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Transport of 5-Aminolevulinic Acid by the Dipeptide Permease in *Salmonella typhimurium*

THOMAS ELLIOTT

Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294

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In a previous search for mutants of *Salmonella typhimurium* that are defective in heme synthesis, one class that is apparently defective in 5-aminolevulinic acid (ALA) uptake (*alu*) was found. Here, I describe the characterization of these mutations. The mutations all map to a single locus near 77.5 min on the genetic map, which is transcribed counterclockwise. Nutritional tests, genetic and physical mapping, and partial DNA sequence analysis revealed that *alu* mutants are defective in a periplasmic binding protein-dependent permease that also transports dipeptides, encoded by the *dpp* operon. The uptake of labeled ALA is defective in *dpp* mutants and is markedly increased in a strain that has elevated transcription of the *dpp* locus. Unlabeled L-leucyl-glycine competes with labeled ALA for uptake. In a strain carrying both a *dpp-lac* operon fusion and a functional copy of the *dpp* locus, the expression of β-galactosidase is not induced by ALA, nor, in a *hemL* mutant, does expression of *dpp* change substantially during starvation for ALA. The dipeptide permease displays a relaxed substrate specificity that allows transport of the important nonpeptide nutrient ALA, whose structure is closely related to that of glycyl-glycine.

Numerous compounds are transported into *Salmonella typhimurium* and other bacterial cells with very high affinity. The bacterial proteins that function in transport include permeases present in the inner membrane and, often, periplasmic or outer membrane components as well. For peptides alone, three different transport systems are known, the dipeptide, tripeptide, and oligopeptide permeases (17). These peptide permeases have overlapping substrate specificities.

In previous work, we isolated more than 170 Mud insertion mutants of *S. typhimurium* that have lost the ability to use exogenous 5-aminolevulinic acid (ALA) to supplement the auxotrophy of a *hemA* mutant (39). The *hemA* gene encodes glutamyl-tRNA reductase, the first committed enzyme in the heme pathway (for a review, see reference 23). Among the mutants isolated in this way were a number with defects in the heme pathway after ALA, as expected. However, many other mutants (termed *alu*) were not defective in heme biosynthesis, and we suggested that these might be defective in the uptake of ALA from the medium.

In this work, I use an uptake assay to show that *alu* mutants cannot transport ALA. Genetic and physical characterization of the *alu* mutants led to the realization that the dipeptide permease encoded by *dpp* is defective in these mutants (1, 30). I suggest that the designation *dpp* be retained. The dipeptide permease transports a wide range of dipeptides, and the close structural similarity of ALA and glycyl-glycine suggests that ALA is a substrate for the dipeptide permease. This idea is strengthened by the finding of competition for transport between ALA and the dipeptide L-leucyl-glycine. Previous studies have found no evidence for regulation of *dpp* expression, and I extend this by showing that a *dpp-lac* fusion is apparently not regulated by ALA.

MATERIALS AND METHODS

Bacteria and phage. All *S. typhimurium* strains were derived from the wild-type strain LT-2; *S. typhimurium* strains used in this study and their sources are listed in Table 1. The basic genetic nomenclature has been described previously (11). The *S. typhimurium* wild type does not carry the *lac* operon. Strain TE1295 (Table 1) was used as the parent strain for isolation of the Mud-J insertions used in this work (39).

Media and growth conditions. Nutrient broth (NB) (Difco), containing 0.5% NaCl, and LB broth (29) were used as maximally supplemented media. E and NCE (no-carbon E) were used as minimal media (6, 37). In minimal medium, glucose and glycerol were added as carbon sources at 0.2%. Difco BiTek agar was added at a final concentration of 1.5% for solid media.

Supplementation with ALA was at 2 μM in minimal medium and 150 μM in rich medium (14); amino acid supplementation was as described previously (10). Hematin was prepared from hemin (Sigma) as described previously (28) and was used at a final concentration of 20 μg/ml. Antibiotics were added to final concentrations in rich or minimal medium, respectively, as follows: chloramphenicol, 20 or 5 μg/ml; kanamycin sulfate, 50 or 100 μg/ml; and tetracycline hydrochloride, 20 or 10 μg/ml. All cultures were grown at 37°C.

Genetic techniques. The high-frequency generalized transducing bacteriophage P22 mutant HT*105/1 int-201* (35) was used for transduction. Phage P22 lysates were prepared as described previously (10).

Transposon nomenclature. Way et al. have constructed several transposition-defective transposons derived from Tn*10* (38). Their defective Tet^r transposon is referred to here as Tn*10d*-Tet. The construction and use of Tn*10d*-Cam have been described previously (13).

Castilho et al. (8) have constructed several phage Mu-derived transposons that can form *lac* operon fusions and are defective for transposition. One of these, MudI1734 (Kan^r *lac*) (8), is used extensively in the present work. For convenience of description, I refer to this Kan^r Mud as Mud-J. Mud-J is missing the Mu A and B genes and lacks Mu transposase. Mud-A refers to a conditionally transposition-

TABLE 1. Bacterial strains

Strain	Genotype[a]	Reference or source
AK3109	*zhh-3109*::Tn*10d*-Tet (for rest of genotype, see reference 25)	34
CH748	Δ(*oppBC*)*250* Δ*leu-3051* Δ*tppB93*	1
CH725	Δ(*oppBC*)*250 tppB16*::Tn*10 dpp-101*::Tn*5*	K. Sanderson (1)
CH726	Δ(*oppBC*)*250 tppB16*::Tn*10 dpp-102*::Tn*5*	K. Sanderson (1)
MS1748	*pyrD121* Δ(*putPA*)*521*	S. Maloy
TR1457	*hisO1242 hisD3749 sufA5*	J. Roth (32)
TR2279	*proAB47 pyrB64 recA1 strA1*	J. Roth
TT9642	*cysG1573*::Mud-A (b)	J. Roth
TT10199	*nadA56*/F'152-2 *nad*$^+$	J. Roth
TT10288	*hisD9953*::Mud-J (b) *hisA9944*::Mud-1 (w)	K. Hughes (22)
TE124-1	*hemL331*::Mud-A (b)	14
TE299-1	Δ*hemL376*	14
TE555	*zhh-1855*::Tn*10d*-Tet	Laboratory collection
TE600	*srl-203*::Tn*10d*-Cam *recA1*	Laboratory collection
TE1059	*hisG10082*::Tn*10d*-Cam	Laboratory collection
TE1295	*hemA60 env-53*	14, 39
TE1630	*dpp-1*::Mud-J (b) *hemA60 env-53*	39
TE1633	*dpp-2*::Mud-J (w) *hemA60 env-53*	39
TE1664	*dpp-7*::Mud-J (b) *hemA60 env-53*	39
TE2730	*putA1309*::Mud-Cam	This study
TE3162	*dpp-1*::Mud-J (b)	P22.TE1630 × LT-2
TE3163	*dpp-2*::Mud-J (w)	P22.TE1633 × LT-2
TE3164	*zhh-6814*::Tn*10d*-Tet	This study
TE3273	*xyl-553*::Tn*10d*-Cam	Laboratory collection
TE3461	*hisD9953*::Mud-Cam *hisA9944*::Mud-1	This study
TE4257	*xyl-551*::Mud-A (b)	This study
TE4259	*xyl-552*::Mud-A (w)	This study
TE4261	*dpp-1*::Mud-A (b)	This study
TE4266	*zhh-3109*::Tn*10d*-Tet	P22.AK3109 × LT-2
TE4295	*dpp-7*::Mud-J (b)	P22.TE1664 × LT-2
TE4712	*hisO1242 hisD3749 sufA5 zhh-1855*::Tn*10d*-Tet	P22.TE555 × TR1457
TE4757	Δ*hemL376 zhh-1855*::Tn*10d*-Tet *dpp-21* (increased *dpp* expression)	This study
TE4758	Δ*hemL376 zhh-1855*::Tn*10d*-Tet *dpp*$^+$	This study
TE4782	Δ(*oppBC*)*250* Δ*leu-3051* Δ*tppB93 hisG10082*::Tn*10d*-Cam	P22.TE1059 × CH748
TE4811	*nadA56 zhh-3109*::Tn*10d*-Tet *xyl-553*::Tn*10d*-Cam/F'152-2 *nad*$^+$	This study
TE4821	*proAB47 pyrB64 recA1 strA1*/F'601 *dpp*$^+$ (Tet^r Cam^r)	This study
TE5067	*dpp-1*::Mud-J (b) *recA1*/F'601 *dpp*$^+$ (Tet^r Cam^r)	This study
TE5068	Δ*hemL376 dpp-1*::Mud-J (b) *recA1 srl-203*::Tn*10d*-Cam/F'601 *dpp*$^+$ (Tet^r Cam^r)	This study

[a] b (blue) and w (white) indicate color on plates containing X-Gal.

defective derivative of the phage Mud1, carrying Amp^r (7), which was constructed by Hughes and Roth (20).

Orienting Mud insertions. The chromosomal orientation of Mud insertions in the *dpp* (*alu*) and *xyl* genes was determined in P22 transductional crosses that scored recombination between a donor Mud-A (Amp^r) and a recipient Mud-J (Kan^r). Amp^r transductants were first screened for those which maintained function of the gene marked by the donor's Mud-A insertion.

For example, a P22 lysate grown on an *xyl*::Mud-A strain was used to transduce various *dpp*::Mud-J recipients selecting Amp^r on MacConkey xylose plates. Amp^r transductants that remained Xyl$^+$ were examined further. Such transductants may arise by recombination of the two Muds to generate a tandem duplication of the interval between them, with Mud-A at the join point (9a). This class is found only if the two parental Muds have the same orientation in the chromosome. Three other events can also generate Amp^r Xyl$^+$ recombinants regardless of the relative orientation of the two Muds: (i) transposition of Mud-A to a new site; (ii) a double crossover using both ends of Mud to substitute the donor Amp^r element with loss of Kan^r, simply converting the recipient Mud-J to Mud-A (22); and (iii) a spontaneous duplication that gives a heterozygote carrying both *xyl*$^+$ and *xyl*::Mud-A (4).

Transposition of Mud-A (class i) is extremely rare and does not interfere in these tests (20). Conversion events (class ii) are easily recognized because such transductants are Kan^s. The key to the method is to distinguish spontaneous duplications (class iii) from Mud-generated ones. Spontaneous duplications are relatively common in this region of the chromosome, as shown by crosses with the same donor *xyl*::Mud-A into the wild-type strain LT-2 as the recipient, in which about 5% of Amp^r transductants were Xyl$^+$. Despite this high background, for all crosses in which the recipient carried Mud-J, the proportion of Amp^r transductants that were Xyl$^+$ was dramatically increased.

With the use of *xyl*::Mud-A as the donor into *dpp*::Mud-J, when the insertions were similarly oriented, more than 90% of all Amp^r transductants were Xyl$^+$ and nearly all of these were Kan^r. That is, recombination between Muds to generate a duplication was much more common than simple inheritance of *xyl*::Mud-A. When the insertions were oppositely oriented, substitution was about as common as simple inheritance; about 50% of Amp^r transductants were Xyl$^+$ and these Xyl$^+$ transductants were nearly all Kan^s. To explain these unusual frequencies, I postulate that the events giving Mud-generated duplications and substitutions may occur frequently with a single transducing fragment. In contrast, the inheritance of *xyl*::Mud-A requires two frag-

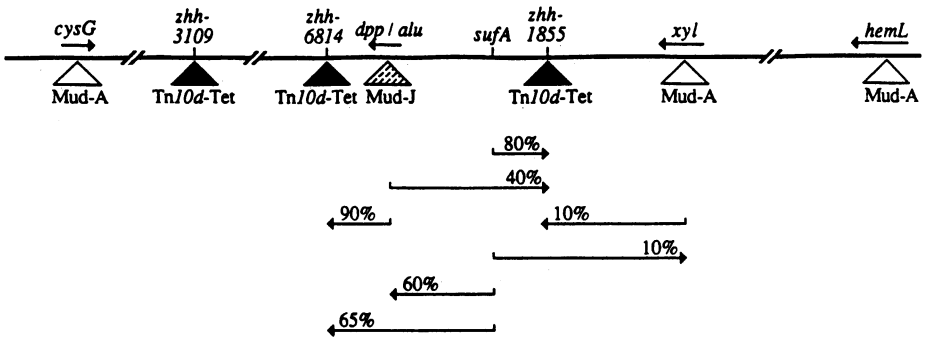

FIG. 1. Genetic linkage map of the *dpp* (*alu*) region. P22 cotransduction frequencies are shown as percentages. The arrowheads point to the marker selected in each cross. Data for the linkage of *sufA* to *xyl* are from reference 32.

ments, because of the large size of Mud relative to the length of P22 transducing fragments (see references 19 and 21 for discussion). Another, possibly related, feature of these crosses is that the number of Mud-generated duplications was much higher when the *lac* genes (7 kb of homology) were used for the recombination event instead of the Mu *c* gene (1 kb of homology).

To confirm the nature of strains identified as carrying Mud-generated duplications, some presumptive duplications were transduced into LT-2. In these crosses, the Amp^r transductants were all Xyl$^+$, as expected, eliminating the possibility of class iii events. Other tests with the back-crossed duplication strains confirmed the instability predicted for Mud-held duplications.

From a series of experiments with *hemL* and *cysG* insertions of known orientation, we conclude that the orientation of *dpp* (*alu*) is the same as that of *xyl* and is counterclockwise on the genetic map. Insertions of Mud-J in *dpp* that express β-galactosidase are all oriented in the same direction, and those which do not express β-galactosidase are all oriented the opposite way. The relative orientations of *dpp*::Mud-J insertions with respect to each other were also confirmed by physical mapping by using the polymerase chain reaction (PCR).

Construction and use of Mud-Cam. Mud-Cam is a small derivative of phage Mu which contains a 1.35-kb *Bam*HI fragment carrying Cam^r, derived from pCJ89 (this Cam^r gene contains the *Eco*RI site [24]). The Cam^r element is inserted between a segment of Mu *attL* which extends from the left end for 1,001 bp up to the *Hin*dIII site (36) and a segment of about 500 bp from Mu *attR*. The Mu DNA was derived from pPH070 (40) after conversion of the *Hin*dIII site which separates *attL* and *attR* to a *Bam*HI site.

Strain TE2730 carries Mud-Cam as an insertion in the *S. typhimurium putA* gene and can be used as a donor for Mud conversions (as discussed above) by using a recipient derived from MS1748 [*pyrD121* Δ(*putPA*)*521*]. Strain TE3461 carries a *hisD9953*::Mud-Cam insertion as well as *hisA9941*::Mud1 and can be used as a donor for transposition by *cis* complementation in the same way as described previously for Mud-J (22).

Construction of an F′ plasmid carrying *dpp*. A composite transposon was used to move the *dpp* region onto the *Escherichia coli* F′ plasmid, F′152-2 *nad*$^+$, by transposition (similar to the method described in reference 33). I constructed a derivative of strain TT10199 (which carries F′152-2) that also carries both *zhh-3109*::Tn*10d*-Tet and *xyl-553*::Tn*10d*-Cam (strain TE4811; Table 1 and Fig. 1). These two Tn*10d* insertions flank the *dpp* locus. A plasmid encoding Tn*10* transposase, pNK972 (38), was introduced by electroporation and, selecting Amp^r, and the pooled transformants were mated with strain TR2279 (*recA* Str^r), selecting Tet^r, Cam^r, and Str^r. Exconjugants were then tested for the presence of the *dpp*$^+$ operon on the F′ plasmid by mating with strain TE1630, selecting Tet^r and Kan^r and screening for complementation of the ALA permease defect. Strain TE4821 carries the F′601 plasmid with Tet^r, Cam^r and *dpp*$^+$ and was used to construct the strains described in Results. The site of insertion has not been determined, nor have the other genes originally present on the F′ plasmid been verified.

ALA uptake assay. Strains were grown overnight in NCE medium with 0.2% glycerol as the carbon source and containing either 20 μg of heme per ml plus 150 μM cystine (for *hemA* strains) or 2 μM ALA (for *hemL* strains). Cultures were diluted 1:50 in the same medium and grown to mid-log phase (A_{600} = 0.5). Cells were harvested by centrifugation in the cold and washed twice in ice-cold NCE salt solution before being resuspended in one-half volume of NCE medium with 0.2% glucose and kept on ice. Cells (75 μl) were preincubated for 10 min at 23°C. The assay was started by the addition of 75 μl of NCE plus 0.2% glucose containing [^{3}H]ALA (2 μCi/nmol; New England Nuclear) to give a final concentration of 10 μM ALA (1.5 nmol of ALA). A 25-μl sample was removed at each time point, and cells were harvested by filtration through a Millipore filter (0.45-μm pore size). Filters were washed three times with 5 ml of NCE salt solution and then dried before determination of radioactivity.

Genetic test for dipeptide utilization. Cultures of strains to be tested for dipeptide utilization were grown overnight in LB medium, harvested by centrifugation, and resuspended in an equal volume of 50 mM $NaPO_4$ (pH 6.8)–0.85% NaCl. Then, 0.1 ml was plated in minimal NCE top agar on an NCE glucose plate which had previously been spread with other required supplements. Compounds to be tested for stimulation of growth were applied to the agar near the edge of the plate, and the plates were observed for growth after 24 and 48 h. Amino acids and dipeptides were obtained from Sigma Chemical Co.

PCR mapping and cloning fragments from insertion mutants. Primers specific for the ends of transposons were used to amplify DNA from strains carrying two insertion mutations. In principle, a mixture of DNAs prepared from each of two single insertion mutants might be used for one PCR in which hybrid molecules carrying both insertions would arise

by "jumping" of incomplete chains between templates followed by amplification (for example, see reference 31). In practice, we constructed double mutants containing both insertions and prepared DNA from them.

DNA was prepared from a 2-ml overnight culture of each strain as described previously (15) and PCR amplified. PCR mixtures contained 10 mM Tris HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100, 200 μM each deoxynucleoside triphosphate, 0.5 μM each primer, 2.5 U of *Taq* DNA polymerase (Promega), and DNA equivalent to 20 μl of overnight culture. The conditions were 4 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C, and finally 8 min at 72°C. The primers were specific for *dpp* (*Hin*dIII site) (CCCAA GCTTG GTT TG AGCCT GGTGG CCAT), Mu *attL* (CCAAG CTTCG T ACTT CAAGT GAATC), Mu *attR* (CCGAA TTCGC ATT TA TCGTG AAACG CTTTC [2]), Tn*10* (GCGGA TCCGA CAAGA TGTGT ATCCA CCTTA AC), and Tn*5* (CTGGA AAACG GGAAA GGTTC CG [26]). Underlined nucleotides are not homologous to the targets. PCR products were examined after electrophoresis on 1% agarose gels by standard techniques (27).

RESULTS

Isolation of Mud-J insertions that block ALA uptake. We have previously described a collection of mutants defective in heme biosynthesis obtained after Mud-J insertion mutagenesis of *S. typhimurium* TE1295 (*hemA60 env-53* [39]). The *hemA* gene encodes glutamyl-tRNA reductase, the enzyme that catalyzes the first committed step of the heme pathway (23). Strain TE1295 cannot make heme, and thus it requires supplementation with heme or with the early intermediate ALA in order to grow on nonfermentable carbon sources. Starting with this strain, insertion mutations that eliminate growth on ALA but still allow growth on heme were found, and many of these insertions define genes of the heme pathway that act after ALA.

However, about half of the mutants in this collection were unusual: the insertions could be transduced into wild-type strain LT-2, in which they had no apparent Hem phenotype. Similarly, the mutants regained a Hem^+ phenotype after receiving a $hemA^+$ allele by transduction even though they retained the original Mud-J insertion, marked by Kan^r. These findings indicated that this class of mutants was not defective in heme synthesis but might be defective in a step required for ALA uptake, probably transport itself. Consistent with this, the mutants all grew on media containing elevated concentrations of ALA (150 μM). Since ALA does not serve as a sole carbon or nitrogen source for *S. typhimurium*, there was no simple genetic test for transport (16). The locus defective in these putative transport mutants was named *alu*, for ALA uptake (39).

Genetic mapping of *alu*. We isolated a set of Tn*10d*-Tet insertions linked to the Mud-J insertion in strain TE1630. One of these, *zhh-6814*::Tn*10d*-Tet, is more than 90% linked to this insertion by phage P22 transduction. (Physical mapping is shown below.) Transduction tests showed that *zhh-6814*::Tn*10d*-Tet is linked to all of the presumptive transport mutations. The map position of this Tn*10d*-Tet was determined by the Hfr method for conjugational mapping of Tn*10* insertions (9). The Tn*10d*-Tet and the linked *alu* mutations map near 77.5 min on the *S. typhimurium* genetic map (34). P22 cotransduction mapping was used to generate the genetic map of this region shown in Fig. 1.

***alu* mutants are defective in the dipeptide permease encoded by *dpp*.** A permease specific for dipeptides, encoded by *dpp*, has been characterized and recently mapped near 78 min in *S. typhimurium* and to a homologous location in *E. coli* (1, 30). This map position is similar to that for the mutants defective in ALA transport. The function of the *dpp* permease depends on a periplasmic binding protein encoded by *dppA*, which has nucleotide and deduced amino acid sequence homologies to the oligopeptide permease gene *oppA*, although the rest of the *dpp* operon sequence has not been determined. Comparison of the nearly identical structures of glycyl-glycine, a good substrate for the dipeptide permease, and ALA strongly indicated that Dpp and the ALA permease might be identical (Fig. 2). Experiments reported below confirm this suggestion. We have therefore renamed the *alu* mutants as alleles of *dpp*, and the mutants are listed accordingly in Table 1 and in the text below.

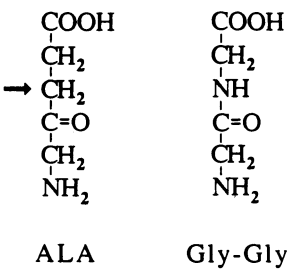

FIG. 2. Structures of ALA and glycyl-glycine (Gly-Gly). The arrow points to the position at which the two compounds differ.

Orientation of the *dpp* locus. The orientations of the *xyl* and *dpp* loci were determined by a modification of the method of Hughes and Roth (21) (see Materials and Methods for details). Mud insertions which lie at separate sites in the chromosome can recombine in transductional crosses. Viable recombinants can be recovered only for crosses in which the two Mud insertions lie in the same orientation in the chromosome; these recombinants carry tandem duplications of the region between the two parental insertions. If the orientation of the Mud is determined in this way, and one knows whether the *lacZ* gene is expressed, the direction of transcription of new genes and operons can be determined by simple genetic crosses by using reference insertions whose orientation is already known.

In these experiments, I used *cysG* (transcribed clockwise) and *hemL* (transcribed counterclockwise) to establish the orientations of *dpp* and *xyl*, which are both transcribed counterclockwise. The orientation of *xyl* shown here is consistent with that shown on the *Escherchia coli* genetic map (5). The map in Fig. 1 is also consistent with data of Riddle and Roth showing that the frameshift suppressor *sufA* lies on the *cysG* side of *xyl* (32). The relative positions of *sufA* and *zhh-1855*::Tn*10d*-Tet shown in Fig. 1 were established by a three-factor cross with *dpp-1*::Mud-J as the selected marker (data not shown).

***dpp* (*alu*) mutants have a defect in ALA transport.** We tested the ability of various strains to transport ALA by measuring the uptake of ^3H-labeled ALA. In Fig. 3A, ALA transport by strain TE1630 (*dpp-1*::Mud-J) is compared with that of the TE1295 parent (dpp^+). ALA transport is severely defective in the *dpp-1*::Mud-J mutant, nearly 10-fold lower than in the wild type. Similar results were obtained for a second *dpp* mutant (12).

I have also isolated a strain with increased transcription of *dpp* due to a closely linked, *cis*-acting mutation, *dpp-21* (12).

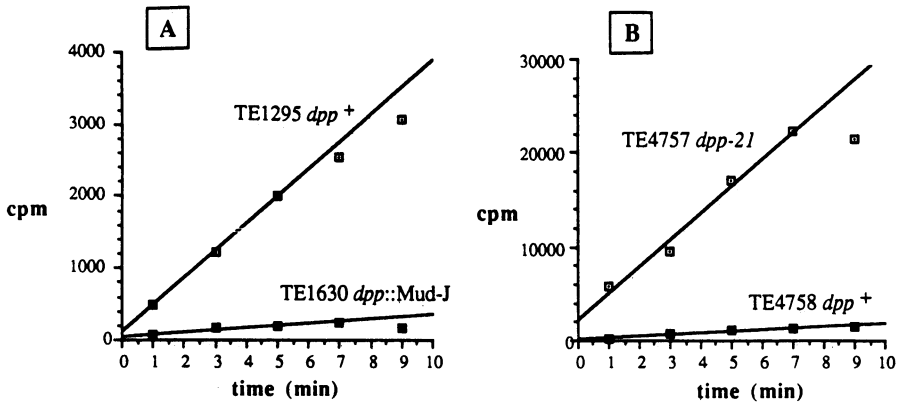

FIG. 3. (A) Uptake of [^{3}H]ALA in a *dpp*$^+$ strain (TE1295) and in the *dpp-1*::Mud-J mutant (TE1630). Normalized uptake values are 569 and 58.5 cpm/min/A_{600} unit for strains TE1295 and TE1630, respectively. (B) Uptake of [^{3}H]ALA in a *dpp-21* strain that overexpresses the dipeptide permease (TE4757) and in a *dpp*$^+$ strain (TE4758). Normalized uptake values are 5,130 and 245 cpm/min/A_{600} unit for strains TE4757 and TE4758, respectively. Note the change of scale for the ordinate axes. Slopes were plotted by using the first three datum points (A) or four datum points (B).

The increased expression is reflected in the activity of β-galactosidase seen in a strain carrying both *dpp-21* and the *dpp-1*::Mud-J insertion. The expression of β-galactosidase in the *dpp-21* mutant is increased about 7.5-fold over that in the wild type during growth in minimal glycerol medium (12). By using a nearby Tn*10d*-Tet insertion, I constructed a strain in which *dpp-21* is linked to a functional *dpp* operon. In Fig. 3B, ALA transport by strain TE4757, which carries *dpp-21* and expresses *dpp* at high levels, is compared with that of the otherwise isogenic *dpp*$^+$ strain TE4758. Increased transcription of *dpp* is reflected in a 21-fold increase in ALA transport measured by the uptake assay. This result strongly suggests that the locus affected in *dpp* mutants functions directly in transport.

Transport of [^{3}H]ALA (10 μM) by strains TE4757 and 4758 was strongly reduced in the presence of the dipeptide L-leucyl-glycine at 10 μM, and ALA transport was eliminated at 50 or 200 μM L-leucyl-glycine (data not shown). This competition for uptake is consistent with the idea that ALA and L-leucyl-glycine are transported by the same permease.

Nutritional tests of ALA and dipeptide transport. I compared known *dpp*::Tn*5* insertions (1) with my Mud-J insertions for their effects on ALA and dipeptide transport, as assayed by nutritional tests. ALA uptake was tested in the TE1295 background (*hemA60 env-53*). Here, *dpp*::Tn*5* insertions resulted in a loss of the ability to grow on minimal glycerol medium supplemented with 2 μM ALA.

Genetic tests of dipeptide transport require a multiply mutant background defective in two other permeases encoded by *opp* and *tpp* which are also active on dipeptides. Strain CH748 [Δ(*oppBC*)*250* Δ*leu-3051* Δ*tppB93*] was obtained from C. Higgins for these experiments. Strain TE4782 [Δ(*oppBC*)*250* Δ*leu-3051* Δ*tppB93 hisG10082*::Tn*10d*-Cam], derived from CH748, was used as the background in which various insertions were compared for their effects on dipeptide transport. I tested the ability of strains to use L-leucyl-glycine and L-leucyl-L-valine to satisfy their leucine requirement and glycyl-L-histidine to satisfy their histidine requirement. The Mud-J insertion mutants, isolated by their effect on ALA transport, were defective in transport of L-leucyl-glycine and L-leucyl-L-valine, similar to authentic *dpp*::Tn*5* mutants. None of the mutants was apparently defective in glycyl-L-histidine transport, in contrast to previous results for *dpp* (1). However, this unexpected result could be explained if free histidine was present in the dipeptide preparation I used.

In P22 transductional crosses, the *dpp*::Tn*5* insertions were both more than 90% linked to the *dpp-1*::Mud-A insertion carried by strain TE4261. (TE4261 was isolated during the orientation crosses described above; in this strain, the Kan^r *dpp-1*::Mud-J insertion has been converted to an Amp^r Mud-A form.) In the crosses of *dpp-101*::Tn*5* × *dpp-1*::Mud-A, the rare class of transductants carrying both insertions, identified by their Amp^r Kan^r phenotype, included many that were light blue on plates containing minimal glycerol plus 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), suggesting that the *dpp*::Tn*5* insertions are polar on the Mud. This light blue color was not changed by the introduction of an F′ plasmid carrying *dpp*$^+$.

Expression of *dpp* (*alu*) is not responsive to ALA levels. I tested whether the expression of *dpp* is responsive to ALA levels by measuring the activity of β-galactosidase in strains carrying a *dpp-lac* operon fusion. Since intracellular effects of ALA might require uptake by the ALA permease, these experiments utilized diploids in which a functional copy of *dpp* was provided by an F′ plasmid. (This plasmid carries Tet^r and Cam^r as selective markers; its construction is described in Materials and Methods.) In one experiment, strain TE5067 (*dpp-1*::Mud-J *recA*/F′ *dpp*$^+$) was grown in minimal glycerol medium in the presence of 2 μM ALA or without ALA. The activity of β-galactosidase was the same in the two cultures (data not shown). In a second experiment, strain TE5068 (Δ*hemL dpp-1*::Mud-J *recA*/F′ *dpp*$^+$) was starved for ALA. The culture grown without ALA showed a twofold increase in β-galactosidase activity, but this small induction probably results from the much lower growth rate of the starving culture (see Discussion). I conclude that neither the presence of ALA in the medium nor starvation for ALA substantially changes the expression of *dpp*.

Physical map of the *dpp* (*alu*) region. I confirmed the relationship between *S. typhimurium dppA* and the previously sequenced *E. coli dppA* gene by using DNA sequence analysis of a cloned fragment of chromosomal DNA derived from the *S. typhimurium dpp-7*::Mud-J insertion. For this

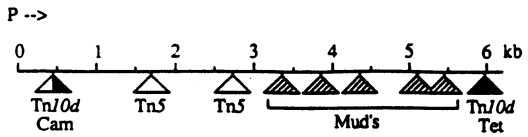

FIG. 4. Physical map of the *S. typhimurium dpp* operon. Transcription is from left to right. PCR was used to determine the approximate positions of various transposon insertions. Numbering is based on the sequence of the closely related *S. typhimurium opp* operon (18); in this scheme, the *dppA* AUG lies at bp 150. The *zhh-6814*::Tn*10d*-Tet at bp 5950 lies just downstream of the *dpp* operon. The *dpp-1*::Mud-J at bp 3850 is in the *dppD* gene, the sequence directly downstream of this insertion is likely to encode an A box for ATP binding (see text).

purpose, the Mud-J was first converted into Mud-Cam. Mud-Cam is a smaller element without the *lac* genes that carries the ends of phage Mu and a Cam^r fragment for selection (see Materials and Methods for details). The Mud-Cam insertion was cloned as a *Hin*dIII fragment into pBR322, and restriction enzyme digests showed that the cloned segment extends approximately 5.5 kb upstream of the *alu-7* insertion. DNA sequencing used a primer homologous to pBR322 to sequence into the cloned segment at the upstream *Hin*dIII side. The sequence (12) is nearly identical to that reported for the region near the *Hin*dIII site at the beginning of the *E. coli dppA* gene (1, 30). A physical map of insertion mutations lying in the *dpp* (*alu*) region was constructed on the basis of the sizes of fragments amplified in PCR with primers specific for the ends of Tn*10*, Tn*5*, and Mu as well as a *dpp*-specific primer (Fig. 4).

It was previously reported that the sequence of *dppA* is homologous to that of *oppA*, the first gene of the *opp* operon which encodes the oligopeptide permease, another binding protein-dependent peptide permease (1, 30). The DNA sequence that I obtained directly downstream of the *dpp-1*::Mud-J insertion (12) can be translated to give an amino acid sequence highly similar to a segment of *oppD* including the region of the conserved A box sequence of ATP-binding proteins (3, 18). The data are consistent with the idea that the *dpp* and *opp* operons are very closely related.

DISCUSSION

In this work, insertion mutations that block ALA transport in *S. typhimurium* have been characterized. Determination of ALA uptake in the mutants confirmed that transport was defective, and overexpression of a functional copy of the locus resulted in a much higher rate of transport. All of the mutations affecting ALA transport map together very near the *dpp* operon which encodes the dipeptide permease (1, 30), and the structural similarity of ALA and glycylglycine strongly suggested that the two transport functions might be the same. The correspondence of the ALA and dipeptide permeases was confirmed by a variety of genetic means and ultimately by physical mapping of insertion mutations and DNA sequencing. These results explain why the concentration of ALA required for effective supplementation of ALA auxotrophs is so much higher in rich than in minimal medium, since dipeptides present in rich medium compete with ALA for transport.

Previous work has shown that expression of the *dpp* operon apparently is not regulated by the availability of peptide substrates, nor is it affected by anaerobiosis, the carbon source, or the nitrogen source (1). The expression of *dppA* in *E. coli* is decreased somewhat by growth with Casamino Acids (30); I have observed a similar effect on the β-galactosidase activity produced by a *dpp-lac* fusion in *S. typhimurium* when comparing NB and minimal glycerol medium (12). I have also tested the effect of ALA on *dpp-lac* expression, in a *dpp*$^+$ background, and find that the presence of ALA does not affect *dpp* expression. A small induction is seen after starvation for ALA, but this effect and the effect of growth medium noted above may reflect a negative correlation between growth rate and *dpp* expression. Since there does not seem to be a connection between ALA as a substrate and regulation of *dpp*, I view the dipeptide permease as simply having a relaxed specificity that allows transport of an important nonpeptide nutrient.

Two genes are required for synthesis of ALA in *S. typhimurium* and *E. coli*, *hemA* and *hemL*. The *hemA* gene encodes glutamyl-tRNA reductase, while *hemL* encodes glutamate-1-semialdehyde (GSA) aminotransferase (for a review, see reference 23). Deletion and insertion mutations of *hemL* are quite leaky, with growth that depends critically on cell density (14). For example, on minimal glycerol agar, growth of large single colonies can be seen close to the mass of cells on a streak plate, yet when cultures are diluted and plated on the same medium, single colonies do not appear. Residual growth of *hemL* mutants is eliminated by mutations in *dpp* that block ALA transport (12). The leakiness of *hemL* compared with *hemA* mutants indicates a bypass of the GSA aminotransferase reaction (whether enzymatic or spontaneous), but, in addition, the growth of *hemL* mutants is promoted by the ability to scavenge ALA from the medium. We suggest that ALA is continually lost from cells and subsequently recovered. Without bona fide deletion mutants, I cannot completely rule out a role for the dipeptide permease in efflux, but I think it unlikely.

Another property of *hemL* but not *hemA* mutants is the ability to form small colonies on rich media (e.g., NB agar [14]). This growth may be explained by the ability of certain amino acids such as arginine to substitute for ALA in allowing growth of *hemL* mutants (12). The mechanism of this supplementation is not understood. The growth of *hemL* mutants on NB agar is not affected by mutations in the dipeptide permease, confirming that transport of ALA is not involved.

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REFERENCES

1. **Abouhamad, W. N., M. Manson, M. M. Gibson, and C. F. Higgins.** 1991. Peptide transport and chemotaxis in *Escherichia coli* and *Salmonella typhimurium*: characterization of the dipeptide permease (Dpp) and the dipeptide-binding protein. Mol. Microbiol. **5**:1035–1047.
2. **Adachi, T., M. Mizuuchi, E. A. Robinson, E. Appella, M. H. O'Dea, M. Gellert, and K. Mizuuchi.** 1987. DNA sequence of the *E. coli gyrB* gene: application of a new sequencing strategy. Nucleic Acids Res. **15**:771–784.
3. **Ames, G. F. L.** 1986. Bacterial periplasmic transport systems: structure, mechanism and evolution. Annu. Rev. Biochem. **55**:397–425.
4. **Anderson, P., and J. Roth.** 1981. Spontaneous tandem genetic duplications in *Salmonella typhimurium* arise by unequal re-

combination between rRNA (*rrn*) cistrons. Proc. Natl. Acad. Sci. USA **78**:3113–3117.
5. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54**:130–197.
6. **Berkowitz, D., J. M. Hushon, H. J. Whitfield, Jr., J. Roth, and B. N. Ames.** 1968. Procedure for identifying nonsense mutations. J. Bacteriol. **96**:215–220.
7. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *in vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA **76**:4530–4533.
8. **Castilho, B. A., P. Olfson, and M. J. Casadaban.** 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. **158**:488–495.
9. **Chumley, F. G., R. Menzel, and J. R. Roth.** 1979. Hfr formation directed by Tn*10*. Genetics **91**:639–655.
9a. **Chumley, F. G., and J. R. Roth.** 1980. Rearrangement of the bacterial chromosome using Tn*10* as a region of homology. Genetics **94**:1–14.
10. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman.** 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61–76.
12. **Elliott, T.** Unpublished observations.
13. **Elliott, T., and J. R. Roth.** 1988. Characterization of Tn*10d*-Cam: a transposition-defective Tn*10* specifying chloramphenicol resistance. Mol. Gen. Genet. **213**:332–338.
14. **Elliott, T., and J. R. Roth.** 1989. Heme-deficient mutants of *Salmonella typhimurium*: two genes required for ALA synthesis. Mol. Gen. Genet. **216**:303–314.
15. **Gay, N. J.** 1984. Construction and characterization of an *Escherichia coli* strain with a *uncI* mutation. J. Bacteriol. **158**:820–825.
16. **Gutnick, D., J. M. Calvo, T. Klopotowski, and B. N. Ames.** 1969. Compounds which serve as the sole source of carbon or nitrogen for *Salmonella typhimurium* LT-2. J. Bacteriol. **100**: 215–219.
17. **Higgins, C. F., and M. M. Gibson.** 1986. Peptide transport in bacteria. Methods Enzymol. **125**:365–377.
18. **Hiles, I. D., M. P. Gallagher, D. J. Jamieson, and C. F. Higgins.** 1987. Molecular characterization of the oligopeptide permease of Salmonella typhimurium. J. Mol. Biol. **195**:125–142.
19. **Hughes, K. T., B. M. Olivera, and J. R. Roth.** 1987. Rec dependence of Mu transposition from P22-transduced fragments. J. Bacteriol. **169**:403–409.
20. **Hughes, K. T., and J. R. Roth.** 1984. Conditionally transposition-defective derivative of Mu d1(Amp Lac). J. Bacteriol. **159**:130–137.
21. **Hughes, K. T., and J. R. Roth.** 1985. Direct formation of deletions and duplications using Mu*d* (Ap, *lac*). Genetics **109**: 263–282.
22. **Hughes, K. T., and J. R. Roth.** 1988. Transitory *cis* complementation: a method for providing transposition functions to defective transposons. Genetics **119**:9–12.
23. **Jahn, D., E. Verkamp, and D. Soll.** 1992. Glutamyl-transfer RNA: a precursor of heme and chlorophyll biosynthesis. Trends Biochem. Sci. **17**:215–218.
24. **Joyce, C. M., and N. D. F. Grindley.** 1984. Method for determining whether a gene of *Escherichia coli* is essential: application to the *polA* gene. J. Bacteriol. **158**:636–643.
25. **Kukral, A. M., K. L. Strauch, R. A. Maurer, and C. G. Miller.** 1987. Genetic analysis in *Salmonella typhimurium* with a small collection of randomly spaced insertions of transposon Tn*10*Δ16Δ17. J. Bacteriol. **169**:1787–1793.
26. **Lupski, J. R., Y. H. Zhang, M. Rieger, M. Minter, B. Hsu, B. G. Ooi, T. Koeuth, and E. R. B. McCabe.** 1990. Mutational analysis of the *Escherichia coli glpFK* region with Tn*5* mutagenesis and the polymerase chain reaction. J. Bacteriol. **172**:6129–6134.
27. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **McConville, M. L., and H. P. Charles.** 1979. Mutants of *Escherichia coli* K-12 permeable to haemin. J. Gen. Microbiol. **113**:165–168.
29. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Olson, E. R., D. S. Dunyak, L. M. Jurss, and R. A. Poorman.** 1991. Identification and characterization of *dppA*, an *Escherichia coli* gene encoding a periplasmic dipeptide transport protein. J. Bacteriol. **173**:234–244.
31. **Paabo, S., D. M. Irwin, and A. C. Wilson.** 1990. DNA damage promotes jumping between templates during enzymatic amplification. J. Biol. Chem. **265**:4718–4721.
32. **Riddle, D. L., and J. R. Roth.** 1972. Frameshift suppressors. II. Genetic mapping and dominance studies. J. Mol. Biol. **66**:483–493.
33. **Roof, D. M., and J. R. Roth.** 1989. Functions required for vitamin B_{12}-dependent ethanolamine utilization in *Salmonella typhimurium*. J. Bacteriol. **171**:3316–3323.
34. **Sanderson, K. E., and J. R. Roth.** 1988. Linkage map of *Salmonella typhimurium*, edition VII. Microbiol. Rev. **52**:485–532.
35. **Schmieger, H.** 1972. Phage P22 mutants with increased or decreased transduction abilities. Mol. Gen. Genet. **119**:75–88.
36. **Symonds, N., A. Toussaint, P. Van de Putte, and M. M. Howe.** 1987. Phage Mu. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
37. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
38. **Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner.** 1984. New Tn*10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. Gene **32**:369–379.
39. **Xu, K., J. Delling, and T. Elliott.** 1992. The genes required for heme synthesis in *Salmonella typhimurium* include those encoding alternative functions for aerobic and anaerobic coproporphyrinogen oxidation. J. Bacteriol. **174**:3953–3963.
40. **Youderian, P., P. Sugiono, K. L. Brewer, N. P. Higgins, and T. Elliott.** 1988. Packaging specific segments of the *Salmonella* chromosome with locked-in Mu*d*-P22 prophages. Genetics **118**: 581–592.