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Deletion of *pgi* Alters Tryptophan Biosynthesis in a Genetically Engineered Strain of *Escherichia coli*

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Deletion of the structural gene for phosphoglucose isomerase (*pgi*) of *Escherichia coli* dramatically alters the path of glucose catabolism by diverting carbon into the hexose monophosphate shunt. The effect of this genetic alteration on the conversion of glucose to tryptophan by strains optimized for the biosynthesis of this amino acid was determined by using ^{13}C-nuclear magnetic resonance spectroscopy in vivo. Pgi$^-$ strains converted glucose to tryptophan almost twice as efficiently as did their Pgi$^+$ counterparts.

In *Escherichia coli*, the biosynthesis of the amino acid L-tryptophan from glucose involves numerous enzymatic steps and the interaction of several interrelated metabolic pathways (Fig. 1). Key steps in each of these pathways are subject to transcriptional and posttranscriptional regulatory mechanisms. The aim of our work has been to utilize a combination of classical genetics and recombinant DNA technology to optimize the conversion of glucose to tryptophan in *E. coli* by systematic circumvention of these regulatory mechanisms.

The first biosynthetic step specific to aromatic biosynthesis is the condensation of erythrose-4-phosphate and phosphoenolpyruvate to form 3-deoxy-D-arabinoheptulosonic acid-7-phosphate (DAHP). Gene expression controls and allosteric inhibition mechanisms operate on each of the three isozymes (DAHP synthetases) catalyzing this step. Similar genetic restraints operate on the utilization of the major branch-point aromatic intermediate, chorismic acid, by each of the pathways specific to tryptophan, tyrosine, and phenylalanine synthesis (for recent reviews, see references 14 and 17).

The final step in tryptophan biosynthesis involves the conversion of indole-3-glycerol phosphate and L-serine to L-tryptophan. The intracellular availability of L-serine can, under certain conditions, become a rate-limiting factor for L-tryptophan synthesis (1).

We have facilitated the synthesis of tryptophan in bacterial strains derived from *E. coli* K-12 W3110 by (i) modifying the genetic regulation and/or increasing the gene dosage of *serA*, *aroG*, *trpE*, *trpD*, *trpC*, *trpB*, and *trpA* (these genes code for key enzymes contributing to tryptophan biosynthesis) and (ii) genetically inactivating *tyrA* and *pheA* (which code for enzymes which divert chorismate away from tryptophan synthesis) and *tna* (which encodes a tryptophan-degrading enzyme). Details of these genetic manipulations are described elsewhere (18).

The purpose of the present study was to use such highly engineered strains to determine whether tryptophan synthesis might be further enhanced by diverting the metabolic flow of carbon away from glycolysis and into the hexose monophosphate (HMP) shunt, thus accelerating the generation of NADPH and of key pentose-derived compounds involved in aromatic amino acid biosynthesis.

We compare the performance of two strains of *E. coli*, one of which carries a deletion in *pgi*, the gene encoding phosphoglucose isomerase. Conversion of ^{13}C-glucose to tryptophan was monitored and quantitated in vivo by using nuclear magnetic resonance (NMR) spectroscopy (1).

MATERIALS AND METHODS

Reagents. All restriction endonucleases and DNA modification enzymes were purchased from New England Biolabs, Beverly, Mass., and used according to the manufacturer's recommendations. Ethyl methanesulfonate, 3-fluorotyrosine and 7-methyltryptophan were obtained from Sigma Chemical Co., St. Louis, Mo. [6-^{13}C]D-glucose (90%) was purchased from MSD Isotopes.

Bacterial strains and plasmids. All bacterial strains are derivatives of *E. coli* K-12 and are listed in Table 1. A *vir* mutant of bacteriophage P1 was used for transductions essentially as described previously (9). The restriction sites referred to in the constructions detailed below are those present in the original published sequences for the respective plasmids and genes.

Plasmid pC501 was selected from a plasmid library prepared from an *Eco*RI digest of *Serratia marcescens* DNA; selection was by complementation of the *trpE* mutation in MV17. Subsequently, MV17(pC501) was treated with ethyl methanesulfonate as described previously (13) and plated on minimal medium containing 7-methyltryptophan (20 μg/ml), and plasmid-linked mutations were selected by retransformation. Feedback insensitivity of the anthranilate synthetase encoded by one such isolate (pC501FBR) was demonstrated by assaying cell extracts in the presence of tryptophan as described previously (7, 18).

Plasmid pD2643 is a 10.4-kb derivative of pBR327 (15) in which a 0.7-kb *Aha*III-generated deletion has removed part of the β-lactamase gene; in addition, pD2643 contains a 7.8-kb *Eco*RI insert consisting of the following (in order, from the β-lactamase gene-proximal site): a 0.48-kb *Eco*RI-*Bgl*II fragment from pKB252 (2), which contains two *lacUV5* promoters in tandem; a 5.8-kb *Bgl*II-*Sal*I fragment from pDB107 (11), which contains *E. coli* genes *trpDCBA* and the *trp* terminator (but not the *trp* promoter, operator, attenuator, or *trpE*); and a 1.6-kb *Sal*I-*Eco*RI fragment which contains the *trpE* gene from pC501FBR. Thus, pD2643 contains the *trpDCBA* genes under *lac* promoter control and

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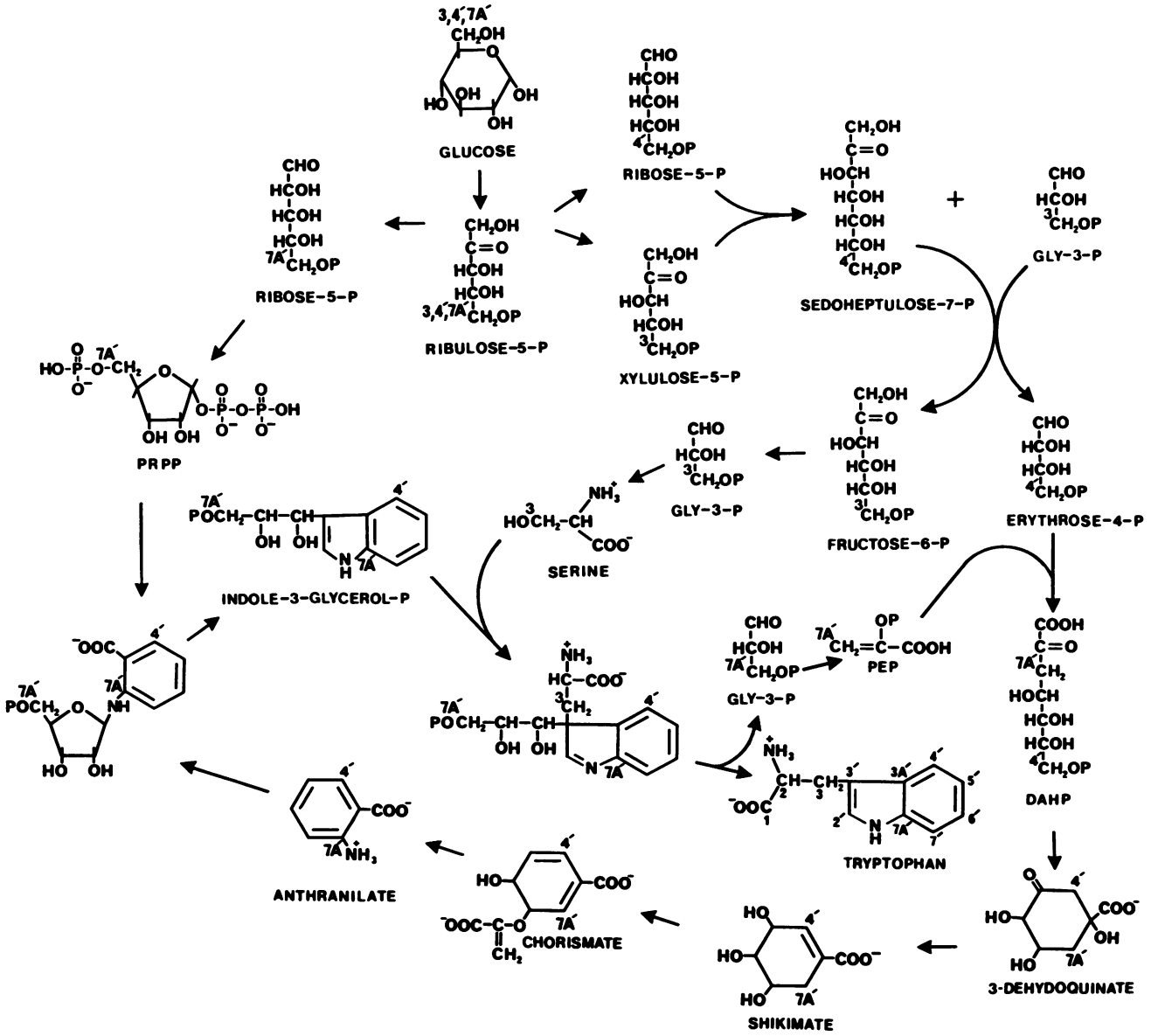

FIG. 1. *E. coli* biosynthetic pathway for the generation of tryptophan from [6-^{13}C]glucose. The specific carbon that becomes the final labelled tryptophan carbon (3, 4′, or 7′) is labelled in each intermediate. All intermediates are not shown, and only one potential route for incorporation of label into the 7A′ position of tryptophan is shown. GLY-3-P, glyceraldehyde-3-phosphate; PEP, phosphoenol-pyruvate.

trpE under the (constitutive) control of the "reverse" *tet* promoter of pBR327.

The *E. coli lacI*, *serA*, and *aroG* genes were each cloned by complementation of strains carrying lesions in these loci with a genomic library generated by partial *Sau*3AI digestion of *E. coli* DNA and ligation into a lambda 1059 vector (8). Subsequently, each of these genes was subcloned into pBR327 or pACYC184 (5). The *aroG* allele from strain MAR13 (7) was rescued by recombination into an *aroG*-carrying plasmid (pC520) by recombination in vivo. The altered properties of the DAHP synthetase encoded by this recombinant (pD2422) were confirmed by assaying cell extracts in the presence of phenylalanine as described previously (7).

Plasmid pD2625 is a 10.5-kb derivative of pACYC184 containing the following: a 0.38-kb *Eco*RI-*Ava*I fragment which includes the *par* locus of pSC101 (12); a 1.1-kb *Sph*I-*Nar*I fragment containing *lacI*; a 1.7-kb *Nru*I-*Eco*RV fragment containing *serA*; a 0.48-kb *Eco*RI-*Bgl*II fragment from pKB252 (2) comprising two *lacUV5* promoters in tandem; and a 1.6-kb *Bgl*II-*Bam*HI fragment which contains the *aroG* gene from pD2422 encoding a feedback-resistant DAHP synthetase. Thus, pD2625 carries *aroG* under *lac* control, and *lacI* and *serA* are expressed from their own native promoters.

Plasmid pD2634 is an 8.4-kb derivative of pHH509 (3) containing a 1.6-kb *Sph*I fragment comprising the *lacI* gene from pD2625. pHH509 belongs to a plasmid compatibility group which is different from that of both pD2643 (ColE1 replicon) and pD2625 (P15A replicon). Strains containing all

TABLE 1. *E. coli* K-12 strains used in this study

Strain	Relevant characteristics	Source or derivation (selection)
AT2471	*tyrA4*	A. L. Taylor via B. Bachmann[a]
B1238	W3110F$^-$ (*argF-lac*)del U169 (*gal-bio*)del (*trp* del61-*intc*226 *trp-lac* W205) *trpR*	Reference 4
KA197	Hfr *pheA*	A. Hoekstra via B. Bachmann[a]
MV17	W3110 (*trpE*)del *tna*	D. Helinski
N1624	*bglC*	M. Gottesman via B. Bachmann[a]
NK6024	*pheA18*::Tn*10*	N. Kleckner via B. Bachmann[a]
TST1	*malE52*::Tn*10*	T. J. Silhavy via B. Bachmann[a]
W3110	F$^-$ IN(*rrnD-rrnE*)*1*	J. Lederberg via B. Bachmann[a]
A103	B1238 Gal$^+$	P1.W3110 × B1238 (Gal$^+$)
A103T	A103 *malE52*::Tn*10*	P1.TST1 × A103 (Tet^r Mal$^-$)
CT10	A103 (*lysC-pgi-malE*)del	Spontaneous Fus^r Tet^s derivative of A103T
C534	A103 *tna* Sal$^+$	P1.C537 × A103 (Sal$^+$ Tna$^-$)
C534TP	C534 *tyrA4 pheA18*::Tn*10*	P1.D2636 × C534 (Tet^r Phe$^-$ Tyr$^-$)
C536	CT10 *tna* Sal$^+$	P1.C537 × CT10 (Sal$^+$ Tna$^-$)
C536TP	C536 *tyrA4 pheA18*::Tn*10*	P1.D2636 × C536 (Tet^r Phe$^-$ Tyr$^-$)
C537	MV17 *tna* Sal$^+$	P1.N1624 × MV17 (Sal$^+$ Tn$^-$)
D2316	C536 *pheA*	C536 × KA197 (Trp$^+$ Phe$^-$)
D2346	D2316 *tyrR trpE*(FBR) *aroG*(FBR)	3FT^r 7MT^r derivative of D2316[b]
D2402	D2346 *tyrA4*	P1(AT2471) × D2346 (Phe$^+$ Tyr$^-$)
D2618	D2402 (*trp-lac*)W205 *tyrB*	Trp$^+$ Lac$^+$ Hpp$^-$ derivative of D2402
D2636	D2618 *pheA18*::Tn*10*	P1(NK6024) × D2618 (Tet^r Phe$^-$ Tyr$^-$)
D2704	C534 *tyrA4* (*pheA*)del	Spontaneous Fus^r Tet^s derivative of C534TP
D2705	C536 *tyrA4* (*pheA*)del	Spontaneous Fus^r Tet^s derivative of C536TP

[a] *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.
[b] 3FT, 3-fluorotyrosine; 7MT, 7-methyltryptophan.

three plasmids showed less than 1% segregation of each plasmid after 20 generations of growth in the absence of antibiotics. In the experiments described here, antibiotics were included as a precaution.

The configuration of the biosynthetic genes in the constructs described above and the choice of regulatory elements were (of the several dozen possibilities tested) found to be most consistent with overall plasmid and strain stability. In preliminary studies with individual genes under *lac* control, gene dosage was shown to correlate with elevated enzymatic activity after induction with isopropyl-β-D-thiogalactopyranoside (IPTG) in the three cases tested: *aroG* (>400-fold), *trpE* (>25-fold), and *trpAB* (>50-fold). Enzymatic activity was not measured in the strains actually used in this work, but elevated synthesis of biosynthetic enzymes was inferred from the ability of D2704—containing all three plasmids—to accumulate approximately 10,000-fold more tryptophan in shake flasks than the parental strain, W3110.

The compatible plasmids pD2625, pD2643, and pD2634 were sequentially introduced into strain D2704 or D2705 by transformation (6) and selection for resistance to chloramphenicol, tetracycline, and ampicillin, respectively, each at 20 μg/ml. Transformants were maintained on medium (Luria broth or appropriately supplemented Vogel-Bonner minimal medium [16]) containing all three antibiotics at the concentration indicated above. During the course of this work, no differences were observed between Pgi$^+$ and Pgi$^-$ strains with respect to plasmid stability (gross structural stability, as determined by restriction mapping, or segregational stability, as determined by loss of antibiotic resistance). Rough copy number estimates of pD2643 and pD2625 obtained by assaying β-lactamase and chloramphenicol acetyltransferase, respectively, also failed to reveal differences between the two strains. However, the mean generation time of the Pgi$^-$ strain was approximately 15 to 25% longer than that of the Pgi$^+$ strain.

NMR spectroscopy. All carbon-13 NMR spectra were obtained on a Varian XL-200 spectrometer equipped with a broad-band probe operating at 50.1 mHz. Fourier-transformed carbon-13 spectra were acquired every 3 h for up to 54 h. All spectra were referenced to the internal β-D-[6-^{13}C]glucose resonance at 61.3 ppm, and further resonance assignments were based on authentic sample chemical shifts and spiking of the in vivo-generated samples with authentic compounds.

Bacteria were grown at 30°C in Vogel-Bonner minimal medium (16) supplemented with 0.4% NZ amine, 1.5% glucose, and 20 μg each of chloramphenicol, tetracycline, and ampicillin per ml. When the cultures reached densities of approximately 1 × 10^8 CFU/ml, IPTG was added to 1 mM and the cultures were used directly for acquisition of NMR spectra. Typically, 6 ml of culture was introduced into a 10-mm NMR tube. Thirty milligrams of D-[6-^{13}C]glucose, dissolved in 1.0 ml of deuterated water, was then added to the culture. During acquisition of spectra, a cell aeration device inserted into the NMR tube supplied air to the culture at approximately 30 ml/min while the NMR tube was spun at 15 rpm as described previously (1).

RESULTS

Figure 2 shows the time-averaged spectra obtained when strain D2704(pD2625, pD2634, pD2643) was grown in the presence of [6-^{13}C]glucose. Spectrum A was taken immediately following addition of the labelled sugar. All resonances appearing in the spectrum can be assigned to natural-abundance carbon signals and enriched C-6 resonances of the alpha and beta glucose in the medium. Spectrum B was

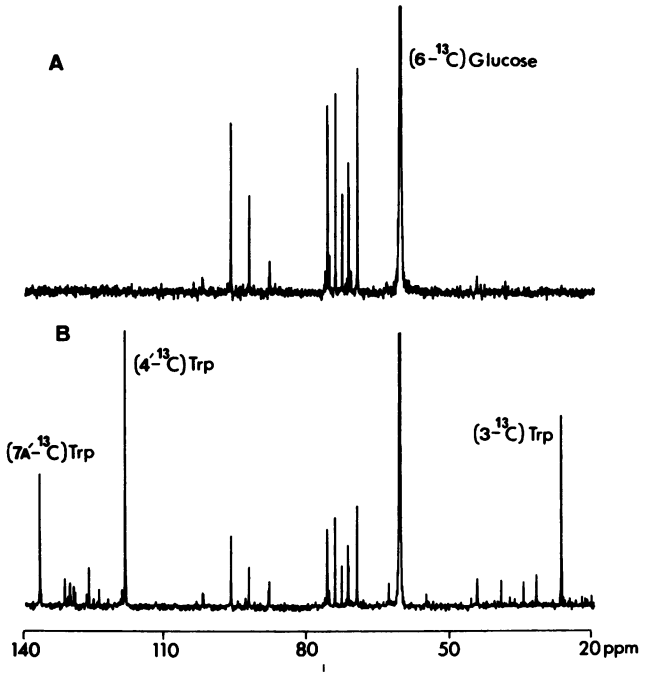

FIG. 2. Conversion of [6-^{13}C]glucose to tryptophan. D2704 (pD2625, pD2634, pD2643) cells were grown in an aerated NMR tube culture while time-averaged spectra were acquired every 3 h. Spectra are plotted in the absolute-intensity mode. The [6-^{13}C]glucose resonances in both spectra are displayed off scale and are actually 28 times more intense than the two most downfield glucose resonances in the top spectrum. (A) Spectrum taken immediately following addition of [6-^{13}C]glucose; (B) spectrum taken 54 h later.

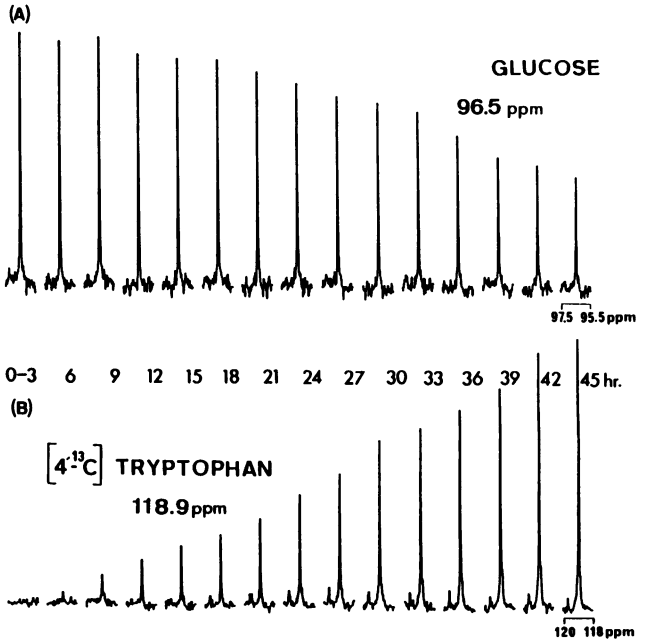

FIG. 3. Glucose depletion correlates with tryptophan accumulation. Conditions were as described in the legend to Fig. 2, except that time-averaged spectra were acquired for 45 h. Spectra expansions show (A) the decrease in the natural-abundance C-1 resonance intensity of β-D-glucose in the medium as a function of time and (B) the incorporation of ^{13}C label from [6-^{13}C]glucose into the C-4′ position of tryptophan as a function of time. All resonances are displayed in the absolute-intensity mode, and the glucose expansions are displayed at 1.5 times the vertical scale of the tryptophan expansions. The final concentration of tryptophan in the NMR tube was 2.0 mg/ml. Resonance intensities are not corrected for nuclear Overhauser effects.

taken 54 h later. The 3, 4′, and 7′ tryptophan carbon resonances are clearly visible at this time, and simultaneously, the glucose carbon signals are reduced. By acquiring intermediate spectra and plotting the decreasing intensity of glucose carbon versus the increase in tryptophan resonances (Fig. 3), a direct estimate of the glucose-to-tryptophan conversion efficiency may be obtained.

This method was used to assess the effect of deleting the structural gene for phosphoglucose isomerase (*pgi*). Strain D2705, which carries such a deletion, converts glucose to tryptophan approximately twice as efficiently as does D2704, the isogenic Pgi$^+$ control (Table 2).

All spectra in the experiments described above were acquired during the logarithmic phase of cell growth (12 to 54 h postinoculation) and covered a period during which approximately 20% of the initial (50 mg/ml) glucose was consumed. This method of estimating strain performance ignores differences in the observed growth rates of the two strains (on glucose as the sole carbon source, D2705 grows more slowly than D2704; see Materials and Methods) but enables us to arrive at what we believe to be more-precise estimates of conversion efficiency.

To ensure that the observed effect on tryptophan accumulation was not a labelling artifact in the *pgi* strain, we confirmed the 2:1 ratio of actual tryptophan accumulated by the two test strains by using conventional chemical assays (in at least four separate experiments [data not shown]). Thus, the increase in conversion efficiency exhibited by strain D2705 results in a corresponding increase in both (i) the average rate of tryptophan production over the 40- to 50-h period of growth monitored for both strains and (ii) the accumulation of final product during that period.

DISCUSSION

The results described above support our prediction that accelerating the flow of carbon through the HMP shunt results in more-efficient conversion of glucose to tryptophan. A separate, independent set of observations had originally indicated this possibility to us. Suitably marked Pgi$^+$ strains of *E. coli* convert gluconate to tryptophan about twice as efficiently as they convert glucose (unpublished data). Thus, the gross metabolic rerouting of carbon, here accomplished by deleting the gene for phosphoglucose isomerase, can have a positive and dramatic effect on the biosynthetic capabilities of this bacterium.

This observation, regardless of the rationale which may be offered to explain it, is likely to have immediate relevance in other applications for engineered *E. coli* strains, most notably in the production of other aromatic compounds.

Although we have not investigated the underlying mechanism(s) for the observed effect of *pgi*, we offer the following hypotheses for further investigation. (i) Increased flow of carbon through the HMP shunt results in the accelerated generation of NADPH, which serves to drive the biosynthesis of aromatic intermediates and glutamate. (ii) Increased synthesis of pentose phosphates could directly affect the supply of two key intermediates in tryptophan biosynthesis:

TABLE 2. Effect of *pgi* deletion on tryptophan synthesis

Strain[a]	Expt no.	Data window[b]	% Glucose consumed	C.E.[c]
D2704 (Pgi$^+$)	1	6–16	21.9	5.2
	2	4–13	20.4	6.3
	3	6–13	21.9	7.2
				(Mean, 6.2 ± 0.7)
D2705 (Pgi$^-$)	1	5–13	23.1	10.6
	2	5–13	18.6	10.2
	3	5–18	18.9	13.1
				(Mean, 11.3 ± 1.2)

[a] Each strain carries the plasmids pD2625, pD2634, and pD2643.

[b] ^{13}C-NMR spectra were acquired continuously during cell growth and time-averaged over successive 3-h periods, each average representing a single datum point. A window of 8 to 14 successive datum points was chosen for each conversion efficiency computation. This window corresponded to the period in each experiment during which a total of ca. 20% of the initial glucose was consumed, in a linear fashion. The actual lapse of time within each window varies (average, 29 h for D2704, 32 h for D2705); see text for rationale.

[c] Efficiency of conversion of [6-^{13}C]glucose to labelled tryptophan (3, 4′, and 7′ carbons combined) was calculated directly from the change in resonance intensities after correcting for the molecular weights of the two compounds. It is expressed as a percentage. In all cases, the ratio of beta to alpha glucose at spectrum 6 was found to lie between 1.60 and 1.77.

5-phosphoribosyl 1-pyrophosphate (PRPP) and erythrose-4-phosphate. Since the addition of these compounds to the growth medium will not result in efficient uptake of them, it might be of interest instead to study the effect of a *pgi* lesion on the synthesis of other aromatic amino acids from glucose. We have not attempted this. A different approach would involve using a clone for the PRPP synthetase gene. For this to result in increased PRPP levels (independent of an increased flow of carbon through the HMP shunt), the normal levels of this enzyme would have to be rate-limiting for PRPP synthesis; we think that this is unlikely.

Our work extends the methodology previously established for the real-time analysis of microbial metabolism (1) to the evaluation of a key genetic modification which alters the overall productivity of a complex biosynthetic pathway. The magnitude of the effect observed (in a strain which is already optimized for tryptophan synthesis) is surprising. In our experience, other, more-logical genetic manipulations (such as raising the gene dosage for additional aromatic and serine biosynthetic genes) show only marginal effects on tryptophan biosynthesis in these highly engineered strains.

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