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Conserving a Volatile Metabolite: a Role for Carboxysome-Like Organelles in *Salmonella enterica*

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Salmonellae can use ethanolamine (EA) as a sole source of carbon and nitrogen. This ability is encoded by an operon (*eut*) containing 17 genes, only 6 of which are required under standard conditions (37°C; pH 7.0). Five of the extra genes (*eutM*, *-N*, *-L*, *-K*, and *-G*) become necessary under conditions that favor loss of the volatile intermediate, acetaldehyde, which escapes as a gas during growth on EA and is lost at a higher rate from these mutants. The *eutM*, *-N*, *-L*, and *-K* genes encode homologues of shell proteins of the carboxysome, an organelle shown (in other organisms) to concentrate CO_2. We propose that carboxysome-like organelles help bacteria conserve certain volatile metabolites—CO_2 or acetaldehyde—perhaps by providing a low-pH compartment. The EutG enzyme converts acetaldehyde to ethanol, which may improve carbon retention by forming acetals; alternatively, EutG may recycle NADH within the carboxysome.

Salmonella enterica can use ethanolamine (EA) as a sole source of carbon and nitrogen under aerobic and anaerobic conditions (25, 29, 30). EA is degraded in two steps (5, 34). The first enzyme (ethanolamine ammonia-lyase) requires the cofactor adenosyl cobalamin (Ado-B_{12}) (6), which is synthesized by *S. enterica* under anaerobic conditions but must be imported under aerobic conditions (17, 31). In this B_{12}-dependent reaction, EA is cleaved to ammonia plus acetaldehyde, which is then converted to acetyl-coenzyme A (acetyl-CoA) by an NAD-dependent oxidoreductase (Fig. 1).

Genetic screens revealed six genes required for the use of EA—*eutA*, *-B*, *-C*, *-D*, *-E*, and *-R*—all located within the 17-gene *eut* operon (20, 30). All other *eut* genes showed no mutant phenotype under standard conditions but seemed likely to contribute to EA use, because the operon (20) is induced by EA plus Ado-B_{12} (28, 37). In keeping with this expectation, several of these extra genes support use of EA under specific nonstandard conditions. The *eutT* gene encodes an adenosyl transferase that forms Ado-B_{12} (the lyase cofactor) from OH- or CN-B_{12} (9, 36). This provides more Ado-B_{12} during periods of high demand and is required in the absence of the general adenosyl-transferase, CobA (36). The EutH protein facilitates ethanolamine diffusion (see below) and is required only at very low levels of EA (23).

Five of the extra genes (*eutS*, *-M*, *-N*, *-L*, and *-K*) encode homologues of proteins that form the carboxysome, an organelle found in cyanobacteria and sulfur-oxidizing bacteria that concentrates CO_2 for fixation by ribulose-bisphosphate carboxylase (RuBisCO) (38). We will refer to the *Salmonella* organelles as carboxysomes, despite the lack of evidence that CO_2 is concentrated or fixed. We suspect that *Salmonella* does both during growth on ethanolamine. These organelles have been referred to elsewhere as "metabolosomes," "enterosomes," "polyhedral bodies," or "bacterial microcompartments." Another set of carboxysome protein homologues is encoded in an operon (*pdu*) that supports catabolism of 1,2-propanediol (1,2-PDL) by a pathway very similar to that for EA (4). The two organelle types form independently, and each type has been visualized during growth on either 1,2-PDL (16) or EA (8, 12). Several enzymes of the 1,2-PDL pathway copurify with carboxysome structures encoded by the *pdu* operon (16), indicating a close association of proteins catalyzing 1,2-PDL metabolism. Carboxysomes serve an unknown purpose in *S. enterica* but seem likely to play the same role in the similar EA and 1,2-PDL pathways and may share mechanistic features with related organelles that support CO_2 fixation in other bacteria.

It was suggested previously that carboxysomes might protect cells from toxic effects of reactive aldehydes in the EA and 1,2-PDL pathways (8, 16, 26, 27, 41). Support for this idea came from the finding that strains defective for *polA* (encoding a DNA repair polymerase) or for synthesis of glutathione (*gsh*) failed to grow on EA. It was suggested that this phenotype reflects lack of a protective reducing agent (for *gsh*) or inability to repair DNA damage caused by aldehyde (*polA*). While toxicity is plausible, inhibition by endogenous acetaldehyde was not demonstrated, and the *polA* and *gsh* mutants used may be supersensitive to levels of acetaldehyde that are not normally toxic. In previous work, we presented evidence that endogenous acetaldehyde can accumulate to toxic levels in *eutE* mutant cells, which lack acetaldehyde oxidoreductase, the second step in EA utilization (23). Thus, the toxicity of endogenous acetaldehyde has been demonstrated or inferred only for mutants that either accumulate very high aldehyde levels or are supersensitive to its effects. We suspect that acetaldehyde toxicity is seldom a problem for wild-type *Salmonella*.

It is suggested here that the primary role of carboxysomes in *S. enterica* is not to protect cells against the toxic effects of aldehyde (which are seen only under extreme conditions) but rather to enhance the use of EA and 1,2-PDL by retaining a volatile intermediate that would otherwise be lost to the gas phase. The aldehyde intermediates in EA and 1,2-PDL (acetaldehdye and propionaldehyde) are volatile and subject to loss as vapor. In trying to learn how carboxysomes work, it seems reasonable to seek a single mechanism that will explain how

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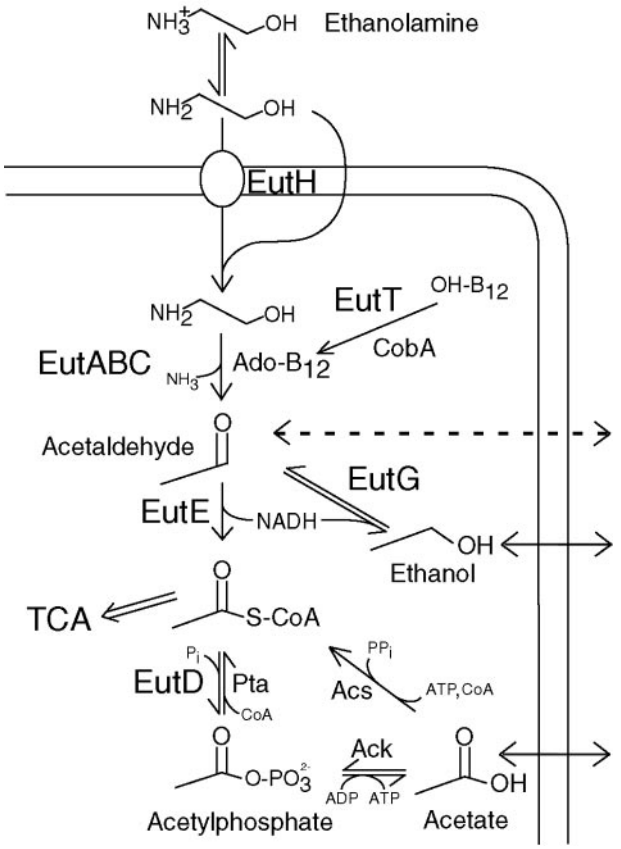

FIG. 1. Basic ethanolamine metabolism. EA can enter the cell by diffusion, either via EutH or by other, unidentified routes. The diagram depicts the known roles of *eut* enzymes in metabolism of EA. The loss of acetaldehyde to the gas phase proposed here is indicated by a dotted arrow.

homologous organelles concentrate CO_2 in support of photosynthesis (in some bacteria) (10, 19) and restrict loss of volatile aldehydes (in enteric bacteria). These aldehydes and CO_2 are both gases whose volatility and loss from solution are affected by pH. A model for carboxysome function is suggested in which the organelle provides a low-pH compartment that favors conversion of aldehydes to less volatile acetals; such a compartment would serve CO_2 fixation (in other bacteria) by helping maintain a high level of dissolved CO_2 gas, the substrate for RuBisCO.

MATERIALS AND METHODS

Strains and crosses. All strains (Table 1) are derivatives of *Salmonella enterica* serovar Typhimurium strain LT2. Transductional crosses were mediated by a high-frequency transducing mutant of phage P22 (HT105 *int*) (35) and performed as described previously (14).

Media and growth conditions. Rich medium (NB) was Difco nutrient broth with added NaCl (0.1 mM). Minimal solid medium for growth assays was 5 mM KH_2PO_4, 5 mM $NaNH_4HPO_4$, 1 mM $MgSO_4$ buffered at various pHs by 50 mM MES [2-(*N*-morpholino)ethanesulfonic acid; pH 5.5 or 6.0], MOPS [3-(*N*-morpholino)propanesulfonic acid; pH 7.0], or Bicine (pH 8.0 or 8.5) as described previously (23). Ethanolamine hydrochloride and sodium succinate (Sigma) served as carbon sources at appropriate concentrations. In the construction of *eutS*, *eutM*, *eutJ*, and *eutG* deletion mutations (see below), glycerol (20 mM; Malinckrodt) was used as a carbon source and EA as a nitrogen source in NCN minimal medium (2). Cyanocobalamin (Sigma) was provided at 150 nM and is referred to simply as B_{12}. Rich medium was solidified by 1.5% agar (EM Science) and minimal media by 1.5% Noble agar (U.S. Biological). To measure growth rates, inocula were 3-ml nutrient broth cultures from single colonies. After overnight growth, cells were pelleted by low-speed centrifugation (Sorval RC5B+ at 4,700 rpm for 10 min), washed in pH 7.0 NCE (no citrate E) minimal medium (2), resuspended in the same medium, and used (35 μl) to inoculate 6 ml of growth medium. Growth was monitored in three parallel cultures as optical density at 650 nm.

Acetaldehyde assay. Acetaldehyde released to the gas phase was measured using a three-part device. (i) Air was forced by an aquarium pump through water to hydrate it. (ii) The hydrated air was delivered through a manifold to the surface of the growth media of six tubes and vented from the tops of theses tube. (iii) The effluent air from each tube was delivered to the bottom of an inclined column containing 3 ml of 0.01% 3-methyl-2-benzothiazolone, which reacts with aldehyde to form an azine derivative that absorbs light from 300 to 360 nm (39). The detection solution was replaced every 30 min, and the optical density at 308 nm was read to determine aldehyde using a standard curve. Quantitative aldehyde trapping was demonstrated using pure acetaldehyde. These assays were performed on cultures growing at 37°C in a shaking water bath (240 rpm) in NCE medium at pH 7.0. The medium included EA (15 mM), B_{12} (150 nM), and glycerol (27 mM) except when noted. In experiments testing *pdu* mutants, 1,2-propanediol (80 mM), sodium pyruvate (40 mM), and B_{12} (150 nM) were added; incubation was at 38°C. For testing the effects of pH on acetaldehyde release (see Fig. 8 and 9), minimal medium was as described for solid media above with reduced phosphate and 50 mM MOPS (at pH 7) or 50 mM Bicine (pH 8) as a buffer.

The cumulative aldehyde release shown below (see Fig. 4 to 6 and 9) was

TABLE 1. Strain list

Strain	Genotype	Source
TR10000	Wild-type LT2	Laboratory collection
TT22815	*eutE356*Δ::FRT (sw)[a]	This work
TT22522	*eutS367*Δ	This work
TT22523	*eutM365*Δ	This work
TT23230	*eutH360*Δ	This work
TT23036	*eutG361*Δ	This work
TT23035	*eutJ362*Δ	This work
TT24801	*eutP369*Δ::FRT (sw)	This work
TT24802	*eutQ370*Δ::FRT (sw)	This work
TT24803	*eutT371*Δ::FRT (sw)	This work
TT24804	*eutD372*Δ::FRT (sw)	This work
TT24805	*eutA373*Δ::FRT (sw)	This work
TT24806	*eutB374*Δ::FRT (sw)	This work
TT24807	*eutC375*Δ::FRT (sw)	This work
TT22569	*eutN366*Δ::FRT (sw)	This work
TT22570	*eutL364*Δ::FRT (sw)	This work
TT22571	*eutK363*Δ::FRT (sw)	This work
TT22971	*metA22 metE551 trpD2 ilv-452 leu pro* (leaky) *hsdLT6 hsdSA29 hsdB strA120*/pKD46 *araC bla oriR101 repA101*(Ts) lambda red (*gam*$^+$ *bet*$^+$ *exo*$^+$)	Don Court (11)
TT19096	*eutJ269*::T-pop	Laboratory collection
TT19097	*eutG270*::T-pop	Laboratory collection
TT20357	*eutS334*::T-pop	Laboratory collection
TT19105	*eutM278*::T-pop	Laboratory collection
	Wild-type LT2	T. A. Bobik
BE87	*pduCDE*Δ	T. A. Bobik
BE184	*pduJ654*Δ	T. A. Bobik
BE185	*pduK655*Δ	T. A. Bobik
BE191	*pduP659*Δ	T. A. Bobik
BE213	*pduB675*Δ::FRT (sw)	T. A. Bobik

[a] sw, swap. The indicated sequence FRT is exchanged for the gene's coding sequence.

calculated as follows. Aldehyde release measurements for each 30-minute period were averaged from three independent cultures. These average values described a graph that depicted aldehyde release per 30-minute period; 80% of these rate measurements had standard deviations of less than 18% of the mean value. This curve was integrated to determine the aggregate release at each point, which is depicted in each figure.

Construction of in-frame *eut* deletion mutants. A chromosomal deletion mutation was constructed for each *eut* gene using linear transformation mediated by the Red functions of phage lambda (13, 43) as described previously (23). Each deletion replaced the coding sequence with a short sequence that did not disrupt the reading frame or translational coupling. The constructed deletions were transduced by P22-mediated crosses into a strain with a wild-type genetic background, and their structures were verified by PCR amplification and sequencing of the junction.

Deletions of the *eutP*, *eutQ*, *eutT*, *eutD*, *eutN*, *eutA*, *eutB*, *eutC*, *eutL*, and *eutK* genes were constructed as described previously (13) using an inserted chloramphenicol resistance (*cat*) cassette. Deletions of the *eutS*, *eutM*, *eutJ*, and *eutG* genes were made by positive selection for removal of a polar mutation in the particular gene. A small linker DNA was used as a template in a PCR with two primers (P1 and P2). The 5′ end of P1 was identical to the first 40 bp of the *eut* gene deleted, and the 3′ end was identical to the 5′ end of the linker. The 5′ end of P2 was reverse complementary to the last 40 bp of the *eut* gene, and the 3′ end was reverse complementary to the 3′ end of the linker. The recipient's polar insertion prevented growth on EA because it reduced expression of the downstream *eutBC* genes. The polar mutation was removed by Red-mediated recombination using the donor fragment described above and selecting for growth on EA as a nitrogen source. The constructed deletion mutant allele had an open reading frame lacking the bulk of the internal coding sequence. All genes deleted in this way were dispensable for growth on EA as a carbon and nitrogen source at pH 7.0 and were located promoter proximal to the *eutBC* genes.

RESULTS

A set of nonpolar deletions of single *eut* genes. A nonpolar deletion mutation was constructed for each of the 16 *eut* structural genes (see Materials and Methods). Each mutant was tested for the ability to use EA as a carbon source on solid medium (40 mM EA, 150 nM CN-B_{12}; 37°C; pH 7.0). Under these standard conditions, *eutA*, *-B*, *-C*, *-D*, or *-E* mutants showed a Eut$^-$ phenotype, and all other *eut* mutants were phenotypically indistinguishable from the wild-type strain LT2. These results (Table 2, pH 7.0) confirm previous conclusions (20, 30) based on point mutations and multigene deletions.

Effects of pH on mutant phenotypes. The single-gene *eut* deletion mutants fell into four phenotypic classes based on their abilities to use ethanolamine as a carbon source on solid media at various nonstandard pH values (Table 2).

Class I mutants failed to use EA at all tested pH values and eliminated the previously described enzymatic functions, EutA, -B, -C, -D, and -E. (Mutants lacking the positive regulatory protein EutR also fell into this class.)

Class II mutants grew at pH 7 or below, but not at pH 8. This class included mutants lacking the carboxysome protein homologues EutM, -N, -L, and -K or the alcohol dehydrogenase EutG. Note that the *eutS* deletion, which lacks another carboxysome shell protein homologue, had no mutant phenotype at high pH under these conditions.

Class III contained only the *eutH* mutant, which showed impaired growth only at low pH as described previously (23).

Class IV mutants behaved like the wild type and showed little or no growth impairment at pH values at or below pH 8. The group included *eutS*, *-P*, *-Q*, *-T*, and *-J* mutants.

These phenotypes are based on qualitative scoring of growth patches on solid minimal medium. The conclusions were verified by growth rate determinations in liquid cultures. Growth rates for several critical strains are presented below.

TABLE 2. Effect of pH on use of ethanolamine as a carbon source

Class	Strain(s)	Growth on ethanolamine as carbon source at pH[a]:				
		5.5	6.0	7.0	8.0	8.5
	Wild-type LT2	4	4	4	4	0
I	*eutA*, *-B*, *-C*, *-D*, and *-E* mutants	0	0	0	0	0
II	*eutM*, *-N*, *-G*, *-L*, and *-K* mutants	4	4	4	0	0
III	*eutH* mutant	0	2	4	4	0
IV	*eutS*, *-P*, *-O*, *-T*, and *-J* mutants	4	4	4	4	0

[a] Numbers represent growth, with 4 being equivalent to that shown by the wild type and 0 indicating no detectable growth after 48 h.

Conservation of a volatile metabolite: a model for carboxysome function. In developing a testable model to explain these results, it seemed likely that external pH might affect EA degradation in two ways. First, it increases the rate of EA influx by raising the concentration of unprotonated ethanolamine, which enters cells by diffusion (23). Second, pH affects the toxicity and volatility of acetaldehyde, an intermediate in EA degradation. Low pH favors reaction of acetaldehyde with alcohols to form less reactive, less volatile acetals. If at high external pH, EA enters and is converted to acetaldehyde faster than acetaldehyde can be consumed, an accumulation is expected. This accumulation could be subject to loss to the gas phase and/or could inhibit growth due to toxic effects.

We propose that external pH affects the rate at which the aldehyde produced escapes from cells and from the medium. Cultures growing aerobically on EA smell strongly of acetaldehyde (the odor of cocktail onions), which suggested initially that this intermediate might be lost to the gas phase at a significant rate. Excessive aldehyde loss might limit the carbon and energy obtainable from ethanolamine. The pH-dependent phenotypes described above would be explained if carboxysomes minimized the loss of aldehyde from cells and therefore became essential when ambient conditions increased the rate of carbon loss above a critical level. The carboxysome might restrict loss by providing a low-pH compartment in which aldehydes are converted to less volatile acetals by combination with alcohols. The need for carboxysomes might be reduced at low outside pH, because aldehyde is retained better in the medium (for reassimilation) or because the intracellular pH drops sufficiently to allow the whole cytosol to play the role of the carboxysome. The model proposes that the principal role of the carboxysome is retention of gases rather than protection of the cell from toxic effects. Some predictions of this model are tested below.

Acetaldehyde vapor is released during growth in the presence of ethanolamine. Air was forced across liquid cultures (see Materials and Methods) and through a trapping solution containing a reagent that reacts with aldehyde to form a colored complex. Cells grown without ethanolamine on acetate, glycerol, or glucose (glucose data not shown) showed no detectable aldehyde release (Fig. 2).

Aldehyde release was detected when ethanolamine and B_{12} were added to medium containing glycerol. Mutations blocking

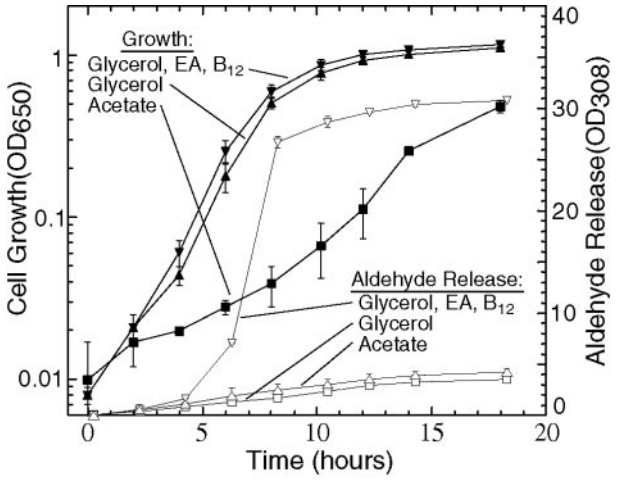

FIG. 2. Growth and aldehyde release by wild-type cells. Growth (closed symbols) and aldehyde release (open symbols) of strain LT2 grown on minimal acetate (squares), on glycerol (triangles, points up), or on glycerol plus ethanolamine and B_{12} (triangles, points down). Minimal medium was NCE salts (pH 7.0), which provides ammonia as a nitrogen source. Cultures were grown at 37°C, and ethanolamine was provided at 30 mM. The error bars represent standard deviations.

the first step in ethanolamine degradation (*eutABC*) prevented this aldehyde release (Fig. 3), suggesting that the assay detects acetaldehyde generated endogenously from ethanolamine.

The wild type and all *eut* mutants grew on glycerol at the same rate with or without added EA; that is, there was no evidence that ethanolamine (or derived acetaldehyde) was toxic under these conditions (Fig. 2 and 3). It should be noted that the *eut* operon is well induced by ethanolamine plus B_{12} in the presence of these carbon sources; glucose reduces induction only about threefold (28). The toxicity of ethanolamine seen previously for a *eutE* mutant growing on succinate (23) required a higher level of EA (40 mM).

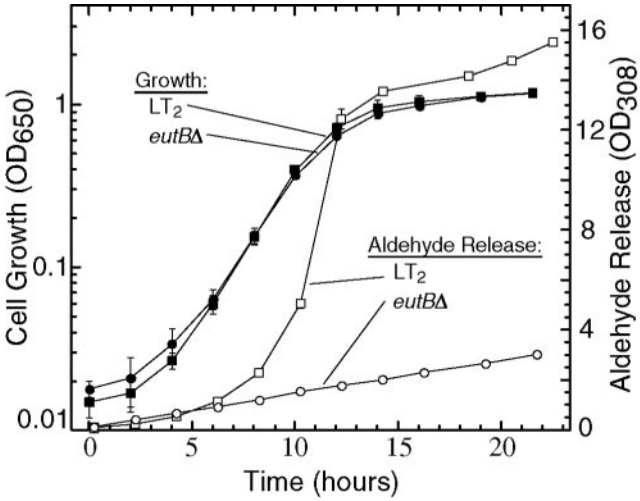

FIG. 3. Aldehyde release requires ethanolamine ammonia lyase. Growth (closed symbols) and aldehyde release (open symbols) of LT2 (squares) and a *eutB* mutant (circles) on minimal glycerol, ethanolamine, and B_{12} medium. Ethanolamine was provided at 15 mM. The error bars represent standard deviations.

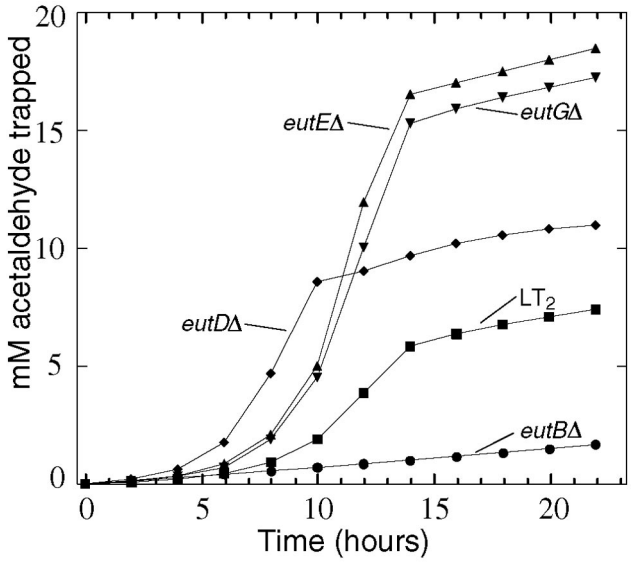

FIG. 4. Mutations that block acetaldehyde metabolism increase aldehyde release. Mutants *eutB* (circles), *eutD* (diamonds), *eutE* (triangles, points up), and *eutG* (triangles, points down) were compared to wild-type LT2 (squares) for aldehyde release during growth on glycerol, ethanolamine, and B_{12}. Ethanolamine was provided at 15 mM.

Pathway blocks increase aldehyde release. Mutants blocked from further metabolism of acetaldehyde (*eutG* or *eutE*) released more acetaldehyde than wild-type cells (Fig. 4). As described above, cells were grown on glycerol with added EA and B_{12}. A slight increase was seen for *eutD* mutants, suggesting some secondary acetaldehyde accumulation in a strain blocked for conversion of acetyl-CoA to acetyl-PO_4 (7, 40). Growth of all strains (on glycerol) was indistinguishable from that of the wild type (Fig. 2 and 3), suggesting that this increased aldehyde level was not detectably toxic.

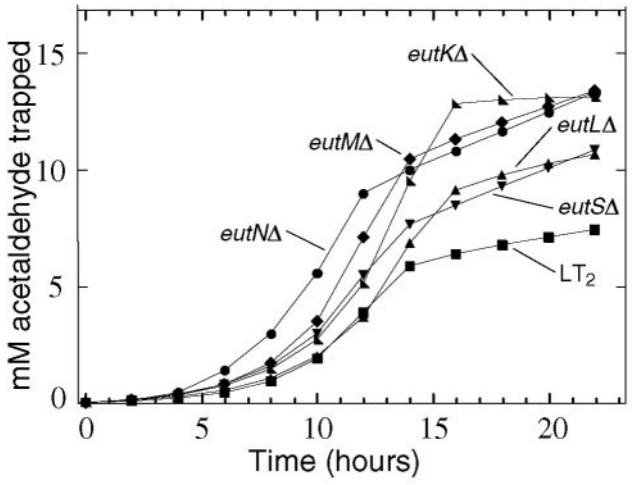

FIG. 5. Aldehyde release increases in carboxysome mutants. Mutants *eutN* (circles), *eutM* (diamonds), *eutL* (triangles, points up), *eutK* (right triangles), and *eutS* (triangles, points down) were compared to wild-type LT2 (squares) for aldehyde release during growth on glycerol, ethanolamine, and B_{12}. Ethanolamine was provided at 15 mM.

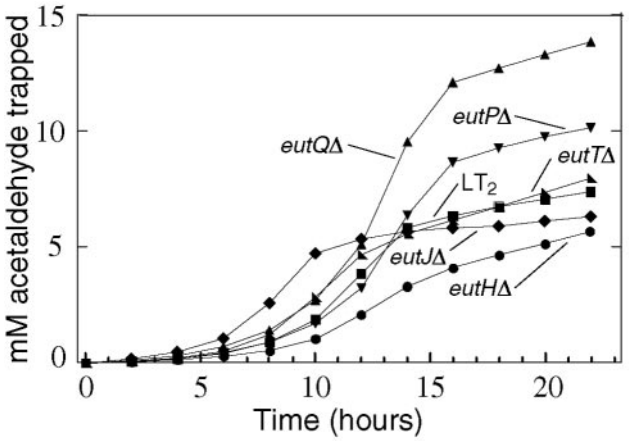

FIG. 6. Effects of other *eut* mutations on aldehyde release. Mutants *eutH* (circles), *eutJ* (diamonds), *eutQ* (triangles, points up), *eutT* (right triangles), and *eutP* (triangles, points down) were compared to wild-type LT2 (squares) for aldehyde release during growth on glycerol, ethanolamine, and B_{12}. Ethanolamine was provided at 15 mM.

Mutants lacking carboxysome proteins released more acetaldehyde. Mutations eliminating the *eutM*, *-N*, *-L*, or *-K* gene increased aldehyde release during growth on glycerol plus EA (Fig. 5). These mutants (and *eutG*) fell into phenotype class II above. The *eutS* mutant, a member of class IV, resembled other carboxysome mutants in aldehyde release even though it showed no obvious growth phenotype at pH 8. The remaining genes in the operon fell into phenotype class III or IV (described above). Mutants lacking EutT (adenosyl transferase) or EutJ (unknown) show about the same level of aldehyde release as the wild type. The EutH (transport) defect reduced release slightly. Mutants lacking EutP and EutQ proteins released more acetaldehyde than the wild type, but the functions of these proteins are unknown (Fig. 6). The growth of these mutants (on glycerol) was identical to that of the wild type (Fig. 2 and 3), so aldehyde release was not toxic at these levels.

The growth phenotype of carboxysome mutants is corrected by restricting diffusion. In the plate growth experiments described above, class II mutants (*eutMNLKG*) failed to use EA on media with a high pH. These tests were made in a standard 37°C incubator with loose petri dish lids, allowing free exchange of gases with the surrounding atmosphere. The effects of this exchange were tested in two ways. First, exchange was limited by incubating plates as described above, but at 30°C. Second, gas exchange was limited (at 37°C) by incubating plates in a closed air-tight box (2.5 liters). The container provided sufficient oxygen to support growth but restricted the loss of gases (such as acetaldehyde).

As seen in Table 3, both reduced temperature and containment allowed wild-type cells to extend their range of growth from pH 8 up to pH 8.5. Restricted exchange also allowed mutants of classes III and IV to grow at pH 8.5 (data not shown). Control experiments (not shown) demonstrated that oxygen was sufficient (full growth on glycerol) and that pH differences did not affect the ability of the wild type to grow on ethanolamine (Table 2) or glycerol (not shown).

More importantly, diffusion limitation corrected the high-pH phenotypes of carboxysome-defective mutants (*eutM*, *-N*, *-K*, and *-L*) and the other mutant of class II, *eutG*. That is, containment extended the growth of these mutants from pH 7 to pH 8.5, and low temperature extended their growth from pH 7 to pH 8. Raising the temperature to 40°C reduced the growth of wild-type cells at pH 6.0 and prevented their growth at pH 7.0 and 8.0 (data not shown). A growth improvement caused by retaining aldehyde vapor is consistent with the mutant phenotype being due to excessive loss of aldehyde and is inconsistent with a phenotype due to aldehyde toxicity.

The *eutG* mutant lacks aldehyde reductase, which converts internal acetaldehyde to ethanol. This mutant had a growth phenotype like that of the carboxysome mutants (see above) and released more aldehyde than the wild type. As described below, the phenotype of *eutG* mutants (like that of carboxysome mutants) is corrected by providing a different carbon source, suggesting that EutG helps to conserve or use aldehyde rather than removing a toxic accumulation. The ethanol produced by EutG may promote formation of acetals within the carboxysome. Alternatively, this enzyme may stimulate EutE (oxidoreductase) by recycling NADH produced within the carboxysome.

If restricting loss of aldehyde to the outside environment improves growth, one might expect acetaldehyde vapor to support the growth of *S. enterica* on medium devoid of any other carbon source. To test this, a *eutB* and a *eutM* mutant were grown in adjacent wells of a 96-well plate. The *eutB* mutant (lacking ethanolamine ammonia-lyase) was given EA plus B_{12}, which does not support its growth but will induce the operon. When a *eutM* mutant (defective in carboxysomes) was grown nearby on glycerol, the neighboring *eutB* cultures still failed to grow. However when the *eutM* mutant was given EA and B_{12} in addition to glycerol, the *eutB* mutant in nearby wells was allowed to grow, presumably on the acetaldehyde liberated by the growing *eutM* mutant (data not shown). In addition, lyase mutants (induced for *eut* by EA and B_{12}) grew on medium when aldehyde vapor was bubbled from an aldehyde solution into the growth medium. The final density of the fed culture depended on the initial concentration of aldehyde in the source solution (data not shown).

Growth on ethanolamine is impaired by increased gas exchange. The model predicts that more rapid exchange of ambient air might increase the loss of acetaldehyde vapor and reduce growth on ethanolamine. This effect should be more severe in mutants impaired in the ability to retain acetaldehyde. To test these predictions, petri plates were incubated

TABLE 3. Growth on ethanolamine with restricted gas exchange

Class	Strains	Conditions	Use of EA as carbon source at pH[a]:				
			5.5	6.0	7.0	8.0	8.5
	Wild-type LT2	37°C, standard plates	4	4	4	4	0
		37°C, confined	4	4	4	4	4
		30°C, standard plates	4	4	4	4	4
II	*eutM*, *-N*, *-G*, *-L*, and *-K* mutants	37°C, standard plates	4	4	4	0	0
		37°C, confined	4	4	4	4	4
		30°C, standard plates	4	4	4	4	0

[a] Numbers represent growth, with 4 being equivalent to that shown by the wild type and 0 indicating no detectable growth.

TABLE 4. Ethanolamine use during rapid gas exchange

Class	Strain(s)	Treatment	Use of EA as carbon source at pH[a]:				
			5.5	6.0	7.0	8.0	8.5
	Wild-type LT2	Standard plates	4	4	4	4	0
		Flushed (O_2, N_2)	4	4	4	0	0
		Flushed (O_2, N_2, CO_2)	4	4	4	4	4
II	*eutG*, *-M*, *-N*, *-L*, and *-K* mutants	Standard plates	4	4	4	0	0
		Flushed (O_2, N_2)	0	0	0	0	0
		Flushed (O_2, N_2, CO_2)	4	4	4	4	4

[a] Numbers represent growth, with 4 being equivalent to that shown by the wild type and 0 indicating no detectable growth.

TABLE 5. Suppression of class I mutants by CO_2

Strain(s)	Conditions	Growth on EA as carbon source at pH[a]:				
		5.5	6.0	7.0	8.0	8.5
LT2	37°C/standard plates	4	4	4	4	0
	30°C/standard plates	4	4	4	4	4
eutA, *-B*, *-C*, *-D*, and *-E* mutants	37°C/standard plates	0	0	0	0	0
	30°C/standard plates	0	0	0	0	0
eutA, *-B*, *-C*, and *-D* mutants	Contained	2	2	0	0	0
	Flushed (CO_2)	3	3	3	2	0
eutE mutant	Contained	0	0	0	0	0
	Flushed (CO_2)	0	0	0	0	0

[a] Numbers represent growth, with 4 being equivalent to that shown by wild type and 0 indicating no detectable growth after 48 hours.

within a chamber that was continuously flushed with various gas mixtures. Flushing plates with 20% or 14% O_2 (balance, N_2) prevented the growth of class II mutants (*eutMNLKG*) at all pH values tested (Table 4). This rapid exchange of the gas phase also prevented the wild type from growing on ethanolamine at pH values above 7. This exchange did not reduce the growth of any strains on glycerol.

Acetaldehyde retention is increased by exposure of cells to CO_2 or benzoate. Addition of CO_2 to the gas mixture used to flush the container (20% O_2 plus 5% CO_2; balance, N_2) allowed the wild type and all mutants to grow across the entire pH range (Table 4; data not shown for class III and IV mutants). Several reasons for the correction were entertained.

The exposure to CO_2 might reduce the pH of the plate. Over the period of these tests, CO_2 reduced the pH of the medium slightly (0 to 0.9 pH units), but subsequent tests with higher buffer concentrations or adjusted pH eliminated the possibility that the effect of CO_2 reflected only changes in the pH of the medium (data not shown).

The CO_2 may have some direct connection to carboxysome function. This was enticing, because the growth defect of photosynthetic organisms lacking carboxysomes is corrected at high CO_2 concentrations (1, 10), leading to the conclusion that the organelles in these organisms concentrate CO_2. This seems unlikely to explain the effects on the use of ethanolamine, in view of the results described below.

The provided CO_2 may enter cells uncharged and reduce the internal pH of the cytosol, replacing the function of the carboxysome. To test this, benzoate was added to the growth medium. Benzoate, like CO_2, can reduce intracellular pH by entering in an uncharged state and releasing a proton into the cytosol (32). Addition of benzoate improved the growth of class II mutants, even when cells were flushed with a gas mixture lacking CO_2. This growth improvement was achieved with no change in the pH of the medium. We suggest that CO_2 (like benozate) corrects the phenotype of carboxysome mutants by reducing intracellular pH.

Suppression of ethanolamine ammonia-lyase (EutBC) mutants by limiting gas exchange. In the course of the experiments described above, it was surprising to note that some class I mutants, which fail to use ethanolamine under standard conditions, were allowed to grow on EA at a rate approaching that of the wild type under conditions that restrict loss of acetaldehyde. The corrected mutants lack either the first degradative enzyme, ethanolamine ammonia-lyase (*eutABC*), or phosphotransacylase (EutD). A mutant lacking the second step (EutE) was not corrected. The correctable mutants used ethanolamine when the plates were incubated in a confined space, when pH was reduced, when CO_2 was added to air flushed over the plates, or when benzoate was included in the medium (Table 5).

This suggested that ethanolamine can be converted to acetaldehyde without EutABC at a rate that supports growth when loss of acetaldehyde is minimized. Perhaps in these mutants acetaldehyde is lost excessively because the alternative enzymes responsible are not associated with the carboxysome. We know little about the inferred alternative pathway except that it requires the proteins AdhP (a homologue of alcohol dehydrogenase) and Ppk (polyphosphate kinase), both encoded outside the operon. This pathway allows ethanolamine to serve as a carbon source, but not as a nitrogen source (22), and is discussed below.

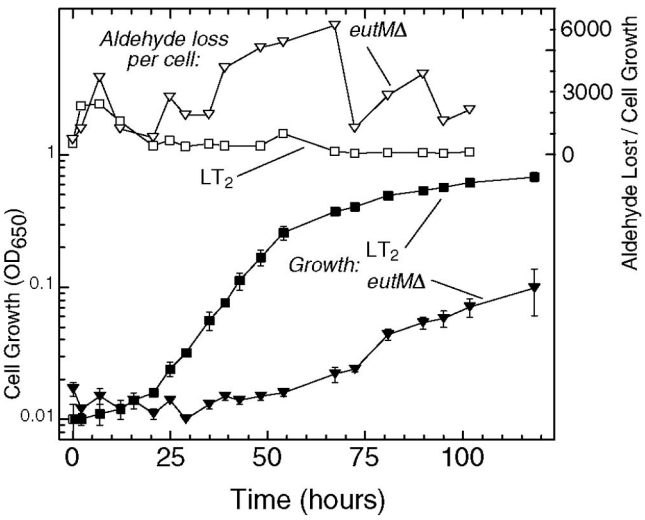

FIG. 7. Aldehyde release during growth on ethanolamine as a carbon source; growth (closed symbols) and aggregated aldehyde release normalized to cell growth (open symbols). Wild-type LT2 (squares) was compared to a *eutM* deletion mutant (triangles, points down). Ethanolamine (40 mM) was the sole carbon source. The error bars represent standard deviations.

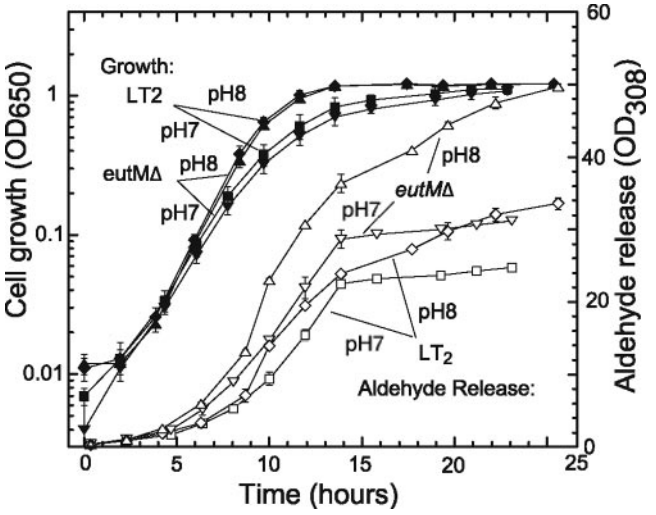

FIG. 8. Effect of pH on aldehyde release. Growth (closed symbols) and cumulative aldehyde release (open symbols) were measured at pH 7 and 8. Wild-type LT2 is depicted by squares at pH 7 and diamonds at pH 8. A *eutM* deletion mutant is depicted at pH 7 (triangles, points down) and pH 8 (triangles, points up). The medium was buffered with 50 mM MOPS (pH 7) or Bicine (pH 8) and contained glycerol, ethanolamine (20 mM), and B_{12}. The error bars represent standard deviations.

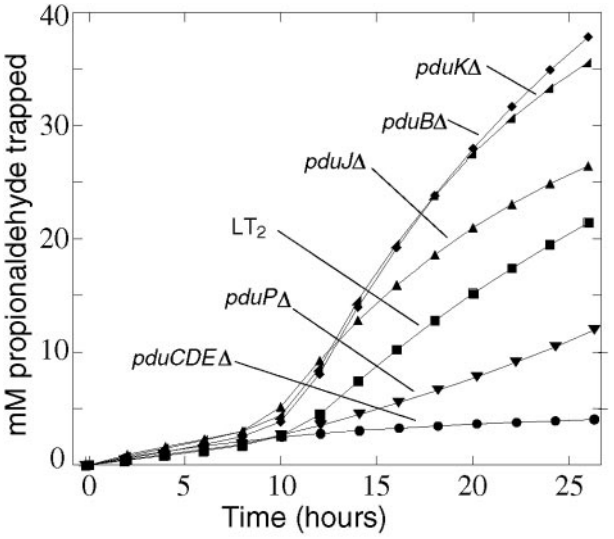

FIG. 9. Aldehyde release by *pdu* mutants. Mutants *pduCDE* (circles), *pduP* (triangles, points down), *pduB* (diamonds), *pduJ* (triangles, points up), and *pduK* (right triangles) were compared to wild-type LT2 (squares) during growth on pyruvate (40 mM), 1,2-propanediol (80 mM), and B_{12}. The cultures were buffered at 7.0 with NCE salts and incubated at 38°C. All strains grew at the same rate (data not shown).

Lack of carboxysomes simultaneously decreases the growth rate and increases acetaldehyde release. In the liquid growth tests described above, aldehyde release was detected while cells grew on an alternative carbon source. To correlate growth impairment with acetaldehyde release, a *eutM* mutant was tested while EA served as the sole carbon source. In liquid medium, this mutant grew more poorly on EA than wild-type cells at pH 7 (Fig. 7). (Note that on solid medium, growth of the *eutM* mutant failed completely only at a slightly higher pH.) To quantify released aldehyde under conditions of reduced growth, release was normalized to the cell number. The growth-impaired *eutM* mutant released more acetaldehyde per cell than the wild type.

Aldehyde release, like growth phenotype, is affected by pH. The effect of pH on aldehyde release correlated with its effect on growth phenotypes scored on plates (described above). The *eutM* mutant was grown in liquid medium on glycerol with added EA/B_{12}, and its aldehyde release was compared with that of wild-type cells at pH 7 and 8. While higher pH increased the aldehyde release of both wild-type and mutant strains, the increase was more pronounced in the mutant. The growth rates were only minimally affected by the pH difference (Fig. 8).

Providing an alternative carbon source corrects all growth phenotypes of carboxysome mutants. Evidence was provided above that acetaldehyde derived from ethanolamine is released during growth on glycerol and has little or no toxic effect even in mutants lacking carboxysome proteins. This suggested that the main role of carboxysomes is to retain acetaldehyde as a carbon source rather than to prevent its toxicity. In further support of this conclusion, the growth defect of class II *eut* mutants at high pH is corrected by the addition of succinate, an alternative carbon source. Succinate also corrects the growth defects caused on solid medium by increased gas exchange (data not shown).

Cells growing on 1,2-propanediol release aldehyde. The model for carboxysome function proposed for EA use would be expected to apply to the parallel propanediol (1,2-PDL) pathway. Growth on 1,2-PDL is associated with production of a carboxysome, and the pathway includes an aldehyde. The first enzyme (PduCED) produces propionaldehyde, which is converted to propionyl-CoA by the second enzyme (PduP). We measured aldehyde release during growth on 1,2-PDL, testing wild-type cells and various mutant strains, including three lacking homologues of carboxysome shell proteins (PduB, -J, and -K). All of the mutants tested were in-frame deletions constructed and generously provided by Thomas Bobik. In these tests, strains were grown on pyruvate plus 1,2-PDL and B_{12}. (Glycerol could not be used as a carbon source because it inhibits propanediol dehydratase [42]).

Mutant and wild-type strains grew similarly on pyruvate with and without 1,2-PDL, indicating no growth inhibition by 1,2-PDL or its derived aldehyde (not shown). The wild-type strain released aldehyde only in the presence of 1,2-PDL, and that release was prevented by the *pduCDE* deletion mutation (Fig. 9), which eliminated propanediol dehydratase. As predicted by the model, three carboxysome mutants tested (*pduJ*, *-K*, and *-B*) released significantly more aldehyde than wild-type LT2. Unexpectedly, a *pduP* mutant, blocked after propionaldehyde, showed less aldehyde release than the wild type rather than more. This result is discussed below.

DISCUSSION

Acetaldehyde, an intermediate in ethanolamine degradation, is both volatile and toxic. Evidence has been presented that the primary function of the carboxysome encoded by *eut* is

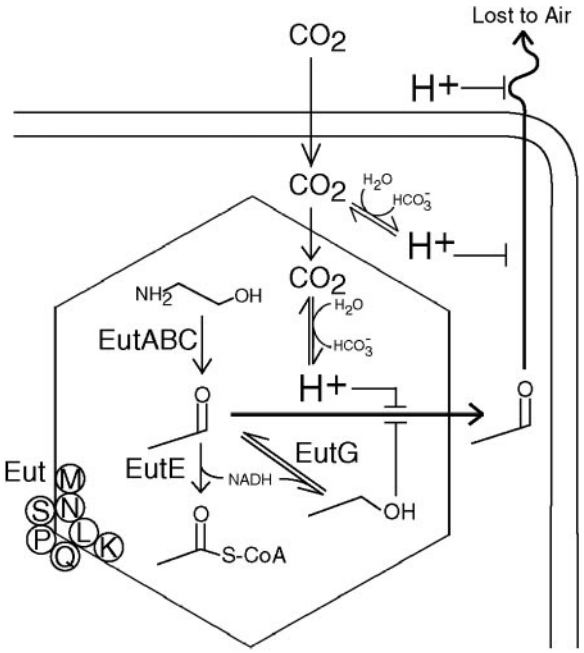

FIG. 10. Proposed role of the carboxysome. It is proposed here that the primary function of a carboxysome is to retain and prevent loss of a volatile metabolite, acetaldehyde in the case of the ethanolamine pathway. Retention of volatile aldehydes (in *Salmonella*) and retention of CO_2 (in other organisms) might be explained if the carboxysome provided a low-pH compartment.

to minimize acetaldehyde loss and improve the efficiency of growth on EA. This function becomes critical under conditions that increase the rate of aldehyde loss. Such conditions include high pH of the medium and rapid exchange of ambient gases. Aldehyde loss is minimized when intracellular pH is reduced by providng CO_2 or benzoate. As expected, phenotypes seen in media with high pH are corrected by restricting gas exchange. All phenotypes attributed to the loss of aldehyde are corrected by providing an alternative source of carbon. These results suggest that the phenotypes reflect a lack of carbon source (due to loss of acetaldehdye) rather than toxic effects of aldehyde. These conclusions are summarized in Fig. 10.

While acetaldehyde is toxic when added at high concentrations to cell cultures (26), the toxicity of acetaldehyde produced endogenously from ethanolamine has been seen only in EutE mutants (23), which lack acetaldehyde oxidoreductase (EutE), and in *polA* and *gsh* mutants, which may be supersensitive to toxic effects of aldehyde (26, 27).

The results presented do not shed light on the mechanisms by which carboxysomes might conserve acetaldehyde—a formidable problem. It seems reasonable that the mechanism used may also explain the ability of similar organelles to concentrate CO_2 in support of photosynthesis (10, 19). Both aldehydes and CO_2 are gases whose volatility and loss from solution are affected by pH. When alcohols are available, low pH should favor the conversion of aldehydes to less volatile acetals, thereby minimizing loss. Low pH favors conversion of carbonate to CO_2 gas, required by RuBisCO in the dark reactions of photosynthesis. The ability of the organelle to maintain a low-pH interior might explain both conservation of aldehyde in *S. enterica* and provision of CO_2 gas in other organisms. An alternative possibility is that the organelle allows entry of CO_2 gas but not carbonate. Organisms that fix CO_2 could use this dissolved gas and help maintain it using carbonic anhydrase, while *S. enterica* might take advantage of the pH drop due to internal carbonate formation. We understand that there is a serious mechanistic problem with this theory in that the organelles are bounded by proteins, not lipids, and appear freely permeable when tested in vitro (10). This problem is faced by all models that propose concentration of substrates or cofactors, retention of intermediates, or sequestration of toxic compounds (8, 16, 41). We can only speculate that the purified organelles behave differently from those inside cells.

The phenotypes reported here parallel phenotypes reported for carboxysome mutants in photosynthetic bacteria, which photosynthesize normally if provided with a higher level of CO_2 (1, 10). In *S. enterica*, ethanolamine phenotypes are also corrected by increased CO_2. We argue here that CO_2 acts in *S. enterica* by reducing the intracellular pH. This may also be true for cyanobacteria, where carboxysome mutant phenotypes might be corrected by providing benzoate rather than CO_2.

It is surprising that the growth phenotype of a *eutG* (ethanol dehydrogenase) mutant is like that of carboxysome mutants—deficient in harvesting carbon and energy from EA. However, *eutG* mutants showed aldehyde excretion like that of *eutE* (aldehyde oxidoreducase) mutants. EutG may simply serve to help recycle electron carriers and balance redox reactions during fermentation by converting excess acetaldehyde to ethanol and NADH to NAD. Recycling of NADH would be particularly important during anaerobic fermentation, which might be expected to occur by conversion of acetyl-CoA to acetate with the production of one ATP and the release of acetate. However, we have been unable to demonstrate anaerobic fermentation of ethanolamine (use as a sole carbon and energy source without an external electron acceptor). It is possible that the reduced nature of ethanolamine and the high flux required for growth might generate more NADH than can be consumed even aerobically. It also seems possible that the NAD available to EutE within the carboxysome is limited and EutG is needed to recycle NADH produced locally by EutE. The phenotype would also be explained if ethanol produced by EutG helped trap acetaldehyde as an acetal within the carboxysome.

Recent results of Starai et al. (40) demonstrate that considerable acetate is produced even during aerobic growth on EA and that EutD (an acetyl phosphotransacylase) contributes to this (7, 8). In this work, *eutD* mutants were found to have no growth defect on ethanolamine, a reasonable finding, since EutD directs carbon toward acetate and out of the cell (Fig. 1). The presence of this gene in the *eut* operon was attributed to the need to recycle CoA when pools of acetyl-CoA are excessive. In contrast, we report here that a similar *eutD* in-frame deletion mutant failed to use ethanolamine under any standard conditions. We attribute the need for this enzyme to a requirement to prevent accumulation of acetyl-CoA and secondarily of acetaldehyde. (EutD mutants excrete as much acetaldehyde as carboxysome mutants.) We suggest that during growth on high levels of ethanolamine, cells convert accumulated excess acetyl-CoA to acetate (which is excreted for later use) rather than risk secondary accumulation of acetadehyde, which is subject to irretrievable loss to the gas phase or conversion to

ethanol (which cannot be used). The difference in observed *eutD* phenotypes needs to be resolved but seems likely to reflect the use of different genetic backgrounds.

The similar pathways for degradation of EA and 1,2-PDL both include an aldehyde, and both operate with the use of carboxysomes. In both systems, aldehyde vapor was detected for wild-type cells and increased in mutants defective for carboxysomes. A key difference in the behaviors of the two pathways was that the oxidoreductase mutant (*eutE*) released more aldehyde (as expected for our model) while the analogous *pdu* mutant (*pduP*) released less. The behavior of the *pduP* mutant might be explained if PduP not only converts propionaldehyde to the CoA derivative, but also interacts directly with dioldehydratase to enhance its activity or prevent its inactivation by aldehyde. This might cause a *pduP* mutation to secondarily reduce dioldehydratase activity in vivo.

The *eutS* gene encodes a distant homologue of carboxysome proteins. Unlike mutants lacking other carboxysome shell proteins, the *eutS* mutant showed no growth defect at high pH on solid medium. Like the other mutants, it did show increased loss of acetaldehyde during growth on glycerol plus acetaldehyde in liquid medium. The EutS protein may make a more subtle contribution to carboxysome structure that is required for ethanolamine use at more extreme conditions. Consistent with this, work on the propandediol (*pdu*) operon (16) has shown that some carboxysome shell proteins can be lost without preventing the formation of an observable carboxysome, while the lack of others leads to structural abnormality or complete absence. Particular shell proteins may provide a specific function without being essential to the formation of the organelle.

A second pathway for ethanolamine use was inferred from the observation that *eutBC* mutants (lacking ethanolamine ammonia-lyase) grow on ethanolamine when high levels of CO_2 or benzoate are provided. We suggest that some other enzyme (perhaps AdhP) initiates the degradation of ethanolamine (outside of carboxysomes), forming acetaldehyde by some disproportionation reaction in which two molecules of ethanolamine yield one acetaldehyde and a diamine or aminoamide that cannot be degraded. The requirement of the second pathway for polyphosphate kinase (Ppk) may reflect the fact that Ppk helps cells tolerate growth with reduced internal pH (24) and therefore may be required under the conditions used here to activate the second pathway.

Assigning a function to carboxysomes helps solve the broader question of why so many genes are required to degrade EA and 1,2-PDL. Seventeen genes support the conversion of ethanolamine (two carbons) to acetyl-CoA and 26 support the conversion of 1,2-PDL (three carbons) to propionyl-CoA. The need for 17 genes to degrade a two-carbon compound seems surprising, especially when one compares the pathway for ethanolamine to that for acetate. Both are two-carbon compounds that are converted to acetyl-CoA, yet acetate use involves only three proteins (Acs, Ack, and Pta), and only one (Acs) is required.

The need for more genes may be explained in part by the volatile intermediates in the EA and 1,2-PDL pathways, which requires the carboxysome structural genes—five in *eut* and seven in *pdu* (15, 20). In addition, the two alcohols are more reduced than acetate, making it necessary to get rid of excess reducing equivalents in some way. The use of a B_{12}-catalyzed first step minimizes this problem by converting an alcohol to an aldehyde with release of H_2O (and NH_3 in the case of EA) and no reducing equivalents. However, B_{12} is both complex to produce and inherently reactive. Two *eut* genes (*eutT* and *eutA*) and four *pdu* genes contribute to the management of B_{12} (3, 18, 21, 33). Thus, the additional *eut* and *pdu* genes may be required to contain a volatile aldehyde intermediate and to allow the use of a reactive cofactor.

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REFERENCES

1. **Badger, M. R., and G. D. Price.** 2003. CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. J. Exp. Bot. **54:**609–622.
2. **Berkowitz, D., J. M. Hushon, H. J. Whitfield, Jr., J. R. Roth, and B. N. Ames.** 1968. Procedure for identifying nonsense mutations. J. Bacteriol. **96:**215–220.
3. **Bobik, T., Y. Xu, R. Jeter, K. Otto, and J. R. Roth.** 1997. Propanediol utilization genes (*pdu*) of *Salmonella typhimurium*: three genes for the propanediol dehydratase. J. Bacteriol. **179:**6633–6639.
4. **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.
5. **Bradbeer, C.** 1965. The clostridial fermentations of choline and ethanolamine. I. Preparation and properties of cell-free extracts. J. Biol. Chem. **240:**4669–4674.
6. **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.
7. **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.
8. **Brinsmade, S. R., T. Paldon, and J. C. Escalante-Semerena.** 2005. Minimal functions and physiological conditions required for growth of *Salmonella enterica* on ethanolamine in the absence of the metabolosome. J. Bacteriol. **187:**8039–8046.
9. **Buan, N. R., S. J. Suh, and J. C. Escalante-Semerena.** 2004. The *eutT* gene of *Salmonella enterica* encodes an oxygen-labile, metal-containing ATP:corrinoid adenosyltransferase enzyme. J. Bacteriol. **186:**5708–5714.
10. **Cannon, G. C., C. E. Bradburne, H. C. Aldrich, S. H. Baker, S. Heinhorst, and J. M. Shively.** 2001. Microcompartments in prokaryotes: carboxysomes and related polyhedra. Appl. Environ. Microbiol. **67:**5351–5361.
11. **Court, D. L., J. A. Sawitzke, and L. C. Thomason.** 2002. Genetic engineering using homologous recombination. Annu. Rev. Genet. **36:**361–388.
12. **Czymmek, K., J. Penrod, E. Kofoid, and J. R. Roth.** Unpublished data.
13. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
14. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **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.
16. **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.
17. **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:**206–216.
18. **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.
19. **Kaplan, A., and L. Reinhold.** 1999. CO_2 Concentrating mechanisms in photosynthetic microorganisms. Annu. Rev. Plant Physiol. Plant Mol. Biol. **50:**539–570.

20. **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.
21. **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.
22. **Penrod, J., T. Fazzio, C. Mace, E. C. Kofoid, and J. R. Roth.** Unpublished data.
23. **Penrod, J. T., C. C. Mace, and J. R. Roth.** 2004. A pH-sensitive function and phenotype: evidence that EutH facilitates diffusion of uncharged ethanolamine in *Salmonella enterica*. J. Bacteriol. **186:**6885–6890.
24. **Price-Carter, M., T. G. Fazzio, E. I. Vallbona, and J. R. Roth.** 2005. Polyphosphate kinase protects *Salmonella enterica* from weak organic acid stress. J. Bacteriol. **187:**3088–3099.
25. **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* sevrovar Typhimurium on ethanolamine or 1,2-propanediol. J. Bacteriol. **183:**2463–2475.
26. **Rondon, M. R., A. R. Horswill, and J. C. Escalante-Semerena.** 1995. DNA polymerase I function is required for the utilization of ethanolamine, 1,2-propanediol, and propionate by *Salmonella typhimurium* LT2. J. Bacteriol. **177:**7119–7124.
27. **Rondon, M. R., R. Kazmierczak, and J. C. Escalante-Semerena.** 1995. Glutathione is required for maximal transcription of the cobalamin biosynthetic and 1,2-propanediol utilization (*cob*/*pdu*) regulon and for the catabolism of ethanolamine, 1,2-propanediol, and propionate in *Salmonella typhimurium* LT2. J. Bacteriol. **177:**5434–5439.
28. **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.
29. **Roof, D. M., and J. R. Roth.** 1988. Ethanolamine utilization in *Salmonella typhimurium*. J. Bacteriol. **170:**3855–3863.
30. **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.
31. **Roth, J. R., J. G. Lawrence, and T. A. Bobik.** 1996. Cobalamin (coenzyme B_{12}): synthesis and biological significance. Annu. Rev. Microbiol. **50:**137–181.
32. **Salmond, C. V., R. G. Kroll, and I. R. Booth.** 1984. The effect of food preservatives on pH homeostasis in *Escherichia coli*. J. Gen. Microbiol. **130:**2845–2850.
33. **Sampson, E. M., C. L. Johnson, and T. A. Bobik.** 2005. Biochemical evidence that the *pduS* gene encodes a bifunctional cobalamin reductase. Microbiology **151:**1169–1177.
34. **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.
35. **Schmieger, H.** 1971. A method of detection of phage mutants with altered transducing ability. Mol. Gen. Genet. **110:**378–381.
36. **Sheppard, D., J. Penrod, T. Bobik, E. Kofoid, and J. Roth.** 2004. Evidence that a B_{12}-adenosyl transferase is encoded within the ethanolamine operon of *Salmonella enterica*. J. Bacteriol. **186:**7635–7644.
37. **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.
38. **Shively, J., and R. English.** 1991. The carboxysome, a prokaryotic organelle: a mini review. Can. J. Bot. **69:**957–963.
39. **Soda, K.** 1967. A spectrophotometric microdetermination of keto acids with 3-methyl-2-benzothiazolone hydrazone. Agric. Biol. Chem. **31:**1054–1060.
40. **Starai, V. J., J. Garrity, and J. C. Escalante-Semerena.** 2005. Acetate excretion during growth of *Salmonella enterica* on ethanolamine requires phosphotransacetylase (EutD) activity, and acetate recapture requires acetyl-CoA synthetase (Acs) and phosphotransacetylase (Pta) activities. Microbiology **151:**3793–3801.
41. **Stojiljkovic, I., A. J. Baumler, and F. Heffron.** 1995. Ethanolamine utilization in *Salmonella typhimurium*: nucleotide sequence, protein expression, and mutational analysis of the *cchA cchB eutE eutJ eutG eutH* gene cluster. J. Bacteriol. **177:**1357–1366.
42. **Toraya, T., T. Shirakashi, T. Kosuga, and S. Fukui.** 1976. Substrate specificity of coenzyme B_{12}-dependent diol dehydratase: glycerol as both a good substrate and a potant inactivator. Biochem. Biophys. Res. Commun. **69:**475–480.
43. **Yu, D., J. A. Sawitzke, H. Ellis, and D. L. Court.** 2003. Recombineering with overlapping single-stranded DNA oligonucleotides: testing a recombination intermediate. Proc. Natl. Acad. Sci. USA **100:**7207–7212.