An Unexpected Route to an Essential Cofactor: *Escherichia coli* Relies on Threonine for Thiamine Biosynthesis

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**ABSTRACT** Metabolism consists of biochemical reactions that are combined to generate a robust metabolic network that can respond to perturbations and also adapt to changing environmental conditions. *Escherichia coli* and *Salmonella enterica* are closely related enterobacteria that share metabolic components, pathway structures, and regulatory strategies. The synthesis of thiamine in *S. enterica* has been used to define a node of the metabolic network by analyzing alternative inputs to thiamine synthesis from diverse metabolic pathways. To assess the conservation of metabolic networks in organisms with highly conserved components, metabolic contributions to thiamine synthesis in *E. coli* were investigated. Unexpectedly, we found that, unlike *S. enterica*, *E. coli* does not use the phosphoribosylpyrophosphate (PRPP) amidotransferase (PurF) as the primary enzyme for synthesis of phosphoribosylamine (PRA). In fact, our data showed that up to 50% of the PRA used by *E. coli* to make thiamine requires the activities of threonine dehydratase (IlvA) and anthranilate synthase component II (TrpD). Significantly, the IlvA- and TrpD-dependent pathway to PRA functions in *S. enterica* only in the absence of a functional reactive intermediate diaminase (RidA) enzyme, bringing into focus how these closely related bacteria have distinct metabolic networks.

**IMPORTANCE** In most bacteria, including *Salmonella* strains and *Escherichia coli*, synthesis of the pyrimidine moiety of the essential coenzyme, thiamine pyrophosphate (TPP), shares enzymes with the purine biosynthetic pathway. Phosphoribosylpyrophosphate amidotransferase, encoded by the *purF* gene, generates phosphoribosylamine (PRA) and is considered the first enzyme in the biosynthesis of purines and the pyrimidine moiety of TPP. We show here that, unlike *Salmonella*, *E. coli* synthesizes significant thiamine from PRA derived from threonine using enzymes from the isoleucine and tryptophan biosynthetic pathways. These data show that two closely related organisms can have distinct metabolic network structures despite having similar enzyme components, thus emphasizing caveats associated with predicting metabolic potential from genome content.

In living cells, a discrete number of enzymes encoded in the genome act in concert to produce diverse characteristic behaviors (phenotypes). In microbes in particular, the metabolic network responsible for these behaviors is robust and can respond to perturbations by reconfiguring the network to maintain the overall function of the organism (1–9). A fundamental question in metabolic systems biology is whether knowledge of the metabolic components can be extrapolated to predict the existence of a specific pathway or network structure. The presence and conservation of metabolic components is easily ascertained by genome analyses and often used to describe the metabolic potential of an organism. Genome annotation that identifies component enzymes has proven valuable for describing central metabolic pathways. It is unclear, however, whether this approach can be extended to describe the higher metabolic network structure that includes atypical metabolites and recruited pathways, many of which provide network plasticity and the resulting robustness. Definition of the higher-order metabolic structure and a solid understanding of the rules that govern its formation are key to elucidating the dynamic roles of metabolic components in different organisms and to refining the information gleaned from genome annotation.

The comparison of thiamine synthesis in the closely related and genetically tractable organisms *Escherichia coli* and *Salmonella enterica* provides an opportunity to address the fundamental question of whether the presence of component enzymes can accurately predict metabolic network structure. The essential cofactor thiamine pyrophosphate (TPP) can be synthesized *de novo* by microbes and plants (10, 11). In most bacteria, including *E. coli* and *S. enterica*, the thiamine biosynthetic pathway shares enzymes with the purine biosynthetic pathway (Fig. 1). Phosphoribosylpyrophosphate amidotransferase, encoded by the *purF* gene, generates phosphoribosylamine (PRA) and is considered the first enzyme in the biosynthesis of purines and the pyrimidine moiety of...
thiamine in both *S. enterica* and *E. coli* (10, 12–14). Biochemical genetic studies in *S. enterica* described the network surrounding thiamine synthesis (specifically PRA) as one in which metabolites can be made by promiscuous enzymes from other metabolic pathways or by enzyme-independent chemical reactions (5, 6, 8, 15–17). *S. enterica* requires PurF for thiamine synthesis on glucose medium, but this requirement can be bypassed by growing this bacterium on different media or by the presence of mutations, both of which allow PRA synthesis (4–6, 8, 15, 18, 19). The alternative mechanisms for the synthesis of PRA and, thus, thiamine, can be activated by (i) increasing cellular ribose-5-phosphate (R5P) levels, (ii) the accumulation of the histidine biosynthetic intermediate 1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboximide (ProFAR), or (iii) the elimination of the RidA deaminase. The former involved the nonenzymatic synthesis of PRA from R5P and was facilitated by the high levels of ammonium in the medium (19). Increased cellular R5P levels that allow PRA synthesis could be generated from (i) the oxidative pentose phosphate (OPP) pathway (18) or (ii) by compromising the activities of ribose-phosphate pyrophosphokinase (PrsA) (7) or indole-3-glycerol phosphate synthase/phosphoribosylanthranilate isomerase (TrpC) (6). Lack of RidA allowed PRA to be formed from threanine and phosphoribosylpyrophosphate (PRPP) in a novel pathway that recruited the enzymes IlvA and TrpD from the isoleucine and tryptophan biosynthetic pathways, respectively (4, 15, 16). RidA deaminates examines, including 2-aminocrotonate, and in its absence, these reactive metabolites accumulate (17). The mechanisms that generate PRA in *S. enterica* are illustrated in Fig. 1. Studies in *E. coli* have presumed that PurF catalyzes the first step in both purine and thiamine synthesis but have not specifically addressed the requirement of this enzyme for thiamine synthesis.

Although *S. enterica* and *E. coli* have unique metabolic capacities encoded in their genomes, the metabolic pathways and regulatory paradigms throughout the central metabolism are conserved between the organisms (20). For instance, each of the proteins mentioned in Fig. 1 are conserved between *S. enterica* and *E. coli* and are >95% identical at the amino acid level. Although the components are conserved, the higher-order metabolic structure derived from these components that could generate metabolic robustness has not been queried in *E. coli*. This study was initiated to determine whether the metabolic network connected to thiamine synthesis was conserved between *S. enterica* and *E. coli*. The work reported herein demonstrated that, in *E. coli*, an IlvA- and TrpD-dependent mechanism of PRA synthesis actively contributes to the thiamine pool despite the presence of functional PurF and RidA proteins. Our results provide evidence that a metabolic network configuration cannot be predicted solely by the presence of the component parts.

**RESULTS**

**Synthesis of PRA for thiamine in *E. coli*.** *S. enterica* and *E. coli* had indistinguishable thiamine requirements, as measured by the growth of thiamine auxotrophs (Table 1). *purF* mutant strains of

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**FIG 1** Mechanisms of PRA synthesis. Metabolic mechanisms capable of contributing to thiamine synthesis (via PRA formation) in the absence of PurF are shown. The reactions that form PRA are denoted i to v. Reactions i, ii, and iii are means to increase R5P, which then allows nonenzymatic formation of PRA when ammonia is in excess, as described in the text. Reactions iv and v are independent of the ammonia concentration. The allosteric inhibition of IlvA by isoleucine is depicted to reflect its importance in the reported work. Abbreviations: PRPP, phosphoribosyl pyrophosphate; PRA, phosphoribosylamine; AIR, amidinate ribotide; OPP, oxidative pentose phosphate pathway; R5P, ribose-5-phosphate; PR-anthranilate, phosphoribosyl anthranilate; ProFAR, 1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4 carboximide.
both *E. coli* and *S. enterica* required a source of purines for growth, but their requirements for thiamine differed. The data in Fig. 2A show the previously characterized growth defect of a *purF* mutant of *S. enterica* on glucose-plus-adenine minimal medium, which is eliminated with exogenous thiamine. In contrast to *S. enterica*, the *purF* mutant of *E. coli* grew as well as the wild-type strain on glucose-plus-adenine medium. These data showed that under these conditions, PurF contributed to the synthesis of thiamine in *S. enterica* but was not necessary in *E. coli*.

Blocking the oxidative pentose phosphate pathway (OPP) with a mutation in *gnd* (6-phosphogluconate dehydrogenase, EC 1.1.1.44) in the *purF* mutant of *S. enterica* abolished residual growth in the absence of thiamine (i.e., thiamine synthesis), as previously reported (18). In contrast, the *purF* *gnd* mutant of *E. coli* maintained significant growth in the absence of thiamine, even after subculturing into fresh medium (Fig. 2). The difference in growth with and without a *gnd* mutation showed that the OPP contributed to the synthesis of thiamine in *S. enterica* but was not necessary in *E. coli*.

Ammonia and R5P contribute to PRA formation in *E. coli*. The results from two experiments supported the conclusion that the OPP contributed to PRA formation in *E. coli* by facilitating the nonenzymatic combination of R5P and ammonia. First, when the *purF* mutant was cultured with glutamine as the sole nitrogen source, its growth on glucose plus adenine decreased to a level comparable to that of the *purF gnd* mutant (Fig. 3A). Second, when excess ammonia was present, PRA formation in the *purF gnd* mutant strain was stimulated by exogenous ribose or R5P (data not shown). Taken together, and based on the precedent in *S. enterica* (19), these data showed that when both R5P and ammonia were in excess, sufficient PRA for thiamine synthesis was synthesized.

The *IlvA*- and *TrpD*-dependent pathway for PRA synthesis functions in *E. coli*. Nutritional studies with each of the 20 common amino acids provided insights into the mechanism of PRA synthesis in the *purF gnd* mutant. The results from these studies showed that PRA formation in the *purF gnd* mutant was stimulated by threonine and inhibited by isoleucine and tryptophan (Fig. 4; Table 2). These nutritional characteristics were reminiscent of the PRA-forming mechanism described in a *purF gnd ridA* mutant of *S. enterica* (4, 15, 16). In the relevant mechanism, threonine dehydratase (*IlvA*) converts threonine to the reactive enamino acid, which is condensed with PRPP by anthra-

### Table 1

*E. coli* and *S. enterica* have the same thiamine requirement.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Growth (avg OD₆₅₀ ± SD) with thiamine concn of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM95 (<em>S. enterica</em>)</td>
<td>thi-918::MudJ</td>
<td>None 0.03 ± 0.01 1 nM 0.32 ± 0.01 10 nM 1.33 ± 0.12</td>
</tr>
<tr>
<td>DM14703 (<em>E. coli</em>)</td>
<td>ΔthiCEFSGH::Kan</td>
<td>0.01 ± 0.01 0.29 ± 0.01 1.28 ± 0.19</td>
</tr>
</tbody>
</table>

*Thiamine auxotrophs of *S. enterica* (DM95) and *E. coli* (DM14703) were grown in minimal medium with the concentrations of thiamine indicated. The results are from three independent cultures after 18 h of growth.

![FIG 2](image1.png) Thiamine-independent growth of *S. enterica* and *E. coli* *purF* and *purF gnd* mutants. (A) Growth of *S. enterica* (circles) and *E. coli* (squares) strains was quantified in glucose-plus-adenine minimal medium. Symbols are filled according to genotype, as follows: gray, wild type (DM1 or DM14520); black, *purF* mutant (DM1936 or DM14545); white, *purF gnd* mutant (DM728 or DM14572). The growth of all strains was restored to the wild-type level with the addition of exogenous thiamine (data not shown). (B) The *purF gnd* mutant of *E. coli* (DM14572) was grown in glucose-plus-adenine minimal medium (black squares). After 15 h, the mutant was subcultured into identical medium and grown for an additional 15 h (white squares). Error bars show standard deviations.

![FIG 3](image2.png) PRA synthesis in *E. coli* is partially dependent on R5P and ammonia. The growth of the *purF* mutant (DM14545) in N⁻⁻⁻ glucose-plus-adenine minimal medium with 1 mM glutamine as a nitrogen source was quantified with (inverted triangles) or without (circles) thiamine provided. The data for thiamine-independent growth of an *E. coli* *purF gnd* mutant (DM14572, squares) are shown for comparison.
nullate synthase component II (TrpD) to form a phosphoribosyl-
nename adduct, the subsequent breakdown of which generates
PRA (Fig. 1) (17). Threonine stimulated PRA formation in this
mechanism by increasing 2-aminocrotonate synthesis, while
isoleucine decreased 2-aminocrotonate production through the allo-
spheric inhibition of IlvA (4, 16). In S. enterica, the 2-ami-
rotonate levels needed to support thiamine synthesis occurred
only in the absence of RldA, which converts 2-aminocrotonate to
2-ketobutyrate for isoleucine biosynthesis. The utilization of this
synthetic mechanism by E. coli, in the presence of a functional
RldA, would demand that the metabolic networks of the two or-
organisms are different, despite the presence of conserved, highly
identical components.

We used genetic approaches to address the role of IlvA in thi-
amine synthesis in E. coli. For this purpose, we took advantage of
the feedback-resistant IlvA1447F variant (with an L-to-F change at
position 447) encoded by the ilvA219 allele (21). A purF gnd mu-
tant lacking ilvA was constructed, and either the ilvA\textsuperscript{+} or the
ilvA219 allele was expressed in trans as the only source of IlvA activity.
Ectopically produced wild-type IlvA and the IlvA1447F protein restored isoleucine synthesis in the purF gnd ilvA
(DM1466) strain, showing that a functional threonine dehydra-
tase activity was associated with both proteins. Both strains were
also proficient in thiamine synthesis, as shown by their growth in
the absence of thiamine (Fig. 5A). However, when isoleucine was
provided in the medium, only the strain expressing the ilvA219
allele in trans (strain DM14684) maintained PRA synthesis that
allowed growth in the absence of exogenous thiamine (Fig. 5B).
The growth of both strains was restored in the presence of isole-
cine when exogenous thiamine was provided (Fig. 5C). These re-
results supported the conclusion that IlvA activity was required for
PRA synthesis and that isoleucine inhibited PRA synthesis via al-
losteric inhibition of IlvA.

PRA synthesis in a purF gnd mutant was inhibited by trypto-
phan, another similarity with the pathway that functions in a purF
mutant of S. enterica (Table 2) (15). In the presence of tryptophan, TrpR represses the transcription of the trp operon
(15, 22). Eliminating TrpR function in the purF gnd mutant had
two effects that supported the involvement of the IlvA- and TrpD-
dependent pathway described above. First, a trpR mutation re-
stored the growth of the purF gnd strain to wild-type levels
on adenine minimal medium. Interestingly, the trpR mutation also
restored growth (i.e., PRA synthesis) on glucose-plus-adenine
medium with limiting nitrogen (data not shown). These results
suggested that the decrease in PRA synthesis caused by the gnd
mutation could be restored not only by increasing nonenzymatic
synthesis (from R5P and ammonia) but by increasing the level of
a tryptophan enzyme(s). Second, exogenous tryptophan no lon-
ger inhibited PRA synthesis in the purF gnd trpR mutant strain
(Table 2), confirming that the relevant effect of tryptophan was on
transcription. When a trpD mutation was introduced in the purF
gnd trpR mutant, the resulting strain failed to synthesize PRA in
adenine-plus-tryptophan minimal medium (Table 2). Taken to-
together, these data showed that TrpD was required for PRA synthe-
sis in a purF gnd mutant of E. coli.

IlvA- and TrpD-dependent PRA synthesis contributes to
the thiamine pool in wild-type E. coli. The contribution of IlvA and
TrpD to thiamine synthesis in the purF gnd mutant suggested that
this pathway could have a role in the wild-type strain. If this were
the case, PRA would include the nitrogen of threonine that is
ultimately incorporated into the 4-amino-5-hydroxymethyl-2-
methylpyrimidin (HMP) moiety of thiamine (Fig. 6A). A strain
(DM14893) that carried wild-type alleles of purF and gnd
was grown in glucose-plus-adenine minimal medium with 15N-
or 14N-labeled threonine. Strain DM14893 carried a thrC mutation
to prevent dilution of the labeled threonine by endogenous syn-
thesis and a mutation in purK to divert metabolic flux toward
HMP biosynthesis and maximize the yield of thiamine. Figures 6B
and C show mass spectrometry (MS) data from positive time of
flight (+TOF) MS analysis of derivatized HMP (2-methyl-4-

\begin{table}[h]
\centering
\caption{TrpD is required for PRA synthesis in E. coli.}
\label{tab:trpD}
\begin{tabular}{lcc}
\hline
Strain & Relevant genotype & Specific growth rate ($\mu$) (final yield [OD$_{600}$]) in medium with: \tabularnewline
\hline
DM14572 & purF gnd & Ade & 0.1 (0.2) & Ade + Trp & 0.7 (0.3) & Ade + Trp + Thi & 0.7 (0.4) \\
DM14729 & purF gnd trpD & NG & 0.6 (0.4) & 0.6 (0.4) & 0.6 (0.4) & 0.6 (0.4) \\
DM14606 & purF gnd trpR & NG & 0.6 (0.4) & 0.6 (0.4) & 0.6 (0.4) & 0.6 (0.4) \\
DM14759 & purF gnd trpR & NG & 0.6 (0.4) & 0.6 (0.4) & 0.6 (0.4) & 0.6 (0.4) \\
\hline
\end{tabular}
\footnote{The E. coli strains listed were grown in glucose minimal medium containing the indicated amino acid(s). The results show the average growth rate and final cell density after 16 h (in parentheses) of three independent cultures. Error between cultures was <10%. NG, no growth; indicates the final cell density was <0.15.}
\end{table}
Amino-5[(ethylthio)methyl]pyrimidine [ETMP] isolated from cells grown in each medium. As expected, the HMP purified from the strain grown in the presence of [14N]threonine generated a single ETMP species with an m/z of 184. When the cells were grown in [15N]threonine, three ETMP species were observed, representing unlabeled ETMP (m/z = 184) or ETMP with one (m/z = 185) or two (m/z = 186) heavy (15N) nitrogens. In total, these data showed that threonine could contribute one or two nitrogen atoms to HMP.

Two strains were generated to probe the contribution of threonine to HMP. A trpR lesion was introduced to eliminate the effect of added tryptophan, which was required to monitor the role of TrpD in this synthesis. Strains DM15000 (thrC purK trpR) and DM15020 (thrC purK trpR trpD) were grown in the adenine-plus-tryptophan minimal medium with [15N]threonine. Figure 7 shows mass spectrometry data from +TOF MS analysis of ETMP isolated from each strain. Three ETMP species were isolated from the first strain (thrC purK trpR), while two ETMP species were present in the strain lacking TrpD. Significantly, in the strain lacking TrpD, the peak with an m/z of 184 was a substantially higher percentage of the total HMP than it was in the strain with a functional TrpD (50% versus 15%, respectively). Qualitatively similar results were obtained in strains with a functional PurK (data not shown). In total, these data supported a scenario in which threonine contributes a nitrogen to HMP via PRA synthesized by the 2-aminocrotonate pathway.

The presence of a doubly labeled peak in the trpD+ strain and the persistence of a singly labeled peak in the absence of TrpD suggested that the nitrogen from threonine was incorporated into HMP by an additional mechanism. Aminomimidazole ribotide (AIR) contains three nitrogen atoms, one from PRA, one from glutamine, and one from glycine (Fig. 6). Threonine is converted to glycine by poorly characterized pathways but has no direct connection to glutamine (23, 24). Based on these considerations, we favor an explanation where [15N]glycine was derived in vivo from [15N]threonine and served as a precursor for HMP. This scenario was supported by the finding that HMP isolated from DM15020 grown with glycine (in addition to adenine and tryptophan) yielded a single ETMP species with an m/z of 184 (data not shown).

FIG 5 Isoleucine inhibits PRA synthesis in E. coli by allosteric inhibition of IlvA. The growth of mutant strains purF gnd ilvA pilvA (DM14683) and purF gnd ilvA pilvA219 (DM14684) is shown. Colonies of each strain were patched to nutrient agar containing chloramphenicol and replica printed to glucose-plus-adenine minimal medium plates containing chloramphenicol (A) and further supplemented with isoleucine (B) or isoleucine and thiamine (C).

DISCUSSION

S. enterica and E. coli are enteric bacteria that have been model organisms for basic genetic, biochemical, and physiological studies for decades. The prevailing perception was that, a few specific pathways notwithstanding, these organisms were similar in metabolic capacity and regulatory organization. This perception has been supported by countless studies defining proteins, pathways, and regulatory systems that are conserved between the organisms. The purpose of this study was to address metabolic network structure and determine whether conservation of metabolic components determines the conservation of metabolic network structure and plasticity. The well-described metabolic network structure around the synthesis of PRA and thiamine in S. enterica provided a model network. Significantly, all of the component enzymes and regulatory proteins implicated in this network were >95% identical between S. enterica and E. coli at the amino acid level (Fig. 1).

The data showed that in E. coli, unlike S. enterica, neither phosphoribosylpyrophosphate amidotransferase (PurF) nor 6-phosphogluconate dehydrogenase (Gnd) was required for thiamine synthesis. This result indicated that there was a significant difference between the organisms in the structure of the metabolic networks involving these gene products and the formation of thiamine. The data showed that, in wild-type E. coli, an IlvA- and TrpD-dependent mechanism for PRA formation contributed to thiamine synthesis as significantly as PurF. This pathway was identified and characterized in S. enterica, where it satisfies a thiamine requirement only in the absence of the RidA hydrolase (4, 15–17). Thus, E. coli depended on a recruited pathway that was first characterized in S. enterica. A number of points were taken from this result. First, while the components of this pathway were present in S. enterica, its function was not detectable in a wild-type strain. The fact that this pathway functions in S. enterica only if the RidA hydrolase is absent and yet it functions in E. coli in the presence of RidA suggests that the cellular milieu of the two bacteria are different. The IlvA- and TrpD-dependent synthesis of PRA depends on the unstable enamine, 2-aminocrotonate. Work in Salmonella strains showed that when RidA was present, the levels of 2-aminocrotonate were not sufficient to support PRA synthesis for thiamine. This was presumed to be due to the conversion of
2-aminocrotonate to 2-ketobutyrate by RidA, but the presence of RidA in *E. coli* means this cannot be the full explanation. In total, the data herein suggested that the metabolic network in *E. coli* has a characteristic(s) distinct from *S. enterica* that allows the IlvA- and TrpD pathway to function in the presence of a functional RidA. An attractive possibility is that the ratio of serine to threonine is different in the two organisms. IlvA uses both amino acids as substrates, and increased availability of threonine could result in higher steady-state levels of 2-aminocrotonate, even in the presence of RidA.

The results of *in vivo* labeling experiments supported the conclusion that in wild-type *E. coli*, in the presence of adenine, the IlvA- and TrpD-dependent pathway contributes significantly to thiamine synthesis. This finding was striking in light of the common assumption that dedicated linear pathways are the dominant form of component synthesis. The natural environment of *E. coli*,
The example herein emphasizes the gap in our understanding of significant differences in the cellular milieus of derivatives of E. coli and S. enterica, its analysis in a ridA mutant strain allowed rapid understanding of the explanation for the E. coli phenotype and, in doing so, suggested significant differences in the cellular milieu of E. coli and S. enterica. The example herein emphasizes the gap in our understanding of how metabolic pieces are organized to generate the complexity and flexibility inherent in the metabolic network, as well as the potential of probing basic metabolic systems to provide fundamental insights into multiple systems.

**MATERIALS AND METHODS**

**Bacterial strains, media, and chemicals.** The strains used in this study are derivatives of S. enterica serovar Typhimurium strain LT2 or E. coli strain K-12 and are described in Table 3. Luria-Bertani (LB) broth and Difco nutrient broth (NB) (8 g/liter) with NaCl (5 g/liter) were used as rich media. No-carbon E medium supplemented with 1 mM MgSO₄ (27–29) and trace minerals (30) was used as minimal medium. Nitrogen- and carbon-free (N⁻C⁻) salts medium (31) supplemented with 1 mM MgSO₄, trace minerals, and 1 (or 5) mM glutamine was used as nitrogen-limiting minimal medium. Glucose (11 mM) or succinate (16.5 mM) was provided as the sole carbon source. Difco BıTek agar was added (15 g/liter) for solid medium. Where indicated in the figure or table legends, additions to the media were used as follows: adenine (0.4 mM), thiamine (100 nM), ribose (20 mM), ribose-5-phosphate (20 mM), glucose-6-phosphate (20 mM), and trace minerals (25, 26). This correlation makes it reasonable to suggest that aspects of the metabolic network have evolved to ensure that thiamine is synthesized even when flux through the purine pathway is dramatically reduced by allosteric inhibition of PurF and transcriptional repression by PurR. This study provides an example of how the modulation and/or enhancement of metabolic plasticity can accomplish a necessary metabolic goal that facilitates the inhabiting of specific niches.

### TABLE 3 Bacterial strains used in the study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM1</td>
<td>S. enterica LT2 wild type</td>
</tr>
<tr>
<td>DM95</td>
<td>S. enterica LT2 thi-918: MudJ⁵⁰⁰</td>
</tr>
<tr>
<td>DM728</td>
<td>S. enterica LT2 purF2085 gnd-181</td>
</tr>
<tr>
<td>DM1936</td>
<td>S. enterica LT2 purF2085</td>
</tr>
<tr>
<td>DM9890</td>
<td>S. enterica LT2 purF2085 gnd-181 trpC3620</td>
</tr>
<tr>
<td>DM14520</td>
<td>E. coli K-12 wild type</td>
</tr>
<tr>
<td>DM14545</td>
<td>E. coli K-12 ΔpurF723::kan</td>
</tr>
<tr>
<td>DM14572</td>
<td>E. coli K-12 purF823 Δgnd-727::kan</td>
</tr>
<tr>
<td>DM14602</td>
<td>E. coli K-12 purF823 gnd-827</td>
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<td>DM14603</td>
<td>E. coli K-12 purF823 gnd-827 ΔaraA790::kan</td>
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<td>DM14661</td>
<td>E. coli K-12 purF823 gnd-827 ΔΔilvA723::kan</td>
</tr>
<tr>
<td>DM14683</td>
<td>E. coli K-12 purF823 gnd-827 ΔΔilvA723::kan pGS22-ilvA</td>
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</tr>
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<td>E. coli K-12 purF823 gnd-827 ΔtrpD1050::cat</td>
</tr>
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<td>E. coli K-12 purF823 gnd-827 ΔtrpD1050::cat ΔtrpR789::kan</td>
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<td>DM15000</td>
<td>E. coli K-12 thrC842 ΔpurK835 ΔtrpR789::kan ΔtrpD1050::cat</td>
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</table>

⁵⁰⁰ MudJ refers to the MudJ1734 transposon (36). pGS22-ilvA and pGS22-ilvA219 contain the loci from S. enterica.
phosphate (0.4 mM), isoleucine (0.3 mM), threonine (0.3 mM), serine (10 mM), tryptophan (0.1 mM), methionine (0.3 mM), glycine (0.13 mM), and histidine (0.1 mM). Antibiotics were used at the following concentrations for rich (or minimal) medium: kanamycin, 50 (12.5 µg/mL) chloramphenicol, 20 (5) µg/mL and ampicillin, 100 (15) µg/mL. [1-15N]threonine was obtained from Cambridge Isotope Laboratories (Tewksbury, MA). All other chemicals were from Sigma (St. Louis, MO).

**Growth quantification.** Cells from overnight cultures in NB medium were pelleted and resuspended in an equivalent volume of saline (0.85% NaCl), and a 0.1-mL aliquot was inoculated into 5 mL of the appropriate minimal medium. Alternatively, a 5-µL aliquot was used to inoculate 195 µL of medium. Growth was quantified in a microplate reader (model EL808; Bio-Tek Instruments). Unless otherwise stated, cell density was measured as the absorbance at 650 nm and growth was reported as the final cell density at an optical density of 650 nm (OD650), reached after 12 h of incubation at 37°C with shaking. When nutritional requirements were measured on solid medium, soft agar overlays were used (32).

**Genetic techniques.** Bacteriophage P1vir was used for transductional crosses. The preparation of P1 phage lysates and the protocol for transductional crosses have been described previously (33). The published protocol was modified such that the phage and cells were incubated for 1 h, washed twice with 1 mL of M9 buffer, and resuspended in 200 µL of M9 buffer before plating on selective medium. Mutant strains were constructed by transducing the relevant mutations from the Keio collection to the appropriate strain (34). The trpD locus in E. coli K-12 was replaced with the cat element using λ-Red-mediated homologous recombination (35).

**Purification of the pyrimidine moiety of thiamine.** A 1- or 0.5-L culture of the appropriate strain in medium with the appropriate nutrients and 0.5 mM threonine (L-15N or 1-15N labeled) was grown to an OD650 of approximately 0.8. The cells were pelleted, and the 4-amino-5-(2-hydroxymethyl)-2-methylpyrimidin (HMP) moiety of thiamine was extracted, derivatized, and purified as described previously (23, 24). In brief, the cell pellet (frozen or fresh) was resuspended in 8 mL of 0.1 M HCl and boiled for 20 min. Cell debris was removed by centrifugation, and the thiamine pyrophosphate was cleaved by ethanol, resulting in the formation of thiazole diphosphate and 2-methyl-4-amino-5[(ethythio)methyl]pyrimidine (ETMP). ETMP was purified by repeated extractions with methylene chloride. The extractions were reduced to dryness with a stream of nitrogen, and the residue containing ETMP was suspended in 30 to 100 µL of double-distilled water as needed. The sample was submitted for positive time of flight (+ TOF) mass spectral analysis at the University of Wisconsin Biotechnology Center.

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**REFERENCES**


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