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LYSINE tRNA IS THE PREDOMINANT tRNA IN MURINE MAMMARY TUMOR VIRUS*

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SUMMARY: The method of aminoacylation and subsequent identification of the esterified amino acids was used to characterize the transfer RNAs in murine mammary tumor virus. Lysine tRNA was the major tRNA in both "free" 4S RNA and "70S-associated" 4S RNA in virus derived from either tissue culture or mouse milk.

Transfer RNAs appear to be ubiquitous in RNA tumor viruses. Specific tRNAs serve as primers, or initiators, of viral DNA synthesis (see ref. 1 for a review). Known properties of primer tRNAs, for a particular virus, include specificity for a single amino acid and strong binding to the viral 35S RNA (2-7). Based on these properties it is possible to identify potential primer tRNAs in RNA tumor viruses (8,9). The technique involves stepwise thermal dissociation of 4S RNA from the 70S and/or 35S genomic RNA, aminoacylation of the released 4S RNA with a mixture of radioactively labeled amino acids, and identification of the tRNAs by analysis of the amino acids released by deacylation of the tRNA. With this technique we showed that $tRNA^{Trp}$ and $tRNA^{Pro}$ were the tRNAs most tightly bound to the 35S RNAs of avian myeloblastosis virus and the AKR MuLV respectively (8,9). A primer function for these tRNAs in these and/or related viruses has been established (1).

The MMTV system is probably more relevant to human cancer than any other RNA tumor virus system. Unfortunately, detailed biochemical characterization of this virus has been hampered because of difficulty in obtaining sufficient amounts of material. Recently, systems for large-scale production of MMTV in vitro have been developed (10-12). We have identified the tRNAs in the virions of MMTV produced in one such system (10) and have compared these

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Abbreviations used are: MuLV, murine leukemia virus; MMTV, murine mammary tumor virus.

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TABLE 1. Analysis of the Specific Amino Acid tRNAs in MMTV Produced in Tissue Culture

Source of 4S RNA	RNA (μg)	Total radioactivity[a] (cpm)	Percentage of total radioactivity identified as:														
			Trp	Lys	His	Arg	Asp	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe
AKR mouse fibroblasts	15	225,660	2.4	15.2	5.7	6.1	11.0	5.9	ND[b]	5.8	4.7	1.4	13.6	4.5	15.3	3.0	2.2	3.3
MMTV "free"	9.6	82,680	0.2	91.2	0.6	0.5	0.8	0.2	ND	0.4	0.4	0.3	1.2	1.8	1.9	0.5	ND	ND
MMTV "70S-associated"																		
65°	c	2,710	7.0	70.2	3.1	5.7	2.3	1.4	ND	ND	ND	ND	ND	6.9	ND	ND	1.4	2.0
65°	d	7,710	2.7	83.4	1.0	0.9	1.8	0.7	ND	ND	ND	ND	1.1	4.9	1.0	2.3	ND	ND
65-100°	c	1,140	4.0	77.3	1.4	5.4	3.5	2.0	ND	ND	ND	ND	ND	ND	ND	ND	2.4	4.0
65-100°	d	3,960	ND	88.5	ND	ND	1.6	1.0	ND	1.5	1.1	ND	ND	4.6	1.0	0.6	ND	ND

[a]Sum of radioactivities eluted from the amino acid analyzer in peak positions corresponding to the indicated amino acids.

[b]ND = none detected.

[c]Derived from ~11 μg 70S RNA; virus lot 1.

[d]Derived from ~42 μg 70S RNA; virus lot 2.

results with our less conclusive data obtained with virus prepared from mouse milk. In both cases MMTV, unlike any other murine RNA tumor virus that we have studied, has $tRNA^{Lys}$ and not $tRNA^{Pro}$ associated with its genome.

MATERIALS AND METHODS

Cells and virus: AKR mouse fibroblasts were grown in this laboratory as previously described (13). MMTVs produced in tissue culture (10) were 1000-fold medium concentrates obtained from the FCRC Viral Resources Laboratory, Frederick Cancer Research Center. MMTV isolated from RIII mouse milk was obtained as a virus pellet from Meloy Laboratories, Inc.

RNA preparation: Cellular and viral RNAs were prepared as previously described (13). "70S-associated" viral 4S RNA was obtained by first heating 70S RNA to 65°, then heating the resulting 35S RNA to 100°. Heating was for 3-5 min in low-salt buffer [0.02 M Tris·HCl (pH 7.6), 0.01 M EDTA, 0.1% SDS]. At each heat step 4S RNA was separated from 35S RNA by sucrose density gradient sedimentation. Because of the minute quantities of "70S-associated" 4S RNA, approximately 10 µg of poly A was added to the gradient pools to facilitate recovery; this poly A does not quantitatively or qualitatively alter subsequent aminoacylation. The RNA preparations were precipitated twice with ethanol to remove SDS, dissolved in water, and lyophilized to dryness.

Aminoacylation: Conditions for aminoacylation and subsequent processing of the tRNA and analysis of the amino acids were as described previously (8,9) except for the following changes: Incubation was for 30 min at 37°. The tritiated amino acid mixture (New England Nuclear) contained, or was adjusted to contain, 16 amino acids, each at a specific activity of 11,400 µCi/µmol and each used at a concentration of 8 µM. Mixed synthetases were prepared from mouse liver and used at 0.65 mg/ml. Counting of the fractions from the amino acid analyzer was about 24% efficient.

RESULTS

The data obtained from aminoacylated AKR fibroblast tRNA show that all synthetases except that for $tRNA^{Glu}$ were active (Table 1). The most striking feature of the tRNAs in tissue culture-produced MMTV, regardless of the fraction, is that $tRNA^{Lys}$ is predominant. No other tRNA exceeds 7% of the total in any fraction, including the "free" 4S RNA (Table 1). Especially noteworthy is the lack of $tRNA^{Pro}$, particularly in the "70S-associated" 4S RNA fractions.

Although the data in Table 2 for MMTV from milk are less complete, they nevertheless are entirely consistent with those in Table 1. These data augment those in Table 1 in that they suggest that neither threonine nor glutamine tRNAs are the major "70S-associated" tRNAs. The deviation in the tRNA composition between the "free" 4S RNA from tissue culture-produced virus and that from milk-derived virus is probably due to a greater contamination of the latter by cellular components.

TABLE 2. Analysis of the Amino Acid tRNAs in MMTV Obtained from Mouse Milk

Source of 4S RNA	RNA (μg)	Total radioactivity[a] (cpm)	Percentage of total radioactivity identified as:															
			Trp	Lys	His	Arg	Asp	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe
MMTV "free"	11.5	142,000	0.6	52.4	2.3	4.2	3.8	1.2	ND[b]	3.5	2.2	1.3	3.1	4.3	7.3	11.0	1.2	1.5
MMTV "70S-associated"																		
65°	c	277	ND	65.0	ND	ND							35.0[d]					
65-100°	c	116	ND	79.4	ND	ND							20.6[d]					

[a]Sum of radioactivities eluted from the amino acid analyzer in peak positions corresponding to the indicated amino acids.

[b]ND = none detected.

[c]Derived from an amount of virus equivalent to 220 ml mouse milk.

[d]Acidic and neutral amino acids were analyzed as the mixture. In addition to the amino acids indicated, the aminoacylation reaction for these samples was supplemented with [^{3}H]threonine and [^{3}H]glutamine, specific activity 2900 and 6100 μCi/μmol, respectively.

DISCUSSION

The tRNA composition of MMTV differs in several aspects from that of other RNA tumor viruses studied. The occurrence of $tRNA^{Lys}$ as practically the only tRNA in the "free" 4S RNA of MMTV is the most extreme example of nonrandom inclusion of tRNA within virions that we have observed to date (1). The capability of producing virus particles deficient in 70S RNA by treating cells with actinomycin D (14) has recently been demonstrated in the MMTV system (15). This system should be useful in determining the influence of the genomic RNA on tRNA inclusion into virus particles. Preliminary data indicate that the isoaccepting species of $tRNA^{Lys}$ in MMTV "free" 4S RNA are quantitatively and qualitatively the same as those in the cell from which the virus was produced. Whether there is a selection for certain $tRNA^{Lys}$ isoacceptors in the "70S-associated" 4S RNA fraction is currently being investigated.

The virtual absence of $tRNA^{Pro}$ in the "70S-associated" 4S RNAs of MMTV is in striking contrast to our results with other murine tumor virus systems, including AKR MuLV, Rauscher MuLV, Moloney MuLV, and Friend MuLV (1). Although it is premature to state that $tRNA^{Lys}$ is the primer in MMTV, it appears certain that $tRNA^{Pro}$ is not. Interestingly, the amount of $tRNA^{Lys}$ associated with the 70S RNA of MMTV is essentially the same as that reported for $tRNA^{Pro}$ in AKR MuLV (9).

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REFERENCES

1. Waters, L. C., and Mullin, B. C. (1977) In: Progress in Nucleic Acid Research and Molecular Biology (W. E. Cohn, ed.), Vol. 20, pp. 131–155, Academic Press, New York.
2. Canaani, E., and Duesberg, P. (1972) J. Virol. 10, 23-31.
3. Harada, F., Sawyer, R. C., and Dahlberg, J. E. (1975) J. Biol. Chem. 250, 3487–3497.
4. Folk, W. R., and Faras, A. J. (1976) J. Virol. 17, 1049–1051.
5. Dahlberg, J. E., Sawyer, R. C., Taylor, J. M., Faras, A. J., Levinson, W. E., Goodman, H. M., and Bishop, J. M. (1974) J. Virol. 13, 1126-1133.
6. Eiden, J. J., Quade, K., and Nichols, J. L. (1976) Nature 259, 245-247.
7. Peters, G., Harada, F., Dahlberg, J. E., Panet, A., Haseltine, W. A., and Baltimore, D. (1977) J. Virol. 21, 1031-1041.
8. Waters, L. C., Mullin, B. C., Bailiff, E. G., and Popp, R. A. (1975) J. Virol., 16, 1608-1614.
9. Waters, L. C. (1975) Biochem. Biophys. Res. Commun. 65, 1130-1136.

10. Fine, D. L., Plowman, J. K., Kelley, S. P., Arthur, L. O., and Hillman, E. A. (1974) J. Natl. Cancer Inst. 52, 1881-1886.
11. Sarkar, N. H., Pomenti, A. A., and Dion, A. S. (1977) Virology 77, 12-30.
12. Howard, D. K., Colcher, D., Teramoto, Y. A., Young, J. M., and Schlom, J. (1977) Cancer Res. 37, 2696-2704.
13. Waters, L. C., Mullin, B. C., Ho, T., and Yang, W.-K. (1974) Biochem. Biophys. Res. Commun. 60, 489-497.
14. Levin, J. G., Grimley, P. M., Ramseur, J. M., and Berezesky, I. K. (1974) J. Virol. 14, 152-161.
15. Sarkar, N. H., Pomenti, A. A., and Dion, A. S. (1977) Virology 77, 31-44.