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Polyproteins Related to the Major Core Protein of Mouse Mammary Tumor Virus

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The mouse mammary tumor virus (MuMTV) contains several low-molecular-weight proteins which, together with the genomic RNA, constitute the core structure of the virion. The most abundant protein in the core is the 27,000-dalton protein (p27), and, by analogy to the type C viruses, this protein probably forms the core shell. In mouse mammary tumor cell lines (GR and Mm5MT) producing MuMTV the major p27 antigenic specificity resides in a large protein, which migrates in polyacrylamide gels as a doublet of 77,000 and 75,000 daltons (p77/75). A series of lower-molecular-weight proteins, p61, p48, p38, and p34, is also present in small amounts and is probably derived by proteolytic cleavage of the p77/75. These proteins have been identified by immunoprecipitation with monospecific antiserum, and their sequence relatedness to p27 has been determined by an analysis of the peptides after trypsin digestion. After a 15-min pulse with [^{35}S]-methionine, all of the p27-related proteins in these cell lines were labeled and, during a subsequent chase, progressively disappeared. The p27 was labeled poorly during the pulse, but the amount of label in this protein increased during the chase. A quantitation of these experiments suggested that the majority of the p27-related proteins were quite rapidly turned over in these cell lines. Hence, if p27 is derived by a progressive proteolytic cleavage mechanism, then the process is inefficient in the GR cells and only moderately efficient in the Mm5MT cells. When MuMTV was isolated from the culture medium of these cells harvested at 5-min intervals, the major p27-related protein was p34. The p27 accounted for only 29% of the anti-p27 serum immunoprecipitable proteins compared to 95% in virus isolated from an 18-h harvest. Incubation of the rapid-harvest virus at 37°C for 2 h resulted in some conversion of p34 to p27. These results suggest that some of the p27 in MuMTV is formed in the virions by proteolytic cleavage of p34.

Several recent studies have shown that oncornavirus structural proteins are synthesized as polyproteins, which are subsequently cleaved by as yet unidentified proteases to yield the characteristic virion structural proteins (1, 7, 8, 12, 13, 19, 30-32, 38, 39; M. J. Hayman, Virology, in press). Previously, we described the presence of a mouse mammary tumor virus (MuMTV) polyprotein in mammary tumor cells, which gave rise upon cleavage to both major virion glycoproteins (7, 8). In this paper we demonstrate the presence of another MuMTV polyprotein which migrates as a doublet with estimated molecular weights of 77,000 and 75,000, respectively, and appears to be the primary polyprotein precursor to the major core protein, p27, of MuMTV (4, 23, 28, 36; R. D. Cardiff, M. J. Puentes, L. J. T. Young, G. H. Smith, Y. A. Teramoto, B. W. Altrock, and T. S. Pratt, Virology, in press). Several other proteins, with molecular weights of 61,000, 48,000, 38,000, and 34,000, are related to the virion p27, as shown by immunoprecipitation with monospecific antiserum and by analysis of the tryptic peptides. However, little p27 was detected in the mammary tumor cell lines replicating MuMTV, and the accumulation of p27 during a chase into extracellular virions was poor when compared to the amount of label found in the presumptive high-molecular-weight precursor molecules. These results show that in two mouse mammary tumor cell lines the majority of the p27-related proteins were turned over rather than undergoing conversion to yield the p27. The cell lines used in this study showed a clear difference in their efficacy to convert the presumptive precursors into p27. Furthermore, evidence is presented to indicate that at least the final stage of the processing can occur after budding of the virions.

MATERIALS AND METHODS

Cell lines. We used two cell lines derived from mouse mammary tumors in these studies. One was derived from the GR mouse strain, as described by

Ringold et al. (26), and was maintained in Eagle minimal essential medium with antibiotics and 10% horse serum. After the initial seeding of the cells, the cultures were maintained in medium supplemented with 10 μg of dexamethasone per ml. The other cell line, Mm5MT, was originally derived from a C3H mouse by Owens and Hackett (21) and kindly supplied as a high-producer clone (11) by Larry Arthur, Frederick Cancer Research Center, Frederick, Md. These cells were maintained in similar medium, except fetal calf serum was substituted for horse serum.

A cell line derived from normal mouse mammary epithelium (NMG) by Owens et al. (22) was used as uninfected controls. The cell line was maintained as described for the Mm5MT line.

Virus production was routinely monitored in the cell lines with the reverse transcriptase assays, using the template-primer poly(rC):oligo(dG)$_{12-18}$ with either Mg^{2+} or Mn^{2+} as divalent cation (6, 10). Use of these cations allows the distinction of type B from type C murine viruses, although a low level of contamination of one in the presence of the other would not be detected. During these experiments the GR maintained a constant level of MuMTV production through 40 passages of the line; however, a substantial level of type C virus was detected after only a few passages of the Mm5MT line.

Virus. [^{35}S]methionine-labeled MuMTV was prepared and isolated as previously described (9).

Antisera. Antiserum to disrupted MuMTV was prepared in rabbits and titrated as previously described (7). The monospecific antisera against p27 were of two sources; one was a generous gift from L. O. Arthur, and the other was prepared by separating the proteins of purified MuMTV by preparative polyacrylamide slab gel electrophoresis. The p27 band was cut from the gel and extracted in 0.1% sodium dodecyl sulfate, dialyzed, and concentrated by lyophilization; 200 μg was inoculated into rabbits as previously described (7, 8).

Preparation of cell extracts. Confluent cultures of GR cells were pulsed with [^{35}S]methionine (300 to 1,000 Ci/mmol) in methionine-free Eagle medium for various times at concentrations indicated in the text. After the pulse the cells were rinsed with complete medium containing 2% serum and chased in the same medium for periods of 20 to 180 min. Whole-cell extracts were prepared and immunoprecipitated as previously described (7, 8).

Protein determinations. The method of Lowry et al. (17) was used for protein determinations, with bovine serum albumin as a standard.

Polyacrylamide gel electrophoresis. The immunoprecipitates obtained from whole-cell extracts were separated on slab gels using the discontinuous method of polyacrylamide gel electrophoresis described by Laemmli (16). In all experiments a 3% polyacrylamide stacking gel and a 10% polyacrylamide running gel were used. Electrophoresis was carried out at 60 V for 1 h and then at 100 V until the bromophenol blue dye marker reached the bottom of the running gel. After electrophoresis the gel slabs were fixed in 10% trichloroacetic acid at 4°C, washed in water for 2 h, and dried under vacuum (8). The labeled proteins were located in the dry gel by autoradiography, using Kodak X-ray film (X-Omat H1). In several experiments the labeled proteins were detected by fluorography as described (3), and Fuji X-ray film was used to locate the labeled proteins. To estimate the molecular weights of the detected proteins, the MuMTV virion proteins (9, 15, 23, 28, 37), bovine serum albumin (65,000), and phosphorylase a (94,000) were used as standards. For the MuMTV proteins the accepted molecular weights of 52,000 for the glycoprotein and 27,000 for the major internal protein were used, although we have previously estimated these proteins at molecular weights of 49,000 and 24,000, respectively (9).

Separation, extraction, and peptide analysis of virus-related proteins. To obtain sufficient amounts of labeled virus-specific proteins from the mammary tumor cell cultures for analysis of the tryptic peptides, one or two roller cultures (Corning, 490 cm^2) of GR cells were labeled with 100 μCi of [^{35}S]-methionine per ml in methionine-free Eagle minimal essential medium or 25 μCi of [^{14}C]arginine and [^{14}C]-lysine per ml in medium free of these amino acids for 1 to 4 h. A cytoplasmic extract was prepared and immunoprecipitated. The washed immunoprecipitate was separated on preparative polyacrylamide slab gels, and the virus-related proteins were localized by autoradiography of the dried gel. The labeled protein bands were cut from the gel and rehydrated, and the extracted proteins were concentrated by trichloroacetic acid precipitation (8). The isolated proteins were then oxidized with performic acid and trypsinized (5). The tryptic digest was spotted onto a CM300 cellulose thin-layer chromatography plate (Machercy-Nagel & Co.), and the peptides were separated in the first dimension by electrophoresis at pH 4.6 (2.5% acetic acid and 2.5% pyridine in water) and in the second by chromatography (*n*-butanol–acetic acid–water–pyridine [75:15:60:60]) (8). The spots were localized by autoradiography using Kodak X-ray film XH-1.

RESULTS

Specificity of antisera. To determine the specificity of the anti-p27 serum, purified [^{35}S]methionine-labeled MuMTV was disrupted with 1% Triton X-100 and 1% sodium deoxycholate and used directly in an immunoprecipitation titration. To a constant amount of the protein solution (approximately 14 μg of viral protein or 130,000 cpm) an increasing amount of antiserum was added. After incubation overnight at 4°C, the immunoprecipitates were washed, dissociated, and separated on a 10% polyacrylamide slab gel as described. An autoradiogram from such a gel is reproduced in Fig. 1 and shows that the anti-p27 serum precipitates the p27 from disrupted virions of MuMTV. Several minor proteins with higher molecular weights were also precipitated; these proteins are most likely incompletely processed polyproteins structurally related to the p27 (see below). To test the antiserum for specificity with the mammary tumor cell lines, a comparison was made using a virus-

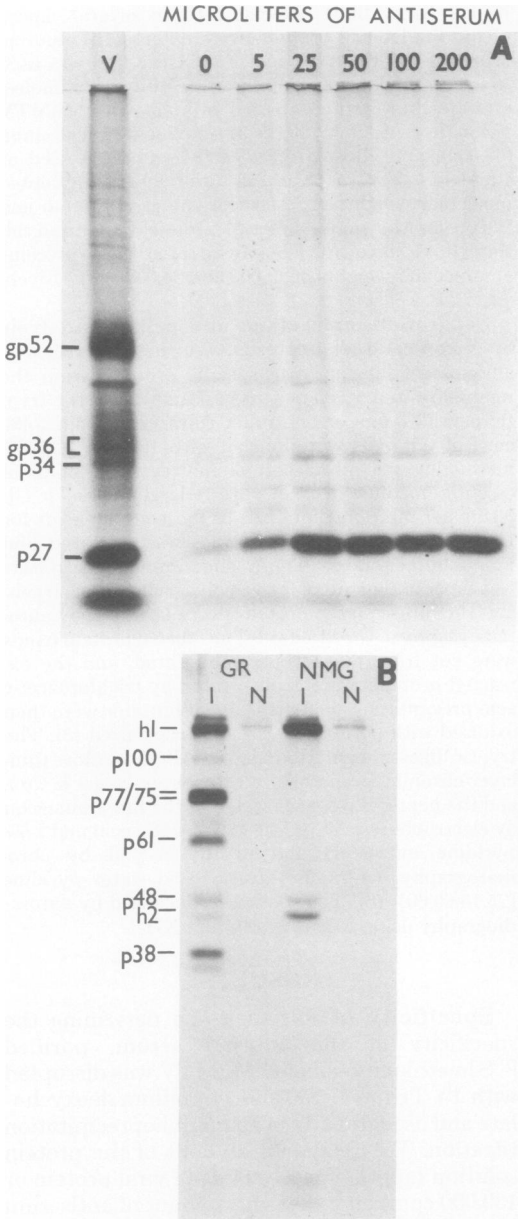

FIG. 1. *(A) Autoradiogram of an anti-p27 serum titration showing potency and specificity. Increasing amounts of antiserum from 5 to 200 μl were added to a constant amount of viral protein (disrupted virus) in a final volume of 500 μl. The immunoprecipitates were processed as described in the text and separated on a 10% polyacrylamide gel. V, Sample of [^{35}S]methionine-labeled MuMTV acting as a standard for the viral proteins. (B) Autoradiogram of two cell extracts immunoprecipitated with anti-p27 serum (I) or normal rabbit serum (N). The GR extract was prepared from one dish of GR cells labeled with 50 μCi of [^{35}S]methionine for 1 h and mixed with one dish of unlabeled NMG cells. The NMG extract was prepared with labeled NMG cells and unlabeled GR cells. h1 and h2 mark the position of host cell proteins that precipitate with the anti-p27 serum, and the numbers are the molecular weights (in kilodaltons) of the specifically immunoprecipitated proteins.*

negative line of NMG cells. A 90-mm dish of the mammary gland cell line and one of GR cells were labeled with [^{35}S]methionine at 50 μCi/ml for 1 h. Each dish of cells was then mixed with a dish of unlabeled cells in a reciprocal manner (e.g., labeled GR cells with unlabeled NMG and vice versa), and the mixed cells were processed as described. The mixing of the cell types was carried out to equalize as near as possible the control and experimental extract preparations. The anti-p27 serum precipitates a series of proteins related to the p27 of the virus (as described in detail below) and two or three host cell proteins. The amount of these host cell proteins varies from undetectable levels (see Fig. 2) to the levels shown in Fig. 1.

Cell-associated viral proteins. To identify the virus-specific proteins and the subset of these proteins antigenically related to the p27, a series of pulse-chase experiments was performed with the GR line. Cell cultures were pulsed with [^{35}S]methionine (100 μCi/ml) for 15 min and chased for periods of 20 min to 2 h. The cell extract from each time point was divided in two and immunoprecipitated with normal rabbit serum or anti-MuMTV serum (Fig. 2A); in the second experiment, the extract was immunoprecipitated with either normal rabbit serum or anti-p27 serum (Fig. 2B). The anti-p27 serum precipitated all the proteins precipitated by the anti-MuMTV serum except for the glycoproteins gp52, gp36, and gp73. These glycoproteins represent the two major virion glycoproteins and their precursor (7, 8).

In the GR cell line the major protein that immunoprecipitates with the anti-p27 serum (Fig. 2B) migrates as a doublet of 77,000 and 75,000 daltons, respectively (p77/75). The immunoprecipitates also contained several proteins with molecular weights ranging between those of p77/75 and p27, as well as two minor proteins with higher molecular weights of about 100,000 and 160,000, respectively. During the 15-min pulse the p77/75 doublet and the lower-molecular-weight proteins of 61,000 (p61), 48,000 (p48), 38,000 (p38), and 34,000 (p34) were labeled with [^{35}S]methionine. In the subsequent chase periods the amount of label in the p77/75, p61, p48, and p38 diminished slowly, whereas the amount of label in p34 remained fairly constant and in some experiments increased slightly. The p27 was not detected in the cells after pulse-labeling, and only minor amounts were found in

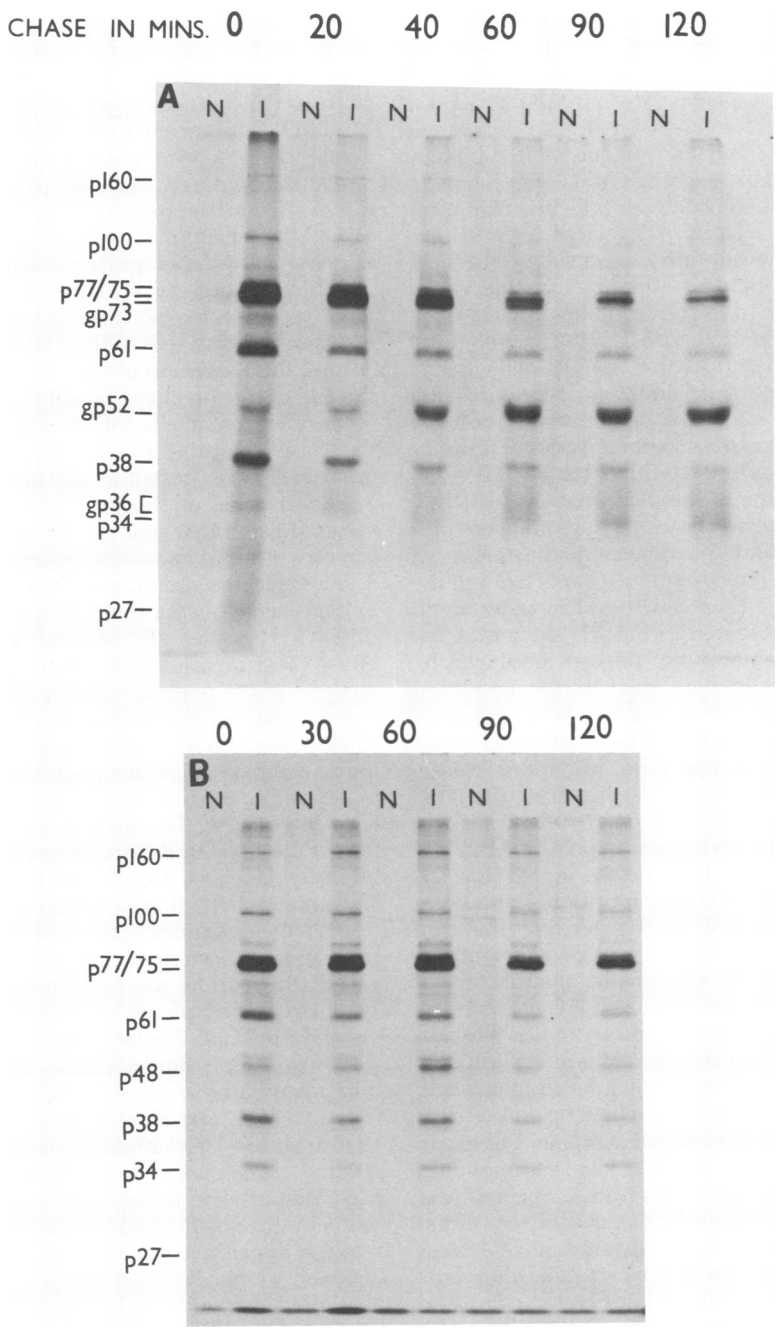

FIG. 2. *Autoradiogram of two pulse-chase experiments performed with GR mammary tumor cell cultures. The cultures were pulsed for 15 min with [^{35}S]methionine at 100 μCi/ml and chased with unlabeled medium containing an excess of cold methionine for the times indicated. Cell extracts were prepared and immunoprecipitated as described in the text. (A) Extracts at each time interval were divided in two and immunoprecipitated with normal (N) or anti-MuMTV serum (I). (B) Cell extracts were immunoprecipitated with normal and anti-p27 serum (I). The washed immunoprecipitated proteins were separated on a 10% polyacrylamide gel and autoradiographed to locate the bands. In (A) the p48 [shown in (B)] is not resolved from gp52 and is not marked.*

the cell extracts of the late chase periods. Furthermore, examination of the tissue culture fluids from the GR cells obtained during the chase, either as particulate material or as immunoprecipitable material, revealed only small amounts of p27 and some of the related higher-molecular-weight proteins (data not shown). This type of kinetic behavior indicates that most of the cell-associated, p27-related proteins are degraded in the cell rather than cleaved to yield p27. To determine whether or not this was a feature specific to the GR cell line, Mm5MT cell cultures were pulsed with [^{35}S]methionine and processed as described for the GR cells in Fig. 2B. The cell extract immunoprecipitates are shown in Fig. 3A and demonstrate that the Mm5MT cells contain a similar series of proteins related to p27 as in the GR line (cf. Fig. 2B). The tissue culture fluids obtained from the Mm5MT cells at the end of the chase periods were also immunoprecipitated to determine the amount of labeled p27-related material exported by the cells during the time course of the experiment. The results presented in Fig. 3B show that relatively small amounts of p27 are produced by the Mm5MT cells compared with the amount of label in the putative precursors (Fig. 3A), indicating that the lack of cell-associated p27 is not due to export in the form of virion release. However, the efficiency at converting the label present in the high-molecular-weight protein into p34 and p27 was greater in the Mm5MT cell line than in the GR line. Assuming that the intensity of film darkening represents a measure of the label present in the gel and that the immunoprecipitation is similarly efficient for each of the related proteins, a quantitation of the amounts of proteins present during the pulse-chase experiments can be made. The autoradiograms from the pulse-chase experiments (Fig. 2B and 3) were scanned densitometrically to estimate the degree of processing and rates of turnover for the p27-related proteins. The graph (Fig. 4) shows the amount of label estimated for each protein plotted against time. The quantity of label (area under each peak in arbitrary units) has been corrected to a unit amount of extract protein and adjusted to relative molar amounts by dividing the amount of label by the estimated number of methionine-containing peptides (see Fig. 6) for each protein. Figure 4A shows that after pulsing the GR cells, most of the label resides in the p77/75, which slowly diminishes during the chase. The other proteins related to p27 (p61, p48, p38, and p34) are all present in smaller amounts than the p77/75 but disappear at about the same rate, indicating that the majority of these proteins are turned over in the cell rather than being processed into p27. However, the amount of p27 increases slowly during the chase, but longer exposures of the autoradiogram are required to detect this protein. In contrast to the GR line, an analysis of the Mm5MT cell line shows that all the intermediates in this line were present in greater relative amounts compared with the p77/75, including the p27, which increases during the chase (Fig. 4B). After the pulse, 5.5% of the specifically immunoprecipitated proteins were present as p27. When compared with the total label present after the pulse, the p27 increases reaching a level of approximately 13% of the molecules (cell and culture fluid associated) by 3 h of the chase. At the end of the 3-h chase period the total amount of specific label in the cells had decreased by 45 to 50%; taking this into account, then 19% of the molecules were present as p27 at this time.

The absence of the gp73 from the anti-p27 serum precipitate is not clearly demonstrated in the comparison of autoradiograms shown in Fig. 2, since this protein migrates immediately below or together with the lower band of the p77/75 doublet. However, the absence of the gp73 in anti-p27 serum precipitates was confirmed by an analysis of the peptides obtained from a tryptic digest of this glycoprotein and p77/75. Figure 5 shows the peptide analysis of gp73 and a schematic comparison of this peptide map superimposed on the peptide map for p77/75 (described later in Fig. 6A). To establish that the proteins immunoprecipitated with anti-p27 serum are related by sequence to p27 of MuMTV, each of the immunoprecipitated proteins was isolated from polyacrylamide gels and digested with trypsin, and a two-dimensional separation analysis of the peptides was performed on thin-layer chromatography plates, as described in Materials and Methods. Figure 6 shows the results of these tryptic peptide analyses. The major protein precipitated from cells with anti-p27 serum is the p77/75, and this protein routinely demonstrates 13 [^{35}S]methionine-labeled peptides. p61 has three spots less (1, 6, and 8), and the p48 contains even fewer of the original 13 spots but contains about 10 additional spots. The protein p48 appears to run as a doublet (Fig. 1 and 2B), and we believe that one of the doublet is a contaminating cell protein which nonspecifically precipitates with the p48 and produces the additional spots. The additional spots in p48 are not derived from gp52, as is apparent from a comparison with the peptide map shown in Fig. 5. The p38 is very similar to the specific p48 pattern, but the p34 contains three spots less than p38 and resembles the p27. Protein p27 has six tryptic peptides (no. 3, 9 through 13) in common with p34. However, the ratio of intensities of these spots, in particular, no. 9 and 10,

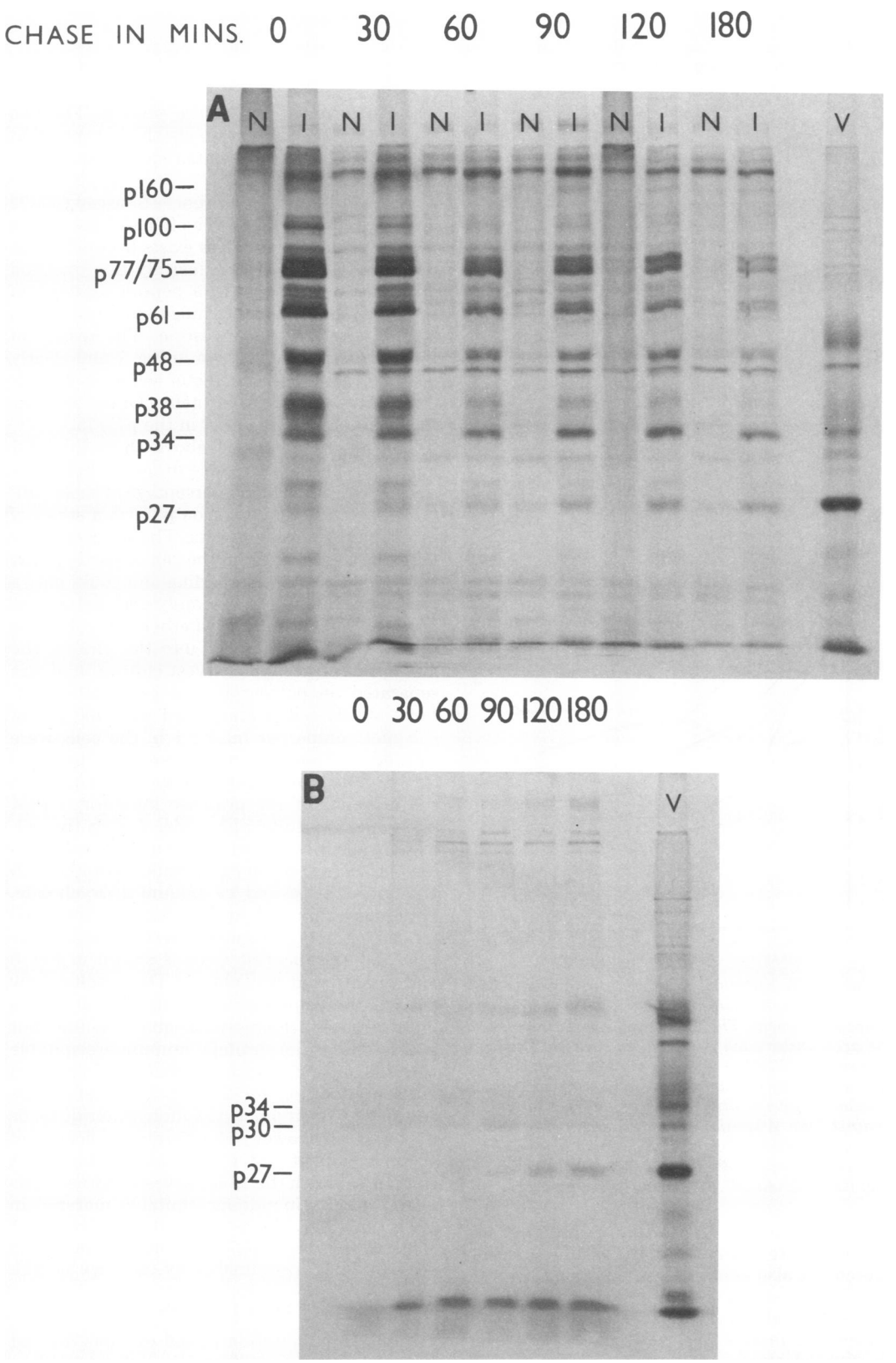

FIG. 3. *Autoradiogram of a pulse-chase experiment performed on the Mm5MT cell line. (A) Cell cultures were treated and processed as described in Fig. 2B, using the anti-p27 serum to immunoprecipitate. (B) In addition, the culture medium was collected during the pulse and chase for each time interval and centrifuged at 100,000 × g for 40 min. The pellets were disrupted with detergent in the presence of enough cold carrier MuMTV to optimize the subsequent immunoprecipitation with anti-p27 serum. The immunoprecipitates from the culture fluids were then processed in a manner similar to that used with the cell extracts. Labeled MuMTV (V) was run as a standard.*

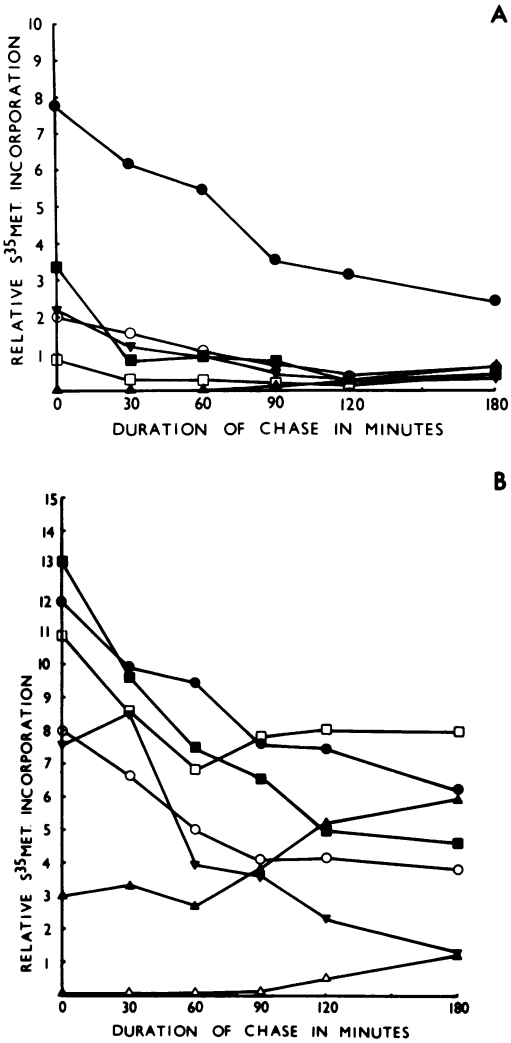

FIG. 4. *Quantitation of p27-related proteins with time. (A) Results obtained from the GR cell pulse-chase experiment. The gel tracks were scanned, and the area under each peak was measured. The area was standardized to a fixed quantity of extract protein, divided by the number of methionine-containing residues to give relative molar amounts, and plotted against time. A similar procedure was performed in (B) for the Mm5MT cells. The symbols represent the following proteins:* (●) *p77/75;* (■) *p61;* (○) *p48;* (▼) *p38;* (□) *p34;* (▲) *p27;* (△) *virion-associated p27.*

varies from p27 to the other five proteins. We are unable to account for these differences at present. It also contains one additional peptide (no. 14) not seen in any of the related proteins. An additional peptide in the final product of processing, in this case p27, could result from the final cleavage, generating a unique methionine-containing peptide derived from the newly exposed terminal end of the protein. The two proteins with molecular weights higher than that of p77/75 also demonstrated spots common to the p27 (data not shown).

The spot intensity differences between p77/75 and p27 reduces the degree of confidence that a close sequence relatedness exists between these proteins. To answer conclusively this question of degree of relatedness, a peptide analysis of the arginine-lysine-containing peptides was performed after trypsin digestion. The results of this comparison are shown in Fig. 7 and clearly demonstrate a high degree of sequence homology between these two proteins. Of the 23 spots found in p27, 22 are found in the p77/75.

The results described above show that p27 antigenic specificities reside in several cell-associated proteins. A small percentage of these proteins may give rise to virion p27 via a series of proteolytic cleavage events. These events may take place exclusively in the cell or partly in the virion after or during budding and could play a functional role in the assembly and maturation process. To determine whether any of these proteins are incorporated into the virions, the virus present in rapid-harvest culture fluid was separated on polyacrylamide gels. Roller cultures of GR cells were pulsed with 100 μCi of [^{35}S]methionine per ml for 1 h, the cells were washed, and 5-min harvests were collected for a further hour. The harvests were placed on ice in the presence of the protease inhibitor, 1 mM phenylmethyl sulfonylfluoride, during the collection. Virus collected in this manner was purified on sucrose density gradients, disrupted with 1% Triton X-100 and 1% sodium deoxycholate, immunoprecipitated, and analyzed as described for cell extracts. A comparison of the rapid-harvest and 18-h-harvest virus is shown in Fig. 8 and quantitated in Table 1. The results show that in the rapid-harvest virus several of the intermediates are present in small amounts but p34 is the major protein immunoprecipitable with the anti-p27 serum. Incubation of a parallel virus preparation for 2 h at 37°C before the addition of phenylmethyl sulfonylfluoride leads to virus with a lower p34–p27 ratio, indicative of a conversion of p34 to p27 (see Table 1). A comparison with the 18-h-harvested virus shows nearly all the immunoprecipitable material in the form of p27. These results demonstrate that some of the virion p27 is formed in the virion after budding, presumably by cleavage of p34.

DISCUSSION

Immunoprecipitation of cell extracts derived from the mammary tumor cell lines, with a monospecific antiserum against MuMTV-p27,

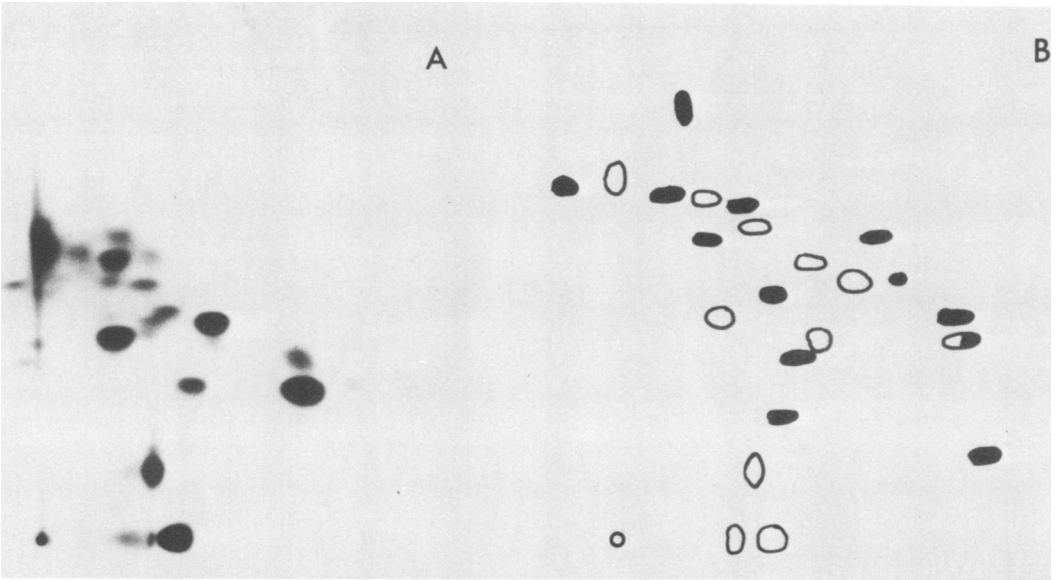

FIG. 5. *Autoradiogram showing the two-dimensional separation of the [^{35}S]methionine-labeled tryptic peptides obtained from gp73 and a schematic comparison of the gp73 and p77/75 tryptic peptide maps. The gp73 was isolated from an anti-MuMTV serum immunoprecipitate of GR cells, which had been separated in a preparative polyacrylamide slab gel. (A) Autoradiogram of the [^{35}S]methionine-labeled peptides from gp73. The electrophoresis was carried out at pH 4.6 from an origin (O) and is shown in the horizontal plane. Chromatography was from the bottom to the top. (B) Schematic representation of the peptide map of gp73 (open spots) superimposed on the peptide map obtained for p77/75 (shown later in Fig. 6) (closed spots).*

results in the isolation of several proteins in the molecular weight range 160,000 to 34,000. Pulse-chase experiments and the analysis of the tryptic peptides obtained from these immunoprecipitated proteins demonstrate that p77/75, p61, p48, p38, and p34 are related by amino acid sequence to p27 of the virion. In the GR cell line p77/75 is the most abundant member of this protein series. However, the situation is different in the Mm5MT cell, in which all proteins are present in similar amounts, with the exception of p27, which only becomes a major component after 2 to 3 h of the chase. The kinetic data show that most of the p27-related proteins synthesized in the mammary tumor cell lines are quite rapidly turned over. Nevertheless, we suspect that the p27 produced by these lines is derived from the p77/75 by a progressive proteolytic processing mechanism. This type of mechanism cannot be directly established from the pulse-chase experiments since the yield of p27 is not quantitative. In fact, there is sufficient incorporation in several of the proteins in the series individually to yield all the accountable p27 by the end of the chase. However, the results are consistent with the idea that p27 is derived by a proteolytic cleavage mechanism, because during the chase the amount of this protein increases whereas all the intermediate proteins rapidly diminish in amount. The synthesis of p27 via a series of proteolytic cleavage events would be consistent with the type of mechanism described for the avian (31, 39; Hayman, in press) and murine (1, 12, 13, 19, 31, 32, 38) type C viruses. In these systems a polyprotein precursor of 76,000 daltons for the avian viruses and 72,000 or 65,000 daltons for the murine viruses appears to be the major viral translation product, giving rise by proteolytic cleavage to the small virion proteins known collectively as the *gag* proteins. Cell-free translation of the virion RNA leads to the synthesis of similar polyproteins, thereby indicating that they represent the major translation product encoding the *gag* proteins (14, 20, 24, 25, 27). Similar in vitro translation studies using the MuMTV RNA demonstrate the synthesis of a 77,000-dalton protein immunoprecipitable with the anti-p27 serum, again consistent with the notion that p77/75 is the major viral gene product in these cell lines which yields the internal proteins of MuMTV (C. Dickson and H. Dahl, unpublished data). A significant difference between the studies reported for the type C viruses and the MuMTV is the amount of viral protein turnover relative to complete processing. The reason for this high degree of turnover is unclear, but it could result from an overproduction of this precursor (p77/75) relative to some rate-

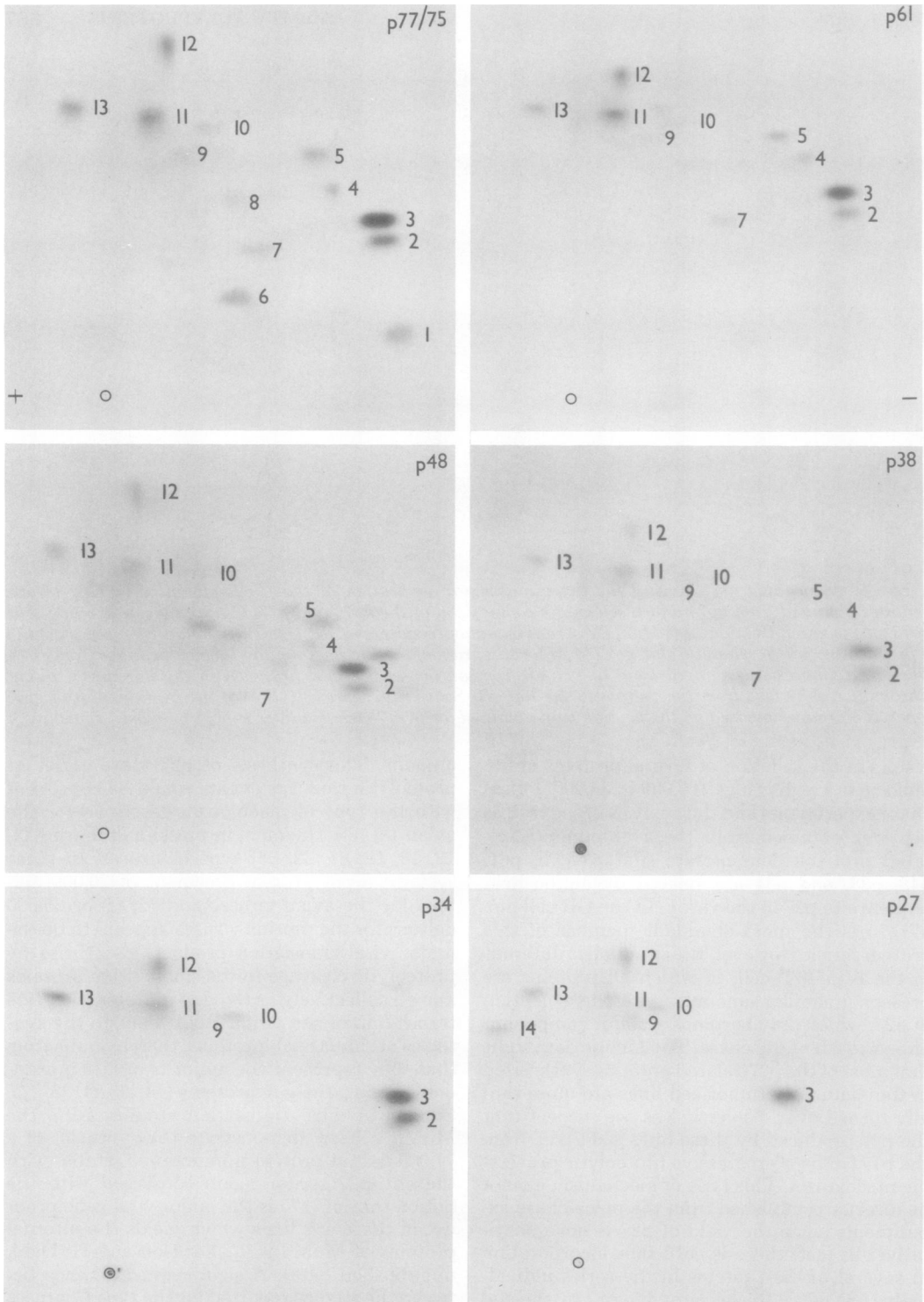

FIG. 6. *Autoradiograms showing the two-dimensional separation of the [^{35}S]methionine-containing tryptic peptides obtained from the proteins isolated by immunoprecipitation with anti-p27 serum.* (○) *marks the origin; electrophoresis was carried out at pH 4.6 shown in the horizontal plane, and chromatography is from bottom to top.*

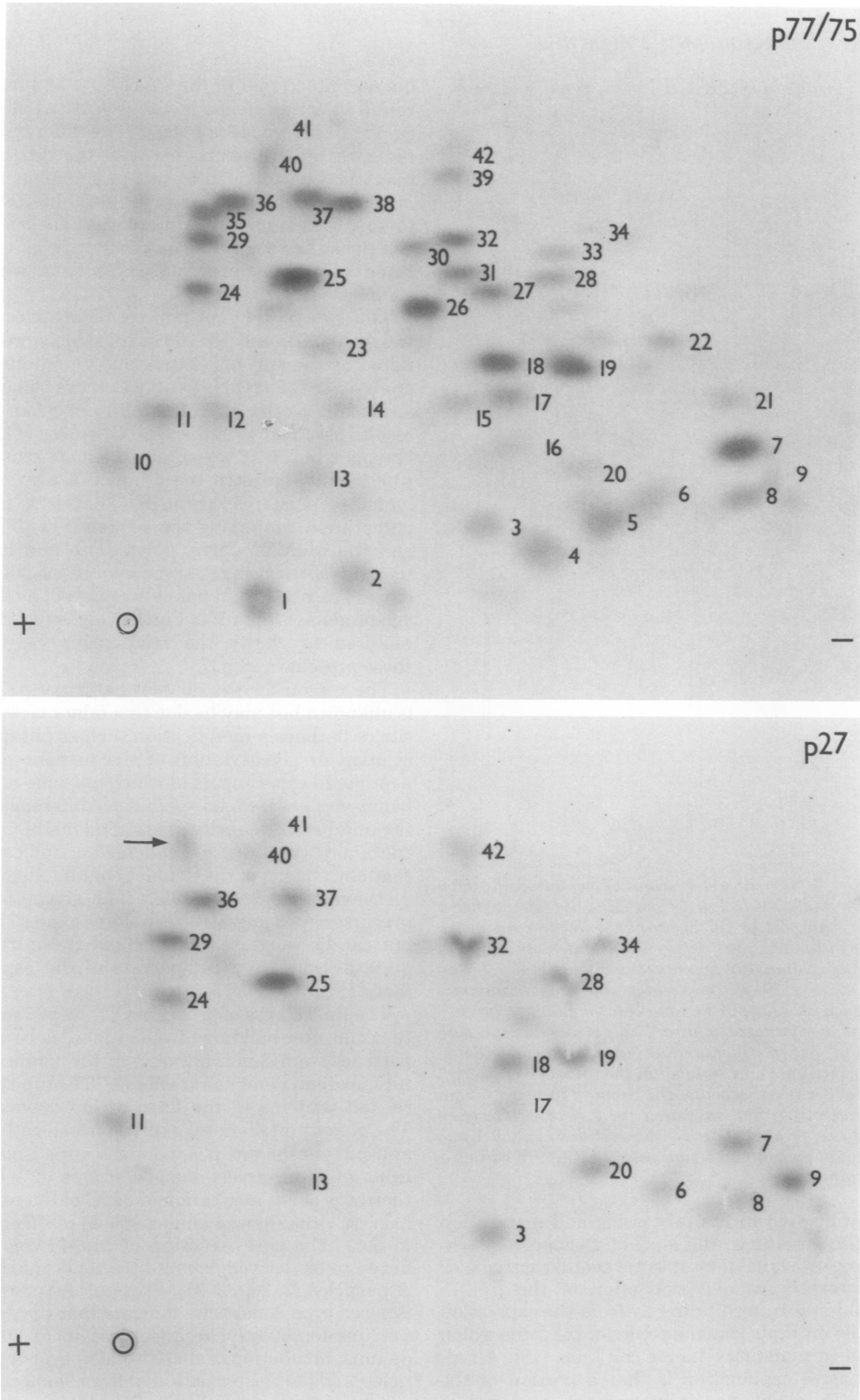

FIG. 7. *[^{14}C]arginine- and [^{14}C]lysine-labeled tryptic peptide maps of p77/75 and p27. The conditions of electrophoresis and chromatography are described in the legend to Fig. 5. Arrow indicates the position of the spot in the p27 map which is not found in p77/75 map.*

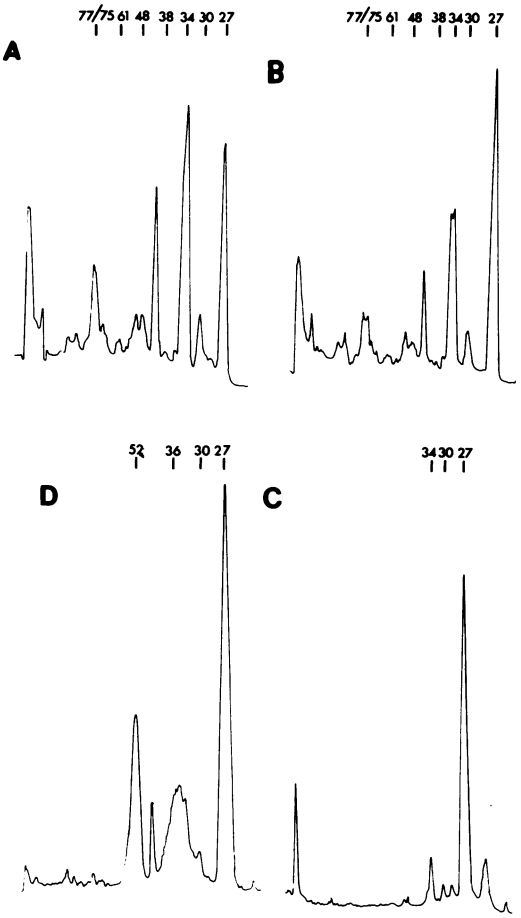

FIG. 8. *Densitometer scans of an autoradiogram of the MuMTV isolated from GR cells after either a rapid harvest or an overnight collection regimens. The virus was purified by sucrose density gradient centrifugation, disrupted with detergents (1% Triton X-100 and 1% deoxycholate), and immunoprecipitated with anti-p27 serum. The immunoprecipitates were then separated in a 10% polyacrylamide slab gel. (A) [^{35}S]methionine-labeled virus harvested at 5-min intervals and isolated in the presence of phenylmethyl sulfonylfluoride. (B) Similar to virus preparation in (A) but incubated for 2 h at 37°C before addition of phenylmethyl sulfonylfluoride. (C) Virus isolated from an 18-h harvest. (D) MuMTV run as a standard.*

limiting step in the processing and maturation of the virus (e.g., the level of glycoprotein synthesis or availability of some cellular enzyme or substrate). An overproduction of the p77/75 could in turn result directly from the expression of the multiple genome copies of the virus which exist in mammary tumor cell lines (18). An alternative explanation is that a portion of the p77/75 could be derived from a defective MuMTV genome, such that an amino acid sequence, functional in the processing and maturation of the proteins, is not recognized at one step and the processing stops. The difference in the rates of processing between the cell lines may also be explained by one or a combination of these possibilities. However, it is interesting to note that despite the differences between the cell lines the rates of virus production as measured by reverse transcriptase activity fall within a similar range.

The two proteins of approximate molecular weight 100,000 and 160,000 are routinely precipitated by the anti-p27 serum, and a preliminary analysis of the tryptic peptides shows some relationship to the p27 of the virion. From the results obtained with the type C viruses (14, 24; Hayman, in press), we would anticipate that the p160 might represent the product of a *gag*-polymerase gene read-through. That is a large polyprotein containing the sequence of p77/75 and the MuMTV-DNA polymerase and functionally representing the precursor to the polymerase. The p100 could possibly represent a cleavage product of the p160. Further investigation is required to clarify the relationship between these proteins and p27.

The reason for the doublet nature of p77/75 is unknown but may be due to a minor cleavage site or perhaps a modification such as phosphorylation or glycosylation of the nascent polypeptide. In experiments in which only one of the bands was isolated, no significant differences in the number or intensity of assigned methionine-containing peptides were detected, indicating that both bands of the doublet contain identical methionine-labeled peptides. The glycoprotein precursor gp73 migrates very close to the p77/75 but can be separated on gels and shown to be quite distinct by a comparison of the peptide maps (Fig. 5).

The final stages of processing of the p27 seems to occur after budding of the virions, since viral particles from 5-min harvests of the tissue culture medium demonstrate about 70% of the p27-related proteins in the form of intermediates. The major protein immunoprecipitated with the anti-p27 serum was p34, whereas virus isolated from an 18-h harvest showed 95% as p27. Furthermore, a 2-h incubation at 37°C of the rapid-harvest virus showed an increase in p27 from 30 to 50%. The core structure of MuMTV is believed to be derived from intracytoplasmic type A particles (2, 29, 33-35). Structural studies on isolated type A particles indicate that they contain predominantly a single protein of 70,000 daltons. Incubation of these isolated type A particles leads to the formation of lower-molecular-weight proteins, including a p25, which is related antigenically to p27 of MuMTV (35). It is pos-

TABLE 1. *Distribution of p27-related proteins immunoprecipitated from purified MuMTV, harvested from cell cultures with different regimens*[a]

Virus prepn	% Total radioactivity in:						
	p77/75	p61	p48	p38	p34	p30	p27
+ PMSF[b]	14.6	2.9	8.1	1.7	41.0	6.0	25.5
2 h at 37°C	10.7	1.5	5.7		29.6	5.3	46.2
18-h harvest					5.0		95.0

[a] Estimated from the areas under each peak shown in Fig. 8.
[b] PMSF, Phenylmethyl sulfonylfluoride.

sible that the p70 of type A particles is the same as, or a cleavage product of, the p77/75 described here. If this is the case, then we may speculate that the core structure of MuMTV probably starts by a self-assembly of the p77/75 into the spheroid structure similar to, or the same as, the intracytoplasmic type A particle. The incorporation of genomic RNA into the structure could initiate the cleavage sequence following an allosteric configurational change in the p77/75, which eventually leads to the condensation of the nucleoid to the electron-dense structure seen in thin-section electron micrographs of the mature virions (2). This maturation would initiate during budding and, as indicated above, be completed after release of the particles from the plasma membrane of the cells. This type of process also implies that the proteases responsible are incorporated into the virion. Recently, postbudding maturation has been implicated in type C virus maturation (40, 41). In the murine system, processing of purified *gag* precursor was demonstrated in vitro with a partially purified low-molecular-weight fraction obtained from disrupted virions (41). Similarly, cleavage of the avian precursor was demonstrated in a cell-free system, and the activity was associated with the virion protein p15 (40).

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