JOURNAL OF BACTERIOLOGY, Oct. 1992, p. 6634–6643
0021-9193/92/206634-10$02.00/0
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Vol. 174, No. 20

Autogenous Regulation of Ethanolamine Utilization by a Transcriptional Activator of the *eut* Operon in *Salmonella typhimurium*

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Received 6 January 1992/Accepted 14 August 1992

The genes required for use of ethanolamine as a carbon and nitrogen source are encoded by a single operon (*eut*) whose expression is induced by the simultaneous presence of both ethanolamine and cobalamin (vitamin B_{12}). The action of B_{12} as an inducer of this operon reflects the fact that this cofactor is required by the degradative enzyme ethanolamine lyase (*eutBC*). The *eutR* gene encodes a protein that activates transcription of the *eut* operon in response to the simultaneous presence of B_{12} and ethanolamine. The *eutR* gene is expressed by a weak constitutive promoter activity (P_{II}) and by the main regulated promoter (P_I). Because it is encoded within the operon that it activates, the EutR protein controls its own production. Initial induction of the *eut* operon by ethanolamine plus B_{12} causes an increase in expression of the *eutR* gene; this increase acts as part of a positive feedback loop that is required for maximal operon expression. Because of this mode of regulation, constitutive regulatory mutations, described here, include mutations that generate new internal promoters and thereby increase the basal level of *eutR* gene expression. In mutants with an increased level of activator protein, each inducer (B_{12} or ethanolamine), presented singly, is sufficient for partial operon induction.

Salmonella typhimurium LT2 uses ethanolamine as the sole source of carbon and nitrogen when B_{12} is available to serve as a cofactor for the first enzyme in the pathway, ethanolamine ammonia lyase (7, 11). Unlike most cofactors, B_{12} is unreliable since it is synthesized only under anaerobic conditions (2, 3, 15, 18). Under both aerobic and anaerobic growth conditions, exogenous B_{12} can be transported into the cell by a mechanism described by Kadner and coworkers (4, 26) for *Escherichia coli*. Cells growing aerobically have B_{12} only if they can obtain it from their environment. In view of the fact that B_{12} may or may not be available, it is logical that transcription of the genes for ethanolamine utilization (the *eut* operon) is induced only when both ethanolamine and the essential B_{12} cofactor are available (23). This pattern of regulation is unusual; we know of no other enzyme whose synthesis requires the presence of its cofactor. This study is an initial characterization of the mechanism whereby two effector molecules serve to induce this operon.

The requirement for both ethanolamine and B_{12} as inducers of synthesis of the ethanolamine ammonia lyase was first shown, in *E. coli*, by Turner and coworkers (5, 6, 25). They have termed this regulatory pattern concerted induction. An *E. coli* regulatory mutant constitutively expresses both ethanolamine ammonia lyase and the second enzyme in the pathway, acetaldehyde dehydrogenase, but the nature of the defect in the *E. coli* mutant is not understood (6, 19, 20).

The genes for degradation of ethanolamine lie in a single operon between the *cysA* and *purC* loci at min 50 of the *S. typhimurium* chromosome (23). The genetic map of this operon is presented in Fig. 1. Complementation tests defined six genes in the operon (24). The *eutB* and *eutC* genes encode the two subunit types of ethanolamine ammonia lyase (ethanolamine→NH_3 plus acetaldehyde), and the *eutE* gene encodes acetaldehyde dehydrogenase, the second enzyme in the degradative pathway (acetaldehyde→acetyl coenzyme A). The *eutD* gene encodes an unknown function that, like acetaldehyde dehydrogenase, is required only for use of ethanolamine as the carbon source. The *eutA* gene is required if cells are to use exogenous cyanocobalamin (commercial vitamin B_{12}) or hydroxy-B_{12} as a source of cofactor for the lyase. Current evidence suggests that the *eutA* function prevents inhibition of lyase by these forms of B_{12} and does this by in part by formation of adenosyl-B_{12}, the true cofactor of ethanolamine ammonia lyase (26a). Null mutations in the *eutR* gene prevent induction of transcription of the operon (23).

Evidence that the *eutR* gene is located within the *eut* operon and encodes a transcriptional activator that mediates activation of the operon in response to ethanolamine plus B_{12} is presented here. Because it is encoded within the operon it activates, the EutR protein controls its own production. The increase in EutR protein level caused by autoinduction appears to be required for maximal operon expression. Several sorts of regulatory mutations affect this control mechanism. Mutations that increase the basal level of *eutR* gene expression appear to partially circumvent the requirement for two effectors; in these mutants, the operon is partially induced by either one of the normal inducers.

MATERIALS AND METHODS

Bacterial strains and transposons. All strains used in this study are derivatives of *S. typhimurium* LT2 (Table 1). Two transposition-defective derivatives of Tn*10*, Tn*10 del16 del17* Tet^r (29) and Tn*10*dCam (14), were used. The Tn*10 del16 del17* Tet^r element is referred to as Tn*10*dTet. Transcriptional fusions were made with two transposition-defec-

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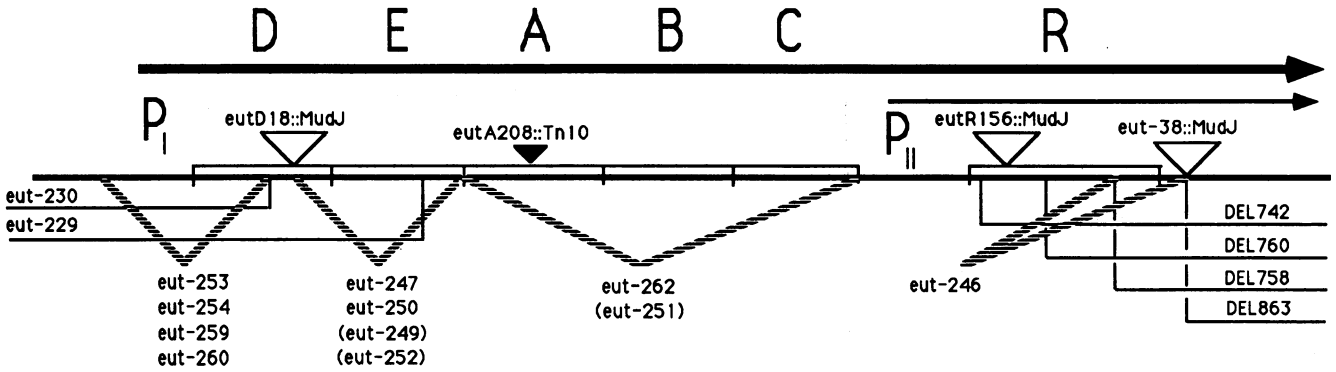

FIG. 1. Genetic map of the *eut* operon. The horizontal line in the middle of the figure represents the chromosome on which are indicated the positions of the deletions and insertion mutations used here. The solid triangle denotes a Tn*10* insertion; open triangles denote insertions of the MudJ(Lac Kn) element, all of which form operon fusions that express the *lacZ* gene from one or both of the *eut* promoters. The arrows above the horizontal line indicate the two transcripts provide by the P_I and P_{II} promoters. Letters above these arrows indicate the *eut* genes. Below the horizontal map are listed the regulatory mutations described here; dashed lines indicate the regions of the map that include the mutations indicated. Mutation numbers in parentheses are assigned to unstable mutations, inferred to be duplications whose join points lie in the designated region.

tive derivatives of the specialized transducing phage Mu dl(Amp^r Lac cts) of Casadaban and Cohen (8). The derivatives MudI 1734 Kan^r (9) and Mud1-8 Amp^r (16) are referred to here as MudJ and MudA, respectively. MudA insertion mutations were converted to MudJ insertion mutations by recombination between sequences common to each element in a cross previously described (17); the insertion site and *lac* operon fusion are unchanged when a MudA prophage is converted to a MudJ prophage. The MudA-to-MudJ conversions were made to eliminate the residual transposition activity of MudA that interferes with use of the *lac* fusions to select regulatory mutations.

Media and growth conditions. The complex medium was nutrient broth (0.8%; Difco Laboratories) with NaCl (0.5%). The minimal medium was the E medium of Vogel and Bonner (28) with glucose (0.2%) as the carbon and energy source. The carbon-free minimal medium was NCE medium (13), and the carbon- and nitrogen-free minimal medium was NCN medium (22). Ethanolamine hydrochloride (0.2%; Aldrich) was used as the carbon source in NCE medium, as the nitrogen source in NCN medium with glycerol (0.2%), or as both the carbon and nitrogen source in NCN medium. Cyanocobalamin (Sigma Chemical) was used as the exogenous B_{12} source (0.1 μg/ml). Amino acids were added to minimal media as required at the concentrations previously described (13). Antibiotics were added to media as previously described (24). Solid medium contained agar (1.5%; Difco) or, when ethanolamine was used as the sole nitrogen source, Noble agar (1.5%; Difco). Cells were grown aerobically at 37°C.

Chromogenic indicator plates for detecting β-galactosidase activity contained NCE medium with glycerol and 25 μg of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) per ml. The X-Gal was dissolved in *N,N*-dimethylformamide before addition to the medium.

Genetic techniques and strain construction. Transductional crosses were performed with the high-frequency, generalized transducing phage mutant P22 HT 105/1 *int-201* (27) as previously described (23). Transductants were purified and made phage free by streaking for single colonies on nonselective green indicator plates (10). Conjugational crosses were performed directly on selective medium as previously described (24).

The plasmids F′ 606 *his*$^+$ *eut*$^+$ and F′ T80 *his*$^+$ (in strains TT14082 and TR35) were used in constructing merodiploid strains. Plasmid F′ 606 was constructed from F′ T80 by addition of the *eut* operon sequences (24). Various *eut*::Tn*10* insertion mutations were placed on plasmid F′ 606 by transduction of strain TT14082 to Tet^r as described previously (24). All F′ plasmids were maintained in strains with a *his* deletion mutation on medium lacking histidine to select against loss of the F′ plasmid. To prevent recombination between plasmid and chromosomal *eut* sequences, the *recA1* mutation was introduced to all strains destined to carry an F′ plasmid. This was done by cotransduction of the *recA1* mutation with the selectable *srl-203*::Tn*10*dCam insertion mutation from donor strain TT11289; *recA* mutant transductants were identified by UV light sensitivity.

Isolation and mapping of regulatory mutations. Regulatory mutants were selected by using a parent strain (TT10271) with a *lac* operon fusion in the first gene of the *eut* operon (*eutD18*::MudJ). Mutant derivatives that expressed the *lacZ* gene of the fusion without one of the two normally required inducers, ethanolamine and B_{12}, were identified. The regulatory mutations were separated from the parental MudJ insertion by transduction into a *eut* deletion mutant, selecting Eut$^+$ transductants. The Eut$^+$ transductants must have acquired donor material to repair the recipient deletion but not the donor's *eut*::MudJ insertion. If the regulatory mutation is located within the deletion, then all Eut$^+$ transductants must acquire the donor regulatory mutation. To test for the presence of the regulatory mutation in the Eut$^+$ recombinants, the *eutD18*::MudA insertion was reintroduced into the Eut$^+$ recombinants by transduction, selecting for Amp^r recombinants on X-Gal indicator medium containing ethanolamine but no B_{12}. If the recipient contains the regulatory mutation, some of the Amp^r transductants will form blue colonies on X-Gal because of constitutive expression of the *eutD18*::MudA fusion. Recipients that do not contain the regulatory mutation will form white transductant colonies on the indicator medium.

To genetically map the location of a regulatory mutation, several different deletion mutations were used and, in each case, at least 60 *eut*$^+$ recombinants were tested for the presence of the regulatory mutation. If all 60 *eut*$^+$ transductants contained the regulatory mutation, the mutation was

TABLE 1. Bacterial strains used

Strain	Genotype
TR35	*his-712 ser-821 arg-501*/F' T80 *his*$^+$
TT10271	*eutD18*::MudJ
TT10654	*eutD18*::MudA
TT10674	*eut-38*::MudA
TT11289	*recA1 srl-203*::Tn*10*dCam
TT11573	*eut-230*
TT11713	DEL743(*cysA1585**MudA**eut-16*)
TT13738	*eutR156*::MudJ
TT14082	*eut-240 hisG10082*::Tn*10*dCam/F' 606 *eut*$^+$
TT14476	*eutD18*::MudJ *recA1 srl-203*::Tn*10*dCam *his-2236*/F' T80
TT14477	*eutD18*::MudJ *recA1 srl-203*::Tn*10*dCam *his-2236*/F' 606 *eut*$^+$
TT14478	*eutD18*::MudJ *eutR205*::Tn*10 recA1 srl-203*::Tn*10*dCam *his-2236*/F' T80
TT14479	*eutD18*::MudJ *eutR205*::Tn*10 recA1 srl-203*::Tn*10*dCam *his-2236*/F' 606 *eut*$^+$
TT14483	*eutD18*::MudJ *recA1 srl-203*::Tn*10*dCam *his-2236*/F' 606 *eutR205*::Tn*10*
TT14484	*eutD18*::MudJ *eutR205*::Tn*10 recA1 srl-203*::Tn*10*dCam *his-2236*/F' 606 *eutD46*::Tn*10*
TT14487	*eutD18*::MudJ *eutR205*::Tn*10 recA1 srl-203*::Tn*10*dCam *his-2236*/F' 606 *eutR205*::Tn*10*
TT14488	*eutR156*::MudJ *recA1 srl-203*::Tn*10*dCam *his-2236*/F' T80
TT14489	*eutR156*::MudJ *recA1 srl-203*::Tn*10*dCam *his-2236*/F' 606 *eut*$^+$
TT14490	*eutR156*::MudJ *eutA208*::Tn*10*dTet *recA1 srl-203*::Tn*10*dCam *his-2236*/F' T80
TT14491	*eutR156*::MudJ *eutA208*::Tn*10*dTet *recA1 srl-203*::Tn*10*dCam *his-2236*/F' 606 *eut*$^+$
TT14492	*eut-38*::MudA *eutA208*::Tn*10*dTet
TT14493	*eutD18*::MudJ *eut-246*
TT14494	*eutD18*::MudJ *eut-247*
TT14495	*eutD18*::MudJ *eut-249*
TT14496	*eutD18*::MudJ *eut-250*
TT14497	*eutD18*::MudJ *eut-251*
TT14498	*eutD18*::MudJ *eut-253*
TT14499	*eutD18*::MudJ *eut-254*
TT14500	*eutD18*::MudJ *eut-255*
TT14501	*eutD18*::MudJ *eut-256*
TT14502	*eutD18*::MudJ *eut-258*
TT14503	*eutD18*::MudJ *eut-259*
TT14504	*eutD18*::MudJ *eut-260*
TT14505	*eut-246*
TT14506	*eut-247*
TT14861	*eut-241 eutR156*::MudJ
TT14838	*eutD18*::MudJ *zfa-3648*::Tn*10 eut-262*
TT14844	*eutR156*::MudJ *eutD45*::Tn*10 eut-247*
TT14845	*eutR156*::MudJ *eutD45*::Tn*10 eut-262*
TT14846	*eutR156*::MudJ *eutD45*::Tn*10*
TT14847	*zfa-3648::Tn10 eut-229*

inferred to map within the deletion or to be greater than 98% linked by P22 transduction to the deletion end point. This cotransduction frequency corresponds to a distance of about 200 bp of DNA, calculated with the formula of Wu (30). Since this is a forced cross, in which a recombination event must occur between the deletion and the donor Mud insertion, the fraction of recombinants inheriting the donor regulatory mutation should reflect the position of the regulatory mutation as a fraction of the distance from the deletion to the *eutD18*::MudJ insertion.

One of the deletions used in the mapping experiments, *eut-230*, enters the operon from the left and includes part of the *eutD* gene; deletions DEL742, DEL760, DEL758, and DEL863 enter the operon from the right. These deletions were included in a genetic map of the *eut* operon previously described (24) and are included in Fig. 1.

One regulatory mutant (*eut-262*) was identified by screening on X-Gal ethanolamine indicator medium for mutants with increased expression of the *eutD*::*lac* fusion following localized mutagenesis of the *eutR* region. Phage was prepared on strain TT14847, which carries a Tn*10* insertion outside the promoter-distal end of the *eut* operon and deletion mutation *eut-229* which enters the operon from the left side and removes the *eutD* and *eutE* genes. This phage lysate was mutagenized with hydroxylamine as described previously (23) and then used to transduce strain TT10271, which carries the mutation *eutD18*::MudJ. Transductants resistant to tetracycline were selected on NCE glycerol ethanolamine medium containing tetracycline, kanamycin, and X-Gal. On this medium one selects for inheritance of the donor Tn*10* element and retention of the recipient *eutD*::MudJ insertion. Inclusion of the donor *eut* deletion ensures that mutations within the operon must lie distal to the *eutE* gene. The *eut-262* mutant was isolated as a blue transductant colony that expressed the *eut*::*lac* fusion despite the presence of only ethanolamine as an inducer.

β-Galactosidase assays. Strains to be assayed for β-galactosidase activity were grown with vigorous shaking in NCE medium supplemented with a carbon source (0.2% glucose or glycerol or 0.6% succinate), ethanolamine (0.2%), and/or B_{12} (0.1 μg/ml); cyclic AMP (cAMP), when used, was added to a final concentration of 5 mM. Cells were harvested at a density of 80 to 110 Klett units by centrifugation and were resuspended in 0.85% NaCl. β-Galactosidase was assayed in permeabilized cells as described by Miller (21). Activity is expressed as nanomoles of product produced per minute per

TABLE 2. Expression of *eutR*::*lac* fusions by the primary promoter (P_I) and the internal promoter (P_{II})

Line no.	Strain	Relevant chromosomal markers[a]	Relevant F′ plasmid marker(s)	β-Galactosidase activity (U) in cells grown in minimal medium supplemented with[b]:				Induction (fold)[c]
				Nothing	EA	B_{12}	EA + B_{12}	
1	TT14488	*eutR156*::*lac*	*his*$^+$	1.2	1.0	1.4	1.6	1
2	TT14489	*eutR156*::*lac*	*his*$^+$ *eut*$^+$	1.6	1.4	1.8	40	25
3	TT14490	*eutA*::Tn*10*dTet *eutR156*::*lac*	*his*$^+$	2.2	2.4	2.8	3.0	1
4	TT14491	*eutA*::Tn*10*dTet *eutR156*::*lac*	*his*$^+$ *eut*$^+$	2.6	2.8	2.8	2.0	1
5	TT10674	*eut-38*::*lac*[d]	None	5.0	5.0	4.8	210	42
6	TT14492	*eutA*::Tn*10*dTet *eut-38*::*lac*[d]	None	8.0	7.8	6.4	8.0	1

[a] All F′ plasmid strains are *recA* mutants. See Table 1 for complete genotype.
[b] The carbon source was glycerol, and the nitrogen source was ammonia. EA, ethanolamine.
[c] Calculated by dividing the activity with ethanolamine plus B_{12} by the activity with neither ethanolamine nor B_{12}.
[d] This *lac* fusion is located promoter distal to the *eutR* gene at the end of the operon.

A_{650} unit of cells. Measured activity in Lac$^-$ strains was 0.2 U or less.

RESULTS

The *eutR* gene product is required only for induction of *eut* operon transcription. It was previously shown that *eutR* mutants fail to induce transcription of the *eut* operon in response to added ethanolamine and B_{12} (23). This suggested that the *eutR* gene might encode a positive activator of transcription that mediates both B_{12} and ethanolamine effects on *eut* operon expression. Alternatively, the *eutR* gene product might catalyze a step of ethanolamine degradation needed for uptake of ethanolamine or synthesis of the true regulatory effector. If the sole function of the *eutR* protein is to activate *eut* operon transcription, then expression of the operon by a *eutR*-independent promoter would permit ethanolamine utilization without the *eutR* function.

To test this possibility, we selected for Eut$^+$ revertants of the *eutR156*::MudJ insertion strain TT13738. Some revertants, designated Eut$^+$(N$^+$C$^-$), could use ethanolamine as a nitrogen source but not as a carbon source; these were isolated at a frequency of approximately 10^{-8} without mutagenesis. These revertants could complete the first step in the ethanolamine degradation pathway (cleavage of ethanolamine to acetaldehyde and ammonia) but not the second step (conversion of acetaldehyde to acetyl coenzyme A). The first step of ethanolamine utilization requires the *eutA*, *eutB*, and *eutC* gene products, while the second step requires the *eutD* and *eutE* gene products (24). Revertants that are able to use ethanolamine as both a carbon and nitrogen source, Eut$^+$(N$^+$C$^+$) revertants, must express all five of the above *eut* genes. No revertants of this second type (frequency of less than 10^{-10}) were found among spontaneous mutants, but a single example (*eut-241*) was obtained after nitrosoguanidine mutagenesis. All of the mutations allowing operon expression independent of EutR function are located in or near the *eut* operon. Six mutations conferring the Eut$^+$(N$^+$C$^-$) phenotype were tested and found to be cotransducible with the *eutR156*::MudJ insertion at frequencies ranging between 48 and 75%; this is consistent with a location within the first portion of the operon, upstream of the *eutABC* genes. The single mutation conferring the Eut$^+$(N$^+$C$^+$) phenotype cotransduced with the *eutR*::MudJ insertion at a frequency of 44%, consistent with a location near the P_I promoter.

In addition to permitting *eutR*-independent ethanolamine utilization, the revertant mutations caused the *lac* genes of the parental *eutR156*::MudJ insertion to be expressed constitutively. In the Eut$^+$(N$^+$C$^+$) revertant, the active promoter must be located to the left of the first gene in the operon (*eutD*), while the new promoters in the Eut$^+$(N$^+$C$^-$) revertants appear to be located within the operon at some point to the left of the *eutA*, *eutB*, and *eutC* genes since they permit use of ethanolamine as a nitrogen source only. Since the Eut$^+$(N$^+$C$^+$) strain is able to use ethanolamine as a carbon source, it must express all *eut* genes; this revertant may have a modified main promoter, allowing it to act without the EutR function, or may have fused the operon to a foreign promoter.

Since all revertants express ethanolamine lyase and one can successfully use ethanolamine as both a carbon and nitrogen source without the *eutR* function, we conclude that the EutR protein plays no essential direct role in transport or degradation of ethanolamine or in generation of the adenosyl-B_{12} cofactor required for lyase activity. We infer that EutR protein plays a purely regulatory role in operon expression.

Inducible transcription of the *eutR* gene from the primary *eut* operon promoter. The *eut* enzymes are encoded by a single transcription unit (*eutDEABC*); insertion mutations within this gene cluster block transcription of genes located farther to the right (promoter distal) as the map is generally presented (23, 24) (Fig. 1). The *eutR* gene is located at the distal end of this *eutDEABC* operon, but previous work did not address how the regulatory gene is expressed.

Because the *eutR* gene is located very close to the *eutC* gene, it seemed possible that the *eutR* gene is included in the *eutDEABC* transcript and expressed from the primary inducible promoter. Transcription of *eutR* was measured by using a chromosomal *eutR*::*lac* operon fusion (*eutR156*::MudJ); a functional *eutR* gene was supplied on a derivative of the F′ *his*$^+$ plasmid which carries the complete *eut* operon of *S. typhimurium* (F′ 606). The latter plasmid was constructed by Tn*10*-mediated transposition of the entire *eut*$^+$ operon onto the F′ *his*$^+$ plasmid (24).

The chromosomal *eutR*::*lac* fusion strain with only the parental F′ *his*$^+$ plasmid showed no induction of β-galactosidase in response to addition of ethanolamine plus B_{12} (Table 2, line 1). However, the same fusion was induced 25-fold in strains with the F′ 606 *his*$^+$ *eut*$^+$ plasmid which could provide the EutR function in *trans* (line 2). Data shown later will demonstrate that the same F′ plasmid with a *eutR* mutation cannot provide for this induction. This inducible expression of the *eutR*::*lac* fusion is dependent on the primary operon promoter (P_I) for the *eutDEABC* genes (Fig. 1). This was shown by adding a polar *eutA*::Tn*10*dTet insertion mutation to the chromosome of the diploid strain to

TABLE 3. Regulation of a *eutD::lac* fusion

Line no.	Strain	Relevant chromosomal markers[a]	Relevant F′ plasmid marker(s)	β-Galactosidase activity (U) in cells grown in medium supplemented with[b]:				Induction (fold)[c]
				No inducer	EA	B_{12}	EA + B_{12}	
1	TT14476	*eutD::lac*	*his*$^+$	0.8	1.0	2.0	310	390
2	TT14478	*eutD::lac eutR*::Tn*10*	*his*$^+$	0.6	0.8	0.8	0.6	1
3	TT14477	*eutD::lac*	*his*$^+$ *eut*$^+$	1.0	2.0	6.0	1,300	1,300
4	TT14479	*eutD::lac eutR*::Tn*10*	*his*$^+$ *eut*$^+$	1.0	1.2	3.2	1,350	1,350
5	TT14484	*eutD::lac eutR*::Tn*10*	*his*$^+$ *eutD*::Tn*10*	1.0	1.2	4.0	620	620
6	TT14487	*eutD::lac eutR*::Tn*10*	*his*$^+$ *eutR*::Tn*10*	1.0	0.6	0.6	0.8	1
7	TT14483	*eutD::lac*	*his*$^+$ *eutR*::Tn*10*	0.6	0.6	0.4	60	100

[a] All F′ plasmid strains are *recA* mutants. See Table 1 for complete genotypes.
[b] The carbon source was glycerol, and the nitrogen source was ammonia in NCE medium. EA, ethanolamine.
[c] The specific activity with ethanolamine plus B_{12} divided by the specific activity without ethanolamine or B_{12}.

block transcription of the *eutR* fusion from the primary promoter. The *eutA* insertion prevented induction of β-galactosidase (lines 3 and 4), indicating that the inducible transcription of *eutR* originates upstream of the *eutA* gene (presumably from the primary promoter).

Expression of the *eutR* gene from the primary promoter was confirmed by using the *eut-38*::MudA insertion. This insertion is located on the promoter-distal side of the *eutR* gene (Fig. 1); since the insertion causes no obvious Eut phenotype, maps distal to *eutR* mutations, and provides regulated β-galactosidase, we infer that it lies in a 3′ untranslated region promoter distal to the *eutR* coding sequences. Unlike the haploid *eutR::lac* fusion strain (Table 2, line 1) and like the *eutR::lac* fusion in strains with an added *eutR*$^+$ gene (line 2), expression of β-galactosidase in the haploid *eut-38*::MudA strain was induced upon addition of ethanolamine plus B_{12} (line 5). Thus, the *eutR* gene appears not to be damaged by the *eut-38*::MudA insertion. The inducible transcription of the *eut-38*::MudA fusion originates from the primary promoter because inducibility was abolished when the polar *eutA*::Tn*10*dTet insertion mutation was introduced (line 6). Since the *eut-38*::MudA element is inserted distal to the *eutR* gene, EutR function can be provided by the intact chromosomal *eutR* gene.

Basal transcription of the *eutR* gene. In addition to the inducible transcription of the *eutR* gene from P_I, we infer the existence of a weak constitutive promoter for the *eutR* gene. We presume that this promoter (P_{II}) is located between the *eutC* and *eutR* genes and provides a basal level of EutR protein that allows the cell to sense the appearance of the inducers.

Polar insertion mutations (Mud and Tn*10* elements) in any of the *eutDEABC* genes do not abolish the *eutR* function in complementation tests, suggesting that the basal expression of the *eutR* gene is independent of the primary operon promoter (P_I) (24). All operon fusions created by Mud insertions in the *eutDEABC* region show *eutR*-dependent induction of β-galactosidase by ethanolamine plus B_{12}; this induction is lost if a *eutR* mutation is added (23). This demonstrates that at least some EutR protein is synthesized when the operon is fully repressed and that this synthesis is not eliminated by any of the insertion elements we have placed promoter proximal to the *eutR* gene. We will describe the basal transcription activity in terms of an internal promoter, located between the *eutC* and *eutR* genes (Fig. 1). However, we cannot eliminate the possibility that all of the Tn*10*dTet and Mud insertion elements we have used introduce a weak promoter that provides *eutR* gene expression. If this were true, the basal transcription of the *eutR* gene in wild-type strains could come from the primary promoter P_I. We cannot distinguish between a single internal promoter and a series of sites within the operon at which a low level of transcription might start.

In strains with a *eutA*::Tn*10*dTet insertion, described above, the *eutR::lac* and the *eut-38::lac* fusions should be expressed only from the internal promoter. The low level of *lac* expression in these strains was similar in the *eutR*$^+$ (Table 2, lines 4 and 6) and *eutR* (line 3) strains, indicating that the internal promoter (P_{II}) does not require EutR function and appears to be constitutively expressed. The β-galactosidase levels measured in the *eut-38*::MudA fusion strain (line 6) are severalfold higher than in the *eutR156*::MudJ strain (line 4); this seems to be simply a characteristic of the particular *lac* fusions.

Positive autogenous regulation of the *eutR* gene. The maximum induced activity of the primary promoter is greater in strains that can induce expression of the *eutR* gene. A chromosomal *eutD18*::MudJ insertion was used to measure primary promoter activity. This insertion blocks transcription of downstream genes (including the *eutR* gene) from the primary promoter; the *eutR* gene is expressed only from the weak constitutive internal promoter. In this strain, the primary promoter showed a 390-fold induction in response to ethanolamine and B_{12}; this induction was completely dependent on an intact *eutR* gene (Table 3, lines 1 and 2).

A larger induction of the primary promoter was observed when *eutR* was expressed by both the internal promoter and the regulated primary promoter. Transcription of the same chromosomal *eutD::lac* fusion was induced 1,300-fold in a strain carrying a second, intact copy of the *eut* operon on plasmid F′ 606 *his*$^+$ *eut*$^+$ (Table 3, line 3). This level is not changed by removal of the chromosomal *eutR* gene (line 4), but it was reduced when transcription of the plasmid *eutR* gene from the primary promoter was blocked by the polar *eutD46*::Tn*10* insertion (Table 3, line 5). Elimination of the *eutR* gene from the plasmid (line 6) abolishes the plasmid's ability to complement a chromosomal *eutR* mutation; the *eutR/eutR* diploid is unable to induce transcription from P_I.

It should be noted that when all EutR protein production is due to the plasmid's internal promoter, the level of induction seen (Table 3, line 5) is higher than that seen for a strain with only a chromosomal copy of *eutR* under the same promoter (line 1). This is probably due to a slightly higher copy number of the *eutR* gene on the F′ plasmid. This further supports the idea that the basal level of *eutR* gene expression provided by the internal promoter provides a

TABLE 4. Catabolite repression of the *eut* operon

Line no.	Strain	Genotype	Promoter(s)[a] for:		β-Galactosidase activity (U) of cells grown in medium supplemented with[b]:					
					Succinate		Glucose		Glucose + cAMP	
			eutR	lacZ	−E&B	+E&B	−E&B	+E&B	−E&B	+E&B
1	TT10654	*eutD*::*lac*	P_{II}	P_I	1	830	1	35	1	960
2	TT14714	*eutD*::*lac cya*::Tn*10*	P_{II}	P_I	ng	ng	1	8	1	500
3	TT14715	*eutD*::*lac crp*::Tn*10*	P_{II}	P_I	ng	ng	1	8	1	8
4	TT10674	*eut-38*::*lac*[c]	P_I, P_{II}	P_I, P_{II}	4	380	3	150	4	260
5	TT14716	*eut-38*::*lac cya*::Tn*10*	P_I, P_{II}	P_I, P_{II}	ng	ng	4	10	4	140
6	TT14717	*eut-38*::*lac crp*::Tn*10*	P_I, P_{II}	P_I, P_{II}	ng	ng	4	10	4	10
7	TT14492	*eut-38*::*lac eutA*::Tn*10*d	P_{II}	P_{II}	8	7	6	6	7	9

[a] Promoter P_I is the primary promoter for the *eut* operon, and promoter P_{II} is the internal promoter.
[b] ng, no growth; E&B, ethanolamine and B_{12}.
[c] This *lac* fusion is located on the promoter-distal side of *eutR* at the end of the operon.

limiting concentration of activator protein and confirms the idea that induction of the *eutR* gene is necessary to achieve full *eut* operon expression.

Extra control regions reduce operon expression. In a strain expressing the *eutR* gene only from the chromosomal internal promoter, induction of the chromosomal *eutD* fusion was reduced by a plasmid that provided a *eut* operon but carried the *eutR205*::Tn*10* mutation (compare lines 1 and 7 in Table 3). This effect may be due to the extra copies of the *eut* primary control region present on the plasmid; these may titrate some of the limiting EutR protein made from the chromosome and reduce the amount of EutR protein reaching the assayed chromosomal control region.

Catabolite repression of the *eut* operon. Catabolite repression was examined by using a *eutD*::MudJ fusion and the *eut-38*::MudA fusion at the 3′ end of the operon. Both fusion strains showed substantially lower levels of induced expression during growth on glucose than during growth on succinate as the carbon source, and catabolite repression of both fusion strains was overcome by adding exogenous cAMP to the glucose growth media (Table 4, lines 1 and 4).

The *cya* and *crp* genes encode the enzyme adenyl cyclase and the catabolite activator protein; these proteins mediate catabolite repression. Insertions of Tn*10* in either gene reduced *eut* operon expression substantially (Table 4, lines 2, 3, 5, and 6). Addition of exogenous cAMP relieved the reduction caused by the *cya*::Tn*10* insertion, as expected if the defect is due to a lack of cAMP (lines 2 and 5), but had no effect in the *crp*::Tn*10* strains (lines 3 and 6). The significant residual expression of the *eut* operon during growth on glucose may explain the ability of *S. typhimurium* to use ethanolamine as the sole nitrogen source even during growth on glucose. However, growth on glucose medium with ethanolamine as the sole nitrogen source is eliminated in *cya* insertion mutants, and growth is substantially slower in *crp* insertion mutants. This suggests that the catabolite activation pathway is required for ethanolamine utilization.

Note that the *eut-38* fusion shows a high induced level of expression in the presence of glucose (Table 4). We suspect that once high levels of EutR protein are produced in the *eut-38* strain by autoinduction, cells may escape the effect of catabolite repression. In the *eutD* fusion strain, EutR protein is produced only by the uninducible internal promoter; these high levels of EutR protein are not attained.

The influence of catabolite repression on the internal promoter was examined by using a *eut-38*::*lac* fusion strain containing an upstream *eutA*::Tn*10*dTet insertion to block transcription of *lacZ* from the primary promoter (Table 4, line 7). No significant difference in internal promoter activity was detected when the strain was grown in media with succinate, with glucose, or with glucose plus cAMP, indicating that the internal promoter is not subject to catabolite repression. Therefore, catabolite repression is most likely mediated via the primary promoter.

Isolation of regulatory mutants. To identify regulatory elements that mediate expression of the *eut* operon in response to ethanolamine plus B_{12}, we isolated mutant derivatives of the *eutD*::*lac* operon fusion strain (TT10271) that are Lac^+ on medium containing ethanolamine but not B_{12}; this strain expresses the *eutR* gene only from the internal promoter. Cells were plated on NCE lactose medium containing ethanolamine; selection was made for growth on lactose as the sole carbon source. Spontaneous Lac^+ derivatives were found at a frequency of approximately 10^{-7}.

One exceptional regulatory mutant (*eut-262*) was identified by screening on X-Gal indicator medium for mutants with increased expression of the *eutD*::*lac* fusion on ethanolamine alone following localized mutagenesis of the *eutABCR* region (as described in Materials and Methods). All of the regulatory mutations described above map in or near the *eut* operon; their Lac^+ phenotype cotransduced with the drug resistance genes of *eut*::Mud and *eut*::Tn*10* elements.

Regulatory mutations affecting the P_I region. The majority of mutants with a Lac^+ phenotype in the presence of only one inducer were found to contain a mutation located in the primary promoter region; the new mutation causes constitutive expression of the *eutD*::*lac* fusion. The operon expression in these mutants (Table 5, lines 3 to 5) is independent of the *eutR* function; the mutants remained Lac^+ when a *eutR205*::Tn*10* insertion was introduced (data not shown). Since operon expression in these mutants does not require EutR function or respond to the standard inducers, it seems likely that mutations of this type (*eut-253*, *eut-254*, and *eut-259*) render the P_I promoter fully constitutive or (more likely) delete the *eut* promoter and fuse the operon to a foreign constitutive promoter of moderate strength.

Seven constitutive mutants, including the three described above and the *eut-260* mutant described below, were mapped. All showed close transductional linkage to the promoter-proximal *eutD18*::MudJ insertion (98 to 100% cotransduction of Lac^+ and Kan^r). The constitutive mutants were crossed with a recipient strain containing a deletion which removes the left end of the operon and extends into the *eutD* gene (*eut-230*). In this cross, Eut^+ transductants

TABLE 5. Effect of regulatory mutations on *eutD::lac* fusion expression

Line	Strain	Relevant markers[a]	cAMP added to medium	β-Galactosidase activity (U) in cells grown in medium with[b]:			
				No inducer	EA	B_{12}	EA + B_{12}
1	TT10271	*eutD::lac*	−	1	1	2	300
2	TT10271	*eutD::lac*	+	1	2	5	700
3	TT14498	*eutD::lac eut-253*	−	60	60	60	60
4	TT14499	*eutD::lac eut-254*	−	110	120	120	110
5	TT14503	*eutD::lac eut-259*	−	70	80	70	70
6	TT14504	*eutD::lac eut-260*	−	25	30	34	500
7	TT14494	*eutD::lac eut-247*	−	1	5	3	1,500
8	TT14494	*eutD::lac eut-247*	+	1	12	3	2,600
9	TT14496	*eutD::lac eut-250*	−	1	6	3	1,500
10	TT14496	*eutD::lac eut-250*	+	1	20	4	2,800
11	TT14838	*eutD::lac eut-262*	−	1	4	16	1,900
12	TT14838	*eutD::lac eut-262*	+	1	5	35	2,000
13	TT14493	*eutD::lac eut-246*	−	1	4	7	340
14	TT14493	*eutD::lac eut-246*	+	1	9	14	900

[a] See Table 1 for complete genotypes.
[b] The carbon source was glycerol, and the nitrogen source was ammonia in NCE medium. EA, ethanolamine.

arose by recombination between the donor *eutD18*::MudJ insertion and the recipient deletion. When the seven regulatory mutants carrying the *eutD18*::MudJ insertion were used as donors in the same cross, all failed to generate Eut^+ recombinants, indicating that the constitutive mutants are defective for the *eutD* structural gene. The results suggest that the constitutive regulatory mutations either generate a new promoter within the *eutD* gene or (more likely) are deletions or insertions which damage the *eutD* gene and fuse the *eut* operon to a foreign promoter.

Another regulatory mutant (*eut-260*) showed an increased basal level but, unlike the mutations described above, showed a further increase in expression to the normal induced level upon addition of the two inducers to the growth medium (Table 5, line 6). These results suggest that this mutation does not remove the main promoter but may have created an additional promoter or allowed the main promoter to act at a low level in the absence of induction.

Regulatory mutations that generate promoters within the operon. Unlike the mutations described above, which permit EutR-independent growth on lactose without any inducer, a few of the regulatory mutants showed a Lac^+ phenotype if either ethanolamine or B_{12} was individually present; these mutants showed no growth on lactose unless at least one inducer was provided. The parent strain requires both ethanolamine and B_{12} to permit growth on lactose.

For one type of mutant, operon expression was inducible to a level that exceeded that of the parent *eutD::lac* fusion strain. Three examples of this type are *eut-247*, *eut-250*, and *eut-262* (Table 5). The induced enzyme level in these mutants is similar to that in strains which are able to increase *eutR* gene expression in the course of operon induction.

The strain used in isolating regulatory mutants (TT10271) carries a *eutD18*::MudJ insertion which exerts a strong polar effect on expression of the promoter-distal *eutEABC* genes as well as on the *eutR* gene. Because of this polarity effect, the parent strain can not use ethanolamine as a nitrogen source. Some regulatory mutations (such as *eut-247* and *eut-250*), identified by their effect on expression of the *eutD::lac* fusion, also enable the parent strain to use ethanolamine as a nitrogen source and thus appear to express the *eutABC* genes. The new promoters provided by these mutations must lie downstream of the polar parental *eutD::lac* fusion (whose expression was scored in isolating the mutants) and upstream of the *eutABC* genes, whose expression is required for growth on ethanolamine. The *eut-247* mutation cotransduced with the *eutD*::MudJ element at a frequency of 85%, and the *eut-250* mutation cotransduced at a frequency of 100%. This suggests that *eut-247* lies in the distal portion of the *eutD* gene or within the *eutE* gene and that *eut-250* is in the distal portion of the MudJ element.

The *eut-262* mutation was generated by localized mutagenesis of the *eutABCR* region. This mutation provides a Lac^+ phenotype for the parental *eutD::lac* fusion and causes an increase in *eutR* gene expression (see below). However, unlike the mutations described above, it does not provide a $Eut(N^+)$ phenotype. The mutation was mapped (as described in Materials and Methods) and was found to recombine with deletion DEL742, which includes the *eutR* gene. Thus, mutation *eut-262* increases *eutR* gene expression but does not provide expression of all of the *eutABC* genes and does not affect the *eutR* gene itself. We suggest that mutation *eut-262* generates a new promoter somewhere within the *eutABC* gene cluster (Fig. 1). The effect of both the *eut-247* and *eut-262* mutations is mediated by functional EutR protein because these strains become Lac^- upon introduction of the *eutR205*::Tn*10* insertion. However, the *eut-247* and *eut-262* mutations recombine with deletion DEL742 and thus do not affect *eutR* gene structure directly. Later we will describe the effect of the *eut-247* and *eut-262* mutations on *eutR* gene transcription.

Three additional mutations that show properties like those described above but were extremely unstable were isolated. Mutations *eut-249* and *eut-252* provide a Lac^+ $Eut(N^+)$ phenotype; however, they revert to a Lac^- Eut^- phenotype with high frequency. Mutation *eut-251* causes a Lac^+ phenotype but does not allow use of ethanolamine as a nitrogen source and therefore does not express *eutABC* genes. Like

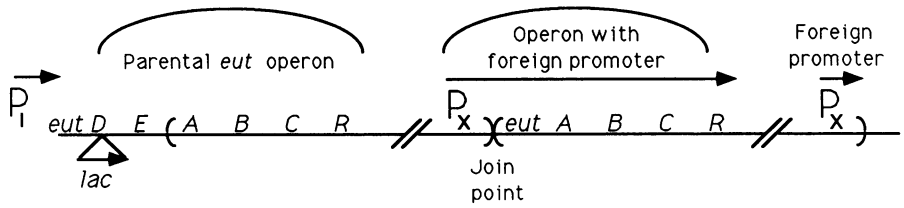

FIG. 2. A duplication that can provide a new promoter for part of the *eut* operon. The duplicated segment is indicated by parentheses. Note that P_X is a foreign promoter that expresses some of the *eut* genes by directing transcription across the duplication join point. One copy of the *eut* operon and one copy of the gene normally expressed by P_X remain intact; the only novel sequence is at the duplication join point.

eut-249 and *eut-252*, it is unstable, reverting to Lac^- with high frequency.

This instability phenomenon has been seen previously for mutants that restore function to promoterless genes. This has been described for the histidine operon (1). In one case, a silent *hisD* gene is activated by fusion to the *argA* promoter at the join point of a duplication (12, 27). It seems likely that the same explanation may account for the unstable *eut* regulatory mutations described above.

We infer that all three unstable mutations are duplications which fuse a foreign promoter to the *eut* operon, allowing expression of genes downstream of the join point (including the *eutR* gene). The proposed structure of these duplications is diagrammed in Fig. 2. In the two Eut(N^+) strains, the fusion join point must be placed so as to activate the *eutABC* genes (i.e., between the *eutD*::*lac* insertion and the *eutA* gene, as depicted in Fig. 2); in the Eut(N^-) mutant the fusion could be located anywhere within the *eutABC* region.

The internal regulatory mutations increase *eutR* gene transcription. We have hypothesized that both the stable and unstable internal regulatory mutations described above exert their effect on the main promoter by increasing the level of *eutR* gene expression. To test this, mutations *eut-247* and *eut-262* were each added singly to a strain with a *eutR*::*lac* fusion (instead of the original *eutD*::*lac* fusion). Both mutations caused a ninefold increase in expression of the *eutR*::*lac* fusion (Table 6). The β-galactosidase activity measured in these strains is due to the native internal promoter plus the novel internal promoters (generated by the regulatory mutations); the primary promoter is not active in the absence of EutR function, and in any case, any residual transcripts are blocked by a *eutD*::Tn*10* insertion.

Elevated EutR allows partial induction by one effector and increased maximal induction. All of the mutations that increase *eutR* gene expression have two additional properties. They allow partial transcriptional activation when only one of the inducers is present, and they allow a higher than normal level of operon induction when both inducers are provided (compare line 1 with lines 7 to 12 in Table 5). Both of these effects were seen earlier in strains whose EutR level was increased by the presence of the F′ 606 eut^+ plasmid (Table 3, compare lines 1 and 3).

A regulatory mutation within the *eutR* gene. One of the regulatory mutations (*eut-246*), whose effect is dependent on the *eutR* activator function, differs in phenotype and map location from the mutations described above. Mutation *eut-246* allows partial induction of the *eutD*::*lac* fusion by a single effector but normal induction in the presence of both inducers (compare lines 13 and 14 with line 1 in Table 5). This suggests that the *eut-246* mutation might alter regulation in a manner that does not involve an increase in *eutR* gene expression (which would increase the maximum induced level).

The *eut-246* mutation was mapped as described in Materials and Methods by using a series of deletions which enter the *eutR* gene from the 3′ end of the operon (Fig. 1). No recombinants were found with any of the deletions which enter the *eutR* gene (DEL742, DEL758, and DEL760). Deletion DEL863 is a constructed $EutR^+$ deletion that extends from the *eut-38*::MudA insertion site rightward to the nearby *cysA* locus (23). Two of 80 recombinants selected for repair of DEL863 deletion did not inherit the donor *eut-246* regulatory mutation, indicating that mutation *eut-246* maps to the left of the end point of deletion DEL863. We infer that the *eut-246* mutation must lie at the extreme downstream end of the *eutR* gene or just outside of the distal end of this gene. It seems likely that this mutation owes its effects to alteration of the *eutR* gene rather than an increase in *eutR* gene expression.

DISCUSSION

Conclusions. Several conclusions are drawn from the results presented here.

(i) The *eut* operon is controlled by a transcriptional activator (EutR) encoded within the operon. A model for the mechanism of regulation is shown in Fig. 3. Null mutations in the *eutR* gene prevent induction of transcription of the operon in response to the availability of ethanolamine plus B_{12}; the

TABLE 6. Effect of internal regulatory mutations on *eutR*::*lac* expression

Strain	Relevant markers	β-Galactosidase activity (U) in cells grown in medium with[a]:			
		No inducer	EA	B_{12}	EA + B_{12}
TT14846	*eutD*::Tn*10 eutR156*::*lac*	2	2	2	2
TT14844	*eutD*::Tn*10 eut-247 eutR156*::*lac*	17	16	19	17
TT14845	*eutD*::Tn*10 eut-262 eutR156*::*lac*	19	19	20	17

[a] Cells were grown in NCE medium with glycerol as the carbon source and ammonia as the nitrogen source. EA, ethanolamine.

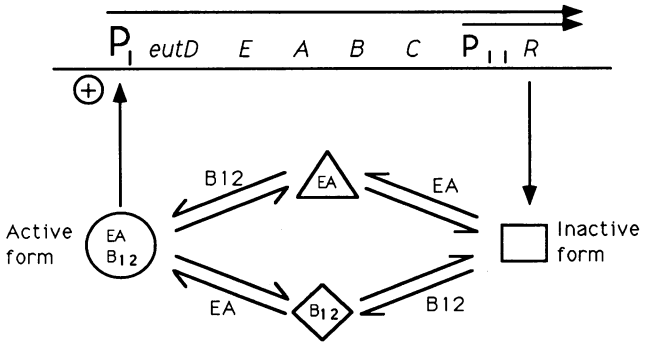

FIG. 3. The EutR regulatory protein responds to two effectors. The model proposes that each effector binds independently. Normally both effectors must be present to stabilize the active conformation and ensure sufficient activator for operon induction. In cells with a high level of regulatory protein, a single effector might generate a large enough pool of an intermediate conformation to lead, by simple equilibrium considerations, to a significant level of active regulator. EA, ethanolamine.

eutR defect can be corrected in *trans* by an F′ *eutR*$^+$ plasmid.

(ii) The EutR protein seems to play a purely regulatory role. Some suppressors of *eutR* mutations express all functions necessary for ethanolamine utilization with no functional *eutR* gene.

(iii) The quantity of EutR protein appears to limit operon expression. Insertion mutations that block transcription of the *eutR* gene from the primary promoter (P_I) leave the *eutR* gene expressed only by a weak constitutive internal promoter and reduce operon expression to 20% of the level seen when *eutR* gene expression is inducible. Mutations that, we argue, generate new internal promoters for a noninducible *eutR* gene increase operon expression.

(iv) Increased expression of the EutR protein allows operon induction by either ethanolamine alone or B_{12} alone. Strains which overexpress *eutR* by virtue of a new internal promoter and strains which carry F′ 606 *eut*$^+$ and also express the *eutR* gene from the primary *eut* operon promoter show significant induction by a single effector.

General points. Fusions of the *lac* operon to the *eutD* gene, to the *eutR* gene, and to an untranslated region promoter distal to the *eutR* gene were used to report operon expression. All fusions show EutR-dependent induction when ethanolamine and B_{12} are available. However, the fusions differ in their basal and fully induced levels of β-galactosidase activity. Several factors may account for this variation. The level of the primary promoter activity is very sensitive to the expression and inducibility of the *eutR* gene. Expression and inducibility of *eutR* varies in each fusion. The position of the fusions relative to the two promoters also influences *lac* expression. Fusions located downstream of the internal promoter show higher basal transcription. The downstream fusions also show a lower maximal induced expression level, possibly due to termination of some of the transcripts originating at the P_I promoter before they reach the end of the operon. In previous experiments we have seen a generally decreasing gradient of induced *eut*::*lac* fusion expression as the fusion point is moved farther downstream in the operon (23). Finally, variation in the structure of individual fusion transcripts could cause differences in transcript stability or translation efficiency; these effects would be a characteristic of each fusion strain.

The fact that either ethanolamine alone or B_{12} alone causes some induction in mutants with elevated *eutR* gene expression implies that ethanolamine and B_{12} both participate in conversion of inactive EutR protein to the form capable of activating transcription. We suggest that the EutR protein may bind either effector singly and thereby stabilize distinct protein conformations that exist in equilibrium with the conformation that activates transcription. This active form may be stabilized by the simultaneous binding of both effectors (Fig. 3). In strains with a high level of EutR protein, the presence of a single effector could lead to an increased level of one of the intermediate conformations. Since these forms are in equilibrium with the active form, the high level of EutR protein could lead, by mass action, to a significant level of the active conformation when only a single effector is present.

Only one of the regulatory mutations described here (*eut-246*) maps in or very near the *eutR* gene. The phenotype of this mutant differs from that of mutations that are thought to cause increased expression of the *eutR* gene. The level of induction achieved in the presence of both inducers is the same level seen for the parental *eutD*::*lac* fusion strain. The results suggest that the *eut-246* mutation may allow the EutR protein (shown in Fig. 3) to assume the active form when either one of the two inducers is present singly.

The model for regulation. The model presented here suggests that a very high concentration of activator protein might allow the operon to be fully induced by only one effector. The regulatory mutations which generate new internal promoters permit weak activation of transcription by a single inducer. These effects should also be seen when *eutR* levels are high as a consequence of a normal induction. That is, once the operon is fully induced (by both effectors), one might expect that the induced state could be maintained by a single effector using the elevated levels of *eutR* function. This could be an important feature of the regulatory mechanism.

During anaerobic growth, the de novo B_{12} biosynthetic pathway produces very little B_{12} (about 60 molecules per cell) (3). Induction of the *eut* operon results in high levels of the B_{12}-dependent enzyme ethanolamine ammonia lyase. When B_{12} is scarce, induction may relax the requirement for B_{12} binding to the *eut* activator and serve to free B_{12} from its inducer role so that it can function as a cofactor for ethanolamine ammonia lyase. We suggest that B_{12} is needed for the initial induction of the operon but that the induced state may be maintained by ethanolamine alone, allowing all of the B_{12} supply to be available for ethanolamine ammonia lyase once induction has been achieved.

ACKNOWLEDGMENTS

We thank David Sheppard for helpful comments on an early version of the manuscript.

This work was supported in part by Public Health Service grant GM34804 to J.R.R.

REFERENCES

1. **Anderson, R. P., and J. R. Roth.** 1978. Tandem chromosomal duplications in *Salmonella typhimurium*: fusion of histidine genes to novel promoters. J. Mol. Biol. **119**:147–166.
2. **Andersson, D. I., and J. R. Roth.** 1989. Mutations affecting regulation of cobinamide biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **171**:6726–6733.
3. **Andersson, D. I., and J. R. Roth.** 1989. Redox regulation of the genes for cobinamide biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **171**:6734–6739.
4. **Bassford, P. J., Jr., and R. J. Kadner.** 1977. Genetic analysis of

components involved in vitamin B_{12} uptake in *Escherichia coli*. J. Bacteriol. **132**:796–805.
5. **Blackwell, C. M., F. A. Scarlett, and J. M. Turner.** 1977. Microbial metabolism of amino alcohols: control of formation and stability of partially purified ethanolamine ammonia-lyase in *Escherichia coli*. J. Gen. Microbiol. **98**:133–139.
6. **Blackwell, C. M., and J. M. Turner.** 1978. Microbial metabolism of amino alcohols. Formation of coenzyme B12-dependent ethanolamine ammonia-lyase and its concerted induction in *Escherichia coli*. Biochem. J. **176**:751–757.
7. **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.
8. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: an in vivo probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA **76**:4530–4533.
9. **Castilho, B. A., P. Olfson, and M. J. Casadaban.** 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. **158**:488–495.
10. **Chan, R. K., D. Botstein, T. Watanabe, and Y. Ogata.** 1972. Specialized transduction of tetracycline resistance by phage P22 in *Salmonella typhimurium*. II. Properties of a high-frequency-transducing lysate. Virology **50**:883–898.
11. **Chang, G. W., and J. T. Chang.** 1975. Evidence for the B12-dependent enzyme ethanolamine deaminase in *Salmonella*. Nature (London) **254**:150–151.
12. **Conner, C., and J. R. Roth.** Unpublished results.
13. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Elliott, T., and J. R. Roth.** 1988. Characterization of Tn*10*d-CAM: a transposition-defective Tn*10* specifying chloramphenicol resistance. Mol. Gen. Genet. **213**:332–338.
15. **Escalante-Semerena, J. C., and J. R. Roth.** 1987. Regulation of cobalamin biosynthetic operons in *Salmonella typhimurium*. J. Bacteriol. **169**:2251–2258.
16. **Hughes, K. T., and J. R. Roth.** 1984. Conditionally transposition-defective derivative of Mu d1 (Amp Lac). J. Bacteriol. **159**:130–137.
17. **Hughes, K. T., and J. R. Roth.** 1988. Transitory cis complementation: a method for providing transposition functions to defective transposons. Genetics **119**:9–12.
18. **Jeter, R. M., 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.
19. **Jones, P. W., and J. M. Turner.** 1984. Interrelationships between the enzymes of ethanolamine metabolism in *Escherichia coli*. J. Gen. Microbiol. **130**:299–308.
20. **Jones, P. W., and J. M. Turner.** 1984. A model for the common control of enzymes of ethanolamine catabolism in *Escherichia coli*. J. Gen. Microbiol. **130**:849–860.
21. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Ratzkin, B., and J. R. Roth.** 1978. Cluster of genes controlling proline degradation in *Salmonella typhimurium*. J. Bacteriol. **133**:744–754.
23. **Roof, D. M., and J. R. Roth.** 1988. Ethanolamine utilization in *Salmonella typhimurium*. J. Bacteriol. **170**:3855–3863.
24. **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.
25. **Scarlett, F. A., and J. M. Turner.** 1976. Microbial metabolism of amino alcohols: ethanolamine catabolism mediated by coenzyme B12-dependent ethanolamine ammonia-lyase in *Escherichia coli* and *Klebsiella aerogenes*. J. Gen. Microbiol. **95**:173–176.
26. **Sennett, K. E., L. E. Rosenberg, and I. S. Mellman.** 1981. Transmembrane transport of cobalamin in prokaryotic and eukaryotic cells. Annu. Rev. Biochem. **50**:1053–1086.
26a. **Sheppard, D., and J. R. Roth.** Unpublished data.
27. **Shyamala, V., E. Schneider, and G. F. Ames.** 1990. Tandem chromosomal duplication: role of REP sequences in the recombination event at the join point. EMBO J. **9**:939–946.
28. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
29. **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.
30. **Wu, T. T.** 1966. A model for three point analysis of random general transduction. Genetics **54**:405–410.