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Intracelluar Trp Repressor Levels in *Escherichia coli*

ROBERT P. GUNSALUS,[1,2]* ANN GUNSALUS MIGUEL,[1]† AND GLEN L. GUNSALUS[3]

Department of Microbiology[1] and the Molecular Biology Institute,[2] University of California, Los Angeles, California 90024, and The Population Council, New York, New York 10021[3]

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A radioimmunoassay for the Trp repressor protein of *Escherichia coli* was developed with antisera raised against purified Trp repressor protein. This assay was used to directly measure the intracellular Trp repressor content in several *E. coli* K-12 and B/r strains. Repressor levels varied from 2.5- to 3-fold in response to L-tryptophan concentration in the growth medium (15 to 44 ng of repressor per mg of protein). Neither cell growth rate nor culture age had a significant effect on repressor concentrations within the cell. Addition of L-tryptophan to the growth medium resulted in lowered intracellular levels of Trp repressor. The absolute amounts of native Trp repressor molecules per cell varied between 120 and 375 dimers in the presence and absence of L-tryptophan in the culture medium, respectively. Assuming an intracellular volume of 7.3 μl/10^{10} *E. coli* cells, the Trp repressor concentration varied from 270 to 850 nM in response to extracellular tryptophan levels. These findings represent the first direct measurements of Trp repressor levels in *E. coli* and confirm the autoregulatory nature of the *trpR* gene.

The Trp repressor of *Escherichia coli* is encoded by the *trpR* gene and negatively regulates gene expression of the unlinked *trp* and *aroH* operons that are involved in cellular L-tryptophan biosynthesis (19, 20, 27). The *trpR* gene has been cloned onto phage and plasmid vectors (14, 22), and the DNA sequence has been determined (13). Its expression has been shown to be autoregulated in response to L-tryptophan levels (13, 16). Protein size predictions from DNA sequence and gel sizing studies of purified Trp repressor protein indicate that the repressor is composed of a single peptide, 108 amino acids in length (M_r, 12,356) (13). The native form of the Trp aporepressor protein is a stable dimer composed of two identical subunits (1, 15).

Estimates of Trp repressor levels in *E. coli* suggest that relatively small amounts of the regulatory protein are present in the cell (23, 26). The values, based on indirect analysis, range from 10 to 30 molecules per cell. Gene fusion methods also have been used to calculate a cellular Trp repressor level; these give a larger value of 150 molecules per cell for *E. coli* cells grown in minimal medium (16). In this report, we examine the effect of L-tryptophan on the intracellular levels of Trp repressor in *E. coli* with a radioimmunoassay that is specific for this regulatory protein.

MATERIALS AND METHODS

Materials. Iodogen was obtained from Pierce Chemical Co. (Rockford, Ill.); ^{125}INa (IMS-300) from Amersham Corp. (Arlington Heights, Ill.); bacitracin, indole acrylic acid, L-tryptophan, and Sephadex G-10 were from Sigma Chemical Co. (St. Louis, Mo.); polyethylene glycol (Carbowax PEG 6000) was from Fisher Chemical Co. (Fair Lawn, N.J.); and goat anti-rabbit immunoglobulin G was from Antibodies, Inc. (Davis, Calif.).

Bacterial strains and plasmids. *E. coli* K-12 strains W3110 (wild type) (2) and LE392 (F^- *hsdR514* [*hsdR hsdM*] *supE44 supE58 lacY1 galK2 galT22 metB1 trpR55* λ^- [17]) and *E. coli B* strain B/r UP1002 (*leu*) (11) were used in the determination of the cellular Trp repressor levels. Plasmid pRPG12, which contains the *trpR*$^+$ gene under bacteriophage lambda p_L control, was previously described (14).

Media and cell growth. Bacterial growth rate was controlled by the composition of the media. Vogel-Bonner minimal E medium (24) was supplemented with 0.2% glycerol, 0.2% glucose, or 0.2% glucose plus 0.2% Casamino Acids (Difco Laboratories, Detroit, Mich.). Individual amino acids and thiamine were supplied at final concentrations of 100 and 1 μg/ml, respectively, when required. The preferred growth medium used was Luria broth plus 0.1% glucose (4). L-Tryptophan was added to a final concentration of 100 μg/ml where indicated. For measurement of Trp repressor levels, cells were grown overnight with shaking at the temperature and in the medium indicated. A sample of the overnight culture was then diluted 200-fold into 100 ml of prewarmed medium in a 500-ml Erlenmeyer flask. The growth rate was determined by measuring the change in A_{600} with a Gilford 300-N spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). The growth rate constant k was calculated from the expression $k = \ln 2/t$, where t is the cell-doubling time in hours. Cells were diluted in a phosphate-buffered salt (PBS) (150 mM NaCl, 10 mM $NaPO_4$ [pH 7]) and held on ice. The cells were then fixed with formaldehyde (0.45%) and counted in a Petroff-Hausser counting chamber. The cell numbers were confirmed by counting in an Electrozone Celloscope particle counter (model 112 CLTH; Particle Data Inc., Elmhurst, Ill.) with a 24-μm orifice. Viable cell numbers were determined by plating in triplicate on Luria broth plates. Cell numbers determined by these methods were in close agreement except for viable cell counts of cultures in stationary phase.

Preparation of cell extracts. Exponential-phase cultures (A_{600} of 0.35 for cells grown on glycerol-containing medium, and A_{600} of 0.8 for all others) were cooled on ice, harvested by centrifugation (10,000 × g for 10 min at 4°C), and suspended in 2.5 ml of PBS. After removal of 50 μl of medium for determination of cell numbers, indole acrylic acid was added to the flask to a final concentration of 200 μM. Cell extracts were prepared by sonication and centrifugation (20,000 × g for 20 min at 4°C). Protein concentration was determined with the Bio-Rad protein assay (Bio-Rad

* Corresponding author.
† Present address: Instituto de Biofisica, Universidade Federal Do Rio De Janeiro, Rio de Janeiro, RJ-20000, Brazil.

Laboratories, Richmond, Calif.), based on the method of Bradford (7).

Preparation of antiserum. Purified Trp repressor was prepared from a pRPG12 plasmid-bearing LE392 strain as previously described (1). Purity of the repressor protein was estimated at greater than 99.5% by sodium dodecyl sulfate-polyacrylamide gel analysis. Trp repressor was used to generate antisera in New Zealand White rabbits as described by Shiigi (18).

Radioiodination of Trp repressor. Radioiodination of purified Trp repressor was performed with Iodogen (10). Iodination tubes were prepared by coating glass tubes (6 by 50 mm) with 5 μg of Iodogen suspended in 50 μl of dichloromethane and drying them at room temperature. Iodination was carried out at room temperature in a total volume of 50 μl consisting of 5 μg of Trp repressor suspended in 0.1 M sodium phosphate buffer, pH 7.2 (NPB), and 1.0 mCi of ^{125}I. After 5 min, iodinated Trp repressor was separated from free iodine by loading the reaction mixture onto a Sephadex G-10 column (10-ml bed volume) which had been washed with 1 ml of 2% bovine serum albumin in NPB and equilibrated with 20 ml of NPB. The sample was eluted with NPB and collected in 0.5-ml fractions. Fractions containing ^{125}I-labeled Trp repressor were pooled, divided into 100-μl aliquots, and stored at −20°C.

Radioimmunoassay for Trp repressor protein. A working solution of rabbit anti-Trp repressor was prepared by diluting antiserum 1/50,000 in PBS (0.15 M NaCl, 10 mM $NaPO_4$ [pH 7.4]) containing 0.2% normal rabbit serum. All other reagents were prepared in PBS containing 0.5% bacitracin (radioimmunoassay buffer). Each assay tube contained 100 μl of ^{125}I-labeled Trp repressor (approximately 10 × 10^3 to 15 × 10^3 cpm per tube) as standard or sample in 300 μl of radioimmunoassay buffer and 100 μl of working solution of anti-Trp repressor. Sample tubes were mixed briefly on a Vortex mixer and incubated for 24 h at 4°C. Goat anti-rabbit immunoglobulin G adequate to precipitate antigen-antibody complex (typically 5 μl) and 50 μl of 12% Carbowax in PBS were added, and the mixtures were incubated for an additional 24 h at 4°C. Tubes were then centrifuged at 2,000 × *g* for 20 min at 4°C, and the supernatants were carefully aspirated before determining the radioactivity in the pellets at 75% counting efficiency (model 5110; Packard Instrument Co., Inc., Rockville, Md.).

Data for the displacement of ^{125}I-labeled Trp repressor by Trp repressor preparations from various sources were compared by simultaneously fitting them to four-parameter logistic curves, with and without constraints, using the computer program ALLFIT (9).

Highly purified Trp repressor (0.91 mg/ml) was used to generate the standard curves from which Trp repressor levels in cell samples were determined. Standard curves were fitted to a four-parameter logistic function, and unknowns were interpolated from the resultant curve with a computer code adapted from Rodbard et al. (21). All standards and unknowns were run in duplicate with multiple dilutions and aliquot sizes. All samples were run in a single assay for the radioimmunoassay results reported in this paper. Trp repressor dimers were considered to be 24,712 M_W (or 2 × 12,356) (1, 15) for expression of results as Trp repressor dimers per cell and normalized to cell counts performed on samples from the same cultures.

RESULTS

Radioimmunoassay and validation. A radioimmunoassay for Trp repressor was developed with antiserum specific for *E. coli* Trp repressor protein. Anti-Trp repressor was used at a final dilution of 1/250,000, which typically bound 30 to 40% of the ^{125}I-labeled Trp repressor. Increasing amounts of purified Trp repressor generated a typical displacement curve (Fig. 1). The midpoint ranged from 0.24 to 0.33 ng of Trp repressor per tube. Over the useful range of the assay (0.05 to 1.5 ng of Trp repressor per tube), the coefficient of variation within the assay was less than 6%, and the minimum detectable dose for duplicate samples was 0.03 ng of Trp repressor per tube. The addition of 0.5% bacitracin to radioimmunoassay buffer was found to decrease nonspecific binding, and the addition of Carbowax at a final concentration of 1% in the second antibody incubation improved assay precision by stabilizing pellet formation. Indole acrylic acid was routinely added before cell disruption to insure that Trp aporepressor remained free of cellular DNA during preparation of cell extracts. In separate experiments we determined that addition of indole acrylic acid to cell extracts or to tubes containing pure Trp aporepressor had no effect on the radioimmunoassay.

Levels of Trp repressor were readily measurable in 1- to 5-μl samples from cell extracts prepared from wild-type *E. coli* grown under various nutrient conditions. Furthermore, these samples exhibited displacement curves parallel to that of purified Trp repressor (Fig. 1). Computer analysis of complete displacement curves generated with purified Trp repressor and from cell extracts confirmed that the curves were parallel and reached the same maximum displacements (data not shown). In contrast, no Trp repressor could be detected in a *trpR* mutant (LE392) known to be defective for *trp* operon repression (Fig. 1). Under these conditions (200 μl of sample) and with the detection limit of the assay, this corresponds to <0.2 Trp repressor dimers per cell.

Two additional experiments were performed to insure that the inability to detect Trp repressor in the *trpR* strain was not due to the presence of material that either (i) interfered with the radioimmunoassay or (ii) caused the destruction of the Trp repressor in this strain. Complete displacement curves were generated with pure Trp repressor in the presence and absence of 30-μl samples of cell extracts prepared from LE392 to test for the presence of materials capable of interfering with the radioimmunoassay. The two displacement curves were indistinguishable. It should be noted that 30-μl portions of all other Trp repressor positive samples measured in this report would yield maximum displacement of ^{125}I-labeled Trp repressor in this assay. The possibility that cell extracts from LE392 might contain factors capable of destroying Trp repressor was also tested. Two identical samples of W3110 cell extract, which would normally fall in the central part of the displacement curve, were mixed with 30-μl samples of extracts from LE392 and radioimmunoassay buffer, left at room temperature for 60 min, and were then assayed by radioimmunoassay for Trp repressor levels. The measurements of Trp repressor levels in W3110 samples in the presence and absence of samples from LE392 yielded identical results. The specificity of the antiserum towards Trp repressor in cell extracts prepared from LE392 and LE392 containing a wild-type copy of the *trpR* gene on pRPG47 was evaluated by Western blotting experiments (unpublished data). A single band corresponding to the Trp repressor protein was observed in lanes containing cell protein from *E. coli* K-12 LE392(pRPG47). This band was absent in lanes containing cell extracts of LE392.

Intracellular levels of Trp repressor. Using the radioimmunoassay for Trp repressor, we examined the absolute levels of Trp repressor in several strains of *E. coli* K-12 and

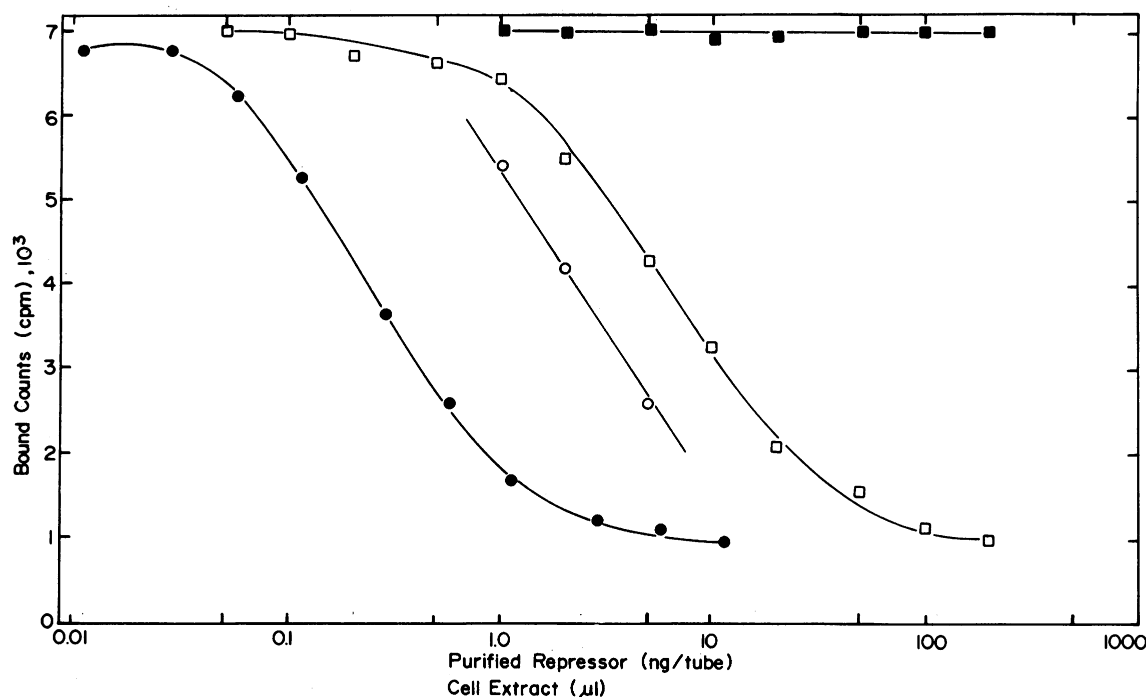

FIG. 1. Displacement of ^{125}I-labeled Trp repressor from anti-Trp repressor by purified Trp repressor and cell extracts from *E. coli* K-12 strain W3110 (2.33 mg/ml), LE392 (2.42 mg/ml), and LE392(pRPG12) (13.4 mg/ml). Pure Trp repressor was expressed as nanograms per tube (●). *E. coli* cell extracts, expressed as microliters of extract, were: W3110 (□), LE392 (■) (expressed as nanoliters of extract), and LE392(pRPG12) (○). Cell extracts were prepared as described in the text.

E. coli B/r. Trp repressor in *E. coli* W3110 varied with L-tryptophan concentration (Table 1). In the absence of added L-tryptophan, the repressor levels were about 44 ng/mg of cell protein, which was threefold higher than the concentration in cells grown in the same medium supplemented with L-tryptophan. In contrast, strain LE392 (*trpR*) had no detectable Trp repressor (i.e., less than 0.1 ng/mg) when cells were grown under identical conditions. Although the biochemical nature of the *trpR* mutation in strain LE392 is unknown, these results suggest that either Trp repressor is not made or that a defective aporepressor peptide is produced that has no cross-reactivity with antiserum to wild-type Trp repressor.

TABLE 1. Levels of Trp repressor in several *E. coli* strains grown in the presence and absence of added L-tryptophan

Strain	Addition of L-tryptophan	Trp repressor level	
		ng/mg[a]	Molecules/cell[b]
W3110 (K-12)	−	44.0	377
	+	15.2	120
LE392 (K-12)	−	<0.1	<0.2
	+	<0.1	<0.2
U1002 (B/r)	−	49.1	155
	+	16.9	59

[a] Repressor levels expressed as nanograms of Trp repressor per milligram of cell protein.

[b] Levels expressed as molecules of native Trp repressor dimer per cell. Cells were grown at 37°C in mineral salts medium plus 0.2% glucose and 0.2% Casamino Acids. Cell numbers were determined as indicated in the text. The number of Trp repressor dimers per cell was calculated by dividing the total number of dimers by the total number of cells where 1 nmol (24.712 µg) equals 6.02×10^{14} molecules of Trp repressor.

When the Trp repressor levels were calculated in terms of the number of molecules of native repressor dimer per cell, the cell contained between 120 and 370 dimers of the regulatory protein (Table 1). Examination of *E. coli* B/r U1002, which is also wild type for the $trpR^+$ gene, shows that the absolute amounts of Trp repressor are equivalent to those of strain W3110 when compared on a protein weight basis but are about one-third to one-half of those seen in the K-12 strain on a molecule-per-cell basis. The range of production of Trp repressor protein in this B/r strain extends to 2.5 times its lowest level, depending on the nutrient composition of the growth medium.

Effect of cell growth rate on Trp repressor levels. To determine if the $trpR^+$ gene expression is growth rate dependent, strain W3110 was inoculated and grown in medium containing different carbon sources. At appropriate density, cells were harvested and broken, and the intracellular levels of Trp repressor were measured by radioimmunoassay. The amount of Trp repressor per cell remained relatively constant over a cell-doubling time of 25 to 120 min when cells were grown with added L-tryptophan (Fig. 2). Omission of L-tryptophan from the medium resulted in the elevated levels of Trp repressor.

Effect of cell growth temperature on Trp repressor levels. To examine the effect of temperature on repressor production in W3110, wild type for $trpR^+$, cells were grown in a basal medium plus 0.2% glucose in either the presence or absence of added L-tryptophan. At appropriate turbidity, cells were harvested by centrifugation, washed, and broken by sonication before radioimmunoassay.

Repressor levels remained relatively constant (35 ng/mg of cell protein) in cells grown in a minimal salts glucose medium without added L-tryptophan (Fig. 3). Addition of L-tryptophan to the culture medium resulted in reduced Trp

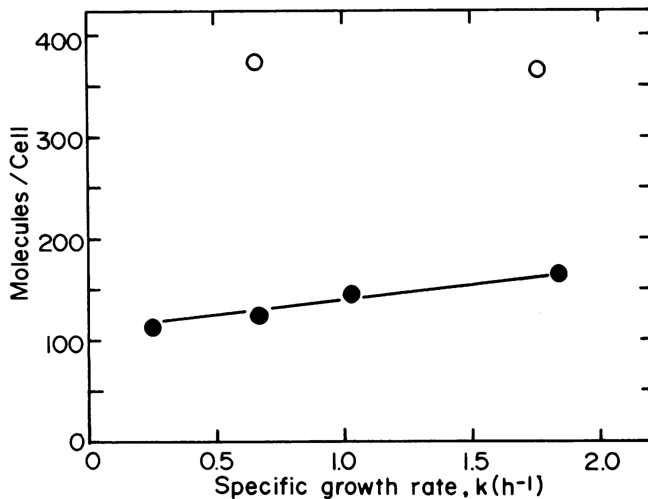

FIG. 2. Amounts of Trp repressor in *E. coli* W3110 at different growth rates in the presence and absence of added L-tryptophan. Measurement of repressor concentration and cell numbers and calculation of the specific growth rate constant (*k*) were as described in the text. The media used were as follows: mineral salts-glycerol; mineral salts-glucose; mineral salts-glucose-Casamino Acids; Luria broth-glucose. Growth was either in the presence (●) or absence (○) of 100 μg of L-tryptophan per ml of medium.

repressor levels, although an increase of intracellular Trp repressor concentration was observed over the increase in growth temperature from 30 to 40°C.

Effect of cell culture density on intracellular Trp repressor levels. To determine if repressor levels in the cell vary significantly at different stages of growth, samples of cells were removed from the growth flask at different cell densities, and the repressor concentration was determined by radioimmunoassay. Trp repressor levels remained relatively constant throughout culture growth (Fig. 4). When compared on a cell protein weight basis, the absolute levels of Trp repressor changed only slightly (less than 20%) during mid- to late-exponential and stationary phases. This was true for both tryptophan-supplemented and non-supplemented cells, although the latter always contained the characteristic two- to threefold-higher Trp repressor levels.

Induction of Trp repressor production in wild-type and overproduced strains. Wild-type cells containing a single copy of $trpR^+$ and grown on a mineral salts and glucose medium had an intracellular Trp repressor concentration (44 ng/mg) corresponding to 0.004% of the total cell protein on a weight basis.

Repressor levels were elevated 100-fold in cells containing a $trpR^+$ expression plasmid. Plasmid pRPG12 (14) contains the $trpR^+$ gene downstream from and under the control of the lambda p_L promoter. When cells transformed with this plasmid were grown under conditions known to induce efficient transcription from the lambda p_L promoter (3), elevated *trpR* gene expression resulted in production of Trp repressor protein at a level of 4,200 ng/mg of protein (Fig. 1). This value corresponds to a Trp repressor concentration of about 0.42% of total cell protein or 100-fold that of wild-type repressor levels. Under noninducing conditions, the presence of this plasmid was apparently not deleterious to cellular reproduction, as indicated by normal cell-doubling times.

The L-tryptophan analog indole acrylic acid is known to derepress *trp* operon expression in *E. coli* (25). It presumably does so by preventing formation of a stable Trp repressor-corepressor complex, thereby preventing a stable repressor-operator DNA complex. We wished to determine if indole acrylic acid has a similar effect on $trpR^+$ gene expression. *E. coli* W3110 was inoculated into a mineral salts and glucose medium supplemented with L-tryptophan at 100 μg/ml and grown to an A_{600} of 0.03. Indole acrylic acid was then added to the culture medium at a final concentration of 0, 10, or 25 μg/ml, and the cells were allowed to grow to a final A_{600} of 0.80. Cells were harvested and prepared for radioimmunoassay. Complete derepression of $trpR^+$ gene expression was observed when indole acrylic acid was present in the growth medium at a final concentration of 10 μg/ml. Addition of this analog resulted in increased Trp repressor levels from a basal level of 15 ng/mg when cells were grown in the presence of L-tryptophan to 35 and 38 ng/mg when indole acrylic acid was present at 10 and 25 μg/ml, respectively. Levels of Trp repressor in indole acrylic acid-treated cells were essentially identical to those observed in control cells grown in the absence of L-tryptophan (35 ng/ml). These data indicate that $trpR^+$ gene expression may be near maximal levels when cells are grown in tryptophan-free medium.

DISCUSSION

We measured the levels of the Trp repressor protein in *E. coli* to determine the relative and absolute amounts of this regulatory protein in the cell. A radioimmunoassay for Trp repressor was developed that is highly specific and sensitive to Trp repressor. The lower limit of detection for Trp repressor was 0.03 ng per tube. The inability to detect Trp

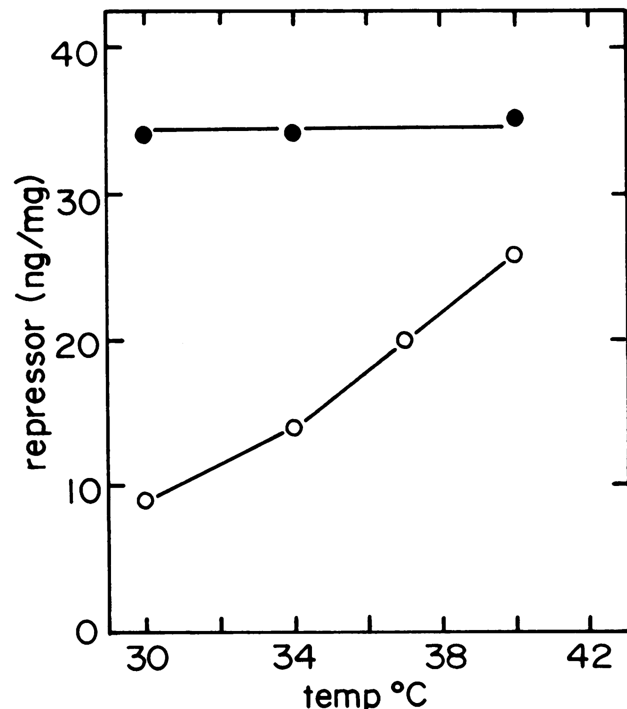

FIG. 3. Influence of growth temperature on Trp repressor concentrations in *E. coli* K-12 strain W3110. Repressor levels were measured in extracts of cells grown at the indicated temperatures in minimal glucose-Casamino Acids medium plus (○) or minus (●) L-tryptophan (100 μg/ml). Repressor levels were expressed as nanograms of repressor per milligram of cell protein.

repressor protein or other cross-reacting material in cell extracts of LE392 (*trpR*) which were defective for the Trp repressor function indicates that the assay is specific. In this strain the Trp repressor level was less than 0.1 ng of cross-reacting material per mg of protein when extracts of 2.6 mg of protein per ml were used. This corresponds to less than 0.2 molecule of Trp repressor per cell, a level well below that measured in wild-type *E. coli* strains. The biochemical product of the *trpR* gene in the LE392 strain is not known. Presumably neither full-length Trp aporepressor polypeptide nor sufficient amounts of an antigenic material (either abnormal repressor or other cross-reacting cell protein) is produced by the cell. With this radioimmunoassay it is possible to routinely screen for Trp repressor in extracts prepared from 5 ml of cell culture grown to mid-log or late-log density and requires less than 5 μl of cell extract (2 mg of protein per ml). Stationary-phase cells may also be used for the assay since the Trp repressor protein levels remained the same (within 20% of mid-exponential-phase cells).

With the Trp repressor protein radioimmunoassay we examined the Trp repressor levels in several *E. coli* strains. Repressor levels varied 2.5- to 3-fold depending on the cell culture medium used. When L-tryptophan was added in excess of that required for cell growth (100 μg/ml) repressor levels were 15 ng/mg of cell protein. Omission of L-tryptophan from the medium resulted in a 2.5- to 3-fold elevation in the levels of Trp repressor. These results are consistent with the initial in vitro observations that the $trpR^+$ gene is autoregulatory (13) and confirm indirect results obtained from in vivo studies of strains containing a *trpR-lacZ* gene fusion in the chromosome (16), in which *trpR* expression was reported to vary four- to five-fold in response to added L-tryptophan. This four- to five-fold range reflects the change in the hybrid Trp aporepressor-β-galactosidase enzyme levels observed in *trpR* strains relative to $trpR^+$ strains grown under high L-tryptophan concentrations; it also apparently reflects the maximum limit of expression possible in cells unable to produce functional Trp repressor protein. Closer examination of the data (16) shows that hybrid β-galactosidase protein levels in a $trpR^+$ background actually varied only 2.7-fold in response to L-tryptophan supplementation, in keeping with the range of Trp repressor concentrations determined directly by radioimmunoassay.

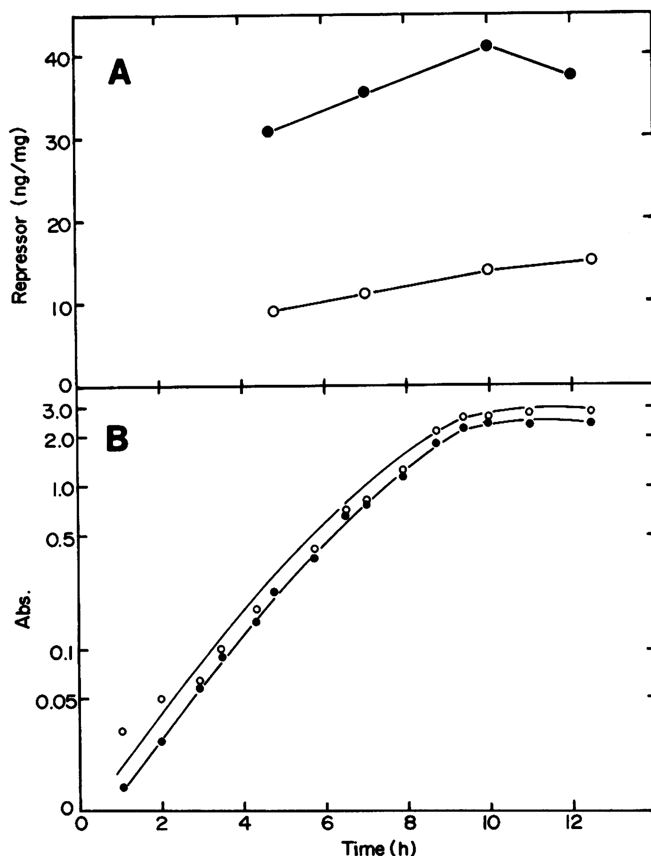

FIG. 4. Levels of Trp repressor in cells harvested at different stages of growth. (A) *E. coli* K-12 strain W3110 was grown in the presence (○) or absence (●) of 100 μg of L-tryptophan per ml in the mineral salts-glucose-Casamino Acids medium. (B) Cell turbidity was measured at A_{600} for the medium with (○) and without (●) L-tryptophan supplement.

The direct radioimmunoassay measurements of intracellular Trp repressor production in *E. coli* do not agree with other reports (5), which indicate that the level of the Trp repressor in haploid strains cannot alter *trpR* expression. These results were based on levels of β-galactosidase resulting from expression of a *trpR-lacZ* operon fusion in mutant and wild-type strains for *trpR*. However, the direct measure of Trp repressor levels in *E. coli* by radioimmunoassay indicates otherwise (Table 1). The Trp respressor levels measured in strain W3110 $trpR^+$ reflect normal $trpR^+$ gene expression from a single copy in the chromosome, and no inference need be made that a particular gene fusion technique is valid.

When the levels of Trp repressor in wild-type cells of strain W3110 were examined on the basis of the number of molecules per cell, between 240 and 750 monomers (M_r, 12,356) of Trp repressor were present (Table 1). These values correspond to 120 and 375 molecules of native Trp repressor per cell, because Trp repressor is known to be a dimer (M_r, 24,712) from gel-sizing and cross-linking studies (1, 15). The lower value represents the steady-state repressor levels in cells grown with high L-tryptophan concentration and reflects the fully repressed conditions for $trpR^+$ operon expression. The higher number is for cells grown in tryptophan-free medium and reflects the maximum level of *trpR* expression. The levels of intracellular Trp repressor are apparently sufficient to properly regulate not only the *trpR* operon but also *trp* and *aroH* operon expression.

The Trp repressor concentrations in *E. coli* K-12 strain W3110 and in *E. coli* strain B/r U1002 are equivalent when compared on a weight basis (nanogram of repressor per milligram of cell protein) (Table 1). However, *E. coli* B/r contains about one-third the amount of Trp repressor in the K-12 strain on a molecule-per-cell basis (Table 1). These results are consistent with the observation that *E. coli* K-12 cells are larger in both length and diameter and therefore have a greater cell volume (2.5-fold) than do B/r cells (12).

Using the measured values for Trp repressor (Table 1) in *E. coli* W3110 and the reported intracellular volume of 7.3 $\mu l/10^{10}$ cells (8), we calculated the molar concentration of Trp repressor protein within the cell. Cells grown in the presence (120 molecules per cell) or absence (375 molecules per cell) of added L-tryptophan had final intracellular Trp repressor concentrations of 270 and 850 nM, respectively.

The number of Trp repressor molecules per cell is in excess of the number of operator DNA sites they are known to bind. It is apparent that cell growth rate does not significantly affect Trp repressor levels within the cell (Fig.

2). It is known, however, that the number of copies of a chromosome per cell, and thus the number of operator DNA sites that the Trp repressor may bind, increases at fast growth rates. Estimates vary from two to three copies at cell-doubling times of less than 40 min to one copy during slower growth. Thus, the number of Trp repressor-specific operator sites on a chromosome available for binding by Trp repressor varies from a low of three (*trp*, *trpR*, and *aroH*, one operator each) to nine or more (three or more copies each). This corresponds to an intracellular operator concentration range in the cell of from 6.8 to 20.5 nM, assuming the cytoplasmic volume cited above. The minimum number of Trp repressor molecules under L-tryptophan-supplemented growth (ca. 120) is clearly sufficient to bind at all operator DNA sites. That excess active Trp repressor is present within the cell is indicated by the nearly full repression of *trp* operon expression (3% of maximum levels) when extra copies of *trp* operator are present (ca. 20 copies of a *trp* operator-containing plasmid) as compared with wild-type *trp* operon expression (1% of fully induced levels) when 1 copy of *trp* operator is present in the chromosome (16). Similar observations have been reported by others (6).

Original estimates of the absolute Trp repressor levels in *E. coli* suggested there were about 10 and 30 molecules per cell, values that were derived from indirect calulations based on assumptions of repressor size and activity (23, 26). However, variation of Trp repressor levels within the cell could not be determined from these studies. More recent estimates have been made with gene fusion methods in which the *trpR* gene was fused to the *lacZ* structural gene (16). Production of the hybrid Trp aporepressor-β-galactosidase protein was shown to be dependent on the *trpR* regulatory signals preceding the structural gene fusion. By calculating the number of hybrid molecules per cell, a value for the number of Trp repressor dimers per cell was derived (150 dimers). This value was based on measurement of hybrid enzyme activity, total protein content of *E. coli* cells, specific activity of the hybrid β-galactosidase, and monomers of protein per unit of activity. This calculation also assumed that the Trp repressor was a dimer, a fact which is now well established (1, 15). Our direct measurements with radioimmunoassay indicate that the Trp repressor levels are about twofold higher (375 dimers) under similar growth conditions (without L-tryptophan). Comparison of these results shows that the gene fusion method for calculating protein levels within the cell is in general agreement with direct radioimmunoassay methods. These results demonstrate that the radioimmunoassay technique, when properly used (16), yields an approximate measure of specific protein in the cell.

The effect of cell growth temperature on Trp repressor levels was also examined in strain W3110, wild type for *trpR*. The production of Trp repressor in cells was found to remain relatively constant (35 ng/mg of cell protein) in cells grown in medium lacking L-tryptophan supplement (Fig. 3). However, addition of L-tryptophan to the culture medium resulted in reduced Trp repressor levels, which varied as a function of temperature over the range of growth temperatures tested (from 30 to 40°C). It is interesting to note the similarity in temperature dependence of Trp aporepressor levels in L-tryptophan-supplemented cells to the marked temperature dependence of L-tryptophan binding to the Trp aporepressor (1). The dissociation constant for corepressor binding increased from 120 to 220 μM with this 10°C rise of temperature. Autorepression of *trpR* gene expression in L-tryptophan-supplemented cells is expected to be partially relieved at higher growth temperatures as the dissociation constant of corepressor-aporepressor is weakened under these conditions.

The Trp repressor radioimmunoassay should be useful in characterizing *E. coli* mutants that are altered for *trpR* gene expression and for Trp repressor structure, the latter class including mutants defective for the corepressor ligand or operator DNA binding functions necessary for normal control of *trp*, *trpR*, and *aroH* operon expression.

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