DNA specificity determinants of *Escherichia coli* tryptophan repressor binding

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We have analyzed the sequence-specific interaction between the *Escherichia coli* tryptophan (Trp) repressor and its operator using challenge phage vectors. These phages, derivatives of *Salmonella* phage P22 that have substitutions of synthetic, symmetric *trp* operators for the P22 *mnt* operator, provide a genetic assay for DNA binding in vivo. Phages carrying operators that retain the determinants of Trp repressor binding efficiently lysogenize cells producing repressor; in contrast, phages with operators missing critical determinants kill such hosts. The binding determinants revealed by this assay corroborate a simple docking model for the Trp repressor–operator interaction postulated from the repressor crystal structure, and account for both the specificity of repressor binding and the ability of Trp repressor to recognize multiple, tandem DNA sites.

[Key Words: Trp repressor; DNA binding; *trp* operator; phage P22]

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The *Escherichia coli* *trpR* gene encodes a *trans*-acting negative regulator of tryptophan biosynthesis (Cohen and Jacob 1959; Yanofsky 1971; Zubay et al. 1972). The product of the *trpR* gene is a small peptide of 108 amino acid residues that assembles as a dimer aporepressor molecule and binds the corepressor, L-tryptophan, to form an active repressor complex (Gunsalus and Yanofsky 1980; Joachimiak et al. 1983; Arvidson et al. 1986). Active Trp repressor binds specifically to at least three targets on the *E. coli* chromosome to regulate initiation of transcription of the *trp* operon (Bennett and Yanofsky 1978), the *aroH* gene (encoding the tryptophan-repressible 3-deoxy-D-arabino heptulosonic acid-7-phosphate synthetase) (Zurawski et al. 1981; Grove and Gunsalus 1987), and the *trpR* gene itself (Gunsalus and Yanofsky 1980). The three operators share extensive sequence homology and are located at different positions relative to their respective promoters (Fig. 1).

The *trp* and *aroH* operators are composed of multiple, tandem Trp repressor binding sites, presumably each of which is bound by a repressor dimer. The *trp* operator has three tandem sites, whereas the *aroH* operator has two sites. The *trpR* operator is the simplest of the three and is presumably bound by a single repressor dimer (Kumamoto et al. 1987). An axis of twofold symmetry divides each of the six natural binding sites into two half-sites. A consensus of the 12 half-sites shows that the 10 central base pairs of each half-site are strongly conserved. Constitutive mutations in the operator controlling *trp* operon expression identify three of these 10

base pairs as critical for Trp repressor binding (Bennett and Yanofsky 1978).

As a first step in identifying the nucleotide base pair/amino acid residue interactions that underlie the binding of Trp repressor to DNA, we have made all possible symmetric pairs of single base pair substitutions at each position of a Trp repressor binding site and have assayed the binding of wild-type Trp repressor to each of these sites in vivo. To assay Trp repressor binding, we have constructed derivatives of phage P22, called challenge phages, that place the expression of the P22 *ant* (antirepressor) gene under the control of each variant repressor binding site.

A challenge phage selectively lysogenizes, and spares from death, host cells producing an activity that binds the substituted operator site. This occurs because the "decision" between lytic and lysogenic development made by a challenge phage is governed by the state of occupancy of the operator controlling transcription of the *ant* gene. Substitution of a synthetic, idealized *trp* operator for the naturally occurring P22 (*mnt*) operator places *ant* expression under the control of Trp repressor. After infection of a sensitive *sup*⁰ host, a challenge phage will establish lysogeny if its operator is bound by Trp repressor, and confer a kanamycin-resistant phenotype upon its host. Alternatively, if the operator controlling *ant* synthesis is free of repressor, a challenge phage will synthesize antirepressor, grow lytically, and efficiently kill its host (Benson et al. 1986).

We have used a set of challenge phages carrying

Figure 1. Trp repressor binds to multiple targets. (a) The DNA sequences of the top strands of the three natural operators controlling the *E. coli* *trp*, *aroH*, and *trpR* operons are shown; numbers indicate the positions of base pairs with respect to the various start points of transcription (see Gunsalus and Yanofsky 1980). The most critical base pairs for repressor binding in each operator half-site are indicated in boldface. Presumably, three Trp repressor dimers contact successive pairs of major grooves of the *trp* operator, and two dimers bind the *aroH* operator, adjacent to faces of the double helix that are rotated with respect to one another by about 270° (Kumamoto et al. 1987). (b) Each half-site of the six repressor binding sites is indicated by an arrow. Base pairs are included in the consensus sequence if they occur in 6 or more of the 12 half-sites; otherwise a position is designated "N". The reference-type sequence is the synthetic *trp* operator sequence used in these studies.

variant *trp* operators derived from reference-type *trp* operators by symmetric single base pair substitutions to define the base pairs within the *trp* operator that are critical for Trp repressor binding. Our results, taken together with the crystal structure of Trp repressor (Schwitz et al. 1985) and data from chemical protection experiments that define the interaction of Trp repressor with naturally occurring operators (Kumamoto et al. 1987), enable us to construct a model for the specificity determinants of the Trp repressor/operator interaction.

Results

A selection for Trp repressor binding

To begin to analyze the Trp repressor–operator binding interaction, we synthesized a symmetric *trp* operator (Fig. 1). This oligonucleotide was introduced into the subcloned regulatory region of the phage P22 *ant* operon on plasmid vector pPY140 to construct plasmid pPY360. As shown in Figure 2, this idealized, symmetric Trp re-

pressor binding site (designated "ref2" in Table 1) is substituted for the *mnt* operator, and is located at the start point of *ant* transcription. Plasmid pPY360 was crossed with P22 Kn9 *arc-amH1605* phage to construct a challenge phage carrying the synthetic *trp* operator in place of the *mnt* operator (Benson et al. 1986). This phage cannot lysogenize a sensitive *sup⁰ trpR⁺* host, indicating that the amount of Trp repressor synthesized from the *Salmonella* chromosome is insufficient to effect full repression of the phage-borne *ant* operon. Because the fractional occupancy of a DNA-binding site increases with increasing repressor concentration, we reasoned that the challenge phage with this idealized *trp* operator should lysogenize a host that overproduces Trp repressor and can saturate this site.

To construct such a host, we transformed a derivative of plasmid pRPG47 (Arvidson et al. 1986), pPY150, into the *sup⁰ Salmonella* host MS1868/F' *lacI^{Q1}*. Plasmid pPY150 (Benson et al. 1986) carries the cloned *E. coli* *trpR* gene under the control of the inducible *lacUV5* promoter; the F' episome carried by the host overproduces Lac repressor and prevents transcription of the *trpR* gene from pPY150 in the absence of inducer. This host allows us to regulate the amount of Trp aporepressor produced from plasmid pPY150 by varying the amount of added inducer of Lac repressor, isopropyl-β-D-thiogalactoside (IPTG).

As shown in Figure 3(a), MS1868/F' *lacI^{Q1}* carrying plasmid pPY150 is efficiently lysogenized, in an IPTG-dependent way, by a challenge phage with an idealized *trp* operator. The efficiency of lysogeny increases over 1000-fold upon induction of *trpR* transcription from the *lac* promoter with IPTG in the presence of excess corepressor. Therefore lysogeny is dependent upon expression of *trpR* from pPY150.

The efficiency of lysogeny is also dependent on the amount of exogenous corepressor. As shown in Figure 3(b), lysogeny is inefficient at low tryptophan concentrations and increases to about 50% at higher tryptophan concentrations. These data show that the amount of active repressor complex is limited by the amount of added corepressor. At low concentrations of IPTG or tryptophan, lysogens grow as small, dark green colonies on green indicator plates. This phenotype indicates that these lysogens are unstable and produce insufficient Trp repressor activity to maintain stable repression of the *ant* gene. At high IPTG and tryptophan concentrations, lysogens form large, white colonies.

A similar bacterial host, MS1868/F' *lacI^{Q1}* carrying pPY158, a plasmid that is missing *trpR* but is otherwise isogenic with pPY150, is efficiently killed by the same challenge phage, even at high levels of IPTG and tryptophan. Therefore, the ability of a bacterial host to survive infection by a challenge phage with the *trp* operator depends solely on the plasmid-borne *trpR* gene.

Construction of Trp challenge phages

Thirty variants of two idealized, symmetric *trp* operators were synthesized and cloned into a unique restriction site of a plasmid carrying the subcloned P22 *ant*

Figure 2. *trp* operators are positioned at the start point of *ant* transcription on challenge phages. The positions of the wild-type *mnt* operator on plasmid pPY97, of substitutions for the *mnt* operator on plasmids pPY190 and pPY140, and of the synthetic, reference *trp* operators in these plasmids are shown with respect to the start point of transcription of the *ant* promoter. The subclone of the *trp* operator (designated ref1 in Table 1) in pPY190 is pPY350; the subclone (of ref2) in pPY140 is pPY360. Operators were cloned into the *SmaI* site of pPY190 as 18-mers, or the filled-in *XhoI* site of pPY140 as 14-mers. pPY350 and pPY360 differ by transitions at positions -4 and +18; these differences have no apparent effect on repressor binding. Differences between the operator regions of these plasmids are indicated in boldface.

control region (either pPY140 or pPY190; Fig. 2). Each of these variant operators differs from one of the two idealized *trp* operators by a pair of symmetric single base pair substitutions (Table 1). Plasmids with cloned operators were transformed into *Salmonella*, and the transformants were infected with P22 Kn9 *arc-amH1605* phage to cross these operators onto phage. Progeny phage that had acquired operator substitutions were selected as virulent recombinants on the host MS1582 (P22 *c2+ mnt+*) (see Benson et al. 1986). Twenty-seven of 31 operator-containing plasmids yielded recombinants having a virulent phenotype. The genotypes of these recombinant phages were confirmed by the detection of restriction fragment length polymorphisms corresponding to each of their genetic markers and by the DNA sequence analysis of each operator on phage DNA (see Table 1).

Operator determinants of Trp repressor binding

Table 1 shows the efficiencies of survival of Trp repressor-producing cells infected with each of the series of challenge phages carrying symmetrically altered *trp* operators in the presence of an excess of corepressor. Efficiencies of survival range from 50% for phages carrying reference *trp* operators, to less than 2×10^{-6} percent for phages carrying a subset of mutant operators. These Trp repressor-producing cells are efficiently lysogenized by phages with variant operators that retain critical contacts for repressor binding, but not by phages with operators that lack critical contacts. Figure 4 summarizes these data.

Base-pair substitutions at four positions (± 6 , ± 5 , ± 4 , and ± 3) within the operator half-site reduce the efficiency of survival of the infected host by more than 1000-fold, and identify these four base pairs as the most critical determinants of Trp repressor binding. A C : G

base pair at position ± 6 in the half-site is absolutely required; challenge phages carrying operators with any other nucleotide base pair at this position evoke cell death. Phages carrying T : A or C : G base pairs at position ± 5 of the operator lysogenize cells with nearly equal, high efficiencies, whereas phages with A : T or G : C changes at this position do not. Phages carrying operators with G : C or T : A base pairs at position ± 4 , or A : T base pairs at position ± 3 , also kill their hosts.

As we have shown above (Fig. 3), when challenge phage infections are carried out under conditions of low exogenous corepressor concentrations, the efficiency of cell survival is low, presumably because there is less active Trp repressor complex in the infected cells. We see more subtle effects of the operator substitutions by measuring efficiencies of survival after challenge at different concentrations of corepressor. Table 1 shows that operators that bind repressor efficiently in the presence of high concentrations of tryptophan may be subdivided further into three classes: operators bound at the lowest concentration of added tryptophan, operators that are not bound at the lowest concentration but are bound at an intermediate concentration, and operators bound only at the highest concentration.

Conclusions

Operator determinants of binding

Previously, we showed that the *E. coli* Trp repressor can function to repress transcription of the P22 *ant* operon when a symmetric *trp* operator site is substituted for the phage *mnt* operator at the start point of *ant* transcription. P22 challenge phages with an idealized, symmetric *trp* operator lysogenize host cells that produce sufficient Trp repressor activity and lyse cells that do not produce sufficient Trp repressor activity (Benson et al. 1986).

Table 1. DNA-binding properties of synthetic *trp* operators

Operator	Sequence	EOS	[tryptophan]		
			1	5	20
ref1	CCGAACTAGTTAACTAGTTCGG	50	+	+	+
10G	<u>G</u> GAACTAGTTAACTAGTTCG	30	+	+	+
10A	<u>A</u> GAACTAGTTAACTAGTTCG	40	+	+	+
10T	<u>T</u> GAACTAGTTAACTAGTTCG	40	+	+	+
9A	C <u>A</u> AACTAGTTAACTAGTTG	30	+	+	+
9T	C <u>T</u> AACTAGTTAACTAGTTAG	20	-	-	+
9C	C <u>C</u> AACTAGTTAACTAGTTGG	30	-	+	+
8G	CG <u>G</u> ACTAGTTAACTAGTCCG	40	+	+	+
8T	CGTACTAGTTAACTAGTACG	nd			
8C	CGC <u>A</u> CTAGTTAACTAGTCCG	nd			
ref2	TCGAACTAGTTAACTAGTTCGA	50	+	+	+
7G	CGAGCTAGTTAACTAGCTCG	40	-	-	+
7T	CGA <u>T</u> CTAGTTAACTAGTTCG	30	-	-	+
7C	CGAGCTAGTTAACTAGGTCG	0.2	-	-	+
6G	CGAAGTAGTTAACTAGTTTCG	$<2 \times 10^{-6}$	-	-	-
6A	CGAA <u>A</u> TAGTTAACTAGTTTCG	$<2 \times 10^{-6}$	-	-	-
6T	CGAA <u>T</u> TAGTTAACTAGTTTCG	$<2 \times 10^{-6}$	-	-	-
5G	CGAACGAGTTAACTCGTTTCG	$<2 \times 10^{-6}$	-	-	-
5A	CGAAC <u>A</u> GTTAACTCGTTTCG	$<2 \times 10^{-6}$	-	-	-
5C	CGAACGAGTTAACTCGTTTCG	30	-	+	+
4G	CGAACTGTTAACTAGTTTCG	$<2 \times 10^{-6}$	-	-	-
4T	CGAACT <u>T</u> GTTAACTAGTTTCG	$<2 \times 10^{-6}$	-	-	-
4C	CGAACTCGTTAACTAGTTTCG	3	-	-	+
3A	CGAACTA <u>A</u> TTAATTAGTTTCG	5×10^{-5}	-	-	-
3T	CGAACTA <u>T</u> TTAATTAGTTTCG	6	-	+	+
3C	CGAACTA <u>C</u> TTAATTAGTTTCG	0.5	-	-	+
2G	CGAACTAGGTA <u>C</u> CTAGTTTCG	20	-	+	+
2A	CGAACTAGATATCTAGTTTCG	0.7	-	+	+
2C	CGAACTAGC <u>T</u> AGCTAGTTTCG	60	-	+	+
1G	CGAACTAGTGCCTAGTTTCG	nd			
1A	CGAACTAGTATCTAGTTTCG	nd			
1C	CGAACTAGTCCAGTTTCG	50	+	+	+

Symmetric operators carried by the P22 *Kn9 arc-amH1605* challenge phages are indicated as either reference operators (ref1 and ref2) or variant operators. Variant operators carry symmetric substitutions (underlined), and are designated by number and variant nucleotide in the top strand of the left operator half-site (e.g., 10G). Positions in the operator half-site are numbered outward from the center of symmetry. To determine the efficiency of survival (EOS) of host MS1868/F'*lacI*^{Q1} (pPY150) infected with a particular challenge phage, cells were first grown overnight in LB medium at 37°C. The overnight culture was diluted 100-fold into LB medium with ampicillin (50 µg/ml) and IPTG (10^{-5} M), and grown to a density of 4×10^8 /ml at 37°C. Phage were added to a final multiplicity of infection of 25, and allowed to adsorb for 20 min at 25°C. Infected cells were diluted and spread on green tryptophan drop-out plates supplemented with ampicillin, kanamycin, tryptophan (40 µg/ml), and IPTG (10^{-5} M). The efficiency of survival is the percent of the titer of cells assayed under these plating conditions divided by the titer of input cells assayed on green plates with ampicillin. At high IPTG and tryptophan concentrations, the efficiency of survival of cells carrying the Trp repressor-producing plasmid is independent of the multiplicity of the challenge phage infection for multiplicities between 10 phage/cell and 100 phage/cell (data not presented). The efficiencies of survival were not determined (nd) for phages carrying operators 8T, 8C, 1G, and 1A, since they lysogenize a host that fails to produce Trp repressor, and these operators are presumably bound by a second endogenous *Salmonella* repressor (unpubl.). Under the same conditions, all but these four phages fail to lysogenize a host carrying plasmid pPY158, an otherwise isogenic plasmid that fails to produce Trp repressor. In a separate set of experiments, cells infected with phage were spread on green tryptophan drop-out plates containing ampicillin, kanamycin, IPTG (10^{-5} M), and either 1, 5, or 20 µg/ml tryptophan. The efficiencies of survival of these "challenges" are scored either as + ($>10^{-2}$) or - ($<10^{-2}$). DNAs from the phage stocks used in these experiments were sequenced to confirm their operator genotypes.

To understand the basis of sequence-specific operator recognition by Trp repressor in vivo, we synthesized an exhaustive series of *trp* operators and recombined them

onto challenge phage vectors. Each *trp* challenge phage contains a variant operator that differs from an otherwise isogenic reference operator by a pair of symmetric

Figure 3. Trp repressor binding to a synthetic *trp* operator depends upon the levels of both aporepressor and corepressor. To determine the dependence of the efficiency of survival upon IPTG concentration, a fresh overnight culture of MS1868/*F'**lacI*^{Q1} (pPY150) was diluted 100-fold into LB medium with ampicillin (50 μ g/ml) and IPTG at the indicated concentrations, and grown to a density of 4×10^8 /ml at 37°C. Phage with the reference-type operator *ref2* were added to a final multiplicity of infection of 25, and allowed to adsorb for 20 min at 25°C. Infected cells were diluted and spread on green tryptophan drop-out plates supplemented with ampicillin, kanamycin, tryptophan (40 μ g/ml), and the indicated concentrations of IPTG. The efficiency of survival is the percent of the titer of cells assayed under these plating conditions divided by the titer of input cells assayed on green plates with ampicillin. To determine the dependence of the efficiency of survival upon tryptophan concentration, MS1868/*F'**lacI*^{Q1} (pPY150) was diluted 100-fold into LB medium with ampicillin (50 μ g/ml) and IPTG (10^{-5} M), grown to a density of 4×10^8 /ml at 37°C, and infected with phage at a final multiplicity of 25. After adsorption for 20 min at 25°C, infected cells were diluted and spread on green tryptophan drop-out plates supplemented with ampicillin, kanamycin, IPTG (10^{-5} M), and the indicated concentrations of tryptophan.

single base pair substitutions. Phages carrying variant operators that retain the nucleotide base pairs required for Trp repressor binding efficiently lysogenize cells producing sufficient Trp repressor, whereas phages carrying variant *trp* operators lacking critical contacts for Trp repressor binding lyse the same host. By comparing the efficiencies of survival of Trp repressor-producing cells after infection with each of this series of Trp challenge phages, we have identified the operator determinants of the sequence-specific binding of Trp repressor.

The idealized *trp* operator extends 9 bp in either direction from its axis of dyad symmetry, since mutations at base pair ± 9 in both half-sites have significant effects on Trp repressor binding at low concentrations of added tryptophan, whereas mutations in the two flanking base pairs (positions ± 11 and ± 10) do not affect the ability of repressor to bind (Table 1). The most critical determinants of DNA recognition reside in four base pairs within each *trp* operator half-site, separated by four central base pairs. Phages carrying operators with nucleotide substitutions at these critical positions fail to lysogenize (Fig. 4). Consistent with these results, Bennett and Yanofsky (1978) have isolated constitutive muta-

tions at positions +4, ± 5 , and -6 within the *trp* operator half-sites that represent four of these eight most critical changes. The G : C base pairs in the natural operators implicated in binding by Kumamoto et al. (1987) include the same G : C base pairs identified as important by our genetic method.

A model for the Trp repressor-operator interaction

The active form of Trp repressor is an interlocked symmetric dimer (Joachimik et al. 1983; Schevitz et al. 1985; Arvidson et al. 1986). Exposed on one face of the dimer are two symmetrically disposed "helix-turn-helix" secondary structures (one for each monomer) characteristic of many specific DNA-binding proteins (Pabo and Sauer 1984; Kelley and Yanofsky 1985; Schevitz et al. 1985). Consistent with the idea that this "helix-turn-helix" substructure of the Trp repressor protein is involved in DNA binding, the majority of dominant negative mutations in the *trpR* gene are located within the coding sequence corresponding to this region of the protein (Kelley and Yanofsky 1985; S. Bass, unpubl.).

A preliminary model for the binding of Trp repressor

Figure 4. A subset of operator mutations interfere with Trp repressor binding. The data from experiments at high corepressor concentration presented numerically in Table 1 are represented in two dimensions. The idealized operator sequence occurs near the top of the diagram, numbered by position from the axis of symmetry (arrow). Immediately below the reference sequence are listed the changes that have negligible effects on repressor binding. Below the reference sequence are listed the changes that reduce the ability of repressor to bind. These changes are grouped into two categories, symmetric mutations that reduce survival 5- to 100-fold (mild) and those that reduce survival more than 1000-fold (severe).

to DNA may be constructed by docking this conserved secondary substructure (as it appears in the crystal structure of the repressor–corepressor complex) to two successive major grooves of B-form DNA (Schevitz et al. 1985). Consistent with this, we have shown that the most critical operator determinants of repressor binding are organized into two 4-bp regions separated by a spacing of 4 bp, a geometry that positions functional groups of the base pairs in these two regions in successive major grooves on the same face of B-form DNA.

Our modeling of the binding of repressor to its operator suggests that two regions of Trp repressor dock closely with the operator: the hydrophilic surface of α -helix D, including residues Gln-68, Arg-69, and Lys-72; and, the proximal end of α -helix E, including residues Ile-79, Ala-80, and Thr-81 (see Fig. 4 in Schevitz et al. 1985). We predict that side-chains of the residues in α -helix D interact with base pairs more distal from the operator dyad, whereas α -helix E side-chains contact more central base pairs. In particular, we suspect that the side-chains of Gln-68, Arg-69, or both, may donate a pair of hydrogen bonds to the O⁶ and N⁷ groups of the guanine of the C : G base pair at position ± 6 . This would account for our observation that a C : G base pair is the only base pair that may be tolerated at this position. Second, a functional group on the side-chains of Arg-69 or Lys-72 may donate a proton to the purine N⁷ at position ± 5 , since we have shown that either a T : A or C : G base pair works at this position. Precedents for these specific interactions come from the models proposed for Cro binding (Ohlendorf et al. 1982; Hochschild and Ptashne 1986) and λ repressor binding (Pabo and Lewis 1982; see also Pabo et al. 1983). Third, the Thr-81 hydroxyl group may form a hydrogen bond with the O⁴

group of thymine of the A : T base pair at position ± 4 , since only A : T and C : G base pairs, which have congruent hydrogen bond donor–acceptor pairs in the major groove, work at this position. Such an interaction might result in a steric clash between the methyl group of Thr-81 and the methyl group of thymine when an A : T base pair is present at adjacent operator position ± 3 , accounting for the observation that an A : T base pair is the only base pair not tolerated at ± 3 . Consistent with this, the mutant Thr-81 $\rightarrow$ Ser-81 repressor has an extended specificity of binding; it binds the operator with an A : T base pair at ± 3 , which wild-type repressor cannot (S. Bass, unpubl.).

Therefore, we predict that specific contacts between Trp repressor and its operator are made with both α -helices D and E of the polypeptide. Although Trp repressor has a conserved helix–turn–helix secondary structure, it may use this structure to recognize DNA in a fundamentally different way than does λ repressor, Cro protein, or 434 repressor, which predominantly use the second helix (Hochschild and Ptashne 1986; Anderson et al. 1987). Consistent with this idea, we find several *trpR* mutations that change amino acids Ile-79, Ala-80, and Thr-81 in α -helix E to other residues, and alter the specificity of recognition of *trp* operators with changes at positions ± 3 and ± 4 . Not one of 24 different amino acid changes at these three residue positions affect recognition specificity at positions ± 5 and ± 6 , which we think are contacted by amino acids in α -helix D. Kelley and Yanofsky (1986) failed to find dominant negative mutations that result in amino acid substitutions in α -helix D, since they used mutagens that preferentially cause G : C $\rightarrow$ A : T transitions, and the corresponding region of the *trpR* gene is particularly A : T-rich. Using site-directed mutagenesis, we have found more than 10 such changes. One of these mutant repressors, Lys-72 $\rightarrow$ Tyr, still binds the reference-type operator, whereas Lys-72 $\rightarrow$ Ala does not, arguing strongly that the side-chain of Lys-72 makes a single, specific hydrogen bond with the operator (S. Bass, unpubl.).

The base pairs on either side of the four critical base pairs in each operator half-site (positions ± 7 and ± 2) do not appear to be involved directly in sequence-specific weak bond formation. However, it is clear from our results that changes in these base pairs can have mild but significant effects on binding (Table 1 and Fig. 4). We suspect that, as is the case for the 434 operator (Kouzelka et al. 1987), changes at base pairs that are not directly involved in specific protein–DNA interactions may alter the local structure of *trp* operator DNA, and thereby affect the formation of weak bonds at neighboring positions.

Genetic analysis of binding specificity

We have shown that, using the challenge phage selection, we can identify the operator determinants of a DNA-binding interaction in a thorough manner. Challenge phages with variant *trp* operators that cannot be bound by the wild-type Trp repressor may be used to select or screen Trp repressor mutants with extended and

altered specificities of binding. Further analyses of such mutants should allow us to test our hypotheses concerning the nature of the specific weak chemical bonds that underlie the Trp repressor–operator interaction.

Materials and methods

Bacterial and phage strains

Bacterial strains are derivatives of *S. typhimurium* LT2 or *E. coli* K12. *Salmonella* strains DB7000 [*leuA-am414 sup⁰*; Susskind et al. 1971] and its *supE* derivative MS1363 (Susskind 1980), used for the permissive growth of P22 *arc-am* phages, have been described. Otherwise isogenic hosts, MS1868 (*sup⁰*) and MS1883 (*supE*), also carry a mutation in the *hsdSB* gene (*r⁻*) that inactivates one of three *Salmonella* restriction systems preventing efficient transformation (Grana et al. 1985). MS1582 is an immune (*c2⁺ mnt⁺*) lysogen of prophage P22 16-*amH1455 sieA44 Ap68tpfr49* in MS1363, used for the isolation of virulent mutants of P22 (Grana et al. 1985). The *trpR* : : Tn10 insertion in strain TT2012 (LT2 *trpR* : : Tn10; from J. Roth) was moved into MS1582 by generalized transduction. Plasmids were constructed in *E. coli* strains JM101 (Messing et al. 1981), C600 (Appleyard 1954), or MM294 (Meselson and Yuan 1968) prior to transformation into *Salmonella* (Lederberg and Cohen 1974). The F'*lac* episome was introduced into MS1868 by conjugal transfer from *E. coli* X90/F'*lacI^{Q1}* (Amann et al. 1983). Challenge phages were constructed by crosses between plasmids and phages (Youderian et al. 1983), and selected as described by Benson et al. (1986).

Media, enzymes, and chemicals

Media and general phage techniques have been described (Levine 1957; Levine and Curtiss 1961; Signer and Weil 1968; Susskind et al. 1971; Botstein et al. 1972; Weinstock et al. 1979; Youderian and Susskind 1980; Youderian et al. 1983).

Green tryptophan drop-out plates consist of M9 medium supplemented to 20 µg/ml L-arginine HCl, L-aspartic acid, L-histidine HCl, L-isoleucine, L-leucine, L-lysine HCl, L-methionine, L-phenylalanine, L-threonine, and L-tyrosine (Sigma); 0.06% alizarin yellow GG and 0.0067% aniline blue (Aldrich); and 0.8% glucose and 1.5% agar (Difco). Ampicillin and kanamycin (Sigma) were added to final concentrations of 50 µg/ml. Plates were also supplemented with L-tryptophan and IPTG (Sigma) at various concentrations as described in the table and figure legends.

Restriction endonucleases, *E. coli* DNA polymerase I large fragment, T4 polynucleotide kinase, and T4 DNA ligase were purchased from New England Biolabs. Deoxyribonucleoside triphosphates and ATP were purchased from P-L Biochemicals. [α -³²P]dATP (700 Ci/mmol), used in DNA sequence analyses, was from ICN. Synthetic *trp* operators were made on an Applied Biosystems Automated DNA Synthesizer (Model 381A) using phosphoramidite substrates (Caruthers 1982).

Plasmids

General procedures used in the construction, selection, and purification of plasmids have been described (Youderian et al. 1982). All plasmids are derivatives of pBR322 (Bolivar et al. 1977).

Plasmid pPY97 (Benson et al. 1986) has the *EcoRII*–*HindIII* fragment of P22 DNA carrying *mnt*, *P_{ant}*, *Omnt*, and the proximal two-thirds of the *arc* gene inserted in the *EcoRI*–*HindIII* “backbone” of pZ150 (Zagursky and Berman 1984), a derivative

of pBR322 carrying the phage M13 origin of replication and packaging initiation sites. Plasmid pPY97 and its derivatives thus create transcriptional fusions of *Pant* to the *tetA* gene of pZ150, yet pPY97 is sensitive to tetracycline due to transcriptional repression by Mnt. Plasmid pPY140 is otherwise isogenic with pPY97, but carries a substitution of a synthetic stretch of DNA including a unique *XhoI* site for the *Omnt* region immediately 3' to the *ant* promoter (Benson et al. 1986; see Fig. 2, below).

Plasmid pPY98 is otherwise isogenic with pPY97, except that it carries the backbone of pZ152 (Zagursky and Berman 1984). Plasmid pPY190, otherwise isogenic with pPY98, carries a substitution of a synthetic stretch of DNA including a unique *SmaI* site for the *Omnt* region immediately 3' relative to the *ant* promoter. pPY190 was derived from pPY98 by site-directed mutagenesis of single-stranded plasmid DNA using a synthetic oligonucleotide (5'-TCTACTATATTCCCGGAATTCCGACCTGTATTGT-3'). The desired product was selected by its ability to confer increased tetracycline resistance upon a sensitive host, due to the substitution of synthetic DNA for *Omnt*, resulting in constitutive expression of the *tetA* gene.

The plasmid source of Trp repressor protein, pPY150, was constructed as described by Benson et al. (1986). It carries the M13 origin from plasmid pZ152 (Zagursky and Berman 1984) and a fusion of the *lacUV5* promoter to the *trpR* structural gene from pRPG47 (Arvidson et al. 1986). Plasmid pPY158 is missing the smaller *BamHI* fragment of pPY150, which contains the entire *trpR* coding sequence.

DNA sequence analyses

Plasmid DNAs and double-stranded phage DNAs were sequenced by the method of Sanger et al. (1977), using an oligonucleotide complementary to the 5' end of the *arc* gene as primer (5'-CGGCATTTTGCTCATTCC-3'). Single-stranded plasmid DNAs were prepared by the method of Zagursky and Berman (1984).

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