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In vivo DNA loops in *araCBAD*: Size limits and helical repeat

(supercoiling/twist/periodicity/linking number deficit/looping energetics)

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ABSTRACT **Formation of a DNA loop by AraC proteins bound at the *araI* and $araO_2$ sites, whose center-to-center distance is 211 base pairs, is necessary for repression of the *araBAD* promoter, P_{BAD}, of *Escherichia coli*. To determine the upper and lower size limits of the loop, we constructed P_{BAD}–reporter gene fusion plasmids with various spacings between *araI* and $araO_2$ and measured their levels of expression. Spacings larger than about 500 base pairs resulted in elimination of detectable repression. No lower limit to spacing was found, suggesting that AraC protein itself possesses significant flexibility and its bending substantially aids formation of small loops. As the spacing between *araI* and $araO_2$ varied, the activity of P_{BAD} oscillated with an 11.1-base-pair periodicity, implying that the *in vivo* helical repeat of this DNA is 11.1 base pairs per turn.**

Regulation of the repressed basal level as well as the induced level of expression of the arabinose operon in *Escherichia coli* involves DNA looping mediated by the AraC protein. AraC proteins bound to two distinct DNA sites and bound to each other to form a DNA loop (1–4). DNA looping in the *ara* system requires that the two binding sites involved in forming the repression loop, *araI* and $araO_2$ (Fig. 1), be on the correct face of the DNA double helix (1, 2). Although the wild-type spacing of 211 base pairs (bp) between *araI* and $araO_2$ gives rise to full repression of P_{BAD}, insertion of 5 bp between the two sites rotates one site halfway around the DNA double helix with respect to the other site and greatly interferes with repression. We interpret this finding as resulting from the torsional stiffness of DNA. Forcing the two misaligned sites to the same side of the DNA, so that looping could occur, would cost about 2.1 kcal/mol (1 cal = 4.184 J) (5) and could interfere with repression if the AraC protein does not have sufficient lateral flexibility to overcome misalignment of its binding sites.

Measurements on bare linear DNA made *in vitro* have yielded values close to 10.5 bp per turn for average DNA (6–8). The helical repeat of DNA *in vivo* apparently has not been measured, but we might expect it to possess a slightly different value because on average such DNA possesses a linking number deficit. This deficit generates supercoiling (9) and, in principle, could also partially untwist the DNA. Indeed, an untwisting caused by a linking number deficit has been observed *in vitro* for supercoiled DNA (10, 11). Another reason for deviation from the canonical value of 10.5 bp per turn might be local supercoiling generated by transcription of adjacent genes (12). As the spacing between *araI* and $araO_2$ is progressively increased or decreased, repression of P_{BAD} should oscillate with a period about equal to the helical repeat of the DNA. Therefore, we have utilized looping in the *ara* system to measure the helical repeat *in vivo* of the DNA of the *ara* operon regulatory region.

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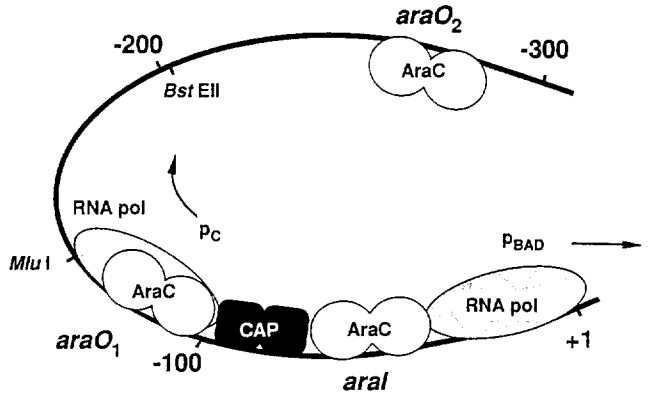

FIG. 1. Protein-binding sites in the L-arabinose *araCBAD* regulatory region. Numbering of base pairs is relative to the P_{BAD} transcription start at position +1. Stippled area, RNA polymerase; open area, AraC protein; solid area, cAMP CAP.

Both upper and lower limits to the DNA loop size ought to exist. As the length of the DNA separating *araI* and $araO_2$ increases to large values, the sites should have increasing difficulty finding one another and looping should decrease. At the other extreme, when the length of DNA separating the sites decreases, the stiffness of the DNA should hold the sites (and the proteins bound to the sites) apart and hinder loop formation. Maximal loop formation ought to occur at some intermediate loop size, much like the maximum that is seen in DNA ring closure or cyclization as a function of DNA length (5, 13, 14).

Several factors other than loop size may also affect DNA looping. Supercoiling may compact DNA and assist loop formation. Proteins other than AraC—for example, HU, IHF, FIS, or catabolite-activating protein (CAP) (15–18)—may bind to and bend the DNA or change its helical repeat. Finally, flexibility of the AraC protein itself could assist formation of small loops.

Here we report experiments showing that the helical repeat of the DNA between *araI* and $araO_2$ is 11.1 bp per turn, that the upper size limit of the *ara* loop is about 500 bp, and that there is no lower size limit to this loop.

MATERIALS AND METHODS

Media, Strains, and General Methods. Media and general methods have been described (19, 20). Plasmid pTD3 contains 440 bp of the *araCBAD* regulatory region on a *Hin*dIII–*Eco*RI fragment and has P_{BAD} driving *galK* of the pKO1 vector (1). Its derivative, pLH2 (4), contains the M13 replication origin at the end of the *galK* structural gene. Plasmid pDL3 was made by replacing the *galK* structural region of pLH2 with the *lacZ* structural region of pPN10 (ref. 21; kindly donated by P. Norton, Tufts University School of Medicine) while maintaining the *galK* leader region. The AraC-overproducing plasmid,

Abbreviation: CAP, catabolite-activating protein.

pDL5, combines the gene coding for AraC under the control of the *lacUV5* promoter (22) and the vector part of pGA44 (23). It has the p15A replication origin that is compatible with the colEI replication origin of pDL3, the chloramphenicol drug-resistance marker, and it synthesizes AraC protein at greater than 100 times the rate of the wild-type chromosomal copy of *araC*. Strains used were the AraC$^-$ strain SH321, its isogenic partner AraC$^+$ SH322, F$^+$ SH322 (called DL100), and AraC$^+$ *cya*$^-$ SH326 (24). Strain DL110 contains the mutation *gyrB225* introduced into SH322 by P1 transduction from AE400 (F$^-$ *trpB gyrB225 tna*::Tn*10 ilvO*; a gift of A. Wright, Tufts University School of Medicine), a derivative of AE199 (25). The linking number deficit in plasmids extracted from this strain was about −0.03, as measured by band counting of topoisomers on chloroquine-containing agarose gels (26). Plasmids from the strain lacking the *gyr* mutation had an average linking number deficit of −0.06.

Construction of Insertion Plasmids. We use the distance between the apparent middle of the inverted sequence symmetries of the *araI* (position −59) and *araO*$_2$ (position −270) sites when discussing the distance between them. Plasmid pTD3 was cut with the restriction enzyme *Bst*EII, treated with the Klenow fragment of DNA polymerase I and alkaline phosphatase, and ligated with *Hae* III fragments of *E. coli* chromosomal DNA smaller than 1000 bp long. The strain SH322 was used for transformation and scoring of insertion candidates on MacConkey galactose-indicating plates. The size of each insertion was determined by electrophoresis of the *Hin*dIII–*Eco*RI segment from the plasmids and comparison to sequenced size markers. Several candidates were chosen for further investigation by sequence analysis, isolation of repression-defective mutations, and investigation of helical twist dependency by addition of 4- or 8-bp insertions.

Small-sized insertions (+13, +15, +17, +19, +21, +23, +25, +29, +31, +33, and +35 bp) were made by inserting oligonucleotides of various sizes into the *Bst*EII site of pLH2 and subsequent insertion of 4 nucleotides in restriction sites contained on the oligonucleotides. Four plasmids having spacing changes of −16, −8, +5, and +11 bp from the wild-type spacing (211 bp) were described in Dunn *et al.* (1). The promoter fragments of these spacing mutants were recloned into the vector part of pDL3 to allow P_{BAD} promoter activities to be measured by β-galactosidase assay.

Construction of Deletion Plasmids. Plasmid pLH2 was cut with *Bst*EII, partially digested with slow BAL-31 exonuclease (27), ligated, and transformed into strain SH322. Approximately 400 candidates were collected, and the sizes of their deletions were determined by comparing their *Hin*dIII–*Eco*RI fragments with known size markers. The candidates having deleted less than 200 bp, which might retain intact *araI* and *araO*$_2$ sites, were chosen for further investigation by sequence analysis, isolation of repression-defective mutations, investigation of helical twist dependency by addition of 4-bp insertions, and *in vivo* footprinting.

Some of the important spacing plasmids discussed later delete the following regions: for a 32-bp spacing, positions −81 to −259; for a 33-bp spacing, positions −82 to −259; for a 34-bp spacing, positions −84 to −260; for a 38-bp spacing, positions −77 to −249; for a 44-bp spacing, positions −82 to −248; for a 49-bp spacing, positions −88 to −249; for a 111-bp spacing, positions −158 to −257; and for a 146-bp spacing, positions −164 to −228. The numbering of positions is relative to the P_{BAD} transcription start at position +1.

Measurement of Promoter Activities. Plasmid constructs possessing an intact CAP binding site were transformed into DL100 cells, whereas plasmid constructs having a defective CAP binding site were transformed into SH326 cells harboring pDL5. The new strains were grown in M10 medium (19) for at least five generations to a density of 2×10^8 cells per ml. The β-galactosidase assays and units described by Miller (28) and the galactokinase assays (24) were used depending on the structural gene of the test plasmids. The typical standard deviations of the β-galactosidase and galactokinase assays were 8% and 11%, respectively.

Other Methods. Detailed methods for the isolation and analysis of repression-defective mutants and *in vivo* footprinting were described (3). Fourier transformation (29) of the repression and induction properties as a function of the *araI*–*araO*$_2$ spacing was performed with a spread-sheet program. For this operation the induced enzyme levels in the spacing plasmids without the CAP binding site (spacing, 32–86 bp) were multiplied by 0.1 before transformation, and the value of the enzyme levels for spacings that were not constructed was taken to be zero. The amplitude (A) as a function of the periodicity (p) was evaluated for p between 9 and 13 bp, where s is the spacing between *araI* and *araO*$_2$ and $E(s)$ is the enzyme level measured for the spacing.

$$A(p) = \sqrt{\left[\sum_s E(s)\sin\left(\frac{2\pi s}{p}\right)\right]^2 + \left[\sum_s E(s)\cos\left(\frac{2\pi s}{p}\right)\right]^2}.$$

The approximate relative energies of loop formation were determined by an analog technique. We measured the work required to bend elastically a series of flexible steel strips with various lengths, obtained from a surveyor's tape, from straight to a conformation in which the ends were a scaled distance of 150 Å apart, corresponding to twice the estimated diameter of AraC protein. During this process the ends were not angularly constrained. This freedom corresponds to flexibility in the protein.

RESULTS

Loop Size Upper Limits. We concentrated on inserting DNA fragments smaller than 2000 bp because separating the *araI* and *araO*$_2$ sites by about 2000 bp yielded only repression-negative derivatives. About 1000 candidates were generated by inserting *Hae* III fragments of *E. coli* chromosomal DNA smaller than 1000 bp long into the *Bst*EII site of pTD3. One hundred and three candidates were repression-positive or partially positive and were examined further. We also chose 41 repression-negative colonies for additional characterization. Fig. 2*a* shows the relationship between the approximate size of the insertion and the degree of repression as shown on indicating plates in which repression-negative colonies were red and repression-positive colonies were white.

In any particular size class of insertions we might expect some to leave *araI* and *araO*$_2$ misoriented with respect to the helical face for repression and others to leave these sites correctly oriented. Hence the most significant sizes with respect to repression or lack thereof in any size region of the graph are those that repress best. Apparently, as the loop size increases above 300 bp, looping or repression becomes increasingly difficult and is lost by about 500 bp. Seven of the 62 different-sized plasmids containing insertions of more than 50 bp were more fully characterized by DNA sequencing and quantitative measurement of repression. Data derived from these plasmids (Fig. 2*b*) agree with their more qualitative characterization.

The plasmid containing the 299-bp spacing repressed P_{BAD} as well as a plasmid containing no insertion, but the insertion of an additional 4 bp at the *Mlu* I site in this plasmid significantly interfered with repression (Fig. 2*b*). Plasmids with a 346-bp separation and a 401-bp separation between *araI* and *araO*$_2$ poorly repressed and their repression was not significantly improved by inserting an additional 4 or 8 bp between *araI* and *araO*$_2$. This indicates that the loop size itself was the reason for the poor repression. The promoter activity on the plasmid with 616 bp between *araI* and *araO*$_2$

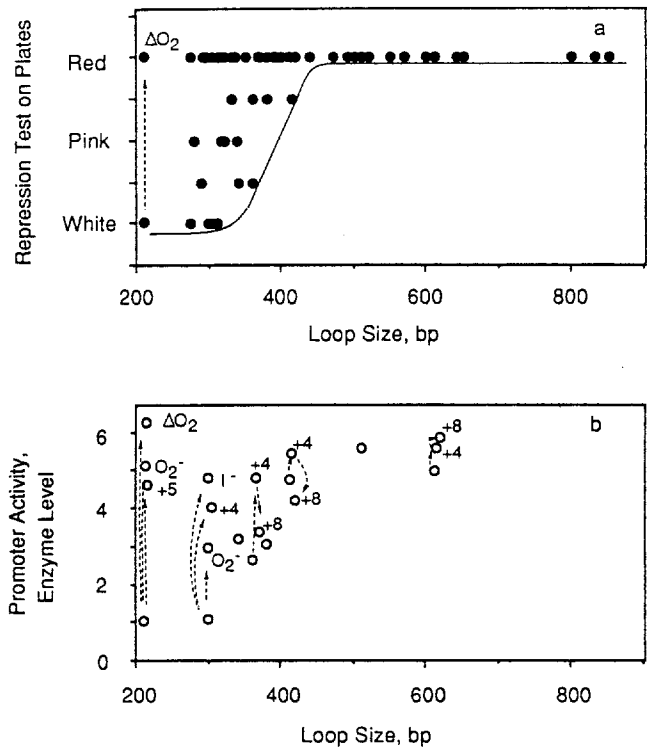

FIG. 2. Repression as a function of loop size. (*a*) Qualitative test of 62 candidates containing insertions between $araO_2$ and *araI*. The plasmids in strain DL100 were streaked on MacConkey galactose-indicating plates and the color of the colonies was recorded after 14 hr of growth. The solid line shows the best repression abilities in each size range. (*b*) Basal level *araBAD* promoter activities in strain DL100 by direct galactokinase assay of seven insertion plasmids and their derivatives that were chosen from the same 62 candidates shown in *a*. Broken lines indicate that subsequent basal level after addition of 4 or 8 bp at the *Mlu* I restriction site, isolation of repression-negative mutants after hydroxylamine mutagenesis of the *araBAD* promoter region (O_2^- and I^-), or deletion of the $araO_2$ site (ΔO_2).

remained repression-negative even after insertion of an additional 4 or 8 bp. These results show that loops in the *ara* system larger than about 500 bp cannot easily be formed with wild-type AraC protein.

Mutations isolated solely on the basis that they reduced repression in the plasmid with the 299-bp loop were located in the *araI* site (G → A at position −43) and the $araO_2$ site (C → T at position −271) or in the RNA polymerase binding site, just as was found by Martin *et al.* (3). This indicates that the *araI* and $araO_2$ sites, and not extraneous sequences on the plasmid, were required for repression in this plasmid.

Loop-Size Lower Limits. Deletions were generated to move $araO_2$ and *araI* closer together, and the candidates retaining intact $araO_2$ and CAP binding sites were further characterized.

A deletion of 100 bp, leaving a loop of 111 bp, showed normal repression. It was a surprise that loops as small as this should form, since significant work is required to bend 100 bp of DNA into a circle. That is, the persistence length of DNA in physiological buffers is about 140 bp (14). Therefore, we tested whether the looping was between *araI* and $araO_2$ by mutational analysis, as was done for the 299-bp spacing plasmid, and found that the *araI* and $araO_2$ sites in a plasmid containing the 146-bp spacing were involved in a small repression loop.

The smallest possible spacing between *araI* and $araO_2$ that will not damage either site or the intervening CAP site is 70 bp. Deleting or damaging the CAP site substantially lowered the repressed and induced levels of P_{BAD} (1, 30), and this would interfere with interpretation of data. In the absence of a functional CAP binding site, however, looping between *araI* and $araO_2$ can be detected by its dramatic reduction of the arabinose-induced levels of P_{BAD} activity when somewhat elevated levels of AraC protein are provided (24).

To examine the ability to form small loops, we constructed another AraC-overproducing plasmid, pDL5, which is compatible with the spacing mutant plasmids so that both the hypersynthesis plasmid and the P_{BAD}–*lacZ* plasmid could be stably maintained in cells. The inducibility of P_{BAD} in this system as a function of the *araI*–$araO_2$ spacing was compared to the repression abilities in the CAP^+ plasmids with the same spacings. As shown in Fig. 3*c*, both inducibility in the CAP^- system and repression in the CAP^+ system show identical loop size dependencies for loops around 140 bp and loops around 230 bp. That is, the maxima and minima of the basal levels occur at the same loop sizes as the maxima and minima of the induced levels. Therefore, we extended our examination of loop formation to spacings of less than 70 bp between *araI* and $araO_2$ when part or all of the CAP site was deleted.

The inducibility of P_{BAD} in the plasmids with the CAP site deleted oscillated as the spacing between *araI* and $araO_2$ varied as shown in Fig. 3*c*. This oscillation retained the same periodicity of about 11 bp and the same phase as the oscillations in the basal level observed with the plasmids

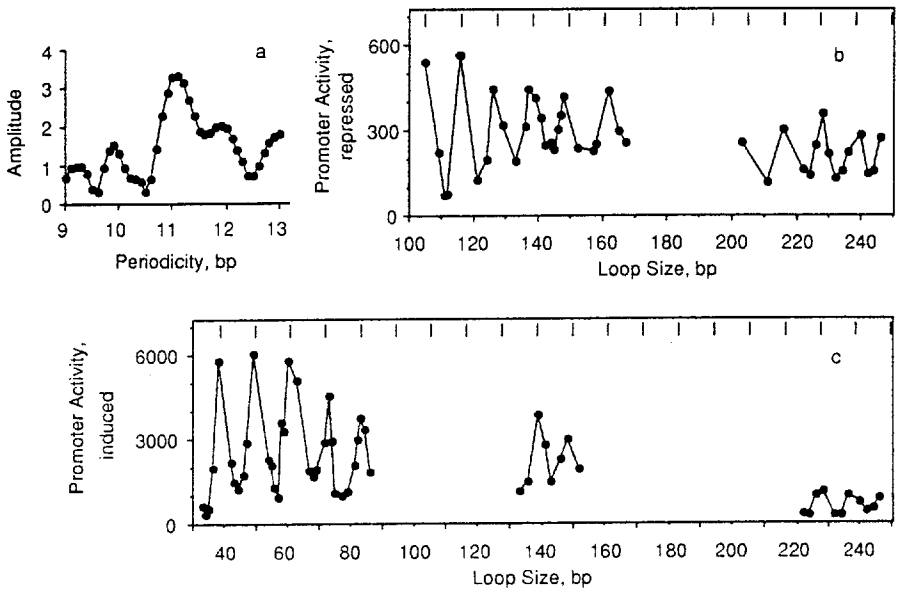

FIG. 3. Change of promoter activities with loop size as measured by the β-galactosidase assay. (*a*) Frequency spectrum of promoter activities computed by Fourier transformation. The 95 points shown in *b* and *c* were used in the analysis. In *b* and *c* the hashmarks at the top are spaced 11.1 bp apart. (*b*) Basal level of the *araBAD* promoter in strain DL100. (*c*) Induced level of the *araBAD* promoter in strain SH326 (cya^- and elevated concentrations of AraC protein).

containing larger loops and retaining the CAP site. Even the small-spacing candidates with separations between $araO_2$ and *araI* of 34, 33, and 32 bp, which leave just the two AraC protein binding sites intact, remained repression-positive. Thus there is no lower limit to the $araO_2$–*araI* loops.

We also examined $araO_2$ involvement in forming these small loops. First, an $araO_2$ point mutant (G → A at position −271) was constructed and its effect on expression was tested in a 44-bp spacing plasmid. This mutation reduced repression by a factor of 2, showing that $araO_2$ is involved in the loop. We used occupancy of $araO_2$ as a second measure of loop formation in the small-loop plasmids. The affinity of $araO_2$ for AraC protein is insufficient for it to be occupied by AraC protein on its own. However, this site is occupied when it can loop to *araI* or $araO_1$ (4). *In vivo* footprinting of $araO_2$ showed it was unoccupied in the repression-minus plasmids with spacings of 38 and 49 bp but was occupied in the repression-positive plasmids with spacings of 33 and 44 bp, consistent with formation of the postulated small loops (data not shown).

Periodicity. Oscillations in repression ability as a function of spacing extend to about 400-bp loops. The hashmarks in Fig. 3 *b* and *c*, which are spaced 11.1 bp apart remain in phase with the oscillations for loops 40–240 bp long, implying that the helical repeat of the DNA *in vivo* between *araI* and $araO_2$ is 11.1 bp per turn. Fourier transformation of the same 95 points (Fig. 3*a*) objectively shows the same result.

Periodicity and Linking Number Deficit. The linking number deficit of *E. coli* DNA could tend to untwist the DNA. Therefore, we tested whether a reduction in DNA gyrase activity would alter the apparent helical repeat in the *ara* system. Twelve plasmids with 222- to 246-bp spacings between *araI* and $araO_2$ were transferred to strain DL110, which is deficient in gyrase activity. In experiments not shown, topoisomer analysis on chloroquine-containing gels of plasmid DNA extracted from these cells showed it to contain about half the linking number deficit as plasmid DNA extracted from the strain used in the previous experiments. The change in the apparent helical repeat in the *ara* system, however, was barely detectable, if present at all, perhaps 2–4 bp in the loop tested (Fig. 4).

Not having remeasured the entire set of spacing plasmids in the gyrase mutant strain, we cannot tell whether the nearest peak shifted 2–4 bp or some more distant peak shifted. To determine whether a more distant peak shifted by more than one cycle, we examined the apparent shift in loops of about half the size. We found a shift of at most 1 or 2 bp

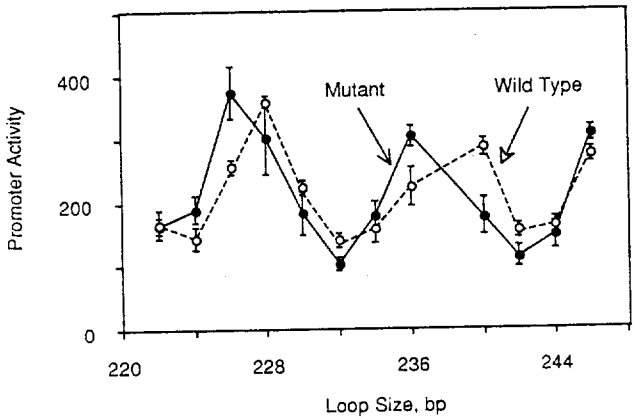

FIG. 4. Basal level of P_{BAD} in a set of insertion plasmids in wild-type strain SH322 (solid circles) and the isogenic gyrase mutant strain DL110 (open circles) derived from five independent cell growth experiments and measurement of the β-galactosidase activity. The points show the average values, and the error bars show the standard deviations of the measurements.

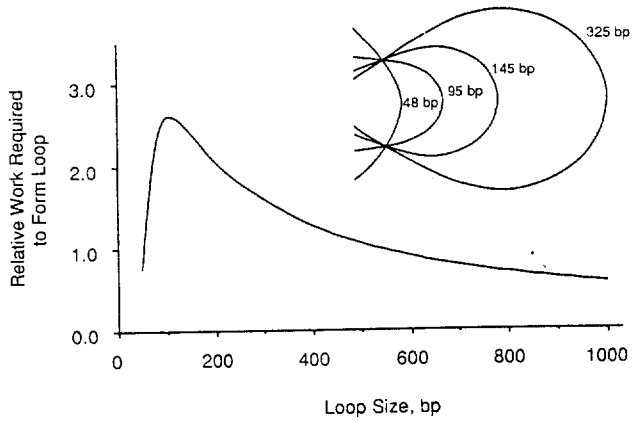

FIG. 5. Approximate shapes of looped DNA and the relative work required for the loop formation.

in a series of 11 plasmids with 121- to 148-bp loops, strongly suggesting that the shift in helical repeat in the gyrase mutant was no larger than 2 bp per 100 bp of loop.

Richardson *et al.* (11) analyzed changes in the helical repeat that were generated by varying the linking number deficit *in vitro*. They found a repeat of 11.07 for $\sigma = -0.061$ and a repeat of 10.95 for $\sigma = -0.031$ very similar to our *in vivo* finding.

Energetics of Loop Formation. The fact that very small loops can form when $araO_2$ and *araI* are correctly oriented suggests that AraC protein must bend significantly in the formation of these loops. Clearly, the actual looping process is aided by the supercoiling present *in vivo* (2, 31, 32), and the results of calculations of the energies depend on the physical model chosen for looping. Nonetheless, as a crude approximation, we determined the approximate relative work necessary to form loops of various sizes if the protein had a fixed diameter but was highly flexible (Fig. 5). As the loop size decreased, the work required for loop formation first increased, then declined. The decline was obtained when the protein bent appreciably to form the loop, ultimately reaching a region of somewhat dubious physical significance.

We cannot easily estimate the propensity to loop as a function of loop size from these considerations, since $K = \exp(-\Delta G/RT)$ and $\Delta G = \Delta H - T\Delta S$. We have estimated relative changes in H but cannot estimate ΔS for loop formation.

DISCUSSION

The L-arabinose operon of *E. coli* has long been known to be positively and negatively regulated by AraC (33). Binding AraC protein at *araI* and at $araO_2$, sites separated by 211 bp, and association of these two proteins to form a DNA loop is necessary for the negative regulation or repression of the *araBAD* promoter P_{BAD} (1–4). As DNA looping appears to be a wide-spread and versatile mechanism utilized in gene regulation, we have explored the parameters of looping in the *ara* system.

We found that separating *araI* and $araO_2$ by more than about 500 bp eliminated detectable repression. At the other extreme, no lower limit to their spacing was found, implying that AraC protein possesses significant flexibility. As the spacing between $araO_2$ and *araI* varies, the level of expression of P_{BAD} oscillates with a period of about 11.1 bp, suggesting that *in vivo* the DNA between *araI* and $araO_2$ possesses a helical repeat of about 11.1 bp per turn.

We are surprised at the relatively small upper limit to the *ara* loop size. In the *deo* system a small amount of looping is detectable when the relevant operator sites are separated by as much as 5000 bp (34). Similarly, the enhancers of eukary-

otic genes likely utilize DNA looping (35) and they often function from distances exceeding 500 bp. Certainly, however, as the distance separating *araI* and $araO_2$ increases, looping must become more difficult because of the lowered concentration of one site in the presence of the other—i.e., for entropic reasons.

As a first approximation to estimating distance effects on looping, we might estimate the concentration of $araO_2$ in the presence of *araI* by assuming the intervening DNA was a long polymer with random angles between stiff segments. This is equivalent to a three-dimensional random walk (36), and yields concentration as a function of the distance ℓ between the sites varying as $\ell^{-3/2}$. If the loop size is increased from 300 to 500 bp, this relationship predicts a decrease in concentration of one site in the presence of the other by 54%. This result is at significant variance with the data presented in Fig. 2 in which the effective concentration must change by much more so that the repression changes from full to undetectable over this range. Presumably the estimation is poor because of the unknown orientation and position requirements of *araI* and $araO_2$ for looping in addition to the unknown effects of DNA supercoiling and binding by other proteins.

Our experimental result that there is no lower size limit for the *ara* loop suggests that AraC protein is flexible. This would have to be a particular flexibility however. On one hand the protein appears free to bend parallel to the DNA. On the other hand, the fact that misorienting *araI* and $araO_2$ interferes with repression implies that the protein is not significantly free to bend in a direction perpendicular to the axis of the DNA. If we assume the protein is completely free to bend in the direction required to form small loops and that it holds the *araI* and $araO_2$ sites a fixed distance apart, we can approximately determine the loop shape and the relative work required for formation (Fig. 5). As we have discussed, larger loops, while energetically more easily formed, are less favored for entropic reasons.

One interesting outcome of the looping energetics calculations is the apparently greater work required to form a loop of about 100 bp (Fig. 5). This maximum may correspond to the relatively poor repression seen for loops of 140–160 bp (Fig. 3*b*).

Our finding of the 11.1-bp periodicity of *ara* P_{BAD} repression raises the question of the structure of DNA *in vivo*. A number of experiments have shown that the helical repeat of linear DNA *in vitro* is about 10.5 bp per turn (6–8). Special sequences can give rise to different helical repeats—e.g., 10.0 ± 0.1 bp per turn with poly(dA)·poly(dT) and 13.6 bp per turn with poly(dG-dC)·poly(dG-dC) (37, 38), but sequence analysis of the *ara* P_{BAD} regulatory region reveals no special base distributions or sequence features with periodicities of 10–12 bp. Additionally, the independence of the oscillation cycle with various sizes of spacing and with various combinations of sequences by random deletions and insertions nearly excludes the possibility that the sequence alone of the *ara* regulatory region is responsible for the 11.1-bp periodicity we observed.

We suspect that the linking number deficit of *E. coli* DNA shifts the average helical repeat from its relaxed value of 10.5 bp per turn to around 11.1. A linking number deficit can generate a torsion that generates supercoiling. This same torsion is in a direction that it could also act to unwind the DNA. In fact, the apparent supercoiling density *in vivo* measured on a plasmid was appreciably less than its probable linking number deficit (9). If the remainder of the linking number deficit were applied to untwisting the DNA, the helical repeat could approach 11 bp per turn.

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1. Dunn, T., Hahn, S., Ogden, S. & Schleif, R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5017–5020.
2. Hahn, S., Hendrickson, W. & Schleif, R. (1986) *J. Mol. Biol.* **188,** 355–367.
3. Martin, K., Huo, L. & Schleif, R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3654–3658.
4. Huo, L., Martin, K. & Schleif, R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5444–5448.
5. Shore, D. & Baldwin, R. (1983) *J. Mol. Biol.* **170,** 957–981.
6. Wang, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 200–203.
7. Rhodes, D. & Klug, A. (1980) *Nature (London)* **286,** 573–578.
8. Tullius, T. D. & Dombrowski, B. A. (1985) *Science* **230,** 679–681.
9. Bliska, J. & Cozzarelli, N. (1987) *J. Mol. Biol.* **194,** 205.
10. Krämer, H., Amouyal, M., Nordheim, A. & Müller-Hill, B. (1988) *EMBO J.* **7,** 547–556.
11. Richardson, S. M. H., Boles, T. C. & Cozzarelli, N. R. (1988) *Nucleic Acids Res.* **16,** 6607–6616.
12. Liu, L. F. & Wang, J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7024–7027.
13. Shimada, J. & Yamakawa, H. (1984) *Biopolymers* **23,** 853–857.
14. Levene, S. & Crothers, D. (1986) *J. Mol. Biol.* **189,** 61–72.
15. Broyles, S. & Pettijohn, D. (1986) *J. Mol. Biol.* **187,** 47–60.
16. Johnson, R., Glasgow, A. & Simon, M. (1987) *Nature (London)* **329,** 462–465.
17. Thomson, J., deVargas, L., Koch, C., Kahmann, R. & Landy, A. (1987) *Cell* **50,** 901–908.
18. Wu, H.-M. & Crothers, D. (1984) *Nature (London)* **308,** 509–513.
19. Schleif, R. & Wensink, P. (1981) *Practical Methods in Molecular Biology* (Springer, New York).
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
21. Norton, P. & Coffin, J. (1985) *Mol. Cell. Biol.* **5,** 281–290.
22. Brunelle, A. (1988) Dissertation (Brandeis University, Waltham, MA).
23. An, G. & Friesen, J. (1979) *J. Bacteriol.* **140,** 400–407.
24. Hahn, S., Dunn, T. & Schleif, R. (1984) *J. Mol. Biol.* **180,** 61–72.
25. DiNardo, S., Voekel, K. A., Sternglanz, R., Reynolds, A. E. & Wright, A. (1982) *Cell* **31,** 43–51.
26. Goldstein, E. & Drlica, K. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4046–4050.
27. Wei, C., Alianell, G., Bencen, G. & Gray, H. (1983) *J. Biol. Chem.* **258,** 13506–13512.
28. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
29. Cantor, C. R. & Schimmel, P. R. (1980) *Biophysical Chemistry* (Freeman, San Francisco), Part 2, pp. 696–697.
30. Dunn, T. & Schleif, R. (1984) *J. Mol. Biol.* **180,** 201–204.
31. Borowiec, J. & Gralla, J. (1987) *J. Mol. Biol.* **195,** 89–97.
32. Whitson, P., Hsieh, W.-T., Wells, R. & Matthews, K. (1987) *J. Biol. Chem.* **262,** 14592–14599.
33. Schleif, R. (1987) *Escherichia coli and Salmonella typhimurium*, eds. Neidhardt, F., Ingrahm, J., Low, K. B., Magasanik, B., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington, DC), Vol. 2, pp. 1473–1481.
34. Dandanell, G., Valentin-Hansen, P., Larsen, J. & Hammer, K. (1987) *Nature (London)* **325,** 823–826.
35. Gluzman, Y. & Shenk, T., eds. (1983) *Enhancers and Eukaryotic Gene Expression* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
36. Jacobson, H. & Stockmayer, W. H. (1950) *J. Chem. Phys.* **18,** 1600–1606.
37. Rhodes, D. & Klug, A. (1981) *Nature (London)* **292,** 378–380.
38. Behe, M., Zimmerman, S. & Felsenfeld, G. (1981) *Nature (London)* **293,** 233–235.