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Research Articles

Direct Evidence for DNA Bending at the Lambda Replication Origin

KENNETH ZAHN AND FREDERICK R. BLATTNER

Replication initiation in bacteriophage lambda appears to require wrapping of origin DNA on an approximately 50 angstrom radius in or around the complex with the initiator protein O. Since short lengths of DNA are not that flexible, it may be that runs of coherently spaced deoxyadenylate residues constitute bend sites in the ori sequence that facilitate the process. Earlier data showed that ori DNA has electrophoretic anomalies characteristic of bend sites and that these are augmented by initiator protein binding. Here origin bending is examined by direct measurement of the ability of polymerized ori sequences to form small circles. The smallest circles observed (84 residues) are compatible with the required radius of curvature. Bend sites within the O protein binding sites, bend sites in the spacers between them, plus the inherent flexibility of non-bent DNA in the origin may all contribute to origin bending. The data also show that a bend site is required for O protein binding to DNA.

BACTERIOPHAGE LAMBDA DNA REPLICATION IS INITIATED bidirectionally at a unique origin site (ori) (*1*), which contains four binding sites (iterons) to which the initiator protein O binds (*2, 3*). Origin DNA exhibits a pronounced curvature in solution (*4*) as indicated by electrophoretic mobility anomalies and O protein binding enhances bending of origin DNA (*5*). Binding of O protein to the origin results in a condensed structure (O-some) (*6*) whose appearance in the electron microscope suggests that DNA is folded or wrapped around protein. Curvature of ori DNA may therefore play a role in O-some formation and ori function.

The mechanism leading to DNA curvature is not understood in detail (*7, 8*). But in a number of cases (*9, 10*), for example the kinetoplast DNA of *Leishmania tarentolae* (*11–13*), clusters of deoxyadenylate (dA) residues punctuating the sequence coherently with the helical pitch induce significant curvature. There are seven

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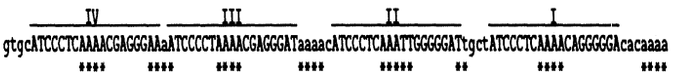
such dA runs in the lambda ori sequence (asterisks below) that we proposed to be responsible for origin curvature. We refer to these sequences as "bend sites."



Four of the bend sites of the origin occur at the central cores of the four O protein binding sites (I to IV overlined and capitalized in the foregoing sequence) in between the symmetrically disposed "half-sites" that contact the protein (*5*). We have suggested (*5*) that these could be the sites where O protein bends its substrate. The bend sites at the iteron cores are not themselves in contact with protein according to chemical probe data (*5*). The other bend sites of the ori region are flanking the binding sites in between or adjacent to the iterons. These could also bend, helping the DNA curve around a small enough radius to form the O-some, promoted by the interaction between adjacently bound O protein subunits.

The electrophoretic mobility test, while offering evidence for DNA bending, did not allow quantitative assessment of the radius of a bend. We have therefore extended the characterization of the origin sequences by assaying the formation of DNA circles during ligation of chemically synthesized variants of the iteron. Systematic bending is expected to greatly increase the cyclization probability of small restriction fragments (*14*). We also consider the question of whether there is a correlation between the ability of these substrates to bend and their ability to bind O protein.

Shore *et al.* (*15*) pioneered the use of the ring closure protocols to study DNA flexibility. Using linear restriction fragments of ϕX174 replicative form thought to be "straight" (computer modeling confirms this), they measured the rate of cyclization in dilute solution as a function of fragment length. They observed that cyclization becomes progressively more difficult as DNA length is reduced below 500 base pairs (bp), reaching zero at some length between 242 and 126 residues. Thus, typically, DNA has difficulty bending at 1.3 degrees per residue and cannot achieve a curvature of 2.8 degrees per residue in solution. In the range of 237 to 254 bp they observed an oscillatory behavior with respect to DNA length having a period corresponding to the helical repeat of DNA (*16*). This indicates that DNA offers resistance to twisting as well as bending distortion.

Ulanovsky *et al.* (*17*) studied ring formation with a 21-residue synthetic oligonucleotide containing a putative bend sequence consisting of dA runs of six and five residues spaced with a 10.5-bp helical repeat. Complementary overhanging sequences were provided to allow polymerization of monomers up to the size needed to close a ring. The dilute DNA concentrations used were comparable to the experiments of Shore *et al.* (*15*) with which they were compared, although a control of putative "non-bent" oligonucleotide DNA was not included. Some circles were formed of substantially smaller radius than the minimum allowed by Shore *et al.* (*15*). Ulanovsky *et al.* (*17*) concluded that the electrophoretic anomalies previously observed were in fact due to sequence-dependent bending of DNA at dA clusters.

To study bending at the lambda origin, we have used a similar approach but with quite different reaction conditions and have included the unbent DNA control. In these experiments we polymerized short (21-residue) linear oligonucleotides containing putative bend sequences from the origin of replication and assayed formation of circles. However, we used high concentrations of oligonucleotide plus a volume excluding agent (10 percent polyethylene glycol) to achieve an effective DNA concentration (about 15 mg/ml) which is on the order of 10^5 times higher than used by Shore *et al.* (*15*). This concentration allows the average distance between 21-bp monomers in solution to approach the distance between their ends if they are considered as rigid rods. The base pair overlaps of our substrates were also chosen to be more stable than the Eco RI sites on the fragments studied by Shore and Baldwin, so that our ligations could be carried out at higher temperature where ligase is more efficient.

The rationale behind these choices is to provide conditions under which the spectrum of circles formed represents a snapshot of molecules whose bend angles approximate the actual range of bends encountered in solution. In order for a linear precursor to be closed under these conditions, its ends must lie on average very close together since, at each stage of reaction, addition of more oligomer subunits competes strongly with circle formation. With dilute conditions, more time is available for cyclization of thermodynamically less favorable configurations.

We argue that the average circle size we observe allows us to calculate the bend angle of the typical molecule in solution; the range of observed circles yields the typical range of bend angles, and the smallest circles give information as to the smallest radius about which the bent DNA is curved with only thermal input of energy. At the very least, our conditions favor formation of large circles and linear polymers, making the finding of small circles a significant indication that highly bent DNA is present.

Design and ligation of ring closure substrates. The three variants of iteron sequence that we examined, substrates I, II, and III, are shown in Fig. 1A. We synthesized 21-residue oligonucleotides corresponding to both strands of each substrate. These were designed to expose seven-nucleotide asymmetric overhangs so as to ensure head-to-tail ligation. All three substrates have the same spacing and sequence of symmetry elements previously demonstrated to be contact points in O protein binding (*5*), but they differ in the disposition of dA clusters.

Substrate I has both iteron core and spacer bend sites so that a 10.5-bp repeat of bend sites is created. This spacing is like the natural origin. Substrate II retains the iteron core bend site, but the spacer is replaced by a mixed sequence so that a 21-bp repeat of bend sites remains. Substrate III has base substitutions that remove all ApA stretches but maintains the spacing and sequence of the protein-contacting elements of the binding site.

The curvature conferred by each sequence was tested by ligating equal amounts of each substrate at high concentration in polyethylene glycol and analyzing the relative amounts and sizes of circular and linear products on two-dimensional acrylamide gels. The first dimension was a standard acrylamide gel, whereas the second dimension contained the intercalating agent chloroquine (*18*). Under these conditions, linear molecules, covalently closed circles, and nicked circles of up to 14 monomer units are well resolved. The molecular size corresponding to each spot was determined by electroelution, denaturation, and electrophoresis on a sequencing gel relative to a marker ladder. The autoradiographs of the two-dimensional gels are presented in Fig. 1B, and the relative amount of each circular species determined by optical densitometry of the films is presented in Table 1.

There was a pronounced inverse relation between the sizes and numbers of circles formed and the number of coherently spaced bend sites per oligonucleotide. Substrate I forms large numbers of small circles with an average size of 147- to 168-bp (that is, seven to eight subunits) and a minimum detectable size of 84 bp (four subunits). Under the conditions of this experiment about 50 percent of the input oligonucleotide was converted to the circular form. Substrate II also forms small circles, but the average circle size is 231 bp (11 subunits) and the minimal detectable circle is 126 bp (six subunits). The total incorporation of substrate II into circles was approximately 10 percent. Substrate III did not form small circles

(≃ 1 percent of the total incorporation). Some radioactivity near the origin of the gel could represent larger circles, but this material was not further characterized.

Estimating the bend angle. The considerable range of circle sizes observed with substrates I and II suggests that these sequences have flexibility superimposed on the static bends. That is, the oligonucleotides can exist in solution at a range of curvatures centered around the equilibrium value of the static bend. Flexibility may reside in the DNA between bend sites as well as in the bend sites themselves.

In view of the high concentration of DNA during ligation, the predominant circles should be composed of molecules with the static bends at or near the equilibrium angle with little or no contribution of DNA flexibility. The minimum and maximum circle sizes provide estimates of the maximal and minimal degree to which the DNA sequence can be bent under thermal input of energy.

To a first approximation, we can estimate bend angles by assuming that the circles are planar and calculate the bend for each monomer subunit as 360 degrees divided by the number of subunits per circle (Table 1). This represents the deviation of the helix from a straight line and is independent of assumptions as to the molecular details of helix bending.

The average circle size for substrate II indicates an equilibrium angle of about 33 degrees for the core bend of the O protein binding site in solution. The average circle size for substrate I is smaller because of the additional spacer bend. If the core bend is assumed to be the same in both molecules, we can estimate the spacer bend at about 15 degrees for a total of 48 degrees per core-spacer subunit. The smallest circles of substrate I consist of four subunits so that there must be enough flexibility to allow deformation from 50 to 90 degrees, which is about 2.0 degrees per base residue. This is a rather large curvature, but not outside the limits of flexibility of DNA determined by Shore *et al.* (*15*) if the entire substrate is assumed to be flexible. This degree of flexibility would be difficult to reconcile with their data if bend sites were rigid and flexibility were confined to the DNA in between them. The smallest circles of substrate II are six subunits, so that the deformation due to flexibility in this case is only 27 degrees per subunit or 1.3 degrees per residue.

The bend is required for O binding. An important question is whether the bend site is necessary for O protein binding. This was tested by measuring the ability of each substrate to bind the O protein as assayed with a gel retardation assay. For technical reasons, the experiment shown in Fig. 2 was done with the amino-terminal half of O protein, which contains the DNA binding site. Its cloning, isolation, and specific binding have been described (*5, 19*). Substrates I and II bind protein perfectly but substrate III, which lacks the bend site is unable to bind O (Fig. 2). Thus, the bendable segment at the center of iteron symmetry is essential for the binding of O protein, but the spacer regions are not.

To ascertain whether the contact residues between DNA and O are the same with the current substrates as had been determined for iteron I (*5*), we determined which phosphate residues of substrates I and II are contacted when the ethylation interference assay is used

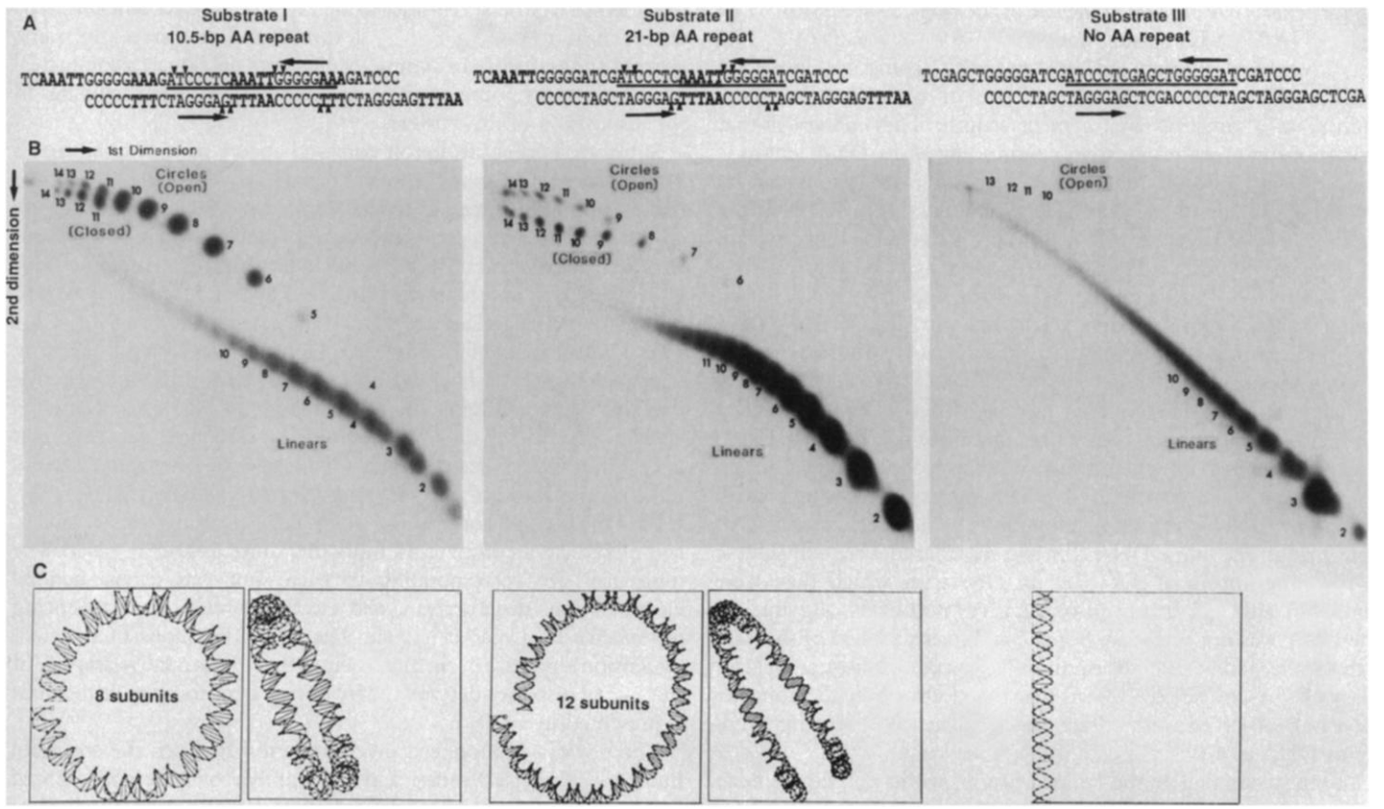


Fig. 1. Ring closure experiments with iteron substrates. (**A**) Substrates: Two tandem copies of each complementary strand of each synthetic oligonucleotide are shown arranged as they would be expected to anneal to form longer duplex DNA segments. Clusters of repeating ApA dinucleotides are shaded, and their repeat spacing is indicated above the sequence. The 19-bp iteron unit is underlined and its 6-bp dyad symmetry elements are indicated by arrows above and below the sequence. Arrowheads indicate the phosphates contacted by O protein. (**B**) Characterization of substrate ligation products; two-dimensional electrophoresis results are shown with sizes of open and closed circles and linear DNA's expressed as number of 21-bp oligomers. Arrows indicate the directions of first- and second-dimension electrophoresis (*41*). (**C**) Helix trajectory plots of iteron substrates; computer predictions of ring closure performed with an 8-subunit oligomer (168 bp) of substrate I and a 12-subunit oligomer of substrate II (252 bp) were made by the method of Levene and Crothers (*24*) with a program obtained from DNASTAR (Madison, Wisconsin) with parameters as discussed in the legend to Table 1. The junction angles were 11 degrees for substrate I and 10 degrees for substrate II. These angles represent values close to the average from the cyclization experiments. Two perspectives are shown for substrates I and II and one for substrate III.

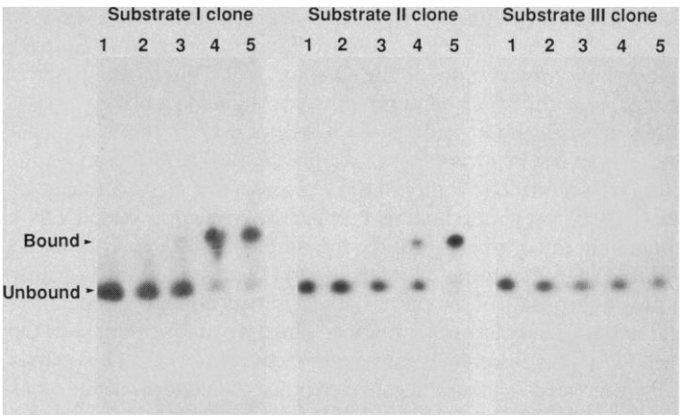


Fig. 2. Binding of O protein to cloned iteron substrates: an autoradiograph of the electrophoresis of O protein–DNA complexes in an acrylamide gel. Cloning of the iteron substrates was achieved by cleavage of the ligated oligomers in Fig. 1 with Mbo I, isolation of the short DNA fragments from 10 percent acrylamide gels, and ligation into the Bam HI site of pBR322. Each gel shows the results of titrating 37 ng of a Nar I–Hind III restriction fragment 5′ end-labeled at the Nar I site with (lanes 1 to 5) 0, 5, 15, 50, and 100 ng of the O protein amino terminus. Arrowheads at the left margin indicate the positions of the DNA-protein complex (bound) and free DNA (unbound). Binding was carried out at 37°C for 20 minutes in 50 μl of 10 m*M* tris-HCl (*p*H 7.4), 20 m*M* KCl, 10 m*M* magnesium acetate, 0.1 m*M* EDTA, 6 m*M* β-mercaptoethanol, and bovine serum albumin at 50 μg/ml. Electrophoresis was carried out for 16 hours at 100 V in 5 percent acrylamide gels containing 25 percent glycerol and a tris-borate buffer.

(*20*). The experiment (Fig. 3) shows that the eight symmetrically disposed phosphate residues on one helical face previously identified (*5*) (arrowheads in Fig. 1A) are the same as the ones contacted in these substrates. Although a minor asymmetric phosphate contact that we observed in iteron I was not observed in these experiments, we conclude that the basic character of the binding is the same regardless of whether the iteron core sequence is AAAAC (iteron I, III, and IV) or AAATT (iteron II and substrates I and II).

Modeling iteron bending and the O protein origin interaction. Although the details of helix deformation involved in bending are not well understood, two types of models have been proposed to explain sequence-dependent bending. In one model, wedges are introduced between adjacent A-A base pairs in a dA run as a result of nonparallel base stacking on one side of the helix (*17*). In the other model, deflection of the helix is postulated to occur at the junctions between dA runs and adjacent DNA segments as a result (*12*) of a nonequivalence of the two DNA strands within the dA run, which is thought to resemble heteronomous DNA (*21*). The electrophoretic studies of Koo *et al.* (*8*) and of Hagerman (*7*) have led to the discovery of sequences that challenge the simple forms of the above models. For example, $(CAAAATTTTG)_n$ bends substantially whereas $(GTTTTAAAAC)_n$ does not (*7*), and $(A_5T_5)_n$ bends whereas $(A_{10}T_{10})_n$ does not (*22*). To accommodate this, Ulanovsky and Trifonov (*23*) have introduced a modification of the wedge model in which the average deviation of each AA dinucleotide is distributed between a 2.3-degree tilt and a 8.7-degree roll. Crothers (*22*) has suggested a junction model in which a run of dA residues induces a junction tilt of 11 degrees at each end and in which junctions between runs of A and runs of T introduce a roll of 11 degrees. All of these models, including the simple ones, yield similar results for the ori sequences since the λ origin does not contain these special sequences.

The junction model was selected for our presentations because it accurately predicted closure of substrates I and II at the correct average circle size. Examples of ring closure experiments performed by the computer with substrates I and II are shown in Fig. 1C. Helix trajectories were plotted by a computer program based on the method of Levene and Crothers (*24*). The picture drawn by the computer by the dot matrix printer also includes connecting lines that correspond to the base paris. This is an approximate model, but it gives a reasonable idea of the DNA trajectory that might exist in the origin.

Figure 4A shows a stereo model of iteron II as it might interact with protein if we assume that the bound form is fully bent. Since the program does not depict flexibility in non-bent DNA, the helix parameters supplied to the computer to make this drawing are those which close the smallest circles observed with substrate II (20 degrees at each end of a dA run). Points of phosphate contact with the protein are indicated by dots between the base pair planes. These occur on one side of the helix, and it appears that the bend at the iteron core does not appreciably disturb the symmetry of these phosphates relative to the dyad axis of the site. If the O protein dimer were to be assembled so that the DNA binding half-sites that interact with the major groove were at an angle, it could easily be explained why O protein binding requires a bendable DNA structure at the center of the binding site and why O protein binding would enhance bending. Although the direction of bending cannot be derived from ring closure results alone, it can be deduced from our nuclease footprinting, ethylation interference, and dimethyl sulfate protection data (*5*). Thus, we have chosen the sign of the angular displacement associated with the bend so that the surface of contact with the O protein is on the inside of the curve. The O protein therefore contacts one face of the helix (as indicated by the phosphate interference data), in two successive major grooves (as deduced from the pattern of dimethyl sulfate protection) (*5*). This is the only way to form a structure with the dimensions of the O-some (*6*).

Figure 4B is a stereo projection of the iteron region of ori with the same helix parameters and display conventions as Fig. 4A. The basic

Table 1. Observed circle sizes, incorporation, and predicted angles. The bend angles per subunit are calculated by dividing the degrees in a circle by the total number of subunits per circle. The number of subunits of 21-bp oligomer per circle is indicated. Bp is the size of the circle in base pairs. Junction angles (J) were used in the DNASTAR program as follows: a tilt angle of +J degrees was introduced at each end of each dA tract (length ≥ 2), except that junctions between dA and dT tracts were assigned a roll of +J degrees and dT tracts followed by dA tracts were assigned a roll of −J degrees. Junction angles needed to close the ends are shown (optimal circles in parentheses). Note the agreement in these values between substrates I and II. The percentage of circles is derived by dividing the integrated optical density of each circle by the sum of the integrated optical density of all circles in the ligation.

Subunits (No.)	Bp (No.)	Angle/subunit (degrees)	Substrate I*		Substrate II†	
			Junction angle (degrees)	Circles (%)	Junction angle (degrees)	Circles (%)
4	84	90	22.5	2.0		
5	105	72	18.0	6.5		
6	126	60	15.0	10.3	20.0	1.8
7	147	51.2	(12.8)	16.3	17.1	6.8
8	168	45	(11.2)	16.2	15.0	6.9
9	189	40	10.0	14.5	13.3	12.6
10	210	36	9.0	12.9	(12.0)	17.3
11	231	32.8	8.2	10.3	(11.2)	18.5
12	252	30	7.5	5.0	10.0	16.7
13	273	28	7.0	4.0	9.3	9.1
14	294	25.6	6.4	2.0	8.5	5.0
15	315	24			8.0	4.0

*Two dA tracts per subunit. †One dA tract per subunit.

form assumed by the DNA is a 300-degree turn, the diameter of which, 110 Å, is remarkably close to that determined from electron microscopic observation of the O-some (*6*). This model predicts that three of the four bound proteins lie roughly in a plane with the DNA curving about them. The GC-rich spacer between iterons I and II does not bend, and hence the first binding unit (iteron I) falls out of the curve. The ability of the O protein to impose a change of about a three-quarter linking number in covalently closed DNA containing the origin (*6*) may be the simple result of an O protein–induced twist that brings all of the bound O protomers into a planar array on the inside of the curve. This twist may also aid in separating the strands for replication initiation. Thus, we predict that, with the help of protein-protein interactions between bound O subunits (*25*), this open structure is converted into the condensed O-some.

DNA bending and flexibility. In these experiments, a graded series of substrates differing only in the disposition of bend sites has been directly compared with respect to ring closure under a consistent experimental protocol. We found a high percentage of cyclization under conditions designed to favor the formation of linear polymers. In fact, circle formation was so efficient that few linears were seen above the ten-subunit size range (Fig. 1, A and B). Although our assay did not require it, a high proportion of circles formed were covalently closed. Either these molecules are reasonably flexible in the torsional dimension or the use of a 21-bp repeat coincidentally allows excellent alignment of the strands with minimal torsional distortion regardless of circle size.

The average circle sizes found suggest that the ori sequences contain static bends. That DNA bends are static was argued by Koo

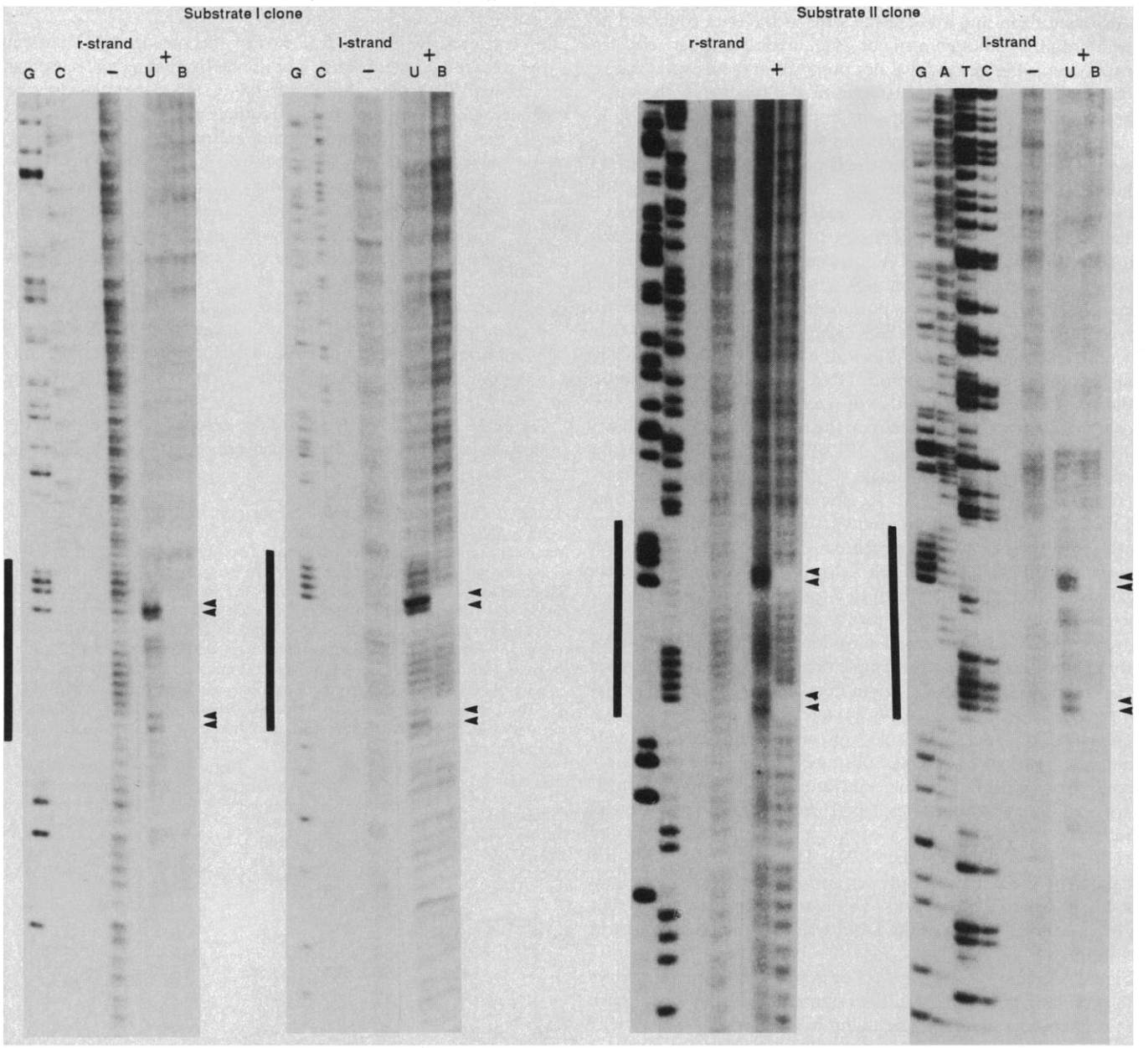


Fig. 3. Ethylation interference with cloned iteron substrates. The ethylation interference method of Siebenlist and Gilbert (*20*) as modified (*5*) was used. The r-strand data were obtained with the 5′ end-labeled Taq I–Sph I fragment and l-strand data with the Nar I–Hind III fragment. In each case, 37 ng of DNA fragment was used. Maxam and Gilbert sequencing standards (G, A, T, C) are run alongside of each ethylation experiment for alignment. Vertical bars indicate the extent of the cloned iteron. Arrowheads indicate the positions of the interfering phosphate groups. Lanes are labeled as follows: (–) no protein was added; (+) 100 ng of O protein amino terminus added; (U) unbound fraction; (B) bound fraction. The fractions (U and B) represent the eluates of the unbound and bound DNA isolated from gels like those shown in Fig. 2.

et al. (*8*) and by Hagerman (*26*) on the basis that coherent phasing of ApA repeats is a prerequisite for electrophoretic anomalies. Additional evidence that sequence-directed bends are static comes from the electron micrographic observations of Griffith *et al.* (*27*) where the curvature of a kinetoplast DNA can be visualized and is consistent from molecule to molecule.

The range of circle sizes that we see suggests, however, that the ori DNA is flexible enough to bend to a considerably smaller radius of curvature than the average dictated by the angle of a static bend. It is not known whether the bend sites themselves are also flexible or whether the ori flexibility is confined to the interstitial segments between bend sites. We suggest that both are flexible to accommodate our smallest circles within the Shore-Baldwin minimal curvature. But the proposal that sequence-dependent bends are rigid is supported by physical studies of Levene *et al.*, which indicate that the bent region from kinetoplast is stiffer than normal DNA (*28*). More data are needed to ascertain how fixed the bent structure of the origin may be. In either case, it is likely that an element of flexibility is critical in the packaging of the O-some by decreasing the radius about which DNA can be curved. The question of whether the existing junction bends are the preferred sites of further protein induced deformation awaits an x-ray crystallographic analysis of the O protein–DNA complexes.

Replication and bending. The relevance of DNA bending to DNA replication is unclear. It has now been implicated, however, in at least five systems—lambda (*4*), SV40 (*29*), R6k plasmid (*30*), plasmid pTI81 (*31*), and yeast autonomously replicating sequences (*32*). Iteron-like sequences are also found at the origins of the lambdoid phages ϕ80 and ϕ82 (*2*). The F plasmid origin (*33*) has four 19-bp initiator protein binding sites with core sequences of AAATT (identical to iteron II and substrates I and II). This suggests that similar bending effects may also occur at these origins. In the case of SV40, a dA run appears to be a determinant of high affinity T antigen binding, and T antigen may also bend this site (*29*). One hypothesis is that replication initiation requires assembly of proteins about a highly condensed structure (*34*). The minimal circle size determined for substrate I (four subunits) would have a diameter of 90 Å, close to the measured size of the condensed O protein–origin complexes observed in the electron micrographs of Dodson *et al.* (*6*). The function of the O protein may be to condense the four iterons of ori into a structure similar to the smallest observable circles. Since the smallest circles formed with substrates I and II are very small proportions of the total circles, we conclude that their formation is energetically unfavored by thermal flexibility of the DNA. Such highly localized DNA bending (or twisting) will, therefore, result in storage of substantial free energy. This stored energy may be used in subsequent stages of the initiation reaction such as strand separation (*25*). Binding of λP protein or a P–dna B protein complex to this condensed structure via the exposed carboxyl terminus of O and consequent DNA unwinding appear to be the next steps in the initiation reaction (*19, 35*).

Protein-DNA interactions and DNA deformation. There are other examples of site-specific DNA binding proteins that appear to bend DNA (*36, 37*), the best characterized being the *Escherichia coli* catabolite gene activator protein (CAP) (*12*). Kotlarz *et al.* (*38*) have demonstrated that CAP increases the rate of cyclization of short pieces of lac operon DNA, presumably because of its ability to bend them. In contrast to the λ origin, CAP binding sites do not bend in the absence of protein (*12*). In CAP binding, the induced bend is estimated to be 90 to 180 degrees (*39*). The CAP binding site, however, does not contain a typical bend site, and therefore considerable difference in mechanism may be indicated. A static bend in DNA seems to be an absolute requirement for binding in the case of the O protein.

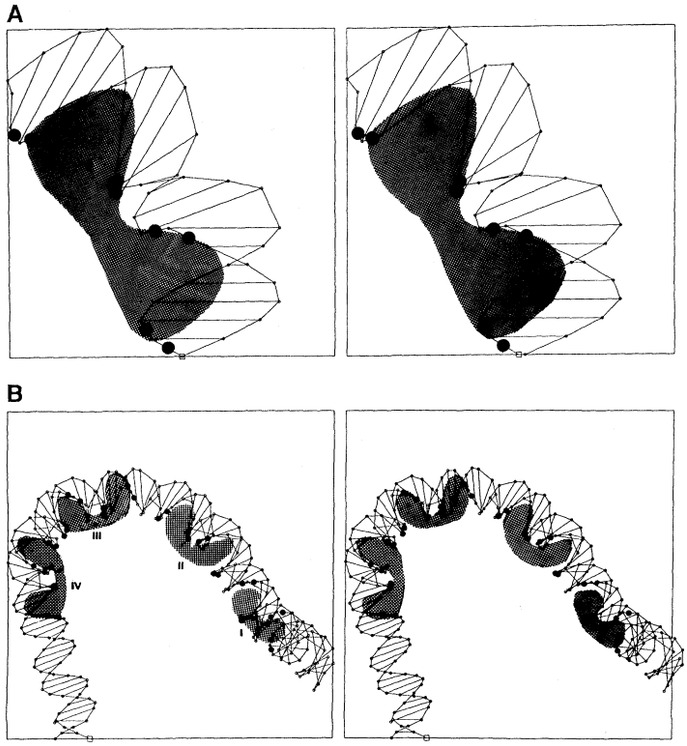


Fig. 4. Helix trajectory plots (stereo) of iteron substrate II and the iteron region of the natural origin and the regions contacted by the O protein. (**A**) Iteron substrate II. Helix parameters are as in Fig. 1C and Table 1, except that the junction tilt angle is −20 degrees. The substrate II sequence ATCCCTCAAATTGGGGGATCG is plotted. The small square at lower margin shows the 5′ end of the sequence, and base pair planes are noted by lines connecting the two complementary strands. Phosphate contacts are shown by large dots between the base pair planes. Shaded areas show only amino-terminal contact portions of an O protein dimer interacting with two successive major grooves of the iteron. The volume of a complete O protein dimer would correspond to a sphere with a diameter 2.7 times the DNA helix diameter. (**B**) Iteron region of the origin. The sequence between coordinates 39013 to 39129 of the λ map (*42*) is plotted. Phosphate contacts are shown as in (A), as are areas of close contact with the O protein (shading). The iterons are numbered I to IV. Helix parameters are the same as those in (A). An O-some composed of four complete O protein dimers packed as a sphere would have a diameter 4.4 times that of the DNA helix and would fill the space surrounded by DNA.

Drew and Travers have studied the detailed path of the DNA helix around nucleosomes reconstituted on *tyrT* DNA (*40*), to which the deduced path of DNA around the O protein (Fig. 4A) bears a striking resemblance. The direction of bending that we predict for the iteron is in agreement with the inner orientation and minor groove compression observed for most of the short dA runs in the nucleosome data. If we use a junction model of bending, it is clear that the base pair tilt at the junctions of the iteron half-sites with the dA run at the iteron core will cause such a compression and bring the half-sites into closer proximity. This compression could also be achieved by base pair roll of the iteron cores into the minor groove as predicted for bending of nucleosome cores (*40*) and for CAP protein (*39*). The minor grooves of the dyad symmetry elements (G/C blocks) are predicted to face out (away from the bound protein), a result also deduced by Drew and Travers (*40*) for nucleosome core structure. Therefore, O binding obeys the "rules" of DNA bending outlined by Drew and Travers (*40*); DNA is deformed in the same specific way whether by protein-induced or other types of deformation.

The striking similarities to DNA bending around nucleosomes suggest that O binding represents an extreme case of the "bendabili-

ty" of a DNA site determining the detailed interaction of a regulatory protein with its DNA substrate. That is, periodicities in base sequence are also used to facilitate the tight bending of DNA as it wraps around an O-some.

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41. Ligations were carried out as follows. Chemically synthesized oligonucleotides (25 μg of each strand) were 5′ end-labeled with [γ-^{32}P]ATP (adenosine triphosphate) (10 μCi/μmol), and 10 units of polynucleotide kinase (BRL) for 30 minutes at 37°C in a total volume of 200 μl. Reactions were extracted with phenol, precipitated with ethanol in the presence of transfer RNA carrier, denatured in formamide, and placed on 10 percent acrylamide DNA sequencing gels. Oligonucleotides were purified from the gels by electroelution. Complementary oligonucleotides were mixed in 50 m*M* NaCl, heated to 65°C, and allowed to cool to room temperature to form hybrids. Ligation conditions were adjusted to those described by Hayashi *et al.* [K. Hayashi, M. Nakazawa, Y. Ishizaki, A. Obayashi, *Nucleic Acids Res.* **13**, 3261 (1985)] in a 30-μl volume: 150 m*M* NaCl, 1 m*M* ATP, 10 m*M* dithiothreitol, 6.6 m*M* $MgCl_2$, 66 m*M* tris-HCl, *p*H 7.4, and 10 percent PEG-6000 (polyethylene glycol) with 1.5 units of T4 DNA ligase (BRL). The PEG increases reaction rate by a factor of about 10, presumably by the excluded volume principle. The reaction was incubated at 37°C for 2.5 hours. Ligation products were extracted with phenol, precipitated with ethanol, and analyzed on a 5 percent acrylamide (25:1, acrylamide:bis ratio) first-dimension gel containing tris-borate buffer and 25 percent glycerol, with electrophoresis at room temperature for 16 hours at 5 V/cm. After autoradiography, the gel segment was excised and placed between slab gel plates and a 7.5 percent acrylamide gel containing chloroquine-phosphate (50 μg/ml) was cast around it. Second-dimension electrophoresis was carried out for 16 hours at 20 V/cm at room temperature with a running buffer containing chloroquine (50 μg/ml). The gel was exposed to x-ray film for 16 hours. Spots corresponding to each species were excised from the second-dimension gel, electroeluted, denatured, and identified by electrophoresis on 10 percent DNA sequencing gels in comparison to the total ligation products. Closed circles were relaxed either by allowing time for strand nicking by radioactive decay or by partial digestion with Mbo I before they were sized.
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