Supporting Information

Kim and Copley 10.1073/pnas.1208509109

SI Materials and Methods

Bacterial Cultures. Bacteria were grown in LB or M9/glucose medium supplemented with kanamycin (20 μg/mL), ampicillin (100 μg/mL), and/or chloramphenicol (35 μg/mL), depending on the antibiotic resistance genes present in the strain. Glucose (0.2% wt/vol) and NH_4Cl (0.1% wt/vol) were added to all M9 media (1). Media described below are designated by LB or M9, followed by K, A, and/or C to indicate inclusion of kanamycin, ampicillin, or chloramphenicol, respectively.

Cultures were grown from a single colony transferred from a freshly streaked LB agar plate into 3 mL of LB containing an appropriate antibiotic until they attained an OD_{600} of 0.5. A 1-mL aliquot was harvested by centrifugation, washed five times with ice-cold PBS (pH 7.4), and resuspended in PBS. Aliquots of washed cells were streaked onto agar plates containing M9/glucose and an appropriate antibiotic, and the plates were incubated at 37 °C. Alternatively, washed cells were diluted in M9/glucose containing an appropriate antibiotic to an initial OD_{600} of 0.001. Cultures (100 μL) were incubated at 37 °C in a 96-well plate, and growth was monitored at 600 nm using a Varioskan plate reader (Thermo Scientific).

Complementation of the Δ*pdxB* strain with genes involved in the serendipitous pathway (*yeaB* and *thrB*) and with *pdxB* as a control was performed using the indicated genes cloned into pTrcHisB as previously described (2).

Preparation of Overexpression Clones. Genomic DNA from *Escherichia coli* BW25113 was used to amplify *serA*, *serB*, *cysE*, *cysM*, and *cysK*. The serine-insensitive allele of *serA* (a1090g, a1091c) was amplified from strain JU430 (2). Primers used for PCR amplification of genes from genomic DNA of *E. coli* BW25113 are listed in Table S1. Genes were amplified using Phusion High Fidelity DNA polymerase (Thermo Scientific). *serA*, *serA* (a1090g, a1091c), *serB*, *cysE*, *cysM*, and *cysK* were cloned into pET46 (EMD Biosciences) by ligation-independent cloning following the manufacturer's protocol (Novagen) under control of an isopropyl-β-D-thiogalactopyranoside (IPTG)–inducible T7 promoter, resulting in incorporation of a His_6 tag at the N terminus of each protein. *ilvBN* and *ilvIH* were cloned into pTrcHisB (Invitrogen) under control of an IPTG-inducible *trc* promoter, resulting in incorporation of an N-terminal His_6 tag on the catalytical subunits. The amplified PCR fragments and the vector were digested with BamHI and EcoRI for *ilvBN* or with XhoI and EcoRI for *ilvIH*, and the digested products were ligated using T4 DNA ligase.

Preparation of Enzymes. A 5-mL culture of LBA was inoculated with a single colony carrying an expression plasmid and grown overnight at 37 °C. The starter culture was transferred to 500 mL of LBA in a 2-L flask, and the culture was incubated with shaking at 200 rpm and 37 °C. After the OD_{600} reached 0.5, the culture was cooled in an ice bath. IPTG was added to give a final concentration of 0.5 mM, except that 0.1 mM IPTG was used to induce overexpression of *ilvBN* and *ilvIH*. The culture was shaken for 3 h at 200 rpm at 30 °C. The ASKA (A Complete Set of *E. coli* K-12 ORF Archive) clone expressing *ilvA* was grown as described above, except that LBC medium was used.

Cells were harvested by centrifugation at 3,500 × *g* at 4 °C for 20 min and washed twice with PBS. Cell pellets were stored at −80 °C before use. Cell pellets were resuspended in Bugbuster (5 mL/g of cells; Novagen, EMD Chemicals,) with added benzonase (final concentration of 25 U/mL; Novagen, EMD Chemicals) and lysozyme (final concentration of 1 mg/mL; Sigma). FAD was added to give a final concentration of 75 μM in all buffers for purification of the two isozymes of acetohydroxyacid synthase, IlvIH and IlvBN, to help retain the small regulatory subunit (3). PMSF was added to a final concentration of 1 mM when *E. coli* K12 AG1, the Δ*ilvB* strain, or the Δ*ilvI* strain was used for protein overproduction. After incubation for 20 min at room temperature, cell extracts were centrifuged at 20,000 × *g* for 20 min at 4 °C. The supernatant was loaded onto a 5-mL Ni-agarose column (Amersham Bioscience Corporation) that had been equilibrated with 50 mM potassium phosphate (pH 6.8) containing 0.5 M KCl and 50 mM imidazole. After the column was washed with the same buffer, bound proteins were eluted with 100 mL of 50 mM potassium phosphate (pH 6.8) containing 0.5 M KCl, followed by a linear gradient from 50 mM to 500 mM imidazole at a flow rate of 5 mL/min. Fractions were collected and analyzed on an SDS-12% polyacrylamide gel.

All enzymes were exchanged into appropriate reaction buffers using a Millipore centrifugal ultra-filtration unit (30,000-Da cutoff) before activity assays. Protein concentrations were determined by Bradford assay using BSA as a standard (4). For multimeric proteins, concentrations reported refer to the concentration of monomer. The purity of each protein was assessed by electrophoresis using an SDS-12% polyacrylamide gel with Coomassie Blue. No contaminating bands were observed for 3-phospho-D-glycerate dehydrogenase (SerC), SerB, CysE, CysM, CysK, IlvBN, and IlvIH. SerA, SerA N364A, and IlvA were judged to be >95% pure by SDS/PAGE.

Assays of Glyceraldehyde 3-Phosphate Dehydrogenase Activity in Cell Extracts. WT *E. coli* was grown to log phase in 500 mL of M9/glucose at 37 °C and harvested by centrifugation at 3,500 × *g* at 4 °C for 16 min when the OD_{600} reached 0.1. Cells were washed twice with ice-cold PBS. A separate batch of culture was treated with 1 mM 3-hydroxypyruvate (3HP) when the OD_{600} reached 0.1 and was incubated for 1 h before harvesting. Essentially no change in OD_{600} was observed after addition of 3HP. A third batch of culture was treated with 1.6 mM serine hydroxamate for 1 h before harvesting. Cell pellets were stored at −80 °C before use. Cell pellets (50 mg) were resuspended in 300 μL of PBS containing protease inhibitor mixture [used to give 1.2 mM AEBSF (4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride), 5 mM EDTA, 0.1 mM Bestatin, 15 μM E-64, and 15 μM Pepstatin A; Research Product International]. After disruption of the cells by sonication (2 × 4 min), samples were subjected to centrifugation at 16,000 × *g* for 10 min at 4 °C to remove particulate material. An aliquot of the supernatant was used to determine the protein concentration using the Bradford assay. Glyceraldehyde-3-phosphate dehydrogenase activity was followed by measuring the formation of NADH at 340 nm in 50 mM potassium phosphate buffer (pH 7.6) at 25 °C with 1 mM NAD, 1 mM glyceraldehyde-3-phosphate, and 1 mg/mL protein.

1. Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning; A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), 2nd Ed.
2. Kim J, Kershner JP, Novikov Y, Shoemaker RK, Copley SD (2010) Three serendipitous pathways in *E. coli* can bypass a block in pyridoxal-5′-phosphate synthesis. *Mol Syst Biol* 6:436.
3. Schloss JV, Van Dyk DE (1988) Acetolactate synthase isozyme II from *Salmonella typhimurium*. *Methods Enzymol* 166:445–454.
4. Bradford MM (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.

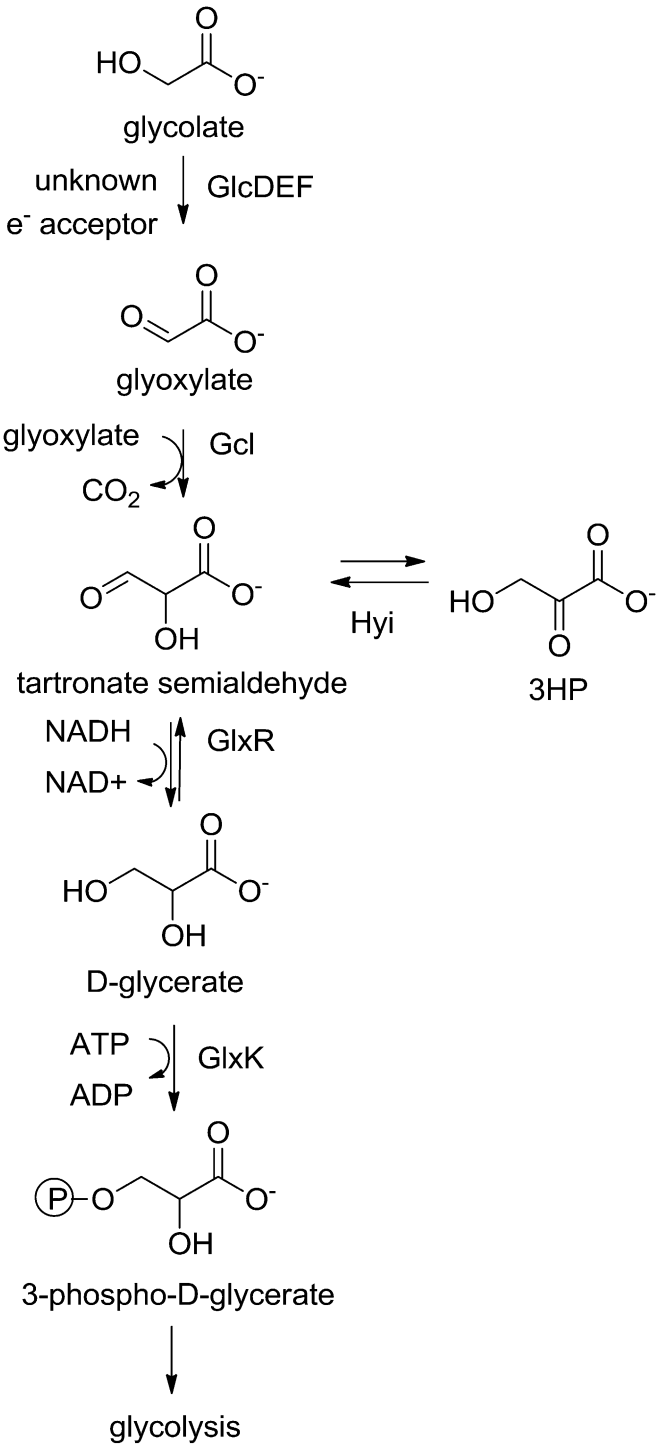

Fig. S1. Glycolate degradation pathway.

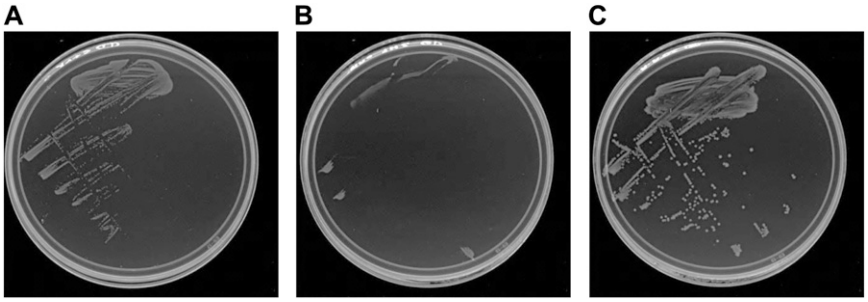

Fig. S2. Growth of WT *E. coli* K12 on glucose alone (*A*), on glucose containing 1 mM 3HP (*B*), and on glucose containing 1 mM 3HP and 20 amino acids (*C*). Plates were incubated for 18 h at 37 °C before pictures were taken. Concentrations of amino acids were as follows: Ala (80 μM), Arg (0.52 mM), Asn (40 μM), Asp (40 μM), Cys (10 μM), Glu (60 μM), Gln (60 μM), Gly (80 μM), His (20 μM), Ile (40 μM), Leu (80 μM), Lys (40 μM), Met (20 μM), Phe (40 μM), Pro (40 μM), Ser (1 mM), Thr (40 μM), Trp (10 μM), Tyr (20 μM), and Val (60 μM).

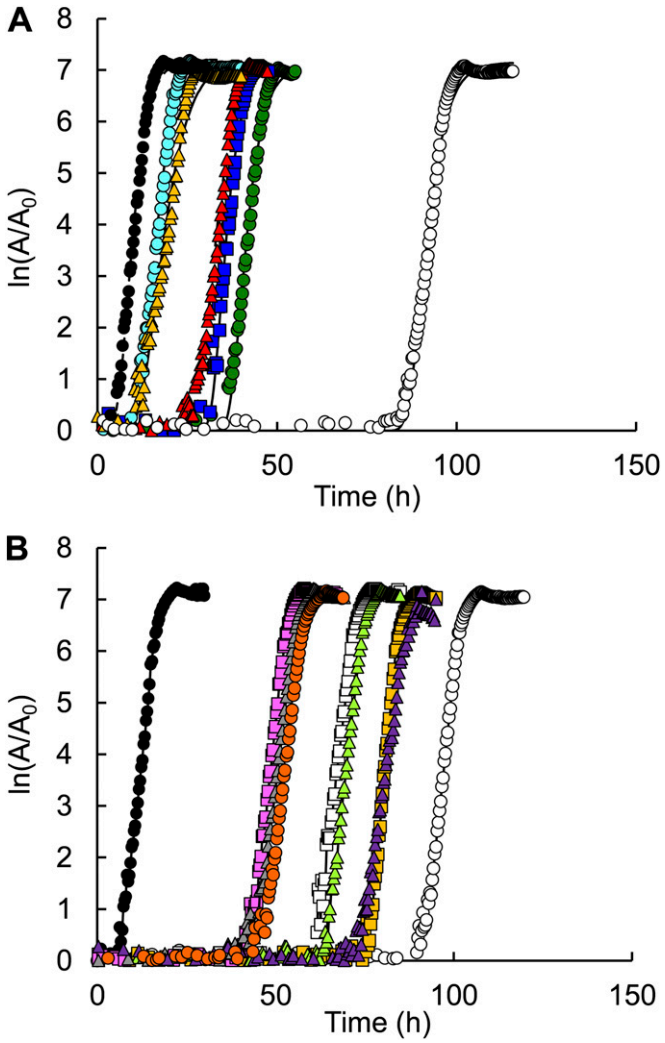

Fig. S3. Growth of *E. coli* K12 on M9/glucose with no supplement (●), with 1.0 mM 3HP (○), and with 1.0 mM 3HP and amino acids as follows: 20 μM Leu (turquoise ○), 10 μM Cys (yellow △), 1 mM Ser (red △), 60 μM Val and 40 μM Ile (blue □), and 40 μM Ile (green ○) (*A*) or 20 μM Met (pink □), 40 μM Lys (gray △), 0.52 mM Arg (orange ○), 10 μM Trp (□), 60 μM Glu (green △), 40 μM Asp + 40 μM Asn (yellow □), and 40 μM Pro (purple △) (*B*). Growth curves with other amino acids not shown above are between that with Pro and that with no supplement. The effect of valine cannot be tested individually because the two isozymes of acetohydroxyacid synthase that are present in *E. coli* K12 are subject to feedback inhibition by valine. Thus, addition of valine shuts down synthesis of all branched chain amino acids. Because leucine is made from valine, the major consequence is inhibition of isoleucine synthesis (1) and the cells cannot grow. Consequently, we tested the effect of valine in the presence of isoleucine. A = OD_{600}.

1. Lawther RP, et al. (1981) Molecular basis of valine resistance in *Escherichia coli* K-12. *Proc Natl Acad Sci USA* 78:922–925.

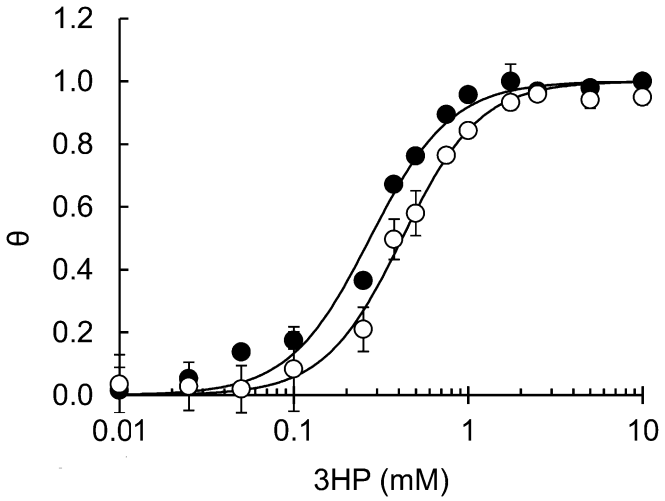

Fig. S4. Inhibition of the α-ketoglutarate reductase activity of SerA (●) and the serine-insensitive mutant N364A SerA (○) by 3HP. The degree of inhibition (θ) is calculated according to the method of Grant et al. (1) by fitting data to the Hill equation $[\theta = [I]^n/([I_{0.5}]^n + [I]^n)]$, where [I] is the concentration of inhibitor, n is the Hill coefficient, and $I_{0.5}$ is the concentration of the inhibitor that produces 50% inhibition.

1. Grant GA, Hu Z, Xu XL (2005) Identification of amino acid residues contributing to the mechanism of cooperativity in *Escherichia coli* D-3-phosphoglycerate dehydrogenase. *Biochemistry* 44:16844–16852.

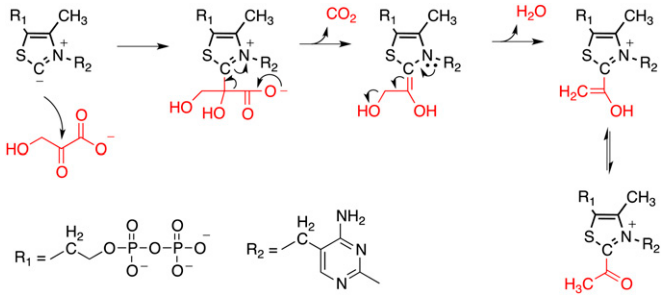

Fig. S5. Postulated mechanism for the formation of acetyl-thiamin pyrophosphate (TPP) adduct with TPP and 3HP.

Table S1. Primers used for PCR amplification of genes and construction of overexpression vectors

Plasmid and overproduced protein		Primers (5′ to 3′) for PCR amplification
pET46-SerA and N364A SerA	Forward	5′-GAC GAC GAC AAG ATG GCA AAG GTA TCG CTG GAG
	Reverse	5′-GAG GAG AAG CCC GGT TAT TAG TAC AGC AGA CGG GCG C
pET46-SerB	Forward	5′-GAC GAC GAC AAG ATG CCT AAC ATT ACC TGG TGC GAC
	Reverse	5′-GAG GAG AAG CCC GGT TAT TAC TTC TGA TTC AGG CTG CCT G
pET46-CysE	Forward	5′-GAC GAC GAC AAG ATG TCG TGT GAA GAA CTG GAA AT
	Reverse	5′-GAG GAG AAG CCC GGT TAG ATC CCA TCC CCA TAC TC
pET46-CysM	Forward	5′-GAC GAC GAC AAG ATG AGT ACA TTA GAA CAA ACA
	Reverse	5′-GAG GAG AAG CCC GGT TAA ATC CCC GCC CCC TGG C
pET46-CysK	Forward	5′-GAC GAC GAC AAG ATG AGT AAG ATT TTT GAA GA
	Reverse	5′-GAG GAG AAG CCC GGT TAC TGT TGC AAT TCT TTC TC
pTrcHisB-IlvBN	Forward	5′-TCA ATT GGA TCC GGC AAG TTC GGG CAC AAC ATC G
	Reverse	5′-TTA TCC GAA TTC TTA TTA CTG AAA AAA CAC CGC
pTrcHisB-IlvIH	Forward	5′-TCA ATT CTC GAG GGA GAT GTT GTC TGG AGC CGA G
	Reverse	5′-TTA TCC GAA TTC TTA TTA ACG CAT TAT TTT ATC G

Table S2. Activity of glyceraldehyde 3-phosphate dehydrogenase in cell extracts

Condition	Activity (μmol/s·mg of protein)
Exponential growth on glucose	82 ± 19
1 h after treatment with 1 mM 3HP	1.9 ± 0.8
1 h after treatment with 1.6 mM serine hydroxamate	28 ± 5