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The challenge-phage assay reveals differences in the binding equilibria of mutant *Escherichia coli* Trp super-repressors *in vivo*

Michael Shapiro+, Dennis N.Arvidson, James Pfau and Philip Youderian*
Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089 and California Institute of Biological Research, 11099 N. Torrey Pines Rd, La Jolla, CA 92037, USA

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ABSTRACT

The phenotypes of four mutant *Escherichia coli* Trp repressor proteins with increased activities have been examined *in vivo* using the challenge-phage assay, an assay based on a positive genetic selection for DNA binding. These proteins, which differ by single amino acid changes from the wild type (Glu13→Lys, Glu18→Lys, Glu49→Lys and Ala77→Val), require less L-tryptophan than wild-type repressor for activation *in vivo*, and are super-aporepressors. However, none of the four mutant repressors binds DNA in a corepressor-independent manner. Three of the four mutant repressors (with Glu→Lys changes) are more active when complexed with tryptophan, and are super-holorepressors. Challenge-phage assays with excess tryptophan rank the mutant holorepressors in the same order as determined by binding studies *in vitro*. Challenge-phage assays with limiting tryptophan reveal additional phenotypic differences among the mutant proteins. These results show that the challenge-phage assay is a robust assay for measuring the relative affinities of specific protein – DNA interactions *in vivo*.

INTRODUCTION

Tryptophan aporepressor, the product of the *Escherichia coli trpR* gene, is an allosteric regulatory protein. Aporepressor binds two molecules of the corepressor ligand, L-tryptophan, to assemble an active holorepressor complex (1). Trp holorepressor binds at least five different specific sites on the *E.coli* genome, to inhibit transcription of the *trp* (2), *trpR* (3), *aroH* (4), *mtr* (5,6) and *aroL* (7) operons.

The binding of Trp repressor to its operators responds in a timely manner to changes in the physiological state of the cell. Unlike coliphage λ repressor, which controls an 'on–off' transcriptional switch, Trp repressor modulates the rate of transcription of the tryptophan biosynthesis (*trp*) operon over a wide range. This rheostatic control is achieved by the binding of multiple Trp repressor dimers to complex, tandem operators (8).

Like the binding of Lac (9,10) and λ (11,12) repressors to their operators, the binding of Trp repressor to its operators is not maximized (13–15). In all three cases, mutations which result in tighter-binding phenotypes have been isolated in both the operator site and the coding sequence for the binding protein. Using a screen for super-repressors, Kelley and Yanofsky (13) isolated four different mutations that increase TrpR activity *in vivo*. When cells with a partial defect in tryptophan biosynthesis are starved for tryptophan, high-level expression of these mutant repressors inhibits growth, under conditions in which high-level expression of wild-type repressor does not. All four mutant proteins are made in wild-type amounts, have been purified, and have been assayed for their abilities to bind tryptophan, the *trp* operator, and non-operator DNA *in vitro* (16–18).

Of these four changes, three (Glu18→Lys, Glu49→Lys and Ala77→Val) have been isolated as second-site revertants of mutations that decrease the activity of Trp repressor (19). The ability of super-repressors to suppress primary site (*trpR*$^-$) mutations is not allele-specific. The changes Glu18→Lys and Glu49→Lys are isolated as suppressors of different primary site mutations (19), and the construction of double mutants with Ala77→Val shows that this change can suppress a number of different primary site mutations as well (unpublished results).

The Ala77→Val change is predicted to replace one hydrophobic side chain with another on the interior of the protein (20–23). In a previous report, we described the detailed analysis of mutants with Ala77 changes, using the challenge-phage assay to measure the DNA-binding activity of Trp repressor *in vivo*. We showed that seven different single amino acid changes of residue alanine 77 result in super-aporepressors, mutant repressors that are more active than the wild-type protein when tryptophan is limiting. With excess tryptophan, many of these super-repressors are not super-holorepressors, but behave like the wild type *in vivo*; Val77 holorepressor behaves like the wild type both *in vivo* and *in vitro* (15,18). On the basis of these and other results, we have deduced that the Ala77→Val change decreases the affinity of aporepressor for non-operator DNA, yet does not affect the binding of holorepressor to operator or non-operator DNA.

* To whom correspondence should be addressed

+ Present address: Department of Biochemistry and Molecular Biology, University of California, Berkeley, CA 94720, USA

The other three super-repressor changes, Glu13→Lys, Glu18→Lys, and Glu49→Lys, are glutamic acid to lysine changes located on the surface of the protein in the multiple crystal structures of repressor (20,21,24,25). Thus, all three Glu→Lys changes are predicted to make the surface of Trp aporepressor more basic. Unlike Val77 holorepressor, the three holorepressors with surface charge changes do not bind operator DNA like wild-type repressor *in vitro*. All three mutant holorepressors show decreased rates of dissociation from operator DNA (18). Presumably, these three changes improve the electrostatics of the holorepressor/operator interaction.

In this paper, we use the challenge-phage assay for DNA binding (14,26,27) to examine these Trp super-repressors. Although two of these proteins result in cell death when overproduced, we can define conditions for challenge-phage assays that permit us to rank the dependence of repression by the mutant proteins on both the amount of aporepressor produced, and the amount of tryptophan required for their activation. Challenge-phage assays in which we vary the level of expression of Trp aporepressor in the presence of excess tryptophan reveal the same order of affinities of these mutant repressors for the *trp* operator as that observed *in vitro*. Challenge-phage assays in which we vary tryptophan concentration rank the activities of these mutant proteins differently. Differences in the results of these two assays indicate that some of these mutant super-repressor changes affect multiple equilibria in the network of interactions between TrpR protein, tryptophan, operator DNA, and non-operator DNA.

MATERIALS AND METHODS

Bacteria, phage, and plasmids

Salmonella typhimurium LT2 strains include MS1363 *leuA-414(am) supE-40* (28), used for the permissive growth of challenge phages, and MS1868/F'*lacI*^{Q} (*leuA-414(am) hsdSB*$^{-}$) (14), used as the host for infection with challenge phage. *Escherichia coli* K12 strain X90/F'*lacI*^{Q} (29), was used as the host for plasmid constructions. The challenge phage used to assay DNA binding *in vivo*, P22 *Kn9 O-ref2 arc-H1605(am)* (14) carries a minimal reference-type *trp* operator, 5' GAACTA-GTTAACTAGTTC 3', controlling P22 *ant* gene expression. Recently, two independent biochemical studies (30,31) have shown that this minimal operator, not an alternative (32), reflects the correct dyad for the binding of repressor dimers. Plasmid derivatives of pRLK13 (13) were the kind gift of Charles Yanofsky. To construct an otherwise isogenic set of plasmids that express wild-type and mutant *trpR* genes from the inducible *lacUV5* promoter, the 434 bp *Bam*HI fragments from mutant derivatives of plasmid pRLK13 encoding aporepressors with the Glu13→Lys, Glu18→Lys, Glu49→Lys, and Ala77→Val changes were cloned into the unique *Bam*HI site of plasmid pPY158 (14). Plasmid pPY150 (14) was used to express the wild-type *trpR* gene.

Challenge-phage assays

Infections with challenge phage were performed as described (14,27). To measure repression as a function of the amount of plasmid-encoded Trp aporepressor required for the efficient lysogenization of the challenge phage, overnight cultures of MS1868/F'*lacI*^{Q} carrying plasmid pPY150 or each mutant plasmid were diluted 100-fold into LB medium with 50 μg/ml ampicillin and various concentrations of IPTG (isopropyl-β-D-thiogalactoside), and grown to a density of 4×10^{8}/ml at 37°C. Each culture was infected with challenge phage P22 *Kn9 O-ref2 arc-H1605(am)* at a multiplicity of 25 phage/cell. After adsorption of the phage for 15 min at 25°C, infected cells were diluted, and each serial dilution was plated on green tryptophan drop-out medium with the same concentration of IPTG, ampicillin (100 μg/ml), kanamycin (25 μg/ml), and tryptophan (100 μg/ml).

To measure repression as a function of the amount of tryptophan corepressor required for the efficient lysogenization of the challenge phage, overnight cultures of MS1868/F'*lacI*^{Q} carrying plasmid pPY150 or each mutant plasmid were diluted 100-fold into LB medium with 50 μg/ml ampicillin and 10^{-6} M IPTG, and grown to a density of 4×10^{8}/ml at 37°C. Each culture was infected with challenge phage P22 Kn9 O-ref2 arc-amH1605 at a multiplicity of 25 phage/cell. After adsorption of the phage for 15 min at 25°C, infected cells were diluted, and each serial dilution was plated on green tryptophan drop-out medium with 10^{-6} M IPTG, ampicillin (100 μg/ml), kanamycin (25 μg/ml), and tryptophan at various concentrations. The numbers of kanamycin-resistant colonies were scored after incubation of the plates at 37°C for 48 h. Frequencies of lysogeny were calculated as the titers of kanamycin-resistant survivors divided by the titers of cells immediately prior to infection with phage (assayed on green plates with 100 μg/ml ampicillin). Values given as frequencies of lysogeny and efficiencies of plating represent the averages of at least six determinations, and varied less than five-fold from experiment to experiment.

To show that mutant and wild-type repressors were made at similar steady-state levels in our challenge-phage assays, mutant proteins made in the *Salmonella* host after induction with IPTG were quantitated using an immunological (Western) assay as described by Arvidson *et al.* (15,33). No differences were found in the levels of wild-type and mutant super-repressor proteins produced after induction with 10^{-6} or 10^{-5} M IPTG (data not shown). In the interpretation of our data, we assume that these proteins are produced at wild-type levels under other conditions of induction.

RESULTS

The challenge-phage assay for Trp repressor binding

To examine the Trp repressor/operator interaction in greater genetic detail, we have measured the binding of Trp repressor to defined operator sites *in vivo*, using the challenge-phage assay (26). Challenge phages are derivatives of temperate phage P22 that afford a positive selection for DNA binding. A challenge phage carries a cloned operator that places the binary developmental switch of P22 under the control of a protein that binds the operator; *trp* challenge phages carry versions of a minimal, consensus binding site for Trp repressor (Figure 1).

To measure the ability of wild-type Trp repressor to bind an operator, a repressor-producing *Salmonella* host is infected with a high multiplicity of a *trp* challenge phage, and the fraction of cells surviving infection is determined. Challenge phages selectively lysogenize host cells that produce a critical level of active holorepressor, and kill cells that do not. For the phage, the developmental choice between lysogenic and lytic pathways depends on the state of occupancy of its *trp* operator. Saturation of the challenge-phage operator with Trp repressor prevents transcription of the P22 *ant* gene, and favors the lysogenic development of the infecting phage and the survival of the infected host cells. Conversely, if a challenge-phage operator is free of

repressor, *ant* transcription leads to lytic P22 development and the death of the infected host. Therefore, the efficiency of lysogeny of a Trp challenge phage is a measure of the relative occupancy of the challenge-phage operator *in vivo*.

The ability of a challenge phage with a *trp* operator to lysogenize a host that produces Trp repressor depends on the concentration of active holorepressor complex, and therefore depends on both the level of Trp aporepressor as well as the level of tryptophan corepressor (14). In our host for the challenge-phage assay, aporepressor is produced at variable levels from the *lacUV5* promoter; this promoter is controlled in turn by Lac repressor. The addition of the inducer, IPTG, to this host results in the inactivation of Lac repressor, and elevated expression of the *trpR* gene. Thus, the level of aporepressor produced by the host is modulated by the addition of different concentrations of IPTG. The level of corepressor in our assays is modulated by the addition of different concentrations of exogenous tryptophan.

To understand the phenotypes of mutant Trp super-repressors, we have used the challenge-phage selection in two different ways. To determine the relative activities of super-repressors as holorepressors, we have varied the level of Trp aporepressor in the presence of saturating tryptophan. We also varied the tryptophan (corepressor) concentration for host cells that produce the same level of aporepressor. This second set of challenge-phage assays provides us with a measure of the relative activities of these mutant super-repressors as aporepressors.

Three mutant super-repressors are super-holorepressors

As shown in Figure 2, our host that produces wild-type Trp repressor (MS1868/F'*lacI*^{Q} (pPY150) is lysogenized by a challenge phage with an idealized operator only when higher levels of Trp repressor are produced; the efficiency of lysogeny increases more than 1000-fold with increasing IPTG concentration. In contrast, three hosts which produce the mutant Glu13→Lys, Glu18→Lys and Glu49→Lys repressors are lysogenized by the challenge phage at lower IPTG concentrations. As shown in Figure 2, the four super-repressors may be ranked in the order Lys49 > Lys18 > Lys13 > Val77 = wild-type by this assay. The relative activities of super-repressors in the presence of excess tryptophan is the same *in vivo* as has been measured by binding studies *in vitro* (18).

This assay is complicated by the finding that, in the presence f high amounts of the inducer, IPTG, cells producing the Lys18 and Lys49 mutant repressors are lysogenized by the challenge-phage more poorly than at lower IPTG concentrations. This is because overproduction of these mutant super-repressors is lethal to the growth of their *Salmonella* host, as is the case for up-mutant λ repressors (11,34). As shown in Figure 3, the efficiency of plating of uninfected hosts producing the mutant Lys18 and Lys49 super-repressors drops at higher concentrations of IPTG.

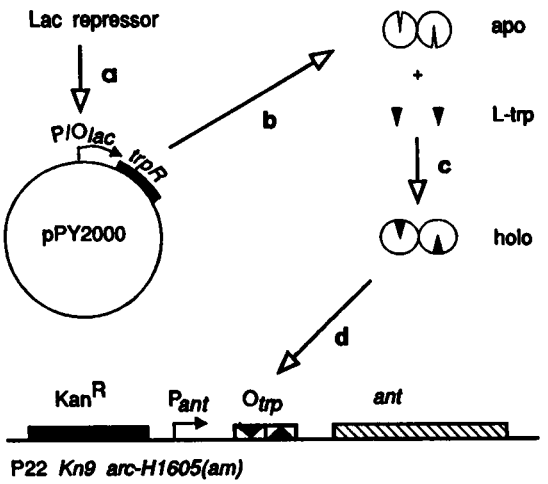

Figure 1. The challenge-phage selection for Trp repressor binding. The fraction of bacteria which survive infection with a *trp* challenge phage depends on multiple factors. (a) The steady-state level of Trp aporepressor (apo) produced from the *lacUV5* promoter on plasmid pPY2000 is regulated by the binding of Lac repressor to the *lac* operator (O_{lac}); thus, the intracellular concentration of TrpR protein increases with increasing concentrations of the inducer, IPTG, added to the medium. (b) The steady-state level of Trp aporepressor can also change due to variations in the rates of degradation and synthesis of messenger RNA and TrpR protein. The mutant aporepressors described in this paper are made at the same steady-state levels as wild-type repressor in the presence of high levels of IPTG. (c) Formation of the holorepressor complex (holo) requires binding of the corepressor, tryptophan (L-trp), and depends on the affinity of aporepressor for tryptophan, as well as other linked equilibria. Because tryptophan is an essential amino acid, some holorepressor will be present *in vivo*, even when tryptophan is not added to the medium. Experimentally, we can drive the conversion of aporepressor to holorepressor by adding an excess of tryptophan to the medium. (d) When holorepressor binds the consensus *trp* operator (O_{trp}) of a challenge phage, it inhibits the transcription of the phage P22 *ant* (antirepressor) gene from its promoter (P_{ant}). The stronger the inhibition of antirepressor expression, the greater the fraction of infected host cells which survive and acquire a kanamycin-resistant (Kan^R) phenotype.

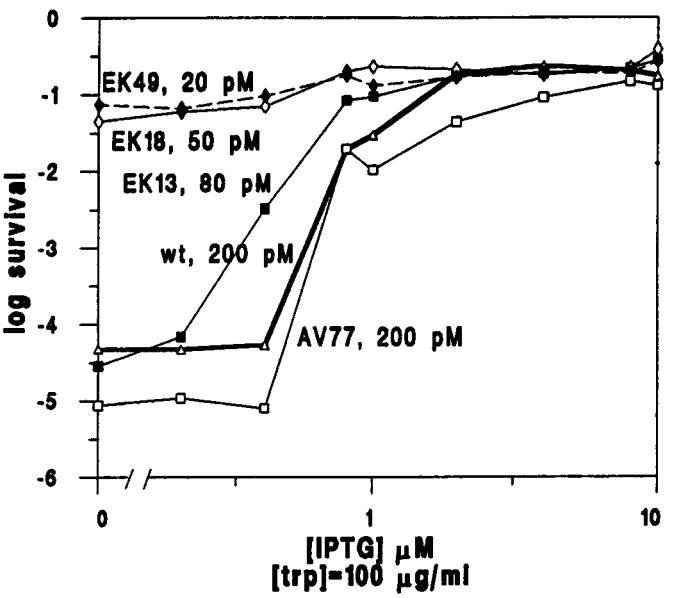

Figure 2. Mutant Trp repressors with Glu→Lys changes are super-holorepressors. *S.typhimurium* host MS1868/F'*lacI*^{Q} cells with derivatives of plasmid pPY150 were infected with a high multiplicity of *trp* challenge phage at different IPTG concentrations, and kanamycin-resistant survivors were assayed as described in Materials and Methods: wt, wild type; EK13, Glu13→Lys; EK18, Glu18→Lys; EK49, Glu49→Lys; AV77, Ala77→Val. The efficiency of lysogeny (log survival, plotted *vs.* log[IPTG]) is a measure of how well the phage-borne reference *trp* operator is occupied by plasmid-encoded Trp repressor. In this experiment, repressor binding *in vivo* is measured as a function of the increasing concentration of intracellular Trp repressor protein in the presence of excess tryptophan (100 μg/ml); identical results were obtained with higher concentrations of exogenous tryptophan. Because corepressor is present in excess under these conditions, these dose-response curves allow us to rank the relative values of K_{app} for each mutant holorepressor. For comparison, values of K_{ATO} for each mutant determined *in vitro* by Hurlburt and Yanofsky (18) are shown.

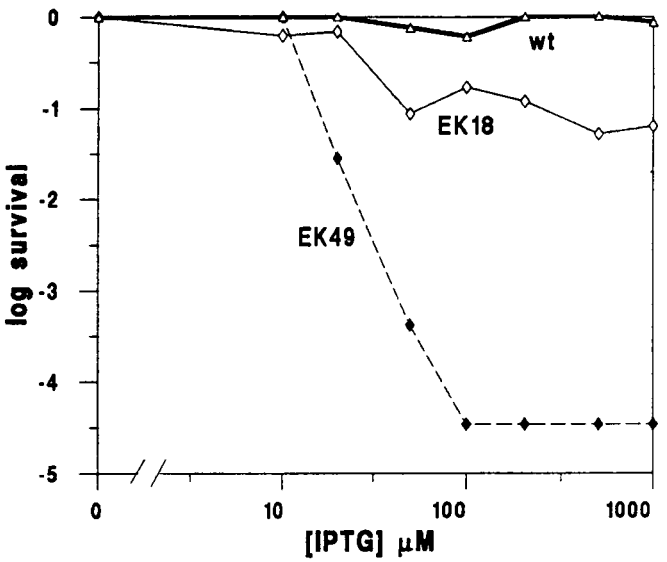

Figure 3. Two mutant Glu→Lys super-repressors are lethal when overproduced. MS1868/F'*lacI*^{Q} cells with derivatives of plasmid pPY150 were grown in LB medium at 37°C to exponential phase, and the efficiencies of plating (log survival) of these cells were measured on green indicator plates with 100 μg/ml ampicillin at different IPTG concentrations (plotted on a log scale), and divided by the efficiency of plating on media without IPTG. Otherwise isogenic hosts producing the mutant Glu13→Lys and Ala77→Val repressors gave results identical to those of the host producing wild-type (wt) repressor.

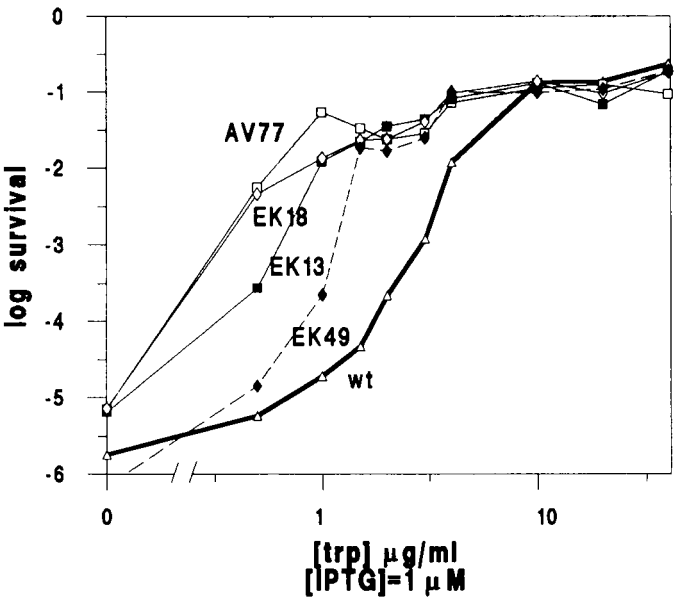

Figure 4. Mutant Trp repressors with Glu→Lys changes are super-aporepressors. The DNA binding activities *in vivo* for mutant repressors depleted for the corepressor, tryptophan, are shown (see legend to Figure 2). In this experiment, repressor binding *in vivo* (log survival, plotted *vs.* log[tryptophan]) is measured as a function of the increasing concentration of exogenous tryptophan, with the level of intracellular Trp repressor protein held constant. Under these conditions, TrpR protein is expected to exist predominantly as aporepressor, not holorepressor. Consequently, mutants with activities higher than the wild type under these conditions are called 'super-aporepressors' However, we do not wish to imply that mutant aporepressor species can effect repression under these conditions.

One possible explanation for why the mutant super-repressors are more active as holorepressors is that they are produced in higher amounts than the wild-type repressor. To rule out this possibility, we induced cells producing the wild-type and each of the mutant super-repressors, determined the relative amounts of repressor produced by each strain by Western immunoblot analysis, and found that all four mutant repressors are made at the same steady-state levels as wild type (data not shown). Thus, these proteins are made in amounts to that of the wild-type repressor in the *Salmonella* host, as well as in an *E.coli* host (18).

All four mutant super-repressors are super-aporepressors

We also assayed how the frequency of lysogeny of hosts that produce each of the mutant aporepressors depends on exogenous tryptophan concentration, after infection with our reference-type challenge phage. As shown in Figure 4, although all of the mutant aporepressors still require tryptophan as corepressor for activation, all of the mutant aporepressors require less tryptophan for activation than wild-type aporepressor. This second type of challenge-phage assay ranks the four super-repressor mutants in a different order: Lys18, Val77 > Lys13 > Lys49 > wild type. In particular, it is important to note that whereas the Lys49 holorepressor is more active than the Lys13 and Lys18 holorepressors, Lys13 and Lys18 proteins are more active than Lys49 when tryptophan (corepressor) is limiting. This result is surprising because all three mutant aporepressors with Glu→Lys changes bind tryptophan with affinities similar to that of wild-type aporepressor *in vitro* (18).

DISCUSSION

The results of challenge-phage infections with excess tryptophan allow us to rank the activities of the four mutant holorepressors in the order: Glu49→Lys > Glu18→Lys > Glu13→Lys > Ala77→Val. Thus, the challenge-phage assay provides us with a robust measure of the relative affinities of specific DNA-binding proteins for their sites *in vivo*. The order of affinities of these mutant Trp holorepressors determined by the challenge-phage assay is the same order that has been determined *in vitro* (18). Similar concordances are observed between the results of challenge-phage assays *in vivo* and measurements *in vitro* of the binding of wild-type Trp repressor to operators with modified base pairs (14,35), the binding of wild-type λ repressor to modified operators (12,36,37), the binding of mutant λ super-repressors to the wild-type operator (11,34,38), the binding of wild-type Cro repressor to modified operators (39,40), and the binding of wild-type and mutant Mnt repressors to wild-type and mutant operators (41,42).

With Trp repressor, a protein that is activated allosterically by the binding of ligand, the challenge-phage assay can provide us with additional information. This is because we can assay repression with the challenge phage under conditions in which the activating ligand for Trp repressor is limiting, and the relative activity of (ligand-free) Trp aporepressor contributes to phenotype. We can model the complexity of a binding situation in which corepressor is limiting, by considering the equilibrium relationships between the different states of intracellular TrpR protein under these conditions (Figure 5).

When tryptophan is saturating, TrpR protein occupies three states, free holorepressor (AT), holorepressor bound to non-specific DNA (ATD), and holorepressor bound to operator DNA (ATO). The activity of a holorepressor *in vivo*, like that of a ligand-independent repressor, is determined by its ability to bind operator DNA in the presence of the competing (non-specific)

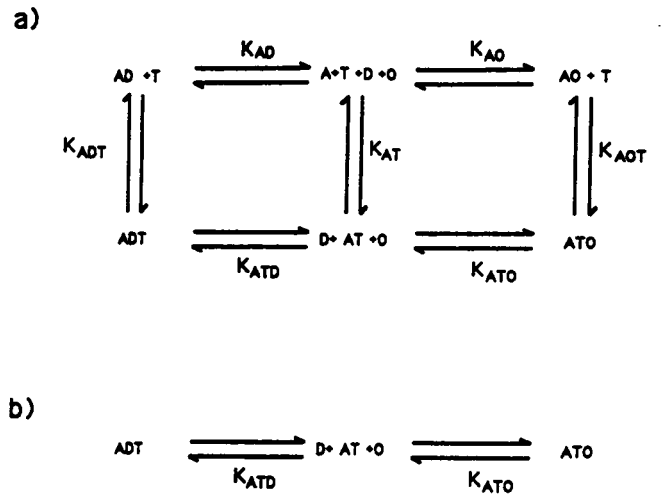

Figure 5. Linked equilibria involved in the assembly of Trp holorepressor/operator complexes. Symbols: A, aporepressor; D, non-specific DNA; O, operator DNA; T, tryptophan. The last letter in subscripts of each of the equilibrium dissociation constants (K's) indicates the species being bound; for examples, K_{AO} refers to the binding of operator DNA (O) by aporepressor (A), and K_{ATD} to the binding of non-specific DNA (D) by holorepressor (AT). When the concentration of intracellular tryptophan is saturating, the cycle of linked equilibria diagrammed in (a) collapses to the 'bottom line' description shown in (b). Unfortunately, because tryptophan is an essential amino acid, conditions described by the corresponding 'top line' may be achieved *in vitro*, but never *in vivo*.

genomic DNA. *In vivo*, what we presume to measure by the challenge-phage assay is the apparent equilibrium dissociation constant of holorepressor, which is related to these constants by the equation,

$$K_{app} = K_{ATO} + (K_{ATO}/K_{ATD})\,[D]$$

Non-specific DNA is in vast excess of operator DNA *in vivo*. Experimentally, if the concentration of genomic (non-specific) DNA is held constant, K_{app} is determined predominantly by the 'specificity ratio', the ratio between specific and non-specific binding constants, $(K_{ATO})(K_{ATD})^{-1}$.

If the steady-state levels of wild-type and mutant Trp repressors are the same, the challenge-phage assay with abundant tryptophan provides us with a relative measure of K_{app} *in vivo*. When corepressor is limiting, the situation is more complex (15). TrpR protein is distributed among at least six different states involving aporepressor (A), tryptophan (T), non-specific DNA (D) and operator DNA (O). The Ala77→Val super-repressor change results in an aporepressor that requires less intracellular tryptophan for activation than wild type, and a holorepressor that has wild-type affinity for the operator (Figure 2). This change does not appear to affect the interaction of holorepressor (AT) with either specific (O) or non-specific (D) DNA, or the interaction of aporepressor (A) with tryptophan (T) (18). The primary effect of the Ala77→Val change is to increase the magnitude of K_{AD}, decrease the magnitude of K_{ADT}, and thereby depopulate the [AD] state. The Ala77→Val change may restrict the ensemble of accessible conformations of the protein to a subset that mimics the set of conformations assumed when tryptophan is bound (15). Thus, the super-aporepressor phenotype of the mutant Val77 protein is not due to the increased affinity of this aporepressor for tryptophan, but rather to its altered interaction with non-specific DNA. This result points out the fact that changes in any of several different combinations of equilibria between aporepressor, tryptophan, specific DNA, and non-specific DNA may lead to a super-aporepressor phenotype: greater activity than the wild-type protein under conditions where intracellular tryptophan is limiting.

Of the three Glu→Lys super-repressor changes, Glu49→Lys results in the greatest increase in the affinity of holorepressor for operator. The primary effect of this change *in vitro* is on the dissociation rate of repressor from operator DNA (17,18). The Glu49→Lys change affects the DNA-binding surface of TrpR protein. The crystal structure of the holorepressor/operator complex predicts that the Lys49 side chain should make new ionic or hydrogen bonds with phosphates or nucleotide functional groups in the minor groove of the central four base pairs of the operator, base pairs that are not contacted by repressor in the wild-type complex (23,25). The interaction of Glu49→Lys holorepressor with the operator is more salt-sensitive than that of wild-type, consistent with the idea that this change creates new electrostatic interactions (17). The observation that the change affects the dissociation rate for complex formation *in vitro* more than the association rate argues that these new, favorable interactions do not participate dramatically in the rate-limiting step for the formation of the repressor/operator complex. Binding of holorepressor, therefore, may proceed along a kinetic pathway that involves at least two steps: a slower, rate-determining step; and, a faster, subsequent step in which Lys49 can contribute to the binding interaction. Alternatively, dissociation of the Lys49 holorepressor may not proceed along the same pathway as association.

Previous studies rank the three mutant holorepressors with Glu→Lys changes in the same order of activity both *in vivo* (13) and *in vitro* (18): Lys49 > Lys18 > Lys13 > wild-type. On the basis of these results, the phenotypes of these changes could be explained solely in terms of their effects on specific binding (K_{ATO}). However, our results that rank the dependence of these mutant repressors on tryptophan for activation complicate this picture, and suggest that at least one of the Glu→Lys changes affects an additional equilibrium involved in repressor binding. This is because the Glu13→Lys and Glu18→Lys mutants are more active than Glu49→Lys when tryptophan is limiting, yet less active than Glu49→Lys when tryptophan is in excess. Examination of the ability of these mutant proteins to bind DNA and tryptophan *in vitro* suggests that the constants K_{AT}, K_{AO}, and K_{ATD} are unchanged (18). If we assume that these three values are constant for the Lys13, Lys18, and Lys49 proteins, then the values of K_{AD} and K_{ADT} must be different for at least one of these mutants.

Three models can account for our data. First, the phenotype of Glu→Lys49 repressor may be due solely to its affects on specific binding (K_{ATO}), and the Glu→Lys13 and Glu→Lys18 changes may increase K_{AD} (like the Ala77→Val change) as well as decrease K_{ATO}. Alternatively, the Glu→Lys13 and Glu→Lys18 changes may affect only specific binding (K_{ATO}), and the Glu→Lys49 change may affect (decrease) both K_{AD} and K_{ATO} This phenotype would be the opposite of that of Ala77→Lys repressor, for which both K_{AD} and K_{ATO} are likely increased relative to wild type (15). Finally, all three changes may improve specific binding (K_{ATO}), and affect K_{AD} to different extents.

Although mutations resulting in both Glu18→Lys and Glu49→Lys changes can be isolated as second-site suppressors of negative dominant mutations in the *trpR* gene, Glu49→Lys restores greater repressor activity to several mutants than

Glu18→Lys (19). Of four changes that improve the binding of λ repressor, two surface Glu→Lys changes predicted either to result in a new phosphate contact or to improve dimerization (Glu34→Lys and Glu83→Lys, respectively) do not suppress a change which results in the loss of base-specific contacts (Lys4→Glu), whereas two changes predicted to create new, additional contacts with operator base pairs do (Gly48→Ser and Gly48→Asn) (11,34,43). Taken together, these results suggest that the Glu49→Lys change may result in new, specific contacts between Trp repressor and its operator.

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REFERENCES

1. Arvidson, D. N., Bruce, C. and Gunsalus, R. P. (1986) *J. Biol. Chem.* **261**, 238–243.
2. Bennett, G. N. and Yanofsky, C. (1978) *J. Mol. Biol.* **121**, 179–192.
3. Gunsalus, R. P. and Yanofsky, C. (1980) *Proc. Nat. Acad. Sci., U.S.A.* **77**, 7117–7121.
4. Zurawski, G., Gunsalus, R. P., Brown, K. D. and Yanofsky, C. (1981) *J. Mol. Biol.* **145**, 47–53.
5. Heatwole, V. M. and Somerville, R. L. (1991) *J. Bacteriol.* **173**, 3601–3604.
6. Sarsero, J. P., Wookey, P. J. and Pittard, A. J. (1991) *J. Bacteriol.* **173**, 4133–4143.
7. Heatwole, V. M. and Somerville, R. L. (1992) *J. Bacteriol.* **174**, 331–335.
8. Kumamoto, A. A., Miller, W. G. and Gunsalus, R. P. (1987) *Genes. Dev.* **1**, 556–564.
9. Pfahl, M. (1981) *J. Mol. Biol.* **147**, 1–10.
10. Sadler, J. R., Sasmor, H. and Betz, J. L. (1983) *Proc. Nat. Acad. Sci., U.S.A.* **80**, 6785–6789.
11. Nelson, H. C. M. and Sauer, R. T. (1985) *Cell* **42**, 549–558.
12. Benson, N., Sugiono, P. and Youderian, P. (1988) *Genetics* **118**, 21–29.
13. Kelley, R. L. and Yanofsky, C. (1983) *Proc. Nat. Acad. Sci., U.S.A.* **82**, 483–487.
14. Bass, S., Sugiono, P., Arvidson, D. N., Gunsalus, R. P. and Youderian, P. (1987) *Genes Dev.* **1**, 565–572.
15. Arvidson, D. N., Pfau, J., Hatt, J. K., Shapiro, M., Pecoraro, F. S. and Youderian, P. (1993) *J. Biol. Chem.* **268**, 4362–4369.
16. Klig, L. S., Crawford, I. and Yanofsky, C. (1987) *Nucleic Acids Res.* **15**, 5339–5351.
17. Klig, L. S. and Yanofsky, C. (1988) *J. Biol. Chem.* **263**, 243–246.
18. Hurlburt, B. K. and Yanofsky, C. (1990) *J. Biol. Chem.* **265**, 7853–7858.
19. Klig, L. S., Oxender, D. L. and Yanofsky, C. (1988) *Genetics* **120**, 651–655.
20. Zhang, R.-G., Joachimiak, A., Lawson, C. L., Schevitz, R. W., Otwinowski, Z. and Sigler, P. B. (1987) *Nature (London)* **327**, 591–597.
21. Lawson, C. L., Zhang, R.-G., Schevitz, R. W., Otwinowski, Z., Joachimiak, A. and Sigler, P. B. (1988) *Proteins* **3**, 18–31.
22. Marmorstein, R. Q. and Sigler, P. B. (1989) In Eckstein, F., and Lilley, D. M. J. (eds), Nucleic Acids and Molecular Biology. Springer-Verlag, Berlin, Vol. 3, pp. 56–78.
23. Luisi, B. F. and Sigler, P. B. (1990) *Biochimica Biophysica Acta* **1048**, 113–126.
24. Schevitz, R. W., Otwinowski, Z., Joachimiak, A., Lawson, C. L. and Sigler, P. B. (1985) *Nature (London)* **317**, 782–786.
25. Otwinowski, Z., Schevitz, R. W., Zhang, R.-G., Lawson, C. L., Joachimiak, A., Marmorstein, R. Q., Luisi, B. F. and Sigler, P. B. (1988) *Nature (London)* **335**, 321–329.
26. Benson, N., Sugiono, P., Bass, S., Mendelman, L. V. and Youderian, P. (1986) *Genetics* **114**, 1–14.
27. Bass, S., Sorrells, V. and Youderian, P. (1988) *Science* **242**, 240–245.
28. Susskind, M. M. (1980) *J. Mol. Biol.* **138**, 685–713.
29. Amann, E., Brosius, J. and Ptashne, M. (1983) *Gene* **25**, 167–178.
30. Carey, J., Lewis, D. E. A., Lavoie, T. A. and Yang, J. J. (1991) *J. Biol. Chem.* **266**, 24509–24513.
31. Haran, T. E., Joachimiak, A. and Sigler, P. B. (1992) *EMBO J.* **11**, 3021–3030.
32. Staacke, D., Walter, B., Kisters-Woike, B., von Wilcken-Bergmann, B. and Muller-Hill, B. (1990) *EMBO J.* **9**, 1963–1967.
33. Arvidson, D. N., Shapiro, M. and Youderian, P. (1991) *Genetics* **128**, 29–35.
34. Benson, N., Adams, C. and Youderian, P. (1992) *Genetics* **130**, 17–26.
35. Mazzerelli, J. M., Rajur, S. B., Iadarola, P. L. and McLaughlin, L. W. (1992) *Biochemistry* **31**, 5925–5936.
36. Sarai, A. and Takeda, Y. (1989) *Proc. Nat. Acad. Sci., U.S.A.* **86**, 6513–6517.
37. Beamer, L. J. and Pabo, C. O. (1992) *J. Mol. Biol.* **227**, 177–196.
38. Nelson, H. C. M. and Sauer, R. T. (1986) *J. Mol. Biol.* **192**, 27–38.
39. Benson, N. and Youderian, P. (1989) *Genetics* **121**, 5–12.
40. Takeda, Y., Sarai, A. and Rivera, V. M. (1989) *Proc. Nat. Acad. Sci., U.S.A.* **86**, 439–443.
41. Youderian, P., Vershon, A., Bouvier, S., Sauer, R. T. and Susskind, M. M. (1983) *Cell* **35**, 777–783.
42. Vershon, A., Youderian, P., Weiss, M. A., Susskind, M. M. and Sauer, R. T. (1985) In Calendar, R. and Gold, L. (eds), Sequence Specificity in Transcription and Translation. Alan R. Liss, New York, pp. 209–218.
43. Hecht, M. H. and Sauer, R. T. (1985) *J. Mol. Biol.* **186**, 53–63.