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Biochemistry

A stationary-phase protein of *Escherichia coli* that affects the mode of association between the *trp* repressor protein and operator-bearing DNA

(repressor-binding proteins/transcriptional control/gene regulation/tryptophan/bacterial physiology)

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ABSTRACT **Highly purified preparations of *trp* repressor (TrpR) protein derived from *Escherichia coli* strains that were engineered to overexpress this material were found to contain another protein, of 21 kDa. The second protein, designated WrbA [for tryptophan (*W*) *r*epressor-*b*inding protein] remained associated with its namesake through several sequential protein fractionation steps. The N-terminal amino acid sequence of the WrbA protein guided the design of two degenerate oligonucleotides that were used as probes in the cloning of the *wrbA* gene (198 codons). The WrbA protein, in purified form, was found by several criteria to enhance the formation and/or stability of noncovalent complexes between TrpR holorepressor and its primary operator targets. The formation of an operator–holorepressor–WrbA ternary complex was demonstrated by gel mobility-shift analysis. The WrbA protein alone does not interact with the *trp* operator. During the stationary phase, cells deficient in the WrbA protein were less efficient than wild type in their ability to repress the *trp* promoter. It is proposed that the WrbA protein functions as an accessory element in blocking TrpR-specific transcriptional processes that might be physiologically disadvantageous in the stationary phase of the bacterial life cycle.**

The *trp* repressor (TrpR) protein of *Escherichia coli* is a homodimer of 108 amino acid residues per subunit. The role of this protein is to respond to the presence of excess L-tryptophan by negatively regulating the transcription of the genes of the tryptophan regulon. The mechanistic basis of regulation is a conformational transition, elicited by the binding of L-tryptophan to TrpR, that greatly enhances the affinity of the protein for several operator targets. Each of the known operators lies within or near promoters that drive the production of proteins important in the biosynthesis or transport of L-tryptophan (reviewed in refs. 1 and 2).

The TrpR system has been intensively studied with the techniques of genetics (3–7), crystallography (8, 9), NMR spectroscopy (10–12), fluorescence spectroscopy (13), and protein chemistry (14). Nonetheless, there remain several unresolved issues in the area of *trp* regulation. Chief among these is the true size and composition of the protein species that engages the *trp* operator under normal physiological conditions. This question was addressed 20 years ago by researchers in three separate laboratories (15–19). Using biochemical approaches, each group obtained a protein preparation of 58–60 kDa that specifically inhibited transcription from the *trp* promoter, in a manner that was dependent on L-tryptophan and a functional *trp* operator. The dimeric TrpR protein that has been studied in recent years, encoded by the well-characterized *trpR* gene, has a molecular mass of 25 kDa (2).

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Among the individual promoters that are subject to control by TrpR there are large *in vivo* differences in amplitude of repression [e.g., 300-fold for the primary *trp* promoter versus 3-fold for the *trpR* promoter (2)]. Yet direct measurements *in vitro* showed that highly purified TrpR engaged three of its cognate operators with essentially identical affinities (20). A similar regulatory anomaly has been reported for ArgR and the promoters of the arginine regulon (21).

Use of immunochemical methods has shown that *E. coli* contains several proteins that are capable of interacting with TrpR (22). In this report we describe the characterization of a member of this series, designated WrbA [for tryptophan (*W*) *r*epressor-*b*inding protein].*

MATERIALS AND METHODS

Bacterial Strains. *E. coli* BL21(DE3) carries, in prophage form, a single copy of the gene for T7 RNA polymerase under the control of the inducible *lac* UV5 promoter (23). *E. coli* DH5α F′ (24) was used as a general-purpose host in cloning and plasmid manipulation. JC7623 (25) was used in the construction of a *wrbA* null mutation. *E. coli* SP1411, a derivative of W3110, has the genotype Δ(*argF–lacZ*)U169, *zah735*::Tn*10*, λBH300. λBH300 is a *cI*$^+$, integration-proficient phage that carries a *trp* promoter/operator–*lacZ* fusion. SP1503 is isogenic with SP1411 except that it has a *wrbA* null mutation.

Plasmids. Plasmids were constructed by standard techniques (26). A 241-bp fragment of DNA (−171 to +70 of the *E. coli trp* operon) bearing the *trp* operator/promoter region was synthesized by PCR using pVH153 (27) as template. The synthetic DNA was inserted into the *Bam*HI/*Eco*RI sites of pBluescript SKII(+) (Stratagene), resulting in plasmid pWPY2. pWPY3 contains a 1.69-kb DNA fragment carrying the *wrbA*$^+$ gene, inserted at the *Hin*dIII site of pBluescript SKII(+). A unique *Bgl* II site was created 96 bp downstream of the ATG codon of *wrbA*$^+$ of pWPY3 by using a site-directed mutagenesis kit (Bio-Rad). This plasmid was named pWPY3M. pWPY5 was constructed by inserting a 1.6-kb *Bam*HI fragment containing the structural gene and promoter of the chloramphenicol acetyltransferase gene into the *Bgl* II site of pWPY3M. This fragment was preparatively isolated from pUC18 CML (28). Plasmid pTrpRT7 contains a semi-synthetic *trpR*$^+$ structural gene, cloned between the *Nde* I and *Bam*HI sites of pET3a. TrpR expression is therefore mediated by T7-specific punctuation elements (23). pLysE encodes T7 lysozyme. By forming a nonproductive complex with T7 RNA polymerase, encoded within the DE5 prophage of the production strain, T7 lysozyme prevents the inappropriate expression of TrpR. Plasmid pVH153 (27) was used in

*The sequence reported in this paper has been deposited in the GenBank database (accession no. M99166).

the *Hpa* I endonuclease protection assay for TrpR–operator interaction.

TrpR Purification. TrpR was overproduced in strain BL21(DE3)/pLysE, pTrpRT7. A single colony, derived from the transformation of *E. coli* BL21(DE3)/pLysE with pTrpRT7, was inoculated into 10 ml of LB containing ampicillin and chloramphenicol (25 μg/ml each). LB medium contains 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, and 1 g of glucose per liter. The LB culture was grown with vigorous aeration for 18 hr at 37°C and then used to inoculate 1 liter of ACH medium [acid casein hydrolysate (ICN), 2 g/liter; glucose, 2 g/liter; vitamin B_1, 1 mg/liter; biotin, 1 mg/liter; salt mixture E of Vogel and Bonner (29)] in a 4-liter flask. Ampicillin was present at 25 μg/ml. The culture was grown with shaking at 37°C to an OD_{660} of 0.6. Isopropyl β-D-thiogalactopyranoside was added (1 mM), and after incubation for an additional 6–8 hr, the cells were harvested by centrifugation. All further operations were carried out at 0–5°C. The cell paste was suspended in grinding buffer (0.5 M Tris, pH 7.4/2 mM EDTA, 0.1 mM dithiothreitol/1 mM 2-mercaptoethanol/1 mM NaCl; 3 ml per g of cells). Typically 50 g of cell paste was processed at a time. After addition of lysozyme (530 μg/ml), phenylmethanesulfonyl fluoride (0.3 mg/ml), and sodium deoxycholate (1 mg/ml), the cells were disrupted by two passages through a French pressure cell (Aminco) operated at 2000 psi (1 psi = 6.89 kPa). Streptomycin sulfate (1 mg/ml) was added slowly with stirring to precipitate nucleic acids. Insoluble materials were removed by centrifugation. The supernatant was heated at 85°C for 10 min and the insoluble proteins were removed by centrifugation. The nondenatured proteins of the supernatant were concentrated by adding solid $(NH_4)_2SO_4$ to 80% saturation, redissolved in TEGD buffer [10 mM Tris, pH 7.9/0.1 mM dithiothreitol/0.1 mM EDTA/5% (vol/vol) glycerol], and dialyzed against the same buffer. The dialyzed material (≈200 mg) was applied to a DEAE-Sepharose 6B (Sigma) column (2.5 × 100 cm). Proteins were eluted with a 200 ml gradient of 0–1 M NaCl. Typically, TrpR was eluted at 0.3 M NaCl, as shown by SDS/PAGE of column fractions. The TrpR-containing fractions were combined, precipitated with $(NH_4)_2SO_4$ (80% saturation), dialyzed against PEGD buffer (5 mM sodium phosphate, pH 7.0/0.1 mM dithiothreitol/0.1 mM EDTA, 5% glycerol), and then subjected to chromatography on a 40-ml hydroxylapatite (Bio-Rad) column previously equilibrated with PEGD buffer. Bound proteins were eluted with a sodium phosphate gradient rising from 5 to 300 mM in 3–4 column volumes. TrpR emerged at 150 mM phosphate. As estimated by SDS/PAGE, about 40 mg of TrpR at >90% purity was obtained.

When it became apparent that TrpR and WrbA tended to cofractionate, a more detailed analysis by SDS/PAGE of the fractions generated by chromatography on hydroxylapatite was carried out. WrbA (see *Results*) was not detectable within TrpR-containing fractions from the trailing edge of the hydroxylapatite chromatogram. These samples were used in studies of the effect of WrbA on DNA binding (see Figs. 3 and 4).

***Hpa* I Endonuclease Protection Assay.** DNA (0.3 μg) from the *trp* operator (*trpO*)-bearing plasmid pVH153 was mixed with purified TrpR and/or purified WrbA (see below). The TrpR level was adjusted so that the *Hpa* I site that bisects *trpO* was 50% protected. The protein–DNA mixture, containing 1 mM L-tryptophan in 20 mM TrisOAc/10 mM $Mg(OAc)_2$/50 mM KOAc/1 mM dithiothreitol at pH 7.9, was incubated at 37°C for 30 min. Then 4 units of *Hpa* I (New England Biolabs) was added. After 2 hr the reactions were analyzed by horizontal gel electrophoresis in 0.8% agarose containing ethidium bromide (1 μg/ml). For further details, see the legend to Fig. 3.

Gel Mobility-Shift and DNase I Footprinting Analysis of Protein–DNA Interaction. pWPY2 (100 μg) was treated with 100 units of *Eco*RI in the presence of 10 units of alkaline phosphatase. The linearized, dephosphorylated DNA was freed of protein by three rounds of phenol extraction and was precipitated with ethanol. The DNA was then digested with *Bam*HI. After electrophoresis in 1% agarose, a 241-bp fragment containing *trpO* was excised and purified with Geneclean (Bio 101, La Jolla, CA). The DNA fragment was labeled with ^{32}P at the *Eco*RI end by standard procedures (26).

In mobility-shift experiments, TrpR and/or WrbA protein, 1 μg of poly(dI-dC)·poly(dI-dC) (Sigma), 1 mM L-tryptophan, and 60 fmol of end-labeled operator-bearing DNA were incubated in 20 μl of 20 mM TrisOAc, pH 7.9/10 mM $Mg(OAc)_2$/50 mM KOAc/1 mM dithiothreitol for 20 min at 37°C. Samples from the incubation mixtures were subjected to 6% PAGE. The gel and running buffer was 10 mM Tris/1 mM EDTA/0.5 mM L-tryptophan at pH 7.0. Autoradiography of the dried gels was carried out with Kodak XAR-70 film.

For DNase I footprinting, a 5-μl aliquot of DNase I working solution [DNase I (Worthington) at 0.5 unit/ml in 12 mM $MgCl_2$] was added to the TrpR/WrbA–DNA mixtures (identical to the mixtures used in mobility-shift experiments). After 2 min at room temperature, digestion was stopped by adding 5 μl of 50 mM EDTA. Proteins were removed by phenol extraction and DNA was precipitated by ethanol and appropriate salts in the presence of glycogen. The precipitated DNA was dissolved in 2 μl of 10 mM Tris, pH 8.0/1 mM EDTA and 2 μl of dye mixture (New England Biolabs) was added. The sample was heated at 90°C for 2 min and applied to a 8 M urea 8% polyacrylamide sequencing gel. The separated fragments were visualized by autoradiography of the dried gel with Kodak XAR-70 film.

Construction of *wrbA* Null Mutation. One microgram of pWPY5 linearized with *Bam*HI was used to transform *E. coli* JC7623 (25). The correct construct was provisionally identified by scoring a group of chloramphenicol-resistant transformants for ampicillin sensitivity. That the resulting isolates bore *wrbA* null mutations was demonstrated by immunoblotting using polyclonal anti-WrbA antibodies (L.N. and R.L.S., unpublished work) and by Southern blotting analysis of genomic DNA (data not shown). The *wrbA* null mutation was transferred into SP1411 by P1 transduction (30). The resulting *wrbA*::*cat* strain (where *cat* is the chloramphenicol acetyltransferase gene) was named SP1503.

RESULTS

Cloning of the Structural Gene for WrbA. When purified TrpR was subjected to SDS/PAGE, in addition to TrpR (12.5 kDa) a second protein, of apparent molecular mass 22–26 kDa, was almost invariably observed (Fig. 1) in different TrpR preparations made over a 5-year period. The second protein, designated WrbA, was transferred electrophoretically from an SDS/polyacrylamide gel to a poly(vinylidene difluoride) membrane and analyzed by sequential Edman degradation. A total of 17 amino acid residues were identified (Fig. 2*A*). No other *E. coli* proteins having any or all of this particular primary structure were found within the GenBank database of protein sequences (as of December 1992). Based on the experimentally determined N-terminal sequence, two degenerate octadecanucleotides were designed (Fig. 2*A*). The synthetic oligonucleotides were purified electrophoretically, end-labeled with ^{32}P, and used as probes for the segment of DNA that encodes WrbA. In Southern blotting experiments on *E. coli* genomic DNA digested with various restriction endonucleases, the presumptive *wrbA* gene was found to lie within a *Hin*dIII fragment of 1.7 kb. A population of *Hin*dIII fragments of this size derived from chromosomal

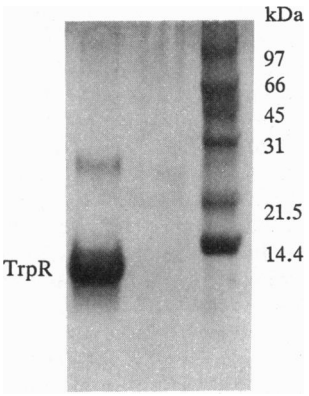

FIG. 1. Copurification of WrbA with TrpR. Left lane, TrpR (pooled hydroxylapatite fractions; see *Materials and Methods*); right lane, protein standards (Bio-Rad). Ten microliters of TrpR solution was mixed with 10 μl of 2× Laemmli sample buffer, heated at 90°C for 5 min, and was subjected to SDS/10% PAGE using a Tricine buffer system (31). Protein was visualized by staining with 2% Coomassie blue R-250 (Sigma) followed by destaining in 10% ethanol/10% acetic acid.

DNA were excised from 1% agarose and inserted into the *Hin*dIII site of phage vector M13mp18. The desired clone was identified by plaque hybridization with the same radiolabeled oligonucleotides (Fig. 2*A*) that had been employed in Southern blotting. Single-stranded DNA was generated from an M13mp18 clone carrying the insert and DNA sequencing was performed. The DNA sequence (Fig. 2*B*) confirmed the sequence of the amino terminus of WrbA (Fig. 2*A*).

WrbA-Mediated Enhancement of TrpR Binding to *trpO*. By PCR an *Nde* I–*Bam*HI fragment of DNA was synthesized that contained the first ATG codon of WrbA plus 120 bp from the downstream side of the inferred termination codon of *wrbA*. The fragment was inserted into pET3a that had been cleaved with *Nde* I and *Bam*HI. The expression of *wrbA* thus became subject to transcriptional and translational control by the T7 promoter and the T7 gene *10* punctuation signals. The resultant plasmid, pWrbAT7, was introduced into a *trpR* mutant strain (SP1514) derived from *E. coli* BL21. High-level production of WrbA was observed when plasmid-bearing cells were grown to saturation in LB medium. For unknown reasons, it proved to be unnecessary to provide T7 RNA polymerase in order to trigger WrbA overproduction. WrbA was purified by a scheme patterned after that of TrpR except that selective heat denaturation was performed at 65°C for 10 min. The purity of WrbA was estimated to be >90% by Coomassie blue staining. During size-exclusion chromatography on a calibrated Superose 12 HPLC gel filtration column (Pharmacia), native WrbA had a mobility characteristic of a dimer (data not shown).

Purified WrbA was tested for its possible effect on the interaction of TrpR holorepressor with *trpO*. In an endonuclease protection assay carried out in the presence of suboptimal levels of TrpR, the WrbA protein was found to increase the effective amount of *TrpR*–operator complex. Full protection by TrpR of a *Hpa* I site within *trpO* was observed in the presence of WrbA under conditions where only half protection takes place in the absence of WrbA (Fig. 3). There was no operator protection by TrpR plus WrbA in the absence of tryptophan (data not shown). A gel mobility-shift study (Fig. 4) provided further support for the notion that WrbA positively affects the formation and/or stability of TrpR–*trpO* complexes. In a tryptophan-dependent fashion, there was at least 6-fold enhancement in the efficacy of TrpR binding to *trpO* when WrbA was present. The mobility of the supershifted species observed in the presence of WrbA (Fig. 4) is consistent with the formation of a ternary complex consisting of operator DNA, TrpR holorepressor and WrbA. In a parallel experiment, an 8-fold molar excess of bovine serum albumin failed to produce the supershifted species in the presence of TrpR (data not shown).

DNase I Footprinting Analysis of TrpR–DNA Complexes. To test whether WrbA altered the nature of the TrpR–operator interaction, DNase I footprinting experiments were carried out. The overall pattern of protection by TrpR of operator DNA was unaltered in the TrpR–operator–WrbA ternary complex (Fig. 5). This result points to an indirect role for WrbA in enhancing the strength of binding of TrpR to its operator, as opposed to direct interaction between WrbA and DNA.

Effect of a *wrbA* Null Mutation on *trp* Promoter Utilization. To investigate the possible functional role of WrbA in TrpR-mediated repression, *E. coli* strains bearing chromosomal *wrbA* null mutations were constructed. This mutation was introduced into a Δ(*lacZ*) strain carrying a single-copy *lacZ*$^+$ reporter gene driven by the *trp* promoter. The levels of β-galactosidase were measured throughout the growth cycle.

A

(Met)·(Ala)·Lys·Val·Leu·Val·Leu·Tyr·Tyr·Ser·Met·Tyr·Gly·His·Ile·Glu·Thr·Met·Ala

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TTN·TAT·TAT·TCN·ATG·TA        CAT·ATT·GAA·ACN·ATG·GC
C    C  C                      C  C  G
                                  A

Probe 6D                      Probe 5D
(128-fold degenerate)         (48-fold degenerate)
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B

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ATGGCTAAAGTTCTGGTGCTTTATTATTCCATGTACGGACATATTGAAACGATGGCACGC
 M  A  K  V  L  V  L  Y  Y  S  M  Y  G  H  I  E  T  M  A  R
GCAGTCGCTGAGGGTGCAAGCAAAGTGGATGGCGCTGAAGTTGTCGTTAAGCGTGTACCG
 A  V  A  E  G  A  S  K  V  D  G  A  E  V  V  V  K  R  V  P
GAAACCATGCCGCCGCAATTATTTGAAAAAGCAGGCGGTAAAACGCAAACTGCACCGGTT
 E  T  M  P  P  Q  L  F  E  K  A  G  G  K  T  Q  T  A  P  V
GCAACCCCGCAAGAACTGGCCGATTACGACGCCATTATTTTTGGTACACCTACCCGCTTT
 A  T  P  Q  E  L  A  D  Y  D  A  I  I  F  G  T  P  T  R  F
GGCAACATGTCCGGTCAAATGCGTACCTTCCTCGACCAGACGGGCGGCCTGTGGGCTTCC
 G  N  M  S  G  Q  M  R  T  F  L  D  Q  T  G  G  L  W  A  S
GGCGCACTATACGGAAAACTGGCGAGCGTCTTTAGTTCCACCGGTACTGGCGGCGGTCAG
 G  A  L  Y  G  K  L  A  S  V  F  S  S  T  G  T  G  G  G  Q
GAACAAACCATCACATCCACCTGGACGACCCTTGCGCATCACGGCATGGTAATTGTCCCT
 E  Q  T  I  T  S  T  W  T  T  L  A  H  H  G  M  V  I  V  P
ATTGCGTACGCAGCGCAGGAATTATTTGACGTTTCACAGGTTCGCGGCGGTACGCCGTAC
 I  A  Y  A  A  Q  E  L  F  D  V  S  Q  V  R  G  G  T  P  Y
GGCGCAACCACCATCGCAGGCGGTGACGGTTCACGCCAGCCCAGCCAGGAAGAACTGTCT
 G  A  T  T  I  A  G  G  D  G  S  R  Q  P  S  Q  E  E  L  S
ATTGCTCGTTATCAAGGGGAATATGTCGCAGGTCTGGCAGTTAAACTTAACGGCTAA
 I  A  R  Y  Q  G  E  Y  V  A  G  L  A  V  K  L  N  G  *
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FIG. 2. (*A*) N-terminal sequence of WrbA as determined by sequential Edman degradation. As revealed by DNA sequencing of the cloned *wrbA* gene, the methionine and alanine residues (in parentheses) are proteolytically removed during or subsequent to translation. The degenerate oligonucleotides (6D and 5D) that were used as probes in cloning and blotting are shown. (*B*) Nucleotide sequence of the *wrbA* gene and the deduced amino acid sequence of the WrbA protein. The entire sequence of a *Hin*dIII fragment (1.7 kb) containing *wrbA* was determined; only those portions relevant to the present paper are shown. The *wrbA* gene lies at coordinate 1076 of the recalibrated map of the *E. coli* genome, as determined by DNA hybridization to members of Kohara's set (42) of phage clones (L.N. and R.L.S., unpublished work).

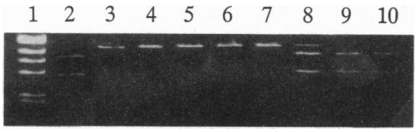

FIG. 3. Effects of WrbA on the binding of TrpR to operator-bearing DNA as determined by *Hpa* I endonuclease protection. Lane 1, DNA markers (phage λ DNA digested with *Bst*EII); lanes 2–7, incubation mixtures containing 0.6 μM TrpR, 1 mM L-tryptophan, and increasing amounts of WrbA (0.04, 0.4, 4, 12, 20, and 28 μM, respectively); lane 8, TrpR control (no WrbA); lane 9, TrpR-negative control lacking both WrbA and L-tryptophan; lane 10, WrbA control (no TrpR); the incubation mixture contained 20 μM WrbA and 1 mM L-tryptophan. TrpR used in this study was essentially free of WrbA (see *Materials and Methods*).

By late stationary phase, there was a 2-fold difference in the β-galactosidase levels of the wild-type and the *wrbA* mutant strain (data not shown). This result supports the idea that the repression of the *trp* operon by TrpR is more efficient in stationary-phase cells that contain WrbA.

DISCUSSION

In recent years it has become apparent that protein complexes, not individual regulatory proteins, are the entities that bind to specific target sequences to turn genes off and on. In *E. coli*, the WrbA protein appears to function as an accessory factor in modulating the interaction of TrpR with its primary operator target.

The characterization of WrbA was greatly facilitated by the fact that preparations of TrpR were almost invariably enriched in this protein. However, there have been previous strong indications from immunochemical work (22) that *E. coli* extracts contain several proteins capable of interacting with TrpR, one of which (WrbA) turned out to be the protein that was characterized in the current study.

It has not been established whether WrbA was complexed with TrpR throughout fractionation. Analytical gel-permeation chromatography studies designed to directly demonstrate stable complexes between TrpR and WrbA were negative, although such experiments did show that WrbA is dimeric (L.N. and R.L.S., unpublished work). It is likely that in the absence of operator-bearing DNA, the affinity of WrbA for TrpR is weak. The high and physiologically abnormal levels of TrpR in extracts of engineered overproduction strains are thought to underlie the persistence of WrbA in our TrpR preparations. There are two published SDS/PAGE analyses of TrpR preparations (32, 33). The previous studies involved the use of extracts that were either prepared from

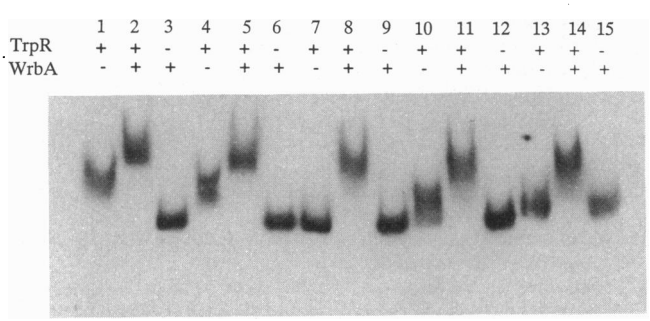

FIG. 4. Mobility-shift analysis of complexes formed between TrpR WrbA and *trp* promoter/operator DNA. All samples contained 1 mM L-tryptophan. TrpR concentrations were 2.4 μM (lanes 1 and 2), 1.2 μM (lanes 4 and 5), 0.6 μM (lanes 7 and 8), 0.3 μM (lanes 10 and 11), and 0.1 μM (lanes 13 and 14). For lanes 3, 6, 9, 12, and 15 incubation mixtures contained only 2.4 μM WrbA protein. TrpR used in this study was essentially free of WrbA (see *Materials and Methods*).

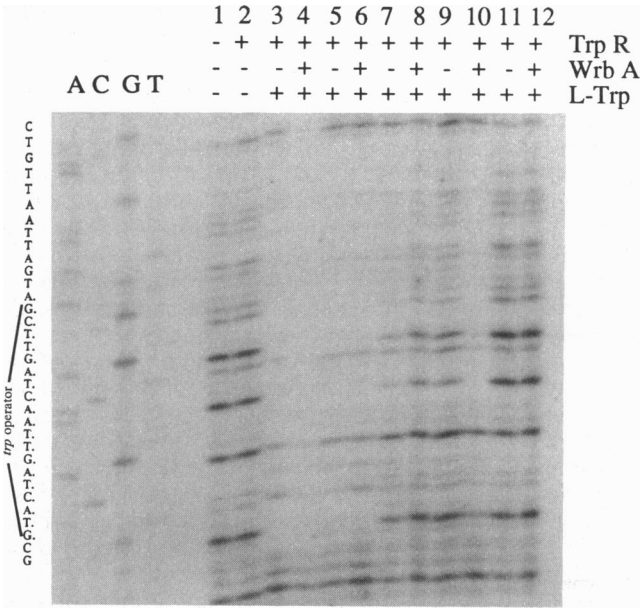

FIG. 5. DNase I footprinting of *trpO* DNA in the presence or absence of WrbA. Lane 1, DNA control; lane 2, TrpR control without L-tryptophan; lanes 3–12, various amounts of TrpR presence or absence of 2.4 μM WrbA. TrpR concentrations were 0.6 μM (lanes 2–4), 0.3 μM (lanes 5 and 6), 0.1 μM (lanes 7 and 8), 0.05 μM (lanes 9 and 10), and 0.01 μM (lanes 11 and 12). No operator protection was observed in the absence of tryptophan (data not shown). TrpR used in this study was essentially free of WrbA (see *Materials and Methods*). Lanes A, C, G, and T show products of sequencing reactions.

isopropyl β-D-thiogalactopyranoside-induced logarithmic-phase cells (32) or from cells which produced TrpR continuously and were harvested in the late logarithmic phase of growth (33). In neither of the photographs of protein gels presented in these papers were there discernible protein bands corresponding to WrbA.

In *Hpa* I endonuclease protection assays and in gel mobility-shift studies, WrbA enhanced the formation and/or stability of complexes between TrpR holorepressor and its primary operator target (Figs. 3 and 4). WrbA alone was unable to interact with operator-bearing DNA. WrbA thus resembles the Bof protein of bacteriophage P1 (34, 35). This protein forms ternary complexes with 13 different operators, provided that the C1 immunity repressor of phage P1 is present.

It is our working hypothesis that WrbA assists in the down-regulation of *trp* mRNA synthesis by blocking the access of RNA polymerase to *trpO*-containing promoters more efficiently than TrpR alone. Whether there are quantitative differences between the other known TrpR-responsive promoters with respect to WrbA-mediated ternary complex formation remains to be tested. This is a matter of particular interest because of the wide variation *in vivo* in amplitude of repression among several TrpR-responsive promoters containing operator targets of essentially identical affinity for the TrpR holorepressor *in vitro* (20).

Within the limits of resolution of DNase I footprinting, the region of *trp* promoter DNA protected by TrpR is the same in the presence or absence of WrbA. It was important to consider the possibility that the footprints might differ in the presence or absence of WrbA, given previous work (36, 37) that suggested that more than one molecule of dimeric TrpR could associate with *trpO*-bearing DNA. The TrpR–WrbA–*trpO* complex was essentially indistinguishable from the TrpR–*trpO* complex with respect to the ability of DNase I to attack phosphodiester bonds in the vicinity of *trpO* (Fig. 5). This result is fully compatible with our observation that WrbA alone does not interact with *trp* promoter-bearing

DNA and suggests that the TrpR–*trpO* complex is the major target species for WrbA.

From a consideration of the geometry of the TrpR–operator complex (9), the most likely surfaces of TrpR that would be available to engage WrbA without affecting DNase I-accessible phosphodiester bonds would lie on the solvent-exposed faces of the A, B, and F helices or on the short polypeptide loops connecting helices A and B or E and F. It should be possible to employ mutagenic procedures to identify amino acid residues within TrpR that may either participate directly in WrbA–TrpR interaction or be essential for whatever conformational changes may be important for the addition of WrbA to the TrpR–*trpO* complex.

The enhanced stability and/or rate of formation of the TrpR–WrbA–*trpO* complex, in comparison to that of the TrpR–*trpO* complex, implies that there is an important physicochemical distinction between the two types of protein–DNA complexes. Because this distinction was not revealed by DNase I footprinting, it will be necessary to employ more incisive chemical or physicochemical methodology in order to achieve a satisfactory understanding of the geometry of the system.

Because WrbA appears to have very low affinity for TrpR in the absence of operator-bearing DNA, it seems reasonable to conclude that TrpR undergoes significant conformational change upon binding to *trpO* and that such an event is important for the entry of WrbA into the ternary complex. Precedent for DNA-mediated conformational change in operator binding proteins comes from crystallographic studies on the Cro protein (38), from spectroscopic studies of the immunity repressor of bacteriophage λ (39, 40), and from analysis by limited proteolysis of the pheremone/receptor transcription factor (also known as MCM1) of yeast (41). Interestingly, the binding of MCM1 to its target sequence is a prelude to the recruitment of ternary complex factors in promoting gene expression.

Separate studies have shown that the *wrbA* gene is preferentially (although not exclusively) expressed in cells that have exited the logarithmic phase of growth. Moreover, the stationary-phase σ factor of *E. coli* (σ^s) must be present for high-level transcription from the *wrbA* promoter to be observed (L.N. and R.L.S., unpublished work). The ability of WrbA to enhance the formation and/or stability of TrpR–operator complexes suggests that one role for WrbA may be to accentuate the shutdown of genes or operons, such as those of tryptophan metabolism, whose expression in stationary-phase cells might be superfluous or even harmful. If this hypothesis is supported by further studies, the TrpR–WrbA system would be an example of a previously unrecognized level of transcriptional regulation of genes bearing recognition sites for TrpR.

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1. Luisi, B. F. & Sigler, P. B. (1990) *Biochim. Biophys. Acta* **1048,** 113–126.
2. Somerville, R. L. (1992) *Prog. Nucleic Acid Res. Mol. Biol.* **42,** 1–38.
3. Kelley, R. L. & Yanofsky, C. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 483–487.
4. Bass, S., Sorrells, V. & Youderian, P. (1988) *Science* **242,** 240–245.
5. Chou, W.-Y. & Matthews, K. S. (1989) *J. Biol. Chem.* **264,** 18314–18319.
6. Hurlburt, B. K. & Yanofsky, C. (1990) *J. Biol. Chem.* **265,** 7853–7858.
7. Arvidson, D. N., Shapiro, M. & Youderian, P. (1991) *Genetics* **128,** 29–35.
8. Schevitz, R. W., Otwinowski, Z., Joachimiak, A., Lawson, C. L. & Sigler, P. B. (1985) *Nature (London)* **317,** 782–786.
9. 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.
10. Lane, A. N. & Jardetzky, O. (1985) *Eur. J. Biochem.* **152,** 395–404.
11. Borden, K. L. B., Bauer, C. J., Frenkiel, T. A., Beckmann, P. & Lane, A. N. (1992) *Eur. J. Biochem.* **204,** 137–146.
12. Tasayco, M. L. & Carey, J. (1992) *Science* **255,** 594–597.
13. Fernando, T. & Royer, C. (1992) *Biochemistry* **31,** 3429–3441.
14. Tsapakos, M., Haydock, P. V., Hermodson, M. & Somerville, R. L. (1985) *J. Biol. Chem.* **260,** 16383–16394.
15. Zubay, G., Morse, D. E., Schrenk, W. J. & Miller, J. H. M. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1100–1103.
16. Shimizu, Y., Shimizu, N. & Hayashi, M. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 1990–1994.
17. Squires, C. L., Rose, J. K., Yanofsky, C., Yang, H.-L. & Zubay, G. (1973) *Nature New Biol.* **245,** 131–133.
18. Rose, J. K. & Yanofsky, C. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3134–3138.
19. Squires, C. L., Lee, F. D. & Yanofsky, C. (1975) *J. Mol. Biol.* **92,** 93–111.
20. Klig, L. S., Carey, J. & Yanofsky, C. (1988) *J. Mol. Biol.* **202,** 769–777.
21. Charlier, D., Roovers, M., Van Vliet, F., Boyen, A., Cunin, R., Nakamura, Y., Glansdorff, N. & Pierard, A. (1992) *J. Mol. Biol.* **226,** 367–386.
22. Somerville, R. L., Zeilstra-Ryalls, J. H. & Shieh, T.-L. (1990) in *Structural and Organizational Aspects of Metabolic Regulation*, eds. Srere, P. A., Jones, M. E. & Mathews, C. K. (Wiley-Liss, New York), pp. 181–197.
23. Studier, F. W. & Moffatt, A. B. (1986) *J. Mol. Biol.* **189,** 113–130.
24. Hanahan, D. (1985) in *DNA Cloning: A Practical Approach*, ed. Glover, D. M. (IRL, Washington, DC), Vol. 1, pp. 109–135.
25. Kushner, S. R., Nagaishi, H., Templin, A. & Clark, A. J. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 824–827.
26. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
27. Helinski, D. R., Hershfield, V., Figurski, D. & Meyer, R. J. (1977) in *Recombinant Molecules: Impact on Science and Society*, eds. Beers, R. F. & Basset, E. G. (Raven, New York), pp. 151–165.
28. Schweizer, H. P. (1990) *BioTechniques* **8,** 612–616.
29. Vogel, H. J. & Bonner, D. M. (1956) *J. Biol. Chem.* **218,** 97–106.
30. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
31. Schägger, H. & von Jagow, G. (1987) *Anal. Biochem.* **166,** 368–379.
32. Paluh, J. L. & Yanofsky, C. (1986) *Nucleic Acids Res.* **14,** 7851–7860.
33. Arvidson, D. N., Kumamoto, A. A. & Gunsalus, R. P. (1987) *UCLA Symp. Mol. Cell. Biol.* **68,** 401–407.
34. Velleman, M., Heinzel, T. & Schuster, H. (1992) *J. Biol. Chem.* **267,** 12174–12181.
35. Lehnherr, H., Velleman, M., Guidolin, A. & Arber, W. (1992) *J. Bacteriol.* **174,** 6138–6144.
36. Kumamoto, A. A., Miller, W. G. & Gunsalus, R. P. (1987) *Genes Dev.* **1,** 556–564.
37. Staacke, D., Walter, B., Kisters-Woike, B., von Wilcken-Bergmann, B. & Müller-Hill, B. (1990) *EMBO J.* **9,** 1963–1967.
38. Brennan, R. G., Roderick, S. L., Takeda, Y. & Matthews, B. W. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8165–8169.
39. Benevides, J. M., Weiss, M. A. & Thomas, G. L., Jr. (1991) *Biochemistry* **30,** 4381–4388.
40. Saha, R., Banik, U., Bandopadhyay, S., Mandal, N. C., Bhattacharyya, B. & Roy, S. (1992) *J. Biol. Chem.* **267,** 5862–5867.
41. Tan, S. & Richmond, T. J. (1990) *Cell* **62,** 367–377.
42. Kohara, Y., Akiyama, K. & Isono, K. (1987) *Cell* **50,** 495–508.