

How Cro and λ -repressor distinguish between operators: The structural basis underlying a genetic switch

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ABSTRACT Knowledge of the three-dimensional structures of the λ -Cro and λ -repressor proteins in complex with DNA has made it possible to evaluate how these proteins discriminate between different operators in phage λ . As anticipated in previous studies, the helix–turn–helix units of the respective proteins bind in very different alignments. In Cro the recognition helices are 29 Å apart and are tilted by 55° with respect to each other, but bind parallel to the major groove of the DNA. In λ -repressor [Beamer, L. J. & Pabo, C. O. (1992) *J. Mol. Biol.* 227, 177–196] the helices are 34 Å apart and are essentially parallel to each other, but are inclined to the major grooves. The DNA is much more bent when bound by Cro than in the case with λ -repressor. The first two amino acids of the recognition helices of the two proteins, Gln-27 and Ser-28 in Cro, and Gln-44 and Ser-45 in λ -repressor, make very similar interactions with the invariant bps 2 and 4. There are also analogous contacts between the thymine of bp 5 and, respectively, the backbone of Ala-29 of Cro and the backbone of Gly-46 of λ -repressor. Otherwise, however, unrelated parts of the two proteins are used in sequence-specific recognition. It appears that similar contacts to the invariant or almost invariant bps (especially 2 and 4) are used by both Cro and λ -repressor to differentiate the operator sites as a group from other sites on the DNA. The discrimination of Cro and λ -repressor between their different operators is more subtle and seems to be achieved primarily through differences in van der Waals contacts at bp 3', together with weaker, less direct effects at bps 5' and 8', all in the nonconsensus half of the operators. The results provide further support for the idea that there is no simple code for DNA-protein recognition.

The two regulatory proteins, Cro and λ -repressor, bind to a series of operator sites on the genome of bacteriophage λ and, together, constitute the “genetic switch” that determines the choice between the lytic and lysogenic modes of phage development (1, 2).

The structures of the Cro protein (3) and the headpiece of λ -repressor, both alone (4) and in complex with operator DNA (5, 6, 15), have been known for some time. Only recently, however, has a complex of the Cro protein with operator DNA been obtained at sufficiently high resolution (unpublished results) to permit a meaningful comparison of the respective complexes. This comparison is the subject of this report.

A related comparison previously has been made between the Cro and repressor operator complexes of phage 434 (7, 8). The 434-Cro and 434-repressor proteins are similar in sequence and structure and bind to their respective operators in a similar, although distinct, fashion. In contrast, with the exception of their respective helix–turn–helix (HTH) motifs, λ -Cro and λ -repressor have substantially different structures, and, as is now seen, bind to DNA in modes that have some

similarities, but in many respects are very different. The 434- and λ -phage operator systems therefore illustrate contrasting modes of achieving a similar biological result.

METHODS

The structures of the λ -repressor “headpiece” (residues 1–92) bound to O_L1 at 1.8 Å resolution (6) and of λ -Cro bound to a pseudo-consensus operator at 3.0 Å resolution (unpublished results) were compared visually. Optimal superposition of corresponding structures was achieved by using the program EDPDB (9). The DNA fragments used in these cocrystals were of similar length and end-type, each consisting of a 19-bp duplex with single base 5' T/A overhangs. The central 17 bps of each fragment contained an operator sequence. In the case of Cro, a pseudo-consensus operator sequence, based on the consensus half-site, was used. This operator was symmetrized in all regions contacted by the protein. As will be shown, Cro-operator complexes are generally symmetric whereas λ -repressor complexes are notably asymmetric. Unless otherwise specified, comments pertaining to Cro interactions apply to both halves of the complex, whereas λ -repressor interactions with the consensus and non-consensus halves are considered separately. Because of differences in crystallographic resolution, solvent molecules were visible in the λ -repressor complex, but not for Cro.

Takeda and coworkers (10, 11) have systematically made all possible “natural” substitutions in the O_R1 operator and determined the effects on the binding of Cro and λ -repressor. Use of an additional “non-natural” thymine to uracil replacement allowed the contribution of the methyl group to be measured. The crystal structure of the Cro- and λ -repressor complexes were analyzed in the context of these thermodynamic binding data. Bp substitutions were model-built into the respective crystal structures by using the graphics program FRODO (12), assuming other parts of the structure remain unchanged.

RESULTS

Overall Comparison of the Complexes. The Cro- and λ -repressor complexes are shown in Fig. 1. Both proteins contain a HTH unit that comprises the α_2 and α_3 helices plus the connection between them. The α_3 helix is more commonly known as the “recognition helix” (13, 14). The HTH of Cro encompasses residues 16–35 and that of λ -repressor includes residues 33–52. Thus, a residue in the λ -repressor HTH that corresponds to a residue in the HTH of Cro can be identified by adding 17.

Both Cro and λ -repressor function as dimers, with the recognition helices of the HTH units inserted into the major groove of the DNA. The DNA-binding headpiece of λ -repres-

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Abbreviation: HTH, helix–turn–helix.

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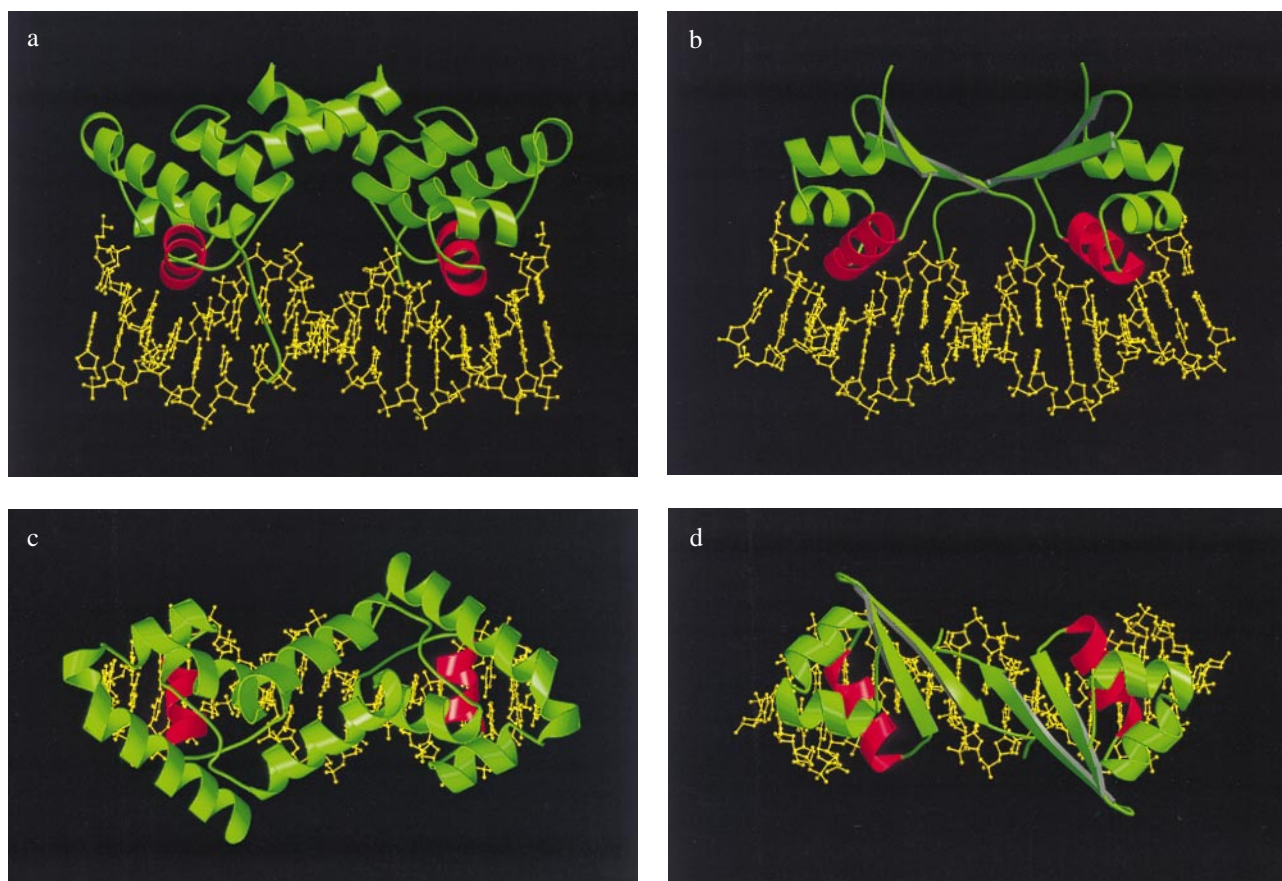


FIG. 1. Comparison of the structures of complexes of λ -repressor (5, 6) and Cro (unpublished results) with operator DNA. In both cases the proteins were crystallized with a 19-bp duplex of which the central 17 bps are shown. Recognition helices are shown in red. (a) Headpiece of λ -repressor bound to DNA. The consensus half is to the left. As can be seen there is substantial asymmetry, especially in the location of the amino-terminal "arm." (b) Cro bound to operator DNA. (c) View down the pseudo 2-fold axis of λ -repressor bound to operator DNA. (d) Related view of Cro bound to operator DNA.

sor lacks the full dimerization interface, but associates via a hydrophobic patch, allowing slight relative shifts on complex formation. Although both monomers of the dimer contain an N-terminal "arm," asymmetric features of the DNA sequence near the middle of the operators, including the central bp, dictate that only the arm on the consensus half makes ordered interactions. The corresponding arm of the nonconsensus half remains predominantly disordered. There is additional global asymmetry within the complex, with the two halves of the dimer aligning slightly differently relative to the operator (6).

The dimer interface of Cro consists of two antiparallel β -strands (Fig. 1b), which presumably allow flexibility. There is also an extensive hydrophobic contact in which Phe-58 of one monomer is buried within the core of the other subunit. The Cro subunits undergo a 55° relative rotation on operator binding relative to the crystal structure of the apo protein (ref. 15 and unpublished results).

As was anticipated from comparison of the Cro and λ -repressor structures (14), stereochemical restrictions prevent the HTH units of the two proteins aligning on the DNA in the same way. The recognition helices of Cro are aligned essentially parallel to the major grooves of the DNA whereas those of λ -repressor are aligned parallel to the bps (Fig. 1). The two recognition helices of λ -repressor are essentially parallel to each other and have a center-to-center distance of 34 Å. In Cro, the recognition helices are tilted relative to each other by 55° and have a center-to-center separation of 29 Å.

Although the consensus half of the operator sequence used in the Cro- and λ -repressor crystal structures are identical, the two proteins induce significantly different conformations in

the DNA (Fig. 1a and b). Cro bends the DNA by approximately 40° whereas λ -repressor causes only slight bending (6, 8). One way to visualize the magnitude of the difference is by superimposing the HTH units on the consensus half of the complex. Within the aligned HTH units the main-chain atoms superimpose with a root-mean-square deviation of 0.57 Å. At the opposite end of the two operators, however, the phosphate groups are displaced by 11 Å.

Interactions with the DNA. Cro directly contacts the seven outermost bps on each end of the 17-bp operator in a highly symmetric manner (Fig. 2). The central three bps are not contacted. For Cro, all direct contacts with bases come from the HTH units, whereas in λ -repressor other regions of the protein also are involved, including the N-terminal arm and the loop following the recognition helix. λ -repressor also contacts 14 bps, but in a much more asymmetric fashion (Fig. 2). Near the middle of the complex, operator-induced structuring of the amino-terminal arm occurs in the consensus half. The λ -repressor subunits also are aligned somewhat asymmetrically on the two halves of the operator, resulting in a different spacing between each recognition helix and the bps.

The locations of the N-termini of the recognition helices relative to the operator bps are very similar in both complexes, allowing nearly identical contacts to be made. In this region, and as argued by Hochschild and Ptashne (16), the first two residues of the recognition helices of both Cro and λ -repressor, which are a glutamine and serine, make similar contacts with bps 2 and 4, the only positions that are invariant in all operator half-sites. As one moves further along the recognition helix, however, there is little if any resemblance between the respec-

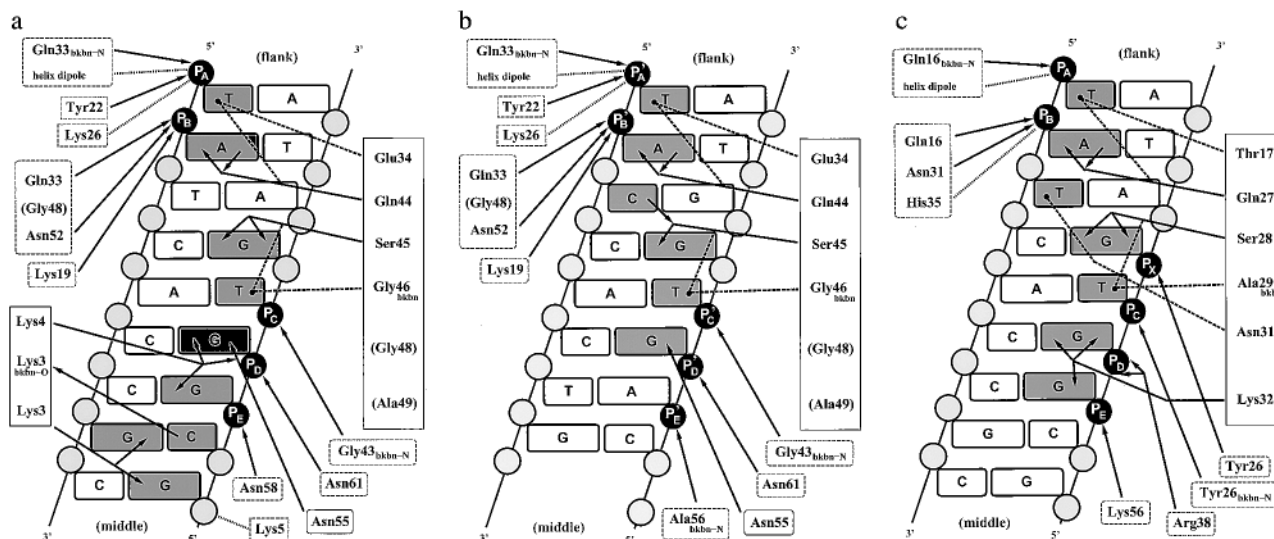


Fig. 2. (a) Schematic diagram showing the contacts made between λ-repressor and DNA on the consensus half of the complex (6). The half-site shown, numbering from top to bottom, consists of bps 1–9 of O_{L1}. When referring to a given base in the text the (+) or (–) strand prefix is added. Also, when referring to the nonconsensus half of the operator a prime is used. For positions that are equivalent in both halves, just the bp number is used. Bases and phosphates contacted by the protein are darkened. Hydrogen bonds are indicated by solid lines, van der Waals contacts by dashed lines, and nonhydrogen bond electrostatic interactions by dotted lines. Residues that make direct contact with bases are enclosed in solid boxes, those that contact the backbone are enclosed in broken boxes. Residues are grouped if they belong to the HTH or N-terminal arm. Residues in λ-repressor, which do not make direct contact with bases, but are analogous to those in Cro which do, are shown in parentheses. (b) Schematic diagram showing the contacts made between λ-repressor and DNA within the nonconsensus half of operator O_{L1} (6). (c) Schematic diagram showing the contacts between Cro and its operator DNA (unpublished results).

tive interactions of Cro and λ-repressor (c.f. ref. 17). The differences are caused by both changes in the amino acid sequence and in the alignments of the respective helices.

Both proteins contact the DNA sugar-phosphate backbone in two regions per half-site (Fig. 2). Interactions made with the phosphate groups of the “outer” region (P_A and P_B) are almost identical in both proteins. P_A receives a hydrogen bond from the main-chain amide of the first residue of the HTH, as well as having a favorable helix dipole interaction. P_B is contacted by the side chains of first and last residues of the HTH. In the Cro complex, an additional interaction with P_B is made by the middle residue of the recognition helix (Asn-31). In contrast, the nature of the “inner” region of contacts differs substantially in the two complexes, although essentially the same set of phosphate groups (P_C to P_E) are involved in each case. Many of the protein residues used to make these interactions lie

outside the HTH region and have little in common. In the Cro complex, the DNA backbone passes through a channel in each protein subunit, becoming substantially shielded from solvent. In the λ-repressor complex, the DNA backbone remains more solvent-exposed.

DISCUSSION

The sequences of the six operators for Cro- and λ-repressor are shown in Table 1. Eight of the 17 positions are invariant in all operators, six of which lie in the consensus half. Only positions 2 and 4 are invariant in all operator half-sites. An analysis of the role of each bp, in the light of the structural and binding data (10, 11) is presented below. In modeling mutant bps into each complex it was assumed that no other structural change takes place. This is clearly an oversimplification. It also should

Table 1. The six operator sites recognized by λ-repressor and Cro

Operator	Sequence	
	Consensus half	Nonconsensus half
O _{R1}	5' T A T C A C C G C C A G A G G T A 3'	A T A G T G G C G G T C T C C A T
O _{R2}	5' T A A C A C C G T G C G T G T T G 3'	A T T G T G G C A C G C A C A C
O _{R3}	5' T A T C A C C G C A A G G A T A 3'	A T A G T G G C G T T C C C T A T
O _{L1}	5' T A T C A C C G C C A G T G G T A 3'	A T A G T G G C G G T C A C C A T
O _{L2}	5' C A A C A C C G C C A G A G A T A 3'	G T T G T G G C G G T C T C T A T
O _{L3}	5' T A T C A C C G C A G A T G G T T 3'	A T A G T G G C G T C T A C C A A
Consensus	5' T A T C A C C G C C A G T G G T A 3'	A T A G T G G C G G T C A C C A T
Number of occurrences	5 6 4 6 6 6 6 5 3 4 5 3 6 3 6 4	
Position	1 2 3 4 5 6 7 8 9 8' 7' 6' 5' 4' 3' 2' 1'	

The sequences of the six operator sites are from Ptashne (2) with O_{L2} aligned so that the bps contacted by the amino-terminal arm of λ-repressor lie in the consensus half, as is the case for all the other operators.

be noted that the crystal structure of Cro is with a symmetrized operator, albeit one to which Cro binds with higher affinity than its natural operators (18). The structure is only of modest (3.0 Å) resolution but does correlate well with the binding data from the asymmetric operator O_{R1} and its variants.

At bp 1, the outermost position of the 17 bp operators (Table 1), Cro and λ-repressor use similar recognition strategies (Fig. 2). Both proteins use the second residue of their HTH units (Thr-17 of Cro; Glu-34 of λ-repressor) to form van der Waals contacts with the methyl group of the thymine. Almost all of the decrease in binding energy of the variants at this position (Fig. 3) can be attributed to the loss of this methyl group.

Bp 2 is invariant in all 12 operator half-sites and plays a critical role in the ability of Cro and λ-repressor to achieve tight binding. This position is not used to discriminate between the natural operators, but to help differentiate each of the operators from the rest of the genome. A virtually identical set of interactions is used by both proteins, involving conserved glutamine residues at the beginning of both helices of the HTH. Ade(+2) is coplanar with the side-chain amide group of the glutamine (Gln-27 of Cro; Gln-44 of λ-repressor), allowing N^ε2 to donate a hydrogen bond to N7 whereas O^ε1 accepts another from N6. These interactions optimally position N^ε2 to donate a second hydrogen bond to the glutamine at the beginning of the α₂-helix (Gln-16 of Cro; Gln-33 of λ-repressor), which in turn forms a hydrogen bonding interaction with a phosphodiester oxygen. Any bp substitution at position 2 is detrimental to the binding of either protein.

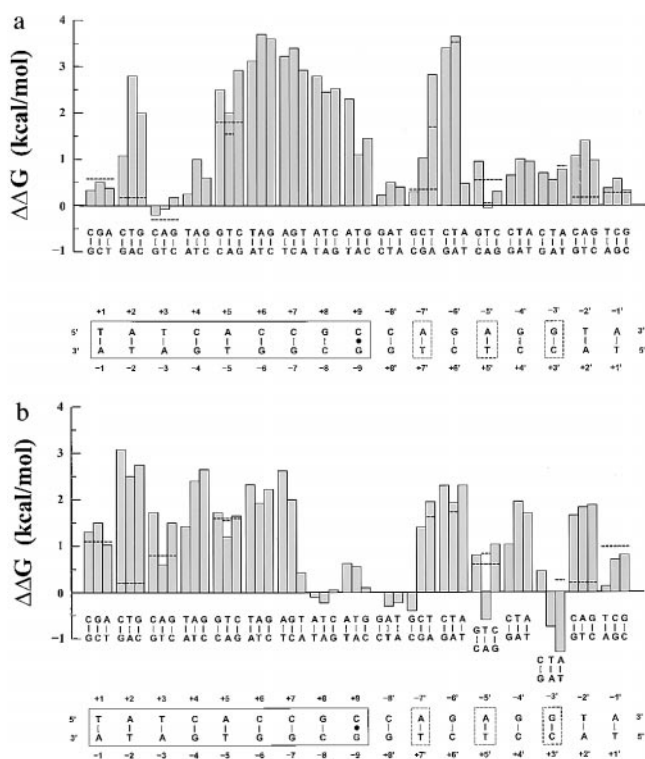


FIG. 3. (a) Relative free energy changes ($\Delta\Delta G$) in the binding of λ-repressor to O_{R1} on base substitution. The figure shows the change in affinity (K_d) that results from each of the three possible substitutions at all 17 sites. The sequence shown at the bottom is that of O_{R1}: the left half represents the consensus-half operator (solid box) and the right half represents the nonconsensus-half operator containing three nonconsensus bps at positions 3', 5', and 7' (dashed boxes). The histogram gives the change in the free energy of binding caused by the indicated bp substitution. The dashed horizontal lines on the histogram that cross over three bars or one bar represent, respectively, the $\Delta\Delta G$ from the replacement of thymine with uracil in O_{R1} or the O_{R1} mutant (redrawn from ref. 10). (b) Analysis of the effects of substitutions on the binding of Cro to O_{R1} (c.f. a) (redrawn from ref. 11).

Bp 3 is a site of major importance in distinguishing between various operators, including O_{R1} and O_{R3} (Table 1; Fig. 3). The preference at this position primarily is determined by interactions, or lack thereof, between the middle residue of the recognition helix (Asn-31 of Cro; Gly-48 of λ-repressor) and the (+3)-base (Fig. 2). Cro exhibits the highest affinity for Thy(+3), which allows a favorable van der Waals contact between the planar face of the side-chain amide group of Asn-31 and the methyl group (3.8 Å). Thymine-to-uracil mutants show this methyl group to favor Cro binding by 0.8 kcal/mol and 1.4 kcal/mol in the consensus and nonconsensus halves, respectively. In addition, Asn-31 is involved in hydrogen bonding interactions with Gln-16, His-35, and a phosphodiester oxygen, part of an extended, operator-induced hydrogen-bonding network along the protein-DNA interface. The resulting immobilization of the asparagine side chain may enhance its ability to discriminate the identity of the (+3)-base. In addition, model building shows that the edge of the aromatic ring of Tyr-26 can make favorable van der Waals contacts with the methyl group of Thy(-3), if present. This is consistent with the observation that whereas TA affords the greatest Cro affinity, AT is favored over CG or GC. This may suggest a functional role in addition to sugar-phosphate interactions for Tyr-26, helping to explain why this residue, known to be unfavorable for stability (19), is retained in Cro. The situation is notably different in λ-repressor where the lack of a side chain at Gly-48, the difference in the orientation of the recognition helix within the major groove, and the global asymmetry of the complex all come into play. In the consensus half, the Thy(+3) methyl group is 4.2 Å from the C^α of Gly-48, a distance too large for a strong van der Waals contact. Virtually no discrimination of bp identity occurs at this position. In the nonconsensus half, the orientation of the recognition helix relative to the bps is somewhat different and model building shows the spacing between C^α of Gly-48 and the methyl group of Thy(+3') to be 5.7 Å, consistent with the observation that a thymine-to-uracil mutation at this position has no impact on binding energy. As noted by Beamer and Pabo (6), the observed preference for CG over all other bps may be explained by an alternative conformation of Ser-45, which can accept a hydrogen bond from N4 of Cyt(+3').

In both complexes, the second residue of the recognition helix, a conserved serine (Ser-28 in Cro; Ser-45 in λ-repressor), interacts with Gua(-4) which is invariant in all 12 operator half-sites. As might be expected, a CG at position 4 is the most-preferred bp for both proteins. In Cro, the hydroxyl of Ser-28 donates a bifurcated hydrogen bond to both the O6 and N7 of Gua(-4). A similar interaction occurs in the consensus half of the λ-repressor complex, with Ser-45 donating a bifurcated hydrogen bond to Gua(-4). In the nonconsensus half, however, the slighted altered position of the helix main chain allows this serine to adopt two different conformations. In one conformation, Ser-45 donates a hydrogen bond primarily to O6 of Gua(-4') and perhaps also to N7. In the other conformation, as mentioned, the hydroxyl of Ser-45 maintains the interaction with O6 of Gua(-4'), while accepting another hydrogen bond from the N4 of Cyt(+3') (6).

Both Cro and λ-repressor prefer AT at position 5, where Ade(+5) is found in all six consensus half-sites, as well as in three of the nonconsensus half-sites. The hydrophobic face of the helical main chain at the third residue of the recognition helix (Ala-29 of Cro; Gly-46 of λ-repressor) forms van der Waals contacts with the methyl group of Thy(-5). This contact is strong in the Cro complex with the methyl group contributing 1.6 kcal/mol of binding energy in each half of the complex. These contacts involve the main-chain nitrogen and C^α of Ala-29 (3.7 Å and 4.0 Å, respectively), and O^γ of Ser-28 (3.9 Å). Similar interactions (1.8 kcal/mol) are made in the consensus half of the λ-repressor complex, predominantly through the main-chain nitrogen and C^α of Gly-46 (both 3.7

Å). In contrast, the methyl group of Thy(-5') in the nonconsensus half has little impact on λ -repressor binding, even though the same types of interactions are used. This appears to be a consequence of slight crowding by the main-chain nitrogen and C α of Gly-46 (3.4 Å and 3.5 Å, respectively) and C β of Ser-45 (3.5 Å). The (+5')-base is not contacted by either protein. However, the methyl group of Thy(+5') enhances binding of both Cro and λ -repressor by about 0.6 kcal/mol, accounting for most of the difference between TA and its isosteric counterpart, CG. This effect is indirect, presumably helping the DNA achieve the required operator-bound conformation.

Bps 6 and 7 are the first to be contacted in distinctly different ways by the two proteins. The extended conformation of Lys-32, the fifth residue of the recognition helix in Cro, allows it to donate hydrogen bonds to both the N7 and O6 of Gua(-6), as well as to N7 of Gua(-7). The strong preference of Cro for CG at bp 6 appears to result from the two hydrogen bonds with Gua(-6). Only guanine can provide the two hydrogen acceptor groups, explaining why other bases, including the isosteric counterpart, adenine, are so disfavored. A variation on this theme occurs at bp 7, where this lysine can reach only the N7 of Gua(-7). In this case N7 of Ade(-7) serves the same role, explaining why Cro strongly prefers either CG or TA over all others. λ -repressor uses a completely different strategy, but to a somewhat similar end. In both halves of the complex, N $^{\delta 2}$ of Asn-55 donates a hydrogen bond to N7 of Gua(-6). In the consensus half, O $^{\delta 1}$ accepts a hydrogen bond from Lys-4, positioning this lysine to donate hydrogen bonds to O6 of Gua(-6), as well as to O6 of Gua(-7) (Fig. 2). Again, because only guanines can provide the required acceptor groups at bps 6 and 7, λ -repressor strongly prefers CG over all other bps at both of these positions (Fig. 3). However, because the N-terminal arm remains disordered in the nonconsensus half, the need for an N7 group on the (-6')-base remains the sole distinguishing determinant at bp 6', consistent with the observation that both CG and TA are strongly preferred. A thymine at (-7') disfavors λ -repressor binding by 1.2 kcal/mol. Model building suggests that this is because of a steric clash between the methyl group and the side chain of Asn-55.

Interactions at the center of the complex are important for the tight binding of λ -repressor, but not for Cro. Neither bp 8 nor 9 is contacted by Cro. However, this is a region of substantial distortion, with bp 8 displaying large propeller twisting, consistent with the very slight preference for TA or AT, which contain just two Watson-Crick hydrogen bonds. As a result of this propeller twisting, an additional hydrogen bond appears to occur between N2 of Gua(-9) and O2 of Cyt(-8). The small preference for CG or GC at bp 9, the middle position of the operator, may derive from the ability to form an additional intra-strand hydrogen bond with one or the other of the flanking bps. In the λ -repressor complex, all of the contacts in this central region come from the N-terminal arm and little if any distinction is made regarding the identity of bp 8'.

CONCLUSIONS

Both Cro and λ -repressor can bind with high affinity to a large number of DNA sites that are not used as natural operators. For example, Cro can tolerate 22 different substitutions in the O_{R1} sequence with a loss in binding affinity of 1 kcal/mol or less (11). λ -repressor is even more forgiving (10). The number of possible substitutions increases rapidly if changes in more than one bp are allowed. It is, therefore, likely that these proteins can form tight complexes with numerous nonoperator sequences. Indeed, the DNA sites for which Cro has the highest affinity are not used as natural operators. The traditional view of DNA being either "operator" or "nonoperator" is clearly an oversimplification. Because the operators in phage λ must bind

both Cro and λ -repressor the number of potential candidates is reduced. For example, TA at bp 7 would be tolerated by Cro, but severely reduces λ -repressor affinity, and is not used in any operator. The consensus half of the natural operators are close to invariant, presumably having evolved to promote tight binding of both proteins. Bps 2 and 4 are the only positions invariant in all half-sites and these are contacted by residues conserved in the two proteins.

Interactions with the DNA backbone contribute to binding and also largely determine the relative orientation of the functional groups on the protein and DNA. The types of base-specific interactions used to select operator (or pseudo-operator) sites from nonspecific DNA differ in character from those used to distinguish between various operators. Recognition of operators in this system relies predominantly on strong, directed interactions, such as multiple hydrogen bonds to a single base or adjacent bases. The loss of such interactions caused by base substitutions is energetically costly. Avoidance of steric clash is also a critical prerequisite for tight binding. van der Waals contacts tend to play a lesser role, except at position +5, where a substantially buried thymine methyl group is favorably contacted by a rigid section of helix main chain.

In contrast, discrimination between various operators relies on more subtle interactions, as is demonstrated by the ways in which Cro and λ -repressor distinguish between O_{R1} and O_{R3}. These two operators differ at just three positions, 3', 5', and 8', all in the nonconsensus half. Of these, bp 3' is most important. Here, Cro prefers the TA(3') of O_{R3} over the CG(3') of O_{R1} by 1.3 kcal/mol, because of the ability of the Asn-31 of Cro to form favorable van der Waals contacts with the Thy(+3') methyl group. The ability to distinguish the identity of the (+3')-base presumably is enhanced by a set of hydrogen bonds that restrict the motion of Asn-31. Such interactions are absent in λ -repressor, where a glycine occupies the position analogous to the asparagine. The 0.8 kcal/mol preference of λ -repressor for the CG(3') of O_{R1} over the TA(3') of O_{R3} appears to be caused by the ability of Ser-45 to adopt an alternative conformation, allowing the formation of a hydrogen bond with Cyt(+3') (6). At bp 5', the mode of interaction is the same for both proteins. Cro and λ -repressor prefer the TA(5') of O_{R1} over the CG(5') of O_{R3} by about 0.8 kcal/mol. The methyl group of Thy(+5'), which is not contacted by either protein, still provides about 0.6 kcal/mol of favorable binding energy in both cases. This is presumably because of indirect effects, such as the ability of the DNA to adopt the required local conformation. The identity of bp 8' has little impact on the binding of either protein. Cro marginally prefers the TA(8') of O_{R3} over the GC(8') of O_{R1} by about 0.3 kcal/mol. Although not directly contacted by Cro, complex formation results in a high degree of propeller twisting at this position. This is consistent with the idea that it is easier to twist against two Watson-Crick hydrogen bonds than against three. The reason for the 0.5 kcal/mol preference of λ -repressor for the GC(8') of O_{R1} over the TA(8') of O_{R3} is not clear. However, λ -repressor binding has been shown to increase methylation at the N7 of Gua(+8') (20), indicating that the local conformation of the DNA may play a role. In summary, in the distinction between O_{R1} and O_{R3}, van der Waals contacts with an immobilized side chain play a central role. Indirect effects that the sequence has on the ability of the DNA to achieve the required conformation also come into play. When a hydrogen bonding interaction is used in distinguishing between operators, the "cost" of its loss is ameliorated by an alternative hydrogen bond.

There are parallels between the comparison of the λ -repressor and λ -Cro complexes described here, and the comparison of the complexes of 434-repressor and 434-Cro (7, 8). 434-Cro and 434-repressor are much more similar to each other than are λ -Cro and λ -repressor, and they present much more similar DNA-binding surfaces to the DNA. Also the bending of DNA, which is modest, is very similar in both cases

(8). Nevertheless, the energetics and the stereochemistry of the interactions differ in each case. Thus, the substitution of a given amino on the recognition helix of 434-Cro need not have the same effect as the same substitution on the recognition helix of 434-repressor (7). In the case of λ -repressor and λ -Cro the differences are much more obvious, both from protein to protein and from complex to complex. In both cases, however, the overall conclusion is the same, namely that there is not a simple one-on-one code for recognition between amino acids and bps (7, 14, 21). Similar DNA sequences can be recognized and differentiated by rather different sets of amino acid side chains organized in different spatial arrangements.

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