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Genomic Complexities of Murine Leukemia and Sarcoma, Reticuloendotheliosis, and Visna Viruses

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The genetic complexities of several ribodeoxyviruses were measured by quantitative analysis of unique RNase T_1-resistant oligonucleotides from 60–70*S* viral RNAs. Moloney murine leukemia virus was found to have an RNA complexity of 3.5×10^6 daltons, whereas Moloney murine sarcoma virus had a significantly smaller genome size of 2.3×10^6. Reticuloendotheliosis and visna virus RNAs had complexities of 3.9×10^6 and 3.5×10^6, respectively. Analysis of RNase A-resistant oligonucleotides of Rous sarcoma virus RNA gave a complexity of 3.6×10^6, similar to that previously obtained with RNase T_1-resistant oligonucleotides. Since each of these viruses was found to have a unique sequence genomic complexity near the molecular weight of a single 30–40*S* viral RNA subunit, it was concluded that ribodeoxyvirus genomes are at least largely polyploid.

The 60–70*S* RNA complex of ribodeoxyviruses contains predominantly two 30–40*S* RNA subunits, having a size of approximately 3×10^6 daltons each (with some size variations between viruses) (13, 27, 28, 33), and is thought to be the viral genome. Several methods have been used in attempts to determine whether the identically sized 30–40*S* RNA subunits of a given 60–70*S* complex contain identical or different RNA sequences. Conflicting results concerning the size of the genome have been obtained from complexity determinations based on RNA-DNA hybridization rates, infectious DNA sizing, and chemical RNA analyses.

Complexity estimates, made on the basis of RNA-DNA hybridization kinetics, have varied from 9×10^6 daltons for Rous sarcoma virus (RSV) (40), Moloney murine leukemia virus (M-MLV) (19), and visna virus (23) to 3×10^6 daltons for avian myeloblastosis virus (3). Using a different approach, Cooper and Temin have analyzed viral sequences which had been transcribed to DNA and integrated into the host genome (10). They reported that the minimum size of integrated, double-stranded reticuloendotheliosis virus (REV) DNA which was infectious was 10×10^6 to 20×10^6 daltons, whereas 6×10^6 dalton double-stranded RSV DNA was infectious, in both cases with single-hit kinetics (10). Therefore, they proposed that REV might have a genome two to three times larger than that of RSV, possibly because RSV genetic information could be complemented by viral information preexisting in the cell whereas REV could not be so complemented.

The 30–40*S* RNA subunits of AKR MLV and of M-MLV have been fractionated into poly(A)-containing and poly(A)-lacking species, raising the question of whether genetically distinct RNA subunits are present in MLVs (25). If these two types of subunits contain different sequence information, the complexity of MLV RNA would be significantly higher than the size of a single 30–40*S* RNA subunit.

Previous complexity estimates based on chemical analyses of avian tumor virus RNA, using a method similar to that of Sinha et al. (38) and Fiers et al. (20), have all been between 2.7×10^6 and 3.5×10^6 daltons (5, 6, 14, 17, 36, 44). It was the purpose of the experiments described here to determine from unique RNase T_1-resistant oligonucleotides the RNA sequence complexities of representatives from ribodeoxyvirus groups not analyzed previously by this method and to compare our results with those cited above. In addition, previous complexity analyses of RSV RNA were extended to RNase A-resistant sequences. The results indicate that the unique sequence genome of M-MLV, Moloney murine sarcoma virus (M-MSV), REV, and visna virus, as well as of RSV, is similar in size to a single 30–40*S* RNA subunit.

MATERIALS AND METHODS

Cells and viruses. NIH-3T3 cells infected by cloned M-MLV were gifts from H. Fan, D. Smotkin, and R. Weinberg. The virus had been cloned by Fan and Paskind as described (19). M-MSV (MLV) clone 3, grown on NRK cells, was derived as described (31). Clone 124 of (R^+T^+) M-MSV-infected TB cells was obtained from J. Ball (1). All murine viruses were propagated in Eagle minimal essential medium supplemented with 10% fetal calf serum (Flow Laboratories), 0.5 μg of amphotericin B (Fungizone) per ml, 100 U of penicillin per ml, and 50 μg of streptomycin per ml.

REV (strain T) was obtained and propagated as described (26). Visna virus was grown on sheep choroid plexus cells as described (22). Cloned Prague strain RSV, subgroup B, was obtained from P. Vogt and propagated on gs^-, chf^- chicken embryo fibroblasts as described (15).

RNA labeling, extraction, and polyacrylamide gel electrophoresis. Murine viruses were labeled with carrier-free ^{32}P (1 mCi/ml; ICN) in Dulbecco modified Eagle medium, (free of phosphate) and supplemented with 2% dialyzed calf serum. Fungizone, penicillin, and streptomycin concentrations were the same as above. Cells were incubated with phosphate-free medium for 1 to 2 h before labeling. After 12 h of labeling, complete media was restored and virus collections were made every 12 h for 2 to 3 days. Labeling with [^{3}H]uridine was as described (31). [^{14}C]uridine (58 mCi/mol; ICN) was added to cells, in minimal essential medium containing 5% fetal calf serum, at a concentration of 3 μCi/ml. After labeling with ^3H or ^{14}C, collections were made for 3 days at 3- to 4-h intervals. Virus was purified and RNA was extracted with phenol and sodium dodecyl sulfate as described (15), with the modification that chloroform, at a volume one-fifth that of phenol, was added during RNA extraction.

Visna virus was labeled with ^{32}P and purified, and its RNA was extracted with lithium dodecyl sulfate and Pronase by method C as described (23). Previously described procedures were used for the preparation of ^{32}P-labeled REV RNA (12), RSV RNA (42), and rRNA (41) which was extracted from M-MLV-infected NIH-3T3 cells.

Electrophoresis of 30–40*S* RNA in 2.1% polyacrylamide gels was as described (15).

RNA fingerprinting and oligonucleotide analysis. 60–70*S* viral RNA, obtained by sucrose gradient sedimentation (15), was precipitated with ethanol twice before RNase digestion. It was then suspended in 0.1 ml of 5 mM Tris-hydrochloride (pH 7.4)–0.5 mM EDTA and heated at 100 C for 45 s. RNA (approximately 20 μg) was digested with 5 U of RNase T_1 (Calbiochem) for 1 h at 37 C.

Digestion with RNase A was in 0.01 ml of buffer containing 0.01 M Tris-hydrochloride, pH 7.4, 1 mM EDTA, 0.3 M ammonium acetate, and 2 mg of RNase A (Worthington) per ml for 2 h at 37 C. After digestion with either RNase, the reaction mixture was lyophilized. The RNase A digest was suspended in water and relyophilized twice. The digested RNA was then suspended in 3 μl of water for application to the cellulose acetate strip (Schleicher and Schuell) used for electrophoresis. Electrophoresis at pH 3.5 in a pyridine-acetic acid buffer was as described by Barrell (4).

A quantitative procedure for transfer from the cellulose acetate strip to the DEAE-cellulose thin-layer plate (Analtech) used for homochromatography was developed. This involved spraying water near the bottom of the plate. The cellulose acetate strip was laid over the wet region and taped it to the back of the plate. Homochromatography was as described (4) except that a 3% dilution of homomixture b was used. The homomixture was incubated at 60 C for 24 h prior to use for better resolution. For autoradiography the plate with the attached cellulose acetate strip was exposed to X-ray film (Kodak NS 54T). Exposure times varied depending on the amount of radioactivity used; 10^6 counts/min of 60–70*S* RNA were exposed for 2 days.

Oligonucleotides were eluted for partial sequencing by the method of Brownlee and Sanger (9). RNase T_1-resistant oligonucleotides were digested with 10 μl of a solution of 0.2 mg of RNase A per ml, 0.01 M Tris-hydrochloride (pH 7.4), and 1 mM EDTA for 2 h at 37 C. Oligonucleotides from RNA originally digested with RNase A were subsequently digested with 5 U of RNase T_1 in 10 μl of 5 mM Tris-hydrochloride (pH 7.4)–0.5 mM EDTA for 4 h at 37 C. Digested oligonucleotides were fractionated by electrophoresis on DEAE-cellulose paper in pyridine-acetic acid (pH 3.5) as described by Barrell (4). Large fragments were eluted, and their base compositions were determined as described (4).

Radioactivity in oligonucleotides was measured by locating spots on a thin-layer plate and scraping them, with 50% glycerol, directly into scintillation vials. They were solubilized by 10 ml of 10% NCS (Nuclear Chicago) in a toluene-based scintillation fluid for 2 h at 60 C and counted.

The total radioactivity remaining on the plate was determined by placing it in 400 ml of 0.4 N KOH in a sealed lucite box for 2 days at room temperature. An aliquot of the solubilized RNA was then counted. By this method 100% of the radioactivity applied to the fingerprint could be recovered.

RESULTS

Complexities of M-MLV and M-MSV RNA. Fingerprint patterns of complete RNase T_1 digests of 60–70*S* RNA from M-MLV and M-MSV are shown in Fig. 1A and B, respectively. The MLV fingerprint looks decidedly more complex than that of MSV since it has many more large, slowly chromatographing oligonucleotides (found in the lower half of the fingerprint).

By eluting large oligonucleotides from a fingerprint, measuring the proportion of the total ^{32}P radioactivity which they contain, and determining their chain lengths by partial sequence analysis, one is able to estimate the complexity of the entire genome, since the specific activity of a unique oligonucleotide

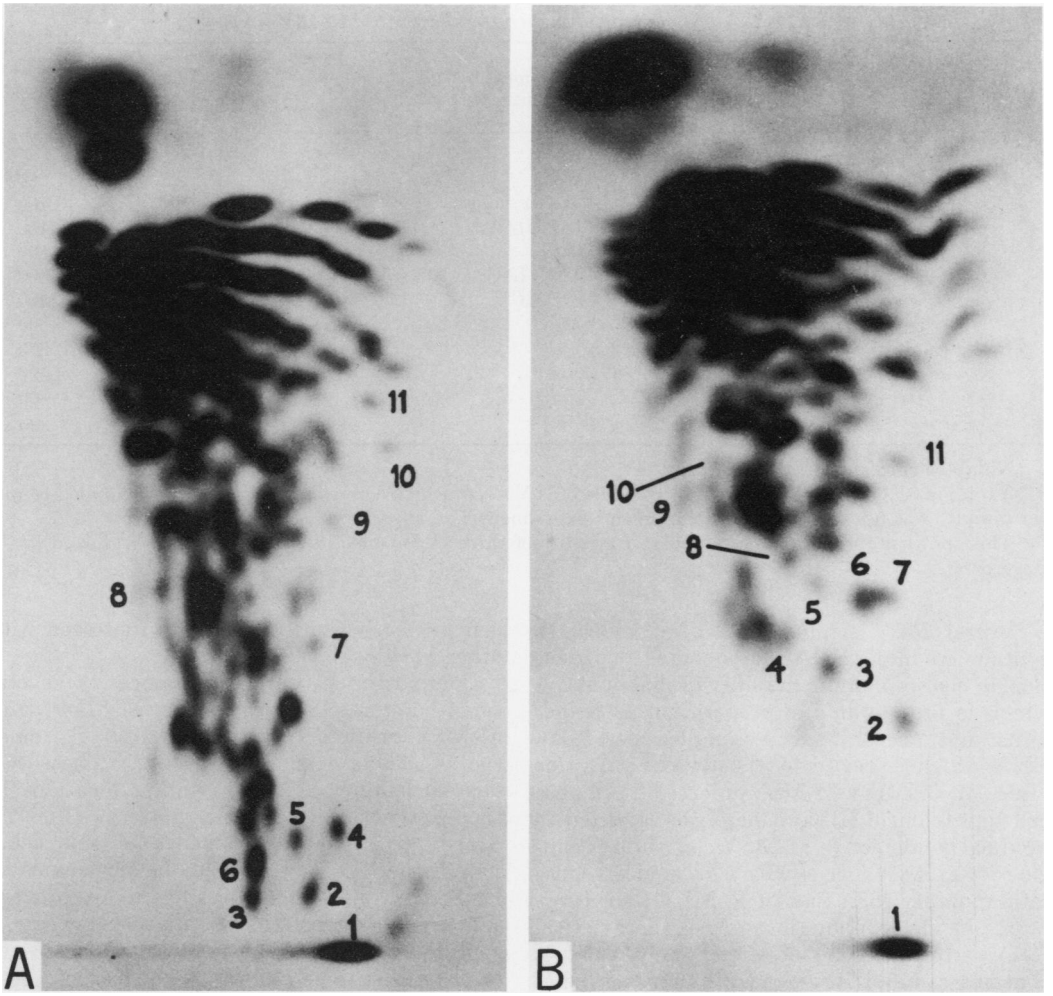

FIG. 1. *Fingerprints of RNase T_1-digested 60-70S RNA from M-MLV (A) and M-MSV clone 124 (B). Electrophoresis was from left to right, and homochromatography was from bottom to top.*

sequence is equal to that of the entire unique sequence genome. This can be represented as:

$$\frac{\text{counts/min}_{\text{oligonucleotide}}}{\text{size}_{\text{oligonucleotide}}} = \frac{\text{counts/min}_{\text{genome}}}{\text{size}_{\text{genome}}}$$

where size is in terms of the number of nucleotides in unique sequences.

The results of such an analysis performed on several oligonucleotides from MLV RNA are shown in Table 1. The complexity estimated as an average of that determined from 10 different oligonucleotides was 3.54×10^6 daltons with a standard deviation of 0.42×10^6. The conversion from nucleotides to daltons is based on an average nucleotide molecular weight of 322 calculated from the base composition determined for 60–70*S* M-MLV RNA (27% C, 24% A, 28% G, and 21% U) and the molecular weights of the bound nucleotides (C = 305, A = 329, G = 345, and U = 306). MLV RNA subunits are similar in size to those of nondefective avian sarcoma viruses (31). The size of the latter RNA has been estimated at 2.4×10^6 to 3.4×10^6 by formamide gel electrophoresis (16) and at 3.0×10^6 to 3.3×10^6 by electron microscopy (13, 28, 33). Therefore, this complexity estimate is similar to, although slightly higher than, the size estimates of 30–40*S* MLV RNA obtained by physical methods (7, 31). The same fingerprint pattern and complexity results were obtained with both the original cloned M-MLV of Fan and Paskind (Fig. 1A, Table 1) and with M-MLV which had been recloned by Smotkin (data not shown).

TABLE 1. *Nucleotide sequence complexity of M-MLV RNA*

Oligonucleotide spot no.[a]	Counts/min (% of total)[b]	Composition (RNase A digestion products)	No. of nucleotides	Genomic complexity (nucleotides)
1	1.47	Poly(A)		
2	0.200	$U_7C_9G(AC)(AAU)$	22	11,000
3	0.183	$U_8C_{10}G(AC)_2(AAC)$	24	13,100
4	0.168	$U_8C_4G(AAC)(AAU)(A_4N)$	22	13,100
5	0.172	$U_8C_{10}G(AAU)$	20	11,600
6	0.557	$U_{10}C_{17}G(AC)_4(AU)(AG)(AAC)_2(A_3G)(A_3N)_2$[c]	58	10,400
7	0.167	$U_8C_3(AU)_2(AG)(A_3C)$	19	11,400
8	0.126	$C_8G(AC)_3$	12	9,500
9	0.104	$U_4C(AU)(A_3G)$	11	10,600
10	0.105	$U_6(AU)(AG)$	10	9,500
11	0.102	$U_8C_2G(AU)$	10	9,800
			Avg = 11,000 ± 1,300	

[a] Numbers correspond to those in Fig. 1A.

[b] A total of 6.11×10^6 counts/min of ^{32}P-labeled MLV RNA, which corresponds to 100% of the applied counts per minute, was used for quantification in this experiment.

[c] This spot has three G residues and is a mixture of three oligonucleotides which were not resolved in the fingerprint.

Several MSV strains have been shown to contain two major RNA components—one identical in electrophoretic mobility to that of MLV, which is present in the preparation as helper virus, and a second species smaller than MLV RNA, which is specific for the defective sarcoma virus (31, 32). The M-MSV-infected TB mouse cell line (clone 124) used here was reported to produce nondefective M-MSV containing a single 30–40*S* RNA species with a lower electrophoretic mobility than that of M-MLV (29). However, our electrophoretic analysis of this viral RNA, after heat dissociation, shows that it contains a major RNA subunit that is smaller (faster migrating) than MLV RNA (Fig. 2). In addition, our MSV preparations consistently had a small RNA peak which co-electrophoresed with MLV RNA subunits. This minor MLV-like RNA species represented 7% of the total radioactivity in the experiment shown in Fig. 2. Since the ratio of MSV RNA to the presumed MLV RNA was so large, a direct oligonucleotide analysis was possible without prior separation from MLV RNA-like components.

The nucleotide sequence analysis of this M-MSV RNA, obtained from clone 124 of Ball (Table 2), gave a complexity of $2.30 \times 10^6 \pm 0.27 \times 10^6$, after correction for radioactivity in the MLV-sized RNA peak. This complexity is significantly smaller than that obtained from M-MLV RNA. This is consistent with the observation that the size of this MSV RNA, estimated by gel electrophoresis, was smaller than that of MLV (Fig. 2). These findings may indicate the presence of a defective rather than a nondefective virus similar to that seen with other MSVs (31, 32).

Comparison of partial sequences of several large oligonucleotides from M-MLV and M-MSV resulted in one compositional isomer (no. 4, Table 1; no. 2, Table 2). These two oligonucleotides also had similar locations in their respective fingerprint patterns (Fig. 1). Since less than one sequence of this chain length per genome would be expected by chance, this finding may suggest some sequence similarities between the two viruses. However, a complete sequence analysis would be necessary to determine whether the sequences of these isomers are identical in the RNA of both viruses.

Complexities of REV and visna virus RNA. The genomic complexity of REV was determined to be $3.88 \times 10^6 \pm 0.58 \times 10^6$ (Fig. 3A, Table 3). This is not significantly greater than the complexity of 3.4×10^6 determined for RSV by the same method (5). Therefore, it seems unlikely that REV has a basically different genome structure than RSV, despite differences observed in transfection experiments using integrated proviral DNA (10).

Visna virus was found to have a complexity of $3.49 \times 10^6 \pm 0.19 \times 10^6$ (Fig. 3B, Table 4) similar to that of the RNA tumor viruses to which it is related. Since the PFU/particle ratio for visna was determined to be less than 10 by the method of Miller et al. (34) (R. Buscho, P. Swoveland, and A. T. Haase, unpublished data), there is a good probability that the biologically active genome was significantly represented in the RNA studied here.

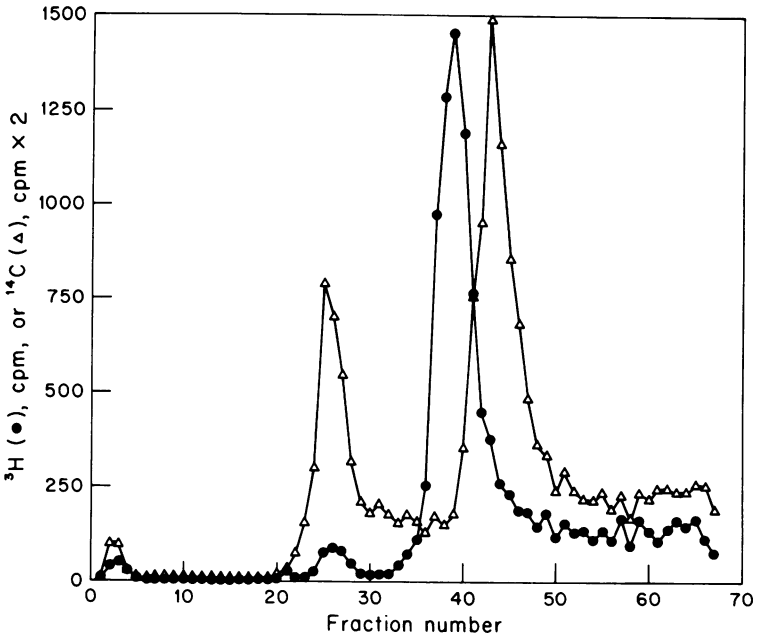

FIG. 2. *Polyacrylamide gel electrophoresis of ^{3}H-labeled M-MSV 124 RNA and ^{14}C-labeled M-MLV (MSV) clone 3 RNA. Both RNAs were heated at 100 C for 60 s prior to electrophoresis in a 2.1% polyacrylamide gel containing sodium dodecyl sulfate. Radioactivity in 1-mm slices was determined. Migration was from left to right.*

TABLE 2. *Complexity of M-MSV RNA*

Oligonucleotide spot no.[a]	Counts/min (% of total)[b]	Composition (RNase A digestion products)	No. of nucleotides	Genomic complexity (nucleotides)[c]
1	1.55	Poly(A)		
2	0.314	$U_6C_4G(AAC)(AAU)(A_4N)$	22	7,000
3	0.290	$U_5C_{10}(AU)(AG)(AAU)$	22	7,590
4	0.288	$U_2C_4(AC)_2(AU)(AG)(AAC)(AAU)$	20	6,940
5	0.197	$UCG(AC)(AU)(AAC)(A_4N)$	15	7,610
6	0.536	$U_{10}C_6G(AC)_3(AAC)(A_3U)(A_2G)$	34	6,340
7	0.268	$U_7C_7(AU)(AG)(AAU)$	21	7,840
8	0.233	$U_2C_7(AC)(AU)_2(AG)$	17	7,300
9	0.146	$UC_6G(AC)_3$	14	9,590
10	0.173	$UC_2G(AC)(AAC)(A_4N)$	14	8,090
11	0.180	$U_5C_3G(AC)_2(AU)$	15	8,330
			Avg =	7,660 ± 900

[a] Numbers correspond to those in Fig. 1B.

[b] A total of 1.28×10^6 counts/min of ^{32}P-labeled 60-70S clone 124 M-MSV RNA was used in this experiment.

[c] To correct for the fraction of the total RNA which migrated with MLV RNA (Fig. 2), this complexity was reduced by 7%, yielding 7,120 ± 840 nucleotides in the MSV genome.

Genomic complexity of RSV based on RNase A-digested RNA. Previous analyses of RSV RNA complexity were based on RNase T_1-resistant sequences which represented about 5% of the RNA (5). To extend these studies to additional sequences, RNase A-resistant fragments were selected. The RNA was digested completely with RNase A (pancreatic), and the resulting digest was fingerprinted as described above. The result is a fingerprint pattern (Fig. 4) with fewer large oligonucleotides than are found after RNase T_1 digestion (5). In addition, many of the spots analyzed were found to be a mixture of two or more unresolved spots since they contained more than one pyrimidine (Table 5). The complexity estimated on the basis of these oligonucleotides, which represented 1.8% of the total RNA, was 3.65×10^6 ±

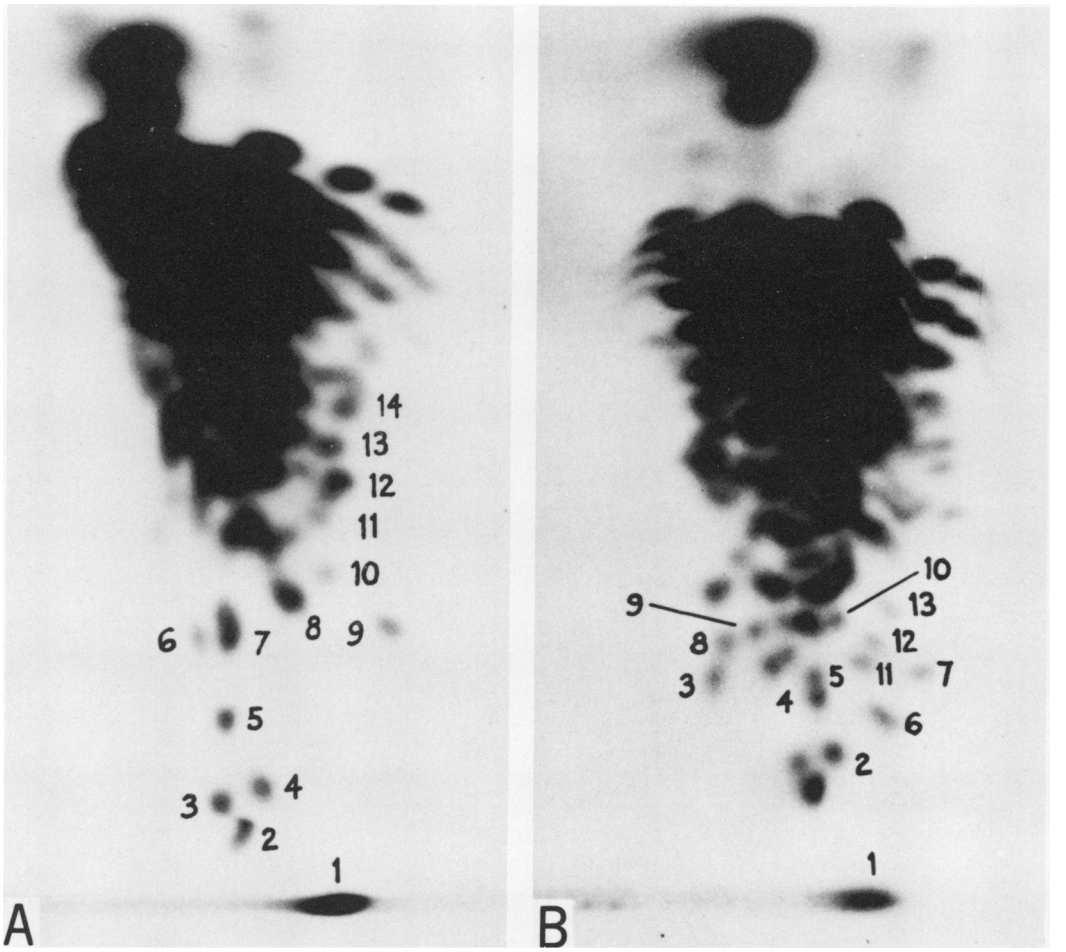

FIG. 3. *Fingerprints of RNase T_1-digested 60-70S RNA from REV (A) and Visna virus (B).*

TABLE 3. *Nucleotide sequence complexity of REV RNA*

Oligonucleotide spot no.[a]	Counts/min (% of total)[b]	Composition (RNase A digestion products)	No. of nucleotides	Genomic complexity (nucleotides)
1	1.39	Poly(A)		
2	0.222	$U_5C_{12}G(AC)_3(AU)_2(AAC)$	31	14,000
3	0.202	$U_3C_{11}(AU)_2(AG)(A_4N)_2$	30	14,800
4	0.209	$U_4C_5G(AC)(AAC)(AAU)(A_3C)(A_4N)$	27	12,900
5	0.194	$U_4C_{11}(AC)_2(AU)(AG)(AAC)$	26	13,400
6	0.150	$UC_6G(AU)(AAC)_2(A_3C)$	20	13,300
7	0.440	$U_8C_{25}G_3(AC)_3(AU)_4(AAC)$	53	12,000
8	0.466	$U_{14}C_{13}G_3(AC)_3(AU)_2(AAC)(AAU)(A_3U)(A_4N)$	55	11,800
9	0.212	$U_8C_5(AU)_2(AG)$	19	8,960
10	0.142	$U_6C_2G(AC)(AU)(A_4N)$	18	12,700
11	0.194	$U_5C_4G(AC)(AU)_3$	18	9,250
12	0.305	$U_{10}C_7G_2(AC)(AU)_3(AAC)(AAU)$	33	10,800
13	0.293	$U_8C_6G_2(AC)(AU)_3(AAU)_2$	30	10,200
14	0.297	$U_8C_3G_2(AC)_3(AU)_6(AAU)$	34	11,400
			Avg = 12,000 ± 1,800	

[a] Numbers correspond to those in Fig. 3A.
[b] A total of 1.38×10^6 counts/min of ^{32}P-labeled 60-70S REV RNA was used in this experiment.

TABLE 4. *Nucleotide sequence complexity of visna RNA*

Oligonucleotide spot no.[a]	Counts/min (% of total)[b]	Composition (RNase A digestion products)	No. of nucleotides	Genomic complexity (nucleotides)
1	1.74	Poly(A)		
2	0.199	$U_2C_4G(AU)(AAU)(A_3C)(A_4C)$	21	10,500
3	0.141	$C_4(AC)_2(AG)(A_4U)$	15	10,600
4	0.172	$U_2C_3G(AC)_2(AAU)(A_7U)$	21	12,200
5	0.171	$U_2C_2G(AC)(AU)(A_3C)(A_5C)$	19	11,100
6	0.176	$U_3C_2G(AC)_2(AU)_3(AAU)$	19	10,800
7	0.135	$U_3(AU)_3(AAU)(AAG)$	15	11,100
8	0.127	$C(AC)(A_4C)(A_4G)$	13	10,200
9	0.137	$UC_3(AC)(AAC)(AAU)(AAG)$	15	10,900
10	0.176	$U_3CG(AC)(AU)_2(A_6C)$	18	10,200
11	0.170	$U_5C_3G(AC)(AAU)(A_4U)$	19	11,200
12	0.134	$U_3C_2(AU)_3(AAG)$	14	10,400
13	0.149	$U_4C_2G(AC)(AU)_2(AAU)$	16	10,700
			Avg =	10,800 ± 600

[a] Numbers correspond to those in Fig. 3B.
[b] A total of 1.22×10^6 counts/min of ^{32}P-labeled 60-70*S* visna RNA was used for quantification.

0.45×10^6. This is in good agreement with the complexity of 3.4×10^6 determined previously for RSV on the basis of RNase T_1-resistant oligonucleotides (5).

If guanine residues were distributed randomly throughout an RNA species, the number of unique RNase T_1-resistant oligonucleotides of a particular size would be directly proportional to the unique sequence complexity of the RNA. Thus, the frequency of occurrence of an oligonucleotide of chain length (n) after RNase T_1 digestion would equal $(G)^2(C + A + U)^{n-1} \times$ (nucleotides/genome), where G, C, A, and U represent the fractional amount of each nucleotide in the total RNA base composition. Using this formula, one can calculate an expected frequency of occurrence of 13 oligonucleotides having a chain length greater than 16 nucleotides if the genome contains 10,000 nucleotides and has a base composition similar to RSV (5). Similarly, after RNase A digestion of a genome this size, one would expect, given a random distribution of pyrimidines, eight different oligonucleotides with a chain length greater than 10 [frequency = $(C + U)^2(A + G)^{n-1} \times$ (nucleotides/genome)](20, 38). The numbers of large RNase-resistant oligonucleotides found for the viruses discussed above (Fig. 1, 3, and 4) are quite compatible with their expected random frequencies of occurrence in a polyploid genome (K. Beemon, Ph.D. thesis, University of California, Berkeley, 1974).

Complexity of 28S rRNA. As a control, mouse 28*S* rRNA was also analyzed (Fig. 5, Table 6). Its fingerprint pattern was considerably simpler than those of the viruses analyzed. The complexity determined for 28*S* rRNA was $2.06 \times 10^6 \pm 0.23 \times 10^6$. Whereas this estimate is 20% higher than the size of 28*S* rRNA determined by gel electrophoresis (30), this discrepancy may be partially due to contaminants in the rRNA preparation. Such contamination with other RNA species would increase the total amount of radioactivity in the preparation, without increasing the radioactivity in 28*S* rRNA oligonucleotides, resulting in a high complexity estimate. Similarly, viral 60–70*S* RNA may be contaminated with nongenomic RNA species in low amounts, including low-molecular-weight RNA species present as 2 to 3% of 60–70*S* RNA (18).

Whereas 100% of the total radioactivity applied to a fingerprint could be recovered by the method described above (Beemon, PhD. thesis), there could be losses in radioactivity in a large oligonucleotide due either to its degradation or to incomplete digestion by RNase T_1. In addition, the chain length of an oligonucleotide would be overestimated if it were contaminated by sequences from neighboring spots. To check this, oligonucleotide chain length was plotted against homochromatography migration distance. Since a linear relationship was found (Beemon, Ph.D. thesis), the oligonucleotide sizes seem fairly accurate.

However, the complexity estimates obtained chemically should probably be considered maximal estimates since any of the possible errors discussed above would increase the complexity. A comparison of molecular weights for viral RNA subunits obtained by either gel electrophoresis or by electron microscopy with RNA complexities obtained by sequence analysis is shown in Table 7. In all cases, the chemical

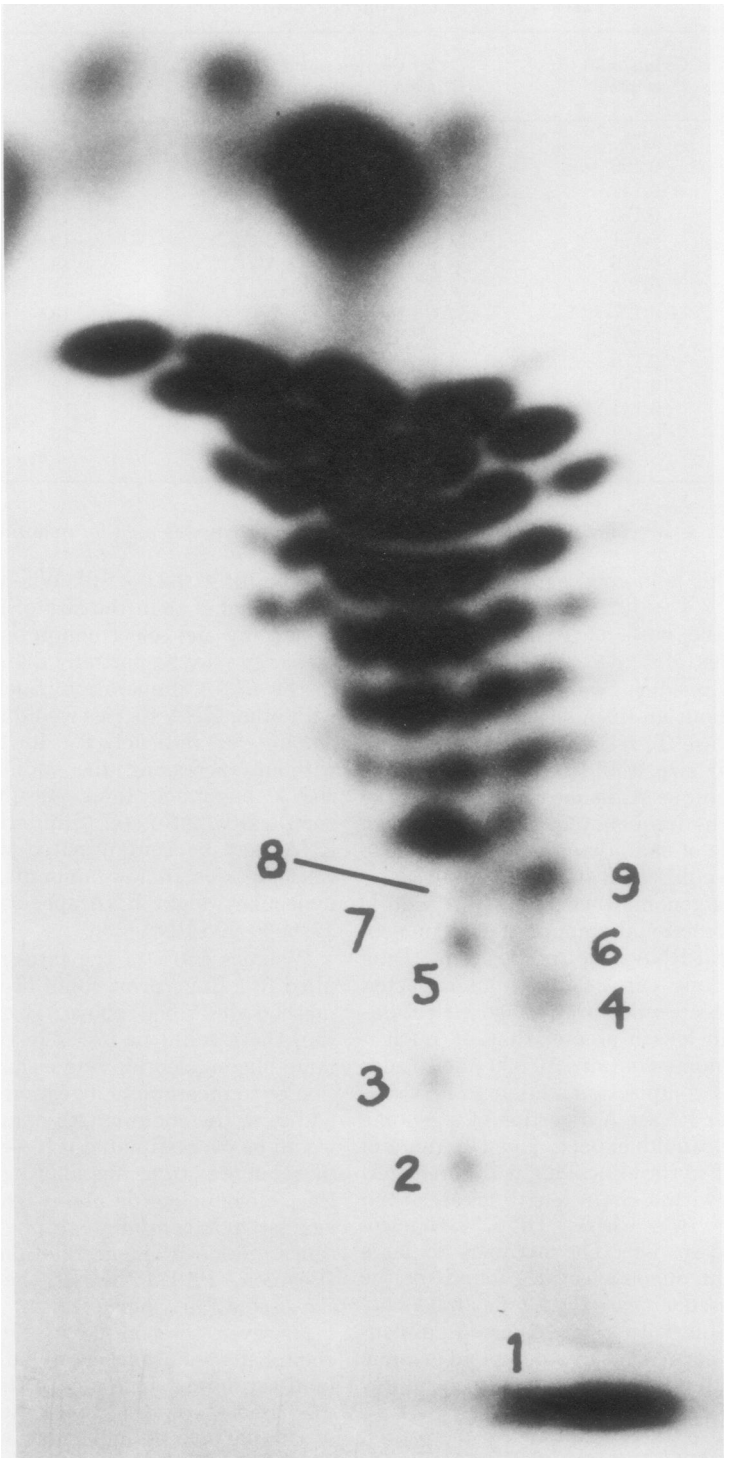

FIG. 4. *Fingerprint of RNase A-digested RNA from the PR-B strain of RSV. The smear of radioactive RNA directly above poly(A) (spot 1) is probably due to partial digestion of poly(A).*

TABLE 5. *Complexity of RSV RNA*

Oligonucleotide spot no.[a]	Counts/min (% of total)[b]	Composition (RNase T_1 digestion products)	No. of nucleotides	Genomic complexity (nucleotides)
1	1.91	Poly(A)		
2	0.148	$CG_3(AAG)(A_3G)(A_4G)$	16	11,000
3	0.130	$G_2(AG)(AAC)(AAG)(A_3G)$	14	10,800
4	0.362	$UCG_{12}(AU)_2(AG)_5(AAG)_2(A_3G)(A_4G)$	43	12,000
5	0.241	$G_5(AAU)(AAG)_3(A_3U)(A_4G)$	26	11,000
6	0.190	$C_2G_9(AG)_3(AAG)_3$	26	13,900
7	0.130	$G_2(AG)_2(AAG)(A_3C)$	13	10,100
8	0.239	$CG_6(AG)_4(AAG)_2(A_3U)_2$	29	12,300
9	0.357	$UCG_6(AU)_2(AG)_6(AAG)$	33	9,320
			Avg = 11,300 ± 1,400	

[a] Numbers correspond to those in Fig. 4.
[b] A total of 6.9×10^6 counts/min of RNA was used for quantification.

complexity is the same or slightly larger than the physical size of a single viral RNA subunit. On this basis, it is concluded that RNA tumor viruses and their close relatives have a unique genome size similar to the size of a single RNA subunit and that their genome is largely polyploid.

DISCUSSION

Although the similarity found between the unique sequence complexity of ribodeoxyviral genomes and the size of a single RNA subunit suggests that each subunit contains a complete genome and that the subunits are identical, these experiments do not indicate how sequences are arranged on each subunit or distributed between subunits. Recent experiments, in which avian tumor virus RNA was partially fragmented and 3'-poly(A)-tagged RNAs of various lengths were selected and fingerprinted, showed that the large RNase T_1-resistant oligonucleotides are ordered identically on each subunit of a virus (41, 42). An approximately linear increase in the number of large oligonucleotides appearing with increasing size of poly(A)-tagged RNA was observed. Therefore, it appears that the RNA sequences are ordered identically on each 30–40*S* subunit and that ribodeoxyviruses are indeed polyploid.

Bolognesi et al. (8) proposed that the RNA tumor virus genome may be partially haploid. They observed that RNase II, a progressive 3' → 5' exonuclease, could digest 50% of the 30–40*S* RNA while leaving the majority of the large RNase T_1-resistant oligonucleotides intact. On this basis, they suggested that the 5' half of the 30–40*S* RNA may be polyploid, as shown by oligonucleotide analysis (5, 6, 36), whereas the 3' half may be partially haploid. However, Bolognesi et al. have failed to show that their RNase II was not contaminated by any endonuclease. Furthermore, the finding of Wang et al. (42) and J. M. Coffin and M. A. Billeter (J. Mol. Biol., in press) that RSV RNase T_1-resistant oligonucleotides are distributed approximately randomly along the 30–40*S* RNA is incompatible with the proposal that these oligonucleotides might all be clustered at the 5' end of the molecule. The proposal of Ihle et al. concerning possible different sequences between subunits of MLV RNA (25) is also incompatible with our evidence for a polyploid genome in which the subunits contain identical sequences.

However, no more than 7% of the total 60–70*S* RNA sequences of each virus has been analyzed. It is possible, therefore, that there are partially haploid regions of the genome linked to the polyploid regions. However, if guanosines were randomly distributed throughout the haploid regions, one would expect to see two to three times as many large RNase T_1-resistant sequences from these regions as from polyploid regions of the same length. None of the viruses studied have shown clear cases of haploid oligonucleotides having one-half to one-third the radioactivity of the major oligonucleotides. If there are haploid regions in the genome, they must either be relatively short or rich in guanosine residues so that no large RNase T_1-resistant oligonucleotides exist.

Genomic complexities around 9×10^6 have been obtained by hybridizing viral RNA with complementary DNA and comparing the rate of hybridization with that of RNA and complementary DNA of known complexity (19, 23, 40). These results may be explained by the observation that such hybridization kinetics can vary with different nucleic acid secondary structures, base compositions, and optimal hybridization conditions (39).

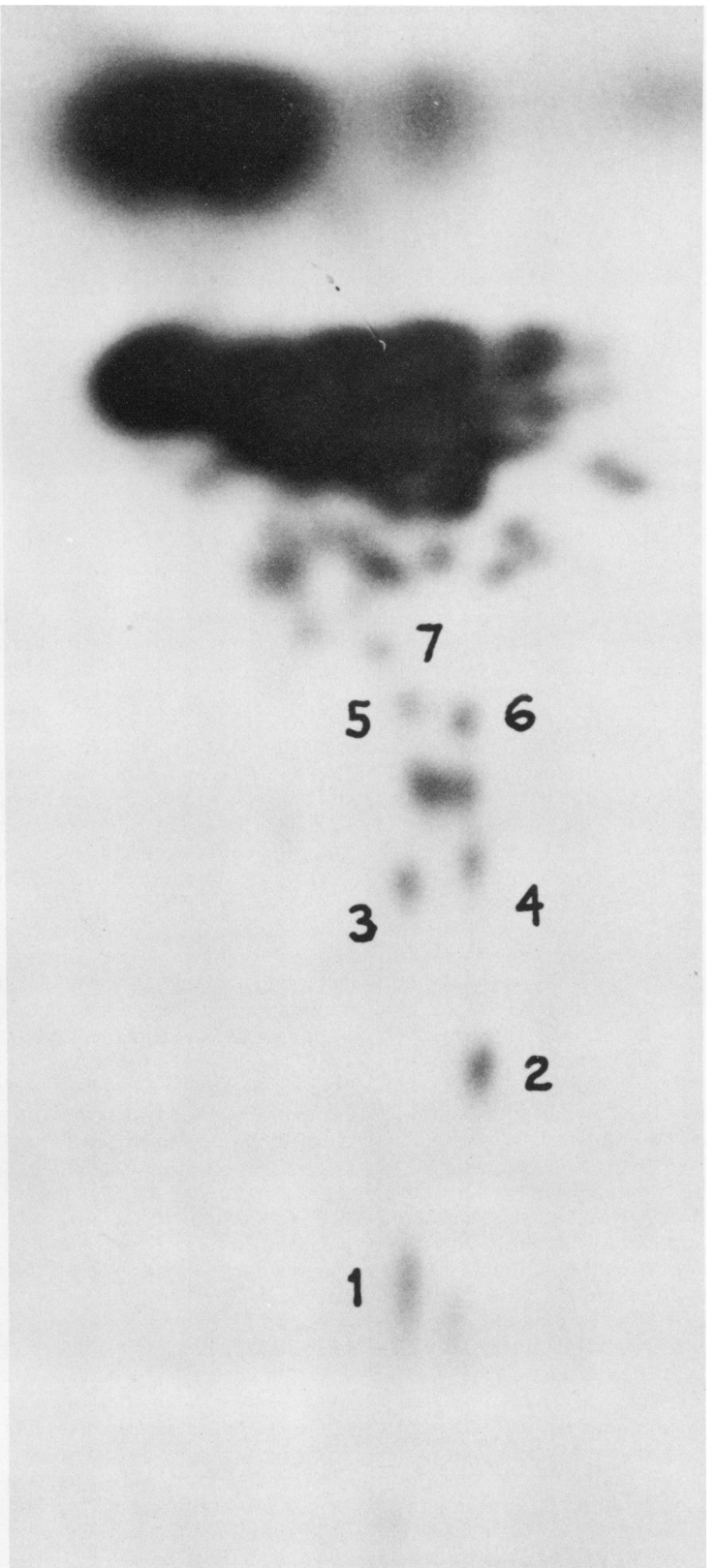

FIG. 5. *Fingerprint of RNase T1-digested 28S ribosomal RNA from mouse NIH-3T3 cells.*

TABLE 6. *Complexity of 28S mouse ribosomal RNA*

Oligonucleotide spot no.[a]	Counts/min (% of total)[b]	Composition (RNase A digestion products)	No. of nucleotides	Genomic complexity (nucleotides)
1	0.388	$U_7C_{14}G(AC)$	24	6,180
2	0.499	$U_6C_6G(AU)_2(AAC)(A_3N)_2$	28	5,600
3	0.381	$U_3C_5G(AC)_2(AU)(AAC)(A_4N)$	23	6,040
4	0.310	$U_3C_5(AC)_2(AU)(AG)(AAC)(AAU)$	22	7,100
5	0.273	$U_5C_5(AU)(AAG)(A_3C)$	19	6,950
6	0.247	$U_4C_5(AU)_2(AG)(AAU)$	18	7,290
7	0.270	$U_3C_3G(AC)(AAC)_2$	15	5,550
			Avg =	6,390 ± 720

[a] Numbers correspond to those in Fig. 5A.
[b] A total of 8.56×10^5 counts/min was used.

TABLE 7. *Comparison of nucleotide sequence complexities and RNA sizes*

RNA source	Nucleotide sequence complexity ($\times 10^{-6}$ daltons)	Size ($\times 10^{-6}$ daltons)[a]	Method of sizing
RSV	3.4 (5)	2.4–3.4	Formamide gel (16)
	3.6	3.0	Electron microscopy (13)
		3.3	Electron microscopy (28)
M-MLV	3.5	2.5	Formamide gel (31)
		3.0	Polyacrylamide gel (7)
M-MSV	2.3	2.0	Formamide gel (J. E. Maisel, unpublished data)
REV	3.9	Similar to RSV	Polyacrylamide gel (24)
Visna	3.5	2.8	Polyacrylamide gel (23)
28S rRNA	2.1	1.7	Polyacrylamide gel (30)

[a] Viral RNA size refers to the molecular weight of 30–40S RNA subunits.

The genomic complexity of a reportedly nondefective isolate of M-MSV (1) was found to be two-thirds that of M-MLV RNA. If both viruses were polyploid, the RNA subunits of MSV should also be smaller than those of MLV, as we observed by gel electrophoresis. This result is in contrast to that of Lo and Ball (29), who reported M-MSV RNA subunits with a lower electrophoretic mobility than M-MLV RNA.

The finding that REV has a polyploid genome leaves unexplained the observation that DNA at least twice the size of the genome from REV-infected cells was required for infection (10). REV proviral DNA may need to be linked to cellular DNA sequences for successful integration as suggested (10). However, it has recently been found that unintegrated REV DNA with a size of approximately 6×10^6 daltons (double stranded) is infectious with single-hit kinetics (E. Fritsch and H. M. Temin, personal communication), consistent with a genome size of 3×10^6.

Recent experiments with visna virus DNA also indicate that the minimum DNA size necessary for infectivity corresponds to the size of a transcript of a single RNA subunit; however, the kinetics were two hit (A. T. Haase, B. Traynor, P. Ventura, and D. Alling, Virology, in press). A possible reconciliation of this finding with the present complexity results is that the visna genome, although the size of a single subunit, might be distributed between two subunits.

A 3×10^6-dalton genome has a coding capacity for only about 3×10^5 daltons of polypeptides, if there are no overlapping genes. The known RNA tumor virus-coded proteins can be accommodated in such a genome if only one polymerase subunit is virus coded and the other, where it exists, is a cleavage product of the viral-coded polymerase subunit (21, 35, 37). Then the viral-coded polypeptides and their approximate molecular weights would be: the RNA-dependent DNA polymerase, 90,000; the major envelope glycoprotein, 70,000; the precursor to the internal proteins, 76,000; and the sarcoma-specific gene product, 40,000 to 50,000; for a total of 276,000 to 286,000 (2).

However, the molecular weights of all proteins associated with purified visna virus add up to 8.8×10^5 (22). Since reconstruction experi-

ments do not suggest the cell-coded proteins are associated with the virions to a detectable degree (22), visna proteins may be generated by overlapping transcription of viral mRNA's or by proteolytic cleavages at different sites in a common polypeptide precursor.

The significance of a polyploid genome is not known. However, it may be required for recombination which has been postulated to involve a heterozygotic intermediate in which different 30–40*S* RNA subunits are linked in the same 60–70*S* complex (43, 45). Alternatively, two identical subunits may be required for transcription of those RNA sequences which are base paired to the viral RNA primer into a complete proviral DNA (11).

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