Annu. Rev. Microbiol. 1996. 50:137–81

COBALAMIN (COENZYME B_{12}): Synthesis and Biological Significance

JR Roth, JG Lawrence, and TA Bobik
Department of Biology, University of Utah, Salt Lake City, Utah 84112

KEY WORDS: B_{12}, propanediol, metabolism, *Salmonella* spp., corrinoids

ABSTRACT

This review examines deoxyadenosylcobalamin (Ado-B_{12}) biosynthesis, transport, use, and uneven distribution among living forms. We describe how genetic analysis of enteric bacteria has contributed to these issues. Two pathways for corrin ring formation have been found–an aerobic pathway (in *P. denitrificans*) and an anaerobic pathway (in *P. shermanii* and *S. typhimurium*)–that differ in the point of cobalt insertion. Analysis of B_{12} transport in *E. coli* reveals two systems: one (with two proteins) for the outer membrane, and one (with three proteins) for the inner membrane. To account for the uneven distribution of B_{12} in living forms, we suggest that the B_{12} synthetic pathway may have evolved to allow anaerobic fermentation of small molecules in the absence of an external electron acceptor. Later, evolution of the pathway produced siroheme, (allowing use of inorganic electron acceptors), chlorophyll (O_2 production), and heme (aerobic respiration). As oxygen became a larger part of the atmosphere, many organisms lost fermentative functions and retained dependence on newer, B_{12} functions that did not involve fermentation. Paradoxically, *Salmonella* spp. synthesize B_{12} only anaerobically but can use B_{12} (for degradation of ethanolamine and propanediol) only with oxygen. Genetic analysis of the operons for these degradative functions indicate that anaerobic degradation is important. Recent results suggest that B_{12} can be synthesized and used during anaerobic respiration using tetrathionate (but not nitrate or fumarate) as an electron acceptor. The branch of enteric taxa from which *Salmonella* spp. and *E. coli* evolved appears to have lost the ability to synthesize B_{12} and the ability to use it in propanediol and glycerol degradation. *Salmonella* spp., but not *E. coli,* have acquired by horizontal transfer the ability to synthesize B_{12} and degrade propanediol. The acquired ability to degrade propanediol provides the selective force that maintains B_{12} synthesis in this group.

0066-4227/96/1001-0137$08.00

CONTENTS

INTRODUCTION

Several questions surround the cofactor cobalamin (coenzyme B_{12}). Its large size and chemical complexity (84, 131) have made the study of its biosynthesis a challenge (9, 10, 146, 147). Cobalamin's uneven distribution among modern life-forms and its proposed prebiotic origins raise questions regarding its general biological significance. Although cobalamin is made by some bacteria and is essential to humans (it was discovered by its ability to cure pernicious anemia), it seems to play no role in the metabolism of plants, fungi, or some bacteria (46, 97, 107, 123). Why do some organisms continue to use this cofactor, whereas others flourish without it?

The discovery of B_{12} synthesis and use in bacteria with well-developed genetic systems has made it possible to apply genetic methods to these broad questions. The synthesis, transport, physiological importance, and evolution of this complex cofactor can be studied in the single organism *Salmonella typhimurium.* We review here the general area of B_{12} metabolism research, with an emphasis on biological uses revealed by genetic studies of enteric bacteria.

Structure of Cobalamin

The magnitude of the synthetic problem can be seen in Figure 1, which diagrams the structure of one form of B_{12}: 5′ deoxyadenosylcobalamin (Ado-B_{12}).

Ado-B_{12} has a molecular weight of 1580, and at least 25 enzymes are uniquely involved in its synthesis. The Ado-B_{12} molecule has three parts: a central ring, an adenosyl moiety, and a nucleotide loop. The central ring is structurally and biosynthetically related to those of heme and chlorophyll. The ring found in B_{12} differs from related rings by its lack of the carbon bridge between the A and D porphyrins (see Figure 1), by the ring oxidation state, by the distribution of ring decorations (methyl, acetamide, and propionamide), and by the central cobalt atom. Ado-B_{12} also has a 5′ deoxyadenosyl moiety serving as its upper (Coβ) axial ligand; the 5′ carbon of this ribose group is joined by a covalent bond to the cobalt within the corrin ring. Homolytic cleavage of the cobalt-carbon bond is central to catalysis of intramolecular rearrangement reactions (154). Cobalt's lower (Coα) axial ligand is the N-7 of dimethylbenzimidazole (Dmb). The Dmb moiety is attached covalently to the corrin ring as part of a nucleotide loop. The nucleotide, 3′ phosphoribosyl-Dmb, is linked through its phosphate to an aminopropanol moiety that is attached to a propionyl group extending from the D porphyrin of the corrin ring.

Distinct forms of cobalamin exist with upper and/or lower ligands that are different from those described above. The cofactor for methyltransferases is methylcobalamin as cofactor, in which the deoxyadenosyl moiety is replaced by a methyl group (145). Cobalamin is prepared commercially with a cyano group as the Coβ ligand; this form (CN-B_{12}) is not found in nature but frequently is used as a nutrient for humans and for bacterial mutants. Alternative forms of B_{12} with different bases in place of Dmb have been identified in various Bacteria and Archaea (35, 63, 72, 156, 157). These corrinoids appear to be isofunctional; no correlation has been made between the nucleotide used as the lower ligand and the function or distribution of a corrinoid (156).

Ancient Origins of Cobalamin

Many authors have suggested that B_{12} was synthesized prebiotically (13) and may have been important to catalysis in the "RNA world." In some bacteria, B_{12} biosynthesis begins with an aminoacyl-tRNA molecule, and an RNA molecule

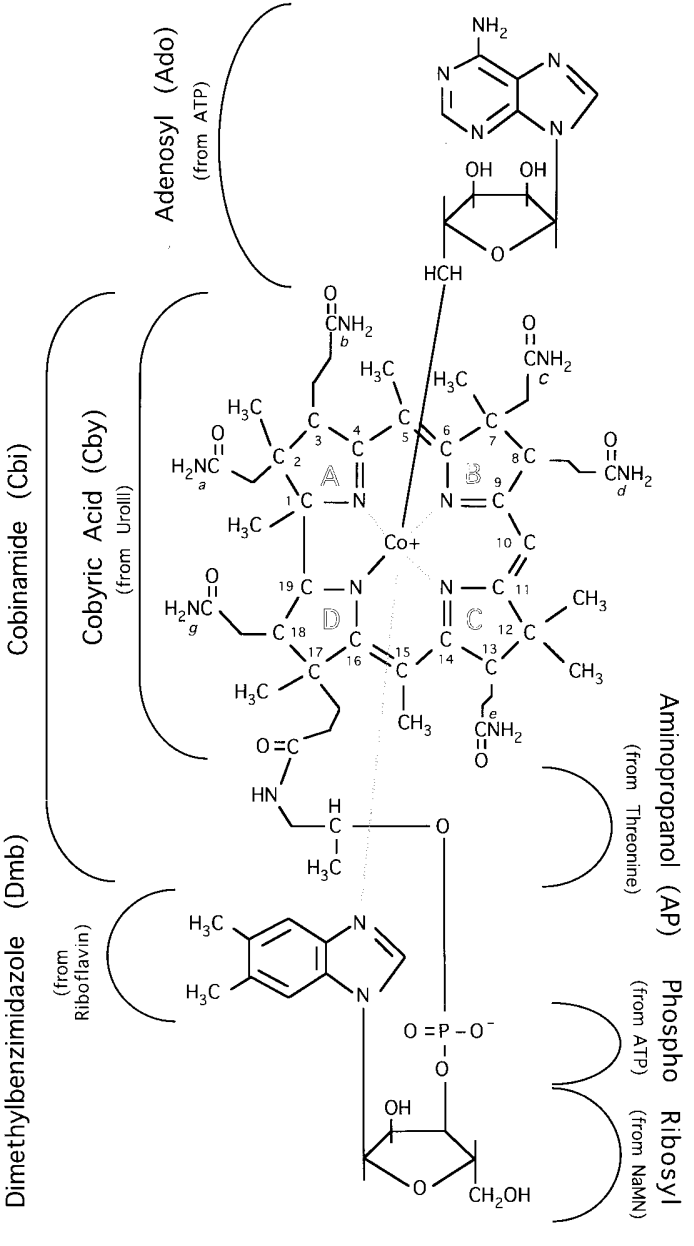

Figure 1 Structure of 5′ deoxyadenosylcobalamin, coenzyme B_{12}. Ring carbons are numbered, and sites of amidations are lettered in italic lower case. Porphyrin rings are designated with capital letters. Name, abbreviation, and source of each moiety are noted.

may play an intimate role in regulating expression of biosynthetic and transport genes (see below). Porphyrins such as those contributing to the B_{12} structure have been synthesized nonenzymatically in so-called primitive earth experiments (85), and Eschenmoser has commented eloquently on how the complex B_{12} structure can be formed by energetically favorable reactions (62).

The cobalamin biosynthetic pathway, and the pathways common to heme, chlorophyll, and siroheme synthesis, may reflect the evolution of energy metabolism (see Figure 2). It seems likely that this pathway first evolved to produce B_{12}, a view supported by considering the structure of UroIII (62), the common precursor of heme, siroheme, chlorophyll, and cobalamin. UroIII is asymmetric in the sense that one of the porphyrin rings is reversed with respect to the others. The reversal may be important for the carbon elimination (ring contraction) that occurs later in cobalamin synthesis (see below). This arrangement indicates that the entire pathway developed initially to serve B_{12} synthesis and later added the branches to siroheme, heme, and chlorophyll.

We suggest below that the initial significance of B_{12} was to support anaerobic fermentation of small molecules by generating internal electron sinks. Later, siroheme allowed inorganic molecules to be used as electron acceptors, which is seen in siroheme's modern role as a cofactor for sulfite and nitrite reductases. Still later arrivals may have been chlorophyll and heme, which allowed biological formation of molecular oxygen and use of oxygen as a respiratory electron acceptor.

Distribution of B_{12} Among Living Forms

According to a widely accepted historical view, B_{12} synthesis is restricted to some Bacteria and Archaea. Many animals (including humans) and protists require B_{12} but apparently do not synthesize it. Plants and fungi are thought to neither synthesize nor use B_{12} in their metabolism (58). Figure 3 superimposes the distribution of B_{12} on a prominent view of the evolutionary relationships between these life-forms.

Figure 3 represents, at best, general conclusions. Some possible exceptions have been documented among algae and legume taxa (119, 170, 171). Some reports of B_{12} in plants and fungi may not have considered adequately the difficulty of excluding bacterial contamination. As better assays are made in a variety of organisms and more rigorous nutritional tests are made, more exceptions may surface.

The distribution pattern shown in Figure 3, even with some exceptions, raises questions regarding the biochemical significance of B_{12} and the metabolic differences among life-forms that allow some to escape the need for B_{12}. Such questions should be kept in mind as we review the general nature of B_{12}-dependent reactions. Later we use studies of bacterial mutant phenotypes and

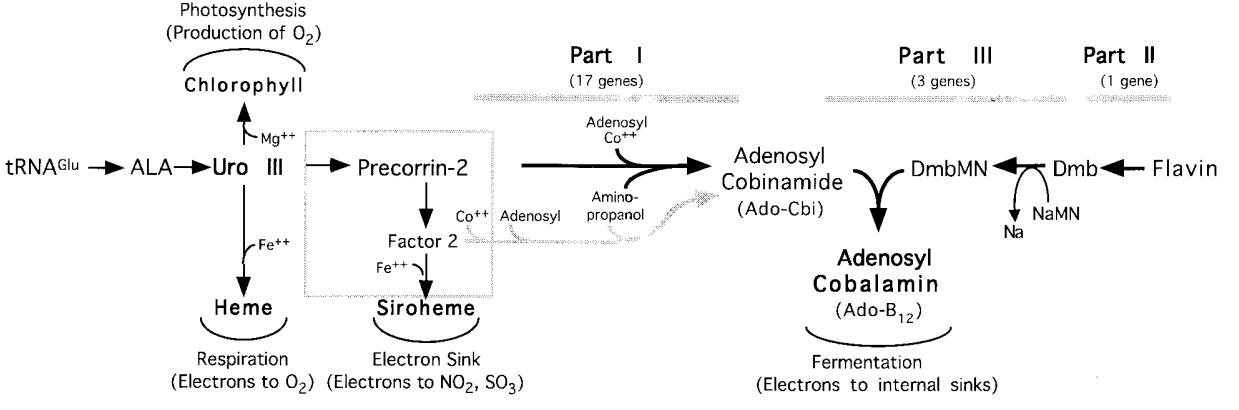

Figure 2 Overview of B_{12} synthetic pathway. Parts I, II, and III are portions of the cobalamin biosynthetic pathway defined by nutritional behavior of mutants (see below). More detailed views of this pathway may be seen in Figure 6 (Part I) and Figure 7 (Parts II and III). The boxed reaction highlights activities provided by the single CysG protein of *S. typhimurium/E. coli* (65, 152); the shaded portion indicates activities of the homologous (CobA) protein of *P. denitrificans* (19, 48). The two pathways leading to Ado-Cbi are the aerobic pathway of *P. denitrificans* (top arrow) and the anaerobic pathway of *S. typhimurium* (bottom arrow). General functions of cofactors are indicated above and below the pathway.

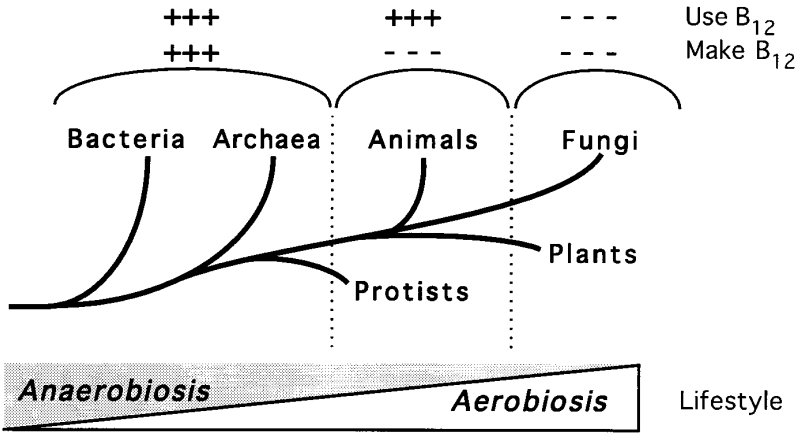

Figure 3 Distribution of cobalamin synthesis and use among living forms. Wedges designate in a general way the evolutionary and current importance of oxygen to organisms in each group.

regulatory behavior of B_{12} synthetic enzymes to illustrate additional puzzles regarding the biological significance of B_{12} within enteric bacteria.

B_{12}-DEPENDENT REACTIONS

In trying to understand the evolutionary history of B_{12}, we have looked for fundamental properties of B_{12}-catalyzed reactions that might explain the evolution and later loss of B_{12} synthesis from various groups. Fermentation may be that common feature. Many of the known B_{12}-dependent reactions in a variety of bacteria support anaerobic fermentation of small molecules. Fermentation, as used in this review, refers to anaerobic growth without an exogenous electron acceptor. In this situation, redox reactions must be internally balanced, and ATP must be produced by substrate-linked phosphorylations. Some reduced compound is excreted to get rid of excess reducing equivalents. In enteric bacteria most fermentable carbon sources are large sugars.

We propose that the original significance of B_{12}, and its remaining primary role in many modern bacteria, may be to support fermentation of small molecules by catalyzing molecular rearrangements that generate both an oxidizable compound and an electron sink for use in balancing redox reactions. In enteric bacteria, this role is seen in the B_{12}-dependent degradation of ethanolamine, propanediol, and glycerol (see Figure 4). In these reactions, the B_{12}-mediated rearrangement generates an aldehyde that can be oxidized and provide ATP; the oxidation reactions can be balanced by reducing a portion of the

aldehyde to an alcohol, which is excreted. The B_{12}-dependent reaction forms the reducible compound by a rearrangement, essentially an internal redox reaction.

In nonenteric bacteria, the B_{12}-dependent amino mutases (specific for glutamic acid, lysine, leucine, or ornithine) catalyze mechanistically similar reactions that support fermentation of these amino acids (154). In methanogens, B_{12} plays a role as a carrier of methane and in essence helps provide a means for getting rid of reducing equivalents.

Thus the earliest use of B_{12} may have been to support anaerobic, fermentative growth at the expense of small molecules. Additional reactions (such as methionine synthesis and nucleotide reduction) appeared as secondary uses. After oxygen and aerobic respiration appeared on earth, many organisms no longer needed to perform fermentations and lost some of their original enzymatic capabilities. The secondary uses such as methyl transfer remained important and enforced a continued requirement for B_{12}. Obligate aerobes and animals appear to require B_{12} to perform these nonfermentative functions. In humans, two B_{12}-dependent reactions are known. Methionine synthetase, a methyl transferase, is presumed to be important primarily in recycling folate and secondarily in producing methionine (3). Methyl malonyl CoA mutase may serve mainly to remove toxic products of lipid breakdown (101).

B_{12}-Dependent Reactions in Enteric Bacteria

The enzymes listed below are found in one or more species of enteric bacteria. The catalyzed reactions are diagrammed in Figure 4.

PROPANEDIOL DEHYDRATASE This enzyme, which converts 1,2-propanediol to propionaldehyde, is found in virtually all enteric bacteria tested except *Escherichia coli* (100, 165). Some bacteria ferment propanediol by oxidizing a portion of the propionaldehyde to provide carbon and energy while reducing the rest to provide an electron sink for balancing redox reactions (114). In *Salmonella* species this process provides energy but no carbon source. Propanediol is encountered frequently by bacteria, because it is produced during anaerobic catabolism of the common methylpentoses, rhamnose and fucose (102).

ETHANOLAMINE AMMONIA LYASE This enzyme converts ethanolamine to acetaldehyde and ammonia (27, 144). Under some conditions, the produced acetaldehyde can serve as a carbon and energy source via acetyl-CoA. Ethanolamine is frequently encountered in nature as part of common lipids, phosphatidyl ethanolamine and phosphatidyl choline.

GLYCEROL DEHYDRATASE This enzyme converts glycerol to β-hydroxypropionaldehyde, which can be reduced to 1, 3 propanediol (1, 71). This reaction balances the reducing equivalents generated by glycerol dehydrogenase.

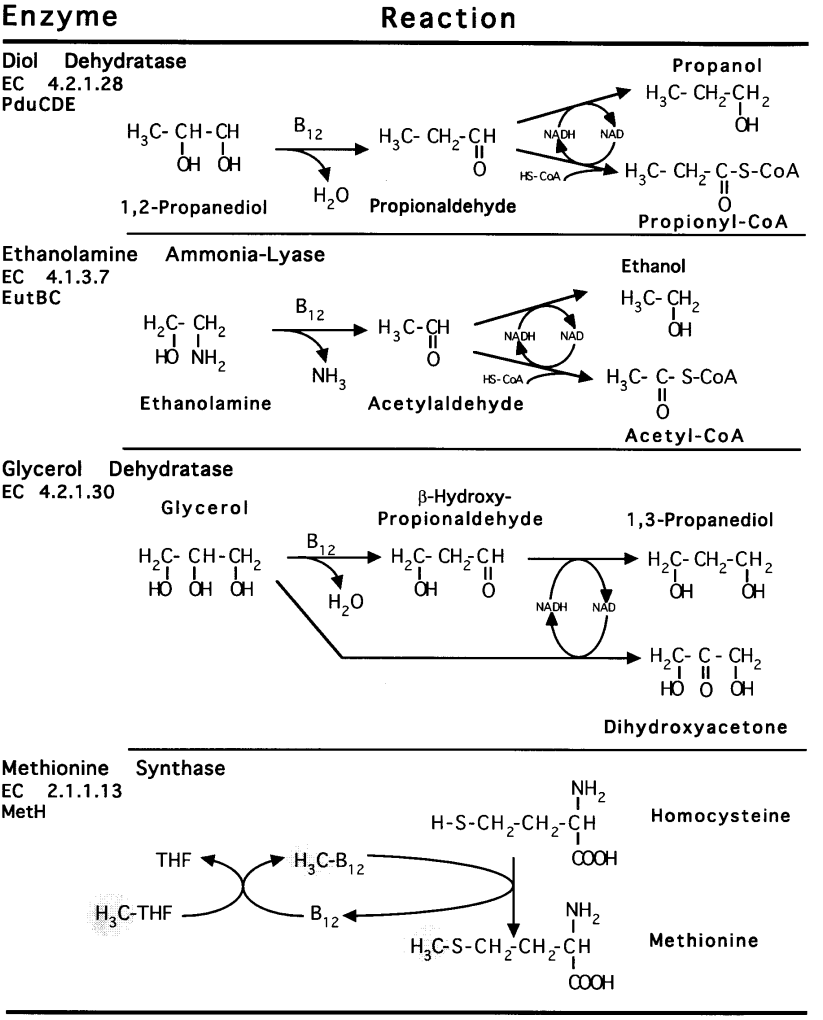

Figure 4 Cobalamin-dependent pathways known for enteric bacteria. Only the first reaction for each pathway employs cobalamin as a cofactor. For each B_{12}-dependent enzyme, the *S. typhimurium* genetic designation is given. THF indicates tetrahydrofolate.

Glycerol dehydratase is common in enterics but is absent from both *Salmonella* spp. and *E. coli* (100).

METHIONINE SYNTHETASE This enzyme transfers a methyl group from methyltetrahydrofolate to homocysteine as the final step in synthesis of methionine and is probably the best-known B_{12}-dependent reaction (57, 162). The lack of this reaction underlies many aspects of human B_{12}-deficiency disorders (3).

In *Salmonella* spp. and in *E. coli*, the B_{12}-dependent methyl transfer reaction is catalyzed by the MetH enzyme, and both organisms produce a second enzyme (MetE) that can catalyze the same reaction without B_{12} (40, 44, 57, 151). The MetH enzyme is used preferentially when B_{12} is available; the alternative MetE enzyme is induced in response to accumulated homocysteine when the MetH enzyme is inactive (175). In bacteria with an alternative, B_{12}-independent enzyme, methionine synthesis is unlikely to be the major selective force maintaining B_{12} synthetic capacity.

EPOXYQUEUOSINE REDUCTASE This enzyme performs the last step in formation of the hypermodified tRNA base, queuosine, found in $tRNA^{tyr}$, $tRNA^{his}$, $tRNA^{asn}$, and $tRNA^{asp}$ (73). The modified base is not essential for bacterial growth under laboratory conditions (113). Although the final reaction has been reported to require B_{12} (73), it proceeds in anaerobically grown *E. coli* cells, which do not make B_{12} (100). We suggest that the reaction may be stimulated by B_{12}, perhaps indirectly, in vivo, but can be catalyzed without this cofactor.

Other Prominent B_{12}-Dependent Reactions

ACETYL-COA SYNTHESIS In many anaerobic bacteria, methyl-corrinoids are involved in acetyl-CoA synthesis via the Wood/Ljungdahl pathway (125, 155, 174). In this pathway, a methyl group is transferred from methyltetrahydofolate via a methyl-corrinoid/iron sulfur protein to CO-dehydrogenase, which synthesizes acetyl-CoA from this methyl group, CO, and coenzyme A (69). A corrinoid plays an analogous role in the energy-yielding metabolism of acetogenic bacteria (103, 125), which synthesize acetate from 2 CO_2 as a means of generating a terminal electron sink.

METHYL TRANSFER IN THE METHANE-PRODUCING ARCHAEA Methyl-corrinoids are essential for formation of methane by the strictly anaerobic methane-producing Archaea (68, 155). Corrinoid proteins play a role in the transfer of methyl groups from methanogenic substrates to the thiol group of the methanogen-specific cofactor, coenzyme M. Different enzymes mediate methyl transfer from alternative methanogenic substrates such as acetate (67), methylamines (36), methanol (95), pyruvate (25) and methyltetrahydromethanopterin, an intermediate of methanogenesis from formate and CO_2 (121, 163). The latter

reaction is analogous to methionine synthesis in that the methyl group is transferred from an intermediate pterin to a thiol group via methyl-B_{12} (121).

Considerable energy is released ($\Delta G'_0$ of approximately -30kJ/mol) by transfer of a methyl group to coenzyme M from methyltetrahydromethanopterin (a natural structural and functional analog of methyltetrahydrofolate) (81, 111, 155, 163). The energy of the transfer to coenzyme M can be recovered by coupling the methyl transfer to extrusion of a sodium ion, which eventually leads to generation of a proton motive force (11, 23). The transferase that achieves this feat is an integral membrane protein (70) composed of eight different subunits (81).

RIBONUCLEOTIDE REDUCTASES Ribonucleotide reductases generate the deoxyribonucleotides needed for DNA synthesis. Four classes of this reductase are known, each with a different cofactor requirement and quaternary structure; this variability is unusual for enzymes that play such key metabolic roles. The Ado-B_{12}-dependent reductases belong to Class II and are found primarily in microorganisms (16, 17). The reaction mechanism and active site structure of the corrinoid-dependent reductase appears remarkably similar to that of the Ado-B_{12}-independent Class I enzyme of *E. coli* (26). In this reductase, Ado-B_{12} serves as a free-radical generator. Other reductases form radicals by alternative means (126).

DEGRADATION OF β-HYDROXY ETHERS AND β-HYDROXY AMINES Corrinoids are implicated in the anaerobic degradation of several compounds thought to be generally recalcitrant to degradation in the absence of oxygen. The compounds include polyethylene glycol (77), triethanolamine (78), and possibly phenoxyethanol (76). The significant chemical feature of these compounds is a hydroxyl group β to an ether, a tertiary amine, or a secondary amine. These reactions are related to those catalyzed by diol dehydratases in that they are intramolecular redox reactions that involve the migration of a hydroxyl group.

METHYLMALONYL-COA MUTASE Methylmalonyl-CoA mutase interconverts (R)-methylmalonyl-CoA and succinyl-CoA. Higher animals require the mutase for degradation of odd-chain-length fatty acids and certain branched-chain amino acids. In humans, mutase deficiency results in an often fatal methylmalonic acidemia (101), and in certain neuropsychiatric symptoms. These symptoms may result from synthesis of abnormal myelin lipids in the presence of accumulated propionyl- and methylmalonyl-CoA (3, 124).

In certain bacterial fermentations, succinate is converted to propionate via the mutase rather than being excreted (128). This pathway allows conservation of a biotin-activated CO_2 that is derived from succinate. In *Streptomyces*

cinnamonensis, the mutase may also play a role in polyketide antibiotic synthesis (14).

GENERAL ASPECTS OF B_{12} SYNTHESIS

In constructing Ado-B_{12}, multiple components are synthesized individually and then assembled (see Figure 2). This pattern is curiously more common for vitamins than for other metabolites. The largest component of B_{12} is the corrinoid ring, synthesized from uroporphyrinogen III (UroIII), a precursor common to heme, siroheme, F430, and cobalamin. Two distinct pathways have been identified (in different bacteria) for conversion of UroIII to the intermediate adenosylcobinamide (Ado-Cbi), which has the fully modified corrin ring with an attached aminopropanol side-chain (see Figure 1). The major difference between the two pathways is in the time of insertion of cobalt (see below).

The nucleotide loop is assembled by first activating the aminopropanol side-chain of Cbi to form GDP-Cbi. The Coα axial ligand, dimethylbenzimidazole (Dmb), is synthesized separately and converted to a nucleotide (DmbMN) by addition of ribose derived from nicotinic acid mononucleotide (NaMN), an intermediate in the synthesis of NAD. Ultimately, Dmb nucleoside is added to the end of the activated isopropanol side-chain to form the nucleotide loop and complete the synthesis of Ado-B_{12}.

Great progress has been made recently in defining the nature of the cobalamin synthetic pathway. This progress is largely the result of genetic identification of biosynthetic genes. Genes were cloned by complementation of these mutants, and cloned sequences were then used to produce enzymes for biochemical analysis. This approach was pursued in *Pseudomonas denitrificans* and in *Salmonella typhimurium* (10, 147). Synthetic genes also have been cloned from *Bacillus megaterium* (34, 173).

Genetic Analysis of B_{12} Synthesis in S. typhimurium

S. typhimurium has genes for biosynthesis and for transport of cobalamin and uses a B_{12} cofactor in at least three reactions. Figure 5 shows the positions of relevant genes in this organism's genetic map. *S. typhimurium* mutants defective in B_{12} synthesis (*cob, cbi*) were isolated in a parental *metE* mutant, since simple cobalamin-deficient mutants have no easily detectable growth phenotype (see below). Strains of *S. typhimurium* with a *metE* mutation require methionine unless they can synthesize (or are given) cobalamin, which is required by the alternative methionine synthetase, MetH. Since *S. typhimurium* synthesizes B_{12} only during anaerobic growth (86), a simple *metE* mutant can grow anaerobically without methionine by producing its own B_{12} and using the MetH enzyme. Starting with such a *metE* strain, mutants unable to synthesize

B_{12} can be identified anaerobically as methionine auxotrophs correctable by B_{12} or one of its precursors (see Table 1).

Mutants can be assigned to one of the three parts of the pathway by anaerobic nutritional tests. These parts are designated I, II, and III in Figure 2, and gene positions are shown in Figure 5. A mutant for Part I of the pathway can make B_{12} if supplied with cobinamide (Cbi). A mutant for Part II can make B_{12} if Dmb is provided. Mutants for Part III lack ability to join Cbi and Dmb and fail to make B_{12} even when both intermediates are provided (see Table 1). Under aerobic conditions, a *cob*$^+$ *metE* mutant behaves like a Part I mutant because early steps in Part I of the pathway are sensitive to oxygen (148).

Most of the B_{12} synthetic genes are located in a single, 20-gene operon that maps near minute 44 of the *S. typhimurium* chromosome (86, 88, 140). Mutations with defects in Part I of the pathway affect one of the first 17 genes, designated *cbi*. Mutations with a Part III defect affect the next two genes, *cobU* and *cobS* (59, 117). Mutations in the last gene of the operon, *cobT*, cause a Part II defect (42, 166). The CobT protein appears to have multiple activities and acts in both Parts II and III of the pathway (see below).

More detailed functional assignments were allowed by identification of *S. typhimurium* homologues of *P. denitrificans cob* genes (140), whose biochemical roles had been defined (described below). Within the Part I region is a three-gene cluster (*cbiNQO*) that is likely to encode a cobalt transport system (140). This assignment was made by sequence comparisons to known transport genes and by finding that the phenotype of mutations mapping in this region can be corrected by a high concentration of cobalt (D Walter, M Ailion, & J Roth, unpublished data).

As more mutations were classified, several (*cobA*, *cobB*, *cobC*, *cobD*, and *cysG*) were found to map outside of the main operon. These unlinked biosynthetic genes appear to contribute to assimilation of exogenous B_{12} precursors or to play secondary roles in some process other than cobalamin de novo synthesis.

Table 1 Growth phenotypes of *S. typhimurium* mutants

	Growth on methionine-deficient medium[a]											
	Aerobic additions						Anaerobic additions					
Genotype	None	Met	Cbi	Dmb	Cbi + Dmb	B_{12}	None	Met	Cbi	Dmb	Cbi + Dmb	B_{12}
wild type	+	+	+	+	+	+	+	+	+	+	+	+
metE	−	+	+	−	+	+	+	+	+	+	+	+
metE metH	−	+	−	−	−	−	−	+	−	−	−	−
metE cob (Part I)	−	+	+	−	+	+	−	+	+	−	+	+
metE cob (Part II)	−	+	−	−	+	+	−	+	−	+	+	+
metE cob (Part III)	−	+	−	−	−	+	−	+	−	−	−	+

[a]A plus sign indicates growth on the indicated medium; a minus sign indicates the lack of growth.

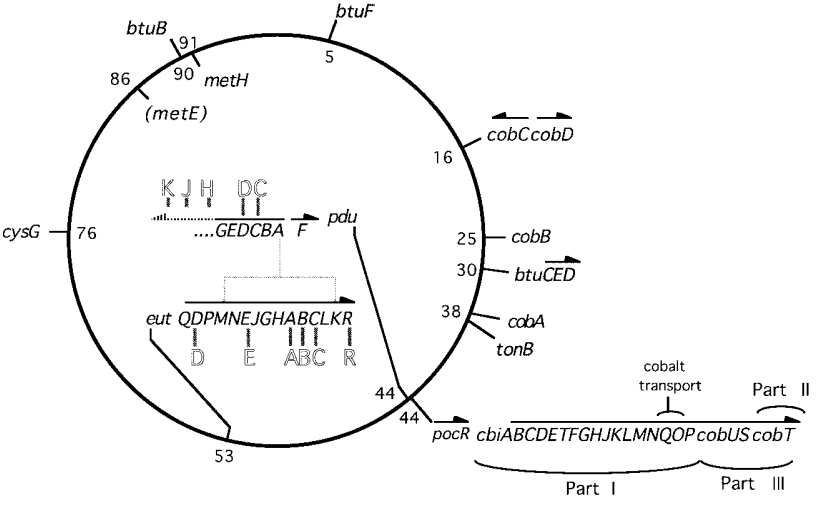

Figure 5 Genes involved in cobalamin synthesis and use in *S. typhimurium.* Map positions are according to Sanderson et al (142). Cobalamin-dependent pathways are positioned on the inside of the map; cobalamin biosynthetic and transport genes are positioned on the outside. Genes involved in cobalamin transport are shaded. Arrows indicate direction of transcription. For the centrally positioned *eut* and *pdu* operons, raised letters indicate genes with an aerobic mutant phenotype; the dashed arrow indicates the unsequenced portion of the *pdu* operon. The functions of genes for Parts I, II, and III of the synthetic pathway (*cbi* and *cob*) are indicated in Figure 2.

Thus the main *cob* operon contains only genes needed for de novo B_{12} synthesis; the unlinked genes may have additional functions that are important even when B_{12} is not being synthesized de novo. The exact functions of some of these unlinked genes is not yet clear. We outline our present understanding of their roles later.

Genetic Analysis of B_{12} Synthesis in P. denitrificans

A large set of cobalamin-defective mutants was isolated both in *Pseudomonas putida* and in *Agrobacter tumefaciens*; the mutants were identified by their inability to degrade ethanolamine unless provided with cobalamin (38). Genomic libraries of *P. denitrificans* were screened for clones that complemented the various mutants. Analysis of the clones revealed four gene clusters (37, 39, 47–49). The locations of these clusters on the *P. denitrificans* chromosome are unknown. The mutants were isolated in the presence of Dmb so that no mutants with simple defects in its synthesis could have been expected. Nevertheless, if the synthesis of Dmb proves to be accomplished by a single multifunctional

enzyme as described below, it is likely that mutants for the Dmb synthetic gene were isolated by virtue their secondary defect in Part III of the pathway.

Comparison of Genes from Salmonella *and* Pseudomonas *spp.*

When DNA sequences of *cob* genes from *P. denitrificans* were compared to those from *S. typhimurium*, many pairs of homologous genes were identified; however, each data set included multiple genes without a homologue in the other species (140). This situation could be explained if each of the two gene identification efforts had missed a different set of genes. It seems more likely that the unmatched genes reflect substantial differences in the biochemistry of the synthetic pathways in the two organisms.

BIOSYNTHESIS OF COBALAMIN

In a monumental effort combining biochemical and genetic work, a detailed picture of the complete biosynthesis of the corrinoid ring in *P. denitrificans* has been developed by studying the activities of proteins produced from individually cloned genes (18–22, 37, 49–51, 164). A similar approach has been taken in *Propionibacterium shermanii* and *S. typhimurium* (135, 143, 149, 152). The results reveal two distinct pathways for corrin ring synthesis; this chemical work has been reviewed recently (10, 147).

Multiple Biosynthetic Pathways to the Corrin Ring

The most extensive analysis of corrinoid ring synthesis was done using *P. denitrificans*, which is able to make B_{12} in the presence of oxygen. In contrast, *P. shermanii* (an anaerobe) and *S. typhimurium* (a facultative anaerobe) make B_{12} only under anaerobic conditions. This difference in lifestyle is reflected in the nature of the B_{12} biosynthetic pathways.

The aerobic pathway of *P. denitrificans* requires at least 20 steps to convert UroIII to Ado-Cbi. These reactions, Part I of the pathway, can be seen in Figure 6. Cobalt insertion, reduction, and adenosylation (Reactions 15–17) occur late in this reaction sequence. This pathway not only proceeds in the presence of oxygen, but includes one step (Reaction 4) that requires oxygen (in vitro) for reoxidation of the enzyme (10). In contrast, both *S. typhimurium* and *P. shermanii* can make Ado-Cbi only in the absence of oxygen. In the anaerobic pathway of these organisms, the initial reactions are oxygen-sensitive and cobalt insertion occurs early in the pathway (109, 110). Thus the cobalamin biosynthetic pathway of one organism may depend on oxygen, whereas the pathway of the others is toxified by oxygen.

In view of these differences, it is not surprising that the two pathways might use nonhomologous enzymes to catalyze some analogous reactions. Most

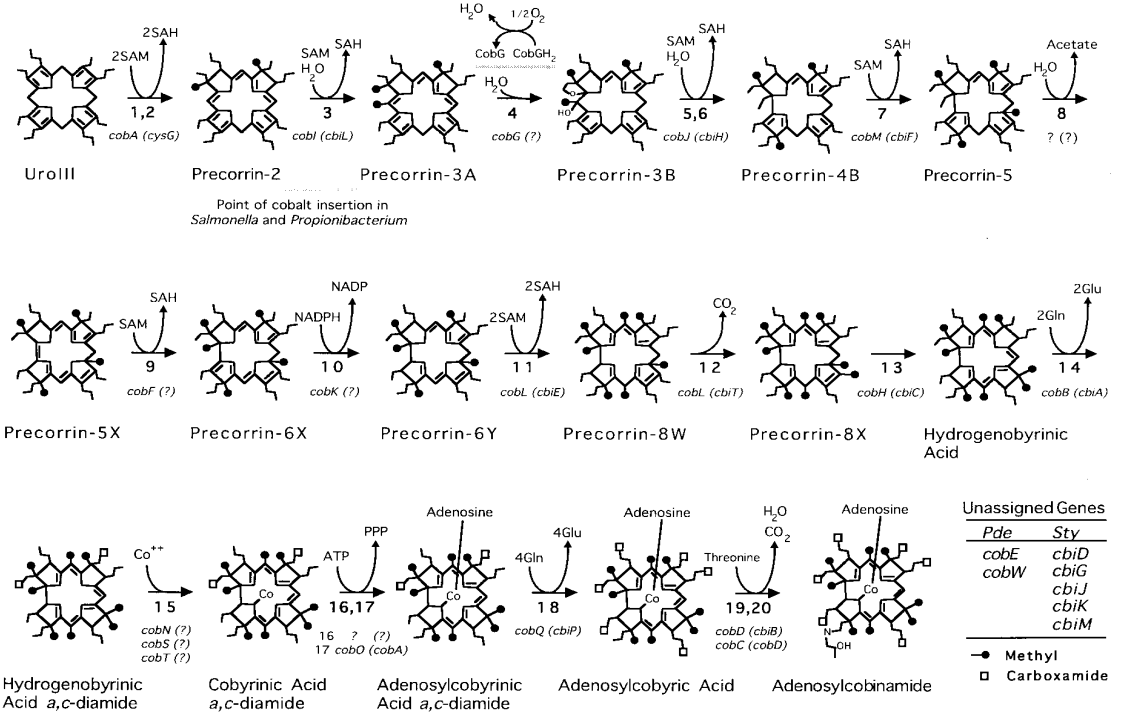

Figure 6 Part I of the aerobic cobalamin biosynthetic pathway. The *P. denitrificans* genes that encode relevant enzymes are listed below each reaction; homologous genes of *S. typhimurium* are in parentheses. Reaction 4 entails the incorporation of an oxygen atom, probably from water, during the reductive lactone formation (10). In this reaction, the enzyme is reduced and can only turn over in the presence of oxygen (52). Reaction 5, the methylation of C17, initiates spontaneous ring contraction (Reaction 6) between C1 and C19 (52). Reaction 7, methylation at C11, leads to the extrusion of acetate (Reaction 8), which can occur spontaneously (10). Reaction 16, reduction of cobalt, is catalyzed by the cobyrinic acid a,c-diamide reductase, whose gene has not been cloned (21). SAM: S-adenosyl methionine; SAH: S-adenosyl homocysteine. No homologues have been identified for any of the unassigned genes in the lower right corner of the figure).

intermediates in the aerobic pathway lack cobalt and, therefore, the upper axial ligand, whereas those in the anaerobic pathway contain cobalt and possibly adenosine. Figure 6 presents the aerobic pathway used by *P. denitrificans.* Note that the *P. denitrificans* genes without a *S. typhimurium* homologue include those involved in the oxygen-dependent step (Reaction 4) and the late cobalt insertion (Reaction 15). Other genes involved in Part I of the pathway but lacking an assigned function are listed in the lower right corner of the figure.

Additional B_{12} pathways may be found since many enteric bacteria can make B_{12} both aerobically (impossible for a pathway of the anaerobic type) and anaerobically (without the oxygen required for the aerobic type) (100). Alternatively these enterics may use the aerobic pathway but are able to regenerate their enzyme for Reaction 4 without molecular oxygen.

The First Enzyme in B_{12} Synthesis

The first two methylations of UroIII are performed by the CysG enzyme of *S. typhimurium*/*E. coli* and by a homologous protein (CobA) of *P. denitrificans.* These reactions generate Precorrin-2, the precursor of both B_{12} and siroheme (see Figures 2 and 6).

The *S. typhimurium* and *E. coli* CysG enzymes are longer than the homologue from *P. denitrificans,* possessing an additional N-terminal region of 201 amino acids. This region appears to be required for catalysis of the additional reactions (ring oxidation and iron insertion) needed for synthesis of siroheme (152). Thus in *S. typhimurium* and *E. coli,* a single protein catalyzes four distinct reactions (see boxed reactions in Figure 2). Certain *S. typhimurium cysG* mutations eliminate B_{12} synthesis but allow continued siroheme production (65). Since these mutants are corrected by exogenous cobalt, CysG protein may also catalyze insertion of cobalt, probably into Factor-2, and thus seems to be responsible for the first reaction unique to B_{12} synthesis by the anaerobic pathway. The multifunctional CysG enzyme of *S. typhimurium* is positioned so as to control the flux of UroIII into three pathways. No evidence has been presented that the activities of this protein vary in response to intracellular conditions. The shorter *P. denitrificans* homologue (CobA; aerobic pathway) appears to catalyze only the two methylation reactions needed to form Precorrin-2; cobalt insertion occurs later in this pathway and is supported by distinct proteins.

Addition of the Deoxyadenosyl Moiety

Adenosyl transfer is required at several points in cobalamin metabolism. During biosynthesis, adenosine is added to a biosynthetic intermediate, leading to synthesis of the intermediate Ado-Cbi. Any assimilated corrinoids are likely to require adenosylation, since the carbon-cobalt bond of cobalamin is unstable and likely to be lost in the extracellular environment. Adenosylation may

also be required by enzyme-bound cofactors lacking the upper ligand. Such enzymes may also recycle cofactors that have lost the adenosyl group as a result of damage by the inherently reactive radicals involved in catalysis.

In *S. typhimurium,* adenosyl transferase mutants (*cobA*) were identified as Part I mutants that map outside of the main operon. Under aerobic conditions *cobA* mutants cannot use the biosynthetic intermediate Cbi as a precursor but can grow when Ado-Cbi is supplied, which suggests a defect in adenosylation. This nutritional pattern was seen when synthesis of B_{12} was scored by the activity of the MetH enzyme, a methyl transferase that requires methyl-B_{12}, not Ado-B_{12}. The results suggest that, under aerobic conditions, adenosylation is a prerequisite for the reactions of Part III of the pathway (see Figure 7); that is, the CobUS enzymes use only Ado-Cbi as a substrate.

Under anaerobic conditions, *cobA* mutations can use Cbi (nonadenosylated) as a precursor for synthesis of a B_{12} form that will support the MetH enzyme. Anaerobiosis may allow the CobU protein to adenosylate Cbi or to process Cbi to form nonadenosylated B_{12} (116); the effect of anaerobic conditions may be simply to permit higher induction of the operon. In *P. denitrificans,* a gene homologous to *cobA* was identified after purification and partial sequencing of a cobalamine adenosyl transferase. This sequence was used to identify the relevant gene from among the sequenced biosynthetic genes (49a).

The CobA enzyme of *S. typhimurium,* in addition to its role in de novo synthesis, appears to adenosylate assimilated CN-B_{12}. This conclusion was reached because a *cobA* mutation prevents repression of the *cob* operon by exogenous CN-B_{12} but does not block repression by Ado-B_{12} (61, 158, 159). Similarly in *E. coli,* the BtuR protein (equivalent to CobA of *S. typhimurium*) was identified by a mutation that causes constitutive expression of the *btuB* (B_{12} transport) gene in cells growing in the presence of a normally repressive level of CN-B_{12}; the *btuR* mutation does not prevent repression by Ado-B_{12}. The regulatory mechanisms of the *cob* operon and the *btuB* gene appear to respond only to Ado-B_{12}, which cannot be made from CN-B_{12} without the CobA adenosyl transferase. These regulatory mechanisms are discussed later. Although adenosylation of enzyme-bound corrinoids has not been demonstrated, genetic evidence suggests that such adenosyl transferases are encoded within the *eut* and *pdu* operons. These operons are also described later.

The Problem of Cobalt Reduction

Cobalt must be reduced to the Co^{I} state prior to addition of an adenosyl group during biosynthesis or in assimilation of a nonadenosylated corrinoid. Cobalt can be reduced chemically in vitro (e.g. by methyl viologen) or biochemically by a system that depends on reduced NADP and a flavodoxin (21, 79, 115). Although proteins able to perform this reduction have been observed, no mutants

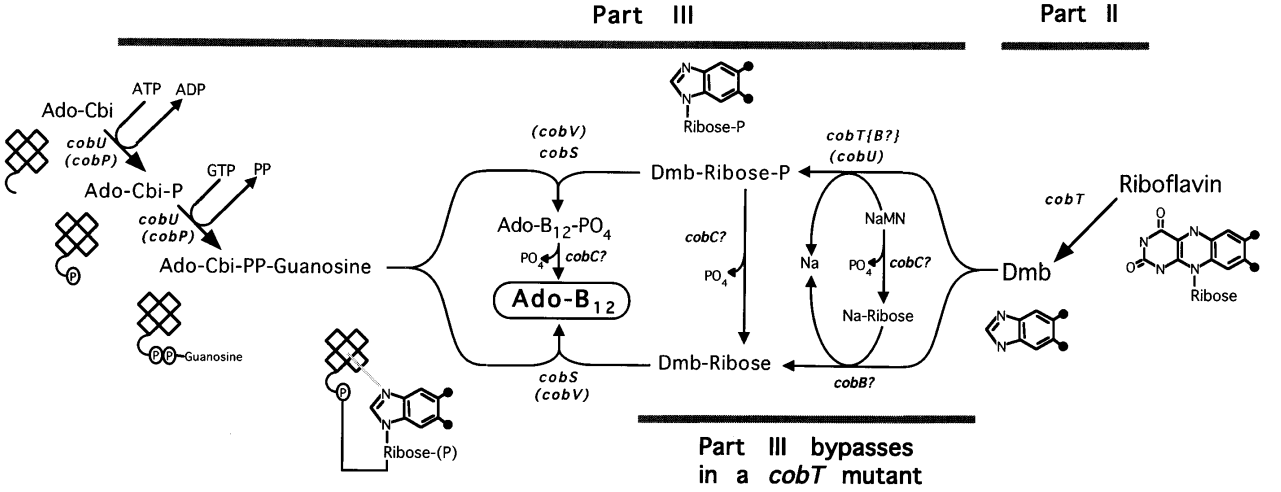

Figure 7 Parts II and III of the cobalamin biosynthetic pathway. Listed genes are those of *S. typhimurium*; homologous genes from *P. denitrificans* are denoted in parentheses. Uncertainties regarding alternative routes from Dmb to Ado-B_{12} are described in the text.

defective in cobalt reduction have been identified to demonstrate biological relevance. The failure to recover such mutants may suggest the existence of multiple reductases or may indicate that a single reductase is also essential to other functions.

Source of the Aminopropanol Side-Chain

Isotope tracer studies in *P. shermanii* and *Streptomyces olivaceus* suggest that the 1-amino-2-propanol side-chain of B_{12} is formed from threonine (98, 104). A simple decarboxylation reaction would generate free 1-amino-2-propanol, which could be attached to cobyric acid (Cby) to form Cbi; however, this decarboxylation has not been demonstrated despite multiple attempts. An alternative route requires oxidation of threonine to amino-ketobutyric acid, followed by decarboxylation to aminoacetone and reduction to yield aminopropanol (112). A third possibility is that threonine may be attached directly to the ring and modified in place (74). The latter possibility would involve no free aminopropanol.

Two classes of mutants appear to be defective in addition of the aminopropanol group: the *cbiB* and *cobD* genes of *S. typhimurium* and the homologous *cobD* and *cobC* genes of *P. denitrificans* (48, 140; C Grabau, unpublished data). These genes have been assigned to Reactions 19 and 20 in the pathway (see Figure 6) based on their ability to use Cbi but not Cby as a B_{12} precursor. The *A. tumifaciens cobD* and *cobC* mutants accumulate cobyric acid (48). *S. typhimurium cobD* mutants can make B_{12} if aminopropanol is provided, which suggests that they are unable to synthesize free aminopropanol (80). However, if threonine is normally attached to the ring before decarboxylation, the growth response of *cobD* mutants could be the result of a scavenging pathway for use of exogenous aminopropanol.

Synthesis of Dimethylbenzimidazole

The synthetic pathway for Dmb has remained elusive. In *P. shermanii*, Dmb is derived from riboflavin, but the synthetic pathway is unknown. A proposed pathway involves five reactions but requires the presence of oxygen (127). This may make sense for *P. shermanii* (an aerotolerant anaerobe), which is reputed to make Dmb only when exposed to oxygen. However, *Salmonella* spp. make B_{12} with a Dmb lower ligand during extended growth under anaerobic conditions, which suggests that Dmb synthesis can occur without oxygen (89). *S. typhimurium* mutants were isolated that could make B_{12} only if supplied with Dmb. These mutants affect a single gene (*cobT*) mapping at the distal end of the *cob* operon (42, 166). The simplest interpretation of these results is that the single CobT protein catalyzes the complete synthesis of Dmb. There are several reasons to believe that the situation is more complex.

First, it seems unlikely that any single enzyme could synthesize Dmb from its probable (but unproved) precursor, riboflavin (see Figure 7). More disturbing, there are mutants of *P. putida* that can be complemented by the *cobU* gene of *P. denitrificans* (a homologue of *Salmonella cobT* gene). These mutants show a phenotypic defect in Part III of the pathway, the joining of Dmb and Cbi (37). Thus two proteins of similar size, showing 33% identity (57% similarity) over their entire length, appear to have different mutant phenotypes in two organisms (42). The *P. denitrificans* enzyme, consistent with its mutant phenotype, was shown to catalyze transfer of ribose phosphate from NaMN to Dmb (37). The *S. typhimurium* CobT enzyme also catalyzes this Part III reaction (166), even though its mutant phenotype shows proficiency in Part III of the pathway. Thus homologous enzymes in two species appear to catalyze the same reaction but have distinct mutant phenotypes; the Part II phenotype of the *S. typhimurium* mutant does not fit with the known enzymological defect.

Escalante-Semerena and coworkers offered an explanation for this discrepancy by proposing that *S. typhimurium* (but not *P. putida*, which was used for the mutant hunts) possesses a second enzyme capable of catalyzing phosphoribosyl transfer to Dmb (166). The alternate enzyme, they suggested, might have a poor affinity for Dmb and thus could act only when a high level of Dmb was supplied exogenously. Their argument was supported indirectly by identification of two classes of mutations (*cobC* and *cobB*); either of these mutations, when combined with a *cobT* mutation, causes a defect in Part III of the pathway. (Single *cobT, cobB,* or *cobC* mutations do not eliminate Part III.) It was suggested that the CobC and CobB enzymes together duplicate the CobT role in Part III of the pathway and thus mask the Part III phenotype expected for *cobT* mutations. Apparently this alternative method for making Dmb nucleosides is not present in *P. putida.* Although this hypothesis explains the phenotype of *cobT* mutants, it does not explain why an enzyme (CobB) would be made that couldn't use ambient levels of Dmb or why the hypothetical Dmb synthetic genes were not affected by any of the many *S. typhimurium cob* mutations.

Later some *cobT* mutants were found to be satisfied by a very low level of exogenous Dmb, whereas others required a 1000-fold higher concentration (42). Deletions of the *cobT* gene require a very high level of Dmb and gain a Part III defect when combined with a *cobB* mutation, showing a CobT role in Part III. Certain point mutants (*cobT*) satisfied by little Dmb retain their Part II phenotype even in the presence of a *cobB* mutation, suggesting that they are proficient in Part III and have a simple (Part II) defect in Dmb production (42).

These results suggested that the normal CobT enzyme might catalyze the complete synthesis of Dmb in addition to performing the ribosyl phosphate

transfer. Although this theory would explain the mutant phenotypes, it still leaves a single enzyme to perform a complex set of mechanistically difficult reactions needed for Dmb synthesis. Possible activities of the *S. typhimurium cobB* and *cobC* genes are discussed below.

Formation of the Dmb Nucleoside: The Phosphate Problem

The CobT enzyme of *S. typhimurium* (and the homologous *P. denitrificans* CobU enzyme) have been shown to catalyze the transfer of Ribose-PO_4 from nicotinic acid mononucleotide (NaMN; an intermediate in NAD synthesis) to Dmb (37, 166). This reaction generates DmbMN, which has a phosphate on the 5′ carbon of ribose. This phosphate poses a problem. Since it is not a part of normal Ado-B_{12}, it must be removed at one of three points in the pathway:

1. The 5′ phosphate could be removed from NaMN before transfer of the ribose to Dmb; it is not clear whether nicotinic acid ribonucleoside (NaR) has ever been tested as a substrate for the ribose transfer reaction.

2. The phosphate could be removed from DmbMN prior to B_{12} completion.

3. DmbMN could be transferred intact to activated Ado-Cbi yielding Ado-B_{12}-PO_4, whose phosphate could be removed as the last synthetic step.

These possibilities are included in Figure 7 with possible *S. typhimurium* gene names. We suspect that in *S. typhimurium*, several of these routes may coexist.

Evidence supports the third possibility in *P. dentrificans*. The CobU enzyme can use NaMN as a phosphoribose donor to produce DmbMN; the CobV enzyme DmbMN can join to Cbi to form Ado-B_{12}-PO_4 (37). This observation suggests that the phosphate could be carried through the joining step, although the joining enzyme is also capable of using Dmb-ribose, which is present in great excess over the nucleotide in cells of *P. denitrificans* (37).

Escalante and coworkers have provided evidence that *S. typhimurium*'s CobC enzyme is a phosphatase that converts DmbMN to Dmb-ribose (DmbR) (118); however, the activity measured was very low, and *P. denitrificans* has no known homologue of the CobC protein. If the CobC enzyme serves the suggested function and is the only route to B_{12}, *S. typhimurium cobC* mutants should show a Part III phenotypic defect, but they do not. Rather, *cobC* mutations show a curious partial defect in B_{12} synthesis that can be corrected by any of the following single nutrients: Cby, Cbi, aminopropanol, or Dmb (M Ailion, D Walter, C Grabau, & J Roth, unpublished data). We suspect that the *cobC* mutation may eliminate only one of several alternative ways of removing the phosphate (see Figure 7).

Completion of Ado-B_{12}

To join Ado-Cbi and the Dmb nucleoside, the aminopropanol group of Ado-Cbi is first activated by two reactions (see Figure 7). The end of the aminopropanol side-chain is phosphorylated (to Ado-Cbi-PO_4), followed by transfer of guanosine phosphate from GTP, with displacement of pyrophosphate, to form Ado-Cbi-GDP (Ado-Cbi-guanosine pyrophosphate) (49). These two reactions are catalyzed by a single enzyme, CobP of *P. denitrificans* or CobU of *S. typhimurium* (49, 117). These reactions activate the end of the aminopropanol side-chain for attachment of the Dmb ribonucleoside at its 3′ position, catalyzed by CobV of *P. denitrificans* or CobS of *S. typhimurium* (37, 117). Attachment generates the completed Ado-B_{12}. As noted above, the joining reaction might use NaMN and yield phophorylated Ado-B_{12}.

TRANSPORT OF B_{12}

The transport of cobalamin into bacterial cells poses two problems. First, the size of cobalamin far exceeds the limit for passage through outer membrane porins of enteric bacteria. Thus entry of B_{12} requires some specific outer-membrane transport system. Second, cobalamin may be present in extremely low quantities in the environment. To transport significant quantities of the cofactor, this outer-membrane transport system must be able to scavenge B_{12} with high affinity and move it into the periplasmic space; other systems can then move it across the inner membrane.

The mechanism of B_{12} transport has been studied extensively in *E. coli* (28) and includes one system for transport across the outer membrane and one for transport across the inner membrane (see Figure 8). Transport mutants were isolated by using MetE mutants, which depend on transported exogenous B_{12} for growth on methionine-deficient medium. Mutants were isolated that require an abnormally high level of exogenous cobalamin to support growth. Transport-deficient mutants fell into four classes (see Table 2) (7, 55, 56).

Transport Across the Outer Membrane

Strains with a *btuB* mutation transport B_{12} only when it is supplied at very high concentration; these mutations map at minute 90 of the genetic map. Mutations with a similar phenotype affect the TonB protein, which participates in several outer-membrane transport systems (see below). The transport defect of *btuC* and *btuD* mutants is corrected by a more modest excess of B_{12} (56); these mutations represent two complementation groups that map at minute 37.

Transport through the outer membrane requires the BtuB protein acting with TonB. This system has a high affinity for vitamin B_{12} and its many derivatives, including adenosylcobalamin and cobinamide (31, 96, 160, 161, 172). Without

Table 2 Phenotypes of cobalamin transport mutants

Genotype	*E. coli*		*S. typhimurium*	
	B_{12} required for Met^+ (nM)	Map[a]	B_{12} required for Met^+ (nM)	Map[a]
metE	0.075	NA	0.075	NA
metE tonB	1000.	28	1000	38
metE btuB	1000.	90	1000	90
metE btuC	0.75	37	0.75	30
metE btuD	0.75	37	0.75	30
metE btuF	NA[b]	NA	0.75	5
metE btuCED btuF	NA	NA	0.75	NA
metE btuB btuCED	ND[c]	NA	100,000.	NA
metE btuB btuF	NA	NA	100,000.	NA

[a]Map position in minutes of the *tonB* or *btu* gene.
[b]Not applicable; *btuF* mutants are not known for *E. coli*.
[c]Not done.

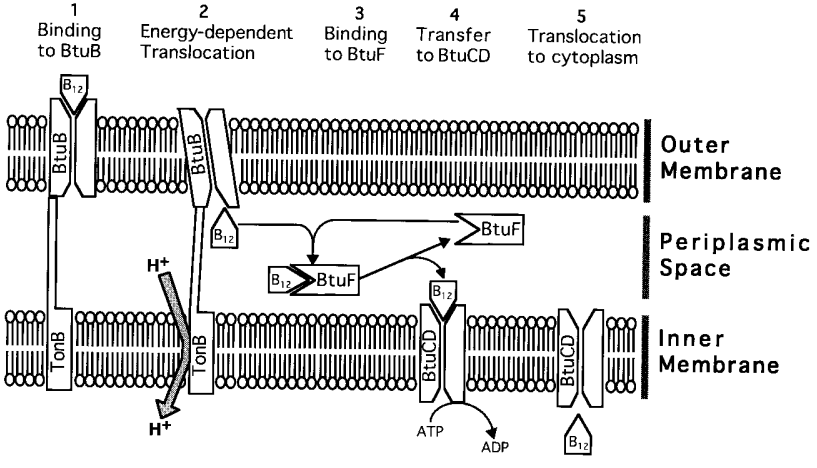

Figure 8 Transport of cobamides. Numbers indicate the inferred sequence of events based on work in *E. coli* (28). While periplasmic binding proteins are thought to act in this process, the BtuF protein is assigned this function only by general phenotypes of *S. typhimurium* mutants (see Table 2 and text).

the BtuB/TonB system, B_{12} penetrates the outer membrane with extremely low efficiency. Once bound to the BtuB protein, B_{12} is moved into the periplasm in an energy-dependent process that requires the TonB protein, which uses proton motive force to drive a structural alteration needed for transport (29, 33). Although the BtuB protein is thought to be associated physically with the TonB protein (12), it can bind B_{12} even in *tonB* mutant strains. Cotransport of calcium is required for successful passage of B_{12} through the outer membrane (30, 32). The *btuB* gene has been cloned and sequenced from *E. coli* (82, 83) and *S. typhimurium* (168).

The BtuB protein provides the binding sites for phage BF23 (8) and for Colicin E (91). The TonB protein also energizes outer-membrane transport systems for iron, including the FepA and FhuA systems (169). Thus the BtuB protein competes with these systems for TonB activity (92, 94).

Transport Across the Inner Membrane

Transport across the inner membrane is provided by the *E. coli* BtuC and BtuD proteins, encoded by the *btuCED* operon at minute 37 (7, 53, 54, 93). The *btuC* and *btuD* genes encode membrane proteins that resemble a family of transport proteins requiring a periplasmic binding protein (75). The BtuD protein has an ATP-binding site. The *btuC* and *btuD* mutant phenotypes are corrected by a modest increase in external B_{12}. The BtuB/TonB system may concentrate B_{12} in the periplasm, facilitating fortuitous transport of B_{12} into the cytoplasm of mutants lacking the inner-membrane transport system.

The central gene of the *btuCED* operon, *btuE*, was found as an open reading frame in the operon DNA sequence but is not required for B_{12} transport (134). The BtuE sequence is not clearly homologous to any known periplasmic binding protein and does not bear a signal sequence (75).

Although a periplasmic B_{12} binding protein (expected for a system of the *btuCD* type) has not been identified in *E. coli*, the *btuF* locus of *S. typhimurium* may encode such a protein (N Clark, J Lawrence, & J Roth, unpublished data). *S. typhimurium* has genetic loci corresponding to the *btuB*, *tonB*, and *btuCED* loci of *E. coli* (108; N Clark, J Lawrence, & J Roth, unpublished data).

In addition, the new *btuF* transport gene of *S. typhimurium* has a phenotype resembling that of *btuC* and *btuD* mutants (see Table 2). The double mutant combination of *btuF* with *btuC* or *btuD* has a transport phenotype indistinguishable from those of the individual mutants, suggesting that all three genes contribute to transport of B_{12} across the inner membrane. In contrast, the combination of a *btuF* (or *btuC* or *btuD*) mutation with a *btuB* mutation causes a transport defect that is more severe than those of the single mutants. This observation suggests two additive functions, one provided by BtuB/TonB (outer-membrane transport) and the other by the BtuC, BtuD, and BtuF proteins (inner-membrane

transport). The BtuCD transport system appears to involve a periplasmic binding protein (172), and BtuF function of *S. typhimurium* is a good candidate for filling this role.

Although the *btuCED* operon appears to be expressed constitutively; the *btuB* gene is regulated as discussed below (6, 90, 106, 168). The cryptic plasmid of *S. typhimurium* carries a cluster of genes that encode the proteins of a pilus; mutant forms of this region of the plasmid affect the rate at which B_{12} crosses the outer membrane in the absence of BtuB function (132, 133).

REGULATION OF B_{12} SYNTHESIS IN *S. TYPHIMURIUM*

The regulatory behavior of any set of genes can provide clues to the role of those genes. Significant questions exist regarding the physiological importance of cobalamin to *S. typhimurium* and *E. coli* (see below). Understanding regulation of the *S. typhimurium cob* operon may shed light on use of B_{12} in this organism.

Control of the cob/pdu *Regulon*

The *cob* operon (encoding B_{12} synthetic enzymes) maps adjacent to the *pdu* operon, which encodes enzymes for propanediol degradation. The two operons are both induced by propanediol using a single regulatory protein. These results indicate that the main role of B_{12} in *S. typhimurium* is in supporting catabolism of propanediol.

Two lines of evidence initially suggested that propanediol was involved in control of the *cob* operon (24, 136). First, propanediol was found to induce transcription of *cob-lac* fusions in cells growing on poor carbon sources. Second, single mutations mapping between the *cob* and *pdu* operons eliminate inducibility of both operons by propanediol. These mutations define the *pocR* gene that encodes a regulatory protein of the AraC-family (43) (see Figure 9). The *pduF* gene, encoding a diffusion facilitator for propanediol (43; P Chen & J Roth, unpublished data), was also found to lie in the region between the *cob* and *pdu* operons (43). There seem to be alternative routes of propanediol entry since *pduF* mutations reduce inducibility of the *cob* and *pdu* operons only by virtue of their polar effect on expression of the *pocR* gene (41).

Two global regulatory systems (Crp/Cya and ArcA/ArcB) affect inducibility of the *cob* and *pdu* operons (2, 4, 60). Both operons are activated aerobically and anaerobically by Crp protein and anaerobically by ArcA protein. Maximum inducibility is seen during anaerobic respiration of a poor carbon source; under these conditions the Crp and ArcA proteins act additively. The control of the regulon depends on five promoters, all located in the central region between the *cob* and *pdu* operons; four of these promoters are activated by the PocR protein (41).

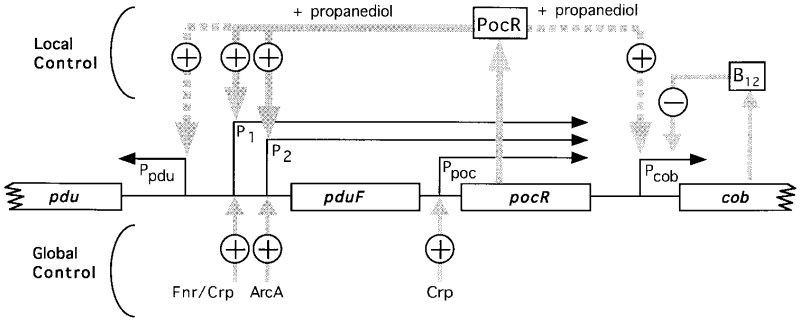

Figure 9 Regulation of the *cob*/*pdu* regulon of *S. typhimurium.* The genetic map describes the region between *pdu* and *cob* operons whose transcripts start at the far sides of the figure. Boxes enclose structural genes. Black arrows designate transcripts (41). Gray arrows indicate regulatory influence; dashed gray arrows indicate the proposal that a higher level of PocR protein may be required to activate these promoters.

The current model for control of this system includes the following features (see Figure 9): The *cob* and *pdu* operons are transcribed divergently from promoters indicated at the far right and left in the figure. These promoters are activated whenever both the PocR protein and propanediol are present. Global control of the two operons is exerted by varying the level of PocR protein. The *pocR* gene is transcribed by three promoters controlled both by global regulatory proteins and by autoinduction (41). The shortest transcript (from Ppoc) appears to be regulated only by Crp/cAMP. The P2 transcript clearly is autoregulated by PocR and, in addition, requires the ArcA protein, which signals a reduced cell interior (41).

Control of the P1 promoter is less certain but may involve either the Fnr protein (responding to a reduced cell interior) or the Crp protein (responding to a shortage of carbon and energy) in addition to the PocR activator. The involvement of these proteins in control of P1 is based heavily on the presence of appropriate binding sites upstream of that promoter.

We propose that the regulon has three states. In the *off* condition (during aerobic growth on glucose), all promoters are at their lowest level; PocR protein is produced by the basal expression levels of promoters P1, P2, and Ppoc. Cells enter a *standby* state when they grow under any set of global conditions appropriate for induction, but without propanediol. These conditions include aerobic growth on a poor carbon source and/or growth without oxygen. These conditions stimulate expression of both the PduF transporter and the PocR regulatory protein, but the *cob* and *pdu* operons remain uninduced. The *standby* expression of PocR can occur from the Ppoc and/or the P1 promoter without

inducer. The two proteins induced in the *standby* state (PduF and PocR) are those needed to sense propanediol. If propanediol appears, the P1 and P2 promoters are induced, increasing the level of PocR protein and placing the system in the *on* state. The resulting high level of PocR/propanediol complex induces expression of the *cob* and *pdu* promoters.

This regulatory pattern suggests several things about the role of B_{12} in *Salmonella* spp. physiology. Induction of the *cob* operon by propanediol (and its coinduction with the *pdu* operon) suggests that degradation of propanediol is a major function of cobalamin in *Salmonella* spp. Global control by Crp/cAMP suggests that B_{12} helps provide a carbon or energy source, consistent with the importance of B_{12} for propanediol degradation. Control by Arc (and perhaps Fnr) suggests that anaerobic expression is important.

Control of the cob *Operon and the* btuB *Gene by Ado-B_{12}*

Independent of the control mechanisms described above, transcription of the *cob* operon, but not the *pdu* operon, is reduced in the presence of the end-product, Ado-B_{12}. This control mechanism shares many features with that of the *btuB* gene, also repressed in response to Ado-B_{12}. These two systems share sequence features with the *P. denitrificans cobP* gene, the first gene of a four-gene *cob* operon. Although the details of these three control mechanisms are uncertain, their common features suggest that all may use an mRNA leader sequence to sense Ado-B_{12} and to effect both translational and transcriptional controls.

The *cob* and *btuB* control mechanisms share the following features (see Figure 10):

1. Both are repressed by Ado-B_{12} and not by CN-B_{12} (105; M Ailion & J Roth, unpublished data).

2. Control seems to alter continuation of message synthesis rather than initiation; for example, repression by Ado-B_{12} is seen even when transcription is initiated at foreign promoters (5, 43, 129; M Ailion & J Roth, unpublished data).

3. Genetic analysis has revealed no protein that might mediate the repressive effects of Ado-B_{12}; all mutations that eliminate control alter the mRNA leader region (106, 130).

4. The leader regions of the two mRNAs (and the region upstream of the *P. denitrificans cobP* gene) share some common sequence features (see Figure 10), including two areas of the leader (Box 1 and Box 2) and a hairpin

structure that occludes the translation initiation site of the adjacent structural gene.

5. In both the *cob* operon of *S. typhimurium* and the *btuB* gene of *E. coli*, translational control of the first gene seems to be an integral part of the control mechanism (106, 129, 130). In these two cases, fusions to distal points in the adjacent gene show both transcriptional and translational control. Fusions to an intermediate region of the gene show translational but not transcriptional control. More proximal fusions show no regulation (see Figure 10).

The above features suggest a mechanism in which a direct interaction occurs between the effector (Ado-B_{12}) and the mRNA leader. This interaction may induce mRNA folding that stabilizes the hairpin, thereby blocking the translational start of the adjacent coding region; this hairpin exerts a translational control. The untranslated coding sequence may lead to message termination at a site within the structural gene, thus providing transcriptional control. Only fusions beyond the inferred transcription termination site can show transcriptional regulation. This model, suggested originally by Lundrigan and colleagues (106), was supported by Richter-Dahlfors and colleagues. This model accounts for the available data but has not been demonstrated fully. In particular, it remains

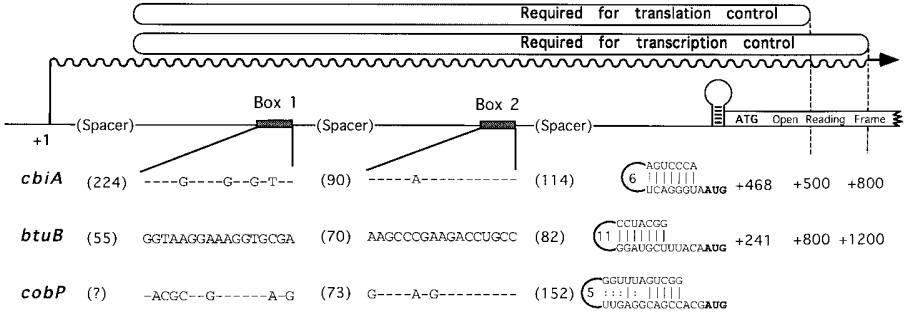

Figure 10 Features of genes repressed by Ado-B_{12}. The three indicated transcripts are the *cob* operon (*S. typhimurium*), the *btuB* gene (*E. coli*), and the *cobPWNO* operon (*P. denitrificans*). Only the first two have been analyzed in detail; the *cobPWNO* features are inferred only by sequence inspection. The transcripts share two separated blocks of sequence similarity within the mRNA leader and a dyad symmetry that may sequester the translation initiation site. Regions required for control were inferred from effects of deletions on *lac* fusions to various points in the adjacent structural gene (106, 130). Bases within the first gene are numbered relative to the transcription start site for the *cbiA* and *btuB* genes. Numbers in parentheses indicate the number of base pairs between the indicated sequence elements.

uncertain how an mRNA might specifically recognize Ado-B_{12} or how that binding might affect mRNA folding.

THE SIGNIFICANCE OF B_{12} FOR ENTERIC BACTERIA

The B_{12} Paradox

The genetic analysis of B_{12} synthesis and use in *S. typhimurium* reveals a problem. Nearly 1% of the *S. typhimurium* genome is dedicated to synthesis or import of B_{12} (see Figure 5), yet a mutant defective only for cobalamin synthesis has no laboratory growth phenotype either aerobically or anaerobically. What selective pressures act in natural populations of *Salmonella* species to maintain this enormous genetic investment?

We have described 26 known genes involved in synthesis of cobalamin and 6 genes providing for its import. This list does not include several predicted functions that have not been identified genetically. Since roughly 4000 genes are likely to make up the *S. typhimurium* genome, nearly 1% of this genome is dedicated to B_{12} acquisition. In addition, this genome includes two large operons (described below) that encode the enzymes needed for B_{12}-dependent utilization of ethanolamine and propanediol. Together these two operons appear to include about 30 genes (see Figure 5). Including the metH gene, a total of at least 63 genes are involved in synthesis and use of B_{12}—representing a huge genetic investment.

In an otherwise wild-type strain, *cob* mutants have no growth phenotype under standard laboratory conditions. The genetics of B_{12} synthesis was made possible by use of a mutant (*metE*) in which methionine synthesis is dependent on B_{12}. Even the two major B_{12}-dependent catabolic pathways do not provide a strong selection for B_{12} synthesis. *S. typhimurium* synthesizes B_{12} only anaerobically, but it requires oxygen to use ethanolamine or propanediol as a sole carbon and energy source.

Even the presence of alternative electron acceptors (nitrate or fumarate) does not allow anaerobic use of these carbon sources. Aerobic use of propanediol or ethanolamine is seen only if B_{12} is provided exogenously, since there is no aerobic B_{12} synthesis. Thus *S. typhimurium* cannot use its endogenous B_{12} (made only anaerobically) to support degradation of propanediol or ethanolamine (requiring oxygen). Exceptions to this behavior are described below.

The large genetic investment in B_{12}, together with the lack of laboratory phenotype for *cob* mutants, raises a paradox. It is clear that any gene not under natural selection will inevitably be lost as a result of mutation accumulation.

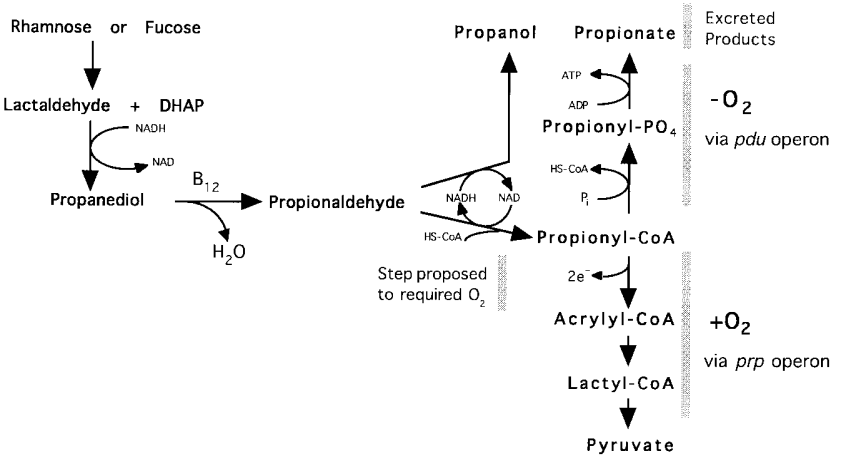

Figure 11 Metabolism of propanediol. Reactions indicated as proceeding without oxygen provide an electron sink and a source of ATP, but not a carbon source. Reactions indicated as proceeding with oxygen provide a carbon and an energy source by respiration using oxygen or tetrathionate (but not nitrate or fumarate) as an electron acceptor (T Bobik & J Roth, unpublished data). DHAP indicates dihydroxyacetone phosphate.

Maintenance of the large collection of genes involved in synthesis and use of B_{12} requires that a strong selective force be imposed on these functions in natural *Salmonella* spp. populations. Standard laboratory phenotypes do not indicate what this selection might be. Understanding the conditions for this selection may provide significant insight into how *Salmonella* spp. live in the real world. Understanding this selection may also be fundamental to understanding the lifestyle of *Salmonella* spp.; the closely related bacterium *E. coli* can neither synthesize cobalamin de novo nor degrade propanediol.

The regulatory behavior of the *cob* operon suggested that B_{12}-dependent utilization of propanediol (possibly under anaerobic conditions) must be a major aspect of the selection for B_{12} production. The pathway for ethanolamine degradation shares many features with that for breakdown of propanediol. To approach the physiological importance of B_{12}, we examine these two pathways.

Metabolism of Propanediol

The first step in this degradative pathway is the B_{12}-dependent diol dehydratase, which produces propionaldehyde (see Figure 11). This aldehyde can

be oxidized to form propionyl-CoA or alternatively can be reduced to form propanol. By forming and excreting propanol, cells can balance internal redox reactions. Under anaerobic conditions, propionyl-CoA can be converted by a phosphotransacylase to propionyl-phosphate, and then by a reversible acetyl-/propionyl-kinase to propionate, producing one molecule of ATP (167). According to this scheme, anaerobic metabolism of propanediol could provide an electron sink (propanol) and a source of ATP but no source of carbon since both propanol and propionate are excreted. Genes needed for conversion of propanediol to propionyl-CoA are encoded in the *pdu* operon (87; T Bobik, Y Xhu, R Jeter, & J Roth, unpublished data; D Walter, M Ailion, & J Roth, unpublished data). This operon may also contain some of the additional genes encoding enzymes that contribute to anaerobic propanediol metabolism (e.g. propionyl-CoA phosphotransacylase, propionate kinase, propanol dehydrogenase).

Under aerobic conditions, *S. typhimurium* can use propionyl-CoA as both a carbon and an energy source, probably by a pathway that includes acrylyl-CoA and lactyl-CoA as intermediates in pyruvate production (66). An operon has been identified that appears to encode enzymes for the aerobic catabolism of propionyl-CoA (and exogenous propionate); this operon maps at minute 8 (J Tittensor, T Bobik, & J Roth, unpublished data).

Metabolism of Ethanolamine

The ethanolamine degradative pathway is directly analogous to that for propanediol. The B_{12}-dependent ethanolamine ammonia lyase produces acetaldehyde (in place of propionaldehyde), which can be oxidized to acetyl-CoA (in place of propionyl-CoA). The acetaldehyde can be also be reduced (to ethanol) to provide an electron sink. Acetyl-CoA can be converted in two reactions to acetate with production of ATP. As for propanediol, anaerobic use of ethanolamine provides ATP and an electron sink but no carbon source. Acetyl-CoA can be utilized aerobically via the TCA cycle. Mutants defective in aerobic use of ethanolamine map in the *eut* operon. Ethanolamine degradation also provides a nitrogen source (see Figure 4), but the regulatory pattern of the operon suggests that this is not its main importance.

Genetic Analysis of the pdu *and* eut *Operons*

Genetic characterization of the propanediol (*pdu*) and ethanolamine (*eut*) operons has employed mutants defective for aerobic use of these compounds as carbon sources with exogenously provided B_{12} (137, 138; D Walter, M Ailion, & J Roth, unpublished data). B_{12} was provided because both propanediol and ethanolamine serve as carbon sources only in the presence of oxygen, which prevents B_{12} synthesis. Complementation tests of the *pdu* and *eut* mutations

revealed five genes in the *pdu* operon and six in the *eut* operon that apparently are needed for aerobic growth on the particular carbon source (diagrammed in Figure 5).

The affected *pdu* genes include those for propanediol dehydratase (*pduCED*) and for propionaldyhyde dehydrogenase. The set of genes affected by *eut* mutations include those for the B_{12}-dependent enzyme ethanol ammonia lyase (*eutBC*) and for acetaldehyde dehydrogenase (*eutE*). Both operons appear to include genes (described below) that may encode B_{12} adenosyl transferases. The genetically identified genes encode functions expected to be necessary for aerobic degradation.

The *pdu* and *eut* operons include many genes that were not detected genetically. The *pdu* operon is about 15 kb in size based on PCR between transposons inserted in the operon, suggesting the existence of about 15 genes (J Lawrence & J Roth, unpublished data), only 5 of which were detected genetically. Only three of the five sequenced *pdu* genes have an aerobic phenotype, and these genes (*pduCED*) encode subunits of the diol dehydratase (T Bobik, Y Xhu, R Jeter, & J Roth, unpublished data). The sequence of the *eut* operon includes 15 genes (64, 153; E Kofoid & J Roth, unpublished data), only 6 of which were detected genetically. (One of these encodes the regulatory protein EutR.)

We suspect that for both operons, the discrepancy is the result of a large number of genes that are not needed under the aerobic conditions used (out of necessity) for mutant hunts. These extra genes are likely to be involved in anaerobic breakdown of these compounds under conditions that have not been fully defined. If conditions can be defined for anaerobic growth at the expense of propanediol and ethanolamine, these conditions may allow the extra genes to show a mutant phenotype. Such anaerobic conditions are likely to provide a selection for B_{12} synthesis that might apply to natural populations. Recent progress toward defining these conditions is described later.

Regulation of B_{12}-Dependent Functions

The *eut* operon is subject to global control by the Crp/cAMP system. The operon is induced by the simultaneous presence of both ethanolamine and Ado-B_{12}; this control is mediated by the EutR protein, a member of the AraC family of positive activator proteins (139, 150; E Kofoid & J Roth, unpublished data). The activator protein appears to recognize both effectors, but this has not been demonstrated directly.

The EutR activator is encoded within the operon and thus induces its own production. The autoinduction circuit avoids competition between the lyase and EutR for binding of a very small pool of Ado-B_{12}, estimated at about 100 molecules per anaerobically growing cell (5). Without autoinduction of EutR, increasing levels of lyase would bind all Ado-B_{12} and limit induction (150).

The global control by Crp/cAMP suggests that the operon is used mainly to provide a carbon and energy source. Inducibility by ethanolamine plus B_{12} suggests that the genes of the operon are required for B_{12}-dependent ethanolamine degradation. The *pdu* operon is regulated coordinately with the *cob* operon, as described above.

Homologues of Carboxysome Proteins

Within both the *eut* and *pdu* operons are reading frames that encode homologues of proteins thought to be carboxysome skin proteins. The carboxysome is an organelle found in photosynthetic bacteria and in sulfur chemolithotrophs that use ribulose bis phosphate carboxylase (Rubisco) to fix CO_2. These organelles may support CO_2 fixation by concentrating CO_2 with Rubisco (inside the organelle) and excluding toxic O_2 (45, 120). Although such organelles have not been seen in enteric bacteria, the existence of these homologues warrants a closer look.

Two genes of the *eut* operon (*eutM* and *eutK*) and one of the *pdu* operon (*pduA*) encode homologues of the CcmK protein of *Synechococcus* sp. (43; E Kofoid & J Roth, unpublished data). The CcmK protein has been reported to be a shell protein of the carboxysome (122). Another *eut* gene (*eutN*) encodes a homologue of a different carboxysome structural protein (CcmL of *Synechococcus* sp.). In addition, the PduB and EutL proteins share significant homology to each other but not to other proteins in the database, suggesting a role common to the two pathways. The five proteins listed above are among those with no aerobic mutant phenotype. We suspect that *S. typhimurium* may assemble some carboxysome-like structure to allow anaerobic metabolism of propanediol and ethanolamine.

Presence of Additional B_{12} Adenosyl Transferases

In addition to the general adenosyl transferase described above (CobA in *S. typhimurium*, CobO in *P. denitrificans*), additional versions of this enzyme may be encoded by the *pdu* and *eut* operons. These enzymes may adenosylate cofactor bound to a particular B_{12}-dependent enzyme. Their substrate could arise by binding of nonadenosylated cofactor or when the functional cofactor loses its adenosyl moiety.

A *eutA* mutant cannot degrade ethanolamine in the presence of a high level of CN-B_{12}; activity is restored if CN-B_{12} is replaced by Ado-B_{12} (138, 139). Lyase is strongly inhibited by CN-B_{12} (15), and the *eutA* activity may be required to reduce the level of toxic CN-B_{12}. A *cobA* (B_{12} adenosyl transferase) mutant cannot grow on ethanolamine with high CN-B_{12}, but it can grow if the level of CN-B_{12} is reduced. This growth of a *cobA* mutant on low CN-B_{12} is

eliminated by addition of a *eutA* mutation; for this reason *eutA* is presumed to encode an adenosyl transferase. Mutants in the *eutD* gene appear to be defective in an adenosyl transferase needed for optimal induction of the operon by CN-B_{12} (150). We speculate that each B_{12}-dependent protein (lyase and regulatory protein) may have an associated adenosyl transferase for adenosylation of protein-bound corrinoids.

In the propanediol operon, *pduH* mutations cause a defect in propanediol utilization on medium with CN-B_{12}, but the defect is corrected if Ado-B_{12} is provided (D Walter, M Ailion, & J Roth, unpublished data). The gene may encode a transferase associated with the propanediol dehydratase.

Possible Solutions to the B_{12} Paradox

Since B_{12} is only made in the absence of oxygen, and its main use appears to be in propanediol metabolism, its main importance is likely to be found in some aspect of anaerobic catabolism of propanediol (and its sister compound ethanolamine). The known metabolism of propanediol and ethanolamine suggests that some benefit (for example, an electron sink and ATP production) might be derived from anaerobic catabolism even when it does not provide a carbon source (see Figure 11).

This theory was tested by measuring the effect of propanediol and ethanolamine on anaerobic growth when a carbon source is provided (as dilute casamino acids). With no added energy source, this amino acid mixture allows very poor anaerobic growth; both propanediol and ethanolamine stimulate the anaerobic growth rate and can do so using endogenously synthesized B_{12} (T Bobik & J Roth, unpublished data). This is a selectable phenotype for B_{12} synthesis that may be important under natural conditions.

In looking for a more striking anaerobic value for B_{12}, we considered various alternative electron acceptors. Although neither nitrate nor fumarate support anaerobic use of propanediol or ethanolamine, a less well-studied acceptor, tetrathionate, allows use of either propanediol or ethanolamine as an anaerobic carbon and energy source. This growth can be supported using endogenously synthesized B_{12} (T Bobik & J Roth, unpublished data). Therefore, anaerobic tetrathionate medium provides conditions under which *S. typhimurium* can both synthesize B_{12} and use it to support growth on these carbon sources.

If such growth conditions are common in the natural habitat of *Salmonella* species, respiration to tetrathionate could provide a selective value for synthesis of B_{12} and solve the paradox of B_{12} use. This observation, however, raises some serious questions of whether tetrathionate, or other polysulfides, are encountered in nature and how they are metabolized to support respiration of carbon sources that cannot be respired using nitrate or fumarate as electron acceptor.

Role of B_{12} in Supporting Growth of Salmonella Species *Within a Host Organism*

Since *S. typhimurium* is discovered frequently by virtue of its pathogenicity, the role of B_{12} may be understandable only in terms of interactions between the bacterium and a metazoan host organism. This possibility has been addressed directly by testing the virulence of isogenic *S. typhimurium* strains with and without a functional *cob* operon (141). These experiments yielded the surprising result that strains unable to make B_{12} are more virulent than are a wild-type strain. This conclusion was seen both for oral and peritoneal routes of infection, suggesting a complex situation in which the host deals with an infecting bacteria differently depending on whether it produces B_{12}. Perhaps by providing B_{12}, *S. typhimurium* establishes a more benign relationship with a host–a relationship in which the invader is tolerated and to which *S. typhimurium* responds less aggressively.

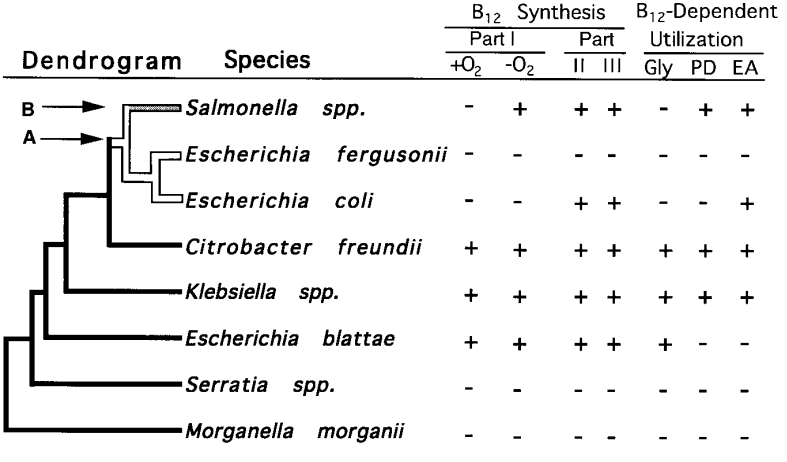

Figure 12 Distribution of cobalamin synthesis and use among enteric bacteria. The dendrogram is based on Lawrence & Roth (100). B_{12} synthesis phenotypes are based on a bioassay of B_{12} production by cells grown with various nutritional supplements (see Table 1) needed to infer active parts of the pathway (Parts I, II, and III). Cobalamin-dependent growth phenotypes are: EA–ethanolamine utilization, PD–propanediol utilization, Gly–glycerol dehydratase activity. Arrow A indicates inferred evolutionary loss of functions (B_{12} Part I, PD, Gly); arrow B indicates acquisition of functions (B_{12} Parts I, II, III, and PD) by a single horizontal transfer.

EVOLUTION OF THE B_{12} SYNTHETIC GENES IN ENTERIC BACTERIA

Analysis of the base sequence of the *S. typhimurium cob* and *pdu* operons suggested that these genes had been acquired by horizontal transfer since the divergence of *Salmonella* species and *E. coli* (99, 100, 140). Multiple isolates of *Salmonella* spp., *E. coli*, and other enteric bacteria were surveyed for their ability to synthesize B_{12}, for possession of sequences closely related to the *S. typhimurium cob* region, and for ability to perform various B_{12}-dependent metabolic functions (see Figure 12).

Virtually all *Salmonella* species tested behaved like *S. typhimurium.* They synthesized B_{12} under anaerobic conditions and possessed a *cob* operon homologous to that of *S. typhimurium.* Virtually all natural isolates showed B_{12}-dependent degradation of propanediol. In contrast, no isolate of *E. coli* synthesized B_{12} de novo, although most possessed genes (*cobUST*) that encoded Parts II and III of the B_{12} synthetic pathway (99). No *E. coli* isolate showed B_{12}-dependent degradation of propanediol. Other enteric taxa synthesized B_{12} both aerobically and anaerobically and were capable of B_{12}-dependent degradation of propanediol (100). Unlike *Salmonella* spp. and *E. coli*, these other enterics showed B_{12}-dependent degradation of glycerol. Although the other enterics synthesized B_{12}, their B_{12} synthetic genes did not show strong homology to the *S. typhimurium cob* operon ($< 70\%$ base sequence identity). These observations are summarized and interpreted in Figure 12.

We propose that the ancestor of most enteric bacteria synthesized B_{12} and used it in degradation of propanediol, glycerol, and ethanolamine under both aerobic and anaerobic conditions. On the lineage leading to *E. coli* and *Salmonella* spp., the common ancestor lost both B_{12} synthesis and the ability to degrade propanediol and glycerol. These losses are still apparent in modern *E. coli* isolates; however, the ancestor of modern *Salmonella* species reacquired by horizontal transfer a chromosomal fragment that includes a B_{12} biosynthetic operon (functional only anaerobically) and the adjacent *pdu* operon. By inheriting these operons, *S. typhimurium* acquired B_{12} synthetic ability and a selectable characteristic to ensure its maintenance.

These results suggest that the selection pressure to maintain B_{12} synthesis varies with the lifestyle of the organism. *E. coli* seems to fill a niche that does not require full de novo B_{12} synthesis, perhaps one in which B_{12} (or Cbi) is prevalent, and ethanolamine (but not propanediol) is an important carbon source. For *Salmonella* spp., the ability to synthesize B_{12} must be strongly selected; its main use may be to degrade propanediol under anaerobic conditions in the presence of a suitable alternative electron acceptor.

ACKNOWLEDGMENTS

This work was supported by research grant GM34804 from the National Institutes of Health.

Literature Cited

1. Abeles RH, Lee HA Jr. 1961. An intramolecular oxidation-reduction requiring a cobamide coenzyme. *J. Biol. Chem.* 236:2347–50
2. Ailion M, Bobik TA, Roth JR. 1993. Two global regulatory systems (Crp and Arc) control the cobalamin/propanediol regulon of *Salmonella typhimurium*. *J. Bacteriol.* 175:7200–8
3. Allen RH, Stabler SP, Savage DG, Lindenbaum J. 1993. Metabolic abnormalities in cobalamin (vitamin B_{12}) folate deficiency. *FASEB J.* 7:1344–53
4. Andersson DI. 1992. Involvement of the Arc system in redox regulation of the *cob* operon in *Salmonella typhimurium*. *Mol. Microbiol.* 6:1491–94
5. Andersson DI, Roth JR. 1989. Mutations affecting regulation of cobinamide biosynthesis in *Salmonella typhimurium*. *J. Bacteriol.* 171:6726–33
6. Aufrère R, Tempete M, Bohin J-P. 1986. Regulation of expression of the gene for vitamin B_{12} receptor cloned on a multicopy plasmid in *Escherichia coli*. *Mol. Gen. Genet.* 205:358–65
7. Bassford PJ Jr., Kadner RJ. 1977. Genetic analysis of components involved in vitamin B_{12} uptake in *Escherichia coli*. *J. Bacteriol.* 132:96–105
8. Bassford PJ Jr., Schnaitman CA, Kadner RJ. 1977. Functional stability of the *bfe* and *tonB* gene products in *Escherichia coli*. *J. Bacteriol.* 130:750–58
9. Battersby AR. 1986. Biosynthesis of vitamin B_{12}. *Acc. Chem. Res.* 19:147–52
10. Battersby AR. 1994. How nature builds the pigments of life: the conquest of vitamin B_{12}. Science 264:1551–57
11. Becher B, Muller V, Gottschalk G. 1992. N^5-methyl-tetrahydomethanopterin: coenzyme M methyltransferase of *Methanosarcina* strain Gü1 is an Na^+-translocating membrane protein. *J. Bacteriol.* 174:7656–60
12. Bell PE, Nau CD, Brown JT, Konisky J, Kadner RJ. 1990. Genetic suppression demonstrates interaction of TonB protein with outer membrane transport proteins in *Escherichia coli*. *J. Bacteriol.* 172:3826–29
13. Benner SA, Ellington AD, Tauer A. 1989. Modern metabolism as a palimpsest of the RNA world. *Proc. Natl. Acad. Sci. USA* 86:7054–58
14. Birch A, Leiser A, Robinson JA. 1993. Cloning, sequencing, and expression of the gene encoding methylmalonyl-coenzyme A mutase from *Streptomyces cinnamonensis*. *J. Bacteriol.* 175:3511–19
15. Blackwell CM, Turner JM. 1978. Microbial metabolism of amino alcohols. Purification and properties of coenzyme B_{12}-dependent ethanolamine ammonia-lyase of *Escherichia coli*. *Biochem. J.* 175:555–63
16. Blakley R. 1965. Cobamides and ribonucleotide reduction. I. Cobamide stimulation of ribonucleotide reduction in extracts of *Lactobacillus leichmannii*. *J. Biol. Chem.* 240:2173–80
17. Blakley R, Barker H. 1964. Cobamide stimulation of the reduction of ribotides to deoxyribotides in *Lactobacillus leichmannii*. *Biochem. Biophys. Res. Commun.* 16:391–97
18. Blanche F, Couder M, Debussche L, Thibaut D, Cameron B, Crouzet J. 1991. Biosynthesis of vitamin B_{12}: stepwise amidation of carboxyl groups *b*, *d*, *e*, and *g* of cobyrinic acid *a*,*c*-diamide is catalyzed by one enzyme in *Pseudomonas denitrificans*. *J. Bacteriol.* 173:6046–51
19. Blanche F, Debussche L, Thibaut D, Crouzet J, Cameron B. 1989. Purification and characterization of *S*-adenosylmethionine:Uroporphyrinogen III methyltransferase from *Pseudomonas denitrificans*. *J. Bacteriol.* 171:4222–31

20. Blanche F, Famechon A, Thibaut D, Debussche L, Cameron B, Crouzet J. 1992. Biosynthesis of vitamin B_{12} in *Pseudomonas denitrificans*: the biosynthetic sequence from precorrin–6y to precorrin–8x is catalyzed by the *cobL* gene product. *J. Bacteriol.* 174:1050–52
21. Blanche F, Maton L, Debussche L, Thibaut D. 1992. Purification and characterization of cob(II)yrinic acid *a,c*-diamide reductase from *Pseudomonas denitrificans. J. Bacteriol.* 174:7452–54
22. Blanche F, Thibaut D, Famechon A, Debussche L, Cameron B, Crouzet J. 1992. Precorrin–6x reductase from *Pseudomonas denitrificans*: purification and characterization of the enzyme and identification of the structural gene. *J. Bacteriol.* 174:1036–42
23. Blaut M, Müller V, Gottschalk G. 1992. Energetics of methanogenesis studied in vesicular systems. *J. Bioenerg. Biomembr.* 24:529–46
24. Bobik TA, Ailion M, Roth JR. 1992. A single regulatory gene integrates control of vitamin B_{12} synthesis and propanediol degradation. *J. Bacteriol.* 174:2253–66
25. Bock A, Prieger-Kraft A, Schönheit P. 1994. Pyruvate–a novel substrate for growth and methane formation in *Methanosarcina barkeri. Arch. Microbiol.* 161:33–46
26. Booker S, Licth S, Broderick J, Stubbe J. 1994. Coenzyme B_{12}-dependent ribonucleotide reductase: evidence for the participation of five cysteine residues in ribonucleotide reduction. *Biochemistry* 33:12676–85
27. Bradbeer C. 1965. The clostridial fermentations of choline and ethanolamine. I. Preparation and properties of cell-free extracts. *J. Biol. Chem.* 240:4669–74
28. Bradbeer C. 1991. Cobalamin transport in *Escherichia coli. Biofactors* 3:11–19
29. Bradbeer C. 1993. The proton motive force drives the outer membrane transport of cobalamin in *Escherichia coli. J. Bacteriol.* 175:3146–50
30. Bradbeer C, Gudmundsdottir A. 1990. Interdependence of calcium and cobalamin binding by wild-type and mutant BtuB protein in the outer membrane of *Escherichia coli. J. Bacteriol.* 172:4919–26
31. Bradbeer C, Kenley JS, di Masi DR, Leighton M. 1978. Transport of vitamin B_{12} in *Escherichia coli.* Corrinoid specificities of the periplasmic B_{12}-binding protein and of energy-dependent B_{12} transport. *J. Biol. Chem.* 253:1347–52
32. Bradbeer C, Reynolds PR, Bauler GM, Fernandez MT. 1986. A requirement for calcium in the transport of cobalamin across the outer membrane of *Escherichia coli. J. Biol. Chem.* 261:2520–23
33. Bradbeer C, Woodrow ML. 1976. Transport of vitamin B_{12} in *Escherichia coli*: energy dependence. *J. Bacteriol.* 128:99–104
34. Brey RN, Banner CDB, Wolf JB. 1986. Cloning of multiple genes involved with cobalamin (vitamin B_{12}) biosynthesis in *Bacillus megaterium. J. Bacteriol.* 167:623–30
35. Brown FB, Cain JC, Gant DE, Parker FJ, Smith EL. 1955. The vitamin B_{12} group. Presence of 2-methyl purines in factors *A* and *H* and isolation of new factors. *Biochem. J.* 59:82–86
36. Burke S, Krzycki J. 1995. Involvement of the "A" isozyme of methyltransferase II and the 29-kilodalton corrinoid protein in methanogenesis from monomethylamine. *J. Bacteriol.* 177:4410–16
37. Cameron B, Blanche F, Rouyez M-C, Bisch D, Famechon A, et al. 1991. Genetic analysis, nucleotide sequence, and products of two *Pseudomonas denitrificans cob* genes encoding nicotinate-nucleotide:dimethylbenzimidazole phosphoribosyltransferase and cobalamin (5′-phosphate) synthase. *J. Bacteriol.* 173:6066–73
38. Cameron B, Briggs K, Pridmore S, Brefort G, Crouzet J. 1989. Cloning and analysis of genes involved in coenzyme B_{12} biosynthesis in *Pseudomonas denitrificans. J. Bacteriol.* 171:547–57
39. Cameron B, Guilot C, Blanche F, Cauchois L, Rouyez M-C, et al. 1991. Genetic and sequence analysis of a *Pseudomonas denitrificans* DNA fragment containing two *cob* genes. *J. Bacteriol.* 173:6058–65
40. Cauthen SE, Foster MA, Woods DD. 1966. Methionine synthesis by extracts of *Salmonella typhimurium. Biochem. J.* 98:630–35
41. Chen P, Ailion M, Bobik T, Stormo G, Roth J. 1995. Five promoters integrate control of the *cob*/*pdu* regulon in *Salmonella typhimurium. J. Bacteriol.* 177:5401–10
42. Chen P, Ailion M, Weyland N, Roth J. 1995. The end of the *cob* operon: evidence that the last gene (*cobT*) catalyzes synthesis of the lower ligand of vitamin B_{12}, dimethylbenzimidazole. *J. Bacteriol.* 177:1461–69

43. Chen P, Andersson DI, Roth JR. 1994. The control region of the *pdu*/*cob* regulon in *Salmonella typhimurium. J. Bacteriol.* 176:5474–82
44. Childs JD, Smith DA. 1969. New methionine structural gene in *Salmonella typhimurium. J. Bacteriol.* 100:377–82
45. Codd GA. 1988. Carboxysomes and ribulose bisphosphate carboxylase/oxygenase. *Adv. Microb. Physiol.* 29:115–64
46. Combe JS. 1824. History of a case of anaemia. *Trans. Med. Chiropr. Soc. Edinburgh* 1:194–203
47. Crouzet J, Cameron B, Cauchois L, Rigault S, Rouyez M-C, et al. 1990. Genetic and sequence analysis of an 8.7-kilobase *Pseudomonas denitrificans* fragment carrying eight genes involved in transformation of precorrin–2 to cobyrinic acid. *J. Bacteriol.* 172:5980–90
48. Crouzet J, Cauchois L, Blanche F, Debussche L, Thibaut D, et al. 1990. Nucleotide sequence of a *Pseudomonas denitrificans* 5.4 kilobase DNA fragment containing five *cob* genes and identification of structural genes encoding *S*-adenosyl-L-methionine:Uroporphyrinogen III methyltransferase and cobyrinic acid *a*,*c*-diamide synthase. *J. Bacteriol.* 172:5968–79
49. Crouzet J, Levy-Schil S, Cameron B, Cauchois L, Rigault S, et al. 1991. Nucleotide sequence and genetic analysis of a 13.1-kilobase-pair *Pseudomonas denitrificans* DNA fragment containing five *cob* and identification of structural genes encoding cob(I)alamin adenosyltransferase, cobyric acid synthase, and bifunctional cobinamide kinase-cobinamide phosphate guanylyltransferase. *J. Bacteriol.* 173:6074–87

49a. Debussche L, Couder M, Thibant D, Cameron B, Crouzet J, Blanche F. 1991. Purification and partial characterization of cob(I)alamin adenosyltransferase from *Pseudomonas denitrificans. J. Bacteriol.* 173:6300–2

50. Debussche L, Couder M, Thibaut D, Cameron B, Crouzet J, Blanche F. 1992. Assay, purification, and characterization of cobaltochelatase, a unique complex enzyme catalyzing cobalt insertion in hydrogenobyrinic acid *a*,*c*-diamide during coenzyme B_{12} biosynthesis in *Pseudomonas denitrificans. J. Bacteriol.* 174:7445–51
51. Debussche L, Thibaut D, Cameron B, Crouzet J, Blanche F. 1990. Purification and characterization of cobyrinic acid *a*,*c*-diamide synthase from *Pseudomonas denitrificans. J. Bacteriol.* 172:6239–44
52. Debussche L, Thibaut D, Cameron B, Crouzet J, Blanche F. 1993. Biosynthesis of the corrin macrocycle of coenzyme B_{12} in *Pseudomonas denitrificans. J. Bacteriol.* 175:7430–40.
53. DeVeaux LC, Clevenson DS, Bradbeer C, Kadner RJ. 1986. Identification of the BtuCED polypeptides and evidence for their role in vitamin B_{12} transport in *Escherichia coli. J. Bacteriol.* 167:920–27
54. DeVeaux LC, Kadner RJ. 1985. Transport of vitamin B_{12} in *Escherichia coli*: cloning of the *btuCD* region. *J. Bacteriol.* 162:888–96
55. di Girolamo PM, Bradbeer C. 1971. Transport of vitamin B_{12} in *Escherichia coli. J. Bacteriol.* 106:745–50
56. di Girolamo PM, Kadner RJ, Bradbeer C. 1971. Isolation of vitamin B_{12} transport mutants of *Escherichia coli. J. Bacteriol.* 106:751–57
57. Drummond JT, Matthews RG. 1993. Cobalamin-dependent and cobalamin-independent methionine synthases in *Escherichia coli*: two solutions to the same chemical problem. *Adv. Exp. Med. Biol.* 338:687–92
58. Duda J, Pedziwilk Z, Zodrow K. 1967. Studies on the vitamin B_{12} content of the leguminous plants. *Acta Microbiol. Pol.* 6:233–38
59. Escalante-Semerena JC, Johnson MG, Roth JR. 1992. The CobII and CobIII regions of the cobalamin (vitamin B_{12}) biosynthetic operon of *Salmonella typhimurium. J. Bacteriol.* 174:24–29
60. Escalante-Semerena JC, Roth JR. 1987. Regulation of cobalamin biosynthetic operons in *Salmonella typhimurium. J. Bacteriol.* 169:2251–58
61. Escalante-Semerena JC, Suh S-J, Roth JR. 1990. *cobA* function is required for both de novo cobalamin biosynthesis and assimilation of exogenous corrinoids in *Salmonella typhimurium. J. Bacteriol.* 172:273–80
62. Eschenmoser A. 1988. Vitamin B_{12}: experiments concerning the origin of its molecular structure. *Angew. Chem. Int. Ed. Engl.* 27:5–39
63. Fantes KH, O'Callaghan. 1955. The effect of *o*-phenylenediamine on the biosynthesis of vitamin B_{12}: a new vitamin B_{12} analogue. *Biochem. J.* 59:79–82
64. Faust LP, Conner JA, Roof DA, Hoch JA, Babior BM. 1990. Cloning, sequencing, and expression of the genes encoding the adenosylcobalamin-

dependent ethanolamine ammonia-lyase of *Salmonella typhimurium*. *J. Biol. Chem.* 265:12462–66
65. Fazzio TG, Roth JR. 1996. Evidence that the CysG protein catalyzes the first reaction specific to B_{12} synthesis in *Salmonella typhimurium*: insertion of cobalt. *J. Bacteriol.* In press
66. Fernández-Briera A, Garrido-Pertierra A. 1988. A degradation pathway of propionate in *Salmonella typhimurium* LT–2. *Biochimie* 70:757–68
67. Ferry J. 1992. Methane from acetate. *J. Bacteriol.* 174:5489–95
68. Ferry J, ed. 1993. *Methanogenesis: Ecology, Physiology, Biochemistry, and Genetics*. New York: Chapman & Hall
69. Ferry J. 1995. CO dehydrogenase. *Annu. Rev. Microbiol.* 49:305–33
70. Fischer R, Gaürtner P, Yeliseev A, Thauer R. 1992. N^5-methyltetrahydromethanopterin: coenzyme M methyltransferase in methanogenic archaebacteria is a membrane-bound protein. *Arch. Microbiol.* 158:208–17
71. Forage RG, Foster MA. 1982. Glycerol fermentation in *Klebsiella pneumoniae*: functions of the coenzyme B_{12}-dependent glycerol and diol dehydratases. *J. Bacteriol.* 149:413–19
72. Ford JE, Holdsworth ES, Kon SK. 1955. The biosynthesis of B_{12}-like compounds. *Biochem. J.* 59:86–93
73. Frey B, McCloskey J, Kersten W, Kersten H. 1988. New function of vitamin B_{12}: cobamide-dependent reduction of epoxyqueuosine in tRNAs of *Escherichia coli* and *Salmonella typhimurium*. *J. Bacteriol.* 170:2078–82
74. Friedman HC, Ford SH. 1976. Vitamin B_{12} biosynthesis: in vitro formation of cobinamide from cobyric acid and L-threonine. *Arch. Biochem. Biophys.* 175:121–30
75. Friedrich MJ, DeVeaux LC, Kadner RJ. 1986. Nucleotide sequence of the *btuCED* genes involved in vitamin B_{12} transport in *Escherichia coli* and homology with components of periplasmic-binding-protein-dependent transport systems. *J. Bacteriol.* 167:928–34
76. Frings J, Schink B. 1994. Fermentation of phenoxyethanol to phenol and acetate by a homoacetogenic bacterium. *Arch. Microbiol.* 162:199–204
77. Frings J, Schramm I, Schink B. 1992. Enzymes involved in anaerobic ethylene glycol degradation by *Pelobacter venetianus* and *Bacteroides* strain PG 1. *Appl. Environ. Microbiol.* 58:2164–67
78. Frings J, Wondrak C, Schink B. 1994. Fermentative degradation of triethanolamine by a homoacetogenic bacterium. *Arch. Microbiol.* 162:103–7
79. Fujii K, Huennekens FM. 1974. Activation of methionine synthetase by a reduced triphosphopyridine nucleotide-dependent flavoprotein system. *J. Biol. Chem.* 249:6745–53
80. Grabau C, Roth JR. 1992. A *Salmonella typhimurium* cobalamin-deficient mutant blocked in 1-amino–2-propanol synthesis. *J. Bacteriol.* 174:2138–44
81. Harms U, Weiss D, Gaürtner P, Linder D, Thauer R. 1995. The energy conserving N^5-methyltetrahydromethanopterin: coenzyme M methyltransferase from *Methanobacterium thermoautotrophicum* is composed of eight different subunits. *Eur. J. Biochem.* 228:640–48
82. Heller K, Kadner RJ. 1985. Nucleotide sequence of the gene for the vitamin B_{12} receptor protein in the outer membrane of *Escherichia coli*. *J. Bacteriol.* 161:904–8
83. Heller K, Mann BJ, Kadner RJ. 1985. Cloning and expression of the gene for the vitamin B_{12} receptor protein in the outer membrane of *Escherichia coli*. *J. Bacteriol.* 161:896–903
84. Hodgkin DC, Kamper J, MacKay M, Pickworth J. 1956. Structure of vitamin B_{12}. *Nature* 178:64–66
85. Hodgson GW, Ponnamperuma C. 1968. Prebiotic porphyrin genesis: porphyrins from electric discharge in methane, ammonia and water vapor. *Proc. Natl. Acad. Sci. USA* 59:22–28
86. Jeter R, Olivera BM, Roth JR. 1984. *Salmonella typhimurium* synthesizes cobalamin (vitamin B_{12}) de novo under anaerobic growth conditions. *J. Bacteriol.* 159:206–16
87. Jeter RM. 1990. Cobalamin dependent 1,2-propanediol utilization by *Salmonella typhimurium*. *J. Gen. Microbiol.* 136:887–96
88. Jeter RM, Roth JR. 1987. Cobalamin (vitamin B_{12}) biosynthetic genes of *Salmonella typhimurium*. *J. Bacteriol.* 169:3189–98
89. Johnson MG, Escalante-Semerena JC. 1992. Identification of 5,6-dimethylbenzimidazole as the coα ligand of the cobamide synthesized by *Salmonella typhimurium*. *J. Biol. Chem.* 267:13302–5
90. Kadner RJ. 1978. Repression of synthesis of the vitamin B_{12} receptor in *Escherichia coli*. *J. Bacteriol.* 136:1050–57

91. Kadner RJ, Bassford PJ Jr. 1977. Relation of cell growth and colicin tolerance to vitamin B_{12} uptake in *Escherichia coli*. *J. Bacteriol.* 129:254–64
92. Kadner RJ, Heller KJ. 1995. Mutual inhibition of cobalamin and siderophore uptake systems suggests their competition for TonB function. *J. Bacteriol.* 177:4829–35
93. Kadner RJ, Liggins GL. 1973. Transport of vitamin B_{12} in *Escherichia coli*: genetic studies. *J. Bacteriol.* 115:514–21
94. Kadner RJ, McElhaney G. 1978. Outer membrane-dependent transport systems in *Escherichia coli*: turnover of TonB function. *J. Bacteriol.* 134:1020–29
95. Keltjens J, Vogels G. 1994. Conversion of methanol and methylamines to methane and carbon dioxide. See Ref. 68, pp. 253–302
96. Kenley JS, Leighton M, Bradbeer C. 1978. Transport of vitamin B_{12} in *Escherichia coli*. Corrinoid specificity of the outer membrane receptor. *J. Biol. Chem.* 253:1341–46
97. Koessler KK, Maurer S, Loughlin R. 1926. The relation of anemia, primary and secondary to vitamin A deficiency. *J. Am. Med. Assoc.* 87:476–82
98. Kurumaya K, Kajiwara M. 1990. Studies on the biosynthesis of corrinoids and porphyrinoids. III. The origin of amide nitrogen of vitamin B_{12}. *Chem. Pharm. Bull.* 38:2589–90
99. Lawrence JG, Roth JR. 1995. The cobalamin (coenzyme B_{12}) biosynthetic genes of *Escherichia coli*. *J. Bacteriol.* 177:6371–80
100. Lawrence JG, Roth JR. 1995. Evolution of coenzyme B_{12} synthesis among enteric bacteria: evidence for loss and reacquisition of a multigene complex. *Genetics* 142:11–24
101. Ledley F. 1990. Perspectives on methylmalonic acidemia resulting from molecular cloning of methylmalonyl CoA mutase. *Bioessays* 12:335–40
102. Lin ECC. 1987. Dissimilatory pathways for sugars, polyols, and carboxylates. In Escherichia coli *and* Salmonella typhimurium*: Cellular and Molecular Biology,* ed. FD Niedhardt, JL Ingraham, KB Low, B Magasanik, M Schaechter, HE Umbarger, pp. 244–84. Washington, DC: Am. Soc. Microbiol.
103. Ljungdahl L, Wood H. 1982. Acetate biosynthesis. In B_{12}, ed. D Dolphin, pp. 165–202. New York: Wiley
104. Lowe DA, Turner JM. 1970. Origin of the D–1-aminopropan–2-ol fragment of vitamin B_{12}. *J. Gen. Microbiol.* 64:119–22
105. Lundrigan MD, Kadner RJ. 1989. Altered cobalamin metabolism in *Escherichia coli btuR* mutants affects *btuB* regulation. *J. Bacteriol.* 171:154–61
106. Lundrigan MD, Koster W, Kadner RJ. 1991. Transcribed sequences of the *Escherichia coli btuB* gene control its expression and regulation by vitamin B_{12}. *Proc. Natl. Acad. Sci. USA* 88:1479–83
107. Minot GR, Murphy WP. 1926. Treatment of pernicious anemia by a special diet. *JAMA* 87:470–76
108. Mojica-a T, Garcia E. 1976. Growth of coliphage BF23 on rough strains of *Salmonella typhimurium*: the *bfe* locus. *Mol. Gen. Genet.* 147:195–202
109. Müller G, Hlineny K, Savvidis E, Zipfel F, Schiedl J, Schneider E. 1990. On the methylation process and cobalt insertion in cobyrinic acid biosynthesis. In *Chemical Aspects of Enzyme Biotechnology,* ed. TO Baldwin, pp. 281–98. New York: Plenum
110. Müller G, Zipfel F, Hlineny K, Savvidis E, Hertle R, Traub-Eberhard U. 1991. Timing of cobalt insertion in vitamin B_{12} biosynthesis. *J. Am. Chem. Soc.* 113:9893–95
111. Müller V, Blaut M, Gottschalk G. 1993. Bioenergetics of methanogenesis. See Ref. 68, pp. 360–406.
112. Neuberger A, Tait GH. 1960. The enzymatic conversion of threonine to aminoacetone. *Biochim. Biophys. Acta* 41:164–65
113. Noguchi S, Nishimura Y, Hirota Y, Nishimura S. 1982. Isolation and characterization of an *E. coli* mutant lacking tRNA-guanine transglycosylase. Function and biosynthesis of queuosine tRNA. *J. Biol. Chem.* 257:6544–50
114. Obradors N, Badía J, Baldomà L, Aguilar J. 1988. Anaerobic metabolism of the L-rhamnose fermentation product 1,2-propanediol in *Salmonella typhimurium. J. Bacteriol.* 170:2159–62
115. Osborne C, Chen L-M, Matthews RG. 1991. Isolation, cloning, mapping, and nucleotide sequencing of the gene encoding flavodoxin in *Escherichia coli*. *J. Bacteriol.* 173:1729–37
116. O'Toole GA, Escalante-Semerena JC. 1993. *cobU*-dependent assimilation of nonadenosylated cobinamide in *cobA* mutants of *Salmonella typhimurium. J. Bacteriol.* 175:6328–36
117. O'Toole GA, Rondon MR, Escalante-Semerena JC. 1993. Analysis of mutants of *Salmonella typhimurium* defective in

the synthesis of the nucleotide loop of cobalamin. *J. Bacteriol.* 175:3317–26
118. O'Toole GA, Trzebiatowski JR, Escalante-Semerena JC. 1994. The *cobC* gene of *Salmonella typhimurium* codes for a novel phosphatase involved in the assembly of the nucleotide loop of cobalamin. *J. Biol. Chem.* 269:26503–11
119. Peston JM. 1977. Leucine 2,3-aminomutase: a cobalamin-dependent enzyme present in bean seedlings. *Science* 195:301–2
120. Pierce J, Carlson TJ, Williams JG. 1989. A cyanobacterial mutant requiring the expression of ribulose bisphosphate carboxylase from a photosynthetic anaerobe. *Proc. Natl. Acad. Sci. USA* 86:5753–57
121. Poirot C, Kengen S, Valk E, Keltjens J, van der Drift C, Vogels G. 1987. Formation of methylcoenzyme M from formaldehyde by cell free extracts of *Methanobacterium thermoautotrophicum.* Evidence for the involvement of a corrinoid-containing methyltransferase. *FEMS Microbiol. Lett.* 40:7–13
122. Price GD, Howitt SM, Harrison K, Badger MR. 1993. Analysis of a genomic DNA region from the cyanobacterium *Synechococcus* sp. strain PCC7942 involved in carboxysome assembly and function. *J. Bacteriol.* 175:2871–79
123. Pruthi RK, Tefferi A. 1994. Pernicious anemia revisited. *Mayo Clin. Proc.* 69:144–50
124. Qureshi A, Rosenblatt D, Cooper B. 1994. Inherited disorders of cobalamin metabolism. *Crit. Rev. Oncol. Hematol.* 17:133–51
125. Ragsdale S. 1991. Enzymology of the acetyl-CoA pathway of CO_2 fixation. *Crit. Rev. Biochem. Mol. Biol.* 26:261–300
126. Reichard P. 1993. From RNA to DNA, why so many reductases? *Science* 260:1773–77
127. Renz P, Hoürig J, Wurm R. 1979. On the biosynthesis of the 5,6-dimethylbenzimidazole moiety of vitamin B_{12}. In *Vitamin B_{12}*, ed. B Zagalak, W Friedrich, 317–22. Berlin: de Gruyter
128. Reťey J. 1982. Methylmalonyl-CoA mutase. In *B_{12}*, ed. D Dolphin, pp. 358–79. New York: Wiley
129. Richter-Dahlfors AA, Andersson DI. 1992. Cobalamin (vitamin B_{12}) repression of the *cob* operon in *Salmonella typhimurium* requires sequences within the leader and the first translated open reading frame. *Mol. Microbiol.* 6:743–49
130. Richter-Dahlfors AA, Ravnum S, Andersson DI. 1994. Vitamin B_{12} repression of the *cob* operon in *Salmonella typhimurium*: translational control of the *cbiA* gene. *Mol. Microbiol.* 13:541–53
131. Rickes EL, Brink NG, Koniuszy FR, Wood TR, Folkers K. 1948. Crystalline vitamin B_{12}. *Science* 107:396
132. Rioux CR, Friedrich MJ, Kadner RJ. 1990. Genes on the 90-kilobase plasmid of *Salmonella typhimurium* confer low-affinity cobalamin transport: relationship to fimbria biosynthetic genes. *J. Bacteriol.* 172:6217–22
133. Rioux CR, Kadner RJ. 1989. Two outer membrane transport systems for vitamin B_{12} in *Salmonella typhimurium. J. Bacteriol.* 171:2986–93
134. Rioux CR, Kadner RJ. 1989. Vitamin B_{12} transport in *Escherichia coli* K12 does not require the *btuE* gene of the *btuCED* operon. *Mol. Gen. Genet.* 217:301–8
135. Roessner CA, Warren MJ, Santander PJ, Atshaves BP, Ozaki S-I, et al. 1992. Expression of 9 *Salmonella typhimurium* enzymes for cobinamide synthesis. Identification of the 11-methyl and 20-methyl transferases of corrin biosynthesis. *FEBS Lett.* 301:73–78
136. Rondon MR, Escalante-Semerena JC. 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.* 174:2267–72
137. Roof DM, Roth JR. 1988. Ethanolamine utilization in *Salmonella typhimurium. J. Bacteriol.* 170:3855–63
138. Roof DM, Roth JR. 1989. Functions required for vitamin-B_{12} dependent ethanolamine utilization in *Salmonella typhimurium. J. Bacteriol.* 171:3316–23
139. Roof DM, Roth JR. 1992. Autogenous regulation of ethanolamine utilization by a transcriptional activator of the *eut* operon in *Salmonella typhimurium. J. Bacteriol.* 174:6634–43
140. Roth JR, Lawrence JG, Rubenfield M, Kieffer-Higgins S, Church GM. 1993. Characterization of the cobalamin (vitamin B_{12}) biosynthetic genes of *Salmonella typhimurium. J. Bacteriol.* 175:3303–16
141. Sampson BA, Gotschlich EC. 1992. Elimination of the vitamin B_{12} uptake or synthesis pathway does not diminish the virulence of *Escherichia coli* K1 or *Salmonella typhimurium* in three model systems. *Infect. Immun.* 60:3518–22

142. Sanderson KE, Hessel A, Rudd KE. 1995. Genetic map of *Salmonella typhimurium*, Edition VIII. *Microbiol. Rev.* 59:241–303
143. Sattler I, Roessner CA, Stolowich NJ, Hardin SH, Harris-Haller LW, et al. 1995. Cloning, sequencing, and expression of the uroporphyrinogen III methyltransferase *cobA* gene of *Propionibacterium freudenreichii* (*shermanii*). *J. Bacteriol.* 177:1564–69
144. Scarlett FA, Turner JM. 1976. Microbial metabolism of amino alcohols. Ethanolamine catabolism mediated by coenzyme B_{12}-dependent ethanolamine ammonia-lyase in *Escherichia coli* and *Klebsiella aerogenes*. *J. Gen. Microbiol.* 95:173–76
145. Schneider Z, Stroiński A. 1987. Methylcobamide-dependent reactions. In *Comprehensive B_{12}*, ed. Z Schneider, A Stroiński, pp. 259–66. Berlin: Gruyter
146. Scott AI. 1990. Mechanistic and evolutionary aspects of vitamin B_{12} biosynthesis. *Acc. Chem. Res.* 23:308–17
147. Scott AI. 1993. How nature synthesizes vitamin B_{12}—a survey of the last four billion years. *Angew. Chemie* 32:1223–43
148. Scott AI. 1994. Recent studies of enzymatically controlled steps in B_{12} synthesis. *Ciba Found. Symp.* 180:285–303
149. Scott AI, Roessner CA, Stolowich NJ, Spencer JB, Min C, Ozaki S-I. 1993. Biosynthesis of vitamin B_{12}. Discovery of the enzymes for oxidative ring contraction and insertion of the fourth methyl group. *FEBS Lett.* 331:105–8
150. Sheppard DE, Roth JR. 1994. A rationale for autoinduction of a transcriptional activator: ethanolamine ammonia-lyase (EutBC) and the operon activator (EutR) compete for adenosyl-cobalamin in *Salmonella typhimurium*. *J. Bacteriol.* 176:1287–96
151. Smith DA, Childs JD. 1966. Methionine genes and enzymes of *Salmonella typhimurium*. *Heredity* 21:265–86
152. Spencer JB, Stolowich NJ, Roessner CA, Scott AI. 1993. The *Escherichia coli cysG* gene encodes the multifunctional protein, siroheme synthase. *FEBS Lett.* 335:57–60
153. Stojiljkovic I, Baümer AJ, Heffron F. 1995. Ethanolamine utilization in *Salmonella typhimurium*: nucleotide sequence, protein expression, and mutational analysis of the *cchA cchB eutJ eutG eutH* gene cluster. *J. Bacteriol.* 177:1357–66
154. Stroiński A. 1987. Adenosylcobamide-dependent reactions. In *Comprehensive B_{12}*, ed. Z Schneider, A Stroinski, pp. 226–59. Berlin: Gruyter
155. Stupperich E. 1993. Recent advances in elucidation of biological corrinoid functions. *FEMS Microbiol. Rev.* 12:349–65
156. Stupperich E, Eisinger H-J, Schurr S. 1990. Corrinoids in anaerobic bacteria. *FEMS Microbiol. Rev.* 87:355–60
157. Stupperich E, Steiner I, Eisinger HJ. 1987. Substitution of coα-(5-hydroxybenzimidazolyl)cobamide (factor III) by vitamin B_{12} in *Methanobacterium thermoautotrophicum*. *J. Bacteriol.* 169:3076–81
158. Suh S-J, Escalante-Semerena JC. 1993. Cloning, sequencing and overexpression of *cobA*, which encodes ATP:corrinoid adenosyltransferase in *Salmonella typhimurium*. *Gene* 129:93–97
159. Suh S-J, Escalante-Semerena JC. 1995. Purification and initial characterization of the ATP:corrinoid adenosyltransferase encoded by the *cobA* gene of *Salmonella typhimurium*. *J. Bacteriol.* 177:921–25
160. Taylor RT, Nevins SMP, Hanna ML. 1972. Uptake of cyanocobalamin by *Escherichia coli* B: corrinoid specificity and relationship of a binder. *Arch. Biochem. Biophys.* 149:232–43
161. Taylor RT, Norrell SA, Hanna ML. 1972. Uptake of cyanocobalamin by *Escherichia coli* B: Some characteristics and evidence for a binding protein. *Arch. Biochem. Biophys.* 148:366–81
162. Taylor RT, Weisbach H. 1973. N^5-methyltetrahydrofolate-homocysteine methyltransferases. In *The Enzymes*, ed. D Boyer, pp. 121–165. New York: Academic
163. Thauer R, Hedderich R, Fischer R. 1993. Reactions and enzymes involved in methanogenesis from CO_2 and H_2. See Ref. 68, pp. 209–52.
164. Thibaut D, Couder M, Famechon A, Debussche L, Cameron B, et al. 1992. The final step in the biosynthesis of hydrogenobyrinic acid is catalyzed by the *cobH* gene product with precorrin–8x as the substrate. *J. Bacteriol.* 174:1043–49
165. Toraya T, Honda S, Fukui S. 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
166. Trzebiatowski JR, O'Toole GA, Escalante-Semerena JC. 1994. The *cobT* gene of *Salmonella typhimurium* encodes the NaMN:5,6-dimethylbenzimidazole phosphoribosyltransferase responsible

for the synthesis of N^1-(5-phospho-α-D-ribosyl)–5,6-dimethylbenzimidazole, an intermediate in the synthesis of the nucleotide loop of cobalamin. *J. Bacteriol.* 176:3568–75

167. Van Dyk TK, LaRossa RA. 1987. Involvement of *ack-pta* operon products in α-ketobutyrate metabolism by *Salmonella typhimurium*. *Mol. Gen. Genet.* 207:435–40
168. Wang B-Y, Bradbeer C, Kadner RJ. 1992. Conserved structural and regulatory regions in the *Salmonella typhimurium btuB* gene for the outer membrane vitamin B_{12} transport protein. *Res. Microbiol.* 143:459–66
169. Wang CC, Newton A. 1971. An additional step in the transport of iron defined by the *tonB* locus of *Escherichia coli*. *J. Biol. Chem.* 246:2147–51
170. Watanabe F, Nakano Y, Tamura Y, Yamanaka H. 1991. Vitamin B_{12} metabolism in a photosynthesizing green alga, *Chlamydomonas reinhardtii*. *Biochim. Biophys. Acta* 1075:36–41
171. Watanabe F, Tamura Y, Stupperich E, Nakano Y. 1993. Uptake of cobalamin by *Euglena* mitochondria. *J. Biochem.* (Tokyo) 114:793–99
172. White JC, di Girolamo PM, Fu ML, Preston YA, Bradbeer C. 1973. Transport of vitamin B_{12} in *Escherichia coli*. Location and properties of the initial B_{12}-binding site. *J. Biol. Chem.* 248:3976–86
173. Wolf JB, Brey RN. 1986. Isolation and genetic characterization of *Bacillus megaterium* cobalamin biosynthesis-deficient mutants. *J. Bacteriol.* 166:51–58
174. Wood H, Ragsdale W, Pezacka E. 1986. The acetyl-CoA pathway: a newly discovered pathway of autotrophic growth. *Trends Biochem. Sci.* 11:14–17
175. Wu W-F, Urbanowski ML, Stauffer GV. 1992. Role of the MetR regulatory system in vitamin B_{12}-mediated repression of the *Salmonella typhimurium metE* gene. *J. Bacteriol.* 174:4833–37