Mutations in *sdh* (Succinate Dehydrogenase Genes) Alter the Thiamine Requirement of *Salmonella typhimurium*

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The goal of our research is to investigate metabolic connections that extend beyond linear biochemical pathways. Thiamine synthesis in *Salmonella typhimurium* serves as a model system for studying these aspects of metabolism. Several unexpected relationships exist between thiamine synthesis and other metabolic pathways in the cell, including, as reported to date, the pentose phosphate pathway, carbon catabolism, and pantothenate biosynthesis (10, 12, 13, 26, 27). In addition, because thiamine is required in small amounts, growth in the absence of thiamine provides a very sensitive assay for endogenous synthesis. Intuitively, pathways with high carbon flux may contribute precursors to low-flux pathways without significant depletion of their intermediates. This may provide the cell with detectable flexibility in supplying its low-level requirements.

Thiamine pyrophosphate (TPP), the biologically active form of thiamine, is a cofactor for metabolic reactions involving the transfer of C2 units, including reactions catalyzed by pyruvate dehydrogenase, pyruvate decarboxylase, and α-ketoglutarate dehydrogenase. TPP is formed by the joining and subsequent phosphorylation of two independently synthesized precursors, 4-methyl-5-β-hydroxyethyl) thiazole phosphate (THZ-P) and 5-aminoimidazole ribotide, which can then be converted to pyrophosphate (HMP-PP) (Fig. 1A). Although analysis of the biosynthetic pathway for THZ-P is in the early stages, labeling studies have suggested that this moiety is derived from cysteine, tyrosine, and 1-deoxy-d-xylulose (7, 9, 15).

Our work is currently focused on the routes of HMP synthesis and their utilization under different growth conditions. Three genetically separable routes have been found to contribute to the synthesis of HMP in *S. typhimurium* (Fig. 1A). The first five steps of the well-characterized purine pathway form 5-aminoimidazole ribotide, which can then be converted to HMP by putative Thi gene products (14, 26). A second pathway to HMP, the alternative pyrimidine biosynthetic (APB) pathway, bypasses the requirement for purine biosynthetic enzymes under some growth conditions (10, 11, 27, 36). Finally, HMP can be generated via the pantothenate-dependent pyrimidine (PDP) pathway. It is important to emphasize that while for simplicity we refer to these routes as pathways, it is possible that some of the metabolites involved are products of side reactions of known enzymes and are not formed by enzymes dedicated to thiamine synthesis.

The PDP pathway appears to bypass function of the PurF enzyme, amidophosphoribosyl transferase (EC 2.4.2.14), suggesting that the product of this pathway is phosphoribosylamine (PRA) (12). Function of the PDP pathway required increased levels of exogenous pantothenate and a source of ribose-5-P, either supplied exogenously or generated by a functional oxidative pentose phosphate pathway (12, 13). The role of pantothenate in thiamine synthesis has remained unclear since its only demonstrated role is to function as a precursor to coenzyme A (CoA) (19).

We report here work initiated to define the role of pantothenate in thiamine synthesis. Mutations that allowed a purF mutant to grow independently of pantothenate or thiamine were isolated. Characterization of these mutations demonstrated that they were lesions in *sdh*, which encodes succinate dehydrogenase (SDH) (Fig. 1B). Evidence is presented that suggests that a block in Sdh causes an effect on thiamine metabolism through an alteration in the succinyl-CoA pool; this effect is distinct from the role of pantothenate in thiamine synthesis. A model for how this alteration allows pantothenate- and thiamine-independent growth is discussed.

Materials and Methods

Bacterial strains, media, and chemicals. All strains used in this study are derived from *S. typhimurium* LT2 and are listed with their respective genotypes in Table 1. The NCE medium of Berkowitz et al. (2) supplemented with 1 mM MgSO4 was used as minimal medium. The following carbon sources were added at the concentrations indicated: glucose, 11 mM; citrate, 11 mM; glycerol, 55 mM. Difco nutrient broth (8 g/liter) with NaCl (5 g/liter) added was used as rich medium. Difco BiTek agar was added to a final concentration of 1.5% for solid medium. When present in the culture media, and unless otherwise stated, the compounds were used at the following final concentrations: adenine, 0.4 mM; thiamine, 0.5 μM; pantothenate, 100 μM; lysine, 0.25 mM; methionine, 0.3 mM; diaminopimelic acid, 0.1 mM; succinate, 4 mM. The final concentrations of the antibiotics in rich or minimal medium were as follows: tetracycline, 20 or 10 μg/ml, respectively; kanamycin, 50 or 125 μg/ml, respectively; ampicillin, 30 or 15 μg/ml, respectively. Unless otherwise stated, all chemicals and enzymes were purchased from Sigma Chemical Co., St. Louis, Mo.

Genetic techniques. (i) Transduction methods. The high-frequency general transducing mutant of bacteriophage P22 (HT105/1, int-20f) (29) was used in all
FIG. 1. Pathway schematics. (A) Biosynthetic pathways involved in thiamine synthesis. Genes whose products are required for selected reactions are indicated next to the relevant arrows. Aside from the purine pathway, specific steps in the synthesis of HMP-PP and THZ-P are unknown. The positive role of pantothenate in the PDP pathway is indicated (12), along with the proposed precursor, ribose-5-P, and the product, phosphoribosylamine (13). The designation of 5-aminoimidazole ribotide as the product of the APB pathway is based on genetic data (27). Abbreviations: PRPP, phosphoribosylpyrophosphate; PRA, phosphoribosylamine; AIR, 5-aminoimidazole ribotide. (B) The TCA cycle and glyoxylate shunt (6). Hashed arrows represent multiple steps and indicate the role of succinyl-CoA in the synthesis of essential compounds.
supplemented only with adenine and tetracycline. The mutations identified in the agitation plates. The Tcr transductants were screened for growth on glucose medium was used to inoculate 5 ml of fresh medium.

were subcultured, 0.15 ml of a full-density culture (or equivalent number of cells) into DM2370 (purF2085 sucA sucCD211) by selecting Km r. The Km r transductants that retained the ::Tn10d(Tc) insertions in strains DM708 as described previously (34). Amplification was performed with Vent (exo-) polymerase (New England Biolabs, Inc., Beverly, Mass.) in a Thermolyne Temp-Ionic thermocycler. PCR conditions were denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1.5 min. This resulted in the amplification of an 870-bp fragment, one end of which was sequenced with the primer to the Tn10d(Tc).

Sequencing. Dideoxy sequencing (28) was performed with a Sequitimer cycle sequencing kit (Epicentre Technologies, Madison, Wis.) and [35S]dATP with a specific activity of 1,000 to 1,500 Ci/mmole (Dupon, Beverly, Mass.). Primers that hybridized to either side of the BamHII site in pBR328 were used to sequence the ends of the insert in pSDH. The −40 and reverse primers for M13mp19 (New England BioLabs, Inc., Beverly, Mass.) in a Thermolyne Temp-Ionic thermocycler. PCR conditions were denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1.5 min. This resulted in the amplification of an 870-bp fragment, one end of which was sequenced with the primer to the Tn10d(Tc).

Sucinate dehydrogenase assays. (i) Preparation of extracts. A 200-ml culture was grown in NCE glycerol medium to the late log phase (100 Klett units), harvested by centrifugation (10,000 × g, 10 min), and washed twice with cold 50 mM potassium phosphate buffer (pH 7.5). The cell pellets were resuspended in 1.5 ml of the same buffer plus 1 mM phenylmethylsulfonyl fluoride (Aldrich Chemical Co., Inc., Milwaukee, Wis.) and disrupted by sonication. Cell debris was removed by centrifugation at 10,000 × g for 30 min at 4°C.

(ii) Sdh assays. Sucinate dehydrogenase activity was measured by the method of Spencer and Guest (31). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.5), 3 mM KCN, 0.23 mM 2,6-dichlorophenol indophenol (DCIP), 2.18 mM N-methylphenazonium methosulfate, and crude extract (up to 0.2 mg of protein) in a final volume of 1 ml. Sample and reference cuvettes were allowed to equilibrate for 1 min, and the reduction was initiated by the addition of 10 mM succinate. The reduction of DCIP (molar extinction coefficient, 21 × 10^3 cm⁻¹) was monitored at 600 nm.

Computer analysis. BLAST (Basic Local Alignment Search Tool) was used to search for homologous DNA and protein sequences from the GenBank and SwissProt databases (1).

RESULTS

Initial observations. Both purF and purF apbA mutants can grow on glucose adenine medium supplemented with either thiamine or pantothenate (13). To identify the role of pantothenate in thiamine synthesis, derivatives of these mutants that could grow in the absence of thiamine or pantothenate were isolated (see Materials and Methods). To ensure that the APB pathway was not responsible for this thiamine-independent growth, we demanded that an apbA mutation (11) not eliminate growth of these mutants on glucose adenine medium. After screening 8,000 transductants, three mutants with the desired phenotype were identified, two in a purF background and one in a purF apbA background. Two of the three mutants contained point mutations, 40 and 46% linked to the selected
Tn10d(Tc) insertion (sdh-201 and sdh-202, respectively). In the third case, the mutant phenotype was 100% linked to the Tn10d(Tc) insertion, and thus the insertion was presumed to be causative [sdh-203::Tn10d(Tc)]. The identification of an insertion mutation was unexpected since the desired phenotype was a gain of function, i.e., a gained ability to make thiamine without exogenous pantothenate.

Lesions in sdh allow pantothenate- and thiamine-independent growth of a purF mutant. Further analysis determined that the three mutations mentioned above were lesions in sdh, which encodes succinate dehydrogenase, an enzyme of the tricarboxylic acid (TCA) cycle (Fig. 1B). Identification of these mutations as alleles of sdh was based on the following results. (i) Mutant phenotypes were complemented by either the sdh or frd genes. We determined that the three mutants described above were unable to use citrate as the sole carbon source for this operon (Fig. 2) (35). Interestingly, although these genes (Fig. 2). Sequence analysis of pFRD1 determined that the respective mutations mapped to the sdh region placed the three insertions at 17 centisomes, linked to a sucCD211::MudJ insertion (19, 20, and 32% linked, respectively). The sdhC203::Tn10d(Tc) insertion was mapped precisely by sequencing a PCR-amplified segment of DNA between this insertion and the gltA1718::MudJ insertion (Fig. 2). Sequence analysis of this fragment with a Tn10 primer determined that the Tn10d(Tc) insertion was within the sdhC gene as indicated in Fig. 2.

The effect of an Sdh block is reduced by an apbA mutation. The stimulatory effect of an sdh mutation on thiamine-independent growth in a purF and a purF apbA genetic background is shown in Fig. 3A and B, respectively. Strains containing the sdh mutation (DM708 and DM2378) grew in the absence of pantothenate or thiamine, i.e., on medium supplemented only with adenine. It was clear from the growth of these strains, however, that an sdh mutation did not completely eliminate the need for exogenous thiamine in the medium, particularly in the apbA derivative. A possible explanation for this growth pattern was that the Sdh block was reducing the amount of thiamine required by the cell, such that the intracellular reserves of TPP accumulated during growth on rich medium (nutrient broth) could sustain a longer period of growth. To address whether the growth of the strains containing sdh mutations was due to a decreased thiamine requirement or increased thiamine synthesis, we subcultured these strains into identical media. Both DM708 (purF sdh) and DM2378 (purF apbA sdh) exhibited greatly reduced thiamine-independent growth after subculturing (Fig. 3C and D). DM708 grew slightly in the absence of

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>SDH activity (nmol/min/mg)</th>
</tr>
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<tbody>
<tr>
<td>DM2358</td>
<td>zbg-8063::Tn10d(Tc)</td>
<td>31.9</td>
</tr>
<tr>
<td>DM2357</td>
<td>zbg-8063::Tn10d(Tc)</td>
<td>1.8</td>
</tr>
<tr>
<td>DM2360</td>
<td>zbg-8081::Tn10d(Tc)</td>
<td>36.3</td>
</tr>
<tr>
<td>DM2359</td>
<td>zbg-8081::Tn10d(Tc)</td>
<td>1.0</td>
</tr>
<tr>
<td>LT2</td>
<td>Wild type</td>
<td>33.1</td>
</tr>
<tr>
<td>DM2361</td>
<td>sdhC203::Tn10d(Tc)</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*Strains were grown in minimal medium containing glycerol as the sole carbon source to the late log phase (100 Klett units) and harvested by centrifugation. Crude extracts were prepared by sonication, and Sdh activity was determined by measuring succinate-dependent DCPIP reduction as described in Materials and Methods.
thiamine (Fig. 3C), but further subculturing eliminated this growth (data not shown). These results suggest that the effect of an Sdh block was at least partially due to a decrease in the cellular thiamine requirement. The more severe phenotype of the \textit{apbA} derivative (DM2378) could be explained if this strain required more thiamine because of loss of residual thiamine synthesis from the APB pathway under these conditions.

The results shown in Fig. 3 also indicate that although the \textit{sdh} mutations were isolated to mimic the effect of pantothenate, their effect was distinct, since both \textit{purF} and \textit{purF apbA} mutants grew continually when pantothenate was added to the medium in place of thiamine (data not shown). This distinction between the effects of exogenous pantothenate and \textit{sdh} mutations on thiamine synthesis was also observed in other experiments (see below).

Although the data described above were consistent with an Sdh block decreasing the amount of thiamine required for growth, the reason for this effect was not clear nor was it clear that this model accounted for all of our results. Subsequent experiments were performed to address these questions.

**Exogenous succinate mimics a block in Sdh.** Since loss of the Sdh enzyme had produced a gain-of-function phenotype, i.e., increased growth in the absence of thiamine, we pursued the idea that this effect was mediated by a metabolite of the Sdh reaction. A mutant blocked in Sdh might be expected to accumulate succinate (Fig. 1B). We found that exogenous succinate allowed both a \textit{purF} mutant (DM1936) and a \textit{purF apbA} mutant (DM587) to grow in glucose adenine medium supplemented with succinate. This growth closely paralleled that of the same strains containing a mutation in the \textit{sdh} gene (Fig. 3A and B); thus, these results were consistent with an \textit{sdh} mutation causing its effect through increased levels of succinate.

When the strains shown in Fig. 4A and B were subcultured into identical media, however, the result was distinct from that seen with strains containing the \textit{sdh} mutation. Although exogenous succinate did not allow thiamine-independent growth of DM587 (\textit{purF apbA}) upon reinoculation, it did allow continuous growth of DM1936 (\textit{purF}) (Fig. 4C and D and data not shown). This result suggested that if sufficient succinate was supplied, a \textit{purF} mutant synthesized enough thiamine via the APB pathway to allow growth in glucose adenine medium. By this scenario, an Sdh block would not result in continued thi-
amine-independent growth because succinate accumulation was not sufficient.

**Exogenous succinate increases thiamine-independent growth of thi mutants.** To directly test whether succinate and/or pantothenate was affecting thiamine metabolism by a reduction in the cellular thiamine requirement, we tested the effect of these compounds on the growth of two defined thiamine auxotrophs that were defective in distinct areas of thiamine synthesis. DM269 contains a mutation in *thiI* (33) and specifically requires the thiazole moiety of thiamine, and DM95 contains a polar mutation in *thiC* and will grow only in the presence of thiamine or TPP (34). The data shown in Table 4 indicate that the growth of both of these thiamine auxotrophs was limited by available TPP when exogenous thiamine concentrations were below 10 nM. When succinate was added to the medium, both DM269 and DM95 grew to higher cell densities under thiamine-limiting conditions. In contrast, exogenous pantothenate did not significantly affect the growth of either mutant. These results suggest that (i) the effect of succinate on the growth of purF derivatives was due to a general effect on the cellular thiamine requirement and was not specifically related to HMP synthesis and (ii) pantothenate did not affect the cellular thiamine requirement and thus had an effect on thiamine metabolism separate from that of succinate.

**A block in SucCD eliminates the effect of succinate.** The results described above were consistent with an *sdh* mutation causing its phenotype by reducing the cellular thiamine requirement through an accumulation of succinate. However, the mechanism by which succinate accumulation had this effect was unclear. One possibility was that succinate accumulation is to serve as a transient intermediate in lysine, methionine, and DAP biosynthesis (16, 25). Exogenous addition of these compounds would be expected to spare succinyl-CoA pools by eliminating the cellular requirement for succinyl-CoA. The effect of exogenous lysine, methionine, and DAP on the thiamine-independent growth of several thiamine auxotrophs is shown in Table 5. Three points can be made from these data. (i) Although some effects on thiamine-independent growth were observed when these compounds were added separately, the most dramatic changes in growth were seen when the three succinyl-CoA-dependent compounds were added together. (ii) As with previous experiments, the thiamine-independent growth observed was more pronounced in DM1936 (*purF*) than in DM587 (*purF apbA*). (iii) Unlike the succinyl-CoA-derived compounds that extended thiamine-independent growth in all of the thiamine-requiring strains, exogenous pantothenate allowed thiamine-independent growth only in *purF* and *purF apbA* mutants.

In a modification of the experiment described above, we

### Table 4. Succinate allows increased thiamine-independent growth of thi mutants

<table>
<thead>
<tr>
<th>Strain (genotype)</th>
<th>Medium</th>
<th>( A_{650} ) after 24 h of growth with thiamine supplement at final concn of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 ( \mu M )</td>
</tr>
<tr>
<td>DM95 (<em>thiC885::MudJ</em>)</td>
<td>Minimal glucose</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>Minimal glucose succinate</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>Minimal glucose pantothenate</td>
<td>1.00</td>
</tr>
<tr>
<td>DM269 (<em>thiI887::Tn10d(Tc)</em></td>
<td>Minimal glucose</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>Minimal glucose + succinate</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>Minimal glucose + pantothenate</td>
<td>1.01</td>
</tr>
</tbody>
</table>

* A 0.15-ml volume of an overnight culture resuspended in saline was used to inoculate 5 ml of the indicated medium. Strains were grown at 37°C in a rotary shaker at 250 rpm. Data shown are representative of three independent experiments.

### Table 5. Compounds that spare succinyl-CoA increase growth of thiamine auxotrophs under thiamine-limiting conditions

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>( A_{650} ) after 24 h of growth in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose Ade</td>
<td>Glucose Ade Lys</td>
</tr>
<tr>
<td>DM1936</td>
<td>purF2085</td>
<td>0.318</td>
</tr>
<tr>
<td>DM587</td>
<td>purF2085 apbA7::Tn10d(Tc)</td>
<td>0.057</td>
</tr>
<tr>
<td>DM95</td>
<td>thiC885::MudJ</td>
<td>0.020</td>
</tr>
<tr>
<td>DM269</td>
<td>thiI887::Tn10d(Tc)</td>
<td>0.141</td>
</tr>
</tbody>
</table>

* A 0.15-ml volume of an overnight culture resuspended in saline was used to inoculate 5 ml of the indicated medium. Strains were grown at 37°C in a rotary shaker at 250 rpm. Data shown are representative of three independent experiments. Abbreviations: Ade, adenine; Met, methionine; Lys, lysine; Pan, pantothenate; Thi, thiamine.
found that addition of succinyl-CoA-derived compounds also stimulated the growth of thi auxotrophs after their growth had ceased because of thiamine starvation. When DM95 and DM269 were grown for 24 h in limiting concentrations (1 mM) of thiamine, their growth ceased at optical densities of 0.175 and 0.245, respectively. The cell densities increased to 0.360 and 0.440, respectively, in another 24 h after addition of lysine, methionine, and DAP, while those of the control culture did not change significantly. This result suggested that TPP-starved cells ceased growth because of the lack of succinyl-CoA-derived metabolites.

**Succinyl-CoA is not required for PurF-independent thiamine synthesis.** Although the results presented above strongly suggested that the accumulation of succinyl-CoA affected growth of thiamine-requiring mutants by altering the cell’s thiamine requirement, the possibility of succinyl-CoA being required for PurF-independent thiamine synthesis had not been ruled out. To test this idea, we constructed a strain unable to synthesize succinyl-CoA by any known route. This triple mutant (DM2408) was defective in the two enzymes able to generate succinyl-CoA, α-ketoglutarate dehydrogenase (SucAB) and succinyl-CoA synthetase (SucCD), in addition to PurF (Fig. 1B). As expected, this triple mutant required adenine, lysine, methionine, and DAP for growth. Significantly, this strain did not require thiamine, despite repeated reinoculations. This result confirmed that succinyl-CoA was not required for PurF-independent thiamine synthesis.

**DISCUSSION**

This work was initiated to identify the role of pantothenate in thiamine synthesis in *S. typhimurium*. We isolated derivatives of a purF mutant that grew in the absence of pantothenate or thiamine and found that they were defective in the reaction catalyzed by the Sdh enzyme. Further characterization of these mutants led us to conclude that the phenotype of purF sdh mutants was not due to pantothenate-independent thiamine synthesis but rather to a decrease in the cellular thiamine requirement. This conclusion was based primarily on two results: (i) growth of strains containing sdh mutations did not continue after subculturing into medium lacking pantothenate or thiamine, and (ii) conditions that imitated the effect of an sdh mutation, i.e., exogenous succinate, or lysine, methionine, and DAP, appeared to have a general effect on the thiamine requirement as evidenced by their ability to increase the growth of various thi mutants under thiamine-limiting conditions.

Based on results described herein, we propose a model to explain how an sdh mutation reduces the amount of thiamine required for growth. The model contains the following key points. (i) The α-ketoglutarate dehydrogenase enzyme is required for synthesis of biosynthetic precursors under aerobic conditions on glucose and sequesters a significant portion of cellular TPP. (ii) Conditions that either increase TPP-independent synthesis of the product of this reaction, succinyl-CoA, or eliminate the need for succinyl-CoA as an essential metabolite decrease the TPP requirement of the cell. (iii) An sdh mutation acts by preventing conversion of the succinate–succinyl-CoA pool into other cellular metabolites, i.e., subsequent TCA cycle intermediates. The Sdh block thus restricts use of succinyl-CoA to biosynthetic purposes, decreasing the need for TPP-dependent synthesis of succinyl-CoA.

One result from this study appeared inconsistent with the model described above. Although a lesion in succinyl-CoA synthetase (SucCD) would be expected to prevent conversion of the succinyl-CoA pool to other metabolites in a way similar to that of an Sdh block, a sucCD mutation did not result in a similar stimulation of the growth of a purF mutant. In fact, the thiamine-independent growth of a purF sdh sucCD mutant was identical to that of a purF mutant (data not shown), suggesting that a functional SucCD enzyme is required for the effect of an Sdh block. An attractive explanation for this result is that there is a source of succinate in the cell other than the TCA cycle, and this succinate contributes to the succinyl-CoA pool. Evidence for an alternative source of succinate has been reported in *Escherichia coli* (4, 5); our results are consistent with this conclusion.

The work described herein provides an explanation for several previous reports in the literature that cite positive effects of succinate or of lysine and methionine on the growth of thiamine auxotrophs (4, 23, 24). Addition of succinyl-CoA-derived compounds stimulated the growth of thi auxotrophs after their growth had ceased because of thiamine starvation. From these results, we concluded that cells starved for TPP stop growing because they lack compounds whose synthesis requires succinyl-CoA (e.g., lysine, methionine, and DAP).

Our results allowed us to conclude that although sdh mutations and pantothenate both stimulate thiamine-independent growth of a purF mutant, their effects on thiamine metabolism are distinct. We present substantial evidence indicating that an Sdh block decreases the cellular thiamine requirement, but our data do not support a similar role for pantothenate. Exogenous pantothenate allowed continuous thiamine-independent growth of purF and purF apbA mutants yet had no effect on defined thi mutants. These results are consistent with pantothenate stimulating thiamine synthesis in the absence of the PurF enzyme, although the mechanism of this stimulation remains unclear.

This study has increased our understanding of the requirement for thiamine in general metabolism. We have presented data showing that the need for α-ketoglutarate dehydrogenase function influences the cellular TPP requirement, even under conditions where this enzyme is strongly repressed (aerobic growth on glucose) (21, 30). In addition, we have shown that a block in another step of the TCA cycle, that catalyzed by succinate dehydrogenase, decreases the need for α-ketoglutarate dehydrogenase function. In addition, our data indicated TPP-starved cells cease growth because they lack essential succinyl-CoA-derived compounds. Thus, while the specific role of pantothenate in thiamine synthesis remains unclear, this work has resulted in an increased understanding of key aspects of thiamine metabolism in *S. typhimurium*.

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