CobB, a New Member of the SIR2 Family of Eucaryotic Regulatory Proteins, Is Required to Compensate for the Lack of Nicotinate Mononucleotide:5,6-Dimethylbenzimidazole Phosphoribosyltransferase Activity in cobT Mutants during Cobalamin Biosynthesis in Salmonella typhimurium LT2*  

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The cobB gene of Salmonella typhimurium LT2 has been isolated and genetically and biochemically characterized. cobB was located by genetic means to the 27-centisome region of the chromosome. Genetic crosses established the gene order to be cobB pepT phoQ, and the direction of cobB transcription was shown to be clockwise. The nucleotide sequence of cobB (711 base pairs) predicted a protein of 237 amino acids length with a molecular mass of 26.3 kDa, a mass consistent with the experimentally determined one of ~28 kDa. The cobB gene was defined genetically by deletions (10), insertions (5), and point mutations (15). The precise location of a Tn10(Tc) element within cobB was established by sequencing. DNA sequence analysis of the region flanking cobB located it 81 base pairs 3' of the potABCD operon, with the potABCD operon and cobB being divergently transcribed. cobB was overexpressed to ~30% of the total soluble protein using a T7 overexpression system. In vitro activity assays showed that cell-free extracts enriched for CobB catalyzed the synthesis of the cobalamin biosynthetic intermediate N1-(5-phospho-α-D-ribosyl)-5,6-dimethylbenzimidazole (also known as α-ribazole-5'-phosphate) from nicotinate mononucleotide and 5,6-dimethylbenzimidazole, the reaction known to be catalyzed by the CobT phosphoribosyltransferase enzyme (EC 2.4.2.21) (Trzebiatowski, J. R. and Escalante-Semerena, J. C. (1997) J. Biol. Chem. 272, 17662-17667). Computer analysis of the primary amino acid sequence of the CobB protein identified the sequences GAGISAESGIRTFR and YTQNID which are diagnostic of members of the SIR2 family of eucaryotic regulatory proteins. Possible roles of CobB as a regulator are discussed within the context of the catabolism of propionate, a pathway known to require cobB function (Tsang, A. W. and Escalante-Semerena, J. C. (1996) J. Bacteriol. 178, 7016-7019).

The genetic analysis of the late steps of cobalamin (Chl) biosynthesis (also known as nucleotide loop assembly) in Salmonella typhimurium (1) has raised important questions about the biochemical capabilities of the enzymes involved (2, 3). The specific step of the nucleotide loop assembly pathway that we address in this paper is the activation of the lower ligand base 5,6-dimethylbenzimidazole (Me₂Bza). This step is catalyzed by the CobT enzyme, which converts Me₂Bza to its 5'-mononucleotide (also known as α-ribazole-5'-phosphate) by transferring the phosphoribosyl group from NaMN to Me₂Bza (Fig. 1) (2, 4).

As predicted by the biochemistry of the CobT reaction, cobalamin biosynthesis in mutants lacking CobT can be restored by providing α-ribazole-5'-phosphate in the medium (4). What was unexpected, however, was the finding that all previously reported Me₂Bza auxotrophs (5) were allelic of cobT (2), including insertions that eliminated CobT completely from cell-free extracts (6). This phenotype was difficult to explain in light of the documented biochemical activity of CobT as the NaMN: Me₂Bza phosphoribosyltransferase (PRTase) (EC 2.4.2.21) (4). This finding basically said that increasing the substrate for CobT would circumvent the lack of this enzyme. To help explain this paradox, we postulated the existence of an alternative enzyme that could catalyze the CobT reaction, with the qualification that such an enzyme would have less affinity for Me₂Bza than CobT to explain the requirement for additional Me₂Bza (2).

In this paper we report the identification of the cobB gene whose product is required to compensate for the lack of NaMN: Me₂Bza PRTase activity in cobT mutants. cobB has been defined genetically by mutation analysis and physically by its nucleotide sequence (GenBank™ accession number U89687). Computer analysis of the primary amino acid sequence of CobB shows it to be a member of the SIR2 family of eucaryotic regulatory proteins.

EXPERIMENTAL PROCEDURES  

Bacteria, Culture Media, and Growth Conditions  

A list of strains, plasmids, and their genotypes are presented in Table I. Culture media composition, concentrations of antibiotics, and nutritional supplements were as reported (2, 5, 7). Increase in cell density

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1 The abbreviations used are: Chl, cobalamin; NB, nutrient broth; MudI, MudI1734; Tn10(Tc); Tn10DEL16DEL7; PRTase, phosphoribosyltransferase; Me₂Bza, 5,6-dimethylbenzimidazole; α-ribazole-5'-phosphate, synthesizes N1-(5-phospho-α-D-ribosyl)-5,6-dimethylbenzimidazole; α-ribazole-5'-phosphate, α-ribazole-5',α-ribazole-5'-phosphate, cyano-cobalamin; Prp, propionate; PRPP, phosphoribosylpyrophosphate; Tc, tetracycline; Tcr, tetracycline-resistant; Cm, chloramphenicol; Km, kanamycin; Km', kanamycin-resistant; pfu, plaque-forming unit; kb, kilobase pair.
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General Location—The general location of cobB in the chromosome was determined by using the Mud-P22 mapping kit of Benson and Goldman (18, 19). Strain JE2445 was grown in NB medium containing tetracycline and plated on freshly prepared Bochner plates (16, 17) to increase the frequency of isolation of Tc strains. Phage P22 lysates were prepared from a collection of 72 strains each containing one MudP or MudQ insertions in a known site of the chromosome. Lysates were prepared after mitomycin C induction as reported (20) and delivered onto plates by means of a multiprong device. Each prong delivered approximately 10 μl of lysate.

Two- and Three-factor Crosses—A more accurate location of cobB was obtained by 2- and 3-factor crosses with genetic markers within the region suggested by the Mud-P22 mapping experiments to contain cobB. Separate 2-factor crosses were performed using phage lysates grown on strain TN257 (lexABCΔ45 pepT::MudJ oxrB-hb-1624:Tn10dTc) as donor and strain JE2445 (cobB1176::Tn10dTc) as recipient, selecting for Km transductants, patching 500 transductants on NB, Km, EGTA, and replica-printing onto NB, Km, Tc, EGTA, and Nb, Km plates to assess the frequency of loss of the cobB1176::Tn10dTc element. All strains used for 3-factor crosses carried mutations metE205 ara-9 and were derived from strain TR6563. E minimal medium containing glucose (11 mM) was used in all crosses (12). (CN)2Cbi and CCBi were at concentrations described above.

Direction of cobB Transcription—The direction of transcription of cobB was determined to be clockwise by the method of Hughes and Roth (21) as described elsewhere (12). The titer of phage lysates (pfu/ml) grown on the appropriate strains was determined as described (7). The cobB1206::MudJ element was converted to cobB1206::MudA as reported (15) to increase the homology between insertions and increase the frequency of recombination between co-infecting Mud elements. cobT mutant JE1857 (cobT cobB+ his+) was co-infected with phage lysates grown on JE3231 (cobB1206::MudA) and JE1391 (hisF9951::MudA) or JE1392 (hisF9954::MudA) each at an approximate multiplicity of infection of 1. Ampicillin-resistant (Ap+) transductants were selected on NB medium containing Ap (30 μg/ml), incubated overnight at 37 °C, and replica-printed to E minimal medium containing glucose (11 mM) as carbon/energy source and methionine (0.5 mM).

Recombinant DNA Techniques

Plasmid Isolation, Plasmid pCOBB1—A plasmid carrying the wild-type allele of cobB was recovered from a sized pool of Sau3A fragments (~9 kb) obtained by partial digestion of the S. typhimurium. The Sau3A fragments were cloned into the BamHI site of the tetA gene of plasmid pBR328 (kindly provided by C. G. Miller, University of Illinois, Urbana). This plasmid is referred to hereafter as pCOBB1, and it is shown in Fig. 2. Phage P22 grown on the clone bank was used to transduce strain KE2607 (cobB1176::Tn10dTc) cobT109::Mud recA1) to Km resistance.
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on NB, Cm, and EGTA medium. Cm’ transductants were replica-printed to E. minimal medium supplemented with glucose, (CN)2Cbi, and EGTA. Transductants able to grow on this medium were identified by their ability to grow on minimal medium containing (CN)2Cbi and Cm. The resulting ~ 1.3-kb fragment was cloned into plasmid pSU19 cut with the same enzymes.

**TABLE I**

| Strains and plasmids |
|----------------------|
| **Strain** | **Genotype** | **Source/Ref.** |
| E. coli DH5αF | F’endA1 hadR17 (r27 m1) supE44 thi-1 recA1 gyrA (Nal’) relA1 Δ(lacZYA-argF)U169 deoR (80dlacD (lacZ)M15) | K. Sanderson via J. Roth|
| S. typhimurium LT2 TR6583 | metE205 ara-9 | C. Miller |
| Derivatives of TR6583 | | |
| JE1391 | cobT111::Tn10/dEL1(6DEL17)hisF951::Mud1–8 | |
| JE1392 | cobT112::Tn10(dTC)hisF954::Mud1–8 | |
| JE1857 | cobT109::Mud11734 | |
| JE2445 | cobB1176::Tn10(dTC) | |
| JE2501 | cobT109::Mud11734 cobB1176::Tn10(dTC) | |
| JE2593 | cobT109::Mud11734 DELcobB1177 | |
| JE2600 | cobT109::Mud11734 DELcobB1184 | |
| JE2607 | cobB1176::Tn10(dTC) cobT109::Mud11734 recA1 pepT7::Mud1(Cm) | |
| JE2761 | cobB1176::Tn10(dTC) pepT7::Mud11734 | |
| JE2699 | cobB1176::Tn10(dTC) | |
| JE2845 | cobB1206::Mud11734 | |
| JE3231 | cobB1206::Mud1–8 | |
| JE3277 | cobT111::Tn10(dTC) cobB1206::MudJ | |
| JE4241 | cobB1176::Tn10(dTC) cobT109::Mud11734 recA1/pCOBB1 | |
| JE4342 | cobB1176::Tn10(dTC) cobT109::Mud11734 recA1/pCOBB2 | |
| JE4343 | cobT109::MudJ DELcobB1184/pCOBB3 | |
| JE4344 | cobB1176::Tn10(dTC) cobT109::Mud11734 recA1/pCOBB4 | |
| JE4345 | cobB1176::Tn10(dTC) cobT109::Mud11734 recA1/pCOBB5 | |
| JE4349 | DEL299hisG-cobBgpG1–2 T7 rpo+ kan’/pCOBB6 | |
| JE4350 | DEL299hisG-cobBgpG1–2 T7 rpo+ kan’/pT7–7 blas+ | |

| Plasmids | Tn10d | -7 |
|----------|------|----|
| pT7-5–6, 7 | Overexpression vectors, bla+ | 35 |
| pGP1–2 | T7 t3 rpo- kan+ | 35 |
| pSU19 | Cloning vector, cat+ | 25 |
| pCOBB1 | ~ 9-kb Sau3A fragment containing cobB+ cloned into the BamHI site of tetA in plasmid pBR328 (bla+ cat+) | |
| pCOBB2 | ~ 5.5-kb EcoRI fragment of pCOBB1 containing cobB+ cloned into pSU19 (cat+) | |
| pCOBB3 | pCOBB2 cobB1176::Tn10(dTC) | |
| pCOBB4 | ~ 1.8-kb Sall fragment of pCOBB2 containing cobB+ cloned into pSU19 (cat+) | |
| pCOBB5 | ~ 1.3-kb Sall-Ndel cobB+ fragment of plasmid pCOBB4 cloned into pSU19 (cat+) | |
| pCOBB6 | ~ 1-kb Sdel-NruI cobB+ fragment of plasmid pCOBB5 cloned into overexpression vector pT7–7 (bla+ | |

a Formerly SA2929.

b Abbreviated in the text as Tn10(dTC).

c Abbreviated in the text as MudA.

d Abbreviated in the text as MudJ.
nucleotide sequence of cobB is available from GenBank™ under the accession number U89687.

Biochemical Techniques

Preparation of Cell-free Extracts—Two-liter cultures of strain JE4349 grown under overexpression conditions (see above) were harvested by centrifugation (10,415 × g) at 4 °C for 10 min with a Sorvall GSA rotor and RC-5B refrigerated centrifuge (DuPont). Cell-free extracts were obtained by sonication using a Sonic Dismembrator model 550 (Fisher). For this purpose, cells were resuspended in 50 mM Tris-Cl buffer, pH 7.5, and sonicated twice at 50% duty at a setting of 3 for 5 min. Cell-free extracts were allowed to cool for 2 min on ice after the first 5-min sonication period. Cell debris was discarded by centrifugation at 43,140 × g at 4 °C using a Sorvall SS34 rotor (DuPont).

Phosphoribosyltransferase (PRTase) Enzyme Activity Assay—In vitro experimental conditions described by Trzebiatowski and Escalante-Semerena (4) for assaying the activity of the CobT protein were used to test for PRTase activity in cell-free extracts enriched for CobB. Cell-free extracts containing high amounts of the CobB enzyme (Fig. 4) were obtained from strain JE4349, a strain carrying a deletion of cobT and the overexpression plasmid pCOBB6. The typical reaction mixture contained NaMN (20 nmol) [2-14C]Me2Bza (40 nmol, specific activity = 31.5 μCi/μmol) glycine/NaOH buffer, pH 10, crude cell-free extract (63 μg of protein) in a final volume of 20 μl. The reaction mixture was incubated at 37 °C for 15 min and terminated by heating to 100 °C for 10 min. Denatured protein was pelleted at room temperature at 4,800 × g for 5 min in a Marathon 13K/M microcentrifuge. Products and reagents in the mixture were resolved by TLC on silica using a CHCl3:MeOH (3:2) solvent system. The product of the reaction, α-ribazole-5′-phosphate, was obtained at the origin and was clearly separated from radiolabeled Me2Bza, hence it became a Cbl auxotroph. The fact that strains with this phenotype were isolated strongly supported our hypothesis for the existence of an alternative phosphoribosyltransferase enzyme that can compensate for the lack of CobT in the assembly of the nucleotide loop of cobalamin in cobT mutants (2, 4). When the cobBI176::Tn10d(Tc) mutation was introduced into the Cbl-proficient strain TR6583 (cbl−cobB−), the resulting strain JE2445 was still able to synthesize Cbl from (CN)2Cbi in the absence of exogenous Me2Bza. This result suggested that cobB function may only be required for AdoCbl biosynthesis under some physiological conditions. We noted, however, a <2-fold but reproducible effect of a mutation in cobB on the doubling time of cultures of cobB mutants even when Cbl was provided in the medium (compare the doubling times of strains JE2445 and TR6583, Table II).

RESULTS

Isolation of cobB Mutants—A total of 30 cobB mutants were isolated. Of these, 10 carried independently isolated cobB deletions, 1 carried a cobB::Tn10d(Tc) insertion, 4 carried a cobB::MudJ insertion, and 15 carried independently isolated, hydroxylamine-generated point mutations.

Cobalamin Biosynthesis (Cob) Phenotype of cobB Mutants—Data presented in Table II illustrate the effect of cobB mutations on Cbl-dependent growth of cobT mutants of S. typhimurium. Unlike strain JE1857 (cobB+ cobT−), strain JE2501 (cobB+ cobT−) was unable to synthesize Cbl from (CN)2Cbi (Fig. 6). However, a 7-day-old culture of strain JE2501 was still able to synthesize Cbl from (CN)2Cbi in the absence of exogenous Me2Bza. This result suggested that cobB function may only be required for AdoCbl biosynthesis under some physiological conditions. We noted, however, a <2-fold but reproducible effect of a mutation in cobB on the doubling time of cultures of cobB mutants even when Cbl was provided in the medium (compare the doubling times of strains JE2445 and TR6583, Table II).

Propionate (Prp) Phenotype of cobB Mutants—We previously reported that insertions in cobB prevented S. typhimurium from using propionate as carbon/energy source (14). We assessed the Prp phenotype of 15 independently isolated cobB point mutants to investigate if this phenotype was due to polar effects of the insertions on a gene downstream of cobB. All point mutants displayed both Cob− and Prp− phenotypes, strongly suggesting that the lack of CobB, and not of a gene 3′ to cobB, was responsible for both of the observed phenotypes. This idea proved to be correct since introduction of plasmid pCOBB5 (carried only the cobB+ gene) into JE2501 (and its recombination-deficient derivative strain JE2607) was sufficient to allow the cell to synthesize Cbl from (CN)2Cbi in the presence of
exogenous Me₆Bza (Table II) and to use propionate as the sole source of carbon and energy (data not shown).

Genetic Mapping—cobB was located to the 27-centisome region of the chromosome using Benson and Goldman’s (18) mapping kit. Two-factor crosses demonstrated that cobB was co-transducible with genetic markers in the 27-centisome region. phoQ was 25% co-transducible with cobB, whereas pepT was 82% co-transducible with cobB. Strain JE2699 (cobB pepT) was constructed for the purpose of determining the gene order of cobB relative to phoQ and pepT using 3-factor crosses. The rare class of recombinant strain (relative frequency of 0.22%, or 1 in 451 analyzed) (Table III) can be explained as the product of four recombination exchanges if the gene order was cobB pepT phoQ. These results were consistent with our sequencing data which placed cobB 3’ to the potABCD whose location relative to pepT and phoQ is known in Escherichia coli (32) and S. typhimurium (33).

Analysis of cobB and Its Product—The nucleotide sequence of cobB and the predicted primary sequence of the protein is shown in Fig. 3. Also included in Fig. 3 is the location of the cobB1176::Tn10d(Tc) element. We have tentatively assigned the AGAG sequence located 11 bp away from the methionine codon as the ribosome-binding site (also known as Shine-Dalgarno). Other putative Shine-Dalgarno sequences are located 16 bp (GAGA) and 29 bp (GAGGA) away from the translation initiation codon. Putative promoter sequences, i.e. the −10 and −35 regions, for cobB were identified 217 and 240 bp 5’ to cobB, respectively; these sequences were separated by 17 bp. Whereas the −35 sequence is close to the consensus sequence TTGACA (4 out of 6 match), the putative −10 sequence is notably away from the consensus sequence TATAAT. It should be emphasized that both the Shine-Dalgarno and promoter sequences are putative, and their role in cobB expression needs to be demonstrated.

We note that the analysis of the cobB sequence showed that this gene lacked its native promoter in plasmid pCOBB1 which was isolated from the gene library. Only 21 bp separated cobB from vector sequence. However, since this plasmid was isolated as capable of complementing the phenotypes of cobB mutants, we inferred that expression of the promoterless cobB was under the control of an unidentified promoter within the vector. No effort was made to identify such a promoter.

Computer comparisons of the cobB and CobB sequences with available gene and protein sequence data bases failed to identify any genes of known function. Interestingly, neither cobB nor CobB showed homology to cobT or CobT. The cobB homolog in E. coli, however, is annotated as a putative member of the SIR2 family of proteins (32). The residues that constitute the core domain of these proteins is underlined in Fig. 3. There are differences in the location of the cobB gene in S. typhimurium and E. coli. In E. coli the potABCD operon and the cobB homolog are separated by 1,424 bp, whereas in S. typhimurium the intervening sequence between these loci is only 81 bp, suggesting that the region 5’ to cobB may also be different in S. typhimurium.

**Table II**

| Strain   | Relevant genotype          | Doubling time (min) on minimal medium* supplemented with (CN),Chi | (CN),Chi,DMB | CNChl |
|----------|----------------------------|---------------------------------------------------------------|--------------|-------|
| TR5683   | cobT' cobB'                | 48                                                           | 54           | 48    |
| JE2445   | cobT' cobB1176::Tn10d(Tc)  | 75                                                           | 84           | 75    |
| JE1857   | cobT109::MudJ cobB'        | NG*                                                          | 54           | 57    |
| JE2501   | cobT109::MudJ cobB1176::Tn10d(Tc) | NG            | 60           |       |
| JE4345   | JE2501/pCOBB5 cobB        | NG                                                          | 61           | 52    |

*NG, no growth.

ASIC Mononucleotide:5,6-Dimethylbenzimidazole PRTase Activity in Cell-free Extracts Enriched for CobB—To facilitate the biochemical analysis of the CobB function, the level of CobB in the cell was increased to ~30% of the total protein by placing cobB under the control of a phage T7 promoter and ribosome binding site (Fig. 4, lane C). Cell-free extracts obtained from the cobB overexpressing strain JE4349 were used to test if CobB had CobT-like NaMN:Me₆Bza PRTase activity. Data presented in Fig. 5, A and B, show a correlation between the presence of CobB in the extract and the synthesis of α-ribazole-5’-phosphate (Fig. 5A, *Rxn. product*). In contrast, assay mixtures containing cell-free extract of control strains that did not carry the overexpression plasmid pCOBB6 failed to synthesize α-ribazole-5’-phosphate as judged by the lack of growth of the indicator strain JE2607 (cobB' cobB') (Fig. 5B, *Rxn. product*). As expected, reaction mixtures lacking a source of protein failed to stimulate growth of the indicator strain. Although these results suggest that CobB has PRTase activity, a demonstration of activity using homogeneous CobB protein is needed.

**DISCUSSION**

CobB, a Putative New NaMN:Me₆Bza Phosphoribosyltransferase Enzyme—The gene encoding the alternative enzyme for CobT has been characterized both physically and genetically. From the data in hand, it appears that cobB is monocistronic and not part of an operon. One interpretation of the genetic and biochemical data presented in this paper would be that cobB encodes a new NaMN:Me₆Bza phosphoribosyltransferase enzyme that is specific for NaMN, and as with CobT, phosphoribosylpyrophosphate (PRPP) does not substitute for NaMN in the reaction.² If CobB has this activity, it is not as efficient an enzyme as CobT. Whereas CobB can satisfy the cell requirement for cobalamin for the purpose of synthesizing methionine, CobB phosphoribosyltransferase activity is not sufficient when the demand for cobalamin is increased, e.g. during growth on ethanolamine or 1,2-propanediol as carbon and energy source. A different interpretation would suggest that CobB is not an enzyme, but it is somehow required for the synthesis of the alternative phosphoribosyltransferase enzyme. This possibility is discussed below.

CobB Is a Member of the SIR2 Family of Eucaryotic Regulatory Proteins—We have previously reported the requirement of CobB for the catabolism of propionate in *S. typhimurium* (14). Although no specific biochemical activity for CobB in this system has been identified so far, we know that a lack of cobB function prevents transcription of the prpBCDE operon which encodes the enzymes responsible for the degradation of propionate in this bacterium (34).³ This observation is interesting for the following reasons. The CobB protein appears to be a...
CobB, a Procaryotic Member of the SIR2 Family of Regulators

The identification of CobB as a member of the SIR2 family of regulators would be consistent with the effect of CobB on the expression of the *prpBCDE* operon. In light of this finding, we are currently addressing the possibility that CobB may not have the alluded enzymatic activity, but instead it may be required for the expression of the alternative PRTase enzyme. Unequivocal assessment of the enzymatic activity of CobB requires homogeneous protein. This work is in progress.

CobB, an Enzyme, Regulator, or Both?—

The presence of PRTase activity in regulatory proteins is not unprecedented. The PyrR protein of *Bacillus subtilis* is a member of a family of proteins that regulate transcription attenuation by binding to mRNA (37–42). PyrR is also a PRPP-dependent uracil phosphoribosyltransferase, but it is not clear whether this activity is needed for the regulatory function of PyrR. In fact, the physiological significance of the uracil phosphoribosyltransferase activity of PyrR in *B. subtilis* is also unclear since at physiological pH this activity is very low (43). In addition, this bacterium contains an alternative enzyme encoded by the *upp*

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**TABLE III**

| Location of cobB relative to nearby markers |
|--------------------------------------------|
| Donor TN2644: phoQ::Tn10d(Cm) pepT::cobB* | Recipient JE2699: pepT::MudJ(Km) cobB::Tn10d(Tc) phoQ* |

| Frequency of other phenotypes of selected (Cm*) transductants* | No. exchanges | No. recombinants | Relative frequency |
|---------------------------------------------------------------|--------------|-----------------|-------------------|
| Km R* Tc                                                      | 2            | 236             | 52.4              |
| Km R Tc                                                       | 4            | 1               | 0.22              |
| Km R* Tc                                                      | 2            | 45              | 10.0              |
| Km S* Tc                                                      | 2 (donor type)| 169             | 37.5              |

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* Cm-resistant transductants were selected on NB medium containing Cm (20 μg/ml) and EGTA (10 mM) after a 2-h preincubation of the bacteria/phage suspension at 30 °C without shaking. The inferred map order was cobB pepT phoQ.

* The abbreviations used are: R, resistant; S, sensitive.
gene (44). It has been suggested that PyrR evolved from an ancestral PRTase in which UMP and PRP-binding sites were retained and an RNA-binding surface arose (45). Unlike PyrR, work presented herein shows that the putative PRTase activity of CobB is physiologically significant. CobB may be the first NaMN-dependent PRTase with regulatory function.

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