conformation of the DNA helix, with hetero- and homodimers bending DNA in opposite orientations. We show that c-Myc and Max also bend DNA and that bending induced by homo- and heterodimers is different.

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## MATERIALS AND METHODS

**Protein Preparation.** Truncated c-Myc-(342–439) and Max cDNA sequences were separately cloned into the pDS vector (obtained from F. Rauscher and T. Curran, Roche, Nutley, NJ) immediately downstream of a sequence encoding a polyhistidine tract. After isopropyl β-D-thiogalactoside induction, proteins were purified from lysates by affinity chromatography over a nickel chelate column (Qiagen, Chatsworth, CA) as described (17). Protein-containing samples were dialyzed against buffers with successively less guanidine and stored in 10 mM Tris·HCl, pH 7.4/100 mM NaCl/1 mM $Na_2$ EDTA/1 mM dithiothreitol/10% (vol/vol) glycerol at −80°C. Full-length c-Myc protein was prepared and purified as described (18). SDS/PAGE was performed under reducing conditions as described (13).

**Circular Permutation Assay.** A CACGTG-containing oligomer (duplex of 5′-TCGACGGGGCACGTGCCCCG-3′) was cloned into the *Sal* I site of the pBEND2 vector (19) (obtained from S. Adhya, National Institutes of Health) to generate pBend-pmb. $^{32}$P-labeled 136-base-pair (bp) DNA fragments containing the CACGTG binding site permuted along the length of the fragment were generated by digestion of this plasmid with *Bgl* II, *Xho* I, *Pvu* I, *Stu* I, and *Bam*HI. Bacterially produced proteins were incubated with 0.5 μg of denatured salmon sperm DNA/1 μg of poly(dI-dC) (Pharmacia) in mobility-shift buffer (MSB = 10 mM Tris·HCl, pH 7.4/80 mM NaCl/1 mM dithiothreitol/5% glycerol) in a final volume of 15–20 μl for 15 min at 42°C and then 15 min at room temperature. Each $^{32}$P-labeled probe (4000–10,000 cpm) was incubated with the protein mixture at room temperature for 15 min, 2 μl of 0.1% bromphenol blue in MSB was added, and aliquots were electrophoresed (15–20 V/cm, 20°C) on 5% or 10% nondenaturing polyacrylamide gels [in 0.5× TBE buffer with 0.01% Nonidet P-40 (Sigma); 1× TBE = 90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3] that had been preelectrophoresed for 30–60 min. Gels were dried and then autoradiographed for 12–60 h.

**Phasing Assay.** Probes were prepared by cloning a CACGTG-containing oligomer (duplex of 5′-CTAGAGGGGCACGTGCCCCG-3′ and 5′-TCGACGGGGCACGTGCCCCT-3′) into the *Xba* I–*Sal* I site of pTK401-26 and

pTK401-28 (provided by T. Kerppola and T. Curran), followed by mung bean nuclease or Klenow fragment of DNA polymerase I treatment of a newly generated *Sal* I site to generate a set of six vectors in which the center of an intrinsic DNA bend is separated by the indicated number of base pairs from the center of the CACGTG binding site. DNA sequence from the CACGTG core binding site to the intrinsic bend in the derivative plasmids was confirmed by the dideoxynucleotide chain-termination method. The respective sequences in the different probes were as follows:

pDW24, CACGTGCCCCGCGCAAAAA;
pDW26, CACGTGCCCCGCACGCAAAAA;
pDW28, CACGTGCCCCGTCGACGCAAAAA;
pDW30, CACGTGCCCCGTCGACACGCAAAAA;
pDW32, CACGTGCCCCGTCGATCGACGCAAAAA;
pDW34, CACGTGCCCCGTCGATCGACACGCAAAAA,

where boldface type indicates nucleotide insertions. DNA probes 280–290 bp long were generated by *Eco*RI/*Hin*dIII digestion of the vectors. Fragments were end-labeled with [$^{32}$P]dATP, incubated with proteins as described above, and electrophoresed on 5% or 7.5% nondenaturing gels.

**Curve Fitting.** Curve fitting was performed using a general curve fitting function (Kaleidagraph, Abelbeck Software) on a Macintosh computer.

**DNA Unwinding.** The ability of c-Myc-(342–439) or Max to unwind DNA was assessed using a modification of the methods of Bodley *et al.* (20). Briefly, proteins (1.6–9.6 μg) were incubated with 0.5 μg of denatured salmon sperm DNA, 0.5 μg of poly(dI-dC) (Pharmacia) in topoisomerase I buffer [50 mM Tris·HCl, pH 7.5/100 mM KCl/10 mM $MgCl_2$/0.5 mM dithiothreitol/0.5 mM EDTA/bovine serum albumin (30 μg/ml)] for 15 min at 20°C; 300 ng of supercoiled plasmid DNA (see below) and 300 ng of topoisomerase I-relaxed plasmid DNA were added for an additional 15-min incubation at 20°C; finally, after addition of 10 units of calf thymus topoisomerase I (BRL) to a final reaction volume of 20 μl, the mixture was incubated for an additional 60–90 min at 20°C. Each reaction was stopped with 5 μl of stop solution [2.5% (wt/vol) SDS/100 mM EDTA] at 20°C and then extracted once with phenol and once with chloroform. Samples were electrophoresed at 4°C in a 0.9% agarose gel in 90 mM Tris/90 mM boric acid/5 mM $MgCl_2$, pH 8.25, at 45 V for 18 h in a horizontal gel apparatus. Doxorubicin (final concentration, 12 μM) was substituted for protein to confirm DNA unwinding (20). Gels were stained with ethidium bromide and then photographed with Polaroid 665 film. Negatives were analyzed on a Bio-Rad model 620 videodensitometer to determine the distribution of DNA topoisomers. The degree of unwinding was determined from the topoisomer distributions by the method of Depew and Wang (21).

Plasmid DNA used in the unwinding assay consisted of (*i*) pBend-pmb (with a single CACGTG site) and pBEND2 as a negative control and (*ii*) pBS-pmb-2 (with two CACGTG sites separated by 136 bp) and pBS-2-2 (which contains two 120-bp repeats of the pBEND2 polylinker without the CACGTG site) as a negative control. These latter plasmids were generated from pBend-pmb and pBEND2, respectively, by isolation of the *Pvu* II restriction endonuclease digestion product (120- to 136-bp fragment) of each, T4 DNA ligase treatment of this fragment to generate a fragment "dimer," and subcloning of this dimer into the *Eco*RV site of pBSIIKS− (Stratagene).

## RESULTS AND DISCUSSION

**Rationale.** DNA binding may be demonstrated by the mobility-shift assay, in which the electrophoretic mobility of a protein-bound DNA fragment is retarded relative to free DNA under nondenaturing conditions (22, 23). The presence of a bend in a DNA fragment is expected to impede movement through the gel matrix, thereby reducing the mobility of the fragment relative to that of a fragment of identical length without a bend (24). Since the mobility of a polyanion is considered to be dependent on the mean square end-to-end distance (25, 26), for fragments of identical length, a bend at the center of the fragment is expected to retard the mobility more than one at the very end of the fragment. By using a set of DNA fragments of identical length with the CACGTG binding site permuted at different sites along the length of the fragment, it is possible to measure the position-dependent effect of bound proteins on electrophoretic mobility by the circular permutation assay. As pointed out by Kerppola and Curran (15), alterations in electrophoretic mobility can be caused by sites of static DNA bends and by sites of increased DNA flexibility with no specific orientation. Such structures are, therefore, referred to as locations of DNA flexure.

**Circular Permutation Analysis.** c-Myc-(342–439) and Max proteins were produced in bacteria and purified (Fig. 1*a*). For c-Myc-(342–439) and Max homodimers and full-length c-Myc–Max heterodimers, complexes bound to probes on which the CACGTG site was located in the middle of the probe migrated more slowly than complexes bound to probes on which the CACGTG site was located on either end (Fig. 1 *b–d*).

The extent of DNA flexure can be determined by plotting the relative probe mobilities as a function of the position of the DNA binding site relative to the end of the probe and then comparing the fragments with the highest and lowest mobilities (Fig. 1*e*). The shape of the plotted curve is inverted with respect to the autoradiograph, since the position of the most central binding site is located furthest from the end of the probe. This is done to obtain an estimate of the mobility of the probe with the binding site centered at the exact end of the fragment, a situation that cannot be evaluated experimentally since it implies the presence of half of a binding site. The amplitude of the function ($A_{CP}$) reflects the difference between the probes with lowest and (theoretically) highest mobility (15). The angle of flexure ($\alpha_F$) can be estimated (19, 27, 28) by the equation $\alpha_F = [2\cos^{-1}(1 - A_{CP})]/k$, where $k$ is a coefficient reflecting factors that influence the relative electrophoretic mobilities (temperature, gel composition, field strength, and fragment length) (15). A set of standards with different intrinsic DNA bend angles (obtained from T. Kerppola and T. Curran) was electrophoresed under the conditions of our assay (5% polyacrylamide gel), and resulted in a coefficient of $k = 0.83$ (data not shown). Thus, truncated c-Myc-(342–439) homodimers (assuming $k = 0.83$) caused an angle of flexure of 53°, Max homodimers caused an angle of 53°, and c-Myc–Max heterodimers caused an angle of 80°. In addition, transcription factors USF, CBF, and TFE3 induce angles of flexure of 55–67° (data not shown). The values for the homodimers are somewhat smaller than the DNA flexure angles reported previously for Jun–Jun (79°) and Fos–Jun (94°) (15) and for CAP, FIS, GalR, Xis, and IHF (70–140°) (19, 28). CI, Cro, and LacI, however, induce flexure angles of 30–62° (19), more comparable to those reported here. The probes themselves showed no significant variations in mobility, suggesting that the CACGTG site and flanking sequences did not contain significant intrinsic DNA bends (data not shown).

**Phasing Analysis.** To further investigate the nature of the flexure induced by c-Myc and Max, we took advantage of the technique of phasing analysis as described by Zinkel and Crothers (29) and extended by Kerppola and Curran (15, 16). This analysis enables discrimination between directed DNA bends and regions of increased DNA flexibility and permits determination of the orientation of a protein-induced DNA bend relative to an intrinsic bend. In phasing analysis, DNA probes are generated such that a stretch of intrinsically bent

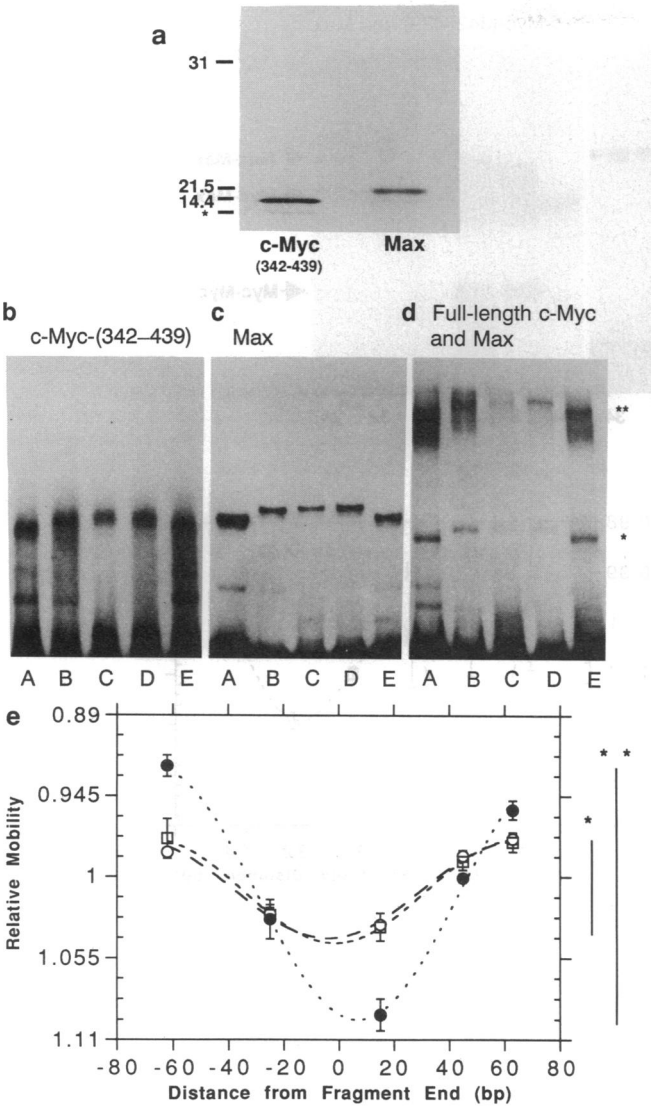


FIG. 1. Circular permutation analysis of DNA flexure. (*a*) SDS/polyacrylamide gel electrophoresis of truncated c-Myc-(342–439) and Max on a 10% gel stained with Coomassie blue. Positions of molecular mass (kDa) markers are indicated on the left. The asterisk indicates the position of dye front. (*b–d*) Electrophoretic mobility-shift circular permutation analysis of c-Myc-(342–439) homodimers (10% gel) (*b*), Max homodimers (5% gel) (*c*), and c-Myc–Max heterodimers (5% gel) (*d*). $^{32}$P-labeled DNA fragments containing the CACGTG binding site permuted along the length of the fragment were generated by digestion with *Bgl* II (lanes A), *Xho* I (lanes B), *Pvu* II (lanes C), *Stu* I (lanes D), and *Bam*HI (lanes E). With full-length c-Myc (*d*), Max was present in both homodimeric (*) and heterodimeric (**) complexes. The reasons for the increased band width of the heterodimeric complexes with probes A and E (*d*) are not clear. All five unbound circular permutation probes have identical mobilities (data not shown). (*e*) The relative mobilities of truncated c-Myc-(342–439) homodimers (open squares), Max homodimers (open circles), and full-length c-Myc–Max heterodimers (solid circles) were plotted as a function of position of the center of the CACGTG binding site relative to the end of the fragment. A cosine curve was fit to the data and the mobility of a hypothetical fragment with half of a binding site at the end was extrapolated. The amplitude of the function (vertical lines on the right) ($\text{mobility}_{\text{high}} - \text{mobility}_{\text{low}}$) was used to determine the angle of flexure according to the equation described in the text (*, amplitude of homodimers; **, amplitude of heterodimer). Each curve represents the data from three (c-Myc–Max heterodimer) or four [c-Myc-(342–439) and Max homodimers] experiments; standard errors are shown as vertical bars. Note that $\text{mobility}_{\text{high}}$ for the heterodimeric complex is not centered on the end of the fragment.

DNA (30, 31) is placed at different distances from a DNA binding site; these distances vary by 1 or 2 bp. A set of probes is thus obtained in which the phasing between the intrinsic bend and the binding site is varied over a single turn of the DNA helix. If binding of protein to the DNA binding site induces a bend in the DNA, the mobility of the probes will vary such that it is lowest when the two bends cooperate to increase the overall extent of bending (because of a decrease in the end-to-end distance) and highest when the bends counteract each other.

The set of unbound probes demonstrated decreasing mobility with increasing length (data not shown). For truncated c-Myc-(342–439) homodimers and Max homodimers, the complex mobilities varied depending on the spacing between the CACGTG binding site and the intrinsic bend (Fig. 2 *a* and *b*). The probe that had the highest mobility with Max homodimers (probe 26) had the lowest mobility with the truncated c-Myc-(342–439) homodimers, and vice versa (probe 32). The pattern of the mobilities of the probes to which truncated c-Myc-(342–439)/Max heterodimers were bound (Fig. 2*c*) was similar to that of the Max homodimers, although the variation in mobilities was less than that of the Max homodimer. Full-length c-Myc–Max heterodimers (Fig. 2*d*) did not cause a significant variation in the mobilities of the different probes. Even though the heterodimeric complex caused significantly greater DNA flexure in the circular permutation assay, phasing analysis demonstrates that bending by the heterodimer is minimal and thus suggests that the Max-induced bend is offset by the presence of c-Myc.

**Relative Orientation of DNA Bending.** To determine the relative orientations of DNA bending induced by different homodimers, the mobilities of the protein–DNA complexes may be plotted as a function of the distance between the centers of the intrinsic bend and the DNA binding site. Normalization of complex mobilities to those of the respective unbound probes demonstrates that the mobilities of the probes separated by a single helical turn are similar (probes 24 and 34) and highlights the differences between the different homodimeric complexes (Fig. 2*e*). The spacer lengths of the probes that gave the lowest mobilities for the different homodimeric complexes differed by 6 bp. By assuming an average of 10.5 bp per helical turn of the DNA helix, this corresponds to a 200° difference in the relative orientations of DNA bends induced by truncated c-Myc-(342–439) and Max, similar to that observed for Fos–Jun and Jun–Jun dimers.

**Absolute Orientation of DNA Bending.** The absolute orientation of bending can be determined from the phasing between the intrinsic DNA bend and the protein-induced bend in combination with the orientation of DNA bending induced by phased A·T tracts, which have been shown to bend DNA toward the minor groove at the center of the A·T tract (29, 31). The center of the middle A·T tract was located 32 bp (approximately three helical turns) from the center of the binding site on probe 32. If a particular dimer bends DNA toward the minor groove, it should cooperate with the intrinsic bend in probe 32 and lead to maximally reduced mobility; if it bends toward the major groove, it should counteract the intrinsic bend maximally in the probe where the sites are separated by half a helical turn (probe 26). Therefore, the Max homodimer appears to bend DNA toward the minor groove (i.e., away from itself) (Fig. 3*a*), whereas the c-Myc-(342–439) homodimer bends DNA in the opposite orientation, in the direction of the major groove (i.e., toward itself) (Fig. 3*b*). Since the c-Myc–Max heterodimer appears not to induce a directed DNA bend, it is hypothesized that the contributions to bending by each half of the heterodimer counteract one another (Fig. 3*c*) and lead to a reduction in the degree of apparent bending. This corresponds to the "jog" or "kink" induced by Fos–Jun heterodimers (15, 16). This is not inconsistent with the circular permutation assay results;

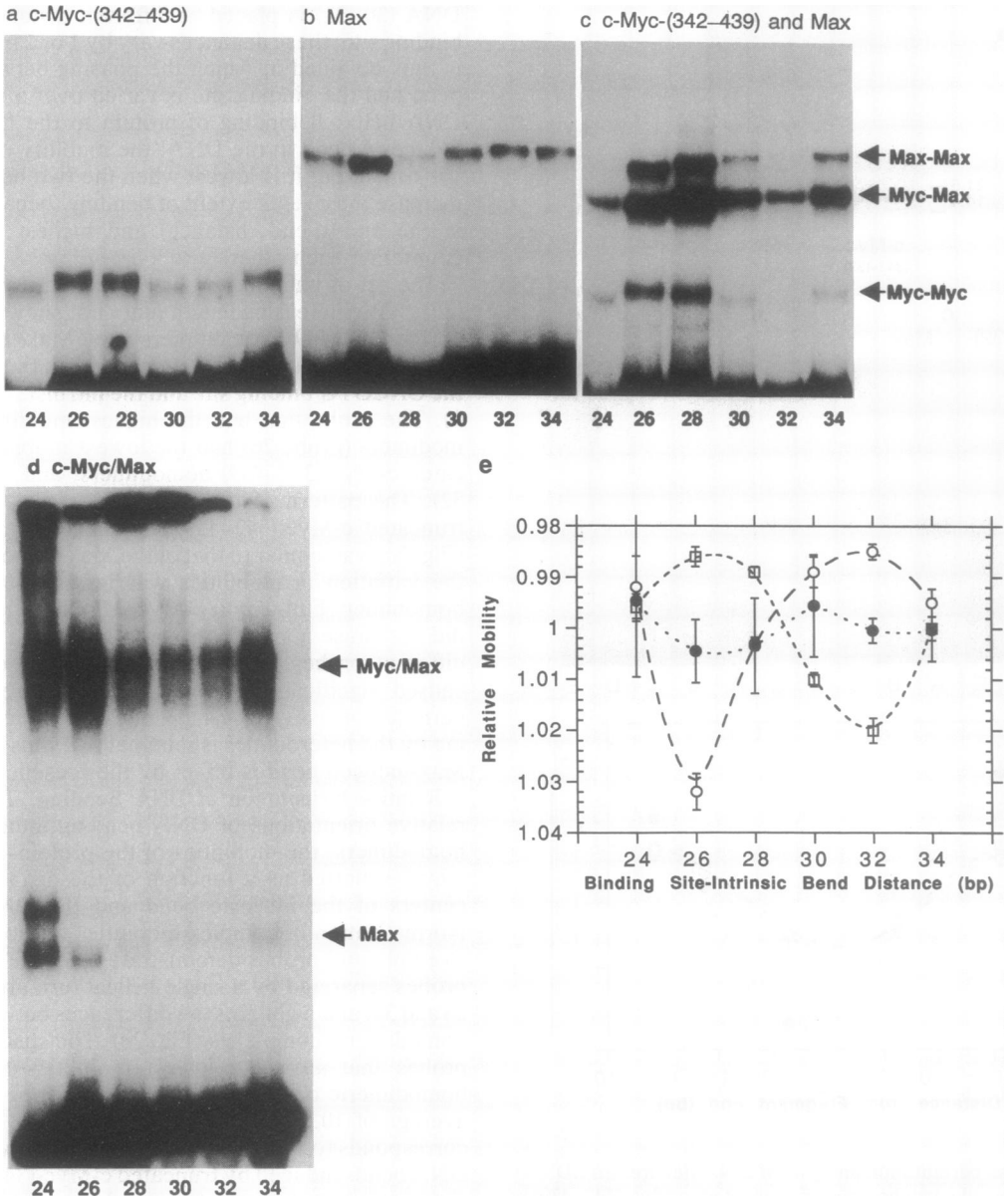


FIG. 2. Phasing analysis of DNA bending. (*a–d*) Electrophoretic mobility-shift phasing analysis of truncated c-Myc-(342–439) (*a*), Max (*b*), truncated c-Myc-(342–439) and Max (*c*), and full-length c-Myc and Max (*d*). Numbers below lanes indicate distance in base pairs between binding site and intrinsic bend in a particular probe. Electrophoresis was performed on 7.5% gels (*a–c*) or a 5% gel (*d*). Free probes have decreasing mobility with increasing length (data not shown). (*e*) Relative mobilities of protein–DNA complexes [open squares, c-Myc-(342–439) homodimers; open circles, Max homodimers; solid circles, full-length c-Myc–Max heterodimers] as a function of spacer length. Mobility of each probe complex was normalized to the mobility of its corresponding unbound probe and then normalized to the average mobility of all probes. Curves for homodimeric complexes represent data from a minimum of six experiments; curve for the heterodimeric complex represents four experiments. Standard errors are shown as vertical bars.

because each half of the heterodimer induces a bend in an opposite orientation, it might be expected that there would be increased flexibility in the region of protein binding. The overall effect at the level of an individual DNA molecule would be an absence of apparent bending.

**DNA Unwinding.** An alternative explanation for the differences observed with phasing analysis is that both c-Myc-(342–439) and Max bend DNA in the same orientation but that one protein is able to locally unwind DNA by one-half of a helical turn, thereby giving the appearance of bending in opposite orientations. To address this possibility, we attempted to demonstrate differential unwinding by c-Myc-(342–439) and Max, by using the pBend-pmb plasmid as a template. Densitometric analysis of the distribution of topoisomers revealed that whereas c-Myc-(342–439) showed no ability to unwind DNA in comparison with doxorubicin, concentrations of Max that were two orders of magnitude higher than those used in the mobility-shift assays caused unwinding of pBend-pmb (data not shown). Max also, however, caused identical unwinding of the pBEND2 plasmid (at these same concentrations), suggesting that this was a nonspecific effect that was independent of the c-Myc/Max core binding site, CACGTG. No unwinding whatsoever was detected with any plasmid when Max was used at concentrations comparable to those used for circular permutation and phasing.

To increase the sensitivity of detection, we performed the unwinding assay with a plasmid substrate containing two specific DNA binding sites. If binding of Max to a single specific DNA site results in a one-half turn of unwinding, it is expected that there would be a difference of one linking number (i.e., one full turn) between the topoisomer distri-

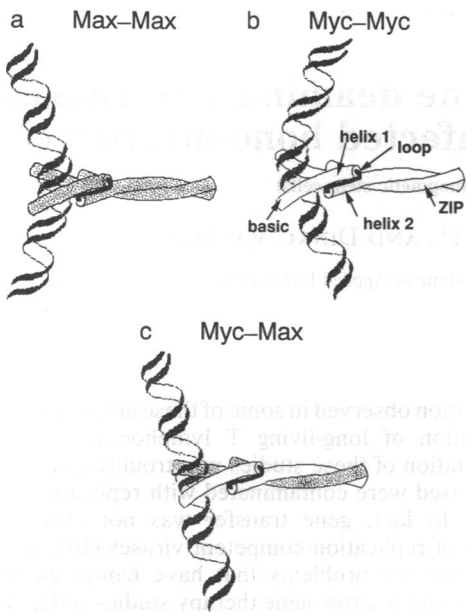


FIG. 3. Model of DNA bending induced by c-Myc and Max. Hypothetical model of the basic helix–loop–helix region (based in part on ref. 32) shows a cylindrical α-helical basic region, continuous with the first helix, in the major groove of double-helical DNA. The solid black line is the loop that links the first and second helices. A longer cylinder, consisting of the second helix in continuity with the leucine-zipper domain, mediates dimerization through a coiled-coil interaction. (*a*) Max (stippled cylinder) homodimer bends DNA away from itself. (*b*) c-Myc-(342–439) (open cylinder) homodimer bends DNA toward itself. (*c*) c-Myc–Max heterodimer causes no appreciable DNA bend.

bution of pBS-pmb-2 (with two CACGTG sites) and pBS-2-2 (identical to pBS-pmb-2 except without CACGTG sites) in the presence of Max. In fact, we found that the topoisomer distributions of these two plasmids determined in three separate assays were indistinguishable in the presence of Max (data not shown). Identical results (i.e., no specific unwinding) were observed with both Max and a FPLC (Mono Q column)-purified Max preparation (a gift of J. Klemm, Massachusetts Institute of Technology, Cambridge, MA). Use of a highly purified Max preparation eliminates the possibility that nonspecific unwinding is caused by a contaminant in the original Max preparation. Although the reason for the nonspecific unwinding is presently unclear, we were unable to demonstrate any specific DNA unwinding by either c-Myc-(342–439) or Max, strengthening the contention that these proteins bend DNA in opposite orientations.

These results support the hypothesis that different dimers are able to bind to the same DNA regulatory element and have distinct effects (16). In addition, they demonstrate that proteins of the basic helix–loop–helix leucine-zipper class of transcription factors are able to bend DNA. Since c-Myc and Max homo- and heterodimers do not bind to DNA in a physically symmetrical fashion, it is conceivable that they might have different effects on transcription. These effects may be mediated by the formation of higher-order protein–DNA complexes. DNA bending may allow for suitable spatial orientation of DNA-bound proteins, enabling appropriate protein–protein interactions necessary for initiation of transcription (29, 33–36); bending in an inappropriate direction could prevent such interactions. Alternatively, the energy stored in a segment of bent DNA might be used at some stage of transcription, possibly to unwind DNA, facilitating the initiation of transcription (34, 35). Finally, the ability of proteins to induce directed bends in DNA might play a role in providing access to nucleosome-bound DNA (37, 38).

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1. Berk, A. J. & Schmidt, M. C. (1990) *Genes Dev.* **4,** 151–155.
2. Gutman, A. & Wasylyk, B. (1991) *Trends Genet.* **7,** 49–54.
3. Johnson, P. F. & McKnight, S. L. (1989) *Annu. Rev. Biochem.* **58,** 799–839.
4. Mitchell, P. J. & Tjian, R. (1989) *Science* **245,** 371–378.
5. Ptashne, M. & Gann, A. F. (1990) *Nature (London)* **346,** 329–331.
6. Dang, C. V. (1991) *Biochim. Biophys. Acta* **1072,** 103–113.
7. Cole, M. D. (1986) *Annu. Rev. Genet.* **20,** 361–384.
8. DePinho, R. A., Schreiber-Agus, N. & Alt, F. W. (1991) *Adv. Cancer Res.* **57,** 1–46.
9. Blackwood, E. M. & Eisenman, R. N. (1991) *Science* **251,** 1211–1217.
10. Prendergast, G. C., Lawe, D. & Ziff, E. B. (1991) *Cell* **65,** 395–407.
11. Blackwell, T. K., Kretzner, L., Blackwood, E. M., Eisenman, R. N. & Weintraub, H. (1990) *Science* **250,** 1149–1151.
12. Prendergast, G. C. & Ziff, E. B. (1991) *Science* **251,** 186–188.
13. Kato, G. J., Lee, W. M. F., Chen, L. & Dang, C. V. (1992) *Genes Dev.* **6,** 81–92.
14. Dang, C. V., Dolde, C., Gillison, M. L. & Kato, G. J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 599–602.
15. Kerppola, T. K. & Curran, T. (1991) *Cell* **66,** 317–326.
16. Kerppola, T. K. & Curran, T. (1991) *Science* **254,** 1210–1214.
17. Abate, C., Rauscher, F. J., III, Gentz, R. & Curran, T. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1032–1036.
18. Watt, R. A., Shatzman, A. R. & Rosenberg, M. (1988) *Mol. Cell. Biol.* **5,** 448–456.
19. Kim, J., Zweib, C., Wu, C. & Adhya, S. (1989) *Gene* **85,** 15–23.
20. Bodley, A., Liu, L. F., Israel, M., Seshadri, R., Koseki, Y., Giuliani, F. C., Kirschenbaum, S., Silber, R. & Potmesil, M. (1989) *Cancer Res.* **49,** 5969–5978.
21. Depew, R. E. & Wang, J. C. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4275–4279.
22. Ceglarek, J. A. & Revzin, A. (1989) *Electrophoresis* **10,** 360–365.
23. Fried, M. G. (1989) *Electrophoresis* **10,** 366–376.
24. Wu, H.-M. & Crothers, D. M. (1984) *Nature (London)* **308,** 509–513.
25. Lerman, L. S. & Frisch, H. L. (1982) *Biopolymers* **21,** 995–997.
26. Lumpkin, O. J., Déjardin, P. & Zimm, B. H. (1985) *Biopolymers* **24,** 1573–1593.
27. Verrijzer, C. P., van Oosterhout, J. A. W. M., van Wepern, W. W. & van der Vliet, P. C. (1991) *EMBO J.* **10,** 3007–3014.
28. Thompson, J. F. & Landy, A. (1988) *Nucleic Acids Res.* **16,** 9687–9705.
29. Zinkel, S. S. & Crothers, D. M. (1987) *Nature (London)* **328,** 178–181.
30. Marini, J. C., Levene, S. D., Crothers, D. M. & Englund, P. T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7664–7668.
31. Crothers, D. M., Haran, T. E. & Nadeau, J. G. (1990) *J. Biol. Chem.* **265,** 7093–7096.
32. Halazonetis, T. D. & Kandil, A. N. (1992) *Science* **255,** 464–466.
33. Gartenberg, M. R., Ampe, C., Steitz, T. A. & Crothers, D. M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6034–6038.
34. Gartenberg, M. R. & Crothers, D. M. (1991) *J. Mol. Biol.* **219,** 217–230.
35. Zinkel, S. S. & Crothers, D. M. (1991) *J. Mol. Biol.* **219,** 201–215.
36. Snyder, U. F., Thompson, J. F. & Landy, A. (1989) *Nature (London)* **341,** 255–257.
37. Taylor, I. C. A., Workman, J. L., Schuetz, T. J. & Kingston, R. E. (1991) *Genes Dev.* **5,** 1285–1298.
38. Workman, J. L., Taylor, I. C. A. & Kingston, R. E. (1991) *Cell* **64,** 533–544.