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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

George Schmitz and Diana M. Downs*

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

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The YjgF/YER057c/UK114 family is a highly conserved class of proteins that is represented in the three domains of life. Thus far, a biochemical function demonstrated for these proteins in vivo or in vitro has yet to be defined. In several organisms, strains lacking a YjgF homolog have a defect in branched-chain amino acid biosynthesis. This study probes the connection between *yjgF* and isoleucine biosynthesis in *Salmonella enterica*. In strains lacking *yjgF* the specific activity of transaminase B, catalyzing the last step in the synthesis of isoleucine, was reduced. In the absence of *yjgF*, transaminase B activity could be restored by inhibiting threonine deaminase, the first enzymatic step in isoleucine biosynthesis. Strains lacking *yjgF* showed an increased sensitivity to sulfometruron methyl, a potent inhibitor of acetolactate synthase. Based on work described here and structural reports in the literature, we suggest a working model in which YjgF has a role in protecting the cell from toxic effects of imbalanced ketoacid pools.

The YjgF/YER057c/UK114 family is a highly conserved class of proteins that is represented in the three domains of life. Members of this protein family share extensive similarities and have been implicated in diverse cellular processes in a number of organisms (17, 24, 28, 39, 40, 46, 47, 50, 67). Though no in vitro activity has been described, in the annotation of various genomes more than 10 different functions have been attributed to members of this family (http://e2f.umbi.umd.edu).

While the phenotypes described for the lack of *yjgF* are diverse, a recurring finding is a defect in isoleucine synthesis (17, 24, 28). Our laboratory identified the *yjgF* locus for its role in the synthesis of phosphoribosylamine in *Salmonella* but suggested that *yjgF* mutants were impaired in a step in isoleucine biosynthesis (17). Subsequently, a report showed that *Saccharomyces cerevisiae* strains lacking the mitochondrial-targeted homolog, YIL051c, displayed less than 3% of wild-type levels of isoleucine-specific transaminase activity (in crude extracts), the last catalytic step of the isoleucine pathway (28).

Structures have been determined for five members of the YjgF/YER057c/UK114 family. In each case, the proteins form homotrimers whose quaternary structures resemble that observed in *Bacillus subtilis* chorismate mutase. Despite this resemblance, none of the YjgF proteins exhibits primary sequence similarity to chorismate mutase, nor are they predicted to perform a similar function (12, 13, 41, 53, 63). Each structure has revealed that a cavity forms at the interface of the three subunits, and this interface is lined with several conserved residues of the signature sequence, [PA]-[ASTPV]-R-[SACVF]-x-[LIVMFY]-x (2)-[GSAKR]-x-[LMVA]-x (5, 8)-[LIVM]-E-[MI], that defines the family. The solution structure of the *Haemophilus influenzae* homolog HI0719 was determined using nuclear magnetic resonance spectroscopy (41). These studies found that 2-ketobutyrate (AKB), the first intermediate in isoleucine biosynthesis, and analogs of its cognate enamine bound within the cavity of HI0719 (41).

In the biosynthesis of isoleucine, AKB is generated as the product of threonine deamination by IlvA. In addition to its role as a dedicated metabolite in the formation of isoleucine, AKB is toxic to *Salmonella* when accumulated to high levels (29, 34). Studies by LaRossa and colleagues have described various metabolic consequences of imbalanced ketoacid pools in *S. enterica*. Much of the work by LaRossa's group involved manipulating the levels of AKB by removing allosteric inhibition of IlvA and/or blocking the subsequent reaction, prior to monitoring cellular phenotypes that correlated with the level of AKB (29, 32–34, 60). The appearance of AKB in both the physical and metabolic studies suggested that the ability of YjgF to bind AKB and similar compounds could be relevant to its role in vivo.

The work presented here was initiated to define how a lack of *yjgF* affects isoleucine biosynthesis in *Salmonella enterica* and to dissect the global role of YjgF in metabolism. Similar to yeast, the absence of a functional *yjgF* in *S. enterica* reduced the cellular activity of transaminase B (IlvE). The previous result was extended with the demonstration that the specific activity of IlvE was reduced, suggesting a posttranslational effect. Data from these studies have suggested that in the absence of YjgF a product of the IlvA reaction modulates the activity of IlvE. A working model for the in vivo role of YjgF is presented and discussed in the context of the results described herein, the structural data in the literature, and the metabolic toxicity of ketoacids.

MATERIALS AND METHODS

Bacterial strains, media, and chemicals. All 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 (8). Tn*10d*(Tc) refers to the transposition-defective mini-Tn*10*(Tn*10*Δ16Δ17) (66).

* 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.

TABLE 1. Bacterial strains

Strain	Genotype
DM1	Wild type
DM3480	*yjgF3*::MudJ[a]
DM4053	*yjgF1*::Tn*10d*(Tc)[b]
DM4210	*apbA7*::Tn*10d*(Tc) *ilvD2654*::MudJ
DM4751	*ilvA219*
DM4955	*pyrB2694*::MudA
DM6012	*zxx9118*::Tn*10d*(Tc) *ilvE3200*
DM6284	*yjgF3*::MudJ
DM6285	Wild type
DM6735	*yjgF3*::MudJ pSU19
DM6736	*yjgF3*::MudJ pIlvE-G1
DM6738	Wild-type pSU19
DM6739	Wild-type pIlvE-G1
DM6757	*purF2085 gnd181*
DM6769	*gnd181*
DM6868	*gnd181 yjgF7*
DM6869	*gnd181*
DM6946	*ilvA219 yjgF3*::MudJ
DM6947	*ilvA219*
DM6968	*gnd181 yjgF7* pIlvE-H1
DM6969	*gnd181* pIlvE-H1
DM6970	*gnd181 yjgF7* pIlvE-H1 pGP1-2
DM6971	*gnd181* pIlvE-H1 pGP1-2
DM7008	*ilvD2654*::MudJ *yjgF7*
DM7009	*ilvD2654*::MudJ
DM7460	*yjgF3*::MudJ pSU19-YjgFa
DM7461	Wild-type pSU19-YjgFa
BL21/λDE3	F^- *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm* (*E. coli*)
TT460	*pyrB692*::Tn*10*

[a] MudJ refers to the Mud1734 transposon (8).
[b] Tn*10*d(Tc) refers to the transposition-defective mini-Tn*10*(Tn*10*Δ*16*Δ*17*) (66).

Unless otherwise indicated, no-carbon E medium (NCE) supplemented with 1 mM $MgSO_4$ (11, 62) was used as minimal medium. Glucose, gluconate, and ribose were used as carbon sources at concentrations of 16, 11, and 16 mM, respectively. Difco nutrient broth (NB; 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, 100 nM; serine, 5 mM; 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; ampicillin, 30 and 15 μg/ml. Unless otherwise stated, chemicals were purchased from Sigma Chemical Co., St. Louis, Mo. Sulfometuron methyl (SMM) was a gift from DuPont Co.

Disk diffusion assays. SMM sensitivity was determined according to the methods of LaRossa and Van Dyk (33) with modifications. Briefly, 1 ml of an overnight NB culture was centrifuged and resuspended in 1 ml of saline solution (0.85% NaCl). Three milliliters of molten (50°C) 0.7% agar was mixed with 0.1 ml (10^8 CFU) of the saline suspension, and the mixture was evenly poured over an NCE plate of minimal medium agar supplemented with glucose (soft agar overlay). Forty micrograms of SMM dissolved in acetone (2 mg/ml) was applied to a 5-mm Whatman no. 3 disk, and the disk was placed on the soft agar overlay. The plate was incubated for 12 h at 37°C, and the zone of inhibition was measured. AKB sensitivity was similarly determined, except that 5 μl of an aqueous solution of 0.67% AKB (330 nmol) was absorbed to a disk.

Genetic techniques. (i) Transduction methods. Transductional crosses were performed using the high-frequency general transducing mutant of bacteriophage P22 (HT105/1; *int*-201) (48, 51). Methods for transductional crosses, purification of transductants from phage, and identification of phage-free transductants have been described elsewhere (14, 42).

(ii) Strain construction. Multiply mutant strains were constructed using standard genetic techniques. When necessary, genetic backcrosses were performed to confirm the presence of a respective allele.

Molecular biology. Construction of plasmid pSU19-YjgFa has been described previously (17). The *ilvE* gene was amplified from *Escherichia coli* K-12 with ORFmer PCR primer pairs purchased from Sigma-Genosys and used according to the company's protocol. The amplification product containing *ilvE* was blunt-end ligated into the *Sma*I site of pSU19 (36), creating pIlvE1-G1. The insert in pIlvE1-G1 was confirmed by complementation and sequencing. The expression of the *ilvE* gene in this construct is under the control of a *lac* promoter.

For protein purification, the *ilvE* gene was amplified from LT2 using primers BluntilvE (5′ ATGACGACGAAAAAAGCTGATTATATTTGG 3′) and XhoIilvECtag (5′ ATTTTATCTCGAGTGAATTTACCGGATC 3′). The XhoIilvECtag primer was designed with a *Xho*I restriction site to facilitate cloning. Amplification was performed by PCR using cloned *Pfu* DNA polymerase. 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 min. The resulting 1-kb fragment was digested with *Xho*I and ligated into the pET20b vector (Novagen, Madison, Wis.) that had been digested with *Eco*RV and *Xho*I. The ligation mix was electroporated into DM6012, and Ap^r transformants that grew on glucose medium lacking isoleucine were obtained. Plasmid DNA was isolated from one transformant and designated pIlvE-H1.

Protein purification. The plasmid pIlvE-H1described above was electroporated into strains DM6868 and DM6869 and Ap^r electroporants were selected, generating DM6968 and DM6969. Subsequently, plasmid pGP1-2, containing a T7 polymerase gene under control of a temperature-inducible promoter (55), was electroporated into strains DM6968 and DM6969, and Ap^r Kn^r transformants were selected at 30°C to generate DM6970 and DM6971. Strains DM6970 and DM6971 were inoculated into 5 ml of NB containing kanamycin and ampicillin at 25 and 15 μg/ml, respectively. The two cultures were allowed to reach full density at 30°C before being inoculated into 1 liter of minimal glucose medium containing kanamycin and ampicillin at 75 and 7.5 μg/ml, respectively. The 1-liter cultures were incubated at 30°C to an absorbance of 0.3 at 650 nm, and expression was induced by a shift to 42°C for 30 min and then incubation at 37°C for 2 h. Cell pellets were resuspended in 40 ml of binding buffer. Cell extracts were generated with a French pressure cell, clarified by centrifugation, and filtered through a 0.4-μm-pore-size filter. The filtered lysates were bound to a Ni^{2+} column and purified using the manufacturer's protocol (Novagen). The purified protein suspensions were concentrated and dialyzed into 100 mM potassium phosphate buffer, pH 8.0. Purified IlvE-His6 was frozen in samples at −80°C.

Western analysis. Western blot analysis was performed according to the methods of Harlow and Lane (25). Anti-IlvE polyclonal rabbit antibodies against purified IlvE-His6 were generated at the University of Wisconsin Animal Care Unit. Anti-IlvE antibodies were precleared against a lysate of strain DM6012, which lacks *ilvE.* Assignment of the *ilvE* band was confirmed by its absence in an *ilvE* null mutant.

Enzyme assays. (i) Threonine deaminase assay. Two milliliters of overnight NB cell cultures was inoculated into 200 ml of minimal glucose medium and incubated at 37°C. The cultures were harvested at 85 Klett units (red filter, ~6 × 10^8 CFU/ml), washed in 20 ml of NCE medium, and resuspended in 4 ml of buffer (50 mM potassium phosphate [pH 7.2], 0.4 mM dithiothreitol). Cell extracts were prepared with a French pressure cell and clarified by centrifugation. Threonine deaminase assays were performed as described previously (29). After derivatization with 2,4-dinitrophenylhydrazine, AKB was quantified as previously described (6). Protein concentrations were estimated by the method of Bradford (5).

(ii) Transaminase B assay. Fifty microliters of overnight NB cell cultures was inoculated into 5 ml of minimal E medium containing a carbon source and, when stated, thiamine. The cultures were incubated at 37°C with shaking until they reached full density (optical density at 650 nm, ~1). The cells were harvested by centrifugation and washed with 1 ml of NCE medium. Cell pellets were frozen at −20°C until use. Cell pellets were resuspended in 0.5 ml of 10 mM potassium phosphate, pH 8.0. To determine the protein concentration for assays, aliquots of the cell suspensions were permeabilized with 10% POPCULTURE (Novagen) and the protein concentration was estimated using a bicinchoninic assay reagent kit (Pierce, Rockford, Ill.).

The transaminase B activity assay was an adaptation of previously described protocols (28, 69). An aliquot of the whole-cell suspension (~35 μg of protein) was added to the reaction mixture and allowed to equilibrate at 37°C for 10 min. The reaction mixture contained 100 mM potassium phosphate (pH 8), 50 μM pyridoxal-5′-phosphate, 10 mM α-ketoglutarate, and 10% POPCULTURE (to permeabilize cells) in a total volume of 200 μl. L-Isoleucine was added to a final concentration of 20 mM to start the reaction. The reaction was allowed to proceed for 20 min at 37°C and stopped with 200 μl of 0.3% 2,4-dinitrophenylhydrazine. Hydrazone formation was allowed to proceed for 5 min at room temperature, prior to extraction with 1 ml of toluene and shaking for 2 min. The two phases were separated by centrifugation, and the aqueous (bottom) layer was removed by micropipette. The toluene layer was washed by adding 0.5 ml of 0.5 N HCl, shaking for 1 min, and separating the phases by centrifugation. A 0.8-ml aliquot of the toluene (top) layer was removed and mixed with 1 ml of 1.5 N

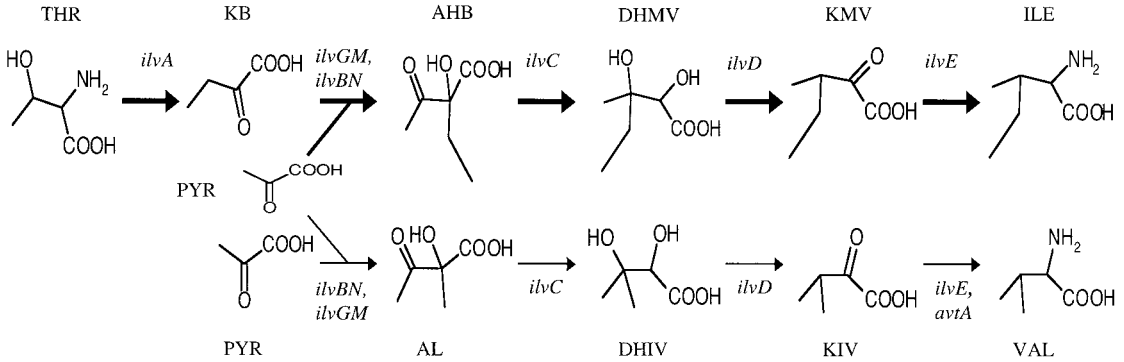

FIG. 1. Isoleucine and valine biosynthetic pathways. The biosynthetic pathways for isoleucine and valine are schematically represented. Genes whose products catalyze the reactions are listed above their respective arrows. Although IlvGM and IlvBN are acetolactate synthase isozymes, IlvBN contributes primarily to Val biosynthesis, and IlvGM contributes to both pathways in cultures grown in glucose (1). Abbreviations: THR, L-threonine; KB, AKB; AHB, 2-aceto-2-hydroxybutyrate; DHMV, 2,3-dihyroxy-3-methylvalerate; KMV, 2-keto-3-methylvalerate; ILE, L-isoleucine; PYR, pyruvate; AL, 2-acetolactate; DHIV, 2,3-dihydroxyisovalerate; KIV, 2-ketoisovalerate; VAL, L-valine.

NaOH, to allow chromophore formation. The A_{540} of the aqueous layer (containing the chromophore) was determined. Known concentrations of DL-α-keto-β-methylvalerate were subjected to the same extraction procedure to generate a standard curve that was used to determine the concentration of product generated. As controls, wild-type cells were assayed without the addition of either L-isoleucine or α-ketoglutarate to the assay and, in both instances, product formation from substrates in the extracts was below detectable levels. Activity levels from permeabilized cells of an *ilvE* mutant, DM6012, were also undetectable by this assay. Transaminase B specific activities are reported in nanomoles per minute per milligram for crude extract and in micromoles per minute per milligram for purified protein preparations.

(iii) β-Galactosidase assays. β-Galactosidase assays were performed according to the method of Miller (70).

RESULTS

Mutants lacking YjgF have reduced transaminase B (IlvE) activity. The two enzymatic steps uniquely required for isoleucine biosynthesis, threonine deaminase (IlvA) and transaminase B (IlvE) (Fig. 1), were assayed in cell extracts and permeabilized cells from wild-type and *yjgF* mutant strains. When cultures were grown in minimal glucose medium, the threonine deaminase activities in wild-type (DM6285) and *yjgF* mutant (DM6284) strains were not significantly different (340 ± 26 and 342 ± 26 nmol/min/mg, respectively [mean ± standard deviation]). In contrast, transaminase B activity differed significantly in the two strains, with the *yjgF* mutant containing ~30% of the activity found in the parental strain (27 ± 5 and 79 ± 4 nmol/min/mg, respectively).

Lack of YjgF exerts a posttranslational effect on transaminase B. The major *ilv* operon (*ilvGMEDA*) in *Salmonella* contains both *ilvA* and *ilvE*. It was unlikely that altered transcription was responsible for reducing IlvE activity in the *yjgF* mutant while leaving IlvA unaffected. Strains containing a transcriptional fusion in the *ilv* operon were constructed and monitored (Table 2). Strains were grown in the presence of valine and leucine (to allow growth), with isoleucine added at three different concentrations to alter expression of the *ilv* operon. These data allowed two conclusions: (i) transcription of the *ilvD2654*::MudJ fusion was regulated as expected, and (ii) the *yjgF* mutation did not cause a decrease in transcription of the *ilv* operon. If anything, lack of *yjgF* appeared to slightly increase transcription of the operon.

The reduced activity of transaminase B in *yjgF* mutant strains could reflect reduced accumulation or reduced specific activity of the IlvE protein. Isogenic strains DM6285 (wild-type) and DM6284 (*yjgF*) were grown in minimal medium to an optical density at 650 nm of ~1. The permeabilized cells were assayed for transaminase B activity prior to being subjected to immunoblot analysis with polyclonal antibodies generated against IlvE. While the IlvE activity was altered (~70% reduction with the *yjgF* mutant), the level of IlvE protein accumulating in the two strains was not detectably different (data not shown).

The specific activity of purified IlvE is dependent on strain background. The results above suggested that the specific activity of the IlvE protein was reduced in a *yjgF* mutant strain. IlvE was overexpressed and purified from the *yjgF* mutant and wild-type strains. After purification, the two protein preparations were assayed for transaminase B activity. As shown in Fig. 2A, the IlvE protein purified from a *yjgF* mutant strain (DM6970) had a specific activity of ~7 μmol/min/mg, while that purified from the wild-type strain had a specific activity of ~14 μmol/min/mg. To confirm the samples were of equivalent purity, each was run on a sodium dodecyl sulfate gel and stained by Coomassie. Such a gel is shown in Fig. 2B and supports the conclusion that the specific activity of the IlvE proteins was affected by the *yjgF* status in the strain from which it was purified. No mobility differences were noted between the two samples. Purification of the IlvE protein (from either genetic background) in the presence of pyridoxal-5′-phosphate (100 μM) did not significantly change the specific activity.

TABLE 2. Expression of the *ilv* operon in a *yjgF* mutant strain

Strain	Relevant genotype	β-Galactosidase activity[a]		
		Low Ile	High Ile	High BCAA
DM7008	*ilvD yjgF*	276 ± 5	150 ± 1	85 ± 5
DM7009	*ilvD*	238 ± 7	110 ± 5	65 ± 4

[a] Data in low and high Ile columns were from cultures grown in 0.3 mM Val and Leu with 0.06 mM and 0.3 mM Ile, respectively. Data in the high BCAA column were from cultures grown in medium with 1 mM (each) Ile, Val, and Leu. BCAA, branch chain amino acids. Values shown are an means ± standard deviations of results from at least two independent cultures. β-Galactosidase activity is reported in Miller units (70). The reporter fusion is in *ilvD*.

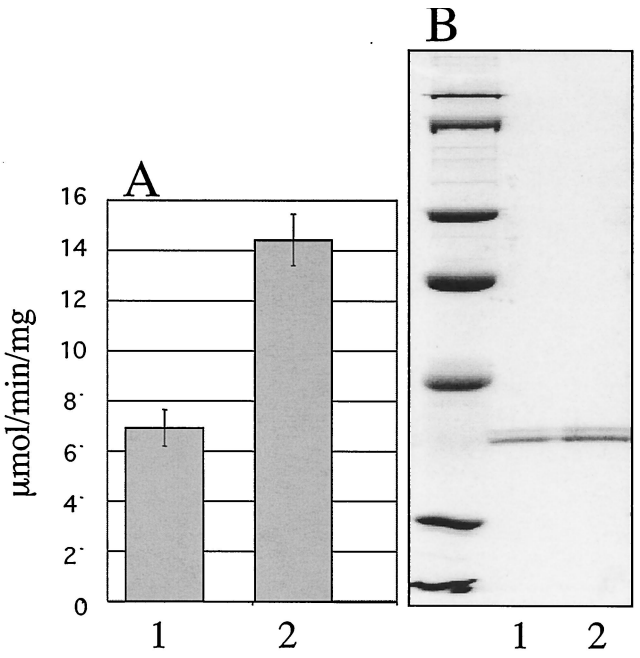

FIG. 2. Distinct specific activity is inherent in purified transaminase B. Transaminase B protein was expressed and purified from *yjgF* mutant (DM6970) and wild-type (DM6971) strain backgrounds. (A) The proteins purified from DM6970 (1) and the wild-type strain (2) were assayed, and the specific activity is shown. (B) Aliquots containing 200 ng of the protein preparations were run in a 12.5% acrylamide gel to assess purity and potential mobility differences. A protein ladder standard was loaded in lane C. The minor band in the doublet is the uncleaved fusion protein that is the initial product of the construct.

In-gel trypsin digests of the purified protein samples were subjected to matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS), and the undigested proteins were subjected to electrospray ionization (ESI) MS analysis at the facility at the University of Wisconsin Biotechnology Center. The initial experiments with these techniques failed to detect a difference in mass between the two protein samples. If a population of the IlvE protein were modified, it is possible that such a modification would not be stable through the MALDI-TOF MS or ESI MS procedures.

Isoleucine restored IlvE activity in *yjgF* mutant strains. The reduced activity of IlvE provided not only a biochemical phenotype for the *yjgF* mutant, but also the predicted defect to explain the serine-sensitive phenotype of a *yjgF* mutant (17). In this scenario, elevating levels of IlvE activity in a *yjgF* mutant should reverse the serine sensitivity and possibly other phenotypes associated with the *yjgF* mutation. To address this possibility, pIlvE-G1 and control plasmid pSU19 were transformed into wild-type and *yjgF* mutant strains to generate strains DM6735, -6736, -6738, and -6739. The IlvE activities of these strains are shown in Fig. 3. It was not possible to test the original premise, since IlvE activity in a *yjgF* mutant failed to reach a wild-type level. However, from the results in Fig. 3, two conclusions were made: (i) the presence of pIlvE increased the level of IlvE activity in both strains by a similar proportion, and (ii) the addition of isoleucine to the growth medium elevated transaminase B activity in the *yjgF* mutant containing the plasmid to the level seen in the equivalent wild-type strain. The latter result indicated that isoleucine not only satisfied the nutritional requirement generated by the *yjgF* mutation (e.g., serine sensitivity) but also eliminated the biochemical defect in IlvE.

Is metabolic flux required for the reduction of IlvE activity in *yjgF* mutants? The results in Fig. 3 suggested a role for isoleucine other than simply satisfying a nutritional requirement. To probe this possibility, strains lacking plasmids were used. Data presented in Table 3 (strain DM6284 data) confirmed that growth in exogenous isoleucine restored IlvE activity in *yjgF* mutant cells to >80% of that found in the wild-type strain. Since it was present in the in vitro assay, isoleucine could not be directly affecting IlvE activity, and an indirect effect was considered. In vivo, isoleucine can reduce metabolic flux through the Ile biosynthetic pathway by allosterically inhibiting IlvA (threonine deaminase) (58). An allele of *ilvA* generating an isoleucine-insensitive protein (*ilvA219*) (29) was used to determine if allosteric inhibition of IlvA was required to restore IlvE activity. Two strains carrying the *ilvA219* allele and that were isogenic at the *yjgF* locus were constructed. The results of analyses involving these strains are shown in Table 3, strains DM6947 and DM6946. When the *ilvA219* mutation was present in the *yjgF* mutant background, isoleucine failed to increase the activity of IlvE.

One interpretation of the above result was that reduced flux through the Ile pathway (caused by allosteric inhibition of IlvA) restored IlvE activity in a *yjgF* mutant. Such an interpretation would be consistent with *yjgF* mutants being sensitive to one or more metabolites of the Ile pathway, with one manifestation of the sensitivity being a decrease in IlvE activity. Two predictions of this hypothesis were tested independently: (i)

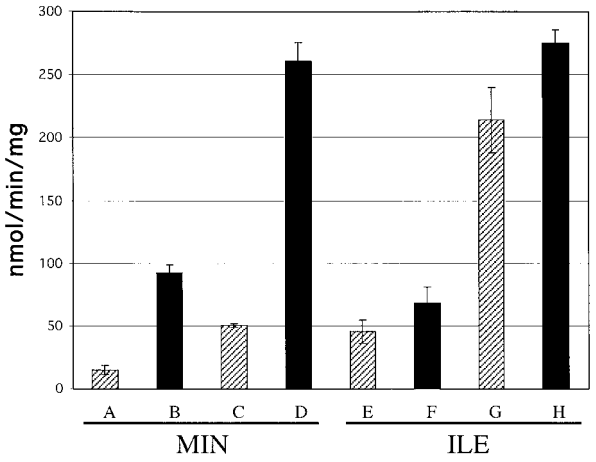

FIG. 3. Overexpression of *ilvE* does not restore transaminase activity in *yjgF* mutants. IlvE specific activity was determined in permeabilized cell extracts of wild-type and *yjgF* mutant strains carrying either empty vector pSU19 or pIlvE-G1. Strains were grown in minimal glucose medium containing 2.5 μg of chloramphenicol/ml and harvested, and transaminase B activity was assayed. Bars A to D represent strains grown in minimal medium. Bars E to H represent strains grown in minimal medium containing Ile. The strains, genotypes, and bar designations are as follows: A and E, DM6735 (*yjgF*/pSU19); B and F, DM6738 (wt/pSU19); C and G, DM6736 (*yjgF*/pIlvE-G1); and D and H, DM6739 (wt/pIlvE-G1). Data from the strains carrying a *yjgF* mutation are marked with hatched bars.

TABLE 3. Feedback inhibition of IlvA activity correlates with IlvE activity in a *yjgF* mutant[a]

Strain	Relevant genotype	IlvE sp act (nmol/min/mg)					
		Ile pathway			Val pathway		
		MIN	AKB	Ile	PYR	KIV	Val
DM6285	Wild type	93 ± 10	81 ± 12	79 ± 3	105 ± 16	83 ± 11	89 ± 6
DM6284	*yjgF*	27 ± 5	79 ± 4	80 ± 8	39 ± 3	29 ± 1	30 ± 9
DM6947	*ilvA219*	117 ± 3	101 ± 7	105 ± 8	111 ± 5	122 ± 12	110 ± 16
DM6946	*ilvA219 yjgF*	25 ± 1	20 ± 0.7	44 ± 11	26 ± 7	38 ± 5	30 ± 0.2
DM4748	*ilvA*	NG[b]	117 ± 9	113 ± 6	NG	NG	NG
DM6143	*ilvA yjgF*	NG	99 ± 15	113 ± 19	NG	NG	NG

[a] Cells were grown in minimal glucose medium containing thiamine (MIN) or were supplemented with 0.3 mM AKB, 2-keto-3-methyl-L-valerate (KMV), isoleucine, pyruvate (PYR), 2-ketoisovalerate (KIV), or valine. Cells were harvested and assayed as described in Materials and Methods. Values shown are means ± standard deviations of three independent cultures.

[b] NG, there was no growth of this strain in the absence of Ile or Ile precursors.

that growth of a *yjgF* mutant would be inhibited by a metabolite of the Ile pathway, and (ii) that exogenous addition of metabolic intermediates would not restore IlvE activity in a *yjgF* mutant.

Strains lacking *yjgF* are sensitive to SMM, but not AKB. The extensive literature detailing the toxicity of accumulating AKB (18, 19, 29, 31–33, 44, 52, 59, 60) and the structural report indicating the binding of this Ile precursor to YjgF in solution (41) suggested that AKB could be the metabolite predicted in item i above. Table 4 shows the sensitivity of relevant strains to addition of AKB and SMM, as measured by disk diffusion assays. SMM is a potent inhibitor of acetolactate synthase isozyme II (*ilvGM*) and thus causes the accumulation of AKB, the product of the preceding reaction (Fig. 1) (30). In comparing the wild-type and mutant strains, the *yjgF* mutant was significantly more sensitive to SMM, consistent with an increased sensitivity to the accumulated AKB. However, the mutant strain was no more sensitive to exogenously added AKB than the wild-type strain. This result was consistent with labeling studies that found no difference between wild-type and *yjgF* mutant strains in accumulation or degradation of AKB (J. L. Enos-Berlage and D. M. Downs, unpublished data). The presence of *yjgF* in multiple copies restored wild-type sensitivity to SMM in the *yjgF* mutant, but it did not increase the level of resistance of the wild-type strain or affect the response of either strain to AKB (data not shown). The expectation is that these two treatments result in the same metabolic imbalance (i.e., accumulation of AKB), making it difficult to interpret these results simply. A class of mutants with a similar pattern of sensitivity has been previously reported (29), but a conclusive explanation for their behavior was not offered. This pattern of sensitivity, in addition to the solution-structure binding studies, was consistent with a model (Fig. 4) in which YjgF bound (and neutralized) a toxic product of IlvA.

IlvA activity, not metabolic flux, correlates with reduced IlvE activity in *yjgF* mutants. Prediction (ii) above anticipates that exogenous addition of metabolic intermediates would not restore IlvE activity in a *yjgF* mutant. Three pairs of strains differing in the allele of *ilvA* present and isogenic at the *yjgF* locus were constructed to probe this prediction. Each strain was grown in minimal medium with the indicated metabolites. IlvE activity was measured, and the data are shown in Table 3. AKB and isoleucine were used as metabolites specific to the Ile pathway, and pyruvate, 2-ketoisovalerate, and valine were used as metabolites specific to the Val pathway. From the data presented in Table 3 several conclusions were made.

First, IlvE activity in the *yjgF* mutant responded specifically to metabolites of the Ile pathway, while metabolites in the parallel Val pathway had no effect. As shown in Table 3, strain DM6284, both AKB and Ile restored IlvE activity to strain DM6284 (*yjgF*). A similar increase in activity was observed when 2-keto-3-methylvalerate, an additional Ile metabolite, was used (data not shown).

Second, the role of isoleucine in restoring IlvE activity was not in its ability to reduce metabolic flux in the Ile pathway. If activity of IlvE were increased by reduced metabolite flux, the addition of AKB (and, similarly, 2-keto-3-methylvalerate [data not shown]) would be expected to exacerbate, or at least maintain, the defect caused by the *yjgF* mutation. Instead, the addition of Ile metabolic intermediates resulted in the same increased IlvE activity as the addition of isoleucine. The restoration in IlvE activity is also not likely to be a general response to the presence of α-ketoacids, since pyruvate and 2-ketoisovalerate did not affect the level of IlvE activity.

Third, the restoration of IlvE activity caused by the addition of AKB was due to its conversion to isoleucine and subsequent allosteric inhibition of IlvA (Table 3, strain DM6946). This conclusion is supported by the inability of either isoleucine or AKB to fully restore IlvE activity in the presence of the *ilvA219* allele, which prevents allosteric inhibition by isoleucine.

Finally, in the absence of IlvA activity, the *yjgF* mutation does not reduce IlvE activity. This conclusion was based on the difference in IlvE activity between strains with wild-type IlvA (DM6284 [*yjgF*]) or constitutively active IlvA (DM6946 [*yjgF ilvA219*]), under growth conditions where wild-type IlvA would

TABLE 4. Lack of *yjgF* exacerbates SMM sensitivity[a]

Strain	Genotype	Zone of inhibition (mm)	
		AKB	SMM
DM6284	*yjgF*	22 ± 1.0	41 ± 2.0
DM6285	Wild type	23 ± 1.0	26 ± 1.0

[a] Sensitivities to AKB and SMM were determined in isogenic *yjgF* strains in top agar overlays on glucose minimal medium. Sterile filter disks with SMM (40 μg) or AKB (300 nmol) were placed on the agar and growth was scored as described in Materials and Methods. Values are means ± standard deviations of three independent cultures.

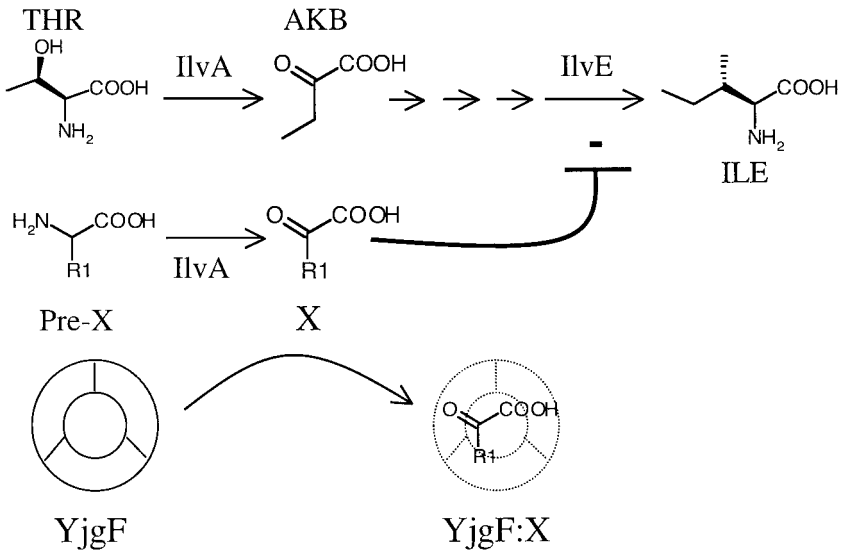

FIG. 4. Working model for the role of YjgF in Ile biosynthesis. The working model for YjgF function with respect to Ile biosynthesis is represented. Specific features of the model are described in the text (see Discussion). This model makes no conclusions about the identity of compound X and considers that it could be AKB, a metabolite of AKB, or a distinct 2-ketoacid.

be inhibited (i.e., growth in the presence of isoleucine). This conclusion was also supported by the fact that when *ilvA* was inactivated by mutation (Table 3, strain DM6143), a *yjgF* mutation failed to reduce the IlvE activity. Although the strength of these conclusions is limited by the inherent metabolic complexity in vivo, the activity of IlvE in a *yjgF* mutant appears to correlate with IlvA activity, not with flux through the Ile pathway. Taken together, we suggest the data presented are consistent with a model in which YjgF binds and sequesters a product of the IlvA reaction.

DISCUSSION

Results from genome sequencing efforts have emphasized the biochemical conservation that exists across the domains of life and have defined broadly conserved protein families often based solely on sequence similarity. The work presented here was initiated to probe the metabolic role of a member of one such family, the YjgF protein in *S. enterica.* This study has contributed to our understanding of the in vivo function of YjgF in *Salmonella* at several levels. The results presented have (i) determined that the specific activity of transaminase B (IlvE) correlates with the presence of *yjgF* in vivo, (ii) demonstrated that expression of *yjgF* protects a cell from SMM toxicity, and (iii) showed that reduced IlvE activity in a *yjgF* mutant correlates with the activity of threonine deaminase but not metabolic flux through the Ile pathway.

The results presented provided insights into the role of the YjgF protein in isoleucine biosynthesis and led to a testable working model that is schematically represented in Fig. 4 and discussed below. This model has four features: (i) IlvA generates a product (X) that may be AKB, a metabolite of AKB, or a distinct 2-ketoacid; (ii) in a wild-type cell, X is neutralized by YjgF; (iii) if not neutralized, X permanently inactivates IlvE; and (iv) IlvA is one of multiple enzymes generating a product that can be bound by YjgF. Central to this model is the inverse correlation of IlvA activity to IlvE activity in a *yjgF* mutant. The inability to find a similar correlation between the known product of IlvA (AKB) and IlvE activity led to the prediction of a second product of IlvA activity. It is anticipated that the suggested product X would look chemically similar to AKB, putatively an α-ketoacid. Finally, the model proposes that product X is responsible for inactivating IlvE, possibly as a suicide substrate.

The increased sensitivity of *yjgF* mutants to SMM but not AKB is consistent with, and provides support for, the model described above (Fig. 4). While the addition of SMM has been shown to cause the accumulation of AKB, any additional products of IlvA would also be expected to accumulate. This expectation is based on the fact that SMM inhibits IlvGM, thus starving the cells for Ile. Under such conditions, IlvA activity will not be inhibited and is likely to be derepressed. In contrast, the addition of exogenous AKB supplements, rather than constricts, the biochemical pathway. In addition, the data presented in Table 3 suggest that exogenous AKB is converted to sufficient Ile to inhibit IlvA, since the presence of the *ilvA219* allele eliminates the effect of AKB as it does that of Ile. Thus, while not the only interpretation, these results could be explained by a detrimental effect of IlvA activity in a *yjgF* mutant.

The working model anticipates a level of promiscuity in both IlvE and IlvA with respect to substrates. There is ample precedent that both IlvA (26, 35, 71) and IlvE (22, 49) interact with molecules other than their substrates in the Ile biosynthetic pathway. In fact, the promiscuity of transaminases (and deaminases) in general has been well documented (2, 3, 37, 38, 43, 49, 64, 65, 68). Further, multiple enzymes have been shown to catalyze side reactions that can be critical for metabolic processes and/or growth phenotypes in the appropriate strain background (7, 45, 54, 57). These examples provide precedent for the suggestion that an activity of IlvA generates a metabolite that has toxic consequences in the absence of YjgF.

It is unlikely that the role of YjgF proteins is solely to protect cells from a toxic by-product(s) of the IlvA reaction, since YjgF homologs exist in mammals that lack threonine deaminase. The deleterious metabolic consequences of unbalanced keto-acid pools in general are common to all organisms (4, 10, 20, 23, 27, 30, 32, 61). We envision YjgF having a broad affinity range and suggest that while YjgF prevents the toxicity of product X, other α-ketoacids could be similarly sequestered. The observation that HI0719, a YjgF homolog, was able to bind α-ketobutyrate, analogs of its enamine counterpart, and α-ketoisovalerate is consistent with this aspect of the model (41).

Accumulation of α-ketoacids occurs in several eukaryotic diseases and disorders, including Friedreich's ataxia, branched-chain ketonuria, tyrosinemia, and tumor biology (cell proliferation) (4, 15, 16, 21, 56). Interestingly, UK114, a YjgF homolog in goats, was found to accumulate in tumor cells and was classified as a tumor antigen (9). The correlation of increased ketoacids and increased UK114 accumulation in tumor cells, in combination with the model proposed here for a role of YjgF in detoxifying the cell from α-ketoacids, is intriguing. Based on the known toxicity of ketoacids and the breadth of phenotypes (functions) reported for YjgF, we suggest a broad global role for YjgF in protecting cells from imbalanced α-ketoacid pools. We suggest that other phenotypes reported for strains lacking *yjgF* could be the consequences of a different α-ketoacid(s) affecting distinct metabolic processes. The data presented here provide a mechanistic model for the protection in one case, involving the Ile biosynthetic pathway.

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REFERENCES

1. **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.
2. **Berg, C. M., M. D. Wang, N. B. Vartak, and L. Liu.** 1988. Acquisition of new metabolic capabilities: multicopy suppression by cloned transaminase genes in Escherichia coli K-12. Gene **65:**195–202.
3. **Berg, C. M., W. A. Whalen, and L. B. Archambault.** 1983. Role of alanine-valine transaminase in *Salmonella typhimurium* and analysis of an *avtA*::Tn*5* mutant. J. Bacteriol. **155:**1009–1014.
4. **Bertrand, M. J., R. Bouchard, G. L. Gauthier, J. P. Bouchard, and A. Barbeau.** 1982. Quantitative metabolic profiling of alpha-keto acids in Friedreich's ataxia. Can. J. Neurol. Sci. **9:**231–234.
5. **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.
6. **Burns, R. O.** 1971. L-Threonine deaminase-biosynthetic (*Salmonella typhimurium*). Methods Enzymol. **17B:**555–560.
7. **Burstein, C.** 1965. Role of transgalactosidations in induction of the lactose operon in Escherichia coli. Bull. Soc. Chim. Biol. (Paris) **47:**1901–1904.
8. **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.
9. **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.
10. **Daniel, J., L. Dondon, and A. Danchin.** 1983. 2-Ketobutyrate: a putative alarmone of *Escherichia coli.* Mol. Gen. Genet. **190:**452–458.
11. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Deaconescu, A. M., A. Roll-Mecak, J. B. Bonanno, S. E. Gerchman, H. Kycia, F. W. Studier, and S. K. Burley.** 2002. X-ray structure of *Saccharomyces cerevisiae* homologous mitochondrial matrix factor 1 (Hmf1). Proteins **48:** 431–436.
13. **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. D **59:**1676–1678.
14. **Downs, D. M., and L. Petersen.** 1994. *apbA*, a new genetic locus involved in thiamine biosynthesis in *Salmonella typhimurium.* J. Bacteriol. **176:**4858–4864.
15. **Eden, A., and N. Benvenisty.** 1999. Involvement of branched-chain amino acid aminotransferase (Bcat1/Eca39) in apoptosis. FEBS Lett. **457:**255–261.
16. **Endo, F., Y. Tanaka, K. Tomoeda, A. Tanoue, G. Tsujimoto, and K. Nakamura.** 2003. Animal models reveal pathophysiologies of tyrosinemias. J. Nutr. **133:**2063S–2067S.
17. **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.
18. **Epelbaum, S., D. M. Chipman, and Z. Barak.** 1996. Metabolic effects of inhibitors of two enzymes of the branched-chain amino acid pathway in *Salmonella typhimurium.* J. Bacteriol. **178:**1187–1196.
19. **Epelbaum, S., R. A. LaRossa, T. K. Van Dyk, T. Elkayam, D. M. Chipman, and Z. Barak.** 1998. Branched-chain amino acid biosynthesis in *Salmonella typhimurium*: a quantitative analysis. J. Bacteriol. **180:**4056–4067.
20. **Falco, S. C., and K. S. Dumas.** 1985. Genetic analysis of mutants of *Saccharomyces cerevisiae* resistant to the herbicide sulfometuron methyl. Genetics **109:**21–35.
21. **Funaro, A., A. L. Horenstein, G. Ghisolfi, B. Bussolati, A. Bartorelli, and G. Bussolati.** 1999. Identification of a 220-kDa membrane tumor-associated antigen by human anti-UK114 monoclonal antibodies selected from the immunoglobulin repertoire of a cancer patient. Exp. Cell Res. **247:**441–450.
22. **Gelfand, D. H., and R. A. Steinberg.** 1977. *Escherichia coli* mutants deficient in the aspartate and aromatic amino acid aminotransferases. J. Bacteriol. **130:**429–440.
23. **Gortz, P., H. Koller, B. Schwahn, U. Wendel, and M. Siebler.** 2003. Disturbance of cultured rat neuronal network activity depends on concentration and ratio of leucine and alpha-ketoisocaproate: implication for acute encephalopathy of maple syrup urine disease. Pediatr. Res. **53:**320–324.
24. **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.
25. **Harlow, E., and D. Lane.** 1988. Antibodies, a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
26. **Hofler, J. G., and R. O. Burns.** 1978. Threonine deaminase from *Salmonella typhimurium.* Effect of regulatory ligands on the binding of substrates and substrate analogues to the active sites and the differentiation of the activator and inhibitor sites from the active sites. J. Biol. Chem. **253:**1245–1251.
27. **Huang, H. M., H. Zhang, H. Xu, and G. E. Gibson.** 2003. Inhibition of the alpha-ketoglutarate dehydrogenase complex alters mitochondrial function and cellular calcium regulation. Biochim. Biophys. Acta **1637:**119–126.
28. **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.
29. **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.
30. **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.
31. **LaRossa, R. A., and T. K. Van Dyk.** 1989. Leaky pantothenate and thiamin mutations of *Salmonella typhimurium* conferring sulphometuron methyl sensitivity. J. Gen. Microbiol. **135:**2209–2222.
32. **LaRossa, R. A., and T. K. Van Dyk.** 1987. Metabolic mayhem caused by 2-ketoacid imbalances. Bioessays **7:**125–130.
33. **LaRossa, R. A., and T. K. Van Dyk.** 1988. Utilization of sulfometuron methyl, an acetolactate synthase inhibitor, in molecular biological and metabolic studies of plants and microbes. Methods Enzymol. **166:**97–107.
34. **LaRossa, R. A., T. K. Van Dyk, and D. R. Smulski.** 1990. A need for metabolic insulation: lessons from sulfonylurea genetics, p. 109–121. *In* Z. Barak, D. M. Chipman, and J. V. Schloss (ed.), Biosynthesis of branched chain amino acids. VCH Balaban, New York, N.Y.
35. **Maeba, P., and B. D. Samual.** 1966. The allosteric threonine deaminase of *Salmonella.* Kinetic model for the native enzyme. Biochemistry **5:**525–536.
36. **Martinez, E., B. Bartolome, and F. de la Cruz.** 1988. pACYC184-derived cloning vectors containing the multiple cloning site and *lacA*α reporter gene of pUC8/9 and pUC18/19 plasmids. Gene **68:**159–162.
37. **Monnier, N., A. Montmitonnet, S. Chesne, and J. Pelmont.** 1976. Transaminase B from *Escherichia coli.* I. Purification and first properties. Biochimie **58:**663–675.
38. **Nowicki, C., G. R. Hunter, M. Montemartini-Kalisz, W. Blankenfeldt, H.**

Hecht, and H. M. Kalisz. 2001. Recombinant tyrosine aminotransferase from *Trypanosoma cruzi*: structural characterization and site directed mutagenesis of a broad substrate specificity enzyme. Biochim. Biophys. Acta **1546:**268–281.
39. **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.
40. **Oxelmark, E., A. Marchini, I. Malanchi, F. Magherini, L. Jaquet, M. A. Hajibagheri, K. J. Blight, J. C. Jauniaux, and M. Tommasino.** 2000. Mmf1p, a novel yeast mitochondrial protein conserved throughout evolution and involved in maintenance of the mitochondrial genome. Mol. Cell. Biol. **20:**7784–7797.
41. **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.
42. **Petersen, L., J. 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.
43. **Powell, J. T., and J. F. Morrison.** 1978. Role of the *Escherichia coli* aromatic amino acid aminotransferase in leucine biosynthesis. J. Bacteriol. **136:**1–4.
44. **Primerano, D. A., and R. O. Burns.** 1982. Metabolic basis for the isoleucine, pantothenate or methionine requirement of *ilvG* strains of *Salmonella typhimurium.* J. Bacteriol. **150:**1202–1211.
45. **Ramos, I., and D. M. Downs.** 2003. Anthranilate synthase can generate sufficient phosphoribosyl amine for thiamine synthesis in *Salmonella enterica.* J. Bacteriol. **185:**5125–5132.
46. **Rappu, P., B. S. Shin, H. Zalkin, and P. Mantsala.** 1999. A role for a highly conserved protein of unknown function in regulation of *Bacillus subtilis purA* by the purine repressor. J. Bacteriol. **181:**3810–3815.
47. **Robello, C., B. Dallagiovanna, J. C. Engel, F. Gamarro, and S. Castanys.** 1998. A new member of YER057c family in *Trypanosoma cruzi* is adjacent to an ABC-transporter. Gene **220:**1–12.
48. **Roberts, G. P.** 1978. Isolation and characterization of informational suppressors in *Salmonella typhimurium.* Ph.D. dissertation. University of California at Berkeley, Berkeley.
49. **Rudman, D., and A. Meister.** 1953. Transamination in *Escherichia coli.* J. Biol. Chem. **200:**591–604.
50. **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.
51. **Schmieger, H.** 1972. Phage P22 mutants with increased or decreased transduction abilities. Mol. Gen. Genet. **119:**75–88.
52. **Shaw, K. J., C. M. Berg, and T. J. Sobol.** 1980. *Salmonella typhimurium* mutants defective in acetohydroxy acid synthases I and II. J. Bacteriol. **141:**1258–1263.
53. **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.
54. **Starai, V. J., I. Celic, R. N. Cole, J. D. Boeke, and J. C. Escalante-Semerena.** 2002. Sir2-dependent activation of acetyl-CoA synthetase by deacetylation of active lysine. Science **298:**2390–2392.
55. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T_7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82:**1074–1078.
56. **Tanaka, K., and L. E. Rosenberg.** 1983. Disorders of branched chain amino acid and organic acid metabolism. McGraw-Hill, New York, N.Y.
57. **Tsang, A. W., and J. C. Escalante-Semerena.** 1998. CobB, a new member of the SIR2 family of eucaryotic regulatory proteins, is required to compensate for the lack of nicotinate mononucleotide:5,6-dimethylbenzimidazole phosphoribosyltransferase activity in *cobT* mutants during cobalamin biosynthesis in *Salmonella typhimurium* LT2. J. Biol. Chem. **273:**31788–31794.
58. **Umbarger, H. E.** 1996. Biosynthesis of the branched-chain amino acids, p. 442–457. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington, D.C.
59. **Van Dyk, T. K., and R. A. LaRossa.** 1990. Prevention of endogenous 2-ketobutyrate toxicity in *Salmonella typhimurium*, p. 123–130. *In* Z. Barak, D. M. Chipman, and J. V. Schloss (ed.), Biosynthesis of branched amino acids. VCH Balaban, New York, N.Y.
60. **Van Dyk, T. K., B. L. Ayers, R. W. Morgan, and R. LaRossa.** 1998. Constricted flux through the branched-chain amino acid biosynthetic enzyme acetolactate synthase triggers elevated expression of genes regulated by *rpoS* and internal acidification. J. Bacteriol. **180:**785–792.
61. **Van Dyk, T. K., and R. A. Larossa.** 1986. Sensitivity of a *Salmonella typhimurium aspC* mutant to sulfometuron methyl, a potent inhibitor of acetolactate synthase II. J. Bacteriol. **165:**386–392.
62. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218:**97–106.
63. **Volz, K.** 1999. A test case for structure-based functional assignment: the 1.2 Å crystal structure of the *yjgF* gene product from *Escherichia coli.* Protein Sci. **8:**2428–2437.
64. **Wang, M. D., L. Buckley, and C. M. Berg.** 1987. Cloning of genes that suppress an *Escherichia coli* K-12 auxotroph when present in multicopy plasmids. J. Bacteriol. **169:**5610–5614.
65. **Wang, M. D., L. Liu, B. M. Wang, and C. M. Berg.** 1987. Cloning and characterization of the *Escherichia coli* K-12 alanine-valine transaminase (*avtA*) gene. J. Bacteriol. **169:**4228–4234.
66. **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.
67. **Weng, M., and H. Zalkin.** 2000. Mutations in the *Bacillus subtilis* purine repressor that perturb PRPP effector function in vitro and in vivo. Curr. Microbiol. **41:**56–59.
68. **Whalen, W. A., and C. M. Berg.** 1982. Analysis of a *avt*::Mu *d*1 (Ap *lac*) mutant: metabolic role of transaminase C. J. Bacteriol. **150:**739–746.
69. **Whittaker, J. J., and J. H. Jackson.** 1980. A permeabilized-cell assay for transaminase B activity in *Escherichia coli* K-12. Anal. Biochem. **105:**133–140.
70. **Winston, F., D. Botstein, and J. H. Miller.** 1979. Characterization of amber and ochre suppressors in *Salmonella typhimurium.* J. Bacteriol. **137:**433–439.
71. **Wood, W. A., and I. C. Gunsalus.** 1949. Serine and threonine deaminases of *Escherichia coli*: activators for a cell-free enzyme. J. Biol. Chem. **181:**171–182.