JOURNAL OF BACTERIOLOGY, Feb. 1998, p. 885–891 Vol. 180, No. 4
0021-9193/98/$04.00+0

The *apbE* Gene Encodes a Lipoprotein Involved in Thiamine Synthesis in *Salmonella typhimurium*

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Received 5 September 1997/Accepted 6 December 1997

Thiamine pyrophosphate is an essential cofactor that is synthesized de novo in *Salmonella typhimurium*. The biochemical steps and gene products involved in the conversion of aminoimidazole ribotide (AIR), a purine intermediate, to the 4-amino-5-hydroxymethyl-2-methyl pyrimidine (HMP) moiety of thiamine have yet to be elucidated. We have isolated mutations in a new locus (*Escherichia coli* open reading frame designation *yojK*) at 49 min on the *S. typhimurium* chromosome. Two significant phenotypes associated with lesions in this locus (*apbE*) were identified. First, *apbE purF* double mutants require thiamine, specifically the HMP moiety. Second, in the presence of adenine, *apbE* single mutants require thiamine, specifically both the HMP and the thiazole moieties. Together, the phenotypes associated with *apbE* mutants suggest that flux through the purine pathway has a role in regulating synthesis of the thiazole moiety of thiamine and are consistent with ApbE being involved in the conversion of AIR to HMP. The product of the *apbE* gene was found to be a 36-kDa membrane-associated lipoprotein, making it the second membrane protein implicated in thiamine synthesis.

An efficient metabolism demands productive interactions between a number of biochemical pathways of both high and low carbon flux. Low-flux pathways, such as those required for vitamin synthesis, provide a sensitive model system for addressing subtle pathway interactions. Recent work with *Salmonella typhimurium* and *Escherichia coli* has shown that the synthesis of at least some vitamins, such as cobalamin (28), pyridoxal phosphate (21, 22), and thiamine (8, 13, 14, 34), is highly regulated and involves input from multiple pathways involved in other aspects of cell metabolism. Our interest lies in identifying metabolic connections which influence thiamine synthesis.

The synthesis of thiamine pyrophosphate requires the condensation of two independently synthesized moieties, 4-amino-5-hydroxymethyl-2-methyl pyrimidine (HMP) and 4-methyl-5-(β-hydroxyethyl) thiazole (THZ) (Fig. 1). Although the biochemical steps for the synthesis of HMP from the purine intermediate aminoimidazole ribotide (AIR) and the independent synthesis of the thiazole moiety have yet to be fully elucidated, a number of genetic loci that are required for thiamine synthesis have been identified (17, 23, 31, 33). The majority of these loci have been implicated in synthesis of the thiazole moiety of thiamine or the condensation of HMP and THZ. A single locus (*thiC*) that is required for HMP synthesis has been identified, although recently mutations in three loci which conditionally affect the synthesis of the pyrimidine moiety have been identified (3, 26). Analysis of one such locus (*apbC*) led to a model proposing redundant pathways for the conversion of AIR to HMP (26). Such a model is consistent with the growing class of conditional HMP auxotrophs. The role of ThiC in this conversion is unclear at this point.

We report here the identification of a new locus, *apbE*, which falls in the class of loci conditionally required for the synthesis of HMP. The *apbE* gene product was found to be a membrane-associated lipoprotein, and mutants with a defect in this locus have a conditional requirement for HMP. Possible explanations for the involvement of a lipoprotein in the biosynthesis of thiamine are discussed.

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MATERIALS AND METHODS

Strains, media, and chemicals. All strains used in this study are derivatives of *S. typhimurium* LT2 and are listed with their genotypes in Table 1. Mu*dJ* is used throughout this paper to refer to the Mu*d* d1734 insertion element (6), and Tn*10d*(Tc) refers to the transposition-defective mini-Tn*10* described by Way et al. (32). NCE medium supplemented with $MgSO_4$ (1 mM) (9) and a carbon source (11 mM) was used as minimal medium. Difco nutrient broth (8 g/liter) with NaCl (5 g/liter) added was used as rich medium. Difco BiTek agar was added to a final concentration of 1.5% for solid medium. Unless otherwise stated, the final concentrations of adenine and thiamine were 0.4 mM and 100 nM, respectively. The final concentrations of antibiotics in rich and minimal media, respectively, were as follows (in micrograms per milliliter): tetracycline, 20 and 10; kanamycin, 50 and 125; ampicillin, 30 and 15; and chloramphenicol, 20 and 4.

Transduction methods. The high-frequency generalized transducing mutant of bacteriophage P22 (HT105/1 *int*-201) (29) was used in all transductional crosses. The method for transduction and subsequent purification of transductants has been previously described (11).

Isolation of *apbE* mutants. Insertions causing a thiamine auxotrophy in a *purF* mutant were isolated by insertion mutagenesis using one of two defective transposons, Tn*10d*(Tc) or Mu*dJ*, as has been described elsewhere (26). After reintroduction of these insertions into DM1936 (*purF*) and confirmation of an appropriate phenotype, genetic linkage groups were determined. One linkage group contained one Mu*dJ* and two Tn*10d*(Tc) insertions and defined a new locus, which we designated *apbE.*

Molecular biology techniques. (i) Identification of *apbE.* The identity of the *apbE* locus was determined by converting *apbE27*::Mu*dJ* into either a Mu*dP*-P22 or a Mu*dQ*-P22 locked-in prophage (5). Standard P22 techniques were used to amplify the nucleotide sequences that flanked the Mu*dP* or Mu*dQ* insertion (36). Primers specific to the ends of Mu*d* (7) were used to sequence this enriched preparation of chromosomal DNA with a Sequitherm cycle sequencing kit from Epicentre Technologies Corporation (Madison, Wis.). [^{32}P]ATP had a specific activity of >6,000 Ci/mol (Dupont, Beverly, Mass.). Computational analysis of the sequence was performed by using BLAST (Basic Local Alignment Search Tool) and the GenBank and Swissprot databases (1).

(ii) Cloning and sequencing of *apbE.* The relative positions of the two Tn*10d*(Tc) insertions in the *apbE* gene were determined by PCR amplification with a Thermolyne Temp-Tronic Thermocycler and Vent exonuclease (*exo*) from New England Biolabs and primers designed from the sequences of *Salmonella typhi ompC* (primer 1, 5′ TATTCCGGCGTACAAATA 3′), *S. typhimurium ada* (primer 3, 5′ GACGGGATCCTTGCGTTTTAATTCTTATCGC 3′), the two loci that flank the *apbE* locus, and a primer specific to the ends of the Tn*10d*(Tc) insertion (primer 2, 5′ GACAAGATGTGTATCCACCTTAAC 3′). Primers 1 and 3 were then used to amplify the *apbE* gene from the wild-type (LT2) *S. typhimurium* chromosome. The resulting 1.15-kb fragment was ligated into pSU19 which had been digested with *Hin*cII to yield pApbE1. Plasmids were introduced into strains by electroporation with an *E. coli* Pulser (Bio-Rad Lab-

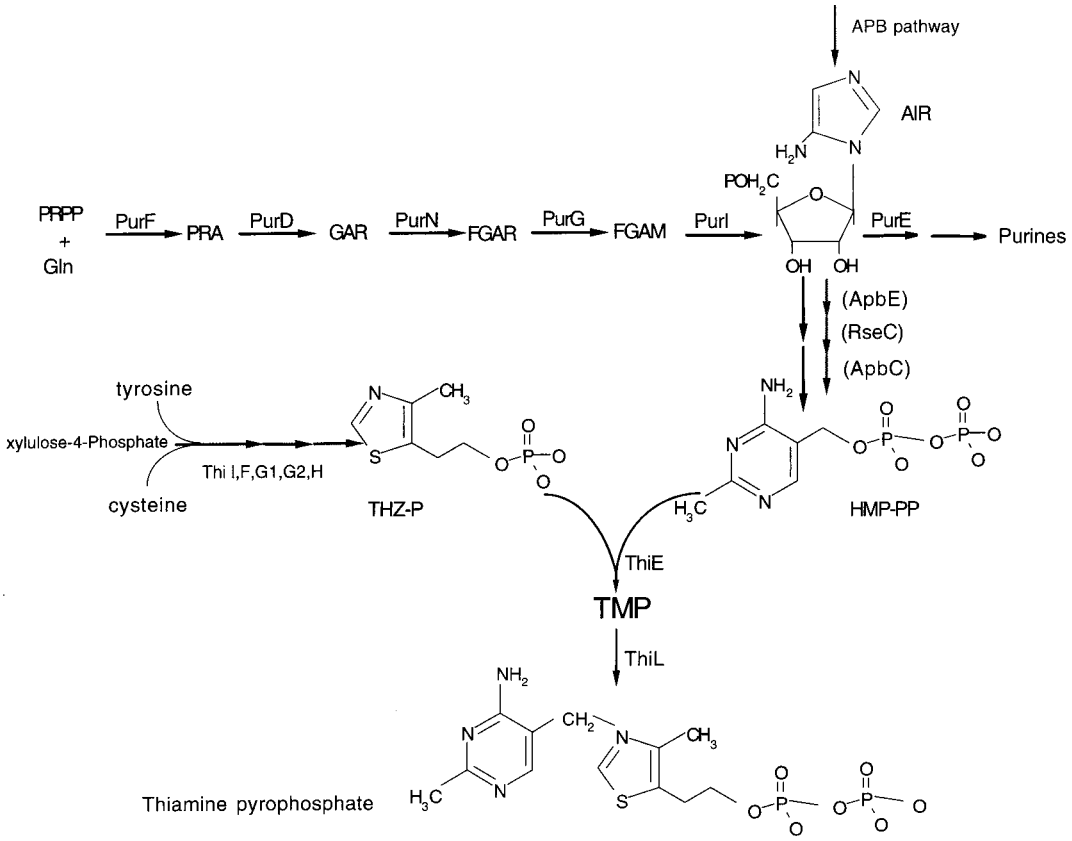

FIG. 1. Schematic representation of the purine and thiamine biosynthetic pathways. Some purine gene products are indicated above the reactions they catalyze. Proposed pathways with an unknown number of reactions are indicated (shaded arrows). ApbE, RseC (3), and ApbC (26) are in paratheses to reflect proposed roles in synthesis of HMP. Abbreviations: PRA, phosphoribosylamine; HMP-PP, HMP pyrophosphate; THZ-P, thiazole phosphate.

oratories, Hercules, Calif.). The sequence of the *apbE* gene was determined by fluorescent-dye nucleotide sequence analysis performed by the University of Wisconsin—Madison Biotechnology Sequencing Center with primers specific to the M13 multiple cloning site of pSU19.

(iii) Overexpression and lipoprotein analysis. A 1.15-kb *Hin*dIII-*Eco*RI fragment from pApbE1 that contained the *apbE* gene was cloned into the pT7-5 overexpression vector so that the *apbE* gene was in the correct orientation to be transcribed by the T7 promoter (30), yielding the construct pApbE3. The pApbE3 construct was electroporated into *E. coli* BL21(DE3), which contains an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible copy of the T7 RNA polymerase gene. Visualization of the *apbE* gene product was achieved by [^{35}S]methionine (specific activity, 1,000 Ci/mmol; New England Nuclear, Du Pont Co., Boston, Mass.) labeling of the overexpressed product by the coupled T7 RNA polymerase-T7 promoter method (30). Labeled protein products were analyzed by 0.1% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (15% polyacrylamide) with the MiniProtean electrophoresis system (Bio-Rad Laboratories) and were visualized with a phosphorimager.

The effect of globomycin (Sankyo Ltd., Tokyo, Japan) on processing of ApbE was determined by overexpressing the *apbE* gene as described above with the procedural exception that the cultures were incubated for 10 min at 37°C with 200 μg of globomycin per ml prior to the addition of 10 μCi of [^{35}S]methionine per ml.

ApbE was labeled with [9,10(*n*)-^{3}H]palmitic acid (specific activity, 40 to 60 Ci/mmol; New England Nuclear, Du Pont Co.) by the procedure described by Jung et al. (19). Overexpression of ApbE was performed as described above, except that [^{3}H]palmitic acid was added in place of [^{35}S]methionine to a final concentration of 10 μCi/ml and the cultures were incubated for 2 h at 37°C. After the labeling, the cellular membranes were lipid extracted according to the method of Fernandez et al. (15). The final air-dried pellet resulting from this extraction was resuspended in 50 μl of loading dye. The membrane proteins were separated by SDS-PAGE (12% polyacrylamide), and autoradiographic detection of [^{3}H]palmitic acid-labeled ApbE was enhanced by using 2,5-diphenyloxazole (PPO) (Aldrich, Milwaukee, Wis.) and Kodak MR film (Eastman Kodak, Rochester, N.Y.).

Phenotypic characterization. The phenotypes of *apbE* mutants were assessed by growth curves for liquid and solid media as previously described (26). The

TABLE 1. Strains and plasmids used

Strain or plasmid	Relevant characteristics	Reference
LT2	Wild type	
DM95	*thiC918*::Mu*dJ*	
DM270	*apbC55*::Mu*dJ*	
DM271	*apbE42*::Tn*10d*(Tc)	
DM763	*apbE27*::Mu*dJ purF2085*	
DM764	*apbE27*::Mu*dJ*	
DM907	*apbE13*::Tn*10d*(Tc) *purF2085*	
DM908	*apbE42*::Tn*10d*(Tc) *purF2085*	
DM1936	*purF2085*	
DM3146	*apbE42*::Tn*10d*(Tc) *purF2085 purE2154*::Mu*dJ*	
BL21(DE3)	F^{-} *ompT hsdS*$_B$(r_B^{-} m_B^{-}) *gal dcm*	
DM3196	BL21(DE3)/pApbE3	
DM3407	BL21(DE3)/pT7-5	
DM3283	*apbE42*::Tn*10d*(Tc) *purF2085*/pApbE1	
pApbE1	*apbE*, 1.15 kb inserted into pSU19 *Hin*cII site; Cm^r	
pApbE3	*Eco*RI-*Hin*dIII fragment from pApbE1 containing *apbE* ligated into pT7-5; Amp^r	
pT7-5	Overexpression vector containing T7-dependent promoter; Amp^r	30
pSU19	Intermediate-copy-number cloning vector; Cm^r	2

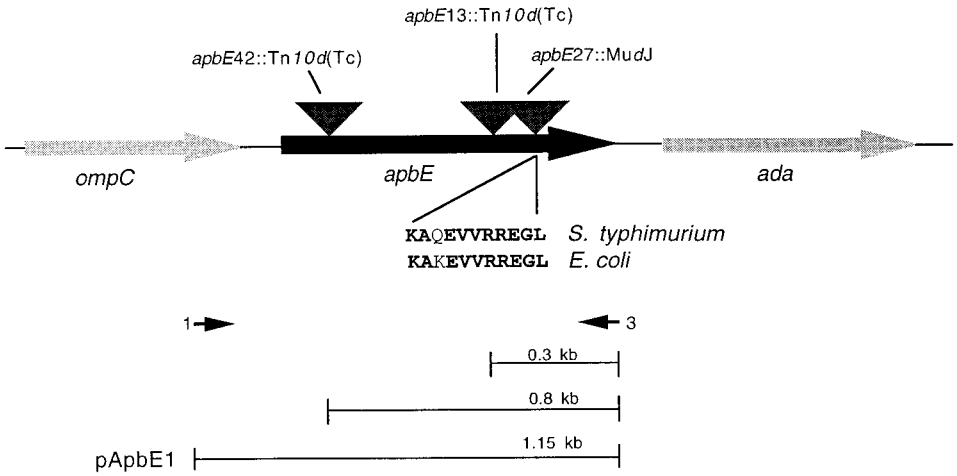

FIG. 2. Relative positions of insertions that define the *apbE* gene. The physical organization of insertions in the *apbE* gene at 49 min is represented. The amino acid sequence of ApbE adjacent to the Mu*d*J insertion is compared to the amino acid sequence of the *E. coli* homolog. Primers used in PCR and/or sequencing analysis are indicated (small arrows). The positions of the Tn*10d*(Tc) insertions were determined by amplification with primer 1 or 3 and primers specific to the ends of the insertion elements. Primers 1 and 3 were used to amplify the gene from the chromosome for cloning and determination of its nucleotide sequence.

growth rate, μ, of liquid cultures was determined by the equation $\mu = \ln(X_T/X_0)/T$, where X is A_{650} and T equals time. In the case of solid medium, soft-agar overlays were used and the compounds to be tested were spotted in the following amounts: thiamine, 20 nmol in 2 μl; THZ, 20 nmol in 2 μl; HMP, 20 nmol in 2 μl; and tyrosine, 80 nmol in 5 μl. The plates were incubated overnight at 37°C before growth was scored.

Nucleotide sequence accession number. The sequence of *apbE* has been submitted to GenBank with accession no. AF035376.

RESULTS

Isolation of *apbE* mutants. In order to define genetic loci involved in the synthesis of HMP, mutant hunts were performed to identify insertions that prevented thiamine synthesis in a *purF* mutant background when gluconate was used as the sole carbon source. Mutations affecting other branches of thiamine synthesis were eliminated by screening prospective mutants for their ability to grow on a variety of carbon sources when supplemented with adenine and HMP. The carbon sources tested included glucose, gluconate, fructose, and glycerol. A series of mutant hunts identified a number of insertions causing the desired phenotype. Linkage analysis determined that the mutations of three phenotypically similar mutants were transductionally linked. These three insertions [*apbE13*::Tn*10d*(Tc), *apbE42*::Tn*10d*(Tc), and *apbE27*::Mu*d*J] defined the *apbE* locus.

Identification of the *apbE* locus. In order to identify the locus disrupted by the above insertions, the Mu*d*J insertion in DM764 (*apbE27*::Mu*d*J) was converted into Mu*d*P-P22 and Mu*d*Q-P22 derivatives and flanking sequences were amplified as has been described previously (5, 36). The nucleotide sequences adjacent to the Mu*d*J insertion were determined by using primers specific to the ends of Mu*d*P and Mu*d*Q. When the amino acid sequences predicted from these sequences were compared with available database sequences, similarity to the amino acid sequence of YojK, a hypothetical protein encoded by an open reading frame between *ompC* and *ada* at 49 min on the *E. coli* chromosome, was revealed (Fig. 2).

The locations of the two Tn*10d*(Tc) insertions [*apbE42*::Tn*10d*(Tc) and *apb13*::Tn*10d*(Tc)] relative to the *apbE27*::Mu*d*J and flanking *ompC* and *ada* genes were determined by using PCR amplification techniques (see Materials and Methods). The sequences between *ompC* or *ada* and each Tn*10d*(Tc) insertion were amplified and visualized by gel electrophoresis to determine the length of the resulting fragment. This analysis determined that both Tn*10d*(Tc) insertions were located promoter proximal to Mu*d*J. The positions of *apbE13*::Tn*10d*(Tc) and *apbE42*::Tn*10d*(Tc) relative to *apbE27*::Mu*d*J were consistent with the observation that both Tn*10d*(Tc) insertions prevented transcription of the promoterless *lacZ* gene in the *apbE27*::Mu*d*J mutant (data not shown). The relative positions of the three insertions in the *apbE* locus and the primers used in their identification are shown in Fig. 2.

Cloning and sequencing of the *apbE* locus. To facilitate identification of the role of its gene product in the synthesis of HMP, the nucleotide sequence of the *apbE* locus in *S. typhimurium* was determined. The *apbE* gene from LT2 was amplified with primers 1 and 3 (Fig. 2), and the fragment was ligated into pSU19. The resulting construct, designated pApbE1 (Fig. 2), was electroporated into strains DM763 (*purF apbE27*::Mu*d*J), DM907 [*purF apbE13*::Tn*10d*(Tc)], and DM908 [*purF apbE42*::Tn*10d*(Tc)]. In each of the resulting strains, wild-type growth was restored on minimal gluconate medium supplemented with 0.4 mM adenine.

After complementation tests verified that pApbE1 contained the desired fragment, the nucleotide sequence of the entire fragment was determined by dye termination sequencing using primers specific to the M13 multiple cloning site of the parent plasmid, pSU19. Since pApbE1 contained a single gene and complemented all defects caused by the *apbE* insertions, we concluded that the lesions in this gene were solely responsible for the phenotypes associated with *apbE* mutations.

ApbE is a lipoprotein. To confirm that *apbE* produced the predicted protein product, we cloned the insert from pApbE1 into pT7-5, resulting in pApbE3, which contained the insert in the correct orientation to express *apbE* from the T7 promoter. This plasmid and an insertless control were electroporated into *E. coli* BL21(DE3), which generated strains DM3196 and DM3407, respectively. These two strains were then subjected to a protocol inducing T7-specific expression (30). Following induction in the presence of [^{35}S]methionine, proteins from the crude extracts were resolved by SDS-PAGE and visualized with a phosphorimager. Two insert-specific products of ap-

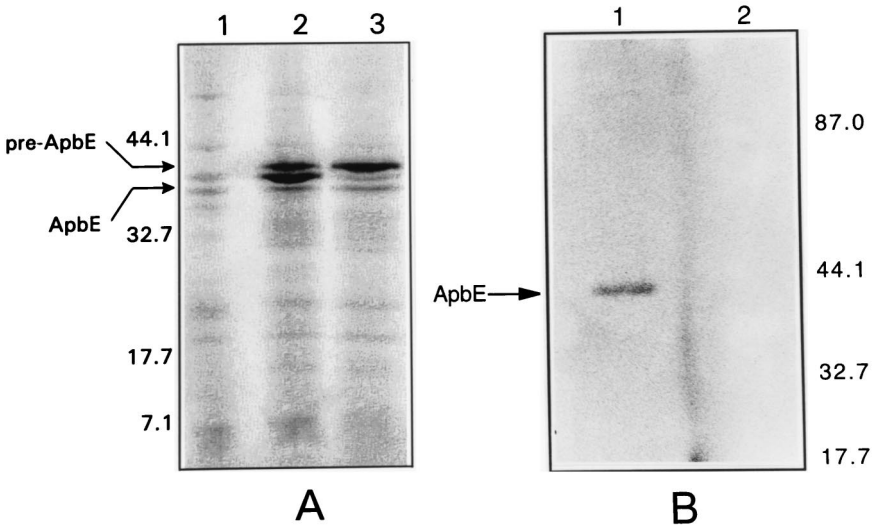

FIG. 3. *apbE* encodes a lipoprotein. (A) [^{35}S]methionine-labeled ApbE and inhibition of its processing by globomycin as analyzed by SDS-PAGE (15% polyacrylamide). Lane 1, DM3407(pT7-5); lane 2, DM3196(pApbE3); lane 3, DM3196(pApbE3) plus globomycin (200 μg/ml). (B) Membrane-associated ApbE was specifically labeled with [^{3}H]palmitic acid and analyzed by SDS-PAGE (12% polyacrylamide). Lane 1, DM3196(pApbE3); lane 2, DM3407(pT7-5). Molecular mass standards (in kilodaltons) for each gel are indicated.

proximately 38 and 36 kDa were revealed, as shown in Fig. 3A, lanes 1 and 2.

Further analysis of the ApbE sequence revealed a potential lipoprotein signal peptide cleavage site. The consensus cleavage site, LXYC, where X and Y are low-molecular-weight residues, followed a short, relatively hydrophobic signal peptide (27) in the predicted protein sequence of ApbE. Cleavage at this site would result in a processed protein of 36.0 kDa, consistent with the lower-molecular-mass band we saw in the specific-labeling experiments. In addition, the predicted amino acid sequence of ApbE contained an aspartate at position 2 in the mature protein, a residue that has been determined to retain lipoproteins in the inner membrane (35). To determine the significance of these sequence motifs, we sought to determine if *apbE* produced a lipoprotein in vivo.

If the *apbE* gene encoded a lipoprotein, sequence analysis suggested that the higher-molecular-mass product in the overexpression was the unprocessed (pre-ApbE) protein and the lower-molecular-mass product was the processed (ApbE) protein. Two experiments were performed to confirm this assignment. First, ApbE was labeled with [^{35}S]methionine in the presence of globomycin. Globomycin is a specific inhibitor of signal peptidase II, the product of the *lspA* gene, which is responsible for the signal peptide cleavage of well-characterized lipoproteins (18). As shown in Fig. 3A, lane 3, globomycin prevented synthesis of the lower-molecular-mass product and resulted in the accumulation of only the higher-molecular-mass species. The sensitivity of ApbE processing to globomycin is consistent with it being a lipoprotein whose signal peptide is processed by signal peptidase II.

Second, overexpression of *apbE* was carried out in the presence of [9,10(*n*)-^{3}H] palmitic acid, the precursor to the attached lipid, which would be expected to be present only in the processed form of a lipoprotein (16). This labeling experiment resulted in the accumulation of a single, membrane-associated, [^{3}H]palmitic acid-labeled product of approximately 36 kDa (Fig. 3B) that was not present in the strain containing the control plasmid. None of the [^{3}H]palmitic acid label was observed in the cytoplasmic fraction from the labeling experiment (data not shown), suggesting a strong interaction between ApbE and the membrane.

Evidence for cross-talk between the THZ and HMP pathways. As expected from the design of the mutant hunts, *apbE* insertions in a *purF2085* background required either thiamine or HMP. A *purF apbE* strain grew in minimal gluconate medium with adenine at a specific growth rate of <0.1 h^{-1}, compared to 0.6 h^{-1} for the parent *purF* strain. When thiamine was added to the medium, the two strains grew equally well (i.e., 0.6 h^{-1}).

When *apbE* insertions were transduced into a wild-type (i.e., Pur^{+}) genetic background, two unexpected phenotypes were noted. First, when grown on fructose, an *apbE* mutant required thiamine. When these mutants were grown on glucose or gluconate, they showed little, if any, requirement for thiamine, unless adenine (0.4 mM) was present in the medium. The effect of the carbon source on the requirement for thiamine was reminiscent of *apbC* mutants and had not been observed with mutants defective in characterized thiamine biosynthetic steps, such as DM95, which is *thiCEFGH* (Table 2). The ability of adenine to cause a thiamine requirement has been reported for other mutants defective in the alternate pyrimidine biosynthetic pathway. The reason for the adenine effect is not yet understood, but it could reflect inhibition of one of the proposed pathways for conversion of AIR to HMP.

The second, and more surprising, phenotype of DM271 (*apbE*) concerned its specific thiamine requirement. Unlike the *purF* derivative (DM908), an *apbE* mutation in a wild-type background resulted in a requirement for either thiamine or both the HMP and the THZ moieties when the strain was grown with fructose as the sole carbon source. This phenotype was also observed with other carbon sources if exogenous adenine was provided. The effect of genetic background on the nutritional requirement caused by an *apbE* mutation is shown in Fig. 4 and was first observed with *apbC* mutants (25).

As shown in Fig. 1, the biosynthetic pathways for the THZ and HMP moieties are distinct; thus, an explanation for a double requirement was not trivial. In consideration of the above experiment, it was possible that the purine source (ad-

TABLE 2. *apbE* mutants are conditional thiamine auxotrophs

Strain	Genotype	Specific growth rate (μ^a) on:					
		Gluconate		Glucose		Fructose	
		Without B_1	With B_1	Without B_1	With B_1	Without B_1	With B_1
LT2	Wild type	0.53	0.61	0.67	0.65	0.55	0.57
DM270	*apbC55*::Tn*10d*(Tc)	0.61	0.52	0.48	0.59	<0.10	0.63
DM271	*apbE42*::Tn*10d*(Tc)	0.51	0.61	0.30	0.46	<0.10	0.58
DM95	*thiC918*::Mu*dJ*	<0.10	0.6	<0.10	0.50	<0.10	0.54

[a] Determined by the equation $\ln(X_T/X_0)/T$, where X is absorbance at 650 nm and T equals time (in hours). Strains were grown in minimal media with the indicated supplements in a 37°C water bath with shaking (250 rpm). Data for a representative experiment are presented. B_1, thiamine.

enine) supplied to satisfy the *purF* mutant was causing the different nutritional requirement. This possibility was ruled out by the finding that strain DM271 (*apbE*) had the same dual requirement in the presence or absence of adenine (data not shown). This result suggested that flux through the purine pathway was responsible for generating the dual HMP and THZ requirement.

During the course of experiments designed to address the connection between the THZ and HMP biosynthetic pathways, we noticed that the thiazole requirement of *apbE* mutants could be satisfied by exogenous tyrosine (data not shown). This was significant, since tyrosine is known to donate its α-carbon and amino group to the thiazole moiety (Fig. 1). Null mutants defective in three of the four loci implicated in thiazole synthesis (*thiG*, *thiH*, and *thiI*) were then tested individually for nutritional correction by tyrosine. Each of these mutants was found to specifically require thiazole (data not shown). However, consistent with other similarities to *apbE* mutants, the thiazole requirement of *apbC* mutants could be satisfied by tyrosine.

If ApbE were essential for the formation of HMP, mutants would be expected to absolutely require the HMP moiety of thiamine, regardless of the carbon source or any additional requirement (e.g., thiazole). In addition to the phenotypes described above, we found that a *purE* insertion mutation eliminated the HMP requirement of strain DM908 (*purF apbE*), as was shown previously for *apbC* mutants (26). This result was illustrated by the specific growth rates of <0.1 and 0.55 h^{-1} for DM908 (*purF apbE*) and DM3146 (*purF apbE purE*), respectively, on adenine gluconate medium. In the case of *apbC*, this suppression was attributed to the accumulation of AIR, which could then be used by a redundant step(s) for HMP synthesis (26). Consistent with this scenario was the finding that in a wild-type (i.e., $PurF^+$) background, a *purE* mutation suppressed the HMP requirement of an *apbE* mutant, but thiazole was still required for growth. This result was found for all growth media on which the *apbE* mutant alone required HMP and THZ, i.e., fructose as the sole carbon source, or addition of exogenous adenine (data not shown). The above phenotypes suggested a regulatory interaction between the biosynthetic pathways for HMP and THZ and were consistent with the previously proposed model for dual pathways for the conversion of AIR to HMP (26).

DISCUSSION

Although the synthesis of thiamine by microorganisms has been studied for several decades, our understanding of the genes, their products, and the biochemistry involved remains incomplete. It is clear that the synthesis of HMP and THZ precedes their condensation and subsequent phosphorylation to thiamine pyrophosphate, but relatively few details about the formation of the two moieties are known.

The contributions of this paper to the understanding of thiamine synthesis in *S. typhimurium* include the following. (i) A new locus, *apbE*, whose gene product is involved in synthesis

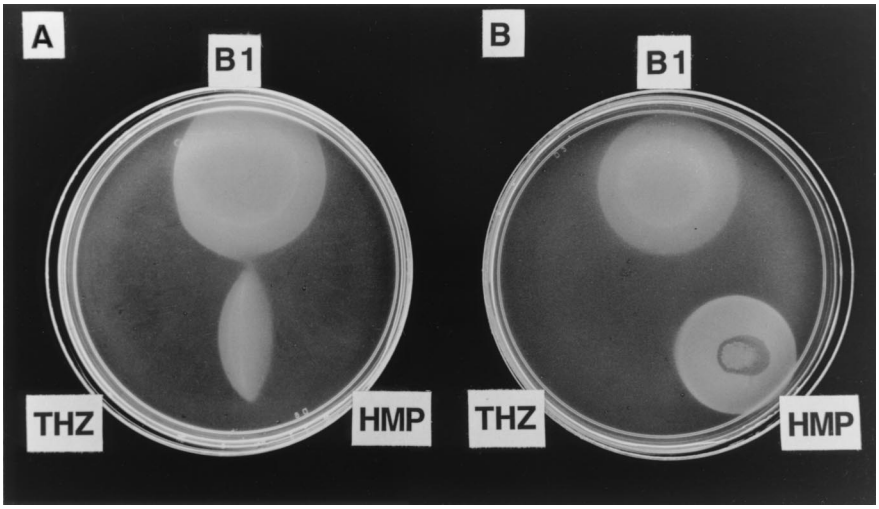

FIG. 4. Growth requirements of *apbE* mutants. Soft-agar overlays were performed on minimal medium supplemented with gluconate and adenine as previously described (12). DM271 (*apbE*) (A) and DM908 (*purF apbE*) (B) cells were plated. The following compounds were spotted (20 nmol each): a 2-μl sample of thiamine (B1), a 2-μl sample of HMP, and a 2-μl sample of thiazole (THZ).

of the pyrimidine moiety of thiamine has been identified. (ii) Phenotypic analysis of *apbE* mutants suggested that metabolic interactions occur between the HMP and THZ biosynthetic pathways and that this interaction depends on flux through the purine pathway. (iii) The biochemical characterization of the *apbE* gene product as a lipoprotein suggests that thiamine synthesis may involve other membrane-associated proteins.

The *apbE* locus is involved in HMP synthesis. Phenotypically, *apbE* mutants are similar to *apbC* mutants (26). The thiamine requirement of both *purF apbE* and *purF apbC* mutants is satisfied by HMP or thiamine. This phenotypic analysis suggests a role for both of these gene products in thiamine synthesis, specifically in synthesis of the HMP moiety. The role of ApbE (and ApbC) in thiamine synthesis is likely to be complementary and not essential, for the following reasons. First, the HMP requirement caused by insertions in *apbE* (in either a wild-type or a *purF* background) can be suppressed with the introduction of a *purE* mutation. Since PurE is involved in carboxylation of AIR to carboxyaminoimidazole ribotide (24), a *purE* mutant would be expected to accumulate AIR. The accumulation of AIR allows HMP synthesis to occur independently of ApbE and ApbC, consistent with the possibility of a second mechanism for the conversion of AIR to HMP, a pathway that requires elevated levels of AIR. A conditional role for ApbE (and ApbC) in thiamine synthesis is supported by the finding that insertion mutations in *apbE* (and *apbC*) cause a thiamine requirement that is carbon source specific in a wild-type genetic background, whereas insertion mutations in dedicated thiamine biosynthetic genes (i.e., *thiE*) cause an absolute thiamine requirement.

We presume that the growth conditions that alter the thiamine requirement of the *apbE* mutant strains, i.e., adenine and the carbon source, so by affecting the function of one of the pathways for the conversion of AIR to HMP. The mechanism and target for such regulation are not yet understood. In addition, the placement of ThiC in the context of the two-pathway model is not clear. We suggest that ThiC serves a nonredundant role in the latter part of the pathway, and experiments to address this prediction are under way.

Metabolic cross-talk between THZ and HMP biosynthetic pathways. The observation that *apbE* and *apbC* mutants (*purF*$^+$) required both moieties of thiamine (HMP and thiazole) was surprising, considering the presumed independence of the two biosynthetic pathways (Fig. 1). Because a *purF apbE* strain no longer required thiazole for growth, it appeared that the coordination of thiazole and HMP synthesis involved flux through the purine pathway. One possible explanation of these phenotypes is that an *apbE* mutation results in the accumulation of an HMP biosynthetic intermediate that inhibits thiazole synthesis. Less of this intermediate would accumulate when AIR synthesis is reduced by disrupting flux through the purine biosynthetic pathway (with a *purF* mutation). The observation that exogenously added tyrosine could satisfy the thiazole requirement of *apbE* mutants may indicate that the point of inhibition in the thiazole biosynthetic pathway is at the step where the α-carbon and amino group of tyrosine are incorporated into the forming thiazole ring. In this model, exogenous tyrosine would overcome this inhibition by driving the proposed reaction.

ApbE is a lipoprotein. From the results presented here, we concluded that ApbE is a lipoprotein, and on the basis of sequence conservation, we predicted that it sorts to the inner membrane. However, because overexpression of membrane proteins often results in an accumulation of the protein in the inner membrane (35), conclusive evidence supporting the localization of ApbE in the inner membrane would require the detection of wild-type ApbE by antibody analysis.

The identification of the *apbE* gene product as a lipoprotein complicates the assignment of a role for ApbE in HMP synthesis. Although biochemical steps for the conversion of AIR to HMP have been proposed, there are no functions obviously attributable to any of the gene products implicated in this pathway. Comparisons of the ApbE sequence with available sequences revealed that the C-terminal two-thirds of RnfF from *Rhodobacter capsulatus* is highly similar to ApbE (30% identical and 50% similar). This finding may provide a clue to the function of ApbE in *S. typhimurium*. The RnfF protein in *R. capsulatus* has been implicated as a membrane anchor that supports a protein complex that might use a chemical gradient to drive a reverse electron flux from some unknown substrate (perhaps NADH) to ferredoxins serving as electron donors to nitrogenase (20). Interestingly, the N-terminal 177 amino acids of RnfF show homology (28% identity and 52% similarity) to RseC, an inner membrane protein that is a regulator of Eσ^E activity in *E. coli* (10). The similarity of RseC to RnfF is particularly intriguing since RseC has also been implicated in the synthesis of HMP in *S. typhimurium* (3). The similarity of both RseC and ApbE to RnfF offers the possibility that RseC and ApbE might be associated in a membrane-bound complex and, together with other, unknown proteins, contribute to the synthesis of the pyrimidine ring of thiamine. In addition, the recent finding that ApbE is involved in cell aggregation and pattern formation indicates that ApbE may be multifunctional, perhaps suggesting additional interactions between quorum sensing and control of metabolism (4). Future work will determine if there exists a membrane complex that is involved in thiamine synthesis.

ACKNOWLEDGMENTS

This work was supported by Hatch grant WIS3734 from the U.S. Department of Agriculture and by NIH grant GM47296 to D.M.D.

We thank Leslie Petersen for the preliminary characterization of the HMP and THZ requirements of *apbE* and *apbC* mutants and Masatoshi Inukai at Sankyo Co., Ltd., Tokyo, Japan, for providing globomycin.

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