RAPID METABOLISM OF MOLONEY LEUKEMIA VIRUS PRECURSOR POLYPEPTIDES IN VIRUS INFECTED SWISS MOUSE EMBRYO CELLS

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Summary: Viral protein synthesis in Moloney murine leukemia virus infected high passage mouse embryo cells was studied utilizing monospecific antisera to the viral core protein p30 and envelope protein gp71. Pulse-chase analysis of [^{35}S]methionine-labeled polypeptides in combination with the demonstration of the presence of either gp71 or p30-specific antigenic determinants in them indicated a 84,000-dalton polypeptide as the precursor of viral glycoproteins and four metabolically unstable polypeptides of approximate molecular weights 88,000, 72,000, 62,000, and 39,000 as the precursors of viral core protein, p30. The p30-containing 88,000 and 72,000-dalton polypeptides were distinctly seen in this system under normal growth conditions. Further, the processing of p30 precursors was very rapid and was complete during a 40 min chase while only partial processing of glycoprotein precursor was observed during the same period.

Recent studies indicate that RNA tumor virus structural proteins are generated by post-translational cleavage of large precursor polypeptides. In avian myeloblastosis virus (AMV) infected cells, the gag (group specific antigen) proteins were shown to be produced by sequential cleavage of four high molecular weight polypeptides of 76,000, 66,000, 60,000, and 32,000 daltons (1). Later studies with cells producing Rauscher murine leukemia virus (RLV) consistently identified two polypeptides of 65,000 and 75,000-80,000 daltons as precursors of murine gag proteins (2-5). These studies also demonstrated an increase in the amount of high-molecular-weight precursor proteins when the cells were grown in the presence of amino acid analogs (3,4) or protease inhibitors (5). Cat thymus cells chronically infected with feline leukemia virus (FLV) contained a 60,000-dalton gag precursor under normal growth conditions and an additional 70,000-dalton precursor protein when L-azetidine-2-carboxylic acid was present in the growth medium (6). The feline and avian virus precursor polypeptides were shown to be processed in 30 to 60 minutes (1,6) while 2 to 4 hours were required for the processing of major proportion of the murine p30 precursors in RLV producing cells (2-5). These differences in the number of precursor polypeptides and their processing rates may reflect variations in the influence of host cell metabolic activity on the processing of precursor polypeptides.

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This communication presents studies on the biogenesis of two major proteins of the gag and env (envelope) genes of Moloney murine leukemia virus in mouse cells newly infected with the virus. Four high-molecuar-weight polypeptides (88,000, 72,000, 62,000, and 39,000 daltons) with p30-specific antigenic determinants and one large gp71-specific polypeptide were detected in pulse-labeled cells. In contrast to other murine virus producing cells (2-5), these precursor polypeptides were rapidly processed in this cell-virus system.

Materials and Methods

Radioactive labeling of cells and virus. High passage Swiss mouse embryo (HPME) cells and Moloney murine leukemia virus (MLV) were obtained through the courtesy of Dr. A.J. Hackett. Cells were grown in Eagle's minimal essential medium (MEM) containing 10% fetal calf serum (FCS) and were infected with MLV as described (7). At 48 h post-infection, cell monolayers (75 cm^2) were incubated in pulse medium (methionine free MEM containing 10% dialyzed FCS) for 30 min and then pulsed for 15 min with 6 ml of pulse medium containing 100 µCi/ml [^{35}S]methionine (328 Ci/mmol, New England Nuclear). Labeling was terminated by washing the monolayers with MEM and the radioactivity was chased by incubation of washed monolayers in normal growth medium (MEM) for the desired periods. At the end of pulse-chase, the monolayers were washed with cold phosphate buffered saline (PBS) and the cells were lysed with PBS containing 1% Triton and 1% sodium deoxycholate (lysis buffer). Pulse-chase labeling of uninfected cell polypeptides was carried out simultaneously under identical conditions. Infected cell monolayers were labeled with [^{3}H]glucosamine in 6 ml of MEM containing 100 µCi/ml of D-[^{3}H] glucosamine (10 Ci/mmol, New England Nuclear). After 4 h of incubation, the radioactive medium was removed, and cells were washed with cold PBS and were lysed as described above.

For radioactive labeling of virus, [^{35}S]methionine or [^{3}H]glucosamine containing media recovered from pulse or short-term labeled cells was filtered through 0.2 µ millipore filters, diluted with an equal volume of MEM containing 10% FCS, and used (25 ml/75 cm^2 monolayers) for the growth of infected cell-monolayers. Following 42 h of incubation virus was purified from the culture medium (8).

Immune Precipitation. Anti-MLV serum was prepared by injecting Nonidet P-40 (0.1%) disrupted MLV into a rabbit (8). Goat antisera to RLV p30 and gp71 were obtained through the Office of Program Resources and Logistics, Viral Oncology Branch, National Institutes of Health. One ml aliquots of cell lysates were centrifuged at 12,000 x g and virus-specific polypeptides present in the supernatant were precipitated with 75 µl of appropriate antisera in the presence of 3 µg of detergent treated MLV (8). Immune precipitates were collected by centrifugation (10,000 x g, 10 min) through 1 ml of 10% sucrose in lysis buffer washed twice with lysis buffer, and finally dissolved in electrophoresis sample buffer (60 mM Tris-HCl, pH 6.9, 5% sodium dodecyl sulfate (SDS), 1% β-mercaptoethanol, 0.002% bromophenol blue, and 15% glycerol).

Polyacrylamide gel electrophoresis (PAGE). The immune precipitates in sample buffer were heated for 5 min at 100°C and electrophoresed in SDS-polyacrylamide gel slabs (9,10). Radioactive protein bands were visualized either by autoradiography (10) or fluorography (11). The molecular weight of virus-specific polypeptides were estimated from the mobility of marker proteins electrophoresed in parallel lanes (10).

Results

Characterization of antisera. Antisera to detergent treated MLV and monospecific antisera to RLV proteins p30 and gp71 which share antigenic determinants with the corresponding MLV proteins (12) were used to specifically precip-

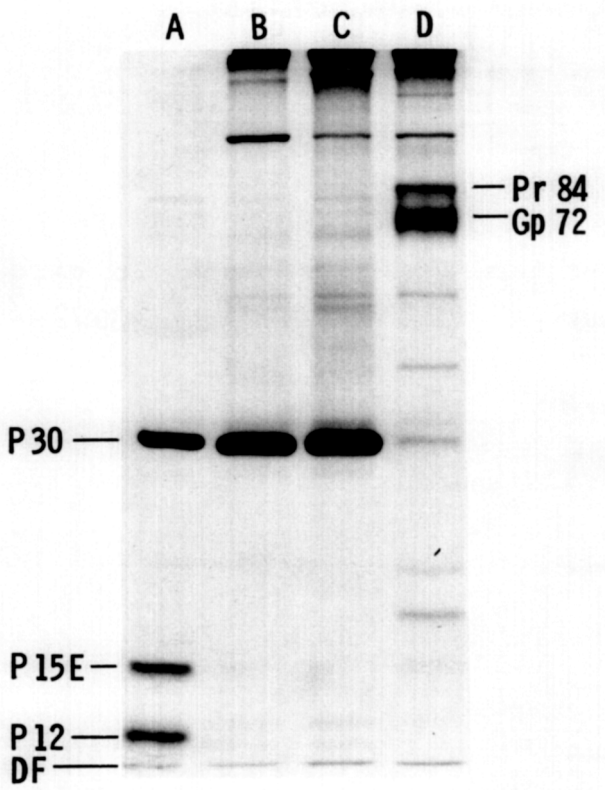

Figure 1. Analysis of MLV-specific polypeptides present in cells labeled for 42 h with [^{35}S]methionine. (A) [^{35}S]methionine-labeled MLV (47,500 cpm). (B) Anti-MLV serum precipitate of proteins present in MLV infected cell lysate labeled for 42 h with [^{35}S]methionine. (C) Cell lysate as in (B), but anti-p30 serum precipitate. (D) Cell lysate as in (B), but anti-gp71 serum precipitate. The immune precipitates containing approximately 50,000 cpm (B-D) were electrophoresed in a 12% polyacrylamide gel slab and fluorographed for 4 days. DF, Dye front.

itate virus-specific polypeptides present in MLV infected cells. The specificity of these antisera were determined by immune precipitation analysis of [^{35}S]methionine-labeled proteins present in MLV infected cells. The anti-MLV and anti-p30 serum precipitates showed a strikingly similar polypeptide composition (Fig. 1B and C); in either case a protein comigrating with viral p30 was found to be the major component in the immune precipitate. Anti-gp71 serum precipitated two major polypeptides of approximate molecular weight 84,000 (pr84) and 72,000 (gp72) from infected cell lysates (Fig. 1D); experiments described in subsequent sections indicate that these proteins are related to viral glycoproteins.

The anti-MLV serum used in this study and the anti-AMV serum preparations used by Vogt et al (1) failed to precipitate glycoproteins from virus infected

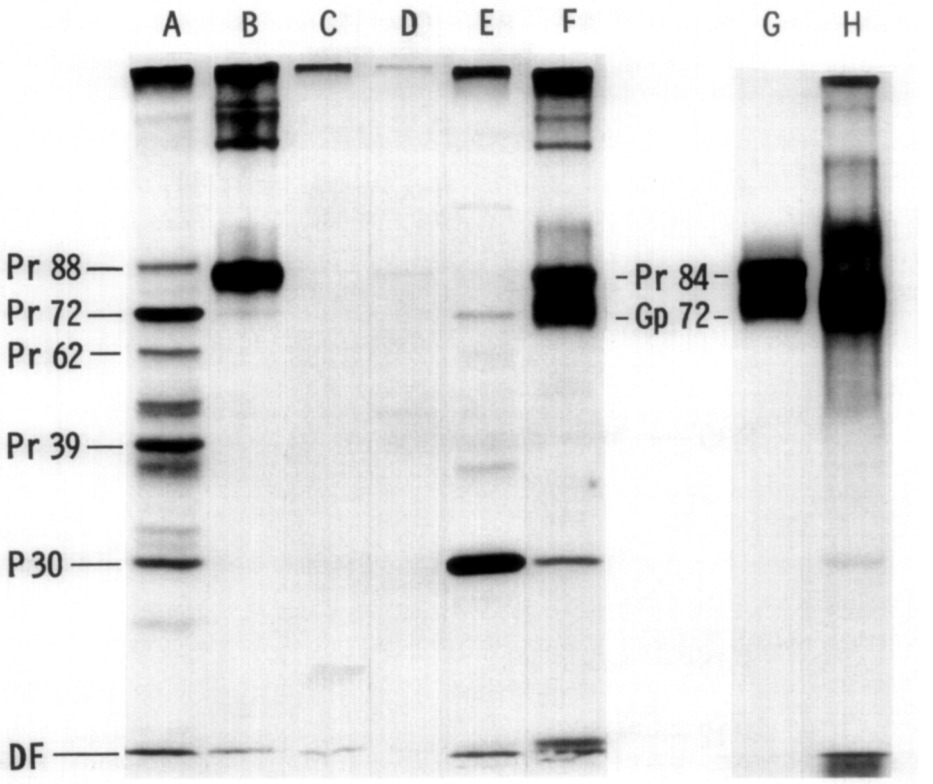

Figure 2. Characterization of MLV-specific polypeptides in pulse-chase experiments. (A) Anti-p30 serum precipitate of proteins present in MLV-infected cell lysate pulse labeled for 15 min with [^{35}S]methionine. (B) Cell lysate as in (A), but anti-gp71 serum precipitate. (C) Anti-p30 serum precipitate of proteins present in uninfected cell lysates labeled as in (A). (D) Cell lysate as in (C), but anti-gp71 serum precipitate. (E) Anti-p30 serum precipitate of proteins present in infected cell lysate pulse labeled for 15 min with [^{35}S]methionine and chased for 40 min in complete medium. (F) Cell lysate as in (E), but anti-gp71 serum precipitate. (G) Anti-gp71 serum precipitate (60,000 cpm) of proteins present in infected cell lysates labeled for 4 h with [^{3}H]glucosamine. (H) [^{3}H]glucosamine-labeled virus (54,300 cpm). The immune precipitates containing 20,000-30,000 (A,B,E,F) or 10,000-12,000 (C,D) cpm were electrophoresed in a 10% polyacrylamide gel slab and fluorographed for 4 days.

cells. The failure of anti-MLV serum to precipitate proteins corresponding to p15E and p12 from infected cell lysates may be due to low antigenicity and/or a low intracellular pool of these proteins.

Pulse-chase analysis of gp71-specific proteins. The results depicted in Fig. 1 showed two virus-specific proteins (p30 and gp72) as predominant components in the immune precipitates of long-term labeled MLV infected cells. In studies described below the mode of formation of these proteins were investigated in pulse-chase experiments. MLV infected cells were pulse-labeled with [^{35}S]

methionine at 48 h post-infection and the radioactivity was chased by incubation of pulse-labeled monolayers in normal growth medium. Virus-specific polypeptides present in pulse or pulse-chase labeled cells were immune precipitated with appropriate antisera and resolved by electrophoresis in high-resolution polyacrylamide gel slabs. Anti-gp71 serum precipitated a major polypeptide of approximate molecular weight 84,000 (pr84) from pulse-labeled cell lysates (Fig. 2B). Protein bands with similar electrophoretic mobility were not present in the anti-p30 serum precipitate of pulse labeled infected cells (Fig. 2A) and in anti-gp71 serum precipitates of pulse-labeled uninfected cells (Fig. 2D). Following a 40 min chase of the label, a second band of gp71-specific proteins (molecular weight 72,000) appeared with concomitant reduction of radioactivity in the 84,000-dalton protein suggesting a precursor role of this polypeptide for gp72 (Fig. 2F). These observations are consistent with the recent findings on the formation of avian (13) and murine (3-5,14, 15) oncornavirus glycoproteins by the way of precursors weighing 80,000 to 90,000 daltons.

Evidence that pr84 and gp72 are related to viral glycoproteins were obtained from the analysis of [^{3}H]glucosamine-labeled proteins present in the MLV infected cells and virus (Fig. 2G and H). Again, these experiments indicate the presence of two gp71-specific polypeptides of approximate molecular weight 84,000 and 72,000 in the immune precipitates of [^{3}H]glucosamine-labeled MLV infected cells (Fig. 2G); the 72,000-dalton polypeptide had the same electrophoretic mobility as the major glucosamine-labeled 72,000 dalton-polypeptide of the virus (Fig. 2H). The comigration in SDS-PAGE of cellular gp71-specific 72,000 dalton-polypeptide with the virion glycoprotein of molecular weight 72,000 and their glucosamine content indicate that gp72 is closely related to viral glycoprotein.

Rapid metabolism of p30 precursors. MLV infected cells pulse-labeled with [^{35}S]methionine for 15 min contained four p30 specific polypeptides of approximate molecular weights 88,000, 72,000, 62,000, and 39,000 (Fig. 2A and 3A). [^{35}S] methionine-labeled polypeptides corresponding to pr88, pr72, pr39,and p30 were not present in the anti-MLV serum precipitates obtained from uninfected cells (Fig. 3E and F). Under chase conditions the level of radioactivity in the high molecular weight virus-specific polypeptides decreased with concomitant accumulation of label in a polypeptide of 30,000 daltons (Fig. 3A-3D). These observations in concert with the presence of p30-specific antigenic determinants in them strongly suggest a precursor role for the 88,000, 72,000, 62,000 and 39,000-dalton polypeptides in the formation of p30. The high-molecular-weight p30 precursors are distinctly seen in this system under normal growth conditions; further, the p30 precursors were rapidly processed since almost complete transition of label from pr88, pr72, pr62, and pr39, to p30 was observed during a 40 min chase of radioactivity in normal growth medium (Fig. 2 and 3).

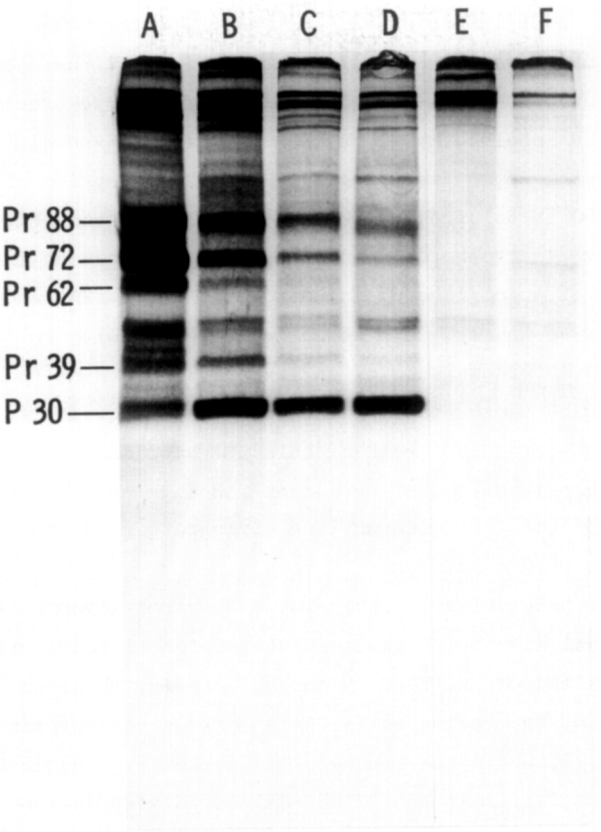

Figure 3. Kinetics of formation of p30 in MLV infected HPME cells. (A) Immune precipitate of proteins present in cell lysate from MLV infected cells pulsed with [^{35}S]methionine for 15 min. (B) As in (A), and chased for 20 min. (C) As in (A), and chased for 40 min. (D) As in (A), and chased for 60 min. (E) Anti-MLV serum precipitate of proteins present in cell lysate from uninfected cells pulsed with [^{35}S]methionine for 15 min. (F) As in (E), and chased for 120 min. *Immune precipitates containing approximately 56,000 cpm were electrophoresed in* a 5-22% polyacrylamide gel slab and autoradiographed for 8 days.

Discussion

Cells newly infected with oncornaviruses are suitable for studies of viral gene expression at different stages of infection and for an understanding of the role of host cells in viral protein synthesis. The results presented in this report demonstrate the advantages of utilizing MLV infected HPME cells for such studies. Two important features of this cell-virus system are (i) the ability of infected cells to synthesize large amounts of high-molecular-weight precursors under normal growth conditions, and (ii) rapid processing of precursor polypeptides in infected cells. In studies with RLV infected cells, it was necessary to use protease inhibitors (5) or amino acid analogs (3,4) to

increase the amounts of high-molecular-weight precursor polypeptides; however, the former compounds interfere with viral protein synthesis (5) and the incorporation of amino acid analogs into the precursors appear to alter the conformation of precursors, resulting in a lower sensitivity of precursors to proteases (3,4). In MLV infected HPME cells, p30 precursors of high-molecular-weight (pr88 and pr72) could be distinctly seen in the absence of added protease inhibitors (Fig. 3A). A p30 precursor polypeptide corresponding to MLV pr88 was not detected in other murine systems under normal growth conditions; the 82,000 dalton-gag precursor that was observed in RLV infected cells (4) when canavanine was present in the growth medium may correspond to MLV pr88. The MLV pr72 may correspond to the RLV precursor weighing 75,000 to 85,000 daltons (2-4) since all these polypeptides migrate below the glycoprotein precursor band in SDS-PAGE; the MLV pr62 may be analogous to RLV pr65 (2-4).

The rapid metabolism of gag and envelope protein precursors in MLV infected cells and the slow processing of these proteins in cells replicating RLV (2-5) and MSV(MLV) (15) imply that the level or availability of specific proteases in these cells may determine the extent of stability of virus-specific precursor polypeptides. Recent studies indicate a role for a trypsin-like protease in the cleavage of an RLV precursor protein, p70 (16) and the involvement of a chymotrypsin-like proteolytic activity in the processing of another RLV precursor protein, Prla (5).

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