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Size of Murine RNA Tumor Virus-Specific Nuclear RNA Molecules

WILLIAM A. HASELTINE[1] AND DAVID BALTIMORE*

Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

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About 1% of the total RNA of cell lines producing murine leukemia virus is virus-specific RNA. About one-third of the virus-specific RNA is located within the nucleus. The size distribution of virus-specific RNA was determined before and after denaturation. Before denaturation, virus-specific RNA sequences sedimented as a heterogeneous population of RNA molecules, some of which sedimented very rapidly. After denaturation, most of the virus-specific RNA had a sedimentation coefficient of 35S or lower, but a small fraction of the nuclear virus-specific RNA sedimented more rapidly than 35S RNA even after denaturation.

The life cycle of RNA tumor viruses involves synthesis of a DNA copy of the RNA genome followed by integration of the viral DNA into the cellular DNA (19). It appears that viral RNA, both messenger and virion, is transcribed from the integrated genome. Both the messenger and virion RNA in productively infected cells are mainly the same strand of RNA that is about 2.6×10^6 daltons, although there may be a fraction of lower-molecular-weight RNA (2, 6, 8, 10, 13, 14, 17, 20, 21).

Because viral RNA is made in the nucleus but functions in the cytoplasm, it is important to know what types of molecules are present in the two fractions. We report here a characterization of the amounts and sizes of nuclear virus-specific RNA. In previous work, the cytoplasmic species were studied and shown to consist mainly of 35S RNA, with lesser amounts of lower molecular weight (8, 10).

MATERIALS AND METHODS

Cell lines. The NRK murine leukemia virus (MuLV)-producing cell line was derived by E. Rothenberg by cloning NRK cells infected at low multiplicity with a cloned stock of Moloney MuLV.

Extraction procedures. Nuclear and cytoplasmic RNA extracts were prepared by harvesting cells grown to half confluence in petri dishes in cold phosphate-buffered saline solution. The cells were washed twice with cold phosphate-buffered saline and resuspended in 5 mM NaCl, 0.5 mM $MgCl_2$, and 5 mM Tris, pH 7.5. After 4 min, the cells were homogenized in a Dounce homogenizer and the nuclei were collected by centrifugation. The nuclei were suspended in 0.1 M NaCl, 0.01 M Tris, pH 7.6, and 0.0015 M $MgCl_2$ containing 1.0% Nonidet P-40 and 0.4% deoxycholate and immediately harvested by centrifugation. ^{32}P-labeled poliovirus RNA was added to the nuclear pellet. The supernatant was added to the first cytoplasmic fraction, and the total cytoplasm was brought to a final concentration of 1% sodium dodecyl sulfate (SDS) and 2 mM EDTA. The nuclei were suspended in 0.5 M NaCl, 0.05 M $MgCl_2$, and 0.1 M Tris, pH 7.4, and treated with 1.0 μg of RNase-free DNase (Worthington Biochemicals Corp.) per ml for 1 min at 37°C. The nuclei retained their intact appearance after this procedure and were contaminated by less than 1% of the cytoplasmic fraction, as judged by the amount of rRNA in the nuclear fraction. Both the nuclear and cytoplasmic fractions were extracted twice at 54°C with an equal mixture of phenol and chloroform-1% isoamyl alcohol and twice more with the chloroform-isoamyl alcohol mixture alone. The RNA extracts were precipitated repeatedly with ethanol.

For determination of the concentration of virus-specific sequences, the cytoplasmic RNA was resuspended in 2× SSC (0.3 M NaCl, 0.03 M sodium citrate), 0.1% SDS, and 2 mM EDTA (hybridization buffer) at a concentration of 1 mg/ml. The nuclear fraction was resuspended in the same volume of buffer as was the cytoplasmic extract from the same batch of cells. Hybridization reactions were 10 μl and contained 2,000 cpm per reaction of [^{3}H]DNA probe synthesized and purified as described by Fan and Baltimore (8).

Gradient analysis. For sucrose gradient analysis, undenatured RNA was resuspended at 0.1 mg/ml in a buffer containing 10 mM Tris-hydrochloride (pH 7.5), 0.1 M NaCl, 1 mM EDTA, and 0.5% SDS (SDS buffer). RNA was denatured by resuspending the pellet in 10 mM EDTA (pH 7.5) at a concentration of 1 mg/ml followed by addition of a 10-fold excess of dimethyl sulfoxide (Me_2SO), and the solution was heated to 60°C for 5 min. This solution was layered directly on 10 to 20% sucrose gradients containing

[1] Present address: Sidney Farber Cancer Center, Harvard Medical School, Boston, Mass. 02115.

80% Me_2SO, 10 mM Tris-hydrochloride (pH 7.5), 10 mM LiCl, and 1 mM EDTA. If the denatured RNA was sedimented on nondenaturing gradients, it was precipitated from the Me_2SO solution with 2 volumes of ethanol and resuspended in SDS buffer. The 15 to 30% nondenaturing gradients were made with SDS buffer. Nondenaturing gradients were spun at 35,000 rpm, 25°C, for 4 h in a Spinco SW41 rotor. Me_2SO gradients were run at 40,000 rpm for 15 h at 30°C in a Spinco SW41 rotor. The gradients were fractionated into 0.5-ml aliquots, and each fraction was precipitated by addition of 1 ml of ethanol. The ethanol precipitates were resuspended in SDS buffer, and 25,000 cpm of [^{3}H]DNA probe was added to aliquots of each fraction for hybridization. After incubation at 65°C for indicated times, the resistance of the [^{3}H]DNA probe to S_1 nuclease was measured as described by Fan and Baltimore (8).

RESULTS

Concentration of virus-specific RNA in the nucleus. We measured the concentration of virus-specific RNA in the nucleus to determine whether the concentration was sufficient to permit analysis of the size distribution of the RNA by hybridization to a labeled complementary DNA probe. For such measurements, cells were fractionated by a modification of the method of Penman (16). Care was taken during the fractionation procedure to ensure that cross-contamination of cellular extracts was low. The nuclear extracts were relatively free of cytoplasmic contaminants as indicated by the presence of less than 1% of the A_{260} of maturer RNA species in the nuclear extracts. The nuclei remained intact during the cell fractionation procedure as judged by their appearance under a phase-contrast microscope. More than 80% of the acid-precipitable RNA labeled in a 10-min [^{3}H]uridine pulse was found in the nuclear fraction (Table 1).

TABLE 1. *Recovery of nuclear RNA during cellular fractionation*[a]

Fraction	^{3}H cpm	% Recovery	^{32}P cpm	% Recovery
Total	450,000	100		
Crude nuclear pellet	360,000	82		
NP-40-DOC-washed nuclei	355,000	79	10,000	100
Crude cytoplasmic fraction	67,000	15		
NP-40-DOC supernatant	5,000	1		
Post-DNase nuclear fraction	334,000	74	8,900	89
Post-phenol nuclear fraction	338,000	75	8,600	86
Ethanol-precipitated nuclear fraction	328,000	73	8,500	85
Recovery from nondenaturing gradients	265,000	58	8,000	80
Recovery from denaturing gradients	315,000	70	8,300	83

[a] Ten microcuries of [^{3}H]uridine (specific activity, 30 Ci/mmol) in 25 ml of Dulbecco modified Eagle medium plus 10% calf serum was layered onto a subconfluent monolayer of NRK (Cl-1) cells (3×10^7 cells). After 10 min the medium was removed and the cells were fractionated as described in Materials and Methods. After the nuclear pellet was washed with Nonidet P-40 (NP-40) and deoxycholate (DOC), 10^4 cpm of ^{32}P-labeled purified poliovirus RNA was added. Portions were removed and precipitated with trichloroacetic acid to determine total trichloroacetic acid counts recovered after each purification step. The recovery of acid-precipitable material after centrifugation on sucrose gradients was determined by precipitating portions of the gradient fractions with trichloroacetic acid.

The nuclear and cytoplasmic extracts of several cell lines producing MuLV were tested for their content of virus-specific RNA by hybridizing their RNA to a labeled DNA probe synthesized by an endogenous reverse transcriptase reaction. Extracts of Moloney MuLV-producing NRK and JLS-V9 lines were tested with the probe synthesized from Moloney MuLV virions. Extracts of JLS-V9 iododeoxyuridine-induced and uninduced lines were tested with a probe synthesized by the induced virus. The hybridization rate of the 70S Moloney MuLV RNA and cytoplasmic RNA of the NRK line producing Moloney MuLV [NRK (Cl-1)] is shown in Fig. 1, in which the initial concentration of RNA multiplied by the time of hybridization (C_rt in mole-seconds/liter) is plotted versus the percentage of DNA in hybrid form. For nuclear RNA, the concentration of RNA used for the abscissa was actually that of the cytoplasmic fraction. Because nuclear and cytoplasmic fractions were suspended in the same volume and the recovery of RNA in the two fractions was greater than 80%, this method reveals directly the relative amounts of viral RNA in the cytoplasm and nucleus of the cell. By comparing the C_rt for 50% hybridization of the various samples to that of the purified 70S viral RNA, it was possible to calculate the dilution of virus-specific RNA by cellular RNA in the various fractions (8). About 1% of the total RNA of productively infected cells was virus specific. There was about twice as much virus-specific RNA in the cytoplasmic fractions as there was in the nuclear RNA. This amounts to about 12,000 copies of 35S RNA equivalents in the cytoplasm and about 6,000 copies in the nucleus. A similar distribution of virus-specific RNA was found in the JLS-V11 line and in JLS-V9 cells induced

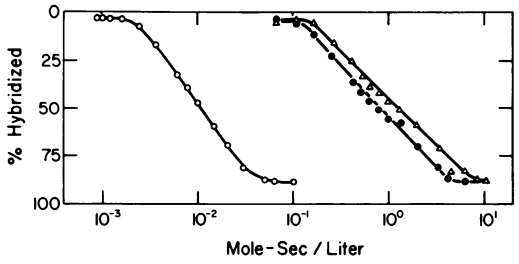

FIG. 1. *Concentration of cytoplasmic and nuclear virus-specific RNA in cells on an NRK line producing Moloney leukemia virus. The curve for 70S RNA (○) was determined for RNA at a concentration of 1.2 μg/ml. The curves for cytoplasmic RNA (●) and nuclear RNA (△) were also determined. The abscissa for the 70S RNA and cytoplasmic RNA (the C_rt value) was calculated in a standard manner (5). For nuclear RNAs the concentration of RNA used to calculate the abscissa was actually that of the cytoplasmic fraction. Because the fractions were suspended in the same volume and the recovery of RNA in the two fractions was greater than 80%, this method reveals directly the relative amounts of viral RNA in the cytoplasm and nucleus of the cell.*

for synthesis of an endogenous virus (Table 2). The amounts of virus-specific RNA in the nucleus and cytoplasm were similar to the amounts found by Green et al. (11) in cells transformed by a murine sarcoma virus. The concentrations of virus-specific RNAs were somewhat higher than those reported for avian cells productively infected by avian C-type viruses (6, 13, 17).

Size distribution of [^{3}H]uridine pulse-labeled RNA on nondenaturing and denaturing sucrose gradients. We investigated the size distribution of pulse-labeled nuclear RNA under denaturing and nondenaturing conditions. These experiments were designed to ensure that our extraction procedures would allow detection of RNA molecules 35*S* and longer.

For analysis of the size distribution of undenatured RNA, NRK (Cl-1) cells were labeled for 10 min with 0.5 mCi of [^{3}H]uridine per ml and fractionated, and the nuclear RNA was purified as described in Materials and Methods. Figure 2a illustrates the size distribution of the undenatured nuclear RNA. The labeled RNA was present throughout the gradient, and much of it sedimented more rapidly than the ^{32}P-labeled 35*S* poliovirus RNA added to the nuclei before the extraction of RNA. This [^{32}P]RNA served as an internal control for the recovery and degradation of RNA during the extraction procedure. About 80% of the added ^{32}P-labeled poliovirus RNA was subsequently recovered from the nuclei, and about 75% of the acid-precipitable [^{3}H]uridine was recovered (Table 1). To determine whether labeled RNA retained its original sedimentation velocity when isolated from the gradient, pooled fractions from the gradient pictured in Fig. 2a were resedimented under identical conditions. The [^{3}H]RNA sedimented with the same velocity as it did originally (Fig. 2b).

To determine whether the rapid sedimentation velocity of [^{3}H]RNA was due to aggregation of smaller RNA molecules, we denatured the pulse-labeled nuclear RNA by heating it to 60°C in a solution containing 90% Me_2SO. Poliovirus double-stranded RNA was completely denatured under these conditions (unpublished data). When a portion of the same nuclear extract as was used for the gradient of Fig. 2a was denatured and sedimented under denaturing conditions, the distribution of [^{3}H]RNA was markedly changed (Fig. 2d). Very little [^{3}H]RNA sedimented more rapidly than 70*S*, and the bulk of the RNA sedimented between 35*S* and 18*S*. The RNA isolated from the pooled fractions of the gradient pictured in Fig. 2d retained its original sedimentation velocity when resedimented under the same conditions (Fig. 2e).

To test whether the RNA that sedimented faster than 35*S* RNA under nondenaturing conditions contained a representative size distribution of RNAs or whether it was enriched for a specific size class of RNA, fractions 8, 9, and 10 of the gradient pictured in Fig. 2a were pooled and separated into three equal portions. One portion was sedimented directly on a nondenaturing sucrose gradient, and a second was denatured by heating in a solution containing 90% Me_2SO and analyzed on a nondenaturing sucrose gradient (Fig. 2c). The remaining portion was denatured and sedimented under de-

TABLE 2. *Concentration of virus-specific RNA in the nuclear and cytoplasmic extracts*[a]

Cell type	% of total RNA	
	Nucleus	Cytoplasm
1. NRK	Undetectable	Undetectable
2. NRK (Moloney MuLV)	0.7	1.3
3. JLS-V9 (Moloney MuLV)	0.4	1.0
4. JLS-V9	0.05	0.07
5. JLS-V9 IUdR[b] induced	0.3	0.7

[a] The amount of virus-specific RNA was determined from the rate of annealing of a DNA probe complementary to Moloney leukemia virus (lines 1 to 3) or to the endogenous virus (lines 4 and 5). One such experiment is pictured in Fig. 1.

[b] IUdR, Iododeoxyuridine.

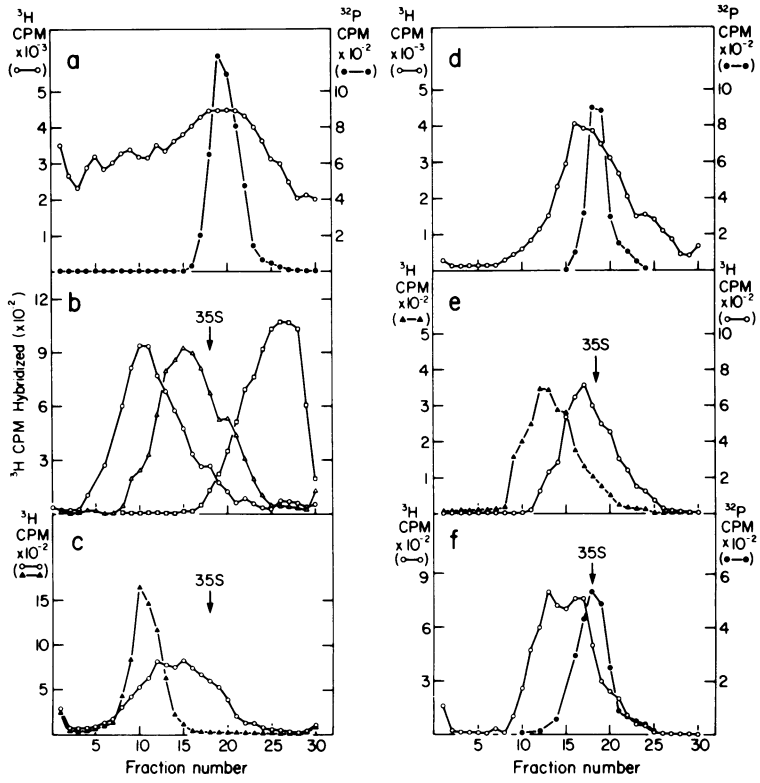

FIG. 2. *Size distribution of [^{3}H]uridine-labeled nuclear RNA under denaturing and nondenaturing conditions. (a) Nuclear RNA labeled with [^{3}H]uridine for 10 min was sedimented on a nondenaturing 15 to 30% sucrose, Tris-buffered 1% SDS gradient. Symbols: (○) [^{3}H]RNA; (●) ^{32}P-labeled poliovirus RNA. Sedimentation was for 3 h at 35,000 rpm in a Beckman SW41 rotor at 22°C. (b) Fractions of nuclear RNA from the gradient pictured in panel a were resedimented on nondenaturing 15 to 30% sucrose, Tris-buffered 1% SDS gradients. Portions of fractions 8, 9, and 10 (○), fractions 14 and 15 (△), and fractions 26 and 27 (□) were precipitated with ethanol and sedimented for 3 h at 35,000 rpm in a Beckman SW41 rotor at 22°C. ^{32}P-labeled poliovirus RNA was added to each sample as a marker. (c) Fractions 8, 9, and 10 of the gradient pictured in panel a were precipitated with ethanol. Part of the sample was resuspended in SDS buffer (△). Another portion was denatured by heating to 60°C for 5 min in 90% Me_2SO, ethanol precipitated, and resuspended in SDS buffer (○). The samples were layered on a 15 to 30% sucrose, Tris-buffered 1% SDS gradient and spun for 3 h at 35,000 rpm at 22°C in a Beckman SW41 rotor. (d) Nuclear RNA labeled with [^{3}H]uridine for 10 min (a portion of the preparation pictured in panel a) was denatured by heating to 60°C in a buffer containing 90% Me_2SO. The denatured RNA was layered onto a 10 to 20% sucrose gradient containing 80% Me_2SO. (○) [^{3}H]RNA. Sedimentation was for 15 h at 35,000 at 30°C in a Beckman SW41 rotor. (●) ^{32}P-labeled poliovirus RNA. (e) Fractions of [^{3}H]uridine-labeled nuclear RNA from the gradient pictured in panel d were precipitated with ethanol, resuspended in a buffer containing 90% Me_2SO, and sedimented on 10 to 20% sucrose gradients containing 80% Me_2SO. Sedimentation was as described in panel d. ^{32}P-labeled poliovirus RNA was added as a marker to each fraction. Symbols: (△) Fractions 11 and 12; (○) fractions 16 and 17. (f) A portion of fractions 8, 9, and 10 of the gradient pictured in panel a was precipitated with ethanol and denatured by heating to 60°C for 5 min in a buffer containing 90% Me_2SO. The RNA was sedimented on a 10 to 20% sucrose gradient containing 80% Me_2SO at 35,000 rpm for 15 h at 30°C in a Beckman SW41 rotor. ^{32}P-labeled poliovirus RNA was included (●). (○) [^{3}H]uridine.*

naturing conditions (Fig. 2f). When denatured and sedimented under either nondenaturing or denaturing conditions, the RNA that originally sedimented rapidly under nondenaturing conditions sedimented as a heterogeneous population of RNA molecules having a lower average sedimentation coefficient. Note also that the [^{3}H]RNA that sedimented very rapidly under nondenaturing conditions did not contain a representative distribution of RNA sizes. It was specifically enriched for those RNA molecules that sedimented more rapidly than the average

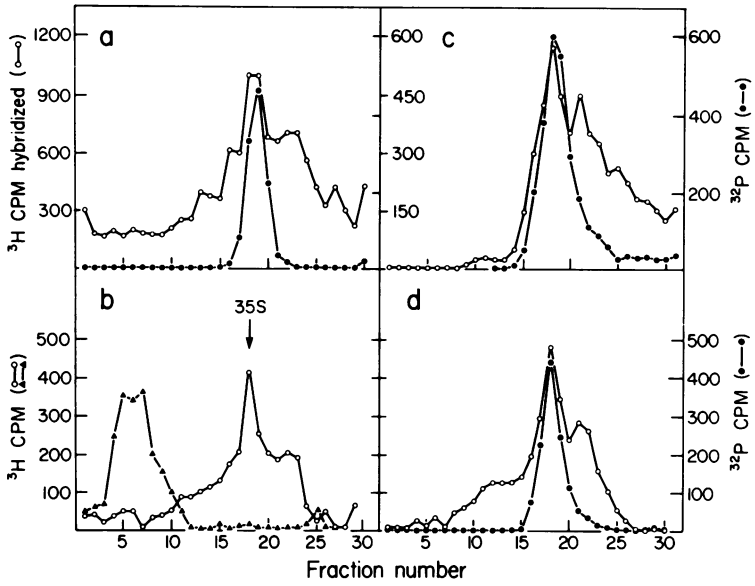

FIG. 3. *Size distribution of Moloney virus-specific RNA under denaturing and nondenaturing conditions. (a) A nuclear RNA extract of a Moloney virus-producing NRK cell line was layered onto a 15 to 30% sucrose, Tris-buffered 1% SDS gradient and sedimented for 3 h at 35,000 rpm in a Beckman SW41 rotor at 22°C. Portions of each gradient fraction were precipitated with ethanol and incubated for 5 h in 50 μl of a buffer containing 2 × SSC, 10 mM EDTA, 0.1% SDS, and 2,500 cpm of a [^{3}H]DNA probe synthesized from Moloney MuLV. The amount of [^{3}H]DNA protected from digestion by S_1 nuclease is shown (○). The size distribution of ^{32}P-labeled poliovirus RNA added to the nuclear pellet after the wash with Nonidet P-40 and deoxycholate is also shown (●). (b) Fractions 5, 6, and 7 of the gradient pictured in panel a were precipitated with ethanol. A portion was resuspended in SDS buffer (△). Another portion was denatured by heating to 60°C for 5 min in a buffer containing 90% Me_2SO, precipitated with ethanol, and resuspended in SDS buffer (○). ^{32}P-labeled poliovirus RNA was added to the initial fraction to serve as a marker. The samples were layered onto 15 to 30% sucrose, Tris-buffered 1% SDS gradients and sedimented for 3 h at 35,000 rpm in a Beckman SW41 rotor. The gradient fractions were precipitated with ethanol and annealed with 2,500 cpm of [^{3}H]DNA probe for 12 h as described in panel a. (c) Nuclear RNA from the same preparation as used in panel a was denatured by heating to 60°C for 5 min in a buffer containing 90% Me_2SO. The RNA was layered onto a 10 to 20% sucrose gradient containing 80% Me_2SO and centrifuged for 15 h at 35,000 rpm at 30°C in a Beckman SW41 rotor. The distribution of virus-specific RNA was determined by incubating portions of each gradient with 2,500 cpm of [^{3}H]DNA probe for 5 h as described in panel a. The amount of ^{3}H probe resistant to S_1 nuclease is plotted (○). ^{32}P-labeled poliovirus RNA added to the nuclei after the wash with Nonidet P-40 and deoxycholate is also shown (●). (d) A portion of fractions 5, 6, and 7 of the gradient pictured in panel a was precipitated with ethanol, denatured by heating at 60°C for 5 min in a buffer containing 90% Me_2SO, and sedimented on a 10 to 20% sucrose gradient containing 80% Me_2SO for 15 h at 35,000 rpm, 30°C, in a Beckman SW41 rotor. Gradient fractions were precipitated with ethanol and then incubated with 2,500 cpm of [^{3}H]DNA probe as described above. The amount of [^{3}H]DNA resistant to digestion by S_1 nuclease is shown (○). ^{32}P-labeled poliovirus RNA was added to the fraction before denaturation (●). The RNA that sedimented more rapidly than 35S, fractions 8 to 14, protected 90% of the [^{3}H]DNA probe from digestion by S_1 nuclease digestion when the hybridization reactions of the separate fractions were allowed to go to completion.*

population under denaturing conditions. This experiment demonstrated that the methods of cell fractionation and RNA extraction used can detect virus-specific RNA chains longer than 35*S* RNA if they exist.

The distribution of ^{3}H-labeled nuclear RNA was the same as that pictured in Fig. 2 when RNA extracts were prepared by using either the proteinase K method described by Fan and Baltimore (8) or the high-salt–urea method of Holmes and Bonner (12) and analyzed on nondenaturing and denaturing sucrose gradients. Results of experiments with pulse-labeled [^{3}H]RNA extracted from HeLa cells and from JLS-V9 cells were very similar to those described above. Others have also reported a decrease in the average length of pulse-labeled nuclear RNA after denaturation (1, 3, 7, 15).

Size distribution of virus-specific nuclear RNA. To measure the steady-state size distri-

bution of virus-specific RNA, we prepared unlabeled nuclear RNA cultures of NRK (Cl-1). This cell line was chosen because of the high content of virus-specific RNA (Table 2) and because the NRK line contains no endogenous virus that has sequences homologous to those of Moloney MuLV (18; Rothenberg and Baltimore, unpublished observations).

To measure the size distribution of virus-specific RNA, unlabeled nuclear RNA was extracted in parallel with the labeled nuclear RNA described in the previous section. The concentration of virus-specific RNA in each of the gradient fractions was determined by the ability of the RNA of each fraction to protect a complementary [^{3}H]DNA probe from digestion by S_1 nuclease. The hybridization reactions were done at RNA excess, at a time sufficiently short so that the amount of ^{3}H probe protected was proportional to the concentration of virus-specific RNA in the sample (8). Figure 3a shows the size distribution of virus-specific RNA when nuclear extracts of the NRK line were sedimented under nondenaturing conditions. Virus-specific RNA was present throughout the gradient, much of it sedimenting more rapidly than the 35*S* ^{3}H-labeled poliovirus RNA marker. However, when the nuclear extracts were denatured in 90% Me_2SO and sedimented under denaturing conditions in gradients containing 80% Me_2SO, very little virus-specific RNA sedimented more rapidly than did the poliovirus RNA (Fig. 3c). The shape of the ^{32}P-labeled poliovirus RNA peak indicates that there was little if any degradation of poliovirus RNA during the extraction and purification procedure. Between 2 and 4% of the virus-specific RNA had a sedimentation velocity of greater than 35*S*.

The native RNA that sedimented more rapidly than 35*S* RNA was enriched in RNA that sedimented faster than 35*S* RNA after denaturation. Figure 3b compares the sedimentation velocity of the rapidly sedimenting RNA of fractions 5, 6, and 7 of Fig. 3a on nondenaturing gradients before and after denaturation. Figure 3d illustrates the size distribution of the same material when it was denatured and sedimented under denaturing conditions. Approximately one-third of this fraction sedimented more rapidly than 35*S*, with a heterogeneous distribution extending up to 60*S*.

The distribution of virus-specific RNA was the same when the nuclear RNA extracts were prepared by the proteinase K method described by Fan and Baltimore (8) or the high-salt–urea method of Holmes and Bonner (12). Treatment of the samples with RNase A before sedimentation completely eliminated hybridization of the probe across the gradients (data not shown).

DISCUSSION

About 1% of the RNA of a cell line continually producing a murine leukemia virus is virus specific, and about one-third of the virus-specific sequences are located within the nucleus. This represents an appreciable fraction, about 5%, of nuclear RNA, based on the assumption that nuclear RNA comprises about one-tenth of the total cellular RNA (4).

We measured the size distribution of this steady-state population of virus-specific RNA molecules under denaturing and nondenaturing conditions. Under nondenaturing conditions, virus-specific nuclear RNA sedimented as a heterogeneous mixture of RNA species, with sedimentation coefficient ranging from 10*S* to over 100*S*. This heterogeneous distribution was different from the distribution of cytoplasmic virus-specific RNA sequences. Cytoplasmic RNA has been shown to consist mainly of 35*S* RNA, with lesser amounts of lower-molecular-weight species (8, 10; Haseltine and Baltimore, unpublished observations). The heterogeneous size distribution of virus-specific RNA must reflect aggregation of RNA species because the size distribution of virus-specific nuclear RNA was markedly altered when the RNA was denatured. After denaturation, the RNA consisted almost entirely of 35*S* RNA and smaller species. The experiments reported here do not distinguish between the possibility that the fraction that sedimented rapidly under nondenaturing conditions represented aggregation between independently transcribed RNA molecules, or the possibility that the virus-specific RNA was part of long primary transcript that was held together by a secondary structure. Whatever the cause of the aggregation, poliovirus RNA added early in the extraction procedure does not enter the aggregates. Pulse-labeled cellular RNA behaved like the virus-specific RNA. Georgieff et al. (9) have reported a similar observation in cells transformed by adenovirus. They found that under nondenaturing conditions adenovirus-specific nuclear RNA sedimented as a heterogeneous mixture of RNA species, some of which had very high sedimentation coefficients. After denaturation, however, the adenovirus-specific RNA sedimented with a sedimentation coefficient of 20*S*.

A small fraction, 2 to 5% of the nuclear RNA, sedimented more rapidly than 35*S* RNA after denaturation. This rapidly sedimenting virus-specific RNA was enriched in RNA that sedi-

mented more rapidly than 35S before denaturation. This fraction may represent incompletely processed precursor RNA molecules.

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