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Subcellular Distribution of Newly Synthesized Virus-Specific Polypeptides in Moloney Murine Leukemia Virus-Infected Cells

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Immune precipitation analysis of pulse-labeled proteins present in subcellular fractions of mouse embryo cells infected with Moloney murine leukemia virus showed the presence of anti-gp70 serum-precipitable viral envelope gene products mainly in the microsomal fraction of these cells. In contrast, anti-p30 serum-specific *gag* (group specific antigen) gene products were found to be distributed in similar amounts in both the microsomal and postmicrosomal supernatant fractions of pulse-labeled cells.

Cells replicating murine leukemia viruses contain two virus-specific RNA species that sediment at 35S and 20S (2, 3, 19). In cell-free systems, 35S RNA directs the synthesis of *gag* proteins (7, 12, 21), and the 20S RNA species codes for the synthesis of viral envelope proteins (12, 18, 21). In our previous studies, the 35S RNA species was detected in both free and membrane-bound polyribosomes and the 20S RNA was observed exclusively in the membrane-bound polyribosomes derived from the microsomal fraction of virus-producing cells (16). In efforts to understand the significance of the above pattern of distribution of virus-specific RNAs, I analyzed the newly synthesized virus-specific proteins present in the microsomal and postmicrosomal supernatant fractions of Moloney murine leukemia virus (MLV)-infected high-passage Swiss mouse embryo cells. The results of these studies indicate a unique subcellular distribution pattern of viral envelope and *gag* gene products that is consistent with the polyribosomal distribution of the virus-specific RNA species coding for these proteins.

The cell-virus system used in this study had been shown to synthesize large amounts of high-molecular-weight virus-specific precursor polypeptides designated according to their molecular weights as Pr88, Pr84, Pr72, Pr62, and Pr39 (15). In this paper, the retrovirus protein nomenclature recommended by the National Cancer Institute Tumor Viral Immunology Workshop (1977) was followed. This new nomenclature is related to our former nomenclature (15) as follows: Pr88 = $Pr88^{gag}$, Pr84 = $Pr84^{env}$, Pr72 = $Pr72^{gag}$, Pr62 = $Pr62^{gag}$, Pr 39 = $Pr39^{gag}$, and gp 74 = gp70. In studies described below, I investigated the intracellular distribution of these virus-specific polypeptides in two subcellular (microsomal and postmicrosomal supernatant) fractions of MLV-infected cells that depict a unique distribution of virus-specific RNA species (16).

The subcellular fractions were prepared by following a simple and rapid procedure that was originally used for the isolation of endoplasmic reticulum membrane fractions (27,000 × *g* cytoplasmic pellet) predominantly containing the mRNA's for secretory proteins (1, 8, 22). The microsomal fraction prepared in this manner showed a higher content of oncornavirus-specific RNAs and proteins than the postmicrosomal supernatant fraction and contained all the cytoplasmic 20S RNAs in addition to the 35S RNA species (16, 23); the latter species was also present in the postmicrosomal supernatant fraction (16). These fractions were not purified further, since prolonged purification procedures result (i) in the degradation of virus-specific RNAs (16) and (ii) in the proteolytic cleavage of newly synthesized virus-specific proteins (unpublished data). Figure 1 shows the immune precipitation analysis of virus-specific polypeptides present in the subcellular fractions of virus-infected cells that were pulse-labeled for 10 min with [^{35}S]-methionine. Anti-p30 serum precipitated five polypeptides ($Pr84^{gag}$, Pr 72^{gag}, $Pr62^{gag}$, $Pr39^{gag}$, and p30), whereas anti-gp70 serum precipitated one polypeptide, $Pr84^{env}$, from pulse-labeled cells; polypeptides with similar electrophoretic mobilities were not precipitated from infected cell lysates by normal rabbit serum (Fig. 1, mid-

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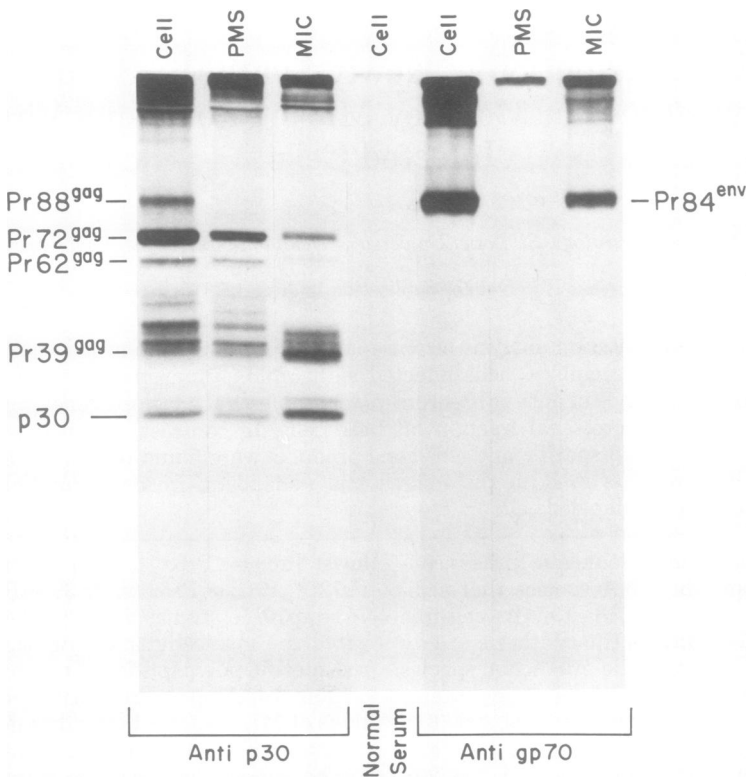

FIG. 1. *Subcellular distribution of MLV-specific polypeptides in pulse-labeled cells. Subconfluent monolayers (75 cm^2) of high-passage Swiss mouse embryo cells were infected with MLV and pulse-labeled for 10 min with 600 μCi of [^{35}S]methionine as previously described (15). At the end of pulse labeling, the cells were washed with cold phosphate-buffered saline, suspended in reticulocyte standard buffer (14), and homogenized, and nuclei were removed (17). The postnuclear supernatant was then centrifuged at 27,000 × g for 5 min to obtain microsomal and postmicrosomal supernatant fractions (1, 8, 14, 16, 22). Samples of the subcellular fractions were mixed with equal volumes of 2× phosphate-buffered saline containing 2% Triton X-100 and 2% sodium deoxycholate and clarified by centrifugation at 12,000 × g, and the virus-specific polypeptides present in the supernatant were precipitated with either anti-p30 or anti-gp70 sera (15). The immune precipitates were dissolved in electrophoresis sample buffer (15), heated for 5 min at 100°C, and subjected to electrophoresis in 12% polyacrylamide gels (15). Radioactive protein bands in the gel were visualized by fluorography (15), and their molecular weights were estimated as previously described (5, 15). PMS, Postmicrosomal supernatant; MIC, microsomes.*

dle lane) or from uninfected cell lysates with anti-p30 or anti-gp70 sera (15). Four of the p30-specific polypeptides (Pr72^{gag}, Pr62^{gag}, Pr39^{gag}, and p30) were present in both the microsomal and postmicrosomal supernatant fractions of infected cells. The fifth polypeptide, Pr88^{gag}, was seen only in the cell lysates. The absence of this polypeptide in the subcellular fractions appears to be due to its rapid processing during the period of cell fractionation. The Pr39^{gag} region in Fig. 1 contained three p30-specific proteins; these might be the intermediate forms of precursors that generate p30. Such intermediates, weighing 40,000 to 50,000 daltons, were previously observed in Rauscher leukemia virus-infected cells (20).

In contrast to the distribution of the *gag* gene products in similar amounts in the microsomal and postmicrosomal supernatant fractions, the envelope gene products were present predominantly in the microsomal fraction of the pulse- or pulse-chase-labeled cells (Fig. 1 and 2). The presence of trace amounts of envelope gene products in the postmicrosomal supernatant fraction (Fig. 2B) might be due to a possible contamination of this fraction with microsomes. Immune precipitation analysis of proteins from MLV-infected cells that were pulse-labeled for

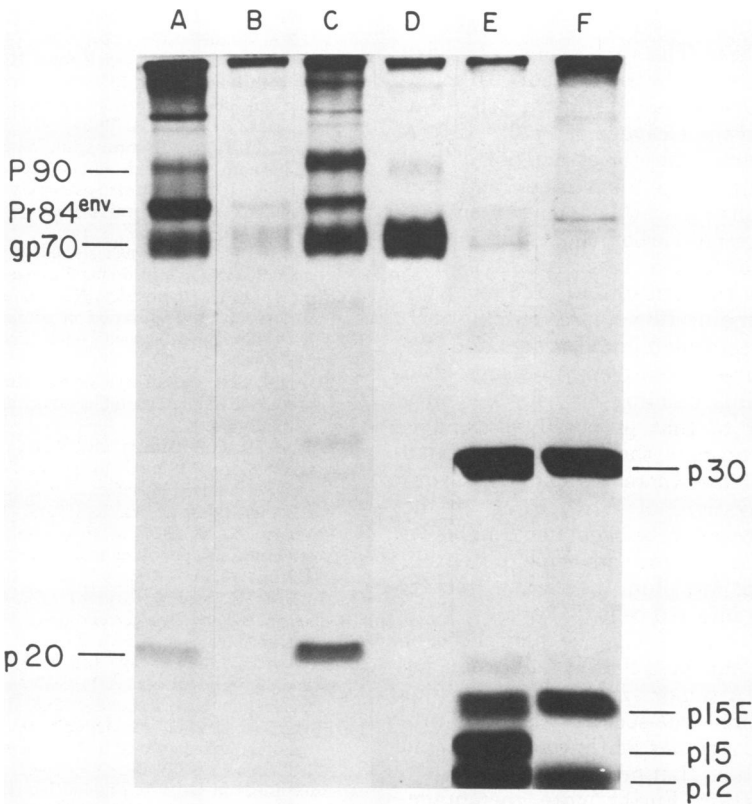

FIG. 2. *Pulse-chase analysis of anti-gp70 serum-specific proteins present in the subcellular fractions of MLV-infected cells. Monolayers of MLV-infected cells were pulse-labeled for 10 min with [^{35}S]methionine. Labeling was terminated by washing the monolayers with normal growth medium, and the radioactivity was chased by incubation of washed monolayers in normal growth medium for 15 min. Details of cell fractionation and immune precipitation analysis of virus-specific proteins are described in the legend to Fig. 1 and in reference 15. (A) Anti-gp70 serum precipitate of proteins present in the lysates of pulse-chase-labeled cells. (B) Immune precipitate of proteins present in the postmicrosomal supernatant fraction of cells labeled as in (A). (C) Immune precipitate of proteins present in the microsomal fraction of cells labeled as in (A). (D) [^{3}H]-glucosamine-labeled virus. (E) [^{3}H]leucine-labeled virus. (F) [^{35}S]methionine-labeled virus.*

10 min with [^{35}S]methionine indicated Pr84^{env} as the predominant component of these cells (Fig. 1). When the pulse-labeled cells were chased for 15 min in normal growth medium, three additional gp70-specific proteins (P90, gp70, and p20) were observed in the total cell lysates and in the microsomal fraction (Fig. 2). gp70 had the same electrophoretic mobility as the [^{3}H]glucosamine-labeled viral envelope protein (Fig. 2D), and a precursor-product relationship between Pr84^{env} and gp70 has been demonstrated (15). The second predominant component, P90, contained antigenic determinants of gp70 as evidenced by its precipitation by anti-gp70 serum. This protein was not characterized further in this study. Additional work, involving kinetic analysis of the extent of labeling of P90 with different sugars and a comparison of tryptic peptides of P90 and Pr84^{env}, is needed to know whether P90 is a fully glycosylated precursor-intermediate of Pr84^{env} analogous to the gp92 of avian oncornavirus-infected cells (11). Alternatively, it is possible that P90 is a disulfide-bonded complex of p15E and gp70 (13). Similar studies are needed to relate p20 to p15E, which is also precipitated by anti-gp70 serum (unpublished data). The cross-reactivity of anti-gp70 serum with p15E is due to the association of p15E with the viral antigen gp70 (20).

The immune precipitation analyses depicted in Fig. 1 and 2 further show that almost all the mature-size viral envelope proteins, and a major proportion of p30 and its low-molecular-weight precursor intermediate Pr39^{gag}, are associated with the microsomal fraction. These results suggest that the processing of both *gag* and enve-

lope gene products may require membrane-associated enzymes. The processing of avian $Pr76^{gag}$ was shown to be associated with membranes, since agents that disrupted cell membranes inhibited the cleavage of $Pr76^{gag}$ (24). A recent study (6) indicates the association of viral envelope proteins in the membrane fraction of pulse-labeled cells replicating avian sarcoma virus. As we suggested earlier, the membrane association of virus-specific macromolecules may be essential for the maturation process of the virus (23). The maturation of virus-specific polypeptides involves proteolytic cleavage and glycosylation and may require enzymes present in microsomes. Virus budding may proceed in a manner similar to that proposed for Sindbis virus (25). Interestingly, the results presented in this report and elsewhere (16) are analogous with the observations of Wirth et al. on the exclusive localization of subgenomic Sindbis virus-specific RNA and viral envelope proteins in the endoplasmic reticulum membrane fraction of Sindbis virus-infected cells (25).

Our previous studies (17, 23) and those of others (3, 4, 10) indicated that in cells producing the murine RNA tumor viruses there was four to six times more virus-specific RNA and proteins in the microsomes and membrane-bound polyribosomes than that present in the free polyribosomal fraction. Furthermore, membrane-bound polyribosomes and the microsomal fraction of these cells contained the subgenomic 20S viral RNA (3, 16) and an RNase III-like activity that may possibly be involved in the generation of the 20S RNA species (9, 14). These observations and the present findings on the localization of the viral envelope gene products in the microsomal fraction are consistent with the notion that the microsomal membrane fraction may play an important role in the replication of RNA tumor viruses.

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