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Processing and Intracellular Transport of Rubella Virus Structural Proteins in COS Cells

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Received February 13, 1990; accepted April 30, 1990

Plasmids encoding rubella virus (RV) structural proteins C-E2-E1, E2-E1, E2, and E1 have been constructed in the eukaryotic expression vector pCMV5. The processing and intracellular transport of these proteins have been examined by transient expression of the cDNAs in COS cells. Compared to alphaviruses, processing of RV glycoprotein moieties occurred relatively slowly and the transport of glycoproteins E2 and E1 to the plasma membrane was inefficient. Indirect immunofluorescence revealed that the majority of RV antigen in transfected and infected COS cells was localized to the Golgi region, including the capsid protein. Accumulation of capsid protein in the juxtanuclear region was determined to be RV glycoprotein dependent. Unlike alphaviruses, RV E1 did not require E2 for targeting to the Golgi where it was retained. E2 was however necessary for cell surface expression of E1. This study revealed that the processing and transport of RV structural proteins is quite different from alphaviruses and that the accumulation of antigens in the Golgi region may be significant in light of previous reports which suggest that RV buds from the internal membranes in some cell types.

INTRODUCTION

Rubella virus (RV) is an enveloped, positive-stranded RNA virus in the family Togaviridae. It is the sole member of the genus Rubivirus and bears striking similarities to the prototype alphavirus Semliki Forest virus (SFV) and Sindbis virus (SV) in terms of genome organization and structural protein expression strategy. Unlike alphaviruses however, RV infection in cultured cells is characterized by relatively long latency periods, slow replication, and limited cytopathology. As well as being able to establish persistent infections in all cell lines tested (Cunningham and Fraser, 1985; Norval, 1979; Stanwick and Hallum, 1974), RV is known to persist in vivo in humans after congenital and postnatal infections (Cooper and Buimovici-Kein, 1985). Moreover initiation of a persistent infection in cultured cells by RV is not dependent upon the presence of defective-interfering particles (Frey and Hemphill, 1988).

The structural proteins of RV are initially translated as a precursor polyprotein (p110) in the order NH2-C-E2-E1-COOH from a 24 S subgenomic mRNA (Oker-Blom, 1984). After synthesis, the polyprotein is cleaved to yield two membrane glycoproteins, E1 (58 kDa) and E2 (42–47 kDa), and a hydrophilic 33-kDa capsid (C) protein (Oker-Blom, 1984; Oker-Blom et al., 1983, 1984). E1 and E2 comprise the spike complexes located on the virion exterior (Vaheri and Hovi, 1972) and are thought to exist as heterodimers (Waxham and Wolinsky, 1983). We have recently demonstrated that insertion of glycoproteins E1 and E2 into the endoplasmic reticulum (ER) is mediated by two independently functioning signal peptides and that two of the p110 cleavages are likely to be catalyzed by signal peptidase (Hobman et al., 1988; Hobman and Gillam, 1989). In alphaviruses, formation of an E1–E2 heterodimer is required for transport of E1 out of the ER lumen to the Golgi and plasma membrane (Kondor-Koch et al., 1982), where budding occurs by binding of nucleocapsids to the membrane-anchored glycoprotein spikes (for review see Garoff et al., 1982). In contrast, electron microscopy studies revealed that RV budding occurs preferentially from internal membranes in BHK cells and at the plasma membrane in Vero cells (Bardeletti et al., 1979; von Bonsdorff and Vaheri, 1969).

We have been studying the processing and intracellular transport of RV structural proteins in mammalian cells and are particularly interested in defining the requirements for transport of the glycoproteins out of the ER and the roles of the structural proteins in the virus assembly process. Previously it has been shown that RV E2 glycoprotein, like its alphavirus counterpart, can be transported to the plasma membrane in the absence of E1 (Hobman and Gillam, 1989; Kondor-Koch et al., 1982). In the present study we have addressed the post-ER fates of the RV glycoproteins in COS cells and also discuss the role of capsid protein in the processing of E1 and E2.
PROCESSING AND INTRACELLULAR TRANSPORT OF RV STRUCTURAL PROTEINS

FIG. 1. Schematic of RV cDNA constructs. Respective portions of the C, E2, and E1 proteins are indicated above the constructs. E and H denote the EcoRI and HindIII restriction sites flanking the 5' and 3' ends of the constructs, respectively. The predicted signal peptides and transmembrane anchors of the glycoproteins are shown as solid and hatched boxes, respectively. N-linked glycosylation sites are indicated by (Y). Translation start sites are marked with arrowheads. Seven amino acids separate the transmembrane region of E2 from the E1 signal peptide. The bar represents approximately 500 nucleotides.

RV cDNAs encoding C-E2-E1, E2-E1, E2, and E1 have been subcloned into a eukaryotic expression vector containing the human cytomegalovirus immediate early gene promoter, which allowed high level transient expression of RV structural proteins in COS cells. Expression of RV structural proteins in transfected cells suggests that unlike SFV and SV E1 glycoproteins, RV E1 is transported to the Golgi complex in the absence of E2, although E2 seems to be required for cell surface expression of E1. The presence of E1 increased the rate of ER to Golgi transport of E2 as monitored by acquisition of endoglycosidase H (endo H)-resistant glycan. Much to our surprise, the rate of E2 processing was slower in the presence of capsid protein, which was found to be concentrated in a juxtanuclear region in transfected COS cells, as well as in RV-infected cells. In pulse-chase experiments with transfected COS cells, we were only able to chase a small fraction of the RV antigen to the cell surface, indicating that the transport of RV glycoproteins was restricted in these cells. Indirect immunofluorescence revealed a high concentration of RV antigen in the Golgi region of transfected and infected cells. Our results suggest that maturation of RV structural antigens is quite different from that of alphaviruses and is consistent with the possibility that assembly of RV particles may begin on internal membranes.

MATERIALS AND METHODS

Plasmid construction

RV cDNAs encoding various combinations of the structural proteins are shown in Fig. 1. All cDNAs were subcloned between the EcoRI and HindIII sites of the expression vector pCMV5 (Andersson et al., 1989) downstream from the human cytomegalovirus immediate early gene promoter. The construction of the RV cDNAs has been previously described (Clarke et al., 1987; Hobman et al., 1988; Hobman and Gillam, 1989), and some of the cDNAs have been renamed for the sake of brevity and clarity. The recombinant plasmids are named as follows: pCMV5-24S encodes all three structural proteins of RV in the order NH2-C-E2-E1-COOH downstream from the promoter in pCMV5 (insert was previously called RV1 (Clarke et al., 1988)); pCMV5-E1 (RV insert was previously called 3'E2E1 (Hobman et al., 1988)); pCMV5 E2E1 (Hobman and Gillam, 1989); pCMV5-E2 (Hobman and Gillam, 1989).

Note: a translation stop signal was introduced into pCMV5E2 by ligating an Xba1 linker encoding stop codons in all three reading frames (pCTAGTCTAGAC-TAG) immediately downstream from the E2 coding region.

Transfection, metabolic labeling, immunoprecipitation, and endoglycosidase digestion

COS cells were transfected with recombinant plasmids and labeled with [35S]methionine and RV-specific proteins were immunoprecipitated as previously described (Hobman and Gillam, 1989). To label cellular proteins with [3H]palmitic acid, transfected COS cells were incubated for 12 hr with 500 µCi of [9,10-3H]palmitic acid approximately 24 hr after transfection. For immunoprecipitation of cell surface antigens, transfected COS cells were labeled with 100 µCi methionine for 30 min, washed with cold phosphate-buffered saline, and treated with 1% normal goat serum at 0° for 10 min to block nonspecific binding of antibodies. Cells were then incubated with human anti-RV serum at 0° for 2 hr and lysed and the immune complexes precipitated with Protein A-Sepharose. The supernatants were incubated with anti-RV serum and Protein A-Sepharose to recover intracellular RV antigen. The immune complexes were washed and the RV antigen was eluted into 100 mM sodium citrate (pH 5.5), 0.15% SDS, 1 mM phenylmethylsulfonyl fluoride at 100° for 5 min and analyzed by SDS–PAGE (Laemmli, 1970). Some immunoprecipitates were digested with endo H as described (Hobman and Gillam, 1989).

Western blotting

COS cell lysates were subjected to SDS–PAGE (Laemmli, 1970) and transferred to nitrocellulose filters. The filters were washed briefly in TBS (0.15 M NaCl; 0.02 M Tris–Cl (pH 7.5)) containing 0.3% Tween
Fig. 2. Pulse-chase analysis of RV structural proteins radioimmunoprecipitated from transfected COS cells using human anti-RV serum. COS cells were pulsed for 30 min with 100 μCi [35S]methionine and chased with Dulbecco’s modified Eagle’s medium containing 2 mM methionine for the indicated time periods prior to lysis. Radiolabeled RV structural proteins and 14C-labeled protein markers (kDa) are included on the gels. Endo H-treated samples are indicated. (A) pCMV5-24S; (B) pCMV5-E2E1; (C) pCMV5-E2; (D) pCMV5-E1. The endo H-resistant E1 species is marked with an arrowhead in the appropriate lanes, as well as the 42-kDa (G) and 39-kDa (ER) forms of E2. Heterogeneous processing of E2 is indicated (*).

20 and blocked for nonspecific binding in TBS containing 4% powdered skimmed milk. Membranes were then incubated with human anti-RV serum (1:200) in this same solution for 2 hr, washed with TBS/0.3% Tween 20, and treated with rabbit anti-human IgG conjugated to horseradish peroxidase (Dako) for 2 hr. Blots were washed as above and developed with 4-chloro-1-naphthol (Bio-Rad). All incubations were performed at room temperature unless otherwise specified.

Immunofluorescence

COS cells were seeded onto poly-L-lysine-coated coverslips, transfected with recombinant plasmids or infected with M33 RV (m.o.i. = 0.1), and processed for the presence of intracellular and cell surface RV antigen 40 hr later as described (Hobman and Gillam, 1989). Where indicated, some cells were first treated with mouse monoclonal antibody to E1, E2, or capsid proteins followed by incubation with tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse serum. To visualize Golgi structures (Virtanen et al., 1980), cells were first incubated with TRITC-conjugated wheat germ agglutinin (WGA) (Sigma), at a concentration of 12 μg/ml before treatment with human anti-RV serum. Incubations were done in reverse order as well, and binding of antibody to the lectins was not observed. Monoclonal antibodies to E2 were kindly provided by Dr. J. Wolinsky (Houston, TX). The anti-C monoclonal was a gift from Dr. J. Safford (Abbott Laboratories). Anti-E1 monoclonals used in this study were also obtained from Dr. Safford or generated in this lab. Cells were examined and photographed with a Zeiss fluorescent microscope using the appropriate filters.

RESULTS

Pulse-chase analysis

RV cDNAs encoding C-E2-E1 (24 S), E2-E1 (E2E1), E2, and E1 alone were employed to determine if lack
FIG. 3. Immunoblot analysis of transfected COS cell lysates. Lysates from COS cells transfected with pCMV5 recombinant plasmids were subjected to SDS-PAGE, transferred to nitrocellulose, and probed with human anti-RV serum followed by rabbit anti-human IgG conjugated to horseradish peroxidase. 42-kDa (G) and 39-kDa (ER) forms of E2 are indicated. Disrupted RV virions were included to indicate the relative positions of the mature structural proteins.

of expression of one or two of the RV structural proteins affected the rate of E2 and E1 glycan processing. Plasmids were transfected into COS cells and labeled RV proteins were immunoprecipitated and treated with endo H to monitor processing of N-linked sugars. Appearance of endo H-resistant sugar moieties on the glycoproteins was taken as evidence that the RV glycoproteins had entered the Golgi complex (Kornfeld and Kornfeld, 1985). Figure 2A shows the results of a pulse-chase experiment using pCMV5-24S-transfected COS cells. After a 30 min pulse with $[^{35}S]$methionine, RV-specific proteins the size of E1 and C were detected, as well as an E2 band of 39,000 D migrating just above the capsid band. Present in much smaller amounts are proteins of approximately 75 kDa, an 85-kDa doublet, and >100 kDa. The 75-kDa protein was subsequently determined to be the resident ER protein BiP by using a monoclonal antibody to BiP (Bole et al., 1986) to immunoprecipitate lysates from transfected COS cells (data not shown). Digestion with endo H reduced the size of E1 and E2 to 51 and 32 kDa, respectively, indicating the presence of high mannose sugars on these proteins. Chase with excess unlabeled methionine resulted in conversion of E2 to a 42-kDa glycoprotein, while the size of E1 and C remained unchanged. The 42-kDa (G) form of E2 was assumed to be a population of E2 that had entered the medial Golgi stacks and been processed by Golgi-specific glycan modifying enzymes, while the 39-kDa (ER) form was thought to be ER or cis-Golgi-associated (Hobman and Gillam, 1989). The endo H digestion product of the 42-kDa E2 glycoprotein was a 37-kDa glycoprotein migrating just above capsid (Fig. 2A). After a 60-min chase period, a fraction of E1 acquired complex sugar moieties evidenced by the appearance of a 53-kDa endo H digestion product (arrowhead in Fig. 2A) just above the 51-kDa E1 band. The mobilities of BiP and >100-kDa proteins were unchanged by endo H, while the 85-kDa doublet proteins obviously contained high mannose sugars, and may correspond to E2–E1 aggregates.

Transport of E1 and E2 into the Golgi stacks occurred more rapidly in pCMVS-E2E1-transfected cells as indicated by the rate of 39- to 42-kDa E2 conversion and appearance of endo H-resistant E1 (arrowhead in Fig. 2B). After a 30-min chase period, almost 50% of E2 was in the 42-kDa Golgi-processed (G) form, and endo H-resistant E1 was clearly visible after this time period (Fig. 2B). In addition, heterogeneous processing of E2 sugars evidenced by smearing in the range 42–46 kDa was observed after increasing lengths of chase period (Figs. 2A and 2B). The presence of 75- and 85-kDa proteins in pCMV5-E2E1-transfected cells indicated that these polypeptides were not RV capsid dependent.

Following a 30-min pulse label, RV-specific proteins from pCMV5-E2-transfected COS cells included 38- and 43-kDa glycoproteins and BiP (Fig. 2C). The most likely reason COS cells containing pCMV5-E2 synthesize a 38-kDa E2 versus a 39-kDa E2 (found in cells transfected with pCMV5-24S or -E2E1) is that there is a stop codon linker engineered into the C-terminal coding region of E2 in pCMV5-E2. Endo H digestion reduced the molecular weight of the 43,000 and 38,000 glycoproteins to 36,000 and 31,000 respectively. Un-
glycosylated E2 is known to be 30–31 kDa in size (Oker-Blom et al., 1983; Clarke et al., 1988; Hobman and Gillam, 1989). The 43-kDa E2 protein was no longer detectable after a 30-min chase with unlabeled methionine, while the 38-kDa protein underwent heterogeneous processing of the N-linked glycans, presumably by Golgi-specific enzymes. The nature of the short-lived 43-kDa protein is uncertain, but may represent a population of E2 that is rapidly transported through the Golgi to the cell surface followed by degradation in the lysosomes (Rizzolo, 1989).

Cells transfected with pCMV5-E1 produced a glycoprotein similar in size to RV virion E1 which could be chased into the Golgi compartments as evidenced by the acquisition of endo H-resistant sugars (arrowhead in Fig. 2D). As in pCMV5-E2-transfected cells, BiP and the unidentified >100-kDa protein were found to coprecipitate with RV antigen from cells expressing pCMV5-E1 (Figs. 2C and 2D). BiP however could not be immunoprecipitated from pCMV5-transfected cells using anti-RV serum alone, indicating that it was not nonspecific binding to the human anti-RV sera (not shown).

Western blot analysis
To examine the steady-state levels of 42-kDa (G) E2 and 39-kDa (ER) E2, lysates from transfected COS cells were separated by SDS-PAGE, transferred to nitrocellulose paper, and probed with human anti-RV serum. E1 from transfected COS cells was of a uniform size and comigrated with the viral counterpart (Fig. 3). The relative amounts of the ER and G forms of E2 in transfected COS cells corresponded well with the pulse-chase data in Figs. 2A–2C. In pCMV5-E2-transfected cells, most of the E2 was in the ER form, whereas in pCMV5-24S and pCMV5-E2E1 expressing cells, the majority of E2 was in the 42-kDa (G) form. These data suggested that the presence of E1 increased the rate of transport of E2 from the ER to the Golgi. Almost no 39-kDa E2 was detected in pCMV5-E2E1-transfected cells suggesting that transport of E2 out of the ER occurs even more rapidly in the absence of capsid protein.

**Glycoproteins F1 and F2 are acylated**

In order to determine which RV proteins became acylated, transfected COS cells were metabolically labeled with [3H]palmitate and the RV proteins isolated by radioimmunoprecipitation and SDS-PAGE. Only proteins corresponding to E1 and E2 were observed to incorporate palmitate (Fig. 4). Three acylated forms of E2 could be detected (39, 42, and 45 kDa) and were presumed to result from differences in glycosylation. Relative levels of 42- and 45-kDa E2 were highest in cells transfected with pCMV5-E2E1 and pCMV5-24S, while cells expressing only E2 contained predominantly 39-kDa E2 (Fig. 4). These data are also consistent with pulse-chase experiments which indicate that processing of E2 occurs more rapidly in the presence of E1 and absence of capsid. E1 seemed to incorporate more palmitate on a molar basis, and was found only as a single 57-kDa species (Fig. 4).

Cell surface immunoprecipitations
To determine which of the RV structural proteins are transported to the plasma membrane, transfected COS cells were processed for cell surface RV antigen as described under Materials and Methods. Figure 5 shows that in live unpermeablized COS cells expressing PC-MV5-24S, only E1 and E2 become accessible to antibody in a time-dependent manner. No capsid protein was detected at the plasma membrane even after a 180-min chase period indicating that this technique allowed distinction between cell surface and intracellular
antigens. The predominant form of E2 at the plasma membrane was the 42-kDa form which is expected since this form of E2 is believed to contain Golgi-processed glycans (Fig. 5). Compared to alphaviral glycoproteins, movement of the RV antigen to the cell surface occurred relatively slowly with the vast majority of E1 and E2 (>90%) still found in the intracellular fraction after the 3-h chase period (Fig. 5). In BHK-21 cells, newly synthesized Sindbis virus glycoproteins are incorporated into virions at the plasma membrane as little as 30 min after synthesis (Erwin and Brown, 1980).

**Immunofluorescence**

Indirect immunofluorescence was used to determine the subcellular location of RV structural proteins in transfected and infected cells. Cells expressing pCMV5-24S and pCMV5-E2E1 displayed strong fluorescence in a juxtanuclear region likely corresponding to the Golgi apparatus as indicated by costaining of this region with WGA (Figs. 6A–6D) (Virtanen et al., 1980). No staining of the plasma membrane in these permeabilized cells was detected; however, RV antigen could be detected on the surface of nonpermeabilized cells using human anti-RV serum (Figs. 6E and 6F). The antigen on the cell surface was found to comprise both E1 and E2 when monoclonal antibodies were used (not shown). No capsid protein was detected on the surface of cells expressing pCMV5-24S (Fig. 6G). COS cells were infected with M33 RV, and also showed strong staining in the Golgi region and limited amounts of RV antigen on the cell surface (Figs. 7A–7C).

RV antigen in cells expressing pCMV5-E2 was found distributed intracellularly throughout FR and Golgi-like regions (Figs. 8A and 8B). As previously reported (Hobman and Gillam, 1989), RV E2 was transported to the cell surface in the absence of E1 (Figs. 8C and 8D). In contrast, E1 from pCMV5-E1-transfected cells localized exclusively to a Golgi-like region and was not found on the cell surface in significant quantities (Figs. 9A–9D). Cotransfection of COS cells with pCMV5-E1 and pCMV5-E2 resulted in the transport of E1 to the cell surface (Fig. 9F), indicating that F2 is required for delivery to the plasma membrane.

Last, monoclonal antibodies to individual RV structural proteins were used to examine the intracellular distribution of RV antigens in transfected and infected COS cells. E1 localized primarily to the juxtanuclear region of M33-infected and pCMV5-24S-transfected cells (Figs. 10A and 10B). In addition to staining the Golgi region, a significant amount of E2 was found in the ER as evidenced by staining of the nuclear envelope and reticular formations by anti-E2 antibodies (Figs. 10C and 10D). The distribution of E1 and E2 in pCMV5-24S-transfected cells was similar to those in M33-infected cells and cells expressing pCMV5-24S (not shown). Infected cells also contained E2 in vesicular structures which seemed to radiate from the juxtanuclear region (Fig. 10D). Surprisingly, capsid protein was concentrated in the Golgi area of M33-infected cells and cells expressing pCMV5-24S (Figs. 10E and 10F). Two control experiments were employed to ensure that the Golgi-specific localization of capsid protein was not artifactual. First, capsid-specific staining was not observed in cells transfected with pCMV5-E2E1 (Figs. 10G and 10H). Second, to determine if the glycoproteins were necessary for the juxtanuclear in-
calization of capsid protein, COS cells were transfected with pSVL-RV2, which encodes the entire capsid gene and the amino-terminal one-third of E2 (Clarke et al., 1988). We found that pSVL-RV2-transfected cells exhibited a diffuse cytoplasmic pattern when stained with anti-capsid antibodies (Fig. 101) and suggest that the concentration of capsid in the Golgi area may be mediated by interaction with the spike glycoproteins which are embedded in the Golgi membranes.

**DISCUSSION**

In the present study we have examined the maturation and intracellular transport of RV structural proteins in COS cells using RV cDNAs which allowed the coordinate and individual expression of C, E2, and E1 proteins. Pulse-chase experiments showed that E2 initially was detected as a 39-kDa glycoprotein containing only endo H-sensitive sugars (Hobman and Gillam, 1989). This high mannose containing glycoprotein was converted into a 42-kDa species by glycan processing enzymes in the Golgi compartments. Although this process occurred in the absence of E1 (Hobman and Gillam, 1989), the rate of ER to Golgi transport of E2 was markedly increased in the presence of E1. In addition, pulse-chase experiments showed that processing of E2 occurred even more rapidly in the absence of capsid protein. These data were also supported by examining stoichiometric levels of RV antigen in transfected cells. Oker-Blom et al. (1983) report that E2 was found exclusively as a 11 kDa protein in infected cells, whereas in virions it exists as a heterogeneous species 42–47 kDa in size. We believe the 42-kDa E2 (G) form of E2 in transfected cells corresponds to the 41-kDa E2 found in infected cells. Expression of a RV E2E1 construct in Sf9 cells by Oker-Blom et al. (1989) revealed also that E2 was synthesized as a 39-kDa high mannose glycoprotein. In contrast to the present study, their E2 could not be demonstrated to acquire endo H-resistant sugars after a 3-hr chase period even though Sf9 cells are able to synthesize endo H-resistant sugars (Jarvis and Summers, 1988). From our results it would appear that processing of RV E2 glycans involves at least two stable intermediates, a 39-kDa high mannose precursor, and a 42-kDa form bearing some complex sugars. Unlike alphaviral E2 glycoproteins, RV E2 is not derived from a larger intermediate glycosylated precursor (Garoff et al., 1982). Previously it was thought that the heterogeneous processing of E2 glycans occurred late in virus maturation (Oker-Blom et al., 1983), although it is clear from our results that virion formation is not necessary for this process to take place. The biological significance of this phenomenon remains to be determined.

We were able to demonstrate that RV glycoproteins produced in transfected cells became acylated with palmitic acid like their virion counterparts (Waxham and Wolinsky, 1985). In addition to a 45-kDa acylated E2, both the 39 and 12 kDa species of E2 incorporated palmitate. Furthermore, the proportions of the high and low molecular weight forms of E2 (low M, = 39 kDa; high M, = 42 and 45 kDa) in the transfected cells correlated well with the E2 processing kinetics from pulse-chase experiments. Cells expressing pCMV5-E2 contained predominantly 39-kDa E2, whereas pCMV5-24S
and pCMV5-E2E1 contained proportionally more of the 42- and 45-kDa species. Covalent attachment of palmitic acid to proteins is a post-translational modification thought to occur immediately prior to exit from the ER (Berger and Schmidt, 1985; Rose and Doms, 1988). However, more recent evidence suggests that this process takes place in a post-ER-pre-Golgi compartment (Bonatti et al., 1989). In light of the latter study, the fact that acylation of RV E1 occurs in the absence of E2 is evidence that, unlike alphaviral E1 glycoproteins (Kondor-Koch et al., 1982). RV E1 can exit the ER without binding to E2.

Indirect immunofluorescence revealed that E1 glycoprotein localized exclusively to the Golgi region in pCMV5-E1-transfected cells. A small fraction of E1 was seen to acquire endo H-resistant sugars which is consistent with the idea that RV E1 does not require E2 for ER to Golgi transport. Using oligonucleotide-di-
rected mutagenesis we have determined that E1 contains three N-linked sugars, and in COS cells only one of the glycans seems to be processed to the complex type (unpublished observations). No E1 was detected on the plasma membrane in significant quantities except when coexpressed with E2. In coronavirus-infected cells, the E1 glycoprotein is targeted to and retained in the Golgi, and is believed to determine the site of virus budding (Rottier and Rose, 1987). Since budding of RV from the Golgi region has been reported in some cells (Bardeletti et al., 1979; von Bonsdorff and Vaheri, 1969), targeting of RV E1 to the Golgi may be important for this process. Although E1 seemed to require binding of E2 for transport to the cell surface, this binding did not seem to involve the formation of intermolecular disulfide bonds (data not shown) as previously reported (Waxham and Wolinsky, 1983), and could occur when E1 and E2 were expressed from different mRNAs. It follows that a possible function of E2 may be to promote movement of the glycoprotein spikes and/or virions from the Golgi to the cell surface.

In the absence of the glycoproteins E2 and E1, capsid was found in the cytoplasm and did not appear to be associated with any particular membrane formations. Cells infected with M33 and cells transfected with pCMV5-24S were found to have capsid concentrated in the juxtanuclear region. We interpreted this to be due to interaction of capsid protein with the cytoplasmic domains of the spike glycoproteins embedded in the Golgi and ER membranes. Kuismanen et al. (1982) reported a similar distribution of capsid protein in cells infected with Uukuniemi virus which is known to bud from Golgi membranes. In contrast, colocalization of capsid protein with the Golgi is not observed in cells microinjected with genes encoding SFV structural proteins in cells microinjected with genes encoding SFV structural proteins or SFV infected cells, unless the cells are first treated with monensin to induce budding at internal membranes (Kondor-Koch et al., 1982; Kuismanen et al., 1982). Presumably during budding of RV there is a capsid–spike interaction, which may account for the capsid-associated delay in E2 and E1 processing in transfected cells. Whether or not capsid protein modulates spike glycoprotein movement in infected cells remains to be determined.

Transport of E2 and E1 to the cell surface was very inefficient in transfected cells resulting in only a small fraction of the total RV-specific antigen arriving to the plasma membrane after a 3-hr chase period. Consistent with the immunofluorescence data, only E1 and E2 (>42 kDa) were transported to the plasma membrane. The amount of RV on the surface of infected cells detectable by immunofluorescence was also very limited. Although comparable amounts of E1 and E2 were delivered to the plasma membrane after 3 hr con-version of E1 glycans to endo H-resistant types occurred more slowly than 39- to 42-kDa E2 conversion in biosynthetic labeling experiments. Coprecipitation of pRIP with E1 and E2 from transfected cells was observed; however the significance of this phenomenon remains to be determined. Due to a shortage of anti-BiP antibody we were unable to determine if the association of pRIP with the RV glycoproteins was transient, or whether a fraction of E1 and E2 was permanently retained in the FR by pRIP.

We have presented evidence which suggests that maturation and intracellular transport of RV structural proteins is quite different from those of alphaviruses, and in at least two respects resembles coronaviruses and Uukuniemi virus which both bud from Golgi membranes. Furthermore, limiting the amount of RV antigen on the surface of infected cells could provide a means to evade immune surveillance, and possibly be a contributing factor to the establishment and maintenance of persistent infection in vivo. Generally the rate-limiting step in transport of proteins through the exocytic pathway is passage from the ER to the Golgi (Lodish, 1988). In the case of RV glycoproteins, the site of accumulation appears to be the Golgi complex. Attaining and maintaining a high concentration of spike glycoproteins in the Golgi membranes may be intrinsic to this virus’s ability to bud from internal membranes in certain cell types.

ACKNOWLEDGMENTS

We are grateful to Dr. David Russell (University of Texas, Dallas, TX) for the pCMV5 vector. We also thank Dr. Aubrey J. Tingle (Department of Pediatrics, University of British Columbia) for supplying human anti-RV serum, Dr. Jerry Wolinsky (University of Texas Medical School, Houston, TX) for anti-E2 monoclonals, and Dr. John Safford (Abbott Laboratories) for supplying anti-C and anti-E1 monoclonal antibodies. We are also grateful to Dr. Frank Tufaro for reading the manuscript and helpful criticisms, and to Mike Weiss for help with microscopy and photography. This work was funded by an MRC operating grant to S.G.T.H. is a predoctoral trainee supported by an MRC biotechnology training studentship.

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