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Identification of Proteins Encoded by the Gazdar Murine Sarcoma Virus Genome by In Vitro Translation and Comparison with Moloney Murine Sarcoma Virus 124

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The gene products of Gazdar murine sarcoma virus (Gz-MuSV) were identified by in vitro translation of Gz-MuSV virion RNA. An overlapping set of proteins with approximate molecular weights of 37,000 (37K), 33K, 24K, and 18K were synthesized from the transforming gene of Gz-MuSV, v-*mos*^{Gz}. In addition, Gz-MuSV-specific RNA directed the in vitro synthesis of a 62K *gag* gene protein and a 37.5K *env* gene-related product. The Gz-MuSV-specific in vitro translation products were compared with the in vitro translation products of M-MuSV 124, an independent isolate with a similar v-*mos* gene. This analysis showed that the 62K Gz-MuSV *gag* gene protein and the 37K, 33K, 24K, and 18K v-*mos*^{Gz} proteins were almost identical to the M-MuSV 124 62K (*gag*) and 37K, 33K, 24K, and 18K (v-*mos*^{Mo}) proteins that we previously identified and characterized. The 37.5K *env* gene product from Gz-MuSV does not have a correlate in the M-MuSV 124 translation products. These results were analyzed in the context of expectations based on similarities and differences in genetic organization of these two viral genomes.

Several different strains of murine sarcoma virus (MuSV) have been isolated from tumors in mice. The most extensively characterized of these viruses is a clonal isolate of Moloney MuSV (M-MuSV 124) which was derived from an original virus stock obtained from a tumor that arose in a BALB/c mouse after injection with Moloney murine leukemia virus (M-MuLV) (2, 22). It is believed that the genome of M-MuSV 124 is the product of a recombination event between M-MuLV and mouse cellular sequences, since the M-MuSV 124 genome contains a block of 1,157 nucleotides which are absent from the parental M-MuLV genome and which can be identified in the genomes of uninfected mouse cells (9, 11–14, 17, 18, 23, 29, 30, 32–34). These cell-derived sequences, defined as the v-*mos*^{Mo} gene, contain the sarcomagenic information of M-MuSV 124 (1, 5–7, 35, 36). The M-MuSV 124 genome is also replication defective as a consequence of several deletions within the *pol* and *env* genes (12, 17, 29, 34).

Gazdar MuSV (Gz-MuSV), a reportedly independent isolate, was derived after serial passage in vivo of a spontaneous tumor which arose in a NZW/NZB F_1 hybrid mouse (15, 16). Heteroduplex mapping (12) and hybridization analysis (24) show that the genome of Gz-MuSV contains a v-*mos* gene (v-*mos*^{Gz}) homologous to the v-*mos*^{Mo} gene of M-MuSV 124. Furthermore, the Gz-MuSV *gag* gene is similar to that of M-MuSV 124. There are, however, two major differences between the genomes. First, Gz-MuSV has no *pol* gene sequences (12), whereas M-MuSV 124 retains two fragments from the parental *pol* gene (12, 17). Secondly, the Gz-MuSV genome has approximately 1,200 nucleotides of *env* sequence immediately 5′ to v-*mos*^{Gz} (12). Nucleotide sequence analysis shows, on the other hand, that the M-MuSV 124 genome contains only 12 nucleotides of coding sequence from the *env* gene on the 5′ side of v-*mos*^{Mo} (11, 30, 33). Although the parental virus of Gz-MuSV is unknown, it seems likely, based on the extensive homology with M-MuSV 124, that Gz-MuSV arose by a similar recombination event between a nonsarcomagenic retrovirus and mouse cellular sequences.

Several groups have carried out in vitro translation experiments with M-MuSV 124 virion RNA to identify v-*mos*^{Mo} gene products (8, 10, 19, 25, 26). In vitro translation of M-MuSV 124 virion RNA preparations which contained full-length genomic RNA as well as naturally occurring subgenomic fragments yielded a product with a molecular weight of 65,000 (65K) synthesized from the 5′ *gag* gene of M-MuSV 124 as well as an overlapping set of v-*mos*^{Mo} proteins, with molecular weights of 37K, 33K, 24K, and 18K, which shared a common COOH terminus

(25, 26). Synthesis of the largest v-*mos* protein, 37K, was initiated at an AUG codon in the M-MuSV 124 *env* gene sequences 12 nucleotides upstream of the v-*mos*^{Mo} insert (26). The 37K v-*mos*^{Mo} protein, therefore, shares its NH_2-terminal five amino acids with the parental MuLV *env* gene product. A v-*mos*^{Mo} protein similar to the 37K in vitro product has been identified in M-MuSV 124-transformed cells (27). Synthesis of the 33K, 24K, and 18K in vitro products, which contain a subset of the sequences in the 37K v-*mos*^{Mo} protein, is initiated at AUG codons within the v-*mos*^{Mo} gene (26). These smaller proteins are not seen in M-MuSV 124-transformed cells (27) and probably result from the ability of the in vitro translation system to initiate synthesis from AUG codons near the 5′ ends of v-*mos*^{Mo}-containing genomic RNA fragments (25).

To identify Gz-MuSV-encoded proteins, we analyzed the in vitro translation products from Gz-MuSV virion RNA and compared them with the in vitro products from M-MuSV 124 virion RNA, which we had previously identified and characterized (25, 26). Based on the homology between the two genomes (12, 24), we expected Gz-MuSV virion RNA to direct the synthesis of a similar in vitro *gag* gene product. It was not clear, however, that the expression of the Gz-MuSV *mos* gene would be similar to that of M-MuSV 124. One possibility was that translation of the Gz-MuSV v-*mos*^{Gz} gene would start at the *env* gene initiator AUG codon and extend through the v-*mos*^{Gz} sequences. This would result in an *env-mos* fusion protein from Gz-MuSV of approximately 74K. There might also be smaller proteins whose synthesis was initiated from internal AUG codons similar to the smaller proteins of M-MuSV. Contrary to these expectations, the only detectable in vitro translation products from the Gz-MuSV v-*mos*^{Gz} gene have approximate molecular weights of 37K, 33K, 24K, and 18K and appear to be indistinguishable from the M-MuSV 124 37K set of in vitro v-*mos*^{Mo} translation products. In addition, in vitro translation of Gz-MuSV virion RNA yields a 62K *gag* gene product, as expected, and a 37.5K protein containing sequences related to the MuLV *env* gene product.

MATERIALS AND METHODS

Virus and RNA. The TB (clone 124) mouse cell line (2) producing the M-MuSV(M-MuLV) complex was obtained from J. Ball, University of Western Ontario, Ontario. The rat cell line (RTG-1) producing the Gz-MuSV(MuLV) complex and a mouse cell line (PLV100) producing only the helper MuLV isolated from the Gz-MuSV(MuLV) stock were obtained from D. Haapala, National Cancer Institute. Virus was purified from medium harvested from the various cell lines as described previously (25). Total RNA was extracted from purified virus, and 60 to 70S RNA was isolated by sucrose gradient centrifugation. The 60 to 70S RNA was precipitated twice with ethanol and lyophilized from water before being taken up at 1 mg/ml and stored in liquid nitrogen. Polyadenylic acid [poly(A)]-containing RNA was selected from Gz-MuSV virion RNA and size fractionated exactly as described previously (25).

In vitro translation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In vitro translation, using the messenger-dependent reticulocyte lysate, was carried out as described previously (3, 25). Small aliquots of virion RNA (1 mg/ml) were heated to 100°C for 1 min and were quickly cooled. Viral RNA was added to the translation mix at a final concentration of 50 μg/ml. [^{35}S]methionine (Amersham Corp.) was used at 1 mCi/ml. Completed translation reactions were analyzed on sodium dodecyl sulfate (SDS)-polyacrylamide gels containing 12.5% acrylamide and 0.1% bisacrylamide as described previously (25). Radioactive proteins were detected by fluorography.

Hybrid arrest translation. The hybrid arrest translation (HART) experiment with cloned recombinant DNA and Gz-MuSV virion RNA was carried out exactly as described previously (26). The recombinant plasmid pMSV-31, used for the HART experiment (see Fig. 3) and the selection experiment (see Fig. 2), has been described elsewhere (33) and contains the entire v-*mos*^{Mo} region between the restriction sites *Xba*I and *Hin*dIII. The molar ratio of pMSV-31 to total Gz-MuSV virion RNA was 3:1 and was sufficient to give maximal inhibition of synthesis of v-*mos*-encoded proteins.

Antisera and immunoprecipitation. Anti-p30 (MuLV) and anti-gp70 (MuLV) sera were kindly provided by H. Fan, The Salk Institute, San Diego. The antiserum against a synthetic peptide consisting of the 12 COOH-terminal amino acids of the v-*mos*^{Mo} gene product has been described elsewhere (26) and is referred to as anti-C-3. Immunoprecipitation of in vitro translation products was performed as described elsewhere (25, 26). In the experiments shown below (see Fig. 4 and 6), 5 μl of translation product was diluted to 150 μl with 0.15 M NaCl–0.01 M sodium phosphate (pH 7.0)–1% Nonidet P-40–1% sodium deoxycholate–0.1% SDS–1% Trasylol and was immunoprecipitated with 1 μl of antiserum. Where indicated, 3 μl of a stock solution (1 mg/ml) of C-3 peptide was incubated with 1 μl of anti-C-3 serum before immunoprecipitation. In every case, the entire immunoprecipitate was analyzed by SDS-polyacrylamide gel electrophoresis.

Selection of Gz-MuSV-specific virion RNA. A cloned recombinant DNA containing v-*mos*^{Mo} sequences (pMSV-31) was immobilized on a nitrocellulose filter and used to select Gz-MuSV-specific virion RNA (see Fig. 2). The experimental procedure is essentially the same as described by Ricciardi et al. (31). Fifty micrograms of *Eco*RI-digested, phenol-extracted pMSV-31 DNA was applied to a nitrocellulose filter (Schleicher and Schuell; 0.45-μm pore size; 24-mm diameter). One-fourth of this filter was used to select Gz-MuSV-specific RNA from 20 μg of total Gz-MuSV virion RNA. Hybridization was carried out at 37°C for 6 h. The filter was washed extensively, and RNA was eluted by boiling for 1 min (×2) in 100 μl of water followed by quick cooling. The eluted RNA was lyophilized and resuspended in 3 μl of water. One microliter was translated in 10 μl of lysate, and 2 μl of

the translation reaction was analyzed by SDS-polyacrylamide gel electrophoresis as described above.

Peptide mapping. Either 3.5 μg of M-MuSV 124 or 3.5 μg of Gz-MuSV virion RNA was translated in 70 μl of reticulocyte lysate, with a final concentration of 4 mCi of [^{35}S]methionine per ml. The entire translation reaction was analyzed on a 1-mm-thick SDS-polyacrylamide slab gel. The bands corresponding to the 62K *gag* and 37K v-*mos* proteins from both M-MuSV 124 and Gz-MuSV were extracted from the dried gels, and chymotryptic peptide mapping of the 37K proteins and tryptic peptide mapping of the 62K proteins were performed as described previously (25, 26).

RESULTS

In vitro translation of Gz-MuSV virion RNA; preliminary identification of Gz-MuSV-specific products. Purified 60 to 70S RNA was isolated from Gz-MuSV, MuLV, or M-MuSV 124 virions. Each viral RNA preparation was heat denatured and translated in the mRNA-dependent reticulocyte lysate in the presence of [^{35}S]methionine, and the products were analyzed by SDS-polyacrylamide gel electrophoresis. The total translation products from Gz-MuSV virion RNA contained a complex array of proteins (Fig. 1B). Unlike M-MuSV 124 (Fig. 1A) (4, 20), Gz-MuSV virion RNA preparations yielded many translation products which comigrated with the translation products from RNA of the helper MuLV (Fig. 1C). Several potential Gz-MuSV-specific products can be distinguished, however, by comparing the sizes of proteins synthesized from Gz-MuSV virion RNA preparations with proteins synthesized from the helper MuLV. By this criterion, the translation products that were unique to Gz-MuSV had approximate molecular weights of 62K, 37.5K, 37K, 33K, 24K, and 18K. The 62K protein from Gz-MuSV migrated slightly faster than the M-MuSV 124-specific 62K *gag* protein. The 37K, 33K, 24K, and 18K translation products appeared to migrate very similarly to the corresponding proteins, which are specific translation products of M-MuSV 124 from the *mos* gene (37K, 33K, 24K, 18K) (25, 26). A protein of approximately 30K (denoted by a star in Fig. 1) appeared, in this experiment, to be unique to Gz-MuSV. This protein was not, however, reproducibly synthesized from different preparations of Gz-MuSV virion RNA. Furthermore, the 30K protein appeared to be unrelated to *gag*, *env*, or *mos* gene products by the criteria outlined in this paper. For these reasons, the 30K protein will not be discussed further.

To identify more rigorously the Gz-MuSV-specific in vitro translation products, the following experiment was carried out. A cloned recombinant DNA containing M-MuSV 124 v-*mos*^{Mo} sequences was hybridized to a Gz-MuSV virion RNA preparation. After extensive washing, the hybridized RNA was eluted and translated in vitro. This procedure specifically selects full-length genomic RNA as well as fragments from Gz-MuSV by virtue of the v-*mos* sequences that are unique to Gz-MuSV but not to the helper MuLV. The translation products of this selected RNA should represent the complete coding potential of the Gz-MuSV genome in the absence of helper MuLV products. Proteins with molecular weights of approximately 62K, 37.5K, 33K, 24K, and 18K were synthesized in vitro from the "selected RNA" (Fig. 2C). This pattern was strikingly similar to the total translation products from M-MuSV 124 virion RNA (Fig. 2B), which contained undetectable levels of helper virus proteins.

(i) Identification of Gz-MuSV in vitro v-*mos*^{Gz}

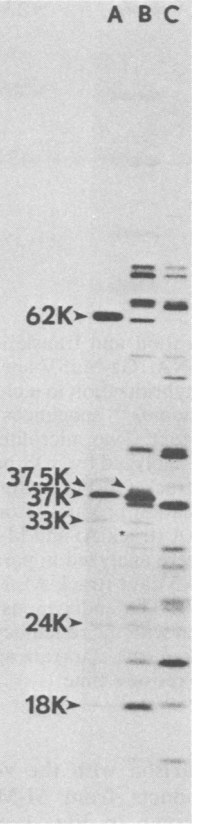

FIG. 1. Translation products from M-MuSV 124, Gz-MuSV, and MuLV virion RNAs. Purified 60 to 70S virion RNAs were heat denatured and translated in the mRNA-dependent reticulocyte lysate as described in the text. One microliter of each translation reaction was analyzed by SDS-polyacrylamide gel electrophoresis and visualized by fluorography. The gel was exposed to film for 2 days. Total translation products from M-MuSV 124 (track A), from Gz-MuSV (track B), and from the helper MuLV cloned from the Gz-MuSV(MuLV) stock (track C) are shown.

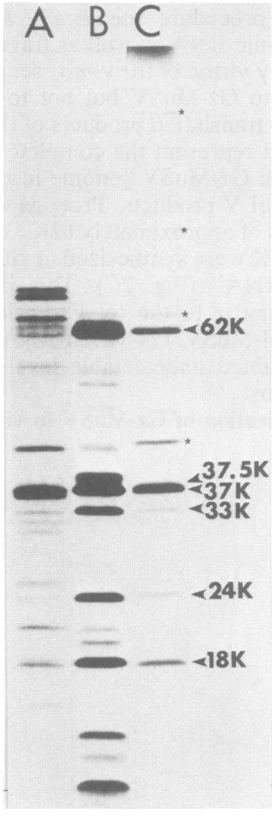

FIG. 2. Purification and translation of Gz-MuSV-specific virion RNA. Gz-MuSV-specific virion RNA was selected by hybridization to a cloned recombinant DNA containing v-*mos*^{Mo} sequences and translated as described in the text. Two microliters of the translation reaction was analyzed by SDS-polyacrylamide gel electrophoresis (track C). For comparison, 1 μl each of the total translation products from unselected Gz-MuSV virion RNA (track A) and M-MuSV 124 virion RNA (track B) were analyzed in parallel. The gel was fluorographed for 3 days (track A), 1 day (track B), or 14 days (track C). The endogenous [^{35}S]methionine-labeled bands seen with the reticulocyte lysate translation system (marked with stars) are apparent in track C due to the long exposure time.

proteins; comparison with the v-*mos*^{Mo} in vitro translation products from M-MuSV 124. The experiments shown in Fig. 1 and 2 identified which of the total translation products from Gz-MuSV virion RNA preparations were derived from Gz-MuSV-specific sequences. To identify proteins synthesized specifically from v-*mos*^{Gz} sequences, a HART experiment was performed. In this experiment, total Gz-MuSV virion RNA was hybridized with a cloned recombinant DNA containing the entire v-*mos*^{Mo} gene from M-MuSV 124. Half of the hybrid preparation was translated directly (hybrid), whereas the other half was denatured before translation (melt). The v-*mos*^{Gz}-specific translation products, i.e., those which were missing from a translation of the hybrid and whose synthesis was restored upon melting the hybrids before translation, had molecular weights of 37K, 33K, 24K, and 18K (Fig. 3, H and M). By this criterion, no proteins larger than 37K were encoded by the Gz-MuSV v-*mos*^{Gz} gene. As a control, a HART experiment carried out in the absence of added DNA did not affect synthesis of any of the Gz-MuSV in vitro translation products (data not shown). These HART results are identical to the results obtained using this same recombinant DNA in a HART experiment with virion RNA from M-MuSV 124 (26).

Since the Gz-MuSV v-*mos*^{Gz} in vitro translation products appeared to be the same sizes as the v-*mos*^{Mo} proteins synthesized from M-MuSV 124 virion RNA, it was of interest to determine whether the v-*mos* proteins from the

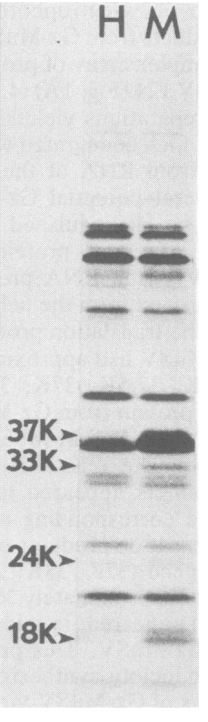

FIG. 3. HART, with a cloned recombinant DNA containing the entire v-*mos* region. HART and gel electrophoresis were carried out as outlined in the text. Two microliters of each translation reaction was analyzed on an SDS-polyacrylamide gel which was subsequently fluorographed for 3 days. Track H shows the translation products from the DNA/RNA hybrid, and track M shows the translation products from the melted DNA/RNA hybrid. Note that the 37K Gz-MuSV-specific protein is not well resolved, on this gel, from the 36K helper MuLV protein.

two viruses also had similar amino acid sequences. We previously showed that an antiserum (anti-C-3) raised against a synthetic peptide corresponding to the COOH-terminal 12 amino acids of a protein predicted from the v-*mos*^{Mo} DNA sequence precipitated the 37K, 33K, 24K, and 18K in vitro v-*mos*^{Mo} proteins from M-MuSV 124 (26). Figure 4B shows that this same serum can immunoprecipitate the 37K, 33K, 24K, and 18K Gz-MuSV v-*mos*^{Gz} proteins. The immunoprecipitation of the v-*mos*^{Gz} proteins by anti-C-3 serum was specifically blocked with an excess of C-3 peptide (Fig. 4C). The v-*mos*^{Gz} in vitro products from Gz-MuSV have, therefore, a similar, if not identical, COOH-terminal amino acid sequence to that of the v-*mos*^{Mo} in vitro products from M-MuSV 124. To examine whether there was further homology, the [^{35}S]methionine chymotryptic peptide map of 37K v-*mos*^{Gz} was compared with the map of 37K v-*mos*^{Mo}. Figure 5 (D, E, and F) shows that the chymotryptic maps of these two proteins are virtually identical. As was the case for M-MuSV 124, the Gz-MuSV 33K, 24K, and 18K in vitro products comprised a subset of the sequences in the 37K protein (data not shown). Only the peptide map of the v-*mos*^{Gz} 37K protein is shown as a representative of this group.

(ii) Identification of Gz-MuSV-encoded *gag* and *env* proteins. To test which, if any, of the Gz-MuSV-specific in vitro translation products were encoded by *gag* or *env* gene sequences, the total translation products from Gz-MuSV were immunoprecipitated with antisera directed against p30, the major MuLV structural protein encoded by the *gag* gene, and antisera against gp70, a glycoprotein derived from the MuLV *env* gene. For comparison, the translation products of the helper MuLV virion RNA were immunoprecipitated in parallel. Four proteins were seen in the immunoprecipitate made with anti-p30 serum from the translation products of Gz-MuSV virion RNA (Fig. 6). One of these proteins corresponded to the Gz-MuSV-specific protein of 62K, whereas the other three were larger proteins that could also be recognized in the translation products from the helper MuLV virion RNA (Fig. 6). Anti-gp70 serum recognized the Gz-MuSV-specific translation product of 37.5K as well as two proteins of approximately 65K and 36K, which appear to be derived from the helper MuLV RNA since they are also precipitated with anti-gp70 serum from the helper MuLV translation products (Fig. 6). Thus, Gz-MuSV-specific sequences contained within Gz-MuSV virion RNA preparations are translated in vitro to produce a 62K *gag* gene protein as well as a 37.5K *env* gene protein. The genomic RNA of M-MuSV 124 codes in vitro for a 62K *gag* gene product, but in contrast, does not yield any *env* gene-related proteins, as expected from the lack of *env* gene coding sequences (25).

Tryptic peptide maps of the Gz-MuSV 62K protein were compared with the M-MuSV 124 62K protein to assess the extent of homology between these two proteins. The two-dimensional tryptic peptide maps of the two *gag* gene products prepared from [^{35}S]methionine-labeled samples were almost identical (Fig. 5A and B). A mixture of the two digests revealed that peptides D and E of M-MuSV 124 run slightly faster in the second dimension than the corresponding peptides of Gz-MuSV (Fig. 5C).

Heteroduplex mapping experiments showed that the Gz-MuSV genome retains the 5′ 1,200 nucleotides of the parental MuLV *env* gene (12). These *env* sequences are located immediately upstream of the v-*mos*^{Gz} insert and could code for a protein of about 40K. To test the possibility that the 37.5K Gz-MuSV-specific *env* gene product was synthesized from these sequences, the

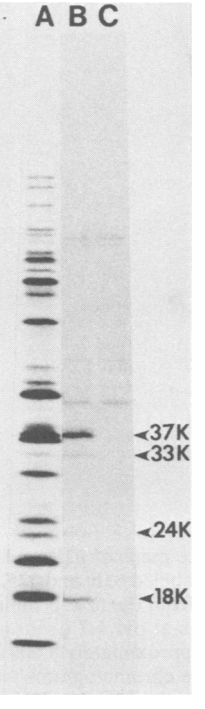

FIG. 4. Immunoprecipitation of Gz-MuSV in vitro translation products with anti-peptide serum. Five microliters of total Gz-MuSV in vitro translation product were immunoprecipitated as described previously either with 1 μl of anti-C-3 peptide serum (track B) or with 1 μl of anti-C-3 peptide serum preabsorbed with 3 μg of C-3 peptide (track C). The entire immunoprecipitate was analyzed on an SDS-polyacrylamide gel. One microliter of the total translation reaction was run in track A. The gel was fluorographed for 1 day (track A) and 3 days (tracks B and C).

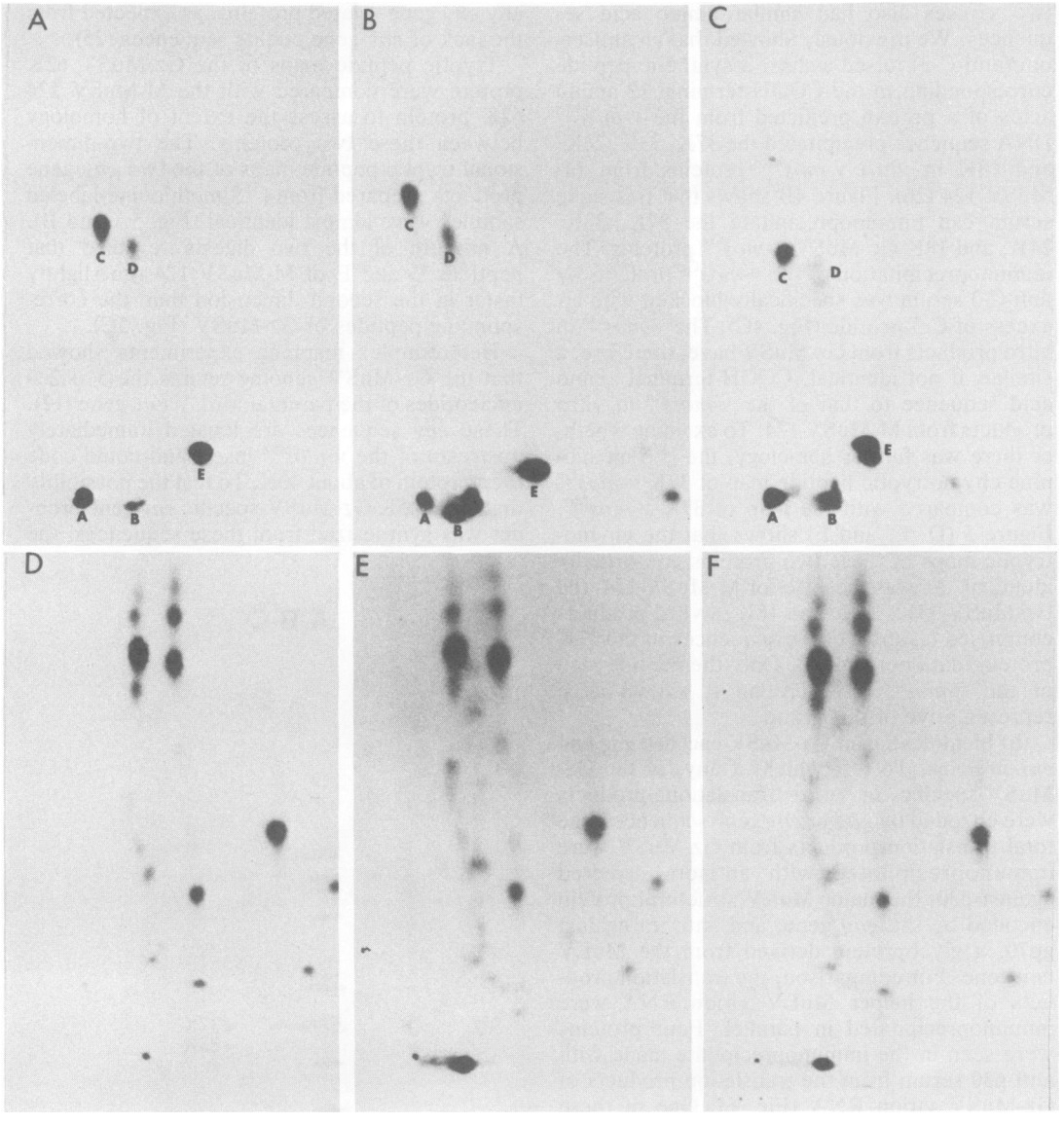

FIG. 5. Peptide maps of 62K and 37K translation products from Gz-MuSV and M-MuSV 124 virion RNAs. [^{35}S]methionine-labeled 62K and 37K in vitro translation products were isolated as described in the text. Tryptic digests prepared from the 62K proteins and chymotryptic digests prepared from the 37K proteins were resolved by electrophoresis at pH 4.7 (from left to right, toward the cathode) and by ascending chromatography (from bottom to top). Approximately 8,000 cpm of 62K protein digests and 10,000 cpm at 37K protein digests were used for each map. The chromatograms were coated with 2-methylnaphthalene containing 0.4% 2,5-diphenyloxazole and exposed to film for 10 days (62K maps) or 15 days (37K maps). (A) Tryptic peptide map of M-MuSV 124 62K, (B) tryptic peptide map of Gz-MuSV 62K, (C) mixture of tryptic digests from M-MuSV 124 62K and from Gz-MuSV 62K, (D) chymotryptic peptide map of M-MuSV 124 37K, (E) chymotryptic peptide map of Gz-MuSV 37K, (F) mixture of chymotryptic digests from M-MuSV 124 37K and from Gz-MuSV 37K.

following experiment was performed. Poly(A)-containing RNA was selected by oligodeoxythymidylic acid cellulose chromatography from heat-denatured Gz-MuSV virion RNA preparations. The poly(A)-selected RNA, which contained full-length genomic RNA as well as a series of polyadenylated fragments, was fractionated by sedimentation through a neutral sucrose gradient, and individual fractions were translated in vitro. We previously used this

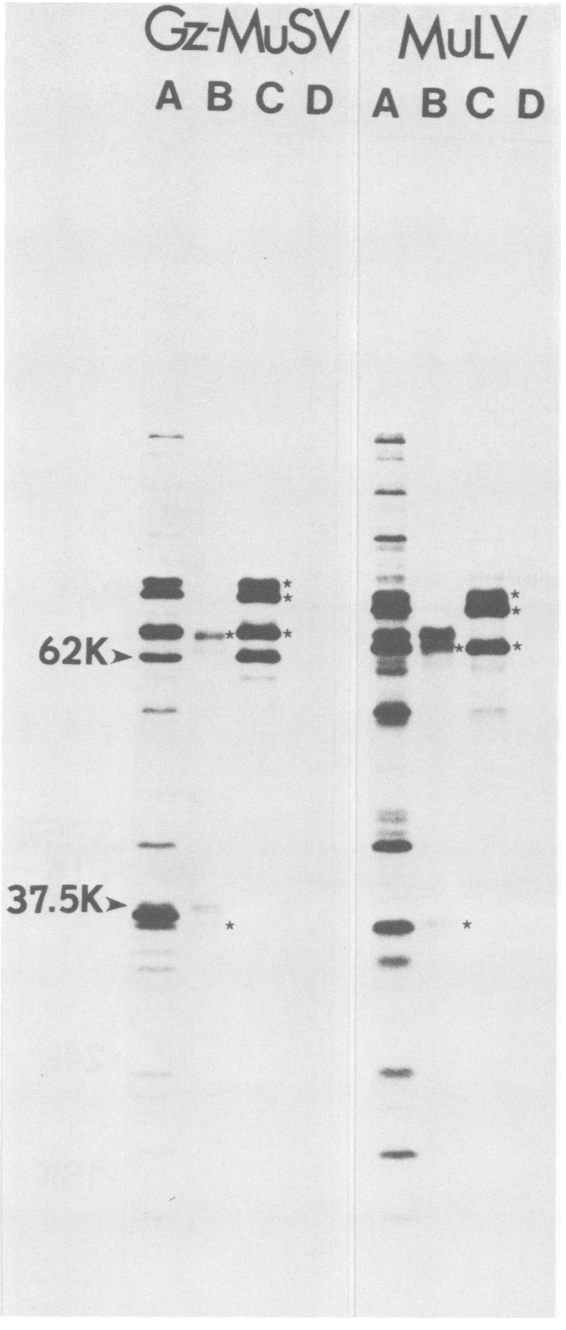

FIG. 6. Immunoprecipitation of Gz-MuSV and helper MuLV translation products with antisera against *gag* and *env* proteins. Five-microliter samples of total Gz-MuSV or total MuLV translation product were immunoprecipitated, as outlined in the text, with 1 μl of anti-gp70 serum (tracks B), 1 μl of anti-p30 serum (tracks C), or 1 μl of normal rabbit serum (tracks D). One-half of each immunoprecipitate was analyzed by SDS-polyacrylamide gel electrophoresis. One microliter of either total Gz-MuSV or total MuLV translation product was run in tracks A. The gel was fluorographed for 2 days. The 62K and 37.5K Gz-MuSV-specific proteins are marked with arrows. For comparison, several proteins, which can be identified in the Gz-MuSV(MuLV) translation products as well as in the translation products from the helper MuLV alone, are denoted by stars.

approach to show that synthesis of the 62K *gag* gene product is initiated at the 5′ end of the M-MuSV 124 genome and that the M-MuSV 124 37K, 33K, 24K, and 18K proteins are synthesized in vitro from RNA fragments containing the 3′ *mos* gene sequences (25). In the present study, based on the structure of the Gz-MuSV genome we expected that the Gz-MuSV 37.5K *env* gene product would be translated in vitro from a polyadenylated genomic RNA fragment of about 3,450 nucleotides, with the Gz-MuSV *env* gene sequences at its 5′ end. The results of this experiment (shown in Fig. 7) demonstrate, as predicted, that the 37.5K *env* protein is synthesized from a polyadenylated RNA of about 24S (circa 3,600 nucleotides). In agreement with conclusions from the experiments presented in section (i), the 37K, 33K, 24K, and 18K v-*mos*^{Gz} products were translated from poly(A)-containing RNAs of 18 to 21S, the size expected for polyadenylated 3′ Gz-MuSV genomic RNA fragments containing v-*mos*^{Gz} sequences at their 5′ ends. In addition, this experiment confirms that the 62K protein is derived from the *gag* gene of Gz-MuSV since it is synthesized primarily from polyadenylated RNA of approximately 32S, the size of Gz-MuSV genomic RNA (12, 24). The larger *gag* gene proteins seen in the translation products of Gz-MuSV virion RNA were synthesized from polyadenylated RNA of approximately 35S, presumably the genomic RNA of the helper MuLV.

DISCUSSION

We previously identified and characterized the gene products from M-MuSV 124 by in vitro translation of virion RNA (25, 26). In these experiments, we identified a 62K *gag* gene protein as well as an overlapping set of v-*mos*^{Mo}-encoded products, with molecular weights of 37K, 33K, 24K, and 18K, which shared a common COOH terminus. In this report the coding potential of the Gz-MuSV genome, an independent MuSV isolate, was analyzed in a similar fashion and the results were compared with the results obtained for M-MuSV 124.

The pattern of protein synthesis from Gz-MuSV virion RNA is much more complex than that seen with M-MuSV 124 virion RNA. This reflects the presence of many proteins synthesized from the genomic RNA of the helper MuLV, which presumably comprises a signifi-

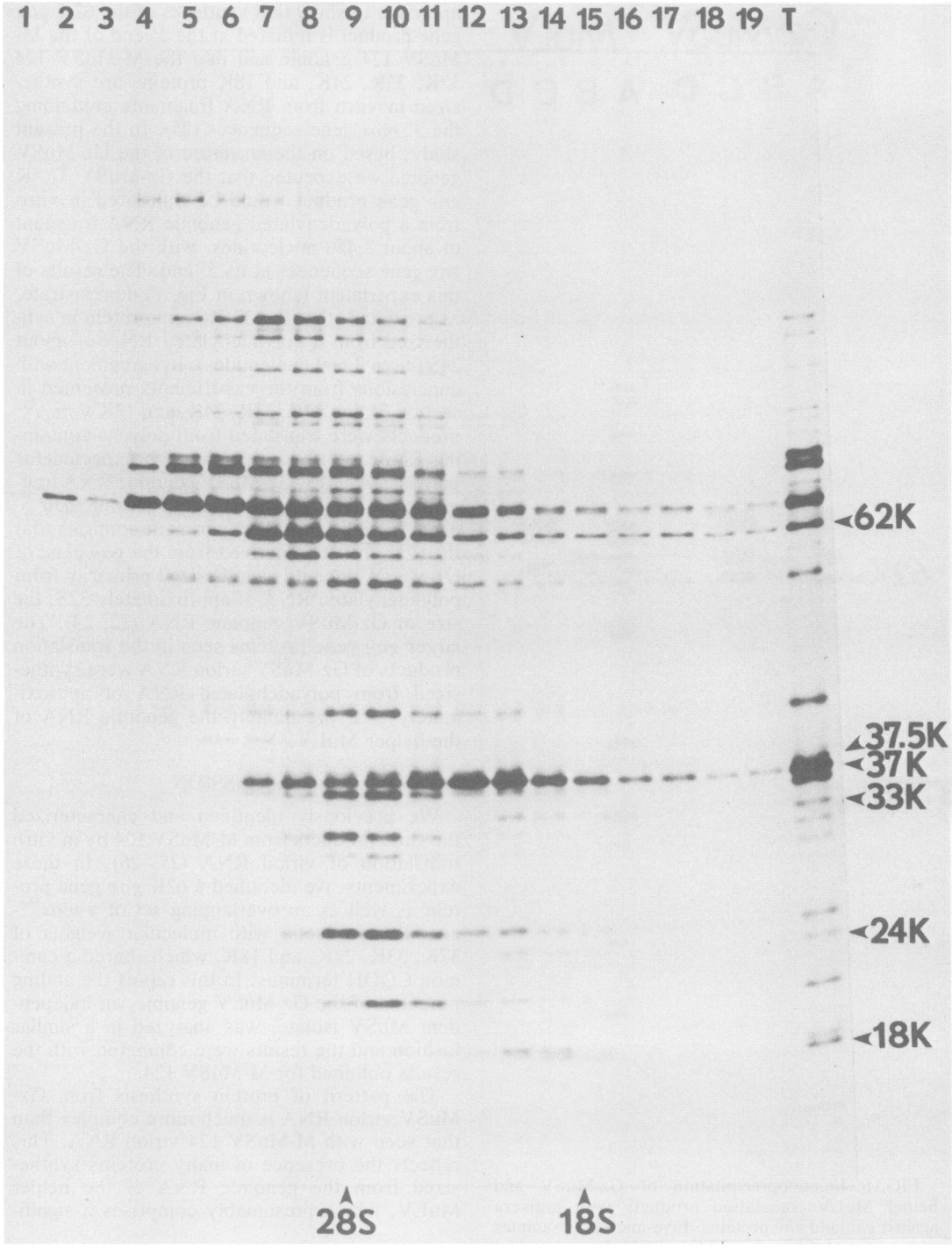

FIG. 7. Size fractionation and translation of poly(A)-containing Gz-MuSV virion RNA. Poly(A)-containing Gz-MuSV virion RNA was isolated from 20 μg of total Gz-MuSV virion RNA and sedimented through a neutral sucrose gradient as described in the text. One-tenth of the RNA in each fraction was translated in vitro in a final volume of 10 μl, and 1.5 μl of each reaction was analyzed by SDS-polyacrylamide gel electrophoresis. The gel was fluorographed for 2 days. Tracks 1 through 19 show translation products from the bottom to the top of the gradient. Track T shows the total translation products of unfractionated Gz-MuSV virion RNA. The positions of 28S and 18S ribosomal RNA run in a parallel gradient are shown.

cant portion of the Gz-MuSV virion RNA preparation. M-MuSV 124 virion RNA, on the other hand, contains very low levels of helper M-MuLV RNA (4, 20) and, consequently, is translated in vitro to yield primarily M-MuSV 124-specific proteins.

Several in vitro products specific to Gz-MuSV can be identified by comparing the total translation products from Gz-MuSV virion RNA with the total translation products from the helper MuLV. The proteins unique to Gz-MuSV have approximate molecular weights of 62K, 37.5K, 37K, 33K, 24K, and 18K. We have also shown by more rigorous criteria that these proteins are unique translation products from Gz-MuSV RNA since they are the only proteins synthesized from RNA selected from Gz-MuSV virion RNA preparations by hybridization with v-*mos* DNA bound to a nitrocellulose filter.

For the purposes of this discussion, a single major difference between the genomic RNA of Gz-MuSV and the genomic RNA of M-MuSV 124 is important. Heteroduplex mapping shows that the Gz-MuSV genome has about 1,200 nucleotides of MuLV-derived *env* gene sequences 5′ to the v-*mos*^{Gz} gene (12), whereas nucleotide sequencing shows that only the most 5′ 12 nucleotides of the *env* gene are retained in the M-MuSV 124 genome (11, 30, 33). We have previously demonstrated that the AUG codon used to initiate synthesis in vitro of the M-MuSV 124 37K v-*mos*^{Mo} protein lies in the *env* gene sequences 5′ to the v-*mos*^{Mo} gene (26). This AUG is the initiator for the parental *env* gene product, and consequently, the 37K v-*mos*^{Mo} protein shares its first five amino acids with the MuLV *env* gene product. Since the Gz-MuSV genome contains roughly 1,200 nucleotides of *env* gene coding sequence between the *env* gene initiator AUG and the beginning of v-*mos*^{Gz} (12), it was possible that the v-*mos*^{Gz} product would be an *env-mos* fusion protein larger than the 37K v-*mos*^{Mo} product. Contrary to this prediction, we found, by HART, that the only Gz-MuSV v-*mos*^{Gz}-specific in vitro translation products had molecular weights of 37K, 33K, 24K, and 18K, exactly the same sizes as the v-*mos*^{Mo} proteins from M-MuSV 124. Furthermore, the Gz-MuSV v-*mos*^{Gz} proteins had the same COOH terminus as that of the counterpart M-MuSV 124 v-*mos*^{Mo} proteins since they were immunoprecipitated with an antiserum specific for the COOH-terminal 12 amino acids of the M-MuSV 124 v-*mos*^{Mo} products. Finally, the two-dimensional [^{35}S]methionine-containing chymotryptic peptide maps of the 37K protein from Gz-MuSV and the 37K protein from M-MuSV 124 were virtually identical. It is possible that we have not detected minor differences between these two proteins since the methionine-containing chymotryptic peptides probably represent only 10% of the protein sequence. We previously demonstrated that the synthesis of the 33K, 24K, and 18K proteins is initiated at AUG codons, which code for internal methionines within the largest 37K v-*mos*^{Mo} product (25, 26). Since Gz-MuSV virion RNA was translated in vitro to give a set of v-*mos*^{Gz} products with similar sizes, it seems likely that the 37K v-*mos*^{Gz} protein has methionine residues at identical positions to those in the v-*mos*^{Mo} 37K protein.

The fact that Gz-MuSV codes for a 37K protein which is apparently identical to that of M-MuSV 124 is inconsistent with the Gz-MuSV genomic organization determined by heteroduplex mapping (12). This apparent discrepancy has recently been resolved by nucleotide sequence analysis (11a). The sequence data show that the Gz-MuSV genome apparently arose by two recombination events such that the v-*mos*^{Gz} sequences first were incorporated into the *env* gene at an identical site to that in M-MuSV 124. This underwent a second recombination event, which resulted in the addition of approximately 1,200 nucleotides of *env* gene sequence, 19 nucleotides to the 5′ side of the first *env-mos* recombination site. Thus, the predicted NH_2 terminus of the 37K v-*mos*^{Gz} protein is the same as the NH_2 terminus of the 37K v-*mos*^{Mo} protein. Since the initiating methionine is removed from the 37K in vitro product (26) this identity is not apparent from the methionine-containing peptide maps of the two 37K proteins (Fig. 5). The open reading frame in the Gz-MuSV *env* gene sequences is separated from the v-*mos*^{Gz} open reading frame by a terminator codon. This result explains the absence of a detectable *env-mos* fusion protein and accounts for the in vitro synthesis of Gz-MuSV v-*mos* products which are identical to the v-*mos*^{Mo} in vitro translation products from M-MuSV 124. The nucleic acid and protein data described above show that Gz-MuSV and M-MuSV 124 encode, in vitro, similar v-*mos* proteins with identical NH_2 and COOH termini. Since these two viruses are reported to be independent isolates (15, 16), it is remarkable that an identical recombination event took place on two separate occasions.

By immunoprecipitation of M-MuSV 124-transformed cells with anti-peptide antiserum, we were able to identify small amounts of a v-*mos*^{Mo}-encoded phosphoprotein of 37K, which is very similar to the 37K in vitro product (27). This was the only v-*mos*^{Mo} product detectable in M-MuSV-transformed cells. In light of the results presented here one might anticipate that a similar protein would be found in cells transformed by Gz-MuSV. We have detected 37K v-*mos*^{Gz} protein in cells transformed by Gz-MuSV, but this protein is present in vanishingly

small quantities and has been impossible to analyze further (J. Papkoff, unpublished data).

The *gag* gene sequences from Gz-MuSV virion RNA were translated in vitro to produce a 62K product which is similar, by peptide mapping, to the M-MuSV 124 62K in vitro *gag* gene product. Consistent with these results, a Gz-MuSV-specific 65 to 68K *gag* protein has been identified in Gz-MuSV–transformed cells (24) and Gz-MuSV virus particles (21, 28). In contrast to the numerous similarities with M-MuSV 124, a 37.5K *env* gene-related protein is the only Gz-MuSV-specific in vitro translation product that does not have a counterpart in the translation products from M-MuSV 124. In light of the heteroduplex mapping experiments and DNA sequence results mentioned above, it seems likely that this protein is synthesized from the 1,200 nucleotides of *env* sequence in the middle of the Gz-MuSV genome. This conclusion is substantiated by the observation that 24S polyadenylated virion RNA contains the primary messenger activity for the 37.5K Gz-MuSV-specific *env* gene protein. We have not been able to identify a similar protein in Gz-MuSV-transformed cells, and the function, if any, that this protein may have is unknown.

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LITERATURE CITED

1. **Andersson, P., M. P. Goldfarb, and R. A. Weinberg.** 1979. A defined subgenomic fragment of in vitro synthesized Moloney sarcoma virus DNA can induce cell transformation upon transfection. Cell **16**:63–75.
2. **Ball, J. K., J. A. McCarter, and S. M. Sunderland.** 1973. Evidence for helper independent murine sarcoma virus I. Segregation of replication-defective and transformation-defective viruses. Virology **56**:268–284.
3. **Beemon, K., and T. Hunter.** 1977. In vitro translation yields a possible Rous sarcoma virus *src* gene product. Proc. Natl. Acad. Sci. U.S.A. **74**:3302–3306.
4. **Beemon, K. L., A. J. Faras, A.T. Haase, P. H. Duesberg, and J. E. Maisel.** 1976. Genomic complexities of murine leukemia and sarcoma, reticuloendotheliosis, and visna viruses. J. Virol. **17**:525–537.
5. **Blair, D. G., W. L. McClements, M. K. Oskarsson, P. J. Fischinger, and G. F. Vande Woude.** 1980. Biological activity of cloned Moloney sarcoma virus DNA: terminally redundant sequences may enhance transformation efficiency. Proc. Natl. Acad. Sci. U.S.A. **77**:3504–3508.
6. **Blair, D. G., M. Oskarsson, T. G. Wood, W. L. McClements, P. J. Fischinger, and G. F. Vande Woude.** 1981. Activation of the transforming potential of a normal cell sequence: a molecular model for oncogenesis. Science **212**:941–943.
7. **Canaani, E., K. C. Robbins, and S. A. Aaronson.** 1979. The transforming gene of Moloney murine sarcoma virus. Nature (London) **282**:378–383.
8. **Cremer, K., E. P. Reddy, and S. A. Aaronson.** 1981. Translational products of Moloney murine sarcoma virus RNA: identification of proteins encoded by the murine sarcoma virus *src* gene. J. Virol. **38**:704–711.
9. **Dina, D., K. Beemon, and P. Duesberg.** 1976. The 30S Moloney sarcoma virus RNA contains leukemia virus nucleotide sequences. Cell **9**:299–309.
10. **Dina, D., and B. Nadal-Ginard.** 1979. Moloney murine sarcoma virions contain subgenomic-length mRNA-like molecules that direct synthesis of sarcoma-specific polypeptides in vitro. Cold Spring Harbor Symp. Quant. Biol. **44**:901–905.
11. **Donoghue, D. J.** Demonstration of biological activity and nucleotide sequence of an in vitro synthesized clone of the Moloney murine sarcoma virus *mos* gene. J. Virol. **42**: 538–546.

11a. **Donoghue, D. J., and T. Hunter.** 1982. A generalized method of subcloning DNA fragments by restriction site reconstruction: application to sequencing the amino-terminal coding region of the transforming gene of Gazdar murine sarcoma virus. Nucleic Acids Res. **10**:2549–2564.

12. **Donoghue, D. J., P. A. Sharp, and R. A. Weinberg.** 1979. Comparative study of different isolates of murine sarcoma virus. J. Virol. **32**:1015–1027.
13. **Frankel, A. E., and P. J. Fischinger.** 1976. Nucleotide sequences in mouse DNA and RNA specific for Moloney sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **73**:3705–3709.
14. **Frankel, A. E., and P. J. Fischinger.** 1977. Rate of divergence of cellular sequences homologous to segments of Moloney sarcoma virus. J. Virol. **21**:153–160.
15. **Gazdar, A. F., H. C. Chopra, and P. S. Sarma.** 1972. Properties of a murine sarcoma virus isolated from a tumor arising in an NZW/NZB F_1 hybrid mouse. I. Isolation and pathology of tumors induced in rodents. Int. J. Cancer **9**:219–233.
16. **Gazdar, A. F., P. S. Sarma, and R. H. Bassin.** 1972. Properties of a murine sarcoma virus isolated from a tumor arising in an NZW/NZB F_1 hybrid mouse. II. Physical and biological characteristics. Int. J. Cancer **9**:234–241.
17. **Hu, S., N. Davidson, and I. M. Verma.** 1977. A heteroduplex study of the sequence relationships between the RNA's of M-MSV and M-MLV. Cell **10**:467–477.
18. **Jones, M., R. A. Bosselman, F. A. Hoorn, A. Berns, H. Fan, and I. M. Verma.** 1980. Identification and molecular cloning of Moloney mouse sarcoma virus-specific sequences from uninfected mouse cells. Proc. Natl. Acad. Sci. U.S.A. **77**:2651–2655.
19. **Lyons, D. D., E. C. Murphy, Jr., S. M. Mong, and R. B. Arlinghaus.** 1980. The translation products of Moloney murine sarcoma virus-124 RNA. Virology **105**:60–70.
20. **Maisel, J., D. Dina, and P. Duesberg.** 1977. Murine sarcoma viruses: the helper-independence reported for a Moloney variant is unconfirmed; distinct strains differ in the size of their RNA's. Virology **76**:295–312.
21. **Maxwell, S., and R. B. Arlinghaus.** 1981. In vitro proteolytic cleavage of Gazdar murine sarcoma virus $p65^{gag}$. J. Virol. **39**:963–967.
22. **Moloney, J. B.** 1966. A virus-induced rhabdomyosarcoma of mice. Natl. Cancer Inst. Monogr. **22**:139–142.
23. **Oskarsson, M., W. L. McClements, D. G. Blair, J. V. Maizel, and G. F. Vande Woude.** 1980. Properties of a normal mouse cell DNA sequence (*sarc*) homologous to the *src* sequence of Moloney sarcoma virus. Science **207**:1222–1224.
24. **Pang, R. H. L., L. A. Phillips, and D. K. Haapala.** 1977. Characterization of Gazdar murine sarcoma virus by nucleic acid hybridization and analysis of viral expression in cells. J. Virol. **24**:551–556.
25. **Papkoff, J., T. Hunter, and K. Beemon.** 1980. In vitro translation of virion RNA from Moloney murine sarcoma virus. Virology **101**:91–103.

26. **Papkoff, J., M. H.-T. Lai, T. Hunter, and I. M. Verma.** 1981. Analysis of transforming gene products from Moloney murine sarcoma virus. Cell **27**:109–119.
27. **Papkoff, J., I. M. Verma, and T. Hunter.** 1982. Detection of a transforming gene product in Moloney murine sarcoma virus transformed cells. Cell **29**:417–426.
28. **Pinter, A., and E. De Harven.** 1979. Protein composition of a defective murine sarcoma virus particle possessing the enveloped type-A morphology. Virology **99**:103–110.
29. **Reddy, E. P., M. J. Smith, and S. A. Aaronson.** 1981. Complete nucleotide sequence and organization of the Moloney murine sarcoma virus genome. Science **214**:445–450.
30. **Reddy, E. P., M. J. Smith, E. Canaani, K. C. Robbins, S. R. Tronick, S. Zain, and S. A. Aaronson.** 1980. Nucleotide sequence analysis of the transforming region and large terminal redundancies of Moloney murine sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **77**:5234–5238.
31. **Ricciardi, R. P., J. S. Miller, and B. E. Roberts.** 1979. Purification and mapping of specific mRNA's by hybridization-selection and cell-free translation. Proc. Natl. Acad. Sci. U.S.A. **76**:4927–4931.
32. **Tronick, S. R., K. C. Robbins, E. Canaani, S. G. Devare, P. R. Andersen, and S. A. Aaronson.** 1979. Molecular cloning of Moloney murine sarcoma virus: arrangement of virus-related sequences within the normal mouse genome. Proc. Natl. Acad. Sci. U.S.A. **76**:6314–6318.
33. **Van Beveren, C., J. A. Galleshaw, V. Jonas, A. J. M. Berns, R. F. Doolittle, D. J. Donoghue, and I. M. Verma.** 1981. Nucleotide sequence and formation of the transforming gene of a mouse sarcoma virus. Nature (London) **289**:258–262.
34. **Van Beveren, C., F. Van Straaten, J. A. Galleshaw, and I. M. Verma.** 1981. Nucleotide sequence of the genome of a murine sarcoma virus. Cell **27**:97–108.
35. **Vande Woude, G. F., M. Oskarsson, W. L. McClements, L. W. Enquist, D. G. Blair, P. J. Fischinger, J. V. Maizel, and M. Sullivan.** 1979. Characterization of integrated Moloney sarcoma proviruses and flanking host sequences cloned in bacteriophage lambda. Cold Spring Harbor Symp. Quant. Biol. **44**:735–745.
36. **Verma, I. M., M. H.-T. Lai, R. A. Bosselman, M. A. McKennett, H. Fan, and A. Berns.** 1980. Molecular cloning of unintegrated Moloney mouse sarcoma virus DNA in bacteriophage lambda. Proc. Natl. Acad. Sci. U.S.A. **77**:1773–1777.