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Pleiotropic Phenotype of an *Escherichia coli* Mutant Lacking Leucyl-, Phenylalanyl-Transfer Ribonucleic Acid-Protein Transferase

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A mutant of *Escherichia coli* that lacks leucyl-, phenylalanyl-transfer ribonucleic acid-protein transferase had diminished activities of L-phenylalanyl-transfer ribonucleic acid synthetase and tryptophanase, grew faster than its parent with aspartic acid as the sole nitrogen source, accumulated higher levels of enterochelin in the medium during iron limitation, and exhibited an abnormal morphology.

Leucyl-, phenylalanyl-transfer ribonucleic acid (tRNA)-protein transferase (EC 2.3.2.6) catalyzes the transfer of leucine, phenylalanine (19), or methionine (9, 17) from aminoacyl-tRNA's into peptide linkage at the NH_2 terminus of specific acceptor proteins. This enzyme accounts for the ribosome-independent amino acid incorporation originally described by Kaji et al. (11) and is specific for acceptors possessing a basic NH_2-terminal residue (12, 18). To define the role of this enzyme in the bacterial cell, we have begun an analysis of transferase-deficient mutants. We have previously shown that a mutant of *Escherichia coli* lacking transferase activity (20) exhibits a four- to fivefold increase in proline oxidase activity (6). We now report that this mutant has pleiotropic physiological defects that revert with the transferase mutation (Table 1).

Concomitant with the loss of leucyl-, phenylalanyl-tRNA-protein transferase in mutant MS845, the specific activity of phenylalanyl-tRNA synthetase was significantly lowered. The activities of 17 other aminoacyl-tRNA synthetases, including those for leucine and methionine, however, were identical in wild-type and mutant strains. In addition to decreased levels of phenylalanyl-tRNA synthetase activity, the specific activity of tryptophanase was decreased in mutant cultures grown in the presence of 1 to 5 mM L-tryptophan. Although mutant tryptophanase was decreased in total activity, the kinetics of induction by tryptophan and repression by glucose were similar in mutant and wild-type cells. Neither phenylalanyl-tRNA synthetase (21) nor tryptophanase (13) purified from mutant cultures functioned as an acceptor of leucine or phenylalanine in the transferase reaction.

In the presence of aspartic acid as the sole nitrogen source, the mutant grew about twice as fast as its parent (doubling times of 2.3 and 4.8 h, respectively). These strains are nonreverting proline auxotrophs and, since proline oxidase is induced by proline (5), this experiment was performed with prototrophic derivatives obtained by introduction of the F128 episome (14). In the presence of tryptophan or 17 other amino acids, the generation time was the same in mutant or wild-type cells. This increased growth rate on aspartate was shown to be independent of aspartate-α-keto-glutarate transaminase (7); the activity of this enzyme was identical in mutant and wild-type strains.

Enterochelin, an iron transport agent, and related derivatives of 2,3-dihydroxybenzoylserine (16), were observed to accumulate in culture filtrates of the mutant. Although increased in amount, the proportions of these compounds detected by thin-layer chromatography (4) were similar in the mutant and its parent. Neither strain was resistant to bacteriophage T1, although resistance to this phage has been associated with other mutations affecting enterochelin levels (8). Enterochelin accumulation, which is regulated by iron (3), was repressed in the mutant at the same iron concentrations as in the wild type.

In addition to these physiological alterations, the mutant MS845 was observed to have an abnormal morphology, appearing as clubs, spheres, or ellipsoids. The growth rate was nearly the same as that observed for wild-type cells, and the irregularly shaped mutant cells were not significantly more sensitive to antibiotics (penicillin, vancomycin, D-cycloserine) or other agents (deoxycholate, crystal violet) that act on the cell wall. Normal morphology

TABLE 1. *Phenotypic defects associated with the transferase mutation*

Strain[a]	Transferase (mU/mg soluble protein)[b]	Phenylalanyl-tRNA synthetase (nmol/min per mg protein at 30°C)[c]	Tryptophanase (nmol/min per mg protein at 37°C)[d]	Enterochelin accumulation (nmol catechol/ml culture fluid)[e]	Morphology
W4977	9.0	6.7	163	130	Rod
MS845	<0.03	2.8	76	250	Irregular
R18	8.0	6.6	180	164	Rod

[a] MS845 was derived from W4977 by nitrosoguanidine mutagenesis; R18 is a spontaneous revertant of MS845 (20).

[b] Soluble extracts were prepared and assayed for phenylalanine transfer activity as previously described (18).

[c] Measured in the same extracts used for transferase assays, as described previously (21).

[d] In toluene-treated cells (2) after growth to mid-log phase in minimal medium containing 0.5% glycerol, 20 mM L-proline, and 2 mM L-tryptophan.

[e] Measured, as described previously (1), after growth for 3 days in minimal medium containing 0.5% glycerol, 20 mM L-proline, and 0.1 mM α,α'-dipyridyl.

was restored by addition of D-alanine or D-glutamic acid to the medium. Alanine racemase (10) and D-alanyl-D-alanine ligase (15) activities were not altered in the mutant.

Since soluble extracts of *E. coli* contain a number of proteins that can be aminoacylated in vitro by leucyl-, phenylalanyl-tRNA-protein transferase (20), it is not surprising that a mutant with altered transferase activity exhibits a widely pleiotropic phenotype. The defects described here persist in the absence of proline oxidase activity and are therefore independent of the increase in proline catabolism previously described (6). Although some of these defects may result from the failure to modify a protein that is directly related to a mutant phenotype, the observation that L-phenylalanyl-tRNA synthetase and tryptophanase are not substrates for the transferase suggests that intermediate acceptors affected by the lack of transferase activity may be several steps removed from the observed phenotypes. That leucyl-, phenylalanyl-tRNA-protein transferase may be specifically associated with the regulation of amino acid metabolism, however, is consistent with the observation that each of the phenotypic defects of the transferase-deficient mutant appears to involve amino acids either as substrates or precursors.

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LITERATURE CITED

1. **Arnow, L. E.** 1937. Colorimetric determination of the components of 3,4-dihydroxyphenylalanine-tyrosine mixtures. J. Biol. Chem. 118:531–537.
2. **Bilezikian, J. P., R. O. R. Kaempfer, and B. Magasanik.** 1967. Mechanism of tryptophanase induction in *Escherichia coli*. J. Mol. Biol. 27:495–506.
3. **Brot, N., and J. Goodwin.** 1968. Regulation of 2,3-dihydroxybenzoylserine synthetase by iron. J. Biol. Chem. 243:510–513.
4. **Bryce, G. F., and N. Brot.** 1972. Studies on the enzymatic synthesis of the cyclic trimer of 2,3-dihydroxy-*N*-benzoyl-L-serine in *Escherichia coli*. Biochemistry 11:1708–1715.
5. **Dendinger, S., and W. J. Brill.** 1970. Regulation of proline degradation in *Salmonella typhimurium*. J. Bacteriol. 103:144–152.
6. **Deutch, C. E., and R. L. Soffer.** 1975. Regulation of proline catabolism by leucyl,phenylalanyl-tRNA-protein transferase. Proc. Natl. Acad. Sci. U.S.A. 72:405–408.
7. **Gunsalus, I. C., and J. R. Stamer.** 1955. Transaminases in bacteria, p. 170–177. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. II. Academic Press, Inc., New York.
8. **Guterman, S. K., and L. Dann.** 1973. Excretion of enterochelin by *exbA* and *exbB* mutants of *Escherichia coli*. J. Bacteriol. 114:1225–1230.
9. **Horinishi, H., S. Hashizume, M. Seguchi, and K. Takahashi.** 1975. Incorporation of methionine by a soluble enzyme system from *Escherichia coli*. Biochem. Biophys. Res. Commun. 67:1136–1143.
10. **Julius, M., C. A. Free, and G. T. Barry.** 1970. Alanine racemase (*Pseudomonas*), p. 171–176. *In* H. Tabor and C. Tabor (ed.), Methods in enzymology, vol. XVIIA. Academic Press Inc., New York.
11. **Kaji, A., H. Kaji, and G. D. Novelli.** 1963. A soluble amino acid incorporating system. Biochem. Biophys. Res. Commun. 10:406–409.
12. **Leibowitz, M. J., and R. L. Soffer.** 1971. Enzymatic modification of proteins. VII. Substrate specificity of leucyl,phenylalanyl-transfer ribonucleic acid-protein transferase. J. Biol. Chem. 246: 5207–5212.
13. **London, J., and M. E. Goldberg.** 1972. The tryptophanase from *Escherichia coli* K12. I. Purification, physical properties, and quaternary structure. J. Biol. Chem. 247:1566–1570.
14. **Miller, H. J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Neuhaus, F. C.** 1962. The enzymatic synthesis of D-alanyl-D-alanine. I. Purification and properties of D-alanyl-D-alanine synthetase. J. Biol. Chem. 237:778–786.
16. **O'Brien, I. G., and F. Gibson.** 1970. The structure of enterochelin and related 2,3-dihydroxy-*N*-benzoylser-

ine conjugates from *Escherichia coli*. Biochim. Biophys. Acta 215:393-402.

17. **Scarpulla, R. C., C. E. Deutch, and R. L. Soffer.** 1976. Transfer of methionyl residues by leucyl, phenylalanyl-tRNA-protein transferase. Biochem. Biophys. Res. Commun. 71:584-589.
18. **Soffer, R. L.** 1973. Peptide acceptors in the leucine, phenylalanine transfer reaction. J. Biol. Chem. 248:8424-8428.
19. **Soffer, R. L.** 1974. Aminoacyl-tRNA transferases. Adv. Enzymol. 40:91-139.
20. **Soffer, R. L., and M. Savage.** 1974. A mutant of *Escherichia coli* defective in leucyl,phenylalanyl-tRNA-protein transferase. Proc. Natl. Acad. Sci. U.S.A. 71:1004-1007.
21. **Stulberg, M. P.** 1967. The isolation and properties of phenylalanyl-ribonucleic acid synthetase from *Escherichia coli* B. J. Biol. Chem. 242:1060-1064.