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λ repressor recognizes the approximately 2-fold symmetric half-operator sequences asymmetrically

(DNA–protein interaction/binding free energy changes/sequence-specific contacts/sequence recognition)

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ABSTRACT **Results of systematic base-substitution experiments suggest that the λ repressor dimer, made of identical subunits, recognizes the "pseudo(2-fold)symmetric" operator sequence asymmetrically. Base substitutions within the consensus half of the operator affect binding more than base substitutions within the nonconsensus half of the operator. Furthermore, changing the nonconsensus base pairs to the consensus base pairs does not increase, but decreases, binding. Evidently, the two subunits of the λ repressor dimer bind to the two halves of the operator differently. This is consistent with the recently determined crystal structure of the complex, which shows that the relative positioning of the amino acids to the DNA bases are slightly different in the two halves of the operator. The sequence-specific interactions indicated by the systematic base-substitution experiments correlate well with the locations of the specific contacts found in the complex. Thus, the amino acids of λ repressor, mainly of α3-helix and the N-terminus arm, seem to directly read-out the DNA sequence by forming specific hydrogen bonds and hydrophobic contacts to the DNA bases. The observed asymmetric recognition suggests that no recognition code governs amino acids and DNA bases in protein–DNA interactions.**

The λ repressor dimer binds to operator DNA by recognizing approximate 2-fold symmetry in each of the six operator sequences of λ DNA O_R1–3 and O_L1–3 (1, 2). Structural studies have shown that each subunit of λ repressor contains a helix-turn-helix motif, which fits into the major groove of the half-operator for specific binding (3–5). The λ repressor dimer also contains two N-terminus arms that wrap around the DNA to contact the central base pairs within the operator (6). The DNA–amino acid interactions of the α3-helix of the helix-turn-helix motif and of the N-terminus arm have been shown to play important roles in the specificity and energetics of binding (4, 5, 7, 8). Lewis *et al.* (5) used model building to predict the sequence-specific contacts that might occur between amino acids and DNA bases.

Recently, Jordan and Pabo (9) determined the structure of the λ repressor–operator DNA complex at high resolution by x-ray diffraction. The crystal structure of the complex shows a number of specific contacts, such as hydrogen bonds and hydrophobic contacts, mainly between the amino acids of the α3-helix and the DNA bases. Several questions confront us: (*i*) How are these specific contacts used to recognize the specific operator sequences? (*ii*) Are these specific contacts alone enough to explain the specific recognition of the operator sequences from other DNA sequences? (*iii*) Are these contacts able to explain the quantitative differences in binding among the six operators? (*iv*) Do some other factors, such as phosphate interactions or changes in DNA conformation, also play major roles in sequence recognition? To answer these questions we must know quantitatively how each of such specific contacts (or some other factors) energetically contributes to specific binding.

Systematic base-substitution studies seem to suit experimental design needs best because such experiments locate precisely sequence-specific interactions within the binding site and at the same time reveal quantitatively how each specific interaction energetically contributes to specific binding (10). When binding of λ repressor was analyzed by systematic base-substitution experiments, λ repressor was found to bind to operator DNA quite asymmetrically with respect to the center of the approximate 2-fold symmetry of the binding site. We analyzed why and how the λ repressor dimer, made of identical subunits, binds to the pseudosymmetric operator sequence asymmetrically and compared our results with the recently determined crystal structure of the complex (9). Based on these analyses we discuss the sequence-recognition mechanism of λ repressor and the implication of this mechanism in protein–DNA interactions generally.

MATERIALS AND METHODS

Operator DNAs. λ operators are thought to be 17 base pairs (bp) long (11), but we synthesized DNA as a 21-mer by adding 2 bp at each end as they occur in the λ DNA sequence (11, 12) to avoid end effects and to see the effects of base substitutions outside the 17-bp operator sequence. Systematic base-substitution experiments were done on the O_R1 operator sequence. Each base pair of the O_R1 21-mer was substituted with 3 other bp, and each thymine was also replaced with uracil. All DNAs were synthesized by using the BioSearch model 8600 DNA synthesizer and purified by gel electrophoresis by the Nucleic Acid and Protein Synthesis group at the Frederick Cancer Research Facility. To form duplex DNA, purified complementary strands were mixed in equal amounts in 10 mM Tris·HCl, pH 7.4/1 mM EDTA/0.1 M NaCl, heated to 90°C and incubated at 45°C overnight. DNAs (1–2 μg) were end-labeled with [γ-^{32}P]ATP (New England Nuclear) using T4 polynucleotide kinase (Bethesda Research Laboratories) (13), and the labeled DNAs were purified by passing them through a Sephadex G-50 column.

λ Repressor. λ repressor was purified to homogeneity from λ repressor overproducer cells (cells containing plasmid pEA300WT) with some modifications of the published procedure (14). Whenever needed, λ repressor was diluted in dilution buffer [50% (vol/vol) glycerol/10 mM Tris·HCl, pH

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Abbreviation: ΔΔ*G*, free energy change relative to O_R1 operator sequence.

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7.4/0.1 M KCl/2 mM $CaCl_2$/0.1 mM EDTA containing bovine serum albumin at 100 μg/ml] just before use.

Filter Binding Assay and Estimation of Free Energy Changes. All filter binding assays were done by using plastic test tubes, essentially as described (10, 15, 16). The K_d values were determined by the saturation experiments. [^{32}P]DNA and varying amounts of λ repressor were incubated in 100 μl of binding buffer [5% (vol/vol) glycerol/10 mM Tris·HCl, pH 7.4/0.1 mM EDTA/50 mM KCl/2 mM $CaCl_2$/0.2 mM dithiothreitol containing bovine serum albumin at 100 μg/ml] at 0°C for 5 min, and the samples were filtered through a nitrocellulose filter (Schleicher & Schuell; BA-85, 0.22 μm) in ≈4–5 sec under suction. Filters were dried and counted by a liquid scintillation counter. Free energy changes relative to O_R1 ($\Delta\Delta G$) were calculated by the equation $\Delta\Delta G = -0.546$ ln (K_d of substituted sequence)/(K_d of O_R1) at 0°C, at which the experiments were done.

RESULTS

Asymmetric Binding of λ Repressor to Operator DNA. Fig. 1 shows the relative affinity changes in terms of $\Delta\Delta G$ to wild-type O_R1, associated with the base or base-pair substitutions at each position of the 17-bp operator sequence. A positive $\Delta\Delta G$ means reduced binding and vice versa. The base-specific interactions of λ repressor are confined within the 17-bp operator sequence. We did not detect any significant affinity changes for substitutions outside of this region. The data show that essentially all substitutions within the 17-bp sequence, including the substitutions of the nonconsensus base pairs at positions 3′, 5′, and 7′ to the consensus base pairs, reduce binding. Exceptions are two substitutions at position 3 and one substitution at position 5′, which slightly increase binding. Large $\Delta\Delta G$ values occur with all the 3 bp substitutions at positions 2, and 5–9 in the consensus half-operator, and with 2 bp substitutions at position 6′ and 1 bp substitution at position 7′ in the nonconsensus half-operator. Base pairs at these positions thus seem to participate in strong interactions. The $\Delta\Delta G$ values are asymmetric with respect to the center of approximate 2-fold symmetry of the binding site, thereby suggesting that the two subunits of λ repressor would interact with the two half-operators differently.

Role of Thymine Methyl Groups in the Specific Binding. The data (Fig. 1) indicate that the substitutions of thymines with uracils at positions +1, −5, +1′, +5′, and +7′ result in $+\Delta\Delta G$ values, and, thus, the methyl groups at these positions probably participate in favorable hydrophobic interactions. In particular, removal of the methyl group of thymine at position −5 results in 1.8 kcal/mol (1 cal = 4.184 J) of free energy change, suggesting that this methyl group is in a very hydrophobic environment in the binding complex. Note that removal of the methyl group of thymine at the 2-fold-related position −5′ [i.e., removal of the methyl from the left half-operator of O_L1, as substitution of T·A with A·T at this position generates O_L1] has little effect on the binding, indicating that this methyl group is in a different environment. The data (Fig. 1) show that removal of the methyl groups of thymines at positions −2, −2′, +3′, and −6′ affects the binding very little, whereas removal of the methyl groups of thymines at positions +3, +5, and +7′ slightly stabilizes the binding, indicating that the methyl groups at the latter positions have either slight steric hindrances or are involved in other weak unfavorable interactions.

Binding of λ Repressor to the Six Operators. We synthesized the six operator sequences all as 21-mers, as they occur in the λ DNA sequence (11, 12) and measured the binding affinities of λ repressor to these operators. As shown in Table 1, λ repressor binds most strongly to O_L1 and O_R1 followed by O_L2, O_L3, O_R2, and O_R3. Using the $\Delta\Delta G$ data (Fig. 1), we calculated the binding affinities of these operators by simply adding the associated $\Delta\Delta G$ for the operator sequences. Table 1 shows that the calculated affinities agree with the measured one, when the strong- and weak-binding sites are properly assigned to the half-operators. This fact suggests that λ repressor binds to all six operators asymmetrically.

Why Does λ Repressor Bind to the Operator DNA Asymmetrically? Agreement between the calculated and measured free energy changes means that the free energy changes for the individual interactions are mostly additive for the binding of λ repressor to the operator (also see below). We used this rule to examine how sequence deviations affect the asym-

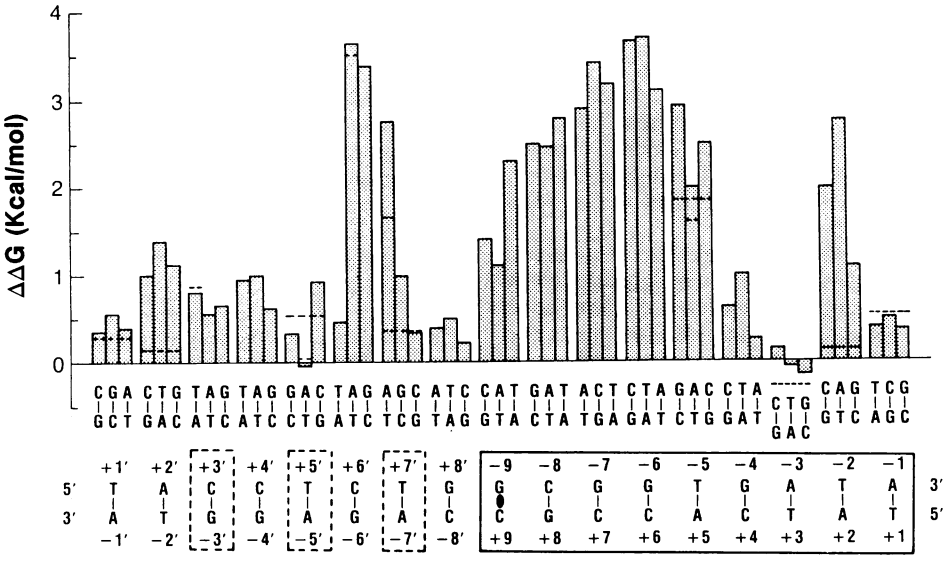

FIG. 1. Relative free energy changes ($\Delta\Delta G$) in the binding of λ repressor to O_R1 upon base substitutions. The affinities (K_d) of λ repressor to wild-type O_R1 and all possible single base-substitution mutants are determined by the filter binding assay; the $\Delta\Delta G$ values are calculated as described in the text. K_d for O_R1 is 10^{-9} M under assay conditions. The sequence shown at bottom is O_R1; the right half represents the consensus half-operator (solid line box), and the left half represents the nonconsensus half-operator containing three nonconsensus base pairs at positions 3′, 5′, and 7′ (broken line box). Each solid bar represents the $\Delta\Delta G$ due to the indicated base-pair substitution. Each dashed line that crosses over three bars or one bar represents the $\Delta\Delta G$ from replacement of thymine of the O_R1 or mutant with uracil, respectively.

Table 1. Binding affinities of λ repressor to the six naturally occurring operators

Operator DNA sequence		ΔΔG, kcal/mol	
		Predicted	Measured
O_L1	TACCACTG[GCGGTGATA]	−0.1	−0.2
O_R1	TACCTCTG[GCGGTGATA]	0.0	0.0
O_L2	TATCTCTGGCGGTGTTG	0.9	0.8
O_L3	AACCATCT[GCGGTGATA]	1.3	1.0
O_R2	CAACACGCACGGTGTTA	2.8	2.0
O_R3	TATCCCTT[GCGGTGATA]	2.0	2.2

All ΔΔG values, relative to O_R1, were predicted simply by adding the ΔΔG values for base alterations according to the ΔΔG data of Fig. 1, assuming that the right half corresponds to the strong-binding site and the left half corresponds to the weak-binding site. Binding measurements were done as described in the text. The consensus sequence is boxed, and base alterations from O_R1 are underlined.

metric binding. We changed 3 nonconsensus bp of O_R1 at positions 3′, 5′, and 7′ to consensus base pairs step by step and measured binding. When calculated free energy changes agreed with the measured change, we interpreted the data to mean that subunit interactions had not changed. We also monitored the change in subunit interactions by measuring the change of ΔΔG due to substitution at position 8′.

The results in Table 2 indicate that the subunit interactions do not change by the double base-pair alterations in any combination, regardless of whether the central base pair is G·C or C·G (sequences 1–7) but do change when all 3 nonconsensus bp are altered concomitantly to the consensus base pairs (sequences 8–10). λ repressor binds to the symmetric consensus operator (sequence 10) more weakly (by +0.5 kcal/mol) than to the O_R1 operator. This value differs from the value predicted for strong binding (ΔΔG = −3.5 kcal/mol) or weak binding (ΔΔG = +1.8 kcal/mol) of both subunits. This result contrasts with the symmetric binding of Cro repressor, which shows highest affinity to the symmetric consensus sequence (10). The above results suggest that the sequence difference in the two half-operators may not be the primary cause of the asymmetric binding. We propose that the asymmetric binding is probably caused by steric clash between the two N termini at the center of the operator and subsequent dislocation of the subunit when the two subunits try to bind to the operator symmetrically. This idea is consistent with the observations that λ repressor binds specifically, though with reduced affinity, to the symmetric operator containing a 1-bp insertion next to the central base pair (sequence 11), whereas λ repressor binds only nonspecifically to O_R1 containing the same 1-bp insertion (sequence 12). λ repressor binds only nonspecifically to O_R1 with a deleted central base pair (sequence 13).

Additivity of Free Energy Changes. Fig. 2 shows the correlation plot between the predicted and measured ΔΔG values for the operator and nonoperator DNAs containing various multiple substitutions. From the results it is clear that ΔΔG values are mostly additive for the specific binding of λ repressor. We showed before that free energy changes are also mostly additive for the specific binding of Cro repressor (10). Additivity of ΔΔG values is significant, because it enables us to predict the binding affinity of λ repressor to any DNA sequence by using the free energy data shown in Fig. 1.

Correlation Between the Sequence-Specific Interactions Indicated by the Systematic Base-Substitution Experiments and the Specific Contacts Found in the Complex. Jordan and Pabo (9) recently determined the structure of the λ repressor–O_L1 operator DNA complex at high resolution by the x-ray diffraction method. Fig. 3 *Left* illustrates the specific contacts in the consensus half-operator, as described in ref. 9 except hydrogen bonds between Lys-4 and guanine at position 7 (see below). Fig. 3 *Right* shows the specific contacts in the nonconsensus half-operator. We generated this figure by analyzing the structure of the complex because Jordan and Pabo (9) barely discussed the nonconsensus contacts, saying that they were similar to contacts in the consensus half-operator. Our analysis seems to confirm asymmetric binding.

The structure of the complex (9) suggests that Gln-44 (first surface amino acid on the α3-helix) forms double hydrogen bonds with the adenine at position 2. This interaction is further stabilized by Gln-33, which hydrogen-bonds to Gln-44 and the phosphate. This extended hydrogen-bonding network appears to correspond to the relatively large free energy changes at position 2, particularly in the consensus half-operator (see Fig. 1). The γ carbon of Gln-44 and the methylene carbon of Glu-34 are very close to the methyl group of thymine at position 1, which seem to participate in hydrophobic contacts (9). Our free energy data are consistent with these interactions (Fig. 1). Ser-45 forms a hydrogen bond to the N-7 of guanine at position −4 in the consensus half-operator, whereas the 2-fold-related Ser-245 seems to interact with the two guanines at positions −3′ and −4′ in the

Table 2. ΔΔG with O_R1 substitutions, insertions, and deletions

No.	DNA sequence Position 1′2′3′4′5′6′7′8′987654321	Base changes from O_R1	ΔΔG, kcal/mol Predicted	Measured
	Multiple substitutions at positions 3′, 5′, 7′, 8′, and 9			
1	TTTACCACTTGCGGTGATAAT	5′c,8′	0.3	0.4
2	TTTACCTCTTCCGGTGATAAT	8′,9	1.9	1.5
3	TTTACCACCGGCGGTGATAAT	5′c,7′c	0.2	0.1
4	TTTACCACCTGCGGTGATAAT	5′c,7′c,8′	0.6	0.3
5	TTTATCACTTGCGGTGATAAT	3′c,5′c,8′	1.1	1.0
6	TTTATCTCCTGCGGTGATAAT	3′c,7′c,8′	1.4	1.3
7	TTTACCACCTCCGGTGATAAT	5′c,7′c,8′,9	2.0	1.8
8	TTTATCACCTGCGGTGATAAT	3′c,5′c,7′c,8′	1.3	>4.5
9	TTTATCACCTCCGGTGATAAT	3′c,5′c,7′c,8′,9	2.7	>4.5
10	TTTATCACCGGCGGTGATAAT	3′c,5′c,7′c (consensus)	(1.8 or −3.5)	0.5
	Insertions and deletion			
11	TTTATCACCGCGCGGTGATAAT	Insertion (consensus 22-mer)	—	2.1
12	TTTACCTCTGCGCGGTGATAAT	Insertion (O_R1 22-mer)	—	>4.5
13	TTTACCTCTG–CGGTGATAAT	Deletion (O_R1 20-mer)	—	>4.5

Underlined bases in the DNA sequence indicate alterations from the O_R1 sequence. The c (after base position) denotes change to the consensus base. ΔΔG values for the respective DNA sequences were predicted by adding ΔΔG values for base alterations according to the data of Fig. 1 or measured as described in the text.

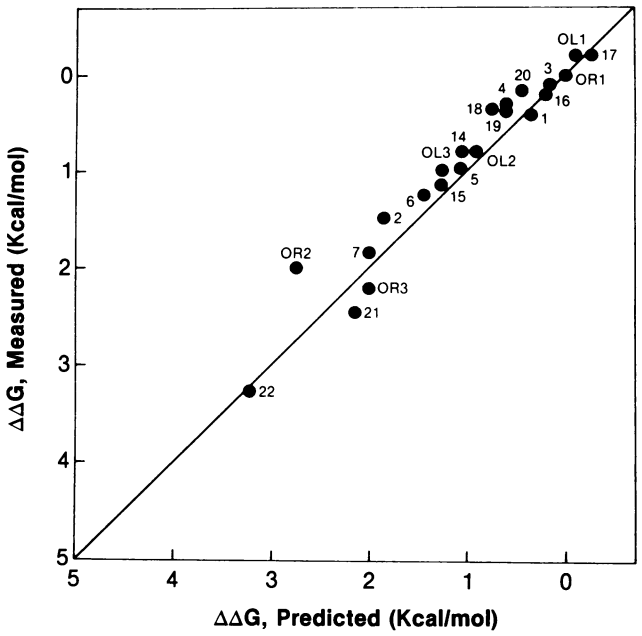

FIG. 2. Correlation plot between the predicted and measured binding free energy changes. Numbers correspond to the sequences in Tables 1 and 2. Additional operator sequences used include the following: 14, TTTACCTCGCGCGGTGATAAT; 15, TTTACCCCTTGCGGTGATAAT; 16, TTTACCTCTGGCGGTATTAAT; 17, TTTACCTCTGGCGGTGAUAAT; 18, TTTACCUCTGGCGGTGATAAT; 19, TTTACCTCUGGCGGTGATAAT; and 20, TTUACCTCTGGCGGTGATAAT (underlined A indicates the position at which the opposite strand contains uracil). Nonoperator DNAs used include 21, TTCAGCATCAGCGGTGATGAT and 22, TTGAACGCCAGCGGTGTGGAT. The 45° straight line shows degree of agreement.

nonconsensus half-operator (9). This result is also consistent with the free energy data, showing that only position 4 participates in the specific interaction in the consensus half-operator, whereas positions 3′ and 4′ participate in the nonconsensus half-operator. The methyl group of thymine at position −5 is surrounded by Gly-46 and the β carbon of Ser-45 in a very hydrophobic environment, whereas the corresponding methyl group of thymine at position −5′ is not in the same environment (because the α3-helix has slightly shifted its position toward the phosphate). Consistent with this, the methyl group of thymine at position −5 is observed to participate in strong hydrophobic interaction, but the methyl group at position −5′ does not do so (see above). Because Ile-54 and Gly-48 are close to the methyl group of thymine at position 3, Jordan and Pabo (9) have suggested that these residues may be involved in hydrophobic contacts. Our free energy data, however, indicate that this methyl group is rather involved in mild steric clash. The amino group of Asn-55 (of the loop after α3-helix) hydrogen-bonds to the N-7 of guanine at position −6, and the ε amino group of Lys-4 (of the N-terminus arm) hydrogen-bonds to the O-6 of the same guanine and the carbonyl oxygen of Asn-55. Although Jordan and Pabo haven't suggested the possibility, our inspection of the structure of the complex suggests that the ε amino of Lys-4 is also in hydrogen-bonding distances to the N-7 and O-6 of guanine at position −7. This hydrogen-bonding network seems to explain the very specific recognition of the base pairs at positions 6 and 7 in the consensus half-operator (Fig. 1). In the nonconsensus half-operator, Asn-255 hydrogen-bonds to the N-7 of guanine at position −6′ and the phosphate. Lys-205 (of the N-terminus arm) hydrogen-bonds mainly to the phosphate but may weakly interact with the N-7 of adenine at position −7′. The free energy data are consistent with these interactions because they show that the purines are equivalent at positions −6′ and −7′, suggesting the specific recognition of the N-7 positions of purines. Although the N-terminus amino acids, Ser-1–Thr-2–Lys-3, are expected to participate in the specific interactions with the central base pairs 8 and 9, neither the positions of these amino acids nor their specific contacts have yet been determined with certainty. Jordan Pabo (9) have temporarily assigned Thr-2 to the guanine at position +8, and Ser-1 to the guanine at position −9, but we suspect the participation of Lys-3 in the specific contact at position 8, because contacts made by threonine or serine are usually weak and may not explain the large free energy change at position 8. The phosphate interactions are similar in the consensus and nonconsensus half-operators, and their locations do not seem to correlate with the base-specific free energy changes (Figs. 1 and 3). λ repressor binds to almost straight B-DNA (9), and, thus, change in DNA conformation does not seem to contribute significantly to the specificity of the binding of λ repressor.

DISCUSSION

The present analysis shows that the λ repressor dimer, made of identical subunits, binds to the pseudosymmetric operator sequences asymmetrically. This is a surprise, because Cro repressor binds to the same sequences symmetrically (10), and dimers of many repressors and activators are generally thought to bind to the palindromic sequences symmetrically (5, 20, 21). The above analysis indicates that (*i*) one subunit interacts more strongly with the consensus half-operator because the amino acids of this subunit participate in both strong hydrogen bondings (partly due to hydrogen-bonding networks) and hydrophobic interactions with the DNA bases, whereas (*ii*) the other subunit interacts only weakly with the nonconsensus half-operator because the amino acids of the latter subunit make fewer isolated specific contacts to the DNA bases. These differences seem to come from the slight difference in geometry of the contact surface of the protein with respect to the contact surface of the DNA major groove in the two half-operators. The differences in geometry are rather small (9), but these differences seem to yield amazingly large differences in binding specificity (see Fig. 1). This relationship indicates the importance of geometry in protein–DNA recognition.

Thus, there are good correlations between the locations of the specific amino acid–base contacts seen in the λ repressor–operator complex and the base-specific affinity changes detected by our systematic base-substitution experiments. Furthermore, we have shown that the affinities of the six operators, or of essentially any DNA sequence, can be predicted by using the $\Delta\Delta G$ data of Fig. 1. These observations strongly suggest that λ repressor recognizes a specific DNA sequence primarily by a direct read-out mechanism. We previously had showed that the amino acids of Cro repressor (in this case, almost exclusively of the DNA recognition α3-helix) also reads-out the DNA sequence by making direct specific amino acid–base contacts (10). Judging from the presence of the extensive specific amino acid–base contacts, 434 repressor (22), 434 Cro repressor (23), and restriction enzyme *Eco*RI (24) also seem to recognize the respective DNA sequences by this direct read-out mechanism. In contrast to these situations, the crystal structure of the *trp* repressor–operator DNA complex (25) showed mostly phosphate interactions and very few specific amino acid–base contacts. Otwinowski *et al.* (25) thus claim that *trp* repressor may recognize the specific DNA sequence indirectly through its effects on the geometry of the phosphate backbone.

The fact that the two identical subunits of λ repressor recognize the very similar half-operator sequences differ-

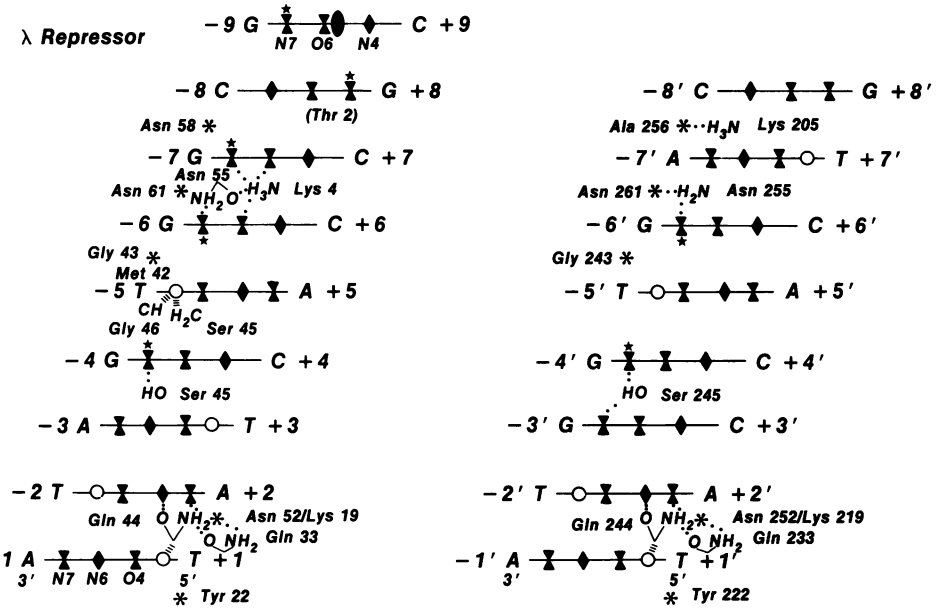

FIG. 3. Specific contacts between amino acids of λ repressor and edges of the DNA bases within the major groove and the phosphate interactions, as seen in the structure of the λ repressor–O_L1 operator DNA complex. (*Left*) Interactions in the consensus half-operator as described by Jordan and Pabo (9), except for hydrogen bonds between Lys-4 and guanine at position 7. (*Right*) Interactions in the nonconsensus half-operator, which is generated by analyzing the structure of the complex in accord with the observed $\Delta\Delta G$. DNA and the functional groups exposed within the DNA major groove are illustrated according to Woodbury *et al.* (17). ⧗, Hydrogen acceptor; ♦, hydrogen donor; ○, thymine methyl; ⋆, N-7 of guanine that is protected from methylation (7, 18, 19); ✱, phosphate that is protected from ethylation upon binding of λ repressor (19); ••, hydrogen bonds; |||, hydrophobic contacts to the methyl group of thymine.

ently raises the question concerning whether there is a recognition code (a code equivalent to the genetic code) between amino acids and DNA bases in DNA–protein interactions (5, 26). Examination of the evidence requires attention to the following: (*i*) In DNA–protein recognition, interactions are highly geometry dependent, as we show here. (*ii*) Interactions between amino acids and bases are very degenerate, and the same amino acid can interact with different bases in a variety of ways. (*iii*) Base pairs are generally only loosely recognized, and specific recognition of base pairs seems to be quite rare (recognition of positions 6 and 7 by λ repressor represents such rare cases; unpublished results). (*iv*) Interactions are context dependent. Jordan and Pabo (9) observed the concerted interactions between Gln-44 and Gln-33 and between Asn-55 and Lys-4 in the λ repressor complex. Aggarwal *et al.* (22) also found a similar extended interaction between two glutamines at the start of helix 2 and helix 3 in the 434 repressor complex. We observed that formation of specific amino acid–base contacts vitally depends on the van der Walls contacts between amino acid side chains in the Cro repressor–DNA interface (Y.T., A.S., and Fred Hausheer, unpublished work). Because of this conflicting evidence, we believe that the existence of a recognition code between amino acids and DNA bases in protein–DNA recognition is very unlikely.

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1. Ptashne, M., Jeffrey, A., Johnson, A. D., Maurer, R., Meyer, B. J., Pabo, C. O., Roberts, T. M. & Sauer, R. T. (1980) *Cell* **19**, 1–11.
2. Johnson, A. D., Poteete, A. R., Lauer, G., Sauer, R. T., Ackers, G. K. & Ptashne, M. (1981) *Nature (London)* **294**, 217–223.
3. Pabo, C. O. & Lewis, M. (1982) *Nature (London)* **298**, 443–447.
4. Lewis, M., Jeffrey, A., Wang, J., Ladner, R., Ptashne, M. & Pabo, C. O. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **47**, 435–440.
5. Pabo, C. O. & Sauer, R. T. (1984) *Annu. Rev. Biochem.* **53**, 293–321.
6. Pabo, C. O., Krovatin, W., Jeffrey, A. & Sauer, R. T. (1982) *Nature (London)* **298**, 441–443.
7. Eliason, J. L., Weiss, M. A. & Ptashne, M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2339–2343.
8. Hecht, M. H., Nelson, H. C. & Sauer, R. T. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2676–2680.
9. Jordan, S. R. & Pabo, C. O. (1988) *Science* **242**, 893–899.
10. Takeda, Y., Sarai, A. & Rivera, V. M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 439–443.
11. Maniatis, T., Ptashne, M., Backman, K., Kleid, D., Flashman, S., Jeffrey, A. & Maurer, R. (1975) *Cell* **5**, 109–113.
12. Humayun, Z., Jeffrey, A. & Ptashne, M. (1977) *J. Mol. Biol.* **112**, 265–277.
13. Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–564.
14. Johnson, A. D., Pabo, C. O. & Sauer, R. T. (1980) *Methods Enzymol.* **65**, 839–856.
15. Kim, J. G., Takeda, Y., Matthews, B. W. & Anderson, W. F. (1987) *J. Mol. Biol.* **196**, 149–158.
16. Riggs, A. D., Suzuki, H. & Bourgeois, S. (1970) *J. Mol. Biol.* **48**, 67–83.
17. Woodbury, C., Hagenbuchle, P. & von Hippel, P. H. (1980) *J. Biol. Chem.* **255**, 11534–11546.
18. Johnson, A. D., Meyer, B. J. & Ptashne, M. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1783–1787.
19. Johnson, A. D. (1980) *Ph.D. thesis* (Harvard University, Cambridge).
20. von Hippel, P. H. (1979) in *Biological Regulation and Development*, ed. Goldberger, R. F. (Plenum, New York), Vol. 1, pp. 279–347.
21. Takeda, Y., Ohlendorf, D. H., Anderson, W. F. & Matthews, B. W. (1983) *Science* **221**, 1020–1026.
22. Aggarwal, A. K., Rodgers, D., Drottar, M., Ptashne, M. & Harrison, S. C. (1988) *Science* **242**, 899–907.
23. Wolberger, C., Dong, Y., Ptashne, M. & Harrison, S. C. (1988) *Nature (London)* **335**, 789–795.
24. McClarin, J. A., Frederick, C. A., Wang, B.-C., Greene, P., Boyer, H. W., Grable, J. & Rosenberg, J. M. (1986) *Science* **234**, 1526–1541.
25. Otwinowski, Z., Schevitz, R. W., Zhang, R.-G., Lawson, C. L., Joachimiak, A., Marmorstein, R. Q., Luisi, B. F. & Sigler, P. B. (1988) *Nature (London)* **335**, 321–329.
26. Ebright, R. H. (1986) in *Protein Structure, Folding, and Design*, ed. Oxender, D. L. (Liss, New York), pp. 207–219.