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# Characterization of Gazdar Murine Sarcoma Virus by Nucleic Acid Hybridization and Analysis of Viral Expression in Cells

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Gazdar murine sarcoma virus (Gz-MSV) and Moloney murine sarcoma virus (M-MSV) are closely related. The complete M-MSV-specific nucleic acid sequences constituted a major portion of Gz-MSV-specific sequences. The MSV-specific sequences in both Gz-MSV and M-MSV genomes shared homology with hamster leukemia virus nucleic acid sequences. Both rat cells (S+L+) and hamster (S+L−) cells expressed two viral proteins of 68,000 and 70,000 daltons. These proteins were immunologically related to p60 purified from m1 virions of M-MSV.

Several murine sarcoma viruses with distinct unique sequences have been isolated (5, 7, 9). Gazdar isolated a murine sarcoma virus (Gz-MSV) from a spontaneous tumor in a NZW/NZB mouse (3). The biological properties of the virus were found to be very similar to Moloney murine sarcoma virus (M-MSV) (2-4). The present investigation was undertaken to compare the nucleotide sequences of Gz-MSV with those in M-MSV by nucleic acid hybridization and analysis of viral expression in cells. In the present communication, we show that Gz-MSV and M-MSV are closely related. A portion of the MSV-specific sequences in Gz-MSV was not found in M-MSV, although the complete MSV-specific sequences in M-MSV were shown to be present in Gz-MSV. In addition, both MSV-specific sequences of both Gz-MSV and M-MSV shared homology with hamster leukemia viruses (HaLV). Gz-MSV did not appear to recombine with rat endogenous virus sequences in spite of its propagation in rat cells. In addition, both the rat and hamster cells infected with Gz-MSV expressed two viral proteins of 68,000 and 70,000 daltons. These proteins were immunoprecipitated by anti-p60 serum prepared from M-MSV(FeLV) virus.

## MATERIALS AND METHODS

**Cell lines and viruses.** Rat tumor-Gazdar (RTG-1:S+L+) cells infected with murine leukemia virus and Gazdar murine sarcoma virus (Gz-MSV/MuLV) and hamster tumor-Gazdar (HTG-2:S+L−) cells infected with defective Gz-MSV(HaLV) were used. F-2833 Graffi hamster cells, infected with chemically induced HaLV, were a gift from Paul Price (Microbiological Associates, Bethesda, Md.). The species of origin of cells were confirmed by karotype and isoenzyme analyses. Moloney MuLV (1869) was propagated in Scl cells. 3B11-IC mouse cells, infected with the m3 isolate of M-MSV and M-MuLV-IC, and B34 hamster cells, infected with hamster leukemia pseudotype of Harvey-MSV (Ha-MSV), were obtained from A. Frankel (National Cancer Institute) and R. Bassin (National Cancer Institute), respectively. Cells were maintained in McCoy 5a medium supplemented with 10% fetal calf serum.

**Virus preparation.** Gz-MSV/MuLV, Gz-MSV(HaLV), M-MSV/MuLV, or M-MuLV supernatant was concentrated 500- to 1,000-fold by polyethylene glycol 6000 precipitation (8%, wt/vol). The virus was then banded at the interface in 25 to 45 discontinuous sucrose gradients as described previously (12). The viral interface was then diluted with equal volume of STE (0.05 M Tris-hydrochloride, 0.1 M NaCl, and 0.001 M EDTA, pH 7.4) for the extraction of viral RNA (vRNA) as described previously (12) or was pelleted in STE for preparation of DNA complementary to vRNA. High-molecular-weight vRNA was fractionated in a 10 to 30% sucrose gradient in STE after centrifugation at 40,000 rpm in an SW41 rotor for 180 min at 12°C.

Rauscher leukemia virus (R-MuLV) from JLS-V9 cells, rat leukemia virus (RaLV) from V-NRK cells, HaLV from F-2833 cells, Ha-MSV(HaLV) from B34 cells, M-MuLV-IC from 3T3-IC-19 cells, and Rous sarcoma virus (RSV) were banded by continuous-flow ultracentrifugation (Electro-Nucleonics Laboratory, Inc.). vRNA was extracted as described previously (12).

**Preparation of cDNA.** Tritiated DNA complementary to Gz-MSV/MuLV vRNA ($cDNA_{Gz\text{-}MSV/MuLV}$) was prepared by the endogenous reverse transcriptase reaction (1). The reaction mixture consisted of (final concentration): dithiothreitol, 0.015 M; $MnCl_2$, 0.0015 M; NaCl, 0.05 M; Tris-hydrochloride (pH 7.8), 0.05 M; Triton X-100, 0.015%; [$^3$H]TTP (specific activity, 40 to 50 μCi/nmol), 0.5 mCi; dATP, dCTP, and dGTP, each at 0.001 M; actinomycin D (Calbiochem), 100 μg/ml; NaF, 0.01 M; cyclic CMP, 50 μg/ml; and virus particles $10^{11}$ to $10^{12}$/ml. The reaction mixture was incubated at 37°C for 4 h. At the end of the incubation, 10% sodium dodecyl sulfate and 0.5 M EDTA (pH 6.5) were added to final concentrations of 1% and 0.01

M, respectively. The reaction mixture, diluted with an equal volume of STE, was then extracted three times with an equal volume of STE-saturated phenol-chloroform (1:1) and then three times with chloroform-isoamyl alcohol (24:1). The aqueous layer was then hydrolyzed in 0.5 N NaOH at 100°C for 10 min. Ten equal volumes of distilled water were added to the sample, and the pH of the solution was adjusted to approximately 7.0 with 6 N HCl. Then, the cDNA was loaded onto a hydroxylapatite column (1 cm by 1 cm ID) equilibrated at 50°C. The column was washed twice with 1 ml of 0.01 M sodium phosphate buffer (pH 6.8; PB), and the cDNA was eluted with 0.14 M PB in 1-ml fractions. The Gz-MSV/MuLV complexes used for cDNA preparation had a six- to eightfold excess of leukemia focus-inducing units to sarcoma focus-forming units as assayed on FG-10 and 3T3-FL cells, respectively. Over 60 and 90% of the 50 ± 6S [$^{32}$P]vRNA from Gz-MSV/MuLV were protected from RNase T1 digestion after hybridization with cDNA to a final $C_rt$ value of 0.5 in cDNA/RNA molar ratios of 2:1 and 8:1, respectively. The size of the cDNA thus synthesized was determined to be 4S to 6S in a 15 to 30% alkaline sucrose gradient.

DNA complementary to M-MSV/MuLV or M-MuLV (1869) vRNA ($cDNA_{M\text{-}MSV/MuLV}$ or $cDNA_{M\text{-}MuLV}$) was prepared similarly.

**Preparation of MSV-specific cDNA sequences (cDNAsarc).** Fractionation of cDNA for MSV-specific sequences was carried out by exhaustive hybridization with vRNA from M-MuLV (1). Tritiated cDNA's prepared from Gz-MSV/MuLV and M-MSV/MuLV were allowed to hybridize with a 40- to 50-fold excess of M-MuLV total vRNA in 0.3 M PB at 63°C for 48 h. At the end of the incubation, the reaction mixture was diluted 1:4 and loaded onto a hydroxylapatite column equilibrated at 45°C, with 1 cm of hydroxylapatite per 30 μg of vRNA used in the hybridization mixture. The column was washed with 0.12, 0.14, and 0.3 M solutions of PB. The 0.12 M fraction was then concentrated on a hydroxylapatite column at 50°C by elution in 0.3 M PB. The $cDNA_{Gz\text{-}MSV/MuLV}$ and $cDNA_{M\text{-}MSV/MuLV}$ were then hybridized with a six- to eightfold excess of high-molecular-weight vRNA from Gz-MSV/MuLV and M-MSV/MuLV, respectively, at 63°C for 20 h in 0.3 M PB. The hybrids were again fractionated on a hydroxylapatite column at 50°C in 0.3 M PB and hydrolyzed in 0.5 N NaOH for 10 min at 100°C. The reaction mixture was adjusted to pH 6 with 6 N HCl. The cDNA was concentrated on a hydroxylapatite column at 50°C by elution in 0.14 M PB. If necessary, the cDNA was further hybridized to M-MuLV vRNA to remove any remaining M-MuLV cDNA nucleotide sequences. The cDNA's fractionated were designated $cDNA_{sarc\text{-}Gz\text{-}MSV}$ and $cDNA_{sarc\text{-}M\text{-}MSV}$ for sequences complementary to Gz-MSV and M-MSV vRNA, respectively.

**Hybridization of vRNA to cDNA.** Hybridization was carried out at 63°C for 20 h in 0.6 M $Na^+$ in 500-μl Eppendorf tubes. The final concentrations of 50 to 70S vRNA and total vRNA in the hybridization mixture were 10 and 20 μg/ml, respectively. Trichloroacetic acid-precipitable 1,500 to 2,000 cpm of cDNA and 500 cpm of MSV-specific cDNA were used in each sample for assay. Homology was assessed by using S1 nuclease (8). The $C_rt$ values were calculated as described by Leong et al. (8).

**Thermal denaturation of cDNA:RNA hybrids.** cDNA:RNA hybrids were prepared by hybridization of 0.6 M $Na^+$ at 63°C to a minimal final $C_rt$ value of 0.8 mol/s per liter. The hybridization mixture was then diluted to a final $Na^+$ concentration of 0.18 M. The hybrids were then incubated at 50, 60, 65, 70, 75, 80, 85, 90, or 95°C for 10 min and cooled at 4°C. The percentage of hybrids dissociated thermally was assessed by using S1 nuclease (8). The melting temperature ($T_m$) is defined as the temperature at which half of the hybrid dissociated above 60°C.

**Expression of viral proteins in cells.** Cells were labeled for 2 h with [$^{35}$S]methionine as described by Robey et al. (13). Cell extracts were prepared and reacted with rabbit antiserum against the ml purified isolate of M-MSV-specific p60 from the feline leukemia pseudotype of M-MSV propagated in P521 feline cells (11). The precipitated polypeptides were then subjected to 6 to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography as described previously (6).

## RESULTS

**Hybridization of $cDNA_{Gz\text{-}MSV/MuLV}$ to vRNA.** To compare the sequences of Gz-MSV with other type C viruses, $cDNA_{Gz\text{-}MSV/MuLV}$ was hybridized to either 50 to 70S vRNA or total vRNA at 63°C in 0.6 M $Na^+$ (Fig. 1). Over 90% of the $cDNA_{Gz\text{-}MSV/MuLV}$ hybridized with 50 ± 6S vRNA from Gz-MSV/MuLV. The $C_rt_{1/2}$ value was estimated to be 0.038 mol/s per liter. In addition, $cDNA_{Gz\text{-}MSV/MuLV}$ hybridized, relative to 50 ± 6S Gz-MSV/MuLV RNA, 92, 73, and 68% with vRNA from M-MSV/MuLV, M-MuLV, and Gz-MSV(HaLV), respectively, indicating close homology between the nucleotide sequences in M-MSV and Gz-MSV. Furthermore, $cDNA_{Gz\text{-}MSV/MuLV}$ hybridized 52, 24, and 22% with R-MuLV, Ha-MSV(HaLV), and

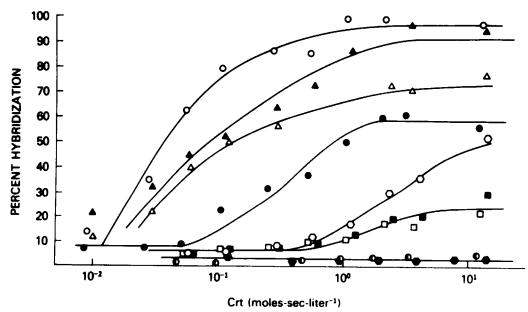


FIG. 1. *Hybridization of $cDNA_{Gz\text{-}MSV/MuLV}$ with vRNA. $cDNA_{Gz\text{-}MSV/MuLV}$ prepared from Gz-MSV/MuLV complexes was hybridized with 50 to 70S vRNA form Gz-MSV/MuLV* (○), *M-MSV/MuLV* (▲), *and M-MuLV* (△), *and with total vRNA from Gz-MSV HaLV* (●), *RLV* (◯), *Ha-MSV (HaLV)* (□), *HaLV* (■), *RSV* (◐), *and yeast* (●) *in 0.6 M $Na^+$ at 63°C as described in the text. The degree of homology was assessed by using S1 nuclease.*

HaLV, respectively. However, $cDNA_{Gz\text{-}MSV/MuLV}$ did not hybridize with RSV vRNA. The complementarity of the $cDNA_{Gz\text{-}MSV/MuLV}$ to vRNA was then tested by thermal dissociation of cDNA:RNA hybrids (Fig. 2). The $T_m$ of $cDNA_{Gz\text{-}MSV/MuLV}$ with homologous vRNA hybrids was determined to be 82.5°C, whereas those for hybrids with Gz-MSV/MSV(HaLV), M-MSV/MuLV, and M-MuLV were 83.0, 81.5, and 81.5°C, respectively, indicating complete complementarity of the sequences. However, the $T_m$ values for hybrids with Ha-MSV(HaLV), HaLV, and R-MuLV were at least 8°C below that of Gz-MSV/MuLV, suggesting mismatches of the sequences in the hybrids.

**Hybridization of $cDNA_{Gz\text{-}MSV/MuLV}$ or $cDNA_{M\text{-}MuLV}$ with vRNA's.** To confirm that Gz-MSV and M-MSV are indeed related and that the MuLV in Gz-MSV/MuLV complexes is that of the M-MuLV type, DNA complementary to vRNA from M-MSV/MuLV or M-MuLV (1869) was synthesized and hybridized with vRNA (Table 1). Tritiated $cDNA_{M\text{-}MSV/MuLV}$ hybridized with Gz-MSV/MuLV and Gz-MSV(HaLV) 93.8 and 73.3%, respectively. In addition, $cDNA_{M\text{-}MSV/MuLV}$ hybridized 76.1, 26.5, 16.5, and 47.2% with M-MuLV, HaLV, Ha-MSV(HaLV), and R-MuLV RNA, respectively.

$cDNA_{M\text{-}MuLV}$ hybridized 93.7 and 89.4% with M-MSV/MuLV and Gz-MSV/MuLV, respectively, indicating that the MuLV in the Gz-MSV/MuLV complex is of the M-MuLV type, if not a variant. This was further substantiated by the inability to detect Gz-MSV/MuLV foci on mouse 3T3 cells infected with M-MuLV, indicating specific interference. Furthermore, $cDNA_{M\text{-}MuLV}$ hybridized 46.5, 24.3, 24.7, and 71.4% with Gz-MSV(HaLV), HaLV, Ha-MSV(HaLV), and R-MuLV RNA, respectively.

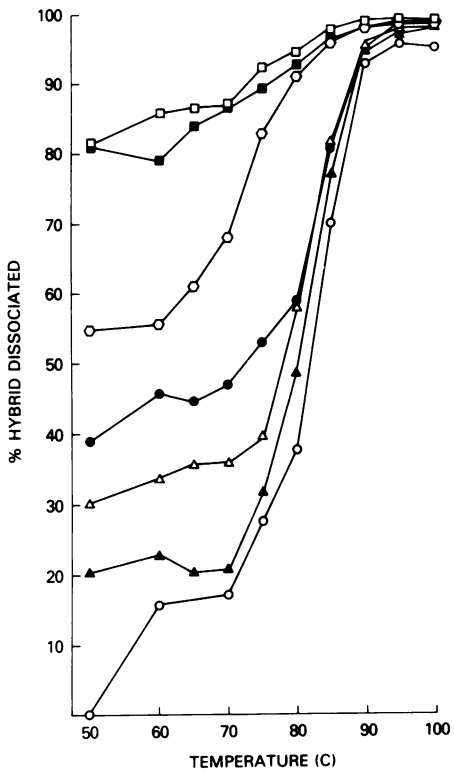


FIG. 2. *Thermal dissociation of cDNA:RNA hybrids. $cDNA_{Gz\text{-}MSV/MuLV}$ was hybridized with 50 to 70S vRNA from Gz-MSV/MuLV (○), M-MSV/MuLV (▲), and M-MuLV (△) and with total vRNA from Gz-MSV (HaLV) (●), Ha-MSV(HaLV) (□), HaLV (■), and RLV (⬡) in 0.6 M $Na^+$ at 63°C to a final C,t of at least 10 mol/s per liter. The $Na^+$ was then diluted to a final concentration of 0.18 M. The samples were then incubated for 10 min at the temperature indicated and cooled at 4°C. The percent hybrids dissociated was assessed by using S1 nuclease. Five hundred trichloroacetic acid-precipitable counts per minute was used in each sample.*

**MSV-specific sequences.** Since M-MSV and Gz-MSV were found to be related, the cDNA's specific for MSV sequences were then fractionated from $cDNA_{Gz\text{-}MSV/MuLV}$ and $cDNA_{M\text{-}MSV/MuLV}$ ($cDNA_{sarc\text{-}Gz\text{-}MSV}$ and $cDNA_{sarc\text{-}M\text{-}MSV}$, respectively) by exhaustive hybridization with M-MuLV RNA for further analysis. $cDNA_{sarc\text{-}Gz\text{-}MSV}$ hybridized, relative to the homologous reactions with Gz-MSV/MuLV, 86.7 and 79.5% to M-MSV/MuLV and Gz-MSV(HaLV) (Table 2), respectively, whereas $cDNA_{sarc\text{-}M\text{-}MSV}$ hybridized completely with Gz-MSV/MuLV and 72.5% with Gz-MSV(HaLV). This suggests that there is about 13% extra MSV-specific sequences in Gz-MSV in addition to those in M-MSV. The failure of $cDNA_{sarc\text{-}Gz\text{-}MSV}$ to hybridize with M-MuLV rules out the possibility that the extra

TABLE 1. *Hybridization of $cDNA_{M\text{-}MSV/MuLV}$ and $cDNA_{M\text{-}MuLV}$ to RNA*

| vRNA | % Homology[a] | |
|---|---|---|
| | $cDNA_{M\text{-}MSV/MuLV}$ | $cDNA_{M\text{-}MuLV}$ |
| M-MSV/MuLV[b] | 100 (85.3)[c] | 93.7 |
| M-MuLV[b] | 76.1 | 100 (79.7)[c] |
| Gz-MSV/MuLV[b] | 93.8 | 89.4 |
| Gz-MSV(HaLV) | 73.3 | 46.5 |
| HaLV | 26.5 | 24.3 |
| Ha-MSV(HaLV) | 16.5 | 24.7 |
| R-MuLV | 47.2 | 71.4 |

[a] 2,000 to 3,000 trichloroacetic acid-precipitable cpm of cDNA was hybridized to vRNA in 0.6 M $Na^+$ at 63°C to a final C,t of at least 10 mol/s per liter. The extent of hybridization was assessed by using S1 nuclease. The percent homology was normalized to 100% with corresponding homologous vRNA. The background hybridizations of cDNA with yeast RNA were found to be 5.4 and 3.6% for $cDNA_{M\text{-}MSV/MuLV}$ and $cDNA_{M\text{-}MuLV}$, respectively.

[b] 50 to 70S vRNA was used.

[c] Actual hybridization value.

TABLE 2. *Hybridization of $cDNA_{sarc\text{-}Gz\text{-}MSV}$ and $cDNA_{sarc\text{-}M\text{-}MSV}$ to vRNA*

| vRNA | $cDNA_{sarc\text{-}Gz\text{-}MSV}$ | | $cDNA_{sarc\text{-}M\text{-}MSV}$ | |
|---|---|---|---|---|
| | % Homology[a] | $T_m$ (°C)[b] | % Homology | $T_m$ (°C) |
| Gz-MSV/MuLV[c] | 100 (±1.4)[d] | 78.5 | 100 (±1.6) | 79.7 |
| Gz-MSV(HaLV) | 79.5 (±1.0) | 77.5 | 72.5 (±1.4) | 79.0 |
| M-MSV/MuLV[c] | 86.7 (±0.2) | 78.5 | 100 (±1.6) | 80.2 |
| M-MuLV[c] | 3.4 (±0.6) | —[e] | 5.8 (±0.5) | — |
| HaLV | 28.3 (±1.3) | 73.2 | 20.5 (±0.3) | 73.0 |
| Ha-MSV(HaLV) | 3.3 (±1.5) | — | 2.1 (±0.2) | — |
| RaLV | 1.8 (±0.4) | — | 1.7 (±0.2) | — |
| R-MuLV | 3.5 (±1.4) | — | — | — |

[a] 500 cpm of cDNA was hybridized with vRNA in 0.6 M $Na^+$ at 63°C to a final $C_rt$ of at least 10 mol/s per liter. The extent of hybridization was assessed by using S1 nuclease. The percent homology was normalized to 100%; the actual hybridizations of $cDNA_{sarc\text{-}Gz\text{-}MSV}$ and $cDNA_{sarc\text{-}M\text{-}MSV}$ to their homologous vRNA were 91.2 and 88.5%, respectively. The background hybridizations of $cDNA_{sarc\text{-}Gz\text{-}MSV}$ and $cDNA_{sarc\text{-}M\text{-}MSV}$ with yeast RNA were 4.4 and 6.4%, respectively.

[b] $cDNA_{sarc\text{-}Gz\text{-}MSV}$ or $cDNA_{sarc\text{-}Gz\text{-}MSV}$ hybridized with vRNA at 0.6 M $Na^+$ at 63°C for 20 h. The $T_m$ values for the hybrids were determined as described in the text.

[c] 50 to 70S vRNA was used.

[d] Variation in percent hybridization.

[e] Not done.

sequences originated from MuLV. In addition, $cDNA_{sarc\text{-}Gz\text{-}MSV}$ did not hybridize with RNA from either Ha-MSV(HaLV) or RaLV from V-NRK cells, indicating that Gz-MSV did not recombine with rat endogenous type C viruses in spite of its propagation in rat cells. Furthermore, $cDNA_{sarc\text{-}M\text{-}MSV}$ did not hybridize with RNA from both Ha-MSV(HaLV) and RaLV. However, both $cDNA_{sarc\text{-}Gz\text{-}MSV}$ and $cDNA_{sarc\text{-}M\text{-}MSV}$ hybridized with HaLV RNA 28.3 and 20.5%, respectively.

Thermal dissociation of cDNAsarc:RNA hybrids indicated that identical MSV-specific sequences were present in both Gz-MSV and M-MSV, since there was no significant difference in $T_m$ values for $cDNA_{sarc\text{-}Gz\text{-}MSV}$ or $DNA_{sarc\text{-}M\text{-}MSV}$ hybrids with RNA from Gz-MSV/MuLV or M-MSV/MuLV (Table 2). However, there was at least a 5°C greater difference in $T_m$ for hybrids of either $cDNA_{sarc}$ with RNA from HaLV than that with homologous vRNA. This suggests that MSV-related, but not identical, sequences are present in HaLV.

**Viral expression in cells.** The viral expression of Gz-MSV in RTG-1(S+L+) and HTG-2(S+L−) cells was analyzed by labeling cell proteins with [$^{35}$S]methionine. Viral proteins were then immunoprecipitated by anti-p60 serum prepared from p60 of M-MSV(FeLV) propagating in P521 cells. An autoradiogram of the labeled proteins after gel electrophoresis showed that, in both RTG-1 and HTG-2 cells, two viral proteins of 68,000 and 70,000 daltons were expressed (Fig. 3). This indicates that these proteins were phenotypic expressions of Gz-MSV in both types of cells regardless of the presence or absence of helper leukemia viruses. However, no p60 was observed as in cells infected with either the m1 or m3 isolate of M-MSV (13). In addition, p30 and another protein of 64,000 daltons were detected in RTG-1.

## DISCUSSION

Gz-MSV was found to be closely related to M-MSV by hybridization studies, as indicated by a high degree of homology between cDNA synthesized from one virus and the RNA of the other. The detection of similar $T_m$ values for the hybrids further substantiates almost, if not completely, identical sequences in these two viruses.

In addition the MSV-specific sequences in M-MSV comprise only a major portion of Gz-MSV-specific genome. This may account for the subtle differences in the biological activities of the two viruses (2–4). Furthermore, in studies where the m1 and m3 isolates of M-MSV were compared, both m1 and m3 specified p60 in cells, but m3 also expressed p70 (13). However, in RTG-1 and HTG-2 cells infected with Gz-MSV, two viral proteins, p68 and p70, respectively, were observed, but no p60 was detected in rat or hamster cells infected with either infectious or defective Gz-MSV. This further substantiates the proposal that there are differences in the Gz-MSV and m1 or m3 isolates of the M-MSV genome, because the viral expression of the gag (common) region of Gz-MSV is different from that in m1 or m3 isolates of M-MSV. p64 and p30 in RTG-1(S+L+) cells are analogous to those observed in cells infected with MuLV (6, 13, 16). This suggests that these two proteins are the phenotypic expressions of MuLV in RTG-1 cells.

The failure of $cDNA_{sarc\text{-}Gz\text{-}MSV}$ to hybridize

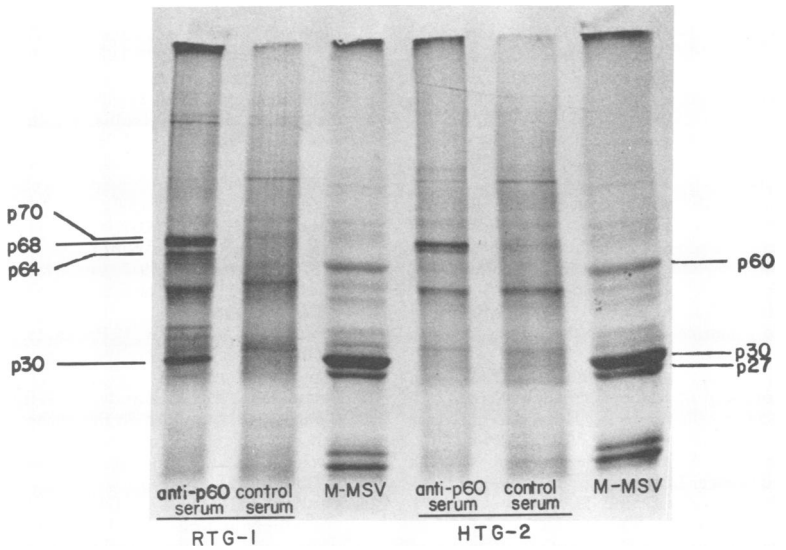


FIG. 3. *Autoradiogram of viral protein. [$^{35}$S]methionine-labeled viral proteins, immunoprecipitated by anti-p60 serum from M-MSV(FeLV), were subjected to 6 to 12% sodium dodecyl sulfate-gel electrophoresis and autoradiography as described in the text. Viral proteins from M-MSV(FeLV) served as markers.*

with RNA from RaLV and Ha-MSV(HaLV) indicates that Gz-MSV did not recombine with rat endogenous type C viral sequences in spite of its propagation in rat cells. This suggests that the integration site of Gz-MSV in rat cells may be different from that of the endogenous type C virus or that a complete set of sequences for cell transformation is so conserved in Gz-MSV that no additional sequences by recombination with rat endogenous viral sequences are required for expression of transformation properties as in the case of Kirsten or Harvey MSV.

$cDNA_{sarc\text{-}Gz\text{-}MSV}$ and $cDNA_{sarc\text{-}M\text{-}MSV}$ hybridized 79.5 and 72.5%, respectively, with vRNA from Gz-MSV(HaLV), although both cDNAsarc's hybridized completely with 50 ± 6S vRNA from Gz-MSV/MuLV. This may be due to the deletion of nucleic acid sequences in the defective Gz-MSV(HaLV) propagated in hamster cells (HTG-2).

Furthermore, both MSV-specific sequences from Gz-MSV and M-MSV hybridized with vRNA from HaLV produced in F-2833 cells, although a lower $T_m$ was detected for the hybrids than for those prepared by hybridization of cDNA with homologous vRNA. This suggests that MSV-specific sequences are related to HaLV nucleotide sequences. The similar degrees of homology and $T_m$ values of the hybrids from $cDNA_{sarc\text{-}Gz\text{-}MSV}$ and $cDNA_{sarc\text{-}M\text{-}MSV}$ with HaLV RNA indicate that the sequences related to HaLV are located in the common MSV-specific sequences shared by both Gz-MSV and M-MSV. In addition, the partial homology between MSV-specific sequences from Gz-MSV or M-MSV with those in HaLV suggests that Gz-MSV, M-MSV, and HaLV may share a common origin for some of the nucleic acid sequences.

Although some HaLV sequences were reported to be present in viruses produced by B34 cells (10), $cDNA_{sarc\text{-}Gz\text{-}MSV}$ and $cDNA_{sarc\text{-}M\text{-}MSV}$ did not hybridize with vRNA from Ha-MSV(HaLV) propagated in B34 cells. This suggests that the HaLV sequences related to MSV-specific sequences are absent in B34 viral particles. Reciprocal hybridization of cDNA complementary to Ha-MSV(HaLV) or HaLV from F-2833 cells with Gz-MSV and M-MSV vRNA is not feasible at present because cDNA could not be synthesized in our laboratory.

In conclusion, Gz-MSV and M-MSV are closely related and, possibly, Gz-MSV is a variant of M-MSV.

## ACKNOWLEDGMENTS

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