DeoR repression at-a-distance only weakly responds to changes in interoperator separation and DNA topology

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ABSTRACT

The interoperator distance between a synthetic operator O_S and the *deoP2O₂-galk* fusion was varied between 46 and 176 bp. The repression of the *deoP2* directed *galk* expression as a function of the interoperator distance (center-to-center) was measured *in vivo* in a single-copy system. The results show that the DeoR repressor efficiently can repress transcription at all the interoperator distances tested. The degree of repression depends very little on the spacing between the operators, however, a weak periodic dependency of 8–11 bp may exist.

INTRODUCTION

The necessity for more than one operator site in order to obtain efficient repression has been well documented for several procaryotic operons. The involvement of DNA looping in this phenomenon was first demonstrated for the *ara* system (1, 2) and later for the *deo* (3, 4, 5) and *lac* operons (6, 7, 8).

In the *deoCABD* operon of *Escherichia coli* the DeoR repressor can bind to three operators O_E , O_1 and O_2 located –886, –607 and –8 bp respectively, upstream of the transcription start of the *deoP2* promoter (5, 9, 10). The *deoR* operator sites O_1 and O_2 which consist of a 16 bp palindromic sequence, overlap the –10 region of *deoP1* and *deoP2*, respectively (5, 11, 12, 13). Two of the three operators are required in order to obtain an efficient DeoR repression while the presence of the third operator increases the repression further by two-three fold (5, 9). We have shown previously that DeoR binds cooperatively *in vivo* when a second operator is placed upstream or downstream of *deoP2*, irrespective of its orientation, and even when the operators have been separated as much as 5000 bp (4, 5). According to the DNA chelate model (or DNA loop model), The octameric DeoR molecule can bind to two or more operator sites at the same time thereby forming a loop in the intervening DNA. The advantage of such loop formation is that once DeoR is bound to one operator, the DeoR molecule is kept in the vicinity of the other operator(s) thereby locally increasing the effective concentration of DeoR.

Proteins that bind DNA through the α -helix-turn- α -helix motif usually bind to one side of the DNA helix. Binding of one protein molecule to two closely spaced operators is therefore most energetically favourable when the two operators are placed on the same side of the DNA helix i.e. the interoperator distance

corresponds to an integral number of helix turns. Conversely, when the operators are placed on opposite sides of the helix, the DNA needs to be twisted in order to form a loop. In the *araBAD* operon an operator 211 bp upstream of the *araI* site was found to be necessary for repression *in vivo*. The effectiveness of this repression was furthermore found to depend on the interoperator distance in a periodic way corresponding to integral number of turns of the DNA helix (1, 14). A similar periodicity of repression has been observed in the *lac* operon both *in vitro* (15, 16) and *in vivo* (17) and with the λ repressor *in vitro* (18, 19) and *in vivo* (20). The DeoR repressor is different from both LacR repressor and AraC because DeoR exists as an octamer in solution while LacR exists as a tetramer and AraC forms a loop through two dimers. DeoR should thus be able to bind four operators at the same time. Therefore, the strong periodic variation depending on the interoperator distance between two operators, observed in both the *ara* and *lac* systems and which has become a diagnostic of loop formation, is not expected to be observed in the *deo* system because of a reduced amplitude. This is the subject of the present work.

MATERIALS AND METHODS

Materials

Restriction endonucleases, T4 DNA ligase, Exonuclease III and DNA polymerase (Klenow fragment) were obtained from Boehringer Mannheim or New England Biolabs and were used as recommended by the manufacturer. Synthetic *Bam*HI (5'CCGGATCCGG), *Hind*III (5'CCCAAGCTTGGG) and *Kpn*I (5'GGGTACCC) linkers were from BRL, PL biochemicals and New England Biolabs, respectively. DE81 filters were purchased from Whatman.

Standard techniques

Plasmid DNA preparation, *E.coli* transformation, isolation of DNA fragments, treatment with enzymes (restriction endonucleases, Klenow enzyme, T4 DNA ligase etc.) was carried out as described by (21) and (22).

Bacterial strains

SØ3431 (F^- , *pro*, *galk2*, *rpsL*, *sup*^o, *clmA1*, *recA56*, *srlC300::Tn10*, *cytR50*) and SØ3432 (F^- , *pro*, *galk2*, *rpsL*, *sup*^o, *metB*, *recA56*, *srlC300::Tn10*, *cytR50*, *deoR51*) which are named *deoR*⁺ and *deoR* respectively, have been described

previously (5). SØ3426 (F^- , *pro*, *galK2*, *rpsL*, *sup*^o, *clmA1*, *recA56*, *srlC300::Tn10*, *cytR50*, *ilv::Tn5*, *cya1039*) and SØ3428 (F^- , *pro*, *galK2*, *rpsL*, *sup*^o, *metB*, *recA56*, *srlC300::Tn10*, *cytR50*, *deoR51*, *ilv::Tn5*, *cya1039*) are the *cya* derivatives of SØ3431 and SØ3432 and are called *cya* and *cya*, *deoR* respectively (23). These strains are *recA* to ensure that the λ -phages only integrate into the λ -attachment site and not by homology. These four strains were lysogenized with the λ -phages listed in Table 1 and single lysogenic derivatives were isolated and tested as previously described (5).

Construction of promoter-galK plasmid fusions

pKH105 was constructed by filling in the ends with Klenow enzyme of a 209 bp *HgaI*-*AvaII* fragment, carrying the *deoP2* region and ligating it into the *SmaI* site of the *galK* promoter cloning vector pKO500 (24). pGD52 (25) is a derivative of pKO500 that contains the 'B-region' upstream of the cloning sites so that it is possible to transfer plasmid fusion to λ gal8 (26) (see fig 1). pGD78 contains the synthetic operator O_S inserted into the *HindIII* and *BamHI* sites of pGD52 whereafter the *HindIII* sites was filled in using Klenow enzyme (this creates a *NheI* site) (27). pGD79-139 was constructed by ligating a 235 bp *BamHI*-*SacI* fragment containing *deoP2* which was isolated from pKH105 into the *BamHI* and *SacI* sites of pGD78. pGD80 was constructed like pGD79-139, but the fragment was inserted into pGD52 instead of pGD78. In pGD88, the O_S operator was destroyed by filling in the *EcoRI* site in the center of O_S using Klenow enzyme (see fig 1). All the plasmids with a varying distance between O_S and O_2 are derivatives of pGD79-139 and are named pGD79-# where the number indicates the center to center distance between O_S and O_2 . See also fig. 1. Transfer of *galK* gene fusions to λ gal8 by *in vivo* recombination was performed as described by (26). λ phages derived from pGD79-# have the same number as the corresponding plasmids (see Table 1) while lGD34 and λ GD50 are derived from pGD80 and pGD88 respectively.

ExonucleaseIII deletions

18 μ g pGD79-139 DNA digested with *BamHI* was incubated with 110 units of exonuclease III (Boehringer) at 23° in 66 mM Tris-HCl, 77 mM NaCl, 5 mM MgCl₂, 10 mM DTT, pH 8.0 in a total volume of 480 μ l. After 5, 10, 15, 20, 25 and 30 minutes 80 μ l was taken out and 8 μ l of 330 mM NaOAc, 500 mM NaCl, 10 mM ZnSO₄, pH 4.4 was added and the mixture was incubated with 60 units of S1 nuclease at 23° for 15 minutes. After ethanol precipitation the DNA was treated with Klenow enzyme to ensure the formation of double stranded DNA, and a *BamHI* linker was ligated. From plasmids with the desired deletions, *BamHI*-*EcoRV* fragments were isolated and inserted into pGD78 creating pGD79-66, pGD79-72 and pGD79-89. All spacer derivatives are described in Table 1. The interoperator distance in the spacer mutants was verified by DNA sequencing (Sequenase kit from United States Biochemical corp.) and/or by comparing the size of the *BamHI*-*SacI* fragments labelled with S³⁵ α -dATP using Klenow enzyme on a DNA sequencing gel (*EcoRV* and *SacI* cut in the vector DNA).

Growth of bacteria and enzyme assays

Cells were grown exponentially at 32° for 5 generations in AB medium (28) with 0.3% glycerol as carbon source supplemented with the appropriate requirements. 0.3% fructose was used as carbon source for the *cya* derivatives. Cells were harvested and

Table 1. Construction of spacer derivatives.

Plasmid (phage)	Starting plasmid/site	Treatment (insert DNA)	Change in operator distance (bp)
pGD79-46	pGD79-139/ Δ (<i>PvuII</i> - <i>MamI</i>)		Δ 83
pGD79-53	pGD79-139/ Δ (<i>BamHI</i> - <i>MamI</i>)	Klenow	Δ 86
pGD79-66	pGD79-139/ <i>BamHI</i>	Exonuclease III	Δ 73
pGD79-69	pGD79-72/ Δ (<i>BamHI</i> - <i>PvuII</i>)	Klenow	Δ 3
pGD79-70	pGD79-66/ <i>BamHI</i>	Klenow	4
pGD79-70 Δ P2	pGD79-70/ Δ (<i>MamI</i> - <i>BglII</i>)	Klenow	Δ deoP2
pGD79-73	pGD79-139/ <i>BamHI</i>	Exonuclease III	Δ 67
pGD79-73	pGD79-69/ <i>BamHI</i>	Klenow	4
pGD79-74	pGD79-66/ <i>PvuII</i>	<i>KpnI</i> linker	8
pGD79-76	pGD79-72/ <i>BamHI</i>	Klenow	4
pGD79-78	pGD79-66/ <i>PvuII</i>	<i>HindIII</i> linker	12
pGD79-80	pGD79-72/ <i>PvuII</i>	<i>KpnI</i> linker	8
pGD79-82	pGD79-78/ <i>BamHI</i>	Klenow	4
pGD79-84	pGD79-80/ <i>BamHI</i>	Klenow	4
pGD79-86	pGD79-82/ <i>HindIII</i>	Klenow	4
pGD79-89	pGD79-139/ <i>BamHI</i>	Exonuclease III	Δ 50
pGD79-83	pGD79-89/ <i>BamHI</i>	Klenow	4
pGD79-87	pGD79-89/ <i>PvuII</i>	<i>KpnI</i> linker	8
pGD79-136	pGD79-139/ Δ (<i>BamHI</i> - <i>PvuII</i>)	Klenow	Δ 3
pGD79-139	Parent plasmid (see methods)		-
pGD79-140	pGD79-136/ <i>BamHI</i>	Klenow	4
pGD79-142	pGD79-147/ Δ (<i>Aap718</i> - <i>BamHI</i>)	Klenow	Δ 5
pGD79-143	pGD79-139/ <i>BamHI</i>	Klenow	4
pGD79-144	pGD79-151/ Δ (<i>BamHI</i> - <i>HindIII</i>)	Klenow	Δ 7
pGD79-147	pGD79-139/ <i>PvuII</i>	<i>KpnI</i> linker	8
pGD79-151	pGD79-139/ <i>PvuII</i>	<i>HindIII</i> linker	12
pGD79-153	pGD79-143/ <i>PvuII</i>	<i>BamHI</i> linker	10
pGD79-157 A	pGD79-139/ <i>PvuII</i>	2x <i>KpnI</i> linker	16
pGD79-161 A	pGD79-157/ <i>BamHI</i>	Klenow	4
pGD79-163	pGD79-139/ <i>PvuII</i>	2x <i>HindIII</i> linker	24
pGD79-167	pGD79-163/ <i>BamHI</i>	Klenow	4
pGD79-171 A	pGD79-161/ <i>SmaI</i> C	<i>BamHI</i> linker	10
pGD79-172 AB	pGD79-161/ <i>SmaI</i> C	<i>HindIII</i> linker	11
pGD79-175 AB	pGD79-171/ <i>BamHI</i>	Klenow	4
pGD79-176 A	pGD79-172/ <i>HindIII</i>	Klenow	4

The λ numbers are the same as the plasmid numbers. The last number in the plasmid and λ names indicates the interoperator distance. Each construction is derived from another construction (starting plasmid/site) by the indicated treatment or ligation of linkers (treatment/insert DNA) resulting in the indicated change in the interoperator distance (Change in the operator distance). The *MamI* site is located in the -35 region of the *deoP2* promoter. A: pGD79-157 and its derivatives contain two 8 bp *KpnI* linkers in tandem, however it turned out that one of the linkers had a two bp insertion. B: In pGD79-172 (and pGD79-176) the 12 bp *HindIII* linker was found only to contain 11 bp. C: The *SmaI* site is created between the two *KpnI* linkers in pGD79-157 and derivatives thereof.

sonicated and galactokinase was assayed as described previously (5). Galactokinase units are expressed as nmol of galactose phosphorylated per minute at 32°C and specific activities are expressed as units of enzyme activity per ml of cells at OD₄₃₆=1.0. All the activities are given as the average of 2-5 independent experiments each determined in triplicate and with a standard deviation of less than 12%.

RESULTS

The plasmid, pGD79-139 was constructed to contain the *deoP2* promoter fused to the *galK* gene and a synthetic operator O_S placed 139 bp upstream of O_2 (see fig. 1). This synthetic operator contains the palindromic operator sequence of O_1 but it has no promoter activity despite the fact that it contains an extended -10 region (TGNTANNNT) which are often found to be relatively independent of a good -35 region (27, 29). It furthermore contains unique *PvuII* and *BamHI* sites immediately following the palindromic sequence which makes it possible to change the distance between O_S and O_2 by DNA manipulations such as insertion or deletion of different DNA fragments.

Figure 1. Structure of the parent plasmid pGD79-139. The arrows on the DNA sequences indicate the palindromic operator sites and +1 is the transcription startsite for *deoP2*. Numbers indicate the centers of the operator sites and the CRP targets relative to the transcription startsite (41).

pGD79-139 carries the 'B-region' upstream of O_S which makes it possible to transfer the entire construction to a $\lambda gal8$ phage by *in vivo* recombination. In *galK* strains lysogenic for such recombinant phages, the *galK* expression originates only from the *deoP2* promoter and hence the effect of interoperator distance on repression of *deoP2* can be measured *in vivo* using a single-copy vector.

Interoperator distances between 136 and 176 bp

The different constructions were performed by ligating 8 bp *KpnI* linker(s) or 12 bp *HindIII* linker(s) into the *PvuII* site of pGD79-139; by filling in the *BamHI* or *HindIII* sites using DNA polymerase (Klenow fragment) or by deleting the DNA between *BamHI* and *PvuII* or sites in the linkers (see Table 1 for details). After *in vivo* recombination, the resulting $\lambda deoP2-galK$ phages were lysogenized into wild type (*deoR*⁺) and *deoR* strains and the *galK* expression was measured. The repressed (*deoR*⁺) and the derepressed (*deoR*) expression for varying interoperator distances is shown in fig 2. As expected, the same expression is obtained in all constructions under derepressed conditions (13.8 units/OD₄₃₆ ± 12%). However, under repressed conditions, the *galK* expression drastically decreases from 14 to 0.34 units/OD₄₃₆ (41 fold repression) when O_S is placed 139 bp upstream of O_2 while *deoP2* alone (one operator site) is repressed only 1.5 fold. Increasing the distance between O_S and O_2 gradually to 176 bp has very little effect on the repressed expression (0.33–0.80 units/OD₄₃₆). Interestingly, no distances were found where the DeoR repression was weak (fold of repression varies from 18 to 55 fold).

Interoperator distances between 46 and 139 bp

Since a very efficient DeoR repression was found at all distances tested so far, the interoperator distance was further decreased by deleting the DNA between the *BamHI* site and O_2 (see fig 1). Four plasmids, pGD79-46, pGD79-66, pGD79-72 and pGD79-89 were used to generate a new series of interoperator constructions by inserting linkers, filling in restriction sites etc. as described above. As a control, the O_S operator was destroyed in pGD79-72 by filling in the *EcoRI* site in the centre of the operator. Deletion experiments in *deoP2* have indicated that the CRP1 target is sufficient for nearly full activation of *deoP2* (after

Figure 2. *GalK* expression of single-copy spacer constructions in the range 136–176 bp. The interoperator distances tested are: 136, 139, 140, 142, 143, 144, 147, 151, 153, 157, 161, 163, 167, 171, 172, 175 and 176 bp. Closed boxes indicate the expression of SØ3432 lysogens (*deoR*) while open boxes indicate the expression of SØ3431 lysogens (*deoR*⁺). ΔO_S indicates the repressed expression of $\lambda GD34$ which only contains one operator site (O_2). The sine curve is chosen arbitrary just to indicate a periodicity. The periodicity of approximately 11 indicated here, is the best visual fit. A log scale is used only to show both curves in the same graph.

deletion of CRP2, *deoP2* retains ca. 80% of its activity (30)). However, when we measured the *galK* expression in lysogens containing the CRP1 consensus sequences, we found a large variation in the promoter strength due to a varying degree of CRP activation of *deoP2* (data not shown). This indicates that also DNA sequences upstream of the CRP1 consensus are important for CRP/cAMP activation of *deoP2*. To avoid differences in CRP activation we used *cya* and *cya*, *deoR* strains where no activation takes place. The *galK* expression from such lysogens is shown in fig 3. Under derepressed conditions the same *galK* expression is obtained in all constructions (0.97 units/OD₄₃₆ ± 12%). The CRP/cAMP activation of *deoP2* is thus 13.8/0.97=15 fold. Under repressed conditions a large variation in *galK* expression is seen for the different spacer derivatives, however, in all constructions, even when the interoperator distance is only 46

Figure 3. *Galk* expression of single-copy spacer constructions in the range 46–139 bp. The interoperator distances tested are: 46, 53, 66, 69, 70, 72, 74, 76, 78, 80, 82, 84, 86, 89, 93, 97 and 139 bp. Closed boxes indicate the expression of SØ3428 lysogens (*cya*, *deoR*) while open boxes indicate the expression of SØ3426 lysogens (*cya*, *deoR*⁺). ΔO_S indicates the repressed expression of λ GD88 in which the O_S operator is destroyed.

Figure 4. Effect of the growth temperature on the repressed expression of SØ3426 lysogens (*cya*, *deoR*⁺). The interoperator distances tested are: 66, 69, 70, 72, 74, 76, 78, 80, 82, 84 and 86 bp. Closed boxes indicate the expression of cells grown at 42°C while open boxes indicate the expression when grown at 32°C.

bp, a stronger repression (3–10 fold) is seen than the repression in the control where O_S is destroyed (1.1 fold). In the range 66–86 bp an oscillatory variation might exist with a periodicity of approximately 8–9 bp.

Effect of the temperature on DeoR repression

Since changes in growth temperature affect both the superhelical tension and the helical pitch of the DNA (31), we wanted to see if changes in the temperature also affect DNA looping. Since the λ -lysogens contain the thermolabile cI857 repressor the cells were first transformed with the plasmid pUN121 which expresses the wild type λ repressor (32). The resulting *cya* strains carrying the spacer derivatives in the 66–86 bp range, were grown at 42°C and the single-copy *galk* expression was measured. In fig 4, the DeoR repressed *galk* expression at both 32° and 42° is

shown as a function of the interoperator distance. The two curves in fig 4 are almost identical indicating that there is no temperature effect on DeoR repression of the *deoP2* promoter *in vivo* in this temperature range.

DISCUSSION

In this paper, the distance between two *deo* operators have been changed systematically in order to find both the minimal distance for cooperative DeoR repression of *deoP2*, and to investigate a possible relation between the extent of DeoR repression and the distance between the two sites as it has been found in other bacterial systems (e.g. the *ara* and *lac* operons).

In vitro, the persistence length of DNA in solution has been estimated to be 150 bp (33, 34, 35). However, the persistence length *in vivo* has been estimated to be only one-fifth of the *in vitro* length (17). DNA loops formed between two operators separated by only 5 to 6 helix turns (52–66 bp) have been observed in the electron microscope with both λ repressor (18) and Lac repressor (15). Also *in vivo* there is good evidence for loop formation in this range (14, 20). Lee and Schleif reduced the distance between two *araC* binding sites to 35–240 bp and found no lower limit for DNA loop formation in this interval.

From the data presented here, DeoR repression is similar to AraC repression with respect to the lack of a lower limit for loop formation. For all interoperator distances tested so far, the second operator efficiently increases the DeoR repression of the primary operator/promoter. In the range 136–176 bp where *deoP2* is activated by CRP/cAMP the DeoR repression is very efficient and varies between 18 and 55 fold. In the range 46–136 bp where there is no CRP/cAMP activation, DeoR can still repress transcription 3–10 fold which should be compared to the 1.1 fold DeoR regulation in the control where the O_S operator is destroyed (i.e. only O_2 is intact). Apparently, the limit for cooperative DeoR repression is below 46 bp or 4 helix turns as it was found in the *araBAD* operon.

With respect to the degree of repression, DeoR regulation is different from the AraC and LacR regulation, as expected from the multimeric structure of the proteins. DeoR exists as an octamer in solution as determined by gelfiltration studies (10, 12, 36) and should therefore be able to bind four operators simultaneously, while one tetrameric LacR molecule (37) or two dimeric AraC molecules (38) only should bind two operators at the same time. Therefore, when the DNA carries two operator sites, there are excess binding sites on DeoR but not on AraC or LacR. In the *ara* system, increasing the distance between *araI* and *araO₂* by half a helical turn from 211 bp to 216 bp almost has the same effect as introducing a mutation in *araO₂* or deleting *araO₂* (14). This effect was even more pronounced when the interoperator distance was decreased to 100–170 bp. A similar strong dependency on the interoperator distance was demonstrated *in vivo* in the *lac* system (17). In the *deo* operon, however, there are no interoperator distances where the repression approaches the level obtained when *deoO_S* is deleted (fig 2 and 3). A simple explanation is that alternative loops may be formed because of the excess of binding sites on the protein. At a given interoperator distance one specific combination of two of the four binding sites might be most favourable. When the distance is increased, e.g. by half a helix turn (5–6 bp), another combination of two binding sites may be favourable and the net result is that no distances seem unfavourable. If the DNA carries three operator sites (as the native *deo* operon), there would be

an even larger number of possible combinations that could be favourable at different distances. A weak flexibility in the DeoR protein would further remove the need for twisting the DNA. In fact, a highly flexible DeoR molecule might itself explain the lack of unfavourable interoperator distances. Instead of twisting the DNA, DeoR could wrap around the DNA. Formation of different loops has been observed in the electron microscope where purified DeoR protein and DNA containing three operators formed both double loops (one DeoR molecule bound to all three operators) and different single loops between only two of the three operators (36).

CRP is known to bend DNA and has been shown to promote the ligation of DNA fragments into circles (39, 40). The insensitivity of the interoperator spacing is found both in the presence (fig. 2) and absence of CRP/cAMP activation (fig 3). We have previously found that both *deoP1* and *deoP2* is regulated cooperatively by DeoR in the absence of CRP/cAMP activation (23). Thus, it is unlikely that the DeoR repression depends on DNA bending although the possibility, that the mechanism of DeoR repression of *deoP2* is different in the presence and absence of CRP/cAMP activation cannot be ruled out.

Does the periodic behaviour observed in fig 2 and 3 then reflect the helical repeat of DNA? The possibility that very weak promoters were created between the two operators during the gene manipulations cannot completely be ruled out, although such activities need to be present in both *cya*⁺ and *cya* strains. This has been tested in the 70 bp construction which has the highest expression under repressed conditions (see fig 3). When the *deoP2* promoter is deleted, the expression decreases down to the average repressed level proving that the high expression is not due to an artificial promoter (data not shown). The excess of binding sites on DeoR relative to the DNA and the resulting possibility of alternative DNA loops can be modelled in terms of sine curves (similar to the curve indicated in fig. 2). If each combination of pairs of binding sites, bound to DNA at different interoperator distances, is represented by a sine curve where the period is the helical repeat of DNA and the amplitude represents the difference between the repressed and derepressed expression, then the observed periodicity is a combination of all the individual binding modes and must therefore have the same period as the individual curves (the helical repeat of DNA). In contrast to the period, the combined amplitude can vary between two limits where the individual curves are exactly in phase or exactly in opposite phase. Only if the curves are in phase (all combinations are favourable or unfavourable at the same distance), a strong amplitude should be obtained. This would require a completely symmetrical DeoR protein. If the curves are in opposite phase, then the amplitude is zero (analogous to the sum: $\sin(x) + \sin(x + \pi)$). All other combinations will give the original period and an amplitude between zero and the amplitude of the in-phase situation. According to this model, the excess of binding sites on DeoR should give rise to a periodic variation corresponding to the helical repeat of DNA, but with an amplitude that depends on the structure, symmetry and flexibility of DeoR. Therefore, the weak 11 bp period indicated in fig. 2 could reflect the helical repeat of DNA and the weakness of the periodicity could indicate that DeoR possesses a relatively high degree of asymmetry and/or flexibility. From the data shown in fig. 2 it is not possible to determine the period more accurately than approximately 11 bp. This is in good agreement with the results of Lee and Schleif who found a period of 11.1 bp (14). In contrast, the shorter interoperator distances (66–86 bp in fig 3

and 4), results in a smaller and more uncertain period of 8–9 bp. In this range, I have independently isolated λ -lysogens in *cya*⁺ derivatives and almost identical *galK* expression levels were obtained in the two different *deoR*⁺ backgrounds indicating that the difference in periodicity between fig 2 and 3 is not due to CRP/cAMP binding (data not shown since the derepressed expression varied because of partial CRP activation).

From the data presented here it is not possible to give any certain value for the helical repeat. Perhaps the isolation of a *deoR* mutant that form tetramers will be helpful for this purpose. The use of a periodic variation as a diagnostic for loop formation is still valid, however, in the light of the results presented here, the reverse is not necessarily the case.

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