JOURNAL OF BACTERIOLOGY, Oct. 1997, p. 6084–6091 Vol. 179, No. 19
0021-9193/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Repression of the *cob* Operon of *Salmonella typhimurium* by Adenosylcobalamin Is Influenced by Mutations in the *pdu* Operon

MICHAEL AILION† AND JOHN R. ROTH*

Department of Biology, University of Utah, Salt Lake City, Utah 84112

Received 2 January 1997/Accepted 29 July 1997

The *cob* operon encodes functions needed for the biosynthesis of adenosylcobalamin (Ado-B_{12}). Propanediol induces transcription of the *cob* operon and the neighboring *pdu* operon, which encodes proteins for the B_{12}-dependent degradation of propanediol. Expression of the *cob* (but not the *pdu*) operon is repressed by exogenous cyanocobalamin. Evidence is provided that *cob* operon repression is signaled by internally generated Ado-B_{12}, which can be formed either by the CobA adenosyltransferase or by an alternative adenosyltransferase (AdoT) that we infer is encoded within the *pdu* operon. Repression is also affected by mutations (AdoB) in the *pdu* operon that map upstream of the inferred *pdu* adenosyltransferase gene. Such mutations allow cobalamin to mediate repression at concentrations 100-fold lower than those needed in the wild type. It is proposed that these mutations eliminate a component of the propanediol dehydratase enzyme complex (PduCDE) and that this complex competes with the *cob* regulatory mechanism for a limited supply of Ado-B_{12}.

The *cob* operon of *Salmonella typhimurium* encodes 20 proteins involved in the synthesis of cobalamin (coenzyme B_{12}) (12, 20, 21, 33). The entire operon is transcribed from a single regulated promoter (9, 28). Induction of the *cob* operon has been shown to depend on a positive regulatory protein, PocR, and its effector, propanediol (9, 30); the PocR protein also regulates the *pdu* operon, which encodes functions needed for the B_{12}-dependent degradation of propanediol (9, 11, 13, 20). Induction of both operons depends on global control by the Crp-cyclic AMP and ArcA-ArcB systems, which permit induction during growth on poor carbon sources and/or during anaerobic growth (1, 3, 4, 18). The general area of B_{12} metabolism in enteric bacteria has been reviewed recently (32).

In addition to this positive control by propanediol, the *cob* (but not the *pdu*) operon is negatively regulated by cobalamin, the end product of the biosynthetic pathway (18). Unlike positive global control of the operon, which appears to be entirely transcriptional, repression by B_{12} appears to affect both transcription and translation, perhaps by a coupled mechanism (27, 29). The *btuB* (B_{12} transport) gene of *Escherichia coli* is repressed by B_{12} in a similar manner (24). Little is known about either mechanism of repression or the role of B_{12} in the process, but it seems possible that cobalamin is recognized directly by an mRNA leader sequence, affecting mRNA folding and the translation initiation site of the first coding sequence. Hunts for mutations in both systems have failed to identify a regulatory protein that might bind cobalamin; all mutations that prevent normal repression by exogenous cyanocobalamin (CN-B_{12}) affect either components of the B_{12} transport machinery, the B_{12} adenosyltransferase (CobA) protein (BtuR in *E. coli*), or the long mRNA leader sequences upstream of the *cob* and *btuB* genes (23, 24, 27, 29, 38).

The CobA protein is an adenosyltransferase which can add a deoxyadenosyl moiety to several corrinoids, including cobinamide and CN-B_{12} (19, 38). Both the *Salmonella* CobA protein and its *Pseudomonas* homolog (CobO) have been purified and demonstrated to have ATP:corrinoid adenosyltransferase activity in vitro (14, 16, 37, 38). In addition to the presence of an adenosyltransferase (such as CobA protein), formation of Ado-B_{12} requires the reduction of the cobalt atom to the Co(I) state. This reduction is done in vitro with chemical reducing agents, but in vivo, it probably requires an enzyme system that has not yet been defined, either genetically or biochemically in *Salmonella*. In *E. coli*, mutations eliminating the CobA adenosyltransferase activity (called BtuR in *E. coli*) prevent repression of the *btuB* gene by exogenous CN-B_{12} (23), suggesting that adenosylcobalamin (Ado-B_{12}) is the true effector of repression. Here we examine the effects of *cobA* and *pdu* mutations on repression of the *Salmonella cob* operon.

* Corresponding author.

† Present address: Department of Genetics, University of Washington, Seattle, WA 98195.

MATERIALS AND METHODS

Bacterial strains and transposons. All of the strains used in this study are derivatives of *S. typhimurium* LT2 (Table 1). The MudJ element is a transposition-defective derivative of phage Mu which, when inserted, creates *lacZ* operon fusions (10). Transposons Tn*10d*Tc and Tn*10d*Cm are transposition-defective derivatives of transposon Tn*10* (17, 40). The *pdu* mutations used here were isolated and described previously (39). Multiply marked strains were constructed by transduction mediated by phage P22 mutant HT105/1 *int-201* (34) as described previously (9).

Media. Rich medium was nutrient broth (0.8%; Difco) with 85 mM NaCl. Minimal medium was NCE medium (6) with 1 mM $MgSO_4$ and one or more of the following carbon and energy sources: Na_2 succinate, 1.0%; Na pyruvate, 0.44%; Na_2 fumarate, 0.32%; and glycerol and DL-1,2-propanediol, 0.2%. Succinate, fumarate, CN-B_{12}, and Ado-B_{12} were from Sigma Chemical Co.; pyruvate and propanediol were from Aldrich Chemical Co.; and glycerol was from Em Scientific. Methionine and histidine were added at standard concentrations (15).

Assay of β-galactosidase. Cells were grown under aerobic or anaerobic conditions as described previously (9). Whenever Ado-B_{12} was present in the growth medium, tubes were wrapped in aluminum foil to exclude light and all manipulations prior to enzyme assay were performed in a dark room. These precautions were taken because Ado-B_{12} is highly sensitive to light and can lose its adenosyl moiety upon illumination. The aluminum foil coverings had no effect on induction of the *cob* operon. Assays were performed on cells subcultured once or twice in minimal medium as described in Results. Activity was determined by the method of Miller (25). Reported data are in Miller units and are the values from single representative experiments; all assays were repeated several times with essentially the same values as those presented.

TABLE 1. Strain list

Strain	Genotype
TT10852	*metE205 ara-9 cob-24*::MudJ
TT14298	*metE205 ara-9 cob-24*::MudJ *cobA367*::Tn*10d*Tc
TT17039	*metE205 ara-9 cob-24*::MudJ *cobA366*::Tn*10d*Cm
TT18117	DEL1077 (*metE*) *ara-9* DEL1715[(*cob-24*) *MudJ*(*zea-3666*)]
TT18118	DEL1077 (*metE*) *ara-9* DEL1715[(*cob-24*) *MudJ*(*zea-3666*)] *cobA367*::Tn*10d*Tc
TT18119	DEL1077 (*metE*) *ara-9* DEL1715[(*cob-24*) *MudJ*(*zea-3666*)] DEL1743 (*his-pduF*)
TT18120	DEL1077 (*metE*) *ara-9* DEL1715[(*cob-24*) *MudJ*(*zea-3666*)] DEL 1743 (*his-pduF*) *cobA367*::Tn*10d*Tc
TT18170	DEL1077 (*metE*) *ara-9* DEL1715[(*cob-24*) *MudJ*(*zea-3666*)] *pdu-216*::Tn*10d*Cm
TT18171	DEL1077 (*metE*) *ara-9* DEL1715[(*cob-24*) *MudJ*(*zea-3666*)] *pdu-216*::Tn*10d*Cm *cobA367*::Tn*10d*Tc
TT18172	DEL1077 (*metE*) *ara-9* DEL1715[(*cob-24*) *MudJ*(*zea-3666*)] *pdu-218*::Tn*10d*Cm
TT19173	DEL1077 (*metE*) *ara-9* DEL1715[(*cob-24*) *MudJ*(*zea-3666*)] *pdu-218*::Tn*10d*Cm *cobA367*::Tn*10d*Tc
TT18174	DEL1077 (*metE*) *ara-9* DEL1715[(*cob-24*) *MudJ*(*zea-3666*)] *pdu-220*::Tn*10d*Cm
TT18175	DEL1077 (*metE*) *ara-9* DEL1715[(*cob-24*) *MudJ*(*zea-3666*)] *pdu-220*::Tn*10d*Cm *cobA367*::Tn*10d*Tc
TT18176	DEL1077 (*metE*) *ara-9* DEL1715[(*cob-24*) *MudJ*(*zea-3666*)] *pdu-222*::Tn*10d*Cm
TT18177	DEL1077 (*metE*) *ara-9* DEL1715[(*cob-24*) *MudJ*(*zea-3666*)] *pdu-222*::Tn*10d*Cm *cobA367*::Tn*10d*Tc
TT18178	*metE205 ara-9 cob-24*::MudJ *pdu-257*
TT18179	*metE205 ara-9 cob-24*::MudJ *pdu-257* *cobA367*::Tn*10d*Tc
TT18180	*metE205 ara-9 cob-24*::MudJ *pdu-265*
TT18181	*metE205 ara-9 cob-24*::MudJ *pdu-265* *cobA367*::Tn*10d*Tc
TT18182	*metE205 ara-9 cob-24*::MudJ *pdu-252*
TT18183	*metE205 ara-9 cob-24*::MudJ *pdu-252* *cobA367*::Tn*10d*Tc
TT18184	*metE205 ara-9 cob-24*::MudJ *pdu-260*
TT18185	*metE205 ara-9 cob-24*::MudJ *pdu-260* *cobA367*::Tn*10d*Tc
TT18186	*metE205 ara-9 cob-24*::MudJ *pdu-261*
TT18187	*metE205 ara-9 cob-24*::MudJ *pdu-261* *cobA367*::Tn*10d*Tc
TT18188	*metE205 ara-9 cob-24*::MudJ *pdu-264*
TT18189	*metE205 ara-9 cob-24*::MudJ *pdu-264* *cobA367*::Tn*10d*Tc
TT18190	*metE205 ara-9 cob-24*::MudJ *pdu-246*
TT18191	*metE205 ara-9 cob-24*::MudJ *pdu-246* *cobA367*::Tn*10d*Tc
TT18803	*metE205 ara-9 cob-24*::MudJ *zec-3796::Tn10d*Tc *pdu-286*
TT18804	*metE205 ara-9 cob-24*::MudJ *zec-3796*::Tn*10d*Tc *pdu-286 cobA366*::Tn*10d*Cm
TT18805	*metE205 ara-9 cob-24*::MudJ *zec-3796*::Tn*10d*Tc *pdu-283*
TT18806	*metE205 ara-9 cob-24*::MudJ *zec-3796*::Tn*10d*Tc *pdu-283 cobA366*::Tn*10d*Cm
TT18807	*metE205 ara-9 cob-24*::MudJ *zec-3796*::Tn*10d*Tc *pdu-238*
TT18808	*metE205 ara-9 cob-24*::MudJ *zec-3796*::Tn*10d*Tc *pdu-238 cobA366*::Tn*10d*Cm

RESULTS

Repression of the *cob* operon is mediated by Ado-B_{12}. Repression of the *cob* operon was examined by assaying β-galactosidase activity in strains carrying an operon fusion. The *lac* operon is placed under transcriptional control of the *cob* promoter. A map of the *pdu-cob* region is in Fig. 1. These fusions were created by inserting a Mud-*lac* element within the *cob* operon (*cbiD24*::MudJ); each fusion strain also carried a deletion of all *cob* operon material promoter distal to the Mud-*lac* insertion. This deletion was used because of suggestions that the included *cobU* gene might encode a protein with adenosyltransferase activity (26). During aerobic growth of wild-type cells on succinate, the *cob* operon was repressed 10- to 20-fold by adding either CN-B_{12} or Ado-B_{12} to the growth medium (Table 2); the same repressive effect was seen when the operon was induced by propanediol. In a *cobA* mutant, repression by CN-B_{12} was reduced during growth without propanediol while repression by Ado-B_{12} was still complete (Table 2). This result suggested that in order to repress the *cob* operon, CN-B_{12} must first be converted to Ado-B_{12} by the CobA adenosyltransferase (19, 37). When propanediol was provided to a *cobA* mutant, the *cob* operon was repressed equally by either CN-B_{12} or Ado-B_{12}. This result suggested that an alternative adenosyltransferase function might be induced by propanediol.

Since propanediol was known to induce the *pdu* operon, we retested repression in strains carrying a deletion mutation (DEL1743) which removes the entire *pdu* operon. This deletion also removes some of the *pduF* gene, reducing PocR levels and leading to a slightly lower maximal level of *cob* operon induction (11). The deletion, tested alone, did not prevent repression by CN-B_{12} but caused stronger repression of the *cob* operon by both CN-B_{12} and Ado-B_{12} (Table 2). A strain carrying both the *pdu* deletion and a *cobA* mutation showed essentially no repression by CN-B_{12} but was strongly repressed by Ado-B_{12} (Table 2). This result suggested that Ado-B_{12} is the actual signal molecule needed for *cob* operon repression. The effects of inducing or deleting the *pdu* operon suggested that the *pdu* operon encodes a second enzyme (a CobA alternative) that can convert CN-B_{12} to Ado-B_{12}. The stronger repression by both CN-B_{12} and Ado-B_{12} seen in the *pdu* deletion mutant will be discussed below.

The strains described above showed similar patterns of repression by CN-B_{12} or Ado-B_{12} during anaerobic growth on pyruvate-fumarate or glycerol-fumarate (Table 3). However, there were two observable differences. When pdu^+ strains were grown aerobically with propanediol, the repressive effects of both CN-B12 and Ado-B12 were rather small and were even smaller in strains with a *cobA* mutation; under anaerobic conditions, repression was stronger and no *cobA* effect was seen (compare Tables 2 and 3). Second, under aerobic conditions, the *pdu* deletion caused much stronger repression by both CN- and Ado-B_{12}; under anaerobic conditions, repression was rather strong and was not increased by a *pdu* deletion (compare Tables 2 and 3). We think these differences may reflect a higher rate of adenosyl transfer under anaerobic conditions; the transfer reaction requires prior reduction of cobalt, which might be facilitated anaerobically. Alternatively, aerobic catalysis by diol dehydratase may destroy Ado-B_{12}. Regardless of these differences, it was clear that, under both aerobic and anaerobic conditions, Ado-B_{12} appears to be required for full repression of the *cob* operon.

Repression by various concentrations of exogenous CN-B_{12} and Ado-B_{12}. The results described in the previous section suggested that strains with a *pdu* deletion were more strongly

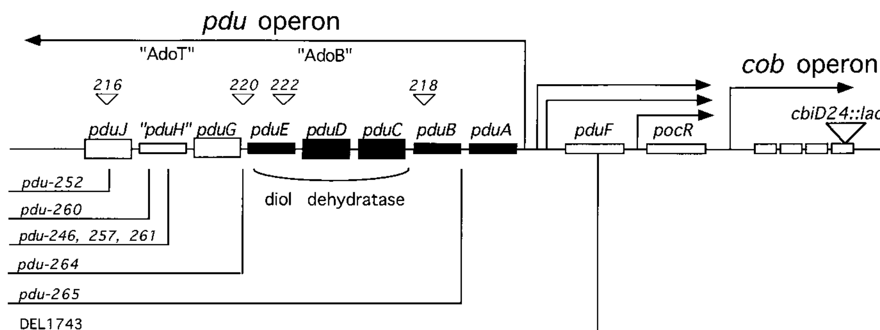

FIG. 1. Genetic map positions of mutations used. The MudJ insertion at the right side of the figure was used to assess transcriptional control of the *cob* operon. Approximate positions of *pdu* insertion and deletion mutations are shown relative to the known genes of the *pdu* operon and the regions thought to encode the AdoT and AdoB activities discussed in this paper. The positions given were inferred from mapping done in several studies (7, 9, 39) and should not be taken as precise locations. Genes represented by filled boxes have been sequenced (8). Genes represented by wider blocks are those for which mutations are known to have a Pdu$^-$ phenotype (39). The region designated "*pduH*" has been inferred to exist based on distribution of deletion endpoints and may include more than one gene.

repressed than the corresponding *pdu*$^+$ strains. Thus, while a deletion of *pdu* did not damage the mechanism of repression, it did appear to affect the sensitivity of that mechanism to Ado-B_{12}; we propose below that the deletion eliminates a protein which competes with the regulatory machinery for Ado-B_{12}. To characterize the dose-response properties of repression by exogenous B_{12}, we assayed the same four strains described above during aerobic growth on succinate with propanediol and various concentrations of CN-B_{12} or Ado-B_{12} (Fig. 2).

As seen in Fig. 2A, maximum repression of the *cob* operon in a *pdu*$^+$ *cobA*$^+$ background occurred at a CN-B_{12} concentration of 75 nM, the concentration used in the initial experiments (Tables 2 and 3). The primary effect of the *pdu* deletion was to shift the apparent K_m of CN-B_{12} for repression; complete repression in the *cobA*$^+$ *pdu* deletion strain was observed at a concentration 100-fold lower than the concentration needed to repress in the isogenic *pdu*$^+$ strain. As seen before, the *pdu* deletion used caused a three- to fourfold reduction in the maximal (unrepressed) expression of the *cob* operon; this reduction has been shown elsewhere to be due to reduction in the level of the PocR regulatory protein, which mediates induction of the operon by propanediol; the PocR protein is not involved in repression by Ado-B_{12} (11).

Also as shown in Fig. 2A, a *cobA* Δ*pdu* double mutant showed little or no repression by CN-B_{12}, even at concentrations several orders of magnitude higher than that needed for repression in wild-type cells; this strain shows repression by Ado-B_{12} (Fig. 2B). It should be noted that in *pdu*$^+$ strains, which can produce Ado-B_{12} by use of a gene in the *pdu* operon, a *cobA* mutation led to a very slight but reproducible increase in the concentration of CN-B_{12} needed for repression (a rightward shift in the titration curve). This suggests that, even in *pdu*$^+$ strains, a *cobA* mutation causes a slight insufficiency in adenosylation of CN-B_{12}. The titration curve for CN-B_{12} repression of *cob* is similar to that reported for repression of the *btuB* gene of *E. coli* (5, 22).

Exogenous Ado-B_{12} effected repression at concentrations similar to those of CN-B_{12} (Fig. 2B). The *pdu* deletion made the *cob* operon several orders of magnitude more sensitive to repression by Ado-B_{12} (as had been seen for CN-B_{12}), while the *cobA* mutation led to a very slight decrease in sensitivity of repression. The Δ*pdu cobA* double mutant was strongly repressed by Ado-B_{12}. As for CN-B_{12}, a slightly higher concentration of Ado-B_{12} was required for repression in the presence of a *cobA* mutation (Fig. 2B). This result may suggest that, even when exogenous Ado-B_{12} is provided, CobA activity helps recycle cobalamin that loses its adenosyl moiety and thereby maintains a maximal pool of internal Ado-B_{12}. The effect of the *cobA* mutation was seen in both the *pdu*$^+$ and Δ*pdu* strains, suggesting that the effect of the *cobA* mutation is independent of the *pdu* operon. The similarity between the CN-B_{12} and Ado-B_{12} titration curves in CobA$^+$ Pdu$^+$ cells suggests that (while adenosylation of B_{12} is required to generate the repression signal) the rate of adenosylation does not limit the conversion of exogenous corrinoids to internal Ado-B_{12}.

Effects of *pdu* mutations on repression of the *cob* operon. The above-described results suggested that the *pdu* deletion has two effects on repression of the *cob* operon by B_{12}. One activity (called AdoT here) encoded within the *pdu* operon appears to contribute to conversion of CN-B_{12} to Ado-B_{12}. Loss of ability to form Ado-B_{12} is detected as inability of CN-B_{12} to mediate operon repression in a *cobA* mutation background. The activity removed by the deletion is most likely

TABLE 2. Repression of the *cob* operon during aerobic growth

Strain	Relevant genotype	β-Galactosidase activity (Miller units) under conditions[a]:					
		Succinate			Succinate-propanediol		
		None	CN-B_{12}	Ado-B_{12}	None	CN-B_{12}	Ado-B_{12}
TT18117	*cbiD::lac*	20	1	1	365	29	32
TT18118	*cbiD::lac cobA*	21	10	2	393	91	114
TT18119	*cbiD::lac* Δ*pdu*	16	1	<1	238	3	2
TT18120	*cbiD::lac* Δ*pdu cobA*	18	18	<1	170	121	5

[a] Cells were grown aerobically at 37°C in NCE medium with methionine and histidine and the indicated sources of carbon and energy. Propanediol was included as an inducer of the *cob-pdu* regulon. None, no addition to the medium. CN-B_{12} and Ado-B_{12} were added at 100 μg/liter (7.5×10^{-8} and 6.3×10^{-8} M, respectively).

TABLE 3. Repression of *cob* during anaerobic growth

Strain	Relevant genotype	β-Galactosidase activity (Miller units) under conditions[a]:								
		Pyruvate-fumarate			Pyruvate-fumarate + propanediol			Glycerol-fumarate		
		None	$CN-B_{12}$	$Ado-B_{12}$	None	$CN-B_{12}$	$Ado-B_{12}$	None	$CN-B_{12}$	$Ado-B_{12}$
TT18117	*cbiD*::*lac*	94	4	4	715	11	9	367	27	35
TT18118	*cbiD*::*lac cobA*	111	31	4	577	10	15	315	23	33
TT18119	*cbiD*::*lac* Δ*pdu*	115	4	4	595	11	10	549	21	19
TT18120	*cbiD*::*lac* Δ*pdu cobA*	131	105	5	543	255	11	464	489	18

[a] Cells were grown anaerobically at 37°C in NCE medium with methionine and histidine and the indicated sources of carbon and energy. Propanediol was included as an inducer of the *cob-pdu* regulon. None, no addition to the medium. $CN-B_{12}$ and $Ado-B_{12}$ were added at 100 μg/liter (7.5×10^{-8} and 6.3×10^{-8} M, respectively).

an adenosyltransferase. Formally, the activity may be a cobalt reductase (also needed for adenosyl transfer); however, in vivo activity of the CobA enzyme without a *pdu* operon demonstrates that a reductase is encoded outside of the operon. Furthermore, if only the reductase is encoded by the *pdu* operon, the results still require the existence of a second transferase acting in strains lacking the CobA activity. We will assume that the operon encodes adenosyltransferase.

A second activity in the *pdu* operon reduces the sensitivity of the *cob* operon to repression; a mutation eliminating this activity allows repression of *cob* by lower concentrations of exogenous $CN-B_{12}$ or $Ado-B_{12}$. We will propose that this activity (called AdoB here) can bind or destroy $Ado-B_{12}$ and thus reduces the amount of $Ado-B_{12}$ available for repression. The *pdu* operon deletion mutation (described above) removed both the AdoT and AdoB activities, resulting both in the failure of $CN-B_{12}$ to repress in a *cobA* background and in increased sensitivity to repression by $CN-B_{12}$ in a $cobA^+$ background. Using these two different phenotypes, we endeavored to identify *pdu* genes responsible for the two activities, using a series of insertion and deletion mutations of the *pdu* operon (Fig. 1).

For each *pdu* insertion or deletion mutation, we constructed a pair of *cob*::*lac* fusion strains with a $cobA^+$ or a *cobA* allele. These strains differ in two ways from those assayed previously. First, none of the *pdu* mutations extends into the *pduF-pocR* region; thus, the *cob* operon is fully induced by propanediol. Second, the *cob* operon downstream of the fusion is not deleted; such a deletion was found to have no effect on repression by B_{12} (data not shown). Each strain was assayed for β-galactosidase activity to score the two phenotypes, AdoT (transferase) and AdoB (binding).

Table 4 shows the effects of several *pdu*::Tn*10*Cm insertion mutations on repression of the *cob* operon. These insertions have been mapped previously and are expected to be polar; hence, each insertion mutation is expected to reduce all operon activities encoded downstream of the insertion site. In an otherwise wild-type background, the *cob* operon is repressed well by either $CN-B_{12}$ or $Ado-B_{12}$ at a high concentration (7.5×10^{-8} M $CN-B_{12}$ or 6.3×10^{-8} M $Ado-B_{12}$) but not by a 10-fold lower concentration of $CN-B_{12}$ (10^{-1} $CN-B_{12}$ column). The *cobA* mutation alone has little effect on repression under these conditions, since propanediol induces the alternative *pdu* adenosyltransferase AdoT. Insertion *pdu-216* has a wild-type repression phenotype and must map downstream of the genes encoding the AdoT and AdoB activities. Insertion *pdu-218* lacks both activities and is inferred to map upstream of both genes. As seen in Table 4, this mutation permits repression by 10^{-1} $CN-B_{12}$ in the $cobA^+$ strain ($AdoB^-$) but is not repressed by $CN-B_{12}$ in a *cobA* background ($AdoT^-$). As predicted by these results, the *pdu-218* mutation maps promoter proximal to the *pdu-216* mutation (39).

Insertion mutation *pdu-220* separates the two activities. A strain with this mutation is not repressed by 10^{-1} $CN-B_{12}$, even in a $cobA^+$ background, and hence is $AdoB^+$; it fails to be repressed by $CN-B_{12}$ in a *cobA* background and hence is $AdoT^-$. Thus, this insertion is expected to map downstream of the gene providing AdoB activity but within or upstream of the gene providing AdoT activity. This mutation maps between the two insertions mentioned above.

Of all the *pdu* insertions assayed, only *pdu-222* gave a phenotype that conflicted somewhat with placement of the gene for AdoB activity upstream of the gene for AdoT activity. This

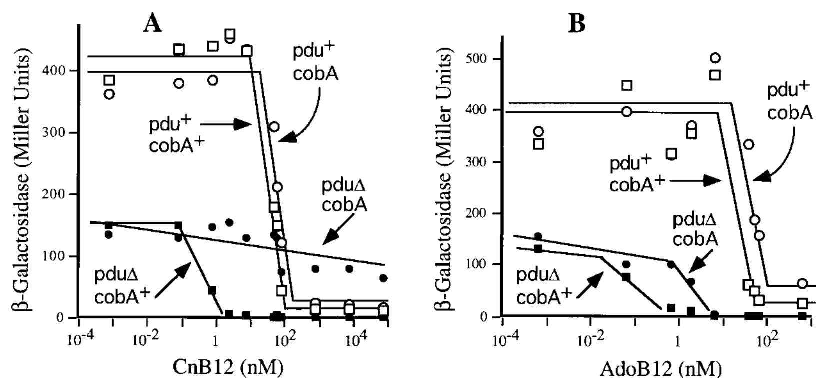

FIG. 2. Aerobic repression of *cob* by different concentrations of $CN-B_{12}$ and $Ado-B_{12}$. Cells were grown on succinate plus propanediol with various concentrations of $CN-B_{12}$ (left) or $Ado-B_{12}$ (right). All strains carried a *lac* operon fusion to the *cob* operon (*cbiD24*::MudJ) and have the region of the *cob* operon distal to the fusion deleted. Strains assayed were TT18117 ($cobA^+$ pdu^+; open squares), TT18118 (*cobA* pdu^+; open circles), TT18119 ($cobA^+$ *pdu*; filled squares), and TT18120 (*cobA pdu*; filled circles). The *cob-lac* fusion used as the reporter was *cbiD24*::MudJ, which is associated with a deletion of all promoter-distal material in the *cob* operon. The *pdu* mutation used was DEL1743, which has the entire *pdu* operon and part of the *pduF* gene removed. The *cobA* mutation used was insertion *cobA*367::Tn*10d*Tc.

TABLE 4. Effects of *pdu* mutations on repression of the *cob* operon

Strain	Relevant genotype[c]	β-Galactosidase activity (Miller units) under conditions[a]: Succinate-propanediol				Phenotype[b]	
		None	10^{-1} CN-B_{12}	CN-B_{12}	Ado-B_{12}	AdoT	AdoB
TT10852	*pdu*$^+$	479	470	110	34	+	+
TT14298	*pdu*$^+$ *cobA*	487	508	204	124	+	+
	Insertion mutations						
TT18170	*pdu-216*	318	324	41	21	+	+
TT18171	*pdu-216 cobA*	234	250	59	38		
TT18172	*pdu-218*	479	67	23	22	−	−
TT18173	*pdu-218 cobA*	527	553	487	31		
TT18174	*pdu-220*	347	244	81	44	−	+
TT18175	*pdu-220 cobA*	220	190	150	61		
TT18176	*pdu-222*	299	45	16	11	±	−
TT18177	*pdu-222 cobA*	303	240	80	11		
	Deletion mutations						
TT18180	*pdu-265*	578	20	18	22	−	−
TT18181	*pdu-265 cobA*	579	603	490	24		
TT18182	*pdu-252*	455	421	44	44	+	+
TT18183	*pdu-252 cobA*	414	381	167	85		
TT18184	*pdu-260*	469	402	35	48	−	+
TT18185	*pdu-260 cobA*	436	481	485	195		
TT18188	*pdu-264*	363	304	19	15	−	+
TT18189	*pdu-264 cobA*	344	345	302	11		
	Point mutations						
TT18803	*pduC286*	430	325	23	14	+	+
TT18804	*pduC286 cobA*	351	318	63	26		
TT18805	*pduD283*	442	16	15	14	+	−
TT18806	*pduD283 cobA*	397	47	26	13		
TT18807	*pduG238*	424	372	52	37	+	+
TT18808	*pduG238 cobA*	458	398	84	34		

[a] Cells were grown aerobically at 37°C in NCE methionine medium with succinate as a carbon and energy source. Propanediol was included as an inducer of the *cob-pdu* regulon. None, no addition; 10^{-1} CN-B_{12}, B_{12} added at 10 μg/liter; CN-B_{12} and Ado-B_{12}, B_{12} added at 100 μg/liter (7.5×10^{-8} and 6.3×10^{-8} M, respectively).

[b] The phenotypes of the inferred adenosylase (AdoT) and Ado-B_{12} binding (AdoB) activities in the *pdu* operon were scored for each *pdu* mutation based on the reported assay values for strains with and without a *cobA* mutation.

[c] All strains carry a *cbiD24*::MudJ insertion that fuses the *cob* operon to the *lac* operon; this fusion is used to report (as the β-galactosidase level) expression of the *cob* operon.

insertion clearly permitted repression by 10^{-1} CN-B_{12} and hence is AdoB$^-$; it showed only a partial loss of repression by CN-B_{12} in a *cobA* background. Thus, its phenotype is scored as AdoT$^{+/-}$. A possible explanation for this behavior is that mutation *pdu-222* directly disrupts a gene required for AdoB activity but provides for expression of AdoT (at reduced levels) with a promoter that emerges from the Tn*10d*Cm element. The Tn*10d*Cm element is known to have a promoter activity that can (at some sites) drive expression of flanking sequences on one side of the transposon (17). Thus, depending on orientation and nearby polarity sites, such an insertion may not be completely polar on downstream genes. To test this possibility, we assayed the effect of insertion *pdu-222* on expression of a downstream *pdu*::*lac* fusion. Although expression was reduced (compared to induced levels with no insertion), there was still substantial expression with the *pdu-222* mutation as measured either on indicator plates or by β-galactosidase assay (data not shown). In contrast, insertion *pdu-220* (AdoT$^-$ AdoB$^+$) did not provide substantial expression of a downstream *pdu*::*lac* fusion. These results are consistent with the hypothesis for *pdu-222* outlined above.

To further confirm that the gene for AdoB activity lies upstream of the gene for AdoT activity, we examined the effects of *pdu* deletions on repression of *cob* (Table 4). These deletions extend into the *pdu* operon from the downstream side and do not remove the *pdu* promoter. Thus, they can be assayed for AdoB and AdoT activities in the same way as the insertion mutations.

Deletion *pdu-265*, which removes most of the *pdu* operon, lacked both activities. Deletion *pdu-252*, which extends only into the downstream part of the operon, did not remove either activity. Both deletions *pdu-260* and *pdu-264* exhibited an AdoT$^-$ AdoB$^+$ phenotype, consistent with the placement of the AdoB gene upstream of the AdoT gene. No deletions with an AdoT$^+$ AdoB$^-$ phenotype were found.

A number of *pdu* point mutations causing an aerobic Pdu$^-$ phenotype have been placed into four complementation groups; DNA sequencing and deletion mapping suggest the existence of more than these four genes in the operon (8, 13, 39). A deletion removing the most downstream complementation group (*pdu-252*) was AdoB$^+$ AdoT$^+$ as shown above, so the genes that encode the AdoB and AdoT activities are presumed to lie upstream of this group. The slightly longer deletions (*pdu-260* and *pdu-264*) eliminated AdoT activity but left AdoB activity intact. These deletions affect a region we have designated *pduH*; we believe that genes lie in this region because it contains many deletion endpoint and insertion mutations, but no point mutations are known here (39).

TABLE 5. Anomalous effects of *pdu* mutations on repression of the *cob* operon

Strain	Relevant genotype[b]	Growth method[c]	β-Galactosidase activity (Miller units) conditions[a]:			
			Succinate-propanediol			
			None	10^{-1} CN-B_{12}	CN-B_{12}	Ado-B_{12}
TT10852	Wild type	1	438	401	83	72
TT14298	*cobA*	1	463	466	248	240
TT18190	*pdu-246*	1	540	355	155	195
TT18191	*pdu-246 cobA*	1	448	454	445	340
TT18186	*pdu-261*	1	454	334	133	190
TT18187	*pdu-261 cobA*	1	474	497	437	329
TT18178	*pdu-257*	1	515	528	41	88
TT18179	*pdu-257 cobA*	1	630	639	558	270
TT18178	*pdu-257*	2	326	ND	294	117
TT18179	*pdu-257 cobA*	2	515	ND	342	669

[a] Cells were grown aerobically at 37°C in NCE medium with methionine on succinate as a carbon and energy source. Propanediol was included as an inducer of the *pdu-cob* regulon. None, no addition to the medium; 10^{-1} CN-B_{12}, B_{12} added at 10 μg/liter; CN-B_{12} and Ado-B_{12}, B_{12} added at 100 μg/liter; ND, not determined.
[b] All strains carry a *cbiD24::lac* fusion that reports expression of the *cob* operon.
[c] Cells were transferred once (1) or twice (2) to minimal media for assays as described in the text.

Point mutations in each of the other complementation groups were tested for AdoB and AdoT activities as described above (Table 4). Based on their complementation behavior, these point mutations should not be polar on downstream functions in the operon (39). The single point mutations in the *pduC* (*pdu-286*) and *pduG* (*pdu-238*) genes had an $AdoB^+$ $AdoT^+$ phenotype. However, mutation *pduD283* had a clear $AdoB^-$ $AdoT^+$ phenotype, suggesting that this gene is required for AdoB activity. The *pduC* and *pduD* genes encode two of the three subunit types of propanediol dehydratase (7); no point mutations are available for the third subunit (*pduE*). Insertion mutation *pdu-222* may affect the *pduE* gene. The results described above suggest that the *pduD* subunit and possibly the *pduE* subunit are responsible for binding Ado-B_{12}. Since none of the *pdu* point mutations tested had an AdoT phenotype, we suspect that this type of point mutation is not included in our collection, possibly because it does not cause a Pdu^- phenotype in the $cobA^+$ strains used for isolating *pdu* mutations (see Discussion).

Certain *pdu* deletions prevent repression of the *cob* operon. Since a deletion mutant lacking the entire *pdu* operon still shows *cob* repression control, no essential component of the *cob* regulatory mechanism is encoded in the *pdu* operon. The above-described data suggest that the *pdu* operon encodes two activities (adenosyltransferase and Ado-B_{12} binding) that modulate the behavior of the mechanism by affecting the level of Ado-B_{12}. In the course of this work, we noticed that several *pdu* mutations practically eliminated repression of the *cob* operon; others eliminated repression under particular growth conditions. These observations are described below. As discussed later, the behavior of these mutants could be explained if they caused the *pdu* operon to actively destroy Ado-B_{12}.

The data in Table 4 were obtained by assaying cells that were grown to full density in rich nutrient broth medium, transferred to minimal medium of the desired type, and grown to mid-exponential phase before being harvested for enzyme assays. The data in Tables 2 and 3 and Fig. 2 were obtained by growing cells in the same way, except that cells were grown to full density in minimal medium and transferred to minimal medium a second time. Levels of induction and repression of *cob* were similar by both growth protocols for wild-type cells, for a *cobA* mutant, and for a strain carrying the large *pdu* deletion mutation (DEL1743).

Two *pdu* deletion mutations (*pdu-246* and *pdu-261*) ending in the *pduH* region (Fig. 1) displayed limited repression (Table 5) when cells were grown under the same single-transfer conditions used to obtain the data listed in Table 4. These two mutants displayed limited repression of *cob* by either CN-B_{12} or Ado-B_{12} in a $cobA^+$ background but virtually no repression by either compound in a *cobA* mutant background. This phenotype might be viewed as due to an increase in AdoB activity.

Another mutant (that with mutation *pdu-257*) appeared to have an $AdoB^+$ $AdoT^-$ phenotype but showed weak repression when it was assayed after a single transfer; this mutant displayed virtually no repression of *cob* if it was assayed after two transfers (Table 5); this was true even with a 10-fold-higher concentration of CN-B_{12} or Ado-B_{12} (data not shown). Induction levels of the *cob* and *pdu* operons by propanediol were not affected by the growth protocol used (data not shown). Some possible explanations of these results will be discussed.

DISCUSSION

We have provided evidence that Ado-B_{12} is the effector for repression of the *cob* operon. Mutations in the *cobA* gene can prevent repression by CN-B_{12} but not by Ado-B_{12}. The CobA protein is known to adenosylate numerous corrinoids (19, 37, 38). Induction of the *pdu* operon corrects the regulatory defect of a *cobA* mutation, suggesting that this operon encodes an additional protein capable of adenosylating exogenous B_{12}. The adenosyltransferase activity of the *pdu* operon is likely to act primarily in generating Ado-B_{12} as a coenzyme for the propanediol dehydratase enzyme. The *eut* operon, which encodes enzymes for B_{12}-dependent degradation of ethanolamine, appears to encode two B_{12} adenosyltransferases (31, 35, 36).

Titration curves for repression of *cob* by exogenous B_{12} are very similar to those for the *btuB* gene of *E. coli*, suggesting that both repression mechanisms respond to similar concentrations of Ado-B_{12}. For both of these mechanisms, it has been suggested that Ado-B_{12} is bound directly by an mRNA leader, without participation of a protein (24, 27, 29); however, in neither case has this binding been directly demonstrated. It is not possible to compare the internal concentrations of B_{12} needed to repress *cob* in our assays with those determined previously by other methods (2), since we did not determine the intracellular concentrations of B_{12} found at the various exogenous concentrations used. Our titration assays also re-

vealed that mutants with *cobA* mutations require a slightly higher concentration of B_{12} to repress *cob* than does the wild type. Since this defect was seen for exogenous Ado-B_{12} as well as for CN-B_{12}, it is possible that the CobA protein plays a role in recycling cobalamins and maintaining an internal Ado-B_{12} pool. The possibility that the CobA protein plays a minor role in B_{12} transport or repression independent of its adenosyltransferase activity is not excluded.

The *pdu* operon appears to encode an activity (designated AdoB) capable of antagonizing the ability of Ado-B_{12} to repress *cob*. Mutations eliminating this activity allow repression at much lower levels of Ado-B_{12}. This activity is distinct from the AdoT activity inferred to adenosylate cobalamin. We have shown that the AdoB and AdoT activities are separable and that AdoT maps downstream of AdoB in the *pdu* operon. The AdoB activity appears to be provided by a component of propanediol dehydratase and may be the binding of Ado-B_{12} or CN-B_{12}, consistent with the observation that loss of AdoB activity has equivalent effects on levels of repression by CN-B_{12} and Ado-B_{12}. The most direct evidence for involvement of dioldehydratase is that a nonpolar point mutation that eliminates propanediol dehydratase activity (*pduD283*) also lacks AdoB activity. Another diol dehydratase mutant (*pduC286*) retained the AdoB activity measured here. Propanediol dehydratase has three subunit types (PduCDE); it seems likely that mutation *pdu-283* affects a component that is required for binding of B_{12} and that mutation *pdu-286* may affect a component needed for enzymatic activity but not needed for binding B_{12}. A similar situation has been observed previously in the *eut* operon; the B_{12}-dependent enzyme ethanolamine ammonia lyase (*eutBC*) competes with the *eut* operon regulatory protein (EutR) for a limited supply of Ado-B_{12}, and lyase mutants have variable effects on this competition (36). The simplest model for AdoB is that propanediol dehydratase and the *cob* repression mechanism compete for a limited supply of Ado-B_{12}.

Here we infer that a B_{12}-adenosyltransferase activity (AdoT) is encoded between the *pduJ* and *pduG* genes of the *pdu* operon. A B_{12} adenosyl transfer activity was assigned to the *pduG* gene based on growth phenotypes of *pdu* mutants (39); mutations in the *pduG* gene show a Pdu^- phenotype that is corrected by added Ado-B_{12}. We present data here that *pduG* mutations do not eliminate AdoT activity, inferred from assays of *cob* operon regulation. This discrepancy could be accounted for if the *pdu* operon encoded two adenosyltransferases, only one of which acted on free B_{12}. While this may seem unlikely, the related ethanolamine (*eut*) operon appears to encode two B_{12} transferase activities (35). The *pdu* operon includes many genes with no aerobic Pdu^- mutant phenotype; the extra genes may be involved only in anaerobic use of propanediol and thus may not have been detected by the aerobic mutant hunts done thus far. The AdoT activity may be encoded by one of these extra genes.

Certain unusual *pdu* mutations with an $AdoB^+$ $AdoT^-$ phenotype prevent essentially all repression of *cob*; they show this behavior only when they are tested following extensive growth. These mutations are deletions extending into the *pduH* region, which includes no point mutations with Pdu^- phenotypes (39). While we do not have an explanation for the effects of deletions in this region, it is possible that these mutants lack proteins that normally form a complex with diol dehydratase; we suggest that in the absence of these proteins, dehydratase can actively destroy Ado-B_{12}. The extended growth period required to eliminate repression in some mutants may allow accumulation of the form of propanediol dehydratase that actively destroys Ado-B_{12}. Cofactor destruction could occur if the abnormal enzyme breaks the cobalt-carbon bond of Ado-B_{12} (required at each catalytic cycle) but releases the products, resulting in loss of the cofactor. Better interpretations of the effects of these mutations on *cob* repression await further characterization of genes in the *pdu* operon and closer study of the diol dehydratase produced by these mutants.

ACKNOWLEDGMENTS

We thank Derek Walter for providing *pdu* mutations and Tom Bobik, Ping Chen, and Jeff Lawrence for helpful discussions.

This work was supported by a grant from the National Institutes of Health (GM34804).

REFERENCES

1. **Ailion, M., T. A. Bobik, and J. R. Roth.** 1993. Two global regulatory systems (Crp and Arc) control the cobalamin/propanediol regulon of *Salmonella typhimurium*. J. Bacteriol. **175:**7200–7208.
2. **Andersson, D.** 1995. Kinetics of cobalamin repression of the *cob* operon in *Salmonella typhimurium*. FEMS Microbiol. Lett. **124:**89–94.
3. **Andersson, D. I., and J. R. Roth.** 1989. Mutations affecting regulation of cobinamide biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **171:**6726–6733.
4. **Andersson, D. I., and J. R. Roth.** 1989. Redox regulation of the genes for cobinamide biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **171:**6734–6739.
5. **Aufrère, R., M. Tempête, and J.-P. Bohin.** 1986. Regulation of expression of the gene for vitamin B_{12} receptor cloned on a multicopy plasmid in *Escherichia coli*. Mol. Gen. Genet. **205:**358–365.
6. **Berkowitz, D., J. M. Hushon, H. J. Whitfield, Jr., J. Roth, and B. N. Ames.** 1968. Procedure for identifying nonsense mutations. J. Bacteriol. **96:**215–220.
7. **Bobik, T., and J. R. Roth.** Unpublished results.
8. **Bobik, T., Y. Xu, R. M. Jeter, K. E. Otto, and J. R. Roth.** Propanediol utilization genes of *Salmonella typhimorium*: three games for the propanediol dehydratase. J. Bacteriol., in press.
9. **Bobik, T. A., M. Ailion, and J. R. Roth.** 1992. A single regulatory gene integrates control of vitamin B_{12} synthesis and propanediol degradation. J. Bacteriol. **174:**2253–2266.
10. **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.
11. **Chen, P., M. Ailion, T. Bobik, G. Stormo, and J. Roth.** 1995. Five promoters integrate control of the *cob/pdu* regulon in *Salmonella typhimurium*. J. Bacteriol. **177:**5401–5410.
12. **Chen, P., M. Ailion, N. Weyand, and J. Roth.** 1995. The end of the *cob* operon: evidence that the last gene (*cobT*) catalyzes synthesis of the lower ligand of vitamin B_{12}, dimethylbenzimidazole. J. Bacteriol. **177:**1461–1469.
13. **Chen, P., D. I. Andersson, and J. R. Roth.** 1994. The control region of the *pdu/cob* regulon in *Salmonella typhimurium*. J. Bacteriol. **176:**5474–5482.
14. **Crouzet, J., S. Levy-Schil, B. Cameron, L. Cauchois, S. Rigault, M.-C. Rouyez, F. Blanche, L. Debussche, and D. Thibaut.** 1991. Nucleotide sequence and genetic analysis of a 13.1-kilobase-pair *Pseudomonas denitrificans* DNA fragment containing five *cob* genes and identification of structural genes encoding cob(I)alamin adenosyltransferase, cobyric acid synthase, and bifunctional cobinamide kinase-cobinamide phosphate guanylyltransferase. J. Bacteriol. **173:**6074–6087.
15. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. A manual for genetic engineering: advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Debussche, L., M. Couder, D. Thibaut, B. Cameron, J. Crouzet, and F. Blanche.** 1991. Purification and partial characterization of cob(I)alamin adenosyltransferase from *Pseudomonas denitrificans*. J. Bacteriol. **173:**6300–6302.
17. **Elliot, 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.
18. **Escalante-Semerena, J. C., and J. R. Roth.** 1987. Regulation of cobalamin biosynthetic operons in *Salmonella typhimurium*. J. Bacteriol. **169:**2251–2258.
19. **Escalante-Semerena, J. C., S.-J. Suh, and J. R. Roth.** 1990. *cobA* function is required for both de novo cobalamin biosynthesis and assimilation of exogenous corrinoids in *Salmonella typhimurium*. J. Bacteriol. **172:**273–280.
20. **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.
21. **Jeter, R. M., and J. R. Roth.** 1987. Cobalamin (vitamin B_{12}) biosynthetic genes of *Salmonella typhimurium*. J. Bacteriol. **169:**3189–3198.
22. **Lundrigan, M. D., L. C. DeVeaux, B. J. Mann, and R. J. Kadner.** 1987. Separate regulatory systems for the repression of *metE* and *btuB* by vitamin B_{12} in *Escherichia coli*. Mol. Gen. Genet. **206:**401–407.

23. **Lundrigan, M. D., and R. J. Kadner.** 1989. Altered cobalamin metabolism in *Escherichia coli btuR* mutants affects *btuB* regulation. J. Bacteriol. **171:**154–161.
24. **Lundrigan, M. D., W. Koster, and R. J. Kadner.** 1991. Transcribed sequences of the *Escherichia coli btuB* gene control its expression and regulation by vitamin B_{12}. Proc. Natl. Acad. Sci. USA **88:**1479–1483.
25. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **O'Toole, G. A., and J. C. Escalante-Semerena.** 1993. *cobU*-dependent assimilation of nonadenosylated cobinamide in *cobA* mutants of *Salmonella typhimurium*. J. Bacteriol. **175:**6328–6336.
27. **Richter-Dahlfors, A. A., and D. I. Andersson.** 1992. Cobalamin (vitamin B_{12}) repression of the Cob operon in *Salmonella typhimurium* requires sequences within the leader and the first translated open reading frame. Mol. Microbiol. **6:**743–749.
28. **Richter-Dahlfors, A. A., and D. I. Andersson.** 1991. Analysis of an anaerobically induced promoter for the cobalamin biosynthetic genes in *Salmonella typhimurium*. Mol. Microbiol. **5:**1337–1345.
29. **Richter-Dahlfors, A. A., S. Ravnum, and D. I. Andersson.** 1994. Vitamin B_{12} repression of the *cob* operon in *Salmonella typhimurium*: translational control of the *cbiA* gene. Mol. Microbiol. **13:**541–553.
30. **Rondon, M. R., and J. C. Escalante-Semerena.** 1992. The *poc* locus is required for 1,2-propanediol-dependent transcription of the cobalamin biosynthetic (*cob*) and propanediol utilization (*pdu*) genes of *Salmonella typhimurium*. J. Bacteriol. **174:**2267–2272.
31. **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.
32. **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.
33. **Roth, J. R., J. G. Lawrence, M. Rubenfield, S. Kieffer-Higgins, and G. M. Church.** 1993. Characterization of the cobalamin (vitamin B_{12}) biosynthetic genes of *Salmonella typhimurium*. J. Bacteriol. **175:**3303–3316.
34. **Schmieger, H.** 1971. A method for detection of phage mutants with altered transducing ability. Mol. Gen. Genet. **110:**378–381.
35. **Sheppard, D., and J. R. Roth.** 1996. Unpublished data.
36. **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.
37. **Suh, S.-J., and J. C. Escalante-Semerena.** 1993. Cloning, sequencing and overexpression of *cobA*, which encodes ATP:corrinoid adenosyltransferase in *Salmonella typhimurium*. Gene **129:**93–97.
38. **Suh, S.-J., and J. C. Escalante-Semerena.** 1995. Purification and initial characterization of the ATP:corrinoid adenosyltransferase encoded by the *cobA* gene of *Salmonella typhimurium*. J. Bacteriol. **177:**921–925.
39. **Walter, D., M. Ailion, and J. Roth.** 1997. Genetic characterization of the *pdu* operon: use of 1,2-propanediol in *Salmonella typhimurium*. J. Bacteriol. **179:**1013–1022.
40. **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.