Three Sizes of Subunits in RNAs from Feline Sarcoma–Leukaemia Virus Mixtures

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SUMMARY

RNA from the Snyder–Theilen feline sarcoma–leukaemia virus complex (ST-FeSV–FeLV) sedimented in a double-peaked band between 50 and 70S, but Gardner–Arnstein (GA) FeSV–FeLV RNA sedimented in a single 70S peak. FeLV isolated from the ST virus mixture contained RNA which sedimented in a 70S band like GA-FeSV–FeLV RNA, but F422 FeLV RNA sedimented more slowly, at 50 to 60S. After thermal denaturation, resedimentation revealed three classes of RNA subunits in ST-FeSV–FeLV RNA: the first class, 35 to 37S, was also found in ST-FeLV and other FeLVS (except F422 FeLV), in the endogenous feline virus, RD114 and in GA-FeSV–FeLV; the second class, 32 to 34S, was similar to subunits in F422 FeLV and minor components of GA-FeSV–FeLV and ST-FeLV; the third class, 25S, was detected only in ST-FeSV–FeLV RNA. Electrophoresis of RNA species in buffered formamide provided evidence that the three classes of RNA subunits distinguishable on the basis of sedimentation rates actually represent three size classes of subunits. The ST virus mixture was shown to contain about equal titres of infectious FeLV and transforming FeSV whereas GA-FeSV–FeLV had at least a 10-fold excess of FeLV over FeSV. These observations are discussed in terms of possible origins of the three sizes of FeSV–FeLV RNA subunits and their relationships to three species of FeSV–FeLV proviral DNA described recently (Sherr et al. 1979).

INTRODUCTION

Recently, we initiated studies on type C viruses from the domestic cat, Felis catus. The origins of feline sarcoma viruses (FeSVs), which are responsible for fibrosarcoma induction, were of particular interest to us. These viruses arise spontaneously in random-bred populations which essentially share the natural human environment (Essex, 1975). Moreover, FeSVs appear to induce neoplastic transformation of human as well as animal cells in cultures (Essex, 1975). Our first objective was to obtain FeSV-specific nucleic acids, both RNAs and complementary DNAs, to facilitate analyses of the genetic information of these viruses.

Earlier studies on RNA tumour viruses have demonstrated that they contain 50 to 70S dimers of the genomic RNA; denaturation of native 50 to 70S RNAs releases monomeric subunits which sediment in the range of 30 to 40S, plus some minor species of less than 10S
(Erikson, 1969; Duesberg & Vogt, 1973; Maisel et al. 1977). FeSVs, like all other mammalian sarcoma viruses, are unable to replicate alone (Sarma & Log, 1973). Therefore, stocks of FeSV always contain associated helper viruses which are often feline leukaemia viruses (FeLVs). Because the RNA subunits of several rodent sarcoma viruses are smaller than those of associated helper viruses (Maisel et al. 1973, 1977; Shih et al. 1978), it was decided to begin by examining the size distributions of RNAs in FeSV–FeLV stocks.

Previous reports on virion RNAs from several isolates of FeLV suggested there might be more size variation for FeLV RNAs than for rodent leukaemia virus RNAs. Results of one study showed that RNAs from different strains of FeLV exhibited differences in electrophoretic mobilities in aqueous acrylamide gels (Whalley, 1973). More recently, the major component of denatured RNA in virions of one particular strain of FeLV, the Rickard F422 isolate, was found to sediment at 28S, more slowly than the 30 to 40S RNAs of other mammalian leukaemia viruses (Brian et al. 1975). These findings raised doubts about the feasibility of distinguishing FeSV RNAs from those of FeLVs on the basis of size. Nevertheless, by examining RNAs from a variety of feline type C viruses, a small RNA species, which could represent the genome of at least one FeSV strain, might be detected.

While these studies were in progress, Sherr et al. (1979) published restriction endonuclease maps of unintegrated proviral DNA of the Snyder–Theilen (ST) strain of FeSV. In that study, proviral DNA from cells infected with ST-FeSV–FeLV virus stocks clearly consisted of three species with different sizes, but denatured RNA from the same virus mixture was reported to contain only two types of subunits with distinct electrophoretic mobilities. In contrast, our studies on denatured ST-FeSV–FeLV RNA have revealed three classes of RNA subunits with apparently distinct sizes. One class of ST-FeSV–FeLV RNA subunits appears to be only slightly smaller than most FeLV subunits and this class is about the same size as RNA subunits from F422 FeLV virions. Detection and characterization of the three ST-FeSV–FeLV RNA subunit species in our work was facilitated by the use of sedimentation methods offering extremely high resolution of large RNAs.

**METHODS**

*Virus and cell sources.* Gardner–Arnstein FeSV–FeLV (Gardner et al. 1970) was kindly supplied by Dr Murray Gardner, School of Medicine, University of Southern California, in the form of an extract of a feline fibrosarcoma from the first in vivo passage of the original isolate. The following were generously provided by Dr Richard Olsen, School of Veterinary Medicine, Ohio State University: Snyder–Theilen FeSV–FeLV (Snyder & Theilen 1969), also as a tumour extract; the F422 cell line (Rickard et al. 1969) producing the Rickard FeLV; and RD114 cells (McAllister et al. 1972). Cloned isolates of FeLV (Sarma & Log, 1973) of subgroups A (MAH strain), B (ST strain) and C (FL-237), and a mink lung cell line (CCL64) were obtained from the American Type Culture Collection. NRK cells (Duc-Nguyen et al. 1966) in the 10th subculture from origin and Kirsten murine sarcoma–leukaemia virus (Kirsten & Mayer, 1967), as homogenate of erythroblastic spleens of Wistar/Furth rats, were gifts of Dr Sandra Panem, School of Medicine, University of Chicago.

*Cell and virus culture.* All cells were grown in medium containing 100 units/ml penicillin and 100 µg/ml streptomycin. F422 cells were grown in sealed flasks in McCoy’s 5A medium modified for suspension culture (Gibco Laboratories, Grand Island, N.Y., U.S.A.) with 10% (v/v) foetal bovine serum (K-C Biologicals, Lenexa, Kan., U.S.A.) in 10% CO₂. For infection with virus, low density cultures were treated with DEAE-dextran (Pharmacia, Piscataway, N.J., U.S.A.) at 30 µg/ml for 30 min before addition of virus. To detect infection with non-transforming viruses, assays for reverse transcriptase [using oligo(dT)].
poly(rA) synthetic primer:template] in 24 h culture supernates were done as described previously (Kimball et al. 1976). Viruses were cloned in microcultures (Microtest II plates, Falcon Plastics, Cockeysville, Md., U.S.A.) infected as described above with a series of dilutions of virus stocks; virus-positive cultures, infected with dilutions for which the majority of cultures were negative, were used as sources of cloned non-transforming viruses.

Radioactive labelling and isolation of RNA. Our procedures for production of radio-labelled virus RNAs have been described (Strauss et al. 1980). Briefly, cells producing virus were incubated for several hours in growth medium supplemented with $^{32}$P-phosphate or $^3$H-uridine. Cells were washed extensively and virus was harvested in serum-free medium at intervals of 3 to 4 h. Detergent and nuclease inhibitors were added directly to serum-free culture supernates, and virus RNA was isolated on an oligo(dT)-cellulose column. After elution from the column and extraction of proteins with phenol, virus RNA can be used directly or concentrated by precipitation with ethanol. For sedimentation or denaturation, RNA was dissolved in 0.01 M-tris-HCl pH 7.4, 0.001 M-EDTA and 0.05% SDS.

Sedimentation analyses of RNA. The conditions for high resolution of RNA species have been published (Strauss et al. 1980). In brief, RNA in 50 μl was layered on to a 12 ml linear gradient of 1 to 15% (w/v) sucrose in 1×TNE buffer pH 7.4 (0.01 M-tris-HCl, 0.1 M-NaCl, 0.001 M-EDTA) with 0.1% SDS. Gradients were subjected to centrifugation in an SW41 rotor (Beckman Instruments, Palo Alto, Calif., U.S.A.) in an oil turbine-driven ultracentrifuge (Sorvall OTD-2, Du Pont Instruments, Newtown, Conn., U.S.A.) equipped with an acceleration-rate controller (Sorvall ARC-1). The rotor was started at the lowest possible rate. Centrifugation was carried out at 20 °C and 36,000 rev/min for 1.75 h for native virus RNA or 4 h for subunit analyses, after which the rotor was braked to 1000 rev/min and then allowed to come to rest without braking. Gradients were collected through the tube bottom and acid-precipitable radioactivity was determined for each gradient fraction as previously described (Kimball et al. 1976).

Electrophoresis of RNA. Electrophoresis in phosphate-buffered formamide was performed as described (Duesberg & Vogt, 1973) with the following modifications. Final concentration of acrylamide–bisacrylamide was 2.6%. To ensure reproducible polymerization at this gel concentration, formamide was deionized immediately before preparation of gels to remove ions produced by spontaneous hydrolysis of the solvent. Electrophoresis using 8 cm gels was carried out at 15 V/cm. To determine radioactivity, 1 mm-thick gel slices were added to 5 ml toluene–Omnifluor with 3% (v/v) Protosol (New England Nuclear, Boston, Mass., U.S.A.) and incubated at 50 °C for 12 h before counting.

RESULTS

Sedimentation analyses of 50 to 70S virus RNAs

To study native 50 to 70S RNAs of FeSV–FeLV mixtures, two homogenates of feline fibrosarcomas were used to infect and transform mink cells with two isolates of FeSV–FeLV, the Snyder–Theilen (ST) and Gardner–Arnstein (GA) strains. To obtain pure FeLV, the ST tumour homogenate was diluted and samples containing less than one transforming (focus-forming) unit (f.f.u.) of FeSV were used to infect several cultures. One of these cultures, which showed no signs of transformation, was used as a source of ST-FeLV free of FeSV.

Virion RNAs were radioactively labelled in cell cultures and then extracted as described in Methods. Sedimentation behaviours were compared directly by mixing two RNA samples, one $^3$H-labelled and the other $^{32}$P-labelled, before sedimentation in sucrose gradients. Fig. 1(a) shows a comparison of $^{32}$P-RNA from the ST-FeSV–FeLV complex
Fig. 1. Comparison of sedimentation behaviour of native 50 to 70S RNAs of feline and murine sarcoma-leukaemia virus mixtures. RNAs were radioactively labelled, isolated and analysed (without dissociation of subunits) by sedimentation in sucrose gradients as described in Methods. (a) ³H-RNA of ST-FeLV (15000 ct/min) and ³²P-RNA of the ST-FeSV-FeLV complex (7500 ct/min). (b) ³H-RNA of F422 FeLV (10000 ct/min) and ³²P-RNA of the GA-FeSV-FeLV complex (6000 ct/min). (c) ³H-RNA of Kirsten MuLV (7500 ct/min) and ³²P-RNA from Kirsten MuSV-MuLV (8500 ct/min). Arrows indicate the positions of 28S and 18S ribosomal ³H-RNA markers in gradients run in parallel. ●— ●, ³H; ○—○, ³²P.

and ³H-RNA from the ST-FeLV. The native ST-FeSV-FeLV RNA sedimented in a double-peaked band with the major peak slightly slower than ST-FeLV RNA which had a sedimentation coefficient of about 70S compared to the 28S RNA marker. In contrast to ST-FeSV-FeLV RNA, ³²P-RNA extracted from the GA-FeSV-FeLV mixture sedimented in a single band at about 70S, like ST-FeLV RNA (Fig. 1b).

Native RNAs from several other isolates of FeLV (cloned MAH, ST, FL-237; Sarma & Log, 1973) also sedimented at about 70S (not shown). The one exception to this general finding was the Rickard F422 strain; RNA from this FeLV was found to sediment more slowly than those of all other FeLVs examined. F422 RNA sedimented at about the same rate as the major peak of ST-FeSV-FeLV RNA (Fig. 1a, b). For reference, native RNAs of the Kirsten murine sarcoma-leukaemia virus (MuSV-MuLV) complex and MuLV were prepared and analysed under the same conditions (Fig. 1c). The bulk of ³²P-RNA from this MuSV-MuLV mixture sedimented in a double-peaked band similar to ST-FeSV-FeLV RNA, while MuLV ³H-RNA migrated in a sharp 70S band. These results suggested that native FeSV-FeLV RNA from the ST isolate, at least, could contain multiple subunit species with different sizes, as is known to be true for the Kirsten MuSV-MuLV RNA (Maisel et al. 1973; Shih et al. 1978).

Analyses of RNA subunits from FeSV-FeLV mixtures

The 50 to 70S RNAs from the two isolates of FeSV-FeLV, the ST-FeLV and the F422 FeLV were isolated from gradients and heated to dissociate subunits. After resedimentation ST-FeSV-FeLV RNA was found to contain three distinct classes of subunits with sedimentation coefficients of 35 to 37S, 32 to 34S and 25S (Fig. 2a). Gradients were calibrated
RNAs of feline viruses

Fig. 2. Comparisons of sedimentation behaviours of subunits from RNAs of feline and rodent sarcoma-leukaemia virus mixtures. Peak fractions of 50 to 70S RNAs were pooled from individual preparative gradients similar to those in Fig. 1. RNAs were precipitated in ethanol as described in Methods and dissolved in 0.01 M-Tris pH 7.4, 0.001 M-EDTA and 0.1% SDS. Pairs of RNAs were mixed, heated in boiling water for 45 s, chilled on ice, and analysed in gradients for subunits as in Methods. (a) $^3$H-RNA of ST-FeSV-FeLV (12500 ct/min) and $^{32}$P-RNA of ST-FeLV (4000 ct/min). (b) $^3$H-RNA of F422 FeLV (10000 ct/min) and $^{32}$P-RNA of GA-FeSV-FeLV (5000 ct/min). (c) $^3$H-RNA from mink cell cytoplasm (5000 ct/min) and $^{32}$P-RNA of Kirsten MuSV-MuLV (4000 ct/min). ●--●, $^3$H; ○----○, $^{32}$P.

with 38S MuLV and 33S MuSV RNAs (Maisel et al. 1977; Shih et al. 1978) and 28S and 18S mink rRNAs, all of which can be resolved under our conditions (Fig. 2c). The fastest sedimenting class of subunits in ST-FeSV-FeLV RNA co-sedimented with ST-FeLV RNA subunits at about 35 to 37S (Fig. 2a). The second class of ST-FeSV-FeLV RNA subunits always appeared in six different preparations as a minor peak trailing just behind ST-FeLV RNA subunits, in the range of 32 to 34S, and this class of ST-RNA sedimented at about the same rate as F422 FeLV RNA subunits which formed a single peak at 32 to 34S (Fig. 2b). These results pointed to the 25S ST-FeSV-FeLV RNA as the most likely possibility for a subunit specific for ST-FeSV RNA.

In contrast to the results with ST-FeSV-FeLV RNA, the GA-FeSV-FeLV RNA was found to contain only two distinguishable classes of subunits, a major peak of 35 to 37S RNA and a minor peak at 32 to 34S (Fig. 2b). Thus, on the basis of sedimentation alone, we were unable to detect any class of subunits specific for FeSV in the GA virus mixture. This is probably due to the presence of much more FeLV than FeSV in this GA virus stock (see below).

RNA subunits from clones of feline viruses

The FeSV-FeLV RNAs described above came from virus stocks which probably contain several serologically distinct strains of FeLV (Sarma & Log, 1973). Therefore, we decided to clone the FeSVs and associated FeLVs to see if the 25S subunit segregated with FeSV transforming activity. Twenty-four microcultures of mink cells were infected with ST-FeSV-FeLV (produced in mink cells) at a multiplicity of about 1 f.f.u./culture. Each culture was examined later for FeSV-induced transformation and for FeLV by testing for virus reverse
transcriptase which is not produced by FeSVs (Porzig et al. 1979a). Ten cultures were transformed by ST-FeSV and eight produced FeLV. This indicated that the ST-FeSV–FeLV stock contained about equal titres of transforming FeSV and infectious FeLV. Several of the cultures transformed by ST-FeSV did not produce FeLV. However, similar cloning experiments with GA-FeSV–FeLV failed to produce any transformed cultures free of FeLV. In fact, all 24 microcultures were infected with FeLV although only two were transformed by GA-FeSV. Therefore, the GA-FeSV–FeLV stock had at least a 10-fold excess of FeLV over FeSV.

To recover the cloned ST-FeSV, it was necessary to superinfect the ‘non-producer’ transformed cells with FeLV which acts as a helper virus for FeSV (Porzig et al. 1979a). One of the ST-FeLV clones from a microculture free of FeSV was used for this purpose. The 50 to 70S virus RNAs were then labelled and isolated as above and RNA subunits were analysed by sedimentation. The cloned mixture of ST-FeSV–FeLV contained both 35 to 37S and 25S RNA subunits (Fig. 3a), although there was less 25S RNA relative to 35 to 37S than in the uncloned parental stock. No distinct peak of 32 to 34S RNA was evident, but the 35 to 37S peak had a shoulder on the trailing side which was also present, although greatly reduced, on the main peak of 35 to 37S subunits from the cloned ST-FeLV (Fig. 3a).

These results further supported the notion that the 25S subunit was ST-FeSV RNA. However, we also examined RNAs of several additional cloned feline type C viruses to see if 25S RNA subunits were present. Subunits from each of these viruses, including the endogenous cat virus, RD114, and three serologically distinct clones of FeLV, sedimented in predominant peaks at 35 to 37S. In comparing pairs of these virus RNAs, several consistent one fraction differences were observed between major peaks in sedimentation profiles (for examples, Fig. 3b, c). No major peaks at less than 35S were observed in any virus stock without FeSV, but minor shoulders at 32 to 34S appeared in some FeLV RNA preparations.
Sizes of RNA subunits with different sedimentation coefficients

The observed differences in sedimentation for various virus RNA components implied mol. wt. differences, but sedimentation behaviour under aqueous conditions may also depend on RNA conformation. To obtain more reliable size comparisons for the ST virus RNA components resolved by sedimentation, subunits were recovered from sucrose gradients and analysed by electrophoresis in buffered formamide. Under these conditions, mobility of RNA molecules is largely independent of original conformation in aqueous solutions (Duesberg & Vogt, 1973; Lehrach et al. 1977). The 38S RNA subunits of Moloney MuLV and 28S mammalian rRNA were used as internal markers. As shown in Fig. 4(a), 35 to 37S RNA from ST-FeSV–FeLV migrated in formamide at the same rate as 38S MuLV subunits. The bulk of the 32 to 34S RNA subunits from the ST virus mixture migrated slightly faster than MuLV RNA, while the 25S subunits moved much faster, almost at the same rate as the 28S rRNA marker (Fig. 4a). Therefore, the three classes of ST-FeSV–FeLV RNA subunits most likely have different sizes. However, precise mol. wt. for these RNA species cannot be determined from these experiments (see Discussion).

The 32 to 34S RNA species from other feline viruses were also analysed for size by electrophoresis in formamide. Peak fractions of 32 to 34S GA-FeSV–FeLV RNA were recovered from a sucrose gradient and mixed with F422 FeLV RNA subunits before electrophoresis (Fig. 4b). The main peak of 32 to 34S GA-FeSV–FeLV RNA migrated at the same rate as F422 RNA subunits, slightly faster than a lesser peak of 35 to 37S GA-FeSV–FeLV RNA which contaminated the 32 to 34S gradient fractions. In a separate gel, 35 to 37S GA-FeSV–FeLV RNA subunits co-migrated with MuLV RNA (not shown). We also examined the minor 32 to 34S component of the cloned ST-FeLV shown in Fig. 3(a). Again, the 32 to 34S gradient fractions contained considerable amounts of 35 to 37S RNA, but a distinct peak of ST-FeLV RNA migrating just ahead of MuLV marker RNA was also observed (Fig. 4c). Thus, all the 32 to 34S components of FeLV or FeSV–FeLV RNAs we examined appear to have sizes similar, if not identical, to that of RNA subunits from virions of F422 FeLV.

The major RNA components from all other virus stocks examined in these studies were found to migrate essentially at the same rate as MuLV subunits. For example, the RNA subunits of RDII 14 virus and one particular cloned FeLV, which appeared to be partially separable by sedimentation (Fig. 3b), showed coincident peaks in electrophoretic analyses (Fig. 4d). Similar results were obtained in further selected analyses of pairs of the Rauscher strain of MuLV, four isolates of FeLV and the RDII 14 virus (not shown). This suggested that the RNAs of a variety of self-replicating mammalian type C viruses have nearly identical mol. wt.

**DISCUSSION**

The original purpose of these studies was to attempt to identify an RNA species that had characteristics appropriate for the genomic RNA of FeSV. The 25S subunits in ST-FeSV–FeLV RNA appear to have properties of mammalian sarcoma virus RNA subunits, including a smaller size than helper FeLV RNAs and the association with ST-FeSV transforming activity in cloning experiments. Furthermore, roughly equal amounts of 25S and 32 to 37S subunits were found in RNA from the ST-FeSV–FeLV stock which was shown to contain about equal titres of FeSV and FeLV. All ST-FeSV–FeLV RNA classes were also observed in virus stocks grown directly from tumour homogenates in feline embryo cells (not shown); therefore, it is not likely that any of these RNAs are derived from mink cells. In summary, it seems most probable from our work that the 25S RNA subunits are ST-FeSV RNA. Recent data on the relative sizes of ST-FeSV and ST-FeLV proviral DNAs (Sherr
Fig. 4. Electrophoresis of virus RNA subunits in phosphate-buffered formamide. (a) Subunits of \(^{3}H\)-RNA from ST-FeSV-FeLV were separated in preparative gradients as in Fig. 2. Fractions at 35 to 37S (i.e. fractions 4 to 6, Fig. 2a), 32 to 34S (fractions 7 to 10) and 25S (fractions 12 to 15) were pooled and precipitated with ethanol. Precipitates were washed once with a small vol. of 75% ethanol in water to remove salts before dissolution in electrophoresis buffer (Duesberg & Vogt, 1973). The three classes of subunits (about 8000 ct/min of each) were run in separate, parallel gels with \(^{32}P\)-RNA markers of Moloney MuLV RNA and 28S rRNA included in each. •—•, 35 to 37S; ■—■, 32 to 34S; ▲—▲, 25S. (b) 32 to 34S subunits of GA-FeSV-FeLV \(^{3}P\)-RNA (8000 ct/min) (e.g. fractions 8 to 10, Fig. 2b) were mixed with denatured 50 to 60S F422 FeLV \(^{3}H\)-RNA (8000 ct/min) in the same gel. (c) 32 to 34S ST-FeLV \(^{3}H\)-RNA (8000 ct/min) (e.g. fractions 8 and 9, Fig. 3a) compared with 38S MuLV and 28S rRNA markers. (d) Subunits from individual gradients similar to those in Fig. 3(b) were mixed together for electrophoresis in one gel. \(^{3}H\)-RNA of FeLV-B (8000 ct/min) and \(^{32}P\)-RNA of RD114 virus (6000 ct/min). The gel in (a) ran for 6 h, those in (b) and (c) for 7 h and that in (d) for 4.5 h. In all panels •—•, ■—■, ▲—▲, \(^{3}H\); ○—○, \(^{32}P\).
et al. 1979) are also consistent with this interpretation. Definitive identification of ST-FeSV RNA subunits should be possible by hybridization with FeSV-specific DNAs (Porzig et al. 1979b; Sherr et al. 1979). Our methods for production of individual virus RNA components should greatly facilitate such experiments.

In the light of recent work on FeSV in other laboratories (Porzig et al. 1979b; Sherr et al. 1979), the more important aspect of our work may be the discovery of 32 to 34S RNAs in several feline viruses. RNA subunits sedimenting in the range of 32 to 34S were present consistently as minor components in 50 to 70S RNAs from ST-FeSV–FeLV and GA-FeSV–FeLV mixtures and a cloned stock of ST-FeLV. RNAs from several other FeLV stocks appeared to contain small amounts of 32 to 34S RNA (e.g. 3H-RNAs in Fig. 3b, c), but we made no attempt to characterize these species further or to establish the reproducibility of their occurrence in different stocks of a given virus strain. The 32 to 34S RNAs of the ST and GA viruses all exhibit about the same sedimentation rate as the only subunit detected in F422 FeLV RNA. F422 RNA subunits were reported to co-sediment with 28S rRNA in 99% dimethyl sulphoxide (Brian et al. 1975), but under our aqueous conditions F422 subunits sediment at approx. 32 to 34S (Fig. 2b), rather like 33S Kirsten MuSV RNA and distinctly slower than the 35 to 37S major component of all other FeLVS examined here.

Direct comparisons of various RNA species by electrophoresis in buffered formamide provided strong evidence that all the 32 to 34S RNA species are about the same size. This size is clearly smaller than all 35 to 38S RNA subunits from self-replicating murine or feline viruses. These 35 to 38S subunits also seem to share a common size. Our data are not sufficient, however, to assign definite mol. wt. to the various RNA components, for several reasons. First, to obtain reliable size estimates of RNAs from relative migration rates in any gel electrophoresis system, it is necessary to demonstrate that several reliable markers and the unknowns behave consistently while critical parameters are varied (Lehrach et al. 1977); we did not vary gel concentration or voltage gradient, two critical parameters for this system. Moreover, the reliability of our markers suffers from two known sources of error. One source derives from the use of 28S rRNA as a convenient migration marker; it is extremely difficult to denature fully and may migrate anomalously fast even under our strongly denaturing conditions (Lehrach et al. 1977). A more important source of error with all large RNA standards is that the sizes of large RNAs are not precisely known. Hu et al. (1977) recently provided a mol. wt. estimate of $3.0 \times 10^6$ for our marker MuLV RNA, based on contour length in electron micrographs; but their size standard was dsDNA. The most definitive source of information for RNA size determinations would be base-sequence data, but complete sequences of large RNAs are not generally available. On the other hand, complete sequences of several DNA molecules are known, DNA structures are simpler and technology for comparing DNA sizes is now quite reliable. Thus, the recent estimate (Sherr et al. 1979) of 8.4 kbp (or mol. wt. about $5.5 \times 10^6$) for a dsDNA provirus of ST-FeLV is probably a more reliable estimate for the size of the FeLV genome than any estimate we could make for FeLV RNA, which seems to have the same size as MuLV RNA. Direct comparisons of RNAs and proviral DNAs, by hybridization, should provide the most efficient means of establishing sizes of retrovirus RNAs.

The origin(s) of the 32 to 34S RNAs cannot be determined from our work. In the case of F422 FeLV, the main subunit species in virions was shown to lack 5'-terminal methylated 'caps' found on other mammalian leukaemia virus RNAs (Thomason et al. 1976); but apparently larger 36S FeLV RNAs were found in the cytoplasm of F422 cells and at low levels in F422 FeLV virions (Conley & Velicer, 1978). These and other observations have raised speculation that most F422 FeLV virions may represent defective interfering particles (Thomason et al. 1978). The similarities in sedimentation rates and sizes among the ST,
GA and F422 32 to 34S RNAs are consistent with a common origin, but there are other possibilities.

The 32 to 34S GA RNA could represent FeSV RNA. From our present data it seems unlikely that we have detected RNA of the GA-FeSV since the GA virus stocks contained a great excess of FeLV. Some of the 32 to 34S RNA species may be analogues of known murine 30S cellular RNAs (Duesberg & Scolnick, 1977; Besmer et al. 1979), production of which can sometimes be induced by infection with a leukaemia virus (Kimball et al. 1980). However, in the case of the ST-FeSV–FeLV 32 to 34S RNA, the most attractive hypothesis for its derivation is that it arose by a deletion of the full-length FeLV genome. The basis for this idea is that Sherr et al. (1979) observed a second form of FeLV proviral DNA, consisting of 6–7 kbp, in ST-FeSV–FeLV-infected cells, in addition to the 8.4 kbp species; this smaller DNA was described as a ‘deletional variant’ of the 8.4 kbp FeLV provirus.

In that same study (Sherr et al. 1979), only two subunit species of ST-FeSV–FeLV virion RNA were resolved by electrophoresis under partially denaturing conditions (10% formamide). However, the slower RNA peak (presumably FeLV subunits) was clearly much broader than the 28S rRNA marker. Since the two FeLV proviral DNAs were found in nearly equal amounts, it seems most likely that the broad virus RNA peak consisted of about equal amounts of 35 to 37S and 32 to 34S RNAs. It is not surprising that we resolved these two RNAs because we have shown previously that our sedimentation method provides resolution of large RNA species equivalent to, if not greater than, acrylamide gels (Strauss et al. 1980).

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