JOURNAL OF BACTERIOLOGY, Aug. 1991, p. 5168–5172
0021-9193/91/165168-05$02.00/0
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Vol. 173, No. 16

Identification and Initial Characterization of the *eutF* Locus of *Salmonella typhimurium*

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Received 1 May 1991/Accepted 14 June 1991

We report the isolation and initial characterization of mutations in the newly described *eutF* locus of *Salmonella typhimurium* LT2. Mutations in *eutF* render a strain unable to utilize ethanolamine as a source of carbon and/or energy and impair growth on ethanolamine as a sole nitrogen source. Strains carrying *eutF* mutations exhibit a 2-order-of-magnitude decrease in transcription of the unlinked *eutDEABCR* operon (50 min), which codes for the enzymes needed to catabolize ethanolamine; have only 10% of the ethanolamine ammonia-lyase activity found in the wild type; and show a marked reduction in the rate of ethanolamine uptake. Deletion mapping and three-factor cross analysis results are consistent with the gene order *cobA trp eutF tonB* at 34 min on the linkage map. We discuss two possible roles for the EutF protein: (i) as an ethanolamine permease or (ii) as a transcription factor required for the expression of the *eutDEABCR* operon.

The amino alcohol ethanolamine is a nonfermentable substrate that can serve as the sole carbon and/or nitrogen source for *Salmonella typhimurium* (6) and *Escherichia coli* (11). The catabolism of ethanolamine is dependent on the availability of adenosylcobalamin (Ado-B_{12}) (2, 5, 6, 11, 19), and because *S. typhimurium* synthesizes Ado-B_{12} de novo only under anaerobic growth conditions (10), cobalamin must be provided exogenously for aerobic growth on ethanolamine to occur (18).

The ethanolamine utilization (*eut*) locus of *S. typhimurium* constitutes an operon comprising six genes (*eutDEABCR*) located at 50 min on the chromosome map (18, 19). Both ethanolamine and Ado-B_{12} are required for the full expression of the *eutDEABCR* operon. This operon encodes all the functions necessary for the catabolism of ethanolamine, except for the function(s) necessary for the transport of ethanolamine into the cell. Roof and Roth (19) showed that any mutation in the *eutDEABCR* operon resulted in a marked decrease in ethanolamine transport. As a result of this work, it was suggested that maximal uptake of ethanolamine may depend on the active degradation of the substrate (19).

In this paper, we report the isolation and initial characterization of mutants unable to utilize ethanolamine. The mutations responsible for this defect map outside of the *eutDEABCR* operon and define a new locus, *eutF*. We discuss two possible roles for the *eutF* gene product in the utilization of ethanolamine: (i) as an ethanolamine permease or (ii) as a transcription factor required for the expression of the *eutDEABCR* operon.

MATERIALS AND METHODS

Bacteria, media, and growth conditions. The bacterial strains used in this study are derivatives of *S. typhimurium* LT2, and their genotypes are listed in Table 1. Nutrient broth (Difco, Detroit, Mich.) (0.8% [wt/vol]) supplemented with 85 mM NaCl was used as a rich medium. Minimal medium E of Vogel and Bonner (25) was supplemented with glucose (11 mM) as the carbon source. No-carbon medium E (NCE) (7) supplemented with ethanolamine was used to investigate growth on ethanolamine as the sole carbon source. No-carbon, no-nitrogen medium (NCN) (17) supplemented with glycerol and ethanolamine was used to investigate growth on ethanolamine as the sole nitrogen source. When present in the culture medium, and unless otherwise stated, the final concentrations of the following compounds were as follows: ethanolamine hydrochloride (Gold Label, ≥99% pure; Aldrich Chemical Co., Milwaukee, Wis.), 27 mM; $MgSO_4$, 1 mM; glycerol, 22 mM; methionine, 0.5 mM; tryptophan, 0.1 mM; cyanocobalamin (CN-B_{12}) or Ado-B_{12} (Sigma Chemical Co., St. Louis, Mo.), 15 nM; and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) (Bachem, Inc., Torrance, Calif.), 40 mg/liter. The final concentrations of antibiotics (micrograms per milliliter) in the rich medium were as follows: tetracycline, 20; ampicillin, 30. Those in the minimal medium were 10 and 15, respectively. For solid medium, Bacto-Agar (Difco) was added to a final concentration of 1.5% (wt/vol). Cultures were incubated at 37°C and shaken vigorously (400 rpm), and growth was monitored with a Spectronic 20D spectrophotometer at 650 nm. All experiments were done under aerobic conditions.

Genetic techniques. (i) Phage P22-mediated transduction. All transductions were performed with lysates of phage P22 (7) carrying the HT*105/1* and *int-201* mutations (22, 23). A multiplicity of infection of approximately 1 was used in all crosses.

(ii) Isolation of a deletion in the *eutF* locus. A deletion in the *eutF* locus was generated in vivo by the method of Bochner et al. (4) as modified by Maloy and Nunn (13). Fusaric acid was dissolved in *N*,*N*-dimethylformamide prior to its addition to the medium. The culture medium was prepared immediately before use, and the plate cultures were incubated at 42°C for 24 h. A Tn*10*DEL*16*DEL*17*Tc^r (Tn*10d*Tc [26]) element inserted between the *trp* and *tonB* loci (JE1121 [24a]) was used to generate deletion DEL*903* (JE1418) (Table 1).

(iii) Isolation of hydroxylamine-generated mutations. Hydroxylamine-generated mutations in *eutF* were isolated by localized mutagenesis (9) as previously described (7). A lysate of phage P22 grown on a strain carrying the selectable marker *trp-3484*::Tn*10d*Tc (JE1291) was chemically mutagenized with hydroxylamine and used as a donor to transduce strain JE1684 (*eutE18*::Mu d1-8) to tetracycline resistance. EutF

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TABLE 1. Strain list[a]

Strain	Genotype	Source
TR6583	LT2 *metE205 ara-9*	J. R. Roth
JE1121	*zdd-3703*::Tn*10*DEL*16*DEL*17*Tc^r	Laboratory collection
JE1418	DEL*903*	
JE1291	*trp-3484*::Tn*10*DEL*16*DEL*17*Tc^r	Laboratory collection
JE1293	*cobA366*::Tn*10*DEL*16*DEL*17*Tc^r	Laboratory collection
JE1372	*pyrF696*::Tn*10*	Laboratory collection
JE1445	DEL*902*	Laboratory collection
JE1684	*eutE18*::Mu dl-8	Roof and Roth (19)
JE1685	*eutE18*::Mu dl-8 derivative of JE1418	
JE1687	*eutE18*::Mu dl-8 *eutF1115* derivative of JE1291	
JE1690	*eutF1115* derivative of JE1291	
JE1692	*eutE18*::Mu dl-8 *eutF1113* derivative of JE1291	
JE1693	*eutF1113* derivative of JE1291	
JE1917	*trp43*	P. Margolin via J. Roth

[a] All strains used were derivatives of *S. typhimurium* LT2. Unless otherwise stated, strains were constructed during the course of this work.

mutants were identified by their X-Gal phenotype (white colonies) on NCE containing glycerol, $MgSO_4$, ethanolamine, CN-B_{12}, tryptophan, and X-Gal.

Enzyme assays. All spectrophotometric measurements were performed with a Perkin-Elmer Lambda 6 UV-VIS spectrophotometer. The Perkin-Elmer UV Data Manager program was used to acquire and analyze data with an Epson Equity II$^+$ computer interfaced with the spectrophotometer.

(i) β-Galactosidase activity assays. β-Galactosidase activity assays were performed by the method of Miller (14) as described elsewhere (7). Units of activity was defined in nanomoles of *o*-nitrophenol produced per minute. Units of β-galactosidase were normalized to cell density measured at 650 nm.

(ii) EAL activity assay. The level of ethanolamine ammonia-lyase (EAL) activity in cells was assayed by a modification of the method described by Sawicki et al. for the determination of aliphatic aldehydes (21). The assay is based on the reaction of aliphatic aldehydes with 3-methyl-2-benzothiazolinone hydrazone (MBTH), which results in a stable adduct with a strong A_{305} ($\varepsilon = 14.7 \times 10^3$ mol^{-1} cm^{-1}). Cultures to be assayed were grown to the mid-log phase in NCE supplemented with glycerol, $MgSO_4$, ethanolamine, tryptophan, and CN-B_{12} (150 nM). Cells were harvested by centrifugation (8,000 × *g* for 10 min), resuspended in cold potassium phosphate buffer (0.35 M, pH 8.0), and broken in a French pressure cell (SLM/Aminco, Urbana, Ill.) at 18,000 lb/in^2. The cell extracts were extensively dialyzed (dilution factor 10^{-5}) against potassium phosphate buffer (0.35 M, pH 8.0) at 4°C. The reaction mixture contained ethanolamine at 200 μmol, potassium phosphate buffer (pH 8.0) at 70 μmol, KCl at 1 mmol, Ado-B_{12} at 30 nmol, and 0.03 to 0.05 mg of protein from the cell extract in a final volume of 333 μl. The reaction was started by the addition of Ado-B_{12} and protected from light to minimize photolysis of Ado-B_{12}. The reaction mixture was incubated at 37°C for 10 min, the reaction was terminated by the addition of potassium citrate (200 μmol, pH 3.6) and MBTH (5 μmol), and the reaction mixture was incubated at 37°C for an additional 10 min. The reaction mixture was brought to a final volume of 1.2 ml with water, and the A_{305} was measured. One unit of EAL activity was defined as the amount of enzyme needed to produce 1 nmol of acetaldehyde per min, and activity was normalized to milligrams of protein.

(iii) Ethanolamine uptake assay. Uptake of radiolabeled ethanolamine was determined by the method of Myers and Malloy (15) with the modifications as described below. Strains to be tested were grown to the mid-log phase in NCE supplemented with $MgSO_4$, CN-B_{12}, glycerol, tryptophan, and ethanolamine. Cells were harvested (8,000 × *g* for 10 min), washed with cold NCE, resuspended in cold NCE supplemented with glycerol (1 mM) and chloramphenicol (50 μg/ml), and stored on ice. Cells were starved for ethanolamine immediately before the assay by being shaken at room temperature for 15 min. The reaction was started by the addition of 0.35 ml of cell suspension (A_{650}, 1.0 to 1.2) to an equal volume of a 2× reaction mixture (glycerol, 2 mM; chloramphenicol, 100 μg/ml; [1,2-^{14}C]ethanolamine, 6.84 μCi [Amersham Corp., Arlington Heights, Ill.; specific radioactivity, 100 mCi/mmol]; and ethanolamine, 20 μM; final volume, 12 ml in NCE [pH 7.0]), and the reaction mixture was mixed rapidly and incubated at room temperature. Samples (100 μl) were removed from the reaction mixture at designated intervals and added to 4 ml of stop buffer (morpholineethanesulfonic acid, 5 mM; Tris, 5 mM; KCl, 300 mM; $HgCl_2$, 2 mM [pH 7.0]). Cells were filtered quickly through 0.45-μm-pore-size nitrocellulose filters (Hoefer Scientific Instruments, San Francisco, Calif.) prewetted with stop buffer by use of a Hoefer filter manifold (model FH 224V), and the filters were rinsed with an additional 4 ml of stop buffer. Filters were dried under a 150-W lamp. Radioactivity retained by the filters was determined by counting for 1 min in a scintillation counter (model 4530) from Packard Instruments, Downers Grove, Ill.

RESULTS

Isolation of EutF mutants. (i) Deletions. A deletion in the *eutF* region (DEL*903*; JE1418) was generated in vivo by the method of Bochner et al. (4) with a Tn*10d*Tc element located between the *trp* and *tonB* loci at 34 min on the *S. typhimurium* linkage map. One of the strains isolated (JE1418; Table 1) was a tryptophan auxotroph and had a functional *cobA* locus (8) but could not use ethanolamine as a sole carbon source when either CN-B_{12} or Ado-B_{12} was provided exogenously. These results suggested that JE1418 (DEL*903*) was unable to utilize ethanolamine because of a deficiency in cobalamin transport caused by the deletion of the nearby *tonB* locus. This possibility was unlikely for two reasons. First, JE1418 could use CN-B_{12} or Ado-B_{12} for the synthesis of methionine at a concentration of 15 nM. A *tonB* mutant strain requires micromolar concentrations of cobalamin to overcome the transport defect (1). Second, JE1418 was sensitive to infection by bacteriophage ES18, which requires a functional *tonB* locus for absorption and subsequent infection of the cell (24). These results suggested that DEL*903* did not delete the *tonB* locus.

Genetic analysis confirmed that the *eutF* locus was missing in deletion DEL*903*. A *pyrF* mutant strain, JE1372, was transduced to *pyr*$^+$ with strain JE1418 (DEL*903*) as the donor. Pyr$^+$ transductants were screened for tryptophan auxotrophs; tryptophan auxotrophy indicated the inheritance of DEL*903*. All the transductants that became tryptophan auxotrophs could no longer utilize ethanolamine as a carbon source (28 of 28), indicating that DEL*903* spanned the *eutF* locus.

(ii) Hydroxylamine-generated mutations. Hydroxylamine-generated mutations in *eutF* were isolated by localized

TABLE 2. Growth of *eutF* mutants on ethanolamine as a carbon or nitrogen source

Strain	Relevant genotype	μ (doublings/h) when ethanolamine was added as a:	
		Carbon source[a]	Nitrogen source[b]
JE1418	DEL*903*	NG	0.10 (0.26)
JE1693	*eutF1113*	NG	0.25 (0.79)
JE1690	*eutF1115*	NG	0.13 (0.32)
TR6583	*eutF*$^+$	0.16	0.33 (0.85)

[a] NCE was supplemented with ethanolamine (27 mM), $MgSO_4$, CN-B_{12}, and tryptophan. NG, no growth.

[b] NCN was supplemented with glycerol, ethanolamine (27 mM), $MgSO_4$, CN-B_{12}, and tryptophan. Numbers in parentheses are the final A_{650} values of the cultures measured after 42 h of growth (all cultures had reached the stationary phase).

mutagenesis. To facilitate the isolation of *eutF* mutants, we took advantage of the lack of expression of a *lacZ* transcriptional fusion to the *eutDEABCR* operon in genetic backgrounds in which the *eutF* gene was defective (see below). Strain JE1684 carries an operon fusion of the *lacZ*$^+$ gene to the *eutDEABCR* promoter and displays an X-Gal$^+$ phenotype on indicator plates containing X-Gal, ethanolamine, and cobalamin. A hydroxylamine-mutagenized lysate of bacteriophage P22 grown on strain JE1291 (*trp-3484*::Tn*10d*Tc) was used as the donor in a transductional cross with strain JE1684 (*eutE18*::Mu d1-8) as the recipient (hereafter referred to as *eutE-lacZ*). We isolated tetracycline-resistant transductants and screened them for mutations which resulted in a lower expression of the *eutE-lacZ* fusion. This protocol effectively eliminated mutations in the *eutDEABCR* operon which would result in the same X-Gal phenotype. Ten independent mutations, 19% cotransducible with the tetracycline resistance marker in *trp*, were isolated. Two of the mutants, JE1693 (*eutF1113*) and JE1690 (*eutF1115*), were selected for further analysis.

EutF phenotype. Table 2 shows the growth phenotypes of *eutF* mutants. These strains did not use ethanolamine as a sole carbon source and retained only a limited ability to utilize ethanolamine as a sole nitrogen source. Growth studies showed that *eutF*$^+$ strains can use ethanolamine as a nitrogen source at concentrations as low as 0.27 mM; all *eutF* mutants tested failed to grow at this concentration of ethanolamine (data not shown). When the concentration of ethanolamine was raised to 27 mM, all mutants grew, and the specific growth rate (doublings per hour [μ]) for cultures grown on ethanolamine as the sole nitrogen source ranged from 0.33 in the wild type to 0.10 in the deletion mutant (JE1418). Cultures of *eutF* mutants did not reach cell densities as high as did the wild type (Table 2). One of the mutants (JE1693 [*eutF1113*]) was unable to use ethanolamine as a sole carbon source but used ethanolamine as a sole nitrogen source at a rate (μ = 0.25) that was only slightly slower than that of the wild-type strain. The behavior of this mutant may have been the result of a mutation which only partially inhibits *eutF* function.

We also investigated the ability of *eutF* mutant strains to grow on acetate as the sole carbon and energy source, since acetate is a final product of ethanolamine catabolism. Growth studies demonstrated that *eutF* mutant strains can utilize acetate as the sole source of carbon and energy at growth rates identical to that of the wild type. These results suggested that *eutF* mutations do not affect the utilization of acetate.

Deletion mapping of the *eutF* locus. Three different classes of deletions were used to identify the position of the *eutF* locus relative to nearby ordered markers at 34 min, *cobA*, *trp*, and *tonB* (20). A mutant (JE1445) with a deletion spanning the *trp* and *cobA* loci did not display a EutF$^-$ phenotype, suggesting that *eutF* does not lie between these two markers. A mutant with deletion *trp43*, a partial deletion of the *trp* operon extending out of *trp* and toward *tonB* (3), did not display a EutF$^-$ phenotype. Finally, JE1418 (DEL*903*) displayed Trp$^-$ and EutF$^-$ phenotypes, but the mutation did not affect either the *cobA* or the *tonB* locus. Taken together, these results located the *eutF* gene between *trp* and *tonB*.

Three-factor cross. A three-factor cross experiment was performed to confirm the location of the *eutF* locus. Donor strain JE1293 (*cobA366*::Tn*10d*Cm *trp*$^+$ *eutF*$^+$) was crossed with recipient strain JE1687 (*cobA*$^+$ *trp-3484*::Tn*10d*Tc *eutF1115 eutE18*::Mu d1-8), and Cm^r transductants were selected. The Tc and X-Gal phenotypes of 960 Cm^r recombinants were determined. Four classes of recombinants were found, as follows (followed by the total number of recombinants and their relative frequency): Tc^s X-Gal$^-$ (721, 75.1%); Tc^r X-Gal$^-$ (166, 17.2%); Tc^s X-Gal$^+$ (62, 6.5%, the donor class); and Tc^r X-Gal$^+$ (11, 1.2%). If the gene order inferred from deletion mapping experiments is correct, the rare class of Cm^r recombinant would be one displaying Tc^r and X-Gal$^+$ phenotypes (this class requires four recombination events to be generated). Such a class of transductant was obtained at a frequency of 1.2%, a frequency lower than those obtained for other classes in the experiment. These data were consistent with the gene order *cobA trp eutF tonB*.

Effect of the *eutF* locus on the transcription of the *eut* operon. In their studies of the transcriptional regulation of the *eutDEABCR* operon, Roof and Roth documented that ethanolamine and cobalamin were necessary for induction (19). To assess any effect that a mutation in *eutF* would have on the expression of the *eutDEABCR* operon, we measured the expression in early-log-phase cultures (A_{650}, 0.2 to 0.3) of the *eutE-lacZ* operon fusion in genetic backgrounds with either a wild-type or a defective *eutF* locus under conditions in which ethanolamine was not required for growth. A strain carrying a deletion of *eutF* (JE1685) showed an 18-fold reduction in the levels of β-galactosidase activity measured (9 U/A_{650} unit) when compared with a strain with a functional *eutF*$^+$ locus (JE1684) (170 U/A_{650} unit; <2 unless both ethanolamine and CN-B_{12} were provided in the medium). Hydroxylamine-generated mutations in *eutF* yielded similar results, although levels of β-galactosidase activity in these backgrounds (*eutF1113* [3 U/A_{650} unit] and *eutF1115* [<2 U/A_{650} unit]) were three- to five-fold lower than those in strains carrying DEL*903*.

A second approach was used to assess the effect of a *eutF* mutation on the transcription of a *eutE-lacZ* operon fusion. The expression of the *eutE-lacZ* operon fusion was determined as a function of the concentration of ethanolamine in strains with a functional or defective *eutF* locus. Induction of the *eutE-lacZ* fusion in a *eutF*$^+$ background was tested for ethanolamine concentrations of up to 56 mM and found to be linear in the range of 0 to 100 μM ethanolamine. In a *eutE-lacZ eutF*$^+$ (JE1684) strain, as little as 25 μM ethanolamine induced levels of β-galactosidase fivefold higher than background levels, and no significant increase in induction was observed at concentrations higher than 1 mM ethanolamine. In contrast, only background levels of β-galactosidase (<2 U A_{650} unit^{-1}) were detected in a *eutE-lacZ eutF1115* (JE1687) strain grown in medium containing up to

TABLE 3. EAL activity in *eutF* mutants

Strain	Relevant genotype	Sp act[a]	% Relative activity
TR6583	*eutF*$^+$	199	100
JE1418	DEL*903*	23	12
JE1690	*eutF1115*	7	4

[a] Expressed in nanomoles of acetaldehyde produced per minute per milligram of protein. Data shown are the averages of two separate experiments, and each determination was performed in duplicate. The values shown are corrected for background.

56 mM ethanolamine, a concentration which exceeds the minimal concentration necessary for induction by >2 orders of magnitude. Taken together, these transcriptional studies indicated that the *eutF* mutation was responsible for the drastic reduction in the transcription of the *eutDEABCR* operon.

Effect of *eutF* on EAL activity. EAL catalyzes the initial step in the catabolism of ethanolamine, an Ado-B_{12}-dependent reaction that yields acetaldehyde and ammonia. We compared the steady-state level of EAL activity present in *eutF* mutants to that present in the wild-type strain (Table 3). The level of enzyme activity present in the mutants was low (10% of the wild-type activity) but was reproducibly detectable. This low level of activity may account for the limited ability of the mutants to use ethanolamine as a nitrogen source (Table 2).

Effect of *eutF* on the uptake of ethanolamine. The possibility that the phenotype of a *eutF* mutant was due to a defect in the transport of ethanolamine was addressed by performing uptake assays with radiolabeled ethanolamine (Fig. 1).

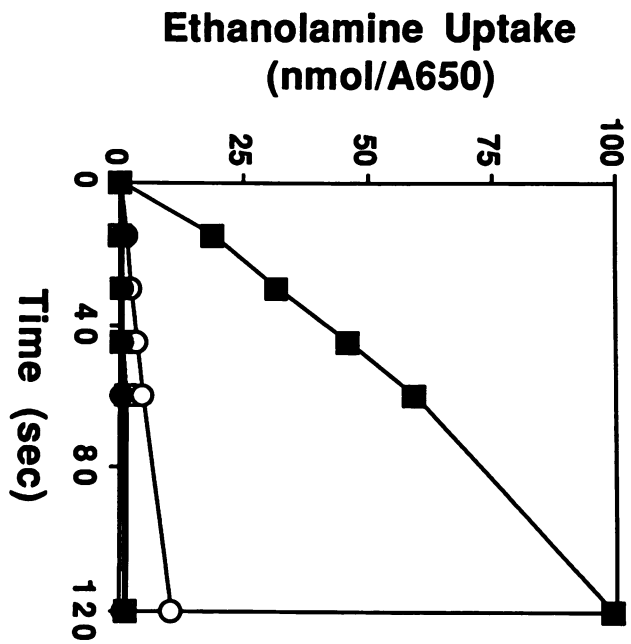

FIG. 1. Determination of the rate of uptake of ethanolamine in EutF and wild-type strains. Cultures at the mid-log phase (A_{650}, 0.2 to 0.3) were used in these experiments. Symbols: ■, TR6583 (wild type); ○, JE1693 (*eutF1113*); ●, JE1689 (*eutF1115*); □, JE1418 (DEL*903*). The calculated rates of uptake (nanomoles of ethanolamine s^{-1} A_{650} $unit^{-1}$) were as follows: wild type, 56.0; JE1693, 4.6; JE1689, 0.3; JE1418, 1.6.

On the basis of these data, it was clear that the transport of ethanolamine by *eutF* mutants was drastically reduced. The rates of uptake were 56 nmol of ethanolamine s^{-1} A_{650} $unit^{-1}$ for the wild type and 1.6 and 0.3 nmol s^{-1} A_{650} $unit^{-1}$ for deletion mutant JE1418 (DEL*903*) and a hydroxylamine-generated mutant (JE1690 [*eutF1115*]), respectively. Strain JE1693 (*eutF1113*), which showed significant growth on ethanolamine as the sole nitrogen source, had a rate of uptake of 4.6 nmol of ethanolamine s^{-1} A_{650} $unit^{-1}$, approximately 10-fold lower than that of the wild type.

The rate of ethanolamine uptake, as well as the transcription of a *eutE-lacZ* operon fusion (see above) and EAL activity (Table 3), were consistently higher in the strain thought to carry a deletion of *eutF* (JE1418 [DEL*903*]) than in the strain carrying a hydroxylamine-generated mutation in this locus (*eutF1115*). It is difficult to explain these results without a molecular analysis of the nature of these mutations.

It is important to note that Roof and Roth (19) reported that the rate of ethanolamine uptake decreased in strains with any lesion in the *eutDEABCR* operon. On the basis of this result, these authors concluded that the transport of ethanolamine may be coupled to its degradation (19). We confirmed their observation by performing ethanolamine uptake assays with strain JE1684 (*eutE18*::Mu d1-8); this strain had no detectable uptake of ethanolamine.

DISCUSSION

We have isolated mutations in the region of 34 min on the *S. typhimurium* chromosome, between the *trp* and *tonB* loci, which define the new genetic locus, *eutF*. A *eutF* mutant cannot use ethanolamine as a sole source of carbon and/or energy and retains only a limited ability to utilize ethanolamine as a sole nitrogen source. Our data are consistent with two possible roles for the EutF protein: (i) as an ethanolamine permease or (ii) as a transcription factor required for the expression of the *eutDEABCR* operon. However, the experimental evidence available at this point is not sufficient to distinguish between these two models.

Roof and Roth (19) showed that any lesion in the *eut DEABCR* operon impaired transport and concluded that degradation and transport of ethanolamine were coupled. Coupling between degradation and transport of a substrate has previously been demonstrated for proline utilization (12, 16, 27). If the *eutF* gene product is a transcription factor required for the expression of the *eutDEABCR* operon, a mutation in this locus would result in decreased expression of the *eutE-lacZ* operon fusion and low EAL activity and consequently abolish the transport of ethanolamine into the cell. However, the data presented are also consistent with the model that *eutF* codes for a permease. That is, a defect in the ethanolamine permease would prevent the uptake of a required coinducer of the transcription of the *eutDEABCR* operon, explaining the 18-fold decrease in expression of the *eutE-lacZ* operon fusion and the low levels of EAL activity measured. Formally, *eutF* could code for a factor required for transcription of the ethanolamine permease and not the actual permease. This model would also be consistent with our results.

Molecular analysis of the *eutF* locus is needed to establish its role in the catabolism of ethanolamine.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM40313 to J.C.E.-S. from the National Institute of General Med-

ical Sciences. G.A.O. is a predoctoral trainee supported by NIH biotechnology training grant 1 T32 GM08349.

We thank D. M. Roof and J. R. Roth for providing us with strains and for valuable comments on this work. We also thank B. M. Cali and J. Silver for critical reading of the manuscript.

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