

Analysis of the Transfer RNA Population of Mouse Mammary Glands Infected with a Latent Mammary Tumor Virus¹

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SUMMARY

Mammary gland transfer RNA's (tRNA's) of C3H mice infected with mammary tumor virus were analyzed in the preneoplastic state and compared to tRNA's of virus-free C3Hf mice and another uninfected strain, C57BL/6, which is completely resistant to cancer.

This quantitative study was based on the ability of each tRNA to fix its corresponding amino acid. The amount of each of the 17 tRNA's tested was identical for the three mammary glands. In addition, tRNA populations during lactation correlated with the amino acids incorporated into the lactoproteins synthesized, which indicates adaptation of the tRNA's to protein biosynthesis.

Qualitative chromatographic studies on reverse phase capillary columns Type 5 of 10 aminoacyl-tRNA's did not reveal any difference in the isoacceptor elution profiles. This shows that no new isoaccepting tRNA is associated with the mammary tumor virus at that stage, and that no viral modification of a host tRNA has occurred.

INTRODUCTION

Many laboratories have shown quantitative or qualitative differences in the tRNA pool of neoplasma and normal tissues (22). It is known, for example, that different types of viruses induce changes at the translation level either by chemical changes of the host tRNA's [chain cleavage, alkylation, oxidation, and reduction of thiolated bases; for a general review, see Sueoka and Kano Sueoka (21)], or by *de novo* synthesis of the tRNA isoacceptors associated with the virus [for review, see Littauer and Inoue (11)]. These alterations, observed during cell differentiation, suggest that the tRNA population could have an essential role in carcinogenesis.

Turkington (23) has shown that the activities of some tRNA methylases increase in mammary carcinoma cells. A change in tRNA methylation is known to cause a change in the chromatographic behavior of the tRNA. Thus hypomethylation of tRNA^{Leu}, tRNA^{Val}, and tRNA^{Phe}

(9, 12, 13) decreases the NaCl concentration necessary to elute them from MAK and reverse phase chromatography Type 2 columns. In contrast, hypermethylation increases the NaCl concentration required to elute these tRNA's.

Therefore, we have compared the tRNA's of MTV-carrier C3H mice in the preneoplastic state, C3Hf mice lacking MTV, and another strain, C57BL/6, lacking MTV and shown to be resistant to carcinogenesis (18). This was done in order to determine whether an isoaccepting tRNA is associated with the latent virus or whether a transcriptional chemical modification of a host tRNA is induced by MTV.

MATERIALS AND METHODS

Eight-month-old C3H, C3Hf, and C57BL/6 mice (Orleans-la-Source, France) were mated and isolated during pregnancy. Twelve days after parturition, when the development of the mammary gland had reached a maximum, the females were killed and the mammary glands were immediately frozen in liquid nitrogen.

tRNA's and aminoacyl-tRNA synthetases were prepared by the method described by Beck *et al.* (1). The synthetases were extracted either from mammary glands or mouse liver. After verifying that the enzymatic activities were of the same order of magnitude in the 2 sources, we have routinely used the liver synthetases.

tRNA's were acylated in a final volume of 500 μ l in the presence of 50 μ Ci ³H-labeled amino acid or 10 μ Ci ¹⁴C-labeled amino acid (C.E.A., Saclay, France). After 30 min of incubation at 37°, a 100- μ l aliquot of the mixture was placed on a Whatman No. 3MM disc, 2 cm in diameter, and was immediately plunged into 10% TCA at 4° and washed in 5% TCA, ethanol, and diethyl ether (see Table 1).

The residual acid-precipitable radioactivity was measured with a liquid scintillation spectrophotometer (Intertech SL-40, Paris, France) in the presence of 5 ml of 4% Omnifluor in toluene (New England Nuclear, Boston, Mass.). The remaining 400 μ l were treated with an equal volume of phenol saturated with water to stop the acylation reaction. After agitation at 4° and centrifugation, the aqueous phase was dialyzed against 5 mM MgCl₂ in 10 mM acetate buffer (pH 4.7) for 2 hr. The solution, which contains the radioactive aminoacyl-tRNA's, was stored at -20°. The aminoacyl-tRNA's were stable for several weeks.

To fractionate the aminoacyl-tRNA isoacceptors, we used reverse phase chromatography in the capillary part of a Pasteur pipet, a technique called micro RPC-5 (7). This fractionation is carried out at pH 4.7 at room temperature

¹ This work was supported by the Ligue Nationale Française contre le Cancer.

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³ The abbreviations used are: tRNA^{Leu}, leucine tRNA (uncharged); Leu-tRNA, leucyl-tRNA (charged); tRNA designation for other amino acids is analogous; MAK, methyl albumin-Kieselguhr; MTV, mammary tumor virus; TCA, trichloroacetic acid; micro RPC-5, reverse phase capillary columns type 5.

Received December 4, 1974; accepted May 25, 1976.

Table 1
Quantitative estimation of lactating C3H, C3Hf, and C57BL/6 mouse mammary gland tRNA's by *in vitro* acylation

Preparative acylation of tRNA was done in 500 μ l of solution containing the following reagent mixture: 2 A₂₅₀ tRNA, 100 μ g enzyme, 50 μ Ci ³H-labeled amino acid or 10 μ Ci ¹⁴C-labeled amino acid (CEA, Saclay, France), 200 μ l stock solution composed of 150 mmoles Tris-HCl (pH 7.4), 50 mmoles KCl, 10 mmoles MgCl₂, 7 mmoles 2-mercaptoethanol, 2 mmoles ATP, 1 mmole CTP, 8 mmoles phosphoenolpyruvate kinase (Boehringer, Mannheim, Germany). After 30 min of incubation at 37°, a 100- μ l aliquot of the mixture was placed on a Whatman No. 3MM disc, 2 cm in diameter, immediately plunged into 10% TCA at 4°, and washed in 5% TCA, ethanol, and diethyl ether. The residual radioactivity was measured with an Intertechnique SL-40 liquid scintillation spectrophotometer in the presence of 5 ml of 4% Omnifluor in toluene (New England Nuclear).

Amino acid	Composition ^a of lactoproteins (mole %)	tRNA acylated (nmoles/mg total tRNA)			Mole % each specific tRNA		
		C3H	C3Hf	C57BL/6	C3H	C3Hf	C57BL/6
Alanine	5.5	2.5	2.3	2.8	7.6	7.2	8.3
Arginine	2.8	1.7	1.8	1.9	5.2	5.6	5.6
Aspartate	7.9	3.3	3.2	3.6	9.9	9.9	10.9
Asparagine							
Glutamate							
Glutamine	19.0	4.3	4.9	4.6	14.4	15.2	13.6
Glycine	3.7	1.5	1.5	1.6	4.5	4.6	4.8
Histidine	2.5	1.1	0.9	1.1	3.3	2.8	3.3
Leucine	9.7	3.0	3.0	2.8	9.1	9.3	8.3
Lysine	6.8	2.2	2.1	2.0	6.6	6.5	6.0
Methionine	2.6	2.0 ^b	2.0 ^b	2.1 ^b	6.0 ^b	6.2 ^b	6.2 ^b
Phenylalanine	4.3	1.5	1.5	1.4	4.5	4.6	4.1
Proline	11.4	0.9 ^c	0.9 ^c	0.8 ^c	2.7 ^c	2.8 ^c	2.5 ^c
Serine	7.8	2.6	2.5	2.8	7.9	7.7	8.3
Threonine	4.6	2.0	2.0	2.2	6.0	6.2	6.5
Tyrosine	4.0	1.2	1.2	1.4	3.6	3.7	4.3
Valine	7.0	2.7	2.3	2.3	8.1	7.1	6.8

^a Calculated from the results obtained by Ribadeau-Dumas *et al.* (17).

^b Combined values for tRNA^{Met} and tRNA^{Met}.

^c Low values because of incomplete acylation.

for about 1 hr. These conditions are suitable for the fractionation of the more unstable aminoacyl-tRNA's (8). In all of our experiments, we have chromatographed aminoacyl-tRNA's of different origin. One tRNA species was labeled with ¹⁴C and the other with ³H; the labeling was reversed in a 2nd experiment. Columns were eluted with a linear NaCl concentration gradient (0.20 to 0.55 M) in a total volume of 8 ml of 5 mM MgCl₂ in 10 mM acetate buffer (pH 4.7). Fractions of 2 drops were collected on Whatman No. 3MM paper discs, plunged into 10% TCA, and treated as indicated above.

RESULTS AND DISCUSSION

Comparative Study of Mouse Mammary Gland tRNA Populations from C3H Mice Infected with MTV and from Virus-free C3Hf and C57BL/6 Mice. To compare the mammary gland tRNA populations of the 3 strains of mice, we measured their capacity to fix 17 amino acids by esterification *in vitro*. Thirty-three nmoles of the total amino acids were fixed on 1 mg of tRNA, which is equivalent to 82% acylation assuming a mean tRNA molecular weight of 25,000; 1 mg thus corresponds to 40 nmoles. We have calculated the percentage of specific tRNA corresponding to each amino acid for the 3 kinds of mammary gland (Table 1). From this quantitative study, it appears that the 17 aminoacyl-tRNA species are present in the same amount in the

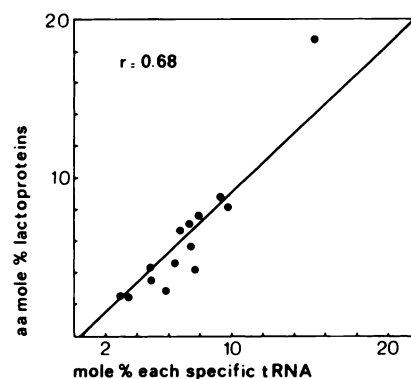


Chart 1. Correlation between *in vitro* acylated mammary gland tRNA frequencies during lactation and amino acid composition of lactoproteins; *r*, correlation coefficient.

C3H, C3Hf, or C57BL/6 glands. The small differences observed for tRNA^{Ala} and tRNA^{Val}, which do not exceed 15 to 20%, are within the limits of experimental error, since for each individual tRNA the acylation rate is about 82 ± 18%.

In all 3 cases, it is possible to establish a correlation between the different tRNA species and the amino acids incorporated in the lactoproteins which are produced in large amount in this tissue during lactation. Chart 1 shows that there is a linear relationship during that period. The

correlation coefficients are: $r_{C3H} = 0.67$; $r_{C57BL/6} = 0.60$; $r_{C3Hf} = 0.68$.

These values are highly significant for 16 of the amino acids but not for proline. These results are consistent with the existence of adaptation of the tRNA population to protein biosynthesis as described by Garel (6) for several highly differentiated biological systems (silk gland of *Bombyx mori* L., reticulocytes, plasmocytes, collagen granuloma, and lens). In the case of the mammary gland, the high percentage of tRNA^{Glu} and tRNA^{Leu} (4, 14) can be related to the high level of glutamate and leucine in the milk casein (17). However, for proline, which represents 9.4% of the amino acids of casein, this relationship cannot be established, probably on account of the low proline tRNA acceptor activity generally observed.

Comparative analysis of the mammary gland tRNA populations by acceptor activity should be investigated further. Indeed, modification in the distribution of the specific aminoacyl-tRNA acceptors, or the appearance of a new isoacceptor, can be concealed at the level of total acceptor activity. Similarly, changes in the tRNA nucleotides do not necessarily affect acceptor activity. However, chromatographic behavior can reveal such modifications; for example, alkyl groups have an effect on the chromatographic mobility of tRNA's. Thus, hypomethylated tRNA's require lower NaCl concentration for elution than do control tRNA's (9, 11, 13). The inverse effect might be expected in the case of the C3H mammary gland tRNA's, which could be hypermethylated through the influence of the MTV virus. Therefore, we have fractionated the tRNA from the 3 sources on micro RPC-5 in order to have a better idea of the changes in tRNA molecules.

Chromatographic Elution Profiles on Micro RPC-5 of Aminoacyl-tRNA's of C3H, C3Hf, and C57BL/6 Mouse Mammary Gland. Chart 2 shows the chromatographic profile of 10 aminoacyl-tRNA's. As indicated in "Materials Methods," we have cochromatographed the tRNA's from different sources, 1 labeled with ³H-labeled amino acid and 1 labeled with ¹⁴C-labeled amino acid. Analysis of this chart shows that the 10 tRNA's studied are identical when we compare C57BL/6, virus-free C3Hf, and C3H mouse mammary gland infected with the virus, before tumor appearance. We found neither a tRNA peak displacement due to structural modification of an isoacceptor nor a difference in the quantitative distribution of the tRNA isoacceptor species following specific inactivation of a given tRNA. Several striking cases of tRNA alteration following viral infection have been described, for example the major tRNA^{Leu} species in *Escherichia coli* infected by phage T₄ (24) or by phage T₂ (10) is cleaved, and thus its acceptor activity and contribution to the biosynthesis of host protein are diminished. Alteration of the isoaccepting tRNA pattern has also been observed after infection with adenovirus type 2 (16), vaccinia virus (2), SV40 (5, 20), or polyoma virus (5). The bases in avian myeloblastosis virus 4 S RNA differ from those found in the host 4 S RNA (15). Furthermore, we failed to find evidence of *de novo* isoacceptor tRNA synthesis induced by MTV, as has been reported for phage T₄ and T₅ (3, 19) and some mammalian viruses (15).

This study indicates that the tRNA's of the mammary

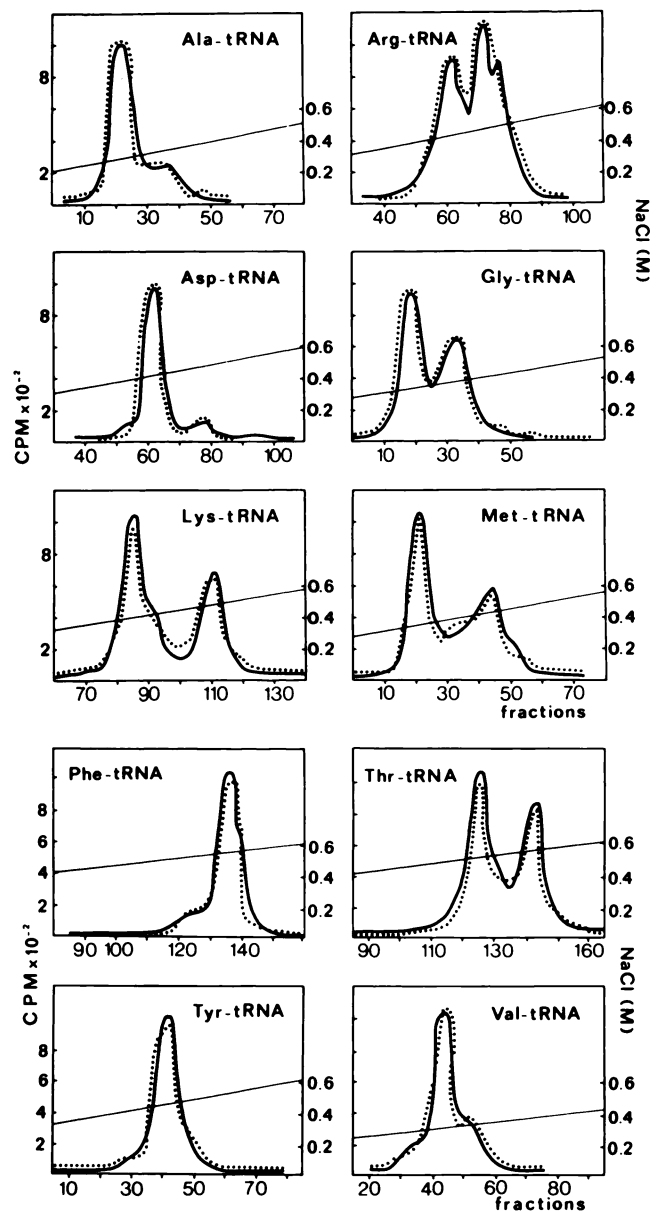


Chart 2. Elution profiles of 10 charged tRNA's from mammary glands of C3H and C3Hf mice (C57BL/6 mice gave the same results in all cases). Samples of ¹⁴C-labeled aminoacyl-tRNA's from C3H mice (····) and ³H-labeled aminoacyl-tRNA's from C3Hf mice (—) were cochromatographed on micro RPC-5 capillary columns (7) with a linear gradient of 0.20 to 0.60 M NaCl in 8 ml of 0.01 M MgCl₂-0.01 M acetate buffer, pH 4.7, at 25°. Two-drop fractions were collected. The labeling was reversed in a 2nd experiment.

gland of C3H infected mice, in the preneoplastic state, are not different from virus-free (C3Hf or C57BL/6) control tRNA's. The activated tRNA methylases observed by Turkington (23) in mammary carcinoma cells only begin functioning just before or during tumor growth. Similarly, if a tRNA is associated with MTV, it cannot preexist (except as pre-tRNA) in the mammary gland tRNA population at the time of analysis. Therefore, the concentration of each tRNA species could be studied before and during the onset of neoplastic transformation. It is not yet possible to determine whether the host tRNA alteration, demonstrated in some

cases following viral infection, plays a direct role in neoplastic cell differentiation or whether it is only the result of some minor phenomenon.

ACKNOWLEDGMENTS

The author wishes to thank Professor C. M. Gros for encouragement, advice, and help.

REFERENCES

1. Beck, G., Hentzen, D., and Ebel, J. P. Essais de Mise en Evidence d'une Régulation Hormonale au Niveau des RNA de Transfert. I. Etude Comparative des RNA de Transfert de Foie de Poule Immature et de Poule en Ponte. *Biochim. Biophys. Acta*, **213**: 55-67, 1970.
2. Clarkson, S. G., and Runner, M. N. Transfer RNA Changes in Hela Cells after Vaccinia Virus Infection. *Biochim. Biophys. Acta*, **238**: 498-502, 1971.
3. Daniel, V., Sarid, S., and Littauer, U. Z. Amino Acid Acceptor Activity of Bacteriophage T₁ Transfer RNA. *Federation Biochem. Soc. Letters*, **2**: 39-41, 1968.
4. Elska, A., Matsuka, G., Matiash, U., Nasarenki, I., and Semenova, N. tRNA and Aminoacyl-tRNA Synthetases during Differentiation and Various Functional States of the Mammary Gland. *Biochim. Biophys. Acta*, **247**: 430-440, 1971.
5. Gallagher, R. E., Ting, R. C., and Gallo, R. C. A Common Change of Aspartyl-tRNA in Polyoma and SV40-Transformed Cells. *Biochim. Biophys. Acta*, **272**: 568-572, 1972.
6. Garel, J. P. Functional Adaptation of tRNA-Population. *J. Theoret. Biol.*, **43**: 211-225, 1974.
7. Hentzen, D., Fournier, A., and Garel, J. P. Separation of Aminoacyl-tRNA's on Capillary Reversed Phase Chromatography Columns. *Anal. Biochem.*, **53**: 299-303, 1973.
8. Hentzen, D., Mandel, P., and Garel, J. P. Relation between Aminoacyl-tRNA Stability and the Fixed Amino Acids. *Biochim. Biophys. Acta*, **281**: 228-232, 1972.
9. Huang, H., Fenrych, W., Pawelkiewicz, and Johnson, B. C. Synthesis of Specific Transfer Ribonucleic Acids during Methionine Starvation in *E. coli* 113-3. *J. Mol. Biol.*, **59**: 307-318, 1971.
10. Kano Sueoka, T., and Sueoka, N. Leucine tRNA and Cessation of *E. coli* Protein Synthesis upon Phage T₂ Infection. *Proc. Natl. Acad. Sci. U. S.*, **62**: 1229-1236, 1969.
11. Littauer, U. Z., and Inoue, H., Regulation of tRNA. *Ann. Rev. Biochem.*, **42**: 439-470, 1973.
12. Littauer, U. Z., Revel, M., and Stern, R. Coding Properties of Methyl-Deficient Phenylalanine Transfer RNA. *Cold Spring Harbor Symp. Quant. Biol.*, **31**: 501-514, 1966.
13. Marmor, J. B., Dickerman, H. W., and Peterkofsky, A. Studies on Methyl-deficient Methionine Transfer Ribonucleic Acid from *E. coli*. *J. Biol. Chem.*, **246**: 3464-3473, 1971.
14. Petrisant, G. Etude de l'Acide Ribonucléique Soluble de Glande Mammaire de Brebis. II. Pouvoir Accepteur. *Bull. Soc. Chim. Biol.*, **51**: 669-689, 1969.
15. Randerath, K., Rosenthal, L. J., and Zamecnik, P. C. Base Composition Differences between Avian Myeloblastosis Virus Transfer RNA and Transfer RNA Soluble from Host Cells. *Proc. Natl. Acad. Sci. U. S.*, **68**: 3233-3237, 1971.
16. Raska, K. J., Frohwirth, J. R., and Schlesinger, R. W. Transfer Ribonucleic Acid in KB Cells Infected with Adenovirus Type 2. *J. Virol.*, **5**: 464-469, 1970.
17. Ribadeau-Dumas, B., Brignon, G., Grosclaude, F. and Mercier, J. C. Séquence Complète de la Caséine β Bovine. *European. J. Biochem.*, **25**: 505-514, 1972.
18. Rudali, G., Goezy, E., Frederic, F., and Apion, F. Susceptibility of Mice of Different Strains to the Mammary Carcinogenic Action of Natural and Synthesis Oestrogens. *Rev. Européenne Etudes Clin. Biol.*, **16**: 425-429, 1971.
19. Scherberg, N. H., and Weiss, S. B. Detection of Bacteriophage T₁ and T₂-Coded Transfer RNA's. *Proc. Natl. Acad. Sci. U. S.*, **67**: 1164-1171, 1970.
20. Sekiya, T., and Oda, K. The Altered Pattern of Transfer RNA in SV-40 Infected and Transformed Cells. *Virology*, **47**: 168-180, 1972.
21. Sueoka, N., and Kano-Sueoka, T. Transfer RNA and Cell Differentiation. *Progr. Nucleic Acid Res. Mol. Biol.*, **10**: 23-55, 1970.
22. Symposium: Transfer RNA and Transfer RNA Modification in Differentiation and Neoplasia. *Cancer Res.*, **31**: 591-721, 1971.
23. Turkington, R. W. Hormonal Regulation of Transfer Ribonucleic Acid and Transfer Ribonucleic Acid-Methylating Enzymes during Development of the Mouse Mammary Gland. *J. Biol. Chem.*, **244**: 5140-5148, 1969.
24. Yudelevich, A. Specific Cleavage of an *E. coli* Leucine Transfer RNA following Bacteriophage T₁ Infection. *J. Mol. Biol.*, **60**: 21-29, 1971.