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An *Escherichia coli* K-12 *tktA tktB* Mutant Deficient in Transketolase Activity Requires Pyridoxine (Vitamin B_6) as well as the Aromatic Amino Acids and Vitamins for Growth

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We show that a *tktA tktB* double mutant, which is devoid of the two known transketolase isoenzymes of *Escherichia coli* K-12, requires pyridoxine (vitamin B_6) as well as the aromatic amino acids and vitamins for growth. This pyridoxine requirement can also be satisfied by 4-hydroxy-L-threonine or glycolaldehyde. These results provide direct evidence that D-erythrose-4-phosphate is a precursor of the pyridine ring of pyridoxine. In addition, they show that the two major *E. coli* transketolase isoenzymes are not required for the biosynthesis of D-1-deoxyxylulose, which is thought to be another precursor of pyridoxine.

Pyridoxine (vitamin B_6) is a direct biosynthetic precursor of pyridoxal 5′-phosphate, an important coenzyme that plays numerous roles in cellular metabolism, especially in pathways of amino acid metabolism (1). Pyridoxine is thought to be synthesized by a branched pathway that leads from the precursors D-1-deoxyxylulose and 4-hydroxy-L-threonine (Fig. 1) (11, 11a, 17). The pathway leading to D-1-deoxyxylulose is not known with certainty, but D-1-deoxyxylulose could result from the condensation of pyruvate and glyceraldehyde-3-phosphate (Fig. 1) by the activity of a transketolase or a pyruvate-dehydrogenase complex-dependent enzyme (2, 11, 11a, 15a, 25). A specific pathway leading from D-erythrose-4-phosphate to 4-hydroxy-L-threonine has been proposed (17, 22). Certain predictions based on this pathway have been confirmed. Genetic evidence showed that the products of *pdxB* and *serC* genes must mediate steps in this branch of the pyridoxine biosynthetic pathway (17). Enzymological experiments confirmed that *pdxB* encodes a 2-hydroxy acid dehydrogenase that uses 4-phosphoerythronate as its substrate (19, 22). The immediate precursor, 4-hydroxy-L-threonine, was shown to meet the pyridoxine requirement of *pdxB* mutants in minimal-plus-glucose medium (7).

One prediction that has not been confirmed directly is that D-erythrose-4-phosphate is the first compound in this branch of the pyridoxine biosynthetic pathway. D-Erythrose-4-phosphate is a central metabolic intermediate in the pentose phosphate pathway and is required for the synthesis of the three aromatic amino acids, L-tryptophan, L-phenylalanine, and L-tyrosine, and the three aromatic vitamins, *p*-aminobenzoate, *p*-hydroxybenzoate, and 2,3-dihydroxybenzoate (Fig. 1) (8, 9, 12, 20, 26). D-Erythrose-4-phosphate is synthesized directly from D-glyceraldehyde-3-phosphate and D-fructose-6-phosphate by a transketolase (Fig. 1) (8, 9, 12). In addition, D-erythrose-4-phosphate is synthesized from sedoheptulose-7-phosphate and D-glyceraldehyde-3-phosphate by a transaldolase (8, 9, 12). However, the latter two substrates are products of a transketolase reaction between D-xylulose-5-phosphate and D-ribose-5-phosphate (Fig. 1) (8, 9, 12). Therefore, the pathway in Fig. 1 predicts that *Escherichia coli* mutants lacking transketolase activity should require pyridoxine as well as the aromatic amino acids and vitamins.

E. coli K-12 contains two transketolase isoenzymes encoded by the *tktA* and *tktB* genes (13, 14). *tktA* corresponds to the original *tkt* transketolase locus of *E. coli* K-10 (13–15). The *tktA* gene product is the major isoenzyme and accounts for about 70 to 90% of transketolase activity in cells grown under the limited number of physiological conditions examined to date (13). *tktB* may be in an operon with *talA*, which encodes the *E. coli* transaldolase (13) (Fig. 1). The cellular activity of the TktB isoenzyme, which shows 74% amino acid identity with the TktA protein, appears to be very low, at least in the few growth conditions tested so far (13). Similarly, there are two transketolase isoenzymes in *Saccharomyces cerevisiae* (21, 23).

We tested the validity of the pathway in Fig. 1 by examining the growth properties of an isogenic set of *tktA*$^+$ *tktB*$^+$ (NU426 [W3110 *sup*(Am) prototroph (17)]), *tktA*::Tn*10* (TX3156), *tktB*::*kan* (TX3157), and *tktA*::Tn*10* *tktB*::*kan* (TX3158) strains (Table 1). The *tktA*::Tn*10* and *tktB*::*kan* mutations originally derived from strains AI80 and AI1122, respectively (13), were moved into the NU426W3110 prototrophic strain by generalized transduction with bacteriophage P1*vir* (17, 22). AI1118, which is the original *tktA tktB* double mutant described previously (13), is in the EJ500 (W3110 *cfs*) background and is not isogenic with NU426.

In our first experiments, P1 transductants were selected on Luria-Bertani (LB) medium supplemented with 1% glycerol, 0.4% vitamin assay Casamino Acids, 200 μM L-tryptophan, 1 μM pyridoxine, 6 μM *p*-aminobenzoate, 6 μM *p*-hydroxybenzoate, 50 μM 2,3-dihydroxybenzoate, and antibiotic (10 μg of tetracycline per ml or 50 μg of kanamycin per ml or both). The *tktA*::Tn*10* and the *tktB*::*kan* single mutants formed colonies of the same size as those of the *tktA*$^+$ *tktB*$^+$ parent when streaked on this medium lacking antibiotic. However, the *tktA*::Tn*10* *tktA*::*kan* double mutant grew very poorly on supplemented or regular LB medium with or without antibiotics compared with the other strains. In addition, the *tktA tktB* double mutant rapidly accumulated fast-growing suppressor mutants on this rich medium. In contrast, the parent and *tktA*::Tn*10*, *tktB*::*kan*, and *tktA*::Tn*10* *tktB*::*kan* mutants readily formed colonies of about the same size on enriched minimal salts (1× E) medium

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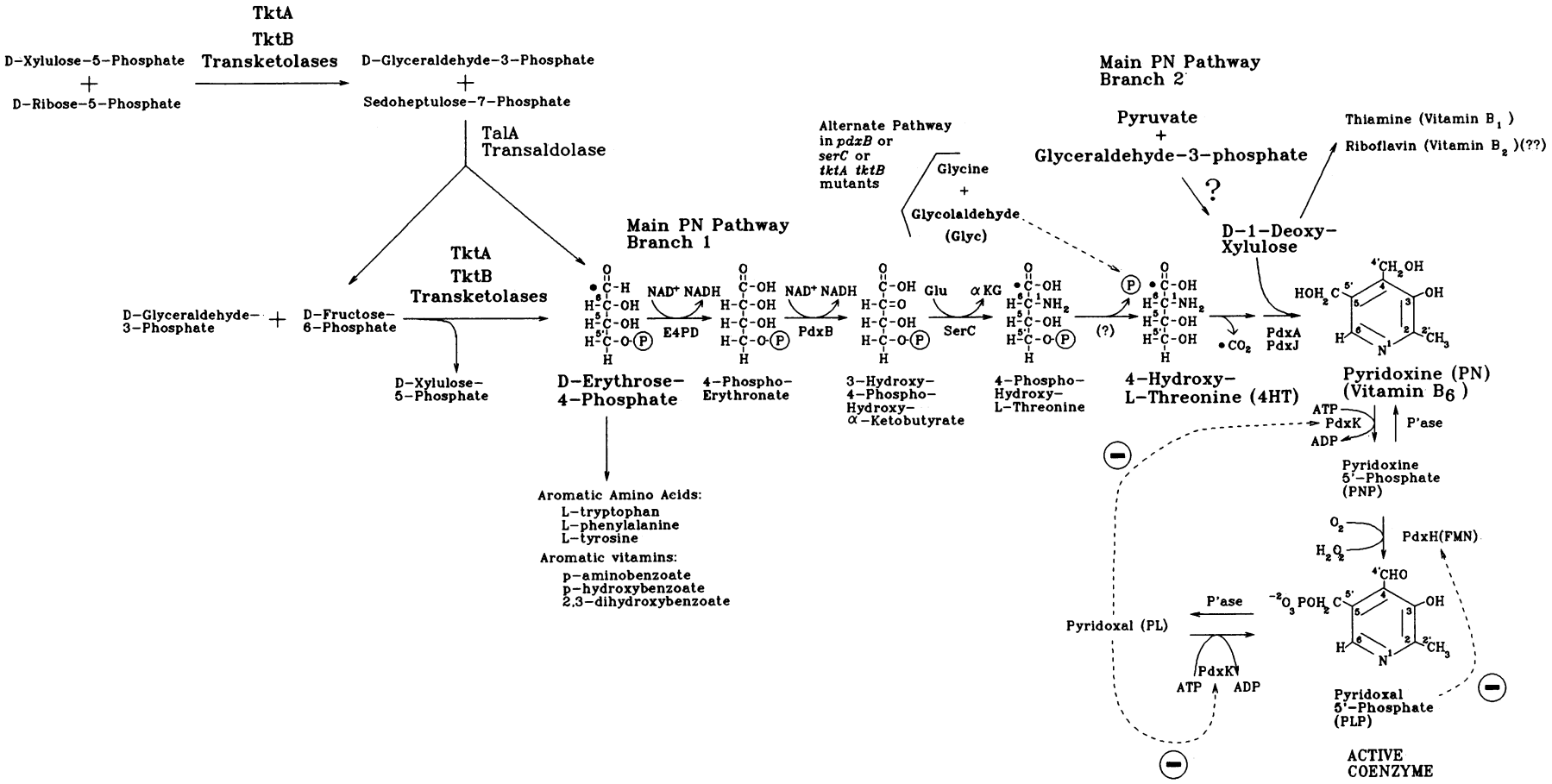

FIG. 1. Pathway of D-erythrose-4-phosphate biosynthesis and proposed branched pathway of pyridoxine (vitamin B_6) (PN) and pyridoxal 5′-phosphate (PLP) biosynthesis in *E. coli* K-12. For the sake of simplicity, most enzymes are designated with symbols corresponding to the genes that encode them. The sugar-phosphate and pentose-phosphate pathway interconversions leading to D-erythrose-4-phosphate are described in references 8, 9, and 12 to 15. In branch 1 of the main PN pathway, E4PD and PdxB are dehydrogenases (17, 19, 22), SerC is a transaminase (17), and "(?)" is a hypothetical phosphatase analogous to SerB (17). The exact steps leading to D-1-deoxyxylulose in branch 2 of the main PN pathway are not known, but they are thought to involve the condensation of pyruvate and glyceraldehyde-3-phosphate (2, 11, 11a, 15a, 25). 4-Hydroxy-L-threonine (4HT) and D-1-deoxyxylulose are likely joined into a pyridine ring by the action of PdxA and PdxJ (11a, 16), and pyridoxine is converted into the coenzyme pyridoxal 5′-phosphate by successive activities of the PdxK kinase and the PdxH oxidase (4, 18). Points of likely negative pathway feedback control are indicated with minus signs. The alternative pathway, which involves a condensation of glycine and glycolaldehyde (Glyc) to form 4-hydroxy-L-threonine, functions only in *pdxB*, *serC*, or *tktA tktB* mutants (references 10, 17, and 24 and this work).

TABLE 1. Growth of *E. coli* transketolase (*tkt*) mutants on different minimal media[a]

Carbon source	Nutrient supplement(s)[a]	Colony diam (mm) of strain[b]: NU426 (*tktA*$^+$ *tktB*$^+$ prototroph)	TX3156 (*tktA*::Tn*10*)	TX3157 (*tktB*::*kan*)	TX3158 (*tktA*::Tn*10* *tktB*::*kan*)	AI1118 (*tktA*::Tn*10* *tktB*::*kan*)
Glucose	None	2.5	2.0	2.5	–[c]	–
Glucose	6 Aro	2.9	2.5	2.5	–	–
Glucose	PN	3.2	2.5	2.5	–	–
Glucose	6 Aro + PN	2.2	2.0	2.2	0.6	0.4
Glucose	6 Aro + PL	3.8	2.5	2.5	0.6	0.6
Glucose	6 Aro + Thi	3.1	2.5	3.0	–	–
Glucose	6 Aro + Ribo	3.0	1.8	2.5	–	–
Glucose	6 Aro + Glyc	3.0	3.0	2.5	0.3	0.2
Glucose	6 Aro + 4HT	2.6	3.0	3.0	0.5	0.2
Glycerol	6 Aro	1.8	1.8	1.8	–	–
Glycerol	6 Aro + PN	1.8	1.5	1.8	0.5	0.9
Glycerol	6 Aro + 4HT	1.6	1.5	1.8	0.5	0.1
Pyruvate	6 Aro	1.6	1.5	1.8	–	–
Pyruvate	6 Aro + PN	1.8	2	2	–	0.6
Pyruvate	6 Aro + 4HT	0.5	1.5	1.6	0.1	0.12
Gluconate	6 Aro	3.0	2.0	2.5	–	–
Gluconate	6 Aro + PN	3.0	2.5	2.5	–	–

[a] 6 Aro is composed of 500 μM L-phenylalanine, 250 μM L-tyrosine, 200 μM L-tryptophan, 6 μM *p*-aminobenzoate, 6 μM *p*-hydroxybenzoate, and 50 μM 2,3-dihydroxybenzoate. PN, pyridoxine; PL, pyridoxal; Thi, thiamine; Ribo, riboflavin; Glyc, glycolaldehyde; 4HT, 4-hydroxy-L-threonine.

[b] Final isolates of the strains were tested for growth on supplemented minimal medium on MacConkey medium containing L-arabinose or D-xylose. As expected, growth of strains containing the *tktA*::Tn*10* but not the *tktB*::*kan* mutation was impaired on these media (13, 14). Fast-growing pseudorevertants of the *tktA tktB* double mutants, TX3158 and AI1118, were observed on media with glucose (but not glycerol) plus 6 Aro plus PN or PL but were not measured.

[c] –, no growth.

supplemented with 0.4% α-D-glucose, 0.4% vitamin assay Casamino Acids, 200 μM tryptophan, the three aromatic vitamins, and pyridoxine. On the enriched minimal medium, no suppressor mutants were evident, even in the presence of antibiotics. Apparently, some compound in rich LB medium severely inhibits bacteria lacking an intact pentose phosphate pathway. For this reason, all transduction and growth experiments were performed with only enriched minimal medium and minimal medium containing the supplements shown in Table 1, respectively.

In final growth experiments, *E. coli* strains were streaked from enriched minimal-plus-glucose medium onto plates containing Difco Bacto agar (1.5%, wt/vol), Vogel-Bonner minimal (1× E) salts (3), and one or more of the following supplements: α-D-glucose (0.4%, wt/vol); glycerol (1%, vol/vol); pyruvate (1%, wt/vol); gluconate (1%, wt/vol); 6 Aro (500 μM L-phenylalanine, 250 μM L-tyrosine, 200 μM L-tryptophan, 6 μM *p*-aminobenzoate, 6 μM *p*-hydroxybenzoate, 50 μM 2,3-dihydroxybenzoate); pyridoxine or pyridoxal (1 μM); thiamine (1.2 μM); riboflavin (13 μM); glycolaldehyde (100 μM); and 4-hydroxy-L-threonine (3 μM). All plates lacked antibiotics. Plates were incubated at 37°C for 2.5 days, and diameters of about 20 well-separated single colonies were measured for each strain. The entire experiment was done twice. Care was taken not to subject the strains to conditions that inhibit growth and allow the accumulation of pseudorevertants.

Growth of the *tktA*::Tn*10* (TX3156) and *tktB*::*kan* (TX3157) single mutants was indistinguishable from that of the NU426 W3110 parent strain on most media tested (Table 1). The *tktA*::Tn*10* mutant did grow slightly more slowly than the NU426 parent and *tktB*::*kan* mutant on some media (e.g., with glycerol as the carbon source [Table 1]), but the differences were not large. By contrast, the *tktA*::Tn*10* *tktB*::*kan* double mutant (TX3158), which lacks both transketolase isoenzymes, failed to grow on minimal-plus-glucose, minimal-plus-glycerol, or minimal-plus-pyruvate medium, even when the six aromatic amino acids and vitamins were supplied (Table 1). Yet the *tktA tktB* double mutant did grow on minimal-plus-glucose medium containing the six aromatic compounds and pyridoxine, pyridoxal, 4-hydroxy-L-threonine, or glycolaldehyde (Table 1). Glycolaldehyde is thought to act as a nonphysiological precursor of 4-hydroxy-L-threonine when the pathway mediated by *pdxB* and *serC* is blocked (Fig. 1) (4, 10, 17, 24). Other vitamins, such as thiamine (vitamin B_1) or riboflavin (vitamin B_2), did not allow growth (Table 1). Pyridoxine and 4-hydroxy-L-threonine also supported growth of the *tktA tktB* double mutant on minimal-plus-glycerol medium containing the six aromatic compounds. Moreover, the original AI1118 (*tktA*::Tn*10* *tktB*::*kan*) double mutant, which is in a different genetic background and was propagated on LB medium during and after mailing, showed the same auxotrophy for pyridoxine, pyridoxal, 4-hydroxy-L-threonine, and glycolaldehyde as TX3158 (Table 1). Together, these data are consistent with the pathway in Fig. 1 and support the notion that D-erythrose-4-phosphate is a direct precursor of 4-hydroxy-L-threonine, pyridoxine (vitamin B_6), and ultimately pyridoxal 5′-phosphate as well as the six aromatic amino acids and vitamins. These data also show that the major *tktA* and *tktB* transketolases are not essential for the biosynthesis of D-1-deoxyxylulose (Fig. 1). If this were the case, then the *tktA tktB* double mutant would grow when supplemented with pyridoxine but not with 4-hydroxy-L-threonine or glycolaldehyde alone. However, it is still possible that a new transketolase which is specific for D-1-deoxyxylulose synthesis but is not part of the pentose phosphate pathway exists.

Table 1 contains data on several additional growth properties of *tktA tktB* transketolase mutants. The TX3158 *tktA tktB* double mutant grew at about the same rate on supplemented glucose as on the glyconeogenic carbon source, glycerol (Table 1). This finding does not support the idea that the major transketolases are necessary for efficient glycolysis in *E. coli*, as was suggested for yeast mutants lacking the major *TKL1*

transketolase (23). However, the growth characteristics of *E. coli tktA tktB* double mutants were strongly strain dependent. Unlike TX3158, the AI1118 *tktA tktB* double mutant did grow much faster on glycerol than on glucose minimal medium (Table 1) and tended to accumulate faster-growing pseudorevertants on minimal-plus-glucose medium containing the six aromatic compounds and pyridoxine (data not shown). As noted above, the AI1118 double mutant was propagated on LB medium and undoubtedly had accumulated additional suppressor mutations before the start of these experiments. Curiously, 4-hydroxy-L-threonine, but not pyridoxine, allowed growth of the TX3158 *tktA tktB* mutant on minimal-plus-pyruvate medium containing the six aromatic compounds (Table 1); we do not have an explanation for this result. Again, TX3158 and AI1118 exhibited strain differences, since AI1118 grew on minimal-plus-pyruvate medium containing either pyridoxine or 4-hydroxy-L-threonine. Finally, neither *tktA tktB* double mutant grew on supplemented gluconate medium (Table 1). This result implies that a sufficient amount of gluconate may be trapped in the pentose phosphate pathway of the *tktA tktB* mutant to inhibit growth, despite the possibility of gluconate utilization by the Entner-Doudoroff pathway (8).

In summary, the findings in this paper extend the well-known central role of D-erythrose-4-phosphate as the precursor to aromatic compounds in bacteria and yeast (Fig. 1) (5, 6, 8, 14, 15, 20, 21, 23, 26). The growth requirement for pyridoxine, pyridoxal, glycolaldehyde, or 4-hydroxy-L-threonine was clearly demonstrated in *E. coli tktA tktB* double mutants lacking all known transketolase activities (Fig. 1; Table 1). It was not established whether D-erythrose-4-phosphate is a precursor to pyridoxine in *S. cerevisiae*, because the culture media used to grow the analogous *TKL1 TKL2* yeast double mutants were supplemented with a vitamin mixture (21). The regulation and roles of the major and minor transketolases in bacteria and *S. cerevisiae* are not well understood (8, 13–15, 21, 23). However, overexpression of a transketolase in *E. coli* led to increased production of 3-deoxy-D-*arabino*-heptulosonate, which is the product of the first step in the common aromatic pathway (5, 6, 20). This result indicates that the in vivo concentration of D-erythrose-4-phosphate might be limiting and therefore might be a potential point of pathway regulation.

The results presented here also show that pyridoxine and pyridoxal 5′-phosphate biosynthesis is linked by a common intermediate to aromatic amino acid and aromatic vitamin biosynthesis. In this regard, two other observations are noteworthy. First, there is an additional genetic link between pyridoxine and aromatic biosynthesis. The *aroA* gene, which mediates the penultimate step in the conversion of D-erythrose-4-phosphate to chorismate (20), is in a superoperon with *serC*, which mediates both pyridoxine and serine biosynthesis (Fig. 1) (17). Second, there appear to be two key shared metabolic intermediates that connect the biosynthesis of pyridoxine (vitamin B_6) and several other vitamins (Fig. 1). D-Erythrose-4-phosphate is a direct precursor of pyridoxine and the three aromatic vitamins, *p*-aminobenzoate, *p*-hydroxybenzoate, and 2,3-dihydroxybenzoate (20, 26) (Fig. 1). The other direct precursor of the pyridine ring of pyridoxine, D-1-deoxyxylulose, is thought to be an intermediate in thiamine (vitamin B_1) and riboflavin (vitamin B_2) biosynthesis (2, 25). Continued genetic and physiological analyses at the pathway and genetic levels should elucidate the role of the two transketolase isoenzymes and the interconnected regulation of vitamin biosynthesis in *E. coli*.

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REFERENCES

1. **Bender, D. A.** 1985. Amino acid metabolism. John Wiley & Sons, New York.
2. **David, S., B. Estramareix, J. C. Fischer, and M. Therisod.** 1982. The biosynthesis of thiamine. Synthesis of [1,1,1,5-H]-1-deoxy-D-threo-2-pentulose and incorporation of this sugar in biosynthesis of thiazole by *Escherichia coli* cells. J. Chem. Soc. Perkin Trans. I **1982:**2131–2137.
3. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. **Dempsey, W. B.** 1987. Synthesis of pyridoxal phosphate, p. 539–543. *In* F. C. Neidhart, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
5. **Draths, K. M., and J. W. Frost.** 1990. Synthesis using plasmid-based biocatalysis: plasmid assembly and 3-deoxy-D-*arabino*-heptulosonate production. J. Am. Chem. Soc. **112:**1657–1659.
6. **Draths, K. M., D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky, and J. C. Lievence.** 1992. Biocatalytic synthesis of aromatics from D-glucose: the role of transketolase. J. Am. Chem. Soc. **114:**3956–3962.
7. **Drewke, C., C. Notheis, U. Hansen, E. Leistner, T. Hemscheidt, R. E. Hill, and I. D. Spenser.** 1993. Growth response to 4-hydroxy-L-threonine of *Escherichia coli* mutants blocked in vitamin B_6 biosynthesis. FEBS Lett. **318:**125–128.
8. **Fraenkel, D. G.** 1987. Glycolysis, pentose phosphate pathway, and Entner-Doudoroff pathway, p. 142–150. *In* F. C. Neidhart, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
9. **Fraenkel, D. G., and R. T. Vinopal.** 1973. Carbohydrate metabolism in bacteria. Annu. Rev. Microbiol. **27:**69–100.
10. **Hill, R. E., P. Horsewood, I. D. Spenser, and Y. Tani.** 1975. Biosynthesis of vitamin B_6. Incorporation of glycolaldehyde into pyridoxal. J. Chem. Soc. Perkin Trans. I **1975:**1622–1627.
11. **Hill, R. E., B. G. Sayer, and I. D. Spenser.** 1989. Biosynthesis of vitamin B_6: incorporation of D-1-deoxyxylulose. J. Am. Chem. Soc. **111:**1916–1917.

11a. **Hill, R. E., and I. D. Spenser.** Biosynthesis of pyridoxine. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, 2nd ed., in press. American Society for Microbiology, Washington, D.C.

12. **Horecker, B. L.** 1968. Pentose phosphate pathway, uronic acid pathway, interconversion of sugars, p. 139–163. *In* F. Dickens, P. J. Randle, and W. J. Whelan (ed.), Carbohydrate metabolism and its disorders. Academic Press, New York.
13. **Iida, A., S. Teshiba, and K. Mizobuchi.** 1993. Identification and characterization of the *tktB* gene encoding a second transketolase in *Escherichia coli* K-12. J. Bacteriol. **175:**5375–5383.
14. **Josephson, B. L., and D. G. Fraenkel.** 1969. Transketolase mutants of *Escherichia coli*. J. Bacteriol. **100:**1289–1295.
15. **Josephson, B. L., and D. G. Fraenkel.** 1974. Sugar metabolism in transketolase mutants of *Escherichia coli*. J. Bacteriol. **118:**1082–1089.

15a. **Julliard, J.-H.** 1992. Biosynthesis of the pyridoxal ring (vitamin B_6) in higher plant chloroplasts and its relationship with the biosynthesis of the thiazole ring (vitamin B_1). C. R. Acad. Sci. Ser. III **314:**285–290.

16. **Lam, H.-M., E. Tancula, W. B. Dempsey, and M. E. Winkler.** 1992. Suppression of insertions in the complex *pdxJ* operon of *Escherichia coli* K-12 by *lon* and other mutations. J. Bacteriol. **174:**1554–1567.
17. **Lam, H.-M., and M. E. Winkler.** 1990. Metabolic relationships between pyridoxine (vitamin B_6) and serine biosynthesis in *Escherichia coli* K-12. J. Bacteriol. **172:**6518–6528.

18. **Lam, H.-M., and M. E. Winkler.** 1992. Characterization of the complex *pdxH-tyrS* operon of *Escherichia coli* K-12 and pleiotropic phenotypes caused by *pdxH* insertion mutations. J. Bacteriol. **174:** 6033–6045.
19. **Pease, A. J., and M. E. Winkler.** 1993. Functional and evolutionary relationships between the *pdxB* and *serA* gene products of *Escherichia coli* K-12, abstr. K-116, p. 280. Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993. American Society for Microbiology, Washington, D.C.
20. **Pittard, A. J.** 1987. Biosynthesis of the aromatic amino acids, p. 368–394. *In* F. C. Neidhart, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
21. **Schaaff-Gerstenschlager, I., G. Mannhaupt, I. Vetter, F. K. Zimmermann, and H. Feldmann.** 1993. *TKL2*, a second transketolase gene of *Saccharomyces cerevisiae*: cloning, sequence and deletion analysis of the gene. Eur. J. Biochem. **217:**487–492.
22. **Schoenlein, P. V., B. B. Roa, and M. E. Winkler.** 1989. Divergent transcription of *pdxB* and homology between the *pdxB* and *serA* gene products in *Escherichia coli*. J. Bacteriol. **171:**6084–6092.
23. **Sundstrom, M., Y. Lindqvist, G. Schneider, U. Hellman, and H. Ronne.** 1993. Yeast *TKL1* gene encodes a transketolase that is required for efficient glycolysis and biosynthesis of aromatic amino acids. J. Biol. Chem. **268:**24346–24352.
24. **Vella, G. J., R. E. Hill, B. S. Mootoo, and I. D. Spenser.** 1980. The status of glycolaldehyde in the biosynthesis of vitamin B_6. J. Biol. Chem. **255:**3042–3048.
25. **Volk, R., and A. Bacher.** 1991. Biosynthesis of riboflavin. The structure of the four-carbon precursor. J. Am. Chem. Soc. **110:** 3651–3653.
26. **Wallace, B. J., and J. Pittard.** 1969. Regulation of 3-deoxy-D-*arabino*-heptulosonic 7-phosphate acid synthetase activity in relation to the synthesis of the aromatic vitamins in *Escherichia coli* K-12. J. Bacteriol. **99:**707–712.