JOURNAL OF BACTERIOLOGY, Apr. 1992, p. 2253–2266
0021-9193/92/072253-14$02.00/0

Vol. 174, No. 7

A Single Regulatory Gene Integrates Control of Vitamin B_{12} Synthesis and Propanediol Degradation

THOMAS A. BOBIK,* MICHAEL AILION, AND JOHN R. ROTH
Department of Biology, University of Utah, Salt Lake City, Utah 84112

Received 10 December 1991/Accepted 2 February 1992

The *cob* operon of *Salmonella typhimurium* encodes enzymes required for synthesis of adenosyl-cobalamin (vitamin B_{12}). The *pdu* operon encodes enzymes needed for use of propanediol as a carbon source, including an adenosyl-cobalamin-dependent enzyme, propanediol dehydratase. These two operons both map near min 41 of the *S. typhimurium* linkage map and are transcribed divergently. Here we report that the *cob* and *pdu* operons form a single regulon. Transcription of this regulon is induced by either glycerol or propanediol. The metabolism of these compounds is not required for induction. Propanediol induces the regulon either aerobically or anaerobically during growth on poor carbon sources. Aerobically glycerol induces only if its metabolism is prevented by a mutational block such as a *glpK* mutation. Under anaerobic conditions, glycerol induces in both *glpK*$^+$ and *glpK* mutant strains during growth on poor carbon sources. A new class of mutations, *pocR*, prevents induction of the *cob*/*pdu* regulon by either propanediol or glycerol and causes a Cob$^-$ Pdu$^-$ phenotype. The *pocR* gene is located between the *cob* and *pdu* operons and appears to encode a *trans*-acting protein that acts as a positive regulator of both operons. Transcription of the *pocR* regulatory gene is induced, even without the PocR protein, during aerobic growth on poor carbon sources and during anaerobic respiration. With the functional PocR protein, transcription of the *pocR* gene is autoinduced by propanediol but not by glycerol. The growth conditions that increase *pocR* gene expression correlate with growth conditions that allow high induction of the *cob*/*pdu* regulon. A model for control of this regulon suggests that the PocR protein is a transcriptional activator of both the *cob* and *pdu* operons and that both glycerol and propanediol can individually serve as effectors of the PocR protein. We suggest that global control mechanisms cause variation in the level of the PocR protein; an increased level of the PocR protein permits higher induction by propanediol or glycerol.

Salmonella typhimurium synthesizes the vitamin B_{12} coenzyme adenosyl-cobalamin (Ado-Cbl) de novo (24). Twenty-five genes that play a role in Ado-Cbl biosynthesis are known (16, 30). The majority of these genes lie in a single large operon, the *cob* operon, that includes three contiguous clusters of functionally related genes (see below and reference 14). Genes of the CobI and CobII clusters are required for biosynthesis of adenosyl-cobinamide (Ado-Cbi) and dimethylbenzimidazole (DMB), respectively. CobIII gene products mediate the covalent joining of Ado-Cbi and DMB to form Ado-Cbl (25). In *S. typhimurium*, de novo vitamin B_{12} synthesis occurs only under anaerobic growth conditions; however, exogenous corrinoids can be taken up and converted to vitamin B_{12} coenzymes both aerobically and anaerobically (18). Previous work suggested that the *cob* operon is induced only anaerobically (redox control) and is stimulated by the complex of the cyclic AMP receptor protein (CRP) with cyclic AMP (cAMP) (3, 17). Rondon and Escalante-Semerena recently found that the *cob* operon can also be induced under aerobic conditions by propanediol (29).

Propanediol utilization is one of the four metabolic functions known to require vitamin B_{12} coenzymes in *S. typhimurium* (23). Ado-Cbl is a cofactor of propanediol dehydratase, which catalyzes dehydration of propanediol to propionaldehyde, the first step of propanediol utilization (23, 32). Propanediol can serve as a sole carbon and energy source for *S. typhimurium* only aerobically, but only if an exogenous corrinoid is provided, since Ado-Cbl is not synthesized de novo under aerobic conditions (24). Mutations causing a defect in utilization of propanediol map in the *pdu* operon, which is adjacent to the *cob* operon. While the *pdu* operon encodes an Ado-Cbl-dependent propanediol dehydratase, other genes of this operon have not been characterized. Induction of the *pdu* operon by propanediol occurs during aerobic growth on a poor carbon source, such as succinate (23).

Here we investigate the system that coregulates the *cob* and *pdu* operons. Both operons appear to be induced by propanediol and by glycerol. In addition, both are subject to global regulation. We propose a model for induction and discuss the paradoxical aspects of the regulatory behavior of this system.

MATERIALS AND METHODS

Bacterial strains and transposons. Strains used in this study were derivatives of *S. typhimurium* LT2 (Table 1). MudA refers to a conditionally transposition-defective derivative of the phage Mu dl (7) that was constructed by Hughes and Roth (21). MudJ refers to phage Mu dl-1734 constructed by Castilho et al. (8). MudA and MudJ elements confer Ap^r and Kn^r, respectively. When any of these transposable Mud elements inserts into a chromosomal operon in the proper orientation, transcription of that operon extends into the *lacZ* gene of the inserted element. By assaying the resulting β-galactosidase one can estimate the activity of the chromosomal promoter. These *lac* operon fusions were used to study transcriptional control of the *cob* and *pdu* operons. Tn*10d*Tc and Tn*10d*Cm are transposition-defective derivatives of transposon Tn*10* that confer Tc^r and Cm^r, respec-

* Corresponding author.

TABLE 1. Strain list

Strain	Genotype
TR6583	*metE205 ara-9*
TT7691	*hisD9952*::MudA
TT9814	*metE205 ara-9 cob-4*::Tn*10* (CobIII)
TT10327	*metE205 ara-9 cob-24*::MudA (CobI)
TT10364	*metE205 ara-9 cob-61*::MudA (CobII)
TT10365	*metE205 ara-9 cob-62*::MudA (CobII)
TT10852	*metE205 ara-9 cob-24*::MudJ
TT10857	*metE205 ara-9 cob-62*::MudJ
TT10858	*metE205 ara-9 cob-66*::MudJ (CobIII)
TT16934	*metE205 ara-9 cob-364*::Tn*10d*Cm (CobI)
TT17073	*DEL1077*(*metE*) *ara-9 cob-66*::MudJ
TT17077	*DEL1077*(*metE*) *ara-9 zeb-3721*::Tn*10d*Tc
TT17078	*DEL 1077*(*metE*) *ara-9 pocR1*::Tn*10d*Tc
TT17085	*DEL 1077*(*metE*) *ara-9 pocR8*::Tn*10d*Tc
TT17089	*DEL1077*(*metE*) *ara-9 pdu-201*::Tn*10d*Tc
TT17095	*DEL1077*(*metE*) *ara-9 pdu-207*::Tn*10d*Tc
TT17104	*metE205 ara-9 pocR12*::MudJ
TT17107	*DEL1077*(*metE*) *ara-9 zeb-3722*::MudJ
TT17119	*metE205 ara-9 cob-24*::MudJ *pocR1*::Tn*10d*Tc
TT17123	*metE205 ara-9 cob-24*::MudJ *pdu-201*::Tn*10d*Tc
TT17130	*metE205 ara-9 cob-24*::MudJ *zeb-3721*::Tn*10d*Tc
TT17131	*metE205 ara-9 pdu-12*::MudJ
TT17135	*metE205 ara-9 pdu-12*::MudJ *zeb-3721*::Tn*10d*Tc
TT17136	*metE205 ara-9 pdu-12*::MudJ *pocR1*::Tn*10d*Tc
TT17144	*DEL1077*(*metE*) *ara-9 hsiC1264*::MudJ
TT17145	*DEL1717*[(*hsiC1264*)*MudJ*(*pdu-12*)]
TT17146	*DEL1718*[(*hsiC1264*)*MudJ*(*pdu-8*)]
TT17147	*DEL1719*[(*hsiC1264*)*MudJ*(*pdu-17*)]
TT17148	*DEL1720*[(*hsiC1264*)*MudJ*(*pdu-28*)]
TT17149	*metE205 ara-9 DEL1721*[(*pocR12*)*MudA*(*cob-62*)]
TT17150	*metE205 ara-9 DEL1722*[(*pocR13*)*MudA*(*cob-62*)]
TT17151	*metE205 ara-9 DEL1723*[(pocR14)*MudA*(*cob-62*)]
TT17152	*DEL1724*[(*hisD9952*)*MudJ*(*pocR12*)]
TT17153	*DEL1725*[(*hisD9952*)*MudJ*(*pocR13*)]
TT17154	*DEL1726*[(*hisD9952*)*MudJ*(*pocR14*)]
TT17155	*DEL1727*[(*hisD9952*)*MudJ*(*zeb-3722*)]
TT17156	*DEL1077*(metE) *ara-9 DEL1728*[(*zeb3722*)*MudA*(*cob-62*)]
TT17158	*metE205 ara-9 DEL1729*[(*pdu-12*)*MudJ*(*cob-61*)]
TT17159	*metE205 ara-9 DEL1730*[(*pdu-8*)*MudJ*(*cob-61*)]
TT17160	*metE205 ara-9 cob-24*::MudJ *cob-640*::Tn*10d*Tc
TT17161	*metE205 ara-9 cob-640*::Tn*10d*Tc (CobI)
TT17166	*metE205 ara-9 pocR12*::MudA
TT17167	*metE205 ara-9 DUP1736*[(*pocR12*)*MudA*(*hisD9952*)]
TT17168	*metE205 ara-9 DUP1736*[(*pocR12*)*MudA*(*hisD9952 pocR8*::Tn*10d*Tc)]
TT17169	*metE205 ara-9 DUP1736*[(*pdu-207*::Tn*10d*Tc *pocR12*)*MudA*(*hisD9952 pdu-207*::Tn*10d*Tc)]
TT17170	*metE205 ara-9 DUP1736*[(*pocR12*)*MudA*(*hisD9952*)] *zig-3723*::Tn*10 glpK7*
TT17171	*metE205 ara-9 DUP1736*[(*pocR12*)*MudA*(*hisD9952*)] *zig-3723*::Tn*10*
TT17174	*metE205 ara-9 DUP1737*[(*cob-24*)*MudA*(*hisD9952*)] *cob-364*::Tn*10d*Cm
TT17175	*metE205 ara-9 DUP1737*[(*pocR8*::Tn*10d*Tc *cob-24*)*MudA*(*hisD9952*)] *cob-364*::Tn*10d*Cm
TT17176	*metE205 ara-9 DUP1737*[(*cob-24*)*MudA*(*hisD9952 pocR8*::Tn*10d*Tc)] *cob-364*::Tn*10d*Cm
TT17177	*metE205 ara-9 DUP1737*[(*pocR8*::Tn*10d*Tc *cob-24*)*MudA*(*hisD9952 pocR8*::Tn*10d*Tc)] *cob-364*::Tn*10d*Cm
TT17178	*metE205 ara-9 cob-24*::MudJ *zig-3723*::Tn*10*
TT17179	*metE205 ara-9 cob-24*::MudJ *zig-3723*::Tn*10 glpK7*
TT17182	*metE205 ara-9 pdu-12*::MudJ *zig-3723*::Tn*10*
TT17183	*metE205 ara-9 pdu-12*::MudJ *zig-3723*::Tn*10 glpK7*
TT17184	*metE205 ara-9 DUP1738*[(*cob-61*)*MudA*(*pdu-12*)]
TT17185	*metE205 ara-9 DUP1738*[(*pocR1*::Tn*10d*Tc *cob-61*)*MudA*(*pdu-12*)]
TT17186	*metE205 ara-9 DUP1738*[(*cob-61*)*MudA*(*pdu-12 pocR1*::Tn*10d*Tc)]
TT17187	*metE205 ara-9 DUP1738*[(*pocR1*::Tn*10d*Tc *cob-61*)*MudA*(*pdu-12 pocR1*::Tn*10d*Tc)]
TT17194	*metE205 ara-9 cob-66*::MudJ *cob-640*::Tn*10d*Tc
TT17195	*metE205 ara-9 cob-62*::MudJ *cob-640*::Tn*10d*Tc
TT17197	*metE205 ara-9 pdu-12*::MudA

Continued

TABLE 1—*Continued*

tively (15, 34). Several *pdu* insertion mutants were kindly provided by Randall Jeter.

Media. The rich medium used was nutrient broth (0.8%; Difco) supplemented with 85 mM NaCl. The minimal medium was either E medium supplemented with glucose (33) or NCE (E medium lacking citrate) (5) supplemented with one or more of the following compounds: D-glucose, 0.2% for aerobically grown cultures and 0.4% for anaerobically grown cultures; glycerol, 0.2%; Na_2 succinate, 1.0%; Na pyruvate, 0.44%; Na_2 fumarate, 0.32%; and DL-1,2-propanediol, 0.2%. Glucose, fumarate, and succinate were from Sigma Chemical Co. (St. Louis, Mo.) Propanediol and pyruvate were from Aldrich Chemical Co. (Milwaukee, Wis.), and glycerol was from Em Scientific (Cherry Hill, N.J.).

Antibiotics were added to nutrient agar as follows: Na ampicillin, 30 μg/ml; chloramphenicol, 20 μg/ml; kanamycin monosulfate, 50 μg/ml; and tetracycline hydrochloride, 20 μg/ml. All antibiotics were from Sigma Chemical. Amino acids, when required, were added to minimal medium at the concentrations recommended previously (13).

Genetic techniques. Transductional crosses were performed with the high-frequency generalized transducing phage mutant P22 HT105/1 *int-201* (31). Cells (2×10^8) were mixed with phage (10^7 to 10^8) and plated directly on selective medium. When drug-resistant transductants (Tc^r Kn^r Ap^r Cm^r) were selected, the cell culture and phage were incubated together nonselectively in liquid for 15 to 45 min before plating on solid selective medium. Transductants were purified and made phage free by streaking for single colonies on nonselective green indicator plates (9). Transductants were tested for phage sensitivity by cross-streaking with the clear-plaque P22 mutant H5.

Screens and selections. The $CobIII^+$ phenotype was scored (in strains carrying a *metE* mutation) as the ability to grow without methionine on E-glucose medium containing 0.2 μg of cobinamide dicyanide (Sigma) per ml and 50 μg of 5,6-DMB (Aldrich) per ml. This method is based on the fact that *S. typhimurium* has alternative enzymes (encoded by *metE* and *metH*) capable of catalyzing the last step in methionine synthesis. The *metH* enzyme depends on vitamin B_{12}; therefore, mutants lacking *metE* function can grow on minimal medium only if they have vitamin B_{12}. Strains that are phenotypically $CobIII^+$ possess the enzymes needed to produce vitamin B_{12} from cobinamide and DMB; these enzymes are expressed both aerobically and anaerobically.

The Cob^+ phenotype was scored (in strains with a *metE* mutation) as the ability to grow anaerobically on E-glucose medium with or without 5 μM $CoCl_2$ (Mallinckrodt Chemicals, St. Louis, Mo.). The growth conditions necessary for de novo synthesis of vitamin B_{12} were provided by an anaerobic chamber (Forma Scientific model 1024) containing an atmosphere of N_2-CO_2-H_2 (89:5:6).

The *pdu* phenotype was scored on MacConkey indicator plates (Difco, Inc.) containing 1% propanediol and 0.2 μg of vitamin B_{12} per ml. On these plates, Pdu^+ strains form red colonies and Pdu^- colonies are white.

β-Galactosidase assays. The β-galactosidase activity in strains grown under specified conditions was assayed as described by Miller (27). For each table, the data presented are derived from a single experiment; each entry is based on a single assay, but the experiments were repeated several times with essentially the same results as those presented. For aerobic growth of cells, an overnight nutrient broth culture, inoculated from a frozen strain, was prepared. This broth culture (0.1 ml) was used to inoculate 2 ml of the desired minimal medium. Cultures were grown to the log phase, and 0.1 ml was used as the inoculum for a second 2-ml culture with similar minimal medium. When the second culture reached log phase, cells were harvested and assayed for β-galactosidase activity. Cultures were grown with shaking in 2 ml of medium in a test tube (15 by 125 mm). For anaerobic growth of cells, 0.1 ml of an overnight nutrient broth culture was used to inoculate 2 ml of minimal medium. This culture was grown aerobically to log phase, brought inside an anaerobic chamber, and used (0.2 ml) to inoculate 5 ml of similar minimal medium in an anaerobic aluminum crimp sealable culture tube (18 by 150 mm) (Bellco Biotechnology, Vineland, N.J.). This medium was previously made anaerobic by incubation in an anaerobic chamber for at least 10 h for gas exchange. After inoculation, tubes were fitted with black rubber stoppers (Bellco), removed from the anaerobic chamber, and sealed. The headspace gas of the tubes was replaced with N_2 by three cycles of evacuation and pressurization (4). Cells were grown to log phase at 37°C with shaking under 2 atm (202 kPa) of N_2 and then assayed for β-galactosidase activity. If N_2 pressure was lost during incubation, assays were repeated.

When strains with duplications were grown for assay of β-galactosidase, loss of duplications was counterselected by supplementing all media with ampicillin; resistance to this antibiotic is encoded by the transposon located between copies of the duplicated segments. Ampicillin was provided at 30 μg/ml in rich medium, 15 μg/ml in minimal medium when cultures were incubated aerobically, and 2 μg/ml in minimal medium when cultures were incubated anaerobically. Cultures of strains with duplications were routinely tested for the presence of haploid segregants by plating for single colonies and replica printing to score the loss of the Ap^r marker between copies of the duplicated segment; reported β-galactosidase activities were obtained from cultures with <5% Ap^s segregants.

Transposon mutagenesis of the *cob/pdu* region. For Tn*10d*Tc mutagenesis, a pool of approximately 100,000 independent Tn*10d*Tc insertion mutants was prepared by transducing a Tn*10d*Tc insertion from an *Escherichia coli* F′ *lac* strain into a recipient strain with a plasmid-encoded Tn*10* transposase (34). The pool of Tc^r transductant clones was used as a donor in transductional crosses with strain TT17073, which contained a CobIII::MudJ insertion. Transductants ($CobIII^+$) were selected and screened for Tc^r recombinants that had inherited a Tn*10d*Tc linked to the CobIII region.

To obtain MudJ insertion mutations in the *cob/pdu* region, a pool of about 100,000 independent MudJ insertions was prepared by the *cis* complementation method of Hughes and Roth (22) and was used as donor in transductional crosses with a recipient strain which contained a CobIII::Tn*10* insertion (TT9814). $CobIII^+$ transductants were selected and scored for coinheritance of Kn^r, encoded by MudJ. The resulting $CobIII^+$ clones were screened for their Pdu phenotype and their ability to synthesize vitamin B_{12} de novo under anaerobic conditions.

Orienting *pocR*::MudJ insertions. The orientation of *pocR*::MudJ(Kn) insertions was determined by transductional crosses that scored recombination between a *pocR*::MudJ(Kn) element and one of two donor CobII::MudA(Ap) insertions of known orientation, *cob-61* (clockwise, TT10364) and *cob-62* (counterclockwise, TT10365). Recombinants inheriting the donor Ap^r were scored for loss of the recipient Kn^r and for acquisition of the $CobIII^-$ phenotype expected for a deletion extending from the *pocR* gene to the CobII region. The orientation of the *pocR*::MudJ element was determined by which of the two donor *cob* insertions could recombine with the recipient *pocR*::MudJ element to generate a deletion (20).

Construction of deletions. The same method used for determining the orientation of Mud elements (see above) was used to construct a set of deletions each extending rightward from an insertion in the *his* operon to the sites of similarly oriented MudJ insertions in *pdu*, *pocR*, or the silent *zeb* region (20). Similarly, we constructed deletions extending leftward from the CobII region to the same MudJ insertions in *pocR* and the *zeb* region. These Mud-directed deletions were used in constructing the deletion map.

Deletions from *his* to *pocR* or to the *zeb* region were constructed by transducing the recipient *hisD9952*::MudA strain (TT7691) with phage grown on strains carrying a *pocR* or *zeb*::MudJ insertion mutation. Deletions from *his* to *pdu* were constructed by transducing recipients carrying a *pdu*::MudA(Ap) insertion with phage grown on an insertion mutant *hsiC1264*::MudJ(Kn) strain (TT17144). Deletions from *pdu* to CobII were constructed by transducing the recipient *cob-61*::MudA strain (TT10364) with phage grown on strains carrying a *pdu*::MudJ insertion. In all of these constructions, donor strains carried a MudJ(Kn) element and the recipients carried a MudA(Ap) element. Transductants (Kn^r) were selected and screened for those that had lost Ap^r and acquired the directed deletion.

Deletions from the *pocR* or *zeb* region to CobII were constructed by transducing recipients containing a *pocR* or *zeb*::MudJ(Kn) insertion mutation with phage grown on a *cob-62*::MudA(Ap) insertion mutant (TT10365). In these crosses, Ap^r recombinants were selected and screened for the loss of the recipient Kn^r phenotype and acquisition of the CobIII phenotype characteristic of the constructed deletion.

Deletion map of the control region. All mapping involved transductional crosses between recipient strains carrying one of the deletions described above (with either a Kn^r or an Ap^r phenotype) and donor strains carrying a simple insertion of Tn*10d*Tc (with a Tc^r phenotype) or Tn*10d*Cm (with a Cm^r phenotype). Transductants resistant to tetracycline (or chloramphenicol) were selected and replica printed to medium with tetracycline (or chloramphenicol) and either ampicillin or kanamycin, depending on whether the deletion recipient had a MudA or a MudJ at the join point. (We use "join point" to designate the site or region between the two copies of the duplicated segment.) Formation of transductants showing both Tc^r (or Cm^r) and either Ap^r or Kn^r phenotypes indicated recombination between the recipient deletion and the donor insertion. A lack (or paucity) of such recombinants indicated that the donor insertion mapped inside the recipient deletion. Crosses were scored as positive for recombination when greater than 5% of the Tc^r transductants also showed the recipient drug resistance. Crosses

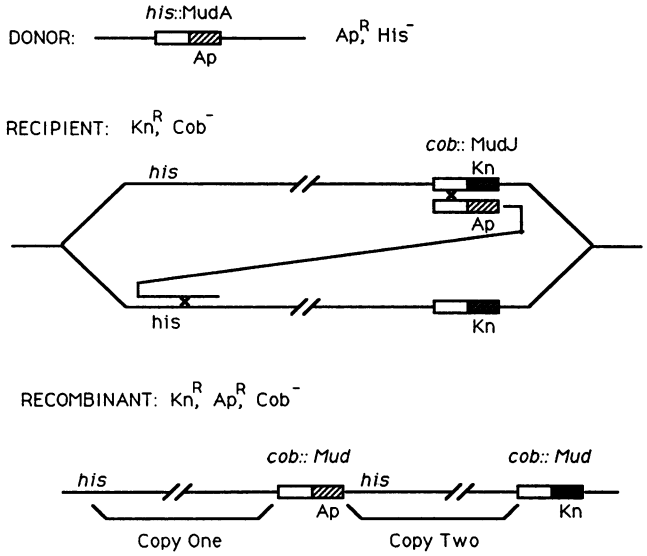

FIG. 1. Construction of duplications. Duplications are constructed by transduction. Selection is made for inheritance of the Ap^r marker within the donor *his*::MudA element, and recombinants that retain a *his*$^+$ phenotype are identified. These arise by the diagrammed exchanges, which include one exchange between the sequences of the donor and recipient Mud elements; the other exchange is between *his* material near the donor insertion and the *his* region of the recipient. The Ap^r recombinants that remain His^+ carry a duplication extending from the site of the donor *his* insertion to the site of the recipient *cob* insertion. The *his* and *cob* regions are not cotransduced by P22 (25).

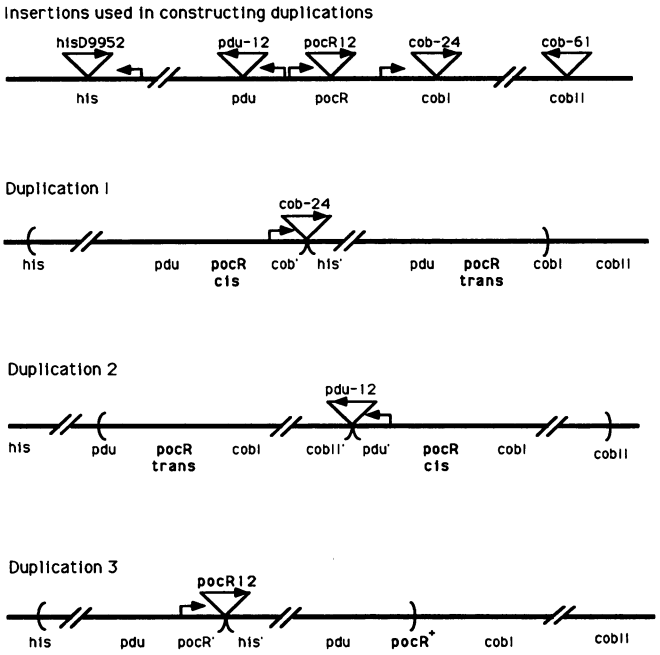

FIG. 2. Duplications used in complementation tests. Merodiploids were constructed by recombination between the inserted elements diagrammed in the top section. The structures of the three duplication types are diagrammed. Various mutant alleles were transduced into these strains to construct the strains described in Tables 5 and 6.

were scored as negative if fewer than 1% of the Tc^r transductants showed the recipient drug resistance. No pair of strains showed intermediate values. The rationale for this mapping method is described in the Appendix.

Construction of tandem chromosomal duplications. A modification of the method of Hughes and Roth (20) was used to construct duplications shown in Fig. 2 (see below).

Duplications with the basic structure of duplication 1 were constructed in several steps by P22 transduction. A *hisD 9952*::MudA(Ap) insertion (TT7691) was crossed into a strain with a *cob-24*::MudJ(Kn) element (TT10852), selecting Ap^r (Fig. 1). Sequences near the donor insertion can recombine with the chromosome at one site (the *his* operon), and sequences within the donor insertion can recombine with the chromosome at the distant site of the recipient Mud insertion within the *cob* operon. Such events can construct a duplication. The phenotype of 8 of 30 Ap^r transductants was Ap^r Kn^r His^+, indicative of a duplication from the *hisD9952* site to the *cob-24* site with a MudA(Ap) element at the join point and a MudJ(Kn) element at the *cob-24* outside end of the duplication (Fig. 1). This duplication was separated from the outside MudJ(Kn) insertion by transduction of the join point into a new recipient (TR6583), selecting Ap^r. The phenotype of six of six transductants was Ap^r Kn^s His^+, indicating that these strains inherited the *his-cob* duplication with the MudA(Ap) at the join point but lacked the outside MudJ(Kn) element. This duplication had the structure of duplication 1 with *pocR*$^+$ alleles at both *pocR* loci (Fig. 2).

To construct duplication 1 with various combinations of *pocR* alleles, a strain with this duplication was used as recipient in a cross with donor strain *pocR8*::Tn*10d*Tc (TT17085), selecting Tc^r Ap^r transductants. All transductants tested (30 of 30) had the phenotype expected of a *his-cob* duplication with an added *pocR*::Tn*10d*Tc insertion (Tc^r Ap^r His^+ Pdu^+). To determine whether the Tn*10d*Tc element was located *cis* or *trans* to the MudA element at the duplication join point, some of these strains were used as transductional donors in crosses with strain TR6583. Inheritance of the marker (Ap^r) at the duplication join point was selected, and approximately 200 transductants were scored for cotransduction of Tc^r. In about 1 of 15 of the strains with a single *pocR*::Tn*10d*Tc element, the Tn*10* element showed about 70% linkage to the MudA element at the duplication join point; in about 14 of 15 of the strains with a duplication, the Tn*10d*Tc element was unlinked to the MudA element at the join point. We inferred that the linked and unlinked Tn*10d*Tc elements were located *cis* and *trans* to the MudA element, respectively.

To construct a duplication with a *pocR8*::Tn*10d*Tc insertion at both *pocR* loci, the transductional cross described above was repeated at a high multiplicity of infection (approximately 5 to 10) and transduction mixtures were plated on MacConkey indicator plates with propanediol, vitamin B_{12}, tetracycline, and ampicillin. The Tc^r transductants could thus be directly screened for their Pdu phenotypes. About 1 of 500 transductants resulting from this procedure was Ap^r Tc^r Pdu^-. To verify that these transductants contained the recipient *his-cob* duplication and a Tn*10d*Tc element at both *pocR* loci, strains were grown nonselectively overnight in nutrient broth. Dilutions of these cultures were plated on nutrient agar, and colonies that developed were printed to rich medium with and without ampicillin or tetracycline. As expected, the MudA element (Ap^r) marker was unstable and the Tc^r phenotype was stable.

In order to block vitamin B_{12} synthesis and prevent

TABLE 2. Induction of the *cob* and *pdu* operons by propanediol

Strain	Genotype	β-Galactosidase level during growth under indicated conditions[a]									
		Aerobic						Anaerobic			
		Glucose		Glycerol		Succinate		Glucose		Pyruvate-fumarate	
		None	PD	None	PD	None	PD	None	PD	None	PD
TT10852	*cob::lac*	3	4	4	7	31	510	2	22	63	590
TT17131	*pdu::lac*	1	3	1	3	<1	1,700	<1	23	1	2,000

[a] Cells were grown at 37°C in NCE medium with methionine and the indicated sources of carbon and energy. None, no addition to the growth medium; PD, propanediol was added.

repression of the *cob* operon by endogenous cobalamin (17), insertion *cob-364*::Tn*10d*Cm was introduced into the strains with the duplications described above. This insertion maps outside the duplicated *his-cob* region and thus prevents vitamin B_{12} synthesis. It was introduced by selecting for resistance to chloramphenicol.

Duplication 2 (Fig. 2) was constructed by a procedure analogous to that for construction of duplication 1. The initial duplication was made by transducing a *cob-61*::MudA(Ap) insertion (TT10364) into a strain (TT17131) with insertion *pdu-12*::MudJ(Kn). The phenotype of 9 of 16 transductants was that expected for a strain with a *cob-pdu* duplication generated by recombination between the Mud elements (Ap^r Kn^r $CobII^+$). This duplication was crossed into TR6583 to eliminate the MudJ element located at the end point of the duplication. A strain carrying duplication 2 was identified as a transductant having the phenotype Ap^r Kn^s Pdu^+. A *pocR1*::Tn*10d*Tc element was then transduced into the *cis* copy, the *trans* copy, or both copies of the *pocR* gene present in duplication 2. The *cis* and *trans* Tn*10d*Tc elements showed 75 and 4% linkage, respectively, to the MudA(Ap) element at the join point of the duplication.

Duplication 3 was constructed by transducing the *hisD 9952*::MudA element from strain TT7691 into a recipient carrying insertion *pocR12*::MudJ (TT17104), selecting Ap^r. Among the transductants, 16 of 30 showed the Ap^r Kn^r His^+ Pdu^- phenotype indicative of a *his-pocR* duplication with a *pocR12*::MudJ insertion at the outside end of the duplicated segment. The join point of this duplication was crossed into strain TR6583. Duplication 3 was carried by an Ap^r Kn^s His^+ Pdu^+ transductant obtained from this cross. Three derivatives of this parental duplication-carrying strain were constructed; each received one of the following mutations. The *pocR8*::Tn*10d*Tc and *pdu-207*::Tn*10d*Tc elements were each introduced by selecting Tc^r Ap^r recombinants. The mutation *glpK7* was introduced into the parent strain by using a linked Tn*10* element as a selective marker (Tc^r).

RESULTS

Coinduction of the *cob* and *pdu* operons by propanediol. Transcription of both operons was induced by propanediol during either aerobic or anaerobic growth of cells on poor carbon sources (Table 2). Induction was determined by measuring the β-galactosidase activity in strains with *cob-lac* and *pdu-lac* operon fusions formed by Mud(Lac) insertions. Neither operon was induced during aerobic growth on glucose or glycerol. During fermentation of glucose, propanediol induced both operons but the maximum level was low. The highest induced level was observed under conditions of anaerobic respiration of pyruvate-fumarate. The inductive effect of propanediol did not require propanediol metabolism; strains with mutations causing a propanediol dehydratase deficiency showed normal induction by propanediol (data not shown).

The aerobic inductive effect of propanediol on the *pdu* operon has been reported previously (23). Previous work suggested that the *cob* operon was not expressed aerobically (3); the induction of the *cob* operon by propanediol under aerobic conditions was surprising. The parallel behavior of the two operons in these experiments and those described below suggest that the *cob* and *pdu* operons constitute a single regulon with a single control mechanism.

Coinduction of the *cob* and *pdu* operons by glycerol. Transcription of both the *cob* and *pdu* operons was induced by glycerol as well as propanediol (Table 3). The aerobic induction by glycerol was observed only in strains unable to metabolize glycerol.

In a *glpK* mutant, which lacks glycerol kinase, glycerol induced both the *cob* and *pdu* operons during aerobic growth on succinate and during anaerobic respiration of pyruvate-fumarate. Since glycerol kinase is required for both aerobic and anaerobic metabolism of glycerol, these results indicated that unmetabolized glycerol stimulated aerobic and anaerobic transcription of both the *cob* and *pdu* operons.

In *glpK*$^+$ strains, aerobic induction of the *cob* and *pdu* operons by glycerol did not occur. This indicated that aerobic glycerol metabolism blocked induction. However, glycerol metabolism did not prevent induction of either operon under anaerobic conditions. Induction was maximal during anaerobic respiration of glycerol-fumarate. Under these conditions, addition of propanediol caused no further induction (data not shown).

Previously it had been seen that the *cob* operon is maximally induced during anaerobic growth on glycerol-fumarate (17), but this was attributed to metabolic consequences of glycerol respiration (3). The finding that glycerol per se induces this regulon is surprising.

Insertion mutagenesis of the *cob/pdu* region. The region between the divergently transcribed *cob* and *pdu* operons was mutagenized with Tn*10d*Tc and MudJ elements (see Materials and Methods). Four classes of mutants were obtained. One class showed a simple Cob^- phenotype, and another showed a simple Pdu^- phenotype. These mutations are presumed to affect the two operons individually. A third class of mutants (*zeb*) had a normal growth phenotype (Cob^+ Pdu^+) but showed slight alterations in expression of the *cob* and *pdu* operons (see below). The fourth class of insertions (*pocR*) showed a pleiotropic Cob^- Pdu^- phenotype. Both the Cob^+ Pdu^+ (*zeb*) and the Cob^- Pdu^- (*pocR*) mutations mapped between the *cob* and *pdu* operons (see below).

A likely reason for the Cob^- Pdu^- phenotype of *pocR* mutants was that they failed to induce both the *cob* and *pdu* operons. Qualitative tests employing MacConkey agar-lac-

TABLE 3. Induction of the *cob* and *pdu* operons by glycerol

Strain	Relevant genotype	β-Galactosidase activity produced during growth under indicated conditions[a]				
		Aerobic, succinate		Anaerobic		
				Pyruvate-fumarate		Glycerol-fumarate (none)
		None	Glycerol	None	Glycerol	
TT10852	*cob*::*lac*	29	14	44	400	1,000
TT17131	*pdu*::*lac*	1	1	1	1,400	2,400
TT17178	*cob*::*lac glpK*$^+$	29	12	47	510	1,100
TT17179	*cob*::*lac glpK* mutant	26	330	36	690	NG[b]
TT17182	*pdu*::*lac glpK*$^+$	1	1	<1	1,600	2,100
TT17183	*pdu*::*lac glpK* mutant	1	33	<1	1,300	NG

[a] All strains were grown in NCE medium with methionine and the indicated sources of carbon and energy. None, no addition to the growth medium; glycerol, an addition to the medium.
[b] NG, no growth.

tose indicator plates suggested that all 11 of the *pocR*::Tn*10d*Tc insertion mutants we isolated were unable to induce the *cob* and *pdu* operons. Four different *pocR*::Tn*10d*Tc mutants were assayed to quantitate the effect of the insertion on expression of the *cob* and *pdu* operons. The results for a representative *pocR*::Tn*10d*Tc insertion are presented in Table 4. During aerobic growth on succinate minimal medium or during anaerobic respiration, the *pocR* mutant showed no induction of either operon by propanediol or glycerol (Table 4, lines 2 and 6). The failure of glycerol to induce the *cob* and *pdu* operons in a *pocR* mutant was also seen in strains with glycerol metabolism blocked by a *glpK* mutation (data not shown).

It should be noted that under anaerobic growth conditions, all *pocR* insertion mutants showed a leaky Cob$^-$ phenotype that was corrected by the addition of $CoCl_2$ to the growth medium. This phenotype is probably due to the fact that *pocR* insertion mutations reduce *cob* operon expression to a level at which Co^{2+} transport, thought to be encoded by the *cbiO* gene within the *cob* operon (30), limits vitamin B_{12} synthesis on medium with no deliberately added cobalt. The *pocR* gene is not directly involved in Co^{2+} transport, since *pocR* mutants have a Cob$^+$ phenotype if one provides for expression of the *cob* operon (1, 2).

The Cob$^+$ Pdu$^+$ class of insertions will be referred to as *zeb*, by the convention for naming insertions by their chromosomal map position (11). The *cob-lac* and *pdu-lac* fusion strains with a *zeb-3721*::Tn*10d*Tc insertion showed about twofold less induction of the *cob/pdu* regulon by propanediol during aerobic growth on succinate (Table 4, lines 3 and 7). The same *zeb* insertion had little or no effect on induction during anaerobic growth on glycerol-fumarate medium. Similar results were obtained with three other *zeb*::Tn*10d*Tc insertions (data not shown).

It is conceivable that *pocR* mutants might fail to express one operon as a secondary consequence of their failure to express the other. To test this, four simple *pdu*::Tn*10d*Tc insertions were tested; none affected regulation of a *cob-lac* fusion. The results obtained with a representative insertion are shown in Table 4 (strain TT17123). Conversely, deletion mutations eliminating the entire CobI region retain a Pdu$^+$ phenotype. Since neither operon is required for expression of the other, we conclude that the Cob$^-$ Pdu$^-$ phenotype of *pocR* mutations is due to impaired expression of both the *cob* and *pdu* operons.

Genetic map of the region between the *pdu* and *cob* operons. By deletion mapping, the following map order was determined: *pdu*, *zeb*, *pocR*, and *cob*. For each Mud(*lac*) insertion mutation type (*pdu*, *zeb*, and *pocR*), two deletions were constructed, one extending leftward to the *his* operon and the other extending rightward to the CobII region (see Fig. 3). Deletion mutants were used as recipients in crosses with donor Tn*10d*Tc (or Tn*10d*Cm) insertion mutations in each of the critical regions. The importance of the deletion pairs is

TABLE 4. Effect of *pocR* and *zeb* mutations on expression of *cob* and *pdu* operons

Strain	Relevant genotype	β-Galactosidase activity activity produced during growth under indicated conditions[a]				
		Aerobic, succinate		Anaerobic		
				Pyruvate-fumarate		Glycerol-fumarate
		None	PD	None	PD	
TT10852	*cob*::*lac*	28	500	59	430	550
TT17119	*cob*::*lac pocR*::Tn*10*	14	14	15	12	30
TT17130	*cob*::*lac zeb*::Tn*10*	26	210	41	250	390
TT17123	*cob*::*lac pdu*::Tn*10*	35	480	55	440	770
TT17131	*pdu*::*lac*	1	1,700	2	970	2,100
TT17136	*pdu*::*lac pocR*::Tn*10*	1	1	<1	<1	<1
TT17135	*pdu*::*lac zeb*::Tn*10*	1	1,000	5	1,100	2,200

[a] Cells were grown at 37°C in NCE medium with methionine and the indicated sources of carbon and energy. None, no addition to the growth medium; PD, propanediol was added.

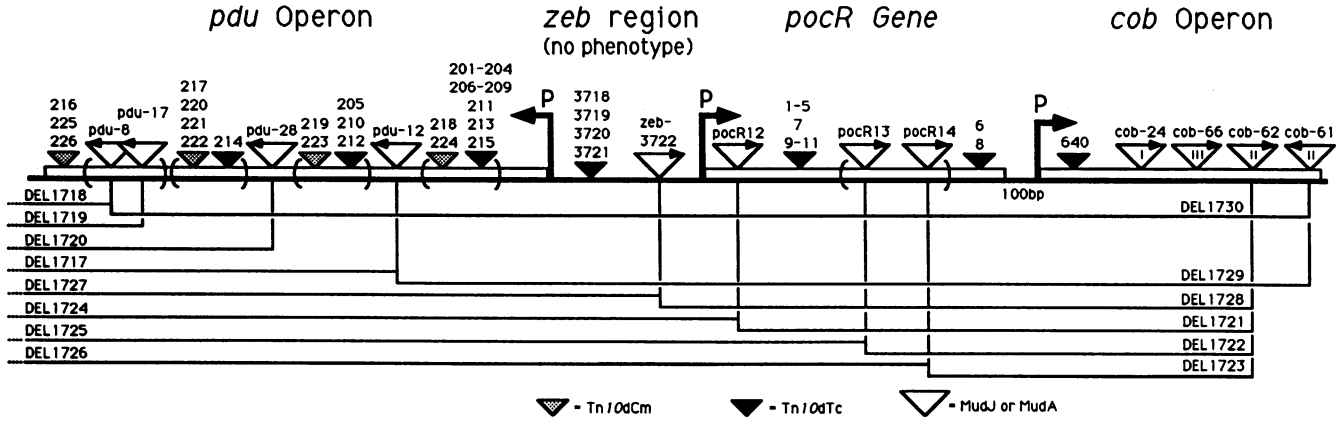

FIG. 3. Genetic map of the control region between the *pdu* and *cob* operons. Allele numbers and the extent of deletion mutations used in mapping are indicated below the chromosome line. Triangles represent insertion mutations used in constructing deletions and in identifying the several regions of the map. Arrows labeled P designate promoters inferred from these studies. Arrows on the open triangles (Mud elements) indicate the direction of transcription of the *lac* operon included in that inserted element. Numbers with each triangle are the allele numbers of individual insertion mutations. Parentheses enclose insertions whose order within the designated interval is uncertain.

explained in the Appendix. Doubly mutant recombinants that carry both the Tc^r (or Cm^r) phenotype of the donor insertion and the Kn^r or Ap^r phenotype of the recipient deletion were selected. This novel mapping method (described in more detail in the Appendix) was required because there was no phenotype to permit selection for wild-type recombinants.

The resulting genetic map is in Fig. 3. Each of the four phenotypic classes of Tn*10d*Tc insertions mapped as a contiguous group, demonstrating a physical clustering of mutations that confer the same phenotype. Since all of the *zeb* and *pocR* insertions mapped outside the longest of the deletions extending from *his* to *pdu*, these regions were placed to the right of the *pdu* operon, as shown in Fig. 3. The *zeb* insertions mapped outside the deletions from *pocR* to CobII; *pocR* insertions mapped inside these deletions. Therefore, the *pocR* region lies to the right of the *zeb* region and immediately to the left of the *cob* operon.

Using deletions extending from several *pocR* insertions to CobII or to *his*, we found that the *pocR*::Tn*10d*Tc insertions mapped in two distinct deletion intervals within the *pocR* gene. The placement of these insertions within the *pocR* gene was confirmed by studies of polarity effects of the Tn*10d*Tc insertions on expression of the *pocR*::*lac* fusions. That is, each *pocR*::Tn*10d*Tc insertion reduces expression of *pocR*::*lac* fusions mapping to its right (Fig. 3). All four *zeb*::Tn*10d*Tc insertions mapped into a single deletion interval. The region between the *pdu* and *cob* operons thus includes the *zeb* region and the *pocR* gene in that order; Tn*10d*Tc and MudJ insertions map at several points in the *pocR* gene (Fig. 3). The fact that *pocR*::Tn*10* insertions block transcription of Mud(*lac*) fusions mapping to their right in the *pocR* gene suggests that the *pocR* gene is transcribed from left to right as the map appears in Fig. 3. This conclusion was confirmed by orienting the *pocR*::Mud insertions (see Materials and Methods); it was found that in these insertions the *lacZ* gene is oriented such that expression requires transcription from left to right in the map in Fig. 3.

Orienting transcription of the *pocR* gene. The direction of transcription of the *cob* operon was previously reported to be counterclockwise and that of the *pdu* operon was reported to be clockwise (23, 25). Here we report that the direction of transcription of the *pocR* gene, like the *cob* operon, is counterclockwise, or left to right as the map is drawn in Fig. 3.

In transductional crosses, transposons at separated chromosomal sites can recombine to construct a deletion if the two elements have the same orientation in the chromosome (12, 20). Crosses (described in Materials and Methods) demonstrated that the *cob-62*::MudA element (transcribed counterclockwise) recombined with the *pocR12*::MudJ element to form a deletion; 28 of 30 transductants inheriting the donor MudA(Ap) element acquired a deletion of the region between the *pocR* and CobII Mud elements. The formation of deletions indicated that these transposons had the same orientation in the chromosome. Since the *lacZ* genes of both insertions are expressed, both target genes must be transcribed in the same direction. The CobII region is known to be transcribed counterclockwise (25). Thus, the *pocR* gene must also be transcribed counterclockwise (left to right in Fig. 3). Counterclockwise transcription of the *pocR* gene was supported by orientation of two additional Lac^+ *pocR*::MudJ insertions; both proved to be in the same orientation as *pocR12*::MudJ.

Orientation of all three *pocR*::MudJ elements was confirmed by individually crossing strains containing these elements with a strain carrying insertion *cob-61*::MudA (Lac^-), oriented clockwise. In each cross, no transductants (0 of 30) inheriting the donor MudA had the phenotype predicted for a deletion formed by recombination between the Mud elements.

Mutations in the *pocR* region can be complemented in *trans*. Tandem chromosomal duplications were used to perform complementation tests demonstrating that the regulatory phenotypes of a *pocR* mutation could be corrected by a wild-type *pocR* gene provided in *trans*. Duplication 1 (Fig. 2) was used to test regulation of the *cob* operon. It has a *cob*::*lac* fusion at the join point (the point or region between the two copies of the duplicated material). The duplication provides two *pocR* genes, one located immediately *cis* to the *cob*::*lac* fusion and the other located far from the *cob*::*lac* fusion, essentially in *trans*. For the complementation tests, *pocR*::Tn*10d*Tc alleles were introduced into strains carrying a *pocR* duplication to construct one derivative that had its sole functional *pocR* allele *cis* to the *cob*::*lac* fusion and a

TABLE 5. Complementation tests of *pocR* mutations

Strain	Duplication type (Fig. 1)	Relevant genotype			β-Galactosidase activity produced during growth under indicated conditions[a]			
		Operon fusion	PocR allele at:		Aerobic		Anaerobic	
			cis	*trans*	Succinate	Succinate + propanediol	Pyruvate-fumarate	Glycerol-fumarate
TT10327	Haploid	*cob*::*lac*	*pocR*$^+$	None	27	450	62	1,050
TT17174	Dupl. 1	*cob*::*lac*	*pocR*$^+$	*pocR*$^+$	46	380	91	710
TT17176	Dupl. 1	*cob*::*lac*	*pocR*$^+$	*pocR*::Tn*10*	41	380	62	710
TT17175	Dupl. 1	*cob*::*lac*	*pocR*::Tn*10*	*pocR*$^+$	37	310	67	650
TT17177	Dupl. 1	*cob*::*lac*	*pocR*::Tn*10*	*pocR*::Tn*10*	20	20	59	72
TT17197	Haploid	*pdu*::*lac*	*pocR*$^+$	None	<1	1,750	<1	2,300
TT17184	Dupl. 2	*pdu*::*lac*	*pocR*$^+$	*pocR*$^+$	<1	1,000	<1	1,150
TT17185	Dupl. 2	*pdu*::*lac*	*pocR*$^+$	*pocR*::Tn*10*	<1	1,100	<1	700
TT17186	Dupl. 2	*pdu*::*lac*	*pocR*::Tn*10*	*pocR*$^+$	<1	1,200	<1	790
TT17187	Dupl. 2	*pdu*::*lac*	*pocR*::Tn*10*	*pocR*::Tn*10*	<1	<1	<1	<1

[a] Cells were grown at 37°C in NCE medium with methionine and the indicated sources of carbon and energy.

second that had the functional *pocR* allele *trans* to the fusion. A third derivative that had two *pocR*::Tn*10d*Tc alleles was constructed. Each duplication-carrying strain contained the insertion *cob-364*::Tn*10d*Cm to prevent endogenous synthesis of vitamin B_{12}, which could have repressed the *cob* operon (17).

Regulation of *cob* operon expression in these strains is shown in Table 5, lines 2 to 5. The regulatory behavior of a haploid strain (TT10327) is also shown by the diploid strains that have at least one *pocR*$^+$ allele, regardless of whether the functional *pocR* allele is located *cis* or *trans* to the assayed *cob-lac* fusion. The strain with no functional *pocR* allele showed little or no induction (Table 5, TT17177), similar to the behavior of a haploid strain with a *pocR* mutation (Table 4, TT17119).

Duplication 2 (in Fig. 2) was used to test the effects of *pocR* on expression of the *pdu* operon. This duplication has a *pdu-lac* fusion at its join point and includes two copies of the *pocR* gene, one copy immediately *cis* to the join point *pdu-lac* fusion being assayed and the other effectively *trans* to the fusion. Strains were constructed with this duplication and a *pocR*::Tn*10* insertion in *cis* or in *trans* to the fusion; a third derivative had *pocR*::Tn*10* at both *pocR* loci. Since the *pdu* operon is not subject to repression by vitamin B_{12} (1), no *cob* insertion was added to these strains.

Regulation of the *pdu* operon in these strains is presented in Table 5, lines 6 to 10. During aerobic growth on succinate plus propanediol and during anaerobic growth on glycerol-fumarate or pyruvate-fumarate plus propanediol, the *pdu* operon showed normal induction in all strains having at least one *pocR*$^+$ allele, regardless of the location of the functional copy with respect to the *pdu*::*lac* fusion. No induction was seen in strains lacking a functional *pocR* allele.

Throughout Table 5, it should be noted that all strains with a duplication of the control region showed a maximal induced level of the *cob/pdu* regulon that is 30 to 85% of that seen in a haploid strain; this is even true for strains with two wild-type PocR$^+$ alleles. This applies to both the *cob* and *pdu* operons, to aerobic and anaerobic conditions, and to strains with either duplication 1 or duplication 2. The basis of this effect is not yet understood.

The above data also demonstrate that there is little read-through transcription from the *pocR* gene into the *cob* operon. The induced level of the *cob* operon is essentially the same regardless of whether PocR$^+$ function is provided by a *pocR* allele located in *cis* or in *trans* to the assayed operon. The presence of a Tn*10* insertion in the proximal *pocR* gene does not reduce *cob* operon expression. Since Tn*10* insertions are strongly polar (26), this is evidence that transcription from the *pocR* gene promoter (which is considerable, as seen below) does not contribute to expression of the adjacent *cob* operon under the conditions tested here.

Regulation of *pocR*. Transcription of the *pocR* gene was examined in a haploid strain containing a *pocR-lac* fusion and in strains containing a duplication with a *pocR-lac* fusion in one copy of the *pocR* locus and either a *pocR*$^+$ or a *pocR8*::Tn*10d*Tc allele at the second *pocR* locus (Table 6). The structure of the strains used is shown in Fig. 2 (duplication 3).

Slight induction of *pocR* transcription occurred during aerobic growth on poor carbon sources (Table 6, aerobic conditions). Transcription of the *pocR* gene increased threefold during aerobic growth on succinate compared with that seen during aerobic growth on glucose. This effect did not require propanediol, glycerol, or a PocR$^+$ allele.

Anaerobiosis also increased *pocR* transcription (Table 6, strain TT17166). Expression of a *pocR-lac* fusion was 3-fold higher during fermentation of glucose than during aerobic growth on glucose, and maximal induction occurred during anaerobic respiration of glycerol-fumarate (17-fold higher than during aerobic growth on glucose). Anaerobic induction did not depend on a PocR$^+$ allele (Table 6, strains TT17166 and TT17168).

Transcription of the *pocR* gene shows autoinduction (about threefold) by propanediol during aerobic growth on succinate and during anaerobic growth on pyruvate-fumarate (Table 6). This is termed autoinduction because it requires a *pocR*$^+$ allele (provided by a genetic duplication which contained both the *pocR-lac* fusion and a *pocR*$^+$ allele). No propanediol induction was seen in a haploid *pocR*::*lac* fusion strain (Table 6, strain TT17166) or in a strain carrying a duplication with a Tn*10* insertion in the second *pocR* allele (Table 6, strain TT17168).

Propanediol metabolism was not required for autoinduction of the *pocR* gene. A strain with a *pdu*::Tn*10* insertion in both copies of the duplicated material lacked the ability to metabolize propanediol but still showed propanediol induction of *pocR* gene transcription (Table 6, strain TT17169).

TABLE 6. Global regulation of *pocR* and autoinduction by propanediol

Strain	Relevant genotypes[a]	β-Galactosidase activity produced during growth under indicated conditions[b]														
		Aerobic								Anaerobic						
		Glucose			Glycerol		Succinate			Glucose			Pyruvate-fumarate			Glycerol-fumarate
		None	PD	Glycerol	None	PD	None	PD	Glycerol	None	PD	Glycerol	None	PD	Glycerol	
TT17166	*pocR*::*lac*	65	50	52	97	100	200	220	160	200	190	210	380	390	370	1,120
TT17167	(*pocR*::*lac*) (*pocR*$^+$)	51	56	62	110	120	225	690	190	190	270	210	380	1,080	320	1,280
TT17168	(*pocR*::*lac*) (*pocR* mutant)	53	55	56	110	120	205	220	150	250	220	220	400	380	330	1,100
TT17169	(*pocR*::*lac*) (*pocR*$^+$) *pdu* mutant	57	53	50	100	130	215	1,000	210	250	220	220	370	1,250	330	1,490
TT17170	(*pocR*::*lac*) (*pocR*$^+$) *glpK* mutant	95	98	100	NG[c]	NG	270	880	310	350	480	360	560	1,200	540	NG
TT17171	(*pocR*::*lac*) (*pocR*$^+$) *glpK*$^+$	92	100	88	160	170	270	880	200	340	440	350	500	1,090	410	1,250

[a] All diploid strains listed carry duplication 3, diagrammed in Fig. 1.
[b] Cells were grown at 37°C in NCE medium with methionine and the indicated sources of carbon and energy. None, no addition to the growth medium; PD and glycerol, propanediol or glycerol, respectively, was added to the medium.
[c] NG, no growth.

In contrast to propanediol, glycerol did not mediate induction of *pocR* gene transcription even in a strain which provided a *pocR*$^+$ allele (Table 6). Similar results were obtained with a *glpK* derivative of the *pocR*$^+$ strain. This indicated that the failure of glycerol to induce *pocR* transcription was not due to aerobic metabolism of glycerol (which prevented aerobic induction of the *cob*::*lac* or *pdu*::*lac* fusion as described above). Although glycerol did not mediate aerobic autoinduction of *pocR* transcription, anaerobic expression of a *pocR-lac* fusion was maximal during growth on glycerol-fumarate, suggesting that anaerobic metabolism of glycerol resulted in strong induction of *pocR* gene transcription. This effect is independent of the PocR function and may be mediated by a global redox control system as suggested previously (3).

Induction of CobIII-*lacZ* and CobII-*lacZ* fusions by propanediol and glycerol. The regulatory effects described above have been observed with a *lac* operon transcribed by the promoter for the CobI region of the *cob* gene cluster. The same effects apply to *lac* fusions to more distal sites within the CobIII and CobII regions of the gene cluster (Table 7). These results suggest that the CobI, CobIII, and CobII genes constitute a single operon regulated as described above.

Transcription of the CobIII and CobII regions was induced by the same growth conditions that induced expression of a CobI fusion, that is, during aerobic growth on succinate plus propanediol and during anaerobic growth on glycerol-fumarate or on pyruvate-fumarate plus propanediol. Note, however, that the uninduced transcription levels of CobIII and CobII fusions are higher than that of the CobI fusion and that the magnitude of the inductive effects is smaller (Table 7, strains TT10857 and TT10858). These results are attributed to weak promoters for the CobIII and CobII genes located within the operon and to a terminator that stops a significant fraction of transcripts originating at the regulated operon promoter at the far left end of the cluster.

To determine whether the internal promoters were regulated by propanediol, we constructed strains containing a CobI, a CobIII, or a CobII MudJ insertion and an upstream CobI::Tn*10d*Tc insertion to block transcription from the left (as seen in Fig. 3). The Tn*10* insertion completely blocks transcription, since it prevents expression of a CobI::*lac* fusion located immediately downstream of the Tn*10* insertion site (Table 7, strain TT17160). Any expression of the distal CobIII and CobII genes in strains with this Tn*10* insertion must be due to the internal promoters.

The *lac* operon fusions in the CobIII and CobII regions are weakly expressed in strains with the CobI::Tn*10d*Tc insertion, but the residual expression is not regulated (Table 7,

TABLE 7. Induction of CobII and CobIII::*lacZ* fusions by propanediol and glycerol

Strain	Relevant genotype	β-Galactosidase activity produced during growth under indicated conditions[a]						
		Aerobic, succinate			Anaerobic, pyruvate-fumarate			Anaerobic, glycerol-fumarate
		None	PD	Induction fold	None	PD	Induction fold	
TT10852	*cob*::*lac* (CobI)	29	650	22	54	460	9	910
TT17160	*cob*::Tn*10* (I) *cob*::*lac* (CobI)	<1	<1	1	<1	<1	1	<1
TT10857	*cob*::*lac* (CobII)	44	280	6	85	510	6	590
TT17195	*cob*::Tn*10* (I) *cob*::*lac* (CobII)	19	12	0.6	33	32	1	30
TT10858	*cob*::*lac* (CobIII)	46	240	5	62	530	8	630
TT17194	*cob*::Tn*10* (I) *cob*::*lac* (CobIII)	24	6	0.25	21	15	0.7	18

[a] Cells were grown at 37°C on NCE medium with methionine and the indicated sources of carbon and energy. None, no addition to the growth medium; PD, propanediol was added.

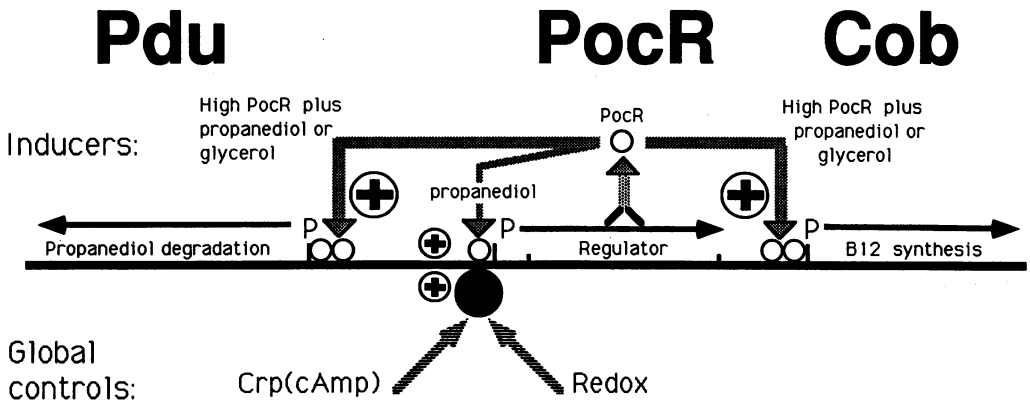

FIG. 4. Model for control of the *cob/pdu* regulon. The model is supported by the data presented here but includes some features that remain speculative. According to the model, expression of the *cob* and *pdu* operons requires the PocR protein. Induction cannot occur unless the level of PocR protein is increased by a combination of global regulatory signals and autoinduction by propanediol. Once the PocR level is high, either glycerol or propanediol can serve as regulatory effectors to promote positive activation of the *cob* and *pdu* operons. The global regulatory systems controlling *pocR* gene expression are not clear but may include CRP-cAMP and a mediator of redox control that senses a reduced cell interior.

strains TT17195 and TT17194). This weak expression from internal promoters is sufficient to account for the CobIII$^+$ CobII$^+$ phenotype of CobI insertion mutants. We infer that the entire *cob* gene cluster is a single operon controlled by the regulatory mechanisms discussed here, which vary transcription of the entire gene cluster by acting at the far left end of the *cob* region as the map is presented in Fig. 3. On the basis of sequence data, this single operon includes about 25 genes (10, 30).

DISCUSSION

We propose a model for transcriptional regulation of the *cob/pdu* regulon (Fig. 4). The key aspects of that model are listed below.

(i) The *pocR* protein acts in *trans* as an activator of the *cob* and *pdu* operons and also activates transcription of its own gene. Insertion mutations in the *pocR* gene prevent induction of the *cob* and *pdu* operons. Such mutations are complemented equally well by a *pocR*$^+$ allele provided either in *cis* or in *trans*. Read-through from the *pocR* promoter is not a major contributor to *cob* operon expression, since a *cob*::*lac* fusion shows full induction in a strain that contains a *pocR*$^+$ allele in *trans* and a strongly polar *pocR* insertion *cis* to the *cob*::*lac* fusion. Autoinduction of *pocR* transcription is supported by the fact that propanediol induces transcription of a *pocR-lac* fusion in a PocR$^+$ merodiploid but not in an isogenic PocR$^-$ strain. Since the *pdu* operon and the *pocR* gene are transcribed divergently and *cob* operon expression is independent of the *pocR* promoter, we infer that the *pdu*, *pocR*, and *cob* regions must be expressed from three distinct promoters. All three are regulated by the PocR protein.

Sequencing data are consistent with the above interpretation. The base sequence of the region between the *pdu* and *cob* operons reveals a reading frame (read left to right as the map in Fig. 3 is drawn) with an inferred protein product that is highly homologous to the *araC* family of transcriptional regulatory proteins (10, 30). Furthermore, Richter-Dahlfors and Andersson have characterized a regulated promoter lying between the *pocR* and *cob* regions (28).

(ii) Both propanediol and glycerol can serve individually as effectors of the *pocR* protein. The *cob* and *pdu* operons are most markedly induced in the presence of either propanediol or glycerol. The metabolism of these inducers is not a requirement for induction. Autoinduction of *pocR* is caused only by propanediol, and this effect is also independent of propanediol metabolism.

It is surprising that either propanediol or glycerol can serve as effectors of PocR for induction of the *pdu* and *cob* operons, yet only propanediol is able to induce *pocR* transcription. This suggests that glycerol and propanediol may stabilize different conformations of the PocR protein or possibly that additional components, such as the mediators of global control, are involved in autoregulation. An alternative interpretation of the data presented is that a complex of propanediol and PocR protein activates *pocR* gene expression. Glycerol would then serve as a PocR effector for induction of the *pdu* and *cob* operons when a sufficient level of PocR protein has been achieved. Both models suggest that the PocR protein binds glycerol and propanediol; these interactions have not yet been directly demonstrated.

(iii) Expression of the *pocR* gene varies in response to global controls as well as autoregulation. In strains with functional PocR protein, propanediol induces *pocR* gene transcription. This autoinduction is seen only during growth on poor carbon sources, suggesting that global controls as well as PocR protein are involved in autoinduction.

In the absence of PocR protein, the level of *pocR* gene transcription increases during growth on poor carbon sources or under anaerobic conditions but propanediol has no inductive effect. Compared with aerobic growth of cells on glucose, aerobic growth on succinate causes a 3-fold increase in *pocR* transcription and anaerobic respiration of glycerol-fumarate causes a 17-fold increase. We suggest that these increases are due to global regulatory mechanisms that act on *pocR* gene transcription independently of the PocR-mediated propanediol induction.

The global control mechanisms at work here have not yet been investigated. Previous studies indicated that both catabolite repression and redox control influence *cob* operon transcription (3, 17). We present evidence that expression of both the *cob* and *pdu* operons requires the PocR protein; induction of this regulon is improved under conditions that increase *pocR* gene expression (see below). Therefore, we propose that CRP-cAMP and redox control are two indepen-

dent global control mechanisms that regulate *pocR* gene transcription. However, neither the direct involvement of CRP-cAMP nor the existence of the redox regulatory protein depicted in Fig. 4 has yet been demonstrated.

(iv) Global control of the PocR protein level mediates global control of the *cob/pdu* regulon. There is a correlation between growth conditions under which *pocR* gene transcription is high and growth conditions under which propanediol and glycerol cause maximal induction of the *cob/pdu* regulon (Tables 2, 3, and 6). Moreover, during aerobic growth of *glpK* strains on succinate, the *cob/pdu* regulon is induced to higher levels by propanediol than by glycerol. This coincides with the fact that propanediol but not glycerol autoinduces transcription of the *pocR* gene. These correlations are consistent with the idea that global control signals increase expression of the *pocR* gene and thereby enhance induction of the *cob/pdu* regulon by propanediol and glycerol. This idea explains why glycerol catabolism prevents aerobic induction of the *cob/pdu* regulon; decreased cAMP levels may prevent adequate expression of the *pocR* gene.

General points regarding the model. Anaerobic induction of the *cob* operon by glycerol per se seems contradictory to the findings of Andersson and Roth (3). They observed maximum operon expression during anaerobic respiration of glycerol-fumarate and concluded that a high level of expression was due not to induction by glycerol but to a highly reduced cell interior achieved by rapid oxidation of glycerol and slow transport of electrons to fumarate. They shifted *glpK*$^+$ and *glpK* mutant strains from glucose to glycerol anaerobically and saw partial induction of a *cob-lac* fusion only in the *glpK*$^+$ strains. From these results the authors suggested that glycerol metabolism was needed for the anaerobic induction of the *cob* operon and that glycerol per se was not an inducer of the *cob* operon.

In this report, we suggest that glycerol is an inducer of the *cob/pdu* regulon but that a high level of PocR protein is also required for induction. We show that anaerobic respiration of glycerol or pyruvate allows a high level of *pocR* gene transcription. Under our experimental conditions, glycerol induction of the *cob* and *pdu* operons was demonstrated in *glpK* cells that were actively engaged in anaerobic respiration of pyruvate-fumarate, a growth condition that leads to relatively high levels of *pocR* gene transcription. We suggest that Andersson and Roth, in shifting a *glpK* mutant from glucose to anaerobic glycerol (3), put cells under starvation conditions that did not allow induction of *pocR* gene expression; therefore, their experiments did not reveal the inductive effect of glycerol.

Previous studies indicated that transcription of the *cob* operon was induced only in the absence of oxygen (17, 24). Subsequently, evidence was presented that a highly reduced cell interior (rather than absence of oxygen per se) was needed for induction (3). In this report, we show that the *cob* operon (and the adjacent *pdu* operon) can also be induced aerobically, but only if cells are grown on a poor carbon source and propanediol (or glycerol) is provided as an inducer. We suggest that CRP-cAMP is a second global regulatory system (in addition to redox control) that can stimulate *pocR* gene transcription and thereby permit induction of the *cob/pdu* regulon.

The *cob* region is a single regulated operon. Previously it was concluded that the *cob* genes were arranged as three independent operons, each including genes of related function (17). This was based on the phenotypes of mutants. CobI insertion mutants are blocked in formation of the intermediate Ado-Cbi but still express the promoter-distal CobIII and CobII gene clusters which are responsible for synthesis of DMB (CobII) and assembly of Ado-Cbi and DMB into the cofactor Ado-Cbl (CobIII) (18, 25). It is now clear that the entire Cob gene cluster (about 25 genes) is a single operon, regulated as a unit, with internal weak constitutive promoters that allow expression of distal genes arranged as logical groups within the operon. This is demonstrated by the data in Table 7 and by additional unpublished data (14).

The paradox of the *cob/pdu* regulatory pattern. At first glance, the regulatory behavior of the *cob/pdu* regulon makes physiological sense. Since Ado-Cbl is required as a cofactor for degradation of propanediol, it seems logical for propanediol to serve as an inducer of the vitamin B_{12} synthetic enzymes as well as the enzymes for propanediol degradation. However, on closer inspection, some problems are presented both by the global regulatory pattern and by the ability of glycerol to serve as an inducer. In fact, all of the described conditions that induce this regulon are apparently futile in that no condition allows cells to use propanediol as a carbon and energy source by means of endogenously synthesized vitamin B_{12}.

S. typhimurium can use propanediol aerobically as a (very poor) source of carbon and energy but cannot synthesize vitamin B_{12} under these conditions, even when the *cob* operon is fully induced (2, 24). It appears that some vitamin B_{12}-synthetic step is oxygen sensitive or that some critical synthetic enzyme (outside the operon) is not induced aerobically. Therefore, aerobic induction of the *cob/pdu* regulon provides enzymes for the degradation of propanediol but does not provide the needed cofactor.

Anaerobic induction of the regulon also appears futile. Under these conditions, vitamin B_{12} is synthesized and the propanediol-degradative genes are induced, but cells are unable to utilize propanediol as a sole carbon and energy source without oxygen to serve as an electron acceptor. Salmonellae cannot ferment propanediol, as do klebsiellae (19), nor can salmonellae use propanediol as a carbon source during anaerobic respiration with alternative electron acceptors such as nitrate or fumarate (6). This apparently paradoxical regulatory pattern may make sense if salmonellae usually grow with oxygen levels insufficient to inhibit vitamin B_{12} synthesis but sufficient to permit respiration of propanediol (and other carbon sources).

Induction of the *cob/pdu* regulon by glycerol is also hard to rationalize. *S. typhimurium* shows no known vitamin B_{12}-dependent metabolism of glycerol, either aerobically or anaerobically. Related enteric bacteria can ferment both glycerol and propanediol by vitamin B_{12}-dependent pathways. In some organisms, a single diol dehydratase is able to perform the dehydration of both propanediol and glycerol (19). The ability of glycerol to induce the *cob/pdu* regulon of *S. typhimurium* may be simply due to the structural similarity of glycerol to propanediol. We suspect that the *cob/pdu* regulatory system makes physiological sense to salmonellae and that some surprises will be forthcoming before the logic is apparent to us.

APPENDIX

Deletion mapping by dominant marker phenotypes. The genetic map of the *cob/pdu* control region presented in this paper was constructed by a method that allows deletion mapping of any chromosomal region, even regions with mutations that cause no counterselectable phenotype. This method could be applied to noncoding regions of the chromosome.

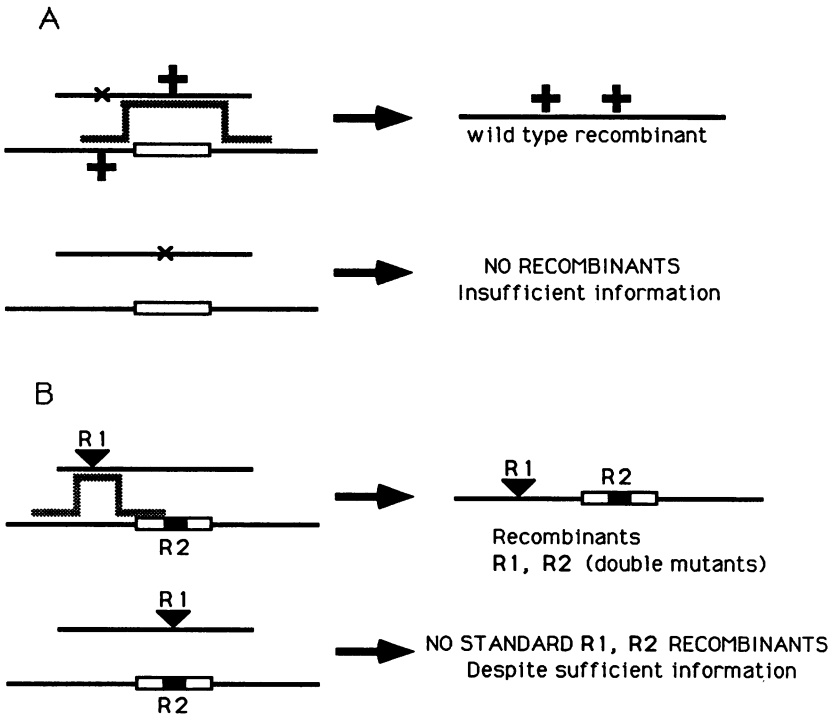

FIG. A1. Deletion mapping. (A) Standard; (B) with dominant mutant phenotypes.

This method was employed here because mutations in the *cob/pdu* control region have no phenotypes to allow selection of wild-type recombinants; therefore, we could not apply the standard deletion mapping method. Mutant phenotypes for *pdu* are scored on MacConkey plates; Pdu^+ strains grow so poorly on minimal propanediol medium with vitamin B_{12} that selection of recombinants is difficult. The *zeb*::Tn*10* insertions have no easily observable phenotypic defect. Mutations in the *pocR* gene have a leaky Cob^- phenotype. The new mapping method employs as selective markers the drug resistance determinants associated with the donor and recipient insertion mutations. We select for inheritance of two dominant mutant alleles rather than the standard counterselection of recessive mutant alleles.

A standard deletion mapping cross is diagrammed in Fig. A1A; both donor and recipient mutations cause a recessive counterselectable phenotype, and one selects wild-type recombinants that lack both parental lesions. If the donor point mutation lies within the region missing from the recipient deletion, no recombinants can form because both parents lack essential information corresponding to the site of the donor mutation. Since the deletion cannot revert, there is no background of revertants and sensitive crosses can be performed to distinguish between mutations lying within the deleted region (no recombinants) and mutations lying outside the deletion (some recombinants). This standard method could not be applied to the *cob/pdu* region.

Instead, we used insertion mutations which add drug resistance determinants to the chromosome. These dominant phenotypes were used as selective markers in the mapping crosses. Deletion mutations were created by recombination between two Mud insertions; the deletion is inseparable from the drug resistance encoded by the Mud element (Ap^r or Kn^r). Donor mutations are insertions of Tn*10* derivatives and therefore provide a drug resistance phenotype (Tc^r or Cm^r) that is inseparable from the insertion mutation.

In scoring recombination between the donor point mutation (insertion) and the recipient deletion, we select for recombinants that inherit both the donor and recipient drug resistance markers. This cross is diagrammed in Fig. A1B. If the donor insertion lies within the recipient deletion, one might expect that the two mutations would be mutually exclusive and double mutants could not form, making this an effective mapping method. However, notice that regardless of the position of the donor mutation, all of the genetic information needed for resistance to both drugs is provided in the cross. Therefore, even if the insertion lies inside the deletion, recombinants will be observed if some genetic event permits addition of the donor element to the recipient chromosome without eliminating the recipient deletion and its associated drug resistance. In practice, such events are observed at a low frequency even when the donor point mutation lies within the recipient deletion. Therefore, all crosses lead to some frequency of recombinants. Two mechanisms for addition of overlapping markers are diagrammed in Fig. A2.

If the donor cell population includes, at low frequency, cells with a duplication whose join point is cotransducible with the donor selective marker, recombination can add the donor marker to the chromosome without removing the deletion which overlaps the site of the donor insertion (Fig. A2A). This event seems to explain the recombinants seen for deletions extending leftward from the *pdu*, *zeb*, or *pocR* locus to the *his* operon. The few recombinants scored in these cases are His^- and show both the donor and recipient drug resistances, but the donor drug resistance is lost with high frequency by segregation of the duplication (exchanges between the repeated cd sequences noted in Fig. A2). The recipient drug resistance is never lost, because the deletion removes homology ab to the left of the donor insertion.

If the recipient strain carries a duplication that includes the site of the deletion mutation, then the donor insertion can replace the deletion in one copy of the duplication while the second copy of the deletion and its drug resistance are retained. This is diagrammed in Fig. A2B. These events appear to explain the few recombinants between donor insertions within the deleted region and deletions extending rightward from the *pdu*, *zeb* or *pocR* locus to CobII. These recombinants are Cob^+ and show both donor and recipient drug resistances, but they frequently lose either one drug resistance marker or the other. Which resistance marker is lost depends on which repeated sequences (ab or cd in Fig. A2) recombine to cause segregation. Recombinants involving the *his-cob* deletions as recipients and donor insertions within the deleted region did not arise by

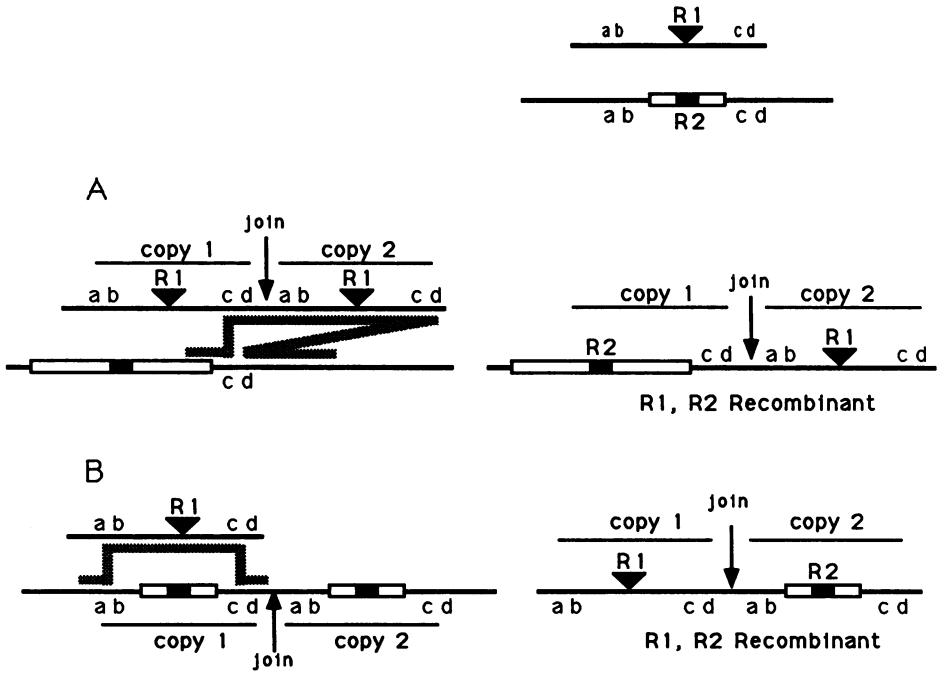

FIG. A2. Nonstandard events that form R1 and R2 recombinants. (A) Duplication in donor; (B) duplication in recipient.

this mechanism because these leftward deletions are too large to be repaired by a single transduced fragment.

Because of the events described above, recombinants are observed in all deletion mapping crosses, regardless of the position of the donor insertion. However, mapping is still possible if one uses pairs of recipient deletions. For each of the critical Mud insertions in the *cob/pdu* region, we constructed a deletion extending to the right (to the CobII region) and another extending to the left (to the *his* operon). The donor mutation must lie within one or the other of these deletions. The recombination events that require involvement of a duplication are understandably rare, since duplications of any particular chromosomal site are typically carried in populations at a frequency of about 10^{-3} to 10^{-4}. All of the donor insertion mutations mapped showed 100- to 1,000-fold more recombinants with one deletion than with the other. The donor mutation was assigned to a location within the deletion whose cross gave fewer recombinants. From all of the crosses yielding few recombinants, the recombinants found invariably showed instability of one of the sorts described above. In addition, such recombinants showed cotransductional linkage between the Mud and Tn*10* elements that was reduced in comparison to the linkage seen in standard haploid strains; this is consistent with the presence of duplications in such recombinants.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institute of General Medical Sciences (R01 GM34804). M.A. was supported in part by the Howard Hughes Undergraduate Initiative Program.

Some of the initial phases of this project were inspired and aided by three high school students (all now undergraduates), Kathleen Treseder (University of Utah), Jeffrey Cook (Occidental College), and Sohail Tavazoie (University of California, Berkeley). The first *pocR* mutants were isolated by Kathleen Treseder.

REFERENCES

1. **Ailion, M. E.** Unpublished data.
2. **Andersson, D. I., and J. R. Roth.** 1989. Mutations affecting regulation of cobinamide biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **171**:6726–6733.
3. **Andersson, D. I., and J. R. Roth.** 1989. Redox regulation of the genes for cobinamide biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **171**:6734–6739.
4. **Balch, W. E., G. E. Fox, L. J. Magrum, C. R. Woese, and R. S. Wolfe.** 1979. Methanogens: reevaluation of a unique biological group. Microbiol. Rev. **43**:260–296.
5. **Berkowitz, D., J. M. Hushon, H. J. Whitfield, Jr., J. R. Roth, and B. N. Ames.** 1968. Procedure for identifying nonsense mutations. J. Bacteriol. **96**:215–220.
6. **Bobik, T.** Unpublished results.
7. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: an *in vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA **76**:4530–4533.
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. **Chan, R. K., D. Botstein, T. Watanabe, and Y. Ogata.** 1972. Specialized transduction of tetracycline resistance by phage P22 in *Salmonella typhimurium*. II. Properties of a high-frequency-transducing lysate. Virology **50**:883–898.
10. **Chen, P., and J. R. Roth.** Unpublished data.
11. **Chumley, F. G., R. Menzel, and J. R. Roth.** 1979. Hfr formation directed by Tn*10*. Genetics **91**:639–655.
12. **Chumley, F. G., and J. R. Roth.** 1980. Rearrangement of the bacterial chromosome using Tn*10* as a region of homology. Genetics **94**:1–14.
13. **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.
14. **Doak, T., and J. R. Roth.** Unpublished results.
15. **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.
16. **Escalante-Semerena, J. C., M. G. Johnson, and J. R. Roth.** 1992. The CobII and CobIII regions of the cobalamin (vitamin B_{12}) biosynthetic operon of *Salmonella typhimurium*. J. Bacteriol. **174**:24–29.
17. **Escalante-Semerena, J. C., and J. R. Roth.** 1987. Regulation of cobalamin biosynthetic operons in *Salmonella typhimurium*. J. Bacteriol. **169**:2251–2258.

18. **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.
19. **Forage, R. G., and M. A. Foster.** 1982. Glycerol fermentation in *Klebsiella pneumoniae*: functions of the coenzyme B_{12}-dependent glycerol and diol dehydratases. J. Bacteriol. **149:**413–419.
20. **Hughes, K., and J. R. Roth.** 1985. Directed formation of deletions and duplications using Mud(Ap, lac). Genetics **109:** 263–282.
21. **Hughes, K. T., and J. R. Roth.** 1984. Conditionally transposition-defective derivative of Mu d1(Amp Lac). J. Bacteriol. **159:**130–137.
22. **Hughes, K. T., and J. R. Roth.** 1988. Transitory *cis* complementation: a method for providing transposition functions to defective transposons. Genetics **119:**9–12.
23. **Jeter, R. M.** 1990. Cobalamin-dependent 1,2-propanediol utilization by *Salmonella typhimurium*. J. Gen. Microbiol. **136:**887–896.
24. **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.
25. **Jeter, R. M., and J. R. Roth.** 1987. Cobalamin (vitamin B_{12}) biosynthetic genes of *Salmonella typhimurium*. J. Bacteriol. **169:**3189–3198.
26. **Kleckner, N., D. Botstein, and J. R. Roth.** 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements. J. Mol. Biol. **116:**125–159.
27. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
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. **Rondon, M. R., and J. 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.
30. **Roth, J. R., S. Kieffer-Higgins, M. Rubenfield, and G. Church.** Unpublished data.
31. **Schmieger, H.** 1971. A method for detection of phage mutants with altered transducing ability. Mol. Gen. Genet. **110:**378–381.
32. **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.
33. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218:**97–106.
34. **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.