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A variant of λ repressor with an altered pattern of cooperative binding to DNA sites

(transcription/linker/spatial range/specificity)

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ABSTRACT **The bacteriophage λ repressor binds cooperatively to pairs of adjacent sites in the λ chromosome, one repressor dimer binding to each site. The repressor's amino domain (that which mediates DNA binding) is connected to its carboxyl domain (that which mediates dimerization and the interaction between dimers) by a protease-sensitive linker region. We have generated a variant λ repressor that lacks this linker region. We show that dimers of the variant protein are deficient in cooperative binding to sites at certain, but not all, distances. The linker region thus extends the range over which carboxyl domains of DNA-bound dimers can interact. In particular, the linker is required for cooperative binding to a pair of sites as found in the λ chromosome, and thus is essential for the repressor's physiological function.**

Crucial to gene regulation is not only the interaction of proteins with DNA but also interaction among the DNA-bound proteins themselves. Interaction among DNA-bound proteins is in turn governed by the strength and specificity of the associations between the proteins and by the relative position of their binding sites on the DNA helix. Thus, it was shown in 1984 that stereospecific alignment of regulatory sites could be critical for gene regulation (1, 2). A number of transcription factors and other DNA-binding proteins have since been shown to require stereospecific alignment of sites in order to be able to interact while binding to DNA (for reviews see refs. 3–6). Although structural motifs that mediate protein–protein interaction have been identified, there is little experimental evidence regarding the general structural features of proteins that might influence the requirement for stereospecific alignment. The presence of flexible interdomain linkers has been found to attenuate the requirement for stereospecific alignment of DNA binding sites. For example, the linker regions of *Saccharomyces cerevisiae* α2 repressor and *Escherichia coli* AraC protein enable these proteins to bind to half-sites at a variety of spacings and orientations (7–9). Here we study the binding to pairs of DNA sites of a mutant bacteriophage λ repressor that lacks a linker region. We find that the linker region is required for cooperative binding of the repressor at some but not all spacings of DNA binding sites. We conclude that the linker increases the spatial range over which interaction between DNA-bound repressors can occur and, in particular, facilitates binding to two sites as found adjacent in the λ chromosome.

Fig. 1*A* depicts the configuration of sites and proteins found at the right operator, O_R, in a λ lysogen. O_R is composed of three adjacent repressor binding sites, O_R1, O_R2, and O_R3, and overlaps P_{RM}, the promoter required for maintenance of lysogeny. O_R1 and O_R2 are separated by 2.3 turns of the DNA helix, center-to-center (assuming 10.5 bp per turn). One repressor dimer is bound to O_R1 and a second is bound to O_R2. The two adjacently bound repressor dimers interact. As a result of this interaction the two repressors bind to O_R1 and O_R2 cooperatively; that is, the interaction increases the apparent affinity of each repressor for its site. The repressor dimer at O_R2 directly activates transcription from the adjacent promoter P_{RM} by interacting with RNA polymerase (10–14). The repressor dimer bound to O_R1 (a strong site) stimulates P_{RM} by helping the second repressor to bind to O_R2 (a weaker site) (15). By ensuring occupancy of O_R2, the cooperative interaction plays a critical role in stimulation of P_{RM} and hence in maintenance of lysogeny.

As shown in Fig. 1 *A* and *B*, the repressor is a two-domain protein. Each monomer is composed of two globular domains connected by a protease-sensitive linker region. The amino domains bear the surfaces of the repressor that contact DNA and interact with RNA polymerase; they also dimerize weakly. The carboxyl domains bear the strong dimerization contacts and mediate cooperative interaction between DNA-bound dimers (15–17). Thus, isolated amino domains are sufficient to bind DNA and stimulate transcription, but do so only when supplied at higher concentrations than the intact protein. High-affinity binding and cooperative binding require the carboxyl dimerization and cooperativity functions, respectively.

Solution NMR experiments indicate that the linker region flexibly connects the amino and carboxyl domains, so that the two domains can rotate somewhat independently of one another (18). This flexibility is thought to account for the ability of repressor dimers to bind with equal cooperativity to sites separated by 1.9, 2.2, and 2.3 turns of the DNA helix, in spite of a 4-bp change in the distance between the sites and a 137° difference in their alignment at these three spacings (19).

In this study we tested whether λ repressor's linker is required for cooperative interaction between repressors binding to sites O_R1 and O_R2. We constructed a repressor derivative lacking a linker region and compared its ability to bind cooperatively to sites on DNA with that of the wild-type repressor. We show that the linker is required for cooperative binding to O_R1 and O_R2 at their normal spacing of 2.3 turns but that it is not required for cooperative binding at spacings of 1.9, 2.0 and 2.1 turns. Thus, by deleting the linker region, we have altered the spatial range over which cooperative interaction between DNA-bound repressor dimers occurs and thereby created a repressor with an altered functional specificity.

MATERIALS AND METHODS

Plasmids Encoding Linkerless Repressor. pUC19cINI, the plasmid from which the linkerless repressor derivative was generated, was made by recloning the *Eco*RI–*Hin*dIII and *Hin*dIII fragments encoding repressor from pDV22 into an *Eco*RI–*Hin*dIII pUC19 backbone and screening for the ori-

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Abbreviation: IPTG, isopropyl β-D-thiogalactopyranoside.

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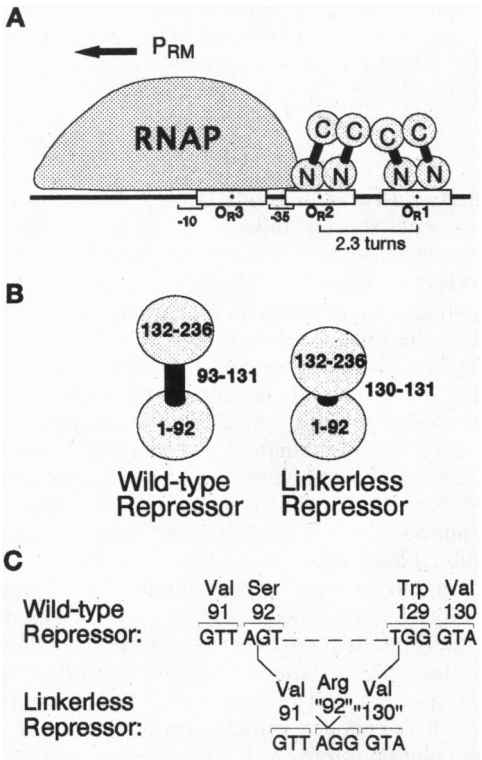

FIG. 1. (*A*) In a λ lysogen, repressor dimers bind cooperatively to O_R1 and O_R2. The repressor dimer bound to O_R2 contacts RNA polymerase (RNAP) and thereby stimulates transcription from P_{RM}. The interaction between the adjacently bound dimers increases the affinity of repressor for O_R2 and thus ensures occupancy of O_R2 under physiological conditions. (*B*) The repressor is a two-domain protein. The amino domain contacts DNA and stimulates transcription at P_{RM}, while the carboxyl domain contains dimer contacts and mediates the cooperative interaction between DNA-bound dimers. The two domains of wild-type repressor are flexibly connected by a 39-aa linker (residues 93–131). The linkerless repressor lacks residues 93–129 and thus is deleted for 37 of the 39 aa of this linker region. (*C*) The upper line shows the portion of the sequence of the wild-type repressor gene that bears the endpoints of the BAL-31 nuclease deletion that generated the linkerless repressor. The lower line shows the new junction between the amino and carboxyl domains that results from that deletion.

entation of the *Hin*dIII fragment that generates an intact repressor gene. These fragments from pDV22 are identical to those in pKB280 (20) except that the *Eco*RI–*Hin*dIII fragment has had an *Nae* I site introduced between codons 111 and 112 of the repressor gene. Thus, pUC19cINI carries the repressor gene under control of a *lacUV5* promoter and with an *Nae* I site between codons 111 and 112. To make a series of derivatives of λ repressor lacking various portions of the linker region, pUC19cINI was digested with *Nae* I and linearized plasmid was gel purified. Aliquots of the purified linear DNA were digested for various times with BAL-31 nuclease. The extent of digestion of the DNA in each aliquot was checked on a gel. The DNA sample that would be expected to give rise to deletions of part or all of the linker was treated with Klenow fragment of DNA polymerase, recircularized with ligase, and screened for its ability to make *E. coli* XA90 cells (F′ *lacI*^{Q1} *lacZ*$^+$*Y*$^+$ *proA*$^+$*B*$^+$/Δ*lac–pro*XIII *ara*$^-$ *nalA argE am thi*$^-$ *rif*^{R}) immune to infection by λKH54 without induction of the *lacUV5* promoter. A plasmid identical to pUC19cINI but encoding an amino-terminal fragment does not confer immunity to λKH54 under these conditions.

For overexpression and purification of the linkerless repressor, the *Hpa* II fragment bearing the linkerless repressor gene was cloned into the *Cla* I site of pEA300 (21), resulting in expression of the gene from the *tac* promoter.

For *in vivo* experiments the *Hpa* II–*Cla* I fragment (with the *Hpa* II end filled in) bearing the linkerless repressor gene and the −10 region of the *lacUV5* promoter was transferred to an *Eco*RI–*Cla* I pBR322 backbone (with the *Eco*RI end filled in) to produce pAA280Δ"−35." The promoter driving expression of repressor on pAA280Δ"−35" is weak because it lacks a −35 region but remains under control of *lac* repressor. When *lac* repressor is inactivated with isopropyl β-D-thiogalactopyranoside (IPTG, 1 mM), this promoter directs the expression of sufficient repressor (either linkerless or wild type) to provide immunity to λKH54.

Plasmids and Bacterial Strains. Templates carrying O_R1 and O_R2 separated by 2.3 and 2.1 turns were made by replacing the *Bgl* II–*Hin*dII fragment of pEM9-O_RP (24) with the synthetic DNAs 5′-GACTATTTTACCACTGGCGGTGATAATGGTTGCATGTA-3′ and 5′-GATCTACATGCAACCATTATCACCGCCAGTGGTAAAATAGTC-3′ (2.3-turn case) or 5′-GACTTTTACCACTGGCGGTGATAATGGTTGCATGTA-3′ and 5′GATCTACATGCAACCATTATCACCGCCAGTGGTAAAAGTC-3′ (2.1-turn case). Each synthetic DNA carries 10 bp of flanking upstream sequence, O_R1, either 7 or 5 bp between O_R1 and O_R2, and the outer base pair of O_R2. In addition, O_R1 bears a point mutation that makes it identical to a consensus operator and increases its affinity for repressor ≈2-fold. O_R3 on the parental plasmid is inactivated by four mutations, such that its sequence is 5′-TACAGCTGCAAGGATA-3′. The resulting constructions, pEM9-O_RP-2.3 and pEM9-O_RP-2.1, were confirmed by sequencing and provided the templates for footprinting experiments.

For *in vivo* experiments, the *Eco*RI–*Hin*dIII inserts from pEM9-O_RP-2.3 and pEM9-O_RP-2.1, with the *Hin*dIII ends filled in, were transferred to an *Eco*RI–*Hin*dIII backbone of pRW283 (22) with the *Hin*dIII end filled in. On the resulting plasmids, pRW283-O_RP-2.3 and pRW283-O_RP-2.1, P_{RM} controls expression of an in-frame fusion of the first 100 bp of the *cI* gene to a small portion of the *lacZ* gene. The two plasmids were recombined *in vivo* with i^{21}RZ11 to generate phage carrying a fusion of P_{RM} and the first 100 bp of the repressor gene to a functional *lacZ* gene (23, 24). Attempts to generate 283-O_RP-2.3 recombinant phage were unsuccessful, so for *in vivo* experiments i^{21}RZ11-O_RP (a gift from Ann Hochschild), which has O_R1 and O_R2 at a separation of 2.3 turns and a quadruply mutant O_R3 but lacks the mutation in O_R1, was used instead. i^{21}RZ11-O_RP-2.1, i^{21}RZ11-O_RP, and i^{21}RZ11O_RPΔO_R1, a phage carrying a P_{RM}–*cI–lacZ* fusion with O_R1 deleted and with a quadruply mutant O_R3 (a gift from Ann Hochschild), were used to make single lysogens of strain X131 (F′lacI^{Q1} *lacZ*::Tn*5* *proAB*$^+$/Δ*lac–pro*XIII *rpsE thi*$^-$ *valR*), resulting in stable single-copy insertion of the O_RP, O_RP-2.1, and ΔO_R1 P_{RM}–*cI–lacZ* fusions into the bacterial chromosome.

β-Galactosidase Assays. Wild-type repressor was produced from pAH280Δ"−35" (24), and linkerless repressor from pAA280Δ"−35" (see above). For determination of the unstimulated level, cells transformed with pBR322 were used.

Overnight cultures of transformants were diluted 1:100 and grown 1.5 hr to an OD_{600} of 0.3–0.6. When needed, IPTG was added at the time of the 1:100 dilution. β-Galactosidase assays (25) were performed in duplicate. Duplicate values differed on average by 6%, and in no case by more than 25%. The values shown for the ΔO_R1 template are averages from three experiments, while the values shown for the 2.3- and 2.1-turn templates are from single representative experiments.

Protein Purification. XA90 bacteria freshly transformed with ptacLR were grown to an OD_{600} of 0.5, induced with 1 mM IPTG, and harvested 5 hr later. Linkerless repressor was purified by method I (26) with the following changes. The bacterial pellet was washed once with lysis buffer prior to sonication. Cells were sonicated in 5 ml rather than 1.7 ml of

lysis buffer per g of cell paste to reduce foaming. Phenylmethanesulfonyl fluoride (1 mM), chymostatin (2 μg/ml), pepstatin (4 μg/ml), leupeptin (5 μg/ml), and benzamidine (1 mM) were present at all steps until after the protein was loaded onto the CM-Sephadex column. NaCl was substituted for KCl. Linkerless repressor remained in the supernatant after the 0.75% (vol/vol) PEI precipitation and was then precipitated with 40% (wt/vol) ammonium sulfate. More than half of the starting amount of linkerless repressor was lost due to precipitation during dialysis of the ammonium sulfate pellet against SB50. Soluble protein was loaded on a CM-Sephadex column. The linkerless repressor was eluted between 150 and 200 mM NaCl. Dialysis against SB50 and consequent precipitation resulted in ≈90% pure linkerless repressor (estimated by visual inspection of a Coomassie-stained gel), free of degradation products. The protein was resuspended at 6 mg/ml in SB with 350 mM KCl.

Footprinting. DNase I footprinting was conducted (15) at 37°C in 200 mM KCl/10 mM Tris, pH 7.0/2.5 mM $MgCl_2$/1 mM $CaCl_2$/0.1 mM EDTA containing bovine serum albumin (100 μg/ml) and chick blood DNA (2.5 μg/ml). For pEM9-O_RP-2.3 and pEM9-O_RP-2.1 the *Eco*RI–*Bst*NI fragment was labeled at the *Eco*RI end. For O_R2 alone, the pBJ300vC (27) *Bgl* II–*Bst*NI fragment was labeled at the *Bgl* II end. All fragments were 3′-end labeled with reverse transcriptase and the appropriate [α-^{32}P]dNTP and purified by PAGE. The concentration of repressor that produced half-maximal protection of bands within a binding site was estimated by visual inspection of the gel.

Calculations. The concentration of repressor dimers at a particular monomer concentration was calculated by using a K_d of 20 nM (28, 29). To calculate the free energy of the cooperative interaction(ΔG_{coop}) from the observed fold increase in affinity of repressor for O_R2 in the presence of O_R1 we used the equations $K_{coop} = (K_{2obs})^2/[(K_1K_2) + K_{2obs}(K_2 - K_1)]$ and $\Delta G_{coop} = 0.616 \ln K_{coop}$, where K_{coop} describes the interaction between DNA-bound repressor dimers, K_1 is the dissociation constant of repressor binding to O_R1, K_2 is the dissociation constant of repressor binding to O_R2, and K_{2obs} is the dissociation constant of repressor binding to O_R2 observed in the presence of O_R1 (see ref. 4). For the linkerless repressor, we used experimentally determined values of $K_2 = 1.6 \times 10^{-7}$ M and $K_1 = 9.2 \times 10^{-9}$ M. In the experiment of Fig. 3, K_{2obs} for the 2.1-turn template was 3.4×10^{-8} M. Although it is possible that deletion of the linker disrupts dimerization, we estimate that a 10-fold decrease in dimerization would introduce only a 15% error into our calculated values of K_{coop} and ΔG_{coop}. For wild-type repressor, we used experimentally determined values of $K_2 = 5.3 \times 10^{-8}$ M and $K_1 = 7.8 \times 10^{-10}$ M.

RESULTS

A Linkerless Repressor Binds DNA. A pool of λ repressor derivatives with shorter linkers was made by digesting a plasmid-borne wild-type repressor gene with BAL-31 nuclease from a restriction site within the linker coding sequence and then religating, transforming, and screening for plasmids with deletions in the linker coding region by restriction analysis. Thirty-six plasmids that were determined to have deletions of part of the linker coding sequence were then screened for ability to confer immunity to phage λ infection. With the plasmid and phage combination we used, only plasmids that encoded repressors that bound with high affinity would confer immunity. Since high-affinity binding of repressor requires both the amino-terminal DNA-binding function and the carboxyl-terminal dimerization function, plasmids encoding both functional amino- and carboxyl-terminal domains would confer immunity while plasmids encoding proteins with deletions that disrupted the function of either domain would not. Four of the 36 plasmids were found to confer immunity. Of these, 1 encoded a protein with a deletion of 37 aa (residues 93–129) while the others encoded proteins with deletions of ≤15 aa. The 37-aa deletion removed all but two of the amino acids of the linker (see Fig. 1). The remainder of the gene was sequenced and found to be wild type. By comparing the behavior of this "linkerless" repressor with that of the wild-type repressor we were able to investigate the role of the linker region in cooperative binding.

Cooperative Binding by the Linkerless Repressor *in Vivo* Depends on the Spacing of Its Binding Sites. We compared the abilities of wild-type and linkerless repressors to bind cooperatively to sites O_R1 and O_R2 in derivatives of O_R that were integrated into the bacterial chromosome. As described above, at O_R, repressor bound to O_R1 (the strong site) helps a second repressor bind to the adjacent site O_R2 (the weak site), and the repressor at O_R2 stimulates transcription from P_{RM}. Thus stimulation of P_{RM} can be used as a measure of occupancy of O_R2 by the repressor. At a given repressor concentration, greater stimulation on a template bearing O_R1 and O_R2 than

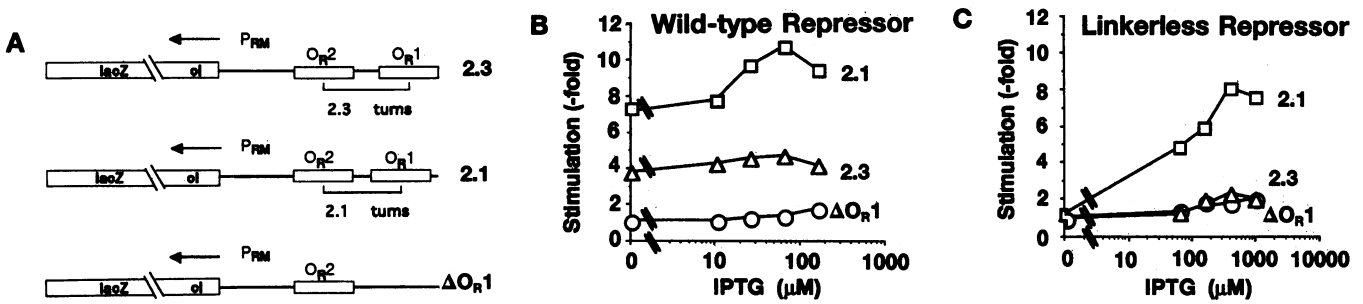

FIG. 2. *In vivo* experiment comparing cooperative binding of linkerless and wild-type repressors to sites 2.1 and 2.3 turns apart. (*A*) Schematic representation of the three templates used in the experiments of *B* and *C*. (*B*) Stimulation of P_{RM} by wild-type repressor as a function of increasing levels of IPTG in the absence (ΔO_R1) or presence of O_R1 2.3 or 2.1 turns from O_R2. (*C*) Stimulation of P_{RM} by linkerless repressor as a function of increasing levels of IPTG in the absence or presence of O_R1 2.3 or 2.1 turns from O_R2. Stimulation of P_{RM} by wild-type repressor is increased on the 2.3- and 2.1-turn templates relative to the ΔO_R1 template. Stimulation of P_{RM} by linkerless repressor is increased on the 2.1- but not the 2.3-turn template relative to the ΔO_R1 template. All templates were integrated into the bacterial chromosome. In each case P_{RM} directed transcription of a *cI–lacZ* fusion and transcription was assayed by measuring β-galactosidase levels. A repressor gene under control of an IPTG-inducible promoter was introduced into the cells on a plasmid. Note that even at 0 μM IPTG there is detectable expression of repressor. Stimulation is expressed as the ratio of the level of transcription in the presence of a repressor-encoding plasmid to the level of transcription in the absence of a repressor-encoding plasmid. One-fold stimulation indicates no increase in transcription over the basal level. The basal levels of transcription from the 2.3, 2.1, and ΔO_R1 templates were 5.3, 1.6, and 5.0 units of β-galactosidase, respectively. When provided at sufficient levels to fully occupy O_R2, wild-type and linkerless repressors stimulated transcription of P_{RM} on the ΔO_R1 template 3.4-fold and 3.0-fold, respectively.

on a template bearing O_R2 but lacking O_R1 is then taken to indicate cooperative binding of repressors to O_R1 and O_R2.

We first examined cooperative binding to sites O_R1 and O_R2 at their normal spacing of 2.3 turns. In the case of the linkerless repressor, stimulation of transcription from P_{RM} on the 2.3-turn template was not increased relative to that observed with the template lacking O_R1 (Fig. 2*C*), indicating that the linkerless repressor binds noncooperatively to O_R1 and O_R2 at this particular spacing. For comparison, stimulation by wild-type repressor was increased 2- to 4-fold on the 2.3-turn template over the entire range of repressor concentrations tested (Fig. 2*B*). Thus the linker appears to be essential for cooperative binding of repressor to O_R1 and O_R2 at their normal spacing.

To investigate whether there were spacings at which the linkerless repressor could bind cooperatively, we next examined cooperative binding of the linkerless repressor to sites separated by 2.1 turns and found that stimulation of transcription by the linkerless repressor was increased significantly by the presence of O_R1 (Fig. 2*C*). Thus it appears that the linker is required for cooperative binding to sites that are 2.3 turns apart but is dispensable for cooperative binding to sites that are 2.1 turns apart.

At the 2.1-turn spacing, cooperative binding of the linkerless repressor was less than that of wild type. Also, at all levels of wild-type repressor, there was greater stimulation of transcription from the 2.1-turn template from the 2.3-turn template. *In vitro* experiments have shown that this difference does not reflect greater cooperativity of binding of wild-type repressor in the 2.1-turn case relative to the 2.3-turn case (see below). We do not understand this difference in activation of transcription from the two templates.

Linkerless Repressor Binds Cooperatively to Sites Separated by 2.1 but Not 2.3 Turns *in Vitro*. To confirm that the differences we observed *in vivo* reflected differences in cooperative binding, we conducted *in vitro* DNase I footprinting experiments measuring binding of purified wild-type and linkerless repressors to templates with sites that were 2.1 and 2.3 turns apart. The affinity of linkerless repressor for O_R2 was not increased when O_R1 was 2.3 turns away but was increased 5-fold when O_R1 was 2.1 turns away (Fig. 3), corresponding to an interaction energy of approximately -1 kcal/mol in the latter case (see *Materials and Methods* for calculations). In a parallel experiment, the affinity of wild-type repressor for O_R2 was increased 30-fold by the presence of O_R1 at either spacing, corresponding to an interaction energy of -2.5 kcal/mol (data not shown).

In further DNase I footprinting experiments, linkerless repressor was found to bind with equal cooperativity to sites separated by 1.9, 2.0, and 2.1 turns of the helix but noncooperatively to sites separated by 1.7, 1.8, and 2.2 turns of the helix (data not shown). Wild-type repressor was found to bind cooperatively to 1.7-, 1.9-, 2.2-, and 2.3-turn templates and noncooperatively to sites separated by 2.5 turns of the helix (data not shown) (binding of wild-type to 1.8- and 2.0-turn

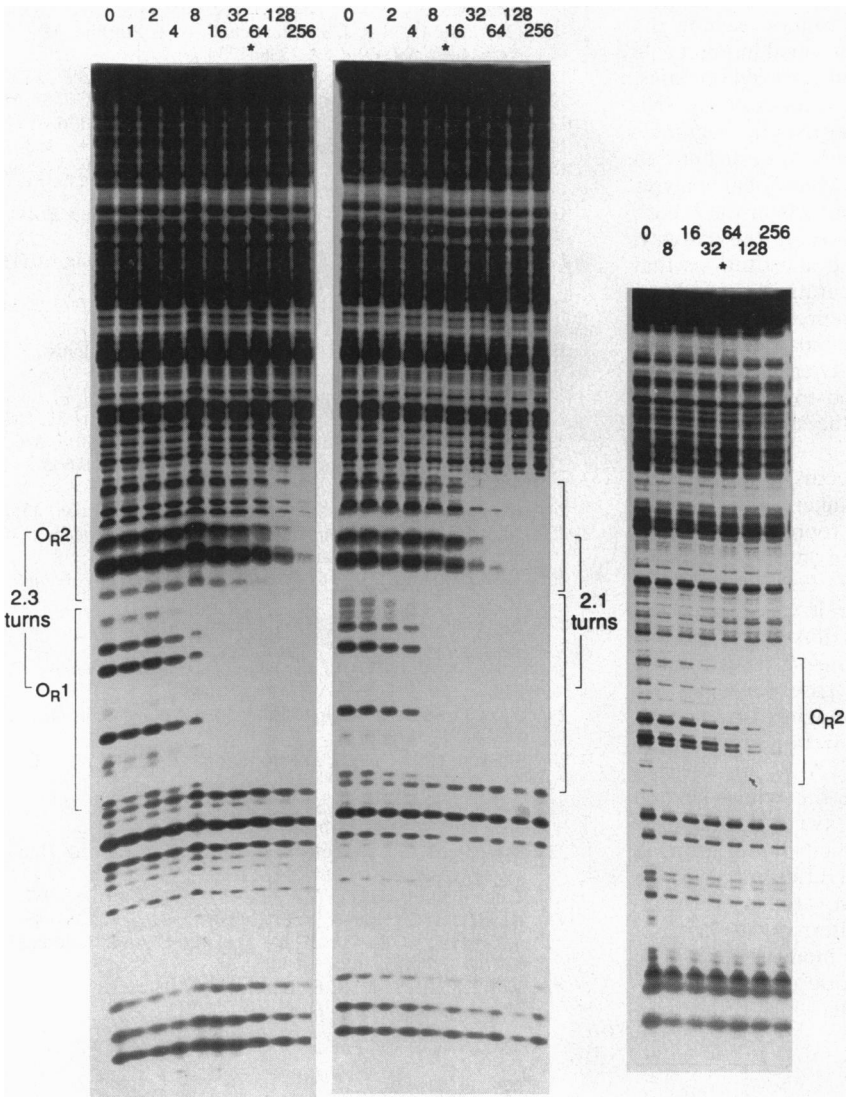

FIG. 3. DNase I protection experiment showing that the linkerless repressor binds cooperatively to sites separated by 2.1 but not 2.3 turns of the helix. Numbers above the lanes indicate units of repressor (1 unit corresponds to 8 nM linkerless repressor monomer). Stars indicate the concentration of linkerless repressor at which O_R2 is half-maximally protected from DNase I cleavage on each of the three templates. Under the conditions of the experiment, the concentration of repressor dimer required to half-maximally protect a site from DNase I cleavage is equal to the affinity (equilibrium dissociation constant, K_d) of repressor for that site.

templates was not tested). Thus, cooperative binding by linkerless repressor exhibits both a diminished spatial range and diminished magnitude relative to that of wild type.

DISCUSSION

In this work we used cooperative binding of bacteriophage λ repressor as a model system to investigate what role a hinge or linker region of a protein might play in the interaction between DNA-bound proteins. We deleted the linker region from λ repressor and compared the behavior of the resulting linkerless repressor with that of wild type repressor when binding to operator sites *in vivo* and *in vitro*.

Our main findings were as follows. First, at the normal spacing of 2.3 turns between sites O_R1 and O_R2, the linkerless repressor failed to exhibit the cooperative interaction that we found with the wild-type repressor. The linkerless repressor also failed to bind cooperatively to sites at the artificial spacings of 1.7, 1.8, and 2.2 turns. Second, when O_R1 and O_R2 were brought into better helical alignment, such that they were separated by 1.9, 2.0, or 2.1 turns, the linkerless repressor did bind cooperatively, although to a lesser degree than wild type. We conclude that a major role of the linker in cooperative binding is to extend the range of distances over which the carboxyl domains of two repressor dimers can reach each other while their amino domains are bound to DNA. This function is essential for the repressor's biological role as a transcriptional regulator.

Because overall the linkerless repressor binds less cooperatively than the wild-type repressor, we cannot exclude the possibility that in addition to the aforementioned indirect role as a flexible tether between the amino and carboxyl domains, the linker may also play a direct role in cooperativity. Oligomerization experiments with purified proteolytic fragments of repressor suggest that the linker region may contribute to dimer–dimer interaction in solution (17). Mutational analyses suggest that if there are cooperativity contacts in the linker, these contacts are less important than those in the carboxyl domain. Specifically, 15 single amino acid substitutions that disrupt or decrease the amount of cooperativity have been isolated; all lie in the carboxyl domain of repressor rather than the linker (24, 30–32). It is possible that the linkerless repressor is somewhat deficient in cooperativity overall because its carboxyl domains are too closely tethered to the amino domains to interact efficiently even when the spacing between operators is optimal.

As a corollary to our findings, it seems plausible that increasing the length of λ repressor's linker would further increase the spatial range over which two repressor molecules bind cooperatively. Such an effect has been observed with two other DNA-binding proteins, the *Eco*R124 HsdS protein and the AraC protein (9, 33). In each case it was found that increasing the length of an interdomain linker enabled the resulting protein to bind to DNA half-sites that were more widely separated than normal. In the AraC case, deprepression of the P_{BAD} promoter by arabinose is thought to rely on an arabinose-induced shortening of the connection between the DNA-binding and dimerization domains of AraC protein (34).

Our comparison of the behaviors of the wild-type and linkerless repressors demonstrates that two variants of a protein with the same DNA-binding specificity but differing in the spacing between domains can respond differentially to the physical arrangement of DNA sites. Such variants of a transcription factor could either arise from alternative splicing of a single gene or be encoded by different members of a gene family. For example, the *Drosophila* homeotic gene Ultrabithorax (*Ubx*) encodes six Ubx protein isoforms which result from alternate splicing of five exons (35, 36). All isoforms share common amino and carboxyl domains: the differential regions consist of different combinations of three small exons, one of which is 9 aa long and two of which are 17 aa long. It is possible that the function of the three internal exons is to set the spacing between the carboxyl domain, which mediates DNA binding, and functional elements in the amino domain and to thereby determine functional specificity, perhaps by altering the pattern of cooperative interactions between Ubx molecules.

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