Proc. Natl. Acad. Sci. USA
Vol. 76, No. 10, pp. 5061–5065, October 1979
Biochemistry

Interactions between DNA-bound repressors govern regulation by the λ phage repressor

(pairwise cooperativity/*cro* protein/DNA binding/amino- and carboxyl-terminal domains/operators and promoters)

ALEXANDER D. JOHNSON, BARBARA J. MEYER, AND MARK PTASHNE

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Contributed by Mark Ptashne, July 26, 1979

ABSTRACT **The λ phage repressor binds cooperatively to the three sites in the right operator (O_R) according to the following pattern. If the DNA is wild type, O_R1 and O_R2 are filled coordinately because of interactions between repressor dimers bound to these two sites. Site O_R3 is filled only at higher repressor concentrations. In contrast, if O_R1 is mutant, O_R2 and O_R3 are filled coordinately because of interactions between repressors bound to these sites. In this case, the affinity of O_R3 is increased and that of O_R2 is decreased relative to the wild type. We infer that a repressor dimer bound to the middle site O_R2 can interact either with another repressor dimer bound to O_R1 (wild-type case) or, alternatively, with one bound to O_R3 (mutant O_R1 case). We argue that these repressor interactions are mediated by protein–protein contacts between adjacent repressor dimers, because the isolated amino-terminal domains of repressor bind to the operator sites noncooperatively. The *cro* protein of phage λ, a second regulatory protein, which recognizes the same three sites in O_R as does repressor, binds noncooperatively. Experiments performed *in vivo* show that regulation of gene expression by repressor can be influenced critically by cooperative interactions. We demonstrate that the effect of repressor in a lysogen on the activity of the promoter P_{RM} can be changed from activation to repression by deletion of O_R1. We explain this effect in terms of the alternative cooperative interactions described above.**

The λ phage repressor is both a negative and a positive regulator of gene transcription. Repressor at the concentration found in typical lysogens turns on transcription of its own gene, *c*I, and turns off transcription of the adjacent oppositely oriented *cro* operon. At high concentrations, repressor also turns off transcription of *c*I (see Fig. 1, and for review, see ref. 1). Repressor mediates these control phenomena by differentially occupying three contiguous sites in the right operator (O_R) of the phage chromosome. At the repressor concentration found in a single lysogen, O_R1 and O_R2 are filled, but O_R3 is largely free. Under these conditions, P_{RM}, the *c*I promoter, is activated and P_R, the promoter of the *cro* operon, is repressed. Only at higher concentrations of repressor is O_R3 fully occupied and P_{RM} repressed (2, 3). λ repressor binds to each of the sites in O_R as a dimer (4, 5). Each repressor monomer (236 amino acids) is composed of two approximately equal-sized domains joined by a connector of 42 amino acids (6–8). Repressor dimers are maintained in solution predominantly by contacts between carboxyl-terminal domains, and amino-terminal domains specifically bind to the operator (8, 9).

The experiments described in this paper show that the pattern of gene control manifested by repressor and outlined above is critically influenced by interactions between adjacently bound repressors. We begin by examining the binding of purified repressor to the three sites in O_R carried on a DNA fragment. We find that operator mutations located in one site can have a significant effect on binding of repressor to other sites, and from the pattern of these effects we infer that pairs of adjacently bound repressors interact. We show that the amino-terminal domain of repressor, isolated after proteolytic cleavage (8), binds noncooperatively to the three operator sites, and we therefore argue that the cooperativity manifested by intact repressor is mediated by protein–protein contacts. We also show that a smaller (66 amino acid) negative regulator encoded by λ, the *cro* protein (10–14), binds to the same three sites in O_R noncooperatively. Finally, in an experiment that analyzes P_{RM} regulation *in vivo*, we describe an example of "action at a distance," which we explain by interactions between contiguous bound repressors.

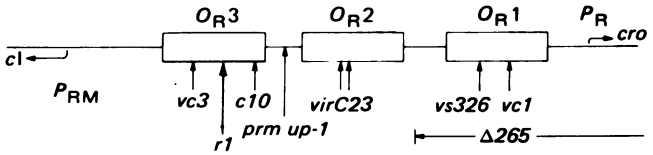

FIG. 1. Portion of the λ genome that includes the right operator (O_R). O_R1, O_R2, and O_R3 are the repressor binding sites, each 17 base pairs long. P_R and P_{RM} are the promoters for genes *cro* and *c*I, respectively, and the transcription startpoints are indicated. Locations of the operator mutations used in this study are shown, as is the P_{RM} promoter mutation *prm up-1*. The wild-type sequence and the base pair changes caused by each mutation are found in refs. 1 and 2. Note that *virC23* is a double mutation and *Δ265* is a deletion.

MATERIALS AND METHODS

Enzymes and Reagents. λ repressor was purified by R. Sauer and the activity was determined as described (15). The amino-terminal domain of repressor, fragment *d* of Pabo *et al.* (8), was prepared by C. Pabo according to that reference. *cro* protein was isolated from *Escherichia coli* strain 294/pTR214 as described by Johnson *et al.* (15). All three proteins were purified to greater than 95% homogeneity. DNase I (code: DP) was purchased from Worthington.

Restriction Fragments and End Labeling. Restriction fragments were isolated from plasmid pKB252 (16) and derivatives of this plasmid that bear mutations in O_R [see Meyer *et al.* (2)] by the method of Maniatis *et al.* (17) and Maxam and Gilbert (18). End labeling with polynucleotide kinase was done as described by Maxam and Gilbert (18). The strategy used to obtain the *Alu/Hha* I 160 fragment (nominal size, 160 base pairs) labeled at the *Alu* end is described in detail by Humayun *et al.* (19). This fragment bears the entire O_R region and was used in most wild-type and mutant DNA binding experiments.

DNase I Protection. DNase I protection buffer consisted of 10 mM Tris·HCl (pH 7.0), 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1 mM EDTA, 200 mM KCl, bovine serum albumin at 100 μg/ml, and chicken blood DNA at 2.5 μg/ml. ^{32}P-Labeled restriction fragments were added such that the operator concentration was ≤ 0.1 nM. To 200-μl reaction volumes were added various amounts of λ repressor, repressor amino-terminal domain, or *cro* protein. After incubation at 37°C for 15 min, DNase I was

added to 2 ng/ml and the mixtures were incubated for 15 min at 37°C. The reactions were stopped with 50 μl of cold 8 M ammonium acetate and tRNA at 300 μg/ml. The DNA was precipitated with ethanol, rinsed, resuspended in 80% (vol/vol) deionized formamide/50 mM Tris–borate (pH 8.3)/1 mM EDTA/0.1% xylene cyanol/0.1% bromphenol blue, and electrophoresed through a 20% acrylamide sequencing gel (see ref. 18). Prefogged Kodak XR-5 film and Du Pont Lightning Plus intensifying screens were used for autoradiography at −70°C (20).

The origins of end-labeled bands produced by DNase I cutting were deduced by comparing the DNase I cleavage products with those produced when the same restriction fragment was subjected to the chemical DNA sequence determination reactions of Maxam and Gilbert (18).

These binding studies were performed under conditions (37°C, 0.2 M KCl) in which the operator concentration (≤ 0.1 nM) was well below the dissociation constant K_d (3 nM) for repressor binding to its strongest site, O_R1. Under these conditions, K_d is approximately equal to the concentration of repressor required to occupy a given site in half the molecules, and this concentration is effectively independent of small variations in the operator concentration [see Riggs *et al.* (21)]. This fact has allowed direct comparison among protection experiments performed with many different alleles of O_R even though the precise operator concentrations were uncertain.

Strains. The *E. coli lac* deletion strain XA10C [*ara*$^-$ Δ(*lac pro*) *nalA*$^-$ *supC*$^-$ *metB*$^-$ *argE-am rif*$^-$ *thi*$^-$] is described by Miller *et al.* (22). Phage strains λ112 *prm up-1 cIsus34*, λ112 *prm up-1 or3-r1 cIsus34*, λ112 Δ*265 prm up-1 cIsus14*, *and* λ112 Δ*265 prm up-1 or3-c10 cIsus14* are described in detail by Meyer *et al.* (2). (See Fig. 1.)

β-Galactosidase Assays. Lysogenic derivatives of *E. coli* XA10C were grown as described (2), and β-galactosidase was assayed (23).

RESULTS

Repressor binding *in vitro*—pairwise cooperativity

The binding of repressor at various concentrations to sites O_R1, O_R2, and O_R3 was studied by using wild-type and various mutant DNAs. The binding was measured by exploiting repressor's ability to protect the DNA it covers from attack by DNase I. The technique used was developed independently in our laboratory as described in *Materials and Methods* and is similar to the "footprinting" method described by Galas and Schmitz (24). In each experiment, a DNA fragment, labeled with ^{32}P at one end and bearing all three operator sites, was incubated with a series of concentrations of repressor and partially digested with DNase. In each case, the products were fractionated according to size by gel electrophoresis and were visualized by autoradiography. In the absence of repressor, DNase I cleavage produces a distinct pattern of bands. In the presence of a bound protein, those bands corresponding to DNA sites covered are absent from the final pattern. We confirmed our previous reports (9, 13, 25) that repressor, its amino-terminal domain, and the *cro* protein bind to the same three sites in O_R. A more detailed analysis showed that the region protected from DNase I cleavage by *cro* protein is slightly shorter (one base at each end of a binding site) than that protected by the other two proteins (unpublished).

A typical experiment using a fragment bearing wild-type O_R is shown in Fig. 2. Inspection of the figure reveals the relative concentration of repressor at which sites O_R1, O_R2, and O_R3 are half-occupied. Table 1 summarizes the data from this and related experiments quantitated as described in the table leg-

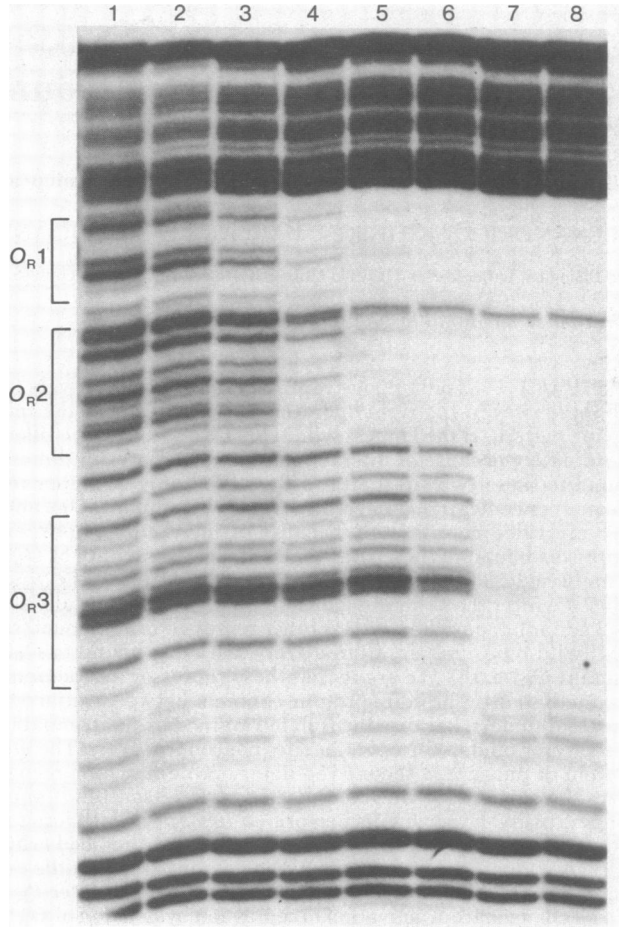

FIG. 2. Visualization of repressor binding to a wild-type O_R template. A DNA fragment bearing wild-type O_R and labeled at one end with ^{32}P (the *Alu/Hha* I 160 fragment of ref. 19) was partially digested with DNase I in the presence of various concentrations of repressor. The products were visualized by autoradiography after electrophoresis through a polyacrylamide gel. The regions of the gel displaying fragments produced by cleavage within the regions O_R1, O_R2, and O_R3 are indicated. The total active repressor concentration (expressed in moles of repressor monomers per liter) in each reaction was as follows: slot 1, 0; slot 2, 3.5 nM; slot 3, 8.8 nM; slot 4, 18 nM; slot 5, 35 nM; slot 6, 88 nM; slot 7, 180 nM; slot 8, 350 nM. We estimate that the experiment provides a measure of the concentration at which each site is filled in half the molecules to an error of less than ±30%. From the total repressor concentration, we calculate the concentration of repressor dimers, the active binding form, using the dimer–monomer dissociation constant K_d = 20 nM (refs. 4 and 5; see also ref. 15). The values in Table 1 are based on the calculated repressor dimer concentrations.

end. The table shows the amount of repressor required to half fill each of the three sites in the wild-type and various mutant DNAs. With DNA containing wild-type O_R, O_R2 was occupied (half filled) at approximately the same concentration as that needed to occupy O_R1, and a 25-fold higher concentration of repressor was required to fill O_R3. In striking contrast are the results with DNA bearing a mutant O_R1 (line 2). In this case, O_R2 and O_R3 were occupied at the same concentration; the apparent affinity of O_R3 for repressor had *increased*, whereas that of O_R2 had *decreased* when compared to the wild-type case. Another O_R1 mutant gave identical results (not shown). Line 3 shows that mutation of site O_R2 had no detectable effect on the affinity of O_R3 but had a small but reproducible (approximately 2-fold) effect on the apparent affinity of O_R1. Line 4 shows that mutation of sites O_R1 and O_R3 dramatically decreased the apparent affinity of O_R2. When O_R1 and O_R2 were

Table 1. Binding of λ repressor to wild-type and mutant O_R DNAs

DNA	Relative repressor concentration O_R3	O_R2	O_R1
O_R^+	25	2	1
O_R1^- (*vs326*)	5	5	—
O_R2^- (*virC23*)	25	—	2
O_R1^- O_R2^- (*Δ265 virC23*)	25	—	—
O_R1^- O_R3^- (*vc1 vc3*)	—	25	—

Values indicate the relative concentration of repressor dimers required to fill the corresponding binding sites on half the wild-type or mutant DNA molecules. These concentrations were deduced from experiments such as the one shown in Fig. 2. The concentration of repressor dimers (≈3 nM) required to half-fill O_R1 on a wild-type DNA was assigned a value of 1, and all numbers in the table are normalized to this value (see legend to Fig. 2). Dashes indicate weak or undetectable binding.

mutant (line 5), the affinity of O_R3 remained that seen in the wild-type case.

We interpret the data of Table 1 as revealing that interactions between pairs of adjacently bound repressors strongly influence the binding of repressor to the three sites in O_R. Strong repressor binding to O_R2 requires binding of another molecule of repressor to O_R1. When sites O_R1 and O_R2 are occupied, binding to O_R3 is weak. In contrast, when repressor is prevented from binding to O_R1 by an operator mutation, the apparent affinity of O_R3 is increased because of cooperative binding to sites O_R2 and O_R3. The data of Table 1 allow us to estimate the standard free energies ($\Delta G = -RT\ln K_d$) of the intrinsic affinity of repressor for each site and of the cooperative effect of neighboring repressors. Thus, for example, the intrinsic affinity of O_R2 is estimated from the equilibrium constant describing binding to that site on the O_R1^- O_R3^- mutant template. Measurements of binding to O_R3 on an O_R1^- O_R2^- mutant template define the intrinsic affinity of that site, and binding to O_R2 and O_R3 on an O_R1^- mutant reveals the cooperative interaction between repressors bound at these two sites.

In summary, the results show that the intrinsic repressor affinity of O_R2 and O_R3 is about 1/15th that of O_R1, and that repressor at O_R2 can interact either with repressor bound to O_R1 or with repressor bound to O_R3, but not with both. The former interaction predominates on a wild-type template, and the latter is revealed if the template is O_R1^-. We estimate that the intrinsic free energy changes upon binding of repressor to the operator sites at 37°C in 0.2 M KCl are: O_R1, 11.6 kcal/mol; O_R2 and O_R3, 10 kcal/mol each (1 kcal = 4.184 kJ). We estimate the net free energy change for the interaction between pairs of adjacently bound repressors to be 2–3 kcal/mol.

Binding of amino-terminal domain of repressor and intact *cro* protein—noncooperative binding

Table 2 shows the results of DNase I protection experiments performed with the isolated amino-terminal domain of repressor. This protein fragment, 92 amino acids long, was prepared by proteolytic cleavage of λ repressor and purified as described by Pabo *et al.* (8). With wild-type O_R, sites O_R2 and O_R3 were filled at the same concentration of amino-terminal fragment, approximately 25-fold higher than that required for filling O_R1. [Note that a much higher concentration of fragment than intact repressor is required to fill O_R1; see Sauer *et al.* (9).] The table also shows that, in contrast to results obtained with intact repressor, mutation of O_R1 had no effect on the binding of fragment to O_R2 and O_R3, and mutation of O_R2 had no effect on the binding of fragment to O_R1 and O_R3. We also found that *cro* protein, like the amino-terminal domain of repressor, bound to the three sites in O_R noncooperatively. That is, mutation of any site in O_R affected the affinity of only that site for *cro* protein (Table 3). The relative dissociation constants on a wild-type template were O_R1:O_R2:O_R3::8:8:1. This result is consistent with our previous observation (13) that O_R3 is the tightest binding site for *cro* protein in O_R.

Table 2. Binding of the repressor amino-terminal domain to wild-type and mutant O_R DNAs

DNA	Relative domain concentration O_R3	O_R2	O_R1
O_R^+	25	25	1
O_R1^- (*vs326*)	25	25	—
O_R2^- (*virC23*)	25	—	1
O_R1^- O_R3^- (*vc1 vc3*)	—	25	—

Values indicate the relative concentration of the amino-terminal domain of repressor required to fill the corresponding binding sites on half the molecules. These concentrations were deduced from experiments similar to that of Fig. 2 but using purified amino-terminal domain of repressor instead of intact repressor. In this case, a concentration of 10 μM amino-terminal monomers was set at 1. The other values are given as the square of the relative concentration because two amino-terminal domains bind to each repressor binding site. Free dimers of this fragment at these concentrations are undetectable (8). Dashes indicate weak or undetectable binding.

We have performed these experiments under a variety of conditions, including 37°C in 0.2 M KCl and 0°C in 0.05 M KCl. Under all conditions examined, repressor showed the same pattern of pairwise cooperativity, and *cro* protein and the repressor amino-terminal domain bound noncooperatively. Moreover, the affinity of *cro* protein for O_R3 was approximately the same under the conditions tested, whereas repressor binds to O_R1 roughly 1000-fold more strongly at the lower temperature and salt concentration (see refs. 5 and 15). One result of this effect is that under the conditions approximating physiological (0.2 M KCl, 37°C), *cro* protein binds to O_R3 approximately as tightly as a repressor dimer binds to O_R1.

Cooperativity and gene control—"action at a distance"

The experiments described above show that there exists a repressor concentration such that (*i*) on wild-type DNA, O_R1 and O_R2 are filled, but O_R3 is largely free, and (*ii*) on DNA mutant for O_R1, O_R2 and O_R3 are filled. Because the former state maximally stimulates P_{RM} and the latter represses it (2, 3), our results lead to the following striking prediction. Mutation of O_R1 should increase the repressibility of P_{RM}; that is, there should be a concentration of repressor that stimulates P_{RM} on a wild-type DNA but that represses that promoter on an O_R1^- mutant DNA. The following experiment, performed *in vivo*,

Table 3. Binding of *cro* protein to wild-type and mutant O_R DNAs

DNA	Relative *cro* protein concentration O_R3	O_R2	O_R1
O_R^+	1	8	8
O_R1^- (*vs326*)	1	8	—
O_R2^- (*virC23*)	1	—	8
O_R1^- O_R3^- (*vc1 vc3*)	—	8	—

Values indicate the relative concentration of *cro* protein required to fill the corresponding binding sites in half the DNAs. These concentrations were deduced from experiments similar to the experiment of Fig. 2 except that the protein was purified *cro* protein. A concentration of 3 nM *cro* protein dimers was set at 1. Dashes indicate weak binding. *vs326* and *vc1* each reduce *cro* protein affinity to 1/5th, and *vc3 and virC23* reduce the affinity to 1/10th and 1/50th, respectively, of the corresponding wild-type site.

demonstrates this effect at the concentration of repressor found in a typical lysogen.

We have described a phage (λ112) that contains a *lacZ* gene whose transcription is directed by the λP_{RM} (1, 3). (The *lacZ*-P_{RM} fusion is substituted for nonessential phage genes, and essential phage genes are controlled by the immunity region from phage 21.) *E. coli* with a deletion of the chromosomal *lacZ* gene and lysogenic for this phage synthesize β-galactosidase only when transcription initiates at P_{RM}. The β-galactosidase levels in such lysogens are a convenient measure of P_{RM} function. Our current experiments utilize various derivatives of λ112 *prm up-1*. *prm up-1* is a single base pair change that renders P_{RM} significantly active in the absence of repressor. Repressor, at the concentration found in a lysogen, stimulates *prm up-1* about 4-fold and at higher concentrations represses it [see Meyer *et al.* (2)]. Binding experiments performed *in vitro* have demonstrated that the mutation *prm up-1* does not significantly affect the affinity of the operator for repressor (not shown).

In the experiment of Table 4, we assayed the activity of *prm up-1* and various mutant derivatives by measuring the synthesis of β-galactosidase in lysogens. In the absence of λ repressor, these mutants all synthesized the same amount of β-galactosidase. The last column shows the effect of a single concentration of repressor, supplied by a second prophage, λ*h*80. The first line shows that *prm up-1* is stimulated about 4-fold at this repressor concentration. Under these conditions, O_R3 is largely vacant; this is shown by the fact that addition of an O_R3^- mutation, which prevents repression of P_{RM} (2, 3), has little effect on the extent to which *prm up-1* is stimulated in this experiment (compare lines 1 and 2 of Table 4). Line 3 shows that on a template mutant in O_R1, *prm up-1* is repressed about 4-fold. Line 4 shows that this repression is relieved by further mutation of O_R3. We interpret these results as showing that at the concentration of repressor found in a lysogen, O_R3 is filled by repressor if O_R1 is mutant, whereas it is largely free if the DNA is wild type. In other words, mutation of O_R1 has an effect at a distance; that is, it increases the affinity of O_R3 for repressor.

DISCUSSION

We have shown elsewhere (2, 3) that maintenance of the λ-lysogenic state requires that, at O_R, repressor be bound predominantly to O_R1 and O_R2. Under this condition, transcription of the lytic functions from promoter P_R is repressed, and transcription of the repressor gene *c*I is stimulated from promoter P_{RM}. Site O_R3, which mediates repression of P_{RM}, is occupied partially if at all. Our current results explain how this

Table 4. Effects of repressor on activity of promoter *in vivo*

Prophage genotype	β-Galactosidase	
	No repressor	Repressor
1. O_R^+	950	3800
2. $O_R1^+ O_R2^+ O_R3^-$	950	4200
3. $O_R1^- O_R2^+ O_R3^+$	900	450
4. $O_R1^- O_R2^+ O_R3^-$	900	1900

Lysogens of four P_{RM}-*lacZ* fusion phages (derivatives of λ112) were assayed for β-galactosidase. One set of these lysogens (right column) contained λ repressor supplied by a second prophage (λ*h*80) integrated at a different chromosomal attachment site. The other set (second column from right) had no λ repressor. All four λ112 derivatives bear the mutation *prm up-1* (Fig. 1). In addition, these phages bear, respectively, the folowing alleles: (1) *c*I*sus34*, (2) *c*I*sus34 or3-r1*, (3) *c*I*sus14* Δ*265*, and (4) *c*I*sus14* Δ*265 or3-c10*. The *c*I*sus* mutations were included to prevent repressor synthesis from the fusion phage. The sites of the operator mutations are shown in Fig. 1. The host XA10c bears a deletion of the *lac* operon. β-Galactosidase activity is expressed in the units of Miller (ref. 23; see also ref. 3).

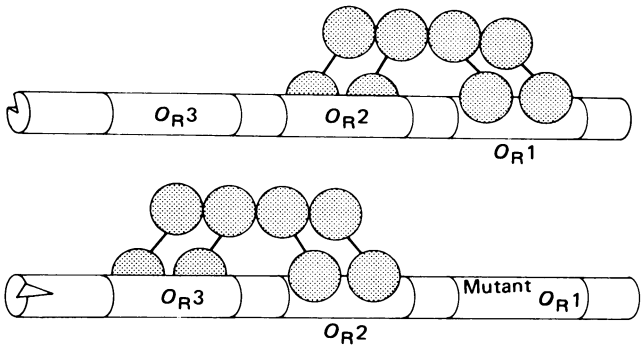

FIG. 3. Representation of repressors bound to O_R. The upper drawing (O_R^+) indicates that, at the concentration of repressor found in a lysogen, O_R1 and O_R2 are each occupied by a repressor dimer and stabilized by a cooperative interaction. The lower drawing (O_R1^-) shows that, at the identical repressor concentration, O_R2 and O_R3 are filled if O_R1 is mutated such that it cannot bind repressor. This is because, in the absence of repressor bound to O_R1, repressors at O_R2 and O_R3 interact. In the wild-type case, P_{RM} is stimulated but in the O_R1^- mutant case, P_{RM} is repressed (see Fig. 1 and Table 4). The figure indicates that the protein–protein interactions are mediated by adjacent carboxyl domains, but the evidence for this is merely suggestive (see text). The lower DNA molecule has been rotated about one-third of a turn relative to the upper one, as indicated by the positions of the notches.

differential occupancy is achieved in a lysogen despite the fact that the intrinsic repressor affinity of O_R1 is approximately 15-fold higher than that of O_R2 and O_R3. We find that repressor binds cooperatively to the sites in O_R according to the following rule. A repressor dimer bound to O_R2 can interact either with another repressor dimer bound to O_R1 or with another bound to O_R3. On a wild-type template, the former interaction predominates, and repressor fills O_R1 and O_R2 coordinately. This interaction precludes the alternate one between repressors bound to O_R2 and O_R3, and hence repressor binds to O_R3 only with the low intrinsic affinity characteristic of that site. In contrast, if no repressor is bound to O_R1, a condition that is obtained with O_R1^- mutants, O_R3 is occupied because of the interaction between repressor dimers bound at O_R2 and O_R3. *In vivo*, this alternate interaction switches the effect on P_{RM} of a single concentration of repressor from positive to negative. Other examples of the effects of these cooperative interactions *in vivo* are discussed by Meyer *et al.* (2). See also Flashman (26), who suggested that repressors bound to O_R1 and O_R2 cooperatively interact.

We ascribe the observed cooperativity to protein–protein interactions and not, for example, to DNA conformational changes transmitted through the helix, because the 92-amino acid amino-terminal fragment of repressor binds to the three sites in O_R noncooperatively. That is, unlike the case with intact repressor, mutations in any of the three sites affect only the affinity of that site for the fragment. The ratio of binding constants is roughly the same as that inferred for the ratio of intrinsic affinities for intact repressor. These facts suggest, moreover, that the cooperative interactions between intact repressor dimers are due to protein–protein interactions between regions of the protein other than the amino-terminal domain. This idea might explain why the interactions occur only pairwise. The carboxyl-terminal domain of repressor consists of 104 residues, and, assuming this domain is spherical, the extremities of two such domains in a dimer bound to O_R2 could not simultaneously contact other repressors bound to both O_R1 and O_R3. Flexibility in the "connector" that joins the repressor domains might orient the carboxyl portion of a repressor bound at O_R2 such that it could contact one neighboring dimer but not another as indicated schematically in Fig. 3.

Why does a repressor dimer bound to O_R2 on a wild-type template cooperatively interact with another bound to O_R1 but not with another at O_R3? That is, given the finding that a repressor dimer at O_R2 can interact with either a repressor bound to O_R1 or another at O_R3, why under equilibrium conditions does O_R3 bind only with its intrinsic affinity? One possibility is that the interaction between repressors at O_R1 and O_R2 is stronger than that between O_R2 and O_R3. In this regard, it should be noted that O_R1 and O_R2 are separated by seven base pairs, and O_R2 and O_R3 are separated by six base pairs.

We have argued elsewhere (13) that the different physiological effects of repressor and *cro* protein are explained in part by the fact that, although the two proteins bind to the same three sites in O_R, they do so with different orders of affinity. This difference depends upon both the intrinsic affinity of each site for each protein and upon the interactions between adjacent repressors. It remains to be seen whether the fact that repressor binds cooperatively to DNA and *cro* protein does so noncooperatively will help explain further details of the different physiological effects of these two proteins. It is clear that, in the case of repressor, interactions between adjacently bound proteins far weaker than those characterizing the interaction of the protein with DNA can have a profound effect on gene control.

We thank R. T. Sauer for purified λ repressor, C. O. Pabo for purified repressor amino-terminal fragment, and A. Jeffrey for help in preparing some of the DNA restriction fragments used in this study. We are grateful to many colleagues, particularly to C. O. Pabo for discussions and to G. Ackers, S. Harrison, T. Maniatis, and N. Kleckner for comments on the manuscript. This work was supported by grants from the National Institutes of Health and the National Science Foundation.

1. Ptashne, M., Backman, K., Humayun, M. Z., Jeffrey, A., Maurer, R., Meyer, B. & Sauer, R. T. (1976) *Science* **194,** 156–161.
2. Meyer, B. J., Maurer, R. & Ptashne, M. (1979) *J. Mol. Biol.*, in press.
3. Maurer, R., Meyer, B. J. & Ptashne, M. (1979) *J. Mol. Biol.*, in press.
4. Chadwick, P., Pirrotta, V., Steinberg, R., Hopkins, N. & Ptashne, M. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **35,** 283–294.
5. Sauer, R. T. (1979) Dissertation (Harvard Univ., Cambridge, MA).
6. Sauer, R. T. & Anderegg, R. (1978) *Biochemistry* **17,** 1092–1100.
7. Sauer, R. T. (1978) *Nature (London)* **276,** 301–302.
8. Pabo, C. O., Sauer, R. T., Sturtevant, J. M. & Ptashne, M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1608–1612.
9. Sauer, R. T., Pabo, C. O., Meyer, B. J., Ptashne, M. & Backman, K. (1979) *Nature (London)* **279,** 396–400.
10. Folkmanis, A., Takeda, Y., Simuth, J. Gussin, G. & Echols, H. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2249–2253.
11. Hsiang, M. W., Cole, R. D., Takeda, Y. & Echols, H. (1977) *Nature (London)* **270,** 275–277.
12. Roberts, T. M., Shimatake, H., Brady, C. & Rosenberg, M. (1977) *Nature (London)* **270,** 274–275.
13. Johnson, A., Meyer, B. J. & Ptashne, M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1783–1787.
14. Takeda, Y. (1979) *J. Mol. Biol.* **127,** 177–189.
15. Johnson, A., Pabo, C. O. & Sauer, R. T. (1979) *Methods Enzymol.* **65,** in press.
16. Backman, K., Ptashne, M. & Gilbert, W. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4174–4178.
17. Maniatis, T., Jeffrey, A. & Kleid, D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1184–1188.
18. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
19. Humayun, Z., Kleid, D. & Ptashne, M. (1977) *Nucleic Acids Res.* **4,** 1595–1607.
20. Laskey, R. A. & Mills, A. D. (1977) *FEBS Lett.* **82,** 314.
21. Riggs, A. D., Suzuki, H. & Bourgeois, S. (1970) *J. Mol. Biol.* **48,** 67–83.
22. Miller, J. H., Ganem, D., Lu, P. & Schmitz, A. (1977) *J. Mol. Biol.* **109,** 275–301.
23. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
24. Galas, D. & Schmitz, A. (1978) *Nucleic Acids Res.* **5,** 3157–3170.
25. Maniatis, T., Ptashne, M., Backman, K., Kleid, D., Flashman, S., Jeffrey, A. & Maurer, R. (1975) *Cell* **5,** 109–113.
26. Flashman, S. F. (1978) *Mol. Gen. Genet.* **166,** 61–73.