# Phenylalanyl Transfer RNA Alteration in Drug Resistant Ehrlich Ascites Tumor Cells (34964)

RODNEY C. RICHIE, MICHELE G. ENGLISH, AND A. CLARK GRIFFIN

*The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Department of Biochemistry, and Baylor College of Medicine, Houston, Texas 77025*

Many investigators have proposed that the transfer ribonucleic acids (tRNAs) may play an important role in the control of protein synthesis and in other regulatory functions within the cell. Withholding a particular amino acid results in decreased synthesis of the tRNA that activates the amino acid. Stent and Brenner (1) and also Kurland and Maaløe (2) reasoned that the uncharged tRNA, denoting a paucity of the amino acid, acts as a specific repressor upon the gene responsible for its synthesis. Although the validity of this hypothesis has been questioned (3, 4), amino acid activation as a prerequisite for RNA synthesis has been demonstrated repeatedly (5, 6).

Vogel (7) has proposed the existence of aminoacyl-tRNA analogues that are preferentially charged under repressive conditions and act with a general repression trigger to block the relative motion of the mRNA and ribosome. Roth and co-workers (8), who have linked histidyl-tRNA with repression of histidine biosynthetic enzymes in *Salmonella,* believe the initiation of translation of the polycistronic histidine message is controlled by a histidine codon in the operator. They envision the histidyl-tRNA as prohibiting, and the uncharged $tRNA^{his}$ as initiating, transcription. As an alternative, the uncharged $tRNA^{his}$ is thought to be altered by acylation into a functional translation inducer. This latter concept is particularly attractive in light of the findings of Yegian *et al.* (9) who noted that many tRNA molecules found in amino acid-deprived conditions (and therefore thought to repress further RNA synthesis) will esterify with such amino acid derivatives as *N*-formyl glycine and *N*-formyl methionine. Vasquez and Monro (10) discovered that certain inhibitors of protein synthesis work specifically upon the aminoacyl tRNA to alter its binding to ribosomal subunits.

In view of the above possible regulatory functions of tRNA it is interesting to speculate the tRNAs may be directly involved in the transformation of normal to malignant tumor cells or in the subsequent behavior of the cancerous cells. Work to support this speculation has been along three lines. First, tRNA changes have been noted with differing carcinogenic stimuli (viral or chemical). Second, tRNA changes have been revealed in comparisons of patterns from normal and neoplastic tissues. Third, tRNA differences have been observed in closely related tumors.

Investigating tRNA changes with respect to carcinogenic stimuli, Miller and Miller (11) have found the potent hepatocarcinogen *N*-acetoxy-2-acetylaminofluorene (*N*-acetoxy-AAF) to react with the guanine component of nucleic acids. Goldman and Griffin (12) have shown that certain of the aminoacyl-tRNA patterns from livers of rats fed diets containing the hepatocarcinogen, 3′-methyl-4-dimethylaminoazobenzene, differ from normal liver controls. Axel *et al.* (13) found that ethionine specifically alters the tRNAs for leucine such that they fail to read their appropriate triplet recognition codons. The alkylation of nucleic acids by nitrosamines and nitrosamides has been reviewed by Magee *et al.* (14). Gefter and Russel (15) found that infection of *Escherichia coli* with the defective transducing bacteriophage 80 $dSU_{III}$ leads to the synthesis of three forms of suppressor tyrosine tRNAs. They differ in the extent of alteration of the base adjacent to the anticodon, and proportional differences

are found in their ability to bind to ribosomes and support *in vitro* protein synthesis. Both Holland *et al.* (16) and Hay *et al.* (17) report entirely new species of tRNAs in tumors produced by SU-40 and herpes viruses, respectively.

Other investigators have revealed differences in tRNA patterns by comparing normal and neoplastic tissue tRNA profiles. Baliga *et al.* (18), Weinstein (19), Goldman *et al.* (20), and Griffin (21) have reported many differences in tRNA profiles between rat liver and Novikoff ascites tumors. Taylor *et al.* (22) similarly noted differences in tRNA patterns between mouse tumors and normal mouse organs.

Transfer ribonucleic acid alterations have also been reported in comparisons between tumors *per se*. Mushinski and Potter (23) and Mach *et al.* (24) have related differences in tRNA patterns from several mouse plasma cell tumors with immunoglobulin variability. They proposed that the gene message coding for the immunoglobulin chain may be altered in translation by regulation of the tRNA molecules available at the transcriptional level.

In view of the excellent resolution of tRNAs achieved by reversed phase chromatography (25, 26) the current study was initiated. Comparisons were made of tRNA fractions from normal mouse liver with Ehrlich ascites mouse tumors both sensitive and resistant to HN2 [nitrogen mustard, methylbis-(*b*-chloroethyl)amine]. Transfer RNA fractions were prepared from washed cells by the method of Brungraber (27). The resistant strain of Ehrlich Lettre' ascites tumor (28) (obtained through the courtesy of Dr. R. J. Rutman) was derived from *in vivo* HN2 treatment. The tumor was grown intraperitoneally in female Swiss mice for 7 days. The cells were initially washed with a buffer (0.14) *M* NaCl, 0.02 *M* dextrose, and 0.04 *M* Tris–HCl, pH 8.5) before homogenization. An aminoacyl synthetase preparation was isolated using the procedure of Goldman *et al.* (20). The tRNAs to be compared were double-labeled with tritium and $^{14}$C-labeled amino acids and resolved by reversed phase chromatography on a column originally described by Weiss and Kelmers (25). Aliquots from each 15-ml fraction were filtered on Millipore filters and counted in a liquid scintillation spectrometer.

Preliminary results indicated that no variation in the tRNA fractions for arginine were seen (Fig. 1). Similarly, the tRNA fractions

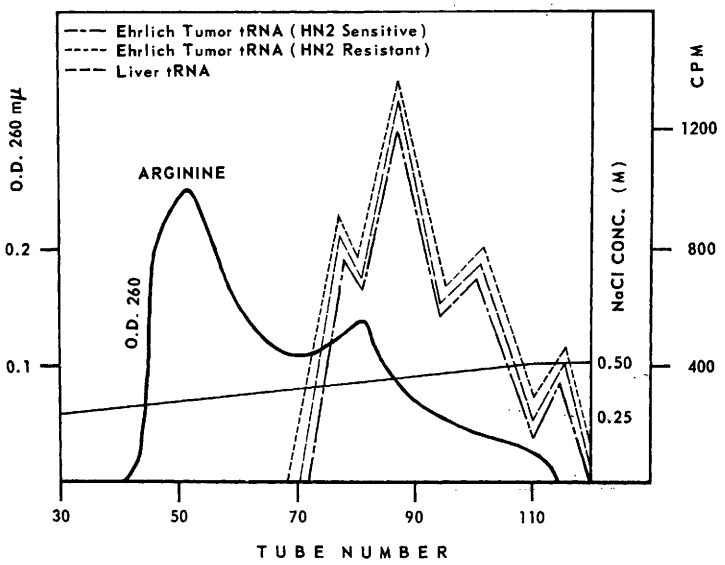


FIG. 1. Comparison of chromatographic profiles of arginyl-tRNAs of Ehrich tumors and normal mouse liver.

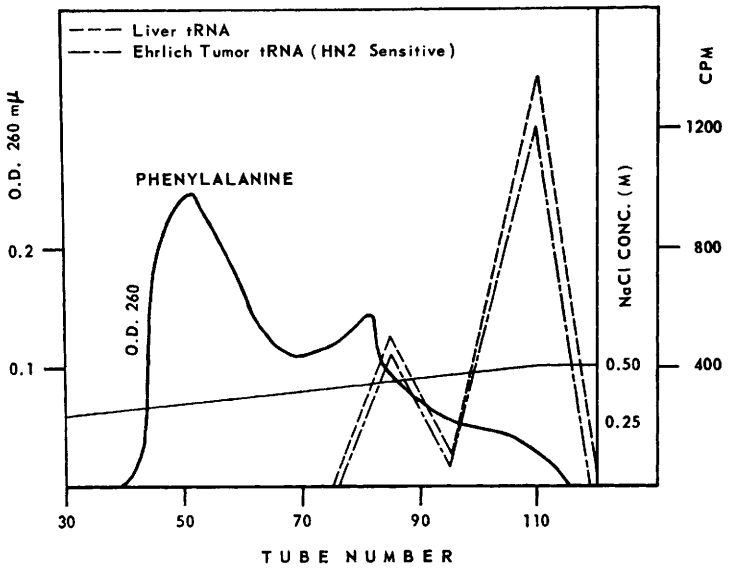


FIG. 2. Comparison of chromatographic profiles of phenylalanyl-tRNAs of Ehrlich tumors and normal mouse liver.

for phenylalanine were identical when the tRNA of Ehrlich tumor cells, sensitive to HN2, were compared with normal mouse liver tRNA (Fig. 2). The major phenylalanyl-tRNA peak in the resistant species, however, eluted 10 fractions early indicating a structural or conformational change in this specific phenylalanyl-tRNA (Fig. 3 and 4). This alteration in the chromatographic behavior of the phenylalanyl-tRNA was observed repeatedly employing many preparations of tumor cells. In the comparisons of the tRNAs for tyrosine the patterns for the resistant and sensitive strains of this tumor were almost identical employing this chromatographic procedure. In contrast, the tyrosyl-tRNA

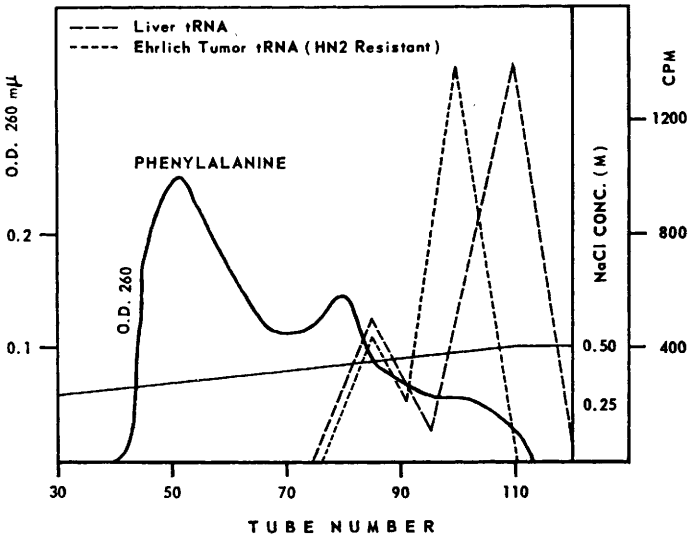


FIG. 3. Comparison of chromatographic profiles of phenylalanyl-tRNAs of Ehrlich tumors and normal mouse liver.

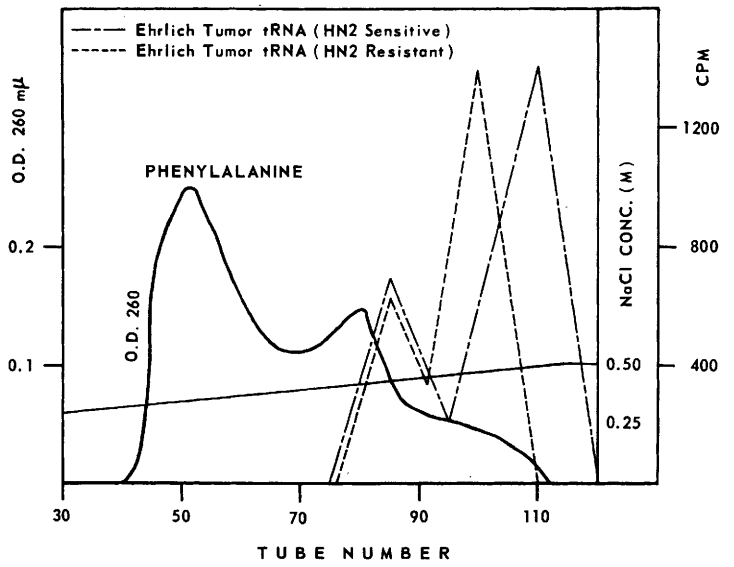


FIG. 4. Comparison of chromatographic profiles of phenylalanyl-tRNAs of Ehrlich tumors and normal mouse liver.

profile of normal mouse liver exhibited a small additional peak not observed in the tumor pattern (Fig. 5). Other investigators have reported differences in the tyrosyl-tRNAs of normal and neoplastic tissues.

Multiple forms of tRNA for the same amino acid (isoaccepting tRNAs) have been previously noted (18). Although some of these different forms have been shown to be merely active and inactive forms of the same tRNA (29, 30), the degeneracy of the tRNA system appears established by the correspondence shown between several isoaccepting tRNAs and the multiple condons for an ami-

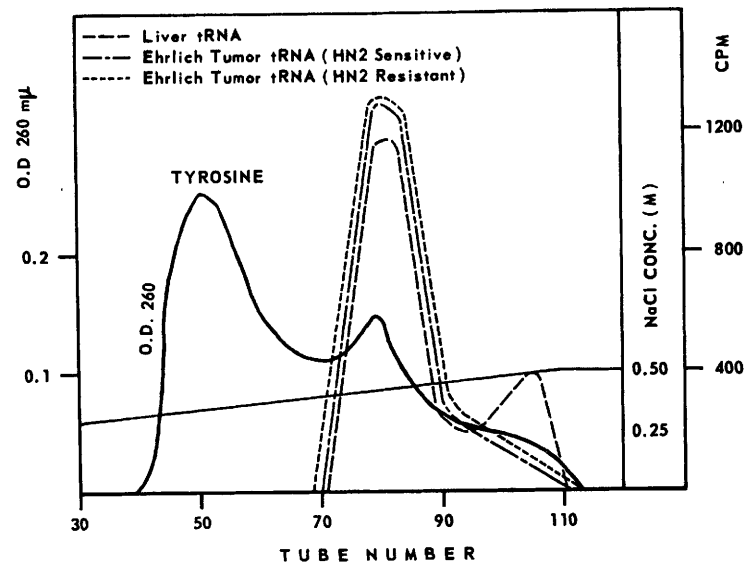


FIG. 5. Comparison of chromatographic profiles of tyrosyl-tRNAs of Ehrlich tumors and normal mouse liver.

no acid (31–33). Whether the four arginyl-tRNAs indicated in this presentation represent four separate tRNAs, each reading a specific codon, was not determined. Recently, Taylor (34) reported that the major form of phenylalanyl-tRNA from an Ehrlich ascites tumor elutes earlier from a MAK column than do phenylalanyl-tRNAs from other tissues. He concluded that the observed modification of the Ehrlich ascites tumor phenylalanyl-tRNA did not affect the acceptance of phenylalanine or the capacity to bind to ribosome in the presence of poly U or poly UC (codon recognition site). Since different column procedures were employed it is difficult to make any direct comparison of the findings reported in this laboratory with those reported by Taylor.

Previous work with the Ehrlich tumors has shown that the HN2 probably exerts its major cytotoxic effects upon the nucleoprotein metabolism and function. Extensive binding between DNA and protein is seen when sensitive cells are treated with the alkylating agent (35), and this binding lessens considerably with the acquisition of resistance. Preliminary evidence based upon HN2 uptake comparisions between resistant and sensitive cells indicates that the altered functioning of the cell's membrane may account, at least in part, for the acquisition of resistance (36).

Rutman and co-workers (35) have confirmed this permeability difference as a source of resistance to alkylating agents and have also pointed to the remarkable repair mechanisms these cells must employ to withstand large single doses of HN2. Assuming these cells are unable to affect a virtually complete repair, the observation of an altered tRNA for phenylalanine presented in this paper may represent a permanently altered gene. Whether such a consistent alteration could have occurred when the resistant strain was developed and whatever its role in the cell's acquisition of resistance must await further investigation.

*Summary.* Phenylalanyl, tyrosyl, and arginyl transfer RNA profiles of normal mouse liver, HN2 sensitive and resistant strains of Ehrlich ascites tumor cells were studied employing reversed phase chromatography. Arginyl-tRNA patterns of the liver and the tumor cells were identical while some minor changes were evident with tyrosyl-tRNAs. Resistance to the HN2 was accompanied by a shift in the phenylalanyl-tRNA pattern.

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1. Stent, G. S., and Brenner, S., Proc. Nat. Acad. Sci. U.S. **47**, 2005 (1961).

2. Kurland, C. G., and Maaløe, O., J. Mol. Biol. **4**, 193 (1962).

3. Morris, D. W., and DeMoss, J. A., J. Bacteriol. **90**, 1624 (1965).

4. Ezekiel, D. H., and Valulis, B., Biochim. Biophys. Acta **129**, 123 (1966).

5. Fangman, W. L., and Neidhardt, F. C., J. Biol. Chem. **239**, 1844 (1964).

6. Eidlic, L., and Neidhardt, F. C., J. Bacteriol. **89**, 706 (1965).

7. Vogel, H. J., 7th Can. Cancer Congr., Honey Harbour, 7, 133. Pergamon Press (1967).

8. Roth, J. R., Silbert, D. F., Fink, G. R., Voll, M. J., Anton, D., Hartman, P. E., and Ames, B., Cold Spring Harbor Symp. Quant. Biol. **31**, 383 (1966).

9. Yegian, C. D., Stent, G. S., and Martin, E. M., Proc. Nat. Acad. Sci. U. S. **55**, 839 (1966).

10. Vasquez, D., and Monro, R. E., Biochim. Biophys. Acta **142**, 155 (1967).

11. Miller, J. A., and Miller, E. C., *in* "Carcinogenesis: A Broad Critique," (The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, ed.), p. 387. Williams and Wilkins, Baltimore (1967).

12. Goldman, M., and Griffin, A. C., Cancer Res., **30** (1970).

13. Axel, R., Weinstein, B. I., and Farber, E., Proc. Nat. Acad. Sci. U. S. **58**, 1255 (1967).

14. Magee, P. N., Craddock, V. M., and Swann, P. F., *in* "Carcinogenesis: A Broad Critique" (The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, ed.), p. 421. Williams and Wilkins, Baltimore (1967).

15. Gefter, M. L., and Russel, R. L., J. Mol. Biol. **39**, 145 (1969).

16. Holland, J. J., Taylor, M. W., and Buck, C. A., Proc. Nat. Acad. Sci. U.S. **58**, 2437 (1967).

17. Hay, J., Subak-Sharpe, H., and Shepherd, W. M., Biochem. J. **103**, 69 (1967).

18. Baliga, B., Srinivasan, P. R., and Borek, E.,

Fed. Proc., Fed. Amer. Soc. Exp. Biol. **27**, 794 (1968).

19 Weinstein, B. I., *in* "Genetic Concepts and Neoplasia" (The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, ed.), pp. 380. Williams and Wilkins, Baltimore (1970).

20. Goldman, M., Johnston, W. M., and Griffin, A. C., Cancer Res. **29**, 1051 (1969).

21. Griffin, A. C., *in* "Advances in Enzyme Regulation" (G. Weber, ed.), Vol. 7, p. 361. Pergamon, New York (1968).

22. Taylor, M. W., Granger, G. A., Buck, C. A., and Holland, J. J., Proc. Nat. Acad. Sci. U.S.A. **57**, 1712 (1967).

23. Mushinski, J. F., and Potter, M., Biochemistry **8**, 1684 (1969).

24. Mach, B., Koblet, H., and Gros, D., Cold Spring Harbor Symp. Quant. Biol. **32**, 269 (1967).

25. Weiss, J. F., and Kelmers, A. D., Biochemistry **6**, 2507 (1967).

26. Yang, W. K., and Novelli, G. D., Biochem. Biophys. Res. Commun. **31**, 534 (1968).

27. Brunngraber, E. F., Biochem. Biophys. Res. Commun. **8**, 1 (1962).

28. Obtained through the courtesy of Dr. R. J. Rutman, Univ. of Pennsylvania, Philadelphia, Pa.

29. Lebowitz, P., Ipata, P., L. Makman, M., Richards, H. H., and Cantoni, G. L., Biochemistry **5**, 3617 (1966).

30. Makman, M. H., and Cantoni, G. L., Biochemistry **5**, 2246 (1966).

31. Nishimura, S., and Weinstein, B., I Biochemistry **8**, 832 (1969).

32. Caskey, C. T., Beaudet, A., and Nirenberg, M. W., J. Mol. Biol. **37**, 99 (1968).

33. Kano-Sueoka, T., Nirenberg, M. W., and Sueoka, N., J. Mol. Biol. **35**, 1 (1968).

34. Taylor, M. W., Cancer Res. **29**, 1681 (1969).

35. Rutman, R. J., Chun, E., and Lewis, F. S., Biochem. Biophys. Res. Commun. **32**, 650 (1968).

36. Klatt, O., Stehlin, J. S., McBride, C., and Griffin, A. C., Cancer Res. **29**, 286 (1969).

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