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Base Composition Differences between Avian Myeloblastosis Virus Transfer RNA and Transfer RNA Isolated from Host Cells

(isotope derivative method/tritium fluorography)

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ABSTRACT **Using a novel chemical tritium derivative method, we have determined the base composition of 4S RNA isolated from an RNA tumor virus, the avian myeloblastosis virus, and from normal and neoplastic host cells. Extensive differences were detected, particularly with respect to the amount of methylated bases in the viral RNA. The viral 4S RNA, which fulfills the criteria for designation as transfer RNA, appears to be derived from a precursor pool that is different from the precursor population of host-cell 4S RNA. These results are discussed in regard to the possible relationship between transfer RNA of avian myeloblastosis virus and cellular transfer RNA.**

RNA tumor viruses such as avian myeloblastosis virus (AMV) and the related Rous sarcoma virus (RSV) contain, in addition to a high molecular weight, single-stranded RNA, small amounts of 18S and 28S ribosomal RNA, 7S RNA, and 4S RNA (1–8). AMV 4S RNA can be acylated with various amino acids (1–3, 6), contains methylated bases (5), and thus consists principally, if not entirely, of transfer RNA. As shown by annealing experiments (8), RSV 4S RNA appears not to be complementary to the RSV genome. Analysis of an alkaline hydrolysate of this viral RNA after *in vivo* labeling with ^{32}P indicates minor chemical differences between the cellular and viral 4S RNA populations (7), with respect to the four major bases: adenine, guanine, cytosine, and uracil. No quantitative data on modified ("minor") bases have, however, been reported thus far for 4S RNA from any RNA tumor virus. 4S RNA from AMV does not have the same amino acid-accepting activity as host-cell 4S RNA (2, 3, 6), and lysine tRNA elution profiles have been shown to differ (9).

We report in this communication major and minor base constitution data for normal chicken liver, myeloblast, and AMV 4S RNA. Base composition was analyzed by a novel isotope derivative method, which involves the stoichiometric incorporation of tritium label into periodate-oxidized enzymatic digests of RNA, chromatographic resolution of such labeled digests into individual radioactive nucleoside derivatives, and evaluation by liquid-scintillation counting (10–14). Due to its sensitivity, this method is capable of assaying minute amounts of RNA (<1 μg) without requiring *in vivo* labeling of the RNA with isotopes.

MATERIALS AND METHODS

Virus Growth and Purification. Avian myeloblastosis virus, Beard's BAI strain A, was propagated by intraperitoneal injection of 1-day-old White Leghorn chickens (15) (Spafas, Inc., Norwich, Conn.). The morphology of peripheral blood was observed by Wright-stained smears. Plasma was obtained by cardiac puncture (heparin was used as an anticoagulant) from chickens with advanced leukemia. The virus was purified by centrifugation of plasma that contained 10^{11}–10^{12} viral particles per ml at 7500 rpm for 10 min in a Sorvall RC-2B refrigerated 4°C centrifuge. The supernatant plasma was layered onto the top of 100% glycerol pads and centrifuged at 5°C for 90 min at 29,000 rpm in a Spinco no. 30 rotor (16). The virion was collected from the top of the glycerol pad and diluted with 0.1 M NaCl–1 mM Tris (pH 7.4)–1 mM EDTA. The virus was purified further after two cycles of differential centrifugation (17).

Isolation of Viral RNA. Viral RNA was isolated from purified preparations of AMV by either of two methods. In the first, purified virus from 300 ml of plasma was extracted according to the procedure described by Travnicek (3). The portion of RNA that was soluble in 2 M NaCl was designated as crude viral tRNA (3), which was further purified by Sephadex column chromatography, as described below. A final yield of 120 μg of purified 4S RNA was obtained.

The second method for extraction of the viral RNA used a buffering system described by Duesberg (18). Purified viral pellets were obtained from 650 ml of plasma. The second method appears to be preferable because of better preservation of m^7G (see *Results* section). 320 μg of purified 4S RNA were obtained after purification by column chromatography.

Extraction of Myeloblast and Liver RNA. Myeloblasts from the circulating blood of leukemic chickens were isolated and purified according to the procedures described by Travnicek *et al.* (19). Sediments of myeloblasts (about 20 g) that were stored at −70°C were crushed while still frozen and added to 400 ml of 0.01 M cacodylate buffer (pH 6.5), containing 0.14 M LiCl, 1 mM $MgCl_2$, and 1.25 mg/ml of bentonite. An equal volume of phenol was added and the mixture was stirred vigorously at room temperature for 15 min. Subsequent procedures were similar to those described above. Livers removed from 3-week-old COFAL-negative (20) chickens (i.e., apparently virus-free) were stored at −70°C before use. The conditions used were identical to those described for myeloblasts.

Gel Filtration of RNA Preparations. Separations of myeloblast and liver RNAs were performed at room temperature with Sephadex G-100. A column of 3.2 × 100 cm was filled with a solution of Sephadex G-100 in 0.05 M ammonium acetate (pH 5.1). Columns were calibrated with known markers

Abbreviation: AMV, Avian myeloblastosis virus.

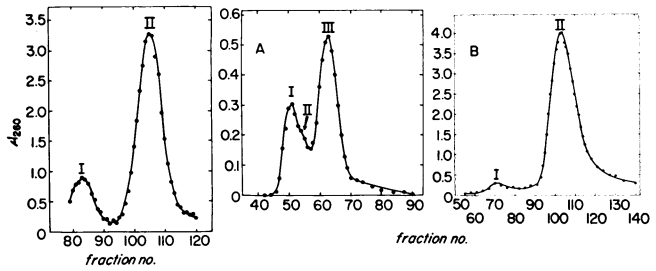

FIG. 1. Column separation on Sephadex G-100 of myeloblast low molecular weight RNA. Column, 3.2 × 100 cm; elution with 0.05 M ammonium acetate (pH 5.1); flow rate, 16 ml/hr. 4-ml fractions were collected and measured for absorbance at 260 nm. About 8 mg of low molecular weight RNA was chromatographed.

Fig. 2*A*. Column separation on Sephadex G-75 of crude viral low molecular weight RNA, isolated by the first method (see text and ref 18): Column, 0.9 × 100 cm; elution with 0.05 M ammonium acetate (pH 5.1); flow rate, 1.6 ml/hr. 0.4-ml fractions were collected and measured for absorbance at 260 nm. 10 A_{260nm} units of 2 M NaCl-soluble extract was applied to the column.

FIG. 2*B*. Column separation on Sephadex G-100 of crude viral low molecular weight RNA, isolated by the second method (see text and ref 19). Column, 0.6 × 100 cm; elution with 0.05 M ammonium acetate (pH 5.1); flow rate, 0.7 ml/hr. 0.17-ml fractions were collected and measured for absorbance at 260 nm. 15 A_{260nm} units of 2 M NaCl-soluble extract were applied to the column.

of Blue Dextran 2000, yeast tRNA, and uracil in all cases. The RNA samples were loaded from the bottom of the column and eluted with the same buffer. 4-ml fractions were collected at a flow rate of 16 ml/hr, and the absorbance was measured at 260 nm. The pooled tRNA fractions were lyophilized, dissolved in 2% potassium acetate (pH 5.1) and precipitated with 2 volumes of 95% ethanol at −20°C. A smaller column (0.9 × 100 cm) of Sephadex G-75 was used at pH 8.8 to purify the viral RNA that was isolated with the buffer as described by Travnicek; a column (0.6 × 100 cm) of Sephadex G-100 was used at pH 7.4 to purify viral RNA that was extracted with NaCl–Tris–EDTA buffer.

Assay of the Activity of Amino-Acid Acceptors. The formation of aminoacyl-tRNA was measured as follows: the reaction mixture contained, in 100 μl final volume: 0.05 M Tris·HCl buffer (pH 8), 5 mM ATP, 5 mM KCl, 0.1 mM [^{14}C]lysine, specific activity 25 Ci/mol, 0.01 M $MgCl_2$, 5 mM mercaptoethanol, 0.3 mg of protein of aminoacyl-tRNA ligases isolated from the livers of COFAL-negative chickens, and 10–50 μg of the tRNA to be measured. Lysine-acceptor activity was chosen for the assay since it had been shown (3) to be present in high activity in viral tRNA. The reaction mixture was incubated at 37°C for 10 min and the formation of [^{14}C]aminoacyl tRNAs (plateau labeling values) was assayed by the conventional Millipore-filter procedure.

Analysis of Base Composition. RNA samples were digested enzymatically to nucleosides and treated with periodate and borotritiide as described (13). Aliquots of the final labeled solution that contained 4–5 μCi of ^{3}H were applied to cellulose thin layers and resolved two-dimensionally; the solvents were (13): A = acetonitrile–ethyl acetate–*n*-butyl alcohol–isopropanol–6 N ammonia (7:2:1:1:2.7, by volume), and B = *t*-amylalcohol–methyl ethyl ketone–acetonitrile–ethyl acetate–water–formic acid [specific gravity 1.2] (4:2:1.5:2:1.5:0.18, by volume). Solvent A was used in the first and solvent B in the second dimension.

After chromatography and film detection (14), the labeled nucleoside derivatives were assayed by liquid-scintillation counting. The base composition was determined according to the method of Randerath and Randerath (13).

RESULTS

The low molecular weight RNAs of AMV, viral-transformed myeloblasts, and chicken liver were fractionated by Sephadex-gel filtration procedures. After chromatography on Sephadex G-100, myeloblast low molecular weight RNAs that are soluble in 2 M NaCl were fractionated into two components (Fig. 1). Good separation was obtained between 5S RNA (peak I) and 4S RNA (peak II), and these elution

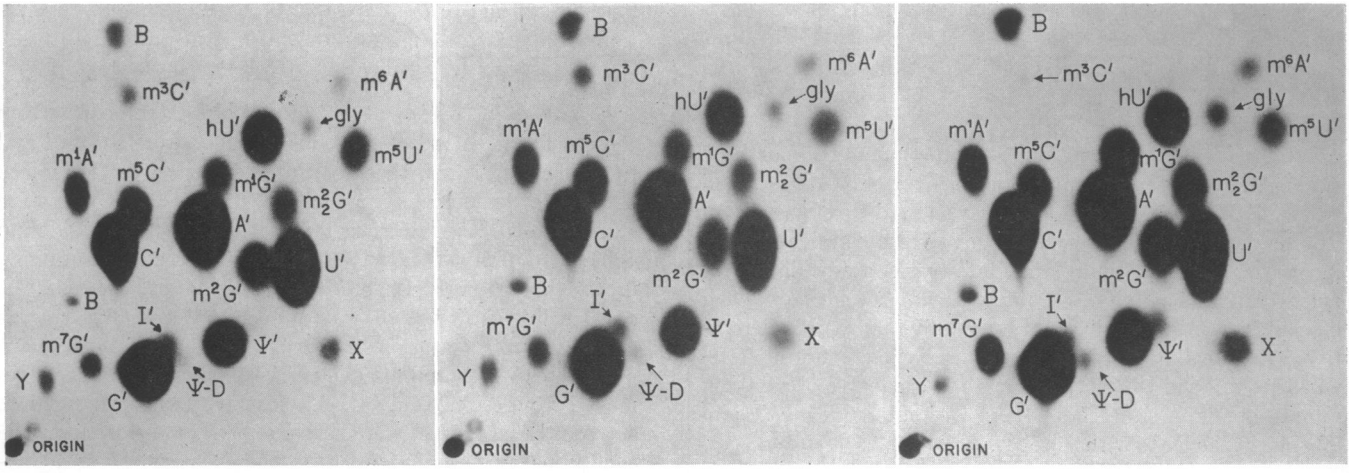

FIG. 3. Fluorograms of maps of tritium-labeled digests of 4S RNA from chicken liver. (*A*), myeloblasts (*B*), and AMV (*C*). RNA was subjected to digestion and labeling as indicated in the text. Labeled digest (4–5 μCi) was applied to a cellulose thin layer and the chromatogram was developed in two dimensions with the solvents mentioned in the *text*. Detection by fluorography at −80°C (14). Exposure for 4 days. *B, X, Y* are compounds not determined or not derived from RNA constituents; *Ψ-D* is a trace of a compound arising from the decomposition of pseudouridine (13); gly is glycerol.

profiles are in agreement with the data of Travnicek (1969). Similarly, satisfactory separations were obtained of the low molecular weight RNA fractions that were isolated from chicken liver. The elution profiles of the low molecular weight fraction of viral RNA, according to the two extraction methods described in *Methods*, are presented in Figs. 2*A* and *B*. The resolution of the viral RNAs shown in Fig. 2*A* by Sephadex G-75 is not as good as the elution profiles obtained with Sephadex G-100 (Fig. 2*B*). The column profile in Fig. 2*A* revealed some high molecular weight RNA fraction, which contaminated the 2 M NaCl extraction. This RNA (peak I) eluted at a region calibrated to be the void volume. The shoulder of this peak, designated as peak II, probably contained the 7S RNA that was previously reported for RSV (8). Peak III emerged at the elution volume expected for tRNA, and could be aminoacylated. A better elution profile was obtained by Sephadex G-100 (Fig. 2*B*), and 7S RNA (peak I) was clearly separated from the 4S RNA-containing region. In all cases, base analyses were performed on the pooled symmetrical regions of each tRNA-containing peak from the virus, myeloblasts, and chicken liver. In addition, the lysine-acceptor activities of viral, myeloblast, and liver tRNA were tested and these data showed the specific activity (nmol/mg of RNA) for viral tRNA to be 1.26; for myeloblast tRNA to be 1.06; and for normal chicken liver to be 2.92. These values (plateau levels) are in agreement with other data that were previously reported (3).

Fig. 3*A*, *B*, and *C* depicts fluorograms (14) of two-dimensional thin-layer chromatograms of tritium-labeled nucleoside trialcohols obtained from 4S RNA of normal chicken liver (*A*), myeloblasts (*B*), and AMV (*C*). Qualitatively, on all three maps the same spots are visible, but quantitative differences are immediately apparent if one compares the intensity of the various spots on Fig. 3*C* (AMV RNA) with the intensity of the corresponding spots on Fig. 3*A* and *B* (host-cell RNA). Since the darkening of the film depends on the radioactivity of a spot [which in turn is directly proportional to the frequency of the particular base in the RNA (11, 13)],

TABLE 1. *Major and minor base composition (mole %) of 4S RNA from chicken liver, myeloblast, and avian myeloblastosis virus*

	Liver		Myeloblasts		AMV	
	Major and minor bases (%)	Minor bases (%)	Major and minor bases (%)	Minor bases (%)	Major and minor bases (%)	Minor bases (%)
A	17.41 ± 0.21*		16.99 ± 0.21*		16.71 ± 0.10*	
G	28.08 ± 0.25		28.62 ± 0.27		27.84 ± 0.06	
C	25.89 ± 0.31		25.84 ± 0.37		27.66 ± 0.14	
U	13.94 ± 0.26		13.68 ± 0.29		12.71 ± 0.25	
m^6A	0.25 ± 0.04	1.77	0.23 ± 0.01	1.57	0.18 ± 0.04	1.20
m^1A	1.11 ± 0.06	7.79	1.08 ± 0.03	7.50	1.18 ± 0.06	8.04
$m^6A + m^1A$	1.36 ± 0.05	9.55	1.31 ± 0.04	9.07	1.36 ± 0.10	9.24
hU	2.85 ± 0.12	20.01	2.93 ± 0.10	20.33	2.52 ± 0.04	17.20
m_2^2G	0.61 ± 0.03	4.26	0.59 ± 0.02	4.09	0.91 ± 0.02	6.27
m^2G	1.42 ± 0.05	9.94	1.39 ± 0.05	9.61	1.69 ± 0.05	11.55
m^7G†	0.56 ± 0.06	3.90	0.56 ± 0.05	3.89	0.98 ± 0.04	6.68
m^1G	0.71 ± 0.06	5.04	0.80 ± 0.05	5.54	1.64 ± 0.04	11.19
m^3C†	0.28 ± 0.01	1.99	0.27 ± 0.00	1.87	0.05 ± 0.00	0.36
m^5C	1.83 ± 0.10	12.82	1.95 ± 0.07	13.53	1.12 ± 0.05	7.65
Ψ	3.59 ± 0.13	25.20	3.63 ± 0.09	25.16	3.82 ± 0.10	26.09
m^5U (rT)	0.75 ± 0.03	5.30	0.72 ± 0.03	4.94	0.39 ± 0.01	2.63
I	0.27 ± 0.07	1.88	0.29 ± 0.05	1.97	0.17 ± 0.03	1.15
glycerol*	0.43 ± 0.09		0.45 ± 0.06		0.46 ± 0.04	
Sum	99.97	99.90	100.02	100.00	100.03	100.01
$\frac{\text{minor bases}}{\text{major bases}}$	0.167		0.170		0.173	
$\frac{\text{methylated bases}}{\text{major bases}}$	0.088		0.089		0.096	
$\frac{m^1G \text{ [mole \%]}}{m^3C \text{ [mole \%]}}$	2.54		2.96		32.80	

* Standard deviation (N = 6)
† Recoveries of m^7G and m^3C as tritium-labeled trialcohols are about 70% and 85%, respectively.
* Glycerol is formed in trace amounts due to a side reaction (13).

TABLE 2. *Base composition (mole %) of hypothetical unmodified precursor RNA populations of 4S RNA from chicken liver, myeloblast, and avian myeloblastosis virus*

	Liver (% ± SD*)	Myeloblasts (% ± SD)	AMV (% ± SD)
A + modified A	19.04 ± 0.13	18.58 ± 0.21	18.23 ± 0.07
G + modified G	31.39 ± 0.18	31.95 ± 0.37	33.05 ± 0.13
C + modified C	28.00 ± 0.33	28.06 ± 0.41	28.83 ± 0.12
U + modified U	21.14 ± 0.21	20.96 ± 0.15	19.43 ± 0.14
Glycerol	0.43	0.45	0.46
Sum	100.00	100.00	100.00
$\frac{A_{total}† + U_{total}}{G_{total} + C_{total}}$	0.676	0.659	0.609

* See Table 1.
† A_{total} = A + modified A, etc.

it is evident from Fig. 3 that AMV 4S RNA contains, for example, more m^1G and less m^3C than does host-cell 4S RNA.

In Tables 1 and 2, quantitative major and minor base-composition data for normal chicken liver, myeloblast, and AMV 4S RNA are presented. Table 1 gives the total base composition (with the exception of isopentenyladenosine, thionucleosides, and 2′-*O*-methylnucleosides), and summarizes data for the minor base composition. In Table 2 we present base composition data pertaining to a hypothetical precursor 4S RNA containing no minor bases. The calculations in this case were done by the addition of percentages obtained from individual maps for each major base and all its modified derivatives. The assumption was made that each derivative is being synthesized from the precursor to which it is most closely related chemically. While this assumption probably is correct for the methylated bases, it may not be correct for such compounds as dihydrouridine or pseudouridine, the biosynthesis of which has not yet been elucidated.

The presence of methylated bases in the AMV 4S RNA cannot be due to contamination by degraded high molecular weight viral RNA because the latter contains few, if any, methyl groups [(5), see also (7)].

The results presented in Tables 1 and 2 may be summarized as follows. (1) Substantial base composition differences exist between AMV 4S RNA and host-cell 4S RNA. (2) Differences between normal chicken liver and tumor-cell (myeloblast) 4S RNA are small or undetectable. (3) The level of modification, i.e., the ratio [Total minor bases (mole %)]/[Total major bases (mole %)] is indistinguishable for all three RNAs. There is no "hyper-modification" of viral or tumor cell RNA, as compared with normal host cell RNA. (4) The level of methylation, i.e., the ratio [Total methylated bases (mole %)]/[Total major bases (mole %)] is similar for the three samples, although the viral RNA appears slightly "hyper-methylated." With the possible exception of m^1G, the tumor RNA is not hyper-methylated in comparison with liver RNA. Similar results have been reported recently for low molecular weight RNA that was isolated from normal brain and brain tumor tissue (21, 22). (5) The differences between viral RNA and host cell—normal as well as neoplastic—RNA are nonrandom: (a) The concentrations of all methylated guanines (m^1G, m_2^2G, m^7G) are elevated in the tumor virus RNA. The most substantial increase—a factor of 2—is in m^1G. (b)

The concentration of inosine is depressed in AMV RNA. (c) The concentrations of all modified pyrimidines (with the exception of pseudouridine) are lower in the tumor-virus RNA than in normal or neoplastic host-cell RNA. The most substantial decrease—a factor of 5–6—is in m^3C. The ratio m^1G/m^3C is over 10-times greater in AMV RNA than in host-cell RNA (Table 1). (6) The concentration of methylated adenine appears to be similar in each of the three samples. m^6A probably is absent from mammalian tRNA (23). Under our reaction conditions, about 15% of m^1A is converted to m^6A (13). The data presented in Table 2 appear to imply that m^6A is also absent from avian tRNA, as well as from AMV tRNA, and that the formation of $[^3H]m^6A'$ may be entirely due to the alkaline rearrangement of m^1A (24). (7) The only modified pyrimidine that is not depressed in AMV RNA is pseudouridine. Its frequency appears slightly elevated in comparison with host-cell RNA. (8) Table 2 shows that AMV 4S RNA contains less "total" A + U and more "total" G + C than host-cell RNA. (9) The increase in methylated guanines in AMV RNA parallels the higher "total" G value, whereas the modification of C is depressed despite a higher "total" C value (Table 2). (10) AMV 4S RNA appears to contain all minor bases thus far identified that are present in host 4S RNA, and there is no evidence for additional bases in the viral RNA. This observation applies to the compounds listed in the tables, and to the unidentified compounds marked X and Y on Fig. 3*A–C* only; not necessarily, however, to isopentenyladenosine, thionucleosides, and 2′-*O*-methylated nucleosides, which were not assayed. We have shown compounds X and Y to arise from the RNA itself and not to be due to the introduction of tritium label into background compounds (Randerath and Randerath, unpublished data).

The results for AMV RNA presented in Tables 1 and 2 were obtained with a preparation extracted at pH 7.4 ("second method," see *Methods*, also Fig. 2*B*). When an earlier batch of AMV was extracted according to the "first method" (see *Methods* and Fig. 2*A*), at pH 8.8, the base composition patterns found were very similar, with the exception of m^7G, the concentration of which was greatly reduced. This loss of m^7G is probably due to the known lability of this compound at alkaline pH (25, 26). It may be noted that the base data for chicken liver and myeloblasts are very similar to those which were previously reported for mammalian brain and brain tumors (21, 22).

DISCUSSION

The base composition of 4S RNA from RNA tumor viruses has previously been analyzed by labeling the viral RNA *in vivo* with ^{32}P, alkaline hydrolysis, and one-dimensional separation of the hydrolysates by paper electrophoresis (5, 7) or column chromatography (27). Data on the composition of the four major bases were obtained in this way for AMV (5), RSV (7), murine sarcoma-leukemia virus (27), and feline leukemia virus (28).

These procedures, however, do not take into account the existence of a large number of modified bases in the tumor virus 4S RNA. ^{32}P-labeled nucleotides containing minor bases will therefore overlap with the major nucleotides during such a one-dimensional separation. The values for the major base composition of 4S RNA from RNA tumor viruses reported in the literature must therefore be regarded as preliminary. Because many modified bases [e.g., m^7G, m^3C, m^1A, hU, but also m^1G, m^2G, and m_2^2G (Randerath and Randerath, unpublished data)] are alkali-labile, the total base composition of 4S RNA cannot be determined with precision from an alkaline hydrolysate. The method used by us avoids pH values higher than 8, and thus leaves the heterocyclic moiety of most minor bases intact. [An exception is 4-thiouridine, which is largely converted to $[^3H]U'$ (13).]

Two-dimensional thin-layer separation after the stoichiometric incorporation of tritium label into an enzymatic RNA digest makes possible a simultaneous determination of the four major and most minor bases present in unfractionated 4S RNA, which is isolated from RNA tumor viruses, as well as from normal and neoplastic host cells. This method has enabled us to report in this communication, for the first time, base-constitution data on major and minor bases for 4S RNA present in an RNA tumor virus.

Several possibilities suggest themselves, in explanation of the base-composition differences between virion tRNA and host-cell tRNA, from normal liver and from infected myeloblasts:

(1) The virion may contain specific methylases (29), and possibly demethylases (although the existence of the latter is hypothetical), that modify the host-cell tRNA found in the virion.

(2) The viral RNA genome may code for new individual tRNAs, via its own reverse transcriptase (30, 31), [or "retroscriptase" (32)], and the host-cell RNA polymerase. In this instance, both the minor and major base changes could be accounted for. No direct evidence for this possibility is presently available.

(3) A selective screening of host-cell tRNAs may occur as the virion pinches off from the host cell. It is possible that the relative amino-acid acceptance pattern for tRNAs may vary in a cell, depending on whether the tRNA is obtained from free cell sap, or from material bound to cell membranes. A plausible explanation for the existence of changes in virion tRNA arises from evidence that tRNAs involved in cell-wall peptidoglycan synthesis in bacteria may be deficient in minor-base constituents, and unable to function in protein synthesis (33). Such tRNAs, if present in avian cells, might play a crucial role in the synthesis of special features of the virion coat, and in the maturation and pinching-off processes.

The data in Table 2 suggest that the hypothetical precursor molecules of the viral and cellular 4S RNAs may differ in major-base composition. Only further analysis will show whether this indicates that some or all viral 4S RNA species are coded for by the viral genome. The data suggest, however, that the viral RNA is not derived simply from bulk cellular 4S RNA or its precursor population by modification reactions that are catalyzed by viral or cellular enzymes.

The striking diminution of m^3C in the virion tRNA invites further comment. This base is among the rarest in the minor-base family, and can be present in only a small fraction (of the order of 25%) of total tRNA molecules in the chicken. Since the concentration of m^3C is decreased to about one-fifth of the normal values in the virion tRNA, a critical shortage of the aminoacyl tRNAs containing this base may be present in the virion tRNA.

Among the tRNAs of several biological species thus far sequenced, only $tRNA^{Ser}_{rat}$ has been found to contain m^3C (34). Curiously, there are two m^3C residues in $tRNA^{Ser}_{rat}$; one is located at the single-stranded tip of the "appendix" loop, and the other in the loop that contains the anticodon. Both of

these locations are potentially strategic in the tertiary structural folding of the tRNA molecule. Furthermore, m^3C is one of the few minor bases that carry a positive charge at physiological pH, and would thus be capable of participating in an ionic interaction. According to a recent hypothesis (35), the functions of the positively charged bases may be to stabilize certain regions of the RNA structure. The finding that serine acceptor activity is greatly reduced or absent in AMV tRNA (3, 6) may be related to our observation of greatly reduced concentrations of m^3C in the tumor virus RNA. In this connection, it is interesting that m^3C is absent in yeast (36) and *E. coli* (37) $tRNA^{Ser}$ in those two positions in which it is found in rat liver $tRNA^{Ser}$.

The multiplicity of minor bases in the virion 4S RNA makes unlikely the possibility that this RNA is simply a degradation product of high molecular weight RNA.

For the future, it would appear interesting to fractionate the tRNAs present in the virion and in the host cells, and to look for differences in minor-base patterns and sequences of individual purified amino-acid acceptor tRNAs. Due to limitations of starting material, this task may be an arduous one. Because avian, murine (27), feline (28), and primate (38) RNA tumor viruses have been shown to contain 4S RNA, it would also appear important to answer the question whether or not analogous differences in 4S RNA base composition exist between all RNA tumor viruses and their respective host cells, in other words, whether we are dealing with a general phenomenon not restricted to AMV.

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