JOURNAL OF BACTERIOLOGY, Apr. 2008, p. 3057–3062 Vol. 190, No. 8
0021-9193/08/$08.00+0 doi:10.1128/JB.01700-07

YjgF Is Required for Isoleucine Biosynthesis when *Salmonella enterica* Is Grown on Pyruvate Medium▽

Melissa R. Christopherson, G. E. Schmitz, and Diana M. Downs*

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

Received 23 October 2007/Accepted 11 February 2008

The YjgF/YER057c/UK114 family of proteins is conserved across the three domains of life, yet no biochemical function has been clearly defined for any member of this family. In *Salmonella enterica*, a deletion of *yjgF* results in a requirement for isoleucine when the mutant strain is grown in glucose-serine or pyruvate medium. Feedback inhibition of IlvA is required for the curative effect of isoleucine on glucose-serine medium. On pyruvate medium, *yjgF* mutants are unable to synthesize enough isoleucine for growth. From this study, we conclude that the isoleucine requirement of a *yjgF* mutant on pyruvate is a consequence of the decreased transaminase B (IlvE) activity that has previously been characterized in these mutants.

The YjgF/YER057c/UK114 family defines a highly conserved class of proteins of approximately 15 kDa. Interest in these proteins of unknown function has led to the elucidation of the high-resolution structures of more than six YjgF homologs spanning the three domains of life (12, 25, 28, 32, 38, 44). Homologs of YjgF bind keto-acids in vitro (6, 32), although no study has clarified the biochemical function of any of these homologs. Eukaryotic members of the YjgF family have been implicated in diverse cellular processes (10, 17, 20, 27, 29, 34). Past results from our laboratory led to a general model in which YjgF removes toxic metabolites (5, 14, 36).

Microbial *yjgF* mutants show an isoleucine requirement under some growth conditions (16, 19, 20). In *Salmonella enterica*, *yjgF* mutant strains are unable to grow with pyruvate as the sole carbon source unless isoleucine is provided (15). On other carbon sources, *yjgF* mutants are prototrophic, but unable to grow in the presence of serine unless isoleucine is also added (16). Serine impacts the activities of both ThrA and IlvA (Fig. 1). The serine sensitivity of *relA* mutants has been attributed to these effects (16, 42). The cause of serine sensitivity in *yjgF* mutant strains is not yet clear, but it is distinct from that in *relA* mutants (16).

Figure 1 schematically represents branched-chain amino acid biosynthetic pathways, relevant regulation, and operon structures. Exogenous isoleucine will satisfy a nutritional requirement and reduce biosynthetic flux by feedback inhibition of IlvA (41). Past work on *yjgF* mutants focused on the two isoleucine-specific biosynthetic enzymes transaminase B (IlvE) and threonine deaminase (IlvA). In *yjgF* mutant strains, IlvE activity was reduced (36). The defect in IlvE activity was shown to be posttranscriptional and dependent on the function of IlvA (36). IlvA activity (i.e., generation of 2-ketobutyrate [AKB]) is not higher in *yjgF* mutants than in the wild type. Unlike other mutants that are sensitive to the inhibition of IlvGM (Fig. 1), *yjgF* mutants are not sensitive to exogenous AKB (24). *yjgF* mutant strains lacking *ilvA* can be grown with AKB as a source of isoleucine with no deleterious effects. These results led us to suggest that a non-AKB product of IlvA accumulates specifically in a *yjgF* mutant and decreases IlvE activity (14, 36) (see Fig. 5). Thus, despite the fact that YjgF has affinity for AKB in vitro (6, 32), we favor a model implicating a non-AKB metabolite.

This study was initiated to understand the isoleucine requirement of a *yjgF* mutant strain on pyruvate and glucose-serine medium. The data obtained show that isoleucine restores growth by distinct mechanisms under the two conditions. We show that on pyruvate medium, a *yjgF* mutant fails to synthesize sufficient isoleucine for growth, resulting in a nutritional requirement for isoleucine. In contrast, the curative effect of isoleucine on glucose-serine medium requires feedback inhibition of IlvA, indicating a regulatory role of isoleucine is allowing growth.

MATERIALS AND METHODS

Bacterial strains, media, and chemicals. Strains used in this study are derivatives of *S. enterica* serovar Typhimurium LT2 and are listed with their respective genotypes in Table 1. MudJ refers to Mud1734, which has been described elsewhere (9). Tn*10*d(Tc) refers to the transposition-defective mini-Tn*10*(Tn*10*Δ*16* Δ*17*) construct (45).

No-carbon E medium (NCE), supplemented with 1 mM $MgSO_4$ (11, 43), trace minerals (2), and 11 mM glucose (or 50 mM pyruvate as indicated) was used as the minimal medium. Difco nutrient broth (NB; 8 g/liter) with NaCl (5 g/liter) was used as the rich medium. Luria broth was used for experiments involving plasmid isolation. 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: serine, 5 mM; and isoleucine, 0.3 mM. The final concentrations of the antibiotics in rich and minimal medium, respectively, were as follows: tetracycline, 20 and 10 μg/ml; kanamycin, 50 and 150 μg/ml; and ampicillin, 30 and 15 μg/ml. The indicator 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) was added at a final concentration of 40 μg/ml.

Growth quantitation. Cells from overnight cultures in NB medium were pelleted and resuspended in an equal volume of saline (0.85% NaCl), and an aliquot (0.2 ml) was used to inoculate 5 ml of the appropriate minimal medium. Unless otherwise specified, growth was measured as the cell density (optical density at 650 nm [OD_{650}]) reached after 12 h of incubation at 37°C with shaking.

Genetic techniques. Transductional crosses were performed using the high-frequency general transducing mutant of bacteriophage P22 (HT105/1 *int-201*) (33, 35). Methods for transductional crosses, purification of transductants from phage, and identification of phage-free transductants have been described elsewhere (13). Multiple-mutant strains were constructed by standard genetic tech-

* Corresponding author. Mailing address: Department of Bacteriology, University of Wisconsin—Madison, 1550 Linden Dr., Madison, WI 53706. Phone: (608) 238-0383. Fax: (608) 262-9865. E-mail: downs@bact.wisc.edu.

▽ Published ahead of print on 22 February 2008.

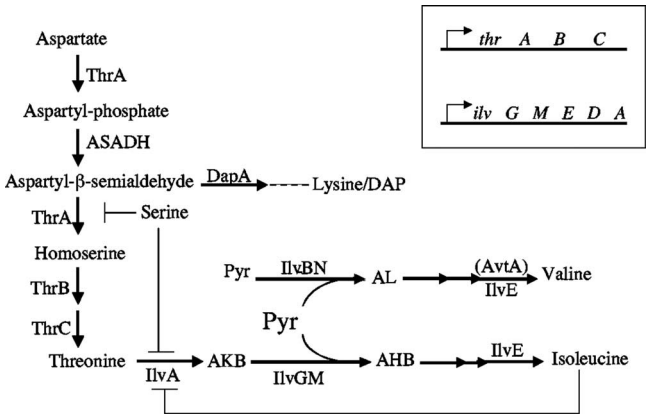


FIG. 1. Branched-chain amino acid biosynthesis. The pathways for branched-chain amino acid biosynthesis are shown schematically. Enzymes are indicated next to the relevant catalytic step. Significantly, only lesions in *ilvA* or *ilvE* prevent isoleucine but not valine synthesis. The *ilvGM* and *ilvBN* genes encode acetolactate synthase enzymes with different substrate specificities. Targets of feedback inhibition by isoleucine and serine are indicated. The inset shows the operon structure of *thr* and *ilv* genes that is significant to the results discussed in the text. Abbreviations: AKB, 2-ketobutyrate; AL, 2-acetolactate; AHB, 2-aceto-3-hydroxybutyrate; Pyr, pyruvate; ASADH, aspartyl-β-semialdehyde dehydrogenase; and DAP, diaminopimelate.

niques. When necessary, genetic backcrosses were performed to confirm the presence of a respective allele.

Molecular techniques. The *ilvA* gene was amplified using the primers N-ter ilvA (ATGGCGGAATCTCAACCTCTG) and C-ter ilvA (GTCTCGCAAAATGAAATGAGTG). Amplification by PCR was done with Herculase (Stratagene) DNA polymerase. The PCR conditions were as follows: denaturation at 95°C for 30 s, annealing at 54°C for 1 min, and extension at 72°C for 1 min. The attenuator region upstream of ThrA was amplified using the primers 3′ upstream ThrA (CCGCCGAACTTCAACACTCGCAT) and 5′ upstream ThrA (AGAGATTACGTCTGGTTGCAAG) under the same PCR conditions described above.

Enzyme assays. (i) Homoserine dehydrogenase (ThrA) assays. Overnight NB cell cultures were diluted 100-fold into 25 ml of minimal glucose medium in 125-ml flasks and were incubated at 37°C with shaking. Cells were harvested at 60 to 80 Klett units (red filter, $\sim 6 \times 10^8$ CFU/ml) by centrifugation at 7,000 × *g* at 4°C, washed in 5 ml cold NCE medium, pelleted again, and frozen at −80°C until use. Cell pellets were thawed on ice, resuspended in 0.5 ml of 50 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS) buffer (pH 7.5)–0.125 mM dithiothreitol, and cells were lysed by sonication at 4°C. Cell extracts were clarified by centrifugation for 20 min at 16,000 × *g*. Generally, extracts had 3 to 4 mg protein/ml. ThrA assays were adapted from the method of Angeles et al. (1) and contained cell extract (15 to 40 μg protein), 50 mM MOPS (pH 7.5), 200 mM KCl, 0.125 mM dithiothreitol, and 0.3 mM $NADP^+$ in a 200-μl total volume. Reactions were initiated with the addition of L-homoserine to a final concentration of 15 mM. The reaction (homoserine to aspartyl-β-semialdehyde) was measured as $NADP^+$ reduction (ΔA_{340}) over time. Specific activity is reported as ΔA_{340} min^{-1} mg^{-1}. Under these conditions, activity was not detectable in a strain containing a *thr-3020*::Tn*10*d(Tc) insertion (data not shown).

(ii) Transaminase B (IlvE) assays. IlvE assays were performed as previously described (30, 36, 40). Cells were permeabilized by sonication. Known concentrations of DL-α-keto-β-methylvalerate were subjected to the extraction procedure to generate a standard curve. Specific activities for transaminase B are reported in nmol min^{-1} mg^{-1} for crude extract.

(iii) Protein concentration. Protein concentration was estimated with bovine serum albumin (BSA) as the standard using a Bradford assay (4).

(iv) Threonine deaminase (IlvA) assays. Cells from overnight cultures grown in NB medium were used to inoculate 50 ml of minimal medium (1:25 dilution) and incubated for 12 h at 37°C. Cells were harvested by centrifugation (7,000 × *g* at 4°C), resuspended in 500 μl 100 mM Tris-HCl (pH 8.0), and disrupted by sonication. Cell extracts were clarified by centrifugation, and enzyme assays were performed as previously described measuring conversion of threonine to AKB by following the formation of the dinitrophenylhydrazone derivative (7). Specific activities for threonine deaminase are reported in nmol min^{-1} mg^{-1} for crude extracts.

(v) SMM disc diffusion assays. Sulfometuron methyl (SMM) sensitivity was determined as previously described (36). When present, isoleucine was added to a final concentration of 240 μM. The numbers reported are an average of two replicates.

(vi) β-Galactosidase assays. β-Galactosidase assays were performed according to Miller (46). Strains harboring the *thr-469*::MudJ fusion were grown in Luria broth (repressing conditions) or in minimal glucose medium supplemented with 180 μM L-threonine (inducing). The numbers reported are an average of three replicates.

RESULTS

A feedback-resistant variant of IlvA distinguishes the *yjgF* mutant phenotypes. Isoleucine is required for *yjgF* mutant strains to grow on glucose-serine medium or pyruvate medium (15). An IlvA variant insensitive to isoleucine inhibition (24) was used to determine whether feedback inhibition was required for the curative effect of isoleucine. Growth of strains DM5970 (*yjgF ilvA219*) and DM3480 (*yjgF*) was assessed on glucose, pyruvate, and glucose-serine media (Fig. 2). YjgF mutant strains containing the *ilvA219* allele responded differently to isoleucine from those carrying a wild-type *ilvA* allele. Normalization to growth on glucose emphasized the difference (Fig. 2B). Isoleucine restored growth of the *yjgF* strain (DM3480) on both media but failed to correct the growth of the *yjgF ilvA219* strain (DM5970) on glucose-serine medium. Thus, feedback inhibition of IlvA was essential for the curative effect of isoleucine in the presence of serine. Isoleucine allowed growth on pyruvate by means that did not require feedback inhibition.

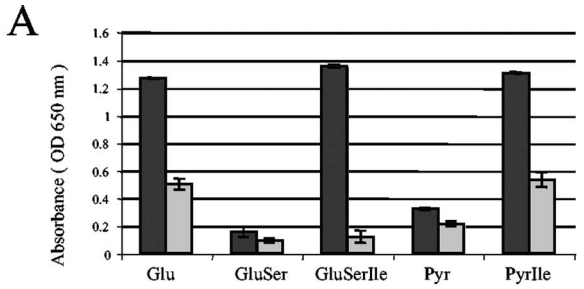
In the growth studies, strain DM4751 (*ilvA219*) was used as a control and reached full density (OD_{650}, ~1.2) in all media (data not shown). Strain DM5970 (*yjgF ilvA219*) failed to reach full density in either medium unless Casamino Acids (0.2%) were added.

Suppressor mutations distinguish between *yjgF* phenotypes. The results above indicated how isoleucine allowed growth. We next probed the cellular adjustments that could be made to allow growth of the *yjgF* mutant in the absence of isoleucine. Spontaneous mutants were selected for growth in the absence of isoleucine. Nine independent mutations were isolated: six on pyruvate and three on glucose-serine. Eight of the nine revertants could be placed in one of two phenotypic classes

TABLE 1. Bacterial strains used in this study

Strain	Genotype[a]
DM3480	*yjgF3*::MudJ
DM4751	*ilvA219*
DM6012	*ilvE3200 zxx-9118*::Tn*10*d(Tc)
DM5970	*ilvA219 yjgF3*::MudJ
DM7607	*yjgF3*::MudJ *gyrA751*
DM7608	*yjgF3*::MudJ *ilvA3211*
DM7609	*yjgF3*::MudJ *thr-1372*
DM7610	*yjgF3*::MudJ *ilvA3210*
DM7612	*yjgF3*::MudJ *thr-1373*
DM7741	*yjgF3*::MudJ *stm0014–7*::Tn*10*d(Tc)
DM7742	*yjgF3*::MudJ *thr-1372* STM0014-7::Tn*10*d(Tc)
DM7825	*yjgF7* STM0014-7::Tn*10*d(Tc) *thr-1372*
DM10009	*yjgF3*::MudJ *ilvA3210 ilvY3212*::Tn*10*d(Tc)
DM10010	*yjgF3*::MudJ *ilvY3212*::Tn*10*d(Tc)

[a] MudJ refers to the Mud1734 transposon (9). Tn*10*d(Tc) refers to the transposition-defective mini-Tn*10*(Tn*10*Δ*16* Δ*17*) construct (45).



B	Glu	GluSer	GluSerIle	Pyr	PyrIle
yjgF	100%	13%	113%	27%	109%
yjgF ilvA219	100%	19%	25%	43%	106%

FIG. 2. The *ilvA219* allele alters the growth of *yjgF* mutants. (A) Growth of strains containing *yjgF* (DM3480 [black bars]) or *yjgF ilvA219* (DM5970 [gray bars]) was monitored at 650 nm after 12 h of growth at 37°C. The carbon source (50 mM pyruvate or 11 mM glucose) and supplements (5 mM L-serine and 0.3 mM L-isoleucine) were present where indicated. (B) Growth was reported as a percentage relative to the strain grown on glucose medium (100%). Abbreviations: Glu, glucose; Ser, serine; Ile, isoleucine; and Pyr, pyruvate.

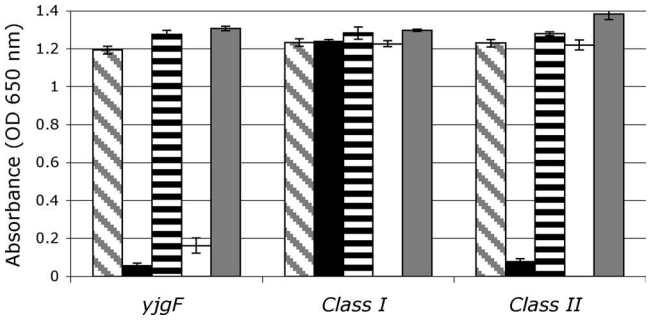


FIG. 3. Suppressor mutations restore growth in the absence of isoleucine. Strains were monitored at 650 nm after 12 h of growth in the indicated medium. Representative strains were grown in 11 mM glucose (diagonally striped bars), 11 mM glucose plus 5 mM serine (black bars), 11 mM glucose plus 5 mM serine plus 0.3 mM isoleucine (horizontally striped bars), 50 mM pyruvate (white bars), or 50 mM pyruvate plus 0.3 mM isoleucine (gray bars). Parental strain DM3480 (*yjgF*) is shown as a control. Data from a representative strain of each class of suppressors are shown (average of three independent cultures).

that are illustrated by the data in Fig. 3. Mutations in class I eliminated the isoleucine requirement on both glucose-serine and pyruvate media. Mutations in class II restored growth only on pyruvate. No mutations were found that restored growth of the *yjgF* mutant on only glucose-serine. (Approximately 200 colonies that grew on glucose-serine were screened.)

The single revertant that was not easily classified restored growth of a *yjgF* strain in the absence of isoleucine after 24 h. The suppressor mutation in this strain (DM7607) was shown to be in *gyrA*. The *gyrA751* allele reduced DNA supercoiling levels and altered the expression of a number of genes by up to twofold (37). Due to the complexity of the transcriptional changes caused by the *gyrA751* allele, its ability to suppress the *yjgF* mutant phenotypes was not pursued.

Of the four class I mutations, two were located in the *dapA* gene (encoding dihydropicolinate synthase) (Fig. 1), one was in the promoter of *dapA*, and one was an allele of *thrA* (data not shown). These mutations were not pursued in this study. The four class II mutations were of two types: two mapped near the *thr* operon and two were alleles of *ilvA*. These mutations are described below.

Suppressor mutations map near the *thr* operon. Class II suppressors were characterized further to understand the need for isoleucine on pyruvate medium. A Tn*10*d(Tc) insertion was isolated by standard techniques (21) and shown to be linked to two of the four suppressor mutations. Sequencing analyses using degenerate primers (8) revealed that the Tn*10*d(Tc) insertion was in STM0014, roughly 10 kb clockwise from the *thrABC* operon (26). The insertion [STM0014-7::Tn*10*d(Tc)] was linked to the *thrA13* and *thrB11* alleles (33 and 24%, respectively), confirming the genomic placement of the insertion.

Increased expression of the *thrABC* operon eliminated the isoleucine requirement of a *yjgF* mutant on pyruvate. Alleles *thr-1373* and *thr-1372* were identified in the attenuator region upstream of the *thrABC* operon (18). Allele *thr-1373* was a T-to-G change at −40 with respect to *thrA*, placing it between the leader peptide and the start of *thrA*. Allele *thr-1372* was a 50-bp deletion (−34 through −83), removing part of the attenuator (18). The locations of these mutations are sufficient to explain the increased expression of the *thr* operon in these mutants. Expression of a *thrABC* operon fusion (*thr-469*::MudJ) in a strain containing *thr-1372* (DM7825) had >5-fold-higher transcription than the isogenic strain under repressing (19 ± 2 versus 316 ± 9 Miller units) or inducing (109 ± 7 versus 537 ± 91 Miller units) conditions. Similar data were obtained in the strain carrying the *thr-1373* allele (data not shown). In other work, homoserine dehydrogenase (ThrA) was assayed in strain DM7742 (*thr-1372*) and found to be elevated threefold compared to the isogenic *thr*$^+$ strain DM7741 (178 ± 30 and 56 ± 14 ΔA_{340}/min/mg protein, respectively). Thus, increasing the expression of the *thr* operon or providing exogenous threonine (data not shown) restored isoleucine-independent growth on pyruvate.

Growth of a *yjgF* mutant on pyruvate medium is impacted by isoleucine biosynthetic flux. The two remaining class II suppressor mutations (in DM7608 and DM7610) were 80% linked to an insertion in the *ilvY* locus: *ilvY3212*::Tn*10*d(Tc). The *ilvY* locus is adjacent to the *ilvGMEDA* operon, and the *ilvY3212*::Tn*10*d(Tc) insertion itself had no discernible growth defect. PCR amplification and sequencing of the *ilvA* gene determined that the suppressor mutations in strains DM7610 and DM7608 were alleles of *ilvA*. Strains DM7610 (*ilvA3210*) and DM7608 (*ilvA3211*) carried G-to-A transitions at bp 424 and 571, respectively. Thus, the *ilvA3210* allele encoded protein variant IlvA^{A142T}, while the *ilvA3211* allele encoded IlvA^{G191S}. IlvA was assayed, and data presented in Fig. 4A show that IlvA activity was decreased 10-fold in a strain carrying the *ilvA3210* mutation. We explored the possibility that the decreased activity of IlvA would indirectly increase total IlvE activity. The finding that IlvE activity was increased twofold in the *ilvA3210* strain suggested a defective IlvA would generate a partial starvation for isoleucine, causing the cell to respond by derepressing the *ilv* genes. To address this further, isogenic strains were grown in minimal medium with and without isoleucine and the level of accumulated IlvE in each was visualized by Western blot analyses. Data in Fig. 4C show that more

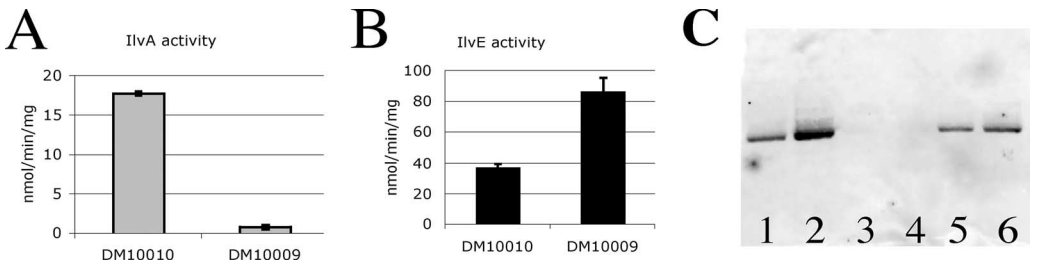


FIG. 4. A suppressor mutation in IlvA decreases threonine deaminase activity. Activities of threonine deaminase (IlvA) (A) and transaminase B (IlvE) (B) were measured in cell extracts from *yjgF* (DM10010) or *yjgF ilvA3210* (DM10009) mutant strains. Strains were grown in the absence of isoleucine, and activities were assayed according to standard protocols (7, 31, 36, 40). (C) Five micrograms of crude cell extracts from DM10009 (lanes 1 and 2), DM6012 (*ilvE*) (lanes 3 and 4), or DM10010 (lanes 5 and 6) cells grown in the presence (lanes 1, 3, and 5) or absence (lanes 2, 4, and 6) of isoleucine was separated on a 12.5% sodium dodecyl sulfate–polyacrylamide gel and detected by Western blot analysis using precleared polyclonal anti-IlvE antibodies.

IlvE accumulated in the *ilvA3210* strain than the wild-type strain grown in minimal medium (lanes 2 and 6). The levels of IlvE that accumulated in the presence of isoleucine were similar in both strains (lanes 1 and 5). The increase in IlvE activity, attributed to increased transcription, suggested the other proteins encoded by the operon (including IlvA) would also be expressed above wild-type levels. The activity of threonine deaminase (IlvA) is thought to be detrimental in a *yjgF* mutant, because it decreases transaminase B (IlvE) activity (36). The above *ilvA* mutations appear to have increased the IlvE activity needed for isoleucine biosynthesis without increasing activity of the IlvA enzyme.

Strains carrying *ilvA3210* retain sensitivity to SMM. SMM is a potent inhibitor of acetolactate synthase isozyme II (*ilvGM*) (Fig. 1 and 5) and causes the product(s) of the preceding reaction to accumulate (22). There is extensive literature describing the toxicity of AKB accumulation after SMM treatment (23, 24). Data in Fig. 4 show that strain DM10009 (*ilvA3210*) has less threonine deaminase (IlvA) activity (i.e., less AKB formed) than strain DM10010 (*ilvA*$^+$). The simple prediction from this result is that the strain carrying *ilvA3210* (DM10009) would be more resistant to SMM than the isogenic strain DM10010. In the absence of isoleucine, *yjgF* strains with a wild-type or *ilvA3210* allele showed similar sensitivity to SMM (inhibition zones of 38 ± 1 and 37 ± 1 mm, respectively). The addition of isoleucine eliminated the zone of inhibition in both strains, indicating the IlvA variant retained sensitivity to feedback inhibition (22).

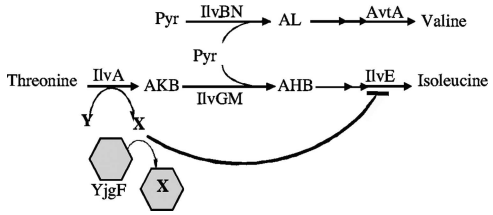


FIG. 5. Expanded working model for YjgF. The working model for YjgF proposed previously (36) has been expanded to incorporate data obtained herein. Parallel pathways for valine and isoleucine biosynthesis are shown. AvtA is transaminase C, which is redundant with IlvE in the formation of valine but not isoleucine. Substrate specificity of the acetohydroxy acid synthase complex I (IlvBN) and II (IlvGM) varies, depending on relative substrate concentration (3), and may result in constriction of flux to isoleucine by favoring valine formation in the presence of pyruvate. IlvA is proposed to generate a product (X) from an unknown substrate (Y). Metabolite X is proposed to decrease IlvE activity (36). YjgF is proposed to bind and/or inactivate X, preventing its accumulation. Abbreviations: AKB, 2-ketobutyrate; AL, 2-acetolactate; AHB, 2-aceto-3-hydroxybutryrate; and Pyr, pyruvate.

DISCUSSION

Studies of the highly conserved YjgF/YER057c/UK114 protein family have yielded varied hypotheses regarding the role of these proteins in physiology. Despite studies in multiple organisms and the existence of numerous high-resolution structures, progress toward understanding the biochemical function of this family of proteins has been slow. We have applied a combination of biochemical and genetic approaches in an effort to define the metabolic role of YjgF. In *S. enterica* specifically, lack of *yjgF* generates a growth requirement for isoleucine on both pyruvate and glucose-serine media. Based on results presented herein, we conclude that isoleucine allows a *yjgF* mutant to grow on pyruvate by satisfying a defect in isoleucine biosynthesis. In contrast, on glucose-serine media, isoleucine must inhibit IlvA to allow growth of a *yjgF* mutant strain (Fig. 2). The mechanism by which serine is toxic to *yjgF* mutants remains to be determined.

Results herein indicated that a *yjgF* mutant strain grown on pyruvate medium has a defect in isoleucine biosynthesis. Elevated pyruvate levels outcompete AKB, leading IlvGM to synthesize 2-acetolactate from two pyruvate molecules instead of condensing pyruvate with AKB to form acetohydroxybutyrate (3) (Fig. 5). Thus, there is a flux bias to valine during growth on pyruvate. We suggest the defect in IlvE caused by lack of YjgF (36), in combination with the effect of pyruvate on biosynthetic flux, generates an isoleucine requirement for growth.

The conclusion that constitutive threonine deaminase activity compromises growth of a *yjgF* mutant was supported by this study. The *yjgF ilvA219* double mutant strain was compromised under all growth conditions. A similar growth defect was found when the constitutively active catabolic threonine deaminase (TdcB) (39) was present in a *yjgF* mutant in *trans* (unpublished data). The data suggest that simple derepression of the *ilvGMEDA* operon is not tolerated in a *yjgF* mutant. It is significant that two suppressor mutations that increased transcription of the operon were defective in IlvA activity rather than transcriptional regulation. Taken together, these results

are consistent with the hypothesis that IlvA generates a toxic side product. Recent biochemical data indicate an IlvA product, distinct from AKB, accumulates in a *yjgF* mutant strain (M. Christopherson, unpublished results). We suggest this product of IlvA is bound and/or sequestered by YjgF (36).

From this study, we conclude that the isoleucine requirement of a *yjgF* mutant strain grown on pyruvate medium is a direct consequence of the decreased IlvE activity in a *yjgF* mutant that we have previously characterized (36) (Fig. 5). As such, this requirement is distinct from the need for isoleucine on glucose-serine, which is not a direct result of lowered IlvE activity, and has yet to be characterized. The clear distinction of the isoleucine requirement will facilitate the design of future experiments to probe the specific biochemical function(s) of YjgF.

ACKNOWLEDGMENTS

We thank undergraduate students Deanna Downs for mapping the *ilvA* suppressor mutations and Ben Bice for the exhaustive screen for a class of mutants that could exclusively suppress the isoleucine requirement of a *yjgF* mutant strain growing on serine medium.

This work was supported by competitive grant GM47296 from the NIH and funds from a 21st Century Scientists Scholars Award from the J. M. McDonnell fund to D.M.D. and a National Science Foundation Graduate Research Fellowship to M.R.C. G. Schmitz was supported as a trainee on the Molecular Biosciences Training Grant from the NIH (GM07215) and an S. C. Johnson Distinguished Fellowship. Deanna Downs was the recipient of undergraduate research fellowships from the Department of Bacteriology, the Hilldale Competition, and Merck and Co.

REFERENCES

1. **Angeles, T. S., P. A. Smanik, C. L. Borders, Jr., and R. E. Viola.** 1989. Aspartokinase-homoserine dehydrogenase I from *Escherichia coli*: pH and chemical modification studies of the kinase activity. Biochemistry **28:**8771–8777.
2. **Balch, W. E., and R. S. Wolfe.** 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. **32:**781–791.
3. **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.
4. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
5. **Browne, B. A., A. I. Ramos, and D. M. Downs.** 2006. PurF-independent phosphoribosyl amine formation in *yjgF* mutants of *Salmonella enterica* utilizes the tryptophan biosynthetic enzyme complex anthranilate synthase-phosphoribosyltransferase. J. Bacteriol. **188:**6786–6792.
6. **Burman, J. D., C. E. Stevenson, R. G. Sawers, and D. M. Lawson.** 2007. The crystal structure of Escherichia coli TdcF, a member of the highly conserved YjgF/YER057c/UK114 family. BMC Struct. Biol **7:**30.
7. **Burns, R. O.** 1968. L-threonine deaminase-biosynthetic (*Salmonella typhimurium*). Methods Enzymol. **17B:**555–560.
8. **Caetano-Annoles, G.** 1993. Amplifying DNA with arbitrary oligonucleotide primers. PCR Methods Appl. **3:**85–92.
9. **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.
10. **Colombo, I., F. Ceciliani, S. Ronchi, A. Bartorelli, and B. Berra.** 1998. cDNA cloning and *Escherichia coli* expression of UK114 tumor antigen. Biochim. Biophys. Acta **1442:**49–59.
11. **Davis, R. W., D. Botstein, J. R. Roth, and Cold Spring Harbor Laboratory.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
12. **Deriu, D., C. Briand, E. Mistiniene, V. Naktinis, and M. G. Grutter.** 2003. Structure and oligomeric state of the mammalian tumour-associated antigen UK114. Acta Crystallogr. Sect. D **59:**1676–1678.
13. **Downs, D. M., and L. Petersen.** 1994. *apbA*, a new genetic locus involved in thiamine biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **176:**4858–4864.
14. **Downs, D. M., G. E. Schmitz, and E. Skovran.** 2005. Probing the complex system of metabolic integration. Prog. Nucleic Acid Res. Mol. Biol. **80:**43–94.
15. **Enos-Berlage, J. L., and D. M. Downs.** 1999. 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. J. Bacteriol. **181:**841–848.
16. **Enos-Berlage, J. L., M. J. Langendorf, and D. M. Downs.** 1998. Complex metabolic phenotypes caused by a mutation in *yjgF*, encoding a member of the highly conserved YER057c/YjgF family of proteins. J. Bacteriol. **180:**6519–6528.
17. **Farkas, A., G. Nardai, P. Csermely, P. Tompa, and P. Friedrich.** 2004. DUK114, the Drosophila orthologue of bovine brain calpain activator protein, is a molecular chaperone. Biochem. J. **383:**165–170.
18. **Gardner, J. F.** 1979. Regulation of the threonine operon: tandem threonine and isoleucine codons in the control region and translational control of transcription termination. Proc. Natl. Acad. Sci. USA **76:**1706–1710.
19. **Goupil-Feuillerat, N., M. Cocaign-Bousquet, J.-J. Godon, S. D. Ehrlich, and P. Renault.** 1997. Dual role of α-acetolactate decarboxylase in *Lactococcus lactis* subsp. *lactis*. J. Bacteriol. **179:**6285–6293.
20. **Kim, J. M., H. Yoshikawa, and K. Shirahige.** 2001. A member of the YER057c/YjgF/UK114 family links isoleucine biosynthesis and intact mitochondria maintenance in *Saccharomyces cerevisiae*. Genes Cells **6:**507–517.
21. **Kleckner, N., R. M. Chalmers, D. Kwon, J. Sakai, and S. Bolland.** 1996. Tn10 and IS10 transposition and chromosome rearrangements: mechanism and regulation *in vivo* and *in vitro*. Curr. Top. Microbiol. Immunol. **204:**49–82.
22. **LaRossa, R. A., and J. V. Schloss.** 1984. The sulfonylurea herbicide sulfometuron methyl is an extremely potent and selective inhibitor of acetolactate synthase in *Salmonella typhimurium*. J. Biol. Chem. **259:**8753–8757.
23. **LaRossa, R. A., and T. K. Van Dyk.** 1987. Metabolic mayhem caused by 2-ketoacid imbalances. Bioessays **7:**125–130.
24. **LaRossa, R. A., 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.
25. **Manjasetty, B. A., H. Delbruck, D. Pham, U. Mueller, M. Fieber-Erdmann, C. Scheich, V. Sievert, K. Bussow, F. H. Neisen, W. Weihofen, B. Loll, W. Saenger, and U. Heinemann.** 2004. Crystal structure of *Homo sapiens* protein hp14.5. Proteins Struct. Funct. Bioinformat. **54:**797–800.
26. **McClelland, M., K. E. Sanderson, J. Spieth, S. W. Clifton, P. Latreille, L. Courtney, S. Porwollik, J. Ali, M. Dante, F. Du, S. Hou, D. Layman, S. Leonard, C. Nguyen, K. Scott, A. Holmes, N. Grewal, E. Mulvaney, E. Ryan, H. Sun, L. Florea, W. Miller, T. Stoneking, M. Nhan, R. Waterston, and R. K. Wilson.** 2001. Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. Nature **413:**852–856.
27. **Melloni, E., M. Michetti, F. Salamino, and S. Pontremoli.** 1998. Molecular and functional properties of a calpain activator protein specific for muisoforms. J. Biol. Chem. **273:**12827–12831.
28. **Miyakawa, T., W. C. Lee, K. Hatano, Y. Kato, Y. Sawano, K. Miyazono, K. Nagata, and M. Tanokura.** 2006. Crystal structure of the YjgF/YER057c/UK114 family protein from the hyperthermophilic archaeon *Sulfolobus tokodaii* strain 7. Proteins **62:**557–561.
29. **Morishita, R., A. Kawagoshi, T. Sawasaki, K. Madin, T. Ogasawara, T. Oka, and Y. Endo.** 1999. Ribonuclease activity of rat liver perchloric acid-soluble protein, a potent inhibitor of protein synthesis. J. Biol. Chem. **274:**20688–20692.
30. **O'Neill, J. P., and M. Freundlich.** 1972. Effect of cyclopentaneglycine on metabolism in *Salmonella typhimurium*. J. Bacteriol. **111:**510–515.
31. **O'Neill, J. P., and M. Freundlich.** 1972. Two forms of biosynthetic acetohydroxy acid synthetase in *Salmonella typhimurium*. Biochem. Biophys. Res. Commun. **48:**437–443.
32. **Parsons, L., N. Bonander, E. Eisenstein, M. Gilson, V. Kairys, and J. Orban.** 2003. Solution structure and functional ligand screening of HI0719, a highly conserved protein from bacteria to humans in the YjgF/YER057c/UK114 family. Biochemistry **42:**80–89.
33. **Roberts, G. P.** 1978. Isolation and characterization of informational suppressors in *Salmonella typhimurium*. Ph.D. thesis. University of California, Berkeley.
34. **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.
35. **Schmieger, H.** 1972. Phage P22-mutants with increased or decreased transduction abilities. Mol. Gen. Genet. **119:**75–88.
36. **Schmitz, G., and D. M. Downs.** 2004. Reduced transaminase B (IlvE) activity caused by the lack of *yjgF* is dependent on the status of threonine deaminase (IlvA) in *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **186:**803–810.
37. **Schmitz, G. E., and D. M. Downs.** 2006. An allele of *gyrA* prevents *Salmo-*

nella enterica serovar Typhimurium from growing with succinate as a carbon source. J. Bacteriol. **188:**3126–3129.
38. **Sinha, S., P. Rappu, S. C. Lange, P. Mantsala, H. Zalkin, and J. L. Smith.** 1999. Crystal structure of *Bacillus subtilis* YabJ, a purine regulatory protein and member of the highly conserved YjgF family. Proc. Natl. Acad. Sci. USA **96:**13074–13079.
39. **Taillon, B. E., R. Little, and R. P. Lawther.** 1988. Analysis of the functional domains of biosynthetic threonine deaminase by comparison of the amino acid sequences of three wild-type alleles to the amino acid sequence of biodegradative threonine deaminase. Gene **63:**245–252.
40. **Taylor, R. T., and W. T. Jenkins.** 1966. Leucine aminotransferase. I. Colorimetric assays. J. Biol. Chem. **241:**4391–4395.
41. **Umbarger, H. E.** 1956. Evidence for a negative-feedback mechanism in the biosynthesis of isoleucine. Science **123:**848.
42. **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.
43. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218:**97–106.
44. **Volz, K.** 1999. A test case for structure-based functional assignment: the 1.2 A crystal structure of the *yjgF* gene product from *Escherichia coli*. Protein Sci. **8:**2428–2437.
45. **Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner.** 1984. New Tn10 derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. Gene **32:**369–379.
46. **Winston, F., D. Botstein, and J. H. Miller.** 1979. Characterization of amber and ochre suppressors in *Salmonella typhimurium*. J. Bacteriol. **137:**433–439.