JOURNAL OF BACTERIOLOGY, Nov. 2004, p. 7635–7644 Vol. 186, No. 22
0021-9193/04/$08.00+0 DOI: 10.1128/JB.186.22.7635–7644.2004

Evidence that a B_{12}-Adenosyl Transferase Is Encoded within the Ethanolamine Operon of *Salmonella enterica*

David E. Sheppard,[1] Joseph T. Penrod,[2] Thomas Bobik,[3]† Eric Kofoid,[2] and John R. Roth[2]*

Department of Biological Sciences, University of Delaware, Newark, Delaware[1]; Section of Microbiology, Division of Biological Sciences, University of California, Davis, Davis, California[2]; and Department of Microbiology and Cell Science, University of Florida, Gainesville, Florida[3]

Received 5 June 2004/Accepted 11 August 2004

Adenosylcobalamin (Ado-B_{12}) is both the cofactor and inducer of ethanolamine ammonia lyase (EA-lyase), a catabolic enzyme for ethanolamine. De novo synthesis of Ado-B_{12} by *Salmonella enterica* occurs only under anaerobic conditions. Therefore, aerobic growth on ethanolamine requires import of Ado-B_{12} or a precursor (CN-B_{12} or OH-B_{12}) that can be adenosylated internally. Several known enzymes adenosylate corrinoids. The CobA enzyme transfers adenosine from ATP to a biosynthetic intermediate in de novo B_{12} synthesis and to imported CN-B_{12}, OH-B_{12}, or Cbi (a B_{12} precursor). The PduO adenosyl transferase is encoded in an operon (*pdu*) for cobalamin-dependent propanediol degradation and is induced by propanediol. Evidence is presented here that a third transferase (EutT) is encoded within the operon for ethanolamine utilization (*eut*). Surprisingly, these three transferases share no apparent sequence similarity. CobA produces sufficient Ado-B_{12} to initiate *eut* operon induction and to serve as a cofactor for EA-lyase when B_{12} levels are high. Once the *eut* operon is induced, the EutT transferase supplies more Ado-B_{12} during the period of high demand. Another protein encoded in the operon (EutA) protects EA-lyase from inhibition by CN-B_{12} but does so without adenosylation of this corrinoid.

The *eut* operon of *Salmonella enterica* encodes enzymes for use of ethanolamine as a carbon and nitrogen source (28, 29). Only 4 of its 17 genes (19, 33), *eutD*, *eutE*, *eutB*, and *eutC*, have been correlated directly with an enzymatic activity known to be required for ethanolamine utilization (9, 29). The operon and metabolic pathway are diagrammed in Fig. 1. A fifth gene, *eutR*, encodes a positive regulatory protein that activates transcription of the *eut* operon in response to the simultaneous presence of ethanolamine plus adenosylcobalamin (Ado-B_{12}) (27, 32). The *eutT* and *eutA* genes described here have not been associated with any specific enzymatic function.

Use of ethanolamine as a carbon or nitrogen source requires Ado-B_{12}, which is the cofactor of ethanolamine ammonia lyase (EA-lyase; EC 4.3.1.7) (2, 6, 8, 12, 28). Ado-B_{12} also serves as a coinducer (with ethanolamine) of *eut* operon transcription (18, 27, 32). A rationale for using Ado-B_{12} as a coinducer is the fact that operon expression is futile without Ado-B_{12} to support EA-lyase and this cofactor (unlike most others) is not always present. This is the case because *S. enterica* synthesizes Ado-B_{12} de novo only under anaerobic conditions (1, 15, 20, 30). Therefore under aerobic conditions, cells can grow on ethanolamine only if the environment can provide either Ado-B_{12} or a suitable precursor. Since Ado-B_{12} is subject to photolysis (4), exogenous precursors are likely to lack the upper (adenosyl) ligand and require internal adenosylation.

It is clear that *S. enterica* can add the upper adenosyl ligand to cobalamins since commercial CN-B_{12}, which lacks this ligand, allows aerobic growth on ethanolamine (28). The CobA enzyme, ATP:cob(I)alamine adenosyl transferase, adenosylates assimilated CN-B_{12} and also contributes to de novo B_{12} synthesis (anaerobic) by adenosylating a biosynthetic intermediate (11, 34). CobA also adenosylates the assimilated precursor cobinamide (Cbi) and converts it to the biosynthetic intermediate Ado-Cbi (11). Another cobalamin transferase (PduO) is encoded within the *pdu* operon and is induced only during growth on propanediol; this transferase is not involved in any of the metabolism described here (17).

Existence of a third adenosyl transferase was suggested by the fact that CN-B_{12} allows a *cobA* mutant to use ethanolamine as a nitrogen source when glycerol is the carbon source (see Table 2), but not with glucose (11). This implied existence of another transferase, perhaps subject to catabolic repression. Evidence is presented that EutT is this transferase and contributes to ability of cells to grow on ethanolamine, especially with low levels of exogenous CN-B_{12}. Another transferase candidate (EutA) helps cells resist inhibitory effects of CN-B_{12} on EA-lyase, but does so without converting CN-B_{12} to the normal cofactor Ado-B_{12}.

MATERIALS AND METHODS

Bacterial strains and transposons. Strains were derived from *S. enterica* (serovar Typhimurium) LT2 (Table 1). Normal and introduced promoters are diagrammed in Fig. 1. The *cobA366*::Tn*10*d(Cm) mutation maps far from the *eut* operon and eliminates the general B_{12} adenosyl transferase (11, 34). An inserted element referred to as *eutA*::P_{int} throughout this paper is the *eutA208*::Tn*10*d(Tc) insertion (28), carrying an outward-directed constitutive promoter, P_{int} (32), created by a point mutation within the Tn*10*d(Tc) element (37). This strong promoter expresses *eut* genes downstream of *eutA*, including EutBC (EA-lyase) and EutR (32). Another Tn*10* derivative (T-POP) (25) inserted in the *eutJ* gene (or in *eutP*) allowed tetracycline-inducible expression of genes downstream of its

* Corresponding author. Mailing address: Section of Microbiology, DBS, University of California, Davis, One Shields Ave., Davis, CA 95616. Phone: (530) 752-6679. Fax: (530) 752-7663. E-mail: jrroth@ucdavis.edu.

† Present address: Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA 50011.

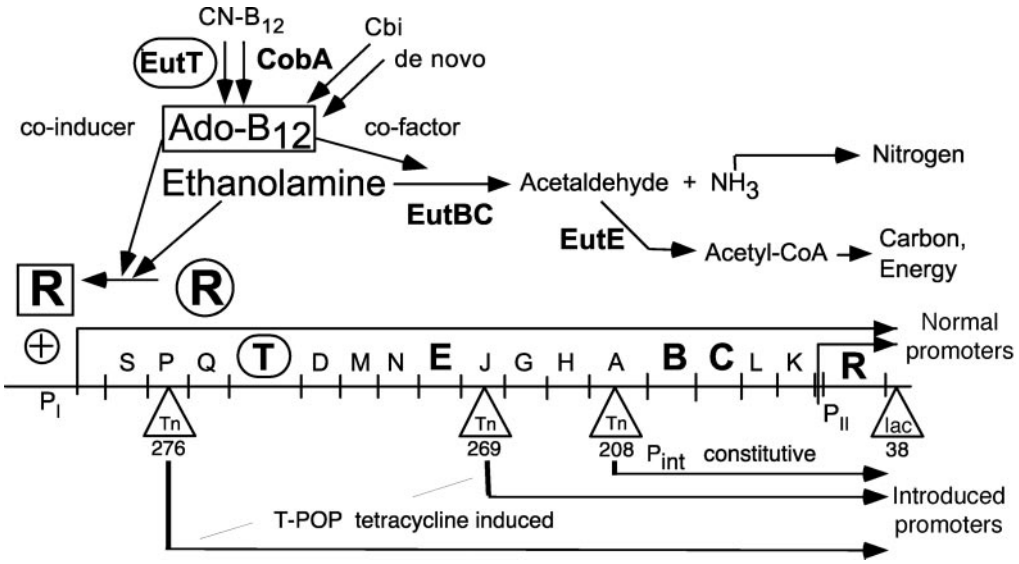

FIG. 1. Pathway and operon for ethanolamine degradation. Ado-B_{12} serves as a coinducer of the operon and cofactor for EA-lyase. Degradation of ethanolamine provides both a carbon source and nitrogen source (30). Cbi (cobinamide) is a corrinoid lacking upper and lower ligands that can be adenosylated by CobA to produce Ado-Cbi, a biosynthetic precursor of B_{12}. All *eut* genes are indicated above the map with the two normal promoters: the major regulated promoter (P_I) and the minor constitutive *eutR* promoter (P_{II}) (19, 27–29). Transposon-associated introduced promoters are below the map.

insertion site. Nonpolar deletion mutants *eutPQTD333* and *eutDM302* were isolated as tetracycline-sensitive Eut$^+$ revertants of T-POP insertions in the *eutQ* or *eutD* genes (19) and a set of constructed single-gene, in-frame deletions will be described (23). The *eut-38*::MudA insertion lies just within the distal end of the *eut* transcript but outside of all reading frames; it reports operon transcription without impairing any *eut* function (19, 27). Other *eut* mutations have been described previously (19, 27).

Media. Rich medium (NB) was nutrient broth (0.8%; Difco Laboratories) supplemented with NaCl (5 g/liter). Minimal media were variants of E medium, which contains citrate. NCE medium lacks citrate (26), and NCN medium lacks both citrate and a nitrogen source (5). Ethanolamine hydrochloride (20.5 mM; Aldrich) was used as a carbon source in NCE medium, as a nitrogen source in NCN medium with glycerol (20 mM), or as a carbon and nitrogen source in NCN medium. CN-B_{12} (80 nM; Sigma Chemical Co.) was the usual exogenous vitamin B_{12} source. Amino acids and purines were added to minimal medium at the concentrations previously recommended (10). NB agar media contained antibiotics at the following concentrations: ampicillin, 30 μg/ml; chloramphenicol, 20 μg/ml; kanamycin, 50 μg/ml; and tetracycline, 20 μg/ml. Solid media were prepared by adding agar (1.5%, Difco) to E, NCN, NCE, or NB medium.

Assay of β-galactosidase. Strains were grown in NCE or NCN medium containing glycerol (0.2%) as a carbon source and ethanolamine (20.5 mM) plus CN-B_{12} (80 nM) as inducers. Following growth to mid-log phase at 37°C on a New Brunswick Shaker, model 50, cells were held at 4°C until assayed. Enzyme activity was determined in chloroform-sodium dodecyl sulfate-permeabilized cells (21). Activity was expressed as nanomoles of nitrophenol produced per minute per optical density at 650 nm (OD_{650}) of cell culture turbidity. All presented values are the average of at least four determinations on two independent cultures.

Cloning the *eutT* gene for expression. The *eutT* coding sequence was amplified by PCR using forward primer GCCGCCAGATCTGATGAACGATTTCATCA CCGAAACGTGG and reverse primer GCCGCCAAGCTTTCATGGCTTCTC TCCCAACCGTTG (17). Template DNA from *S. enterica* was prepared with a Bio-Rad Aquapure genomic DNA isolation kit. Polymerase for PCR was a mixture of *Pfu* and *Taq* polymerases (7:1 [unit/unit]). The PCR products were cloned into the T7 expression plasmid pTA925 with BglIII and HinDIII (17). The DNA sequences of selected clones were verified. Plasmids pTA925 (no *eutT* insert) and pTA1019(*eutT* insert) were introduced into expression strain *Escherichia coli* BL21 DE3RIL (Stratagene, La Jolla, Calif.), to form strains BE119 and BE205, which were used for EutT production. Adenosyl transferase activity was assayed as described previously (17).

Overexpression of EutT and preparation of cell extracts. EutT expression strains were grown at 30°C with shaking in 1,000-ml baffled Erlenmeyer flasks containing 500 ml of Luria broth supplemented with 25-μg/ml kanamycin. Cells were grown to an OD_{600} of 0.6 to 0.8. At that density, protein expression was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. Cultures were shaken for an additional 3 h at 30°C, placed on ice for 5 min, and collected by centrifugation for 5 min at 4,650 × *g* (maximum) using a Beckman J2-HS centrifuge and a Beckman JLA10.500 rotor. The cell pellet was frozen at −80°C until used. Cell pellets were resuspended in 50 mM potassium phosphate buffer at pH 7, containing 50 mM NaCl, and cells were disrupted in a 5-ml French pressure cell (SLM Aminco, Urbana, Ill.). Cell debris and unbroken cells were pelleted by centrifugation at 31,000 × *g* (maximum) with a Beckman JA20 rotor. The supernatant served as the soluble fraction for enzyme assays and polyacrylamide gel electrophoresis. The pellet obtained by centrifugation was extracted with Bacterial Protein Extraction Reagent II (B-PERII; Pierce, Rockford, Ill.) according to the manufacturer's directions, but with the following modifications. The B-PERII solution used for the first extraction was supplemented with the protease inhibitor phenylmethylsulfonyl fluoride at a concentration of 100 μg/ml. The B-PERII solution used for the second extraction was supplemented with DNase at a concentration of 20 μg/ml. Inclusion bodies were washed twice with 10 ml of a 1/20 dilution of B-PERII and then resuspended in 50 mM potassium phosphate pH 7.0 containing 50 mM NaCl; this suspension was used for assay of inclusion bodies (17).

Growth inhibition by CN-B_{12}. A *eutA* mutant fails to grow on ethanolamine as a carbon source, regardless of the cobalamin form provided. It also fails to use ethanolamine as a nitrogen source when CN-B_{12} is provided at a high level (80 nM) but does grow if Ado-B_{12} is given. The Ado-B_{12}-stimulated growth is inhibited by high CN-B_{12} levels (29). We show here that growth on ethanolamine as a nitrogen source is allowed (inhibition is avoided) if the level of CN-B_{12} is reduced to 20 nM. To measure this inhibition, overnight nutrient broth cultures were diluted 1/100 into 200 μl of NCN medium containing glycerol (0.2%), ethanolamine (20.5 mM), and CN-B_{12} at 0, 20, 80, or 140 nM. Cultures were incubated, with aeration, at 37°C in a Bio-Tec automated plate reader. Three parallel cultures were grown at each concentration of CN-B_{12}, and OD_{650} turbidity was measured every hour.

Effect of *cobA* and *eutT* mutations on growth rates. Three independent NB cultures of each strain were inoculated from single colonies grown on NB plates. After overnight growth, cells were pelleted, washed in minimal medium (pH 7.0), and used (35 μl of final suspension) to inoculate 6-ml cultures in minimal medium. Liquid minimal medium was a mixture of 5 mM KH_2PO_4, 5 mM $NaNH_4HPO_4$, and 1 mM $MgSO_4$ buffered at pH 7.0 by 50 mM MOPS (morpholinepropanesulfonic acid). The carbon source was 41 mM ethanolamine hydrochloride (0.04%). Cyanocobalamin concentrations are indicated later. Minimal medium also contained biotin; Ca-*d*-pantothenic acid, nicotinamide, and

TABLE 1. Bacterial strains used in this study

Strain	Genotype
BE119	*E. coli ompT hsdS* (r_B^- m_B^-) *dcm*$^+$ Tet^r *gal* λ(DES) *endA* DE3[*argU ileY leuW* Cam^r]/pTA925 (T7 expression vector without insert)
BE205	*E. coli ompT hsdS* (r_B^- m_B^-) *dcm*$^+$ Tet^r *gal* λ(DES) *endA* DE3[*argU ileY leuW* Cam^r]/pTA1019 (T7 expression vector with *eutT* insert)
TT10000	LT2 wild type
TT10674	*eut-38*::MudA
TT17300	*eutA208*::Tn*10*d(Tc)P_{int} *eutR156*::MudJ
TT17306	*eutA208*::Tn*10*d(Tc)P_{int}
TT17310	*eutE56 eut-38*::MudA
TT17312	*eutA114 eut-38*::MudA
TT17319	*eutD101 eut-38*::MudA
TT17320	*eutQ18*::Mu dJ *eutA208*::Tn*10*d(Tc) P_{int}
TT20024	*eutE56 eut-38*::MudA *cobA366*::Tn*10*d(Cm)
TT20025	*eutA111 eut-38*::MudA
TT20026	*eutA111 eut-38*::MudA *cobA366*::Tn*10*d(Cm)
TT20033	*eutQ18*::MudJ *eutA208*::Tn*10*d(Tc)P_{int} *cobA366*::Tn*10*d(Cm)
TT20039	*eutE163*::MudJ *eutA208*::Tn*10*d(Tc)P_{int}
TT20041	*eutE163*::MudJ *eutA208*::Tn*10*d(Tc)P_{int} *cobA366*::Tn*10*d(Cm)
TT20042	*eut-38*::MudA *cobA366*::Tn*10*d(Cm)
TT20043	*eutT11*::MudA *eutA208*::Tn*10*d(Tc)P_{int}
TT20044	*eutT11*::MudA *eutA208*::Tn*10*d(Tc)P_{int} *cobA366*::Tn*10*d(Cm)
TT20045	*eutP171*::MudJ *eutA208*::Tn*10*d(Tc)P_{int} *cobA366*::Tn*10*d(Cm)
TT20046	*eutP171*::MudJ *eutA208*::Tn*10*d(Tc)P_{int}
TT20047	*eutG3*::MudA *eutA208*::Tn*10*d(Tc)P_{int}
TT20054	*eutE10*::Mud *eutA208*::Tn*10*d(Tc)P_{int} *cobA366*::Tn*10*d(Cm)
TT20069	*eutE6*::MudA *eutA208*::Tn*10*d(Tc)P_{int}
TT20071	*eutE10*::MudA *eutA208*::Tn*10*d(Tc)P_{int}
TT20078	*eutT86 eut-38*::MudA *cobA366*::Tn*10*d(Cm)
TT20079	*eutT86 eut-38*::MudA
TT20114	*cobA366*::Tn*10*d(Cm) *cobR4 cob24*::MudJ *metE205 ara-9 zeb-1845*::Tn*10*
TT20124	*eutQ18*::MudJ *eutJ269*::Tn*10*(Tc)T-POP
TT20125	*eutQ18*::MudJ *eutJ269*::Tn*10*(Tc)T-POP *cobA366*::Tn*10*d(Cm)
TT20126	*eutT11*::MudA *eutJ269*::Tn*10*(Tc)T-POP
TT20127	*eutT11*::MudA *eutJ269*::Tn*10*(Tc)T-POP *cobA366*::Tn*10*d(Cm)
TT20128	*eutE10*::MudA *eutJ269*::Tn*10*(Tc)T-POP
TT20129	*eutE10*::MudA *eutJ269*::Tn*10*(Tc)T-POP *cobA366*::Tn*10*d(Cm)
TT20136	*eutDM*302 *eut-38*::MudA
TT20137	*eutDM*302 *eut-38*::MudA *cobA366*::Tn*10*d(Cm)
TT20138	*eutPQTD*333 *eut-38*::MudA
TT20139	*eutPQTD*333 *eut-38*::MudA *cobA366*::Tn*10*d(Cm)
TT24803	*eutT371*::FRT(Δ)
TT25127	*cobA366*::Tn*10*d(Cm)
TT25128	*cobA366*::Tn*10*d(Cm)*eutT371*::FRT

pyridoxine HCl at 4×10^{-4}% (wt/vol); and thiamine and riboflavin at 2×10^{-5}% (wt/vol) and trace metals as previously described (24). Cultures were aerated slightly by shaking at 240 rpm in tubes standing upright. Growth was monitored by observing OD_{650} on a Spectronic 20D+ spectrophotometer.

RESULTS

Evidence for a CobA-independent route of cobalamin adenosylation. Mutants of *S. enterica* lacking the CobA adenosyl transferase cannot use ethanolamine as a nitrogen source when provided with CN-B_{12} and glucose as a carbon source (11). This failure is due to inability to convert CN-B_{12} to the required EA-lyase cofactor, Ado-B_{12}; growth is restored if Ado-B_{12} replaces CN-B_{12}. We noted that CN-B_{12} allowed *cobA* mutants to use ethanolamine as a nitrogen source when glycerol rather than glucose provided carbon (Table 2, top row) or when ethanolamine provided both nitrogen and carbon. This implied that an adenosyl transferase (other than CobA) was expressed during growth on poor carbon sources. Conditions under which this additional activity was inferred were ones that induce the *eut* operon (27, 32), suggesting that a gene in the *eut* operon might encode the inferred transferase.

Preliminary evidence for two *eut* genes that might provide Ado-B_{12}. Several *eut* mutations were combined with a *cobA* mutation and tested for their effect on the inferred ability to adenosylate cobalamin. When CN-B_{12} was provided aerobically, only *eutT* and *eutA* point mutants failed to use ethanolamine as a nitrogen source on glycerol, Eut(N$^-$). Below we characterize these *eutT* mutations and then return to *eutA*.

In an otherwise wild-type strain, available *eutT* mutations (all nonsense types) eliminated growth on ethanolamine as the sole carbon source even when Ado-B_{12} is provided (19). This is due to their polar effect on expression of the downstream genes for EA-lyase (*eutB* and *eutC*), which is essential for use of ethanolamine. However, these simple *eutT* mutants can use ethanolamine as a nitrogen source, demonstrating that their level of EA-lyase is sufficient for the less-demanding task of supplying nitrogen (Table 2, rows 1 to 4). In strains lacking *cobA*, *eutT* mutations eliminate use of ethanolamine as a ni-

TABLE 2. Aerobic growth phenotypes of *eut* point and deletion mutants

Strain	Relevant genotype		Eut(N) growth phenotype[a]			No. of mutations examined[b]
	eut	*cobA*	None	+CN−B_{12} (0.08 μM)	+Ado−B_{12} (0.08 μM)	
TT20042	*eut*$^+$	−	−	+	+	
TT10674		+	−	+	+	
TT20078	*eutT86*(Am)	−	−	−	+	9[c]
TT20079		+	−	+	+	
TT20026	*eutA111*	−	−	−	+	2[e]
TT20025		+	−	(−)[d]	+	
TT20138	*eutPQTD333*(Δ)[f]	−	−	−	+	1
TT20139		+	−	+	+	
TT20136	*eutDM302*(Δ)[f]	−	−	+	+	1
TT20137		+	−	+	+	
TT20064	*eutD101*(Am)	−	−	+	+	3[g]
TT17319		+	−	+	+	
TT20024	*eutE56*	−	−	+	+	3[h]
TT17310		+	−	+	+	

[a] Growth tests were done in aerobic liquid medium containing glycerol as a carbon source and ethanolamine as a nitrogen source with indicated additions; growth was scored after 2 days.
[b] Several different mutations in each gene were tested; all mutations in the same gene exhibited the same phenotype. Full genotypes are in Table 1.
[c] All known *eutT* mutants are nonsense mutations (19). The alleles tested were *eutT67* [*gln229*(Am)], *eutT74* [*gln529*(Am)], *eutT75* [*gln379*(Oc)]*eutT77*[*gln181*(Am)], *eutT78* [*gln402*(Op)], *eutT86* [*pro227*(Leu)] [*gln229*(Am)], *eutT88*[*gln184*(Am)], *eutT90*[*gln184*(Am)], and *eutT104*[*trp374*(Op)].
[d] This strain has a Eut(N$^+$) phenotype at 0.02 μM CN-B_{12} but fails to grow at 0.08 μM CN-B_{12}; this inhibition phenotype will be described later.
[e] The alleles tested were *eutA111* and *eutA114*.
[f] In-frame nonpolar deletion mutations (19).
[g] The alleles tested were *eutD53*[gln103(Oc)], *eutD101*[gln4656(Am)] and *eutD124*[*arg518*(Op)].
[h] The alleles tested were *eutE56, eutE65,* and *eutE79.*

trogen source with provided CN-B_{12}, a Eut(N$^-$) phenotype. This defect is corrected by providing Ado-B_{12} (Table 2). The Eut(N$^-$) phenotype on CN-B_{12} was caused by nonpolar deletion mutations that remove *eutT* (*eutPQTD333*) but was not seen in *eutT*$^+$ strains carrying a nonpolar deletion (*eutDM302*) or in strains with a polar point mutation downstream of the *eutT* gene (e.g., *eutD101*). Since the Eut(N$^-$) phenotype of *eutT* mutants on CN-B_{12} was corrected by providing either Ado-B_{12} or a functional CobA transferase activity, EutT was a prime candidate for the *eut*-specific adenosyl transferase.

Genetic evidence that EutT contributes to cobalamin adenosylation in vivo. The cofactor Ado-B_{12} is required to induce the *eut* operon and to serve as cofactor for EA-lyase. Thus, if *eutT* mutants fail to make Ado-B_{12}, both induction and enzyme activity should be impaired. These processes were tested independently in strains with insertion *eutA*::P_{int} (32), which constitutively expresses the EA-lyase genes (*eutBC*). Fusions of *lac* to the *eut* operon upstream of *eutA* reported operon induction (Table 3). Operon induction was tested on glycerol-ammonia medium, where no EA-lyase activity is required. All *cobA*$^+$ strains showed induction. However, in *cobA* mutants, no induction was seen for Mud insertions that eliminated EutT activity either directly or by polarity (*eutP*, *eutQ*, or *eutT*; Table 3, rows 3 to 6). Inducibility was normal in strains with a *eutE*::Mud fusion, distal to the *eutT* gene (Table 3, rows 7 and 8). The induction defect in strains lacking EutT (and CobA) was corrected by Ado-B_{12} (Table 3, right column). Thus, EutT allows CN-B_{12} to induce the operon without CobA and is therefore inferred to contribute to conversion of CN-B_{12} to the inducer Ado-B_{12}.

Further evidence for a role of EutT in Ado-B_{12} production was its effect on EA-lyase-dependent growth. In the strains used above, EA-lyase (EutBC) was expressed constitutively from a strong promoter (P_{int}) within the inserted *eutA*::P_{int} element. Since EA-lyase is the only enzyme required to produce ammonia from ethanolamine, these strains can use ethanolamine with or without operon induction but do so only if cofactor Ado-B_{12} is available (32). Mud insertions that eliminate EutT expression (those in the *eutP*, *eutQ*, or *eutT* genes) caused a Eut(N$^-$) phenotype in the absence of CobA (Table 3, first six rows), whereas a *eutE*::Mud insertion (distal to *eutT*) is Eut(N$^+$) (Table 3, last two rows). The *eutT* growth defect on CN-B_{12} was corrected by Ado-B_{12} or by restoring a *cobA*$^+$ allele (Table 3). Similar results were seen for point mutations in genes *eutT*, *eutD*, and *eutE* (data not shown). Thus, EutT served to provide Ado-B_{12} by the criterion of ethanolamine growth, as well as operon induction. (Effects of a simple in-frame *eutT* deletion on growth will be shown later.)

Assays of EutT adenosyl transferase activity. The genetic evidence above suggested that EutT was a B_{12} adenosyl transferase. This conclusion was confirmed by transferase assays. Since assay of the CobA and PduO adenosyl transferases requires overproduction of the protein (34), the EutT protein was produced in a T7 expression system (17). A strain (BE205) with *eutT* inserted in the expression plasmid produced relatively large amounts of a 30.2-kDa protein in both the soluble

TABLE 3. Two tests of EutT-mediated Ado-B_{12} production, induction and activity

Strain	Genotype[a]		Eut(N) growth phenotype[b]			*eut* operon induction (β-galactosidase activity)[c]					
	eut	*cobA*	None	+CN-B_{12}	+Ado-B_{12}	None	+EA	+EA +CN-B_{12}	Fold increase by CN-B_{12}[d]	Effect of *CobA*$^{+}$[e]	+EA +Ado-B_{12}
TT20045	*eutP171*::*lac* (EutT^{-})	−	−	−	+	2.7	3.9	6.2	1.6	21	130
TT20046		+	−	+	+	1.6	4.6	130	28		110
TT20033	*eutQ18*::*lac* (EutT^{-})	−	−	−	+	9.8	32	46.	1.4	28	1350
TT17320		+	−	+	+	21	56	1300	23		960
TT20044	*eutT11*::*lac* (EutT^{-})[f]	−	−	−	+	5.5	18	27.	1.5	34	590
TT20043		+	−	+	+	6.0	20	910	46		590
TT20041	*eutE163*::*lac* (EutT^{+})[g]	−	−	+	+	5.8	29	960	33	0.9	740
TT20039		+	−	+	+	5.1	30	850	29		760

[a] All strains contain the *eutA*::P_{int} mutation that eliminates EutA protein and highly expresses the EutBC (lyase) and EutR (transcription activator) proteins (Fig. 2A). Complete genotypes are in Table 1.
[b] Growth phenotypes were scored + or − by replica-printing cell patches from an NB plate to an NCN plate with glycerol as a carbon source and ethanolamine as a nitrogen source.
[c] Cells were grown on NCE medium containing glycerol as a carbon source and ammonia with additions as shown. β-Galactosidase activity was determined as described in Materials and Methods.
[d] Induction caused by CN-B_{12}.
[e] This is the ratio (*cobA*$^{+}$/*cobA* mutant) of operon induction in strains isogenic except for the *cobA* mutation.
[f] Essentially the same results were seen with *eutT17*::Mud-*lac*.
[g] Essentially the same results were seen with all other Mud-*lac* insertions located promoter distal to *eutT* (Fig. 2A). MutA insertion alleles tested were *eut-6* (between *eutN* and *eutE*), *eutE12*, *eutE10*, *eutE1*, *eutJ26*, and *eutH25*. The MudJ alleles tested were *eut-168* (between *eutD* and *eutM*) *eutE163*, and *eutE181*.

and inclusion body fractions; this protein was not found in a strain (BE119) whose expression plasmid lacked *eutT*. Extracts of strains expressing *eutT* (BE205) showed 50- to 100-fold more transferase activity than the control strain (BE119) (Table 4). These assays were done exactly as described previously and used OH-B_{12} (reduced to the CobI form) as an adenosyl-accepting substrate (17). It should be noted that a substantial fraction of adenosyl transferase activity sedimented with cell debris and may be present as inclusion bodies or associated with membranes. These results demonstrate that EutT protein has ATP:cob(I)alamin adenosyl transferase activity; the phenotypes above show that this activity is relevant in vivo.

Phenotypes of *eutA* mutations. Like *eutT* mutants, *eutA* mutants failed to use ethanolamine as either a carbon or nitrogen source when standard levels of CN-B_{12} (80 nM) were provided. It was shown earlier that growth on ethanolamine as a nitrogen source was restored when Ado-B_{12} replaces CN-B_{12}, but not when Ado-B_{12} and high CN-B_{12} were supplied together (29), suggesting an inhibitory effect of CN-B_{12}. A lower CN-B_{12} concentration (20 nM) allowed *eutA* mutants to use ethanolamine as a nitrogen source. The inhibition of growth by high levels of CN-B_{12} agrees with the previous in vitro demonstration that purified EA-lyase (EutBC) is inhibited by CN-B_{12} (3, 16, 31). Thus EutA seems to protect against inhibition by CN-B_{12}, reminiscent of reactivating factors that remove inappropriate cofactors from other B_{12}-dependent enzymes (22, 35, 36). Since EutA might protect EA-lyase by adenosylating excess CN-B_{12}, it was tested further as a candidate for an adenosyl transferase.

TABLE 4. ATP:cob(I)alamin adenosyl transferase activity

Strain[a]	Plasmid[b]	Gene expressed	Fraction[c]	Sp act (nmol/min/mg)[d]
BE119	pTA925	Vector only	Soluble	0.3
BE119	pTA925	Vector only	Inclusion bodies	0.4
BE205	pTA1019	EutT	Soluble	33.2
BE205	pTA1019	EutT	Inclusion bodies	21.1

[a] Complete genotypes are in Table 1.
[b] Plasmid construction is described in Materials and Methods.
[c] Preparation of soluble and inclusion body fractions for enzyme assay is described in Materials and Methods.
[d] ATP:cob(I)alamin specific activities were determined as described in Materials and Methods. The values shown are the averages of three trials.

EutA protein does not provide an adenosylation function. The above tests of EutT function were done in strains that carried the *eutA*::P_{int} insertion and thus lacked EutA function, showing that EutA protein is not required for EutT transferase activity. To test whether EutA can provide adenosylation independent of EutT, the *eutA* gene was expressed from a tetracycline-inducible promoter (T-POP) inserted in the *eutJ* gene; this promoter also expressed EA-lyase (*eutBC*) genes needed for growth on ethanolamine. In this strain, ability to convert CN-B_{12} to Ado-B_{12} could be assessed as ability to grow on ethanolamine as a nitrogen source (which is independent of operon induction). Alternatively, Ado-B_{12} production can be scored by operon induction (which does not require EA-lyase activity). Promoter proximal insertions of Mud-*lac* in the *eutQ*, *eutT*, or *eutE* genes were added to the *eutA*::P_{int} strain in order to block expression of upstream genes and as reporters of operon induction. The *eutQ* and *eutT* insertions eliminate EutT expression, while the *eutE*::MudJ insertion leaves *eutT* expression intact. As seen in Table 5, high-level expression of the *eutA* gene (from the *eutJ*::T-POP promoter) does not provide Ado-B_{12} in strains that lack EutT and CobA. That is, EutA could not substitute for the EutT or CobA adenosyltransferases in supporting either induction or EA-lyase-dependent growth. We conclude that EutA does not provide an adenosyl transferase activity and must provide resistance to the

TABLE 5. The EutA protein cannot provide adenosyl transferase activity

Strain	Genotype[a]		Eut(N) growth phenotype[b]			Operon induction (β-galactosidase activity)[c]					
	eut insertion	*cobA*	None	+CN-B_{12}	+Ado-B_{12}	None	+CN-B_{12}	Fold increase by CN-B_{12}[d]	Effect of CobA[e]	+Ado-B_{12}	Fold increase by Ado-B_{12}[f]
TT20125	*eutQ18*::*lac* (EutT$^-$A$^+$)	−	−	−	+	5.7	5.8	1.0	240	1,200	220
TT20124		+	−	+	+	5.6	1,400	250		1,300	230
TT20127	*eutT11*::*lac* (EutT$^-$ A$^+$)	−	−	−	+	4.2	3.8	0.9	190	640	150
TT20126		+	−	+	+	5.3	710	135		670	130
TT20129	*eutE163*::*lac* (EutT$^+$ A$^+$)	−	−	+	+	4.2	680	160	1.2	870	210
TT20128		+	−	+	+	6.3	900	140		920	150

[a] All strains carry (distal to the *lac* fusion) a *eutJ*::T-POP insertion, which expresses the *eutGHABCR* genes in the presence of tetracycline. Complete genotypes are in Table 1.
[b] All strains were tested for growth on NCN glycerol medium with 20.5 mM ethanolamine, 0.08 μM CN-B_{12} or Ado-B_{12}, and 2-μg/ml tetracycline.
[c] All strains were grown on NCE glycerol NH_3 medium with 20.5 mM ethanolamine, 0.08 μM CN-B_{12} or Ado-B_{12}, and 2-μg/ml tetracycline. β-Galactosidase activity was determined as described in Materials and Methods.
[d] Induction caused by CN-B_{12}; large induction is due to high expression of EutR from T-POP.
[e] This is the ratio (*cobA*$^+$/*cobA* mutant) of operon induction in strains isogenic except for the *cobA* mutation.
[f] Induction caused by Ado-B_{12}.

inhibitory effect of high CN-B_{12} in some other way. Additional tests of this inhibition are below.

Inhibition of *eutA* mutants by CN-B_{12}. The inhibitory effect of CN-B_{12} (80 nM) on *eutA* mutants noted above was relieved at lower concentrations of CN-B_{12} (20 nM), which allowed these mutants to grow on glycerol using ethanolamine as a nitrogen source. The growth inhibition by high CN-B_{12} is not due to a failure to express the operon, since normal induction was seen in *eutA* mutant strains by 80 nM CN-B_{12} (in combination with ethanolamine). We presume that CN-B_{12} inhibits growth by inhibiting EA-lyase directly as seen previously for the pure enzyme (2). However this sensitivity seemed to require other genes in the *eut* operon.

A role of other genes in sensitivity to CN-B_{12} was demonstrated with the *eutA*::P_{int} insertion (described above), which disrupts *eutA* and expresses EA-lyase (EutBC) from a constitutive promoter. A strain with this insertion grew on ethanolamine as a nitrogen source (with 20 nM CN-B_{12}) but was inhibited by a higher concentration (80 nM) of CN-B_{12}. The inhibition by high CN-B_{12} was abolished by an insertion in the promoter proximal *eutQ* gene, but not by an insertion in the *eutG* gene. This suggested that one or more genes upstream of *eutG* (*eutS*, *-P*, *-Q*, *-D*, *-T*, *-M*, *-N*, *-E*, or *-J*) must be needed for sensitivity. Consistent with this, a *eutR* mutation, which prevents expression of these genes, eliminated sensitivity to high CN-B_{12}. (As described above, the strains used have no EutA function and express EA-lyase [EutBC] constitutively from the P_{int} promoter.)

To identify the gene or genes necessary for inhibition by CN-B_{12}, a series of in-frame deletions of individual *eut* genes were added to the *eutA*::P_{int} mutant. The sensitivity of the parent strain to high CN-B_{12} (Fig. 2A) appears as delayed growth of liquid cultures. This sensitivity was not relieved by deletion of *eutT* (adenosyl transferase) or the *eutD*, *eutE*, or *eutJ* genes (encoding metabolic enzymes acting after acetaldehyde). Growth of the *eutT* and *eutE* mutants is shown in Fig. 2B and C. Inhibition by high CN-B_{12} was eliminated by removal of any one of the *eutM*, *-N*, *-P*, *-Q*, or *-S* genes. Growth of the *eutM* deletion mutant was typical and is shown in Fig. 2D. The EutM, -N, and -S proteins are homologues of carboxysome shell proteins; the functions of EutP and -Q are unknown. These effects are discussed below. It should be noted that delayed growth caused by high CN-B_{12} occurs in tests reported in Table 3, but growth is scored after 48 h in these tests so the inhibition does not interfere.

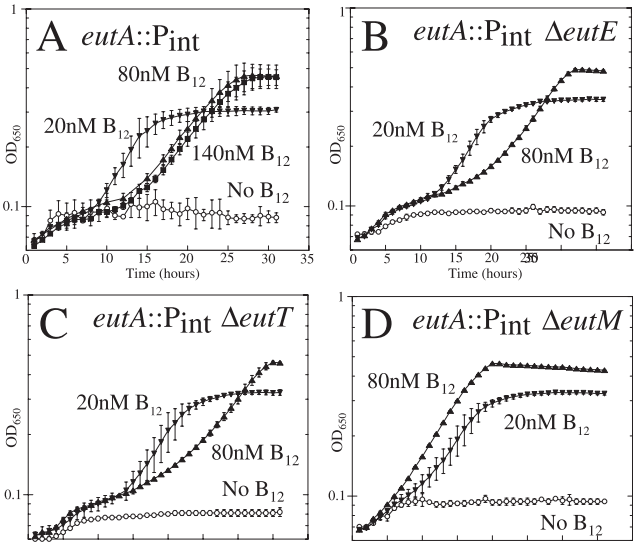

FIG. 2. Genes required for inhibition of *eutA* mutants by CN-B_{12}. Mutants lacking EutA function can grow aerobically on ethanolamine as a nitrogen source when supplied with a low level of CN-B_{12} but are inhibited by higher concentrations of CN-B_{12} (A). This sensitivity requires expression of some *eut* genes upstream of the *eutA* gene (*eutS*, *-P*, *-Q*, *-M*, and *-N*) but not others (*eutT*, *-D*, *-E*, *-J*, *-G*, and *-H*). All strains carry the *eutA*::P_{int} insertion and therefore express EA-lyase constitutively. Added mutations are constructed nonpolar deletion mutations. (B) Δ*eutE*. (C) Δ*eutT*. (D) Δ*eutM*. The responses shown are typical of mutations in the two groups. Growth rates were determined with a Biotec automated plate reader.

TABLE 6. Initiating operon induction with EutT as the sole Ado-B_{12} source

Strain	Genotype[a]		*eut* operon induction (β-galactosidase activity)[b]						
	eut	*cobA*	None	+EA	Fold increase by EA[c]	+EA + CN-B_{12}	Fold increase by CN-B_{12}[d]	+EA + Ado-B_{12}	Fold increase by Ado-B_{12}[e]
TT20042	*eut-38*:Mud*lac* (Eut$^+$)[f]	−	NA	6.0		11 (34)	1.8 (5.6)	110 (74)	19 (12)
TT10674		+	NA	5.8		240 (164)	42 (19)	150 (110)	25 (19)
TT20129	*eutE10*:Mud*lac eutJ269*::T-POP	−	NA	4.2		680	160	870	210
TT20128		+	NA	6.3		900	140	920	150
TT20127	*eutT11*::Mud*lac eutJ269*::T-POP	−	NA	4.2		3.8	0.9	640	150
TT20126		+	NA	5.3		710	130	670	130
TT20054	*eutE10*:Mud*lac eutA*::P_{int}	−	3.6	36	10.0	1,300	37	690	19
TT20071		+	3.5	27	7.7	920	34	590	22
TT20044	*eutT11*::Mud*lac eutA*::P_{int}	−	5.5	18	3.3	27	1.5	590	33
TT20043		+	6.0	20	3.3	910	46	590	30

[a] Complete genotypes are in Table 1.
[b] Strains were grown on NCE glycerol medium; 20.5 mM ethanolamine, and either 80 nM CN-B_{12} or 80nM Ado-B_{12}. Strains with a T-POP insertion were grown with 2-μg/ml tetracycline. NA, not assayed. Numbers in parentheses are for strains grown without NH_3; ethanolamine must serve as a nitrogen source.
[c] Induction by ethanolamine.
[d] Induction by CN-B_{12} plus ethanolamine over that seen for ethanolamine alone.
[e] Induction by Ado-B_{12} plus ethanolamine over that seen for ethanolamine alone.
[f] This strain has a functional *eut* operon with a *lac* operon fusion to the transcribed region distal to all genes.

Testing autogenous induction of the *eut* operon by EutT and EutR. Evidence is described above that EutT and CobA (but not EutA) contribute to formation of Ado-B_{12}, the inducer and required cofactor for ethanolamine degradative enzymes. This raises the question of the relative roles of EutT and CobA during normal growth on ethanolamine. The positive regulatory protein EutR is encoded within the *eut* operon (Fig. 1) and thus serves to activate its own production (autogeneous regulation) (27, 32). This autoinduction circuit is required for maximal operon expression (27, 32). The EutT protein also contributes to its own expression by producing Ado-B_{12}, a coinducer of the operon. Thus a strain with a *cobA* mutation and an uninduced *eutT* in the operon should not convert CN-B_{12} to Ado-B_{12} and therefore should show no induction of its *eut* operon. However, this situation might be unstable if a momentary stochastic expression of the operon provided enough EutT adenosyl transferase to initiate an autocatalytic induction (i.e., if a tiny bit of EutT produces enough Ado-B_{12} to supply more EutT and launch a full induction).

To test this, *cobA* strains with three different preinduced levels of EutT were examined for operon induction by CN-B_{12} as the Ado-B_{12} precursor (Table 6). The first two rows describe a normal *eut* operon with *lac* genes that were fused at a point within the operon distal to all structural genes (*eut-38*::*lac*), leaving EutT expression dependent on induction. With CobA (row 2), normal induction is seen, but without CobA, uninduced EutT allows very little induction (1.8-fold) by CN-B_{12} ethanolamine on NH_3-glycerol-ethanolamine medium. A slightly greater induction by CN-B_{12} (fivefold) was seen when ethanolamine must provide the nitrogen source (Table 6, row 1, numbers in parentheses). Previous work has revealed no effect of nitrogen limitation on *eut* operon transcription (27, 32). We suggest that when ethanolamine must provide a nitrogen source, the only cells that grow are those that have stochastically achieved a slightly higher operon expression level, which can be maintained by the produced EutT enzyme.

To test the effect of a higher basal level of EutT on operon inducibility, a *eutJ*::T-POP insertion was added to strains carrying various *eut-lac* reporter fusions; the T-POP insertion allows tetracycline-inducible expression of downstream genes including *eutR*. Increasing the level of EutR, is known to allow modest operon expression without regulatory effectors (27, 32). In *eutT*$^+$ strains expressing *eutR* from the *eutJ*::T-POP (Table 6, rows 3 and 4), ethanolamine alone did not induce operon induction with or without CobA, but ethanolamine plus CN-B_{12} caused full operon induction independent of CobA (compare rows 3 and 4). The full induction depended on the *eutT*$^+$ gene in strains lacking *cobA*: that is, when the reporter disrupted *eutT*, no induction was seen without CobA (rows 5 and 6). Thus a very slight increase in basal operon expression provided enough EutT (and adenosylated CN-B_{12}) for full operon induction by CN-B_{12} independent of CobA.

A still higher basal (uninduced) level of operon expression is seen in strains that express *eutR* from the stronger P_{int} promoter (Table 6, rows 7 to 10). When such strains had a functional *eutT* gene (rows 7 and 8), ethanolamine alone caused a slight induction but ethanolamine plus CN-B_{12} caused full induction independent of CobA (compare rows 7 and 8). This CobA-independent induction depended on EutT (compare rows 9 and 10 to 7 and 8). In all of the strains above, Ado-B_{12} induced the operon even when both CobA and EutT are limiting (see right column).

Physiological roles of EutT and CobA enzymes in ethanolamine metabolism. The above conclusions raise the question of the relative importance of EutT and CobA to growth on ethanolamine. To test this, an in-frame (nonpolar) deletion of the *eutT* gene was constructed and placed in strains with and without a *cobA* mutation. These strains were compared for

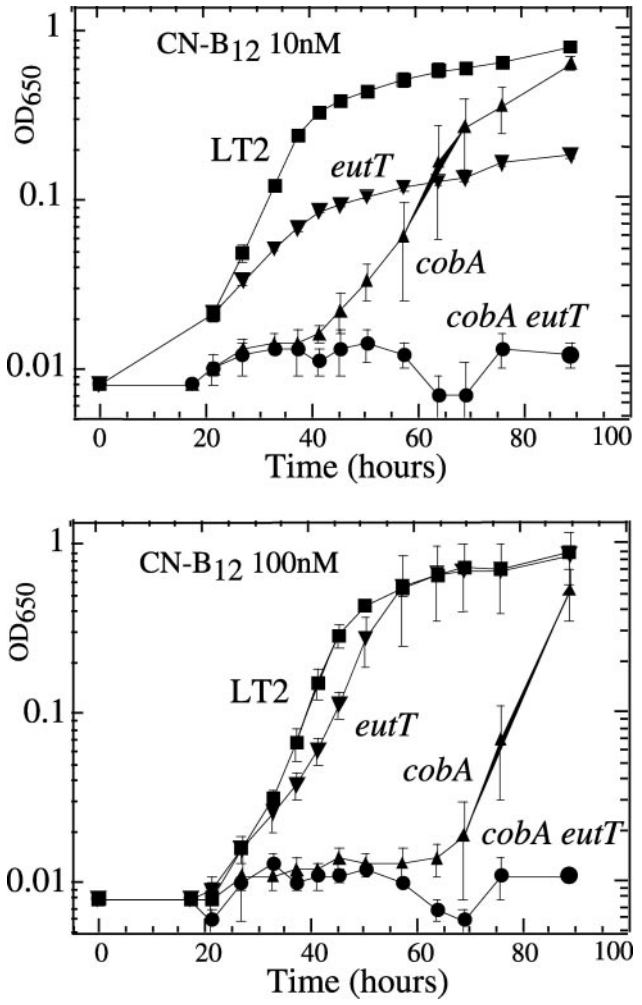

FIG. 3. Effects of *cobA* and *eutT* mutations on use of ethanolamine as a carbon source. Growth and media are described in Materials and Methods. In panel A, CN-B_{12} was added at 10 nM. In panel B, CN-B_{12} was added at 100 nM. All cultures were grown under low aerobic conditions at 30°C. The isogenic strains used were TT10000 (wild type; squares), TT25127 (*cobA*; upward triangles), TT24803 (*eutT*; downward triangles), and TT25128 (*cobA eutT*; circles).

their ability to grow on ethanolamine (Fig. 3). Lack of EutT caused little impairment of growth on high levels of exogenous B_{12}, but reduced growth rate at low B_{12} levels. Lack of CobA alone delayed growth initiation regardless of B_{12} level, presumably by making it difficult to induce the operon, as described above.

Evidence that EutT adenosyl transferase does not contribute to de novo B_{12} synthesis or assimilation of cobinamide. The CobA adenosyl transferase acts on several substrates, including CN-B_{12} or the incomplete corrinoid cobinamide (Cbi; the corrinoid ring lacking both the upper and lower ligands); CobA also acts on some biosynthetic intermediate (perhaps cobyric acid) leading to production of Ado-Cbi, the precursor of Ado-B_{12} (11). The experiments above demonstrated that EutT adenosylates assimilated CN-B_{12} but do not test its ability to use Cbi or a biosynthetic intermediate.

EutT, unlike CobA, does not adenosylate either Cbi or the biosynthetic intermediate. Strains for these tests expressed *eutT* from the tetracycline-inducible promoter in a *eutP*::TPOP insertion (19). Production of B_{12} in vivo was monitored by using a *metE* mutant, whose synthesis of methionine depends on the alternative B_{12}-dependent enzyme MetH (reviewed in reference 30). Results are outlined below.

First, a *metE* mutant can grow aerobically without methionine if Cbi is provided as a B_{12} precursor. This growth occurs because CobA enzyme adenosylates Cbi to Ado-Cbi, which can be converted to B_{12} (11). A *metE cobA* double mutant lacks the CobA adenosyl transferase and cannot produce B_{12} from Cbi; therefore, it cannot grow on minimal medium without added methionine (or B_{12}). This defect was not corrected by inducing EutT, suggesting that (unlike CobA) EutT cannot adenosylate assimilated Cbi.

Second, a *metE* mutant can grow anaerobically on minimal medium because B_{12} is synthesized de novo under these conditions. The de novo B_{12} pathway depends on adenosylation of an intermediate by CobA (11). A *metE cobA* double mutant cannot synthesize B_{12} de novo and fails to grow anaerobically on minimal medium. This defect was not corrected by expressing EutT, suggesting the EutT cannot adenosylate the intermediate in the normal biosynthetic pathway.

DISCUSSION

Ethanolamine metabolism requires Ado-B_{12} as a coinducer (with ethanolamine) of operon transcription and as a cofactor for the first enzyme (EA-lyase). Under natural conditions, Ado-B_{12} is not always available because it is synthesized only anaerobically and adenosylated corrinoids may not be present in all aerobic environments (30). By requiring Ado-B_{12} (plus ethanolamine) for induction of the *eut* operon, cells ensure that this gene system (17 proteins) is expressed only if both its substrate and required enzyme cofactor are present.

Induction of *eut* operon transcription is autocatalytic in the sense that the positive regulatory protein EutR is encoded within the operon that it controls (27). This arrangement is thought to be needed because the EutBC (EA-lyase) and EutR (regulator) proteins compete for binding Ado-B_{12} (32). By producing these proteins at a constant ratio (from genes in the same operon), the cell can respond to a wide range of Ado-B_{12} levels and remain sensitive to induction at all levels of operon expression; this can be achieved with very little investment in EutR protein prior to induction. The EutT adenosyl transferase adds a second autocatalytic element to this regulatory circuit by contributing to the level of a coinducer of the operon (Ado-B_{12}). Because of this circuit, cells need maintain only a minimal pool of Ado-B_{12} (made by CobA)—just sufficient to initiate *eut* operon induction. As the operon (and its *eutT* gene) is induced, EutT may supplement the Ado-B_{12} level during high-demand growth on ethanolamine.

The three cobalamin adenosyl transferases, CobA, PduO, and EutT, play distinct biological roles. The CobA enzyme may be the basal housekeeping activity. It supports de novo B_{12} synthesis (anaerobically) and can adenosylate the assimilated B_{12} precursor cobinamide, allowing its conversion to Ado-B_{12}. This basal Ado-B_{12} level initiates *eut* operon induction. The EutT enzyme adds Ado-B_{12} to support full induction and allows full growth ability during the period of high demand. The

PduO enzyme is thought to serve an analogous function during growth on propanediol, in which CobA seems to play a very minor aerobic role. The *pdu* operon is induced by propanediol alone (with no need for cofactor), and mutants lacking CobA or PduO singly show only slightly impaired growth on propanediol using CN-B_{12}, while the *cobA*, *pduO* double mutant fails to grow (17). It is not known whether PduO can substitute for CobA in de novo B_{12} synthesis or in assimilation of cobinamide.

It is surprising that the three cobalamin adenosyl transferases of *S. enterica*, EutT, PduO, and CobA, show no obvious amino acid sequence similarity (17). This could either reflect independent evolutionary origins or such extensive divergence from a common ancestor that homology is not apparent. If the three adenosyl transferases are derived from a common ancestor, their divergence may have been driven by the need (for PduO and EutT) to form close interactions with distinct groups of proteins contributing to the two pathways. The function of both these pathways is associated with formation of carboxysomes (7, 13, 14, 23).

The EutA protein appears to protect EA-lyase from inhibition by excessive CN-B_{12} (29). Evidence is provided that this protection does not involve conversion of inhibitory CN-B_{12} to Ado-B_{12}. It seems likely that EutA serves as a reactivating factor that removes damaged or inappropriate cofactor from the enzyme, as has been shown for several B_{12}-dependent enzymes (22, 35, 36). It is curious that while purified EA-lyase is strongly inhibited by CN-B_{12} (2) in vitro, this inhibition depends in vivo on the EutM, -N, -P, -Q, and -S proteins. While the functions of EutP and -Q are not known, the EutM, -N, and -S proteins resemble structural proteins or the carboxysome, an organelle thought to contain enzymes of the ethanolamine pathway. Thus sensitivity of *eutA* mutants to CN-B_{12} may require an intact carboxysome. We suggest that adenosylation (by both CobA and EutT) may occur primarily outside this compartment, and CN-B_{12} that enters the compartment may escape adenosylation and inhibit EA-lyase. Disruption of the carboxysome may make it impossible for excessive CN-B_{12} to escape adenosylation. Alternatively, these proteins may form complexes with EA-lyase that increase its sensitivity to CN-B_{12}.

ACKNOWLEDGMENTS

This work was supported by NIH grant GM34804 to J.R.R.

We thank Tom Fazzio, Chad Rappleye, Marian Price-Carter, Chris Mace, and Peter Anderson for helpful suggestions and conversations.

ADDENDUM IN PROOF

Evidence that EutA removes CN-B_{12} from lyase was published recently (K. Mori, R. Bando, N. Hieda, and T. Turaya, J. Bacteriol. **186:**6845–6854, 2004).

REFERENCES

1. **Andersson, D. L., and J. R. Roth.** 1989. Mutations affecting regulation of cobinamide biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **171:**6726–6733.
2. **Babior, B. M.** 1969. The mechanism of action of ethanolamine deaminase. I. Inhibition by coenzyme B_{12} analogues. J. Biol. Chem. **244:**2917–2926.
3. **Babior, B. M., T. J. Carty, and R. H. Abeles.** 1974. The mechanism of action of ethanolamine ammonia-lyase, a B12-dependent enzyme. J. Biol. Chem. **249:**1689–1695.
4. **Babior, B. M., H. Kon, and H. Lecar.** 1969. The mechanism of action of ethanolamine. V. The photolysis of enzyme-bound alkylcobalamins. Biochemistry **8:**2662–2889.
5. **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.
6. **Blackwell, C. M., and J. M. Turner.** 1978. Microbial metabolism of amino alcohols: purification and properties of coenzyme B_{12}-dependent ethanolamine ammonia-lyase of *Escherichia coli.* Biochem. J. **175:**555–563.
7. **Bobik, T. A., G. D. Havemann, R. J. Busch, D. S. Williams, and H. C. Aldrich.** 1999. The propanediol utilization (*pdu*) operon of *Salmonella enterica* serovar Typhimurium LT2 includes genes necessary for formation of polyhedral organelles involved in coenzyme B_{12}-dependent 1,2-propanediol degradation. J. Bacteriol. **181:**5967–5975.
8. **Bradbeer, C.** 1965. The clostridial fermentations of choline and ethanolamine. II. Requirement for a cobamide coenzyme by an ethanolamine deaminase. J. Biol. Chem. **240:**4675–4681.
9. **Brinsmade, S. R., and J. C. Escalante-Semerena.** 2004. The *eutD* gene of *Salmonella enterica* encodes a protein with phosphotransacetylase enzyme activity. J. Bacteriol. **186:**1890–1892.
10. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. A manual for genetic engineering advanced bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
11. **Escalante-Semerena, J. C., S.-J. Suh, and J. R. Roth.** 1990. *cobA* function is required for both de novo cobalamin biosynthesis and assimilation of exogenous corrinoids in *Salmonella typhimurium.* J. Bacteriol. **172:**273–280.
12. **Faust, L. R., and B. M. Babior.** 1992. Over expression, purification, and some properties of the Ado Cbl-dependent ethanolamine ammonia-lyase from *Salmonella typhimurium.* Arch. Biochem. Biophys. **294:**50–54.
13. **Havemann, G. D., and T. A. Bobik.** 2003. Protein content of polyhedral organelles involved in coenzyme B_{12}-dependent degradation of 1,2-propanediol in *Salmonella enterica* serovar Typhimurium LT2. J. Bacteriol. **185:**5086–5095.
14. **Havemann, G. D., E. M. Sampson, and T. A. Bobik.** 2002. PduA is a shell protein of polyhedral organelles involved in coenzyme B_{12}-dependent degradation of 1,2-propanediol in *Salmonella enterica* serovar Typhimurium LT2. J. Bacteriol. **184:**1253–1261.
15. **Jeter, R., B. M. Olivera, and J. R. Roth.** 1984. *Salmonella typhimurium* synthesizes cobalamin (vitamin B_{12}) de novo under anaerobic growth conditions. J. Bacteriol. **159:**206–213.
16. **Joblin, K., A. Johnson, M. Lappert, and O. Wallis.** 1976. Coenzyme B12 dependent reactions. Part IV. Observations on the purification of ethanolamine ammonia-lyase. Biochim. Biophys. Acta **452:**262–270.
17. **Johnson, C. L. V. J., E. Pechonick, S. D. Park, G. D. Havemann, N. A. Leal, and T. A. Bobik.** 2001. Functional genomic, biochemical, and genetic characterization of the *Salmonella pduO* gene, an ATP:cob(I)alamin adenosyltransferase gene. J. Bacteriol. **183:**1577–1584.
18. **Jones, P. W., and J. M. Turner.** 1984. A model for common control of enzymes of ethanolamine metabolism in *Escherichia coli.* J. Gen. Microbiol. **130:**849–860.
19. **Kofoid, E., C. Rappleye, I. Stojiljkovic, and J. Roth.** 1999. The 17-gene ethanolamine (*eut*) operon of *Salmonella typhimurium* encodes five homologues of carboxysome shell proteins. J. Bacteriol. **181:**5317–5329.
20. **Lawrence, J. G., and J. R. Roth.** 1995. The cobalamin (coenzyme B_{12}) biosynthetic genes of *Escherichia coli.* J. Bacteriol. **177:**6371–6380.
21. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Mori, K., T. Tobimatsu, T. Hara, and T. Toraya.** 1997. Characterization, sequencing, and expression of the genes encoding a reactivating factor for glycerol-inactivated adenosylcobalamin-dependent diol dehydratase. J. Biol. Chem. **272:**32034–32041.
23. **Penrod, J., K. Czymmek, D. Sheppard, E. Kofoid, and J. Roth.** Unpublished results.
24. **Price-Carter, M., J. Tingey, T. A. Bobik, and J. R. Roth.** 2001. The alternative electron acceptor tetrathionate supports B_{12}-dependent anaerobic growth of *Salmonella enterica* serovar Typhimurium on ethanolamine or 1,2-propanediol. J. Bacteriol. **183:**2463–2475.
25. **Rappleye, C. A., and J. R. Roth.** 1997. A Tn*10* derivative (T-POP) for isolation of insertions with conditional (tetracycline-dependent) phenotypes. J. Bacteriol. **179:**5827–5834.
26. **Ratzkin, B., and J. Roth.** 1978. Cluster of genes controlling proline degradation in *Salmonella typhimurium.* J. Bacteriol. **133:**744–754.
27. **Roof, D. M., and J. R. Roth.** 1992. Autogenous regulation of ethanolamine utilization by a transcriptional activator of the *eut* operon in *Salmonella typhimurium.* J. Bacteriol. **174:**6634–6643.
28. **Roof, D. M., and J. R. Roth.** 1988. Ethanolamine utilization in *Salmonella typhimurium.* J. Bacteriol. **170:**3855–3863.
29. **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.
30. **Roth, J. R., J. G. Lawrence, and T. A. Bobik.** 1996. Cobalamin (coenzyme B12): synthesis and biological significance. Annu. Rev. Microbiol. **50:**137–181.

31. **Scarlett, F. A., and J. M. Turner.** 1976. Microbial metabolism of amino alcohols. Ethanolamine catabolism mediated by coenzyme B_{12}-dependent ethanolamine ammonia-lyase in *Escherichia coli* and *Klebsiella aerogenes.* J. Gen. Microbiol. **95:**173–176.
32. **Sheppard, D. E., and J. R. Roth.** 1994. A rationale for autoinduction of a transcriptional activator: ethanolamine ammonia-lyase (EutBC) and the operon activator (EutR) compete for adenosyl-cobalamin in *Salmonella typhimurium.* J. Bacteriol. **176:**1287–1296.
33. **Stojiljkovic, I., A. J. Bäumler, and F. Heffron.** 1995. Ethanolamine utilization in *Salmonella typhimurium*: nucleotide sequence, protein expression, and mutational analysis of the *cchA cchB eutJ eutG eutH* gene cluster. J. Bacteriol. **177:**1357–1366.
34. **Suh, S.-J., and J. C. Escalante-Semerena.** 1995. Purification and initial characterization of the ATP:corrinoid adenosyltransferase encoded by the *cobA* gene of *Salmonella typhimurium.* J. Bacteriol. **177:**921–925.
35. **Tobimatsu, T., H. Kajiura, M. Yunoki, M. Azuma, and T. Toraya.** 1999. Identification and expression of the genes encoding a reactivating factor for adenosylcobalamin-dependent glycerol dehydratase. J. Bacteriol. **181:**4110–4113.
36. **Toraya, T., and K. Mori.** 1999. A reactivating factor for coenzyme B12-dependent diol dehydratase. J. Biol. Chem. **274:**3372–3377.
37. **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.