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Size of Virus-Specific RNA in B-34, a Hamster Tumor Cell Producing Nucleic Acids of Type C Viruses from Three Species

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B-34 is the designation of a hamster tumor-derived cell line induced by the Harvey sarcoma virus. This cell line produces virions which contain structural proteins common to endogenous hamster viruses and nucleic acid sequences of hamster, mouse, and rat origin. The sedimentation characteristics of the intracellular virus-specific RNA was determined in sucrose gradients after treatment with dimethylsulfoxide by molecular hybridization using complementary DNA of strict virus specificity. Hamster virus-specific RNA sedimented at 35*S* (major peak) as is characteristic of productive infection by type C leukemia viruses of other species. Rat virus-specific RNA sedimented at 30*S* which is characteristic of the sarcoma virus-related genome found in nonproducer cells transformed by Kirsten sarcoma virus. Both Harvey and Kirsten sarcoma viruses contain a related but not necessarily identical 30*S* rat-specific component which is also found in normal cultured rat cells. Mouse cells producing Harvey sarcoma virus also contain a rat-specific 30*S* RNA. Mouse virus-derived sequences also sedimented at 30*S* in B-34 cells and in a similar size range in Harvey virus-infected mouse cells. The possiblility that the mouse and rat-derived sequences are present on a single 30*S* RNA species which would then be related to sarcomagenic potential is one attractive hypothesis suggested by these data.

In 1968 Bassin et al. (2) reported the derivation of a cell line, "B-34," from the 25th hamster transplant generation of a tumor induced by fragments from a mouse tumor induced by the Harvey strain of murine sarcoma virus (MSV). B-34 cells produced a filterable sarcomagenic agent for hamsters but not mice, and in this respect the virus is similar to a number of other sarcomagenic viruses recovered from hamster tumors induced by other strains of MSV (10, 12, 16, 17). Immunologic tests established that the B-34 virus contained structural proteins of endogenous hamster type C (4, 14) viruses and that even in sensitive radioimmunoassays no evidence of mouse viral proteins, specifically the major internal protein (p30), could be found (4). Nevertheless, nucleic acid sequences common to endogenous viruses of both hamsters and mice were demonstrated by molecular hybridization in B-34 virus (15). The mouse sequences in B-34 are highly related to Moloney virus based on thermal elution profiles of viral RNA-complementary DNA (cDNA) hybrid molecules from hydroxylapatite columns (H. Okabe, unpublished data). The latter technique permits distinction among mouse type C viruses with relatively large differences in midpoint of the thermal elution profile (H. Okabe, R. V. Gilden, and M. Hatanaka, Int. J. Cancer, in press; 8).

The Harvey sarcoma virus (H-SV) itself was derived from plasma filtrates of one of six leukemic litter mate outbred Chester Beatty rats inoculated at birth with Moloney leukemia virus (9). The plasma filtrates induced sarcomas in newborn rats, hamsters, and mice. Molecular hybridization analyses of H-SV showed the presence of nucleic acid sequences common to endogenous mouse and certain rat type C viruses even after prolonged passage on mouse cells (19). In this context, H-SV resembles the Kirsten sarcoma virus (KiSV) (20) which also arose on rat passage of leukemogenic mouse virus (11). H-SV and KiSV contain only mouse virus structural proteins and are thus more accurately described as H-SV(MLV) and KiSV(MLV). Based on the history of origin it was not surprising to find evidence of rat virus nucleic acid sequences in B-34 (15).

In previous studies we have found that the Moloney strain of MSV (M-MSV) genome in nonproducer (NP) tumor cells or cells transformed by M-MSV was expressed in the form of

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RNA molecules sedimenting at 30S (24, 25) as opposed to helper virus subunits which sediment at 35S (23–25). The KiSV-specific sequences found in KiSV-transformed NP cells also sedimented at 30S and could be found in normal rat cells as well (21, 26, 27). The rat type C viruses we have analyzed contain both 35S and 30S subunits which are distinct in hybridization specificity. These results imply that the sequences related to the sarcomagenic viruses, M-MSV and KiSV, reside exclusively in 30S subunits.

In the present studies, the size distribution of sequences related to hamster, mouse, and rat viruses in B-34 cells was determined by hybridization with cDNA of strict virus specificity.

MATERIALS AND METHODS

Cells. The following mouse cells were used: BALB/3T3; K-234, a BALB/3T3 cell transformed by KiSV (1); 58-2T, a K-234 cell activated to produce KiSV and murine leukemia virus (MLV) (26); and H-SV(MLV)-BALB/3T3, which is a BALB/3T3 cell infected with H-SV(MLV). The H-SV(MLV)-BALB/3T3 cell produced a 10- to 100-fold excess of leukemia virus based on results of focus-forming and XC assays (R. Klein and M. Hatanaka, unpublished data). The rat cells used in the present study were designated NRK-9 (13), a normal rat kidney (NRK) cell spontaneously activated to produce an endogenous rat leukemia virus (RaLV); 78A1 (7), a rat embryo fibroblast transformed by the M-MSV and producing M-MSV(MLV); and 5053 (5), a clonal cell line of Fischer rat embryo cells. The virus produced by 78A1 cells is primarily M-MLV based on hybridization studies and biological focus-forming and XC assays (23).

The following hamster cells were used: a tumor cell line derived from the Graffi hamster strain which was found to produce HaLV (G-HaLV) after treatment with dimethylbenzanthracene (6); B-34 (2), a hamster cell transformed by H-SV as described in detail above; and BHK, which was obtained from Flow Laboratories, Inc. (Rockville, Md.).

Preparation of total cell RNA. Total cell RNA was prepared as described previously (23) and further purified to remove residual possible contamination of DNA by Cs_2SO_4 density gradient centrifugation (27).

Preparation of [^{3}H]DNA. [^{3}H]DNA products were prepared from RaLV, M-MSV(MLV), and G-HaLV by the endogenous reverse transcriptase reaction as described previously (27). These cDNA products were designated RaLV [^{3}H]DNA, M-MSV(MLV) [^{3}H]DNA, and G-HaLV [^{3}H]DNA, respectively. 58-2TS [^{3}H]DNA, which hybridizes specifically with RNA sequences of the KiSV genome, was prepared as described previously (27). The specific activity of the cDNA preparations averaged 1.8×10^7 to 2.6×10^7 counts/min per μg of DNA as calculated from the specific activity of [^{3}H]TTP, assuming equimolar amounts of the four deoxynucleotides. The transcripts used protected 80% of ^{32}P-labeled viral 70S RNA from RNase digestion after hybridization with 10- to 20-fold excess [^{3}H]DNA (27). More than 60% of NRK-9-derived RaLV [^{3}H]DNA thus prepared did hybridize with rat cell DNA but not with mouse cell DNA (Tsuchida, Gilden, and Hatanaka, unpublished data) as previously seen with a RaLV from RPL cells (22).

Hybridization. All hybridization reactions were performed in 2× SSC (SSC = 0.15 M NaCl and 0.015 M sodium citrate) and 40% formamide at 45 C for 48 h. The extent of hybridization was assayed using S-1 nuclease as described previously (26).

Size of virus-specific RNA. The whole cell RNA was treated with dimethylsulfoxide (Me_2SO) as described (21, 23, 24) and layered on a 15 to 30% sucrose gradient in NTE buffer (0.01 M Tris-hydrochloride [pH 7.0], 0.1 M NaCl, 0.001 M EDTA) containing 0.5% $NaDodSO_4$. The RNA was centrifuged for 5 to 6 h at 36,000 rpm at 20 C in the SW41 rotor in a Spinco model L2-65 ultracentrifuge. After centrifugation, fractions were collected from the bottom of the tube. Absorbancy at 260 nm was measured, and RNA was precipitated with 2 volumes of ethanol in the presence of 50 μg of yeast tRNA per ml. RNA collected by low speed centrifugation was dissolved in 0.1 ml of 0.1× SSC. A portion of each fraction was hybridized with [^{3}H]DNA, and the extent of hybridization was assayed as described above.

RESULTS

Rat sequences in B-34 cellular RNA. The [^{3}H]DNA transcript of NRK-9 virus detects both 35 and 30S RNA species in NRK-9 cells (21). This transcript shows no hybridization with uninfected hamster or mouse cells (Fig. 1A) or with cells actively replicating endogenous viruses of either species (e.g., Fig. 1 of reference 21). In contrast, virus-free rat cells, mouse cells transformed nonproductively by KiSV, and mouse cells infected with H-SV all contain RNA sequences homologous to NRK-9 DNA (Fig. 1A). The percentage of hybridization at saturation was ~20% with each of these cellular RNA preparations compared to ~75% with NRK-9 RNA. This difference is accounted for by the differential specificity of 35 and 30S subunits and the absence of the 35S RNA species in all but the cells producing NRK-9 (21). B-34 cellular RNA gave ~20% hybridization with NRK-9 cDNA, in agreement with the earlier report of rat sequences in B-34 viral RNA (15). The specificity and size characteristics of this RNA will be described in later sections.

Mouse sequences in B-34 cellular RNA. The [^{3}H]DNA transcript of M-MSV(MLV) gave ~70% hybridization with cellular RNA from both the homologous virus-producing cell line and BALB/c cells infected with H-SV(MLV) (Fig. 1B). This transcript did not

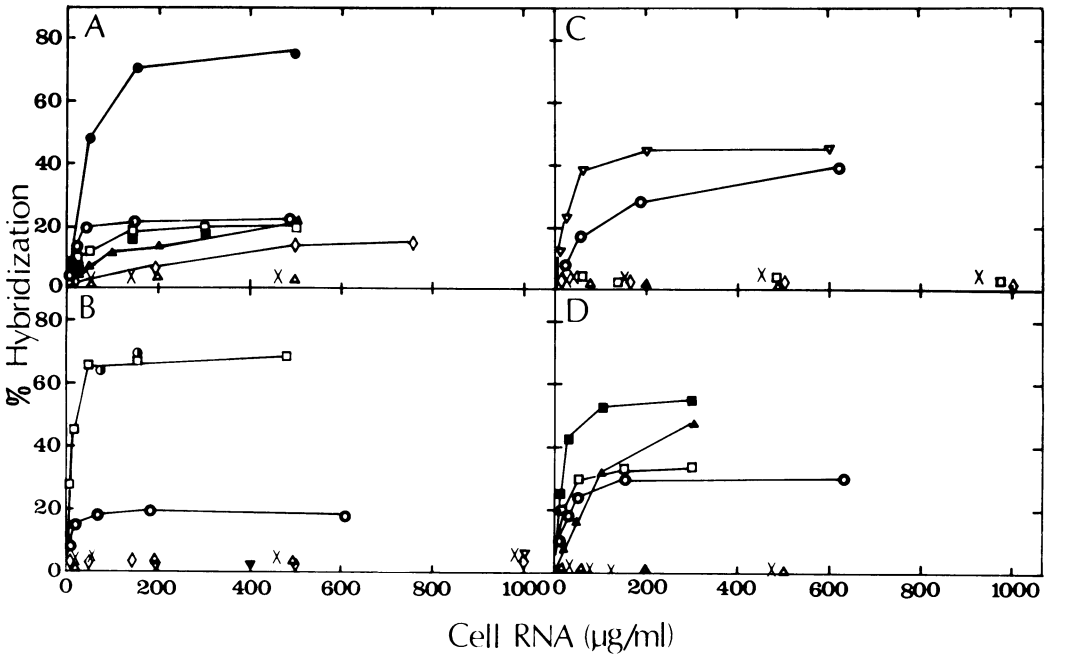

FIG. 1. *Hybridization-saturation of [³H]DNA probes with various cellular RNA preparations. Five hundred counts/min of RaLV [³H]DNA (A), M-MSV(MLV) [³H]DNA (B), G-HaLV [³H]DNA (C), and 58-2TS [³H]DNA (D) were hybridized with increasing amounts of cellular RNA from NRK-9 (●); B-34 (○); BHK (×); BALB/3T3 (△); 5053 normal rat (◇); K-234 (▲); H-SV(MLV)-BALB/3T3 cell RNA (□); 58-2T (■); 78-A1 (◑); and G-HaLV cells (▽). The values shown are the actual percentage of input counts after subtraction of background (no RNA in the incubation mixture, ~25 count/min). All incubations were made in 100-μl volumes.*

hybridize with normal mouse, rat, or hamster cellular RNA (Fig. 1B). While our unpublished experiments indicate small amounts of rat-specific sequences in viral RNA preparations prepared from 78A1 cells, the cDNA transcripts are only representative of mouse virus sequences. This transcript was reactive with RNA from the B-34 cell line, and in other experiments (H. Okabe, unpublished data) B-34 viral RNA was found to give a thermal elution profile with Moloney virus DNA which was indistinguishable from the homologous Moloney system.

Hamster sequences in B-34 cellular RNA. The G-HaLV transcript has previously been shown not to hybridize with mouse or rat viruses (15). This transcript hybridized to a similar final extent (~40%) with cellular RNA of the G-HaLV and B-34 cell lines and was nonreactive with uninfected hamster, mouse, and rat cells, and with mouse cells producing H-SV (Fig. 1C).

The reaction patterns of the three transcripts establish that sequences corresponding to endogenous viruses of three species can be detected in B-34 cellular RNA.

KiSV-specific sequences in B-34 cells. The preparation of a transcript with specificity for the 30S component of KiSV has been described (27). This involved activation of virus from KiSV-transformed NP mouse cells and removal by absorption of that portion of the cDNA reactive with Rauscher virus. This transcript (58-2TS) is nonreactive with uninfected mouse or hamster cells and also cells producing endogenous viruses of these species (Fig. 1D). Hybridization is obtained with normal rat, virus-producing rat, mouse cells producing or transformed by KiSV and H-SV, and also B-34 cells. Of interest was the finding that the KiSV-specific cDNA gave a final extent of hybridization with K234 and 58-2T cells (50 to 55%) which was significantly higher than that obtained with H-SV-infected cells or B-34 cells (30%). Since the rat-derived sequences in the latter two cells are expected to be highly related, this indicates a possible difference between rat-derived sequences of KiSV and H-SV. These results show that nucleic acid sequences of rat origin which are associated with H-SV are found in B-34 cells.

Size of the various RNAs in B-34 cells. Previous studies with cells infected with MLV indicate the presence of RNA species sediment-

ing at 35 and 20S, whereas 35S is the predominant species of genomic RNA in purified virions (23). Hybridization of B-34 cell RNA with the G-HaLV DNA (Fig. 2A) gave a similar result to that seen with MLVs in that the major species detected sedimented at 35S; in addition, lower-molecular-weight RNA was also detected. In contrast, the three other transcripts (58-2TS [^{3}H]DNA, RaLV [^{3}H]DNA, and M-MSV(MLV) [^{3}H]DNA) all hybridized with an RNA species which sedimented at 30S. Thus, the rat-specific sequences appear similar to that seen in KiSV NP cells in that 35S RNA is not found. The M-MSV(MLV) transcript also detected only a 30S RNA in B-34 cells in contrast to H-SV(MLV)-infected mouse cells where 35, 28 to 30, and 20S RNAs are detected by this transcript (Fig. 2B). Both RaLV and 58-2TS transcripts detect a 30S RNA in the H-SV-infected cells (Fig. 2B), indicative of rat-derived se-

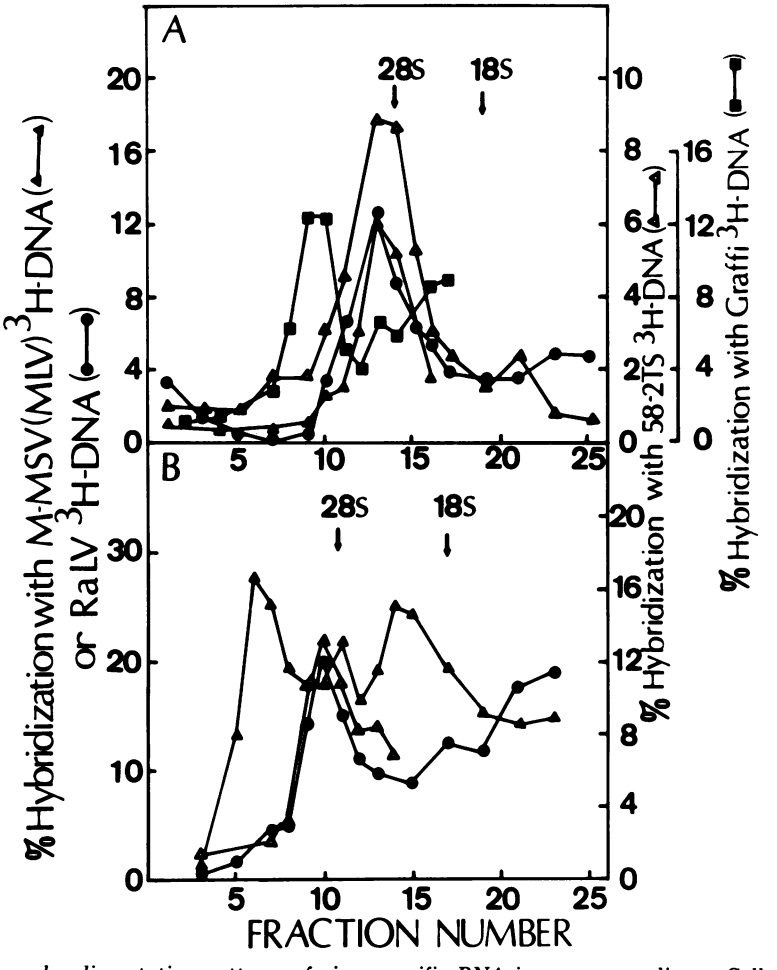

FIG. 2. *Rate zonal sedimentation patterns of virus-specific RNA in sucrose gradients. Cellular RNA was isolated from (A) B-34 or (B) H-SV(MLV)-infected BALB/3T3 and layered on a 15 to 30% sucrose gradient in NTE buffer containing 0.5% sodium dodecyl sulfate and centrifuged at 20 C in a Spinco SW41 rotor for 6 h at 36,000 rpm. The amounts of RNA loaded on the gradients were 5.0 absorbance at 260 nm units for B-34, and 1.6 absorbance at 260 nm units for H-SV(MLV)-infected BALB/3T3. Fractions were collected from the bottom, and absorbance at 260 nm was measured. RNA was precipitated with 50 μg of carrier yeast tRNA per ml and dissolved in 100 μl of 0.1× SSC. (A) Portions (6, 10, 5, and 10 μl) of each fraction were hybridized with M-MSV(MLV) [^{3}H]DNA (1,620 counts/min), RaLV [^{3}H]DNA (832 counts/min), G-HaLV [^{3}H]DNA (514 counts/min), and 58-2TS [^{3}H]DNA (352 counts/min), respectively. (B) Portions (10, 10, and 5 μl) of each fraction were hybridized with M-MSV(MLV) [^{3}H]DNA (1,620 counts/min), RaLV [^{3}H]DNA (694 counts/min), and 58-2 TS [^{3}H]DNA (373 counts/min), respectively.*

quences. Table 1 summarizes the size of virus-specific RNA in three rodent sarcoma and leukemia viruses.

DISCUSSION

The present report confirms previous observations (15) that B-34 cells contain nucleic acid sequences of hamster, mouse, and rat origin. These cells and the virions produced therefrom contain structural proteins common to endogenous hamster type C viruses, and even in highly sensitive radioimmunoassays no mouse or rat specificities were detected (4). Cellular RNA homologous to an endogenous hamster virus had the size characteristics of replicating virus in other species, i.e., a major component sedimenting at 35*S* (21, 23), and also components with lower *S* values. The 35*S* genomes of mouse, rat, and hamster viruses are expected to direct synthesis of virion structural proteins (at least p30) since in the cases where they are not found, e.g., nonproducer cells in heterologous species, such proteins have not been found. The sequences in KiSV and H-SV related to rat viruses sedimented at 30*S* and could be detected with a cDNA preparation which was specific for the KiSV genome. This cDNA was nonreactive with 35*S* RNA of rat type C viruses but did form hybrids with a 30*S* RNA species found in normal rat cells (21). Thus, the current data show that the H-SV and KiSV genomes are related in terms of size and hybridization specificity although the extent of hybridization of KiSV cDNA with H-SV RNA was lower than for homologous reactions. The difference in reaction extent indicates either absence of certain sequences in H-SV or evolutionary divergence of related sequences in the two 30*S* RNAs. These possibilities are being tested with reciprocal hybridizations and hydroxylapatite thermal elution chromatography. In a somewhat analogous situation, Scolnick et al. (18) found that Moloney virus passaged through rat cells could incorporate KiSV-specific sequences into 60 to 70*S* RNA. The virus however did not contain rat-specific p12 polypeptide and very low amounts of rat-specific p30. This result is in agreement with our conclusions that the 30*S* KiSV-specific sequences of rat origin do not direct synthesis of type C virus structural proteins even when incorporated into virions. In the case of M-MSV where a 30*S* RNA subunit is also detected in denatured viral RNA and NP transformed cells (25), hybridization experiments indicate (in contrast to the rat system) considerable homology to helper virus sequences (23–25). As with the rat 30*S* RNA subunit, MSV 30*S* RNA apparently does not direct the synthesis of structural proteins common to the helper virus. The size relationship seen in sarcoma virus subunits from two species is presumably not coincidental but should have some bearing on sarcomagenic potential.

TABLE 1. *Characterization of major RNA subunits of rodent sarcoma and leukemia viruses*

Viruses	Size of RNA subunit homologous to:			
	M-MLV	R-MLV	RaLV	HaLV
M-MSV	30*S*[a]	ND	—[b]	ND[c]
	26–28*S*[d]	ND	—[e]	ND
KiSV	ND	—[f]	30*S*[b]	—
H-SV	30*S*	ND	30*S*	ND
MLV	35*S*[g]		—[b]	—
RaLV	—[h]	—[b]	35*S* + 30*S*[b]	—[h]
HaLV		—[h]	—[h]	35*S*

[a] Reference 25.
[b] Reference 21.
[c] ND, Not done.
[d] Reference 24.
[e] Homology was not detected.
[f] Reference 27.
[g] Reference 23.
[h] Reference 15.

Based on the models of formation of both KiSV and H-SV (19, 20), and on previous observations of size differences between helper (35*S*) and sarcoma-specific (30*S*) subunits, 35*S* hamster and 30*S* rat sequences were expected in B-34 cells. The origin of H-SV from Moloney-MuLV (9) suggested the retention but not the size characteristics of mouse sequences in B-34 cells. Thermal elution experiments gave results consistent with a high degree of relationship to Moloney virus, and interestingly these sequences seem to be preferentially transcribed in endogenous reverse transcriptase reactions in B-34 (15). This is one clear case where hybridization and immunological assays gave disparate results, and thus emphasizes the need for precise virus histories.

The present experiments show that the mouse virus-derived sequences present in B-34 cells also reside on a 30*S* RNA subunit and that an RNA species (mouse specific) of this approximate size can be detected in H-SV-infected mouse cells. This result is striking since Moloney leukemia virus contains only 35*S* RNA subunits (23). The possible explanations are: (i) low levels of 30*S* RNA in the original MLV; (ii) loss of sequences from 35*S* MLV in the course of virus passage; and (iii) recombination of MLV and rat sequences to give a single 30*S* molecule with both specificities. The data are consistent with a stable recombination (point 3) at the

time of origin of H-SV which was retained when this genome was rescued in vivo in the hamster. This initial recombinational event may then be the sufficient basis of sarcomagenic potential as opposed to simple incorporation of 30*S* rat "cellular" RNA into virions as can occur simply on passage of virus in rat cells (18). Our KiSV-specific cDNA (58-2TS DNA) does not detect MuLV-derived sequences in KiSV or H-SV 30*S* RNA because it has been prepared by a procedure which removes all MuLV-related DNA (27).

In keeping with other observations, the lack of an intracellular 35*S* mouse virus RNA species in B-34 cells seems an adequate descriptive reason for failure to detect MuLV proteins in this cell line.

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