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STUDIES ON THE STRUCTURE OF AVIAN MYELOBLASTOSIS VIRUS (AMV) RNA. II. INTEGRITY OF THE RNA IN DEPENDENCE ON RNA ISOLATION AND VIRUS PROPAGATION

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Summary. — While sedimentation analysis revealed no basic changes in the sedimentation chatacteristics of 60-70 S avian myeloblastosis virus (AMV) RNA isolated under different conditions from two sources, electron microscopy exhibited differences in the length distributions of this RNA. Based on the length distribution profiles, phenol extraction showed a higher degradation effect in comparison with direct gradient centrifugation of viral lysates. Proteinase K appeared to be a more suitable nuclease inhibitor than diethylpyrocarbonate during the isolation of AMV RNA. Fundamental differences between length distributions of 60-70 S RNA from plasma AMV and that from AMV propagated in cultured leukaemic myeloblasts were found. Precipitation with ethanol had no effect on the length distribution of AMV RNA. Nor did storage of leukaemic chicken plasma at −70° C and one thawing substantially influence the lengths of AMV RNA. The heterogeneous length distributions of 60-70 S AMV RNA molecules with preferential size-classes were independent of virus propagation and RNA isolation. The results support the idea of preferential splitting of AMV RNA at specific sites.

Key words: avian myeloblastosis virus; integrity of 60-70 S AMV RNA; sedimentation and electron microscopic analysis; length distribution profiles

Introduction

The genome of retroviruses is represented by the single-stranded (ss) RNA with a sedimentation coefficient of 60-70 S in native state and 30-40 S after denaturation (Robinson *et al.*, 1965; Duesberg, 1968; Tooze, 1973; Riggin *et al.*, 1973, 1975). Retroviral RNA is very sensitive to mechaniclal and enzymatic degradation (Erikson, 1969; Cheung *et al.*, 1972; Scheele and Hanafusa, 1972; Stoltzfus and Snyder, 1975; Chen *et al.*, 1980) and that it is rather difficult to obtain an intact 60-70 S viral RNA (Coffin, 1979) suitable for electron microscopic analysis. The accurate length measurement of ss

viral DNA molecules requires either strongly denaturing conditions (Heine *et al.*, 1975; Jacobson and Bromley, 1975; Chi and Bassel, 1975) or the presence of the bacteriophage T4 gene-32 protein (Delius *et al.*, 1974). Under strongly denaturing conditions some "hidden" breaks in the retroviral RNA can be revealed (Chi and Bassel, 1975). From this point of view, the high-molecular-weight RNAs of avian retroviruses seem to be generally more sensitive than are RNAs of retroviruses from mammals (Kung *et al.*, 1975, 1976; Bender and Davidson, 1976). The integrity of the viral genome may depend on both the conditions of RNA isolation and purification (Scheele and Hanafusa, 1972; Chen *et al.*, 1980) and virus maturation (Bader and Steck, 1969; Cheung *et al.*, 1972; Stoltzfus and Snyder, 1975). The maturation process leads to the stabilization of the 60-70 S RNA complex (Korb *et al.*, 1976), but the "old" virus particles may contain labilized RNA with ss breaks (Smith, 1974).

The intactness of the RNA from retroviruses is very important for studying the physical nature of their genomes. Therefore we compared the length distribution of AMV RNA in dependence on the conditions of RNA isolation and virus propagation. The RNA was isolated from purified AMV particles by two different methods: either by phenol-sodium dodecyl sulfate (SDS) extraction or by direct gradient centrifugation from viral lysates, in the presence of either proteinase K (Hayward, 1977), or diethylpyrocarbonate (DEPC) (Fedorcsák *et al.*, 1969; Yoshida *et al.*, 1979) as inhibitors of nucleases. To analyze the effect of the virus stage on RNA integrity, we studied RNA isolated from fresh or frozen chicken leukaemic plasma virus and from virus propagated in leukaemic myeloblast culture. Furthermore, we investigated the stabilization by ultraviolet (UV) irradiation of genomic RNA inside the virions (Sen and Todaro, 1977).

Materials and Methods

Virus. AMV was isolated from fresh and frozen plasma of leukaemic chickens or from virus-containing media (6 hr collections) of a virus-producing culture of leukaemic myeloblasts. Virus-containing medium was thawed and clarified twice by centrifugation at 3000×g for 30 min. The virus originating from leukaemic chicken plasma or culture media was purified in the same way, as described previsously for plasma virus (Korb and Heine, 1978).

Ultraviolet (UV) irradiation of AMV. Virus suspension (from 150 ml of blood plasma) in 10 ml of TN buffer (0.1 mol/ l NaCl, 0.05 mol/l TRIS, pH 7.5) was UV-irradiated from a source 5 cm above the liquid surface (output 3×10^4 J/cm^2 per sec) for 2 min at 0° C. The RNA was isolated from UV-irradiated AMV by phenol extraction or by direct sucrose gradient centrifugation of viral lysates.

Isolation of AMV RNA. (i) Phenol-SDS extraction. AMV RNA was isolated from plasma virus, from virus produced by cultured myeloblasts or from UV-irradiated AMV by phenol-SDS extraction as described elsewhere (J. Štokrová *et al.*, 1982). (ii) *Direct sucrose gradient isolation.* Plasma AMV or UV-irradiated AMV were resuspended in TN buffer pH 8 (0.1 mol/l NaCl, 0.05 mol/l TRIS, phH 8), gently homogenized and lysed by SDS in the presence of proteinase K or DEPC. Final concentration of SDS was 1% in both cases and that of proteinase K or DEPC was 500 μg/ml and 20 μl/ml, respectively. Suspension with proteinase K was incubated for 1 hr at room temperature and layered on a 10—30% glycerol gradient in TN buffer, pH 8, with 0.05% SDS. Suspension protected by DEPC was placed on the same gradient in a separate tube. Centrifugation was carried out in an SW 50.1 Spinco rotor at 49 000 rev/min for 40 min at 20° C.

Fractions were analyzed for A_{260}, and the material sedimenting at 60-70 S was pooled, precipitated with ethanol, resuspended in TN buffer, pH 8, and prepared for electron microscopy.

Denaturation of 60-70 S AMV RNA. Preparations of 60-70 S AMV RNA in TN buffer, pH 8, were denatured by heating at 73° C for 3 min. After cooling in ice, the solution was layered on a 10—30% sucrose gradient in TNA buffer, pH 7.5 (0.1 mol/l NaCl, 0.05 mol/l TRIS and 0.001 mol/l EDTA) with 0.1% SDS. Centrifugation was carried out in a Spinco SW 41 rotor at 39 000 rev/min for 4.5 hr at 20° C. Fractions were analyzed for A_{260} and pooled as described for isolation of 60-70 S RNA.

Electron microscopy. AMV RNA was prepared by the basic protein film technique (Kleinschmidt and Zahn, 1959) modified for strongly denaturing conditions (Robberson *et al.*, 1971) as described (Štokrová *et al.*, 1982). The RNA samples were examined in a Jeol 100 B electron microscope at a magnification of 10 000 and an accelerating voltage of 60 kV.

Length measurement and molecular weight determination. Length measurements were made on prints after a 5- to 8-fold photographic enlargement using a Hewlett-Packard 9864 A Digitizer equipped with a Hewlett-Packard 9830 calculator. The myeloblastic 18 S rRNA was used as the length standard. The molecular weights were calculated on the basis of linear density estimated from the length of this rRNA under our spreading conditions and its published molecular weight (Korb and Heine, 1978).

Results

Sedimentation analysis of 60-70 S AMV RNA isolated by phenol-SDS extraction

Total RNAs extracted by the phenol-SDS method from (1) frozen chicken leukaemic plasma AMV, (2) UV-irradiated AMV from frozen chicken leukakaemic plasma, and (3) AMV produced in leukaemic myeloblast culture were sedimented in a sucrose gradient as described in Materials and Methods. The 60-70 S AMV RNA sedimented identically in all cases, but there were differences between fast-sedimenting and slow-sedimenting components (Fig. 1).

The sedimentation profile of RNA isolated from AMV from frozen chicken leukaemic plasma revealed the presence of a main peak containing the 60-70 S RNA in the position of fractions from 4 to 11 (Fig. 1-I). In the position of fractions from 14 to 17, 18 to 20 and 21 to 23, RNAs corresponding to 28 S, 18 S and 4 S were present. We observed a slight shoulder on the side of the fast-sedimenting components. It could have been due to the aggregation of plasma virus RNA molecules with some residual protein. The 28 S, 18 S and 4 S RNAs are the generally observed low-sedimenting AMV RNA components (Heine *et al.*, 1975; Chi and Bassel, 1975).

After UV-irradiation of AMV, the RNA sedimentation profile was similar to that of RNA from untreated plasma virus. The position of the 60-70 S RNA from the irradiated AMV was the same but a higher amount of fast- and slow-sedimenting material was observed (Fig. 1-III).

In the case of RNA isolated from myeloblast culture virions, the position of 60-70 S RNA corresponded to that shown in Fig. 1-I, but a higher amount of the slow-sedimenting components was found (Fig. 1-II).

To verify the integrity of 60-70 S RNA during phenol extraction, the 60-70 S RNAs isolated from plasma AMV or leukaemic myeloblast culture AMV were heated to 73° C for 3 min and analyzed by sedimentation. The

sedimentation profile was used as a criterion of 60-70 S RNA intactness. The sharpness of the 30-40 S RNA component and the amount of slow-sedimenting material depended on the conditions used for both virus propagation and RNA isolation (Fig. 2). Only the intact 60-70 S RNA was used for electron microscopic analysis.

Sedimentation analysis of 60-70 S AMV RNA isolated by gradient centrifugation of viral lysates

To protect the high-molecular-weight AMV RNA against possible degradation during phenol deproteinization, the 60-70 S RNA was isolated directly from viral SDS-lysates by sucrose gradient centrifugation in the presence of proteinase K or DEPC as described in Materials and Methods. RNAs from either untreated or UV-irradiated AMV frozen chicken leukaemic plasma was analysed.

RNA isolated by direct gradient centrifugation from UV-irradiated AMV revealed the typical sedimentation profile (Fig. 3) and no differences were

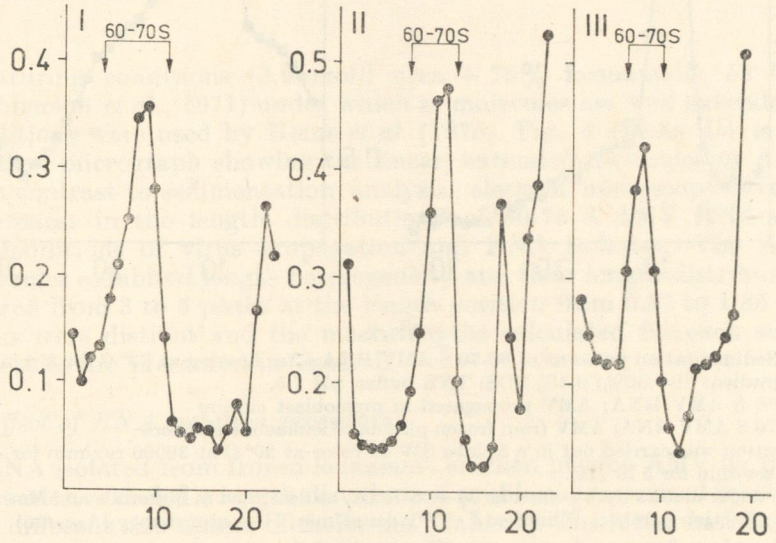

Fig. 1.

Sedimentation profiles of AMV RNA extracted from AMV by phenol treatment
Sucrose gradient 10—30%; 0.1% SDS; TNE buffer, pH 7.5.
I — AMV from frozen plasma of leukaemic chickens
II — AMV produced in myeloblast culture
III — AMV from frozen plasma of leukaemic chickens, UV-irradeated virus
Centrifugation was carried out in a Spinco SW 41 rotor at 20° C, at 38000 rev/min for 160 min (I, III), or at 38000 rev/min for 130 min (II). Fractions under arrows were pooled as 60-70 S RNA for electron microscopic analysis.
Abscissae: fraction number; ordinates: absorbancy (A_{260} nm)

observed when compared with that of phenol-SDS extracted RNA. However, when untreated AMV from frozen leukaemic chicken plasma was used for RNA isolation in the presence of proteinase K, the sedimentation profile of this RNA revealed a high amount of slowly sedimenting material. The amount of the latter was as high as 68% of the total RNA, while the 60-70

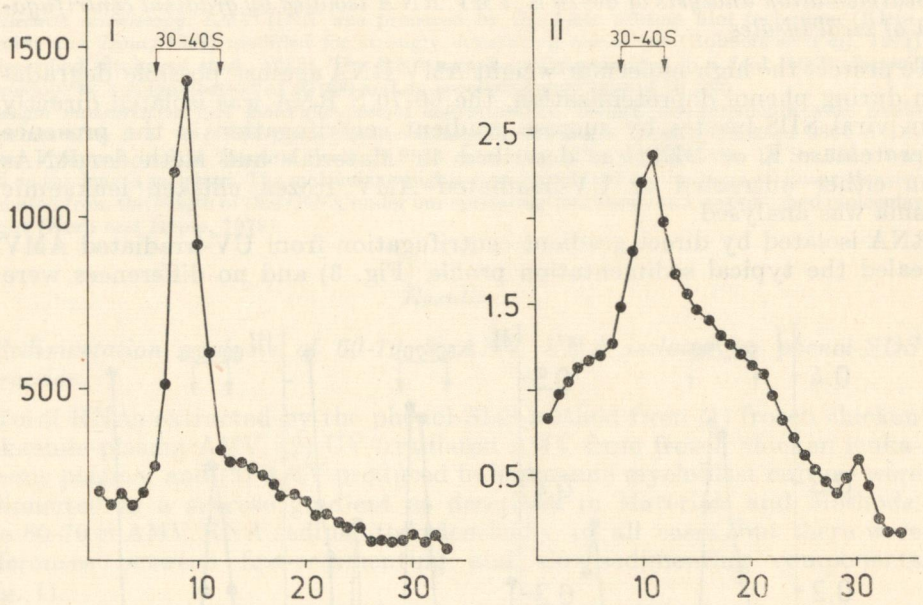

Fig. 2.

Sedimentation patterns of 60-70 S AMV RNA after heating at 73° C for 3 min
Sucrose gradient 10—30%; 0.1% SDS; TNE buffer, pH 7.5.
I — 60-70 S AMV RNA; AMV propagated in myeloblast culture
II — 60-70 S AMV RNA; AMV from frozen plasma of leukaemic chickens
Centrifugation was carried out in a Spinco SW 41 rotor at 20° C at 39000 rev/min for 4.5 hr (I) or 38000 rev/min for 5 hr (II).
Fractions under arrows were pooled as 30-40 S RNA, as described in Materials and Methods.
Abscissae: fraction number; ordinates: I — ^{32}P count/min; II — absorbancy (A_{260} nm).

RNA component constituted 32% only. When DEPC was used instead of proteinase K, the sedimentation profile was comparable except that in this case the amount of the material sedimenting faster than the 60-70 S RNA was as high as 32% of total RNA.

Electron microscopic analysis

We studied the length distribution of high-molecular-weight RNAs isolated by different methods from AMV either propagated in experimental chickens or in cultures of myeloblast. The RNA was visualized under strongly

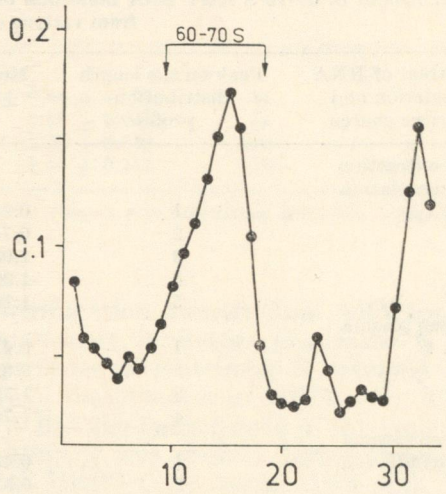

Fig. 3.
Sedimentation profile of AMV RNA isolated by direct gradient centrifugation from lysates of UV-irradiated virus
Proteinase K was used as a nuclease inhibitor. Glycerol gradient 10–30%; 0.05% SDS; TN buffer, pH 8. Centrifugation was carried out in a Spinco SW 50.1 rotor at 49000 rev/min and 20° C for 40 min.
Abscissa: fraction number; ordinate: absorbancy (A_{260} nm).

denaturing conditions (3.9 mol/l urea + 78% formamide; 53° C, 30sec) (Robberson *et al.*, 1971) under which ss molecules are well extended. Similar conditions were used by Heine *et al.* (1975). Fig. 4 (Plate II) is a typical electron micrograph showing the linear, extended molecules of AMV RNA.

In contrast to sedimentation analysis, electron microscopy revealed some differences in the length distributions of 60-70 S AMV RNA depending on conditions of virus propagation and RNA isolation. The AMV RNA molecules exhibited length heterogeneity and their length distribution profile showed from 3 to 5 peaks at the length position from 0.37 to 1.85 μm. These peaks were distinct and the mean lengths calculated for each size class of molecules are presented in Table 1.

Effect of RNA isolation procedure

RNA isolated from frozen leukaemic chicken plasma AMV by phenol-SDS extraction revealed a length distribution profile characterized by the presence of 5 different size classes of molecules (Table 1). The latter had mean lengths of 0.46, 0.75, 1.03, 1.38, and 1.76 μm. These size classes of molecules differed to some extent in the number and proportion in different plasma pools. The amount of molecules which fell into the highest size class (1.76 μm) corresponding to the 35 S varied from 5 to 11%. When this RNA was prepared by direct gradient centrifugation of AMV lysate, the amount of molecules in this highest size class was considerably higher (30%). It appears that phenol extraction led to partial degradation of ss AMV RNA, resulting in accumulation of molecules of the lower size classes. In our hands the method of direct gradient centrifugation thus gave better preservation of intact RNA molecules.

Table 1. Length of 60-70 S AMV RNA molecules obtained by two different methods of isolation from various sources

Method of RNA isolation and virus source	Peak on the length distribution profile	Mean length ±SM (μm)	% of molecules	No. of molecules measured
Phenol extraction				
Frozen plasma				
exp. 1	1	0.46 ± 0.074	15	511
	2	0.75 ± 0.076	22	
	3	1.03 ± 0.066	20	
	4	1.38 ± 0.091	29	
	5	1.76 ± 0.105	11	
Frozen plasma				
exp. 2	1	0.42 ± 0.098	28	251
	2	0.82 ± 0.137	31	
	3	1.33 ± 0.128	32	
	4	1.75 ± 0.077	5	
Frozen plasma				
UV irradiation	1	0.43 ± 0.102	24	411
	2	0.83 ± 0.136	37	
	3	1.29 ± 0.137	39	
Frozen				
culture fluid	1	0.50 ± 0.122	28	331
	2	0.79 ± 0.049	20	
	3	1.09 ± 0.149	51	
Direct sucrose gradient centrifugation				
Proteinase K				
Fresh plasma before ethanol				
precipitation	1	0.54 ± 0.135	49	298
	2	1.18 ± 0.109	20	
	3	1.51 ± 0.077	18	
	4	1.80 ± 0.116	10	
Fresh plasma after ethanol				
precipitation	1	0.58 ± 0.179	45	480
	2	1.09 ± 0.108	20	
	3	1.45 ± 0.078	12	
	4	1.82 ± 0.168	21	
Frozen plasma	1	0.46 ± 0.129	20	410
	2	0.89 ± 0.106	24	
	3	1.28 ± 0.110	23	
	4	1.76 ± 0.188	30	
Frozen plasma				
UV irradiation	1	0.49 ± 0.124	31	327
	2	0.88 ± 0.109	29	
	3	1.29 ± 0.138	40	
Frozen plasma UV irradiation plus 2 extractions by phenol	1	0.37 ± 0.072	12	180
	2	0.70 ± 0.109	40	
	3	1.11 ± 0.149	47	

Table 1, cont.

DEPC				
Frozen plasma	1	0.46 ± 0.135	36	176
	2	0.86 ± 0.079	19	
	3	1.23 ± 0.143	39	
	4	1.85 ± 0.141	5	

Per cent of molecules falling into individual size classes was calculated from the total No. of molecules measured.

With RNA isolated by gradient centrifugation directly from AMV lysates, we examined the effects of the ribonuclease inhibitors proteinase K and DEPC. While RNA isolated in the presence of proteinase K revealed 30% of molecules in the highest size class, in the presence of DEPC only 5% of molecules were in this length category. But the mean length of the molecules falling into the highest size class category was slightly higher, when the RNA was isolated in the presence of DEPC (1.85 versus 1.75 μm). This result showed that proteinase K is suitable for the isolation of high-molecular-weight AMV RNA, despite the variations in spreading caused by the presence of DEPC.

Comparison of the RNAs isolated from frozen and fresh plasma virus revealed length distribution profiles with four peaks. The mean lengths of the highest molecular size class was slightly higher (1.82 μm) in the RNA isolated from fresh plasma AMV than that of RNA from frozen plasma AMV (1.76 μm). Surprisingly, a shift of the molecular length to the lower size classes was detected in RNA from fresh plasma virus. These results indicate that storage of leukaemic chicken plasma for several months at −70° C and one thawing appear to have little effect on integrity of high-molecular-weight AMV RNA.

To clarify the effect of ethanol precipitation on the length and structure of viral RNA (Erikson, 1969), the RNAs of AMV from fresh leukaemic chicken plasma before and after ethanol precipitation were subjected to electron microscopic analysis. No substantial differences in the mean length of individual size classes or in their proportion were found.

Table 2. Properties of 60-70 S RNA molecules obtained by phenol extraction of AMV frozen leukaemic chicken plasma

Modal length ± SD (μm)	Molecular weight*	S value**
0.46 ± 0.075	0.74 ± 0.12 × 10^6	19
0.75 ± 0.076	1.20 ± 0.12 × 10^6	24
1.03 ± 0.066	1.65 ± 0.11 × 10^6	28
1.38 ± 0.091	2.21 ± 0.15 × 10^6	32
1.76 ± 0.105	2.82 ± 0.15 × 10^6	36

* Assuming a mass of 1.6 × 10^6 daltons/μm as found with 18 S myeloblast rRNA.

** S value was calculated according to the equation: molecular weight = 1550 × $(S)^{2.1}$ (Spirin, 1963).

Erikson (1969) and Bader and Steck (1969) suggested that protein may participate in the structure of the high-molecular-weight AMV RNA. To test this possibility, we analyzed AMV RNA isolated from the UV-irradiated virions (described in Materials and Methods). UV-irradiation, as shown by Sen and Todaro (1977), stabilizes the linkage between protein and RNA. Electron microscopic analysis of RNA from UV-irradiated virions revealed the disappearance of highest molecular size class with the presence of only 3 peaks on the length distribution profile. The results were independent on the method of RNA isolation used. In the preparation of UV-irradiated RNA, aggregates of different lengths without structural specificity were observed in addition to the linear molecules.

Effect of conditions of virus propagation

We used AMV from blood plasma of leukaemic chickens or from media of myeloblast cultures to check any possible differences in the structure of genomic RNA from these viral sources. We found fundamental differences between the two viral RNAs. The main difference concerned the mean length of molecules falling into the highest size class which was 38% shorter in the RNA of AMV obtained from culture media (1.09 versus 1.76 μm). In this case only 3 size classes were present and the individual peaks on the length distribution profile were less distinct (Table 1). In our hands leukaemic myeloblast cultures produced AMV that contained less intact RNA in comparison with plasma virus.

Calculation of molecular weight

Electron microscopic analysis of 60-70 S AMV RNA under strongly denaturing conditions revealed a heterogeneous population of molecules with preferential size classes, in agreement with the data reported by Chi and Bassel (1975) and Heine *et al.* (1975). The linear density used for the calculation of molecular weight was estimated by means of 18 S rRNA from chicken myeloblasts (Korb and Heine, 1978) spread simultaneously with AMV RNA. In our conditions this linear density was 1.6 MD per 1 μm. The molecular weights calculated for each size of RNA isolated from frozen plasma AMV by the phenol SDS-method are shown in Table 2. The presence of individual size classes in the population of 60-70 S AMV RNA studied was essentially the same as in the preparation of 30-40 S AMV RNA released by heating from the 60-70 S complex isolated from plasma AMV by the phenol-SDS procedure (Heine *et al.*, 1975).

Discussion

The high-molecular-weight AMV RNA was examined by both sedimentation and electron microscopic analysis. In the present study we paid attention to two important factors affecting the integrity of the AMV RNA: the conditions of virus propagation and RNA isolation procedures. The high-

molecular-weight RNA of retroviruses is labile and sensitive to mechanical degradation and splitting by nucleases (Erikson, 1969; Bader and Steck, 1969; Scheele and Hanafusa, 1972). This is especially true when the structure of virus particles is disrupted and proteins are removed from the viral RNA (Chen *et al.*, 1980). In this case the degradation resulting in a small number of nicks in viral RNA need not affect the sedimentation characteristics of 60-70 S RNA, but may manifest itself only under strong denaturation conditions (Chi and Bassel, 1975). In this respect it is well known that the age of virions plays an important role in the stabilization of the 60-70 S AMV RNA complex (Stoltzfus and Snyder, 1975).

Our results obtained by electron microscopic analysis of 60-70 S AMV RNA under strongly denaturing conditions revealed significant differences between the length distribution profiles for viral RNA molecules isolated by different methods from virions propagated in vitro and in vivo, even though no substantial differences in the sedimentation characteristics of these RNAs were found.

As for the influence of the isolation procedure on the length of RNA molecules and proportion of their size classes, a significant difference was found between phenol-extracted viral RNA and that isolated by direct gradient centrifugation of viral lysates. Average length of RNA molecules and their number in each size class (see Table 1) indicate a higher degradation after phenol treatment. This may be explained either by the influence of mechanical forces or time-dependent degradation during the phenol extraction. A similar effect was described during phenol extraction of rat liver RNA (Liu *et al.*, 1978).

Direct comparison of two nuclease inhibitors, proteinase K and DEPC, revealed a stronger protective effect of proteinase K during the isolation of AMV RNA. In the presence of proteinase K, 30% of the RNA molecules fell into the highest size class, in contrast to only 5% of the molecules in this length category when the DEPC was used.

We found only small differences between RNA of AMV isolated from fresh and frozen chicken blood plasma. This showed that the plasma had a high protective effect. We therefore conclude that one thawing of leukaemic plasma stored at −70° C does not influence significantly the high-molecular-weight RNA of AMV present in it. Nor did ethanol precipitation affect the length of AMV RNA. We found neither aggregates as supposed by Erikson (1969), nor degradation products.

The age-dependent properties of the virions represent another important factor affecting the length of AMV RNA. This was confirmed by comparing the RNA isolated from virions produced in vivo (pool of plasma from 60 chickens) and in vitro (cultured leukaemic myeloblasts — 6 hr virus). RNA isolated from AMV from these two sources revealed differences in the length distribution profiles. RNA obtained from "younger" virus, produced in culture of leukaemic myeloblasts, exhibited a length distribution profile in which the mean length of the highest size class was significantly shorter than that of plasma virus RNA. This difference suggests two possible explana-

tions. One is the special internal arrangement of viral RNA in "younger" AMV. In this case, the structural properties of RNA allow the formation of extremely stable base-paired ds regions too short to be identified by electron microscopy (Davidson, 1978), which seemingly shorten the ss molecules. This assumption may be supported by comparing the proportions of mean length of individual molecular size classes of RNA from leukemic plasma virus and from "younger" myeloblast culture virus. The proportions of mean length of individual size classes were very similar in both cases. The other explanation is based on the presumption of a different stability of the RNA from this "younger" virus (Stoltzfus and Snyder, 1975) and its sensitivity to mechanical or enzymatic degradation. The chemical bonds in RNA molecules of avian retroviruses are stabilized during the maturation process (Korb *et al.*, 1976). This fact could explain the observed "bad" length distribution profile of RNA from myeloblast culture virus. However, the rate of the maturation proces of retroviruses is high and therefore it is rather unlikely to expect some continuation of the maturation proces in respect to stabilization of RNA molecules after 6 hr (Cheung *et al.*, 1972; Korb *et al.* 1976). On the other hand, some differences in localization and activity of cellular nucleases resulting in different degradation of RNA molecules in these two compared cases cannot be excluded. Further, the virions are not present in the extracellular space (plasma virus) or in culture fluid for the same length of time. This could have resulted in a different nicking of AMV RNA molecules in these two cases.

We also considered the possibility that the difference in the length of RNA molecules between AMV produced by myeloblasts in culture and AMV from leukaemic chicken plasma could depend on the different proportion of individual viruses in the AMV viral complex. However, recent investigations (Duesberg *et al.*, 1980; Chen *et al.*, 1980; Souza *et al.*, 1980) of a defective AMV and its helper ivrus genomes revealed a 7.8-kilobase RNA for the defective AMV component that was only 5-10% smaller than the 8 to 8.5 kilobase helper viral RNA. Since in our case the difference in mean length of highest size class between RNA from plasma AMV and RNA from AMV produced by leukaemic myeloblasts was approximately 30%, we assume that the difference cannot be explained by a different composition of the AMV viral complex.

In all cases studied the AMV RNA represented a heterogeneous population of molecules with preferential size classes (Table 1). Similar size classes based on mathematical analysis were reported for 30-40 S AMV RNA (Heine *et al.*, 1975). Several peaks on the lengths histograms of avian retrovirus RNAs were also published by others (Jacobson and Bromley, 1975; Chi and Bassel, 1975). Our results support the suggestion that AMV RNA is preferentially split at specific sites along the viral genome (Heine *et al.*, 1975; Korb and Štokrová, 1979, 1980). Since a heterogeneous length distribution was found for AMV RNA independently of virus propagation and RNA isolation, we conclude that it is a property of AMV RNA. It cannot be excluded that the differences in the genomes of AMV and its helper viruses MAV-1 and MAV-2

may contribute to this heterogeneity. Further experiments with separated helper viruses are needed to elucidate this point.

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