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Post-translational Processing of the Glycoproteins of Lymphocytic Choriomeningitis Virus

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Intracellular events in the synthesis, glycosylation, and transport of the lymphocytic choriomeningitis virus (LCMV) glycoproteins have been examined. We have shown by *N*-glycanase digestion that LCMV strain Arm-4 bears five oligosaccharides on GP-1 and two on GP-2. By pulse–chase labeling experiments in the presence of drugs which inhibit N-linked oligosaccharide addition and processing we demonstrate that addition of high mannose precursor oligosaccharides is necessary for transport and cleavage of the viral GP-C glycoprotein. Moreover, in the presence of tunicamycin which inhibits *en bloc* addition of these mannose-rich side chains, virus budding was substantially decreased and infectious virions were reduced by more than 1000-fold in the supernatant medium. Incubation in the presence of castantospermine, which permits addition of oligomannosyl-rich chains but blocks further processing, restored transport and cleavage of GP-C and maturation of virions. Finally, by temperature block experiments we have determined that maturation of GP-C oligosaccharides to an endoglycosidase H resistant form precedes cleavage to GP-1 and GP-2. The latter process is most likely to occur in the Golgi or post-Golgi compartment.

INTRODUCTION

Lymphocytic choriomeningitis virus (LCMV), the prototype member of the Arenaviridae, has provided investigators with a wealth of information about virus–host interaction. In the mouse, LCMV can establish a range of diseases from acute, lethal choriomeningitis to lifelong persistent infection. Through the study of these diseases several fundamental concepts have evolved including tolerance and immune complex disease (reviewed in Buchmeier *et al.*, 1980), virus alteration of specialized or luxury functions of differentiated cells (Oldstone *et al.*, 1984; Klavinskis *et al.*, 1988), and the requirement for major histocompatability complex in expression of antiviral cytotoxic T-cell killing and virus clearance (Zinkernagel and Doherty, 1974; Zinkernagel and Welsh, 1976).

The structure of LCMV is the best characterized of the arenaviruses. There are three major structural proteins, a nucleocapsid protein (NP, M_r 63 kDa), and two glycoproteins, GP-1 (M_r 44 kDa) and GP-2 (M_r 35 kDa). In addition, there are at least two quantitatively minor proteins, L (M_r 200 kDa) which is presumed to be a viral RNA dependent RNA polymerase, and a minor 12–14,000 M_r polypeptide, termed Z, which may constitute a zinc binding protein (Salvato *et al.*, 1989; Buchmeier and Parekh, 1987). Glycoproteins GP-1 and GP-2 are post-translationally cleaved from a cell-associated mannose-rich precursor, GP-C (M_r 75–76 kDa) (Buchmeier and Oldstone, 1979). Most of our work to date has focused on the antigenic structure of these glycoproteins. The major glycoprotein, GP-1, has at least four B-cell epitopes, two of which bind neutralizing antibodies. GP-2 has three overlapping epitopes (Parekh and Buchmeier, 1986), of which two are conserved among the arenaviruses (Weber and Buchmeier, 1988; Buchmeier *et al.*, 1981).

Little is known about post-translational processing of arenavirus glycoproteins. The proteolytic cleavage site of GP-C is apparently a paired basic amino acid sequence, Arg–Arg at amino acids 262–263 (Buchmeier *et al.*, 1987), and cleavage is mediated by a cellular protease. A similar precursor glycopeptide has also been identified for Pichinde (Harnish *et al.*, 1981), Lassa (Clegg and Lloyd, 1983), and Tacaribe viruses (Gimenez *et al.*, 1983; Franz-Fernandez *et al.*, 1987); however, Tacaribe contains only one structural glycoprotein.

An understanding of the biosynthesis, processing, and transport of the LCMV glycoproteins may aid in interpreting aspects of the viral biology. For example, in persistent LCMV infections selective modulation of glycoprotein expression has been reported in infected cells (Welsh and Buchmeier, 1979) and tissues (Oldstone and Buchmeier, 1982), but the mechanism of regulation remains unclear. Moreover, recent studies have described cytotoxic T-cell epitopes on the glycoproteins of LCMV (Whitton *et al.*, 1988). Based on current knowledge of the role of class I MHC in the endogenous pathway of antigen presentation, it is likely that association between class I and glycoprotein or a frag-

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ment derived from it occurs within the intracellular transport pathway. Finally, little is known about interaction between the LCMV glycoproteins and cell-surface viral receptors and the mechanism of viral entry into cells. Studies reported here address two basic aspects of post-translational processing of LCMV: N-linked glycosylation of the glycoproteins, and requirements for and kinetics of subsequent transport, trimming and proteolytic cleavage of GP-C, GP-1 and GP-2.

MATERIALS AND METHODS

Virus and cell culture

The virus used throughout these experiments, LCMV Armstrong clone 4 (Arm-4), was plaque purified from a stock of Armstrong CA-1371 (Parekh and Buchmeier, 1986; Wright *et al.,* 1989). Working stocks were prepared by infecting BHK-21 cells at a multiplicity of infection (m.o.i.) of 0.1 and harvesting the supernatants 48 hr later. Virus was purified by polyethylene glycol precipitation followed by banding on 10–40% (v/v) renograffin-76 (Squibb Diagnostics) gradients (Buchmeier and Oldstone, 1979).

Glycosylation inhibitors

Tunicamycin (TUN), deoxymannojirimycin (DMJ), and swainsonine (SSN) were purchased from Boehringer-Mannheim, and castanospermine (CSP) and *N*-methyl-deoxynojirimycin (NM-DNJ) from Genzyme. Stock solutions of TUN were prepared in DMSO. Stock solutions of DMJ, SSN, and NM-DNJ were prepared in culture medium. In immunofluorescence experiments inhibitors were added at the time of infection and maintained for the duration. For metabolic labeling, inhibitors were added to infected cultures for 24 hr before labeling and retained throughout the labeling and chase periods. Optimal drug concentrations used were predetermined by titration of their inhibitory activity in BHK-21 cells. Concentrations used were TUN, 0.5 μg/ml; CSP, 80 μg/ml; NM-DNJ, 2 m*M;* DMJ, 2 m*M;* SSN, 0.5 μg/ml. Control cultures for TUN were incubated in the presence of equivalent concentrations of DMSO.

Immunofluorescence

Indirect immunofluorescence of permeabilized cells was done on BHK-21 cell coverslips infected 24 hr earlier at an m.o.i. of 1.0 (Buchmeier *et al.,* 1981). For surface immunofluorescence, infected BHK cells were trypsinized, then stained with anti-LCMV monoclonal antibodies (MAb) and fluorescein-labeled sheep anti-mouse IgG. Other studies have established that LCMV antigens on the surfaces of infected cells resist trypsin treatment (Buchmeier *et al.,* 1981).

Immunoprecipitation and polyacrylamide gel electrophoresis (PAGE)

BHK-21 cells were infected at an m.o.i. of 1.0 for 48 hr, then pulse labeled for 1 hr in methionine-free Dulbecco's medium (Flow Laboratories) containing 60 μCi/ml L-[^{35}S]methionine (Amersham). Cells were pretreated for 24 hr with glycosylation inhibitors at the indicated concentrations in methionine-free media before labeling. Cells were chased as indicated in medium containing glycosylation inhibitors and a 10-fold excess of cold L-methionine. Lysates were prepared in a buffer containing 20 m*M* Tris, 137 m*M* NaCl, 1 m*M* $CaCl_2$, 0.5 m*M* $MgCl_2$, 1% (v/v) NP-40, 10% (v/v) glycerol, and 1% (v/v) aprotinin, then cleared by centrifugation at 14,000 rpm for 15 min. Cell equivalents of control and test lysates were incubated with antibody at a final dilution of 1:500 for 45–60 min at 22°, after which 80 μl of washed protein A–Sepharose CL-4B beads (Sigma) were added. Tubes were incubated for 30 min with continuous shaking, then precipitates were collected by centrifugation and washed three times in wash buffer (100 m*M* Tris, 500 m*M* LiCl) before resuspending in 20 μl buffer containing 1% 2-ME, 2% SDS. Endoglycosidase H (EH) digestion was done on immune-precipitated viral proteins. Briefly, each precipitated sample was digested for 2 hr at 37° with 0.005 units of EH. Samples were heated to 100° for 2 min, then loaded onto 10% polyacrylamide gels (Laemmli, 1970). ^{14}C-methylated protein markers (Amersham) were run on each gel. After electrophoresis gels were fixed in 7% acetic acid, 20% methanol, washed with distilled water, incubated in Autofluor (National Diagnostics) for 30 min, then dried and exposed to film at −70°. For temperature block experiments, chases were carried out at the indicated temperatures in individual water baths.

Western blotting

Purified virus (200 μg) was digested with 0.5 units peptide *N*-glycosidase F (PNGF), a gift from Dr. J. Elder, Scripps Clinic and Research Foundation, for varying lengths of time to remove N-linked carbohydrates. Samples were then separated by SDS–PAGE, electrophoretically transferred to nitrocellulose, and immunoblotted with MAb specific for either GP-1 (67.5) or GP-2 (33.6).

Electron microscopy

Virus-infected and uninfected control monolayer cultures of BHK-21 cells were detached by scraping with a rubber policeman, then pelleted at 500 *g.* Pellets were fixed in 2.5% buffered glutaraldehyde and epon embedded. Thin sections were prepared, stained with osmium tetroxide, and examined in an Hitachi electron microscope.

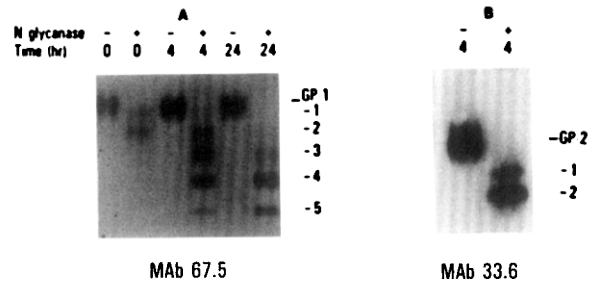

FIG. 1. Enumeration of oligosaccharide side chains on the LCMV GP-1 and GP-2 glycoproteins. Purified LCMV Arm-4 was digested with PNGF as described under Materials and Methods. Samples of the digest were removed immediately after adding all reagents (0 time) and at 2, 4, and 24 hr as indicated. Polypeptides were resolved on 10% (A) or 12.5% (B) SDS–PAGE gels and viral proteins visualized by Western blotting using GP-1-specific (MAb 67.5) or GP-2-specific (MAb 33.6) MAb.

RESULTS

Glycosylation of the major glycoprotein of LCMV

We have previously observed that the precursor molecule, GP-C, was labeled heavily with 2-[^{3}H]mannose, whereas the mature GP-1 and GP-2 glycoproteins contained relatively little mannose but were labeled with [^{3}H]galactose and [^{3}H]fucose (Buchmeier and Oldstone, 1979; Buchmeier and Parekh, 1987). These results suggested that GP-C contained high mannose oligosaccharides which were trimmed to a complex form prior to post-translational proteolytic cleavage to GP-1 and GP-2. In order to determine the extent of glycosylation of each glycoprotein, purified virus was digested with PNGF for various intervals before separation by SDS–PAGE and Western blotting with GP-1- or GP-2-specific antisera. Digestion produced a ladder of bands representing polypeptide chains with successively fewer oligosaccharide side chains. By this method we were able to demonstrate a total of six bands which reacted with GP-1 antibody, indicating that Arm-4 GP-1 contained five N-linked carbohydrates (Fig. 1A). One of these bands, labeled -1 on Fig. 1A, was present only momentarily after addition of PNGF and comigrated with the fastest migrating portion of the undigested GP-1 band, suggesting that native GP-1 may actually be a mixture of polypeptide core chains with four and five oligosaccharides.

Duration between synthesis and cleavage of GP-C

In order to estimate the time between synthesis and cleavage of GP-C, replicate cultures were pulsed for 5 min with [^{35}S]Met, then chased for intervals of 15, 30, 60, 90, 120, 180, 270, or 360 min before immunoprecipitation with antibody to GP-2. The results of such a pulse–chase experiment are illustrated in Fig. 2. GP-C was evident immediately after pulsing but GP-2, indicative of GP-C cleavage, was not evident until 90 min chase had elapsed. In additional experiments focusing on the 60- to 120-min chase interval we have observed the first appearance of GP-2 at 75 min (data not shown).

Post-translational cleavage of GP-C requires prior glycosylation

In experiments carried out to characterize the two neutralizing epitopes on GP-1, it was found that folding of the GP-C precursor required N-linked glycosylation (Wright *et al.*, 1989). In order to investigate the role of glycosylation in post-translational processing, we utilized the glycosylation inhibitors TUN, DMJ, SSN, CSP, and NM-DNJ to examine the requirement for N-linked glycosylation in more detail. BHK-21 cells were infected with Arm-4, then incubated with the indicated inhibitor for 24 hr prior to pulse labeling for 1 hr with [^{35}S]methionine. Labeled cells were then chased in the presence of drug for 4 hr and immunoprecipitated with MAb 33.6 as above. In the presence of TUN there was only a slight reduction of the band representing unglycosylated GP-C and no concomitant appearance of a faster migrating band indicative of unglycosylated GP-2. We next examined the effect of inhibition of oligosaccharide trimming on cleavage using the inhibitors CSP, NM-DNJ, DMJ, and SSN. CSP and NM-DNJ inhibit glucosidases I and II and prevent trimming of terminal glucose residues from the core oligosaccharide. DMJ and SSN inhibit mannosidases I and II, respectively, and prevent trimming events that occur in the Golgi (for review see Elbein, 1987). In the presence of these trimming inhibitors, cleavage of GP-C was observed (Fig. 3, data for SSN not shown). Quantitative estimates of the proportion of the incorporated radioactivity in GP-C and GP-2 in pulse and chase samples were made on the basis of densitometer scans of exposed films. Table 1 summarizes the analysis of the experiment de-

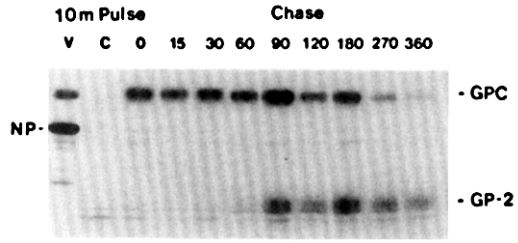

FIG. 2. Pulse–chase labeling of LCMV GP-C. BHK-21 cells were infected with LCMV-Arm (m.o.i. 0.1). After 24 hr cultures were pulse labeled for 10 min with ^{35}S-translabel (200 μg/ml). GP-C and GP-2 were immunoprecipitated using MAb 33.6 as described under Materials and Methods and analyzed on a 10% SDS–polyacrylamide gel. Cleavage of GP-C to yield GP-2 was first evident at 90 min in this experiment. V, pulse-labeled lysate incubated with guinea pig polyclonal antiserum to LCMV.

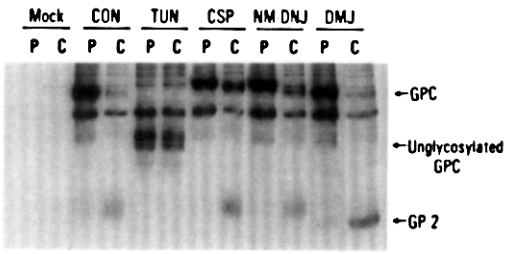

FIG. 3. GP-C is not cleaved when glycosylation is prevented by incubation in the presence of TUN. Infected BHK cells were pulsed and chased in the presence of various inhibitors of glycosylation as described under Materials and Methods, then immunoprecipitated with MAb 33.6 that binds to both glycosylated and unglycosylated GP-C and GP-2. Appearance of GP-2 was indicative of cleavage of the GP-C precursor polypeptide.

picted in Fig. 3. The scan confirmed the observation that cleavage failed to occur in the TUN-treated cultures. Cleavage occurred in the presence of CSP, NM-DMJ, and DMJ, although CSP appeared to exhibit a slight inhibitory effect on processing as indicated by greater retention of label in GP-C in the chase samples. Thus, while addition of N-linked oligosaccharides is necessary for cleavage, processing is tolerant of variations in the extent of trimming.

Cleavage of GP-C occurs in the Golgi or post-Golgi compartment

The data indicated that in the absence of glycosylation, cleavage failed to occur because the protein was not transported to the site of cleavage, or perhaps because the protein, although transported, was not folded in a conformation appropriate for proteolysis. To determine the intracellular location of GP-C cleavage we examined the temporal relationship between cleavage and trimming by pulse–chase under conditions of reduced temperature which have been shown to halt the transport of viral proteins in defined compartments of the cell (Matlin and Simons, 1983; Saraste and Kuismanen, 1984; Saraste *et al.*, 1986; Balch and Keller, 1986; Copeland *et al.*, 1988). At 15°, proteins exit the rough endoplasmic reticulum, but are halted at a pre-Golgi compartment. At 20° proteins reach the trans-Golgi, as determined by the transition to complex carbohydrate, but are not transported to the cell surface. Infected cells were pulsed for 1 hr at 37°, then chased at 15, 20, and 37° for 4 hr. Lysates were then immunoprecipitated with MAb 33.6 to determine whether cleavage had taken place. There was no evidence for cleavage at either 15 or 20° (Table 2). At both temperatures uncleaved GP-C remained EH sensitive, indicating that GP-C did not reach the medial Golgi. To define the location more precisely, we repeated the experiments chasing at 15, 26 and 37° in order to find the lowest temperature where GP-C acquired EH resistance, and this occurred at 26°. As evident in Fig. 4, there was a small amount of GP-2 present when cells were chased at 15°, and this was likely due to cleavage of GP-C during the 1-hr pulse period. The bands increased markedly at 26 and 37°. During the pulse, and at both 15 and 26°, GP-C was sensitive to EH, as indicated by a shift in migration relative to control lanes. However, at 37°, GP-C was EH resistant, indicating addition of terminal sugars. At all temperatures, GP-2 was resistant to EH. These results indicate that cleavage of GP-C to GP-1 and GP-2 must occur after trimming, that is, in the medial or trans-Golgi or later. Although cleavage had occurred at 26°, a portion of GP-C was still EH sensitive, indicating that transport past the medial Golgi compartment was incomplete. At all temperatures, some full-length GP-C acquired EH resistance, suggesting that cleavage was not essential for transport of GP-C to the medial Golgi.

Production of infectious virus is reduced in the presence of TUN

The results of the above experiments indicated that GP-C was cleaved in the medial or trans-Golgi or later, and that in the absence of N-linked glycosylation GP-C was not cleaved. It seemed likely that this was because the unglycosylated proteins were not transported to the site of cleavage, but we could not exclude the possibility that abnormal folding precluded recognition by

TABLE 1

QUANTITATIVE DISTRIBUTION OF LABEL BETWEEN GP-C AND GP-2 IN THE PRESENCE OF INHIBITORS OF GLYCOSYLATION

Inhibitor	Sample	Percentage of GP-C + GP-2 Label[a]	
		GP-C	GP-2
Control	Pulse[b]	98	2
	Chase[c]	40	60
TUN	Pulse	100[d]	0
	Chase	100	0
CSP	Pulse	100	0
	Chase	75	25
NM-DNJ	Pulse	100	0
	Chase	50	50
DMJ	Pulse	99	1
	Chase	25	75

[a] Distribution of label in GP-C and GP-2 was estimated by quantitative densitometry of two autoradiographic exposures of the gel shown in Fig. 3. Total area under GP-C + GP-2 bands was normalized to 100%. Percentage distribution of exposure under each peak is shown.

[b] Pulse label was for 1 hr with [^{35}S]methionine (60 μCi/ml) in Met-free medium.

[c] Chase was for 4 hr in the presence of a 10-fold excess of unlabeled methionine.

[d] Present as unglycosylated GP-C.

TABLE 2

QUANTITATIVE DISTRIBUTION OF LABEL BETWEEN GP-C AND GP-2 AT VARIOUS TEMPERATURES

Sample	Temperature	Endo H	Percentage of GP-C and GP-2 Label[a]	
			GP-C	GP-2
Pulse[b]	37°	—	100	0
Chase[c]	15°	—	94	6
Chase	20°	—	95	5
Chase	26°	—	83	16
Chase	37°	—	54	46
Pulse	37°	+	96	4
Chase	15°	+	94	6
Chase	20°	+	93	7
Chase	26°	+	78	22
Chase	37°	+	54	46

[a] Distribution of label in GP-C and GP-2 was estimated by quantitative densitometry of two autoradiographic exposures of the gel shown in Fig. 4. Total area under GP-C + GP-2 bands was normalized to 100%. Percentage distribution of exposure under each peak is shown. Data were pooled from two experiments.

[b] Pulse labeling was for 1 hr with 60 μCi/ml [^{35}S]methionine in Met-free medium.

[c] Chase period was 4 hr at the indicated temperature with 10-fold excess of [^{35}S]methionine.

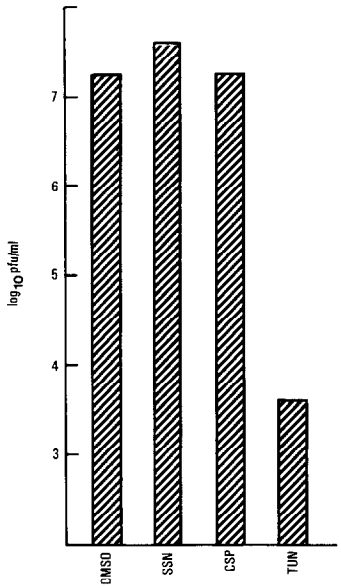

FIG. 5. Replication of LCMV is reduced in the presence of TUN. BHK cells were infected with LCMV, then incubated for the duration of infection with TUN, CSP, SSN, or DMSO (control). Media were changed once after 24 hr and supernates were collected at 48 hr and assayed for infectious virus.

the appropriate protease. As another measure of the effect of glycosylation inhibitors on transport, we assayed the production of infectious virus grown in the presence of TUN, SSN, or CSP. Supernates from infected BHK-21 cells were titered 48 hr after infection. Medium containing the drugs was changed once at 24 hr, so the data represent virus released in the last 24 hr of infection. Only TUN significantly reduced the amount of virus produced (Fig. 5).

TUN prevents transport of LCMV glycoproteins

To determine whether the reduction in infectivity was due to the production of noninfectious particles, or a failure to bud, we attempted to purify virus particles from infected cultures grown in TUN. We were unable to band virus in renograffin gradients prepared from supernatants of TUN-treated cultures. We also examined virus budding by electron microscopy and found that budding was inhibited in the presence of TUN, suggesting that the unglycosylated viral proteins were not reaching the surface of the cell (Fig. 6). This result was confirmed by immunofluorescence staining of viral proteins on the surfaces of infected BHK cells. We used a MAb against GP-1 which stained control virus-infected cells and also reacted with unglycosylated glycoproteins in permeabilized cells treated with TUN. This MAb failed to detect any viral protein on the surfaces of TUN-treated cells (Fig. 7). Viral glycoproteins were evident at the surfaces of infected cells treated with the trimming inhibitors CSP and SSN in agreement with infectivity data. Thus we concluded from these studies that transport of the unglycosylated proteins to the cell surface was blocked.

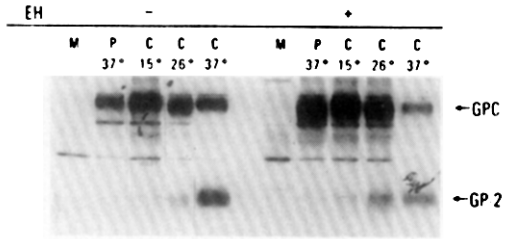

FIG. 4. Cleavage of GP-C occurs in the Golgi or post-Golgi compartment. Infected BHK cells were pulsed for 1 hr at 37°, then chased for 4 hr at the specified temperatures, before immunoprecipitation with MAb binding GP-C and GP-2. Half of each sample was treated with EH after immunoprecipitation as described under Materials and Methods. The other half was incubated in EH buffer without enzyme for the same time before SDS–PAGE.

DISCUSSION

Results presented in this paper address basic aspects of glycosylation and proteolytic cleavage of the LCMV glycoprotein precursor, GP-C, and how these processes affect transport of viral proteins within the infected cell. We have shown that the mature structural glycoproteins, GP-1 and GP-2, of the Arm-4 strain of LCMV bear five and two N-linked complex carbohy-

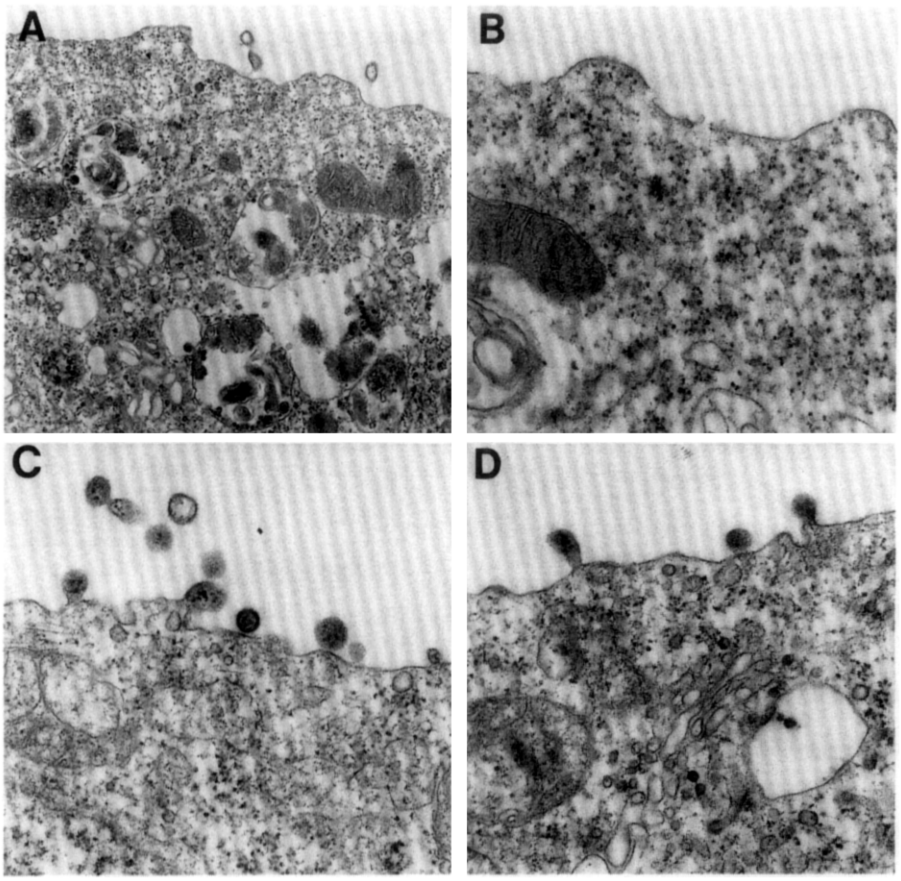

FIG. 6. Budding of LCMV is inhibited in the presence of TUN. Monolayers of BHK cells were infected with LCMV and grown for 24 hr in the presence of TUN or DMSO (control) before fixation and examination by EM. No virions were evident budding from tunicamycin-treated cells (panels A and B), while numerous budding virions were seen in control cultures (panels C and D). Magnified 30,000×.

drates, respectively. In previous studies we have shown that radiolabeled mannose and glucosamine were preferentially incorporated into GP-C, while the GP-1 and GP-2 cleavage products were labeled with glucosamine, galactose, and fucose but contained little residual mannose, suggesting that GP-C was a high mannose precursor (Buchmeier and Oldstone, 1979; Buchmeier and Parekh, 1987). When the role of glycosylation in post-translational events was examined, we found that appropriate folding of the glycoprotein, indicated using MAb which bind to conformational epitopes, failed to occur without glycosylation (Wright *et al.*, 1989). In the present study, we have demonstrated that cleavage and transport of GP-C also failed to occur when glycosylation was blocked by TUN.

To determine how prevention of glycosylation affected cleavage, we needed to establish the temporal sequence of oligosaccharide trimming and proteolytic cleavage of GP-C. As noted, GP-1 and GP-2 bear complex carbohydrates, but GP-C contains predominantly mannose-rich core sugars. A quantitatively minor fraction of GP-C does however contain fucose and galactose, indicative of mature N-linked oligosaccharides. Transition from high mannose carbohydrate to complex carbohydrate, measured by loss of sensitivity to EH and acquisition of terminal sugars, occurs in the medial Golgi (Balch and Keller, 1986). The results of our pulse–chase experiments suggest that cleavage occurs approximately 75–90 min after synthesis and after transport to the medial Golgi. These conclusions were strengthened in temperature block pulse–chase experiments. When infected cells were incubated at temperatures known to halt protein transport at defined compartments within the cell, we never observed EH sensitivity in the cleaved GP-2 molecule, indicating that transition to complex carbohydrates occurred before cleavage. Moreover, by temperature block experiments we localized the site of cleavage to the Golgi apparatus. In cells pulsed and then chased at 20°, no cleavage of GP-C was observed. When the chase was performed at 26°, a temperature which halts transport in the medial/trans-Golgi, we observed cleavage similar to that seen in cells chased at 37°. The reported sites of cleavage of precursor glycoproteins of other enveloped RNA viruses vary. In this respect, LCMV resembles most closely the paramyxoviruses, where the

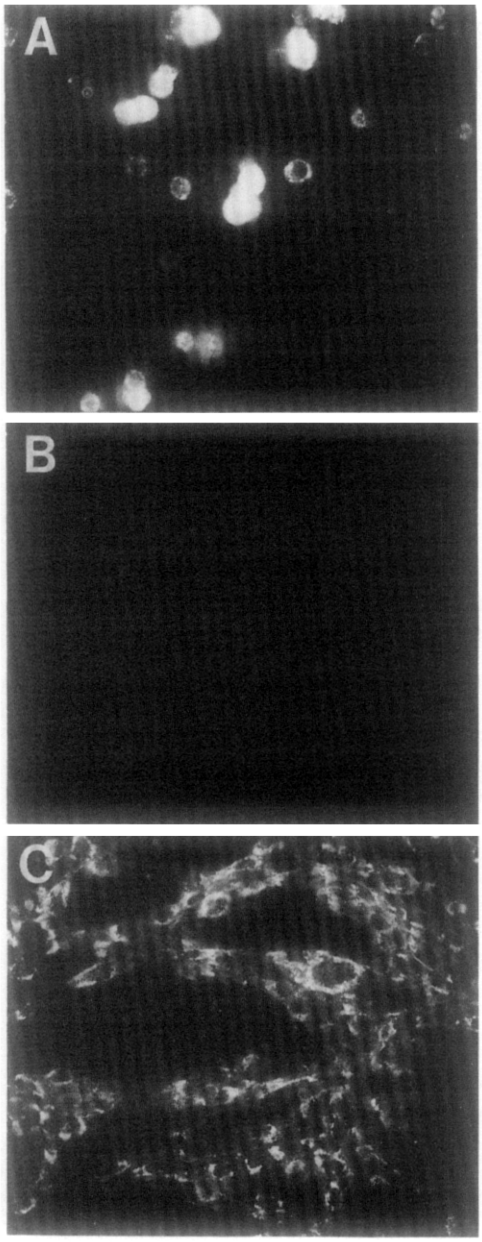

FIG. 7. LCMV glycoproteins are not transported to the cell surface when glycosylation is blocked. BHK cells were infected, then incubated in the presence of TUN for the duration of infection. Living cells were assayed for surface expression of GP-1 using MAb 67.2. Control cells expressed GP-1 (A); TUN-treated cells did not (B). Infected BHK monolayers also incubated in TUN were permeabilized with acetone, then stained to visualize intracellular GP-C (C).

F proteins are cleaved in the trans-Golgi or the immediate trans-Golgi compartment (Sato *et al.*, 1988; Yamada *et al.*, 1988; Morrison *et al.*, 1985; Nagai *et al.*, 1976). However, for measles virus, unlike LCMV, cleavage apparently continues at the cell surface (Yamada *et al.*, 1988).

There are two likely possibilities to explain how prevention of glycosylation interferes with cleavage of GP-C. Either the unglycosylated GP-C polypeptide chain aggregates in the RER and does not reach the site of cleavage or cleavage is dependent on a conformation which is not expressed in the unglycosylated molecule. Our results indicate that under conditions of TUN block, unglycosylated, uncleaved GP-C does not reach the cell surface. Prevention of GP-C transport is reflected by both lack of viral glycoprotein staining at the cell surface and a failure to produce virions by budding at the plasma membrane. Similar findings have been reported for feline and murine leukemia virus (Polinoff *et al.*, 1982; Pinter *et al.*, 1984), Mason-Pfizer monkey virus (Chatterjee *et al.*, 1981), Junin virus (Padula and de Martinez Segovia, 1984), bovine herpes virus (van Grunen-Littel-van den Hurk and Babiuk, 1985), and vesicular stomatitis virus (VSV) (Leavitt *et al.*, 1977). For the latter, it was suggested that failure of transport was due to protein aggregation in the RER (Gibson *et al.*, 1978, 1979). Unglycosylated envelope proteins of measles virus (Sato *et al.*, 1988) and Sendai virus (Mottet *et al.*, 1986) also may remain in the RER. We observed accumulation of unglycosylated GP-C within cells in a form which was not recognized by antibodies to conformational epitopes on GP-C and GP-1 (Wright *et al.*, 1989). We attempted to demonstrate transport of unglycosylated GP-C in cells held at 30°, conditions under which unglycosylated VSV G protein is transported, but neither GP-C transport nor virion production was observed. On the basis of the accumulated evidence, it is likely that unglycosylated GP-C aggregates in the RER or pre-Golgi compartment and thus never reaches the site of proteolysis.

To determine whether transport and cleavage were dependent upon oligosaccharide chain structure, we assessed cleavage in the presence of a variety of inhibitors of oligosaccharide trimming. For some viruses, addition of the core oligosaccharides alone is not adequate for transport, and additional trimming of sugar moieties is required. Specifically, prevention of trimming of the outermost glucose moieties on the core oligosaccharide (Glc_3 Man 5–9 $GlcNAc_2$) with either CSP or DNJ inhibits transport of the envelope glycoproteins of murine retroviruses (Pinter *et al.*, 1984), murine hepatitis virus (Repp *et al.*, 1985), and VSV (Schlesinger *et al.*, 1984), but has no effect on the transport of HA of influenza (Romero *et al.*, 1983; Burke *et al.*, 1984; Elbein *et al.*, 1984) or of the envelope glycoprotein of RSV (Bosch and Schwarz, 1984). The same drugs reduced infectivity of Sindbis not by preventing transport but by preventing cleavage of the glycoprotein precursor (Schlesinger *et al.*, 1985; McDowell *et al.*, 1987). For LCMV, we found that addition of the core oligosaccharide alone without any further processing was sufficient to allow transport, cleavage, and production of infectious particles.

Although we can correlate the absence of cleavage with reduced infectivity in these experiments, we do not know whether cleavage is necessary for transport to the plasma membrane. We do occasionally see small amounts of a glycoprotein comigrating with GP-C associated with mature virions, and others have reported a molecule the same size as GP-C on the surfaces of infected cells (van der Zeijst *et al.,* 1983), suggesting that cleavage may not be an absolute requirement for viral maturation. Relatively few experiments have been conducted in viral systems where the need for cleavage has been examined in the absence of drugs such as TUN or monensin that also effect transport. When cleavage of a glycoprotein precursor activates fusion activity of the subunits, as is the case for many enveloped RNA viruses, absence of cleavage would reduce infectivity. This is true for Sendai virus (Scheid and Choppin, 1974, 1976), mammalian influenza viruses grown in avian cells (Klenk *et al.,* 1975; Lazarowitz and Choppin, 1975; Kawaoka *et al.,* 1984), and HIV-1 (McCune *et al.,* 1988). Heretofore, the glycoproteins of LCMV have not been associated with membrane fusion activity. Efforts to further define the biosynthetic pathways of the LCMV glycoproteins and their interaction in the virion structure are under way.

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