Mutations Affecting Aromatic Amino Acid Transport in *Escherichia coli* and *Salmonella typhimurium*

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SUMMARY

A genetic locus, *aroT*, located between *chr* and the *trp* operon in *Salmonella typhimurium*, and similar genes, *aroR* and *aroS*, near the *trp* locus of *Escherichia coli*, were found to be involved in the transport of aromatic amino acids. Genetic lesions at these loci cause a variable diminution in uptake and accumulation of aromatic amino acids, alanine and glycine compared with the wild type. The F'*trp* episome carries the *aroR* locus. Curing an *E. coli* strain of the F'*trp* episome which covers a chromosomal deletion from *cysB* through the *trp* operon and *tonB* regions, results in a 60 to 80 % decrease in tryptophan uptake. The introduction of F'*trp* into a *trp* operon-deleted *S. typhimurium* of low transport ability restores transport ability, suggesting that *aroT* in this organism may be homologous with *aroR* in *E. coli*. In *E. coli*, tryptophan accumulation is normally increased by prior growth in L-tryptophan, while in *S. typhimurium* it is repressed. In both genera, the *trpR* gene appears to have no effect on the tryptophan transport capabilities in response to changes in the concentration of L-tryptophan in the medium. Tryptophan transport in the *S. typhimurium* F'*trp* hybrid was subject to repression, while in the *E. coli* strain which carries F'*trp* covering the equivalent chromosomal deletion, an increase in tryptophan accumulation was shown after growth in L-tryptophan-supplemented medium.

INTRODUCTION

In *Escherichia coli* K12 and *Salmonella typhimurium*, the amino acid tryptophan is transported by two systems: a general aromatic transport system with K_m about 1×10^{-7} M, and a specific non-inducible transport system for tryptophan with a K_m of about 1×10^{-6} M (Ames, 1964; Ames & Roth, 1968; Piperno & Oxender, 1968; Brown, 1970). A third inducible tryptophan transport system was reported in *E. coli* by Boezi & DeMoss (1961) with a K_m value for tryptophan of 1×10^{-5} M. The latter system was absent under the growth conditions used in our study since it was repressed by the glucose in our growth medium. A genetic locus coding for a component of the general aromatic transport system (*aroP*) has been located. The relative gene order in the *aroP* region is *leu-aziA-aroP-aceE* in both *S typhimurium* (Langley & Guest, 1974) and *E. coli* (Guest, 1974). The genetic regions responsible for the three specific aromatic amino acid transport systems have not been found.

Increasing the concentration of tryptophan in the medium from 20 to 100 μg/ml enhances the growth of some slow-growing *trp-chr* deletion strains of *S. typhimurium* (Corwin *et al.* 1966). One possibility, that this effect was due to increased penetration of the required amino acid at the higher concentration, prompted a study of tryptophan transport in these strains. As a result, genetic lesions which result in lowered tryptophan transport were

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Table 1. *Escherichia coli strains*

Strain	Genetic loci*	Source
W1485	F⁻ wild type	
T3	*trpE, tnaA*	Boezi & DeMoss (1961)
DB1	*trpE, tnaA, aroS*	Indole acrylic acid-resistant mutant of T3
DB1/F′trp	*trpE, tnaA, aroS/F′trp⁺ cysB⁺ aroR⁺ tonB⁺ colI⁺, V⁺, B⁺*	Conjugation with *E. coli* KY113
KY113	*trp-tonB-cysB-aroR* deletion/F′trp⁺, colI⁺, V⁺, B⁺, cysB⁺, aroR⁺, tonB⁺	L. Baron
X7700	F⁻ *proA, proB, lac, ø80dlac, ara, thi, malB,* Str^R	W. Reznikoff
X7800	F⁻ *proA, proB, lac, ø80dlac, ara, thi, malB, trpR,* Str^R	W. Reznikoff

* Gene symbols: *aroR* and *aroS* denote mutations affecting transport of aromatic amino acids, glycine and alanine. The episome, F′trp ⁺cysB⁺ aroR⁺ tonB⁺ colI⁺, V⁺, B⁺, is referred to as F′trp. All other symbols are those recommended by Taylor & Trotter (1972).

Table 2. *Salmonella typhimurium strains*

Strain	Genetic loci*	Source
LT2	Wild type	P. Margolin
SUPX11	*supX-cysB* deletion	P. Margolin
5	*supX, leu-500, ara9*	P. Margolin
2	*supX, leu-500, ara9*	P. Margolin
OABE130	*trpOABE* deletion	P. Margolin
PM458	*trpA512* deletion, *cysB529, leu-500*	P. Margolin
PM566	*trpABEDC167* deletion, *cysB529, leu-500*	P. Margolin
SUPX33	*trpOAB-supX* deletion	Wuesthoff & Bauerle (1970)
BEDC107	*trpBEDC-chr-aroT* deletion	Wuesthoff & Bauerle (1970)
ABEDC101	*trpABEDC-chr-aroT* deletion	Wuesthoff & Bauerle (1970)
BEDC43	*trpBEDC* deletion	Wuesthoff & Bauerle (1970)
A49	*trpA49*	Wuesthoff & Bauerle (1970)
A50	*trpA50*	Wuesthoff & Bauerle (1970)
E171	*trpE171*	Margolin & Bauerle (1966)
MTR1	*trpR782 cysB517*	Bauerle & Margolin (1966)
BEDC107/F′trp	*trpBEDC-chr-aroT* deletion/F′trp⁺ cysB⁺ tonB⁺ colI⁺, V⁺, B⁺ aroR⁺	Conjugation with *E. coli* KY113

* Gene symbols: *aroT* denotes the mutation affecting transport of aromatic amino acids, glycine and alanine. The episome F′trp⁺, cysB⁺, aroR⁺ tonB⁺ colI⁺, V⁺, B⁺, is referred to as F′trp. All symbols are those recommended by Sanderson (1972). The strains from Margolin and his group are all derived from the wild type LT2.

mapped near the *trp* operon in both *E. coli* and *S. typhimurium* (Thorne & Corwin, 1970). Subsequent studies have shown that the transport of phenylalanine and tyrosine, as well as alanine and glycine, is also lowered. The loci, which may or may not be homologous in the two genera, have been named *aroT* in *S. typhimurium* and *aroR* and *aroS* in *E. coli*. Their effect on aromatic amino acid transport is similar to *aroP* lesions (Ames, 1964; Brown, 1970). These studies were extended to include the role of tryptophan transport regulation by *trpR* (Thorne & Corwin, 1971). This report describes the genetic mapping, regulation and kinetics of tryptophan transport by *aroT* strains of *S. typhimurium* and *aroR* and *aroS* strains of *E. coli*.

METHODS

Bacteria. The *Escherichia coli* K12 and *Salmonella typhimurium* LT2 strains used are listed in Tables 1 and 2 respectively. In comparing results for transport systems that are inducible or repressible, care was taken to delineate the strains used (preferably isogenic ones) and the growth conditions. Of the *E. coli* strains used, T3 and DB1 are isogenic (DB1 being an indole acrylic acid-resistant mutant of T3), and X7700 and X7800 *trpR* are isogenic *E. coli* K12 strains. All the *S. typhimurium* strains received from P. Margolin were derived from LT2 and are well characterized (Table 2).

Phage. Virulent P1 *vir* (P1 *vs*) phage was obtained from L. Baron. P1 *vir* lysates were prepared by a confluent lysis technique (Adams, 1959). Phage T1 was prepared by the soft agar method described by Adams (1959). Phage P22 lysates were prepared according to the method of Margolin (1963)

Media. Rich medium was the LC broth of Luria & Burrous (1957), Penassay broth or nutrient broth (Difco). The minimal medium (medium A) was modified from Davis & Mingioli (1950), and contained (g/l): K_2HPO_4, 10·5; KH_2PO_4, 4·5; $(NH_4)_2SO_4$, 1·0; $MgSO_4$. $7H_2O$, 0·1. When 2 g of glucose was added to this medium it was termed minimal glucose (MG) medium. Amino acids were added as required at 20 μg/ml unless otherwise noted. When derepression of transport was sought in tryptophan-requiring strains, 2 μg/tryptophan/ml rather than 20 μg/ml were added.

Genetic methods. Transductions with P1 *vir* and P22 were carried out as described by Lennox (1955) and Margolin (1963), respectively. Sensitivity to phage T1 was routinely tested by cross-streaking on LC agar. Mating experiments were done according to the method of Falkow *et al.* (1964).

Isolation of mutants resistant to indole acrylic acid. An *E. coli* culture of T3 grown overnight in Penassay broth was diluted 1:15 with fresh broth and allowed to grow to 1×10^7 cells/ml. A 5 ml sample of the culture was centrifuged, washed twice and resuspended in 0·5 ml of 0·1 M-sodium citrate buffer pH 5·0. The cells were then treated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) according to the method of Adelberg, Mandel & Chen (1965). The NTG-treated cells were spread on MG plates containing 100 μg indole acrylic acid and 1 μg tryptophan/ml. The plates were incubated at 37 °C for 3 to 4 days. Isolated colonies were picked, replica-plated on to fresh analogue-supplemented plates and assayed for tryptophan transporting ability. A representative strain DB1 (*trpE, tnaA, aroS*) was picked. This strain is not a rapid tryptophan excretor, because supernatant fluid from the mutant culture did not alter the extent of labelled tryptophan uptake by the parental strain T3. During isolation of the mutants the colonies were not surrounded by haloes of 'feeding' bacteria.

Ethidium bromide curing of F′trp episome. Cells harboring the F′*trp* episome were grown overnight in nutrient broth and diluted to 10^4 cells/ml in fresh nutrient broth pH 7·6. Ethidium bromide was added to the diluted cells in a concentration range of 30 to 0·03 μg/ml (modification of the method of Bouanchaud, Scavizzi & Chabbert, 1968). All cultures were allowed to grow overnight at 37 °C. Cultures with 60 to 80 % of the growth of controls grown without ethidium bromide were replated on to nutrient agar plates. Isolated colonies were screened for Cys Trp phenotype by replicating on to single amino acid-supplemented MG plates.

Transport assay. Transport of amino acids was estimated as the amount of radioactively-labelled compound accumulated by cells transferred to medium containing chloramphenicol. Under these conditions, the label was not incorporated to any appreciable extent into protein

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or other trichloroacetic acid-insoluble material, but was accumulated within the cell. This allows direct measurement of the initial rate of uptake. The growth medium contained sufficient glucose to inhibit the formation of tryptophanase by the *E. coli* strains which are tryptophanase positive (Freundlich & Lichstein, 1960). *Salmonella typhimurium* LT2 and its derivatives do not utilize aromatic amino acids either as carbon or as nitrogen sources (Ames, 1964). Therefore, the uptake of radioactivity could be used as a measure of tryptophan transport unaffected by tryptophan metabolism.

The bacteria were grown overnight at 37 °C, with shaking, in MG medium supplemented with 20 μg L-tryptophan/ml. Initially studies were performed using exponentially-growing cells; values obtained were in agreement with those using overnight (16 to 17 h) cells. Derepression of the tryptophan transport system in salmonellae was achieved by omitting L-tryptophan or adding only 2 μg/ml. Cells were harvested by centrifuging at 25 °C, washed and resuspended in medium A to a concentration of 1×10^9 cells/ml. One ml of washed cells (1·5 mg wet mass/ml) was then added to reaction mixture containing 12 μmol $MgCl_2$, 15 μmol tris-HCl buffer pH 8, and 250 μg chloramphenicol/ml, into a final volume of 7·0 ml. Radioactive amino acid was added at zero time and the mixture incubated at 25 °C. Samples (1 ml) were taken at 15 and 20 s and at 1, 2, 4 and 6 min, or as indicated, and filtered through membrane filters (Millipore type HA, 0·45 μm pore size). The filtered cells were washed immediately with 5 ml medium A (25 °C), dried and counted in a butyl-PBD–toluene mixture (7 g PBD, from CIBA/l toluene) in a Beckman-LS 200B liquid scintillation counter. Controls were included in which 1 ml of cells without radioactive amino acid was filtered, followed by 1 ml of the same radioactive amino acid solution as that used in each transport assay. Filtering took 5 s. The c.p.m. values in the controls, always less than the 15 s experimental values, were taken to represent the amino acid bound to the membrane filter and to the cells, and were subtracted from the experimental values. Data in this paper represent the mean values of three or more experiments, run on different days. The transport data are presented as either the initial rate of transport (30 s to 1 min) expressed as μmol/g cells/min, or the 6 min accumulation level expressed as the concentration of the amino acid in cell water, based on the calculation of Broda (1968) that 10^8 cells contain 1×10^{-4} ml of water. A ratio of the radioactivity concentration inside the cell (G_{in}) to the radioactivity concentration outside the cell (G_{ex}) is presented to demonstrate the accumulation capabilities of the various strains after 6 min incubation.

Chemicals. Analytical reagent grade inorganic chemicals were used throughout. Organic chemicals were the highest grade commercially available. Radioactive amino acids obtained from New England Nuclear Corp., Boston, Massachusetts, U.S.A., included DL-[³H]tryptophan (185 mCi/mmol), L-[³H]tryptophan (33 mCi/mmol), L-[³H]tyrosine (20 mCi/mmol), L-[³H]phenylalanine (135 mCi/mmol), L-[¹⁴C]leucine (5000 mCi/mmol), L-[¹⁴C]isoleucine (250 mCi/mmol), DL-[¹⁴C]lysine (7·1 mCi/mmol), L-[¹⁴C]alanine (11 mCi/mmol), and [¹⁴C]glycine (4 mCi/mmol). Indole acrylic acid was obtained from Sigma, NTG from Aldrich Chemical Co., New Jersey, U.S.A., scintillator butyl-PBD from CIBA Corp., Summit, New Jersey, and filters from Millipore. Ethidium bromide was generously given by Dr R. Herrmann.

<div align="center">RESULTS</div>

<div align="center">*Tryptophan uptake in E. coli K12 and S. typhimurium LT2*</div>

Figure 1 illustrates the time course of tryptophan uptake by strains DB1 (an *E. coli* transport-deficient strain), its parental strain T3, the transport-deficient *S. typhimurium* BEDC107, and its parental strain LT2. In order to demonstrate optimal transport and
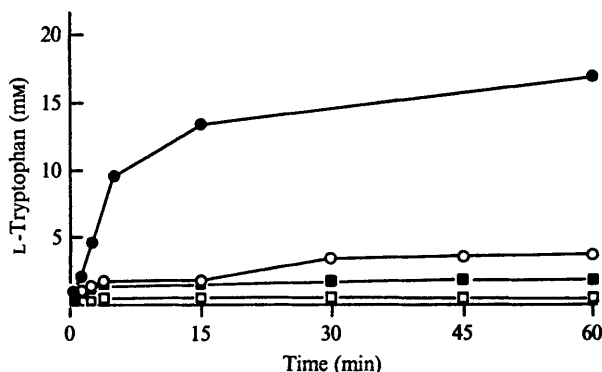
Fig. 1. Time-course of tryptophan uptake in *E. coli* strains (●) T3 and (○) DB1, and *S. typhimurium* strains (■) LT2 and (□) BEDC107. The *E. coli* strains were grown overnight in MG medium supplemented with 20 μg/L-tryptophan/ml. The Salmonella strain LT2 was grown overnight in MG without L-tryptophan, and BEDC107 was grown in MG medium supplemented with 2 μg tryptophan/ml. Cells were incubated with L-[³H]tryptophan at a final concentration of 7·1 μM (33 mCi/mmol) and uptake was expressed as the intracellular concentration of radioactive material.

Table 3. *Tryptophan uptake in E. coli and S. typhimurium. Comparison of apparent Michaelis constants, maximum reaction velocities, and concentration ratios*

Cells were grown overnight in MG medium with tryptophan supplementation at 20 μg/ml unless otherwise noted, i.e. (–) none, (2) 2 μg/ml.

	Genotype	K_m*(μM)	V_{max}* (μmoles/g cell/min)	G_{in}/G_{ex}†
E. coli				
WI485	Wild type	1·4	1·19	6820
T3	*trpE* (parent of DB-1)	3·0	1·29	5350
DBI	*trpE aroS*	1·4	0·35	2400
S. typhimurium				
LT2	Wild type	1·3	0·50	510 1200 (–)
BEDC107	*trp BEDC, chr, aroT*	1·0	0·08	104 225 (2)

* K_m and V_{max} values were derived from Lineweaver–Burk plots of (*V*) the initial rate of transport (30 s to 1 min) and (*S*) the ³H-L-tryptophan concentrations over the range 0·43 to 19·7 μM.

† Ratio of c.p.m. in bacterial cell water to initial concentration outside. G_{in} is the c.p.m. accumulated during 6 min incubation; G_{ex} is the initial external concentration of tryptophan (i.e. 0·43 μM L-[³H]tryptophan).

accumulation by the two genera, the two *E. coli* strains were grown in media supplemented with L-tryptophan, while the two *S. typhimurium* strains were grown with 2 μg tryptophan/ml or no amino acid. The maximal rate of tryptophan uptake occurs from 30 s to 1 min, except in the *E. coli* parental strain T3 which maintains this rate for 4 to 6 min. Under saturating conditions (i.e. radioactive substrate concentration $> 2 \times K_m$ of transport for T3) a final level of accumulation was reached in 15 min by T3 and in 6 min by the other three strains. The plateau levels were maintained relatively constant for 1 h.

Saturation kinetics

Lineweaver–Burk plots of the initial rate of uptake at varying L-tryptophan concentrations (a range of 0·43 to 19·7 μM) yielded Michaelis constants for these *E. coli* and *S. typhimurium* strains (Table 3). G_{in}/G_{ex} ratios were also determined, to indicate the accumulation
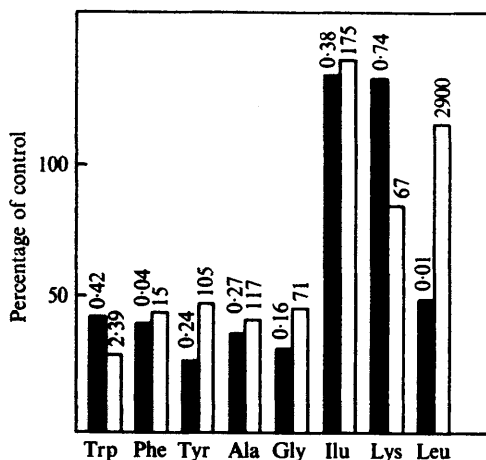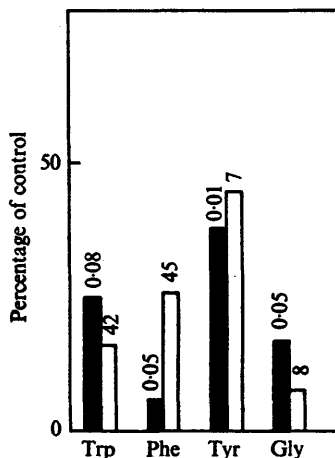
Fig. 2   Fig. 3

Fig. 2. Transport of aliphatic, basic and aromatic amino acids by *E. coli* transport-deficient strain DB1. Data given are percentages relative to a value for the *E. coli* parental strain T3 of 100 %. The solid bars represent percentage initial rate of uptake (μmol/g cells/min). The open bars represent percentage G_{in}/G_{ex} ratio. Actual rate and ratio values for DB1 appear at the bases of these bars. Both strains were grown overnight in MG medium supplemented with 20 μg/tryptophan/ml. Symbols of radioactive amino acids used (and their specific activities) are: Ala, L-[^{14}C]alanine (11·5 μM, 11 mCi/mmol); GLY, [^{14}C]glycine (11·5 μM, 4 mCi/mmol); Ile, L-[^{14}C]isoleucine (10·9 μM, 250 mCi/mmol); Leu, L-[^{14}C]leucine (0·01 μM, 5000 mCi/mmol); Lys, DL-[^{14}C]lysine (10 μM, 7·1 mCi/mol); Phe, L-[^{3}H]phenylalanine (9·1 μM, 135 mCi/mmol); Trp, L-[^{3}H]tryptophan (7·1 μM, 33 mCi/mmol); Tyr, L-[^{3}H]tyrosine (7·1 μM, 20 mCi/mmol).

Fig. 3. Transport of aromatic amino acids and glycine by *S. typhimurium* transport-deficient strain BEDC107. Data given are percentages relative to a value for the *S. typhimurium* parental strain LT2 of 100 %. The solid bars represent percentage initial rate of uptake (μmol/g cells/min) while the open bars represent percentage G_{in}/G_{ex} ratio. Actual rate and G_{in}/G_{ex} values for BEDC107 appear at the bases of these bars. Strain LT2 was grown overnight in MG medium without L-tryptophan, while BEDC107 was grown in MG medium supplemented with 2 μg L-tryptophan/ml. For symbols of radioactive amino acids used and their specific activities, see Fig. 2.

ability of each strain. These ratios demonstrate the five-fold difference in accumulation ability of *E. coli* K12 wild type and *S. typhimurium* LT2. Mutation at the *aroT* or *aroS* regions results in an analogous loss of transport activity in both species. *Escherichia coli* DB1 accumulated to a level 45 % of that of its parent, T3. In *S. typhimurium*, the permease-deficient strain BEDC107 accumulated only 20 % of that of wild-type LT2, regardless of the level of tryptophan in the growth medium.

V_{max} values were 4 to 6 times lower in the transport-deficient mutants, DB1 and BEDC107, while their apparent dissociation constants (K_m) were in the same range as the parental wild-type strains of their respective genera.

Uptake of aromatic and other amino acids by E. coli

In the presence of a 20-fold excess of each of 17 amino acids, only phenylalanine and tyrosine interfered markedly with the uptake of DL-[^{3}H]tryptophan in *E. coli* T3 (Thorne, 1972). The aromatic amino acid uptake of DB1, the transport-deficient strain, and its parental strain T3 were compared using eight radioactively-labelled L or DL amino acids (Fig. 2). Strain DB1 showed the greatest impairment in tryptophan transport of the amino acids tested, as reflected in a G_{in}/G_{ex} ratio of 28 % compared with control strain T3. The initial rates of transport and G_{in}/G_{ex} ratios as compared to control T3 were all below 50 % for
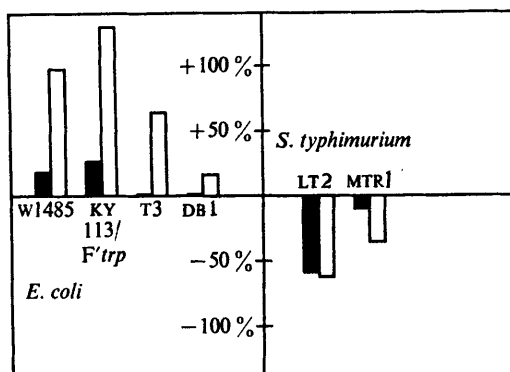
Fig. 4. Effect of growth in L-tryptophan-containing media on tryptophan transport. Cells grown overnight in MG medium alone, or MG medium supplemented with 2 or 20 μg tryptophan/ml, were assayed as described in Methods using L-[³H]tryptophan (7·1 μM, 33 mCi/mmol). Initial rates and accumulation levels were calculated. The solid bars represent percentage change in initial rate (μmol/g cells/min) and the open bars represent percentage change in accumulation (μmol/g cells). *Escherichia coli* data given are percentages relative to a value for *E. coli* K12 W1485 of 100 % (initial rate when grown without tryptophan was 1·11 μmol/g cells/min, accumulation was 3·60 μmol/g). *Salmonella typhimurium* data given are percentages relative to a value for *S. typhimurium* LT2 of 100 % (initial rate when grown without tryptophan was 0·39 μmol/g cells/min, accumulation was 1·31 μmol/g cells).

phenylalanine, tyrosine, alanine and glycine. The initial rates and G_{in}/G_{ex} ratio of DB1 for isoleucine and lysine were similar to the parental strain T3. Although a low concentration of leucine was used in these assays (0·01 μM), the low initial rate for leucine uptake by DB1 was consistently found. The G_{in}/G_{ex} ratio for leucine uptake of DB1, however, appears similar to parental T3.

Uptake of aromatic and other amino acids by S. typhimurium

In *S. typhimurium*, the transport defects of strain BEDC107 were examined (Fig. 3). Comparison of strain BEDC107 with wild-type LT2 revealed defects in transport of the aromatic amino acids and glycine. Rates and accumulation ratios, expressed as percentages of those of the control strain LT2, were all well below 50 %.

Study of leucine transport by various *trp-chr* deletion strains of *S. typhimurium* revealed the presence of a locus affecting leucine transport on the side of the *chr* marker distal to the *trp* operon (Thorne & Corwin, 1972). This locus is thought not to be involved in aromatic amino acid transport. L-[¹⁴C]alanine transport assays showed this amino acid to be poorly transported in all Salmonella strains tested compared with *E. coli* (Thorne, 1972).

Regulation of aromatic transport in E. coli

In *E. coli* the aromatic permease is not controlled by the tryptophan regulator gene, since the presence or absence of the *trpR* gene has no effect on permease regulation. This was shown by the identical uptake of DL-[³H]tryptophan by the two isogenic *E. coli* K12 strains x7700 and x7800, which differ only by a *trpR* mutation. Maximal rates of uptake after growth in the presence of 20 μg tryptophan/ml were 0·34 and 0·36 μmol/g cells/min, while the accumulations inside the cells after 6 min incubation were 1·63 and 1·72 μmol/g cells respectively. Thus the *trpR* gene does not play a role in the regulation of tryptophan transport.
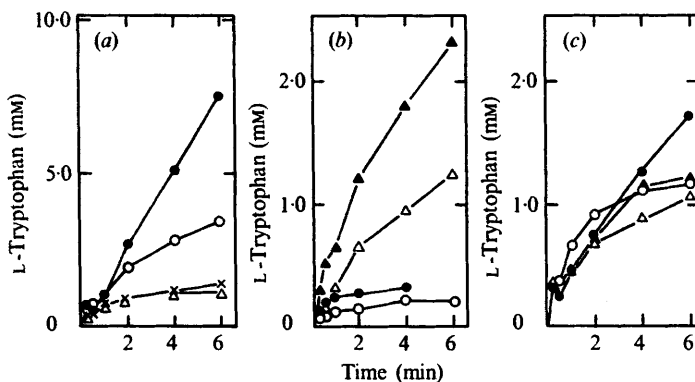
Fig. 5. Effect of *E. coli* F'*trp* episome on tryptophan uptake by various *E. coli* and *S. typhimurium* strains. (*a*) L-[³H]tryptophan (7·1 μM, 33 mCi/mmol) uptake (expressed as the intracellular concentration of radioactive material) into: ●, *E. coli* KY113/F'*trp* grown overnight in MG + 20 μg L-tryptophan/ml; ○, *E. coli* KY113/F'*trp* grown overnight in MG; ×, KY113 (cured) grown overnight in MG + 20 μg L-tryptophan/ml; △, KY113 (cured) grown overnight in MG medium + 2 μg L-tryptophan and 20 μg cysteine/ml. (*b*) L-[³H]tryptophan (7·1 μM, 33 mCi/mmol) uptake (expressed as the intracellular concentration of radioactive material) into: ▲, Salmonella hybrid BEDC107/F'*trp* grown overnight in MG medium; △, BEDC107/F'*trp* grown overnight in MG medium + 20 μg L-tryptophan/ml; ●, BEDC107 grown in MG + 2 μg L-tryptophan/ml; ○, BEDC107 grown in MG + 20 μg L-tryptophan/ml. (*c*) L-[³H]-tryptophan (7·1 μM, 33 mCi/mmol) uptake (expressed as the intracellular concentration of radioactive material) into: ●, *E. coli* DB1 grown overnight in MG + 20 μg L-tryptophan/ml; ○, DB1 grown overnight in MG + 2 μg L-tryptophan/ml; ▲, DB1/F'*trp* grown overnight in MG; (△) DB1/F'*trp* grown overnight in MG + 20 μg L-tryptophan/ml.

Following growth with tryptophan, *E. coli* strains KY113/F'*trp* and W1485 showed 50 % increases and T3 a 33 % increase in the amounts of tryptophan accumulated after 6 min incubation (Fig. 4). The initial uptake rates were only slightly affected by growth on tryptophan, thus appearing constitutive. These results suggest that tryptophan accumulation can be increased by growth in the presence of this amino acid.

Regulation of aromatic transport in S. typhimurium

Transport by the wild-type *S. typhimurium* LT2 was regulated by prior growth in the presence of tryptophan (Fig. 4). Repression of tryptophan uptake resulted after growth in tryptophan-supplemented media. The accumulation of tryptophan by the *trpR* strain MTR1 was still significantly repressed and therefore regulated by tryptophan. Thus, as in *E. coli*, the *trp* operon regulator system of *S. typhimurium* does not appear to control tryptophan uptake.

Mapping of the aroR, aroS and aroT loci affecting aromatic amino acid uptake in E. coli and S. typhimurium

Escherichia coli strain KY113 contains a very large chromosomal deletion of the *cysB-trp-tonB* region. This genetic lesion is compensated for in this cell by the presence of the episome F'*colI*, *V*, *B*⁺ *cysB*⁺ *trp*⁺ *tonB*⁺ (referred to below as F'*trp*) which covers the genetic gap on the chromosome. Ethidium bromide removal of the episome results in an endogenote with a deletion extending through the *trp* operon and neighbouring *ton B* and *cysB* regions. Curing of the episome was assessed easily, since these strains upon curing regained a requirement for cysteine and tryptophan, and became resistant to phage T1 and indole acrylic acid (IA),

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                              trp
         chr              C D E  B A O   supX  cysB
    ──────┼─┼──────────┼─┼┼─┼─┼─┼──────┼─┼───┼─┼──────
                          E 171  A 50      5
                          0·17  0·27     0·27
                                A 49
                                0·36      0·32
                    BEDC 43    A 458     sup X 11
                    0·14(0·32)  0·22     0·18(0·46)
                              sup X 33
                                0·32
                             OABE 130
                                0·22
                             PM 566
              ABEDC 101        0·23
              0·09(0·00)
         BEDC 107
         0·03(0·09)
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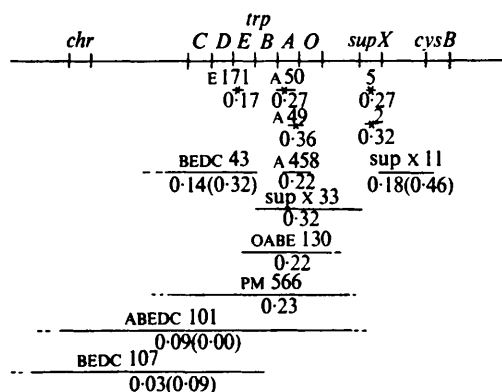
Fig. 6. The *chr-trp-supX-cysB* portion of the *S. typhimurium* chromosome. The lines below the chromosome represent the approximate extent of the deletions in the mutant strains listed above the line except where an × through the line denotes a point mutation. Below the line are the initial rates of L-tryptophan uptake of each strain, expressed as μmol/g cells/min (30 s to 1 min values). The rates in parentheses are for cells grown overnight in MG medium supplemented with 2 μg L-tryptophan/ml; otherwise, cells were grown in media with 20 μg tryptophan/ml. Assays were performed with L-[^{3}H]tryptophan at a final concentration of 7·1 μM (33 mCi/mmol). Each value is the average of two or more experiments run on separate days. Symbols: *chr*, chromium ion sensitivity; *trp*, tryptophan operon; *supX*, suppressor of *leucine-500* operator mutation; *cysB*, cysteine regulation.

the latter suggesting that the gene for aromatic amino acid uptake might lie within the chromosomal deletion.

Transport of tryptophan in KY113 before and after curing provided the first clue to the location of *aroR* (Fig. 5a). After curing the strain of the F'*trp* episome, the amount of tryptophan transported by the cells was reduced by 80 % compared with the hybrid. The initial rate of uptake and intracellular level after 6 min in the cured strain were the same whether the cells had been grown with 2 or 20 μg tryptophan/ml. Thus the decrease in rate of uptake and accumulation in the cured strains is not the result of repression of transport by growth in tryptophan, but is due to the alteration of a component required for tryptophan transport that is coded for by a gene on the F'*trp* episome. This gene has been termed *aroR*.

To determine the location of the *aroR* gene of *E. coli* more precisely we made use of various deletion mutants of *S. typhimurium* LT2 that had been isolated and identified by Margolin and his group (Table 2). This is a feasible approach because the genetic map in *S. typhimurium* is analogous to that of *E. coli* in the region of the *trp* operon. The five genes of the *trp* operon are aligned in the same sequence in both genera. Although the operons show opposite orientation with respect to other chromosomal markers, the relationship of the *trp* operon to closely linked genes is identical in the two genera (Sanderson, 1972; Taylor & Trotter, 1972). The *E. coli tonB* locus is also analogous to the *chr* locus in *S. typhimurium* since *tonB* point mutants WD38 and 169 are also sensitive to chromium ion (Thorne, 1972).

The genetic map of the Salmonella strains studied is shown in Fig. 6 together with the initial rates of L-[^{3}H]tryptophan uptake in these strains. The various strains with point mutations and deletions which cover the *trp* operon and extend through *supX* and *cysB* all have initial rates of tryptophan uptake which are similar to that of wild-type LT2 (0·32 μmol/ g cells/min). The lowest initial rates of transport were exhibited by BEDC107 and ABEDC101, even under derepressed conditions (growth in 2 μg tryptophan/ml). Both ABEDC101 and

BEDC107 carry deletions extending through the *chr* locus (Corwin *et al.* 1966). These results suggest that a locus exists, on the *chr* side of the *trp* operon, which is involved in aromatic amino acid transport; we have termed this gene *aroT*.

To determine the location of the *aroT* region in relation to the *chr* (chromium ion sensi·tivity) and *trp* loci, strain BEDC107 (*trpBEDC, chr, aroT*) was transduced to chromium resistance (Chr^R) with a P22 lysate from wild-type strain LT2. Chr^R transductants were tested for tryptophan requirement and rate of uptake of this amino acid. All 20 Chr^R Trp$^+$ transductants tested were able to take up tryptophan at rates identical to wild type, LT2. Chr^R Trp$^-$ transductants were also found, indicating that either the recipient strain actually possesses a double deletion or that partial diploids were formed. Nineteen of the 20 Chr^R Trp$^-$ transductants tested retained low transport ability. This result, coupled with the normal rate of uptake of the various *chr-trp-supX-cysB* deletion strains (Fig. 6), suggest that in *S. typhimurium* the *aroT* region lies between *chr* and the *trp* operon. However, as the transduction data cannot be interpreted with certainty, the exact location of *aroT* remains unknown.

The results of the following intergeneric mating give added evidence for the presence of a gene coding for a transport component in the F'*trp* episome. The BEDC107 strain of *S. typhimurium* has a low derepressed rate of transport and low steady-state accumulation of tryptophan when grown on 2 μg tryptophan/ml. After conjugation with *E. coli* cells harbouring the F'*trp* episome, the *S. typhimurium* F'*trp* hybrid had increased transport ability (Fig. 5*b*). This suggests that the *E. coli aroR* gene is homologous with the *S. typhimurium aroT* gene. As mentioned previously, in *S. typhimurium* transport was repressed by prior growth in MG–tryptophan medium. This regulation exerted control over the expression of the *E. coli* F'*trp aroR* gene, because prior growth in MG–tryptophan medium caused repression of both the transport rate and accumulation level. Even under derepressed conditions (no tryptophan supplement) the *S. typhimurium* F'*trp* hybrid had a transport rate and an accumulation level which was considerably below the normal *E. coli* range (note the difference in scale between Fig. 5*a* and Fig. 5*b* and *c*). This result may indicate the presence of a tryptophan transport regulator gene in *S. typhimurium* which causes repression of transport after prior growth in tryptophan, and controls the expression of the *E. coli* episomal genetic message.

The results of the intra-generic mating are more difficult to interpret (Fig. 5*c*). When F'*trp* was transferred into DB1, the early transport rates were changed very little. It seems very likely therefore that the genetic lesion (*aroT*) in *S. typhimurium* BEDC107 and the probably homologous region *aroR* in the cured *E. coli* KY113, which are correctable by the F'*trp* episome, are different from the lesion (*aroS*) in *E. coli* DB1 which is not so correctable. Thus the genetic lesion in DB1 must lie outside the range of DNA covered by the F'*trp* episome.

Evidence that the mutational locus in *E. coli* DB1 lies near the *trp* operon was supplied by P1 transduction. The indole acrylic acid (IA)-resistant strain DB1 (*trpE, tnaA, aroS*) was transduced to Trp$^+$ with a P1 lysate from the wild-type *E. coli* K12 strain W1485. Of the 57 Trp$^+$ transductants tested, 22 were IA sensitive. Therefore the frequency of co-transduction of IA sensitivity with tryptophan requirement was 38 %. The exact location of DB1 relative to the *trp* region in *E. coli* remains to be determined, for although *aroS* is co-transducible with *trp* it lies outside the region contained in F'*trp*.

The possibility that the uptake-deficiency of *E. coli* DB1 and/or *S. typhimurium* BEDC107 is due to a cell wall defect caused by a mutation involving *galU* (linked to *trp*) can be ruled out, since these strains are galactose positive and in addition DB1 can absorb phage P1;

neither of these characters can be shown by *galU* mutants (Shapiro, 1966; Rapin & Kalckar, 1971).

<div align="center">DISCUSSION</div>

This study presents evidence that general aromatic amino acid transport and uptake of glycine and alanine is impaired in *aroR*, *aroS* and *aroT* strains of *E. coli* and *S. typhimurium*, respectively, caused by genetic lesions near the *trp* operon and the *tonB* or *chr* loci. The transport defects displayed by these mutations are similar to the *aroP* mutations in *E. coli* (Brown, 1970) and *S. typhimurium* (Ames, 1964). In *E. coli*, mutation in *aroP* results in roughly a 75 % decrease in transport of labelled aromatic amino acids by whole cells. In-hibition studies indicate that in *E. coli*, the general aromatic transport system *aroP* has a high affinity for phenylalanine, tyrosine and tryptophan and lower affinity for cysteine, leucine, alanine, methionine, histidine and aspartic acid.

In our study, aromatic amino acid transport by *aroT*, *aroR*, *aroS* strains appears similar to that of *aroP*. Of the other neutral and basic amino acids which were directly tested, uptake of alanine and glycine were also impaired.

Both the cured *E. coli* KY1113 and *S. typhimurium* BEDC107 deletions termed *aroR* and *aroT* respectively have their tryptophan transport and IA phenotype restored to normal by the presence of the F'*trp* episome. Transduction studies and rates of uptake of various *chr-trp-supX-cysB* deletion strains suggests that the transport defect of *S. typhimurium aroT* strain BEDC107 is located between *trp* and *chr*. Thus the *aroT* and *aroR* mutation can be located on the *tonB* or *chr* side of the *trp* operon, and results in impaired transport of labelled aromatic amino acids in *E. coli* K12 and *S. typhimurium*. The IA^R character of *E. coli* strain DB1 (*aroS*) is co-transduced with *trp*, and if the transport defect of DB1 (which is similar to the cured IA^R KY1113 deletion strain) is directly related to its IA resistance, then the genetic locus *aroS* is also close to the *trp* operon although outside the region contained in F'*trp*. Exact correlation of the resistance phenotype with the transport defect was not made. The possibility remains of DB1 being a double mutant, especially since it was produced by NTG mutagenesis (Guerola, Ingraham & Cerdá-Olmedo, 1971). Location of the *aroT*, *aroR* and possibly *aroS* regions near the *trp* operon raises the question of whether there are two or more genetic loci coding for separate components functioning in general aromatic amino acid transport. This may be so, since the *aroP* mutations and the *aroS* mutation were isolated on the basis of resistance to three different analogues.

The similarities in K_m of wild-type and *aroT* and *aroS* strains suggest that the mutations do not alter the structure of a binding protein involved in transport. The decrease in the V_{max} of DB1 (*aroS*) and BEDC107 (*aroT*) may be associated with loss of transport components or with alterations in the cell wall around the transport components which cause the trans-port reactions to proceed more slowly. Binding proteins may play a role in aromatic amino acid transport. Guroff & Bromwell (1970) have reported the isolation of a phenylalanine-binding protein from *Comamonas* sp. Such structural defects may lead to loss of transport components from the periplasmic space due to an incomplete cell wall, thereby contributing to a smaller number of transport proteins capable of reacting with the substrate.

A preliminary study of *S. typhimurium* strains possessing different defects in their lipo-polysaccharide structure (SL1181, SL896, SL1197, SL1034, HN300 and HN308; see Gemski & Stocker, 1967; Nakae & Nikaido, 1971) indicated that strains with *rfbT*, *rfaL* and *galU* mutations had 14 to 38 % lower initial rates and accumulation levels of tryptophan uptake than the wild-type LT2 (Thorne, 1972). While only the tryptophan transport ability of these rough strains was assayed, one might suspect that such lipopolysaccharide defects may have

a generalized effect involving other transport systems as well. It may be noted that DB1 transports glycine and alanine poorly and BEDC107 has a deficient glycine uptake. The presence of *galU* mutations was ruled out in both strains, although other cell-wall defects cannot be precluded.

Other studies have shown that the area around the *trp-tonB* loci in *E. coli* contains genes for a number of bacterial cell-surface components. Mutations in these regions lead to deficiencies in transport of iron (Cox *et al.*, 1970; Wang & Newton, 1969*a*, *b*, 1971) and resistance to phages T1, ϕ80 and colicins B, I, and V (Gratia, 1966; Signer, 1966; Yanofsky & Lennox, 1959). Our mapping data have revealed regions in both *E. coli* and *S. typhimurium* in this same area concerned with the ability to transport the aromatic amino acids, glycine, and alanine. There is also evidence of a different region affecting leucine transport ability on the side of the *chr* locus distal to the *trp* operon (Thorne & Corwin, 1972).

An example of a mutation with a missing surface component associated with a pleiotropic transport effect has been demonstrated in *ctr* strains of *E. coli* (Wang, Morse & Morse, 1969; Dahl, Wang & Morse, 1971). These are defective in the uptake of various carbohydrates and of tryptophan, and are located 20 min from the *trp-tonB* area. The *ctr* mutational effect on tryptophan uptake was confined to the specific inducible transport system for tryptophan.

Comparison of tryptophan transport by E. coli and S. typhimurium. The ability to transport tryptophan is lower in wild-type *S. typhimurium* than in wild-type *E. coli* K12, even under conditions of maximal transport activity. Either the salmonella normally contain a mechanism responsible for maintaining a relatively low level of tryptophan transport by regulating the number of transport carriers, or perhaps differences in the cell envelope of the salmonellae are sufficient to provide more of a physical barrier to passage of amino acids (Robbie & Wilson, 1969), or there could be a different amount of metabolic energy made available for active transport. Significantly, the capacity for tryptophan transport in *S. typhimurium* harbouring the F'*trp* episome from *E. coli* never achieved levels found in *E. coli* harbouring the episome, but remained at the lower level of wild-type salmonellae.

Comparison of regulation of tryptophan transport and trp operon. Somerville (1966) reported the regulation of expression of episomal *E. coli* tryptophan biosynthetic enzymes in strains of *S. typhimurium* whose chromosomes were deleted through the *trp* operon. The response to regulation in the *S. typhimurium* F'*trp* hybrid was the same as that in the *E. coli* wild type. Thus, although regulation of *trp* operon structural genes is similar in the two genera, this is not the case with the aromatic transport system. Our findings indicate that the *trpR* locus, which regulates the *trp* structural genes, appears to have no effect on tryptophan transport in *E. coli* and *S. typhimurium*. The two genera differ in their response to L-tryptophan supplementation. In *E. coli*, accumulation of tryptophan is normally enhanced by growth in tryptophan, while in *S. typhimurium*, accumulation is depressed by such growth conditions.

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