JOURNAL OF VIROLOGY, Nov. 1984, p. 630–637
0022-538X/84/110630-08$02.00/0

Vol. 52, No. 2

Evidence for Post-Translational Glycosylation of a Nonglycosylated Precursor Protein of Herpes Simplex Virus Type 1

TERESA COMPTON AND RICHARD J. COURTNEY*

Department of Microbiology and Program in Cellular, Molecular, and Developmental Biology, University of Tennessee, Knoxville, Tennessee 37996-0845

Received 29 May 1984/Accepted 8 August 1984

Incubation of herpes simplex virus type 1-infected Vero and HEp-2 cells at a reduced temperature (34°C) enhanced the detection of the nonglycosylated precursors (pgB97 and pgC75) to the gB and gC glycoproteins in the cytoplasmic and nuclear fractions. Relative to the fully glycosylated and high-mannose forms detected, the nonglycosylated precursors were the predominant components associated with the nuclear fraction of infected cells. Furthermore, addition of protease inhibitors to the fractionation buffers did not affect the distribution or abundance of the nonglycosylated precursors, suggesting that the presence of pgB97 and pgC75 was not the result of proteolysis. When infected Vero or HEp-2 cells were harvested at various times postinfection, the nonglycosylated precursors were detected after the initial appearance of the high mannose components (pgB110 and pgC105). In Vero cells, pgB97 and pgC75 were detected simultaneously at 8 h postinfection, whereas detection was not apparent in HEp-2 cells until 20 h postinfection. Conditions which favored detection of appreciable amounts of nonglycosylated precursors provided an unique approach to probe possible post-translational modifications in the absence of inhibitors of glycosylation. In nuclear fractions isolated from cycloheximide-treated HEp-2 or Vero cells, numerous discrete gC-immunoreactive bands migrating with decreased electrophoretic mobility relative to the nonglycosylated precursor pgC75 were observed. This series of one to four additional bands was eliminated by digestion with endoglycosidase H, and the appearance of these bands was blocked by the addition of tunicamycin. Collectively, the data suggest that high-mannose core oligosaccharides may be added to the nonglycosylated precursor of the gC glycoprotein of herpes simplex virus type 1 in a post-translational fashion.

Enveloped viruses have played a predominant role in studies designed to elucidate mechanisms involved in the biogenesis of membrane proteins. Viral glycoproteins presumably utilize the same biosynthetic pathway employed by cellular plasma membrane proteins. The single membrane protein of the vesicular stomatitis virus, G protein, has been used to study such processes. Such studies have demonstrated that glycoproteins are synthesized by membrane-bound ribosomes and are cotranslationally inserted into the lumen of the endoplasmic reticulum (ER) (for reviews, see references 30 and 44). Shortly after penetration of the polypeptide into the lumen of the ER, a small hydrophobic leader sequence is removed (4). Two asparagine-linked (N-linked) oligosaccharide chains are added to G protein (22) in the form of a branched oligosaccharide precursor containing three glucose residues, nine mannose residues, and two *N*-acetyl glucosamine residues. This high-mannose chain is preformed on a dolichol phosphate lipid carrier which is localized in the ER (38). The oligosaccharide chain is then transferred en bloc to the nascent polypeptide. Both chains are added to G protein by the time the polypeptide is ca. 70% complete; thus, high-mannose core oligosaccharides are thought to be added in a cotranslational fashion. The polypeptide is then transported by unknown mechanisms to the Golgi apparatus (3), in which the high-mannose chains are processed to the complex or mature oligosaccharide chains via a concerted set of reactions (34).

Numerous studies have focused on the biochemical characterization of the herpes simplex virus type 1 (HSV-1) glycoproteins, and much information has been generated in recent years concerning the synthesis, processing, and localization of these proteins within the infected cell. There are four major antigenically distinct species of HSV-1, designated gB, gC, gD, and gE (1, 11, 32), which are present on the virion envelope as well as virtually all membranes of the infected cell (12, 33). The HSV-1 glycoproteins acquire N-linked oligosaccharides (28), and subsequent processing steps (6, 12, 41) may occur in the Golgi apparatus in a manner similar to that described for the G protein of vesicular stomatitis virus. Studies with endo-β-*N*-acetylglucosaminidase H (endo H) have demonstrated the presence of partially glycosylated precursors (i.e., containing high-mannose cores), which have been designated pgB110, pgC105, pgE65, and pgD52 (1, 31, 32, 41). Furthermore, several criteria have been used to demonstrate that the gC glycoprotein contains serine- or threonine-linked (O-linked) oligosaccharides (19, 25, 26, 42) which are thought to be acquired in the Golgi apparatus (19).

Studies on the distribution of the HSV-1 glycoproteins have demonstrated that the high-mannose precursors of gB, gC, and gD are the predominant components of the nuclear fraction of infected cells (7). The occurrence of high-mannose-type glycoproteins in the nuclear fraction has been explained by studies which have shown that the glycoprotein content of the nuclear membrane is very similar to the rough ER (17, 21). In addition, in studies on lectin binding, it has been found that the nuclear membrane and the ER strongly bind concanavalin A, which is specific for mannose residues, but not wheat germ agglutinin, which is specific for sialic acid (36). Furthermore, glycoproteins of the nuclear membrane are thought to be synthesized en loco rather than transported from a distal ER (2). It seems likely that the nuclear membrane, which is juxtaposed with the ER in many sites, has all the components necessary for the initial glycosylation event.

* Corresponding author.

In experiments in which the effect of variable culture conditions on the distribution of HSV-specific glycoproteins within the infected cell are studied, the observation was made that significant amounts of the nonglycosylated precursors to the gB, gC, and gD glycoproteins were detected in cells cultured at a reduced temperature of 34°C. Subsequent experiments were designed to examine possible post-translational modifications to the nonglycosylated precursors in the absence of any inhibitor of glycosylation. Although high-mannose core oligosaccharide chains are thought to be added to viral proteins in a cotranslational fashion, the data presented in this report are the first to suggest that this event may also occur post-translationally under certain circumstances.

MATERIALS AND METHODS

Cells and viruses. HEp-2 and Vero cells were grown in Eagle medium supplemented with 10% fetal calf serum and 0.075% sodium bicarbonate. The KOS strain of HSV-1 was grown in human embryonic lung fibroblasts (MRC-5), and all virus titrations were conducted in Vero cell monolayers (5).

Infection of cells. Monolayers of Vero or HEp-2 cells cultured in dishes (100 mm) were infected at a multiplicity of 10 PFU/ml. After a 1-h absorption at 34°C, the inoculum was removed, and maintenance medium containing 2% serum was added. Unless otherwise indicated, all incubations were carried out at 34°C.

Harvesting of cells and cell fractionation. At the appropriate time postinfection, cells (2×10^7) were scraped into the medium with a rubber policeman and washed twice with phosphate-buffered saline. Cytoplasmic and nuclear fractions were obtained by the modified Penman procedure (8, 27, 40). Washed cell pellets were suspended in 10 mM Tris (pH 7.4)–10 mM KCl–1.5 mM $MgCl_2$ (reticulocyte standard buffer) at 10^7 cells per ml, allowed to swell on ice for 15 min, and disrupted in a Dounce homogenizer (20 strokes). The crude lysate was centrifuged at $250 \times g$ for 6 min. The supernatant (cytoplasmic fraction in 2 ml) was removed, and the pellet was washed in one-half the original volume of reticulocyte standard buffer, followed by washing with 0.5% deoxycholate–1% Tween 40. The resulting pellet was washed two successive times with one-half the original volume of reticulocyte standard buffer. Nuclear pellets were suspended in glass-distilled water (0.2 ml) and stored at −20°C. When indicated, phenylmethylsulfonyl fluoride (PMSF) (Sigma Chemical Co., St. Louis, Mo.) or tosyllysyl chloromethyl ketone (TLCK) (Sigma) was added immediately upon scraping the cells from the dish at a final concentration of 1 mM. These inhibitors were present throughout the cell fractionation procedure.

SDS-PAGE and immunoblotting. Details of the methods used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) have been previously described (29). All slab gels were 7% bisacrylamide. Efforts were made to load into each well approximately equal amounts (25 to 30 μg) of protein derived from the cytoplasmic or nuclear fractions. The proteins resolved by SDS-PAGE were transferred to nitrocellulose paper (Schleicher & Schuell, Inc., Keene, N.H.), and immunoautoradiography was performed as previously described (7, 37). Hyperimmune monospecific rabbit antisera to gB or gC were used throughout and were prepared as previously described (10).

Endo H digestion. Endo H digestion was performed essentially as described previously (41). Briefly, nuclear fractions were made to 0.8% SDS and boiled for 2 min. Samples to be treated with and without endo H were diluted to 0.2% SDS and made 50 mM with respect to sodium citrate (pH 5.5). Endo H (6 μl at a 0.5-IU/ml concentration) was added to samples, whereas controls were treated with water. After incubation at 37°C for 4 h, the samples were precipitated with a 10× volume of ice-cold acetone and centrifuged at $13,000 \times g$ for 8 min. The pellet was suspended in 1% SDS–1% mercaptoethanol–0.5 M urea and stored at −20°C until analysis by SDS-PAGE.

RESULTS

Detection of nonglycosylated precursor proteins of gB and gC in Vero and HEp-2 cells. In studies designed to determine the distribution of the HSV-1 glycoproteins and their precursors associated with the cytoplasmic and nuclear fractions of infected cells, we repeatedly observed the presence of significant amounts of the nonglycosylated precursor components. HSV-1-infected HEp-2 or Vero cells cultured at 34 or 37°C were harvested and separated into the cytoplasmic and nuclear fractions. Analysis by SDS-PAGE followed by immunoblotting with either anti-gB or anti-gC serum revealed the presence of gB- and gC-reactive components with apparent molecular weights of 97,000 and 75,000, respectively (Fig. 1). Previous studies have shown these components to represent the nonglycosylated precursors of gB and gC and are designated pgB97 and pgC75 (41). The data indicate that relative to the amount of the fully glycosylated (gB and gC) and high-mannose (pgB110 and pgC105) components that were detected, the nonglycosylated precursors (pgB97 and pgC75) were the predominant forms associated with the nuclear fraction. It was also noted that incubation of the infected cells at a reduced temperature (34°C) appeared to increase the amount of pgB97 and pgC75 detected in the cytoplasmic and nuclear fractions. This effect of temperature on the distribution and relative amount of the nonglycosylated precursor detected, compared with the amount of the fully glycosylated and high-mannose forms present, was most notable in the cytoplasmic fraction. Specifically, pgB97 and pgC75 were readily detected in the cytoplasmic fraction of either cell type incubated at 34°C; however, at 37°C, the nonglycosylated precursors were only detected in Vero cells. The relative proportion of pgB97 and pgC75 compared with the high-mannose precursor and the fully processed forms also varied among cell types. For example, the nonglycosylated precursors were detected in the nuclear fraction of a human fibroblast cell line (MRC-5) when cultured at 34°C; however, none could be detected in cells cultured at 37°C (data not shown). These data demonstrate that in certain cell lines, the nonglycosylated forms of the HSV-1-specific glycoproteins are synthesized and represent the predominant form of the glycoprotein associated with the nuclear fraction relative to the fully glycosylated and high-mannose components.

In a recent study by Zezulak and Spear (43), the authors reported that HSV-specific glycoproteins produced in Vero cells were subject to limited proteolysis upon their extraction from infected cells. Furthermore, it was reported that similar proteases were not active in HEp-2 cells. pgC75 and pgB97 were detected in HEp-2 as well as Vero cells (Fig. 1). In other studies, infected Vero cells were harvested and fractionated in the presence of PMSF (an inhibitor of serine proteases) or TLCK (an inhibitor of papain and trypsin) to determine whether the protease inhibitor affected the appearance of the nonglycosylated protein. The distribution and proportion of the nonglycosylated precursors in HSV-1-infected Vero cells was unchanged by the addition of protease inhibitors (Fig. 2). A similar experiment was performed

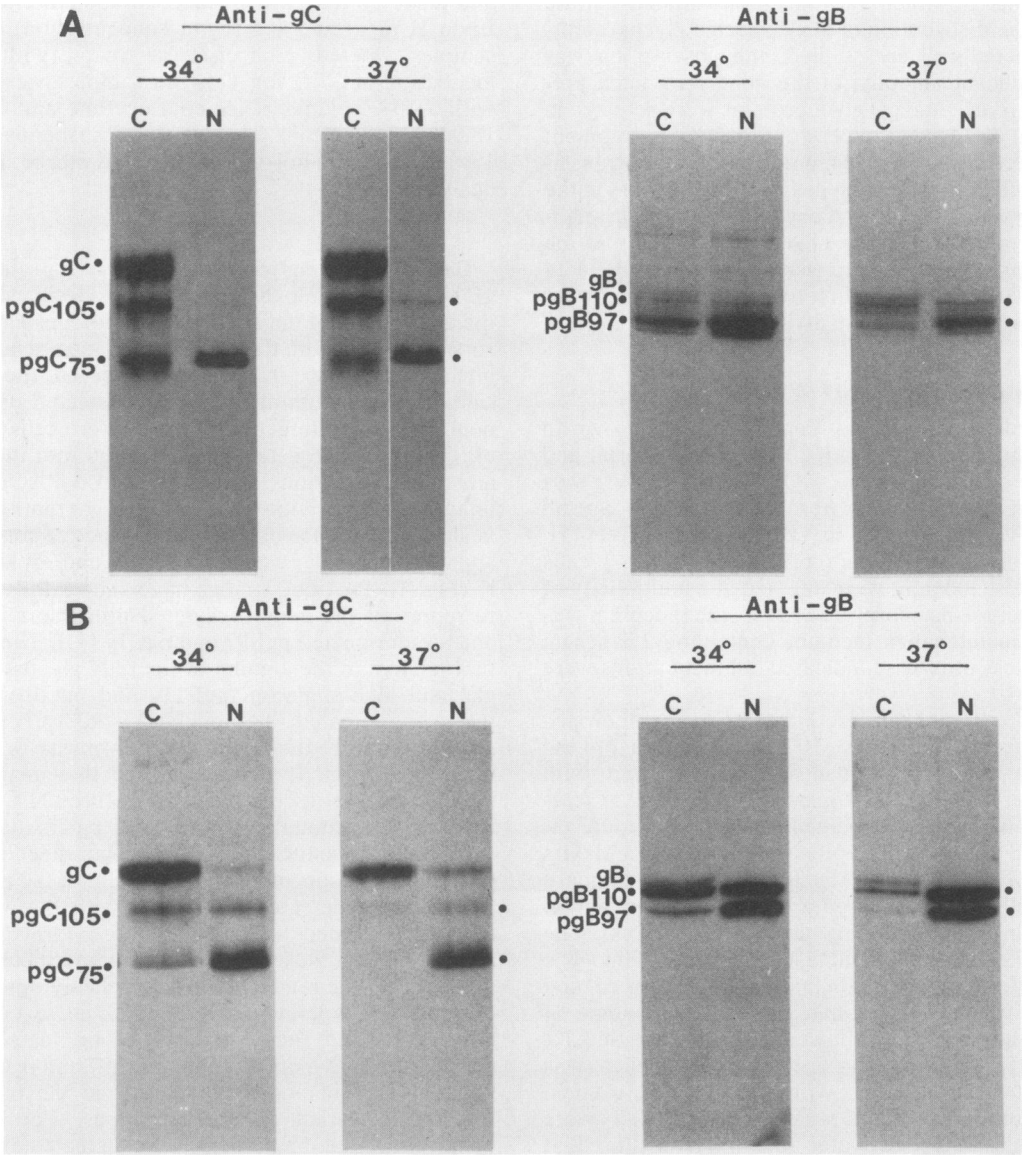

FIG. 1. Distribution of HSV-1 glycoprotein components immunoreactive with anti-gB and anti-gC in Vero (A) and HEp-2 (B) cells. HSV-1-infected Vero and HEp-2 cells were incubated at 34 or 37°C. At 15 h postinfection (Vero cells) and 22 h postinfection (HEp-2 cells), the cultures were harvested and fractionated into cytoplasmic (C) and nuclear (N) fractions. After analysis by SDS-PAGE, the resolved proteins were transferred to nitrocellulose sheets and reacted with either anti-gC or anti-gB serum.

with HEp-2 cells in which PMSF, TLCK, or pepstatin A (an inhibitor of acid proteases) was used. As was observed in the Vero cell fractions, no alteration in the distribution or relative proportion of pgC75 and pgB97 was detected (data not shown). These results, together with data presented below, indicate that the nonglycosylated precursors do not arise as a result of proteolysis.

Kinetics of the appearance of pgB97 and pgC75. To determine when the nonglycosylated precursors of the gB and gC glycoproteins were detectable in infected cells relative to the other species, infected cells were harvested at various times postinfection followed by cell fractionation. Analysis of nuclear fractions of Vero cells (Fig. 3) by immunoblotting revealed that the high-mannose precursors were detected first, with pgB110 detected at 4 h postinfection and pgC105 detected at 6 h postinfection. These findings are consistent with the results of previously published studies on the kinetics of synthesis of HSV-1 glycoproteins (6, 7). In contrast, the nonglycosylated precursors pgB97 and pgC75 were detected at 8 h postinfection. Although the gB and gC glycoproteins are synthesized at different times postinfection and most likely belong to separate kinetic classes (39; T. Compton and R. J. Courtney, Virus Res., in press), the appearance of the nonglycosylated precursors in the nuclear fraction was simultaneous. The concurrent appearance of pgB97 and pgC75 was also observed in HEp-2 cells (Fig. 4); however, in this cell line, nonglycosylated precursors were not detected until 20 h postinfection. The late appearance of the nonglycosylated precursors explains why previous studies (7) did not detect significant amounts of pgB97 and pgC75 in nuclear fractions which were obtained from cells 18 h after infection. Thus, it appears that the time in the replication

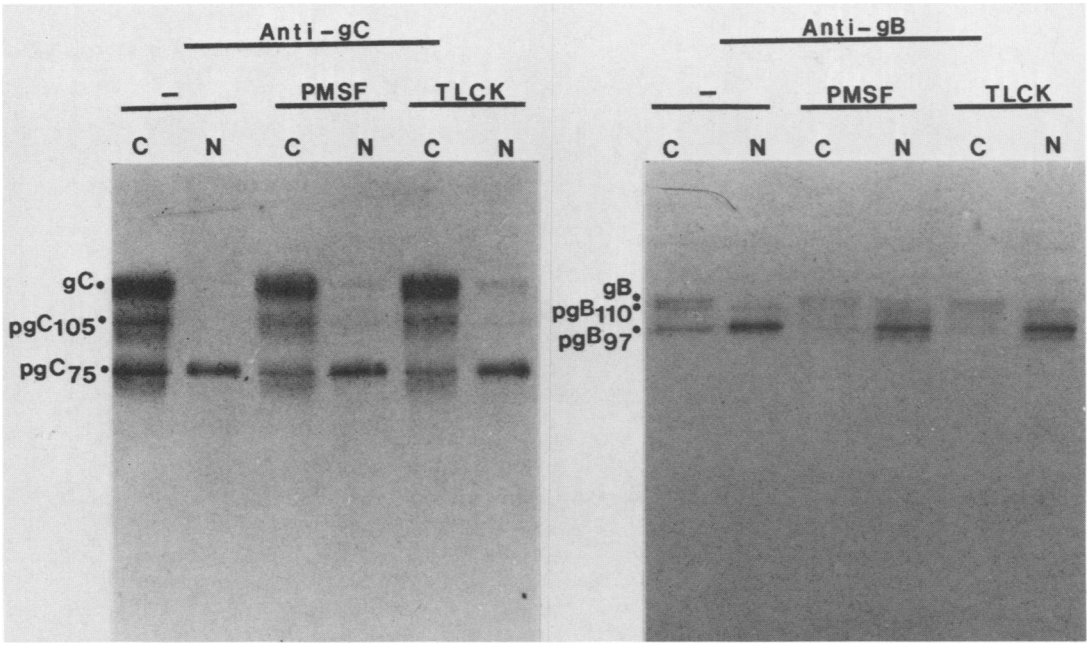

FIG. 2. Effect of protease inhibitors on the proportion of pgC75 and pgB97 in Vero cells. HSV-1-infected Vero cells were harvested at 14 h postinfection. Immediately after the cells were scraped from the culture dish, PMSF (1 mM) or TLCK (1 mM) was added and was present throughout the cell fractionation procedure. Cytoplasmic (C) and nuclear (N) fractions were analyzed by SDS-PAGE followed by immunoblotting with anti-gC or anti-gB serum.

cycle in which significant amounts of the nonglycosylated precursors accumulate within infected cells varies with the cell line used.

Post-translational modifications to nonglycosylated precursors. Conditions which allowed detection of appreciable amounts of a nonglycosylated precursor protein in the absence of inhibitors of glycosylation provided a unique approach to probe possible post-translational modifications of the proteins involved. The experimental design was to inhibit protein synthesis by the addition of cycloheximide (CX) at a time when significant quantities of the nonglycosylated precursors were detectable. These cultures were then shifted to an elevated temperature (39°C) for various periods of time (20 to 120 min) and then harvested. The results of such an experiment with HSV-1-infected Vero cells are shown in Fig. 5. As can be observed in the immunoautoradiograph, multiple discrete bands migrating with decreased electrophoretic mobility relative to pgC75 were detected in the CX-blocked samples. In cells harvested 20 min after shift-up, two additional bands were observed, whereas from 40 to 120 min, as many as four additional bands were detected. Furthermore, if CX was added to cultures which

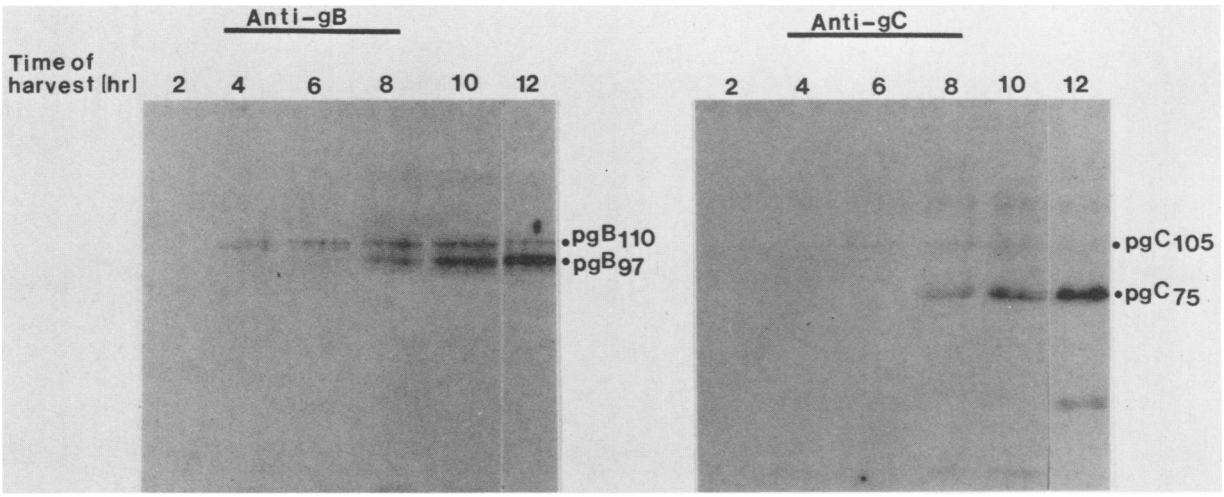

FIG. 3. Kinetics of the appearance of pgB97 and pgC75 in the nuclear fraction of Vero cells. Monolayers of Vero cells were infected with HSV-1 and cultured at 34°C. At various times postinfection (2 through 12 h), cultures were harvested and fractionated into cytoplasmic and nuclear fractions. Nuclear fractions were disrupted by sonication and analyzed by SDS-PAGE followed by immunoblotting with either anti-gB or anti-gC serum.

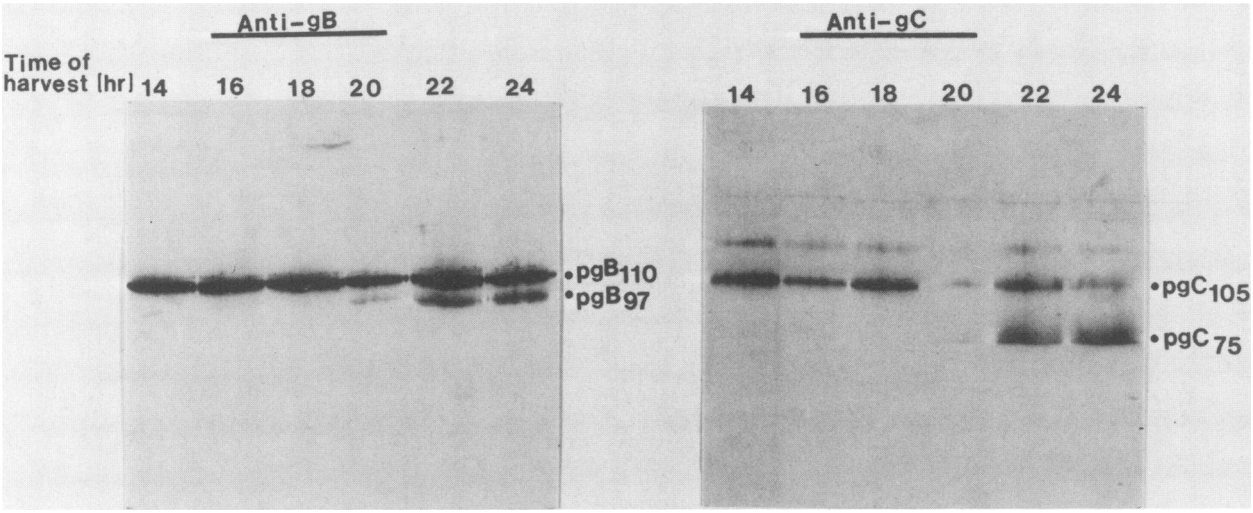

FIG. 4. Kinetics of appearance of pgB97 and pgC75 in the nuclear fraction of HEp-2 cells. Monolayers of HEp-2 cells were infected with HSV-1 and cultured at 34°C. At various times postinfection (14 through 24 h) cultures were harvested and fractionated into cytoplasmic and nuclear fractions. Nuclear fractions were disrupted by sonication and analyzed by SDS-PAGE followed by immunoblotting with either anti-gB or anti-gC serum.

were then allowed to remain at 34°C, this "ladder effect" of bands was also observed. In contrast, no species migrating with an apparent increased molecular weight was detected if CX was omitted. A similar effect was observed in HEp-2 cells (Fig. 6). In cultures which were treated with CX and shifted to 39°C or kept at 34°C, one to four additional bands were observed; however, if CX was omitted from a culture which was shifted to 39°C, it was difficult to discern the appearance of additional bands. The implications of these experiments are twofold. First, the nonglycosylated precursors which accumulated in the nuclear fraction of infected cells may be post-translationally modified if protein synthesis is halted. Second, an increase in the temperature of incubation is not essential, although data presented in Fig. 5 and 6 suggest that it facilitates the processing. This effect could not be rigorously documented for pgB97 due to the limited resolution possible between the 97,000- and 110,000-molecular-weight species of gB.

Discrete shifts in electrophoretic mobility of HSV-1 glycoproteins have been attributed to subtle differences in glycosylation (6, 19). To determine whether the observed series of multiple bands in CX-blocked samples was due to glycosylation of the nonglycosylated precursor, two approaches were

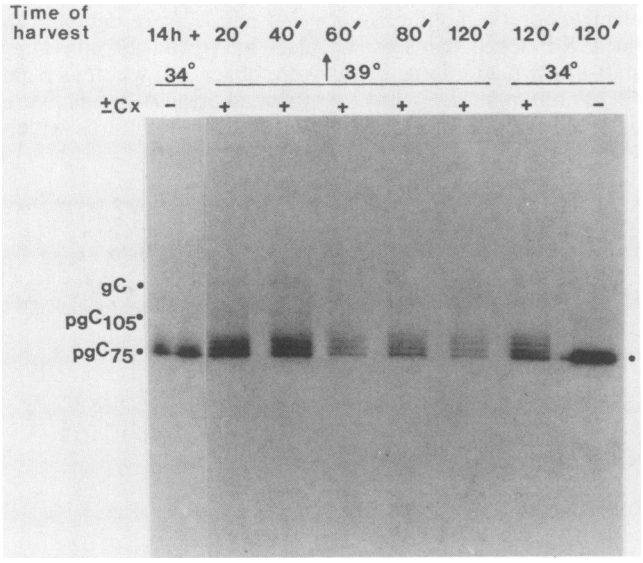

FIG. 5. Effect of CX on the nonglycosylated precursor of HSV-1 gC in Vero cells. Vero cells were infected with HSV-1 and incubated at 34°C. At 14 h postinfection, one culture was harvested and fractionated, whereas parallel cultures were treated with CX (50 μg/ml) and shifted to 39°C or allowed to remain at 34°C. At various periods of time after the addition of CX (20 through 120 min), the cultures were harvested and fractionated. Nuclear fractions were electrophoresed by SDS-PAGE and immunoblotted with anti-gC serum.

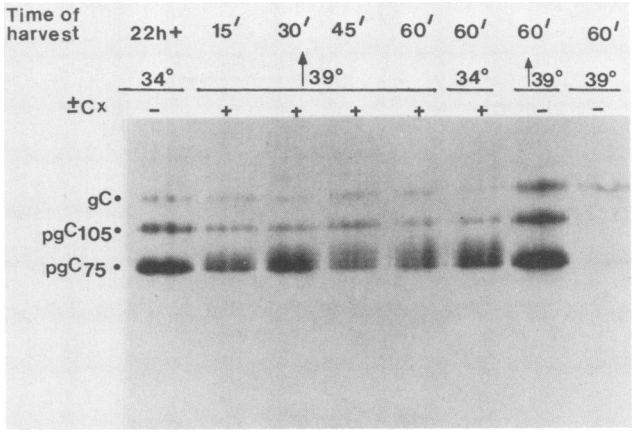

FIG. 6. Effect of CX on the nonglycosylated precursor of HSV-1 gC in HEp-2 cells. HEp-2 cells were infected with HSV-1 and incubated at 34°C. At 22 h postinfection, one culture was harvested and fractionated, whereas parallel cultures were treated with CX (50 μg/ml) and shifted to 39°C or allowed to remain at 34°C. At various periods of time after the CX block (15 through 60 min), the cultures were harvested and fractionated. Nuclear fractions were analyzed by SDS-PAGE followed by immunoblotting with anti-gC serum.

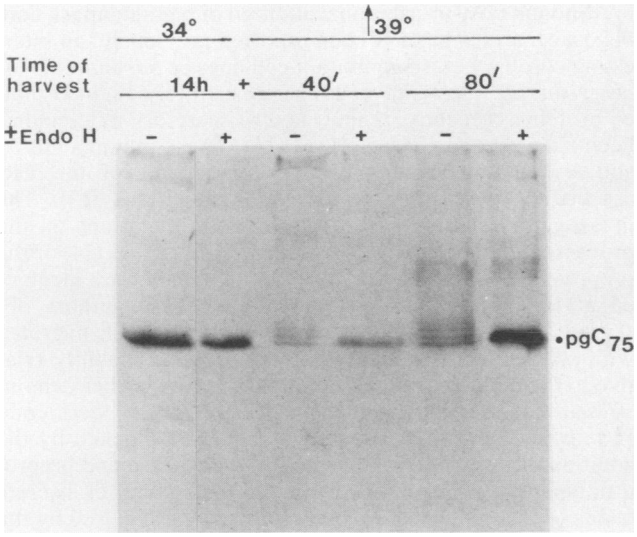

FIG. 7. Endo H digestion of CX-blocked Vero cells. HSV-1-infected Vero cells were cultured at 34°C. At 14 h postinfection, one culture was harvested, whereas two parallel cultures were treated with CX and shifted to 39°C for 40 or 80 min. After cell fractionation, nuclear fractions were subjected to digestion by endo H or treated as controls. Analysis was by SDS-PAGE followed by immunoblotting with anti-gC serum.

employed. The first approach involved the use of the endoglycosidase, endo H, which is known to selectively cleave N-linked oligosaccharides of the high-mannose type but not of the complex type (35). Infected Vero cells were treated with CX at 14 h postinfection for 40 or 80 min, harvested, and fractionated into cytoplasmic and nuclear fractions. Aliquots of nuclear fractions were then subjected to digestion with endo H. The data presented in Fig. 7 demonstrate that the four additional bands migrating above pgC75 were eliminated by endo H digestion, suggesting the presence of high-mannose core sugars. The second approach was to add the antibiotic tunicamycin at the time of the CX block. Tunicamycin inhibits the production of *N*-acetylglucosamine-pyrophosphoryl dolichol (18), which is the first step in the synthesis of lipid-linked oligosaccharides (38). Tunicamycin blocked the production of the slower-migrating species, whereas a predominant band comigrating with pgC75 persisted in both the cytoplasmic and nuclear fractions (Fig. 8). In addition, these data also demonstrate that the same discrete bands are detected in the cytoplasmic fraction. Taken together, the data suggest that N-linked high-mannose core sugars may be added to the nonglycosylated precursor of gC in a post-translational fashion.

DISCUSSION

The findings of this study are threefold. (i) The nonglycosylated precursor of the gB and gC glycoproteins is detectable in the cytoplasmic and nuclear fractions in the absence of inhibitors of glycosylation in Vero and HEp-2 cells. (ii) Relative to the other glycosylated forms, the nonglycosylated precursors of gB and gC are the predominant components associated with the nuclear fraction of infected cells. (iii) The nonglycosylated precursor of the gC glycoprotein of HSV-1 may be post-translationally modified by the addition of high-mannose core sugars.

Variable effects of the temperature of incubation on membrane proteins have been previously reported. The nonglycosylated precursor of G protein synthesized in the presence

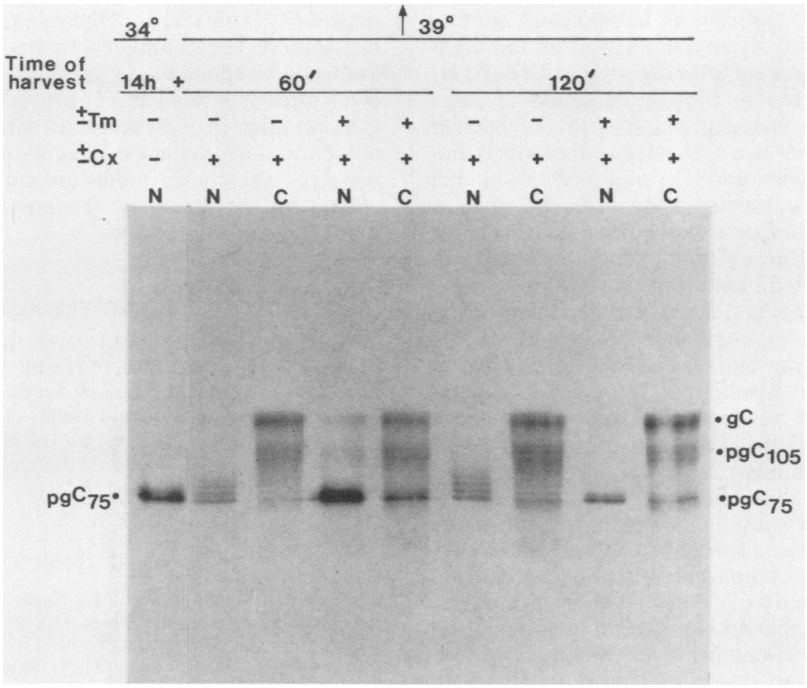

FIG. 8. Effect of tunicamycin on the appearance of multiple bands in Vero cells. HSV-1-infected Vero cells were incubated at 34°C until 14 h postinfection. At 14 h postinfection, one culture was harvested, whereas four cultures were treated with CX (50 μg/ml). Two of the CX-blocked cultures were also treated with tunicamycin (10 μg/ml). All cultures were shifted to 39°C for 60 or 120 min. After fractionation, cytoplasmic (C) and nuclear (N) fractions were analyzed by SDS-PAGE followed by immunoblotting with anti-gC serum.

of tunicamycin in cells incubated at 30°C has been shown to have different physical properties from the nonglycosylated G protein synthesized at 37°C (16). Additionally, in a recent study in which a sensitive trypsin assay was used, Matlin and Simons (24) have reported that incubation of cells at a reduced temperature prevented transfer of the hemagglutinin glycoprotein of influenza to the cell surface. Other studies have shown that temperatures between 15 and 20°C selectively block certain steps in the endocytic pathway (9, 23). The results presented here demonstrate that nonglycosylated precursors of HSV-1 glycoproteins may be more readily detected in cells which were incubated at a reduced temperature.

Several lines of evidence suggest that the nonglycosylated precursors pgB97 and pgC75 do not appear as a result of proteolysis of the high-mannose precursor. Although no one approach could be considered totally definitive, when one considers all the observations, it seems unlikely that proteolysis plays a role in the appearance of these components. First, no effect on the detection of the nonglycosylated precursors was observed when certain protease inhibitors were used. Although the inhibitors used do not represent an all-inclusive selection, it does cover a spectrum of such protease specificities as serine type, papain-trypsin type, and acid type. Second, if the components were the result of proteolysis, one would predict them to be glycosylated. We were not able to detect any carbohydrate label associated with pgC75 and pgB97; however, the components which appear in the ladder pattern can be metabolically labeled with [^{3}H]mannose (T. Compton and R. J. Courtney, manuscript in preparation). Third, the pgC75 component is insensitive to endo H digestion, suggesting that this component is not derived from proteolysis of the high-mannose precursor pgC105. In addition, the pgC75 component which is putatively glycosylated during CX treatment is sensitive to endo H digestion. Finally, the pgC75 and pgB97 components have the same electrophoretic mobility as components synthesized in the presence of tunicamycin as well as the high-mannose-containing component after digestion with endo H.

The mechanism involved in the accumulation of large proportions of nonglycosylated precursors in the nuclear fraction as compared with the cytoplasmic fraction is unknown. One possible explanation is a limitation of dolichol phosphate in the nucleus-associated glycosylation apparatus. This limitation can be particularly prominent in some cell types (Vero and HEp-2) but not in others (MRC-5). Although increasing the temperature of incubation to 39°C facilitated the processing events, the crucial parameter to the detection of the slower-migrating bands was the addition of CX to inhibit additional protein synthesis. It is possible to speculate that the nucleus-associated glycosylation apparatus is overburdened with newly synthesized protein, such that reduced transfer of the lipid-linked oligosaccharide occurs until protein synthesis is halted. The inefficient transfer of high-mannose core sugars to the nascent polypeptide apparently does not occur to the same extent in the cytoplasmic fraction. Although some nonglycosylated precursor was detected in the cytoplasmic fraction (especially at 34°C) and the series of bands was detected after the addition of CX (Fig. 8), the proportion of pgC75 was small as compared with that associated with the nuclear fraction. Recent studies designed to measure the amount of lipid-linked oligosaccharide in the various cell lines cultured at reduced and elevated temperatures have provided support for this hypothesis (Compton and Courtney, manuscript in preparation).

Although post-translational addition of high-mannose core oligosaccharides has not been reported previously, an interesting corollary exists in mutant cell lines of *Saccharomyces cerevisiae*. *S. cerevisiae* cells secrete a variety of glycosylated proteins. Recently, a new class of secretory mutants has been isolated and characterized (14). These mutants (*sec53* and *sec59*) appear to code for components of the ER-associated polypeptide translocation apparatus (13). The investigators studied the effects of these mutations on the processing of the secretory glycoprotein invertase (14) or the prepro-α-polypeptide (20). When mutant cells were incubated at the nonpermissive temperature, these authors observed a series of multiple discrete bands which migrated with progressively decreased electrophoretic mobility relative to the nonglycosylated precursor. It was further demonstrated that the higher-molecular-weight species were endo H sensitive, and their appearance could be blocked by the addition of tunicamycin. For both the invertase and prepro-α-polypeptide, the authors attributed the number of discrete bands to the number of carbohydrate chains acquired by the polypeptide. In our study, we observed one to four additional bands which may possibly represent the addition of one to four high-mannose chains. Analysis of the nucleotide sequence encoding the gC glycoprotein has revealed that there are eight potential N-linked glycosylation sites, seven of which lie in the extracytoplasmic domain of the polypeptide (15). It is possible that all glycosylation sites would not be accessible to the acceptance of oligosaccharide chains due to conformational differences between a fully synthesized polypeptide versus one that is being glycosylated cotranslationally.

The addition of high-mannose oligosaccharides is known to occur as the polypeptide chains are being synthesized. The data reported here provide evidence that this event may occur in a post-translational fashion under special conditions. This process does not appear to be totally temperature dependent, and the nonglycosylated precursor of gC does not acquire the complete number of carbohydrate chains known to be added to gC, as judged by the molecular weight. Even after an extended incubation time after the addition of CX, no species was detected which comigrated with the authentic high-mannose precursor of 105,000 molecular weight. Pulse-chase studies are currently in progress which should further document the post-translational addition of high-mannose core sugars.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant CA24564 from the National Institutes of Health. T.C. is a predoctoral fellow supported by National Research Service Award Training grant T32 AI07123 from the National Institutes of Health.

We thank Krista Dearstone for technical assistance, Jackie Pratt for expert assistance in the preparation of the manuscript, and Joel Richter for critical review.

LITERATURE CITED

1. **Baucke, R. B., and P. G. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. V. Identification of an Fc-binding glycoprotein. J. Virol. **32**:779–789.
2. **Bergmann, J. E., and S. J. Singer.** 1983. Immunoelectron microscopic studies of the intracellular transport of the membrane glycoprotein (G) of vesicular stomatitis virus in infected Chinese hamster ovary cells. J. Cell Biol. **97**:1777–1787.
3. **Bergmann, J. E., K. T. Tokuyasu, and S. J. Singer.** 1981. Passage of an integral membrane protein, the vesicular stomati-

tis virus glycoprotein, through the Golgi apparatus en route to the plasma membrane. Proc. Natl. Acad. Sci. U.S.A. **78**:1746–1750.
4. **Blobel, G., P. Walter, C. N. Chang, B. M. Goldman, E. H. Erikson, and V. E. Lingapappa.** 1979. Translocation of proteins across membranes: the signal hypothesis and beyond. Symp. Soc. Exp. Biol. **33**:9–36.
5. **Bone, D. R., and R. J. Courtney.** 1974. A ts mutant of herpes simplex virus type 1 defective in the synthesis of the major capsid protein. J. Gen. Virol. **24**:17–27.
6. **Cohen, G. H., D. Long, and R. J. Eisenberg.** 1980. Synthesis and processing of glycoproteins gD and gC of herpes simplex virus type 1. J. Virol. **36**:429–439.
7. **Compton, T., and R. J. Courtney.** 1984. Virus-specific glycoproteins associated with the nuclear fraction of herpes simplex virus type 1-infected cells. J. Virol. **49**:594–597.
8. **Courtney, R. J.** 1976. Herpes simplex virus protein synthesis in the presence of 2-deoxy-D-glucose. Virology **73**:286–294.
9. **Dunn, W. A., A. L. Hubbard, and N. N. Aronson, Jr.** 1980. Low temperature selectively inhibits fusion between pinocytic vesicles and lysosome during heterophagy of ^{125}I-asialo fetuin by the perfused rat liver. J. Biol. Chem. **255**:5971–5978.
10. **Eberle, R., and R. J. Courtney.** 1980. Preparation and characterization of specific antisera to individual glycoprotein antigens comprising the major glycoprotein region of herpes simplex virus type 1. J. Virol. **35**:902–917.
11. **Eberle, R., and R. J. Courtney.** 1980. gA and gB glycoproteins of herpes simplex virus type 1: two forms of a single polypeptide. J. Virol. **36**:665–675.
12. **Eisenberg, R. J., C. Hydrean-Stern, and G. H. Cohen.** 1979. Structural analysis of precursor and product forms of type-common envelope glycoprotein D (CP-1 antigen) of herpes simplex virus type 1. J. Virol. **31**:608–620.
13. **Ferro-Novick, S., W. Hansen, I. Schauer, and R. Schekman.** 1984. Genes required for completion of import of proteins into the endoplasmic reticulum in yeast. J. Cell Biol. **98**:44–53.
14. **Ferro-Novick, S., P. Novick, C. Field, and R. Schekman.** 1984. Yeast secretory mutants that block formation of active cell surface enzymes. J. Cell Biol. **98**:35–43.
15. **Frink, R. J., R. Eisenberg, G. Cohen, and E. K. Wagner.** 1983. Detailed analysis of the portion of the herpes simplex virus type 1 genome encoding glycoprotein C. J. Virol. **45**:634–647.
16. **Gibson, R., S. Schelsinger, and S. Kornfeld.** 1979. The nonglycosylated glycoprotein of VSV is temperature-sensitive and undergoes intracellular aggregation at elevated temperatures. J. Biol. Chem. **254**:3600–3607.
17. **Harris, J. R.** 1978. The biochemistry and ultrastructure of the nuclear envelope. Biochim. Biophys. Acta **515**:55–104.
18. **Heifetz, A., R. W. Keeran, and A. D. Elbein.** 1979. Mechanism of action of tunicamycin on the UDP-Glc NAc:dolichylphosphate transferase. Biochemistry **18**:2186–2192.
19. **Johnson, D. C., and P. G. Spear.** 1983. O-linked oligosaccharides acquired by herpes simplex virus glycoproteins in the Golgi apparatus. Cell **32**:987–997.
20. **Julius, D., R. Schekman, and J. Thorner.** 1984. Glycosylation and processing of prepro-α-factor through the yeast secretory pathway. Cell **36**:309–318.
21. **Kawasaki, T., and J. Yomashina.** 1972. Isolation and characterization of glycopeptides from rat liver nuclear membrane. J. Biochem. **72**:1517–1525.
22. **Li, E., I. Tagas, and S. Kornfeld.** 1978. Structure of the lipid-linked oligosaccharide precursor of the complex-type oligosaccharides of the vesicular stomatitis virus G protein. J. Biol. Chem. **253**:7762–7770.
23. **Marsh, M., E. Bolzau, and A. Helenius.** 1983. Penetration of Semliki Forest virus from acidic prelysosomal vacuoles. Cell **32**:931–940.
24. **Matlin, K. S., and K. Simons.** 1983. Reduced temperature prevents transfer of a membrane glycoprotein to the cell surface but does not prevent terminal glycosylation. Cell **34**:233–243.
25. **Olofsson, S., J. Blomberg, and E. Lycke.** 1981. O-glycosidic carbohydrate peptide linkages of herpes simplex virus glycoproteins. Arch. Virol. **70**:321–329.
26. **Olofsson, S., I. Sjoblom, M. Lundstrom, S. Jeansson, and E. Lycke.** 1983. Glycoprotein C of herpes simplex virus type 1: characterization of O-linked oligosaccharides. J. Gen. Virol. **64**:2735–2747.
27. **Penman, S.** 1966. RNA metabolism in the HeLa cell nucleus. J. Mol. Biol. **17**:117–130.
28. **Pizer, L. I., G. H. Cohen, and R. J. Eisenberg.** 1980. Effect of tunicamycin on herpes simplex virus glycoproteins and infectious virus production. J. Virol. **34**:142–153.
29. **Powell, K. L., and R. J. Courtney.** 1975. Polypeptides synthesized in herpes simplex virus type 2 infected HEp-2 cells. Virology **66**:217–228.
30. **Sabatini, D. D., G. Krebich, T. Morimoto, and M. Adesnik.** 1982. Mechanism for the incorporation of proteins in membranes and organelles. J. Cell Biol. **92**:1–22.
31. **Serafini, C.-F., and G. Campadelli-Fiume.** 1981. Studies on benzhydrazone, a specific inhibitor of herpesvirus glycoprotein synthesis. Size distribution of glycopeptides and endo-β-*N*-acetyl glucosaminidase-H treatment. Arch. Virol. **70**:331–343.
32. **Spear, P. G.** 1976. Membrane proteins specified by herpes simplex viruses. I. Identification of four glycoprotein precursors and their products in type 1-infected cells. J. Virol. **17**:991–1008.
33. **Spear, P. G., and B. Roizman.** 1972. Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpesvirion. J. Virol. **9**:143–159.
34. **Tabas, I., S. Schlesinger, and S. Kornfeld.** 1978. Processing of high-mannose oligosaccharides to form complex-type oligosaccharides on the newly synthesized polypeptides of the VSV G protein. J. Biol. Chem. **253**:716–722.
35. **Tarentino, A. L. F., and Maley.** 1974. Purification and properties of an endo-β-acetyl glucosaminidase from *Streptomyces griseus*. J. Biol. Chem. **249**:811–817.
36. **Torrisi, M. R., and P. P. DaSilva.** 1984. Compartmentalization of intracellular membrane glycocomponents is revealed by fracture-label. J. Cell Biol. **98**:29–34.
37. **Towbin, H., T. Staehlin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. **76**:4350–4354.
38. **Waechter, C. J., and W. J. Lennarz.** 1976. The role of polyprenol-linked sugars in glycoprotein synthesis. Annu. Rev. Biochem. **45**:95–112.
39. **Wagner, E.** 1983. Transcription patterns of HSV infections, p. 239–270. *In* G. Klein (ed.), Advances in viral oncology, vol. 3. Raven Press, New York.
40. **Warner, J. R., P. M. Knopf, and A. Rich.** 1963. A multiple ribosomal structure in protein synthesis. Proc. Natl. Acad. Sci. U.S.A. **49**:122–129.
41. **Wenske, E. A., M. W. Bratton, and R. J. Courtney.** 1982. Endo-β-*N*-acetyl glucosaminidase H sensitivity of precursors to herpes simplex virus type-1 glycoproteins gB and gC. J. Virol. **44**:241–248.
42. **Wenske, E. A., and R. J. Courtney.** 1983. Glycosylation of herpes simplex virus type 1 gC in the presence of tunicamycin. J. Virol. **46**:297–301.
43. **Zezulak, K. M., and P. G. Spear.** 1984. Limited proteolysis of herpes simplex virus glycoproteins that occurs during their extraction from Vero cells. J. Virol. **50**:258–262.
44. **Zilberstein, A., M. D. Snider, and H. F. Lodish.** 1981. Synthesis and assembly of the vesicular stomatitis virus glycoprotein. Cold Spring Harbor Symp. Quant. Biol. **46**:785–805.