A Novel Metabolic Cycle Catalyzes Glucose Oxidation and Anaplerosis in Hungry Escherichia coli*

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Complete oxidation of carbohydrates to CO₂ is considered to be the exclusive property of the ubiquitous tricarboxylic acid cycle, the central process in cellular energy metabolism of aerobic organisms. Based on metabolism-wide in vivo quantification of intracellular carbon fluxes, we describe here complete oxidation of carbohydrates via the novel P-enolpyruvate (PEP)-glyoxylate cycle, in which two PEP molecules are oxidized by means of acetyl coenzyme A, citrate, glyoxylate, and oxaloacetate to CO₂ and one PEP is regenerated. Key reactions are the constituents of the glyoxylate shunt and PEP carboxykinase, whose conjoint operation in this bi-functional catabolic and anabolic cycle is in sharp contrast to their generally recognized functions in anaplerosis and gluconeogenesis, respectively. Parallel operation of the PEP-glyoxylate cycle and the tricarboxylic acid cycle was identified in the bacterium Escherichia coli under conditions of glucose hunger in a slow-growing continuous culture. Because the PEP-glyoxylate cycle was also active in glucose excess batch cultures of an NADPH-overproducing phosphoglucose isomerase mutant, one of this new central pathway may be the decoupling of catabolism from NADPH formation that would otherwise occur in the tricarboxylic acid cycle.

Structuring of cellular networks into pathways with distinct functions is pivotal for comprehension of “textbook” biochemistry. The ability of existing model pathways to portray flux through complex metabolic networks, however, is an open question that is just beginning to be addressed theoretically (1–3) and experimentally (4, 5). Microbial growth on the most abundant carbon-source glucose represses transcription of metabolic functions that are required on alternative carbon sources. This universal phenomenon is referred to as catabolite repression and includes a number of mechanistically distinguishable but physiologically related regulation mechanisms (6, 7). Although catabolite repression is strong under conditions of feast with excess glucose, microbes typically thrive under conditions of starvation (absence of nutrients) or hunger (suboptimal supply of nutrients) in their natural environments (8, 9). This metabolic state of hunger, between optimal growth and starvation, can be studied in glucose-limited continuous (chemostat) cultures with very low glucose concentrations at a rate of growth that is controlled by the experimenter. Catabolite repression is absent under the severe glucose limitation in slow growing chemostat cultures (9–11), and, as a consequence, increased in vivo activity of repressed metabolic enzymes is often observed with advanced methods of metabolic flux analyses based on 13C-labeling experiments (4, 5, 12, 13).

Here we elucidate metabolic impacts of severe and absent catabolite repression during growth of Escherichia coli in glucose-excess batch cultures and glucose-limited chemostat cultures. Direct analytical interpretation of 13C-labeling patterns in proteinogenic amino acids was used to establish the network topology of active reactions and to quantify the relative contributions of converging pathways to the formation of target metabolites (14, 15) (Fig. 1). In addition to this local biochemical analysis, we quantified in vivo reaction rates by comprehensive isotope labeling balancing with a computer model that maps metabolic fluxes to all available 13C-labeling and physiological data (16, 17). These network analyses provide a system-wide perspective on carbon traffic in E. coli that is at present the exclusive methodology to quantify in vivo activity of the identified P-enolpyruvate (PEP)-glyoxylate cycle in central metabolism.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions—E. coli MG1655 (λ−F rph−1; Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany) cultures of 0.8 liter were grown in a 2-liter bioreactor (LH-Inceltech) under fully aerobic conditions at 37 °C and pH 7.0 (18). Labeling experiments with a phosphoglucose isomerase deletion mutant of MG1655 were done with fully aerobic 50-ml cultures in 500-ml shake flasks on a rotary shaker at 37 °C. Batch cultures were grown in M9 minimal medium (15) supplemented with 0.6 g/liter [U-13C]glucose (>98%; Isotec, Miamisburg, OH) and 2.4 g/liter natural glucose as the sole carbon source in bioreactors, or 1.5 g/liter [U-13C]glucose and 1.9 g/liter natural glucose in shake flasks. An exponentially growing preculture in the same medium was used to inoculate, with a starting optical density at 600 nm (A₆₀₀) below 0.005. Biomass for GC-MS and in vitro enzyme analysis was harvested during the mid-exponential growth phase at A₆₀₀ 1–1.5.

Continuous cultures in the chemostat mode were grown in a modified M9 medium (12) with 5 g/liter glucose as the growth-limiting component at constant dilution rates between 0.12–0.4 h⁻¹. The fermentation volume was kept constant by a weight-controlled pump. The 13C-labeling experiment was initiated after the chemostat culture was in a physiological steady state after about five culture volume changes. The feed medium was then replaced for one volume change by an identical medium that contained 1 g/liter [U-13C]glucose and 4 g/liter natural glucose.

Analytical Procedures—Cellular dry weight, glucose, acetate, CO₂, and O₂ concentrations were determined as described before (18). Crude cell extracts for in vitro enzyme assays were prepared from pellets of 10-ml culture aliquots. Pellets were resuspended in 2 ml of 0.9% (w/v) NaCl and 10 mM MgSO₄ and disrupted by sonication on ice for 5 min at 60 W. Isocitrate dehydrogenase (EC 1.1.1.42), PEP carboxylase (EC 4.1.1.31), and PEP carboxykinase activities were monitored spectrophotometrically in proteinogenic amino acids was used to establish the network topology of active reactions and to quantify the relative contributions of converging pathways to the formation of target metabolites (14, 15) (Fig. 1). In addition to this local biochemical analysis, we quantified in vivo reaction rates by comprehensive isotope labeling balancing with a computer model that maps metabolic fluxes to all available 13C-labeling and physiological data (16, 17). These network analyses provide a system-wide perspective on carbon traffic in E. coli that is at present the exclusive methodology to quantify in vivo activity of the identified P-enolpyruvate (PEP)-glyoxylate cycle in central metabolism.

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1 The abbreviations used are: PEP, P-enolpyruvate; GC-MS, gas chromatography-mass spectrometry.
2 F. Canonaco and U. Sauer, unpublished data.
by centrifuging the crude cell extracts for 45 min at 150,000 × g. Isocitrate lyase (EC 4.1.3.1) activity was monitored by following the rate of NADPH production or NADH consumption at 340 nm, assuming an extinction coefficient of 6.2 mM cm⁻¹. Acetyl-CoA was added at 1 mM as an allosteric activator of the PEP carboxykinase assay. Contaminating oxidases were removed by centrifuging the crude cell extracts for 45 min at 150,000 × g. Isocitrate lyase (EC 4.1.3.1) activity was monitored by following the rate of NADPH production or NADH consumption at 340 nm, assuming an extinction coefficient of 6.2 mM cm⁻¹. The protein content in crude extracts was determined with the biuret reaction.

**Determination of Physiological Parameters**—In batch culture, specific uptake and secretion rates were determined during the exponential growth phase as the coefficients of the substrate or product concentration versus biomass concentration divided by the growth rate. In chemostat culture, dilution and thus growth rate are constant. Hence, specific consumption and production rates were determined from the concentration difference in the feed medium (or air) and the culture (or gas) effluent, normalized to the steady-state biomass concentration. The macromolecular biomass composition for metabolic flux analysis was calculated from the linear correlation of cellular protein and RNA content with growth rate (18). The corresponding fractions were extrapolated from the experimental data of E. coli in glucose-limited chemostat cultures (12). Thus, the respective calculated relative fractions of protein and RNA in total biomass were 51.1 and 26.7% during unrestricted growth and 70.1 and 4.7% during glucose-limited growth at a dilution rate of 0.12 h⁻¹. The remaining fraction of biomass was assigned to minor macromolecules and was assumed to be independent of the growth rate (12).

**Metabolic Network Analysis**—Mass spectrometry of amino acids from hydrolyzed biomass followed exactly a protocol published previously (15). Mass spectra of the derivatized amino acids were corrected for the natural abundance of all stable isotopes. In the chemostat culture, the fraction of partially 13C-labeled biomass (f) was calculated according to a first-order washout kinetic: 

\[ f_{\text{lab}} = 1 - e^{-\frac{t}{D}} \]

where D is the dilution rate, and t is time. Previously described algebraic equations were then used to calculate ratios of intracellular fluxes using so-called metabolic flux ratio analysis by GC-MS (15). Briefly, flux ratio analysis compares the 13C-labeling pattern in two or three intracellular metabolites that serve as building blocks for proteinogenic amino acids (5, 14). This biochemical approach quantifies the relative contribution of converging pathways or reactions to several metabolic intermediates from mass distributions in amino acids, thus providing direct evidence for a particular flux. The approach used here is different from previous mathematical models that typically describe the relationship between isotope ratios and the parameters of one or few pathways or cycles (22, 23): it provides a more comprehensive view with several independent flux ratios from different metabolic nodes (15, 24).

A comprehensive isotopomer model of bacterial central carbon and amino acid metabolism (12, 16) was used to estimate intracellular net fluxes from experimentally determined fluxes in and out of the cell, the macromolecular biomass composition, and the mass isotopomer distributions in the amino acids. For this purpose, we augmented the previously described bioreaction network of E. coli (12) with the glyoxylate shunt (Fig. 1). The Entner-Doudoroff pathway was not included in the network model because it was found to be inactive in separate experiments with 100% [1-13C]glucose (15) under the conditions investigated here (data not shown). The isotopomer model was used to simulate expected GC-MS measurements as a function of a given flux distribution that was randomly chosen. The best-fit intracellular fluxes were then estimated by minimizing the deviation between experimentally determined and simulated MS data, using an iterative scheme for minimization of the χ² error criterion with a simulated annealing search algorithm (16). The resulting calculated set of fluxes, which also includes exchange fluxes, can be taken as the best estimate for the metabolism-wide flux distribution and thus the intracellular in vivo reaction rates (4, 16, 17). In contrast to the strictly local interpretation of selected MS data by flux ratio analysis, isotopomer balancing thus affords an integrative and complementary interpretation of all 13C-labeling and physiological data (12). The uniqueness of a flux solution was verified by estimating at least five independent solutions with equal error criteria that were obtained from random starting points.

**RESULTS**

**Metabolic Flux Ratio Analysis by GC-MS—E. coli** wild-type MG1655 was grown under severe catabolite repression in batch culture with excess glucose and gradually decreasing catabolite repression in glucose-limited chemostat cultures at growth rates between 0.4–0.1 h⁻¹, respectively. Extracellular concentrations of the catabolite-repressing sugar glucose were above 1 g/liter in the batch experiment and below the detection limit of 20 mg/liter in all chemostats, which is well below the concentration that exerts cAMP-mediated catabolite repression (9–11). The physiological consequence was quantitative conversion of glucose to CO₂ and biomass in the chemostat cultures but significant overflow metabolism to acetate in the batch culture (data partly shown in Table 1). Although about 20% of the available carbon was secreted as acetate, the biomass yield on glucose was significantly higher in the batch culture.

To elucidate the metabolic impact of catabolite repression, independent ratios of converging carbon fluxes to intracellular metabolites were quantified by GC-MS analysis of 13C-labeling pattern in proteinogenic amino acids (15). The flux ratios obtained for the batch culture were consistent with those reported previously from NMR-based (14, 25) and MS-based (15) flux ratio analysis: (i) moderate contribution of the pentose phosphate pathway to glucose catabolism (PEP from pentoses); (ii) balanced contribution of catabolic and anabolic fluxes to oxaloacetate synthesis; and (iii) absence of glyoxylate shunt activity (Fig. 2). A very different mode of metabolic operation was identified in the slow-growing chemostat culture (Fig. 2). First, about 20% of the PEP molecules originated from oxaloacetate through the catabolite-repressed gluconegoric PEP carboxykinase (Fig. 2). Second, only a negligible fraction of oxaloacetate originates from PEP, thus demonstrating the absence of the reverse PEP carboxykinase reaction and, unexpectedly, also the absence of the anaplerotic PEP carboxylase that replenishes tricarboxylic acid cycle intermediates consumed for amino acid biosynthesis. Third, about 80% of the oxaloacetate molecules were derived through the glyoxylate shunt in a unidirectional fashion because no isocitrate molecules originated from succinate and glyoxylate. This complete switch in anaple-
The PEP-glyoxylate Cycle in Escherichia coli

Table I
Physiological parameters of batch and glucose-limited chemostat cultures of E. coli

| Growth condition | Specific growth rate | Specific glucose uptake rate | Specific O₂ uptake rate | Specific CO₂ formation rate | Specific acetate formation rate | Biomass yield on glucose |
|------------------|----------------------|------------------------------|-------------------------|-----------------------------|-------------------------------|-------------------------|
| Batch            | 0.85 ± 0.05          | 11.3 ± 0.2                   | 18.8 ± 0.8              | 19.6 ± 0.5                   | 7.1 ± 1                       | 0.42 ± 0.05             |
| Chemostat        | 0.12 ± 0.01          | 2.3 ± 0.1                    | 8.7 ± 0.8               | 9.0 ± 0.5                    | <0.5                          | 0.29 ± 0.03             |

Fig. 2. METAFor analysis in batch and chemostat culture. Origin of metabolic intermediates during growth of E. coli in batch culture (white bars) and glucose-limited chemostat culture at a growth rate of 0.12 h⁻¹ (gray bars), as determined by metabolic flux ratio analysis by GC-MS from [U-¹³C]glucose experiments. The values presented quantify the relative contribution of one reaction or pathway to a given metabolite; in the case of oxaloacetate from PEP, this is the anaplerotic reaction catalyzed by PEP carboxylase. The remaining fraction must then be obtained from alternative reactions, in this case from malate via the tricarboxylic acid cycle enzyme malate dehydrogenase. The fraction of oxaloacetate from glyoxylate could not be determined (n.d.) for the batch culture because of the extensive origin of oxaloacetate from PEP.

Catabolite Repression of PEP Carboxykinase and the Glyoxylate Shunt—To verify the in vivo evidence for the unexpected anaplerotic switch, we determined in vitro activities of the catabolite-repressed PEP carboxykinase and isocitrate lyase (6, 26, 28) and of the normal glucose metabolism enzymes PEP carboxylase and isocitrate dehydrogenase (Table II). Consistent with previous reports (12, 25), the more rapidly growing chemostat cultures at growth rates of 0.2 and 0.4 h⁻¹ were similar to batch cultures, as would be expected from catabolite repression under these conditions (9–11). In sharp contrast, in vitro activities of the catabolite-repressed enzymes were severalfold higher in slow-growing chemostat than in batch culture, which agrees favorably with the in vivo flux ratios (Fig. 2). In vitro activities of the competing enzymes isocitrate dehydrogenase and PEP carboxylase were lower or at detection levels, respectively, in this chemostat culture (Table II). It should be noted that, as seen in Table II, reduced isocitrate dehydrogenase activity is consistent with the 4-fold lower activity during growth on acetate compared with glucose (29). This reduction is a prerequisite for glyoxylate shunt operation, because the dehydrogenase affinity for isocitrate is much higher than the lyase affinity (22).

Metabolic Net Fluxes—The above in vivo and in vitro biochemical results demonstrate the drastic anaplerotic switch between batch and slow-growing chemostat culture. Moreover, significant conversion of oxaloacetate to PEP by means of PEP carboxykinase in the chemostat culture (Fig. 2) reveals glyoxy-

late shunt activity beyond anaplerosis. To quantify the extent of in vivo glyoxylate shunt activity, we estimated intracellular net fluxes in a system-integrating approach by quantitative balancing of all GC-MS and physiological data within a comprehensive isotopomer model of metabolism (12, 16). Notably, pyruvate carboxylase was not excluded from this model; thus, the analysis was not biased by a false assumption. Although the absolute molar fluxes were much higher in the rapidly growing batch culture, the relative distribution of these fluxes into the pentose phosphate pathway and glycolysis was similar in both cultures (data not shown). Below PEP, however, a dramatically different distribution of fluxes was obvious (Fig. 3). Consistent with the biochemical results, almost no flux was catalyzed by the anaplerotic PEP carboxylase from PEP to oxaloacetate in the chemostat culture, but a significant molar flux of 1.0 mmol g⁻¹ h⁻¹ in the reverse direction was catalyzed by PEP carboxykinase (Fig. 3B). The anaplerotic function of PEP carboxylase was fully complemented by the glyoxylate shunt. In its anaplerotic mode of operation, the glyoxylate shunt diverts isocitrate from the tricarboxylic acid cycle to prevent the loss of acetyl-CoA, but the molar flux of 1.5 mmol g⁻¹ h⁻¹ through the shunt was in 3-fold excess of the biosynthetic precursor withdrawal of oxaloacetate and 2-oxoglutarate. In combination with the PEP carboxykinase, this extensivitive activity of the glyoxylate shunt constitutes a cycle for complete oxidation of PEP to CO₂. Therefore, the chemostat flux distribution presented in Fig. 3B is a superposition of two simultaneously operating metabolic cycles that contribute equally to complete oxidation of carbohydrates: the well known tricarboxylic acid cycle and the new PEP-glyoxylate cycle (Fig. 4).

PEP-glyoxylate Cycle Activity in an NADPH-overproducing Phosphoglucone Isomerase Mutant—Whereas the PEP-glyoxylate cycle clearly has a catabolic function under the carbon hunger conditions investigated here, the potential metabolic benefit of using this cycle for catabolism is not directly apparent, because it has a similar stoichiometry and an equal number of enzymatic reaction steps as the tricarboxylic acid cycle and oxidative phosphorylation (27), the potential for more ATP equivalents in the PEP-glyoxylate cycle may be reduced formation of the biosynthetic reducing equivalent NADPH. Hence, we performed ¹³C-labeling batch experiments with a phosphoglucone isomerase deletion mutant and the parent in shake flask cultures, with glucose as the sole carbon source and growth rates of 0.22 and 0.67 h⁻¹, respectively. As reported before (15, 34), the pentose phosphate pathway was used extensively in the mutant (Fig. 5), thereby producing NADPH in excess of the biosynthetic requirements.
Additionally, however, the mutant exhibited a PEP-glyoxylate cycle flux profile akin to that of the parent in the slow-growing chemostat culture: (i) an increased fraction of PEP originating from oxaloacetate via PEP carboxykinase; (ii) absence of pyruvate carboxylase activity as evidenced by the complete absence of oxaloacetate originating from PEP; and (iii) a high fraction of oxaloacetate derived through the glyoxylate shunt (Fig. 5).

Significant in vivo flux through the glyoxylate shunt was consistent with a 6-fold higher in vitro activity of isocitrate lyase and 4-fold lower isocitrate dehydrogenase activity in the mutant when compared with the parent in-batch culture, 115 and 100 μmol min⁻¹ g⁻¹ (protein), respectively. Consistent with our earlier results (15, 34), we found no in vitro activity of the PEP-glyoxylate cycle in batch cultures of a phosphoglucone isomerase that was derived from E. coli W3110 (data not shown). Although the in vitro isocitrate lyase activity of a W3110 mutant was significantly higher than in the parental W3110, we found unaltered high isocitrate dehydrogenase activity (data not shown). Because the dehydrogenase affinity for isocitrate is much higher than the lyase affinity (22, 29), the

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**TABLE II**

In vitro enzyme activities in crude cell extracts of batch and glucose-limited chemostat cultures of E. coli

| Enzyme                 | Specific enzyme activity  |
|------------------------|---------------------------|
|                        | Batch 0.12 h⁻¹ | Chemostat 0.2 h⁻¹ | Chemostat 0.4 h⁻¹ |
|                        | μmol min⁻¹ g protein⁻¹ |
| Isocitrate lyase       | 20 ± 3 "         | 300 ± 7           | 109 ± 9          |
| Isocitrate dehydrogenase| 1424 ± 23       | 411 ± 18          | 1125 ± 20        |
| PEP carboxylase        | 199 ± 21         | 15 ± 21           | 26 ± 23          |
| PEP carboxykinase      | 12 ± 14          | 127 ± 15          | 53 ± 14          |

" Average and deviation from duplicate experiments.

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**Fig. 3.** Net flux analysis in batch and chemostat culture. Molar net fluxes (mmol g⁻¹ h⁻¹) in batch culture (A) and in glucose-limited chemostat culture at a growth rate of 0.12 h⁻¹ (B) of E. coli. The arrows are drawn in proportion to the flux. Mass spectral data of proteinogenic amino acids from cultures grown on a mixture of 20% [U-¹³C]glucose and 80% natural glucose and the physiological data of Table I were used for the flux estimation. Fluxes to biomass building blocks are indicated by the short arrows.

**Fig. 4.** Stoichiometries of two alternative cycles for complete oxidation of PEP. The tricarboxylic acid cycle (A) and the PEP-glyoxylate cycle (B). Large solid arrows indicate reactions that are used twice per turn of the cycle. Gene names are shown in italics.
apparent absence of isocitrate dehydrogenase down-regulation may explain why the batch cultures of a W3110 phosphoglucose isomerase mutant (15, 34) and the slow-growing chemostat cultures of wild-type W3110 (13) do not seem to use the PEP-glyoxylate cycle extensively.

**DISCUSSION**

By using enzymatic methods and isotopic tracer experiments, we demonstrate the activity of a novel bi-functional cycle that catalyzes anaplerosis and complete oxidation of PEP to CO₂ in *E. coli*, a function that was considered exclusive to the tricarboxylic acid cycle. The key enzymes of this cycle are PEP carboxykinase as well as isocitrate lyase and malate synthase of the glyoxylate shunt. In addition to their generally recognized functions in gluconeogenesis and anaplerosis (26, 27), respectively, we demonstrate their joint catabolic function in the PEP-glyoxylate cycle (Fig. 4B). The PEP carboxykinase activity is essential for the catabolic mode of operation, because more oxaloacetate is generated than is required for biosynthesis, and this surplus cannot be oxidized directly in the tricarboxylic acid cycle.

A somewhat similar cycle was described in *E. coli* during growth on two-carbon compounds that involved also PEP carboxykinase and malate synthase (35). Because this dicarboxylic acid cycle does not contain isocitrate lyase and citrate synthase, it is specific for the oxidation of compounds that are metabolized via glyoxylate. The PEP-glyoxylate cycle, in contrast, could potentially serve as a general catabolic route for all three-or higher-carbon compounds that are degraded to PEP or pyruvate. The PEP-glyoxylate cycle is different from the dicarboxylic acid and tricarboxylic acid cycles, and there is no requirement for an additional anaplerotic reaction in the PEP-glyoxylate cycle. We could not detect any in *vivo* or *in vitro* evidence for the operation of a second anaplerotic reaction in the chemostat culture. For the chemostat culture investigated here, about one-third of the molar flux through the PEP-glyoxylate cycle was used for anaplerosis, and two-thirds were used for catabolic oxidation to CO₂ (Fig. 3B).

Consistent with the succinate requirement of PEP carboxylase mutants during growth on glucose, we did not find even trace activity of the PEP-glyoxylate cycle in wild-type batch cultures or the more rapidly growing chemostat cultures. Circumstantial evidence suggests the absence of catabolite repression as an important factor for activity of this cycle, because the key enzymes are subject to this regulation (6, 26). At least cAMP-dependent catabolite repression is absent at the low growth rate in the glucose-limited chemostat used here (9–11). The underlying genetic control mechanism may be Cra-mediated catabolite repression, which induces transcription of all three key enzymes at the low intracellular concentrations of fructose phosphates (7) that are present in such *E. coli* chemostat cultures (36). Because Cra-mediated catabolite repression does not depend on the extracellular glucose concentration, it could also explain PEP-glyoxylate cycle activity in batch cultures of the slow-growing MG1655 phosphoglucose isomerase mutant. We cannot, however, exclude the possibility of a suppressor mutation in this mutant. Absence of Cra-mediated catabolite activation of phosphofructokinase, glyceraldehyde-3-P dehydrogenase, and pyruvate kinase (6, 7) would also be consistent with about 3-fold lower glycolytic fluxes in the slow growing wild-type chemostat culture and in batch cultures of the mutant. Strain-specific degrees of catabolite repression (10) may explain the apparent variations in glyoxylate shunt activity that was observed in batch cultures of an *E. coli* B strain (37) but was absent in other strains during slow, glucose-limited growth (12, 38).

One function of the PEP-glyoxylate cycle might be redox-cofactor balancing, because it was active in batch cultures of an NAPDH-overproducing phosphoglucose isomerase mutant. Specifically, the PEP-glyoxylate cycle adds potential metabolic flexibility to redox metabolism by effectively decoupling catabolic carbon flow from NAPDH formation that would occur in the parallel tricarboxylic acid cycle. This function is redundant with the presumed function of the soluble transhydrogenase UdhA (34). In *vivo* transhydrogenase activities were identical in batch cultures of MG1655 and its phosphoglucose isomerase mutant. Because the assay cannot distinguish between the membrane-bound and the soluble transhydrogenase isoforms, it remains unclear whether UdhA and/or the PEP-glyoxylate cycle contribute alone or in combination to redox-cofactor balancing. Although the PEP-glyoxylate cycle was identified under environmental conditions of glucose hunger in *E. coli*, it could potentially play a role during conditions that lead to a higher formation than consumption of NADPH, e.g. growth on alternative substrates or when little biomass is synthesized (5, 12). Because of the ubiquity of the key enzymes in microbes, it is not unreasonable to speculate that this new catabolic cycle operates also in other microbes.

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