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Biochemistry

Identification of the messenger RNAs coding for the *gag* and *env* gene products of the murine mammary tumor virus

(translation *in vitro*/molecular hybridization/immunoprecipitation/type B oncornavirus)

GANES C. SEN, STEVEN W. SMITH, STUART L. MARCUS, AND NURUL H. SARKAR

Laboratory of Molecular Virology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

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ABSTRACT **Full-length (35S) genomic RNA from murine mammary tumor virus (MuMTV) was translated *in vitro*, using a reticulocyte lysate system, into proteins of 105,000, 75,000, 65,000, 35,000, and 27,000 daltons. These proteins were all immunoprecipitable with a monospecific antiserum to the major viral core protein, p27, but not with antiserum to the major viral envelope glycoprotein, gp47. Translation *in vitro* of RNA of about 24S size extracted from MuMTV yielded proteins similar in size and immunoreactivity to the products of the 35S RNA translation. Polyadenylylated RNA isolated from an MuMTV-producing cell line was fractionated according to size by velocity sedimentation and subsequently hybridized to MuMTV complementary DNA probes. These studies identified at least three size classes (35S, 24S, and 14–18S) of intracellular MuMTV-specific RNA. The 35S intracellular RNA was translated into MuMTV-specific proteins identical in size and immunoreactivity to the products of the virion-derived 35S RNA. On the other hand, translation of the intracellular 24S RNA fraction resulted in the synthesis of proteins, of which two (of about 70,000 daltons) could be immunoprecipitated with anti-gp47 serum, but not with anti-p27 serum. From these data we conclude that MuMTV core and envelope proteins are synthesized from two different mRNAs with approximate sizes of 35S and 24S, respectively. Our results also imply that the intracellular 24S mRNA is synthesized by a process more complex than simple cleavage of the 35S RNA.**

Murine mammary tumor virus (MuMTV) is the only known RNA tumor virus that specifically causes mammary tumors in mice. Production of MuMTV *in vivo* is thought to be physiologically controlled by steroid hormones (1, 2). In tissue culture cell lines established from mouse mammary tumors, MuMTV synthesis is enhanced by treatment with dexamethasone, a synthetic steroid (3). MuMTV is a type B RNA tumor virus composed of six major structural proteins and some additional minor polypeptides, including the reverse transcriptase (RNA-dependent DNA polymerase). The two major glycoproteins (gp47 and gp34) are associated with the viral membrane and the other four major polypeptides (p27, p23, p16, and p12) are core constituents (4).

It has been well established for the type C RNA tumor viruses that the structural proteins are synthesized as high molecular weight polyproteins that are subsequently cleaved and processed to give rise to the mature viral proteins (for review, see ref. 5). The viral glycoproteins and the internal core proteins are derived from two different polyproteins referred to as the *env* and *gag* gene products, respectively. Recently it has been shown that MuMTV proteins are synthesized in an analogous fashion (4, 6, 7). By pulse–chase experiments two proteins with molecular weights of 75,000 and 70,000 have been identified as the intracellular precursors for the *gag* and *env* proteins, respectively.

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In the case of type C viruses, it has also been established that viral 35S genomic RNA contains information for the synthesis of the major proteins constituting the virus, namely, the core proteins, the glycoproteins, and the reverse transcriptase (5). The avian sarcoma viral RNA also codes for a *src* protein responsible for the transformation of fibroblasts *in vitro* and for the induction of sarcoma *in vivo*. However, when the full-size virion RNA or intracellular viral specific RNA of similar size is translated in an exogenous *Xenopus* oocyte system or an *in vitro* cell-free protein-synthesizing system, only the *gag* proteins and a *gag-pol* polyprotein are synthesized. It has been reported that virion RNA of smaller size classes can be translated into the *src* protein product (5). The *env* proteins are translated only from a virus-specific intracellular mRNA of about 21 S in size. For MuMTV no information is available regarding the identity of the mRNAs for different viral proteins.

We report here the translation *in vitro* of MuMTV virion RNAs. By hybridization with a representative MuMTV cDNA probe, we have identified at least three size classes of MuMTV-specific intracellular RNAs. Intracellular RNA of 35S size class was translated into MuMTV core proteins, while 24S size class RNA was translated into MuMTV envelope proteins.

MATERIALS AND METHODS

Cell Culture. MuMTV-producing GR cells (8) were grown in monolayer culture in Eagle's minimal essential medium supplemented with 10% calf serum. Confluent cultures were treated with dexamethasone at 1 μg/ml for the production of MuMTV. The cells were grown in the presence of [^{3}H]uridine at 50 μCi/ml (1 Ci = 3.7×10^{10} becquerels) for preparing RNA-labeled MuMTV.

Virus Purification. Virus was purified from the culture fluid by using the following method. The culture fluid was first clarified by centrifugation at 10,000 × *g* for 10 min. The supernatant was then layered on a cushion of 60% sucrose in TNE buffer (10 mM Tris·HCl, pH 7.5/100 mM NaCl/1 mM EDTA) and centrifuged at 100,000 × *g* for 90 min. The virus layer at the top of the cushion was removed and layered onto a preformed linear gradient of 25% to 60% sucrose in TNE buffer. This was centrifuged at 100,000 × *g* for 3 hr. The virus banding at a density of 1.17 g/ml was removed and stored at 0°C.

Isolation of Viral and Cellular RNAs. RNA was extracted from the virus by incubation with self-digested Pronase and phenol/chloroform extraction (9). Total cytoplasmic RNA was extracted from cells that had been treated with dexamethasone for 18 hr, following published procedures (10). Poly(A)-containing RNA was selected from this total RNA by two consecutive passages through an oligo(dT)-cellulose column (11).

Abbreviations: MuMTV, murine mammary tumor virus; cDNA, complementary DNA; TNE, Tris/NaCl/EDTA.

Sedimentation Analysis of Viral and Cellular RNA. Viral RNAs or cellular poly(A)-containing RNAs were separated according to size by centrifugation through a preformed linear gradient (10–30%, vol/vol) of glycerol in TNE. Before centrifugation the RNA was heated to 100°C for 1 min in a buffer containing 10 mM Tris·HCl at pH 7.5 and 1 mM EDTA. The glycerol gradients were centrifuged at 4°C and 200,000 × *g* for 7 hr.

Preparation of MuMTV-Specific Complementary DNA (cDNA). MuMTV-specific, single-stranded cDNA was synthesized by reverse transcription of MuMTV subunit genomic RNA with avian myeloblastosis virus DNA polymerase on calf thymus DNA oligonucleotide primers. Reaction conditions for the synthesis of cDNA were similar to those of Stavnezer and Bishop (12).

Nucleic Acid Hybridization. Hybridization of RNA with [^{3}H]cDNA was carried out under mineral oil in 0.6 M NaCl and percent hybridization was determined by using single-strand-specific nuclease. For the determination of RNA C_0t values (C_rt) (product of RNA concentration in moles of nucleotide per liter and incubation time in seconds), corrections were made for salt concentration (9).

***In Vitro* Translation.** Micrococcal nuclease-treated rabbit reticulocyte lysate was prepared according to Pelham and Jackson (13). Reaction mixtures for translation included calf liver tRNA at 100 μg/ml and [^{35}S]methionine at 250 μCi/ml. The incubations were for 1 hr at 30°C.

Immunoprecipitation. Immunoprecipitation followed published procedures, using *Staphyloccus aureus* and monospecific antisera to MuMTV gp47, p27, and p12 (4). Characterizations of antisera to gp47 and p27 sera have been reported earlier (4). Production and specificity of anti-p12 serum will be described elsewhere.

Polyacrylamide Gel Electrophoresis and Fluorography. Electrophoresis on a 15% acrylamide gel and fluorography of the gel were performed by using standard procedures (13). Proteins for molecular weight markers were labeled with iodo[^{14}C]acetamide (14).

RESULTS

Isolation of MuMTV virion RNA and its translation *in vitro*

MuMTV RNA was extracted from the virus, heated to 100°C for 1 min, and subjected to sedimentation velocity analysis in glycerol gradients. Fig. 1*A* shows the size distribution of virion RNA isolated from freshly prepared virus and Fig. 1*B* shows the corresponding pattern for RNA isolated from an aliquot of purified virus that had been stored at 0°C for 30 days. The majority of virion RNA from freshly harvested virus sedimented as a 35S component (Fig. 1*A*), whereas RNA from an aliquot of purified, aged virus represented a more heterogeneous population (Fig. 1*B*).

RNA of about 35S size was recovered from fractions 9 and 10 (Fig. 1*A*) and from fractions 8 and 9 (Fig. 1*B*) and RNA of about 24S size was recovered from fractions 14 and 15 (Fig. 1*B*). Aliquots of each RNA pool were translated in a reticulocyte lysate system. Products from the translation of 35S virion RNAs recovered from the experiments described in Fig. 1 *A* and *B* were identical and are shown in lane 2 (Fig. 2*A*). Translation products of the 24S virion RNA are shown in lane 3 (Fig. 2*A*). Both the RNAs were translated into proteins of similar sizes, the most prominent proteins being of about 105,000, 75,000, and 65,000 daltons. Proteins of about 35,000 and 27,000 daltons were also present though not visible in this figure. A distinct band of about 180,000 daltons is visible in lane 2 but not in lane 3. However, none of these proteins were detected if the incubation mixture was not supplemented with viral RNA (data not shown).

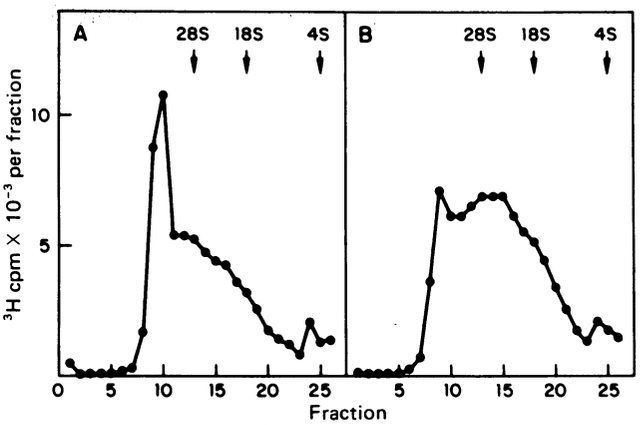

FIG. 1. Analysis of [^{3}H]uridine-labeled MuMTV virion RNA on glycerol gradient. (*A*) RNA extracted from freshly prepared virus. (*B*) RNA extracted from virus stored at 0°C for 30 days. Arrows indicate the positions of RNAs of known sizes, analyzed under identical conditions. Amounts of virion RNA analyzed in experiments *A* and *B* were 71,700 cpm and 87,000 cpm, respectively.

In order to establish that the products translated from both the 35S and the 24S RNAs, as shown in Fig. 2*A*, were antigen-

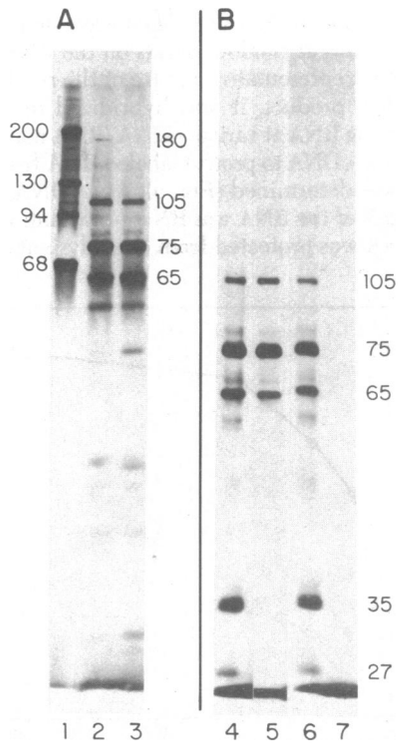

FIG. 2. Fluorographs of MuMTV virion RNA translation products after analysis by polyacrylamide gel electrophoresis. Gel *A* was not electrophoresed as long as gel *B*. ^{14}C-Labeled proteins of known molecular weights (myosin, β-galactosidase, phosphorylase B, and bovine serum albumin) were analyzed in lane 1. ^{14}C-Labeled MuMTV proteins were used as markers for the low molecular weight proteins (not shown). Lanes 2 and 3 contained translation products of 35S RNA and 24S RNA, respectively. Lanes 4 and 5 show the translation products of the 35S RNA precipitated with anti-p27 and anti-p12 sera, respectively (lane 5 was exposed for 5 times longer than lane 4.) Lanes 6 and 7 show the translation products of the 24S RNA precipitated with anti-p27 and anti-gp47 sera, respectively. Numbers on the side indicate the molecular weights (in thousands) of the proteins.

ically related to MuMTV envelope or core proteins, the translation products were immunoprecipitated with monospecific antisera to MuMTV gp47, p27, and p12 (Fig. 2*B*). Immunoprecipitates obtained with anti-p27 serum from the 35S RNA translation products contained the 105,000-, 75,000-, 65,000-, 35,000-, and 27,000-dalton proteins (lane 4). It should be noted that more of the 35,000- and 27,000-dalton proteins are visible in lane 4 than in lane 2. This is probably due to their preferential concentration by immunoprecipitation. When anti-p12 serum was used, however, for immunoprecipitation, mainly the 105,000-, 75,000-, and 65,000-dalton proteins were precipitated (lane 5). These results indicate that 105,000-, 75,000-, and 65,000-dalton proteins contain antigenic determinants of both p27 and p12, whereas proteins of 35,000 and 27,000 daltons do not have the antigenic determinants of p12. No proteins were precipitated with anti-gp47 serum (data not shown). Translation products of 24S virion RNA after immunoprecipitation with anti-p27 and anti-gp47 sera are shown in lanes 6 and 7, respectively. All the proteins that were translated were precipitated by anti-p27 serum, whereas none of them was precipitated with anti-gp47 serum. It should be mentioned that, under similar conditions, anti-gp47 serum precipitated a protein that was translated from intracellular 24S mRNA (see Fig. 5).

Identification of MuMTV-related intracellular mRNA

In order to determine the size classes of MuMTV-related intracellular RNA, we used cDNA probes that were representative of the entire viral genome. The probes were synthesized on MuMTV genomic RNA, using oligonucleotide DNA primers to initiate synthesis at random points on the RNA molecules (12). To test the representative nature of the resulting single-stranded cDNA product, it was hybridized to ^{125}I-labeled MuMTV genomic RNA at various cDNA/RNA mass ratios, and the ability of the cDNA to protect labeled RNA from digestion with RNase was determined (Fig. 3). At a cDNA/RNA mass ratio of 1, >60% of the RNA was RNase-resistant, while >90% of labeled RNA was protected from hydrolysis at a ratio of 4.

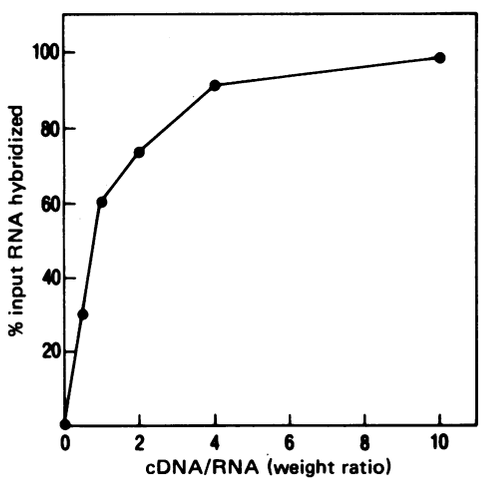

FIG. 3. Protection of ^{125}I-labeled MuMTV RNA from RNase digestion by hybridization with MuMTV cDNA. RNA was isolated from C3H mouse-derived MuMTV and iodinated to a specific activity of approximately 130 × 10^6 cpm/μg. The cDNA concentration was kept constant (to maintain identical C_0t values during annealing), and the RNA concentration was varied. Hybridization was carried out at 68°C in 50-μl volumes in 0.6 M NaCl to a C_0t of 1 mol sec/liter. After hybridization, samples were diluted to 1 ml, and 0.5-ml aliquots were removed, one of which was treated with pancreatic RNase (10 μg/ml) and T1 RNase (1 unit/ml) for 1 hr at 37°C. Trichloroacetic acid-precipitable cpm were determined in the presence of yeast RNA at 1 mg/ml as carrier.

This result indicates that the MuMTV genome appears well represented in the cDNA probes ($cDNA_{rep}$) prepared by this procedure. On the other hand, this $cDNA_{rep}$ did not hybridize at all with RNA extracted from MuMTV-negative liver tissue.

Total cytoplasmic RNA was isolated from GR cells that had been treated with dexamethasone at 1 μg/ml for 18 hr. In order to enrich for presumptive mRNA populations, the poly(A)-containing RNA was selected from this RNA by two successive passages of the RNA through an oligo(dT)-cellulose column under appropriate conditions (11). The poly(A)-containing RNA was analyzed by centrifugation through a glycerol gradient as shown in Fig. 4. RNA from an aliquot of each fraction was hybridized to a fixed amount of MuMTV $cDNA_{rep}$, and the amount of S1 nuclease resistant cDNA was measured. By this analysis, we could identify MuMTV-specific poly(A)-containing RNA of at least three distinct size classes: 35S, 24S, and about 14–18S. Similar results were obtained when the RNA was analyzed on a sucrose gradient containing 0.1% sodium dodecyl sulfate and hybridized with MuMTV $cDNA_{rep}$.

In vitro translation of intracellular poly(A)-containing RNA into MuMTV proteins

In order to determine whether the MuMTV-specific intracellular RNA of different sizes might serve as different mRNAs, we attempted to identify the proteins they code for by translation *in vitro*. RNA from alternate gradient fractions from the experiment shown in Fig. 4 was recovered by ethanol precipitation and translated in the reticulocyte lysate system. Equal aliquots of the ^{35}S-labeled translation products of each fraction were immunoprecipitated with both anti-p27 and anti-gp47 serum and analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. When RNAs recovered from gradient fractions 6, 8, 10, 14, and 16 were individually translated *in vitro* and the translation products were immunoprecipitated with anti-p27 serum, the patterns shown in Fig. 5*A* were obtained. It is clear that fractions 6, 8, 10, and 14 all contained mRNA that could be translated into proteins precipitable with anti-p27 serum. By densitometric determination of the intensity of the protein bands from a lower-exposure fluorograph, it was found that the amount of p27-related protein translated from the RNA of fraction 8 was at least 6-fold more than that which

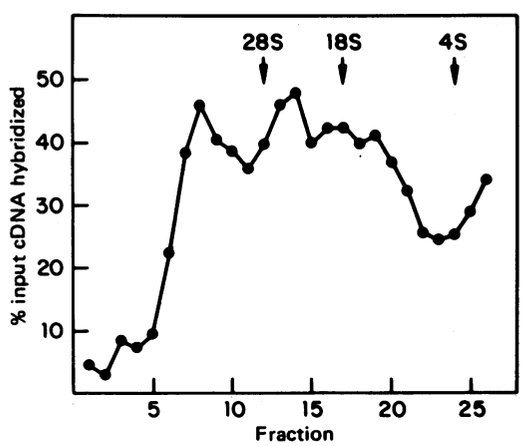

FIG. 4. Size analysis of MuMTV-specific intracellular RNA. Poly(A)-containing RNA (30 μg) from GR cells was analyzed on a 12-ml glycerol gradient. An aliquot from each fraction was hybridized with MuMTV-specific [^{3}H]cDNA to a C_rt value of 1 mol sec/liter. Trichloroacetic acid-precipitable ^{3}H-labeled material was determined after treatment with S1 nuclease. Arrows indicate the positions of RNAs of known sizes analyzed under identical conditions.

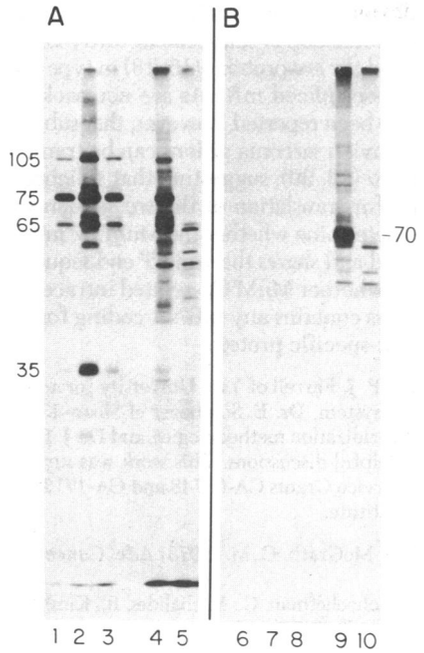

FIG. 5. Analysis of translation products of intracellular RNA of different sizes. (*A*) Translation products precipitated with anti-p27 serum; (*B*) translation products precipitated with anti-gp47 serum. *A* and *B* show results from two different gels. RNAs translated in this experiment were recovered from the gradient fractions of the experiment shown in Fig. 4. Lanes 1 and 6 were from fraction 6, lanes 2 and 7 were from fraction 8, lanes 3 and 8 were from fraction 10, lanes 4 and 9 were from fraction 14, and lanes 5 and 10 were from fraction 16.

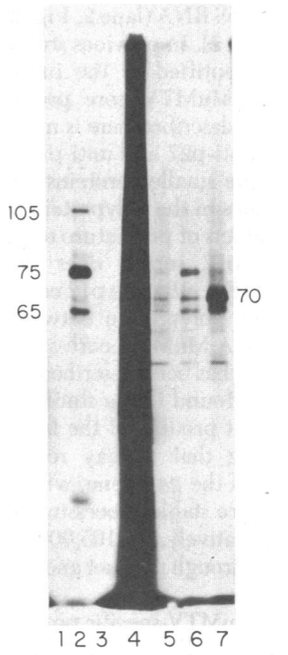

FIG. 6. Analysis of translation products of the intracellular 35S and 24S RNAs. Lanes 1, 2, and 3 show the translation products of the 35S RNA after precipitation with normal serum, anti-p27 serum, and anti-gp47 serum, respectively. Lanes 4, 5, 6, and 7 show the translation products of the 24S RNA without any immunoprecipitation and precipitated with normal serum, anti-p27 serum, and anti-gp47 serum, respectively.

could be translated from the RNA of any other fraction (data not shown). Because the translations were done under conditions of limiting mRNA concentrations, it may be inferred, therefore, that fraction 8 contained the highest amount of mRNA coding for p27-related proteins. This was also one of the peak fractions identified by hybridization with cDNA (Fig. 4). Thus, both virion RNA and intracellular mRNA coding for the synthesis of core proteins seem to have the same 35S size.

Translation of RNA from fractions 6, 8, and 10 did not produce any detectable product immunoprecipitable with anti-gp47 serum (Fig. 5*B*). However, a protein of about 70,000 daltons was precipitated from the translation products of RNA from fraction 14 (24S). Because this protein is precipitated with only anti-gp47 serum, it most probably represents a precursor to gp47. The intracellular MuMTV glycoprotein precursor molecule has also been identified as a protein of 70,000 daltons (4, 6, 7). These results combined with the results from our hybridization experiment suggest the existence of an intracellular MuMTV-specific mRNA of about 24 S that codes for the glycoprotein precursor. When RNAs from gradient fractions 18, 20, and 22 (Fig. 4) were translated and immunoprecipitated with normal serum, anti-p27 serum, or anti-gp47 serum, no MuMTV-specific translation products could be identified.

In order to examine the two apparent MuMTV intracellular mRNA populations more critically, RNAs from the two peak fractions, namely 8 (35S) and 14 (24S) were again translated *in vitro*, and the translation products from each fraction were immunoprecipitated with normal, anti-p27, and anti-gp47 sera. All the immunoprecipitates were electrophoresed in a single gel (Fig. 6). All the proteins synthesized by the 35S RNA (data not shown) were precipitated with anti-p27 serum (lane 2), none with normal serum (lane 1) or with anti-gp47 serum (lane 3). This is very similar to the patterns obtained with virion 35S RNA. In the case of intracellular 24S mRNA, a great number of proteins are synthesized (lane 4), only two of which (having molecular weights of about 70,000) were specifically precipitated with anti-gp47 serum (lane 7). The normal serum did not precipitate significant quantities of these proteins (lane 5); anti-p27 serum, however, precipitated a small amount of the 75,000-dalton protein (lane 6).

Therefore, GR tissue culture cells producing MuMTV appear to contain two viral mRNA populations of differing molecular weights and translation potentials. The larger, genome-sized, mRNA (35S) codes for the *gag* gene product and the smaller (24S) mRNA codes for the remaining structural glycoproteins, the *env* gene products.

DISCUSSION

The experiments reported here were carried out in order to examine the *in vitro* translation potential of MuMTV virion RNA and to identify MuMTV-specific intracellular mRNAs. The translation products of the various RNAs were identified by virtue of their molecular weights and antigenic properties. MuMTV RNA isolated from freshly prepared or frozen virions was found to sediment as a 70S species (data not shown) that, upon heating at 100°C for 1 min, appeared to generate, in addition to the 35S full-length genomic RNA (15), a heterogeneous population of RNA shorter than the 35S component. It is possible that the subgenomic size RNAs were generated during isolation of the virus, during the extraction of viral RNA or both, because RNA prepared from virus preparations that have been pelleted, or frozen, or stored for more than one week at 0°C consistently contained more of the shorter RNAs. It should be mentioned, however, that, using identical procedures, we have isolated RNA consisting only of the 35S and 4S size from murine leukemia viruses.

The full-sized MuMTV virion RNA of 35S size and the half-genome size 24S virion RNA both translate *in vitro* into 105,000-, 75,000-, 65,000-, 35,000-, and 27,000-dalton proteins. However, a protein with a molecular weight of 180,000 was

translated from the 35S RNA (lane 2, Fig. 2), but not from the 24S RNA (lane 3, Fig. 2). In previous studies, a 75,000-dalton protein has been identified as the intracellular precursor polyprotein for the MuMTV core proteins (4, 6, 7). The 75,000-dalton protein described here is most probably identical to it, because both anti-p27 and anti-p12 sera recognize this component, while the smaller proteins observed may all be cleavage intermediates in the polyprotein processing pathway or they may be products of premature termination of translation. Because anti-p12 serum does not precipitate the 35,000-dalton band (Fig. 2*B*), the p12 core protein is presumably cleaved off the polyprotein between the 65,000- and 35,000-dalton stages. A MuMTV core-specific polyprotein of about 100,000 daltons has been described from experiments *in vivo* (4, 7). We have found that a similar protein of 105,000 daltons is a prominent product of the *in vitro* translation experiments, indicating that it may represent the primary translation product of the *gag* gene, while the 75,000-dalton protein may be a more stable processing intermediate in the cellular milieu. Alternatively, the 105,000-dalton protein could be an aberrant read-through product and not a precursor to the 75,000-dalton protein.

A 180,000-dalton MuMTV-specific protein has been detected by labeling of whole cells and immunoprecipitation with anti-p12 serum (unpublished data). The 180,000-dalton protein translated from the 35S RNA *in vitro* could be the same protein. It may be the MuMTV counterpart of the "*gag-pol*" product identified for type C viruses (5). In type C viruses it has been shown that the *gag* gene is located at the 5′ end of the virion RNA (5). By analogy with these viruses, because virion-derived MuMTV 24S RNA translates into *gag* gene products but not the 180,000-dalton putative "*gag-pol*" product, we may infer that the *gag* gene is located within the 5′ half of the MuMTV genome and that the *pol* gene may extend beyond the midpoint of the virion RNA towards the 3′ end.

GR cells stimulated with dexamethasone contain steady-state levels of MuMTV-related RNA that are about 0.2% of the total cellular RNA (8). We therefore used this cell line to identify size classes of intracellular MuMTV RNA and, through *in vitro* translation analysis, to determine whether the different size classes of RNA represent mRNAs coding for different proteins. The resolution of the intracellular MuMTV-specific mRNAs of different size classes as determined by hybridization with $cDNA_{rep}$ and subsequent translation suggested that the viral core proteins and the envelope proteins are synthesized from two different mRNA populations.

Although the mRNA used for core protein synthesis is of full genome length, the reason(s) for the lack of translation of sequences coding for the envelope proteins remains unknown. However, because 24S virion RNA does not translate into the envelope proteins whereas the 24S mRNA does, the apparent size of RNA cannot be the only factor determining the nature of its translation products. The possibility that *env* sequences have been selectively degraded or deleted from the 24S virion RNA has not, however, been ruled out. Specific splicing of different regions of the virion RNA has been shown to be necessary for the production of intracellular mRNAs coding for the *env* proteins and the *src* proteins (16–18) in type C viruses, and presumably these spliced mRNAs are not packaged into the virus. It has also been reported, however, that subgenomic RNA (18–20S) from avian sarcoma virions can be translated into *src* proteins *in vitro* (19, 20), suggesting that splicing may not be a prerequisite for translation of the *src* sequences. It will be interesting to determine whether the MuMTV intracellular 24S mRNA is spliced and shares the same 5′ end sequences with the 35S RNA, and whether MuMTV-related intracellular RNA of 14–18S size class contains any mRNA coding for an as yet unidentified virus-specific protein.

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1. Nandi, S. & McGrath, C. M. (1973) *Adv. Cancer Res.* **17,** 353–414.
2. Schlom, J., Schochetman, G., Michalides, R., Kimball, P., Drohan, W. & Colcher, D. (1977) in *Recent Advances in Cancer Research,* ed. Gallo, R. C. (CRC, Cleveland, OH), pp. 89–120.
3. Parks, W. P., Scolnick, E. M. & Kozukowski, E. H. (1974) *Science* **184,** 158–160.
4. Racevskis, J. & Sarkar, N. H. (1978) *J. Virol.* **25,** 374–383.
5. Stephenson, J. R., Devare, S. G. & Reynolds, F. H., Jr. (1978) *Adv. Cancer Res.* **27,** 1–53.
6. Schochetman, G., Long, C. W., Oroszlan, S., Arthur, L. & Fine, D. L. (1978) *Virology* **85,** 168–174.
7. Dickson, C. & Atterwill, M. (1978) *J. Virol.* **26,** 660–672.
8. Ringold, G. E., Lasfargues, E. Y., Bishop, J. M. & Varmus, H. E. (1975) *Virology* **65,** 135–147.
9. Varmus, H. E., Quintrell, N., Medeiros, E., Bishop, J. M., Nowinski, R. C. & Sarkar, N. H. (1973) *J. Mol. Biol.* **79,** 663–679.
10. Perry, R. P., Kelley, D. E. & LaTorre, J. (1974) *J. Mol. Biol.* **82,** 315–331.
11. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
12. Stavnezer, J. & Bishop, J. M. (1977) *Biochemistry* **16,** 4225–4232.
13. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67,** 247–256.
14. Smith, A. E., Smith, R. & Paucha, E. (1978) *J. Virol.* **28,** 140–153.
15. Duesberg, P. H. & Blair, P. B. (1966) *Proc. Natl. Acad. Sci. USA* **55,** 1490–1497.
16. Mellon, P. & Duesberg, P. H. (1977) *Nature (London)* **270,** 631–634.
17. Rothenberg, E., Donoghue, D. J. & Baltimore, D. (1978) *Cell* **13,** 435–451.
18. Cordell, B., Weiss, S. R., Varmus, H. E. & Bishop, J. M. (1978) *Cell* **15,** 79–91.
19. Beemon, K. & Hunter, T. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3302–3306.
20. Kamine, J. & Buchanan, J. M. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 2011–2015.