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Accelerated Publications

Physical Properties of DNA in Vivo As Probed by the Length Dependence of the *lac* Operator Looping Process†

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Received February 29, 1988; Revised Manuscript Received April 5, 1988

ABSTRACT: Plasmid constructs containing a wild-type (O^+) *lac* operator upstream of an operator-constitutive (O^c) *lac* control element exhibit a length-dependent, oscillatory pattern of repression of expression of the regulated gene as interoperator spacing is varied from 115 to 177 base pairs (bp). Both the length dependence and the periodicity of repression are consistent with a thermodynamic model involving a stable looped complex in which bidentate *lac* repressor interacts simultaneously with both O^+ and O^c operators. The oscillatory pattern of repression with distance occurs with a period approximating the helical repeat of DNA and presumably reflects the necessity for proper alignment of interacting operators along the helical face of the DNA. In the length regime examined, the presence of the upstream operator enhances repression between 6-fold and 50-fold depending upon phasing. This reflects a torsional rigidity of DNA in vivo that is consistent with in vitro measurements. The oscillatory pattern of repression is best fit with a period of either 9.0 or 11.7 bp/cycle but not 10.5 bp/cycle. This periodicity is interpreted as reflecting the average helical repeat of the 40-bp interoperator region of plasmid DNA in vivo, suggesting that the local helical repeat of DNA in vivo may differ significantly from 10.5 bp/turn. The *apparent* persistence length needed to fit the data (a_{app}) is only one-fifth the standard in vitro value. This low value of a_{app} may be due in part to DNA bending induced by catabolite activator protein (CAP) bound to its site between the interacting operators. Quantitative analysis of the dependence of loop formation on the interoperator spacing can thus be used to characterize the physical properties of the intervening DNA sequence in vivo.

The phenomenon of action-at-a-distance, i.e., the ability of a region of DNA to affect processes that occur hundreds to thousands of base pairs away, is of general importance in eucaryotic systems. Recent studies in several procaryotic transcriptional regulation systems have indicated that a similar phenomenon occurs in the *gal* (Irani et al., 1983; Majumdar & Adhya, 1984), *ara* (Dunn et al., 1984; Martin et al., 1986), *deo* (Dandanell & Hammer, 1985), *lac* (Besse et al., 1986; Mossing & Record, 1986; Krämer et al., 1987, 1988; Borowiec et al., 1987; Hsieh et al., 1987; Whitson et al., 1987b), and *glnA* (Reitzer & Magasanik, 1986) operons of *Escherichia coli*. Various mechanisms have been proposed to explain the ability of distant regions of DNA to influence each other, but recent evidence, primarily obtained for procaryotic systems, strongly implicates formation of a stable DNA loop [for a review, see Ptashne (1986)]. Evidence for loop formation in vitro includes the appearance of an altered pattern of nuclease sensitivity in DNA involved in highly bent loops (Hochschild & Ptashne, 1986; Krämer et al., 1987; Borowiec et al., 1987), direct observation of loops in electron micrographs (Krämer et al., 1987; Thêveny et al., 1987), and the observation of an oscillatory pattern of increased and decreased repression as operators are moved into and out of phase with one another (Dunn et al., 1984; Hochschild & Ptashne, 1986; Krämer et al., 1987, 1988).

The work presented here is a quantitative analysis of the effect of variation of the interoperator spacing on *lac* repression in vivo. The oscillatory dependence of repression on interoperator spacing that we observe strongly supports the quantitative model of DNA loop formation between *lac* operators in vivo proposed by Mossing and Record (1986) and provides a means of evaluating the helical periodicity and apparent lateral and torsional stiffness of DNA in vivo. These and other physical properties of DNA have been thoroughly examined in vitro [e.g., Wells and Harvey (1988)], but little information is available regarding their magnitudes or variability in vivo. This study demonstrates the use of the DNA looping assay to deduce the physical properties of DNA in vivo.

Materials and Methods

Strains and Media. All experiments were performed in *E. coli* strain HB101 (F^-, *hsd*S20 *rec*A *rps*L *lac*Y *leu pro gal*K2) containing the plasmid pi^Q (pACYC184 with an R1 insert

† This work was supported by a grant from the NSF (CHE85-09625) to M.T.R. and by an NSF graduate fellowship to G.R.B.
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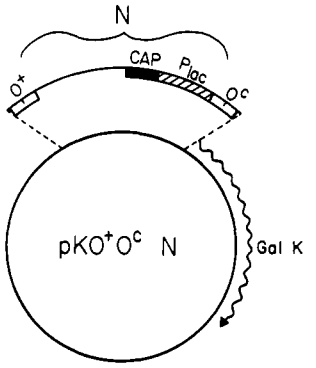

FIGURE 1: Schematic diagram of pKO^+O^c plasmids used. The control region for expression of galK is shown in expanded form and includes an O^+ *lac* operator *N* bp upstream of an O^c *lac* operator, which overlaps the *lac* promoter. Upstream of the *lac* promoter is the CAP binding site. Not shown is an additional binding site for CAP protein upstream of O^+, which we expect to have no effect on the looping process. Construction of the plasmids is described in the text.

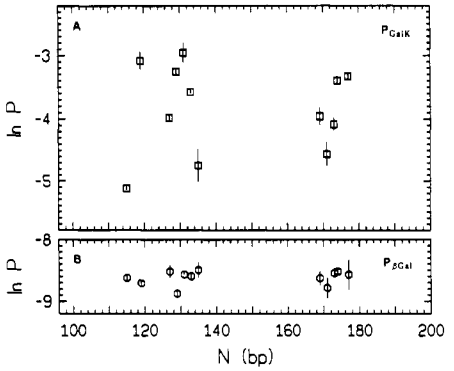

FIGURE 2: Degree of repression (*P*) of the pKO^+O^c plasmid (P_{galK}; A) and the chromosomal *lac* operon ($P_{\beta gal}$; B) as a function of the plasmid interoperator spacing (*N*). The corresponding values for the plasmid without any upstream operator (pKO^c) are $\ln P_{galK} = -1.1 \pm 0.3$ and $\ln P_{\beta gal} = -8.50 \pm 0.01$. Error bars represent the range of duplicate determinations. (N = 115, 119, 127, 129, 131, 133, 135, 169, 171, 173, 174, and 177).

containing the i^Q gene; a gift of L. Regan) and a pKO^+O^c plasmid (described below). Cultures were grown at 37 °C in 1× MOPS [3-(*N*-morpholino)propanesulfonic acid] minimal medium (Neidhardt et al., 1974) supplemented with casamino acids (0.2%), glycerol (0.2%), thiamin (5 μg/mL), ampicillin (50 μg/mL), and tetracycline (12.5 μg/mL).

Interoperator Spacing Variant Plasmid (pKO^+O^c). The plasmid previously designated O^+O^c283 (Mossing & Record, 1986) was used as the parent plasmid for creation of a set of BAL-31 deletion mutants. [The operator constitutive mutant is that previously designated as IIIb (Smith & Sadler, 1971) or 666 (Maquat et al., 1980) and has A replaced by G at position +8 relative to the start point of transcription.] Fine scale alterations in the spacing *N* (bp) between the O^+ and O^c operators were obtained by insertion of oligonucleotide linkers of different lengths (New England Biolabs). Figure 1 is a schematic representation of the plasmids used in this study. Note the presence of a binding site for the catabolite activator protein (CAP) between the operators. The nomenclature is such that pKO^+O^cN has *N* bp separating the center of the regulatory O^c operator from the center of the upstream O^+ operator.

Assays. Overnight cultures were diluted 1:200 into duplicate flasks containing fresh medium with and without 1 mM isopropyl β-D-thiogalactoside (IPTG). After four to five cell doublings (at an absorbance at 550 nm of 0.6–0.9), cells were chilled on ice, harvested by centrifugation, sonicated, and then assayed for β-galactosidase (βgal) (Miller, 1972), galactokinase (galK), and total cellular protein (Adams & Hatfield, 1984). The ratio of the −IPTG enzyme activity (E_{-IPTG}) to the +IPTG activity (E_{+IPTG}) defines the degree of repression *P* ($P \equiv E_{-IPTG}/E_{+IPTG}$) for the regulatory operator under study (Sadler & Novick, 1965). Control experiments on some of the cultures revealed that plasmid copy numbers (as determined by cell lysis, agarose gel electrophoresis, and densitometric scanning of the gel photo against standards) were higher by a factor of approximately 1.5 in uninduced cultures as compared to induced cultures, independent of which pKO^+O^c plasmid was used. Galactokinase assay results were corrected for these differences in copy number. (This correction has no effect on comparisons of *P* values between constructs with different interoperator spacings.)

RESULTS

Distance-Dependent Effect of an Upstream O^+ Operator on the Degree of Repression of a Promoter-Proximal O^c Operator. Mossing and Record (1986) observed that an upstream O^+ operator increased the degree of repression of a promoter-proximal O^c operator on a plasmid in vivo. We have investigated the effect of interoperator distance (*N*) on the enhancement of repression of the O^c operator over the range 115–177 bp. In all, 12 constructs were examined, including sets of five constructs in the ranges 127 bp ≤ *N* ≤ 135 bp and 169 bp ≤ *N* ≤ 177 bp. For each *N*, duplicate determinations of the degrees of repression of the pKO^+O^c plasmid (P_{galK}) and of the chromosomal *lac* operator ($P_{\beta gal}$) were performed. Results are presented in Figure 2. In the strain investigated, the degree of repression of the chromosomal *lac* operator ($P_{\beta gal}$) was essentially independent of the presence of the upstream operator on the plasmid and the value of *N* (Figure 2B).

In contrast, Figure 2A shows that the effect of the upstream O^+ operator on repression of the O^c operator (P_{galK}) is highly dependent upon the interoperator distance *N*. The enhancement of repression in plasmids containing an upstream operator relative to pKO^c ranges from over 50-fold at N = 115 bp to approximately 6-fold at N = 131 bp. Near N = 130 bp the value of P_{galK} is at a local maximum, and near N = 171 bp a local minimum is observed. It is apparent that the upstream O^+ operator exhibits a complex, apparently oscillatory modulating effect on repression of the O^c operator.

Thermodynamic Model for Repression. Regulation of expression of the *lac* genes by *lac* repressor is believed to be under thermodynamic control in vivo, whereby the equilibrium level of occupancy of the promoter-proximal operator by *lac* repressor determines the level of repression (von Hippel, 1979). In a thermodynamic model, the degree of repression *P* is a function of the apparent repressor–operator binding constant K_{app} and the effective repressor concentration (activity) R_e in the vicinity of the operator (Sadler & Novick, 1965; Mossing & Record, 1986)

$$P = (1 + K_{app}R_e)^{-1} \quad (1)$$

Modulation of R_e by Upstream O^+ (Looping Model). Although the modulation of repression by the upstream O^+ operator may be interpreted thermodynamically as arising from the effect of the O^+ operator on K_{app} and/or on R_e, the model of DNA loop formation between operators suggests that the primary effect of the upstream operator is to increase the local effective repressor concentration R_e. It is in the context of this model that we analyze the data.

An interpretation of the data of Figure 2, for which ample biophysical precedent exists, ascribes the modulation of repression by the upstream O^+ operator to the effect of *bidentate* repressor bound to that operator on the effective concentration

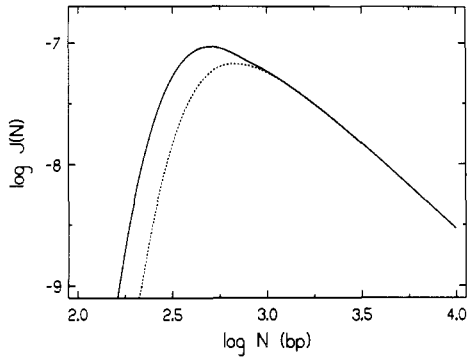

FIGURE 3: Calculated ring closure probabilities (J, in M) as a function of DNA length (N, in bp) calculated from Shimada and Yamakawa (1984a). The curve for small N was generated by using their eq 37–39, 70, and 73; for large N eq 50 and 69 were used, and interpolation formulas C1 and C2 were used for intermediate values. The values for the Poisson's ratio and persistence length were those used by Shimada and Yamakawa (1984a) to fit the data of Shore and Baldwin (1983a). The upper (solid) curve represents the behavior of the predicted local maxima in repression; the lower (dashed) curve represents the local minima. The actual curve describing the data would oscillate between these two bounds with a period equal to the DNA helical repeat. The oscillations in this complex curve are far too numerous to be clearly represented on this plot.

of repressor (R_e) in the vicinity of the O^c operator. As proposed by Mossing and Record (1986)

$$R_e = R_f + R_N \tag{2}$$

where R_f is the free concentration (activity) of repressor and where R_N is the additional contribution to the local concentration of repressor in the vicinity of the O^c operator resulting from the presence of a bound bidentate repressor at the upstream O^+ operator (N bp distant). Note that in calculating the effective concentration (R_e) one must consider not only repressor molecules with both DNA-binding sites vacant but also those with one site occupied by DNA but with a second site available for DNA binding. For bound protein to contribute to R_e, it clearly is necessary for it to be bidentate, i.e., to have an additional binding site capable of interacting with the O^c operator in cis (determining R_N by looping of the intervening N bp DNA segment) or in trans (contributing to R_f).

The quantitative model of DNA looping makes specific predictions for the dependence of repression on interoperator spacing. This model is based on the analogy of interoperator DNA loop formation to cyclization of DNA restriction fragments and phage DNA molecules (Shore & Baldwin, 1983a; Horowitz & Wang, 1984; Wang & Davidson, 1966; Shimada & Yamakawa, 1984a). This experimental and theoretical work on DNA cyclization showed there to be two components to the dependence of cyclization on contour length: (1) a characteristic overall "bell-shaped" envelope with its maximum at an optimum length determined by the DNA lateral stiffness (cf. Figure 3) and (2) a periodicity of cyclization with respect to length (modulating the overall length dependence), which reflects the helical repeat of DNA.

The data in Figure 2 demonstrate the periodicity of cyclization expected for a looping model. It can also be inferred that if a bell-shaped envelope is to describe the data, as expected by analogy with DNA cyclization, then the global maximum in repression (corresponding to the global minimum in P_{galK}) must occur at a value of $N < 115$ bp, the lower limit accessible to us as a result of the presence of the binding site for CAP protein between the two operators. Since the maximum in DNA cyclization probability in vitro (0.05 M NaCl, 0.01 M $MgCl_2$, pH 7.5) occurs at DNA lengths of 500–600 bp (cf. Figure 3), then DNA with a bound CAP protein in vivo behaves as if it is significantly more readily cyclized than DNA in vitro.

Description of the Oscillatory Pattern of Repression Using the Twisted Wormlike Chain Model. Yamakawa and Stockmayer (1972) developed a quantitative theory of the cyclization process for chain polymers. The theory is based on the wormlike chain model of Kratky and Porod (1949) and the statistical analysis of ring closure probabilities of Jacobson and Stockmayer (1950). Inclusion of a requirement for torsional alignment led to a statistical mechanical theory of the twisted wormlike chain, which was used by Shimada and Yamakawa (1984a) to calculate cyclization probabilities as a function of molecular length. Using the cyclization data of Shore and Baldwin (1983a), Shimada and Yamakawa (1984a) determined the persistence length (a), torsional rigidity constant (C), and helical repeat (h) for DNA in vitro. These values [a = 132–140 bp, $C = (2.4–3.6) \times 10^{-19}$ erg cm, and h = 10.46 bp/turn] (Shimada & Yamakawa, 1984b) are in close agreement with similar values measured for bulk DNA in vitro by other methods. Our experiments involve the length dependence of DNA loop formation, which is in many ways analogous to cyclization, and for that reason we adopt this model to extract the analogous in vivo quantities from the data in Figure 2.

Generation of a theoretical fit to analyze our data on repression as a function of interoperator spacing requires the evaluation of four quantities: the repressor concentration in the *E. coli* cytoplasm (R_f), the apparent lateral "bending" stiffness of DNA in vivo, the torsional stiffness of DNA in vivo, and the helical repeat of DNA in vivo. The parameters needed to generate curves to fit the data of Figure 2 and their evaluation are discussed below.

Calculation of Repressor Concentrations (R_N, R_f, R_e). The following (iterative) algorithm was employed to extract the contributions of R_f and R_N to the effective repressor concentration R_e from measured P values. Since P_{galK}, in the thermodynamic model of plasmid O^c repression (cf. eq 1), is a function only of the O^c binding constant (K_{O^c}, which in the pure looping model is not a function of the presence or position of upstream operators) *and* of the effective repressor concentration (R_e), comparison of values of P_{galK} for two interoperator spacings N1 and N2 yields the ratio of effective concentrations for these two constructs

$$\frac{R_{e,N1}}{R_{e,N2}} = \frac{(1 - P_{N1})(P_{N2})}{(1 - P_{N2})(P_{N1})} \tag{3}$$

From these *relative* effective repressor concentrations we were able to infer the overall shape of the Shimada–Yamakawa (SY) curve of R_N vs N that would ultimately be needed to fit the data, i.e., a curve with a global maximum at a value of N less than 115 bp and with gently decreasing local maxima over the range 115 bp $\leq N \leq$ 177 bp. The values of a and C needed to generate this initial curve were determined by trial and error.

Once an initial curve of the proper overall shape had been generated, an initial estimate for the value of $R_{N,115}$ was read from the curve. Since for pKO^c (the plasmid lacking an upstream operator) $R_N = 0$, we can write

$$P_{galK,115} = \frac{1}{1 + K_{O^c}R_{e,115}} \qquad P_{galK,pKO^c} = \frac{1}{1 + K_{O^c}R_{f,pKO^c}} \tag{4}$$

These two equations were used to solve for R_{f,pKO^c} with the definition $R_{e,115} = R_{N,115} + R_{f,115}$ (eq 2) and the experimental observation that $R_{f,115} \approx R_{f,pKO^c}$ since $P_{\beta gal,pKO^c} = P_{\beta gal,115}$. This

value of R_{f,pKO^c} (180 nM) was then used to calculate apparent values of K_{O^c} and K_{O^+} from P_{galK,pKO^c} and $P_{\beta gal,pKO^c}$, respectively, using eq 1 with $R_{f,pKO^c} = R_{e,pKO^c}$. With these values ($K_{O^c} = 1.3 \times 10^7$ M^{-1} and $K_{O^+} = 2.0 \times 10^{10}$ M^{-1}), which we assume to be strain invariant, eq 1 was used to calculate R_e and R_f for all N, with R_N values calculated by using eq 2. The values of R_N were superimposed on the initial SY curve, and the parameters (a, C, h) were refined to give the best visual fit to the data. The average value of R_f was 220 ± 30 nM.

The high value of R_f deserves comment, since it is at or near the total repressor concentration originally reported in an i^Q strain (Müller-Hill et al., 1968; Jobe et al., 1972). (1) The total concentration of repressor in our i^Q strains may be significantly higher than that reported by Müller-Hill et al. (1968) (100 repressors/cell ≃ 170 nM) due to gene dosage effects. The original studies of i^Q were conducted with the i^Q gene present on the chromosome and/or F' factors that are regulated at 1–2 copies/cell. We observe that the pi^Q plasmid in our strains is present at approximately 20–30 copies per cell (data not shown). (2) The value of R_f represents the thermodynamic activity of free repressor rather than its actual concentration. There is ample precedent for thermodynamic activities of proteins at physiological concentrations that exceed their molar concentration by 10–100-fold due to extreme excluded volume effects (macromolecular crowding: Minton, 1983; Fulton, 1982, and references cited therein). (3) The in trans effect of nonspecifically bound bidentate repressor may increase the apparent free repressor concentration above the unbound repressor concentration. Therefore, a repressor concentration (R_f) of greater than 200 nM is not unreasonable.

The high repressor concentration in our i^Q strain may explain the lack of a coupling of the plasmid and chromosomal P values, as was previously observed by Mossing and Record (1986) in a strain carrying the i^Q allele on an F' factor. In that case chromosomal and plasmid repression appeared to be coupled through the free repressor concentration, whereas in the strain used in this study the repressor appears to be in sufficient excess to mask the effect of changes in the extent of binding of repressor to the plasmid operators on chromosomal repression.

Lateral Rigidity (*Bending Stiffness*). The value of the persistence length was varied to obtain a curve where the envelope of the local maxima in repression decreased gently with increased interoperator separation in the range 115–177 bp, as required to fit the data. These curves are presented in Figure 4, where a persistence length of approximately 30 bp was used. This apparent persistence length (a_{app}) is much less than the 150-bp value measured for DNA in vitro (Hagerman, 1985, and references cited therein). We must emphasize that this *apparent* persistence length is related to the bending stiffness of the DNA but is not actually a true persistence length. Its interpretation is complicated by a number of factors that may influence the overall bending stiffness of the DNA (see Discussion). For this reason the value of a_{app} cannot be compared directly to values measured in other ways, but will be quite useful when compared with values determined under other conditions by the same technique.

Torsional Rigidity. The torsional rigidity was varied to fit the amplitudes of the experimentally observed oscillations in repression. The best fit was obtained for an apparent torsional rigidity of $C_{app} = 1.1\ (\pm 0.3) \times 10^{-19}$ erg cm. The value of C_{app} calculated in this way represents a lower bound since possible flexibility of the protein is not considered.

Helical Repeat. The helical repeat was fit by fixing a minimum in the curve at $N = 130.5$ and determining the values

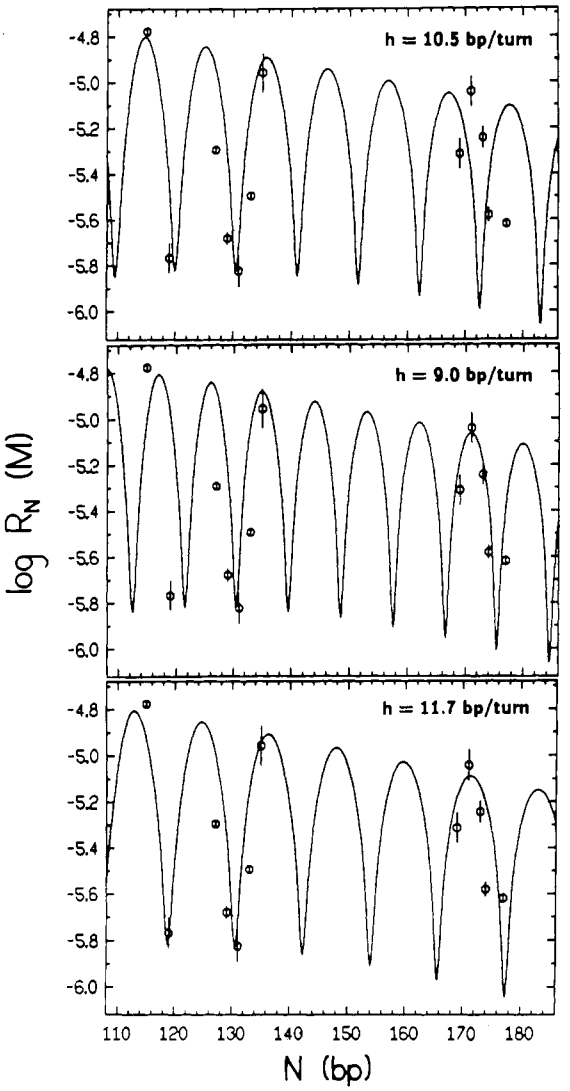

FIGURE 4: The local concentration R_N plotted on a logarithmic scale as a function of N, the interoperator distance, for three choices of the helical repeat, h: Curves were generated by using 37–39, 70, and 73 of Shimada and Yamakawa (1984a) as described in the text. Fitting parameters are $C_{app} = 1.1\ (\pm 0.3) \times 10^{-19}$ erg cm, $a_{app} = 30$ bp, and $h = 10.5$, 9.0, or 11.7 bp/turn. Values of R_N were calculated as described in the text. Error bars represent the range of duplicate determinations. All curves were constrained to have a minimum at $N = 130.5$, thus defining the phase of the curve to agree with the strong apparent minimum in the data.

of h that gave a maximum near $N = 171$. The best values of h, as shown in Figure 4, were $h = 9.0 \pm 0.3$ and 11.7 ± 0.3 bp/turn. (The error estimates correspond to the ranges of values that were visually acceptable.) These values represent the average helical repeat for the DNA segment that distinguishes pKO^+O^c 171 from pKO^+O^c 131 (see Table I). Note that the CAP binding site is located outside the region over which the helical repeat determination applies, so that bound CAP should not influence the measurement of the helical repeat.

Discussion

The results presented here support the quantitative looping model proposed by Mossing and Record (1986) to explain the ability of upstream *lac* operators to enhance repression at the primary operator. The oscillation in repression with a period approximating the helical repeat of DNA is a hallmark of the looping phenomenon (Dunn et al., 1984; Hochschild & Ptashne, 1986; Krämer et al., 1987, 1988). Fitting the data

Table I: Differences in DNA Sequences of pKO$^+$O^{c}171 and pKO$^+$O^{c}131

Position relative to the start site of transcription of lacP (+1):													-83
pKO$^+$O^{c}171	...CACAG	GAAAC	AGCTA	TGACC	ATGAT	TACGG	ATTCA	CTGGC	CCCAA	GCTCG	CGGAT	CCGCG	AGCTT...
pKO$^+$O^{c}131	...CACAG	-----	-----	-----	-----	-----	-----	-----	-CCAA	GCT--	CGGAT	CCG--	AGCTT...

to the theory of Shimada and Yamakawa (1984a) provides valuable information regarding the physical properties of DNA in vivo. The period of the oscillation can be interpreted as the DNA helical repeat. The amplitude of the oscillations reflects the torsional rigidity of DNA. The overall length dependence of repression provides an estimate of the apparent lateral (bending) stiffness of DNA in vivo. Each of these quantities is discussed below.

Lateral Rigidity. Interpretation of the *apparent* persistence length of 30 bp for this DNA segment in vivo is complicated due to a number of differences between the model used to obtain the value (a uniform flexible polymer) and the actual DNA segment studied (which contains a bound CAP protein on a supercoiled plasmid), as well as the inherent differences between DNA looping and DNA cyclization. For looping to occur, the operators need only be brought to within the distance spanned by a repressor tetramer (Borowiec et al., 1987) rather than the much smaller distance required for base pair formation. It is also probable that loop formation requires less net curvature of the helix axis than ring closure, since a DNA molecule undergoing ring closure must bend a full 2π radians to come into proper alignment.

DNA supercoiling should facilitate the looping process due to the net compaction of the DNA and resultant increase in the local concentration of DNA segments. The in vitro data of several groups support this hypothesis (Whitson et al., 1987a; Borowiec et al., 1987; Krämer et al., 1988). The unique properties of a plectonemically interwound supercoiled DNA may also influence these results (Bliska & Cozzarelli, 1987). In addition, for the constructs examined, it is probable that the binding of CAP to its binding site between the interacting operators distorts the interoperator DNA (Wu & Crothers, 1984; Liu-Johnson et al., 1986; Kolartz et al., 1986); this bending is expected to influence loop formation. The bend could either facilitate or inhibit loop formation depending upon the relative phasing of the primary operator and the CAP-induced DNA bend. Recently Dripps and Wartell (1987) have proposed that DNA bending by CAP may increase the local concentration of the ends of a 144-bp fragment more than 100-fold, pointing out the potential magnitude of this effect. It is also possible that some stress-induced structure transition might result during loop formation that would increase the apparent lateral flexibility. The observation of an altered pattern of chemical or nuclease sensitivity in looped DNAs suggests that such structural transitions may occur (Borowiec et al., 1987; Hochschild & Ptashne, 1986; Krämer et al., 1987). Although supercoiling and CAP-induced DNA curvature prohibit a straightforward interpretation of a_{app} (measured by this technique) in terms of inherent DNA bending stiffness, it is probable that any variation of a_{app} with supercoil density and/or the placement of the CAP site will be interpretable in the context of the model.

Torsional Rigidity. In addition to supporting the model of DNA loop formation in vivo, the periodic oscillation of repression with interoperator spacing can also be used to infer the torsional properties of DNA in vivo. This helical periodicity will only result if the protein requires that the interacting operators be aligned in a certain relative orientation along the helical face of the DNA. Should the protein have enough inherent flexibility to allow even fully misaligned operators to be bound without significant energetic costs, then a helical periodicity is not expected. As pointed out by Schleif (1987), this appears to be the case in the binding of the yeast transcriptional activation proteins GAL4 (Brent & Ptashne, 1985) and possibly GCN4 (Hope & Struhl, 1986). However, in the case of *lac* repressor binding to multiple sites, a helical periodicity is observed both in vitro (Krämer et al., 1987, 1988) and in vivo (this work), which implies that repressor has at least some geometric requirements for simultaneous interaction with two operators. It is also expected that the amplitude of the oscillations will reflect the degree to which misalignment can be tolerated; the more flexible the DNA (and/or protein), the less difference in repression between aligned and misaligned pairs of operators.

It is thus the amplitudes of these oscillations that can be quantitated to determine the torsional rigidity constant. Values of a and C were determined that gave rise to a curve with the appropriate amplitudes with the additional constraint on a that the curve exhibit the proper overall shape. The observed value ($C = 1.1\ (\pm 0.3) \times 10^{-19}$ erg cm) is in good agreement with in vitro measurements of $C = (1.3\text{–}4.1) \times 10^{-19}$ erg cm (Thomas et al., 1980; Millar et al., 1982; Shimada & Yamakawa, 1984a,b; Barkley & Zimm, 1979), especially considering that the value obtained here represents a lower bound to the value of C since it neglects possible protein flexibility.

Helical Repeat. The average helical repeat observed for the 40 bp of DNA that distinguishes pKO$^+$O^c 171 from pKO$^+$O^c 131 is not 10.5 bp/turn but appears closer to either 9.0 or 11.7 bp/turn. The sequence of the DNA over which the measured helical repeat applies is shown in Table I. Although it is possible that an unusual DNA structure in the 40-bp segment is the cause of the apparent altered helical repeat, there are no patterns in this sequence to suggest any such structure [e.g., Koo et al. (1986) and Ulanovsky et al. (1986)], and we make the most straightforward interpretation of these results as an altered helical repeat.

The value of the helical repeat of B-DNA in vitro is well established as 10.4–10.6 bp/turn (Wang, 1979; Rhodes & Klug, 1980; Tullius & Dombroski, 1985; Shore & Baldwin, 1983b; Horowitz & Wang, 1984), but the value in vivo is less easily determined. Attempts have been made to determine the helical repeat of the DNA in partially reconstituted systems with the result that the helical repeat is altered when bound in a nucleosome (Klug & Lutter, 1981) or saturated with *E. coli* histone-like protein HU (Broyles & Pettijohn, 1986). The data of Broyles and Pettijohn (1986) show a helical repeat of 8.5 bp/turn for DNA saturated with protein HU, which would suggest that the helical repeat of 9.0 bp/turn is closer to the actual value in vivo. In contrast, the data of Reitzer and Magasanik (1986) demonstrate that insertion of 12 bp between interacting sites retains the function of *gln*Ap2 transcriptional activator whereas insertion of 8 bp decreases its activity somewhat. A helical repeat of 11.7 bp/turn is more consistent with their data, although the differences in the systems under

study preclude any firm conclusion.

We note that the difference between a global helical repeat of either 11.7 or 9.0 bp/turn in vivo and 10.5 bp/turn in vitro would give rise to a very large net change in superhelix density (σ) upon purification of plasmid DNA from cells. For either helical repeat the predicted change in superhelix density is inconsistent with the known superhelix density in vitro (e.g., $\sigma = -0.06$; Courey & Wang, 1983) and the estimated unrestrained superhelix density in vivo ($\sigma \cong -0.03$; Pettijohn & Pfenninger, 1980; Sinden et al., 1980). This implies that the measured h cannot be the average helical repeat throughout the entire plasmid and must only apply to the particular 40-bp segment under study. The difference between the helical repeat of the 40-bp segment under study here and reasonable estimates for the average helical repeat for the whole plasmid indicates that the variability of h must be quite dramatic in vivo (or that some unusual structure is being induced in the interoperator DNA during loop formation). It will be very interesting to examine other sequences cloned between operators to see how h varies with sequence.

It must be emphasized that the values of a, C, and h we obtain using these equations are fundamentally fitting parameters that may or may not be as simply interpreted as the corresponding quantities for DNA in vitro. Specifically, the presence of a CAP-induced bend or a stress-induced distortion of the DNA between the two interacting operators may affect the value of the apparent persistence length (a_{app}) dramatically. This apparent persistence length not may reflect the inherent lateral rigidity of this DNA and thus is not a true intrinsic persistence length, but comparison of its value to the apparent persistence length in the absence of CAP-induced bending can give very useful information regarding the severity of the induced structural alteration in vivo. It must also be mentioned that any parameters obtained from the entire data set will represent *average* values, as we implicitly assume a uniform polymer in application of the model. Specifically, the helical repeat we obtain is an average for the 41 bp over which the measurement applies.

Of the parameters evaluated, the helical repeat is most reliable as it is essentially model independent. The torsional rigidity represents a lower limit to the true value, and the apparent persistence length may reflect the effects of CAP-induced DNA bending and supercoiling in addition to the inherent DNA bending stiffness in vivo. These latter quantities will be of most use when compared to analogous values determined for other DNA segments (or under other conditions) by the same method. Recently, the existence of such unusual DNA structures as cruciforms and Z-DNA has been demonstrated in vivo (Panayotatos & Fontaine, 1987; Jaworski et al., 1987). These experiments involved the assay of an enzymatic activity in vivo (an endonuclease and a methylase, respectively) to distinguish the "normal" from the unusual DNA structure. In a similar way the *lac* looping assay can also be used to assess the physical state of DNA in vivo. The role of CAP-induced DNA bending and DNA supercoiling is currently under investigation using this approach.

Acknowledgments

We thank N. Cozzarelli, A. Eschenlauer, M. Krebs, H. Yamakawa, and the referees for their valuable comments and S. Aiello for careful preparation of the manuscript.

Added in Proof

Recent work of Krämer et al. (1988) on loop formation between *lac* operators in vitro has shown that the helical repeat of DNA is altered on a supercoiled molecule. Although this demonstrates the potential for a supercoiling effect on the helical repeat, we note that no reasonable amount of supercoiling can be the sole explanation for the helical repeat we observe in vivo.

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