Glycopeptides of Murine Leukemia Viruses

III. Glycosylation of *env* Precursor Glycoproteins

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We have compared the glycopeptides obtained after extensive pronase digestion of the *env* precursors (PrENV proteins) of ecotropic, xenotropic, and dual-tropic murine leukemia viruses. Two glycopeptide size classes, having molecular weights of approximately 2,200 and 1,500, were shown to be associated with the PrENV proteins of all murine leukemia viruses studied. Glycopeptides associated with the *env* precursors were totally susceptible to endo- β -*N*-acetylglucosaminidase H. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of partial endo- β -*N*-acetylglucosaminidase H digestion products of the *env* precursor of dual-tropic mink cell focus-forming virus (MCF 247) revealed the presence of seven bands, suggesting that six glycosylation sites were present on the precursor molecule. The MCF 247 PrENV protein had been previously shown to be accessible to lactoperoxidase-catalyzed radioiodination on the surface of infected cells. The cell surface PrENV molecules had the same electrophoretic mobility as pulse-labeled PrENV protein, and after endo- β -*N*-acetylglucosaminidase H treatment a similar shift in electrophoretic mobility was observed for the cell surface PrENV protein and the pulse-labeled precursors, a finding which indicated that the PrENV protein located on the cell surface also possessed only mannose-rich oligosaccharides. These results indicated that the *env* precursor glycoproteins of dual-tropic viruses had the unusual property of migrating to the cell surface without undergoing the normal oligosaccharide processing and proteolytic cleavage events that had been observed for ecotropic and xenotropic murine leukemia virus glycoproteins.

The primary product of translation of the murine leukemia virus (MuLV) envelope (*env*) gene is a polyprotein (PrENV protein) which undergoes glycosylation during synthesis and is subsequently cleaved to yield gp70 and p15(E) (3, 12, 18). Although this sequence of events appears to be the general pathway of synthesis and processing of MuLV *env* gene products, the kinetics of cleavage of the PrENV protein into gp70 and p15(E) varies greatly from one host range class to another (4). Further, a fraction of the population of PrENV protein of dual-tropic viruses is not processed into gp70 and p15(E), but is transported to the cell surface as un-cleaved precursor (4).

It has been demonstrated previously that gp70's of MuLV's from different host range classes (7, 8, 15), as well as avian retrovirus glycoproteins (6), possess both mannose-rich and complex oligosaccharide side chains. The patterns of glycosylation observed for gp70's of MuLV's were distinctive for members of different host range classes, were constant for several viruses from each class, and were not altered

significantly when viruses were grown in different host cells (8). These findings suggested that the structure of the gp70 oligosaccharides is determined by the polypeptide backbone and that glycosylation sites on this molecule are conserved within each host range class. The experiments reported in this paper were designed to investigate whether the structural differences observed in the oligosaccharide side chains of various gp70 molecules were reflected in the glycosylation patterns of their respective PrENV proteins. We also wished to determine whether the glycosylation pattern of the PrENV protein of viruses from each host range class was related to the kinetics of cleavage of this molecule and whether the oligosaccharide side chains of dual-tropic-virus PrENV protein expressed on the cell surface resembled those of the intracellular PrENV or of gp70.

MATERIALS AND METHODS

Virus and cells. The origins of the viruses used have been previously described (8). Virus producer cell lines in either mouse fibroblasts (SC-1) or mink

lung fibroblasts (CCL64) were provided by P. V. O'Donnell, Memorial Sloan-Kettering Cancer Center. Cultured AKR mouse leukemia cells (AK2D) were provided by J. Biedler, Memorial Sloan-Kettering Cancer Center; cultures were maintained as previously described (5, 13).

Radiolabeling of cell cultures. Virus-infected cell cultures, with the exception of AK2D leukemia cells, were radiolabeled for 2 h with [^3H]glucosamine (New England Nuclear) at a concentration of 70 $\mu\text{Ci}/\text{ml}$ in Eagle medium supplemented with 5% fetal calf serum or with [^3H]mannose (New England Nuclear) at a concentration of 140 $\mu\text{Ci}/\text{ml}$ for 2 h in the same medium. AK2D cell cultures were labeled for 16 instead of 2 h. For amino acid labeling, cells were labeled for 15 min with 80 μCi of [^3H]leucine per ml in Eagle medium lacking leucine and chased for 4 h in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum. Radiolabeling of the cell surface proteins with ^{125}I was carried out by the lactoperoxidase method previously described (4).

Isolation of *env* gene products. Cell extracts were prepared as described previously (4). The *env* gene products were immunoprecipitated by the addition of goat anti-Rauscher MuLV gp70 serum (provided by R. Wilsnack, Huntington Research Center) or by rabbit anti-Rauscher MuLV p15(E) serum (provided by E. Fleissner, Memorial Sloan-Kettering Cancer Center) followed by the addition of Formalin-killed *Staphylococcus aureus* cells (9) to precipitate the immune complexes. The immunoprecipitated proteins were solubilized and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 7.5% slab gel as described previously (4). After electrophoresis, the slab gel was fixed and processed for fluorography (1), which was carried out on dried gels with Kodak XR-2 X-ray film at -70°C . Gels containing ^{125}I -labeled *env* gene products were dried and autoradiographed directly with Cronex intensifying screens at -70°C .

Preparation and analysis of glycopeptides. Gel segments containing the radiolabeled glycoproteins were rehydrated, and the glycopeptides were prepared by pronase digestion as described previously (8) and chromatographed on Bio-Gel P-6 with [^{14}C]glucosamine-labeled A/WSN(H0N1) influenza virus marker glycopeptides (11).

Endo-H treatment of glycopeptides. Glycopeptides were treated with endo- β -*N*-acetylglucosaminidase H (endo-H; Miles Research Products) as described previously (7, 8).

Endo-H treatment of ^{125}I - and [^3H]leucine-labeled glycoproteins. Immune precipitates were suspended in 0.1 ml of 0.07 M sodium citrate (pH 5.5) and treated with 0.02 U of endo-H for 24 h at 37°C (8). The endo-H-treated *env* gene products were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described above.

RESULTS

Glycosylation of the *env* gene precursors of ecotropic and xenotropic MuLV's. In previous studies of the pronase derived glycopep-

tides of a number of MuLV's from different host range classes, we have demonstrated that the gp70's from the different host range classes have distinguishable glycosylation patterns (8). To determine if the oligosaccharides linked to the PrENV proteins also exhibited such differences, we analyzed the glycopeptides of PrENV proteins coded for by ecotropic, xenotropic, and dual-tropic MuLV's. Initially we compared the PrENV glycopeptides of an ecotropic (69E5) MuLV and a xenotropic (69X9) MuLV derived from the same AKR mouse (14). Cultures of 69E5-infected SC-1 mouse fibroblasts and 69X9-infected mink lung cells were labeled for 2 h with [^3H]mannose, and PrENV proteins were isolated by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described in Materials and Methods. The glycopeptides were digested with pronase and neuraminidase, and the resulting glycopeptides were chromatographed on a Bio-Gel P-6 column. Two distinct size classes, with molecular weights of 2,200 and 1,500, were resolved from the 69E5 (Fig. 1A) and 69X9 (Fig. 1B) proteins. A distinct shoulder detectable between fractions 47 and 55 in the 69E5 PrENV protein profile was not evident in the 69X9 PrENV protein profile. The elution profiles of [^3H]glucosamine-labeled precursors were similar in that two glycopeptide size classes, of 2,200 and 1,500 daltons, were resolved in each case (data not shown). To determine whether the oligosaccharide side chains detected on the PrENV proteins of 69E5 and 69X9 were high mannose (type II) or complex (type I), their sensitivities to endo-H were examined. Endo-H effectively cleaves asparagine-linked oligosaccharides containing cores of more than three mannose residues (type II), but does not cleave complex oligosaccharides (19). The elution profiles of the endo-H-treated 69E5 and 69X9 PrENV glycopeptides (Fig. 1C and D) showed that all of the oligosaccharides in both profiles were completely sensitive to endo-H. Thus, the PrENV proteins of ecotropic and xenotropic viruses contained two major size classes of oligosaccharide side chains, both of which were of the high-mannose type.

Glycosylation of the *env* gene polypeptide of dual-tropic MuLV. The glycopeptides of the PrENV proteins of three AKR dual-tropic viruses were examined. Two of the isolates, MCF 247 and SC37, have been shown to code for PrENV proteins which are indistinguishable from one another by partial digest mapping analysis. The PrENV proteins of these dual-tropic viruses are expressed on the surface of infected fibroblasts. The third dual-tropic virus, 2169-4, codes for a PrENV protein that is similar but

FIG. 1. Analysis of the [^3H]mannose-labeled glycopeptides of the *env* precursors of 69E5 and 69X9 MuLV's by gel filtration on Bio-Gel P-6 before and after endo-H treatment. (A) Glycopeptides of 69E5 PrENV protein before endo-H treatment. (B) Glycopeptides of 69X9 PrENV protein before endo-H treatment. (C) Glycopeptides of 69E5 PrENV protein after endo-H treatment. (D) Glycopeptides of 69X9 PrENV protein after endo-H treatment. [^{14}C]glucosamine-labeled glycopeptides of influenza virus A/WSN grown in MDBK cells were cochromatographed as internal molecular weight markers (14), and their elution positions are indicated by arrows in this and subsequent figures. V_0 , Void volume; V_T , total volume.

not identical to that of the AKR xenotropic virus 69X9 and is not detectable on the surface of infected fibroblasts (N. G. Famulari and P. V. O'Donnell, submitted for publication).

We characterized the glycopeptide patterns of the PrENV proteins of these viruses to assess the involvement of oligosaccharide side chains in the increased intracellular stabilities of the PrENV proteins of dual-tropic MuLV's as compared with those of ecotropic and xenotropic MuLV's and in the cell surface localizations of the PrENV proteins of certain dual-tropic iso-

lates. The elution profiles of SC-1/MCF 247 (Fig. 2A) and mink/MCF 247 (Fig. 2B) glycopeptides were very similar; two distinct glycopeptide size classes, of about 2,200 and 1,500 daltons, were resolved. The oligosaccharide side chains of the SC-1/MCF 247 PrENV protein contained only high-mannose (type II) glycopeptides, as shown by their sensitivity to cleavage by endo-H (Fig. 2C); similar results were obtained with the PrENV protein of mink/MCF 247 (data not shown). The glycopeptide patterns of the PrENV proteins of SC37 and 2169-4 vi-

ruses isolated from SC37- and 2169-4-infected mink cells (Fig. 3A and B) closely resembled those of MCF 247 PrENV proteins grown in SC-1 or mink cells, although the two glycopeptide size classes were present in somewhat different relative amounts on the PrENV protein of SC37 virus. The major MuLV *env* gene product synthesized by AKR MuLV's has been shown to be indistinguishable from that of MCF 247 or SC37 virus by partial digest mapping (4). This dual-tropic-virus PrENV protein also was observed

to contain two major glycopeptide size classes, of 2,200 and 1,500 daltons (Fig. 3C).

The above results indicated that the *env* gene polyproteins coded for by viruses from three MuLV host range classes, ecotropic, xenotropic, and dual-tropic, had similar glycopeptide compositions. Further, the sizes and endo-H sensitivities of the glycopeptides did not appear to be affected by the host cells in which the viruses were replicating.

Glycosylation of *env* precursors ex-

FIG. 2. Analysis on Bio-Gel P-6 of the [^3H]mannose-labeled glycopeptides of PrENV proteins from SC-1/MCF 247 MuLV and mink/MCF 247 MuLV before and after endo-H treatment. (A) Glycopeptides of SC-1/MCF 247 MuLV before endo-H treatment. (B) Glycopeptides of mink/MCF 247 MuLV before endo-H treatment. (C) Glycopeptides of SC-1/MCF 247 MuLV after endo-H treatment. V_0 , Void volume; V_T , total volume.

FIG. 3. Analysis of the [^3H]glucosamine labeled *env* precursor glycopeptides of SC-1/SC37(A), mink/2169-4(B), and AK2D(C) MuLV's on Bio-Gel P-6. V_0 , Void volume.

pressed on surfaces of infected cells. Pulse-chase analysis has shown that MCF 247 virus PrENV protein synthesized in infected fibroblasts and dual-tropic-virus PrENV protein expressed in a cultured AKR leukemia have increased stabilities relative to 69E5 and 69X9 PrENV proteins (4, 5). Further, PrENV protein has been shown to be expressed on the surface of MCF 247-infected fibroblasts and on the surface of AKR leukemia cells (4, 5). We wished to determine whether the oligosaccharides associated with the cell surface dual-tropic-virus PrENV protein were processed after transport from the endoplasmic reticulum. Since gp70 of MCF 247 virus contains both complex and high-mannose oligosaccharides (8, 15), it was possible that the long-lived PrENV molecules and those expressed at the cell surface might resemble gp70 in their oligosaccharide components.

Initially we observed that the glycopeptides of MCF 247 PrENV protein isolated from cells which had been labeled with [3 H]glucosamine or [3 H]mannose for 18 h were endo-H sensitive and were of the same size class as PrENV glycopeptides labeled for 2 h (data not shown). This result suggested that the carbohydrate side chains of certain dual-tropic-virus PrENV molecules remained of the high-mannose type throughout the lifetime of the molecule. To examine the glycopeptides of long-lived PrENV proteins and PrENV proteins expressed on the cell surface directly, two types of experiment were carried out. The first was designed to determine whether the population of PrENV molecules which are stable to chase possess complex oligosaccharides. Replicate cultures of MCF 247 virus-infected mink cells were pulse-labeled for 15 min with [3 H]leucine or pulse-labeled and chased for 4 h. Cell extracts were prepared, and PrENV protein was isolated by immunoprecipitation with rabbit anti-Rauscher MuLV p15(E) serum and *S. aureus*. The PrENV protein was eluted from the immune complexes and divided into two aliquots, one of which was treated with endo-H and one of which was not. The effects of endo-H digestion on pulse-labeled and pulse-labeled and chased PrENV protein were compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 4A). The untreated PrENV protein from both the pulse-labeled and the pulse-chase samples (tracks 1 and 3, respectively) migrated with a molecular weight of approximately 80,000. The major endo-H cleavage products of both the pulse-labeled sample (track 2) and the pulse-chase sample (track 4) migrated at a molecular weight of approximately 70,000. These results indicated that no significant shift from high-mannose to complex oligosaccharides

FIG. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of endo-H-digested MCF 247 PrENV protein. (A) MCF 247 virus-infected mink cells were radiolabeled with [3 H]leucine for a 15-min pulse or a 15-min pulse followed by a 4-h chase and the PrENV protein was isolated by immunoprecipitation with rabbit anti-Rauscher MuLV p15(E) serum. The isolated PrENV protein was divided into two aliquots; one was treated with 0.02 U of endo-H for 24 h, and one was mock treated. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 7.5% slab gels (6) was carried out on the resulting protein species. Tracks: 1, PrENV protein pulse-labeled for 15 min and mock treated; 2, PrENV protein pulse-labeled for 15 min and treated with endo-H; 3, PrENV protein pulse-labeled and chased for 4 h and mock treated; 4, PrENV protein pulse-labeled and chased for 4 h and treated with endo-H. (B) Enlargement of tracks 1 and 2 of (A). Note the six minor bands in addition to completely digested PrENV species (major band). This result suggests the presence of six high-mannose oligosaccharide side chains on MCF 247 PrENV protein.

took place during a 4-h chase. Further, endo-H treatment of the PrENV protein remaining after 6- and 8-h chase periods also resulted in a cleavage product of approximately 70,000 daltons (data not shown). It should be noted that endo-H treatment was not cleaving PrENV into gp70 and p15(E), but rather causing a shift in electrophoretic mobility due to removal of high-mannose oligosaccharides.

It was possible to count the intermediate products of endo-H cleavage of the pulse-labeled sample (see Fig. 4B for enlargement of Fig. 4A, tracks 1 and 2). Seven bands were detectable, one comigrating with untreated PrENV and six

cleavage products. Such a pattern was compatible with the conclusion that there were six high-mannose side chains present on the PrENV protein. It is likely that the presence of intermediate bands in lane 2 represented incomplete digestion rather than the presence of endo-H-resistant oligosaccharides, since processing to yield endo-H-resistant structures would have been more likely in the pulse-chase sample (lane 4), and in this lane almost all of the oligosaccharides were apparently cleaved.

The experiment presented in Fig. 5 was carried out to determine whether PrENV protein expressed on the cell surface contained complex carbohydrate chains. A comparison was made of the endo-H cleavage products of PrENV protein labeled by lactoperoxidase-catalyzed radioiodination of intact cells and of PrENV protein labeled in a 15-min pulse with [3 H]leucine. Rabbit anti-Rauscher MuLV p15(E) serum was used to isolate PrENV protein from cell extracts; the PrENV protein was then eluted from the immune complex and treated with endo-H. The

FIG. 5. Endo-H digestion of surface-labeled PrENV protein from MCF 247 virus-infected mink cells radiolabeled by lactoperoxidase-catalyzed iodination of intact cells or by pulse-labeling for 15 min with [3 H]leucine. *env* gene products were isolated by immunoprecipitation with either goat anti-Rauscher MuLV gp70 serum or rabbit anti-Rauscher MuLV p15(E) serum. PrENV protein isolated by anti-p15(E) serum was then treated with 0.02 U of endo-H for 24 h. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out in 7.5% slab gels (6). Tracks: 1 and 5, PrENV protein and gp70 isolated from 125 I-labeled cells by immunoprecipitation with anti-gp70 serum; 2, PrENV protein isolated from 125 I-labeled cells by immunoprecipitation with anti-p15(E) serum and digested with endo-H; 3, PrENV isolated from pulse-labeled cells with anti-p15(E) serum and mock-treated; 4, PrENV isolated from pulse-labeled cells with anti-p15(E) serum and digested with endo-H.

PrENV protein radioiodinated on the cell surface was found to be cleaved by endo-H to a protein of approximately 70,000 daltons (Fig. 5, track 2) that comigrated with the endo-H-cleaved PrENV protein labeled in a 15-min pulse of [3 H]leucine (track 4). These results indicated that the oligosaccharides of the *env* gene precursor expressed on the cell surface were only of the high-mannose type. The observation that the untreated PrENV protein labeled in a 15-min pulse (track 3) comigrated with that labeled by surface radioiodination (tracks 1 and 5) was consistent with this conclusion.

DISCUSSION

In previous studies we demonstrated significant differences in the glycosylation patterns of the gp70 molecules coded for by the *env* genes of ecotropic, xenotropic, and dual-tropic MuLV's (8). The similarity in glycosylation of gp70 within each host range class suggested that glycosylation sites are conserved. One of the objectives of the present study was to determine whether the glycosylation patterns of the *env* precursors were also distinguishable for the MuLV's from different host range classes. In every case, for the three different host range classes studied, we observed that the *env* precursors always possessed two glycopeptide size classes, having molecular weights of 2,200 and 1,500. In contrast, the ecotropic-virus gp70 glycoproteins possess four different glycopeptide size classes, with molecular weights of approximately 5,100, 2,900, 2,200, and 1,500; xenotropic- and dual-tropic-virus gp70's contained the 2,900-, 2,200-, and 1,500-dalton classes, but are deficient in the 5,100-dalton glycopeptide size class. Glycopeptides in the 5,100- and 2,900-dalton size classes were shown to be complex, whereas the 2,200- and 1,500-dalton glycopeptides were of the high-mannose type (7, 8). The glycopeptides associated with the *env* precursors from all of the host range classes studied were observed to be sensitive to endo-H, indicating that both the 2,200-dalton and the 1,500-dalton glycopeptides were high-mannose (type II) glycopeptides.

The 5,100-dalton glycopeptide, designated G₁, appears to be associated only with ecotropic-virus gp70. The large size of this oligosaccharide is unusual, and its structure and the pathway by which it is synthesized remain to be characterized. The present results indicate that the *env* precursor glycoprotein of 69E5, an ecotropic MuLV previously shown to possess G₁ on its gp70 (8), contains only endo-H-sensitive oligosaccharides. These high-mannose oligosaccharides are similar in size to those linked to the

PrENV proteins of other MuLV's, with the exception of a minor size class of 2,900 daltons. Whether this size class is involved in the synthesis of G_1 remains to be determined. Since endo-H cleaves only *N*-asparagine-linked high-mannose glycopeptides (19), it may be concluded that the precursor of G_1 is also *N*-asparagine linked to the polypeptide. A glycopeptide associated with embryonal antigens having similar size characteristics to G_1 also was shown to be *N*-asparagine linked (10). The present results rule out the possibility that G_1 could represent a glycopeptide with two oligosaccharides attached in such close proximity that pronase could not cleave the peptide backbone, since if this were the case, such a pronase-resistant glycopeptide should also have been obtained from the corresponding PrENV molecule.

It has been reported that the oligosaccharide side chains present on the PrENV protein of Moloney MuLV (20) and AKV-1 and MCF 247 MuLV's (15) are of the high-mannose type. Whereas it had been suggested that the *env* precursors of Moloney MuLV possessed only two glycosylation sites (20), our results suggest that the dual-tropic MCF 247 PrENV protein possesses at least six glycosylation sites. This conclusion is based on the observation of a series of new bands after endo-H treatment, which are intermediate between the fully glycosylated and unglycosylated protein. A similar observation was used to estimate the number of glycosylation sites in the glycoproteins of mouse mammary tumor virus (2) and other MuLV's (16). Analysis of the tryptic glycopeptides of the PrENV protein of MCF 247 virus also indicates that there are as many as seven glycosylation sites (M. Kemp, unpublished data). Moreover, Schultz and Oroszlan (17) have reported that the *env* precursor of Rauscher MuLV is reduced in electrophoretic mobility from an apparent molecular weight of 85,000 to a molecular weight of 68,000 in the presence of tunicamycin, which completely blocks glycosylation of asparagine residues. If the oligosaccharides of PrENV proteins have an average molecular weight of 2,000, as indicated by the present results, then these data would be compatible with the presence of eight or nine glycosylation sites on the PrENV glycoproteins.

The *env* precursors of ecotropic, xenotropic, and dual-tropic MuLV's were previously shown to differ in intracellular stability (4, 5). In pulse-chase experiments, the PrENV protein of MCF 247 virus is much more stable than the precursor glycoproteins of ecotropic or xenotropic viruses. However, the glycosylation patterns of the *env* precursors of dual-tropic viruses are very similar to those of ecotropic and xenotropic viruses.

Therefore, the types of oligosaccharide side chains present on the dual-tropic-virus PrENV protein are probably not responsible for the resistance of this protein to cleavage into gp70 and p15(E).

The previous finding of the MCF 247 virus glycoprotein precursor on the cell surface was unusual when compared with results obtained with other MuLV's (4). We have obtained evidence that the MCF 247 virus PrENV protein present on the cell surface contains only type II oligosaccharides, whereas the processed MCF 247 gp70 molecules contain type I as well as type II oligosaccharides (8). These results suggest that the signal(s) for intracellular transport of glycoproteins can function independently of those for the final processing of the oligosaccharides. It will be of interest to investigate the mechanism by which a population of MCF 247 PrENV molecules can migrate to the cell surface without encountering the enzymes involved in the processing of oligosaccharides.

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LITERATURE CITED

1. 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.* **46**:83-88.
2. Dickson, C., and M. Atterwill. 1980. Structure and processing of the mouse mammary tumor virus glycoprotein precursor Pr73^{env}. *J. Virol.* **35**:349-361.
3. Famulari, N. G., D. L. Buchhagen, H.-D. Klenk, and E. Fleissner. 1976. Presence of murine leukemia virus envelope proteins gp70 and p15(E) in a common polypeptide of infected cells. *J. Virol.* **20**:501-508.
4. Famulari, N. G., and K. Jelalian. 1979. Cell surface expression of the *env* gene polyprotein of dual-tropic mink cell focus-forming murine leukemia virus. *J. Virol.* **30**:720-728.
5. Famulari, N. G., J.-S. Tung, P. V. O'Donnell, and E. Fleissner. 1980. Murine leukemia virus *env* gene expression in preleukemic thymocytes and leukemia cells of AKR mice. *Cold Spring Harbor Symp. Quant. Biol.* **44**:1281-1288.
6. Hunt, L. A., S. E. Wright, J. R. Etchison, and D. F. Summers. 1970. Oligosaccharide chains of avian RNA tumor virus glycoproteins contain heterogeneous oligomannosyl cores. *J. Virol.* **29**:336-343.
7. Kemp, M. C., S. Basak, and R. W. Compans. 1979. Glycopeptides of murine leukemia viruses. I. Comparison of two ecotropic viruses. *J. Virol.* **31**:1-7.
8. Kemp, M. C., N. G. Famulari, P. V. O'Donnell, and R. W. Compans. 1980. Glycopeptides of murine leukemia viruses. II. Comparison of xenotropic and dual-tropic viruses. *J. Virol.* **34**:154-161.
9. Kessler, S. W. 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody absorbent; parameters of the interaction of antibody-antigen complexes with protein A. *J. Immunol.* **115**:1617-1624.
10. Muramatsu, T. G., G. Gachelin, M. Damonville, C. Delarbe, and F. Jacob. 1979. Cell surface carbohy-

- drates of embryonal carcinoma cells: polysaccharidic side chains of F9 antigens and of receptors to two lectins, FBP and PNA. *Cell* **18**:183-191.
11. Nakamura, K., and R. W. Compans. 1978. Glycopeptide components of influenza viral glycoproteins. *Virology* **86**:432-442.
 12. 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.
 13. O'Donnell, P. V., and E. Stockert. 1976. Induction of G_{IX} antigen and gross cell surface antigen after infection by ecotropic and xenotropic murine leukemia viruses in vitro. *J. Virol.* **20**:545-554.
 14. O'Donnell, P. V., E. Stockert, Y. Ohata, A. B. Deleo, and L. J. Old. 1980. Murine leukemia virus-related cell surface antigens as serological markers of AKR ecotropic, xenotropic, and dualtropic viruses. *Cold Spring Harbor Symp. Quant. Biol.* **44**:1255-1264.
 15. Rosner, M. R., L. S. Grinna, and P. W. Robbins. 1980. Differences in glycosylation patterns of closely related murine leukemia viruses. *Proc. Natl. Acad. Sci. U.S.A.* **77**:67-71.
 16. Rosner, M. R., J.-S. Tung, N. Hopkins, and P. W. Robbins. 1980. Relationship of G_{IX} antigen expression to the glycosylation of murine leukemia virus glycoproteins. *Proc. Natl. Acad. Sci. U.S.A.* **77**:6420-6424.
 17. Schultz, A. M., and S. Oroszlan. 1979. Tunicamycin inhibits glycosylation of precursor polypeptide encoded by *env* of Rauscher murine leukemia virus. *Biochem. Biophys. Res. Commun.* **86**:1206-1213.
 18. Shapiro, S. Z., M. Strand, and J. T. August. 1976. High molecular weight precursor polypeptides to structural proteins of Rauscher murine leukemia virus. *J. Mol. Biol.* **107**:459-477.
 19. Tarentino, A. L., and F. Maley. 1975. A comparison of the substrate specificities of endo- β -N-acetylglucosaminidases from *Streptomyces griseus* and *Diplococcus pneumoniae*. *Biochem. Biophys. Res. Commun.* **67**:455-462.
 20. Witte, O. N., and D. F. Wirth. 1979. Structure of murine leukemia virus envelope glycoprotein precursor. *J. Virol.* **29**:735-743.