TRANSFER RNAs ASSOCIATED WITH THE 70S RNA OF AKR MURINE LEUKEMIA VIRUS*

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SUMMARY: The specificity of the aminoacyl-tRNA synthetase and tRNA interaction was used to identify the amino acid tRNAs most tenaciously bound to the 70S RNA of the AKR murine leukemia virus. Of the 17 amino acid tRNAs tested for, proline tRNA was the major one which was dissociated from the viral RNA at temperatures above 60°.

Tryptophan tRNA has been shown to serve as a primer for *in vitro* DNA synthesis in Rous sarcoma virus (RSV) (1-3) and in avian myeloblastosis virus (AMV) (4). Thus, a precedent for a potential primer function for tRNA *in vivo* has been established. Studies in avian systems all show that the active primer is present in the 4S RNA subfraction which is thermally dissociated last from the 70S RNA (1,5). Though very much less is known concerning the template-primer interactions in murine RNA tumor virus systems (6), 4S RNA in association with 70S RNA has been reported (7-10). Analogy with the avian systems would indicate that in the murine systems also the "70S-associated" 4S RNA might be functionally important. In order to further study the interactions between viral 35S RNA and 4S RNA we have tried to devise methods which would also be applicable to those tumor viruses available only in limited quantity. Using AMV as a model system we showed interaction between specific cellular 4S RNAs and viral 35S RNA *in vitro* (4,10). We further showed that tryptophan tRNA was the major tRNA component to hybridize to AMV 35S RNA and that the hybrid formed is an efficient

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template-primer for reverse transcription in vitro (4). We have also demonstrated an unique association of tryptophan tRNA and AMV 35S or 70S RNA in the virion which provides added biological significance to our findings based on in vitro hybridization. This was done by taking advantage of the specificity of the tRNA aminoacylation reaction and by the use of the amino acid analyzer to show that the last amino acid tRNA to be thermally dissociated from AMV 35S RNA is tryptophan tRNA (manuscript submitted for publication). In this communication we report results, obtained by application of the same technique to the AKR murine leukemia virus, which indicate a strong association between AKR 35S RNA and proline tRNA.

METHODS

RNA preparation: 4S RNA from AKR mouse embryo cells and total RNA from AKR virus were prepared as previously described (10). Methods for growing the cells and producing the virus were also as reported (10). "Free" viral 4S RNA is that obtained in the total RNA extracted from the virus and was separated from 70S RNA by sucrose density gradient sedimentation as described (10). "70S-associated" viral 4S RNA was obtained by heating 70S RNA first to 60° and then by heating the 70S and/or 35S RNA again to 80°. At each step 4S RNA was separated from 70S and/or 35S RNA by sucrose density gradient sedimentation.

The "70S-associated" 4S RNA released at 60° is referred to as 60° 4S RNA and that released upon further heating as 60-80° 4S RNA. Dissociation at either 60° or 80° was accomplished by heating for 3 min followed by quick-cooling. Two different salt concentrations were used for dissociation. In high salt (0.01 M Tris·HCl, pH 7.6; 0.1 M NaCl; 0.001 M EDTA and 0.1% SDS) 70S to 35S RNA conversion is only 60-75% complete at 60°. In low salt (0.02 M Tris·HCl, pH 7.6; 0.01 M EDTA and 0.1% SDS) 70S RNA is completely converted to 35S at 60°. All dissociations at 80° were done in low salt to insure complete 4S RNA dissociation. To remove SDS each 4S RNA sample was twice precipitated with ethanol, dissolved in water and lyophilized to dryness.

Aminoacylation: Reaction mixtures, 125 or 250 µl, were incubated at 37° for one hr and contained the following components: 50 mM Tris·HCl, pH 7.6; 50 mM KCl; 2 mM 2-mercaptoethanol; 4 mM ATP; 12 mM Mg acetate; 17 tritium-labeled amino acids (as indicated in the Figure and Table) custom packaged by New England Nuclear, each at a specific activity of 1300 µCi/µmole, each used at 9 µM; the indicated 4S RNA samples; and mixed synthetases prepared from mouse Krebs II ascites tumor cells (11) at 0.48 mg/ml.

At the end of the reaction period, radioactive amino acids were separated from ^{3}H-aminoacyl-tRNAs by DEAE-cellulose chromatography at pH 4.5 and at 4°. Bound ^{3}H-amino acids were released from the tRNA by incubation for 2 hrs at 37° in 0.01 M $(NH_4)_2CO_3$ and were analyzed on an amino acid analyzer (12). Fractions of approximately one ml each were

collected directly from the columns, bypassing the ninhydrin system. The total radioactivity in each sample was determined by liquid scintillation counting after the addition of four ml of Aquasol. Counting was about 10% efficient.

RESULTS

Experiments preliminary to these showed that greater than 70% (approximately 1350 pmoles/A_{260}) of the theoretical maximum amount of tRNA in a 4S RNA preparation from AKR cells was aminoacylated under the conditions used. The distribution of amino acid tRNAs in AKR mouse embryo cells is shown in Figure 1A and is tabulated in Table 1. The presence of active synthetases for each of the 17 amino acids tested is indicated. The reproducibility of the method is seen by comparing the results from AKR samples 1 and 2 (Table 1). The amino acid tRNA distribution in the AKR cells, as determined by this method, is distinguishably different from that seen in a variety of chicken cells (manuscript submitted for publication).

In AKR "free" 4S RNA the amino acid tRNA distribution is different from that observed in the cell (Figure 1B and Table 1). Most notable are relative increases in lysine, histidine, proline and glycine tRNAs and decreases in tryptophan, threonine and/or serine, valine, leucine, tyrosine and phenylalanine tRNAs. No definitive statement can be made regarding the methionine tRNA content of AKR "free" 4S RNA from this experiment because a subsequent analysis showed the radioactive amino acid mixture to be deficient in methionine. Methionine and to a lesser extent tryptophan have proved to be unstable in the mixture (in 0.01 N HCl) stored at -40°. It is advisable to use amino acid mixtures without these amino acids and to add them as supplements from known stocks at the time of aminoacylation.

There is little difference in the amino acid tRNA composition of the "70S-associated" 4S RNA which dissociates at 60° in either high or low salt (Table 1). Relative to the distribution in AKR cells, the "70S-associated" 60° 4S RNA is characterized by significant increases in the proportions of tryptophan, arginine, and glutamate tRNAs (Figure 1C and

TABLE 1. COMPARISON OF THE SPECIFIC AMINO ACID tRNAs IN AKR MOUSE EMBRYO CELLS AND IN AKR MURINE LEUKEMIA VIRUS 4S RNAs.

Source of RNA	RNA (μg)	Total radioactivity[a]	Percentage of total radioactivity identified as: Trp	Lys	His	Arg	Asp	Thr and Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe
Cellular 4S RNAs																		
AKR mouse embryo cells (1)[b]	35	73,120	2.3	11.8	3.3	18.2	3.6	17.4	2.2	3.0	2.4	2.5	14.6	1.8[c]	3.3	9.4	1.7	2.5
AKR mouse embryo cells (2)	18	48,070	2.3	12.5	2.9	17.5	2.8	17.2	1.4	2.5	2.4	1.1	13.2	7.3	2.7	9.8	1.8	2.4
Viral 4S RNAs																		
"Free" 4S RNA	28	43,210	1.1	22.0	11.1	24.3	2.5	5.1	1.2	13.3	8.2	2.2	3.0	0.5[c]	3.1	1.4	0.5	0.5
"70S-associated" 4S RNA																		
High salt 60°	d	10,390	6.9	20.0	3.0	37.6	1.7	7.8	8.2	2.8	2.1	1.0	0.6	0.3[c]	4.0	3.8	0.1	0.2
High salt 60-80°	d	1,350	4.4	5.9	ND	8.5	0.9	3.6	6.6	59.8	1.4	5.0	ND	ND[c]	1.0	3.0	ND	ND
Low salt 60° (1)	d	9,700	7.4	19.9	2.4	34.6	2.0	8.1	9.1	2.7	2.3	0.7	1.6	0.4[c]	4.2	4.1	0.2	0.2
Low salt 60° (2)[e]	d	8,000	7.5	21.2	1.4	39.3	1.6	9.6	4.6	1.6	2.0	0.7	0.7	0.9	4.0	4.9	ND	ND
Low salt 60-80° (1)	d	890	3.8	6.5	1.3	10.8	ND	6.4	6.8	54.5	0.9	3.9	ND	ND[c]	2.0	3.0	ND	ND
Low salt 60-80° (2)[e]	d	720	4.4	8.4	ND	12.0	1.3	8.0	5.5	49.6	1.7	2.6	ND	0.9	2.2	3.4	ND	ND

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

[b] Numbers in parentheses are to indicate different RNA samples.

[c] Non-saturating concentrations of methionine in aminoacylation reaction.

[d] The high salt 60° and 60-80° samples and the low salt 60° and 60-80° samples (1) were derived from equivalent portions of 70S RNA (110 μg each); the low salt samples (2) were derived from 90 μg 70S RNA.

[e] These samples (2) were prepared from a different virus preparation than were samples (1).

ND = none detected.

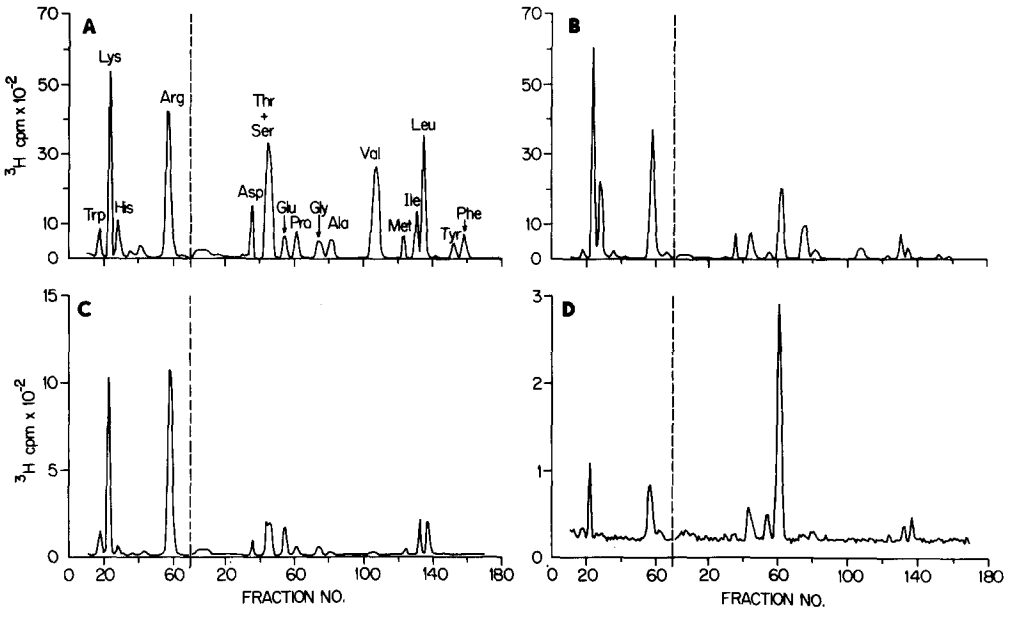

Fig. 1. Analysis of the amino acids obtained from in vitro aminoacylated cellular and viral RNAs. A) AKR mouse embryo cell 4S RNA, B) AKR "free" viral 4S RNA, C) AKR low salt 60° viral 4S RNA, D) AKR low salt 60-80° viral 4S RNA.

Table 1). Likewise relative to that in the cell, the proportions of valine, methionine, tyrosine and phenylalanine tRNA are decreased in this fraction.

In the "70S-associated" 4S RNA which dissociates between 60° and 80°, tryptophan, lysine, arginine, threonine and/or serine, glutamate, proline, glycine, alanine, isoleucine and leucine tRNAs were repeatedly demonstrated (Figure 1D and Table 1). However, none with the exception of proline tRNA are present at a level greater than 12%. Proline tRNA comprises 50% to 60% of the identified amino acid tRNAs which dissociate from AKR 70S and/or 35S RNA between 60° and 80°.

DISCUSSION

Our previous studies (4,10) utilizing reversed-phase chromatography [RPC-5] indicated that the cellular 4S RNAs which hybridized to AMV 35S RNA might be the same as those which hybridized to AKR 35S RNA. Subsequently we demonstrated

that a major portion of chick cell tRNA which hybridized in vitro with AMV 35S RNA was tryptophan tRNA (4). At the same time we indicated that tryptophan tRNA was not a major component of the hybridizable tRNA in the AKR mouse cell tRNA - AKR viral 35S RNA system (4,10). Our conclusions from these experiments are confirmed by the results presented here in which tryptophan tRNA is shown not to be a major portion of the tRNA associated with AKR 70S RNA. Unlike RSV and AMV the tryptophan tRNA content of AKR "free" 4S RNA is very low, an observation which has been reported for another murine virus system (3).

Regarding proline tRNA, it is interesting to note that although it is in relatively high concentration in AKR "free" 4S RNA, it is low in the 60° "70S-associated" 4S RNA fraction. Thus the high content of proline tRNA in the 60-80° 4S RNA fractions but not in the 60° fractions strongly indicates an unique association between it and AKR 35S RNA. We are currently attempting to purify proline tRNA from mouse tissue in order to determine if it, like tryptophan tRNA in the AMV (4) and RSV (13) systems, will prime reverse transcription of AKR 35S viral RNA in vitro. We have preliminary results to indicate that in Rauscher leukemia virus, as in AKR virus, proline tRNA is the major RNA component released from 35S RNA between 60° and 80°. These results suggest that tRNA molecules capable of priming viral RNA directed DNA synthesis in vitro might be species specific.

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