Polyprotein Precursors to Mouse Mammary Tumor Virus Proteins

S. J. ANDERSON,¹ R. B. NASO,^{1*} J. DAVIS,² AND J. M. BOWEN²

Department of Biology¹ and Department of Molecular Carcinogenesis and Virology,² The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Received for publication 16 February 1979

Mouse mammary tumor virus (MMTV) derived from the culture medium of GR cells contained seven proteins, identified as gp55, gp33, p25, pp20, p16, p12, and p10. The major viral phosphoprotein was the 20,000-molecular-weight protein, pp20. Immunoprecipitation of cytoplasmic extracts from pulse-labeled GR cells identified three MMTV *gag*-specific proteins, termed Pr78^{gag}, Pr110^{gag}, and Pr180^{gag+}. These intracellular polyproteins were precipitable from cytoplasmic extracts by antisera to virions p25 and p12 but not by antisera to gp55. The major intracellular *gag*-specific precursor polyprotein, Pr78^{gag}, contained antigenic determinants and tryptic peptides characteristic of p25, p12, p10, and presumably pp20. This precursor is presumably derived from nascent chain cleavage or rapid posttranslational cleavage of the larger intracellular precursor-like protein, designated Pr110^{gag}. Pr110^{gag} contained all but one of the leucine-containing tryptic peptides of Pr78^{gag}, plus several additional peptides. In addition to Pr78^{gag} and Pr110^{gag}, monospecific antisera to virion p12 and p25 were also capable of precipitating from pulse-labeled cells a small amount of a 180,000-molecular-weight precursor-like protein, designated Pr180^{gag+}. This large polyprotein contained nearly all of the leucine-containing tryptic peptides of Pr78^{gag} and Pr110^{gag} plus several additional peptides. By analogy to type C viral systems, Pr180^{gag+} is presumed to represent a *gag-pol* common precursor which is the major pathway for synthesis of MMTV polymerase. Immunoprecipitation of cytoplasmic extracts from pulse-labeled cells with antisera to gp55 identified two *env*-specific proteins, designated gPr76^{env} and gP79^{env}. The major *env* precursor, gPr76^{env}, could be labeled with radioactive glucosamine and was shown to contain antigenic determinants and tryptic peptides characteristic of gp55 and gp33. A minor glycoprotein, gP79^{env}, contained both fucose and glucosamine and was precipitable from cytoplasmic extracts with monospecific serum to gp55. It is suggested that gP79^{env} represents fucosylated gPr76^{env} which is transiently synthesized and cleaved rapidly into gp55 and gp33.

The precursor-product relationships of virus-specific proteins during the replication of murine and avian type C retroviruses have been well established (2, 6, 13). The presence of mouse mammary tumor virus (MMTV) polyprotein precursors in mouse mammary tumor cells has also been reported (8-11, 28, 30, 34, 35). In this paper, we describe the further characterization of MMTV proteins and the intracellular precursors from which they are derived. Specific immunoprecipitation of intracellular precursors and analysis of trypsin-digested MMTV proteins by column chromatography confirmed that viral proteins p25, p12, and p10 are derived from an intracellular precursor polyprotein, Pr78^{gag}, which migrates as a doublet of approximately 78,000 molecular weight. In addition, however, we report here on the presence of two larger

polyproteins, termed Pr110^{gag} and Pr180^{gag+}, which also contain the antigenic properties and peptide sequences characteristic of the MMTV core proteins. Both of these precursors are shown to contain extra peptide sequences not accounted for by their content of known core proteins. We have also identified what we believe to be a fucosylated form of the MMTV glycoprotein precursor, gPr76^{env}. This minor polyprotein, termed gP79^{env}, contains both the antigenic properties and peptide sequences of MMTV gp55 and gp33 and can be labeled with either [³H]glucosamine or [³H]fucose.

MATERIALS AND METHODS

Cell cultures and virus. MMTV was purified from the culture medium of a GR mouse mammary adenocarcinoma cell line (23, 32). The cells were propagated

in Dulbecco modified Eagle medium (GIBCO, Grand Island, N.Y.) with a glucose concentration of 4,500 mg/liter, supplemented with 10% fetal calf serum (GIBCO), insulin at 10 μ g/ml, dexamethasone at 1 μ g/ml, and gentamicin at 50 μ g/ml (DHG+DI medium). Production of MMTV was monitored routinely by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of sucrose gradient-purified virus (density, 1.16 to 1.18 g/ml) labeled with [3 H]leucine.

Virion polypeptide labeling. The GR cells grown to confluency in 490-cm² plastic roller flasks were used 14 days after subculturing. Cells were labeled by incubation in 50 ml of methionine-free DHG+DI medium to which had been added 2.5 mCi of [35 S]methionine (910 Ci/mmol). After a 24-h incubation, 25 ml of nonradioactive complete DHG+DI medium was added, and the incubation was continued for an additional 24 h. The medium was then removed from the cells and clarified of debris by centrifugation at 16,300 $\times$ g for 10 min. Crude virus pellets were obtained by centrifugation of the culture fluids at 80,000 $\times$ g for 2 h. The virus pellets were suspended in buffer containing 0.01 M Tris-hydrochloride (pH 7.5), 0.1 M NaCl, and 1 mM EDTA (TNE) and banded by centrifugation at 38,000 $\times$ g for 16 h through a 15 to 60% sucrose gradient in TNE. Virus banding at 1.16 to 1.18 g of sucrose per ml was diluted in TNE and pelleted by centrifugation at 80,000 $\times$ g for 2 h.

For comparison, virion proteins were sometimes labeled with 5 mCi of [3 H]leucine (30 Ci/mmol) in medium at 50% the usual concentration of amino acids or with [32 P]phosphate in phosphate-free medium.

Antisera. Antisera to MMTV proteins were prepared in rabbits (1, 13) by injection of preparations of viral proteins. Gradient-purified MMTV disrupted with Nonidet P-40 and sodium deoxycholate (0.5%) was used to prepare anti-MMTV sera. Viral proteins from detergent-disrupted virus were also separated into glycoprotein and nonglycoprotein fractions by chromatography on concanavalin A (J. Davis, manuscript in preparation). Antisera to viral gp55, p25, and p12 were made using proteins isolated by isoelectric focusing of concanavalin A-chromatographed MMTV glycoproteins and nonglycoproteins (Davis, in preparation). Anti-p25 serum was also prepared, using p25 purified by SDS-PAGE. When indicated, antisera were preabsorbed with cytoplasmic extracts of Rauscher murine leukemia virus (RLV)-infected NIH Swiss mouse cells (JLS-V16) or with extracts of GR cells grown in the absence of dexamethasone (1, 25). Antisera were tested for specificity by precipitation of detergent-disrupted [3 H]leucine virus and analysis of the precipitates by SDS-PAGE and autoradiography.

Labeling and extraction of cytoplasmic viral proteins. Cytoplasmic proteins were labeled by the addition of 2.0 mCi of [35 S]methionine (910 Ci/mmol), 5 mCi of [3 H]methionine (40 Ci/mmol), or 5 mCi of [3 H]leucine (45 Ci/mol) in 20 ml of Earle balanced salt solution to cells grown to confluency in 150-cm² plastic culture flasks. Cells were then lysed immediately or subjected to a chase period by removal of the isotope and subsequent incubation in DHG+DI medium. Isolation of cell-associated viral proteins by immunopre-

cipitation of cytoplasmic extracts has been described previously (1, 25). Immunoprecipitates were pelleted by low-speed centrifugation and washed twice in buffer containing Nonidet P-40 and sodium deoxycholate (1, 25). When indicated, immunoprecipitation was aided by absorption of the immune complexes with the Cowan I strain of *Staphylococcus aureus* (17, 29). Long-term pulsing of 4.5 h with [3 H]glucosamine (38 Ci/mmol) or [3 H]fucose (24 Ci/mmol) was done in 10 ml of DHG+DI per flask.

SDS-PAGE. SDS-PAGE of immunoprecipitated cytoplasmic and virion proteins was performed in 11.25% or continuous-gradient 6 to 12% polyacrylamide slab gels containing SDS and using the Tris-glycine buffer system as previously described (1, 25). Gels containing tritiated samples were treated with dimethyl sulfoxide-PPO (2,5-diphenyloxazole) (5) and exposed to preflashed X-ray film (20).

Tryptic digestion and column chromatography. Bands showing radioactivity on autoradiograms were cut from gels, and the proteins were extracted and digested with trypsin (1). The resulting polypeptide fragments were separated by ion-exchange chromatography on Chromobead type P resin (Technicon Corp., Inc., Tarrytown, N.Y.) (1) with a logarithmic gradient buffer of acetic acid and pyridine. Fractions were collected sequentially, and the buffer was evaporated at 95°C. Triton X-100-containing scintillation fluid (1) was added, and the radioactivity in each fraction was measured in a Packard scintillation counter.

Nomenclature. In this study, we are using a recently adopted nomenclature for oncornaviral polypeptides agreed upon at a National Cancer Institute-sponsored Tumor Viral Immunology Workshop held in Arlington, VA., on 8-9 March 1977. The terminology had been previously introduced (15) and applied to MMTV proteins (27). The estimated molecular weights of the virus-specific proteins observed in these studies are in close agreement with the results of Nusse et al. (27) and approximate the results of others (8-11, 30). In these studies, gp55 and gp33 are often referred to as gp52 or gp49 and gp36 or gp34, respectively. Virion p25 is likewise referred to as p27 or p24, and similar minor variations in the smaller viral proteins can be noted. When published values differ from estimates of the protein molecular weights presented in this paper, published values will be followed by our estimates in parentheses.

RESULTS

SDS-PAGE of viral proteins. Electrophoresis of MMTV obtained from the culture medium of radioactively labeled GR cells separates proteins of approximately 55,000 (gp55), 33,000 (gp33), 25,000 (p25), 20,000 (pp20), 16,000 (p16), 12,000 (p12), and 10,000 (p10) molecular weights, as determined by comparison with the proteins of RLV (Fig. 1). Labeling with [35 S]methionine or [3 H]leucine indicated that each of these viral proteins contained methionine as well as leucine. The SDS-PAGE profiles of MMTV derived

FIG. 1. SDS-PAGE of the proteins of MMTV and RLV. Virus labeled with [^3H]leucine, [^{35}S]methionine, or [^{32}P]phosphate was purified by isopycnic gradient centrifugation, and viral proteins were separated on an 11.25% polyacrylamide slab gel. (A) MMTV labeled with [^3H]leucine (10,000 cpm); (B) MMTV labeled with [^{35}S]methionine (10,000 cpm); (C) RLV labeled with [^3H]leucine (15,000 cpm); (D) MMTV labeled with [^3H]leucine (10,000 cpm); (E) MMTV labeled with [^{32}P]phosphate (3,000 cpm). Virus bands are visualized by fluorography as described in Materials and Methods.

from GR mouse mammary tumor cells lacked any protein bands migrating parallel to the p30 of RLV. Therefore, we concluded that release of type C retrovirus by GR cells was negligible. Other minor bands were present in virus, the most evident being those of approximately 50,000 and 29,000 molecular weight.

Labeling of virion proteins with [^{32}P]phosphate yielded bands in SDS-PAGE that migrated parallel to most of the major proteins of a [^3H]leucine-labeled MMTV preparation, with the exception of p12 (Fig. 1, lane E). Judging by the appearance of the radiogram corresponding to this gel, the protein of 20,000 molecular weight is the major phosphorylated protein. We therefore have designated this protein pp20 (S. J. Anderson and R. B. Naso, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, S270, p. 257).

Characterization of antisera. Antisera to viral proteins were used to precipitate virus-specific proteins from pulse-labeled GR cells. Before this, these sera were characterized by their ability to precipitate isotopically labeled proteins from detergent-disrupted virions. Results of

these serum characterizations are shown in Fig. 2. Anti-gp55 serum, under these conditions, precipitated only gp55 from detergent-disrupted virions, whereas anti-p25 serum precipitated only p25, with trace amounts of smaller viral proteins. Anti-p12 serum precipitated p12 and trace amounts of p25 from detergent-disrupted virus (results not shown).

Immunoprecipitation of virus-specific proteins from infected cells. Precipitation of pulse-labeled viral proteins from cells with either anti-p25 or anti-p12 serum resulted in recovery of radioactive proteins of approximately 78,000 ($\text{Pr}78^{\text{gag}}$), 110,000 ($\text{Pr}110^{\text{gag}}$), and 180,000 ($\text{Pr}180^{\text{gag+}}$) molecular weight (Fig. 3, lanes A and D, respectively). The $\text{Pr}78^{\text{gag}}$ protein appeared as a closely migrating doublet characterized by a sharp lower band under a hazy upper band. The $\text{Pr}110^{\text{gag}}$ and $\text{Pr}180^{\text{gag+}}$ bands were minor compared with the $\text{Pr}78^{\text{gag}}$ bands. Analysis by SDS-PAGE of the immunoprecipitable cytoplasmic proteins labeled by a 15-min pulse followed by a chase of 1 or 3 h revealed a gradual decrease in radioactivity of the large precursors

FIG. 2. Characterization of antisera against MMTV proteins. [^3H]leucine-labeled MMTV was solubilized, and equal portions were immunoprecipitated by (A) anti-gp55, (B) anti-p25, or (C) anti-MMTV serum. Immunoprecipitates were analyzed by SDS-PAGE in 11.25% gels and scintillation autoradiography as described in Materials and Methods.

(lanes B and C, and E and F), concomitant with increased radioactivity in virions released from the cells (not shown). Only an extremely small amount of label appeared during the chase as intracellular p25 (lane C), and no intracellular p12 was apparent. Immunoprecipitation of cell extracts from similarly pulse-labeled and chase-incubated cells with antiserum to gp55 revealed the presence of a protein of 76,000 molecular weight, termed gPr76^{env} (lane G). This protein was also reduced in specific activity during the chase period, but its removal was concomitant with the appearance of label in the gp55 region of the gel.

Several background bands representing non-specific precipitation are also evident in this figure. They can be recognized by their ability to be precipitated by all of the sera and by the rather limited changes in these bands during chases.

Labeling of MMTV precursor polypeptides with [^3H]glucosamine or [^3H]fucose. Figure 4 shows the SDS-PAGE profile of intracellular virus-specific proteins labeled with [^3H]glucosamine or [^3H]fucose and immunopre-

cipitated with anti-MMTV, anti-p25, or anti-gp55 serum. [^3H]glucosamine was incorporated into gPr76^{env}, gp55, and gp33 (lanes A and C), but these proteins could not be precipitated by anti-p25 serum (lane B). These three proteins also could be labeled with [^3H]fucose (lanes D and F). In addition to gPr76^{env}, however, [^3H]fucose appeared to be incorporated into a protein of approximately 79,000 molecular weight, termed gP79^{env}. Again, anti-p25 serum did not recognize any [^3H]fucose-labeled proteins (lane E). The relative absence of this fucosylated protein in experiments labeling with [^3H]leucine (see Fig. 3) or [^3H]glucosamine suggested that gP79^{env} is present in very low amounts and is apparently fucose rich. It is also obvious that gp55 and gp33 labeled better with [^3H]fucose, than with [^3H]glucosamine under the labeling conditions used.

Tryptic digestion and chromatographic analysis of virion core proteins and their intracellular precursors. Virion and cytoplasmic proteins were labeled with [^{35}S]methionine, [^3H]methionine, or [^3H]leucine as described above. Radioactive proteins were separated by SDS-PAGE and located by radiofluorography. Regions corresponding to bands in the autoradiograms were cut from the gels, and the proteins in these slices were digested with trypsin (1, 24, 33). Polypeptide fragments were separated by ion-exchange column chromatography (1). Eluted radioactive counts per fraction are shown (Fig. 5 and 6).

Figure 5A-C shows the peptide maps of p25, p12, and p10 cochromatographed with Pr78^{gag} peptides. The precursor-product relationship is obvious from the maps of overlapping peptides. The region of eluent with comigration peaks corresponding to all three viral proteins may represent methionine-arginine or methionine-lysine dipeptides present in each protein. One major peak and several minor peaks in Pr78^{gag} are unaccounted for by the three viral proteins analyzed. These regions, in addition to the void volume of the column, represent peptides which suggest the presence of a fourth viral protein, presumably pp20, in Pr78^{gag}.

Figure 6A-C shows the peptide map of [^3H]leucine-labeled Pr180^{gag+}, Pr110^{gag}, and Pr78^{gag}, precipitated from cytoplasmic extracts with anti-p25 serum. The maps show that Pr110^{gag} contained all but one of the peptides (panel C, asterisk) characteristic of Pr78^{gag} plus seven other peptides (panel B, asterisks) not found in Pr78^{gag}. Pr180^{gag+} likewise contained all but one of the leucine-containing peptides characteristic of Pr110^{gag} (missing peptide in fraction 11, panel B) and was characterized by approximately five

FIG. 3. Synthesis and cleavage of MMTV precursor polyproteins. Cultures of GR cells were pulse-labeled for 15 min with 100 μ Ci of [3 H]leucine per ml (A, D, and G) and chase-incubated for 1 h (B, E, and H) or 3 h (C, F, and I). Equal portions of the cytoplasmic extracts were immunoprecipitated with anti-p25 (A-C), anti-p12 (D-F), and anti-gp55 (G-I) sera. Immunoprecipitates were analyzed as described for Fig. 2. Equal volumes of the solubilized immunoprecipitates were applied to the gel.

additional peptides not found in either Pr78^{gag} or Pr110^{gag}.

DISCUSSION

SDS-PAGE analysis of MMTV proteins labeled with [35 S]methionine indicates that all the viral proteins contain methionine. Of the six to seven virus-specific proteins identified, the protein of 20,000 molecular weight, termed pp20 (Anderson and Naso, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, S270, p. 257) was shown to be most heavily phosphorylated, in agreement with the results of other investigators (27, 33). Most, if not all, of the other viral proteins are also phosphorylated, but to a lesser extent than pp20 (28, 33; Anderson and Naso, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, p. 257). Results from the immunoprecipitation analyses presented here confirm that gp55 and gp33 arise from a common intracellular precursor, termed gPr76^{env} (10, 11, 34, 35; Anderson and Naso, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, p. 257). The MMTV glycoproteins and their major intracellular precursor gPr76^{env} can be labeled

well with [3 H]leucine or [3 H]glucosamine. However, labeling with [3 H]fucose indicates that synthesis of gp33 and gp55 may involve a minor, high-molecular-weight and heavily fucosylated polyprotein termed gP79^{env}. The major glycoprotein precursor, gPr76^{env}, does not label as well with [3 H]fucose as it does with [3 H]glucosamine and may be fucose deficient. It is suggested that gP79^{env} represents fucosylated gPr76^{env} which is transiently made and cleaved rapidly into gp55 and gp33. Similar results have been observed in the synthesis of Moloney leukemia virus gp70 (T. Gordon Wood, personal communication). In the case of the leukemia virus glycoprotein, the higher-molecular-weight glycosylated polyprotein (gP93^{env}) can be labeled efficiently with radioactive fucose, whereas the major glycoprotein precursor gPr83^{env} lacks fucose. The leukemia virus glycoprotein (gp70) and the MMTV glycoproteins contain both glucosamine and fucose.

Similar analyses confirm that a 78,000-molecular-weight protein doublet, Pr78^{gag}, has antigenic determinants in common with at least two

FIG. 4. Synthesis of MMTV glycoproteins and glycopolyproteins. GR cells were labeled for 4.5 h with [^3H]glucosamine (A–C) or [^3H]fucose (D–F). Equal portions of the cytoplasmic extracts were immunoprecipitated with anti-gp55 (A and D), anti-p25 (B and E), or anti-MMTV (C and F) sera. Immunoprecipitates were analyzed as described in Fig. 2.

of the core proteins of MMTV (p25 and p12). Tryptic peptide mappings confirm the precursor-product relationship of Pr78^{gag} and p25 and, in addition, indicate that Pr78^{gag} shares peptides in common with the p12 and p10 of the virus. These results are similar to those of Dickson and Atterwill (9). Again by analogy to RLV, the *gag* precursor of MMTV may be expected to contain four viral proteins, including the major phosphoprotein pp20. We have been unable to obtain enough radioactivity in pp20 to analyze its methionine-containing peptides. This is presumably due to its low methionine content relative to the other viral proteins (see Fig. 1, lane B). Other workers have shown that the major MMTV *gag* precursor, Pr73^{gag} (Pr78^{gag}), is phosphorylated to yield pPr75^{gag} (27). This phosphorylated *gag* precursor presumably corresponds to the upper band in the Pr78^{gag} doublet reported here. This is consistent with other results concerning the phosphorylation of RLV *gag* precursor (pPr65^{gag}) and suggests that pp20 may be present in Pr78^{gag} and phosphorylated initially while in that precursor form (R. B. Naso, W. L. Karshin, Y. H. Wu, and R. B. Arlinghaus, submitted for publication). Trypsin digestion of each of the [^{35}S]methionine-labeled Pr78^{gag} bands yields identical peptide maps when analyzed by ion-exchange chromatography (results not shown).

Chase experiments generally indicate that gPr76^{env} is more rapidly turned over in cultured GR cells than is Pr78^{env}. Chasing permits the appearance of isotopically labeled gp55 in the cell, presumably via cleavage of gPr76^{env} or gP79^{env}, or both. Chase incubation, however, does not result in the appearance of significant amounts of p25 or other *gag* proteins in the cell, even though Pr78^{gag} appears to chase and viral proteins appear in virus particles in the chase medium. These results suggest an inefficient precursor processing, but they may also reflect the precursor-product relationship between the A particles and B particles of MMTV (37). Tanaka (38) has shown that intracytoplasmic A particles represent the pronucleocapsids of MMTV. A particles are composed of polypeptides of approximately 70,000 molecular weight which carry the antigenicities of the virion core proteins, p25, p15 (p12), and p7 (p10). Incubation of A particles resulted in the generation of the core proteins of MMTV (38). The P70 observed by Tanaka presumably is analogous to the Pr78^{gag} identified in this study. The cleavage products of this precursor, therefore, would not be present in the cell until the A particles mature to B particles. The extremely low levels of p25 in infected cells suggest that this maturation occurs coincident with or shortly after budding of the virus. The previous work by Dickson and Atter-

FIG. 5. Ion-exchange chromatography of trypsin-digested $[\text{}^3\text{H}]$ methionine-labeled Pr78^{gag} cochromatographed with similarly digested $[\text{}^{35}\text{S}]$ methionine-labeled p25 (A), p12 (B), or p10 (C). Identities of some of the peptides as to their presence in viral proteins are indicated above each peak. The arrows mark the elution of the column with 2 M pyridine acetate (13).

FIG. 6. Ion-exchange chromatography of trypsin-digested [^3H]leucine-labeled Pr78^{gag}, Pr110^{gag}, and Pr180^{gag}. Results were plotted on the same graph based on alignment of [^{14}C]tyrosine-labeled tryptic peptides of RLV p30 (arrows) which were used as an internal marker in each chromatography run (12). The heavy bars mark elution of the column with 2 M pyridine acetate.

will also suggest this possibility (9). In these characteristics, the replication of MMTV is distinguished from that of type C virus replication. In the latter, intracellular levels of virion p30 are evident even during relatively short chase incu-

bations (1, 5), and *gag* precursor cleavage can be shown to proceed normally in the cell even when virus assembly or budding is prevented with interferon treatment (36; Y. H. Wu and R. B. Naso, manuscript in preparation). Similarly, type C *gag*- and *env*-specific precursors synthesized during the S phase of synchronized cells are cleaved in the cell before virus release during the subsequent mitosis (26).

Continuing the analogy with murine type C viruses, Long et al. (21) have observed that MMTV p14 (p12) binds efficiently to single-stranded DNA and may, therefore, be analogous to murine type C virus p10 (4, 14). The further comparisons of MMTV p25 with RLV p30 as the major core protein, MMTV pp20 with RLV pp12 as the major virion phosphoprotein (16, 28), and MMTV p10 with RLV p15 as the only virion envelope-associated polypeptide coded for by the *gag* regions of their respective virion genomes indicate further analogy (7, 36). The order of *gag* proteins in the RLV *gag* precursor, Pr65^{gag}, has been shown to be H₂N-p15-pp12-p30-p10-COOH (3, 24, 31). An analogous order for MMTV *gag* precursor, Pr78^{gag}, is predicted to be H₂N-p10-pp20-p25-p12-COOH.

Recently, Dahl and Dickson (8) translated the genomic RNA of MMTV in a cell-free translation system derived from mouse L cells and rabbit reticulocytes. They identified among the translation products proteins of 77,000, 110,000, and 160,000 molecular weights. All three proteins were precipitable by antiserum to the major core protein of MMTV and the 110,000-dalton protein contained methionine-labeled peptides characteristic of the 77,000-dalton protein plus additional peptides. Our results indicate the existence of three similar precursor-like polyproteins, Pr78^{gag}, Pr110^{gag}, and Pr180^{gag}, in infected cells. The precursor-product relationships of these polyproteins to the viral core proteins is concluded based on their antigenic specificities, peptide compositions, and kinetics of appearance and removal during pulse-chase studies. The presence of the complete core protein complement in Pr78^{gag} suggests that this MMTV-specific precursor is analogous to the 65,000-dalton precursor protein, Pr65^{gag}, of murine type C viruses. The primary translation product of the type C virus *gag* gene, however, is a molecule of 80,000 daltons, Pr80^{gag}, which contains all the core sequences plus additional information which may represent a virus-specific protease (1, 12, 40). We suggest that the MMTV-specific Pr110^{gag} polyprotein represents the primary translation product of the MMTV *gag* gene and is, therefore, analogous to the type C viral Pr80^{gag}. In addition to core and envelope proteins, MMTV also contains a RNA-dependent

DNA polymerase (22). In murine type C viral systems, the 70,000 to 80,000-dalton polymerase is synthesized via a 180,000 to 200,000-dalton precursor polyprotein, Pr200^{gag-pol}, which contains both core proteins and polymerase antigenicities and peptide sequences (18, 19). We suggest that the MMTV-specific Pr180^{gag+} is analogous to the type C viral Pr200^{gag-pol} and represents the primary precursor to MMTV polymerase in the infected cell. Further studies on MMTV polymerase and Pr180^{gag+} will be necessary to establish this presumed relationship.

ACKNOWLEDGMENTS

We thank Maria Elena Leroux and Norma Mindiola for expert technical assistance.

S.J.A. is the recipient of a Rosalie B. Hite fellowship, and R.B.N. is a Leukemia Society of America Scholar. This work was supported by a grant from the American Cancer Society (NP 245) and a Public Health Service grant (RR5511-14) from the National Institutes of Health.

ADDENDUM IN PROOF

While this manuscript was in press, Dickson and Atterwill (Cell 17:1003-1012, 1979) reported the composition of two GR-MMTV precursor polyproteins, Pr77^{gag} and p110^{gag}. They also observed a viral polyprotein, termed p30, which apparently contains all of the peptides of p14 (p12) and additional peptides unique to Pr110^{gag}. Due to the complexity of the two-dimensional maps of p110^{gag} and Pr77^{gag}, however, several of the peptide spots reported to be unique to p110^{gag} appeared to comigrate with peptides of Pr77^{gag}, confounding attempts to assign some of these peptides uniquely to p110^{gag}. Peptide analysis of p30 and other intermediate-sized polyproteins suggested that the gene order in P110^{gag} is p10-pp21-p27-p14-X, with X representing the unique p110^{gag} peptides. Based on the size differential of Pr77^{gag} and p110^{gag}, one would expect the peptides representing X to approach 33,000 molecular weight. If p30 contains p14, then only 16,000 molecular weight of protein remains as p110^{gag}-specific peptides. This suggests that about 17,000 molecular weight of protein unique to p110^{gag} remains unaccounted for. Dickson and Atterwill's map of p110^{gag} does appear to contain several peptides unique to p110^{gag} but absent in p30. The placement of these sequences at N-terminal or C-terminal ends of p110^{gag} remains speculative.

LITERATURE CITED

1. Arcement, L. J., W. L. Karshin, R. B. Naso, and R. B. Arlinghaus. 1977. 'gag' polyprotein precursors of Rauscher murine leukemia virus. *Virology* 81:284-297.
2. Arlinghaus, R. B., R. B. Naso, G. A. Jamjoom, and L. J. Arcement. 1976. Biosynthesis of Rauscher leukemia viral proteins, p. 293-309. In *Animal virology*, vol. 4. ICN-UCLA Symposium on Molecular and Cellular Biology. Academic Press, New York.
3. Barbacid, M., J. R. Stephenson, and S. A. Aaronson. 1976. 'gag' gene of mammalian type C RNA tumor viruses. *Nature (London)* 262:554-559.
4. Bolognesi, D. P., R. Luftig, and J. H. Shafer. 1973. Localization of RNA tumor virus polypeptides. I. Isolation of further virus substructures. *Virology* 56:549-564.
5. Bonner, W. M., and R. A. Laskey. 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* 45:83-88.
6. Butterworth, B. E. 1976. Proteolytic processing of animal virus proteins. *Curr. Top. Microbiol. Immunol.* 77:1-41.
7. Cardiff, R. D., N. J. Puentes, L. J. T. Young, G. H. Smith, Y. A. Teramota, B. W. Altrock, and T. S. Pratt. 1978. Serological and biochemical characterization of the mammary tumor virus with localization of p10. *Virology* 85:157-167.
8. Dahl, H.-H. M., and C. Dickson. 1979. Cell-free synthesis of mouse mammary tumor virus Pr77 from virion and intracellular mRNA. *J. Virol.* 29:1131-1141.
9. Dickson, C., and M. Atterwill. 1978. Polyproteins related to the major core protein of the mouse mammary tumor virus. *J. Virol.* 26:660-672.
10. Dickson, C., J. P. Puma, and S. Nandi. 1975. Intracellular synthesis of mouse mammary tumor virus polyproteins: indication of a precursor glycoprotein. *J. Virol.* 16:250-258.
11. Dickson, C., J. P. Puma, and S. Nandi. 1976. Identification of a precursor protein to the major glycoproteins of mouse mammary tumor virus. *J. Virol.* 17:275-282.
12. Dittmar, K. J., and K. Moelling. 1978. Biochemical properties of p15-associated protease in an avian RNA tumor virus. *J. Virol.* 28:106-118.
13. Eisenman, R., and V. Vogt. 1978. The biosynthesis of oncornavirus proteins. *Biochim. Biophys. Acta* 473:187-239.
14. Fleissner, E., and E. Tress. 1973. Isolation of a ribonucleoprotein structure from oncornaviruses. *J. Virol.* 12:1612-1615.
15. Jamjoom, G. A., V. L. Ng, and R. B. Arlinghaus. 1978. Inhibition of maturation of Rauscher leukemia virus by amino acid analogs. *J. Virol.* 25:408-412.
16. Karshin, W. L., L. J. Arcement, R. B. Naso, and R. B. Arlinghaus. 1977. Common precursor for Rauscher leukemia virus gp69/71, p15(E) and p12(E). *J. Virol.* 23:787-798.
17. Kessler, S. W. 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. *J. Immunol.* 115:1617-1624.
18. Kopchick, J. J., G. A. Jamjoom, K. F. Watson, and R. B. Arlinghaus. 1978. Biosynthesis of reverse transcriptase from Rauscher murine leukemia virus by synthesis and cleavage of a 'gag-pol' read-through viral precursor polyprotein. *Proc. Natl. Acad. U.S.A.* 75:2016-2020.
19. Kopchick, J. J., W. J. Karshin, and R. B. Arlinghaus. 1979. Tryptic peptide analysis of 'gag' and 'gag-pol' gene products of Rauscher murine leukemia virus. *J. Virol.* 30:610-623.
20. Laskey, R. A., and A. D. Mills. 1975. Quantitative film detection of ³H and ¹⁴C in polyacrylamide gels by fluorography. *Eur. J. Biochem.* 56:335-341.
21. Long, C. W., R. Berzinski, and R. V. Gilden. 1977. Immunologic studies of the low-molecular-weight DNA binding protein of murine oncornaviruses. *Int. J. Cancer* 19:843-850.
22. Marcus, S. L., N. H. Sarkar, and M. J. Modak. 1976. Purification and properties of murine mammary tumor virus DNA polymerase. *Virology* 71:242-254.
23. Muhlbock, O. 1965. Note on a new inbred mouse-strain, GR/A. *Eur. J. Cancer* 1:123-124.
24. Murphy, E. C., Jr., and R. B. Arlinghaus. 1978. Tryptic peptide analyses of polypeptides generated by premature termination of cell-free protein synthesis allow a determination of the Rauscher leukemia virus gag gene order. *J. Virol.* 28:929-935.
25. Naso, R. B., L. J. Arcement, W. L. Karshin, G. A. Jamjoom, and R. B. Arlinghaus. 1976. A fucose-deficient glycoprotein precursor to Rauscher leukemia virus gp69/71. *Proc. Natl. Acad. Sci. U.S.A.* 73:2326-2330.
26. Naso, R. B., and R. L. Brown. 1977. Synthesis and cleavage of Rauscher leukemia virus precursor proteins in synchronized cells. *Virology* 82:247-251.

27. Nüsse, R., F. A. M. Asselbergs, M. H. L. Salden, R. J. A. M. Michalides, and H. Bloemendal. 1978. Translation of mouse mammary tumor virus RNA: precursor polypeptides are phosphorylated during processing. *Virology* **91**:106-115.
28. Pal, B. K., R. M. McAllister, M. B. Gardner, and P. Roy-Burman. 1975. Comparative studies on the structural phosphoproteins of mammalian type C viruses. *J. Virol.* **16**:123-131.
29. Premkumar-Reddy, E., S. G. Devare, R. Vasuder, and P. S. Sarma. 1977. Simplified radioimmunoassay for viral antigens: use of *Staphylococcus aureus* as an adsorbent for antigen-antibody complexes. *J. Natl. Cancer Inst.* **58**:1859-1861.
30. Racevskis, J., and N. H. Sarkar. 1978. Synthesis and processing of precursor polypeptides to murine mammary tumor virus structural proteins. *J. Virol.* **25**:374-383.
31. Reynolds, R. K., and J. R. Stephenson. 1977. Intracistronic mapping of the murine type C viral 'gag' gene by use of conditional lethal replication mutants. *Virology* **81**:328-340.
32. Ringold, G., E. Y. Lasfargues, M. J. Bishop, and H. E. Varmus. 1975. Production of mouse mammary tumor virus by cultured cells in the absence and presence of hormones: assay by molecular hybridization. *Virology* **65**:135-147.
33. Sarkar, N. H., E. S. Whittington, J. Racevskis, and S. L. Marcus. 1978. Phosphoproteins of the murine mammary tumor virus. *Virology* **91**:407-422.
34. Schochetman, G., C. W. Long, S. Oroszlan, L. Arthur, and D. L. Fine. 1978. Isolation of separate precursor polypeptides for mouse mammary tumor virus glycoproteins and nonglycoproteins. *Virology* **85**:168-174.
35. Schochetman, G., S. Oroszlan, L. Arthur, and D. L. Fine. 1977. Gene order of the mouse mammary tumor virus glycoproteins. *Virology* **83**:72-83.
36. Shapiro, S. Z., M. Strand, and A. Billiau. 1977. Synthesis and cleavage processing of oncornavirus proteins during interferon inhibition of virus release. *Infect. Immun.* **16**:742-747.
37. Smith, G. H. 1978. Evidence for a precursor-product relationship between intracytoplasmic A particles and mouse mammary tumor virus cores. *J. Gen. Virol.* **41**:193-200.
38. Tanaka, H. 1977. Precursor-product relationship between nonglycosylated polypeptides of A and B particles of mouse mammary tumor virus. *Virology* **76**:835-850.
39. Van de Ven, J. M., A. J. M. Vermorken, C. Onnekink, H. P. J. Bloemers, and H. Bloemendal. 1978. Structural studies on Rauscher murine leukemia virus: isolation and characterization of viral envelopes. *J. Virol.* **27**:595-603.
40. Yoshinaka, Y., and R. B. Luftig. 1977. Properties of a p70 proteolytic factor of murine leukemia viruses. *Cell* **12**:709-719.