Specificity determinants for the interaction of λ repressor and P22 repressor dimers

Frederick W. Whipple, Natalie H. Kuldell, Lynn A. Cheatham, and Ann Hochschild

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts, 02115 USA

The related phage λ and phage P22 repressors each bind cooperatively to adjacent and separated operator sites, an interaction that involves a pair of repressor dimers. The specificities of these interactions differ: Each dimer interacts with its own type but not with dimers of the heterologous repressor. The two repressors exhibit significant amino acid sequence homology in their carboxy-terminal domains, which are responsible for both dimer formation and the dimer-dimer interaction. Here, we identify a collection of amino acid substitutions that disrupt the protein-protein interaction of DNA-bound λ repressor dimers and show that several of these substitutions have the same effect when introduced at the corresponding positions of P22 repressor. We use this information to construct a variant of the λ repressor bearing only six non-wild-type amino acids that has a switched specificity; that is, it binds cooperatively with P22 repressor, but not with wild-type λ repressor. These results identify a series of residues that determine the specificities of the two interactions.

[**Key Words:** λ repressor; P22 repressor; cooperativity; protein-protein interactions; DNA looping; transcriptional regulators]

Received February 14, 1994; revised version accepted April 12, 1994.

Transcriptional regulation in both prokaryotes and eukaryotes involves the interaction of both adjacently and nonadjacently bound regulatory proteins. In the case of nonadjacently bound molecules, the interaction involves the formation of a DNA loop (Adhya 1989; Hochschild 1990; Schleif 1992). Both homologous and heterologous pairs of regulators participate in these interactions. Although some of these protein-protein interactions are relatively strong, occurring off as well as on the DNA, others are detectable only when the interacting partners are appropriately positioned on DNA, or when the protein concentrations are artificially elevated. Little is known about the structural nature of these weaker interactions.

The phage λ repressor, which is both a repressor and an activator of transcription, binds cooperatively to adjacent operator sites on the phage chromosome (Ptashne 1992). Cooperative DNA binding involves an interaction between pairs of dimers, each of which binds to a single operator site, as shown in Figure 1a. A repressor dimer bound at the high affinity operator O_R1 interacts with a second dimer, thereby stabilizing its association with the lower affinity operator O_R2 (Johnson et al. 1979). That dimer in turn interacts with RNA polymerase to activate transcription from promoter P_{RM} (Guarente et al. 1982; Hochschild et al. 1983; Bushman et al. 1989; Kuldell and Hochschild 1994; Li et al. 1994). As shown in Figure 1, repressor is a two-domain protein; the

amino-terminal domain contacts the DNA and interacts with RNA polymerase, whereas the carboxy-terminal domain mediates both dimer formation and the dimer-dimer interaction (Pabo et al. 1979).

λ repressor also binds cooperatively to artificially separated operators provided they are phased so as to lie on the same side of the DNA helix (Hochschild and Ptashne 1986). This interaction has an unexpected effect on P_{RM} transcription when O_R1 is positioned several integral turns of the DNA helix upstream from O_R2 (Hochschild and Ptashne 1988). As illustrated in Figure 1b, when a dimer bound at O_R2 interacts with a second dimer bound some distance away, it is unable to stimulate transcription efficiently from P_{RM} . However, when the spacing between operators precludes this interaction, repressor dimers bind independently to the two operators and, provided the concentration of repressor is high enough to ensure that O_R2 is occupied, efficient stimulation of P_{RM} transcription is observed.

The carboxy-terminal domain of λ repressor mediates three distinct functions: dimer formation, the dimer-dimer interaction, and also self-cleavage (Sauer et al. 1990). Like the bacterial LexA repressor, which regulates the expression of genes involved in the SOS response to DNA damage (Little and Mount 1982), the lambdoid phage repressors undergo proteolytic cleavage at a specific site when the bacterial RecA protein is activated by exposure of the cell to DNA-damaging agents (Roberts

Figure 1. The effect of adjacently and nonadjacently bound λ repressor dimers on P_{RM} transcription. (A) The arrangement of molecules at the right operator (O_R) in a λ lysogen. Repressor dimers are bound cooperatively to O_{R1} and O_{R2} , the carboxy-terminal domain mediating the interaction of the adjacently bound dimers. Transcription from P_R is repressed, and the dimer bound at O_{R2} interacts with RNA polymerase to stimulate transcription from P_{RM} . (B) The inhibition of repressor-stimulated transcription that occurs when the dimer bound at O_{R2} interacts with a nonadjacently bound dimer. When the operators are separated by a nonintegral number of turns of the DNA helix (top), the dimer bound at O_{R2} is able to stimulate transcription from P_{RM} , but when the operators are separated by an integral number of turns (bottom) the dimers interact and stimulation is abolished.

and Roberts 1975; Sauer et al. 1982a). This cleavage reaction separates the amino-terminal domain from the carboxy-terminal domain and, in the case of the phage repressors, results in prophage induction (for review, see Roberts and Devoret 1983). The reaction, though dependent on activated RecA protein in vivo, occurs spontaneously in vitro at high pH in the absence of RecA protein (Little 1984); a conserved serine located in the carboxy-terminal domain makes the intramolecular attack at a specific site within the linker that connects the two domains (Slilaty and Little 1987).

The λ repressor shares its two-domain structure with the repressors of the related lambdoid phages P22 and

434. Like λ repressor, the P22 and 434 repressors bind cooperatively to adjacent operators on the phage chromosome and also to artificially separated operators (Johnson et al. 1981; Poteete and Ptashne 1982; Valenzuela and Ptashne 1989; D. Valenzuela, pers. comm). In the case of the λ and P22 repressors, mutants have been identified previously that are specifically defective for cooperative DNA binding (Hochschild and Ptashne 1988; Valenzuela and Ptashne 1989; Benson et al. 1994). As expected, they bear amino acid substitutions within the carboxy-terminal domain.

Here, we report isolation of 14 λ repressor mutants that are unable to interact when bound at separated operators, and we show that they are also unable (or less able) to interact when bound at adjacent operators. The amino acid substitutions carried by these mutants enable us to identify several positions at which the wild-type residue plays an essential role in mediating cooperative DNA binding. More than half of these amino acid substitutions affect residues conserved in the carboxy-terminal domains of λ and P22 repressor; we introduce several of these at the corresponding positions of P22 repressor and show that they also eliminate the interaction of nonadjacently bound P22 repressor dimers. Finally, we use the information obtained from the analysis of the λ and P22 repressor mutants to construct a λ repressor variant bearing just six non-wild-type residues that interacts specifically with P22 repressor dimers.

Results

Isolation of λ repressor mutants specifically defective for cooperative DNA binding

We used a previously described genetic screen to identify λ repressor mutants that are unable to interact when bound at nonadjacent operators in vivo (Hochschild and Ptashne 1988). This screen is based on the observation that repressor, if provided at an appropriately high concentration, stimulates transcription from promoter P_{RM} poorly when O_{R1} and O_{R2} are separated by an integral number of turns of the DNA helix, but stimulates P_{RM} transcription efficiently when the sites are separated by a nonintegral number of turns (see Fig. 1b). Repressor mutants that are unable to interact when bound to separated operators activate transcription efficiently whether O_{R1} and O_{R2} are phased so as to lie on the same side of the helix or not. One such mutant was isolated previously and shown to bind noncooperatively to both separated and adjacent operators in vitro (Hochschild and Ptashne 1988). To isolate additional mutants, we mutagenized a plasmid directing the synthesis of enough repressor to occupy O_{R2} independently. The mutagenized plasmid DNA was introduced into a strain harboring a P_{RM} -*lacZ* fusion on its chromosome with O_{R1} centered 5.9 turns of the DNA helix upstream of O_{R2} and multiple base pair substitutions in O_{R3} (see Materials and methods). Transformants containing wild-type λ repressor form pale blue colonies on indicator plates con-

taining XG. Transformants containing cooperativity mutants were identified by their darker blue color.

Using two mutagenesis methods, which targeted either the entire repressor gene or only the carboxy-terminal 77 codons (see Materials and methods), we obtained 14 mutants bearing single amino acid substitutions, 3 of which originally occurred in combination with a second substitution (see Table 1). Table 1 also includes two mutants that were not uncovered in the genetic screen (see footnote a). We then performed β -galactosidase assays with cells bearing either the 5.9-turn or an otherwise similar 5.5-turn template to quantitate the levels of *lacZ* expression (i.e., P_{RM} transcription) in the presence of each of the mutants [Table 2 (A)]. In contrast to wild-type repressor, most stimulated P_{RM} transcription equally well irrespective of the spacing between O_{R1} and O_{R2} . A few exceptions, such as N148D and G199S, retained some ability to interact when bound to the integrally spaced operators as reflected in decreased stimulation from the 5.9-turn template relative to the 5.5-turn template.

Dimer formation by the cooperativity mutants

Our screen was designed to permit the isolation of repressor mutants specifically defective for cooperative

Table 1. λ Repressor cooperativity mutants

Substitutions		Mutagenesis method	Independent isolates
amino acid	base pair		
N148D	AAT-GAT	— ^a	N.A.
S149F	TCC-TTC	<i>mutD</i>	2
S159N	AGC-AAC	<i>mutD</i>	1
E188K	GAG-AAG	<i>mutD</i>	1
K192N ^b	AAG-AAT	PCR	1
R196G ^b	AGG-GGG	PCR	1
R196M	AGG-ATG	— ^a	N.A.
D197G	GAT-GGT	PCR	1
S198N ^b	AGC-AAC	PCR	1
G199D	GGT-GAT	<i>mutD</i>	3
G199S	GGT-AGT	<i>mutD</i>	1
F202S	TTT-TCT	PCR	1
Y210N	TAC-AAC	<i>mutD</i>	1
M212T	ATG-ACG	PCR	1
S228N	AGT-AAT	<i>mutD</i>	1
T234K	ACG-AAG	PCR	3

^aMutant R196M was received from J. Hu as a double mutant, together with F189L on a derivative of repressor-encoding plasmid pFG600. The R196M allele was then moved to our vector pLR2. Mutant N148D was constructed by site-directed mutagenesis.

^bSubstitutions K192N and S198N were originally isolated together, and substitution R196G was originally isolated together with a second change, V168A. K192N, S198N, and R196G were subsequently introduced individually into plasmid pLR1 using site-directed mutagenesis; each was found to result in a cooperativity defect.

Table 2. Stimulation of P_{RM} by λ repressor mutants bound to templates bearing nonadjacent operators

Repressor plasmid	Fold stimulation ^a	
	in-phase operators (5.9 turns)	out-of-phase operators (5.5 turns)
A. pLR1-wt	1.5	7.8
pLR1-N148-D	5.8	10.9 ^b
pLR1-S149-F	10.1	11.6
pLR1-S159-N	8.4	11.4
pLR1-E188-K	11.3	13.0
pLR1-K192-N	10.4	11.3
pLR1-R196-G	10.0	11.9
pLR1-S198-N	10.9	12.2
pLR1-G199-S	3.9	10.3
pLR1-G199-D	10.9	11.7
pLR1-Y210-N	11.9	11.8
pLR1-S228-N	10.7	11.4
pLR2-wt	2.3	7.7
pLR2-R196-M	10.4	11.5
pLR2-D197-G	11.4	12.5
pLR2-F202-S	11.0	12.5
pLR2-M212-T	10.1	11.5
pLR2-T234-K	9.1	11.7
pLR1-P158-T	1.2	7.0
B. pA3B2-wt	1.3	8.5
pA3B2- λ v1-5;148	1.9	9.0
pA3B2- λ v1-5	10.0	11.5

β -Galactosidase activity was measured in cells containing a P_{RM} -*lacZ* fusion with O_{R1} positioned 5.9 turns of the DNA helix upstream of O_{R2} (in-phase operators) or with O_{R1} positioned 5.5 turns upstream of O_{R2} (out-of-phase operators). The two strains, AH5.9 and AH5.5, were transformed with plasmids pLR1 or pLR2 (A) or pA3B2 (B) encoding wild-type or mutant λ repressor proteins. pLR1 and pLR2 are identical except that the latter bears a deletion just downstream of the *cl* gene (see Fig. 2). pLR1 was used for *mutD*-dependent mutagenesis and pLR2 was subsequently constructed to facilitate PCR mutagenesis (but see Table 1, footnote b). pA3B2 is a pACYC184-based vector that carries the same operon fusion as pLR2 (see Materials and methods). Dimerization mutant P158T, which is not defective for cooperative DNA binding, was included as a control.

^aFold stimulation is the ratio of β -galactosidase activity in cells containing the stated plasmid to that in cells containing a plasmid that does not encode repressor (pLR1 Δ cl or pA3H Δ cl). The absolute activities in the absence of repressor ranged from 2.1 to 2.5 Miller units. For each vector type (pLR1, pLR2, or pA3B2), data are shown for a single representative experiment. In repeated trials the fold stimulation by wild-type repressor ranged from 1.3 to 2.3 and 7.7 to 11.0 on the 5.9- and 5.5-turn templates, respectively, and the rank order for mutants with residual function was always consistent.

^bIn general, the mutants activated transcription from the 5.5-turn template somewhat more effectively than did wild-type repressor (see also Fig. 3). We suggest the following explanation: When wild type repressor binds to O_{R2} on this template, cooperativity might lead to the occasional occupancy of the mutant O_{R3} site (which contains mutations in only one of its half-sites). Because binding to O_{R3} represses P_{RM} transcription, this pattern of site occupancy would reduce somewhat the magnitude of P_{RM} stimulation. In the case of the cooperativity mutants, however, occupancy of O_{R2} would not facilitate binding to O_{R3} , and full stimulation would be achieved.

DNA binding. In particular, it ensures that mutant candidates must be able to dimerize sufficiently well to bind O_{R2} and activate P_{RM} transcription at the intracellular concentration of repressor used. To detect any possible defects in dimer formation, it was necessary to lower the concentration of repressor. We therefore introduced the 5.5-turn P_{RM} - $lacZ$ fusion into a strain that expresses high levels of lac repressor. Because our plasmids express λ repressor under the control of a lac operator (see Fig. 2), we were able to vary the concentration of λ repressor in this strain by growing the cells in the presence of various concentrations of IPTG. Mutants with dimerization defects should bind O_{R2} (and therefore stimulate P_{RM} transcription) less efficiently than wild-type repressor at low concentrations of IPTG.

The results of these experiments are summarized in Table 3, and the data from some representative assays are shown in Figure 3. As a control, we assayed a mutant (P158T) that is not defective for cooperative DNA binding but dimerizes ~ 50 -fold more weakly than wild-type repressor, as measured *in vitro* (Gimble and Sauer 1989). Most of the mutants we isolated had no detectable defect in dimer formation. There were three exceptions; S159N manifested a moderate defect, whereas S228N and T234K manifested defects comparable to that seen with P158T. We note that substitutions S228N and T234K lie in the vicinity of a previously identified substitution, E233K, that also reduces dimerization (Gimble and Sauer 1985), but does not weaken the interaction of nonadjacently bound dimers (data not shown).

Western blot analysis confirmed that all of the mutant proteins were present at the same concentration as wild-type repressor in the strain utilized in the experiments of Figure 3 (data not shown). This strain carried a *recA*⁻ mutation; Western blot analysis using an otherwise isogenic *recA*⁺ strain revealed that one of the mutants (S159N) was present at $\sim 50\%$ the level of the wild-type reference. This observation suggests that mutant S159N underwent some RecA-mediated proteolytic cleavage even in the absence of conditions that activate RecA protein (see Discussion).

Figure 2. Structure of repressor-encoding plasmids derived from pBR322. Repressor genes, truncated *lac* promoter-operator regions, and relevant restriction sites are shown (not to scale).

Table 3. Summary of activities of λ repressor mutants

Allele	Dimer-dimer interaction		Dimerization
	nonadjacent operators	adjacent operators	
Wild type	+++	+++	+++
S149F	-	+	+++
S159N	+	+	++
E188K	-	+	+++
K192N	-	-	+++
R196G	-	+	+++
R196M	-	+	+++
D197G	-	-	+++
S198N	-	-	+++
G199D	-	-	+++
F202S	-	-	+++
Y210N	-	-	+++
M212T	-	+	+++
N148D	++	++	+++
G199S	++	++	+++
S228N	-	-	+
T234K	(+)	-	+
P158T	+++	N.D.	+

Data from Table 2 and from experiments identical to those of Figs. 3 and 4 are summarized. Mutants were assigned scores (+++ for fully functional to - for completely defective) based on their abilities to interact when bound at nonadjacent and adjacent operators, and to dimerize. These abilities were assessed by performing β -galactosidase assays in liquid cultures (see Table 2 footnotes, and legends to Figs. 3 and 4). Mutant T234K exhibited a barely detectable degree of interaction when bound at nonadjacent operators and was therefore given a conditional plus score [(+)]. Among the mutants scored as - in column 2, three (K192N, D197G, and Y210N) were slightly less active on the two-site template than on the single-site template at every assay point, indicating that the substitutions they bear abolish cooperativity totally. The others were slightly more active on the two-site template than on the single-site template at one or two data points, but the magnitude of these effects was too small to be certain that they are significant.

Cooperative binding of the mutants to adjacent operators

The mutants were also assayed for their abilities to bind cooperatively to adjacent operators *in vivo*. We again made use of a pair of P_{RM} - $lacZ$ fusions: One bears only operator O_{R2} and the other bears both O_{R1} and O_{R2} at their natural positions adjacent to P_{RM} . (Both templates bear the same mutant O_{R3} site as the templates described above.) As for the dimerization assays described above, we measured P_{RM} activity as a function of intracellular repressor concentration. The results of these assays are summarized in Table 3, and the data from representative experiments are presented in Figure 4. The presence of O_{R1} adjacent to O_{R2} enables wild-type repressor to stimulate P_{RM} transcription efficiently even at low concentrations. In contrast, for eight of the mutants, the magnitude of P_{RM} stimulation was unaffected by the

Figure 3. Dimerization of wild-type and mutant λ repressors. The relative abilities of wild-type and mutant λ repressors to dimerize were assessed by measuring activation of promoter P_{RM} at several protein concentrations in a strain (FW15) in which cooperative DNA binding is precluded because of the out-of-phase spacing of operator sites O_{R1} and O_{R2} . Plasmids present were pLR1 (wild type; ■), pLR1 derivatives K192N (□), S159N (△), P158T (○), or pLR1ΔCI (+). Protein concentrations were controlled by varying the concentration of IPTG in the medium. The values for wild type and P158T were obtained by averaging the results of two experiments; the actual values differed from the averages by <0.6 units at each data point. Mutants K192N and S159N, as well as all other mutants defective for cooperative DNA binding, activated P_{RM} more efficiently than did wild-type repressor, an effect that became increasingly noticeable as the concentration of repressor was raised. For an explanation, see Table 2 footnote.

presence of the second site; we infer that these mutants are completely unable to bind cooperatively to separated and adjacent operators. As expected, the two mutants (N148D and G199S) that retained a significant ability to interact when bound at nonadjacent operators were only partially defective in binding cooperatively to adjacent operators. Finally, several mutants that manifested little or no ability to interact when bound at separated operators retained some residual ability (though less than did N148D and G199S) to bind cooperatively to adjacent operators; these included S149F, S159N, E188K, R196G, R196M, and M212T. Previous *in vitro* measurements indicate that wild-type λ repressor exhibits stronger cooperativity when bound at adjacent than at nonadjacent operators (Hochschild and Ptashne 1986); it is not surprising, therefore, that the adjacent-site assay would be a more sensitive indicator of residual function.

Analysis of four conserved residues and one nonconserved residue implicated in the cooperative binding of both λ and P22 repressors

As shown in Figure 5, the carboxy-terminal domains of λ repressor and P22 repressor are homologous; at 51 of 105

positions they bear identical residues. Of the 16 amino acid substitutions listed in Table 1, 9 affect conserved residues. One of these (E188K) had previously been isolated at the corresponding position of P22 repressor in a screen for P22 repressor mutants unable to bind cooperatively to separated operators (Valenzuela and Ptashne 1989). To ask whether other conserved residues identified in our screen were also implicated in the cooperative binding of P22 repressor, we used site-directed mutagenesis to introduce several of the amino acid substitutions found in the λ repressor cooperativity mutants into the corresponding positions of P22 repressor. We assayed the resulting P22 repressor mutants for their abilities to bind cooperatively to separated operators using a previously described DNA template that bears the *lacZ* gene and an artificial *lacUV5* promoter with a low-affinity P22 operator between its -10 and -35 hexamers and a high-affinity P22 operator 5.0 turns of the DNA helix upstream (Valenzuela and Ptashne 1989; see Fig. 6). The binding of P22 repressor to the low-affinity site represses *lacZ* transcription, and cooperative DNA binding to the integrally spaced operators results in enhanced occupancy of this site and, hence, enhanced repression. Figure 6 shows that unlike wild-type repressor, each of the

Figure 4. Cooperative binding to adjacent operator sites by wild-type and mutant λ repressors. Activation of promoter P_{RM} was measured as a function of repressor concentration in strains bearing O_{R2} and O_{R1} in their native adjacent positions (closed symbols, strain X131-parGA) or O_{R2} alone (open symbols, strain X131-BB). Plasmids present were pLR2 (circles), pLR2-D197G (squares, top), or pLR2-R196M (squares, bottom). Protein concentrations were controlled by varying the concentration of IPTG in the medium.

Figure 5. Carboxy-terminal domain sequences of λ repressor and P22 repressor. The amino acid sequences of the carboxy-terminal domains of λ repressor and P22 repressor are shown (dots indicate amino acid identities). Sequence alignment is according to Sauer et al. (1982b). Cooperativity mutants identified or used in this study bear amino acid substitutions at highlighted positions. Residues involved in the switched-specificity mutant λv1-5;148 are boxed. (*) Residues implicated in auto-cleavage; (≠) positions of amino acid substitutions that affect dimerization.

P22 repressor mutants that we constructed failed to mediate increased repression on the two-site template relative to a template bearing just the low-affinity operator. These P22 repressor mutants bear changes of residues D179, F184, and Y192 (corresponding to λ residues 197, 202, and 210, respectively).

Among the P22 repressor cooperativity mutants previously isolated, one bore a change of a nonconserved res-

idue (D131) to the residue found at the corresponding position of λ repressor (Valenzuela and Ptashne 1989; see Fig. 5). To ask whether this residue was also implicated in the interaction of λ repressor dimers, we used site-directed mutagenesis to introduce the reciprocal change into λ repressor (N148D). As shown in Tables 2 and 3, this change partially disrupted the interaction of both nonadjacently and adjacently bound dimers.

P22 repressor allele	Fold repression	
	in-phase operators	single operator
wild type	110.4	8.7
D179G	5.1	5.1
F184S	5.1	4.9
F184G	4.7	4.3
Y192N	4.6	4.6
Y192I	5.3	5.1

Figure 6. Repression by P22 repressor mutants bound to templates bearing one or two operators. The templates utilized carry a synthetic lacUV5 promoter (with no lac operator present) bearing a P22 operator (Or2) between its -35 and -10 hexamers and either no upstream operator (strain DV59) or a strong P22 operator positioned 5.0 turns of the DNA helix upstream (strain DV72). Fold repression is the ratio of β-galactosidase activity in cells containing a plasmid that does not encode repressor (pLR1Δcl) to that in the same cells containing pPR2 expressing wild-type or mutant versions of P22 repressor. Cells were grown at 30°C in the presence of 100 μM IPTG. Absolute activities in the no-repressor case were 4230 and 4400 Miller units in strains DV72 and DV59, respectively. Wild-type P22 repressor exhibited stronger repression in the control (single operator) strain than did any of the cooperativity mutants. We suspect that this difference may be attributable to the presence of some pseudo-operator in the vicinity that allows wild-type P22 repressor to bind cooperatively to the two sites.

A λ repressor variant that interacts with wild-type P22 repressor

The fact that corresponding amino acid substitutions affect the interaction of both λ repressor and P22 repressor dimers encouraged us to attempt to construct a λ repressor variant that would interact with wild-type P22 repressor. To permit detection of such an interaction, we modified the template described in the preceding section by placing a strong λ operator either 5.0 or 5.4 turns of the DNA helix upstream of the low-affinity P22 operator (see Fig. 7). We anticipated that any λ repressor variant able to interact with P22 repressor would contribute to repression on the in-phase (5.0-turn), but not the out-of-phase (5.4-turn), template.

We performed β-galactosidase assays to measure transcriptional repression in the presence of P22 repressor only, or both P22 repressor and either wild-type λ repressor or a synthetic variant. The results of these experiments are shown in Figure 7A. In the presence of P22 repressor only, lacZ expression was repressed ~4× relative to the level measured in the absence of any repressor. The magnitude of this repression was approximately the same on the two templates. In the presence of both P22 repressor and wild-type λ repressor there was a slight increase in the magnitude of the repression to ~6×; this increase was only observed with the in-phase template. However, when wild-type λ repressor was replaced with a synthetic variant (λv1-5;148) bearing P22 residues at just six positions, the magnitude of the repression increased substantially to ~19×; again, this increase was observed only with the in-phase template (Fig. 7A, line 3).

This λ repressor variant was constructed by replacing codons 196–204 and codon 148 of λ repressor with the corresponding codons from the P22 repressor sequence

Repressor proteins		Fold repression		
		in-phase operators	out-of-phase operators	
A	---	P22 wt	4.1	4.6
	λ wt	P22 wt	5.8	4.5
	λ v1-5; 148	P22 wt	19.3	4.7
	λ v1-5	P22 wt	6.4	5.0
	λ v1-4; 148	P22 wt	5.1	4.3
	λ v1-3; 148	P22 wt	4.0	3.6
	λ v1-2; 148	P22 wt	4.3	3.9
	λ v1; 148	P22 wt	5.2	3.4
B	λ wt	---	1.0	0.9
	---	hybrid	2.5	2.5
	λ wt	hybrid	16.4	2.7
	λ v1-5; 148	hybrid	3.1	2.4

Figure 7. Repression by heterologous repressors bound to templates bearing a low-affinity P22 operator and a high affinity λ operator. The templates utilized bear a synthetic *lacUV5* promoter with the embedded P22 O_{R2} site (see Fig. 6 legend) and a strong λ operator (O_{R1}) positioned 5.0 or 5.4 turns of the DNA helix upstream (strains FW40 and FW42, respectively). β -Galactosidase activities were measured in cells containing two compatible plasmids, one (pA3B2) that encoded λ repressor or a variant thereof and the other that encoded P22 repressor (pPR2, A) or the hybrid repressor (pFW7, B). Each of these plasmids directs expression of moderate levels of repressor (see Materials and methods). Fold repression is the ratio of β -galactosidase activity in cells containing control plasmids pLR1 Δ cl and pA3H Δ cl to that in the same cells containing the indicated repressors. Cells were grown in the presence of 300 μ M IPTG. Absolute activities in the absence of repressors (4010 ± 470 and 3670 ± 590 Miller units in strains FW40 and FW42, respectively) varied somewhat in individual experiments, but repression ratios were reproducible (4.1 ± 0.5 and 4.6 ± 0.6 with P22 repressor alone).

(see Fig. 5). The replacement of region 196–204, which contains a cluster of residues implicated in the dimer–dimer interaction, results in the introduction of five P22 residues into the λ sequence (at positions 196, 198, 200, 201, and 204). We refer to these positions using the numerals 1–5 and, accordingly, call this λ repressor variant λ v1-5;148. To ask whether the change at codon 148 was critical, we reintroduced the wild-type residue at this position to create variant λ v1-5. Unlike the original variant, this variant did not mediate a significant increase in repression over that seen with wild-type λ repressor (Fig. 7A, line 4). We also tested four additional variants bearing P22 residues at positions 148 and: 196–201, 196–200, 196–198, and 196; none mediated any more repression than did wild-type repressor.

We performed two additional tests to confirm that λ repressor variant λ v1-5;148 is a switched specificity mutant. First, we measured its ability to interact with its own type. Using the 5.9- and 5.5-turn λP_{RM} –*lacZ* reporter strains described above, we found that pairs of λ v1-5;148 dimers did interact, whereas pairs of λ v1-5 dimers, (which were unable to interact with P22 repressor dimers), did not [Table 2 (B)]. Second, we measured the ability of variant λ v1-5;148 to interact with the carboxy-terminal domain of wild-type λ repressor. For this purpose, we made use of a hybrid repressor bearing the DNA-binding domain of P22 repressor and the carboxy-terminal domain of λ repressor (see Figs. 2 and 8). Using the DNA template bearing the λ operator upstream of the low-affinity P22 operator, we found, as expected, that wild-type λ repressor, but not the variant, was able to interact with the hybrid repressor (Fig. 7B). To eliminate the possibility that this apparent failure was due to inefficient dimerization and consequently to poor site occupancy by variant λ v1-5;148, we tested its ability to dimerize. Using the same assay as that shown in Figure 3, we found that this variant dimerized as efficiently as wild-type λ repressor (data not shown). Thus, variant λ v1-5;148, having gained the ability to interact with P22 repressor and also lost the ability to interact with the carboxy-terminal domain of λ repressor, is a switched-specificity mutant.

Discussion

The amino-terminal domains of the lambdoid phage repressors bind DNA using a conserved module, the familiar helix–turn–helix motif (Aggarwal et al. 1988; Jordan and Pabo 1988). The specificities of these protein–DNA interactions are determined largely by solvent-exposed residues in the second α -helix, known as the recognition helix. In the case of the 434 and P22 repressors, this was elegantly demonstrated by replacing specific residues in the recognition helix of 434 repressor with the corresponding residues from the recognition helix of P22 repressor; the resulting 434 repressor variant bound specifically to P22 operators (Wharton and Ptashne 1985).

In this study we have focused on a carboxy-terminal domain-mediated function, the protein–protein interaction of pairs of λ repressor dimers and also P22 repressor dimers. The two repressors exhibit considerable amino acid identity in their carboxy-terminal domains, the structures of which are still unknown (see Fig. 5). However, although each dimer interacts with its own type, λ repressor dimers and P22 repressor dimers interact with one another only weakly, if at all, when bound to an appropriately spaced pair of heterologous operators (see Fig. 7, line 2). We have replaced specific residues in the carboxy-terminal domain of λ repressor with the corresponding residues from the carboxy-terminal domain of P22 repressor and created a λ repressor variant that interacts with P22 repressor dimers, but not with the carboxy-terminal domain of wild-type λ repressor (see Fig. 8). We suggest that these amino acid substitutions, in

Figure 8. The interactions of wild-type λ repressor or the switched-specificity λ repressor variant. Wild-type λ repressor does not interact with wild-type P22 repressor but does interact with the hybrid repressor bearing the carboxy-terminal domain of λ repressor. In contrast, the switched-specificity λ repressor variant interacts with wild-type P22 repressor but fails to interact with the hybrid repressor.

analogy with those implicated in the helix–swap experiment described above, lie within a conserved module that mediates the protein–protein interaction of the lambdoid phage repressors.

Our switched-specificity λ repressor variant bears just six non-wild-type residues occurring at positions 148, 196, 198, 200, 201, and 204. We do not know whether all of these substitutions are required. We have directly demonstrated that substitutions N148D and Q204K are required by constructing variants bearing just the other five substitutions; neither was able to interact with P22 repressor dimers. In the case of position 148, this result was anticipated because replacement of asparagine-148 of λ repressor with aspartate and of aspartate-131 of P22 repressor with asparagine significantly weakened the homotypic interaction of the respective repressor dimers. Similarly, replacement of arginine-196 in λ repressor with methionine (the residue found at the corresponding position of P22 repressor) disrupted the interaction of λ repressor dimers, suggesting that substitution R196M is also likely to be required in the switched-specificity variant. Thus, the set of essential specificity-determining residues includes residues 148, 204, and probably 196 and may or may not also include residues 198, 200, and 201.

The argument that a common structure underlies the cooperative binding of λ and P22 repressor dimers is supported also by mutations that alter conserved residues. At four conserved positions (188, 197, 202, and 210 in λ repressor) we have shown that the same amino acid change that disrupts the interaction of λ repressor dimers also disrupts the interaction of P22 repressor dimers. The P22 repressor mutant corresponding to λ repressor mutant E188K (E170K) was isolated previously (Valenzuela and Ptashne 1989). In the other three cases (D197G, F202S, and Y210N), we introduced the change into P22 repressor and showed that it resulted in a cooperativity defect.

Our λ repressor mutants bear amino acid substitutions

at 14 different positions in the carboxy-terminal domain, 8 of which were also identified by Benson et al. (1994), who used an elegant genetic selection to isolate a set of λ repressor mutants unable to bind cooperatively to adjacent operators. Based on the character of the wild-type residues and, in some cases, on the nature of the substitutions, we think it likely that many of the affected residues lie at the protein surface. Ten are either charged or hydrophilic: N148, S149, S159, E188, K192, R196, D197, S198, S228, and T234. In addition, charged substitutions have been isolated at seven positions: N148D, G199D, and T234K in this study, S198R, M212R, and S228R in Benson et al. (1994), and E188K in both studies. Because these substitutions do not prevent dimer formation, we argue that they do not disrupt the overall folded structure of the domain, as they might be expected to do if they involved buried residues. In the case of G199, a second charged residue is also tolerated: Mutant G199R is neither defective for dimerization nor for cooperative binding to DNA (N. Kuldell, unpubl.).

Although we isolated amino acid substitutions extending from position 148 to 234, half of the affected residues lie in the region 192–210, including substitutions at four consecutive positions from 196 to 199. We suggest that this region is likely to play a particularly important role in mediating cooperativity; the fact that all but one of the residues changed in our switched-specificity λ repressor variant lie within a subregion extending from position 196 to 204 is consistent with this proposal. Circular dichroism measurements indicate that λ repressor's carboxy-terminal domain is largely made up of β -sheet (E. Rivera, M. Weiss, and A. Hochschild, unpubl.), and the amino acid sequence suggests that region 192–210 may contain two turns, which both bear residues critical for the dimer–dimer interaction. Residues 196–199 are likely to adopt a type I β -turn (Wilmot and Thornton 1988), and residues 207–210 conform to a consensus (NPXY) derived for the internalization signals of certain coated pit receptors; this motif has been shown to adopt a reverse-turn conformation in solution (Bansal and Gierasch 1991). In the case of residues 207–210, this prediction is particularly strong because the same motif is also present in both the P22 and 434 repressors.

Each of these putative turns contains a conserved residue (D197 and Y210, respectively) that appears to play an essential role in the dimer–dimer interaction. Replacement of D197 (D179 in P22 repressor) with glycine eliminated the homotypic interaction of both λ and P22 repressor dimers, suggesting that the side chain of D197 is critical. Likewise, replacement of Y210 with asparagine, histidine, cysteine, or serine (see also Benson et al. 1994), or the corresponding P22 residue with asparagine or isoleucine, eliminated the interaction of the respective repressor dimers, suggesting that the side chain of Y210 (Y192 in P22 repressor) is also critical. The first putative turn also includes two nonconserved residues (R196 and S198); we have argued that at least one of these (R196) is likely to help determine the specificity of the interaction (see above).

The only specificity-determining residue identified in

our study that does not lie in the region 196–204 is N148. However, an independent line of evidence suggests that residue 148 lies in close proximity to this region. Analysis of the intramolecular cleavage reaction has revealed that a pair of conserved residues (S149 and K192 in λ repressor) are critical, with the hydroxyl group of the serine making the proteolytic attack while the lysine functions as a base catalyst to deprotonate the serine hydroxyl (for review, see Little 1993). The interaction of K192 and S149 places residue 148 in the vicinity of the 192–210 region and suggests that essential residues such as N148, K192, R196, and D197 may form part of a continuous surface at the dimer–dimer interface. Although replacement of S149 with phenylalanine also disrupted the dimer–dimer interaction, we know that the serine side chain is not required for the interaction; another mutant, S149A, though unable to undergo intramolecular cleavage, interacts normally when bound at both adjacent and separated operators (data not shown).

Our data indicate that the three carboxy-terminal domain activities are separable. Mutants have been identified (e.g., P158T) that dimerize poorly but, when dimerized, manifest no defect in cooperative binding to adjacent or separated operators. Conversely, most (though not all) of the mutants isolated on the basis of their inability to interact when bound at separated operators dimerize normally (see Table 3). Moreover, as mentioned above, mutant S149A is unable to undergo RecA-facilitated intramolecular cleavage but is defective neither in dimer formation nor in cooperativity. Nevertheless, residues implicated in each of the three functions are interspersed and, in some cases, overlapping (see Fig. 5). Particularly striking is the clustering of residues critical for cooperativity with those that carry out intramolecular cleavage. Residue K192, which when changed to asparagine eliminated the interaction of both adjacently and nonadjacently bound dimers, may in fact play a direct role in both processes. We suggest that the two processes may share a common structural basis. RecA-facilitated cleavage is a conserved process that presumably places a set of constraints on the carboxy-terminal domain structures of the lambdoid phage repressors. Perhaps the three-dimensional fold thus dictated also provides the structural basis for the dimer–dimer interaction, the specificity of which is determined by nonconserved residues that are displayed on a common structural framework.

This hypothesis is supported by another amino acid substitution that affects both cooperativity and RecA-facilitated cleavage. We observed that cooperativity mutant S159N was present at reduced levels in a *recA*⁺ strain but at wild-type levels in an otherwise isogenic *recA*[−] derivative. We confirmed that this was because of increased susceptibility to RecA-facilitated cleavage by performing phage spot tests in the presence or absence of the inducing agent mitomycin C (Gimble and Sauer 1985). When comparing a *recA*⁺ strain bearing the S159N mutant with one bearing wild-type repressor, we found that it was much more sensitive to superinfecting λ phage in the presence but not in the absence of mito-

mycin C (L.A. Cheatham, unpubl.). Mutants exhibiting this phenotype (called Ind^s mutants) have been described previously (Cohen et al. 1981; Gimble and Sauer 1989). One class derives its Ind^s character from a reduced ability to form dimers, the monomer being the substrate for cleavage. However, mutant S159N dimerizes more efficiently than several other mutants that were not present at reduced levels in our *recA*⁺ strain; the basis for its Ind^s phenotype could either be an intrinsically more efficient cleavage reaction or an increased affinity for RecA protein. Similar Ind^s mutants, whose increased susceptibilities to autocleavage are not related to dimerization, have been described in the case of the homologous bacterial repressor LexA (Kim and Little 1993).

It is likely that the conserved structural features that allow λ repressor dimers to interact with P22 repressor dimers also characterize the carboxy-terminal domains of other lambdoid phage repressors. For example, the phage 434 repressor, which like the λ and P22 repressors, binds cooperatively to adjacent and separated operators (Johnson et al. 1981; D. Valenzuela pers. comm.), shows an even higher degree of amino acid sequence similarity with P22 repressor in its carboxy-terminal domain than does λ repressor (Sauer et al. 1982b). Another lambdoid phage, HK022 (Dhillon and Dhillon 1976; Oberto et al. 1989), encodes a repressor that has recently been shown to bind with a very high degree of cooperativity to adjacent operators (Carlson and Little 1993). In addition, certain observations suggest that HK022 repressor may participate in long-range interactions during the phage life cycle (Carlson and Little 1993). It will be interesting to learn what structural features account for the higher degree of cooperativity achieved by the HK022 repressor as compared with the λ , P22, and 434 repressors.

Cooperative binding to DNA has been demonstrated for a growing number of transcriptional regulatory proteins in both prokaryotes and eukaryotes (see, e.g., LeBowitz et al. 1989; Li et al. 1991; Pedersen et al. 1991, 1992; Kim and Little 1992; Somers and Phillips 1992; Weiss et al. 1992; Cleary et al. 1993; Jiang and Levine 1993; Mak and Johnson 1993; Porter et al. 1993; Vershon and Johnson 1993; Wilson et al. 1993; see also McKnight and Yamamoto 1992). Specificity is achieved not only by the interaction of these proteins with specific sites on the DNA but also by their interactions with one another. In some cases, such as the lambdoid phage repressors, these interactions involve identical molecules, and in other cases they involve heterologous molecules. We have chosen to focus on a relatively simple example involving identical molecules to begin to probe the structural requirements for such an interaction. Genetic and structural analyses have led to the characterization of structural motifs that mediate DNA binding (for reviews, see Harrison 1991; Pabo and Sauer 1992), as well as motifs that mediate protein dimerization or oligomerization (for reviews, see Alber 1992; Chakerian and Matthews 1992; Baxevanis and Vinson 1993; see also Ferre-D'Amare et al. 1993). Likewise, it may be possible eventually to describe other motifs that mediate DNA-dependent protein–protein interactions.

Materials and methods

Media and stock strains

LB liquid media and plates were prepared as described by Miller (1972). Strains NK5031 (F' *lacZ*ΔMS265 *suIII*⁺ *Nal*^R), X131 (F' *lacI*^{q1} *lacZ*::Tn5 *proA*⁺ *B*⁺/Δ(*pro*, *lac*)_{XM} *rpsE* *thi*[−] *Val*^R), JM101 (F' *traD*36 *lacI*^q *lacZ*M15 *proA*⁺ *B*⁺/*supE* *thi*[−] Δ(*lac*–*proAB*), and the *mutD* strain were from our laboratory collection. Strain CC114^{iq} was constructed by mating an F' 128 *lacI*^q *lacZ*::Tn5, a gift from J. Hu (Texas A&M University, College Station), into strain CC114 (MC1061 *lacZ*_{amY14} *argE*_{am} *rif*^R *recA* *srI*::Tn10), a gift from D. Boyd (Harvard Medical School, Boston, MA).

Plasmids

Plasmid pLR1 (see Fig. 2 for this and other repressor-encoding plasmids) is a derivative of pBR322 that bears the λ repressor gene (*cI*) controlled by a truncated *lacUV5* promoter–operator region. It directs the expression of a level of λ repressor ~10 times that found in a λ lysogen when *lac* repressor is not present (data not shown). In a strain containing *lac* repressor, expression of the *cI* gene can be modulated by the addition of various concentrations of IPTG to the medium. pLR1 was constructed by filling out the *Hind*III site in the pBR322 vector portion of p280Δ-35 (Hochschild and Ptashne 1988) with DNA polymerase I Klenow fragment and religating. Plasmid pLR2 was constructed to facilitate mutagenesis of the 3' end of the *cI* gene. It was made from pLR1 by changing the sequence immediately downstream of the *cI* gene from TGATCGCAAGGT to TGACCTAGGATCC (underline indicates the stop codon) and deleting the DNA between the *Bam*HI site thus introduced (italics) and the *Bam*HI site within the pBR322 vector. pLR2 directs expression of somewhat higher levels of λ repressor than does pLR1 (N.H. Kuldell, unpubl.). Plasmid pLR1Δ*cI* is a deletion derivative of pLR1 lacking DNA between the *Nsi*I site at codon 56 of the *cI* gene and a *Pst*I site downstream of the gene.

Plasmid pPR2 is derived from pLR1Δ*cI* and contains the P22 repressor gene (*c2*) under the control of its own copy of a truncated *lacUV5* promoter–operator region. Its construction was performed in several steps but is equivalent to excision of the promoter–operator region and the entire P22 *c2* gene from plasmid pTP15 (Poteete and Roberts 1981) on a 770-bp fragment beginning at the *Msp*I site in the *lacUV5* promoter, and insertion into the backbone of pLR1Δ*cI* cleaved with *Cl*aI and *Bam*HI.

Plasmid pFW7 directs expression of a hybrid repressor consisting of the amino-terminal domain of P22 repressor fused at the Ala–Gly bond that is the target of RecA-mediated autocleavage to the carboxy-terminal domain of λ repressor. It was constructed from derivatives of pPR2 and pLR2 into which translationally silent mutations were introduced creating *Ngo*MI restriction sites at P22 repressor codons Ala-94–Gly-95 and λ repressor codons Ala-111–Gly-112, respectively. This crossover point was chosen to minimize the possibility of disrupting function of either domain. The appropriate *Ngo*MI fragment was excised from the pLR2 derivative and ligated to the *Ngo*MI-cleaved backbone of the pPR2 derivative.

Plasmids pA3B2 and pA3HΔ*cI* are pACYC184 derivatives that carry the operon fusions from pLR2 and pLR1Δ*cI*, respectively. pA3B2 bears the *Eco*RI–*Bam*HI λ repressor fragment of pLR2 inserted into pACYC184 between the *Hind*III and *Bst*YI sites. pA3HΔ*cI* contains a fragment corresponding to the region between the *Eco*RI site and the filled-in *Hind*III site of pLR1Δ*cI* inserted into the backbone of pACYC184 cleaved with *Hind*III

and *Hinc*II. In both constructions, all *Eco*RI and *Hind*III overhanging ends were filled in with DNA polymerase I Klenow fragment.

Plasmids pA3B2-v1-2, -v1-3, -v1-4, and -v1-5 consist of pA3B2 in which DNA containing the λ repressor codons R196 through S198, Q200, V201, and Q204, respectively, have been replaced by the corresponding base pairs of P22 DNA. To construct them, a *Bcl*I restriction site present in the λ repressor gene (at codons 194–196) was introduced into the P22 repressor-coding sequence at the corresponding position, and *Hae*III and *Dra*I sites present in the P22 repressor gene (at codons 181–182 and 184–186, respectively) were introduced into the λ repressor-coding sequence at their corresponding positions. DNA fragments bearing these changes were obtained by PCR amplification of appropriate portions of plasmids pLR2 and pPR2 using primers that introduced the desired changes. pA3B2-v1-2 and pA3B2-v1-5 were constructed in several steps by ligating the appropriate restriction fragments together. pA3B2-v1-3 and pA3B2-v1-4 were made in a similar way using PCR primers that contained additional mismatches to create the Q200R and V201K changes. Mutation N148D was then moved into or out of these plasmids as appropriate by standard subcloning techniques. All constructions were verified by sequencing.

All the repressor-bearing plasmids described above direct the synthesis of sufficient protein to confer immunity to superinfection by the appropriate clear phage mutant (either λ*cI*[−] or λi21clear) as assayed by cross-streak tests.

Indicator strains

All indicator constructs were assembled on plasmids and then recombined in vivo onto bacteriophage λRZ11 (Yu and Reznikoff 1984), which bears a λ*cI*⁸⁵⁷ (temperature sensitive) immunity region, or onto derivatives of λRZ11 bearing the immunity region of either phage 21 (*imm*21) or phage 434 (*imm*434). Recombinant phages were then integrated in single copy into the chromosome of an appropriate host strain by lysogeny as described previously (Hochschild and Ptashne 1988).

Strains AH-5.9 and AH-5.5 have been described (Hochschild and Ptashne 1988). They bear the *lacZ* gene under the control of a modified λP_{RM} promoter region in which O_R3 was inactivated by changing its sequence from TATCACCGCAAGGGATA to TACAGCTGCAAGGGATA, and O_R1 was moved to a position 5.9 or 5.5 turns of the DNA helix upstream of O_R2. The latter manipulation utilized a *Hinc*II restriction site that overlaps O_R2 and resulted in a G → A change at the most promoter–distal base of O_R2. [Note that in Hochschild and Ptashne (1988) the mutant O_R3 sequence was reported erroneously as TACAGC-TGCAAGGATA]. These promoter–*lacZ* fusions were recombined onto phage λRZ11*imm*21 and integrated into the chromosome of the *lacI*[−] strain NK5031.

Strain FW15 bears the 5.5-turn version of the phage described above integrated into the chromosome of the *lacI*^q strain CC114^{iq}.

Strain X131-BB (O_R2 only) bears the same construct as AH-5.5 except that O_R1 was removed by deletion of DNA between a *Bam*HI site in the spacer between O_R1 and O_R2 and a *Bgl*II site 98 bp upstream of the center of O_R1. Strain X131-parGA (O_R1 and O_R2 in their natural positions) bears the parent construct used to generate the 5.5- and 5.9-turn constructs into which the G → A substitution at the edge of O_R2 has been introduced by site-directed mutagenesis. These constructs were crossed onto phage λRZ11*imm*21 and integrated into the chromosome of the *lacI*^q strain X131.

Strains DV59 and DV72, derived from plasmids pDV59 and pDV72, have been described (Valenzuela and Ptashne 1989).

They bear the *lacZ* gene under the control of a promoter that consists of the *lacUV5* –10 and –35 hexamers with a P22 O_R2 operator site in the spacer region. In addition, DV72 bears a strong P22 operator site (O_1) located 5.0 helical turns upstream of O_R2 . These constructs were crossed onto phage λ RZ11 and integrated into the chromosome of the *lacI^q* strain JM101.

Strain FW40 bears the same construct as DV72 except that the P22 O_1 operator was replaced by λO_R1 (operator spacing 5.0 turns of the DNA helix). O_R1 was provided on a *EcoRI*–*HincII* fragment from plasmid pEM9O_RP (Hochschild and Ptashne 1988) bearing a short *Bam*HI adapter sequence (CAGACG-GATCC) at the *HincII* end, which was inserted into the *EcoRI*–*Bam*HI-cleaved backbone of pDV59. For the FW42 construct (operator spacing 5.4 helical turns), the *Bam*HI site was subsequently filled in with DNA polymerase I Klenow fragment and religated. These constructs were crossed onto phage λ RZ11imm434 and integrated into the chromosome of the *lacI^q* strain CC114^{iq}.

Mutagenesis

Mutagenesis of the λ repressor gene was performed by two different methods. For *mutD* mutagenesis, plasmid pLR1 was passaged through our *mutD* strain, transformed into strain AH-5.9, and plated on indicator plates containing the chromogenic lactose analog X-gal. Plasmid DNA purified from dark blue colonies was then analyzed by subcloning various restriction fragments into a nonmutagenized version of pLR1 and identifying the reconstructed plasmids associated with the mutant phenotype. Alternatively, the region between the *Hind*III (at codon 158) and *Bam*HI sites of plasmid pLR2 was mutagenized by PCR as described (Zhou et al. 1991), and ligated plasmid DNA was transformed into strain AH-5.9 as described above. For both methods, the entire segment of DNA subjected to mutagenesis was sequenced to identify individual mutations.

Site-directed mutagenesis was performed using the Bio-Rad Mutagen phagemid mutagenesis kit, or by a PCR technique, in which one of the PCR primers introduced a specific change near a restriction site. PCR DNA was cleaved with appropriate restriction endonucleases, and the plasmids were reconstructed by standard cloning techniques. In both cases, the final constructs were verified by sequencing the entire region subjected to mutagenesis.

Enzyme assays

β -Galactosidase assays were as performed as described by Miller (1972). Cells were grown in supplemented BU medium as described, with carbenicillin (50–100 μ g/ml) or chloramphenicol (30 μ g/ml) or both added as appropriate. For strains FW40 and FW42, kanamycin (30 μ g/ml) was also added. IPTG was added as stated in the figure legends.

Acknowledgements

We are very grateful to D. Valenzuela for providing us with the P22 operator-bearing templates and to J. Hu for providing us with λ repressor mutants R196M and S149A. We also thank them for many helpful discussions. We thank V. Podolny for excellent technical assistance in sequencing many of the mutant repressor genes and performing site-directed mutagenesis. We thank L. Ko, J.K. Joung, C. Petosa, A. Hirsh, and K. Forbes for additional plasmid constructions and site-directed mutagenesis. We are grateful to M. Ptashne and G.P. Hochschild for their comments on the manuscript.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Adhya, S. 1989. Multipartite genetic control elements: communication by DNA loop. *Annu. Rev. Genet.* **23**: 227–250.
- Aggarwal, A.K., D.W. Rodgers, M. Drott, M. Ptashne, and S.C. Harrison. 1988. Recognition of a DNA operator by the repressor of phage 434: A view at high resolution. *Science* **242**: 899–907.
- Alber, T. 1992. Structure of the leucine zipper. *Curr. Opin. Genet. Dev.* **2**: 205–210.
- Bansal, A. and L.M. Gierasch. 1991. The NPXY internalization signal of the LDL receptor adopts a reverse-turn conformation. *Cell* **67**: 1195–1201.
- Baxevanis, A.D. and C.R. Vinson. 1993. Interactions of coiled coils in transcription factors: Where is the specificity? *Curr. Opin. Genet. Dev.* **3**: 278–285.
- Benson, N., C. Adams, and P. Youderian. 1994. Genetic selection for mutations that impair the co-operative binding of lambda repressor. *Mol. Microbiol.* **11**: 567–579.
- Bushman, F.D., C. Shang, and M. Ptashne. 1989. A single glutamic acid residue plays a key role in the transcriptional activation function of lambda repressor. *Cell* **58**: 1163–1171.
- Carlson, N.G. and J.W. Little. 1993. Highly cooperative DNA binding by the coliphage HK022 repressor. *J. Mol. Biol.* **230**: 1108–1130.
- Chakerian, A.E. and K.S. Matthews. 1992. Effect of lac repressor oligomerization on regulatory outcome. *Mol. Microbiol.* **6**: 963–968.
- Cleary, M.A., S. Stern, M. Tanaka, and W. Herr. 1993. Differential positive control by Oct-1 and Oct-2: Activation of a transcriptionally silent motif through Oct-1 and VP16 corecruitment. *Genes & Dev.* **7**: 72–83.
- Cohen, S., B. Knoll, J.W. Little, and D.W. Mount. 1981. Preferential cleavage of phage λ repressor monomers by *recA* protease. *Nature* **294**: 182–184.
- Dhillon, T.S. and E.K.S. Dhillon. 1976. Temperate coliphage HK022. *Jpn. J. Microbiol.* **20**: 385–396.
- Ferre-D'Amare, A.R., G.C. Prendergast, E.B. Ziff, and S.K. Burley. 1993. Recognition by Max of its cognate DNA through a dimeric b/HLH/Z domain. *Nature* **363**: 38–45.
- Gimble, F.S. and R.T. Sauer. 1985. Mutations in bacteriophage λ repressor that prevent RecA-mediated cleavage. *J. Bacteriol.* **162**: 147–154.
- . 1989. λ repressor mutants that are better substrates for RecA-mediated cleavage. *J. Mol. Biol.* **206**: 29–39.
- Guarente, L., J.S. Nye, A. Hochschild, and M. Ptashne. 1982. Mutant lambda phage repressor with a specific defect in its positive control function. *Proc. Natl. Acad. Sci.* **79**: 2236–2239.
- Harrison, S.C. 1991. A structural taxonomy of DNA-binding domains. *Nature* **353**: 715–719.
- Hochschild, A. 1990. Protein–protein interactions and DNA loop formation. In *DNA topology and its biological effects* (ed. N.R. Cozzarelli and J.C. Wang), pp. 107–138. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Hochschild, A. and M. Ptashne. 1986. Cooperative binding of λ repressors to sites separated by integral turns of the DNA helix. *Cell* **44**: 681–687.
- . 1988. Interaction at a distance between λ repressors disrupts gene activation. *Nature* **336**: 353–357.

- Hochschild, A., N. Irwin, and M. Ptashne. 1983. Repressor structure and the mechanism of positive control. *Cell* **32**: 319–325.
- Jiang, J. and M. Levine. 1993. Binding affinities and cooperative interactions with bHLH activators delimit threshold responses to dorsal gradient morphogen. *Cell* **72**: 741–752.
- Johnson, A.D., B.J. Meyer, and M. Ptashne. 1979. Interactions between DNA-bound repressors govern regulation by the λ phage repressor. *Proc. Natl. Acad. Sci.* **76**: 5061–5065.
- Johnson, A.D., A.R. Poteete, G. Lauer, R.T. Sauer, G.K. Ackers, and M. Ptashne. 1981. λ repressor and cro—Components of an efficient molecular switch. *Nature* **294**: 217–223.
- Jordan, S.R. and C.O. Pabo. 1988. Structure of the lambda complex at 2.5 Å resolution: Details of the repressor-operator interactions. *Science* **242**: 893–899.
- Kim, B. and J.W. Little. 1992. Dimerization of a specific DNA-binding protein on the DNA. *Science* **255**: 203–206.
- . 1993. LexA and λ CI repressor as enzymes: Specific cleavage in an intermolecular reaction. *Cell* **73**: 1165–1173.
- Kuldell, N. and A. Hochschild. 1994. Amino acid substitutions in -35 recognition motif of σ^{70} that result in defects in phage λ repressor-stimulated transcription. *J. Bacteriol.* **176**: 2991–2998.
- LeBowitz, J.H., R.G. Clerc, M. Brenowitz, and P.A. Sharp. 1989. The Oct-2 protein binds cooperatively to adjacent octamer sites. *Genes & Dev.* **3**: 1625–1638.
- Li, M., H. Moyle, and M.M. Susskind. 1994. Target of the transcriptional activation function of phage λ CI protein. *Science* **263**: 75–77.
- Li, R., J.D. Knight, S.P. Jackson, R. Tjian, and M.R. Botchan. 1991. Direct interaction between Sp1 and the BPV enhancer E2 protein mediates synergistic activation of transcription. *Cell* **65**: 493–505.
- Little, J.W. 1984. Autodigestion of lexA and phage lambda repressors. *Proc. Natl. Acad. Sci.* **81**: 1375–1379.
- . 1993. LexA cleavage and other self-processing reactions. *J. Bacteriol.* **175**: 4943–4950.
- Little, J.W. and D.W. Mount. 1982. The SOS regulatory system of *Escherichia coli*. *Cell* **29**: 11–22.
- Mak, A. and A.D. Johnson. 1993. The carboxy-terminal tail of the homeodomain protein $\alpha 2$ is required for function with a second homeo domain protein. *Genes & Dev.* **7**: 1862–1870.
- McKnight, S.L. and K.R. Yamamoto, eds. 1992. *Transcriptional regulation*, Book 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Miller, J.H. 1972. *Experiments in molecular genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Oberto, J., R.A. Weisberg, and M.E. Gottesman. 1989. Structure and function of the *nun* gene and the immunity region of the lamdoid phage HK022. *J. Mol. Biol.* **207**: 675–693.
- Pabo, C.O. and R.T. Sauer. 1992. Transcription factors: Structural families and principles of DNA recognition. *Annu. Rev. Biochem.* **61**: 1053–1095.
- Pabo, C.O., R.T. Sauer, J.M. Sturtevant, and M. Ptashne. 1979. The λ repressor contains two domains. *Proc. Natl. Acad. Sci.* **76**: 1608–1612.
- Pedersen, H., L. Sogaard-Andersen, B. Holst, and P. Valentin-Hansen. 1991. Heterologous co-operativity in *Escherichia coli*: The CytR repressor both contacts DNA and the CRP protein when binding to the *deoP2* promoter. *J. Biol. Chem.* **266**: 17804–17808.
- Pedersen, H., L. Sogaard-Andersen, B. Holst, P. Gerlach, E. Bremer, and P. Valentin-Hansen. 1992. cAMP-CRP activator complex and the CytR repressor protein bind co-operatively to the *cytRP* promoter in *Escherichia coli* and CytR antagonizes the cAMP-CRP-induced DNA bend. *J. Mol. Biol.* **227**: 396–406.
- Porter, S.C., A.K. North, A.B. Wedel, and S. Kustu. 1993. Oligomerization of NTRC at the *glnA* enhancer is required for transcriptional activation. *Genes & Dev.* **7**: 2258–2273.
- Poteete, A.R. and M. Ptashne. 1982. Control of transcription by the bacteriophage P22 repressor. *J. Mol. Biol.* **157**: 21–48.
- Poteete, A.R. and T.M. Roberts. 1981. Construction of plasmids that produce phage P22 repressor. *Gene* **13**: 153–161.
- Ptashne, M. 1992. *A genetic switch*. Cell Press and Blackwell Scientific Publications, Cambridge, MA.
- Roberts, J.W. and R. Devoret. 1983. Lysogenic induction. In *Lambda II* (ed. R.W. Hendrix, J.W. Roberts, F.W. Stahl, and R.A. Weisberg) pp. 123–144. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Roberts, J.W. and C.W. Roberts. 1975. Proteolytic cleavage of bacteriophage lambda repressor in induction. *Proc. Natl. Acad. Sci.* **72**: 147–151.
- Sauer, R.T., S.R. Jordan, and C.O. Pabo. 1990. λ Repressor: A model system for understanding protein-DNA interactions and protein stability. *Adv. Protein Chem.* **40**: 1–61.
- Sauer, R.T., M.J. Ross, and M. Ptashne. 1982a. Cleavage of the λ and P22 repressors by *recA* protein. *J. Biol. Chem.* **257**: 4458–4462.
- Sauer, R.T., R.R. Yocum, R.F. Doolittle, M. Lewis and C.O. Pabo. 1982b. Homology among DNA-binding proteins suggests use of a conserved super-secondary structure. *Nature* **298**: 447–451.
- Schleif, R. 1992. DNA Looping. *Annu. Rev. Biochem.* **61**: 199–223.
- Slilaty, S.N. and J.W. Little. 1987. Lysine-156 and serine-119 are required for LexA repressor cleavage: A possible mechanism. *Proc. Natl. Acad. Sci.* **84**: 3987–3991.
- Somers, W.S. and S.E.V. Phillips. 1992. Crystal structure of the *met* repressor-operator complex at 2.8 Å resolution reveals DNA recognition by β strands. *Nature* **359**: 387–393.
- Valenzuela, D. and M. Ptashne. 1989. P22 repressor mutants deficient in co-operative binding and DNA loop formation. *EMBO J.* **8**: 4345–4350.
- Vershon, A.K. and A.D. Johnson. 1993. A short, disordered protein region mediates interactions between the homeodomain of the yeast $\alpha 2$ protein and the MCM1 protein. *Cell* **72**: 105–112.
- Weiss, V., F. Claverie-Martin, and B. Magasanik. 1992. Phosphorylation of nitrogen regulator I of *Escherichia coli* induces strong cooperative binding to DNA essential for activation of transcription. *Proc. Natl. Acad. Sci.* **89**: 5088–5092.
- Wharton, R.P. and M. Ptashne. 1985. Changing the binding specificity of a repressor by redesigning an α -helix. *Nature* **316**: 601–605.
- Wilmot, C.M. and J.M. Thornton. 1988. Analysis and prediction of the different types of β -turn in proteins. *J. Mol. Biol.* **203**: 221–232.
- Wilson, D., G. Sheng, T. Lecuit, N. Dostatni, and C. Desplan. 1993. Cooperative dimerization of Paired class homeo domains on DNA. *Genes & Dev.* **7**: 2120–2134.
- Yu, X.-M. and W.S. Reznikoff. 1984. Deletion analysis of the CAP-cAMP binding site of the *Escherichia coli* lactose promoter. *Nucleic Acids Res.* **12**: 5449–5464.
- Zhou, Y., X. Zhang, and R.H. Ebricht. 1991. Random mutagenesis of gene-sized DNA molecules by use of PCR with Taq DNA polymerase. *Nucleic Acids Res.* **19**: 6052.

Specificity determinants for the interaction of lambda repressor and P22 repressor dimers.

F W Whipple, N H Kuldell, L A Cheatham, et al.

Genes Dev. 1994, **8**:

Access the most recent version at doi:[10.1101/gad.8.10.1212](https://doi.org/10.1101/gad.8.10.1212)

References

This article cites 57 articles, 21 of which can be accessed free at:
<http://genesdev.cshlp.org/content/8/10/1212.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

Focused on your science.