Complex Metabolic Phenotypes Caused by a Mutation in *yjgF*, Encoding a Member of the Highly Conserved YER057c/YjgF Family of Proteins

JODI L. ENOS-BERLAGE, MARK J. LANGENDORF, AND DIANA M. DOWNS*

*Department of Bacteriology, University of Wisconsin—Madison,
Madison, Wisconsin 53706*

The oxidative pentose phosphate pathway is required for function of the alternative pyrimidine biosynthetic pathway, a pathway that allows thiamine synthesis in the absence of the PurF enzyme in *Salmonella typhimurium*. Mutants that no longer required function of the oxidative pentose phosphate pathway for thiamine synthesis were isolated. Further phenotypic analyses of these mutants demonstrated that they were also sensitive to the presence of serine in the medium, suggesting a partial defect in isoleucine biosynthesis. Genetic characterization showed that these pleiotropic phenotypes were caused by null mutations in *yjgF*, a previously uncharacterized open reading frame encoding a hypothetical 13.5-kDa protein. The YjgF protein belongs to a class of proteins of unknown function that exhibit striking conservation across a wide range of organisms, from bacteria to humans. This work represents the first detailed phenotypic characterization of *yjgF* mutants in any organism and provides important clues as to the function of this highly conserved class of proteins. Results also suggest a connection between function of the isoleucine biosynthetic pathway and the requirement for the pentose phosphate pathway in thiamine synthesis.

The increasing number of completed genome sequences has resulted in the identification of new families of hypothetical proteins whose function has yet to be established. The lack of existing mutants defective in these conserved proteins suggests novel, complex, or subtle phenotypes. Through our work on thiamine synthesis in *Salmonella typhimurium*, we have isolated mutants defective in the recently identified YER057c/YjgF protein family. Our data suggest that defects in this protein result in complex phenotypes involving thiamine and isoleucine biosynthesis.

Thiamine pyrophosphate (TPP) serves as an essential cofactor for a number of metabolic reactions involving the removal or transfer of C_2 units. Despite the important role of TPP in cellular metabolism, its synthesis and regulation are not well understood in any organism. TPP is formed from two precursors, 4-methyl-5-(β-hydroxyethyl)thiazole phosphate (THZ-P) and 4-amino-5-hydroxymethyl-2-methylpyrimidine pyrophosphate (HMP-PP). These compounds are joined and subsequently phosphorylated as shown in Fig. 1A. Although many of the enzymatic steps in both the THZ-P and HMP-PP pathways have not been clearly defined, the major precursor molecules for both of these compounds have been determined by labeling studies (17, 20, 28, 29). In particular, the purine pathway intermediate, aminoimidazole ribotide (AIR), has been shown to provide all of the atoms in HMP (28, 50, 51).

Although the involvement of the purine pathway in the synthesis of HMP is clear, there is substantial genetic and biochemical evidence indicating that the first enzyme of the purine pathway, phosphoribosylpyrophosphate amidotransferase (PurF) (EC 2.4.2.14), is not required for HMP synthesis in *S. typhimurium* under all conditions. Mutants defective in *purF* are able to grow in the absence of thiamine when glucose is used as a carbon source if pantothenate is also supplied in the medium (23). Similarly, *purF* mutants do not require thiamine when grown on a number of nonglucose carbon sources, such as gluconate or ribose (54). The pathway responsible for synthesis of HMP independent of the PurF enzyme has been defined as the alternative pyrimidine biosynthetic (APB) pathway (21, 54); recent biochemical data suggest that phosphoribosylamine (PRA), or a derivative, is an intermediate in this pathway (24).

Significant progress in our understanding of the APB pathway has been made by the isolation and characterization of mutants unable to synthesize thiamine in a *purF* background. One class of mutants, designated *apbA*, was defective in a pantothenate biosynthetic enzyme (ketopantoate reductase [PanE]) (32, 33), consistent with previous results implicating a role for pantothenate in PurF-independent thiamine synthesis (23). A second class of these mutants was defective in the oxidative pentose phosphate pathway, affecting either glucose-6-phosphate dehydrogenase (Zwf) or gluconate-6-phosphate dehydrogenase (Gnd) (25, 54). Addition of ribose-5-phosphate (ribose-5-P) restored function of the APB pathway in these mutants, suggesting that the role of these enzymes in HMP synthesis was to supply ribose-5-P. These results led to the model shown in Fig. 1A which implicates ribose-5-P and an amine donor as precursors to PRA. Repeated attempts have failed to identify either the predicted PRA-forming activity or mutants defective in this step (27). There are several possible explanations for this. It is possible that the correct substrates have not been identified and/or that the PRA-forming activity is required for another cellular function.

In this report, we describe the isolation and characterization of mutations that allow function of the APB pathway in the absence of the pentose phosphate pathway. These mutations were found to disrupt a previously uncharacterized open reading frame (ORF) encoding a hypothetical 13.5-kDa protein. We have designated this gene *yjgF* based on homology to the respective ORF in *Escherichia coli*. The YjgF protein belongs to the YER057c/YjgF protein family, a class of proteins of

* Corresponding author. Mailing address: Department of Bacteriology, University of Wisconsin—Madison, Madison, WI 53706. Phone: (608) 262-2914. Fax: (608) 262-9865. E-mail: downs@macc.wisc.edu.

TABLE 1. Bacterial strains

Strain	Genotype
LT2	Wild type
DM574	purF2085 gnd175::Tn10d(Tc)
DM730	purF2085 gnd181 apbA1::MudJ
DM1936	purF2085
DM1231	purF2085 gnd174::MudJ
DM1247	purF2085 gnd181 apbA1::MudJ yjgF1::Tn10d(Tc)
DM1248	purF2085 gnd181 apbA1::MudJ yjgF2::Tn10d(Tc)
DM2944	purF2085 pyrB2694::MudA yjgF1::Tn10d(Tc)
DM2946	purF2085 pyrB2694::MudA yjgF2::Tn10d(Tc)
DM3215	purF2085 gnd181 apbA7::Tn10d(Tc)
DM3477	purF2085 gnd181 apbA7::Tn10d(Tc) yjgF3::MudJ
DM3478	purF2085 gnd181 apbA7::Tn10d(Tc) yjgF4::MudJ
DM3479	purF2085 gnd181 apbA7::Tn10d(Tc) yjgF5::MudJ
DM3480	yjgF3::MudJ
DM3488	purF2085 gnd181 pgi::Tn5
DM3489	purF2085 gnd181 pgi::Tn5 yjgF1::Tn10d(Tc)
DM3502	purF2085 zwf23::MudJ
DM3503	purF2085 zwf23::MudJ yjgF1::Tn10d(Tc)
DM3660	yjgF3::MudJ/pT7-5YjgF1
DM4053	yjgF1::Tn10d(Tc)
DM4440	relA21::Tn10d(Tc)
DM4442	yjgF1::Tn10d(Tc) hisT290::Tn5
DM4762	purF2085 gnd174::MudJ ilvA595::Tn10
DM4763	purF2085 gnd174::MudJ
DM4765	purF2085 gnd174::MudJ ilvA219
DM4767	purF2085 gnd174::MudJ yjgF2::Tn10d(Tc)
DM4769	purF2085 gnd174::MudJ yjgF2::Tn10d(Tc) ilvA219
DM4772	purF2085 gnd175::Tn10d(Tc) yjgF3::MudJ
DM5027	yjgF3::MudJ/pSU19YjgFa
DM5031	BL21/λDE3/pT7-5YjgFa
DM5032	BL21/λDE3/pT7-6YjgFa
BL21/λDE3	F⁻ ompT hsdSB(rB⁻ mB⁻) gal dcm (E. coli)
TT9534	pyrB2694::MudA
TT10288	hisD9953::MudJ his-9944::MudA
TV086	ilvA595::Tn10
TV088	ilvA219

unknown function that exhibit striking conservation across a wide range of organisms. Characterization of these mutants revealed that they also were sensitive to the presence of serine in the medium, exhibiting a requirement for isoleucine under this condition. The phenotypes caused by *yjgF* mutations suggest that the YjgF protein may be involved in regulation or function of the isoleucine biosynthetic pathway. Further, results suggest a connection between isoleucine biosynthesis and function of the APB pathway in thiamine synthesis.

MATERIALS AND METHODS

Bacterial strains, media, and chemicals. Unless otherwise noted, all strains used in this study are derivatives of *S. typhimurium* LT2 and are listed with their respective genotypes in Table 1. MudJ and MudA refer to the MudI1734 and Mud1-8 transposons, respectively, which have been described elsewhere (11, 39). Tn*10*(Tc) refers to the transposition-defective mini-Tn*10*(Tn*10*Δ*16*Δ*17*) (67).

Unless otherwise indicated, no-carbon E medium supplemented with 1 mM MgSO₄ (18, 66) was used as minimal medium. Carbon sources were used at a concentration of 16 mM, with the exception of pyruvate (24 mM), glycerol (55 mM), and acetate (40 mM). Difco nutrient broth (8 g/liter) with NaCl (5 g/liter) was used as rich medium. Luria broth was used for experiments involving plasmid isolation and protein overexpression. Difco BiTek agar was added (15 g/liter) for solid medium. When present in the culture media, and unless otherwise stated, the compounds were used at the following final concentrations: adenine, 0.4 mM; thiamine, 0.5 μM; pantothenate, 100 μM; serine, 4 mM; isoleucine, 0.3 mM. The final concentrations of the antibiotics in rich and minimal medium were as follows: tetracycline, 20 and 10 μg/ml, respectively; kanamycin, 50 and 125 μg/ml, respectively; and ampicillin, 30 and 15 μg/ml, respectively. Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.

Genetic techniques. (i) Transduction methods. Transductional crosses were performed by using the high-frequency general transducing mutant of bacteriophage P22 (HT105/1, *int-201*) (58). Methods for transductional crosses, purifi-

cation of transductants from phage, and identification of phage-free transductants have been described elsewhere (22, 54).

(ii) Mutant isolation. A P22 lysate grown on a pool of >70,000 cells containing random Tn*10*d(Tc) insertions was generated as described elsewhere (40, 43) and mutagenized with hydroxylamine (18, 38). This lysate was used to transduce DM730 (*purF2085 gnd181 apbA1*::MudJ) to tetracycline resistance (Tc^r) on nutrient agar plates. The Tc^r transductants were screened for growth on glucose medium supplemented with adenine and tetracycline. Upon reconstruction, the mutants isolated in this screen were found to grow weakly on glucose-adenine medium but were indistinguishable from a *purF* mutant (DM1936) on glucose medium supplemented with adenine and pantothenate. MudJ insertion mutants were isolated as follows. A P22 phage lysate grown on TT10288, which contains a MudJ construct that allows independent transposition events by *cis* complementation (41), was used to transduce DM3215 [*purF2085 gnd181 apbA7*::Tn*10*d (Tc)] to kanamycin resistance (Kn^r). Kn^r transductants were screened for those that could grow on glucose medium supplemented with adenine, pantothenate, and kanamycin. All putative mutations [Tn*10*d(Tc) and MudJ insertions] were reconstructed into the parent strain to confirm the observed phenotype. Although an *apbA* mutation was present in the strains used for mutant isolation, the phenotypes described are independent of this mutation.

(iii) Construction of strains containing the *ilvA* feedback-resistant mutation. A P22 phage lysate grown on TV086 [*ilvA595*::Tn*10*] was used to transduce DM1231 (*purF2085 gnd174*::MudJ) to Tc^r, creating DM4762 [*purF2085 gnd174*::MudJ *ilvA595*::Tn*10*(Tc)]. DM4762 was subsequently transduced with a P22 phage lysate grown on either TV088 (*ilvA219*) or LT2 (*ilvA^+*). Transductants that grew on glucose medium supplemented only with adenine and thiamine, i.e., Ile⁺, were saved and tested to confirm they were Tc^s, creating strains DM4765 (*purF2085 gnd174*::MudJ *ilvA219*) and DM4763 (*purF2085 gnd174*::MudJ *ilvA^+*). The *yjgF2*::Tn*10*d(Tc) insertion was then introduced via P22 transduction into DM4765 and DM4763 by using DM1248 [*purF2085 gnd181 apbA1*::MudJ *yjgF2*::Tn*10*d(Tc)] as a donor and selecting for Tc^r.

Phenotypic characterization. Nutritional requirements of mutants were tested in solid medium by using agar overlays and in liquid medium by using growth curves as previously described (54). Data presented are representative of at least three independent experiments. Compounds that corrected the serine sensitivity of *yjgF* mutants were identified by spotting pools of overlapping compounds on solid medium (18).

Molecular biology techniques. (i) Plasmid manipulation and recombinant DNA techniques. Standard methods were used for DNA restriction endonuclease digestion and ligation. All restriction enzymes and ligase were purchased from Promega (Madison, Wis.). Plasmid DNA was isolated with the QIAprep spin plasmid kit, and DNA fragments requiring purification were purified with the Qiaquick gel extraction kit (Qiagen, Chatsworth, Calif.). Plasmids were transferred between strains by electroporation with a Bio-Rad (Richmond, Calif.) *E. coli* pulser as suggested by the manufacturer. Primers for PCRs and sequencing were generated by the University of Wisconsin Biotechnology Center-Nucleic Acid and Protein Facility (Madison, Wis.) and Genosys Biotechnologies, Inc. (The Woodlands, Tex.).

(ii) Localization of *yjgF* mutations by PCR amplification. The Tn*10*-I (5′ GACAAGATGTGTATCCACCTTAAC 3′) and MuR (5′ GCTTTCGCGTTT TTCGTG 3′) primers, specific to the ends of the Tn*10*d(Tc) and MudJ insertions, respectively, were used to PCR amplify the DNA between these insertions in DM2944 [*purF2085 yjgF1*::Tn*10*d(Tc) *pyrB2694*::MudA] and DM2946 [*purF2085 yjgF2*::Tn*10*d(Tc) *pyrB2694*::MudA] as described previously (68). Amplification was performed by using Vent (Exonuclease-minus) polymerase (New England Biolabs) in a Thermolyne Temp-Tronic thermocycler. For DM2944, PCR conditions were as follows: denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1.5 min. For DM2946, PCR conditions were as follows: denaturation at 95°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 1.75 min. The Tn*10*d(Tc) proximal end of the 1.1-kb fragment resulting from each PCR amplification was sequenced by using the pyrBI-1 primer (5′ ATTTGGGTTGGTTATGAGAT 3′).

(iii) Cloning of *yjgF*. The *yjgF* gene and adjacent sequences were amplified from LT2 by using primers specific to the ends of the *mgtA* and *pyrI* genes, XbamgtA-1 (5′ GCTCTAGAGCGCATATGATCCGTACCCGC 3′) and XbapyrI-1 (5′ GC TCTAGAGCTCGCCCTCAAATGCAAATAC 3′), respectively. Primers were designed with *Xba*I restriction sites at the ends to facilitate cloning. Amplification was performed as described above. PCR conditions were as follows: denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. The resulting 1.4-kb product was digested with *Xba*I and ligated into the pT7-5 overexpression vector (61) also digested with *Xba*I. The ligation mix was electroporated into DM3480, and Ap^r electroporants that grew on glucose medium containing serine were obtained. One plasmid that contained the 1.4-kb insert was designated pT7-5YjgF1.

Following sequence analysis (see below), it was found that an additional ORF (besides *yjgF*) was present between *mgtA* and *pyrBI* in *S. typhimurium*. To obtain a single-gene clone of *yjgF*, the *yjgF* gene was amplified from the pT7-5YjgF1 plasmid by using the XbapyrI-1 primer (above) and a primer specific to sequence immediately following the *yjgF* gene, yjgF-end (5′ CTGTTGGTGCCCGAACC 3′). PCR conditions were denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1.5 min. The resulting 600-bp fragment was

blunt end ligated into the pSU19 vector (48) previously digested with *Hin*cII, creating pSU19YjgFa.

To construct a single-gene clone for overexpression of YjgF, pSU19YjgFa was digested with *Eco*RI and *Pst*I. The resulting 600-bp fragment containing the *yjgF* gene was gel purified and ligated into pT$_7$-5 and pT$_7$-6 digested with the same restriction enzymes, creating plasmids pT$_7$-5YjgFa and pT$_7$-6YjgFa, respectively. Restriction fragment digestion patterns with either *Pvu*II/*Xmn*I or *Hind*III/*Xmn*I confirmed that these two constructs contained the *yjgF* gene in opposite orientations with respect to the T$_7$ promoter. pT$_7$-6YjgFa contained the *yjgF* gene in the correct orientation for T$_7$-dependent expression.

(iv) DNA sequencing. The 1.4-kb insert of pT$_7$-5YjgF1 was sequenced by the University of Wisconsin Biotechnology Center-Nucleic Acid and Protein Facility. Primers in addition to the XbamgtA-1 and XbapyrI-1 primers above were designed as sequence was determined. The additional primers used for sequencing were mgtA-2 (5′ GCCAGCGTGCTTAATCC 3′), pyrBI-2 (5′ GGTCGATCCG AAAACCG 3′), and yjgF promoter (5′ GCCTTCACTAATGGTACG 3′). The entire 1.4-kb insert was sequenced at least three times.

Computer analysis. DNA sequence was analyzed with BLAST (Basic Local Alignment Search Tool) (1). The DNASTAR alignment program (Madison, Wis.) was used to line up homologous proteins.

Overexpression of YjgF. The overexpression plasmids pT$_7$-5YjgFa and pT$_7$-6YjgFa were constructed as described above. These plasmids were electroporated into *E. coli* BL21/λDE3, generating strains DM5031 and DM5032, respectively. These strains contain the T7 RNA polymerase on a lambda lysogen under the control of an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible promoter. For induction, strains were grown with shaking at 37°C to 60 Klett units in Luria broth containing ampicillin. IPTG (Fischer Biotech, Chicago, Ill.) was added at a final concentration of 400 μM, and incubation was continued at 37°C for 3 h. After this time, 1 ml of cells was removed, pelleted, and resuspended in 100 μl of cracking buffer (3). Proteins were subsequently separated by sodium dodecyl sulfate (SDS)–15% polyacrylamide gel electrophoresis (PAGE) by the Bio-Rad MiniProtean electrophoresis system and visualized by Coomassie blue staining.

Stringent response assays. Cultures of LT2, DM3480 (*yjgF3*::MudJ), and DM4440 (*relA21*::Tn*10*) were grown overnight in nutrient broth (NB) medium and then subcultured (1/25 dilution) into the same medium. After growth at 37°C to an optical density at 600 nm (OD$_{600}$) of 0.15 to 0.2, two 0.6-ml aliquots were removed to prewarmed test tubes. Lysine hydroxamate (49) (Sigma) was then added at a final concentration of 400 μg/ml to induce amino acid starvation (time = −5 min). At t = 0, 5 μl of ^{32}P$_i$ (H$_3$PO$_4$, 1 mCi/ml; DuPont) was added. One hundred-microliter aliquots were subsequently removed into 3 ml of ice-cold 10% (wt/vol) trichloroacetic acid (TCA) at t = 5, 15, 30, 45, and 60 min. The TCA-precipitatable material was filtered, washed, and counted as described elsewhere (34). Control cultures were monitored by OD$_{600}$, and counts were corrected for optical density at each time point. Lysine hydroxamate inhibited cell growth of all of the strains to the same extent, as determined by growth rates.

Nucleotide sequence accession number. The nucleotide sequence of *yjgF* has been submitted to GenBank under accession no. AF095578.

RESULTS

Isolation of mutations that suppress the requirement for Gnd in thiamine synthesis. Both *purF* and *purF apbA* mutants can grow on glucose minimal medium containing adenine and pantothenate due to PurF-independent thiamine synthesis, defined as the APB pathway (Fig. 1A) (25). When a *gnd* mutation is introduced into these strains, it causes a thiamine requirement, presumably because thiamine synthesis is disrupted. To further investigate the role of the pentose phosphate pathway in thiamine synthesis, mutations that allowed PurF-independent thiamine synthesis in the absence of Gnd were isolated. A P22 phage lysate grown on a pool of Tn*10*d(Tc) insertions was treated with hydroxylamine and used to transduce DM730 (*purF2085 apbA1*::MudJ *gnd181*) to tetracycline resistance. Derivatives from this cross that grew on glucose minimal medium supplemented with adenine and pantothenate were saved. Seven mutants with the desired phenotype were isolated from a screen of 16,000 Tc^r transductants. Four of these mutants were assumed to be *purF$^+$* since they grew on minimal medium, and one was assumed to be *gnd$^+$* because it was highly linked to a known *gnd* insertion. The remaining two mutants, DM1247 and DM1248, were further characterized. The Tn*10*d(Tc) insertions in both of these mutants were found to be 100% linked to the respective phenotype by P22 trans-

duction, and thus the insertions were presumed to be causative of the growth phenotype.

A second mutant hunt was performed to identify MudJ insertions that caused the same phenotype as that described above. In this hunt, a P22 lysate grown on a strain that allows random insertion of MudJ transposons was used to transduce DM3215 [*purF2085 apbA7*::Tn*10*d(Tc) *gnd181*] to kanamycin resistance. Twelve mutants that could grow on glucose-adenine-pantothenate medium were identified by screening 16,000 transductants. By the criteria described above, 4 of the 12 were assumed to be *purF$^+$*. The MudJ insertions in the remaining mutants were tested for linkage to the Tn*10*d(Tc) insertions in strains DM1247 and DM1248. Three of the isolates, DM3477, DM3478, and DM3479, contained MudJ insertions that were >95% linked by P22 transduction to the Tn*10*d(Tc) insertions in both DM1247 and DM1248. This result suggested that these five insertions were disrupting the same locus, and further analysis of this class of mutants was performed.

Insertion mutations disrupt a gene designated *yjgF*. The map location of the Tn*10*d(Tc) insertions in DM1247 and DM1248 was determined in several steps. Derivatives of DM1247 and DM1248 that were sensitive to tetracycline were isolated by the method of Bochner (6, 47). In each strain, it was possible to identify Tc^s derivatives that were auxotrophic for pyrimidines. This result suggested that the Tn*10*d(Tc) insertions were near a gene required for pyrimidine synthesis. After testing linkage to a number of known pyrimidine loci, we found that the Tn*10*d(Tc) insertions in both DM1247 and DM1248 were 99% linked by P22 transduction to an insertion in *pyrB* (*pyrB2694*::MudA; TT9534). This result placed both Tn*10*d(Tc) insertions at 96.8 min on the *S. typhimurium* chromosome. To determine the precise location of the insertions, the DNA between the Tn*10*d(Tc) insertion and the *pyrB*::MudA insertion in strains DM2944 [*purF2085 yjgF1*::Tn*10*d(Tc) *pyrB2694*::MudA] and DM2946 [*purF2085 yjgF2*::Tn*10*d(Tc) *pyrB2694*::MudA] was amplified by PCR. Sequence analysis of the resulting 1.1-kb fragment by using a primer to the C terminus of *pyrI* determined that the Tn*10*d(Tc) insertion in DM2944 was within a previously uncharacterized ORF in *E. coli* designated *yjgF* (Fig. 2). Sequencing results demonstrated that the Tn*10*d(Tc) insertion in DM2946 was in precisely the same location (data not shown).

Cloning and sequencing of *yjgF*. The DNA between *mgtA* and *pyrI* in *S. typhimurium* was PCR amplified with primers to the C termini of these genes. The resulting 1.4-kb fragment was cloned into the pT$_7$-5 vector, creating pT$_7$-5YjgF1 (Fig. 2). This plasmid complemented the phenotypes (see below) caused by a *yjgF* mutation. Sequence analysis of the entire 1.4-kb insert in pT$_7$-5YjgF1 indicated that it contained two small ORFs, designated *orfA* (447 bp) and *yjgF* (387 bp) (Fig. 2). The gene structure in *S. typhimurium* shown in Fig. 2 is similar to that of *E. coli*, the exception being that this region in *E. coli* does not contain *orfA*. A BLASTX (1) database search indicated that the predicted OrfA protein showed significant homology (from amino acids 1 to 43) to hypothetical proteins in a number of insertion sequences, including a putative transposase. To confirm that the insertions in *yjgF* caused the respective phenotypes because of disruption of *yjgF* (and not polarity on *orfA*), a single-gene clone of *yjgF*, pSU19YjgFa (Fig. 2), was constructed as described in Materials and Methods. This clone also complemented the phenotypes caused by the *yjgF* insertion mutations, indicating that the phenotypes observed were due to loss of the *yjgF* gene.

The predicted size of the YjgF protein was 128 amino acids (13.5 kDa). A BLASTX database search with *S. typhimurium* YjgF revealed that this protein belonged to a family of small
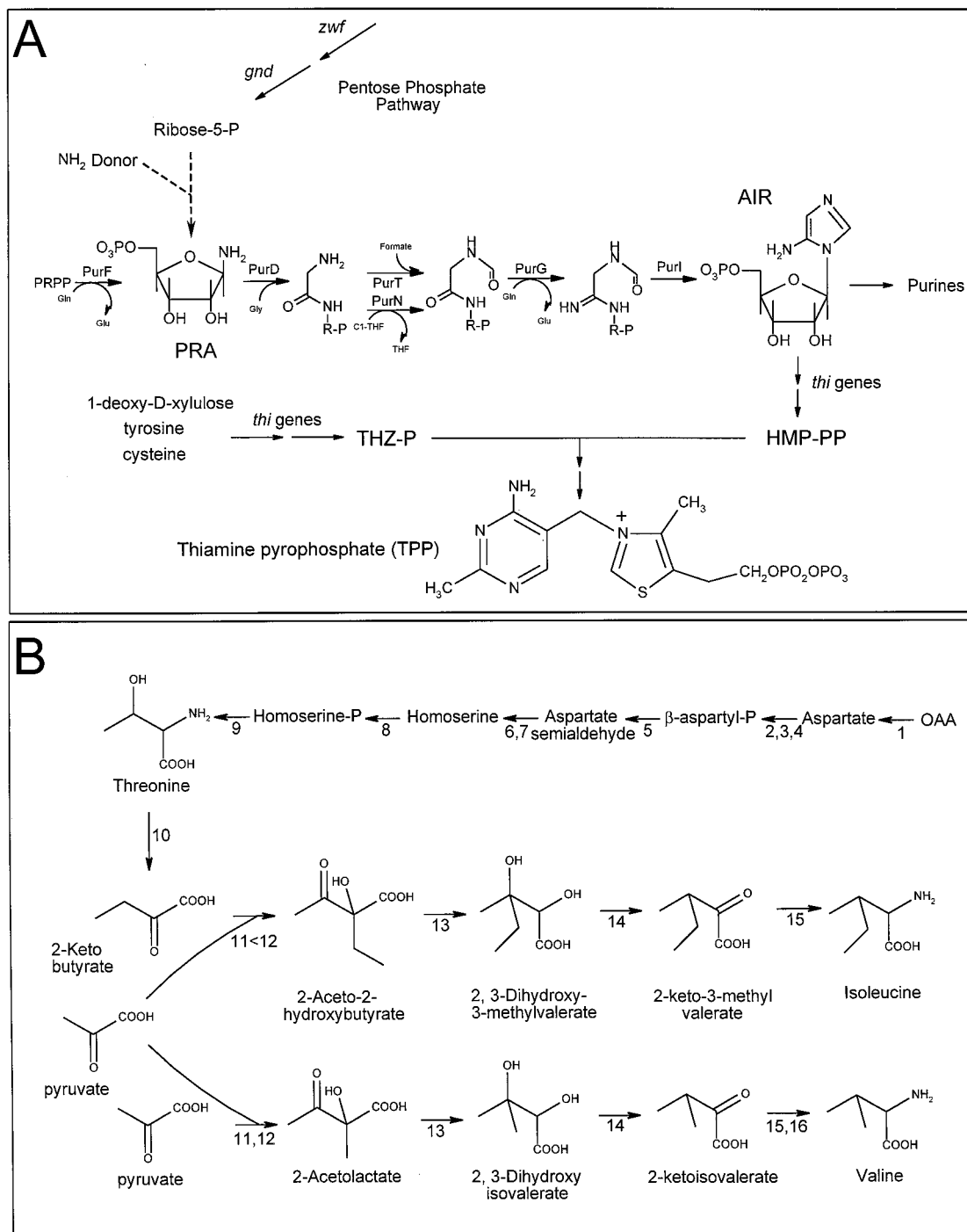
FIG. 1. Pathway schematics. (A) Biosynthetic pathway for TPP. The involvement of the purine pathway in HMP-PP synthesis is shown with structural intermediates prior to the AIR branch point. Arrows denoted with dotted lines represent proposed steps. Reactions involved in the conversion of AIR to HMP-PP and in the synthesis of THZ-P have not been clearly defined. Genes whose products are required for selected reactions are indicated next to the relevant arrows. Abbreviations: R-P, ribose-5-phosphate, PRPP, phosphoribosylpyrophosphate. (B) Biosynthetic pathways for the branched-chain amino acids isoleucine and valine. Enzymes that catalyze specific steps are as follows: 1, aspartate transaminase; 2, 3, and 4, aspartate kinases I, II, and III, respectively; 5, aspartate semialdehyde dehydrogenase; 6 and 7, homoserine dehydrogenases I and II, respectively; 8, homoserine kinase; 9, threonine synthase; 10, threonine deaminase; 11 and 12, acetohydroxy acid synthases I and II, respectively; 13, acetohydroxy acid isomeroreductase; 14, dihydroxy acid dehydratase; 15, transaminase B; 16, transaminase C. OAA, oxaloacetic acid.

proteins of approximately 15 kDa designated the YER057c/ YjgF family. These proteins are highly conserved across a wide range of organisms. Numerous homologues exist in bacteria, e.g., *E. coli* hypothetical proteins YjgF and YhaR (5), *Haemophilus influenzae* hypothetical protein HI0719 (30), *Bacillus*

subtilis hypothetical protein YabJ (52), *Helicobacter pylori* putative translation initiation inhibitor (62), *Lactococcus lactis* AldR (35), *Aquifex aeolicus* hypothetical protein (19), *Myxococcus xanthus* DfrA, *Rhizobium* sp. strain NGR234 hypothetical 13.3-kDa protein Y4SK (31), and *Azotobacter vinelandii*
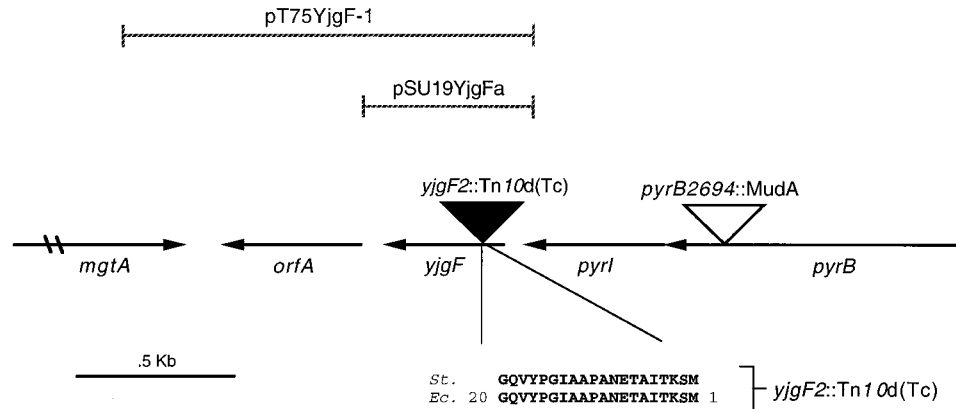
FIG. 2. Location of the *yjgF2*::Tn*10*d(Tc) insertion. A schematic representation of the 96.8-min region of *S. typhimurium* (*St.*) is shown. The *yjgF2*::Tn*10*d(Tc) insertion was mapped within the *yjgF* gene based on homology with *E. coli* (*Ec.*). DNA between the *yjgF2*::Tn*10*d(Tc) insertion and the *pyrB2694*::MudA insertion was amplified by PCR. Sequences flanking the *yjgF2*::Tn*10*d(Tc) insertion were determined, and the predicted amino acids are shown. Sequence analysis of the region flanking the *yjgF1*::Tn*10*d(Tc) insertion placed it at the same location. Amino acids conserved between the two organisms are in boldface, and numbers represent amino acids in *E. coli* YjgF protein. The inserts contained in the pT$_7$-5YjgF1 and pSU19YjgFa plasmids are indicated by the hatched lines. The *mgtA* gene is not drawn to scale.

hypothetical protein in the *vnfA* 5′ region (42). In addition, these proteins are highly conserved in cyanobacteria (*Synechocystis* strain 6803 hypothetical 13.8-kDa protein) (13), fungi (*Saccharomyces cerevisiae* chromosome V hypothetical protein YER057c and chromosome IX hypothetical protein YIL051c), and higher eukaryotes (*Caenorhabditis elegans* hypothetical 19.6-kDa protein, mouse heat-responsive protein 12, rat 14.5-kDa translational inhibitor protein [46, 53], human 14.5-kDa translational inhibitor protein [57], and goat UK114 tumor antigen [12]). Noticeably absent from this list was a representative from the archaea, despite the fact that several genomes have been completely sequenced (9, 44, 59). The homologues listed here ranged from 97% similar (*E. coli*) to 43% similar (goat). Figure 3 shows the deduced amino acid sequence of *S. typhimurium* YjgF compared to several of the known homologues. The YER057c/YjgF protein family is defined by a highly conserved signature motif located at the C terminus of these proteins, consisting of the following amino acids: P-[AT]-R-[SA]-X-[LIVMY]-X$_2$-A-X-L-P-X$_4$-[LIVM]-E. The *S. typhimurium* YjgF protein matches this consensus sequence exactly between amino acids 103 and 120.

Despite the high conservation of the YER057c/YjgF protein family, little is known about the function of these proteins. Studies with two of the eukaryotic proteins (rat and human) have indicated tissue-specific expression of YjgF and upregulation upon cellular differentiation (46, 53, 57). In addition, these two proteins inhibited in vitro translation in a rabbit reticulyte lysate system.

Overexpression of YjgF. To confirm that the ORF designated *yjgF* produced a protein of the predicted size, the DNA insert in pSU19YjgFa (Fig. 2) was cloned into the T$_7$ overexpression vectors, pT$_7$-5 and pT$_7$-6, creating pT$_7$-5YjgFa and pT$_7$-6YjgFa, respectively. Restriction enzyme digestion patterns indicated that pT$_7$-6YjgFa contained the *yjgF* gene in the correct orientation for expression from the T$_7$ promoter. Both plasmids were electroporated into an *E. coli* strain containing the gene for T7 RNA polymerase on a lambda lysogen (BL21/λDE3), generating strains DM5031 and DM5032, respectively. These strains were subjected to a protocol that induced T7-specific expression as described in Materials and Methods. Proteins of induced crude cell extracts were resolved by SDS-PAGE and visualized by Coomassie blue staining. As shown in Fig. 4, extracts from DM5032 (lane A) contained a prominent band not observed in DM5031 (lane B). Calculations of the

molecular mass of the overexpressed protein from diluted samples indicated that the additional band was approximately 12 kDa, which corresponded well with the predicted molecular mass of 13.5 kDa for YjgF.

The YjgF homologues from rats and humans had the unusual property of being soluble in 5% perchloric acid (PCA) and 10% TCA, respectively (46, 53, 57). We therefore tested whether the *S. typhimurium* YjgF protein was acid soluble. The induced crude cell extract from the overexpressing strain was extracted with 1.25, 2.5, and 5% PCA and 2.5, 5, and 10% TCA. Precipitated proteins were removed by centrifugation,
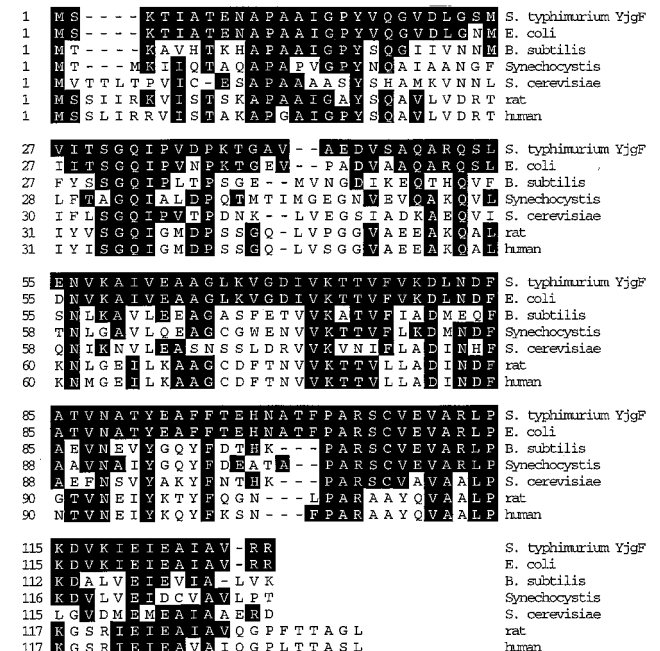

FIG. 3. The YjgF protein is highly conserved. The DNASTAR alignment program was used to align several proteins homologous to YjgF of *S. typhimurium*. Regions shaded in black represent amino acids that are conserved with respect to YjgF. Proteins in the alignment include *E. coli* YjgF, *B. subtilis* YabJ, *Synechocystis* hypothetical 13.8-kDa protein, *S. cerevisiae* YER057c, rat 14.5-kDa translational inhibitor protein, and human 14.5-kDa translational inhibitor protein.
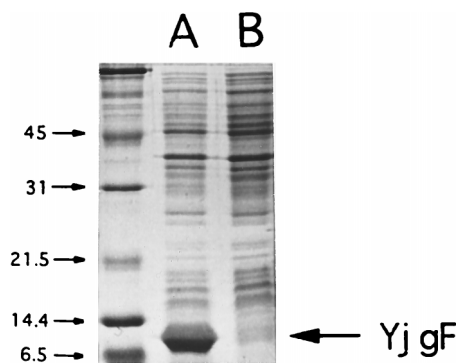
FIG. 4. Overexpression of YjgF. Overexpression of YjgF was performed by using the T7 overexpression system as described in Materials and Methods. Proteins were separated by SDS-PAGE and visualized by Coomassie blue staining. Extracts from DM5032 and DM5031, which contain plasmids pT$_7$-6YjgFa and pT$_7$-5YjgFa, respectively, are shown in lanes A and B, respectively. pT$_7$-6YjgFa contains the insert in the proper orientation for YjgF expression from the T7 promoter. Numbers at left indicate molecular mass in kilodaltons.

and those remaining in solution were resolved by SDS-PAGE as described above. Staining with Coomassie blue revealed several faint protein bands of high molecular masses in each of the acid extractions, but no significant bands of approximately 14 kDa. This result suggested that the *S. typhimurium* YjgF protein, unlike the eukaryotic homologues, was not highly soluble in PCA or TCA.

A *yjgF* mutation suppresses the requirement for the pentose phosphate pathway in thiamine synthesis but not carbon catabolism. A *gnd* mutation eliminates function of the APB pathway when strains are grown either on glucose-adenine-pantothenate medium or on a number of nonglucose carbon sources supplemented with adenine, including gluconate (54). We tested whether *yjgF* mutations alleviated the requirement for Gnd in thiamine synthesis under all conditions. As expected, DM4772 [*purF2085 gnd175*::Tn*10*d(Tc) *yjgF3*::MudJ] grew on glucose-adenine-pantothenate medium while the parent strain, DM574 [*purF2085 gnd175*::Tn*10*d(Tc)], did not. When these two strains were grown in gluconate-adenine medium, growth was similarly restored in the *yjgF* derivative (DM4772) (Fig. 5A). Similar phenotypic tests performed with DM3502 (*purF2085 zwf23*::MudJ) and DM3503 [*purF2085 zwf23*::MudJ *yjgF1*::Tn*10*d(Tc)] determined that *yjgF* mutations suppressed the effect of *zwf*

mutations in a similar manner (data not shown). Thus, insertion mutations in *yjgF* eliminated the requirement for the pentose phosphate pathway in the function of the APB pathway.

To test the simple explanation that *yjgF* mutations caused their effect by activating a new enzyme(s) that performed the same functions as did Gnd and/or Zwf, we addressed whether *yjgF* mutations could suppress the requirement for one of these enzymes in carbon catabolism. DM3488 (*purF2085 gnd181 pgi*::Tn*5*) was unable to utilize glucose as a sole carbon source because both the pentose phosphate pathway and the glycolytic pathway were blocked. We found that *yjgF* mutations did not restore the ability of the respective strain [DM3489; *purF2085 gnd181 pgi*::Tn*5 yjgF1*::Tn*10*d(Tc)] to utilize glucose as the sole carbon source. This result indicated that *yjgF* mutations did not suppress the requirement for the pentose phosphate pathway in carbon catabolism.

It was a formal possibility that *yjgF* mutations allowed thiamine-independent growth because of a reduction in the cellular TPP requirement, rather than restored synthesis. Conditions that reduce the cellular TPP requirement can be identified based on their ability to allow growth of *thi* auxotrophs on lower concentrations of thiamine (26). When the *yjgF* mutations were introduced into known thiamine auxotrophs, they did not alter the amount of thiamine required for growth (data not shown). These results suggested that the *yjgF* mutation caused its effect by restoring thiamine synthesis.

Mutations in *yjgF* cause a conditional requirement for isoleucine. In the course of experiments performed to phenotypically characterize DM1247 [*purF2085 apbA1*::MudJ *gnd181 yjgF1*::Tn*10*d(Tc)], we found that this strain was hypersensitive to the presence of serine when grown on minimal medium-based medium. When 8 μmol of L-serine was spotted on a plate overlaid with DM1247, a large zone of inhibition was observed. Further testing determined that any strain defective in YjgF, regardless of genetic background, exhibited a similar serine sensitivity. The serine sensitivity caused by the *yjgF* mutation is shown quantitatively in Fig. 5B and C. Growth of DM3480 (*yjgF3*::MudJ) on minimal glucose medium was completely inhibited in the presence of 4 mM serine (Fig. 5C), while growth of the wild-type strain (LT2) was unaffected (Fig. 5B). Titration experiments determined that serine caused this severe growth defect when present at final concentrations greater than or equal to 500 μM (data not shown).

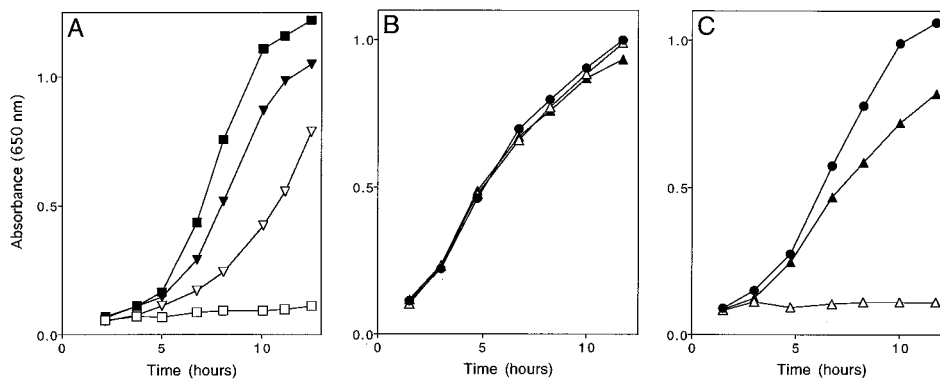Compounds that corrected the serine sensitivity of a *yjgF*

FIG. 5. Phenotypes caused by a *yjgF* mutation. Growth curves were obtained from strains grown at 37°C as described in Materials and Methods. (A) Suppression of the requirement for Gnd in thiamine synthesis. Growth of DM574 [*purF2085 gnd175*::Tn*10*d(Tc)] in gluconate-adenine medium and gluconate-adenine-thiamine medium is indicated by open and solid squares, respectively. Growth of DM4772 [*purF2085 gnd175*::Tn*10*d(Tc) *yjgF3*::MudJ] in the same medium is indicated by open and solid inverted triangles, respectively. (B and C) Sensitivity to exogenous serine. Shown are growth of LT2 (wild type) (B) and growth of DM3480 (*yjgF3*::MudJ) (C) in minimal glucose medium (solid circles), glucose medium supplemented with serine (open triangles), and glucose medium supplemented with serine and isoleucine (solid triangles).

mutant were identified by spotting pools containing over-lapping compounds on minimal serine plates overlaid with DM3480 (18). Two compounds were found that clearly corrected serine-inhibited growth of DM3480: isoleucine and threonine. Correction of growth by isoleucine is shown quantitatively in Fig. 5C. The sensitivity of DM3480 to serine and correction by isoleucine or threonine (an intermediate in isoleucine synthesis) suggested that the *yjgF* mutation was exacerbating a condition designated "serine toxicity" that was first observed in *E. coli* over 40 years ago (2, 15, 16, 55, 56). Wild-type *E. coli* K-12 undergoes a transient cessation in growth in response to 1.5 mM serine (15, 65); this sensitivity was more severe in different genetic backgrounds (14). In all cases, the serine toxicity was corrected by isoleucine. Serine is thought to induce starvation for isoleucine by negatively affecting isoleucine biosynthesis in several ways. There is evidence that serine inhibits two enzymes required for the synthesis of isoleucine, L-threonine deaminase (55) and homoserine dehydrogenase I (no. 10 and 6, respectively, in Fig. 1B) (36, 37). In addition, serine may lead to increased levels of pyruvate through its catabolism to pyruvate and feedback inhibition of its own synthesis (serine is derived from 3-phosphoglycerate, a precursor to pyruvate) (60). Elevation of pyruvate leads to isoleucine restriction due to the kinetic parameters of the acetohydroxy acid synthase isozymes (4). Increased pyruvate outcompetes α-ketobutyrate for available enzyme (no. 11 and 12 [Fig. 1B]), resulting in valine and leucine synthesis at the expense of isoleucine synthesis. To further characterize the serine sensitivity observed with DM3480, we tested the effects of intermediary metabolites of the isoleucine biosynthetic pathway (Fig. 1B) on serine inhibition. Growth of DM3480 in the presence of serine was restored by isoleucine, α-ketobutyrate, threonine, or homoserine, but not by aspartate (data not shown). These results parallel those observed with *E. coli* (37) and are consistent with at least one of the causes of isoleucine starvation by serine being inhibition of homoserine dehydrogenase I (no. 6 [Fig. 1B]).

Growth of DM3480 on minimal medium in the presence or absence of serine was examined when a variety of compounds were used as the sole carbon source. Growth of DM3480 was sensitive to serine and corrected by isoleucine on all carbon sources tested, including gluconate, sorbitol, xylose, ribose, pyruvate, glycerol, citrate, succinate, fructose, galactose, and acetate (data not shown). Growth of LT2 was not inhibited by serine under any of these conditions. Significantly, when DM3480 was grown with either pyruvate or glycerol as sole carbon source, the strain showed a significant requirement for isoleucine even in the absence of serine. The fact that *yjgF* mutants were starving for isoleucine even in the absence of serine suggested that these growth conditions may have introduced increased restrictions on the isoleucine biosynthetic pathway. Levels of pyruvate are likely to be elevated under these two growth conditions and may be contributing to isoleucine restriction as described above.

A *yjgF* mutation does not cause serine sensitivity by the same mechanism as does a *relA* mutation. Mutations exacerbating serine sensitivity have been isolated previously in *E. coli* (14), the most well-characterized being the *relA* mutation (64, 65). *relA* mutants are defective in (p)ppGpp synthetase and consequently are defective in the stringent response (10). A *relA* mutation seems to cause serine sensitivity by preventing derepression of the *ilv* genes, a natural way to avoid growth inhibition by serine (64). The serine sensitivity of a *relA* mutant was suppressed by mutations, including *hisT* (7, 8), that resulted in constitutive expression of the *ilv* genes (64). The results of two experiments suggested that the *yjgF* mutation
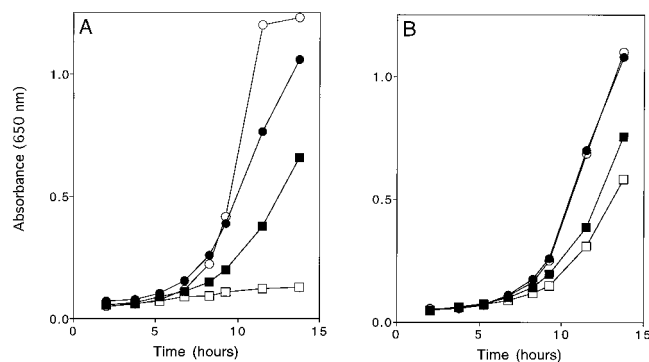

FIG. 6. Flux through the isoleucine biosynthetic pathway is important for restoration of thiamine synthesis by a *yjgF* mutation. Growth curves were obtained from strains grown at 37°C as described in Materials and Methods. Shown are growth of DM4767 [*purF2085 gnd174*::MudJ *yjgF2*::Tn*10*d(Tc)] (A) and growth of DM4769 [*purF2085 gnd174*::MudJ *yjgF2*::Tn*10*d(Tc) *ilvA219*] (B) in minimal gluconate medium supplemented with adenine (solid squares); adenine and isoleucine (open squares); adenine and thiamine (solid circles); and adenine, thiamine, and isoleucine (open circles).

was not acting in the same manner as a *relA* mutation. First, we tested if a *yjgF* mutant was defective in the stringent response by measuring stable RNA accumulation in starved and unstarved cultures as previously described (34). We found that stable RNA synthesis (as measured by $^{32}P_i$ incorporation into TCA-precipitable material) was dramatically decreased in response to starvation in both DM3480 (*yjgF3*::MudJ) and LT2 but, as expected, not in DM4440 (*relA21*::Tn10). $^{32}P_i$ was incorporated into TCA-precipitable material at rates of 1,608, 1,748, and 1,264 cpm/OD_{600}/min in unstarved cultures of LT2, DM4440, and DM3480, respectively. When these cultures were starved for amino acids, the incorporation rates of LT2 and DM3480 were significantly decreased, to 206 and 217 cpm/OD_{600}/min, respectively. The rate of incorporation into DM4440 remained high, at 1,112 cpm/OD_{600}/min. These results indicated that, unlike a *relA* mutant, a *yjgF* mutant was not defective in the stringent response. Second, when we constructed a *hisT yjgF* double mutant, the resulting strain, DM4442 [*yjgF1*::Tn*10*d(Tc) *hisT290*::Tn5], was as sensitive to serine as the parent strain, DM4053 [*yjgF1*::Tn*10*d(Tc)]. This result suggested that the cause of serine sensitivity in a *yjgF* mutant was not due to an inability to derepress the *ilv* genes.

Flux through the isoleucine biosynthetic pathway is important for a *yjgF* mutation to suppress the requirement for Gnd in thiamine synthesis. As shown in Fig. 5A, a *yjgF* mutation allowed a *purF gnd* double mutant to grow in the absence of thiamine. During routine phenotypic experiments, we found that exogenous isoleucine significantly reduced the ability of the *yjgF* mutation to restore thiamine synthesis in this strain. Figure 6A shows the growth of DM4767 [*purF2085 gnd174*::MudJ *yjgF2*::Tn*10*d(Tc)] in gluconate-adenine and gluconate-adenine-thiamine medium in the presence and absence of isoleucine. Although isoleucine increased the growth of DM4767 slightly when thiamine was included, it inhibited growth in the absence of thiamine. Isoleucine feedback inhibits its own synthesis by inhibiting activity of threonine deaminase (IlvA, no. 10 [Fig. 1B]), which is the first dedicated step to isoleucine biosynthesis (63). Therefore, one possible explanation for the effect of isoleucine described above was that carbon flux through the isoleucine biosynthetic pathway was important for the restoration of thiamine synthesis by the *yjgF* mutation. To test this possibility, we constructed a derivative of DM4767 that contained a feedback-resistant mutation in *ilvA*, DM4769 [*purF2085 gnd174*::MudJ *yjgF2*::Tn*10*d(Tc) *ilvA219*]. The *ilvA219* muta-

tion results in a threonine deaminase enzyme that is resistant to feedback inhibition by isoleucine (45). As shown in Fig. 6B, DM4769 was able to grow in the absence of thiamine even when isoleucine was included in the medium. Thus, isoleucine did not interfere with the effect of the *yjgF* mutation in a strain resistant to feedback inhibition by isoleucine. These results suggested that flux through the isoleucine biosynthetic pathway was important for the *yjgF* mutation to restore thiamine synthesis.

DISCUSSION

In this study, we describe the isolation and characterization of mutations in a previously uncharacterized ORF, designated *yjgF*. Mutations in *yjgF* caused multiple phenotypes. These mutations eliminated the requirement for the oxidative pentose phosphate pathway in PurF-independent thiamine synthesis. In addition, *yjgF* mutants appeared to be compromised in their ability to synthesize isoleucine, as indicated by their hypersensitivity to conditions that restrict isoleucine biosynthesis, e.g., increased levels of serine and/or pyruvate. This result is in agreement with previous work indicating that growth of the corresponding mutant in *L. lactis* (*aldR*) was slowed twofold if isoleucine was omitted from the medium (35).

Data presented here suggest a link between the effect of a *yjgF* mutation on thiamine synthesis and the apparent defect that this mutation causes in isoleucine synthesis. Results suggest that carbon flux through the isoleucine biosynthetic pathway is important for a *yjgF* mutation to restore thiamine synthesis. The following general model is proposed to explain these results. We suggest that the *yjgF* mutation results in the partial block of at least one step in isoleucine biosynthesis (by an as yet undefined mechanism) subsequent to the reaction catalyzed by threonine deaminase (IlvA, no. 10 [Fig. 1B]). This block results in starvation for isoleucine when growth conditions, i.e., increased levels of serine and/or pyruvate, further restrict isoleucine biosynthesis. In addition, we propose that the defect in isoleucine biosynthesis causes accumulation of a compound, likely an isoleucine biosynthetic intermediate or derivative, that is important for restoration of thiamine synthesis in the absence of the oxidative pentose phosphate pathway. When isoleucine is added to the medium, accumulation of the compound (and the corresponding restoration of thiamine synthesis) is prevented by feedback inhibition of the pathway at IlvA. Introduction of an *ilvA* feedback-resistant mutation thus abolishes isoleucine's inhibitory effect.

The above model predicts that an isoleucine biosynthetic intermediate, or a derivative, suppresses the requirement of the pentose phosphate pathway in thiamine synthesis. There are several possibilities for how this could occur. Since ribose-5-P also corrects the requirement, the simplest explanation is that the relevant intermediate increases ribose-5-P levels independently of the oxidative pentose phosphate pathway. The compound may be involved in regulating flux through the nonoxidative pathway or another metabolic pathway in which ribose-5-P is a precursor or intermediate. Such scenarios would be consistent with the ability of a *yjgF* mutation to suppress the requirement for Gnd in thiamine synthesis but not carbon catabolism. Alternatively, the intermediate might allow function of the APB pathway despite low ribose-5-P levels. For example, the relevant metabolite might increase levels of the predicted PRA-forming enzyme or enhance its activity, thus compensating for decreased ribose-5-P. The proposed model for how a YjgF defect affects thiamine synthesis (through the accumulation of an isoleucine biosynthetic intermediate) predicts that isoleucine intermediates added exogenously might also suppress the requirement for the pentose phosphate path-

way. Significantly, in a preliminary test of this prediction, none of the intermediates that we tested, including homoserine, threonine, and α-ketobutyrate, restored thiamine synthesis in a *purF gnd* mutant. Additional genetic and biochemical tests are currently under way to test this proposed model.

This study represents the first detailed phenotypic characterization of mutants defective in the YER057c/YjgF protein family in any organism and provides important clues as to the function of this highly conserved class of proteins. Results presented here suggest that the YjgF protein, or alternatively, the product of the YjgF protein if it functions as an enzyme, is important for appropriate function and/or regulation of the isoleucine biosynthetic pathway. Comparing the levels of isoleucine biosynthetic intermediates and enzymes in wild-type strains and *yjgF* mutants will be informative in this regard.

REFERENCES

1. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403–410.
2. Amos, R. H., and G. N. Cohen. 1954. Amino acid utilization in bacterial growth. J. Biochem. 57:338–343.
3. Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.). 1997. Current protocols in molecular biology, vol. 2. John Wiley & Sons, Inc., New York, N.Y.
4. Barak, Z., D. M. Chipman, and N. Gollop. 1987. Physiological implications of the specificity of acetohydroxy acid synthase isozymes of enteric bacteria. J. Bacteriol. 169:3750–3756.
5. Blattner, F. R., G. Plunkett III, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao. 1997. The complete genome sequence of *Escherichia coli* K-12. Science 277:1453–1474.
6. Bochner, B. R., H. Huang, G. L. Schieven, and B. N. Ames. 1980. Positive selection for loss of tetracycline resistance. J. Bacteriol. 143:926–933.
7. Bresalier, R. S., A. A. Rizzino, and M. Freunlich. 1975. Reduced maximal level of derepression of the isoleucine-valine and leucine enzymes in *hisT* mutants of *Salmonella typhimurium*. Nature 253:279–280.
8. Bruni, C. B., V. Colantuoni, L. Sbordone, R. Cortese, and F. Blasi. 1977. Biochemical and regulatory properties of *Escherichia coli* K-12 *hisT* mutant. J. Bacteriol. 130:4–10.
9. Bult, C. J., O. White, G. J. Olsen, L. Zhou, R. D. Fleischmann, G. G. Sutton, J. A. Blake, L. M. Fitzgerald, R. A. Clayton, J. D. Gocayne, A. R. Kerlavage, B. A. Dougherty, J.-F. Tomb, M. D. Adams, C. I. Reich, R. Overbeek, E. F. Kirkness, K. G. Weinstock, J. M. Merrick, A. Glodeck, J. L. Scott, N. S. M. Geoghagen, J. F. Weidman, J. L. Fuhrmann, D. Nguyen, T. R. Utterback, J. M. Kelley, J. D. Peterson, P. W. Sadow, M. C. Hann, M. D. Cotton, K. M. Roberts, M. A. Hurst, B. P. Kaine, M. Borodovsky, H.-P. Klenk, C. M. Fraser, H. O. Smith, C. R. Woese, and J. C. Venter. 1996. Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. Science 273:1058–1073.
10. Cashel, M., D. R. Gentry, V. J. Hernandez, and D. Vinella. 1996. The stringent response, p. 1458–1496. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington, D.C.
11. Castilho, B. A., P. Olfson, and M. J. Casadaban. 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. 158:488–495.
12. Ceciliani, F., L. Faotto, A. Negri, I. Colombo, B. Berra, A. Bartorelli, and S. Ronchi. 1996. The primary structure of UK114 tumor antigen. FEBS Lett. 393:147–150.
13. Chavez, S., J. C. Reyes, F. Chauvat, F. J. Florencio, and P. Candau. 1995. The NADP-glutamate dehydrogenase of the cyanobacterium *Synechocystis* 6803: cloning, transcriptional analysis and disruption of the *gdhA* gene. Plant Mol. Biol. 28:173–188.
14. Cosloy, S., and E. McFall. 1970. L-Serine-sensitive mutants of *Escherichia coli* K-12. J. Bacteriol. 103:840–841.
15. Cosloy, S., and E. McFall. 1973. Metabolism of D-serine in *Escherichia coli* K-12: mechanism of growth inhibition. J. Bacteriol. 114:685–694.

16. **Danchin, A., and L. Dondon.** 1980. Serine sensitivity of *Escherichia coli* K 12: partial characterization of a serine resistant mutant that is extremely sensitive to 2-ketobutyrate. Mol. Gen. Genet. **178:**155–164.

17. **David, S., B. Estramareix, J. C. Fischer, and M. Therisod.** 1982. The biosynthesis of thiamine. Synthesis of [1,1,1,5-²H₄]-1-deoxy-D-*threo*-2-pentulose and incorporation of this sugar in biosynthesis of thiazole by *Escherichia coli* cells. J. Chem. Soc. Perkin Trans. **1:**2131–2137.

18. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

19. **Deckert, G., P. V. Warren, T. Gaasterland, W. G. Young, A. L. Lenox, D. E. Graham, R. Overbeek, M. A. Snead, M. Keller, M. Aujay, R. Huber, R. A. Feldman, J. M. Short, G. J. Olson, and R. V. Swanson.** 1998. The complete genome of the hyperthermophilic bacterium *Aquifex aeolicus*. Nature **392:**353–358.

20. **DeMoll, E., and W. Shive.** 1985. Determination of the metabolic origin of the sulfur atom in thiamine of *Escherichia coli* by mass spectrometry. Biochem. Biophys. Res. Commun. **132:**217–222.

21. **Downs, D. M.** 1992. Evidence for a new, oxygen-regulated biosynthetic pathway for the pyrimidine moiety of thiamine in *Salmonella typhimurium*. J. Bacteriol. **174:**1515–1521.

22. **Downs, D. M., and L. Petersen.** 1994. *apbA*, a new genetic locus involved in thiamine biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **176:**4858–4864.

23. **Downs, D. M., and J. R. Roth.** 1991. Synthesis of thiamine in *Salmonella typhimurium* independent of the *purF* function. J. Bacteriol. **173:**6597–6604.

24. **Enos-Berlage, J. L., and D. M. Downs.** Biosynthesis of the pyrimidine moiety of thiamine independent of the PurF enzyme (phosphoribosylpyrophosphate amidotransferase) in *Salmonella typhimurium*: incorporation of stable isotope-labeled glycine and formate. Submitted for publication.

25. **Enos-Berlage, J. L., and D. M. Downs.** 1996. Involvement of the oxidative pentose phosphate pathway in thiamine biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **178:**1476–1479.

26. **Enos-Berlage, J. L., and D. M. Downs.** 1997. Mutations in *sdh* (succinate dehydrogenase genes) alter the thiamine requirement of *Salmonella typhimurium*. J. Bacteriol. **179:**3989–3996.

27. **Enos-Berlage, J. L., and D. M. Downs.** 1996. Unpublished results.

28. **Estramareix, B., and S. David.** 1990. Conversion of 5-aminoimidazole ribotide to the pyrimidine of thiamine in enterobacteria: study of the pathway with specifically labeled samples of riboside. Biochim. Biophys. Acta **1035:**154–160.

29. **Estramareix, B., and M. Therisod.** 1972. Tyrosine as a factor in biosynthesis of the thiazole moiety of thiamine in *Escherichia coli*. Biochim. Biophys. Acta **273:**275–282.

30. **Fleischmann, R. D., M. D. Adams, O. White, R. A. Clayton, E. F. Kirkness, A. R. Kerlavage, C. J. Bult, J.-F. Tomb, B. A. Dougherty, J. M. Merrick, J. F. Weidman, C. A. Phillips, T. Spriggs, E. Hedblom, M. D. Cottom, T. R. Utterback, M. C. Hanna, D. T. Nguyen, D. M. Saudek, R. C. Brandon, L. D. Fine, J. L. Fritchman, J. L. Fuhrmann, N. S. M. Geoghagen, C. L. Gnehm, L. A. McDonald, K. V. Small, C. M. Fraser, H. O. Smith, and J. C. Venter.** 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science **269:**496–512.

31. **Freiberg, C., R. Fellay, A. Bairoch, W. J. Broughton, A. Rosenthal, and X. Perret.** 1997. Molecular basis of symbiosis between *Rhizobium* and legumes. Nature **387:**394–401.

32. **Frodyma, M. E., and D. M. Downs.** 1998. ApbA, the ketopantoate reductase enzyme of *Salmonella typhimurium*, is required for the synthesis of thiamine via the alternative pyrimidine biosynthetic pathway. J. Biol. Chem. **273:**5572–5576.

33. **Frodyma, M. E., and D. M. Downs.** 1998. The *panE* gene, encoding ketopantoate reductase, maps at 10 minutes and is allelic to *apbA* in *Salmonella typhimurium*. J. Bacteriol. **180:**4757–4759.

34. **Gaal, T., and R. Gourse.** 1990. Guanosine 3′-diphosphate 5′-diphosphate is not required for growth rate-dependent control of rRNA synthesis in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**5533–5537.

35. **Goupil-Feuillerat, N., M. Cocaign-Bousquet, J.-J. Gocon, S. D. Ehrlich, and P. Renault.** 1997. Dual role of α-acetolactate decarboxylase in *Lactococcus lactis* subsp. *lactis*. J. Bacteriol. **179:**6285–6293.

36. **Hama, H., T. Kayahara, M. Tsuda, and T. Tsuchiya.** 1991. Inhibition of homoserine dehydrogenase I by L-serine in *Escherichia coli*. J. Biochem. **109:**604–608.

37. **Hama, H., Y. Sumita, Y. Kakutani, M. Tsuda, and T. Tsuchiya.** 1990. Target of serine inhibition in *Escherichia coli*. Biochem. Biophys. Res. Commun. **168:**1211–1216.

38. **Hong, J. S., and B. N. Ames.** 1971. Localized mutagenesis of any specific small region of the bacterial chromosome. Proc. Natl. Acad. Sci. USA **68:**3158–3162.

39. **Hughes, K. T., and J. R. Roth.** 1984. Conditionally transposition-defective derivative of Mu d1 (Amp Lac). J. Bacteriol. **159:**130–137.

40. **Hughes, K. T., and J. R. Roth.** 1985. Directed formation of deletions and duplication using Mu*d* (Ap, Lac). Genetics **109:**263–282.

41. **Hughes, K. T., and J. R. Roth.** 1988. Transitory *cis* complementation: a method for providing transposition functions to defective transposons. Genetics **119:**9–12.

42. **Joerger, R. D., M. R. Jacobson, and P. E. Bishop.** 1989. Two *nifA*-like genes required for expression of alternative nitrogenases by *Azotobacter vinelandii*. J. Bacteriol. **171:**3258–3267.

43. **Kleckner, N., J. R. Roth, and D. Botstein.** 1977. Genetic engineering *in vivo* using translocatable drug resistant elements. J. Mol. Biol. **116:**125–159.

44. **Klenk, H. P., R. A. Clayton, J. F. Tomb, O. White, K. E. Nelson, K. A. Ketchum, R. J. Dodson, M. Gwinn, E. K. Hickey, J. D. Peterson, D. L. Richardson, A. R. Derlavage, D. E. Graham, N. C. Kyrpides, R. D. Fleischmann, J. Quackenbush, N. H. Lee, G. G. Sutton, S. Gill, E. F. Kirkness, B. A. Dougherty, K. McKenney, M. D. Adams, B. Loftus, J. C. Venter, et al.** 1997. The complete genome sequence of the hyperthermophilic sulphate-reducing archaeon *Archaeoglobus fulgidus*. Nature **390:**364–370.

45. **LaRossa, R., T. K. Van Dyk, and D. R. Smulski.** 1987. Toxic accumulation of α-ketobutyrate caused by inhibition of the branched-chain amino acid biosynthetic enzyme acetolactate synthase in *Salmonella typhimurium*. J. Bacteriol. **169:**1372–1378.

46. **Levy-Favatier, F., L. Cuisset, B. Nedelec, L. Tichonicky, J. Kruh, and M. Delpech.** 1993. Characterization, purification, and cDNA cloning of a rat perchloric-acid-soluble 23-kDa protein present only in liver and kidney. Eur. J. Biochem. **212:**665–673.

47. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. **145:**1110–1112.

48. **Martinez, E., B. Bartolome, and F. de la Cruz.** 1988. pACYC184-derived cloning vectors containing the multiple cloning site and *lacAa* reporter gene of pUC8/9 and pUC18/19 plasmids. Gene **68:**159–162.

49. **Negre, D., J.-C. Cortay, P. Donini, and A. J. Cozzone.** 1989. Relationship between guanosine tetraphosphate and accuracy of translation in *Salmonella typhimurium*. Biochemistry **28:**1814–1819.

50. **Newell, P. C., and R. G. Tucker.** 1968. Biosynthesis of the pyrimidine moiety of thiamine. Biochem. J. **106:**279–287.

51. **Newell, P. C., and R. G. Tucker.** 1968. Precursors of the pyrimidine moiety of thiamine. Biochem. J. **106:**271–277.

52. **Ogasawara, N., S. Nakai, and H. Yoshikawa.** 1994. Systematic sequencing of the 180 kilobase region of the Bacillus subtilis chromosome containing the replication origin. DNA Res. **1:**1–14.

53. **Oka, T., H. Tsuji, C. Noda, K. Sakai, Y.-M. Hong, I. Suzuki, S. Munoz, and Y. Natori.** 1995. Isolation and characterization of a novel perchloric-acid soluble protein inhibiting cell-free protein synthesis. J. Biol. Chem. **270:**30060–30067.

54. **Petersen, L. A., J. L. Enos-Berlage, and D. M. Downs.** 1996. Genetic analysis of metabolic crosstalk and its impact on thiamine synthesis in *Salmonella typhimurium*. Genetics **143:**37–44.

55. **Rasko, I., and L. Alfoldi.** 1971. Biosynthetic L-threonine deaminase as the origin of L-serine sensitivity of *Escherichia coli*. Eur. J. Biochem. **21:**424–427.

56. **Rowley, D.** 1953. Inhibition of *E. coli* strains by amino-acids. Nature **171:**80–81.

57. **Schmiedeknecht, G., C. Kerkhoff, E. Orso, J. Stohr, C. Aslanidis, G. M. Nagy, R. Knuechel, and G. Schmitz.** 1996. Isolation and characterization of a 14.5-kDa trichloroacetic-acid-soluble translational inhibitor protein from human monocytes that is upregulated upon cellular differentiation. Eur. J. Biochem. **242:**339–351.

58. **Schmieger, H.** 1972. Phage P22 mutants with increased or decreased transduction abilities. Mol. Gen. Genet. **119:**75–88.

59. **Smith, D. R., L. A. Doucette-Stamm, C. Deloughery, H. Lee, J. Dubois, T. Aldredge, R. Bashirzadeh, D. Blakely, R. Cook, K. Gilbert, D. Harrison, L. Hoang, P. Keagle, W. Lumm, B. Pothier, D. Qiu, R. Spadafora, R. Vicaire, Y. Wang, J. Wierzbowski, R. Gibson, N. Jiwani, A. Caruso, D. Bush, et al.** 1997. Complete genome sequence of *Methanobacterium thermoautotrophicum* ΔH: functional analysis and comparative genomics. J. Bacteriol. **179:**7135–7155.

60. **Stauffer, G. V.** 1996. Biosynthesis of serine, glycine, and one-carbon units, p. 506–513. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington, D.C.

61. **Tabor, S.** 1990. Expression using the T7 RNA polymerase/promoter system, p. 16.2.1–16.2.11. *In* P. A. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. C. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. Wiley, New York, N.Y.

62. **Tomb, J.-F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzegerald, N. Lee, M. D. Adams, E. K. Hickey, D. E. Berg, J. D. Gocayne, T. R. Utterback, J. D. Peterson, J. M. Kelley, M. D. Cotton, J. M. Weidman, C. Fujii, C. Bowman, L. Watthey, E. Wallin, W. S. Hayes, M. Borodovsky, P. D. Karp, H. O. Smith, C. M. Fraser, and J. C. Venter.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:**539–547.

63. **Umbarger, H. E.** 1996. Biosynthesis of the branched-chain amino acids, p. 442–457. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular

biology, 2nd ed., vol. 1. ASM Press, Washington, D.C.

64. **Uzan, M., and A. Danchin.** 1978. Correlation between the serine sensitivity and the derepressibility of the *ilv* genes in *Escherichia coli relA⁻* mutants. Mol. Gen. Genet. **165:**21–30.

65. **Uzan, M., and A. Danchin.** 1976. A rapid test for the *relA* mutation in *E. coli*. Biochem. Biophys. Res. Commun. **69:**751–758.

66. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*:

partial purification and some properties. J. Biol. Chem. **218:**97–106.

67. **Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner.** 1984. New Tn*10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. Gene **32:**369–379.

68. **Webb, E., F. Febres, and D. M. Downs.** 1996. Thiamine pyrophosphate (TPP) negatively regulates transcription of some *thi* genes of *Salmonella typhimurium*. J. Bacteriol. **178:**2533–2538.