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Genetic Characterization of the *pdu* Operon: Use of 1,2-Propanediol in *Salmonella typhimurium*

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Salmonella typhimurium **is able to catabolize 1,2-propanediol for use as the sole carbon and energy source; the first enzyme of this pathway requires the cofactor adenosyl cobalamin (Ado-B_{12}). Surprisingly, *Salmonella* can use propanediol as the sole carbon source only in the presence of oxygen but can synthesize Ado-B_{12} only anaerobically. To understand this situation, we have studied the *pdu* operon, which encodes proteins for propanediol degradation. A set of *pdu* mutants defective in aerobic degradation of propanediol (with exogenous vitamin B_{12}) defines four distinct complementation groups. Mutations in two of these groups (*pduC* and *pduD*) eliminate propanediol dehydratase activity. Based on mutant phenotypes, a third complementation group (*pduG*) appears to encode a cobalamin adenosyl transferase activity. No function has been assigned to the *pduJ* complementation group. Propionaldehyde dehydrogenase activity is eliminated by mutations in any of the four identified complementation groups, suggesting that this activity may require a complex of proteins encoded by the operon. None of the mutations analyzed affects either of the first two genes of the operon (*pduA* and *pduB*), which were identified by DNA sequence analysis. Available data suggest that the *pdu* operon includes enough DNA for about 15 genes and that the four genetically identified genes are the only ones required for aerobic use of propanediol.**

Nearly 1% of the genome of *Salmonella typhimurium* is devoted to the synthesis and uptake of cobalamin. An additional 1% of the genome is involved in metabolic pathways that require cobalamin (33). However, vitamin B_{12} is completely dispensable in laboratory cultivation of *Salmonella*. This large genetic investment in vitamin B_{12} and its use suggests that B_{12}-dependent metabolism is important to natural populations of *Salmonella*. Understanding this metabolism may shed light on the natural history of *Salmonella.*

The main use of B_{12} in *Salmonella* appears to be as a cofactor for propanediol dehydratase, the first enzyme in propanediol degradation. This conclusion is based on the fact that the *cob* operon, encoding the B_{12} biosynthetic genes, is induced by propanediol and is coregulated with the *pdu* operon, encoding genes for propanediol degradation (5, 31). The two operons map close together at min 41 of the genetic map (20), and induction of both by propanediol is mediated by a single regulatory protein, PocR, encoded by a gene that maps between the two operons (5, 31). Induction of this *cob*/*pdu* regulon is permitted by the additive effects of two global control systems (1): the Crp protein signals the need for carbon or energy, and the ArcA protein signals anaerobiosis or a reduced cell interior (2, 19). This control pattern suggests that perhaps the main role of B_{12} in *S. typhimurium* is in propanediol metabolism and that propanediol could serve as a useful carbon and energy source which is frequently valuable under anaerobic conditions. Natural populations of *S. typhimurium* are likely to encounter propanediol frequently because it is made as a by-product during degradation of the common plant sugars rhamnose and fucose (27).

The laboratory growth behavior of *Salmonella* and the regulatory pattern of the *cob*/*pdu* regulon appear paradoxical in that *Salmonella* is only known to use propanediol as the sole carbon source under aerobic conditions (20), while vitamin B_{12} is synthesized only under anaerobic conditions (21). Even more peculiar is the fact that both operons are inducible under aerobic conditions (when B_{12} synthesis is impossible). The resolution of this paradox may provide insights into the anaerobic lifestyle of *Salmonella.*

The metabolic pathway envisioned for propanediol degradation is shown in Fig. 1 (42). Propanediol is initially dehydrated to propionaldehyde by the action of the vitamin B_{12}-dependent propanediol dehydratase. Propionaldehyde can then be oxidized to propionyl-coenzyme A (propionyl-CoA) by propionaldehyde dehydrogenase. The subsequent reactions described as anaerobic are known to occur in *Salmonella*, but it is not clear what specific enzymes are required for use of propanediol or which of the indicated activities are provided by proteins encoded by the *pdu* operon. Under aerobic conditions, propionyl-CoA can be further metabolized to pyruvate by a pathway that is thought to include intermediate conversion to acrylyl- and lactyl-CoA (16) by using enzymes encoded in the *prp* operon (17, 38).

Under anaerobic conditions, propionaldehyde and propionyl-CoA may be metabolized differently than they are under aerobic conditions. Propionaldehyde can be reduced to propanol (29), which is excreted, and propionyl-CoA can be split by phosphotransacylase to form propionyl phosphate. Excretion of propanol into the medium could provide cells with an electron sink to help balance redox reactions during fermentative growth. Propionylkinase can cleave propionyl phosphate to give ATP and propionate, which is excreted. Anaerobic metabolism of propionyl-CoA by these two reactions (which may also proceed aerobically) would fix one molecule of inorganic phosphate and generate one ATP. In the absence of an electron acceptor, these reactions may be the only way of processing propionyl-CoA. Thus, propanediol would provide no source of carbon; all products would be excreted as either propanol or propionate. Under such fermentative conditions, propanediol could provide only an electron sink and a source

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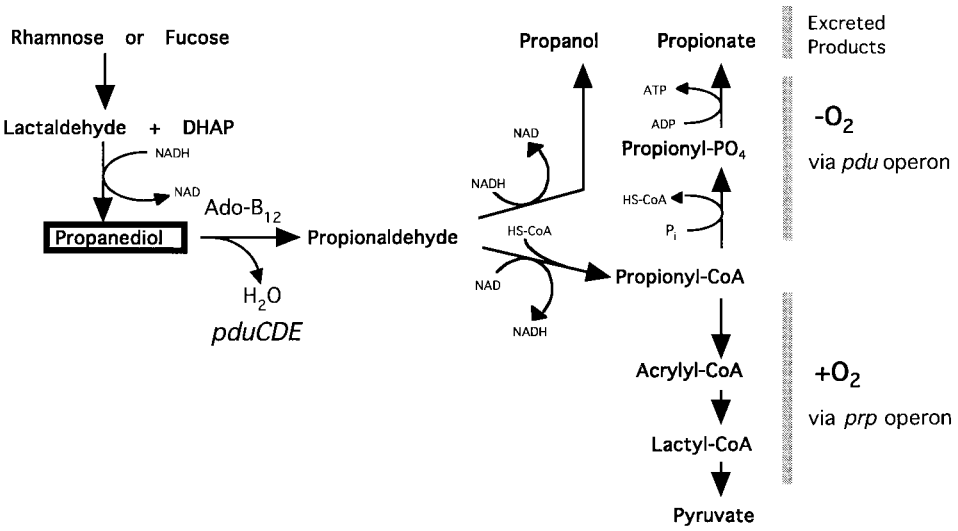

FIG. 1. Pathway proposed for anaerobic and aerobic catabolism of propanediol in *Salmonella.*

of ATP. One resolution of the paradox described above is that the *pdu* operon may have evolved to provide only these things and not a carbon source.

This outline of propanediol metabolism is likely to be incomplete. The first two genes in the *pdu* operon (*pduA* and *pduB*) are homologous to different proteins thought to contribute to the shell of carboxysomes, bacterial organelles that play a role in CO_2 fixation in some bacteria (9, 23, 30). No CO_2 fixation is involved in the scheme presented here, and carboxysomes have not been reported in enteric bacteria. Furthermore, evidence has been presented that *Escherichia coli* can degrade propionyl-CoA by a second route which involves CO_2 fixation and the B_{12}-dependent methylmalonyl-CoA mutase (15, 34). Although this is an attractive possibility, we have seen no evidence for this pathway and (to our knowledge) the methylmalonyl-CoA mutase has never been assayed in *E. coli* or *Salmonella*. PCR analysis suggests that the *pdu* operon includes about 15 kb of DNA and thus could encode many activities beyond those envisioned by the above description (25). Possible additional functions might support the recent preliminary observation that propanediol can provide a source of carbon and energy during anaerobic respiration when tetrathionate (but not nitrate or fumarate) is available as an electron acceptor (4).

To approach these questions, we have begun a genetic characterization of the *pdu* operon. Using production of acid as an assay for propanediol degradation, we isolated *pdu* mutations, constructed a deletion map, and classified mutations functionally by complementation tests and enzyme assays. Results suggest that the operon encodes at least some of the activities predicted by the outline in Fig. 1 but must also include additional genes that are not required for aerobic use of propanediol.

MATERIALS AND METHODS

Bacterial strains and transposons. All strains used are derivatives of *S. typhimurium* LT2 (Table 1). The Tn*10d*Tc and Tn*10d*Cm elements are transposition-defective transposons that confer tetracycline and chloramphenicol resistance, respectively (13, 44). The MudJ element is a transposition-defective phage Mu derivative, Mud1-1734, which confers kanamycin resistance (8).

Media and growth conditions. The rich medium was nutrient broth (0.8% Difco) supplemented with 85 mM NaCl. The minimal medium was E medium supplemented with glucose (0.2%) (43). The carbon-free minimal medium was NCE (3). In solid NCE medium, sorbitol was used at a concentration of 0.2%. In liquid NCE medium, succinate was used at a concentration of 1%, 1,2-propanediol (a racemic mixture) was used at a concentration of 0.2%, and cyanocobalamin (CN-B_{12}) was used at a concentration of 15 nM.

MacConkey acid indicator plates supplemented with 1,2-propanediol (1% by use of a racemic mixture) and CN-B_{12} (150 nM) were used to test the *pdu* phenotype. On this medium, colonies of Pdu^+ strains are red and those of Pdu^- strains are white. The acid produced may be formed aerobically (by reactions encoded by *prp* genes) or anaerobically as propionate as indicated in Fig. 1. When necessary, MacConkey agar was supplemented with adenosyl cobalamin (Ado-B_{12}) by spreading 100 μl of a 1.5 mM Ado-B_{12} solution directly onto the plate.

All manipulations involving Ado-B_{12} were carried out in the dark because of the photolability of the Co-C bond of Ado-B_{12}. The lactose utilization (Lac) phenotype was screened on MacConkey agar supplemented with lactose (1%). On this medium, Lac^+ strains form red colonies. Amino acids were added at concentrations recommended previously (12). Antibiotics were added at the following concentrations: Na ampicillin, 30 μg/ml; chloramphenicol, 20 μg/ml; kanamycin monosulfate, 50 μg/ml; tetracycline HCl, 20 μg/ml; streptomycin, 200 μg/ml.

Genetic techniques. Transductional crosses were performed with phage P22 HT105/1 *int-201* (35). Approximately 10^8 cells were mixed with approximately 10^7 phage and allowed to incubate nonselectively in liquid medium at 37°C for 15 to 90 min before being plated on solid selective media. Transductant colonies were purified and made phage-free by streaking on nonselective green indicator plates (12).

Two methods were used for conjugational crosses. For routine strain construction, an overnight liquid culture of the donor strain was diluted 1,000-fold and 30 μl of the resulting suspension was mixed with 100 μl of an overnight liquid culture of the recipient strain. This mixture was plated on nonselective solid media and allowed to incubate for 2 days at 37°C. The resulting lawn was replica printed to selective media and incubated for an additional 2 days at 37°C.

A second conjugation method, used for complementation tests, permitted many crosses to be done on the same plate. A patch of each of the donor strains (carrying an F′ plasmid) was made on rich medium and incubated overnight at 37°C. This plate was then replica printed to rich plates spread with 100 μl of an overnight liquid culture of the recipient strain. These replica plates were incubated for 2 days at 37°C, and the resulting lawns were replica printed to selective media and incubated for an additional 2 days at 37°C. Growth was seen only in patches which yielded diploids expressing selected phenotypes provided by the plasmid and the recipient strain. In most cases, the plasmid strain conferred chloramphenicol resistance and the recipient strain was streptomycin resistant.

Isolation of insertion mutations downstream of the *pdu* operon. A pool of approximately 100,000 random Tn*10d*Tc insertions was used as a transductional donor in crosses with recipient strain TT17108, which carries a Tn*10d*Cm insertion in the *pdu* operon. Transductants were selected as tetracycline-resistant clones on MacConkey propanediol-vitamin B_{12} plates, which permit visual screening of the Pdu phenotype. Transductants with a Tc^r Pdu^+ phenotype had coinherited a pdu^+ allele with a nearby Tn*10d*Tc insertion. Two insertion mutations (*zec-3796*::Tn*10d*Tc and *zec-3797*::Tn*10d*Tc) mapped clockwise (promoter distal) to the *pdu* operon and showed approximately 30 and 24% cotransduction, respectively, with mutation *pdu-216*::Tn*10d*Cm. These were used in later experiments.

TABLE 1. List of strains

Strain	Genotype
LT2	Wildtype
TR6625	*strA1*
TT10852	*metE205 ara-9 cob-24*::MudJ
TT11855	*metE205 ara-9* DEL299(*hol*$^+$ *his phs pdu cob*)
TT16462	*srl-203*::Tn*10d*Cm
TT16463	*recA1*
TT17076	*ara-9* DEL1077(*metE*) *pduF354*::Tn*10d*Tc
TT17108	*metE205 ara-9 pdu-216*::Tn*10d*Cm
TT17131	*metE205 ara-9 pdu-12*::MudJ
TT17679	*metE205 ara-9 strA1* Δ*pdu-258*
TT17680	*metE205 ara-9 strA1* Δ*pdu-242 cbiA700*::*cam*
TT17681	*metE205 ara-9 strA1* Δ*pdu-245*
TT17682	*metE205 ara-9 strA1* Δ*pdu-240 cbiA700*::*cam*
TT17683	*metE205 ara-9 strA1* Δ*pdu-265*
TT17684	*metE205 ara-9 strA1* Δ*pdu-243*
TT17685	*metE205 ara-9 strA1* Δ*pdu-264*
TT17686	*metE205 ara-9 strA1* Δ*pdu-257*
TT17687	*metE205 ara-9 strA1* Δ*pdu-253*
TT17688	*metE205 ara-9 strA1* Δ*pdu-267*
TT17689	*metE205 ara-9 strA1* Δ*pdu-261*
TT17690	*metE205 ara-9 strA1* Δ*pdu-256*
TT17691	*metE205 ara-9 strA1* Δ*pdu-246 cbiA700*::*cam*
TT17692	*metE205 ara-9 strA1* Δ*pdu-254*
TT17693	*metE205 ara-9 strA1* Δ*pdu-260*
TT17694	*metE205 ara-9 strA1* Δ*pdu-268*
TT17695	*metE205 ara-9 strA1* Δ*pdu-248 cbiA700*::*cam*
TT17696	*metE205 ara-9 strA1* Δ*pdu-252*
TT17697	*metE205 ara-9 strA1* Δ*pdu-241 cbiA700*::*cam*
TT17698	*metE205 ara-9 strA1* Δ*pdu-262*
TT17699	*metE205 ara-9 strA1* Δ*pdu-249*
TT17700	*metE205 ara-9 strA1* Δ*pdu-255*
TT17701	*metE205 ara-9 strA1* Δ*pdu-251*
TT17702	*metE205 ara-9 strA1* Δ*pdu-244 cbiA700*::*cam*
TT17903	*phs-201*::Tn*10 cob-363*::Tn*10d*Cm *nadA219*::MudJ
TT17905	*phs-201*::Tn*10 cob-363*::Tn*10d*Cm *nadA219*::MudJ/F′152/pNK2881
TT17906	*ara-9* DEL1077(*metE*) DEL1743(*his phs pdu*) *strA1 nadA219*::MudJ
TT17907	*ara-9* DEL1077(*metE*) DEL1743(*his phs pdu*) *strA1 nadA219*::MudJ/F′607
TT17908	*metE205 ara-9* DEL299(*hol*$^+$ *his phs pdu cob*)/F′607 *pdu*$^+$
TT17929	*metE205 ara-9 zec-3796*::Tn*10d*Tc *cbiA700*::cam
TT17930	*metE205 ara-9 zec-3797*::Tn*10d*Tc *cbiA700*::cam
TT17931	*metE205 ara-9 zec-3796*::Tn*10d*Tc
TT17932	*metE205 ara-9 zec-3797*::Tn*10d*Tc
TT17936	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-227*
TT17937	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-228*
TT17938	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-229*
TT17939	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-230*
TT17940	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-231*
TT17942	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-233*
TT17943	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-234*
TT17944	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-235*
TT17945	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-236*
TT17946	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-237*
TT17947	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-238*
TT17948	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-270*
TT17949	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-271*
TT17950	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-272*
TT17951	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-273*
TT17952	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-274*
TT17953	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-275*
TT17956	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-278*
TT17957	zec-3796::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-279*
TT17958	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-283*
TT17959	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-281*
TT17961	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-284*
TT17962	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-285*
TT17963	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-286*
TT17964	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-287*
TT17966	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-289*
TT17967	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-290*
TT17968	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-292*
TT17969	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-293*
TT17970	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-294*
TT17971	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-295*
TT17972	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-296*
TT17973	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-297*
TT17974	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-298*
TT17975	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-299*
TT17976	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-300*
TT17979	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-303*
TT17981	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-305*
TT17984	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-308*
TT17985	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-309*
TT17986	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-310*
TT17987	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-311*
TT17988	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-312*
TT17989	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-313*
TT17992	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-316*
TT17993	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-317*
TT17994	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-318*
TT17995	zec-*3796*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-319*
TT17998	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-322*
TT18000	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-324*
TT18002	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-326*
TT18003	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-327*
TT18004	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-328*
TT18005	pduF354::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-329*
TT18006	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-330*
TT18007	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-331*
TT18009	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-333*
TT18010	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-334*
TT18011	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-335*
TT18012	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-336*
TT18013	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-337*
TT18014	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-338*
TT18016	*pduF354*::Tn*10d*Tc *srl-203*::Tn*10d*Cm *pdu-340*

Isolating deletion mutants of the *pdu* region. Strains with a Tn*10d*Tc insertion downstream of the *pdu* region (TT17929, TT17930, TT17931, and TT17932) were subjected to the Bochner selection for Tc^s derivatives (7, 28). This selection was performed at 42°C as suggested by Kelly Hughes (18). Survivors with a Tc^s Pdu$^-$ phenotype were kept as likely deletions extending from the insertion element into the *pdu* operon. Since the parental Tn*10* derivatives are defective for transposition, these deletions are presumed to be of spontaneous origin rather than transposition induced. Two of the parent strains, TT17929 and TT17930, carry the constructed insertion mutation *cbiA700*::Cm. In screening deletion strains derived from these two parents, scoring the Cm^r phenotype allowed us to quickly determine whether a deletion extended beyond the first gene of the *cob* operon. A total of 24 deletions which extend into the *pdu* operon from the distal end were isolated.

Isolation of *pdu* point mutants. Phage grown on *pdu*$^+$ strains carrying a Tn*10d*Tc insertion either upstream or downstream of the *pdu* operon (TT17931 or TT17076) was mutagenized with hydroxylamine by the method of Hong and Ames as described by Davis et al. (12). The mutagenized phage was then used in transductional crosses with a recipient strain carrying a Tn*10d*Cm insertion in the genes for sorbitol utilization (TT16462). This marker was present to permit later introduction of a linked *recA* mutation. The transduction crosses were performed on MacConkey propanediol-vitamin B_{12} plates with tetracycline to simultaneously select Tc^r transductants and permit visual scoring of their Pdu phenotypes. A total of 63 Tc^r Pdu$^-$ mutants were isolated; all are still Srl$^-$ and Cm^r.

Transposition of the *pdu*$^+$ region onto an F′ plasmid. An F′ plasmid carrying the entire *pdu* operon was constructed by transposition as diagrammed in Fig. 2. Flanking Tn*10* elements, placed on both sides of the *pdu* operon, cooperated to allow transposition of the intervening chromosomal region to an F′ plasmid. A

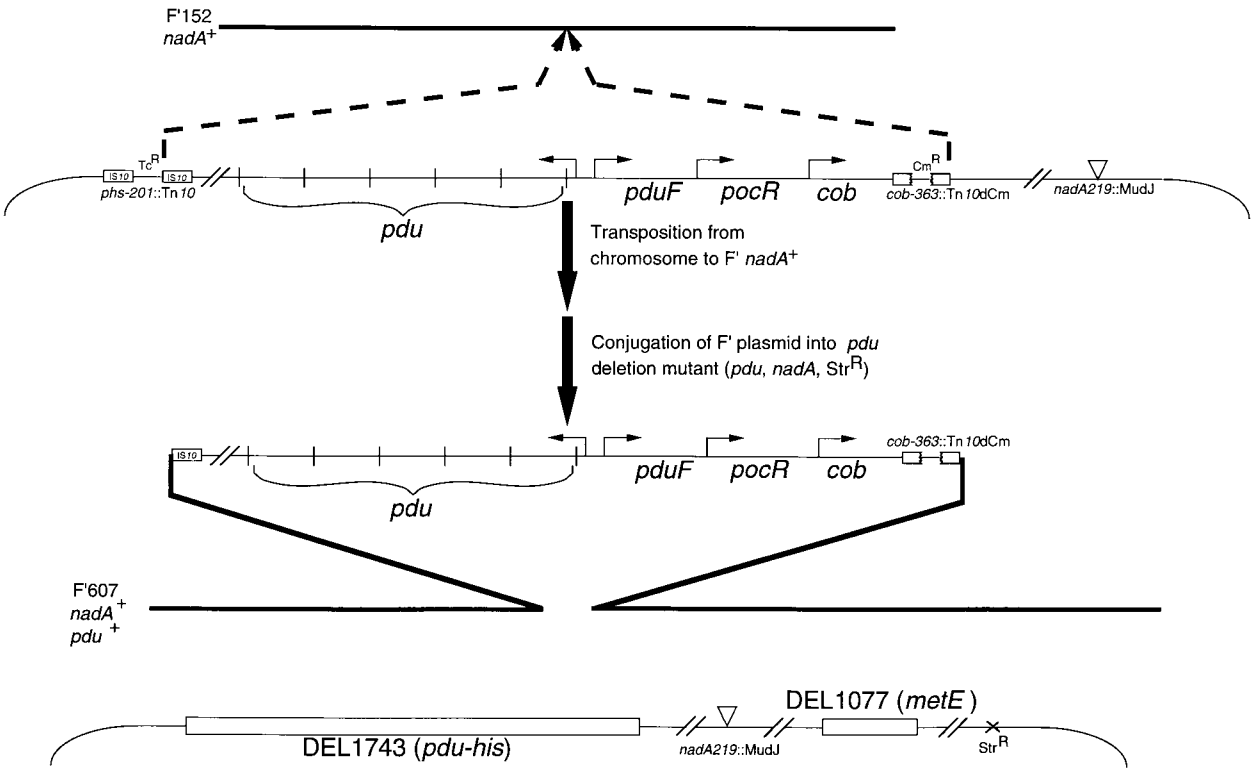

FIG. 2. Construction of plasmid F′607 used for complementation testing and deletion mapping. The chromosomal insertion mutation *phs-201*::Tn*10* (left of the *pdu* operon) is a complete Tn*10* element flanked by two intact IS*10* elements in inverse orientation (boxes). The *cob-363*::Tn*10d*Cm insertion mutation (right of the *pdu* operon) is a transposition-defective Tn*10* derivative lacking the inside ends of both IS*10* elements (boxes with a jagged inside edge). The desired plasmid was formed by transposition of the *pdu* region from the chromosome to plasmid F′152. The transposed region is between the right end of the *cob-363*::Tn*10d*Cm element and the inside end of one IS*10* element of insertion *phs-201*::Tn*10*. The final plasmid includes the entire wild-type *pdu* operon and encodes chloramphenicol but not tetracycline resistance.

strain (TT17903) that carried the two flanking insertions (*phs-201*::Tn*10* and *cob-363*::Tn*10d*Cm) and an unlinked *nadA219*::MudJ(Kn) insertion was constructed. Plasmid F′152, which carries the *nadA*$^+$ gene of *E. coli*, was introduced into this strain by selecting NadA$^+$ exconjugants. To promote transposition of the *pdu* region onto F′152, a plasmid (pNK2881) encoding Tn*10* transposase was transduced into this strain, selecting for Ap^r; this generated strain TT17905. The transposase encoded by this plasmid has reduced target site specificity (22).

In strain TT17905, the chromosomal segment (IS*10*-*pdu*$^+$-Tn*10d*Cm) is, in effect, a new composite transposon. The transposable region extends from the inside end of IS*10* (within the *phs-201*::Tn*10* element) to include the entire *cob-363*::Tn*10d*Cm element. This transposable region includes the entire *pdu* operon and provides a Cm^r Tc^s phenotype. To detect transposition of this region to the F′152 plasmid, strain TT17905 was used in a conjugational cross with a Str^r His$^-$ NadA$^-$ Pdu$^-$ recipient strain (TT17906); selection was made for transfer of Cm^r, and the donor was counterselected by streptomycin. Exconjugants were screened for those that coinherited Pdu$^+$ with the Cm^r phenotype. We saved one such exconjugant (TT17907) which carried an F′ plasmid (designated F′607) which had acquired the *pdu* operon; this plasmid was able to cotransfer the Nad$^+$ Cm^r and Pdu$^+$ phenotypes. Plasmid F′607 was then introduced into a strain (TT11855) which carries a *his pdu cob* deletion (DEL299) and can synthesize histidine if given the precursor histidinol. The plasmid was transferred by selecting for Cm^r exconjugants on minimal histidinol medium. Donors are counterselected because they cannot use histidinol; recipient cells grow only if they receive the Cm^r determinant. This final strain (TT17908) was used to construct derivatives used in complementation testing.

Recombination and complementation tests of *pdu* point mutations with deletions. Point mutations (*pdu*) were moved onto the F′607 plasmid of strain TT17908 by cotransduction with insertion *zec-3796*::Tn*10d*Tc, located downstream of the *pdu* operon, or with insertion *pduF354*::Tn*10d*Tc. All deletion strains were transduced to Str^r with phage grown on TR6625 (*strA1*). Derivatives of plasmid F′607 carrying a *pdu* point mutation were then transferred by conjugation into *pdu* deletion strains (Str^r) by selecting Cm^r and counterselecting the donor with streptomycin. The behavior of the resulting diploids allows both deletion mapping and complementation testing as described in Results.

Complementation testing of *pdu* point mutations. Tests of the ability of point mutations to complement each other were done in a *recA* mutant background in which no recombination was possible. All point mutations were moved onto the F′607 plasmid by cotransduction with a linked Tn*10d*Tc insertion. Each of the original chromosomal *pdu* point mutants (all Pdu$^-$ Srl$^-$) was made Str^r (by transduction from strain TR6625) and RecA$^-$ (selecting Srl$^+$ by use of a *srl*$^+$ *recA* donor strain). Finally, derivatives of plasmid F′607 carrying a *pdu* point mutation were conjugated into a series of *strA1 recA1* strains carrying a *pdu* point mutation, selecting Cm^r and with Str^r as the counterselecting marker. The *pdu* phenotypes of the merodiploids were then scored on MacConkey propanediol-vitamin B_{12} agar.

Propanediol dehydratase assays. Propanediol dehydratase was assayed by the method of Toraya et al. (40). In this assay, propionaldehyde produced by the reaction is detected by monitoring formation of a color complex formed with 3-methyl-2-benzothiazolanone hydrazone (MBTH-HCl). This complex was detected by monitoring A_{305}. Liquid cultures (20 ml) of the strain to be assayed were grown to full density in NCE liquid medium supplemented with propanediol, succinate, CN-B_{12}, and any other necessary nutritional supplements. The cells were harvested by centrifugation, washed once in 70 mM potassium phosphate buffer (pH 8.0), and resuspended in 2 ml of the same buffer. Cells were disrupted by sonication with a Branson 450 sonifier for 1 min 15 s on setting 5 at a 50% duty cycle. Cell debris was removed by centrifugation, and the supernatant was shielded from light and stored at −70°C. To determine the protein content of the cell extract, Pierce Coomassie blue reagent was used with bovine serum albumin as the standard. The propanediol dehydratase assay mixture contained 1,2-propanediol (0.2 M), KCl (0.05 M), potassium phosphate buffer (pH 8.0; 0.035 M), and Ado-B_{12} (15 μM) in a total volume of 1 ml. To start the reaction, 5 to 20 μl of cell extract was added. The reaction was terminated by the addition of 1 ml of 0.1 M potassium citrate buffer (pH 3.6) and 0.5 ml of 0.1% MBTH-HCl. After no less than 15 min, an additional 1 ml of H_2O was added, and the A_{305} was read. The extinction coefficient for the product was determined to be 11.4×10^3 M^{-1} cm^{-1}. Because of the photolability of the Co—C bond of Ado-B_{12}, the entire assay procedure was carried out in the dark.

Propionaldehyde dehydrogenase assay. The propionaldehyde dehydrogenase assay was adapted from an assay for ethanol dehydrogenase described by Cunningham and Clark (10). The cell extract was made as described above except that 50 mM MOPS (morpholine propanesulfonic acid) buffer was substituted for the potassium phosphate buffer. The assay was performed in 1 ml of 50 mM

CHES (2-[*N*-cyclohexylamino]ethanesulfonic acid) buffer (pH 9.5) containing the following: 1 μmol of dithiothreitol, 75 nmol of NAD^+, 100 nmol of Li-CoA, and an appropriate amount of cell extract. The reaction was started by adding 10 μl of 1 M propionaldehyde. The conversion of NAD^+ to NADH was then monitored by measuring the increase in A_{340}.

PCR. To localize endpoints of deletions within the region of known sequence of the *pdu* operon (9), the PCR was performed with pairs of primers that could amplify upstream regions of the *pdu* operon. Template DNA was derived from various deletion mutants, and the following oligonucleotides were used as PCR primers (see Fig. 5): PduC-1 (starts at the left end of the known sequence, at bp 1 of the reported sequence, with its 3′ end directed toward the *pdu* promoter), PduB-1 (starts with bp 840 and also extends to right), and PduA-1 (starts with bp 1266 of the *pduA* sequence and extends right). These primers were each used in combination with primer PduF-1, which directs replication to the left and starts with bp 1800 at the left end of the *pduF* gene. For any primer pair, production of a PCR product indicated that the deletion mutation did not extend into or across the interval between the sites of the two primers used; lack of a PCR product indicated that the deletion removed at least the region complementary to the promoter-distal primer.

PCRs were run in thin glass capillaries with an Air Thermo-Cycler (Idaho Technology). Each 20-μl reaction mixture contained the following: deoxynucleoside triphosphates 2 mM each, 2 mM $MgCl_2$, 50 mM Tris (pH 8.3), 0.25 ng of bovine serum albumin per ml, 0.5 mM cresol red, 2% sucrose, 0.5 μM each primer, and 0.8 U of *Taq* polymerase. DNA templates were provided by picking a bacterial colony with a platinum wire and adding cells directly to the reaction mixture. PCR parameters were as follows: 30 cycles with dissociation at 94°C, annealing at 50°C, and extension at 72°C with an extension time of 1 min. PCR products were visualized on 0.8% agarose gels by ethidium bromide staining. PCR primers were designed by use of the published sequence of the *pduB* and *-C* genes (9) and made by Bob Schackmann at the University of Utah Sequencing Facility.

RESULTS

Isolation of *pdu* mutants. Because *S. typhimurium* grows very slowly on propanediol, *pdu* mutants were detected by use of MacConkey indicator plates. On such plates (containing propanediol and vitamin B_{12}), both Pdu^+ and Pdu^- strains grow rapidly; Pdu^+ colonies are red, and Pdu^- colonies are white. The red color of Pdu^+ colonies is due to a pH change caused either by excreted propionate (generated by the anaerobic reactions outlined in Fig. 1) or by some end product of aerobic degradation of propionyl-CoA using the Prp enzymes (perhaps pyruvate). In principle, this color test allows detection of defects in most functions known to be involved in propanediol degradation. Using colony color as the identification criterion, we isolated 63 hydroxylamine-induced point mutants and 24 mutants carrying deletions that extended into the *pdu* operon from the downstream end. See Materials and Methods for details.

Isolation of an F′ *pdu*$^+$ plasmid by transposition. For use in complementation and deletion mapping, we constructed a plasmid that carries the entire *pdu* operon. Transposable elements were placed on either side of the *pdu*$^+$ region, and the intervening chromosomal segment was allowed to transpose to an F′ plasmid (see Materials and Methods) (Fig. 2). By using a linked Tn*10d*Tc insertion as a selective marker, *pdu* point mutations were added to this plasmid.

Operon mapping and complementation tests involving deletions. Even wild-type cells show very slow aerobic growth on propanediol. Because of this, tests of Pdu phenotypes for mapping experiments were done with MacConkey indicator plates that score production of acid from propanediol. Plasmids carrying *pdu* point mutations were introduced into strains carrying *pdu* deletion mutations. These deletions remove material from the promoter-distal end of the operon and thus allow expression of the remaining *pdu* genes from the normal *pdu* promoter.

Each of these diploids was patched or replica plated onto MacConkey indicator medium containing propanediol and CN-B_{12}. Solid red growth indicated that the two mutations complemented and affected different genes. A white patch with red papillae indicated that the two mutations did not complement but could recombine to form occasional Pdu^+ clones. A uniform white color indicated that the mutations present in the merodiploid showed neither complementation nor recombination. These responses are diagrammed in Fig. 3.

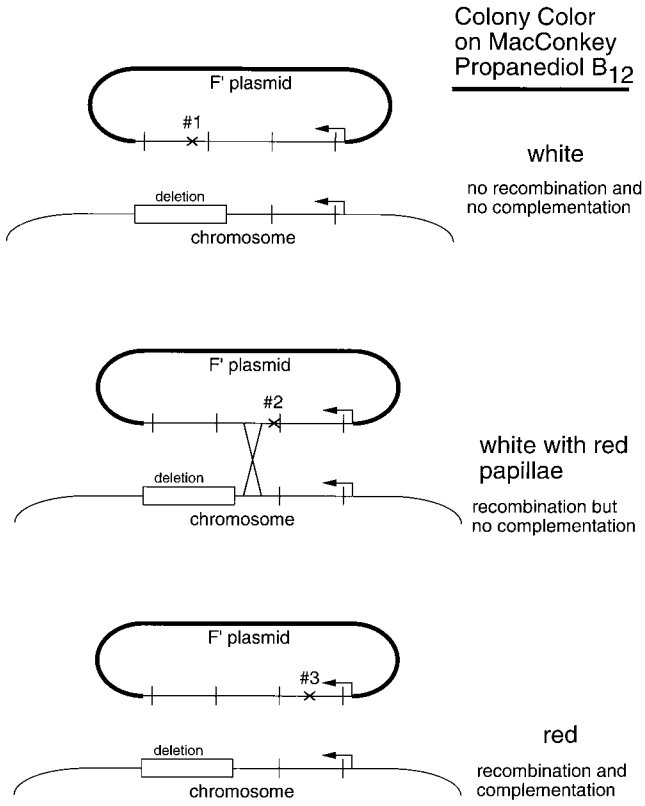

FIG. 3. Complementation testing and deletion mapping using a *pdu* merodiploid. All diploids showed one of the three depicted behaviors when plated on MacConkey medium containing propanediol and cobalamin. A white colony (or patch) lacking red papillae indicated that the point mutation (on the plasmid) neither complemented nor recombined with the chromosomal deletion. A white colony or patch with red papillae indicated that the point mutation did not complement but could recombine with the chromosomal deletion. A red colony indicated that the point mutation complemented (and presumably also recombined) with the chromosomal deletion. To increase the frequency of recombination events, all diploids, after 1 day of growth on selective media, were exposed to UV light for 8 to 10 s (20 J/m^2) and allowed to grow overnight before being scored for the presence of Pdu^+ papillae.

Deletion map of the *pdu* operon. Figure 4 presents the results of deletion mapping. If either complementation or recombination was seen, the point mutation on the plasmid was assumed to lie outside of the region removed by the promoter-distal chromosomal deletion. In general, the apparent positions of insertion mutations and polar point mutations agreed closely with the previous data (5).

The sensitivity of the mapping method is low because recombinants were scored visually rather than by positive selection. This means that in some cases, diploids involving point mutations or insertions that fail to complement a deletion and lie outside of that deletion close to the deletion endpoint may show no complementation and give rise to recombinants (red papillae) at a frequency too low to detect. Thus, the true position of such mutations may be somewhat to the right of the position indicated in Fig. 4. This problem does not interfere with the main point of the map construction, which is to put the point mutations into a general order which is helpful in inter-

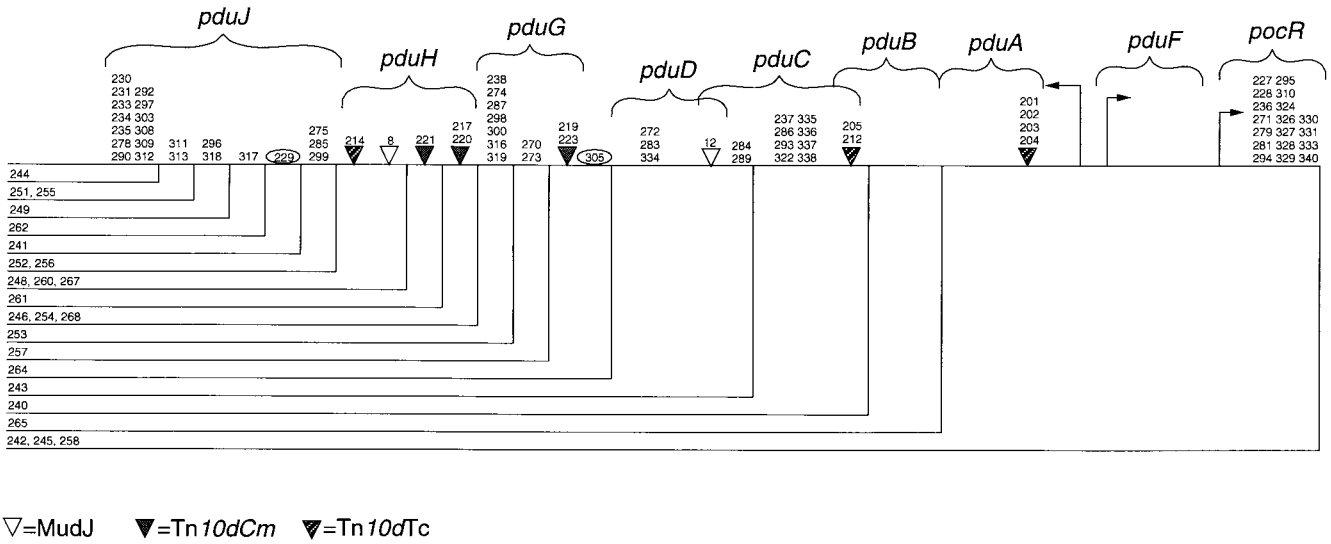

FIG. 4. Deletion map of the *pdu* region. Triangles represent insertion mutations, and the numbers above them are their allele numbers. Numbers without triangles are allele numbers of point mutations (top of figure) or deletion mutations (left side of figure). The relative order of mutations within a deletion interval is not known. Previous sequencing data determined that the insertion mutations *pdu-201*, *pdu-202*, *pdu-203*, and *pdu-204* are all directly downstream of the promoter (9). The direction of transcription (right to left in this map) was also determined previously (5). Positioning the right endpoints of deletions *pdu-243*, *-240*, *-265*, and *-245* was based (at least in part) on PCR results shown in Fig. 5. The two circled mutations (*pdu-305* and *pdu-229*) are the only two whose positions in the deletion map contradict their gene assignments, based on complementation tests. This discrepancy is probably due to insensitivity of the nonselective recombination tests used.

preting the complementation tests (see below). The mapping disagreed with grouping of point mutations into complementation groups for only two strongly polar point mutations. In mapping insertion mutations, the low sensitivity was a particularly serious problem because insertions are absolutely polar and recombination is required to determine whether the insertion lies inside or outside a deletion. Because of this problem, the map positions of several of the insertion mutations in Fig. 4 differ slightly from their positions in a previously published map based on selective crosses (5).

The point mutations at the right side of the map affect the *pocR* gene. Expression of the *pdu* operon requires the positive regulatory protein PocR, which is encoded by a gene lying between the *pdu* and *cob* operons. Because *pocR* mutants are white on MacConkey indicator plates, *pocR* point mutations are likely to be included in our set of Pdu$^-$ mutations. To determine whether any of our point mutations affect the *pocR* regulatory gene, a *cob-24*::MudJ *lac* fusion was introduced into the *pdu* point mutants; only *pocR* mutations affect expression of a *cob*::*lac* fusion (5). Expression of this *cob*::*lac* fusion was impaired by any of the *pdu* mutations mapping at the right side of the *pdu* region (Fig. 4); the structure of these doubly mutant strains was verified by confirming that they were both Pdu$^-$ and Cob$^-$ Kan^r. This suggests that all point mutations in the furthest-upstream deletion interval affect the *pocR* gene.

Correlation of the genetic map with available sequence. The promoter-proximal (right-most) 1,370 bp of the *pdu* operon have been sequenced and shown to include two open reading frames (*pduA* and *pduB*) and the beginning of a third (*pduC*), as diagrammed in Fig. 5 (9). Previous data showed that four Tn*10* insertion mutations (*pdu-201*, *pdu-202*, *pdu-203*, and *pdu-204*) were all located at a single site, 50 bp downstream of the *pdu* promoter, outside of the first open reading frame, *pduA* (9). All deletions that recombine with these insertions must end within the *pdu* transcript.

To see whether any mutations map within the *pduA*, *pduB*, or *pduC* gene, we used PCR to localize deletion endpoints on the physical map. As shown in Fig. 5, a single primer within the *pduF* gene (directing replication to the left) was used in combination with each of three primers complementary to various

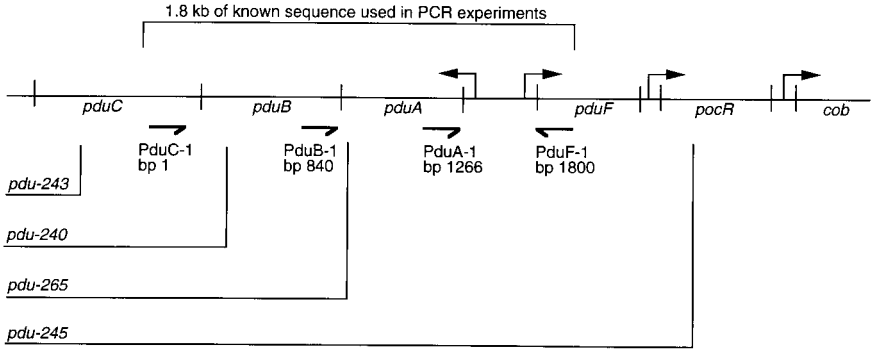

FIG. 5. Primers used in PCR experiments. The PCR primers are shown as half arrows below the chromosome; arrows above chromosomes designate promoters. The positions of a few deletion endpoints as determined by these PCR experiments are also shown.

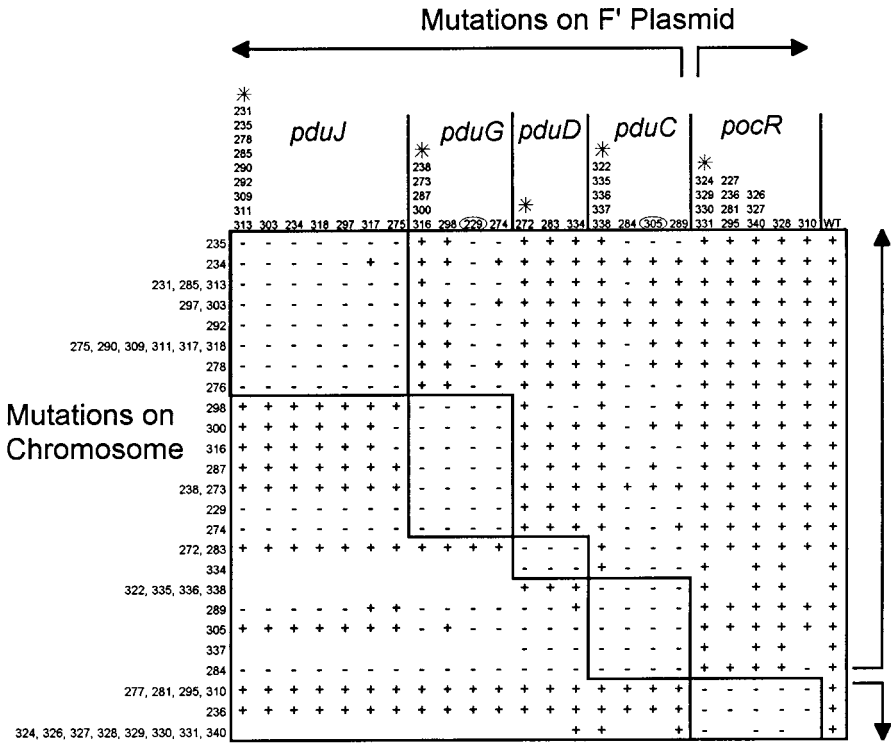

FIG. 6. Complementation tests of point mutations. Mutations on the F′607 plasmid are listed across the top of the figure, and mutations in the chromosome are listed vertically on the left. A "+" indicates that the diploid had a Pdu$^+$ phenotype; a "−" indicates that the diploid had a Pdu$^-$ phenotype; a blank indicates that the diploid was not constructed. Boxes enclose responses of mutation pairs that are concluded to affect the same gene. Blocks of genes are in order of their position in the deletion map with the two (circled) exceptions. Gene assignments are given above the complementation table. Mutation sets marked with an asterisk contributed most heavily to defining the group, usually because of being apparently nonpolar. The direction of transcription of the *pdu* operon is from right to left and from bottom to top (see arrows above and on the right of the figure).

points in the known sequence. The first primer marked the promoter-distal end of the known sequence within the *pduC* gene; the second and third marked the far right (promoter-proximal) end of the *pduB* and *pduA* genes. If a given reaction produced the appropriately sized PCR product, the deletion is assumed to have an endpoint outside the amplified interval. If no product was made, the deletion is assumed to remove at least the downstream primer site.

The deletion mutation *pdu-245* must extend past the *pduA* gene since it failed to give a product with any of the three primer pairs. Deletion *pdu-265* gave a product only with primer pair PduA-1/PduF-1 and thus most likely ends within the *pduA* gene. The *pdu-240* deletion mutant gave PCR products with all primer pairs except PduC-1/PduF-1 and thus must end within the *pduB* gene or at the upstream end of the *pduC* gene. An insertion mutation known to eliminate dehydratase function, *pdu-12*::MudJ, gave a product (of the size expected for the wild type) with all three primer pairs and must therefore map within or downstream of the *pduC* gene.

All mutations recombining with deletion *pdu-265* are in the *pocR* gene. No *pdu* point mutation maps between the endpoints of deletions *pdu-240* and *pdu-265*. The most promoter-proximal group of point mutations within the *pdu* operon mapped between the endpoints of deletions *pdu-243* and *pdu-240* and thus lie within or downstream of the *pduB* gene. These upstream *pdu* point mutations all lack diol dehydratase activity (described later). Since the inferred amino acid sequence of the PduC but not the PduB protein shows homology to the diol dehydratases of *Citrobacter freundii* or *Klebsiella oxytoca* (6, 11, 39), we propose that the most upstream group of point mutations within the operon are in the *pduC* gene and that no point mutations affect either the *pduA* or the *pduB* genes; we presume that these genes have no mutant phenotype under the conditions used. While it is possible that we simply did not find this class, the large number of mutations in the flanking *pocR* and *pduC* genes suggests that we would have found this class if it showed a phenotype.

Complementation testing of *pdu* point mutations. The results of complementation tests between point mutations are shown in Fig. 6. In the figure, point mutations are arranged in groups corresponding to their order in the deletion map. With only two exceptions (circled allele numbers in Fig. 4 and 6), mutations assigned to one complementation group appear as a contiguous block of point mutations in the genetic map. Mutations within any particular gene are arranged to most clearly show the complementation patterns. Asterisks mark columns of mutations that were most important for defining a complementation group; these are mutations whose behaviors suggested that they are not polar on distal genes. Mutations in Fig. 6 that fail to complement mutations in more than one gene are presumed to be polar (nonsense or frameshifts). One pair of mutations assigned to the *pduJ* gene showed complementation with each other but not with other *pduJ* point mutations. This intragenic complementation may reflect activity of a multimeric protein assembled from the two mutant subunit types.

At least five genes appear to be required for acid production by propanediol degradation. One of these complementation groups corresponds to the known *pocR* regulatory gene, leaving four complementation groups within the *pdu* operon downstream of the *pduA* and *pduB* genes. The complementation

TABLE 2. Propanediol dehydratase activities of selected strains[a]

Strain	Relevant genotype	Activity (nmol/min/mg)
TT16462	*pdu*$^+$	1,960
TT11855	DEL299 (*his-pdu-cob*)	<1
TT17989	*pduJ313*	780
TT17944	*pduJ235*	2,100
TT17985	*pduJ309*	494
TT17974	*pduG298*	1,740
TT17992	*pduG316*	930
TT17958	*pduD283*	<1
TT18010	*pduD334*	<1
TT18013	*pduC337*	<1
TT17961	*pduC284*	<1
TT18005	*pdu-329* (*pocR*)	26
TT17955	*pdu-277* (*pocR*)	<1

[a] Liquid cultures of the strains were grown in NCE liquid medium supplemented with propanediol, succinate, CN-B_{12}, and required nutritional supplements.

TABLE 3. Propionaldehyde dehydrogenase activities of selected strains grown under inducing conditions[a]

Strain	Relevant genotype	Activity (nmol/min/mg)
LT2	*pdu*$^+$	362
LT2 (uninduced)	*pdu*$^+$	<1
TT11855	DEL299 (*his-pdu-cob*)	<1
TT17908	DEL299/F′607 *pdu*$^+$	565
TT10852	*pdu*$^+$ *cob-24*::MudJ	736
TT16462[b]	*srl-203*::Tn*10d*Cm	469
TT17989	*pduJ313*	<1
TT17944	*pduJ235*	<1
TT17985	*pduJ309*	<1
TT17974	*pduG298*	<1
TT17992	*pduG316*	<1
TT18010	*pduD334*	<1
TT18013	*pduC337*	<1
TT17961	*pduC284*	<1
TT18005	*pdu-329* (*pocR*)	<1
TT17955	*pdu-277* (*pocR*)	<1

[a] Liquid cultures were grown to full density in NCE liquid medium supplemented with propanediol, succinate, CN-B_{12}, and any other required nutritional supplements. The strain marked uninduced was grown in the same medium without propanediol.

[b] This *pdu*$^+$ strain is isogenic with all of the *pdu* mutants listed in the table.

groups corresponding to *pduJ* and *pocR* genes are very heavily populated with mutations compared with genes in the central region. This probably does not reflect the relative sizes of these *pdu* genes but is more likely due to the fact that point mutations were isolated by local mutagenesis based on their linkage to a Tn*10* insertion mutation either upstream or downstream of the operon. Thus, the two ends of the *pdu* region were, in effect, more heavily mutagenized than the central region of the operon. Only three point mutations define the *pduD* complementation group, and eight point mutations define complementation groups corresponding to the *pduC* and *pduG* genes.

The *pduC* and *pduD* genes encode the vitamin B_{12}-dependent propanediol dehydratase. To determine which of the complementation groups correspond to the propanediol dehydratase genes, *pdu* point mutants were assayed for expression of propanediol dehydratase. The results of these experiments are summarized in Table 2. Strains having activities less than 3% of the wild-type activity were classified as not having a functional dehydratase enzyme. All *pdu* point mutations assigned to the *pduC* and *pduD* genes had little or no dehydratase activity. This suggests that the B_{12}-dependent propanediol dehydratase is encoded by the *pduC* and *pduD* genes and that it has at least two subunits. After determining the sequence of the *pdu* operon, Bobik et al. (6) demonstrated that a third gene is also required for dehydratase activity. The third gene is *pduE*, lying just downstream of *pduD* and *pduC*. All three open reading frames must be supplied to *E. coli* to allow formation of dehydratase activity, suggesting that the third gene is essential for activity. The *pduE* gene was not detected in the genetic experiments described here.

Point mutations from the *pocR* complementation group also had little or no dehydratase activity, as expected for mutations unable to transcribe the *pdu* operon. Point mutations in all other complementation groups showed normal dehydratase activity.

The *pdu* operon encodes a protein required for propionaldehyde dehydrogenase activity. To determine whether any of the complementation groups correspond to a gene for propionaldehyde dehydrogenase, various strains were assayed for this activity (Table 3 and Fig. 1). Wild-type *Salmonella* (LT2) showed propionaldehyde dehydrogenase activity only when grown under conditions known to induce the *pdu* operon, suggesting that this activity is encoded within the operon. A deletion mutant lacking the entire *pdu* operon failed to produce the activity, but the activity was restored by addition of the F′607 *pdu*$^+$ plasmid. Individual *pdu* point mutations were assayed to try and assign this activity to a particular gene. Oddly, all of the *pdu* point mutants tested (regardless of their complementation group) were defective for dehydrogenase. This may suggest that the activity requires formation of a multisubunit complex. Alternatively, the dehydrogenase may be encoded elsewhere but require a product of the operon (e.g., propionaldehyde) for induction.

The *pduG* gene may encode a protein involved in the adenosylation of B_{12}. Mutants lacking the CobA protein, which adenosylates CN-B_{12} to form Ado-B_{12}, still show a Pdu$^+$ phenotype. This is of interest because Ado-B_{12} is the required cofactor for propanediol dehydratase. To determine whether a B_{12} adenosylation function might be encoded by the *pdu* operon, we tested *pdu* point mutants to see if any showed a Pdu$^-$ phenotype (with CN-B_{12}) that was corrected when Ado-B_{12} was substituted for CN-B_{12}. Five of the eight *pduG* point mutants became phenotypically Pdu$^+$ on MacConkey propanediol plates with 100 μl of a 1.5 mM Ado-B_{12} solution; these mutants were all Pdu$^-$ on medium with CN-B_{12}. Mutant *pduG229* was not corrected by Ado-B_{12}, and mutation *pduG274* appeared to be only partially corrected. Complementation studies revealed that both of these mutations were polar on distal genes, so their failure to be corrected can be explained by a loss of multiple Pdu functions. Mutant *pduG316* was also not corrected by Ado-B_{12} but is not polar; it is not clear why this mutation failed to show correction by Ado-B_{12}. None of the tested *pdu* point mutations in other genes were corrected by Ado-B_{12}.

All of the Ado-B_{12}-correctable *pduG* mutations were isolated and tested in a genetically *cobA*$^+$ strain. This suggests that the Ado-B_{12} produced by the CobA protein is insufficient or unavailable to the degradative enzyme. A parallel situation has been observed for the *eut* (ethanolamine) operon (32).

Inferring the existence of the *pduH* gene. Several insertion mutations (*pdu-8*, *-214*, *-217*, *-220*, and *-221*) mapped in a contiguous region that includes multiple deletion endpoints. This region included no point mutations. The finding of multiple different insertions and deletion endpoints suggests that

the affected region is large. We infer that this region includes at least one gene (designated *pduH*) which is nonessential for aerobic propanediol degradation. Point mutations for this activity could not be detected, and the Pdu^- phenotype of the detected insertions and deletions in the region may be due to polarity or loss of downstream *pdu* genes.

DISCUSSION

Mutants defective in aerobic degradation of propanediol (*pdu*) were isolated and characterized by complementation tests, deletion mapping, and enzyme assays. Point mutations within the *pdu* operon defined four complementation groups corresponding to genes which were necessary for aerobic, B_{12}-dependent production of acid by propanediol degradation. Two of these complementation groups (*pduC* and *pduD*) include point mutations which eliminate propanediol dehydratase activity. This suggests that there could be at least two separate subunits of the propanediol dehydratase enzyme. Previous work on the diol dehydratase from *K. oxytoca* (previously called *Klebsiella pneumoniae*) showed that purified dehydratase may include five distinct subunits (41). Recent purification of this *Klebsiella* enzyme and sequencing of its coding region demonstrate that the enzyme is comprised of three subunit types which are necessary and sufficient for dehydratase activity (39). Thus, the five-protein complex identified previously may represent a higher aggregate of Pdu proteins, only some of which are essential to dehydratase activity.

In *Salmonella*, sequencing and expression of cloned genes demonstrate that (as for *Klebsiella*) three genes are necessary and sufficient for production of active dehydratase (6). The genetic analysis described here identified only two of the three genes encoding dioldehydratase subunits. We have reserved the *pduE* designation for the genetically unidentified third open reading frame. The failure to find *pduE* mutants (and the paucity of *pduD* mutants) may reflect the fact that both of these genes are small.

The *cobA* gene encodes an enzyme that catalyzes adenosylation of B_{12} (14, 37). Strains with a *cobA* mutation are phenotypically Pdu^+ even though Ado-B_{12} is the cofactor for the propanediol dehydratase. This suggests the existence of a second Ado-B_{12} transferase that is specific for dioldehydratase or is induced only in the presence of propanediol. Many of the *pduG* point mutants were corrected by exogenously supplied Ado-B_{12}, suggesting that the PduG protein may be involved in adenosylation of B_{12}. Since these *pduG* mutations have a Pdu^- phenotype even in a $cobA^+$ strain, we infer that the adenosylation activity provided by CobA enzyme alone is insufficient to support a Pdu^+ phenotype.

We have not assigned a function to the *pduJ* gene. The *pdu* phenotype scored here is acid production from propanediol. Assuming that propionate is the acid produced (Fig. 1), the pathway for conversion of diol to acid is catalyzed by diol dehydratase, propionaldehyde dehydrogenase, phosphotransacylase, and propionylkinase. Two enzymes thought to catalyze the last two reactions are encoded by the *pta* and *ack* genes, respectively (26). Mutants in these genes do not impair the Pdu^+ (acid production) phenotype of an otherwise-wild-type strain, making it likely that analogous enzymes are encoded within the *pdu* operon. The *pduJ* gene might encode either of these activities or the propionaldehyde dehydrogenase activity, which was missing from mutants in all complementation groups.

The existence of the *pduH* gene was inferred from the distribution of mutations in the genetic map. Five insertion mutations (*pdu-214*, *pdu-8*, *pdu-221*, *pdu-217*, and *pdu-220*) mapped to a continuous cluster of deletion intervals in which no point mutations mapped. The existence of an extensive silent region is suggested by identification of multiple deletion endpoints and insertion sites within the region. We have tentatively designated this silent gene *pduH*. We infer that this region of the *pdu* operon encodes one or more enzymes that are not required for the Pdu^+ (aerobic acid production) phenotype.

Although propanediol does not serve as the sole carbon and energy source under standard anaerobic laboratory conditions, we presume that *Salmonella* must be able to degrade propanediol to good advantage under some set of natural anaerobic conditions. This presumption is suggested by the facts that B_{12} is produced only in the absence of oxygen and that expression of the synthetic genes is induced by propanediol. Preliminary results suggest that propanediol supports anaerobic growth when tetrathionate (but not nitrate or fumarate) is used as an alternative electron acceptor (4). Thus, in assigning functions to *pdu* genes, we may also need to identify functions that might be useful under these anaerobic conditions.

The diagram in Fig. 1 proposes that in the absence of oxygen, propanediol could provide an electron sink (by reduction of propionaldehyde) and a source of ATP (by conversion of propionyl-CoA successively to propionyl phosphate and propionate). The diagrammed reactions do not provide an anaerobic carbon source since all carbon atoms are excreted as either propanol or propionate. Additional functions are likely to exist since propanediol can provide an anaerobic carbon source in the presence of tetrathionate.

Consistent with the ideas described above, PCR analysis of the *pdu* operon suggests that the *pdu* operon is large enough to encode about 15 proteins (25), of which we have detected only 4 by genetic defects in aerobic production of acid from propanediol. Here we provide evidence that the *pduA* and *pduB* genes as well as the inferred *pduH* gene are not needed for aerobic acid production and may be required for anaerobic degradation.

The existence of genes in the *pdu* operon which are not required for aerobic acid production by propanediol degradation is not unprecedented. The *eut* operon, which encodes enzymes needed for B_{12}-dependent degradation of ethanolamine by a very similar pathway, encodes (in addition to a regulatory protein) 15 proteins, only 6 of which have a mutant phenotype for aerobic degradation of ethanolamine (24, 32, 36). This operon includes two homologs of the *pduA* gene and at least one for the *pduB* gene. Mutations in the *pduA* and *pduB* genes (and in their *eut* homologs) have no aerobic phenotype, and the proteins they encode have homology to proteins found in other bacteria, where they are thought to contribute to the shell of the bacterial carboxysome, an organelle supporting CO_2 fixation. This suggests that anaerobic degradation of ethanolamine and propanediol may involve currently unknown pathways for utilization of these compounds; these pathways may require formation of a carboxysome-like compartment.

The *pdu* operon appears to encode a propionaldehyde dehydrogenase function. This activity is present in wild-type *S. typhimurium* only when the *pdu* operon is induced and is eliminated by deletions of the operon and by point mutations in any of the several complementation groups defined here. To explain this, we suggest that the proteins encoded by genes of the *pdu* operon form a complex which is required for propionaldehyde dehydrogenase function. All tested *pdu* mutants prevent formation of a functional complex and therefore result in a lack of propionaldehyde dehydrogenase activity. The protein directly responsible for dehydrogenase activity remains un-

identified, but the PduJ protein is a likely candidate. It is interesting to note that initial work on propanediol dehydratase of *K. oxytoca* indicated that under some conditions, the activity was associated with a complex including five different subunits (41). Later work showed that only three of these subunits are essential for dehydratase function, making it possible that enzymes such as those discussed above are members of the larger complex.

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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. I.** Involvement of the Arc system in redox regulation of the *cob* operon in *Salmonella typhimurium*. Submitted for publication.
3. **Berkowitz, D., J. M. Hushon, H. J. Whitfield, J. R. Roth, and B. N. Ames.** 1968. Procedure for identifying nonsense mutations. J. Bacteriol. **96:**215–220.
4. **Bobik, T. A.** Personal communication.
5. **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.
6. **Bobik, T. A., R. Jeter, Y. Xhu, and J. Roth.** Unpublished data.
7. **Bochner, B. R., H.-C. Huang, G. L. Schieven, and B. N. Ames.** 1980. Positive selection for loss of tetracycline resistance. J. Bacteriol. **143:**926–933.
8. **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.
9. **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.
10. **Cunningham, P., and D. Clark.** 1986. The use of suicide substrates to select mutants of *Escherichia coli* lacking enzymes of alcohol fermentation. Mol. Gen. Genet. **205:**487–493.
11. **Daniel, R., and G. Gottschalk.** Nucleotide sequence of the *dha* regulon from *Citrobacter freundii* (accession no. U09771). Unpublished data.
12. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **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.
14. **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.
15. **Evans, C., B. Sumegi, P. Srere, D. Sherry, and C. Malloy.** 1993. [^{13}C] Propionate oxidation in wild type and citrate syntase mutant *Escherichia coli*: Evidence for multiple pathways of propionate utilization. Biochem. J. **291:** 927–932.
16. **Fernández-Briera, A., and A. Garrido-Pertierra.** 1988. A degradation pathway of propionate in *Salmonella typhimurium* LT-2. Biochimie **70:**757–768.
17. **Hammelman, T., G. O'Toole, J. Trziebiatowski, A. Tsang, D. Rank, and J. Escalante-Semerena.** 1996. Identification of a new *prp* locus required for propionate catabolism in *Salmonella typhimurium* LT2. Mol. Microbiol. **6:**1491–1494.
18. **Hughes, K., J. Roth, and B. Olivera.** 1991. A genetic characterization of the *nadC* gene of *Salmonella typhimurium*. Genetics **127:**657–670.
19. **Iuchi, S., V. Chepuri, H.-A. Fu, R. Gennis, and E. C. C. Lin.** 1990. Requirement for terminal cytochromes in generation of the aerobic signal for the *arc* regulatory system in *Escherichia coli*: study utilizing deletions and *lac* fusions of *cyo* and *cyd*. J. Bacteriol. **172:**6020–6025.
20. **Jeter, R. M.** 1990. Cobalamin-dependent 1,2-propanediol utilization by *Salmonella typhimurium*. J. Gen. Microbiol. **136:**887–896.
21. **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.
22. **Kleckner, N., J. Bender, and S. Gottesman.** 1991. Uses of transposons with emphasis on Tn*10*. Methods Enzymol. **204:**139–180.
23. **Kofoid, E.** Personal communication.
24. **Kofoid, E., C. Rappleye, I. Stojiljkovic, D. Sheppard, L. Warnick, G. Plunkett, and J. R. Roth.** Unpublished data.
25. **Lawrence, J.** Personal communication.
26. **LeVine, S. M., F. Ardeshir, and G. F.-L. Ames.** 1980. Isolation and characterization of acetate kinase and phosphotransacetylase mutants of *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **143:**1081–1085.
27. **Lin, E. C. C.** 1987. Dissimilatory pathways for sugars, polyols, and carboxylates, p. 244–284. *In* F. D. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
28. **Maloy, S. R., and W. D. Dunn.** 1981. Selection for loss of tetracycline resistance in *Escherichia coli*. J. Bacteriol. **145:**1110–1112.
29. **Obradors, N., J. Badía, L. Baldomà, and J. Aguilar.** 1988. Anaerobic metabolism of the L-rhamnose fermentation product 1,2-propanediol in *Salmonella typhimurium*. J. Bacteriol. **170:**2159–2162.
30. **Price, G. D., S. M. Howitt, K. Harrison, and M. R. Badger.** 1993. Analysis of a genomic DNA region from the cyanobacterium *Synechococcus* sp. strain PCC7942 involved in carboxysome assembly and function. J. Bacteriol. **175:** 2871–2879.
31. **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.
32. **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.
33. **Roth, J. R., J. G. Lawrence, and T. A. Bobik.** Cobalamin (coenzyme B_{12}): synthesis and biological significance. Annu. Rev. Microbiol., in press.
34. **Roy, I., and P. Leadlay.** 1992. Physical map location of the new *E. coli* gene *sbm*. J. Bacteriol. **174:**5763–5764.
35. **Schmeiger, H.** 1971. A method for detection of phage mutants with altered transducing ability. Mol. Gen. Genet. **110:**378–381.
36. **Stojiljkovic, I., A. Bäumler, and F. Heffron.** 1995. Ethanolamine utilization in *Salmonella typhimurium*: nucleotide sequence protein expression and mutational analysis of the *cchA cchB eutJ eutG eutH* gene cluster. J. Bacteriol. **177:**1357–1366.
37. **Suh, S., and J. Escalante-Semerena.** 1995. Purification and initial characterization of the ATP:corrinoid adenosyl transferase encoded by the *cobA* gene of *Salmonella typhimurium*. J. Bacteriol. **177:**921–925.
38. **Tittensor, J., T. A. Bobik, and J. R. Roth.** Unpublished results.
39. **Tobimatsu, T., T. Hara, M. Sakaguchi, Y. Kishimoto, Y. Wada, M. Isoda, T. Sakai, and T. Toraya.** 1995. Molecular cloning, sequencing, and expression of the genes encoding adenosylcobalamin-dependent diol dehydrase of *Klebsiella oxytoca*. J. Biol. Chem. **270:**7142–7148.
40. **Toraya, T., K. Ushio, S. Fukui, and H. P. C. Hogenkamp.** 1977. Studies on the mechanism of the adenosyl cobalamin-dependent diol dehydratase reaction by the use of analogs of the coenzyme. J. Biol. Chem. **252:**963–970.
41. **Toraya, T., and S. Fukui.** 1981. Diol dehydrase, p. 233–262. *In* D. Dolphin (ed.), B_{12}, vol. 2. John Wiley & Sons, Inc., New York, N.Y.
42. **Toraya, T., S. Honda, and S. Fukui.** 1979. Fermentation of 1,2-propanediol and 1,2-ethanediol by some genera of *Enterobacteriaceae* involving coenzyme B_{12}-dependent diol dehydratase. J. Bacteriol. **139:**39–47.
43. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218:**97–106.
44. **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.