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cobB Function Is Required for Catabolism of Propionate in *Salmonella typhimurium* LT2: Evidence for Existence of a Substitute Function for CobB within the 1,2-Propanediol Utilization (*pdu*) Operon

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The *cobB* function of *Salmonella typhimurium* LT2 was defined in vivo as an alternative activity for the nicotinic acid mononucleotide:5,6-dimethylbenzimidazole phosphoribosyltransferase enzyme (CobT), which is involved in the assembly of the nucleotide loop of cobalamin in this bacterium (J. R. Trzebiatowski, G. A. O'Toole, and J. C. Escalante-Semerena, J. Bacteriol. 176:3568–3575, 1994). In this paper we document that, independent of their inability to substitute for CobT function, *cobB* mutants are unable to use propionate as a carbon and energy source. A plasmid carrying only a wild-type copy of *cobB* complemented the cobalamin biosynthesis and propionate catabolism phenotypes of *cobB* mutants, indicating that a lack of CobB was responsible for both phenotypes. We demonstrate the existence of a function encoded by the 1,2-propanediol utilization (*pdu*) operon, which when induced by 1,2-propanediol compensated for the lack of CobB during propionate catabolism but failed to compensate for CobT in the assembly of the nucleotide loop of cobalamin in a *cobB cobT* double mutant.

On the basis of genetic evidence, we defined *cobB* as a locus encoding a function that compensated for the lack of nicotinic acid mononucleotide:5,6-dimethylbenzimidazole (DMB) phosphoribosyltransferase enzyme (CobT) during the assembly of the nucleotide loop of cobalamin (CBL) (Fig. 1) (18). It was suggested that CobB could have a role elsewhere in metabolism because *cobB* mutants, with wild-type copies of all known CBL biosynthetic genes (6, 7, 10, 12), were not blocked in their ability to synthesize CBL.

Recently, we completed the physical characterization of *cobB*, whose nucleotide sequence indicated that it was a previously uncharacterized gene of *Salmonella typhimurium* LT2 located at 27 centisomes, downstream of the putrescine transport (*pot*) operon (data to be presented elsewhere).

CobB is required for the catabolism of propionate. We investigated the possible role of CobB in the catabolism of several carbon and energy sources. For this purpose, 0.1 ml of a 10^6 dilution of overnight cultures of strains JE2845 (*cobB1206*::MudI1734, hereafter referred to as *cobB1206*::MudJ) and TR6583 ($cobB^+$) grown in nutrient broth was plated onto no-carbon E (NCE) minimal medium (4) supplemented with one of several compounds known to support growth of *S. typhimurium*. Compounds tested included glucose (11 mM), glycerol (22 mM), pyruvate (50 mM), acetate (50 mM), citrate (50 mM), succinate (50 mM), propionate (30 mM), and 1,2-propanediol (1,2-PDL) (50 mM). Medium containing 1,2-PDL as the sole source of carbon and energy was supplemented with cyano-CBL (CN-CBL, 20 nM) because 1,2-PDL dehydratase (EC 4.2.1.28), the first enzyme of the 1,2-PDL catabolic pathway, is a cobamide-dependent enzyme. In the absence of CBL, 1,2-PDL is not catabolized by *S. typhimurium* under aerobic conditions. Growth was assessed after 96 h of incubation at 37°C under aerobic conditions.

Unlike strain TR6583, strain JE2845 failed to grow on medium containing 1,2-PDL (with or without CN-CBL in the medium) or on medium containing propionate as a carbon and energy source (Table 1). Both strains, however, grew on glucose, glycerol, pyruvate, acetate, citrate, and succinate. Since 1,2-PDL is thought to be catabolized to propionate (17), we concluded that the lack of CobB function affected propionate rather than 1,2-PDL breakdown.

The negative effect of the *cobB1206*::MudJ mutation on propionate catabolism is not due to polarity. To address the possibility that the observed inability of strain JE2845 was due to polar effects of the *cobB1206*::MudJ insertion on a gene involved in propionate breakdown, plasmid pCOBB5 (19) carrying only a wild-type copy of *cobB* cloned into vector pSU19 (11) was introduced into strain JE2845 (*cobB1026*::MudJ). As a positive control, plasmid pCOBB5 was introduced into strain JE2501 [*cobT109*::MudJ *cobB1176*::Tn*10*DE*16*DE*17*, hereafter referred to as *cobB1176*::Tn*10d*(Tc)]. The propionate and CBL biosynthetic phenotypes of the resulting strains were assessed. The presence of pCOBB5 restored the ability of strain JE2845 to grow on propionate. In the absence of plasmid pCOBB5, strain JE2845 failed to grow in medium containing propionate as a carbon and energy source even after 90 h of incubation; a derivative of JE2845 carrying pCOBB5 (strain JE4052) grew on propionate with a doubling time of 8.6 h and reached full density (A_{650} = 0.8) after 60 h of incubation.

Plasmid pCOBB5 also restored the ability of JE2501 to make CBL from its precursors cobinamide and DMB. Synthesis of CBL from these precursors was assessed under aerobic conditions in medium containing glucose as a carbon and energy source. Strain JE2501 failed to grow even after 20 h of incubation, while a culture of the derivative of strain JE2501 carrying pCOBB5 (strain JE4051) grew with a doubling time of 0.65 h and reached full density (A_{650} = 0.9) after 10 h of incubation.

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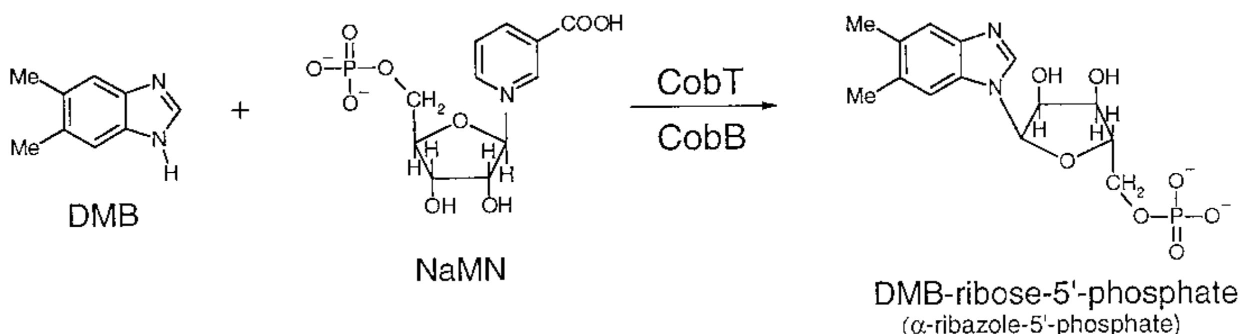

FIG. 1. Synthesis of DMB-ribose-5′-phosphate (α-ribazole-5′-phosphate). CobT is the nicotinic acid mononucleotide (NaMN):DMB phosphoribosyltransferase that catalyzes the synthesis of α-ribazole-5′-phosphate. Genetic data suggest that CobB substitutes for CobT in the reaction.

These results demonstrated that the lack of CobB protein was solely responsible for the propionate and CBL phenotype of strains JE2845 and JE2501.

1,2-PDL-inducible function compensates for the lack of CobB during the catabolism of propionate. Also shown in Table 1 is the growth behavior of strain JE2845 (*cobB1206*::MudJ) in medium containing a mixture of propionate and 1,2-PDL but lacking CBL. It is important to emphasize that the observed growth could not be attributed to the utilization of 1,2-PDL as a carbon and energy source, since CBL was not provided exogenously and the culture was grown under aerobic conditions, under which *S. typhimurium* is unable to synthesize CBL de novo (5, 10, 13). The presence of 1,2-PDL in the medium allowed growth with a doubling time of 8.4 h; in the absence of added 1,2-PDL no growth was observed after 90 h of incubation. We concluded that growth on propionate was due to a 1,2-PDL-inducible function that compensated for the lack of CobB. The lack of growth observed in medium containing only 1,2-PDL supported the conclusion that 1,2-PDL catabolism was not responsible for the observed growth in medium containing propionate and 1,2-PDL. The lack of growth on 1,2-PDL and CN-CBL supported to the hypothesis that 1,2-PDL is metabolized via the propionate catabolic pathways in this bacterium.

A gene product that allows a *cobB* mutant to metabolize propionate is encoded by the 1,2-PDL utilization (*pdu*) operon. Expression of the 1,2-PDL utilization (*pdu*) operon of *S. typhimurium* is regulated at the transcriptional level by the PocR regulatory protein in response to 1,2-PDL in the environment (1, 3, 9, 13, 14). Two lines of evidence support the conclusion that a function that allows *cobB* mutants to use propionate as a carbon and energy source is encoded by the *pdu* operon. First, strain JE3983 [*cobB1206*::MudJ *pocR106*::Tn*10d*(Tc)], defective in the synthesis of both the CobB and the PocR proteins, failed to grow on propionate even when 1,2-PDL was included in the medium (Table 1, lines 2 and 3). This result was consistent with a lack of expression of the *pdu* operon. Second, strains JE2506 (*metE205 ara-9 pdu-8*::MudJ) and JE2507 (*metE205 ara-9 pdu-12*::MudJ), carrying independently isolated MudJ elements somewhere within *pdu*, also failed to grow on propionate when 1,2-PDL was added to the medium (data not shown). These results raised the possibility that both MudJ elements were inserted in or were polar on the expression of the *pdu* gene encoding the function that allows *cobB* mutants to grow on propionate. We predicted that if this function were encoded within *pdu*, missense *pdu* mutants should display a propionate-proficient (Prp^+) or propionate-deficient (Prp^-) phenotype. To address this prediction, mutations were introduced into the *pdu* operon by chemical mutagenesis with hydroxylamine. For this purpose, a lysate of phage P22 (HT *105/1 int-201*) (15, 16) grown on strain JE3607 [*zeb-6400*::Tn*10d*(Cm) *cobB1206*::MudJ pdu^+] was mutagenized with hydroxylamine as described elsewhere (4, 8); strain JE3607 is phenotypically Prp^+. Mutagenized phage was used as a donor to transduce strain JE2445 [*cobB1176*::Tn*10d*(Tc)] to chloramphenicol resistance on nutrient broth plates containing chloramphenicol (20 mg/ml). Cm^r transductants were replica printed onto NCE medium containing 1,2-PDL (50 mM) and CN-CBL (20 nm) and onto NCE medium containing glycerol (22 mM). A total of 135 Cm^r transductants unable to grow on 1,2-PDL–CBL medium were purified as previously described (2), and their ability to grow on propionate when 1,2-PDL was present in the medium was assessed. Of these mutants, 115 (85%) grew on propionate–1,2-PDL medium while 20 (15%)

TABLE 1. Mediation of the effect of 1,2-PDL on the Prp phenotype of *cobB* mutants by the PocR regulatory protein

Line	Strain	Relevant genotype[a]	Growth[b] on NCE minimal medium with:				
			Prp	Prp–1,2-PDL	1,2-PDL	1,2-PDL–CN-CBL	Glycerol
1	TR6583	$cobB^+$ $pocR^+$	+	+	−	+	+
2	JE2845[c]	*cobB* $pocR^+$	−	+	−	−	+
3	JE3983[d]	*cobB pocR*	−	−	−	−	+
4	JE1857[e]	*cobT* $cobB^+$	+	+	−	+	+

[a] All strains were derivatives of strain TR6583 (*metE205 ara-9*) and contained a wild-type copy of the *pdu* (propanediol utilization) operon.
[b] The final concentration of carbon or energy was as follows: propionate (Prp), 30 mM; 1,2-PDL, 13 mM; glycerol, 22 mM; CN-CBL, 20 nM. In all cases, a plus sign represents a colony of 1.5 to 2.0 mm in diameter after 96 h of incubation at 37°C. Results are averages from 10 colonies randomly selected from a plate containing approximately 100 colonies. A minus sign indicates that no measurable colonies were formed after 96 h of incubation at 37°C.
[c] *cobB1206*::MudJ.
[d] *pocR106*::Tn*10d*(Tc).
[e] *cobT109*::MudJ.

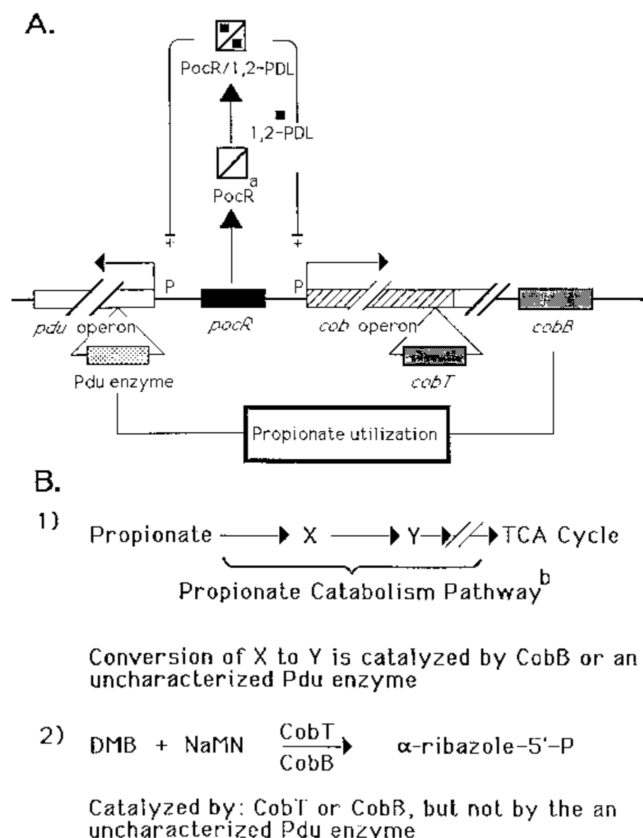

FIG. 2. Summary of findings. (A) PocR–1,2-PDL complex formation results in the activation of transcription of the *cob/pdu* regulon, resulting in the synthesis of an undetermined number of Pdu proteins and 20 CBL biosynthetic proteins, including CobT. *cobB* expression is unaffected by 1,2-PDL in the medium (19). (B) (Reaction 1) Synthesis of α-ribazole-5′-phosphate is catalyzed by either CobT or CobB but not by the Pdu product that can substitute for CobB in propionate breakdown. TCA, tricarboxylic acid. (Reaction 2) An undefined step of the propionate catabolism pathway is catalyzed when either CobB or an uncharacterized protein encoded by the *pdu* operon are synthesized. NaMN, nicotinic acid mononucleotide.

did not. These results were anticipated, since the *pdu* operon contains several genes (14a).

To rule out the possibility that the observed phenotype was due to a lack of expression of *pdu* brought about by inactivation of the *pocR* gene, plasmid pPOCR100 [*pocR*$^+$, in vector pSU18(Km^r) (11)] was introduced into these strains and into *pocR* mutant JE1939 [*pocR106*::Tn*10d*(Tc)] as a positive control. Three of the *pdu* point mutants displaying a Prp$^-$ phenotype were complemented by plasmid pPOCR100, indicating that in those strains, the lesions were located within *pocR*. The remaining 17 *pdu* Prp$^-$ mutants were not complemented by pPOCR100 (*pocR*$^+$); thus, they were inferred to carry lesions in *pdu*.

We concluded that the class of *cobB pdu* double mutants unable to grow on propionate–1,2-PDL carried mutations in the *pdu* gene encoding the function that allows *cobB* mutants to use propionate as a carbon and energy source. Introduction of plasmid pCOBB5 (*cobB*$^+$) into the *pdu cobB*$^+$ Prp$^-$ strains restored their ability to grow on propionate, indicating that the function encoded in *pdu* was not required for the breakdown of propionate. In addition, the fact that TR6583 (*prp*$^+$) was able to grow on propionate in the absence of 1,2-PDL to induce *pdu* expression indicated that the *pdu* function that restores propionate catabolism in *cobB* mutants was not required for the breakdown of this fatty acid.

The Pdu function that allows *cobB* mutants to grow on propionate does not restore CBL biosynthesis in a *cobT cobB* double mutant. Induction of *pdu* expression by 1,2-PDL did not restore the ability of strain JE2501 [*cobT109*::MudJ *cobB1176*::Tn*10d*(Tc)] to convert cobamide to CBL in the absence of DMB in the medium (data not shown). This suggested that none of the Pdu gene products could compensate for the lack of CobT and CobB in the assembly of the nucleotide loop of CBL in a *cobT cobB* double mutant. A control experiment showed that a *cobT* mutant was able to grow on propionate as efficiently as the *cobT*$^+$ strain TR6583 (Table 1, line 4).

Conclusions. Figure 2 summarizes our findings as follows. (i) The CobB protein is required for the catabolism of propionate in *S. typhimurium* LT2 (Fig. 2A). (ii) An uncharacterized, 1,2-PDL inducible function encoded by the 1,2-PDL utilization (*pdu*) operon allows a *cobB* mutant to use propionate as a carbon and energy source (Fig. 2A and B, reaction 1). (iii) The Pdu protein that allows a *cobB* mutant to grow on propionate does not compensate for the lack of CobT or CobB during CBL biosynthesis in a *cobT* or *cobT cobB* double mutant (Fig. 2B, reaction 2); CobT is not required for propionate catabolism in this bacterium.

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