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Physiological Studies of Tryptophan Transport and Tryptophanase Operon Induction in *Escherichia coli*

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***Escherichia coli* forms three permeases that can transport the amino acid tryptophan: Mtr, AroP, and TnaB. The structural genes for these permeases reside in separate operons that are subject to different mechanisms of regulation. We have exploited the fact that the tryptophanase (*tna*) operon is induced by tryptophan to infer how tryptophan transport is influenced by the growth medium and by mutations that inactivate each of the permease proteins. In an acid-hydrolyzed casein medium, high levels of tryptophan are ordinarily required to obtain maximum *tna* operon induction. High levels are necessary because much of the added tryptophan is degraded by tryptophanase. An alternate inducer that is poorly cleaved by tryptophanase, 1-methyltryptophan, induces efficiently at low concentrations in both *tna*$^+$ strains and *tna* mutants. In an acid-hydrolyzed casein medium, the TnaB permease is most critical for tryptophan uptake; i.e., only mutations in *tnaB* reduce tryptophanase induction. However, when 1-methyltryptophan replaces tryptophan as the inducer in this medium, mutations in both *mtr* and *tnaB* are required to prevent maximum induction. In this medium, AroP does not contribute to tryptophan uptake. However, in a medium lacking phenylalanine and tyrosine the AroP permease is active in tryptophan transport; under these conditions it is necessary to inactivate the three permeases to eliminate *tna* operon induction. The Mtr permease is principally responsible for transporting indole, the degradation product of tryptophan produced by tryptophanase action. The TnaB permease is essential for growth on tryptophan as the sole carbon source. When cells with high levels of tryptophanase are transferred to a tryptophan-free growth medium, the expression of the tryptophan (*trp*) operon is elevated. This observation suggests that the tryptophanase present in these cells degrades some of the synthesized tryptophan, thereby creating a mild tryptophan deficiency. Our studies assign roles to the three permeases in tryptophan transport under different physiological conditions.**

Escherichia coli uses several mechanisms to regulate the expression of its tryptophan (*trp*) operon and control the rate of tryptophan biosynthesis. The most important of these are repression, transcription attenuation, and feedback inhibition (29). Their combined action permits the bacterium to vary the rate of tryptophan production over a several thousand-fold range. Since tryptophan is costly to produce, efficient shutdown of synthesis is advantageous to the bacterium whenever the amino acid is present in its environment. Consistent with this conclusion, most organisms that feed on other organisms have lost the capacity to synthesize tryptophan.

E. coli is capable of efficiently transporting tryptophan from its environment. It can use tryptophan as a sole source of carbon or nitrogen because of the action of the inducible enzyme tryptophanase, which degrades tryptophan to indole, pyruvate, and ammonia. Tryptophanase can also catalyze the synthesis of tryptophan from indole and serine or cysteine (24). To achieve its tryptophan uptake objectives, *E. coli* synthesizes three tryptophan permeases, designated Mtr, TnaB, and AroP (2, 3, 7, 10, 12, 17, 20, 22, 28). Mtr and TnaB are tryptophan specific, whereas AroP also transports phenylalanine and tyrosine. The genes for these permeases have been cloned and sequenced (4, 6, 10, 13, 21, 22), and the sequences of their predicted polypeptide products suggest that each functions as a transmembrane protein (21). Comparison of the amino acid sequences of Mtr and TnaB revealed that they are members of the same protein family (21); this family also includes the tyrosine-specific permease TyrP (10, 21).

The regulatory responses of the operons encoding these permeases and the different transport specificities and affinities of the permeases raise questions about the unique features of each in transporting tryptophan. Knowledge of these features should contribute to our understanding of tryptophan metabolism and how it is influenced by amino acid levels in the environment of the bacterium. In this report we describe physiological experiments performed under a variety of growth conditions. Our principal objective was to determine the role of each of the permeases in tryptophan transport. We examined mutants with inactivating alterations in each of the permeases, and we constructed strains with all possible combinations of these alterations. Tryptophan uptake was assessed by exploiting the fact that the extent of induction of the tryptophanase (*tna*) operon varies directly with the capacity to transport tryptophan. We observed that in a medium containing all of the amino acids the TnaB permease was most important for tryptophan uptake. However, the Mtr permease also contributed to tryptophan transport. In a medium lacking phenylalanine and tyrosine, all three permeases were active in transporting tryptophan. The Mtr permease was found to be principally responsible for transporting indole, the degradation product of tryptophanase action. The TnaB permease was shown to be essential for growth on tryptophan as the sole carbon source. Expression of the tryptophan permeases increased retention of newly synthesized tryptophan. However, cells with high levels of tryptophanase and the TnaB permease experienced a mild tryptophan deficiency when they were

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TABLE 1. Bacterial strains

Strain	Relevant genotype	Source or reference
SVS1144	W3110 *bgl-551* Δ(*lac-argF*)U169 (λSVS44)	25
Derivatives of SVS1144[a]		
CY15200	*tnaA2*	This study
CY15201	*tnaA270*::Tn*5*	This study
CY15202	*tnaB271*::Tn*5*	This study
CY15203	*mtr*	This study
CY15204	*aroP*	This study
CY15205	*tnaA2 tnaB271*::Tn*5*	This study
CY15206	*mtr tnaA270*::Tn*5*	This study
CY15207	*mtr tnaB271*::Tn*5*	This study
CY15208	*mtr aroP*	This study
CY15209	*aroP tnaA270*::Tn*5*	This study
CY15210	*aroP tnaB271*::Tn*5*	This study
CY15211	*mtr aroP tnaA270*::Tn*5*	This study
CY15212	*mtr aroP tnaB271*::Tn*5*	This study
CY15213	Δ*trpEA2*	This study
CY15214	Δ*trpEA2 tnaA2*	This study
CY15215	Δ*trpEA2 tnaB271*::Tn*5*	This study
CY15216	Δ*trpEA2 mtr*	This study
CY15217	Δ*trpEA2 aroP*	This study
CY15218	Δ*trpEA2 mtr tnaB271*::Tn*5*	This study
CY15219	Δ*trpEA2 mtr aroP*	This study
CY15220	Δ*trpEA2 aroP tnaB271*::Tn*5*	This study
CY15221	Δ*trpEA2 mtr aroP tnaA2*	This study
CY15222	Δ*trpEA2 mtr aroP tnaB271*::Tn*5*	This study
CY15223	Δ*trpE5 tnaA2*	This study
CY15224	Δ*trpE5 mtr aroP tnaA2*	This study
CY15225	Δ*trpE5*	This study
CY15226	Δ*trpEA2 tnaA270*::Tn*5*	This study

[a] Strains were derived from SVS1144 by introducing the appropriate mutant gene(s) by P1 transduction.

transferred to a tryptophan-free medium. These findings provide possible explanations for the existence of the three tryptophan permeases in *E. coli*.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* strains employed in this study and their relevant genetic characteristics are listed in Table 1. Mutants with the *tnaA2* mutation, initially designated T_3C, lack tryptophanase and form very little TnaB permease (3). All of the mutant strains are derivatives of strain SVS1144, which was described previously (25). This strain has a deletion of the *lac* operon and is a single lysogen carrying λ SVS44 (25). In λ SVS44, *lacZ* has been fused in frame to the beginning of *tnaA* to give the transcription-translation fusion *tnaA'-'lacZ* (25). This λ construct lacks *tnaB*; therefore the fusion provides a β-galactosidase reporter system in which the promoter and regulatory region of the *tna* operon drive production of a tryptophanase–β-galactosidase fusion protein.

Media and growth conditions. Vogel and Bonner minimal medium (27) and morpholinepropanesulfonic acid (MOPS) minimal medium (25) were employed in this study. These media were supplemented as indicated in the figure legends and tables footnotes with either glucose (0.2 or 0.3%), a catabolite-repressing carbon source, or one of three non-catabolite-repressing carbon sources (0.2% glycerol, 1% acid-hydrolyzed casein [lacking tryptophan], or 0.2% potassium succinate) plus the five amino acids glycine (0.25%) and serine, threonine, aspartate, and glutamate (100 μg/ml each). The composition of the succinate medium was based on the results of experiments designed to determine the nonaromatic amino acids, which, when added to succinate, would stimulate the growth of SVS1144. All liquid culture experiments were performed with shaking at 37°C in a New Brunswick shaker water bath.

Enzyme assays. β-Galactosidase assays were performed on permeabilized cells exactly as described by Miller (16). The β-galactosidase units reported are Miller units (16). Tryptophanase was assayed similarly (19) with the colorimetric substrate *S*-(*O*-nitrophenyl)L-cysteine (26) instead of *o*-nitrophenyl-β-D-galactopyranoside. The units of tryptophanase were calculated as follows: $100 \times [A_{470} - 1.7\ (A_{550})]/(t$ [hours] $\times A_{600} \times$ volume [ml] of cells). The values reported generally are percentages of the activity of appropriate controls.

Anthranilate synthase enzymatic activity was determined fluorometrically by using Triton X-100-permeabilized cells as described previously (14). Each assay sample contained cells equivalent to 400 Klett units (absorbance, 660 filter) of the respective culture. One unit of anthranilate synthase activity is defined as the activity responsible for the synthesis of 0.4 nmol of anthranilate by 400 Klett units of cells in 20 min at 37°C.

RESULTS

Relative induction of the *tnaA* operon and a *tnaA'-'lacZ* translational fusion. Studies on the mechanism of tryptophan induction of the *tna* operon have established that induced expression is due to a transcription antitermination mechanism that allows RNA polymerase to bypass Rho factor-dependent transcription termination sites located in the leader region of the operon (9, 25). Although the mechanism of tryptophan-induced antitermination is unknown, it has been demonstrated that transcription attenuation is the sole mechanism of specific regulation of the *tna* operon (9, 25).

Maximal induction of the *tna* operon in media that do not contain a catabolite-repressing carbon source requires high concentrations of tryptophan. Acid-hydrolyzed casein, an amino acid mixture lacking tryptophan, is a nonrepressing carbon source, as is succinate plus the five nonaromatic amino acids (glycine, serine, threonine, aspartate, and glutamate). The addition of tryptophan to media containing either of these carbon source mixtures leads to a rapid increase in the expression of the *tna* operon (Fig. 1). In our initial experiments we compared the induction of the *tna* operon and a single-copy chromosomal *tnaA'-'lacZ* reporter gene translational fusion, driven by the tryptophan-responsive *tna* operon promoter-leader region, as a function of tryptophan concentration in the two media. The expression of the two operons was determined by measuring both β-galactosidase and tryptophanase activities. The expression of both operons increased in parallel as a function of increasing tryptophan concentration in both media (Fig. 1). Enzyme levels had not reached a plateau at the highest tryptophan concentration tested. Although the expression of both operons increased in parallel, the relative uninduced level of β-galactosidase was higher than the uninduced level of tryptophanase in either medium. A possible explanation for this difference is the existence of a weak constitutive upstream promoter in the λ *tnaA'-'lacZ* construct. We examined this possibility by transferring the *tnaA'-'lacZ* operon to λ RS45 (23), downstream from several transcription terminators, and incorporating the modified lambda into

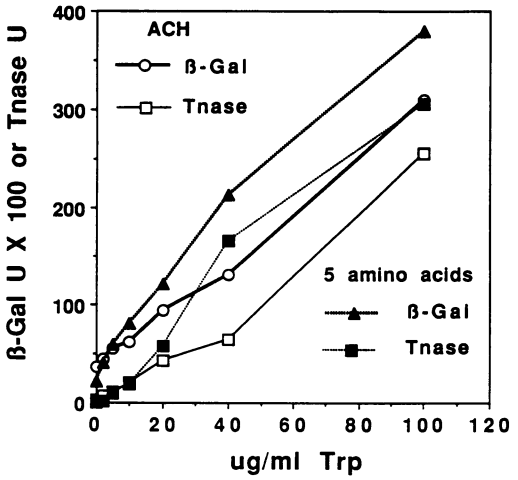

FIG. 1. Comparison of induction of the *tna* operon and the *tnaA'-'lacZ* translational fusion operon. ACH, MOPS minimal medium (26) plus 1% acid-hydrolyzed casein; 5 amino acids, Vogel and Bonner minimal medium (27) plus 0.2% potassium succinate, 0.25% glycine, and 100 μg each of serine, threonine, aspartate, and glutamate per ml. Cells were grown overnight in the indicated media and then diluted 1:100 in fresh medium with the tryptophan supplement indicated. Cultures were grown with shaking at 37°C to an A_{600} of 0.5 to 0.8. Cultures were chilled on ice and assayed for β-galactosidase (β-Gal) and tryptophanase (Tnase) as described in Materials and Methods.

the chromosome. The basal level of β-galactosidase observed with the latter construct was reduced only slightly, whereas the induced level was unaffected. Thus we cannot explain the basal level discrepancy.

Tryptophan and 1-methyltryptophan induction in *tnaA*$^+$ and *tnaA* strains. The high levels of tryptophan required for maximum induction of the *tna* operon could be due to limited tryptophan uptake and/or rapid degradation of intracellular tryptophan by tryptophanase, the product of *tnaA* of the *tna* operon. To explore this possibility, we compared induction in *tna*$^+$ and *tnaA* strains with either L-tryptophan or L-1-methyltryptophan (18) as the inducer.

The data in Fig. 2 document the importance of degradation of tryptophan and the *tnaB* transport function on the effectiveness of a given concentration of inducer. In the acid-hydrolyzed casein medium (Fig. 2A), the *tnaA* mutant was maximally induced by low levels of tryptophan, but induction was poor. Induction was poor because the *tnaA* mutation employed is polar on the expression of the *tnaB* permease (3) and, as shown below, because the *tnaB* permease is required in this medium for full induction by tryptophan. The requirement for high levels of tryptophan for maximal induction in the *tna*$^+$ strain can be attributed to tryptophanase-mediated degradation of tryptophan.

In acid-hydrolyzed casein medium, 1-methyltryptophan was equally effective as an inducer in the *tnaA*$^+$ strain and the *tna* mutant strain (Fig. 2B). This is to be expected, since 1-methyltryptophan is hydrolyzed very slowly by tryptophanase. Approximately 1 μg of 1-methyltryptophan per ml was sufficient for half-maximum induction. Induction by 1-methyltryptophan is not as dependent as tryptophan induction on *tnaB* activity (see below).

In the succinate medium with the five amino acids, induction by tryptophan was efficient in the *tnaA* mutant; approximately 2 μg/ml was needed for half-maximal induction (Fig. 2C). In the wild-type strain, induction increased as the tryptophan concentration was increased, up to 100 μg/ml. We interpret these results to indicate that in this medium tryptophan was efficiently transported into the cell in both the wild type and the *tnaA* mutant, but that much of the tryptophan taken up by the wild type was degraded to the noninducer indole. In contrast to the results obtained with the acid-hydrolyzed casein medium, where tryptophan induction is limited by the polar effect of the *tnaA2* mutation on *tnaB* expression, tryptophan induction is not dependent on TnaB function in this medium (see below). When 1-methyltryptophan was used as the inducer in this medium (Fig. 2D), induction was efficient in both strains.

These findings demonstrate the importance of both transport and tryptophanase action on the effectiveness of tryptophan as an inducer of the *tna* operon.

Measurement of induction in relation to permease genotype in different growth media. We wished to determine which permeases were responsible for *tnaA'-'lacZ* induction in the two media, in response to the inducers tryptophan and 1-methyltryptophan. The expression of the *tnaA'-'lacZ* fusion was measured in strains with all possible combinations of mutations affecting the TnaB, Mtr, and AroP tryptophan permeases (Fig. 3). Both the uninduced and induced levels of β-galactosidase fusion protein were measured. The *tnaA* and *tnaB* mutant alleles employed have Tn*5* insertions (Table 1), whereas, as mentioned above, the *tnaA2* mutation is polar on *tnaB* expression (3). The *tnaA*::Tn*5* allele also appears to be polar on *tnaB* expression. The *mtr* and *aroP* mutants used were defective, uncharacterized mutants isolated in this laboratory.

In the acid-hydrolyzed casein medium (Fig. 3A), tryptophan induction was most dependent on *tnaB* expression and was unaffected by mutations in *mtr* or *aroP*. In the double mutants, an additive effect of the *mtr* mutation was evident; induction was virtually eliminated in the *mtr tnaB* double mutant. Consistent with the conclusion that in this medium *tnaB* function is largely responsible for tryptophan uptake and hence *tnaA'-'lacZ* induction, the *mtr aroP* double mutant showed normal induction. We believe that in the acid-hydrolyzed casein medium the phenylalanine and tyrosine present largely saturate the transport capacity of AroP, limiting tryptophan transport by this permease. In addition, in an acid-hydrolyzed casein medium *aroP* expression is reduced (2, 5, 28).

In the acid-hydrolyzed casein medium, any one of the permeases could be inactivated without affecting induction by 1-methyltryptophan (Fig. 3B). Only when both *mtr* and *tnaB* were altered was induction prevented. The Tn*5* insertion in *tnaA* apparently reduced *tnaB* expression appreciably; hence the *mtr tnaA* double mutant probably had little TnaB activity. There was full induction in the *aroP tnaB*, *aroP tnaA*, and *mtr aroP* double mutants, suggesting that in the acid-hydrolyzed casein medium either the Mtr or TnaB function would suffice for 1-methyltryptophan transport. Here again, *aroP* was ineffective as a tryptophan transport system in this medium. Although the tryptophan-activated *trp* repressor represses transcription of the *mtr* operon about 15-fold (11, 22), 1-methyltryptophan does not activate the *trp* repressor (15). Hence, Mtr levels would be expected to be higher in the presence of 1-methyltryptophan than with tryptophan.

In the succinate medium with five amino acids, any one of the three permeases can mediate tryptophan induction (Fig. 3C). However, the contribution of *mtr* appears to be minimal, since the *aroP tnaB* double mutant showed only 40%

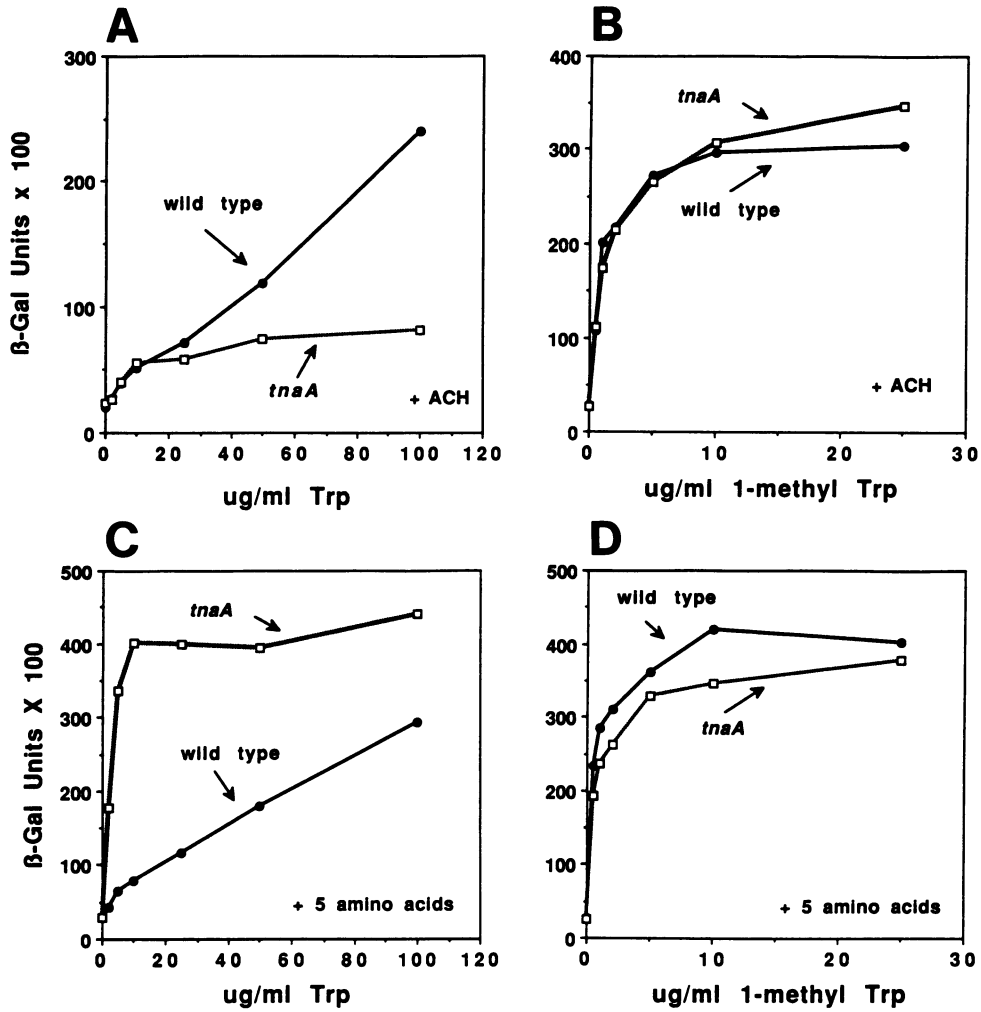

FIG. 2. Tryptophan and 1-methyltryptophan induction in *tnaA* mutant and wild-type strains. Strains SVS1144 and CY15201 (*tnaA270*::Tn*5*), which are lysogenic for λ*tnaA'-'lacZ*, were grown overnight in the indicated medium lacking tryptophan or 1-methyltryptophan. Cultures were diluted 1:100 in the indicated medium and grown at 37°C with shaking to an A_{600} of 0.5 to 0.8. Cultures were chilled on ice and assayed for β-galactosidase (β-Gal) activity as described in Materials and Methods. The media are as described in the legend to Fig. 1.

induction and the *mtr aroP tnaB* triple mutant showed ca. 20% induction. Only when all three permeases were inactivated was induction by 1-methyltryptophan appreciably affected in this medium (Fig. 3D).

From the results in Fig. 3 it is apparent that, in the presence of acid hydrolyzed casein, AroP is ineffective as a tryptophan permease. This ineffectiveness is due in part to the reduced activity expected in this medium and to the blocking of tryptophan permease activity by phenylalanine and tyrosine (2, 5, 28). To prove that this ineffectiveness is due to the aromatic amino acids in acid-hydrolyzed casein, we examined tryptophan induction in the same set of mutants in the medium containing succinate plus five amino acids and supplemented with phenylalanine and tyrosine (Fig. 4). Comparison of the data in Fig. 3 and 4 shows that, in the presence of the phenylalanine and tyrosine supplement, induction was defective in the *mtr tnaB* double mutant. This finding demonstrates that the effectiveness of AroP as a tryptophan permease is markedly reduced by the presence of the other aromatic amino acids (2).

Tryptophan leakage in mutants lacking the three permeases. In addition to transporting tryptophan from its environment, the tryptophan permeases of *E. coli* may play a second role: that of maintaining a high intracellular tryptophan concentration when there is no source of extracellular tryptophan. To examine this possibility, we performed experiments designed to determine whether permease function influenced the retention of biosynthetically produced tryptophan. Triple permease-positive and -negative cells that lacked tryptophanase (*tnaA2*) and a functional anthranilate synthase (Δ*trpE5*) were mixed with cells that lacked the entire *trp* operon (Δ*trpEA2*) and hence did not produce any of the tryptophan biosynthetic enzymes. The cell mixture was cultured in a glucose medium in the presence of either excess tryptophan or a low concentration of anthranilate. Anthranilate will support the growth of the Δ*trpE5* mutant but not the Δ*trpEA2* mutant. Only if the Δ*trpE5* mutant secreted some of the tryptophan it synthesized from anthranilate would the Δ*trpEA2* strain be able to grow. Cell mixtures were cultured for over four generations, and the

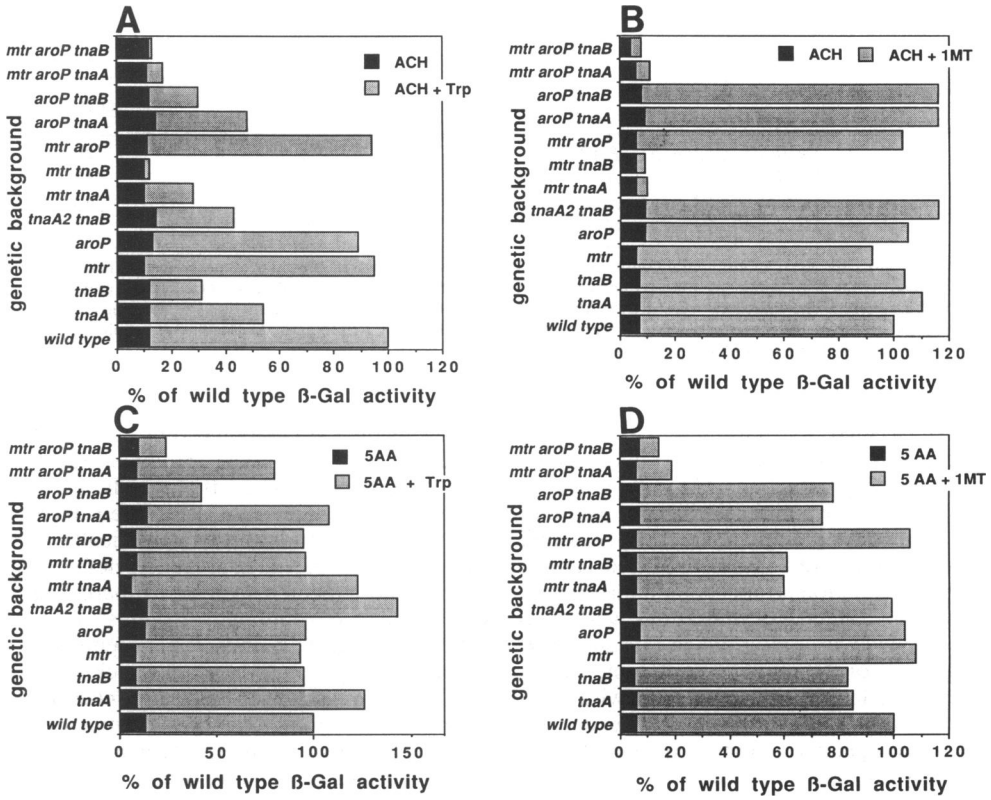

FIG. 3. Induction of β-galactosidase (β-Gal) in prototrophic strains containing various combinations of permease mutations. β-Galactosidase was expressed from a λ lysogen; the λ genome contained the promoter-regulatory region followed by a *tnaA'-'lacZ* translational fusion. All strains used (CY15201 through CY15212) were derivatives of SVS1144 (Table 1). Inocula were grown overnight in the medium indicated in the absence of tryptophan or 1-methyltryptophan. Inocula were diluted 1:100 in the indicated media and grown at 37°C with shaking to an A_{600} 0.5 to 0.8. The cultures were chilled on ice and assayed for β-galactosidase activity as described in Materials and Methods. The media were as described in the legend to Fig. 1 and were supplemented with tryptophan (100 μg/ml) or 1-methyltryptophan (1MT; 10 μg/ml) where indicated. Values presented are the percent of values obtained with the control culture, SVS1144. AA, amino acids.

proportion of the two types of mutants was determined in the final cultures. As can be seen from the results of the control experiment (Table 2), in the presence of a high concentration of tryptophan the proportion of the progeny that consisted of *trp* operon deletion (Δ*trpEA2*) cells was unaffected by mutations in the three permease genes. However, on low levels of anthranilate, cell mixtures containing permease-positive and -negative Δ*trpE5* strains behaved differently. In the mixture with the permease-positive Δ*trpE5* strain there was a marked reduction in the proportion of cells of the *trp* operon deletion type. This result indicates that permease activity may result in the retention of biosynthetically produced tryptophan or may facilitate the recapture of secreted tryptophan from the environment. We did not determine which of the permeases was responsible for the observed effect.

Effect of tryptophanase action on tryptophan utilization by permease mutants. Despite the effectiveness of catabolite repression in downregulating the expression of the *tna* operon of *E. coli* (Table 3), *trp* operon deletion strains that can synthesize tryptophanase accumulate indole during growth in the presence of glucose and tryptophan (unpublished observation). This indole is produced from tryptophan by the tryptophanase that these cells contain (Table 3). Although tryptophanase can synthesize tryptophan from indole, the favored direction of the action of this enzyme is

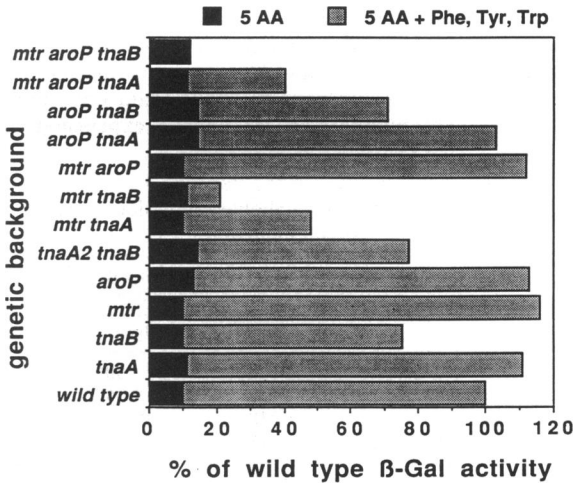

FIG. 4. Effect of added phenylalanine and tyrosine on tryptophan induction in the succinate medium containing 5 amino acids (AA). The experiment was like those described in the legend to Fig. 3, with the same strains. The media were as described in the legend to Fig. 1. Phenylalanine and tyrosine were added at 100 μg/ml each. β-Gal, β-galactosidase.

TABLE 2. Competition for tryptophan produced biosynthetically[a]

Strains mixed	Relevant genotypes	Growth supplement	% *ΔtrpEA2* progeny	
			Inoculant	After ≥4 doublings
CY15223 + CY15213	*ΔtrpE5 tnaA2* + *ΔtrpEA2*	Trp	40	33
CY15224 + CY15213	*ΔtrpE5 tnaA2 mtr aroP* + *ΔtrpEA2*	Trp	58	49
CY15223 + CY15213	*ΔtrpE5 tnaA2* + *ΔtrpEA2*	Anth	40	5.6
CY15224 + CY15213	*ΔtrpE5 tnaA2 mtr aroP* + *ΔtrpEA2*	Anth	58	29

[a] Cultures were grown to the early log phase in minimal medium plus 0.2% glucose and 20 μg of tryptophan per ml with shaking at 37°C. Cells were sedimented at room temperature and washed twice with single-strength minimal medium lacking tryptophan. The cultures were diluted, and equal amounts were mixed to give a final cell density of 2×10^7/ml. The mixtures were grown for ≥4 generations in minimal-glucose medium containing either tryptophan (20 μg/ml) or anthranilate (Anth; 4 μg/ml), as indicated. These mixed cultures were diluted and plated on nutrient agar. Colonies were replicated to minimal agar containing 4 μg of anthranilate per ml to distinguish between *ΔtrpE5* and *ΔtrpEA2* colonies.

degradation (24). We wished to determine whether the presence of the various tryptophan permeases influenced the ability of a cell to grow on tryptophan. Three tryptophan-containing media were tested: minimal-glucose medium, minimal-glucose medium plus phenylalanine and tyrosine, and minimal-glucose medium plus acid-hydrolyzed casein. The growth of *trp* operon deletion strains containing various combinations of permease and *tnaA* mutations was then examined (Table 4). Strains with mutations in *mtr* and *aroP* were incapable of growth on tryptophan-containing media supplemented with phenylalanine and tyrosine or with acid-hydrolyzed casein. In addition, these strains grew poorly on the minimal-glucose-tryptophan medium. The inability to use tryptophan is clearly due to the degradative action of the tryptophanase that is produced. This conclusion is based on the finding that growth of the *mtr aroP tnaA2* strain was not inhibited by the aromatic supplements (Table 4). The most likely explanation of these observations is that tryptophan is transported poorly into permease-negative strains and that, when it does enter, it is degraded to indole by the tryptophanase that these cells contain. Apparently, catabolite repression reduces the tryptophanase activity of glucose-grown cultures to a level that is inadequate for the synthesis of sufficient tryptophan from the indole that is generated. In addition, since *mtr* function appears to be essential for efficient indole uptake (see below), indole generated from tryptophan probably is not taken up by *mtr* mutant cells. These growth tests were repeated in tryptophan prototrophs in the absence of tryptophan. The permease mutations did not limit growth of prototrophic strains (data not shown).

Effect of tryptophanase on *trp* operon expression. Cells growing on non-catabolite-repressing carbon sources supplemented with tryptophan produce high levels of tryptophanase (Table 3). When these cells are shifted to a growth medium lacking tryptophan, their tryptophanase can degrade some portion of biosynthetically produced tryptophan and create a partial tryptophan deficiency. This deficiency would be expected to lead to increased production of the tryptophan biosynthetic enzymes. To test this possibility, nutritional growth shift experiments were performed with isogenic strains producing or lacking tryptophanase. Cultures were grown in media containing a non-catabolite-repressing carbon source supplemented with tryptophan, washed, resuspended, and grown in the same medium lacking tryptophan. Samples were taken at frequent intervals, and the cells were harvested and assayed for anthranilate synthase activity (Fig. 5). Immediately after the shift to a tryptophan-free medium, *trp* operon expression was greater in the *tna*$^+$ strain (SVS1144) than in the *tnaA2* mutant strain (CY15200). We presume that the increased expression indicates that after the shift the intracellular tryptophan concentration was lower in the *tna*$^+$ strain. Note that by about 10 min all cultures had responded maximally, albeit to different extents. Apparently the tryptophanase content of a bacterium does influence the expression of the *trp* operon.

TABLE 3. Effect of catabolite repression on *tna* operon induction[a]

Addition (μg/ml)	β-Galactosidase U in the following medium:		
	Glucose[b]	Glycerol[c]	ACH[d]
None	22	506	2,150
Tryptophan (20)	320	2,350	7,750
Tryptophan (100)	318	10,620	28,600
1-Methyl tryptophan (10)	178	15,640	24,870

[a] Strain SVS1144 was grown with shaking at 37°C overnight in each of the three media in the absence of tryptophan. Each overnight culture was diluted 1:100 into fresh medium with the addition indicated and grown with vigorous shaking to an A_{600} of 0.5 to 0.8. The cultures were chilled on ice and assayed for β-galactosidase as described in Materials and Methods.
[b] Minimal medium containing 0.3% glucose.
[c] Minimal medium containing 0.2% glycerol.
[d] MOPS-minimal medium containing 1% acid-hydrolyzed casein.

TABLE 4. Requirements for growth of a *trp* operon deletion strain[a]

Strain	Relevant genotype[b]	Growth on glucose-tryptophan[c] plus:		
		No other addition	Phe + Tyr[d]	CH[e]
CY15213		++	++	++
CY15214	*tnaA2*	++	++	++
CY15215	*tnaB*	++	++	++
CY15216	*mtr*	++	++	+
CY15217	*aroP*	++	++	++
CY15218	*mtr tnaB*	++	++	+
CY15219	*mtr aroP*	+	−	−
CY15220	*aroP tnaB*	++	++	++
CY15221	*mtr aroP tnaA2*	++	++	++
CY15222	*mtr aroP tnaB*	+	−	−

[a] Each of the strains listed was streaked on minimal-glucose-tryptophan agar plates. Single colonies from each plate were picked, suspended in saline, and streaked on the media indicated. The plates were scored for growth visually after 48 h at 37°C.
[b] All strains are *ΔtrpEA2*.
[c] 0.2% glucose plus 20 μg of tryptophan per ml.
[d] 50 μg of phenylalanine and 50 μg of tyrosine per ml.
[e] 0.5% acid-hydrolyzed casein.

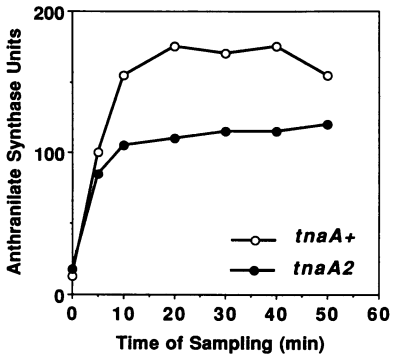

FIG. 5. Effect of a nutritional shift on anthranilate synthase levels in *tnaA* mutant and wild-type strains. Strains SVS1144 (*tnaA*$^+$) and CY15200 (*tnaA2*) were grown overnight at 37°C with shaking in MOPS-minimal medium plus 1% acid-hydrolyzed casein. These cultures were used to inoculate flasks containing the same medium supplemented with 100 μg of tryptophan per ml, and the cultures were grown to approximately 2×10^8 cells per ml. The cells were sedimented, washed twice in minimal medium, and resuspended at a density of 2×10^8 cells per ml in MOPS-minimal medium plus 1% acid-hydrolyzed casein (lacking tryptophan). Samples (400 Klett units) were taken at the times indicated and chilled on ice. The cells in each sample were collected by centrifugation, washed, and resedimented. The pellets were stored frozen at −80°C. Cell samples were thawed, permeabilized by Triton X-100 treatment, and assayed for anthranilate synthase as described in Materials and Methods.

Permease requirements for tryptophanase-dependent growth on indole. In strains lacking the *trp* operon and hence all of the tryptophan biosynthetic enzymes, growth on indole as a tryptophan precursor is dependent on the induction of the *tna* operon and the conversion of indole to tryptophan by tryptophanase. Indole will not support growth of a *tna*$^+$ *trp* operon deletion strain in the presence of a catabolite-repressing carbon source (8). In addition, indole as such does not induce the expression of the *tna* operon. Therefore both a non-catabolite-repressing carbon source and an inducer must be provided to obtain growth on indole. A variety of tryptophan analogs will replace tryptophan as an inducer, although they will not substitute for tryptophan in permitting growth. Thus the analogs 4-, 5-, and 6-methyltryptophan are effective inducers when added simultaneously with an appropriate concentration of indole. Each of these analogs also inhibits growth, presumably by blocking charging of tryptophan onto tRNA, by false feedback inhibition, or by being incorporated into protein in place of tryptophan. The analog 1-methyltryptophan is an efficient inducer (18, 19); in fact, it is the most efficient inducer we have tested (Fig. 2). In addition, it does not inhibit growth. Several experiments were performed to examine the metabolism of this analog. Comparisons of the in vitro hydrolysis of equimolar concentrations of L-tryptophan and L-1-methyltryptophan by tryptophanase indicated that 1-methyltryptophan was hydrolyzed at about 1% the rate of tryptophan hydrolysis (data not shown). Consistent with this finding, cells grown in the presence of 1-methyltryptophan degraded only some of the 1-methyltryptophan to 1-methylindole. In vitro charging analyses were performed with highly purified tryptophanyl tRNA synthetase, partially purified $tRNA^{Trp}$, and 1-methyltryptophan (data not shown). The 1-methyltryptophan was not detectably charged onto $tRNA^{Trp}$, nor did it significantly inhibit charging of labeled L-tryptophan (data not shown). The relative resistance of 1-methyltryptophan to hydrolysis and the inability of 1-methyltryptophan to function as a substrate for tryptophanyl tRNA synthetase explain its effectiveness as an apparent semigratuitous inducer of the *tna* operon.

Any of the tryptophan permeases, or some other permease, could be responsible for transporting indole into the cell. To determine whether loss of the *mtr*, *tnaB*, or *aroP* function would prevent indole utilization, *trp* operon deletion strains with individual permeases inactivated by mutation were examined for growth on indole plus 1-methyltryptophan. It was shown above (Fig. 2) that in strains lacking any one of the permeases the *tna* operon was induced by 1-methyltryptophan. It was observed that only when the *mtr* locus was inactivated was growth on indole plus 1-methyltryptophan prevented. We conclude that Mtr is principally responsible for indole transport in *E. coli*.

Permease requirements for growth on tryptophan as the sole carbon source. Strains that do or do not lack tryptophanase or the TnaB tryptophan permease were tested for their ability to grow on purified (Noble) agar containing Vogel and Bonner minimal medium and 0.5% L-tryptophan as the sole carbon source. The following strains were examined: SVS1144 (*tnaA*$^+$*B*$^+$), CY15201 (*tnaA270*::Tn*5*), CY15202 (*tnaB271*::Tn*5*), CY15213 (Δ*trpEA2 tnaA*$^+$*B*$^+$), CY15226 (Δ*trpEA2 tnaA270*::Tn*5*), and CY15215 (Δ*trpEA2 tnaB271*::Tn*5*). Significant growth of only the two *tnaA*$^+$*B*$^+$ strains was observed after 3 days of incubation at 37°C. These findings establish that functional tryptophanase and TnaB permease are both required for the utilization of tryptophan as the sole carbon source.

DISCUSSION

E. coli and related bacteria synthesize multiple transport systems to scavenge specific amino acids from their environment (1, 20). Tryptophan, in addition to its structural and functional role in proteins, can be degraded to indole, pyruvate, and ammonia, and the pyruvate and ammonia can be used as carbon and nitrogen sources (24). Three permeases mediate tryptophan uptake; they are designated Mtr, TnaB, and AroP (6, 10, 12, 20, 28). AroP also transports phenylalanine and tyrosine. In the studies described in this report we evaluated the contributions of the three permeases to tryptophan uptake by measuring tryptophan induction of the *tna* operon of *E. coli*. Considerable information is available on the regulation of the operons encoding the three transport proteins (5, 9, 10, 11, 22, 25, 28). Expression of the *mtr* operon is repressed by the *trp* repressor in excess tryptophan (10, 11, 22) and activated by phenylalanine and the *tyrR* product (22). *tnaB*, as the second gene in the *tna* operon, is subject to catabolite repression, and its expression is induced appreciably by tryptophan (7, 9, 25). The *aroP* operon is transcriptionally repressed about threefold by the *tyrR* product in the presence of acid-hydrolyzed casein (5).

The extent of induction of the *tna* operon is a function of the intracellular tryptophan concentration. This fact allowed us to assess tryptophan transport, albeit indirectly, by measuring *tna* operon expression. Expression of the *tna* operon was measured in two ways. Tryptophanase, the product of *tnaA*, was assayed directly. In addition, a tryptophanase-β-galactosidase fusion protein was assayed in strains containing a single integrated copy of a *tnaA'-'lacZ* translational fusion. Two media were used in these studies; one contained acid-hydrolyzed casein as the sole source of carbon,

whereas the other contained succinate plus five nonaromatic amino acids (glycine, serine, threonine, aspartate, and glutamate). Comparative studies indicated that both the *tna* operon and the *tnaA'-'lacZ* fusion operon responded equally to the inducer in both media. We also analyzed tryptophan induction in a *tnaA* mutant strain, since tryptophanase degrades tryptophan to indole, a noninducer. Two inducers were examined: tryptophan and 1-methyltryptophan. Both inducers were effective in the two media, but much higher concentrations of tryptophan were required for comparable induction in tna^+ strains. This requirement was shown to be due to the degradative action of tryptophanase. 1-Methyltryptophan was a very effective inducer in $tnaA^+$ and *tnaA* mutant strains. This observation is consistent with the finding that 1-methyltryptophan is a relatively poor substrate for tryptophanase.

Tryptophan induction was measured in strains containing all possible combinations of mutant permease genes and in strains altered in *tnaA*. In the acid-hydrolyzed casein medium, tryptophan uptake was most dependent on *tnaB* expression; i.e., in the *tnaB* mutant induction was only 30% of that in the control, whereas in the *mtr aroP* double mutant induction was equivalent to that in the wild-type strain. In strains with defects in both *mtr* and *tnaB*, there was essentially no induction—presumably because there was no uptake of tryptophan. When 1-methyltryptophan was used instead of tryptophan as an inducer in the acid-hydrolyzed casein medium, mutations in both *mtr* and *tnaB* were required to eliminate induction. We interpret this result as reflecting the fact that 1-methyltryptophan is a more effective inducer than tryptophan; hence, even in the *tnaB* mutant sufficient analog was taken up to induce β-galactosidase formation. In the succinate medium containing five amino acids, a defect in *tnaB* alone was insufficient to limit tryptophan uptake. In this medium, AroP is probably largely responsible for transporting tryptophan, since the medium lacks phenylalanine and tyrosine. To confirm this interpretation, we examined induction in this medium in the presence of phenylalanine and tyrosine. Under these conditions, the *mtr tnaB* double mutant was defective in induction. We conclude that added phenylalanine and tyrosine block much of the tryptophan transport that is normally attributable to the action of AroP.

In the succinate medium with five amino acids, induction was eliminated only in strains defective in producing all three transport proteins.

Role of the tryptophan permeases in tryptophan retention. In addition to their role in transporting amino acids present in the environment, permeases may facilitate the retention or recapture of newly synthesized amino acids. To determine whether the tryptophan permeases of *E. coli* influence tryptophan utilization, we made appropriate mixtures of permease-positive and -negative cells and examined their relative growth on tryptophan and on a precursor of tryptophan, anthranilate. It was observed that permease-positive cells were more effective than permease-negative cells in utilizing newly synthesized tryptophan. We also found that in the presence of excess tryptophan the three permeases did not confer a significant growth advantage. Apparently, in excess tryptophan in the medium employed, permease activity was not essential for growth on tryptophan.

Effect of tryptophanase and tryptophan permeases on growth on glucose-tryptophan medium. During growth in glucose-tryptophan medium the cellular level of tryptophanase is low because of catabolite repression. Nevertheless the *tna* operon is induced and *trp* operon deletion strains accumulate some indole, indicating that the tryptophanase that is produced is capable of degrading some of the tryptophan that is available. This observation raises the following question: does tryptophanase action in glucose-tryptophan medium affect tryptophan utilization, and, if so, to what extent is the effect permease dependent? A second important question is, do high levels of tryptophanase have an effect on the expression of the biosynthetic *trp* operon? To answer the first question, we examined the growth of permease mutants that were tryptophanase positive or negative. To increase the sensitivity of the test, we performed the growth tests in a *trp* operon deletion strain. In this strain, if tryptophanase degraded tryptophan to indole, the indole could only be converted back to tryptophan by the condensation of indole with serine or cysteine, catalyzed by tryptophanase. We observed that growth on glucose-tryptophan was poor in the *mtr aroP* and *mtr aroP tnaB* strains. More importantly, growth of the same strains was prevented by adding either tyrosine plus phenylalanine or acid-hydrolyzed casein to the glucose-tryptophan medium. Growth was restored when the *mtr aroP* strain also was *tnaA*, proving that tryptophanase action in the double permease mutant was responsible for growth prevention. Our interpretation of these results is that in strains capable of forming tryptophanase tryptophan is degraded despite the presence of glucose. We believe that TnaB cannot compensate for the defective Mtr and AroP permeases because the level of TnaB is too low in glucose media. We also determined whether growth was inhibited in a prototrophic tryptophanase-positive strain lacking the two permeases; no inhibition was noted.

Effect of tryptophanase content on *trp* operon expression. To examine the effect of the presence of high levels of tryptophanase on *trp* operon expression, we measured anthranilate synthase levels after a shift from growth in a high-tryptophan medium to growth in its absence. Both media contained a non-catabolite-repressing carbon source. We observed that *trp* operon expression was slightly higher in cells containing tryptophanase than in those lacking it. We believe that the tryptophanase degraded some of the tryptophan that was produced biosynthetically. This presumably forced the cell to form higher levels of the *trp* operon polypeptides in an effort to maintain an intracellular tryptophan concentration that was sufficient for a maximal growth rate.

Tryptophanase-dependent growth on indole. On non-catabolic-repressing carbon sources, strains lacking tryptophan synthetase activity can use indole as a source of tryptophan in reactions catalyzed by tryptophanase. However, indole is not a *tna* operon inducer; therefore, to obtain growth of strains lacking tryptophan synthetase a *tna* operon inducer must be provided along with indole. We examined the effects of tryptophan permease mutations on indole utilization in an acid-hydrolyzed casein medium in the presence of 1-methyltryptophan as an inducer. We observed that only *mtr* mutations prevented growth on indole plus 1-methyltryptophan. In this medium, the induction of tryptophanase formation was maximal in strains lacking any one of three permeases. We conclude that of the three permeases only Mtr is effective in transporting indole. This transport activity probably is important to overall metabolic efficiency, because cells with tryptophanase activity will degrade tryptophan to indole. This indole must be transported into the cell and reconverted to tryptophan by the action of either tryptophan synthetase or tryptophanase.

Conclusions. *E. coli* can synthesize three permeases that transport tryptophan from its environment. Each, acting

alone in an appropriate growth medium, can concentrate sufficient intracellular tryptophan to induce the expression of the *tna* operon. However permease formation and function are markedly influenced by the growth medium. One high-affinity permease, Mtr, which is negatively regulated by the *trp* repressor and positively regulated by the TyrR product (10, 11, 22), can transport indole as well as tryptophan. A second high-affinity permease, AroP, which is negatively regulated by the *tyrR* product (5), transports phenylalanine and tyrosine as well as tryptophan. Synthesis of the third tryptophan permease, TnaB, which has a low affinity and a high capacity for tryptophan, is efficiently regulated by both tryptophan-induced transcription antitermination and catabolite repression (9, 25). This permease is essential for growth on tryptophan as the sole carbon source. The different properties of the three tryptophan permease systems allow *E. coli* appreciable latitude in coping with a variety of environments while adjusting its metabolism of tryptophan. For a general discussion of the effects of other mutations and varied nutritional supplementation on tryptophan transport, the reader is referred to an article by Heatwole and Somerville (10).

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