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Localization to the Golgi Complex of Uukuniemi Virus Glycoproteins G1 and G2 Expressed from Cloned cDNAs

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The membrane glycoproteins G1 and G2 of Uukuniemi virus, a bunyavirus, accumulate in the Golgi complex (GC) during virus infection. These proteins have therefore been considered to be good models for studying the intracellular transport to and retention in the GC. In this study, I have used indirect immunofluorescence to localize in COS cells the Uukuniemi virus glycoproteins G1 and G2 expressed together or separately from cloned cDNAs with use of simian virus 40-based vectors. When expressed together from the full-length cDNA, G1 and G2 were correctly translocated, processed, and targeted to the GC, indicating that the information for GC targeting resides in the proteins. When the proteins were expressed separately, G1 was transported to the GC and retained there. In contrast, G2 could not be detected in the GC but was most probably retained and finally degraded in the endoplasmic reticulum. However, in cells cotransfected with G1 and G2 cDNAs, the proteins could both again be found in the GC. These results suggest that G1 is responsible for targeting to and retention of the Uukuniemi virus glycoproteins in the GC. G2 would thus accumulate in the GC by virtue of its binding to G1.

Intracellularly maturing enveloped viruses have been used as tools to study membrane traffic in animal cells. Bunyaviruses belong to the category of intracellularly maturing viruses. Both morphological and expression studies have shown that these viruses bud into smooth-surfaced vesicles in or near the Golgi complex (GC) (20, 21). To analyze the basis for this site of maturation, we have studied the biosynthesis and intracellular transport of the two membrane glycoproteins G1 and G2 of Uukuniemi virus, a member of the genus *Phlebovirus* within the family *Bunyaviridae*. We have previously shown that G1 and G2 are transported during virus infection from the endoplasmic reticulum (ER) to the GC, where they are retained and accumulate, causing a progressive vacuolization of the GC (5, 10–13). It is believed that the accumulation of G1 and G2 in the GC determines the site of virus maturation and that the information required for the retention in the GC may be contained in the structure of G1 and/or G2 (20).

Uukuniemi virus has a tripartite single-stranded RNA genome of negative polarity (22, 23). The middle-sized M segment encodes an 110,000-Da precursor (p110) to the envelope glycoproteins G1 (75 kDa) and G2 (65 kDa) (32). The complete sequence of the M RNA has been determined from cDNA (26). The virion RNA contains an open reading frame of 3,024 nucleotides, corresponding to 1,008 amino acids. This is sufficient to encompass the p110 precursor, which has previously been shown to be cotranslationally cleaved in the ER into G1 and G2 (10, 32). From the nucleotide sequence and the N-terminal amino acid sequences of G1 and G2, it was concluded that both G1 and G2 are preceded by a 17-residue-long signal sequence. Furthermore, both G1 and G2 have at their C-terminal end a hydrophobic stretch of amino acids (19 and 27 residues, respectively) that anchors the proteins to the lipid bilayer (21, 26). Both proteins are oriented in the lipid bilayer as class I membrane proteins, with N termini facing the ER lumen and C termini facing the cytoplasm. G1 and G2 each have four sites for N-linked glycosylation. In virions, these glycans are endoglycosidase H (endo H) resistant in G1, whereas G2 contains mainly endo H-sensitive glycans (19). Both proteins are very cysteine rich, each containing 26 residues. This suggests a crucial role for the cysteines in creating the proper three-dimensional structure of the proteins. Despite having the same number of cysteines, G1 folds much faster than G2 in the ER. G1 and G2 heterodimerize in the ER and are then slowly transported to the GC, where they are incorporated into budding virus particles (10, 18).

We are using G1 and G2 of Uukuniemi virus as models to study the biosynthesis of GC-specific proteins (21). The aim of our studies is to map a specific GC retention signal and to understand how membrane proteins in general are targeted and retained in the GC. Therefore, we have constructed cDNA clones encoding G1 and G2, both together and separately, and analyzed their expression in COS cells by using simian virus 40 (SV40)-based vectors.

Construction of recombinant plasmids for expression studies. The full-length cDNA clone encoding G1 and G2 was created by joining the inserts from two partly overlapping cDNA clones pUM40 and pUM 187 (26) combined at the unique *Nde*I site (positions 733 to 738) with a fragment made by polymerase chain reaction (PCR) that includes the translation termination codon (Fig. 1A). PCR was done under standard conditions (28). The 5′-end poly(C) tail, added during the initial cloning step, was removed by oligonucleotide-directed mutagenesis (34), and all recombinant DNA manipulations were performed according to standard methods (29). These steps created a full-length cDNA that in an in vitro transcription-translation system (Promega Corp., Madison, Wis.) consistently resulted in the synthesis of an about 180-amino-acid-long polypeptide that could be immunoprecipitated with a G1-specific monoclonal antibody (data not shown). Sequencing of several different cDNA clones and comparison of them with the sequence obtained from direct virion RNA sequencing using reverse transcriptase revealed that an A residue at position 469 was missing from all examined clones. This resulted in a frameshift and prema-

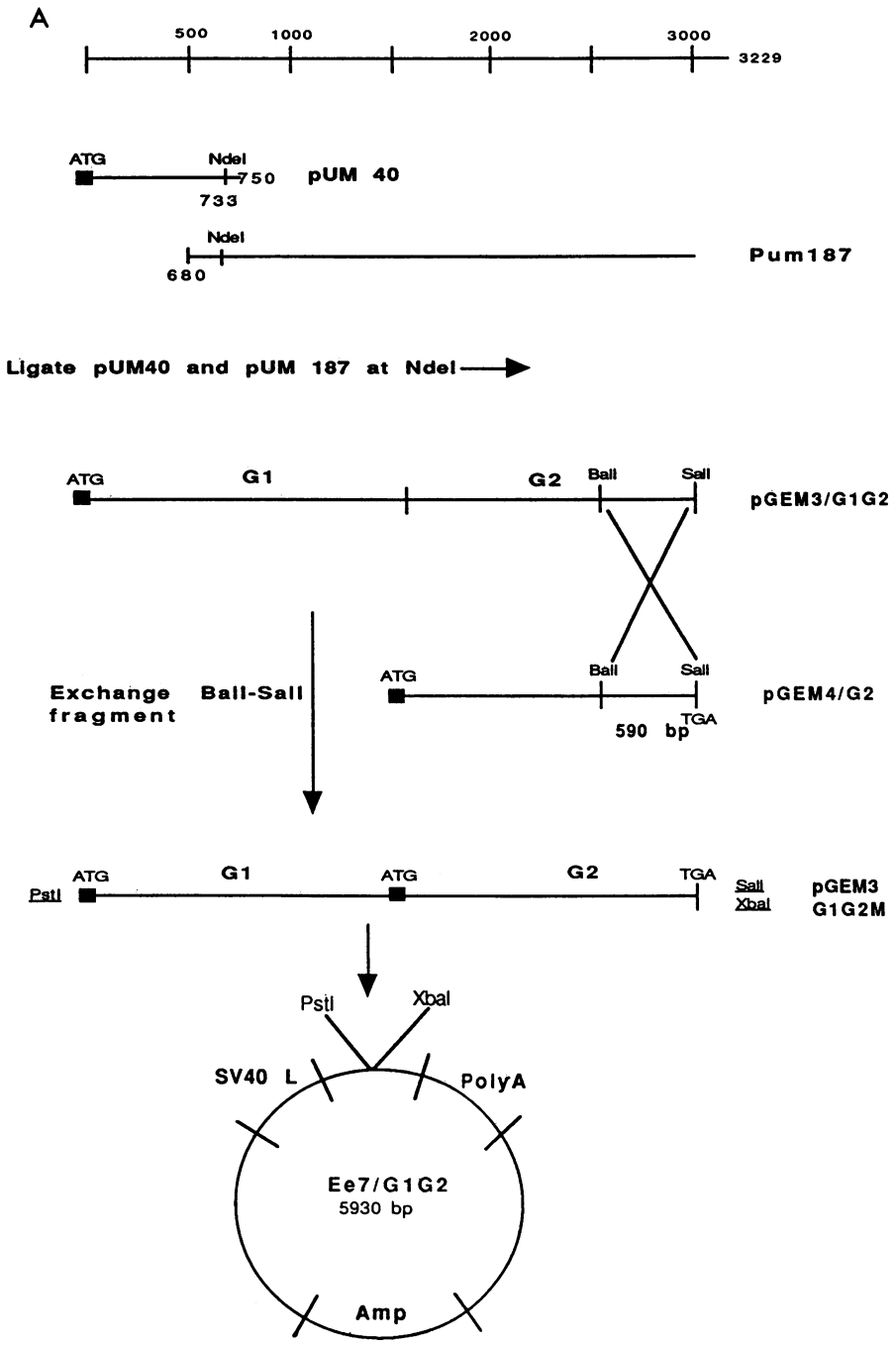

FIG. 1. Schematic representations of the construction of a full-length G1G2 clone and cloning of it into the expression vector Ee7 (A) and construction of a complete G2 clone and cloning of it into the expression vector pSV23 (B). The different constructs are described in the text.

ture termination at a downstream stop codon. I also found that there was a T residue at position 461 in the published sequence that did not exist in the virion RNA. This apparent sequencing mistake compensated for the deleted A residue and erroneously gave an open reading frame. The previously reported sequence between positions 456 and 473 (5′-GCA ATT GCC AGA CCT GAG ACA-3′) (26) should therefore read 5′-GCA ATG CCA GAC CAT GAG-3′. This changes the amino acid sequence from -Ala-Ile-Ala-Arg-Pro-Glu- to Ala-Met-Pro-Asp-His-Glu- (the changed nucleotides and amino acids are underlined). Since no functional full-length clones could be obtained by regular cDNA cloning, the missing A residue at position 469 was introduced by site-directed mutagenesis, yielding the clone pGEM3/G1G2M. This resulted in a translation product of correct size (data not shown). The accuracy of the manipulations was checked by sequencing according to a modified dideoxy-chain termination method using double-stranded cloned cDNA as the template and Sequenase (United States Biochemical Corp.). The *Pst*I and *Xba*I fragment from pGEM3/G1G2M was

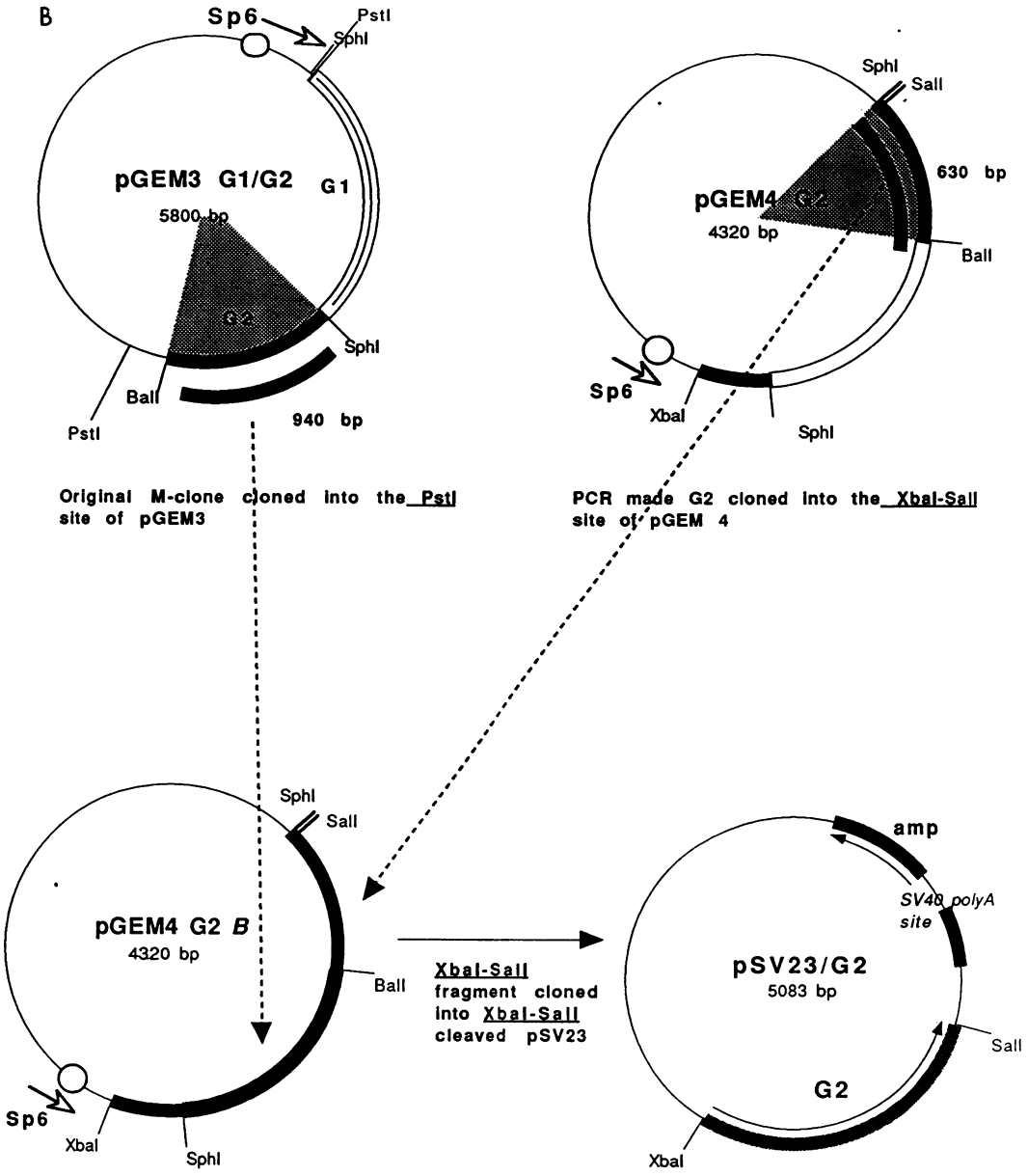

FIG. 1—*Continued.*

isolated and cloned into the *Pst*I and *Xba*I-cleaved expression vector Ee7 (a gift from P. E. Stephens, Celltech Ltd., Slough, Berkshire, United Kingdom) (31) (Fig. 1A).

During the course of the work, I also checked the nucleotide sequence of the entire M cDNA and found the following additional sequencing errors: in the 3′ noncoding region, a T residue at position 3048 should be a C, and two A residues at positions 3153 and 3179 do not exist and should be omitted. In the G2 coding region at positions 1582 to 1586, the reported sequence CCCTC should read CCTCC, changing the amino acids from -Ala-Leu- to -Ala-Ser-. Thus, considering the above corrections, the correct length of the M RNA segment is 3,229 residues instead of the previously reported 3,231 (26).

To generate a cDNA encoding G1 alone, the G2 part from the full-length clone was deleted by cleavage with *Stu*I (position 1848) and *Hin*cII (in the polylinker region of the plasmid), religated, and cloned. The resulting plasmid, pGEM3/G1, encodes the whole of G1, the region between G1 and G2, and the first 98 amino acids of mature G2. This construct should result in the proper processing by the signal peptidase at the N terminus of G2 and give rise to an authentic G1. For cloning into the expression vector pSV23 (a gift from D. Huylebroeck, Innogenetics, Ghent, Belgium), the coding region for G1 was excised by *Hin*dIII and *Xba*I digestion, and the fragment was cloned into the corresponding sites of the vector downstream of the early SV40 promoter, yielding plasmid pSV23/G1.

To generate an insert encoding G2, PCR was used to amplify the whole of the G2 coding sequence, including the internal signal peptide region preceding G2. An AUG codon in an optimal translation initiation context (9) was introduced

in frame at the N terminus by introducing an extra G after the start methionine between positions 1497 and 1498. This gave rise to a valine before the threonine at position 1500, two amino acids before the predicted signal sequence of G2. Because of errors in the PCR reaction, the amplified DNA encoding the complete G2 could not be used as such; rather, it was constructed from fragments from the full-length clone and PCR-made G2, as shown in Fig. 1B. Plasmid pGEM3/G1G2 and the PCR-amplified pGEM4/G2 were each cleaved with *Sph*I (in the polylinker region of the plasmid) and *Bal*I (position 2460) and the 940- and 630-bp fragments, respectively, were isolated, and were ligated with the *Sph*I-cleaved vector pGEM4/G2, creating pGEM4/G2B. The complete coding region for G2 is encompassed in the 1,500-bp *Xba*I-*Sal*I fragment, which was cloned into the *Xba*I- and *Sal*I-cleaved pSV23. The resulting plasmid was named pSV23/G2 (Fig. 1B).

Expression of G1 and G2 from the same cDNA. For expression of G1 and G2 from the same cDNA, 20 μg of plasmid G1G2/Ee7 and, as a control, 20 μg of the vector Ee7 were transfected into 70% confluent COS cells by electroporation using a Gene Pulser (Bio-Rad) (16). A capacitance of 960 F and voltage setting of 0.22 kV were used, giving a time-constant readout of about 30 ms. Expression of G1 and G2 was monitored 40 h after transfection in fixed cells permeabilized with Triton X-100 by indirect immunofluorescence using rabbit anti-G1/G2 antibodies (18) followed by rhodamine-conjugated swine anti-rabbit immunoglobulin G (Dakopatt). Bright fluorescence could be visualized in the juxtanuclear region in 2 to 10% of G1G2/Ee7-transfected cells (Fig. 2a), whereas cells transfected with Ee7 showed no detectable fluorescence (data not shown). No staining was observed at the plasma membrane in nonpermeabilized cells (data not shown). The immunofluorescence pattern was similar to that seen in Uukuniemi virus-infected cells (Fig. 2e) (11, 12). To confirm that the observed intracellular location corresponded to the presence of the glycoproteins in the GC, double staining using anti-Golgi antibody CTR433 (8) (a gift from Michel Bornens, Centre de Génétique Moléculaire, Gif-sur-Yvette, France), which specifically stains the medial GC, followed by fluorescein isothiocyanate-conjugated anti-mouse antibody (BIOSYS, Compiegne, France) was performed. The results seen in Fig. 2b show that the patterns obtained with the two antibodies are very similar, suggesting that G1 and G2, when expressed from cloned cDNA, accumulate in the GC. The intracellular localization of the proteins was unchanged after treatment with 50 μg of cycloheximide (Sigma) per ml for 3 h, indicating that G1 and G2 are retained in the GC and are not rapidly degraded (Fig. 2c and d). These results show that the information for targeting to as well as retention in the Golgi apparatus is contained in the glycoproteins themselves.

G1 and G2 are correctly processed and glycosylated. To study whether p110 was correctly processed into G1 and G2, cells transfected with G1G2/Ee7 or Ee7 were metabolically labeled and analyzed. The procedure was essentially as described by Persson and Pettersson (18). After being labeled with 100 μCi of [^{35}S]methionine (Amersham Corp.) per ml between 25 and 40 h after transfection, the cells were solubilized with Triton X-100 in 50 mM Tris-HCl (pH 8.0) and then subjected to immunoprecipitation with a polyclonal G1-specific antibody known to precipitate the complex between G1 and G2 (18). The reduced and alkylated precipitates were analyzed on a sodium dodecyl sulfate (SDS)–10 to 15% polyacrylamide gradient slab gel under reducing conditions (14). Bands comigrating with authentic G1 and G2 precipitated from virus-infected cell lysates were identified (Fig. 3, lane 3). These bands were absent from immunoprecipitates from mock-transfected cells (lane 5).

To analyze whether the proteins had acquired complex N-linked glycans, immunoprecipitated G1 and G2 were subjected to endo H treatment by incubating the immunoprecipitates with 0.25 U of the enzyme (Boehringer Mannheim) per ml overnight before SDS-polyacrylamide gel electrophoresis (PAGE). As shown in Fig. 3 (lane 4), G2 was endo H sensitive, giving most likely rise to three faster-migrating species ($G2_s$). G1 apparently remained endo H resistant, in accordance with previous results (10). As noted before, G1 acquires endo H-resistant glycans, while G2 remains largely endo H sensitive during virus infection and in virions (10, 19). Thus, the results obtained in this study suggest that the glycans of G1 and G2 expressed from cDNA are processed to the same extent as during normal infection. In conclusion, these results indicate that G1 and G2 are properly cleaved, processed, and targeted to the GC when expressed from a cDNA copy.

G1 expressed alone can be targeted to the GC. To investigate the fate of G1 and G2 expressed separately, COS cells were first transfected with pSV23/G1. Intracellular immunofluorescence staining with a polyclonal G1-specific antibody 40 h later showed bright fluorescence in the Golgi region (Fig. 2f). The staining pattern did not change after treatment with cycloheximide for 3 h (data not shown). Double-staining with the Golgi marker resulted in a similar fluorescence pattern (Fig. 2g). No plasma membrane staining could be detected in nonpermeabilized cells (data not shown). These results suggest that G1 can be targeted to and retained in the GC without the expression of other viral proteins including G2.

To study whether G1 in the absence G2 was properly translated and processed, pSV23/G1-transfected COS cells were metabolically labeled with [^{35}S]methionine and then subjected to immunoprecipitation and SDS-PAGE. As shown in Fig. 3 (lane 2), a weak G1 band (asterisk) comigrated with the viral counterpart (lane 1).

Thus, G1 expressed alone is properly translocated, cleaved, and, interestingly, also targeted to the GC when expressed from a cDNA copy.

G2 requires coexpression with G1 to be targeted to the GC. To monitor the fate of G2 expressed alone, COS cells were transfected with plasmid pSV23/G2 and 40 h later subjected to immunofluorescence staining with a G2-specific polyclonal antibody. Occasionally some intracellular staining could be seen, probably localized to the ER. The weak staining made photographic visualization impossible. No staining could be detected on the plasma membrane. However, when pSV23/G2 was cotransfected with pSV23/G1, both proteins could be detected, using either G1- or G2-specific polyclonal antibodies, in the juxtanuclear region representing the GC, as judged from the similar staining patterns with the Golgi marker (Fig. 2h and i). These results suggest that in order for G2 to be targeted to and retained in the GC, an interaction with G1 is needed.

The low level of transient expression has made any further biochemical analyses difficult. A similar low frequency has been observed for the E1 glycoprotein of the coronavirus MVH-A59, using an SV40-vector that allows abundant expression of the G protein of vesicular stomatitis virus (25). I have explored several different SV40-based vectors as well as different transfection methods, without any major differences in the outcome. However, preliminary studies with

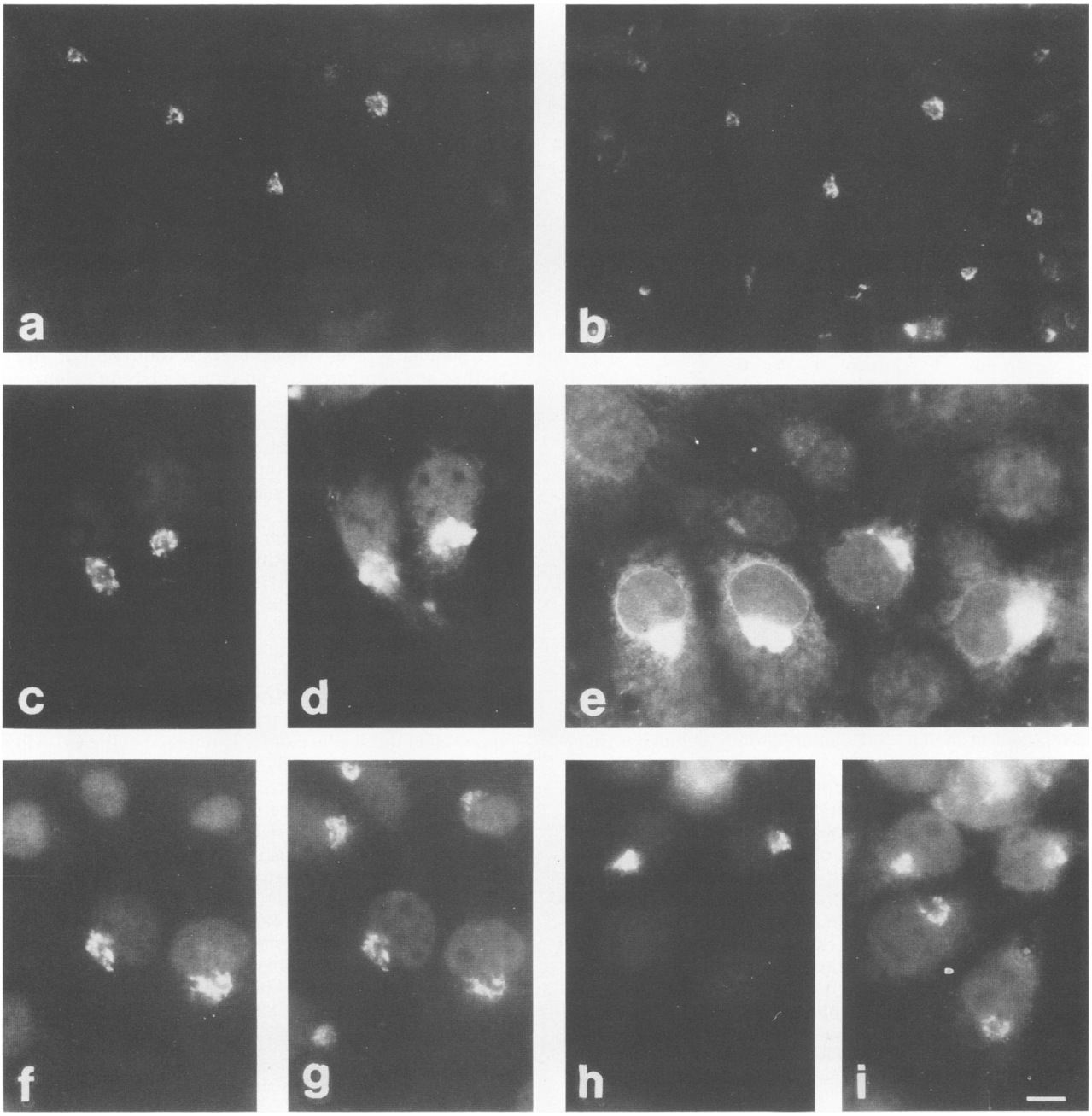

FIG. 2. Double immunofluorescence staining of COS cells transfected with G1 and G2. Forty hours after transfection by electroporation, the cells were stained with rabbit anti-G1G2 antibodies followed by rhodamine-conjugated swine anti-rabbit immunoglobulin G (a, c, and f). For double staining, monoclonal anti-GC antibody CTR433 and fluorescein isothiocyanate-conjugated anti-mouse antibody were used (b, d, g, and i). A typical Golgi-like staining similar to that seen in virus-infected cells (e) can be seen with the full-length construct G1G2/Ee7 (a and b), with G1 expressed alone from G1/pSv23 (f and g), and with G1 and G2 coexpressed from G1/pSV23 plus G2/pSV23 (h and i). The cells were stained with anti-G2 antibodies in the coexpression study. Treatment with cycloheximide for 3 h did not change the localization of G1 and G2 when the proteins were expressed from the G1G2/Ee7 construct (c and d). Bar, 10 μm.

recombinant vaccinia viruses have shown considerably higher expression levels, essentially with the same results.

Previous studies on the biosynthesis of Uukuniemi virus G1 and G2 showed that G1 is transported from the ER faster than G2 to the site of virus budding in the GC (10). This finding suggested that G1 and G2 might be transported from the ER to the GC independently of each other. Recent studies on the intracellular transport of G1 and G2 using pulse-chase and subcellular fractionation have shown that G1 and G2 of Uukuniemi virus form heterodimers shortly after synthesis (18). Despite their similar size, number of N-linked glycans, and number of cysteines, G1 was found to fold much faster than G2. Apparently, only properly folded glycoproteins are dimerization competent. The results showed that the proteins in virus-infected cells are transported together to the GC.

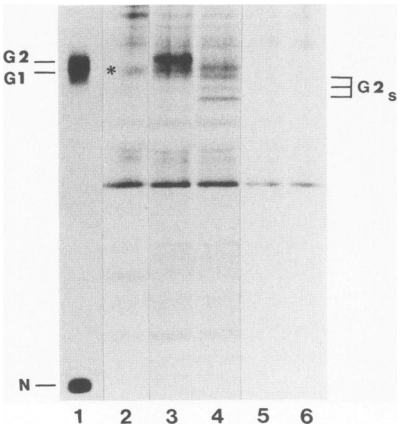

FIG. 3. Immunoprecipitation of metabolically labeled G1 and G2 expressed in COS cells. The lysates from G1/pSV23 (lane 2)- and G1G2/Ee7 (lane 3 and 4)-transfected and labeled cells were analyzed by SDS-PAGE (10 to 15% polyacrylamide gel) under reducing conditions. In lane 2, the G1 protein comigrating with authentic G1 precipitated from virus-infected cell lysates (lane 1) is indicated with an asterisk. Lanes 3 and 4 show the effect of endo H treatment on G1 and G2. The drug did not affect the mobility of G1 but resulted in three endo H-sensitive G2 species ($G2_s$) migrating faster than untreated G2. Lanes 5 and 6 show mock-transfected cells without and with endo H treatment.

However, expression of G1 and G2 separately from cDNA clones, as done here, shows that G1 does not necessarily need to form a complex with G2 in order to be targeted to and retained in the GC. G1 can apparently achieve a transport-competent conformation in the ER. In contrast, the results obtained for Hantaan virus (another bunyavirus) G1 and G2 show that neither G1 nor G2 was able to exit the ER on its own (27). Only coexpression resulted in proper Golgi localization. A small fraction of G2 of Punta Toro virus (a phlebovirus) can assemble into a homodimer and be transported to the plasma membrane (2), whereas a soluble (anchor-minus) G2 was retained in the GC when coexpressed with G1, also suggesting that G1 contains the information for Golgi localization (3). The results obtained in this study indicate that G2 of Uukuniemi virus is unable to exit the ER on its own. Preliminary results using a vaccinia virus expression system have also confirmed that G2 remains in the ER (unpublished results). Most likely, G2 fails to assemble into a proper oligomeric complex and is prevented from progressing through the secretory pathway, resulting in eventual degradation in the ER. The low expression level observed for G2 can be due partly to rapid degradation. Retention in the ER could possibly be mediated by the C-terminal sequence -KVKKS-COOH (26). It has been shown that transmembrane proteins can be retained in the ER by a motif of lysine at the third position and a positively charged amino acid at either the fourth or fifth position from the C terminus (7, 30). Coexpression with G1 restored the Golgi localization, supporting the notion that G2 has to interact with G1 to mature structurally. The folding and oligomerization with G1 could, as a result of architectural editing, mask the ER retention signal of G2 and make the proteins competent to leave the ER. The glycoproteins G1 and G2 would be guided as a heterodimer to the GC by the Golgi targeting signal residing in G1.

It is noteworthy that depending on the proteins under study and the expression systems used, quite different results may be obtained. Both the nature of the polypeptide chain and of cellular factors and conditions determine the folding rate of the protein (1). The mechanisms by which proteins are folded and subunits are assembled into oligomers in the ER, and the rules governing competence to exit the ER, are still poorly understood (6, 24). In most cases, proper folding and oligomerization of subunits have to occur in the ER before export can take place. However, it is conceivable that certain proteins that form parts of oligomers (like G1 of Uukuniemi virus and its dimerization with G2) may acquire a transport-competent conformation when expressed in the absence of its partner(s). Also, transport competence may be a relative property, in that an altered conformation may substantially slow down the transport rate rather than completely block exit from the ER.

In summary, the transient expression of G1 and G2, together and separately, from cloned cDNAs as monitored by immunofluorescence and immunoprecipitation has been studied in this investigation. The results suggest that the information for Golgi localization may be contained in the G1 glycoprotein. Expression of G1 and G2 from a full-length cDNA resulted in their transport to and accumulation in the GC, as evidenced by their colocalization with a marker for the GC. Immunofluorescence studies on vaccinia virus-expressed G1 and G2 of Rift Valley fever virus (33), Punta Toro virus (2–4, 15), and Hantaan virus (17, 27) have also demonstrated that signals for Golgi localization reside within the G1-G2 glycoprotein complex. Importantly, as shown here, G1 expressed alone also localized to the GC, while G2 expressed alone was retained in the ER. Coexpression of G1 and G2 from separate cDNAs again resulted in the transport of G2 to the GC. The fact that G1 of Uukuniemi virus expressed in the absence of G2 is retained in the GC will now allow a more detailed study to pinpoint a possible retention signal. The nature of this signal is still open. It may be a linear amino acid sequence or a domain created by protein folding. Arrest in the GC may also be caused by the lateral interaction of glycoproteins, resulting in the exclusion of the proteins from transport vesicles.

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REFERENCES

1. **Braakman, I., H. Hoover-Litty, K. R. Wagner, and A. Helenius.** 1991. Folding of influenza hemagglutinin in the endoplasmic reticulum. J. Cell Biol. **114**:401–411.
2. **Chen, S.-Y., and R. W. Compans.** 1991. Oligomerization, transport, and Golgi retention of Punta Toro virus glycoproteins. J. Virol. **65**:5902–5909.
3. **Chen, S.-Y., Y. Matsuoka, and R. W. Compans.** 1991. Golgi complex localization of the Punta Toro virus G2 protein requires its association with the G1 protein. Virology **183**:351–365.
4. **Chen, S.-Y., Y. Matsuoka, and R. W. Compans.** 1991. Assembly and polarized release of Punta Toro virus and effects of brefeldin A. J. Virol. **65**:1427–1439.
5. **Gahmberg, N., E. Kuismanen, S. Keränen, and R. F. Pettersson.** 1986. Uukuniemi virus glycoproteins accumulate in and cause morphological changes of the Golgi complex in the absence of

virus maturation. J. Virol. **57**:899–906.
6. **Hurtley, S. M., and A. Helenius.** 1989. Protein oligomerization in the endoplasmic reticulum. Annu. Rev. Cell Biol. **5**:277–307.
7. **Jackson, M. R., T. Nilsson, and P. Peterson.** 1990. Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. EMBO J. **9**:3153–3162.
8. **Jasmin, B. J., J. Cartaud, M. Bornens, and J. P. Changeux.** 1989. Golgi apparatus in chick skeletal muscle: changes in its distribution during end plate development and after denervation. Proc. Natl. Acad. Sci. USA **86**:7218–7222.
9. **Kozak, M.** 1989. The scanning model for translation: an update. J. Cell Biol. **108**:229–241.
10. **Kuismanen, E.** 1984. Posttranslational processing of Uukuniemi virus glycoproteins G1 and G2. J. Virol. **51**:806–812.
11. **Kuismanen, E., B. Bång, M. Hurme, and R. F. Pettersson.** 1984. Uukuniemi virus maturation: immunofluorescence microscopy with monoclonal glycoprotein-specific antibodies. J. Virol. **51**: 137–146.
12. **Kuismanen, E., K. Hedman, J. Saraste, and R. F. Pettersson.** 1982. Uukuniemi virus maturation: accumulation of virus particles and viral antigens in the Golgi complex. Mol. Cell. Biol. **2**:1444–1458.
13. **Kuismanen, E., J. Saraste, and R. F. Pettersson.** 1985. Effect of monensin on the assembly of Uukuniemi virus in the Golgi complex. J. Virol. **55**:813–822.
14. **Maizel, J. V. J.** 1971. Polyacrylamide gel electrophoresis of viral proteins. Methods Virol. **5**:179–246.
15. **Matsuoka, Y., T. Ihara, D. H. L. Bishop, and R. W. Compans.** 1988. Intracellular accumulation of Punta Toro virus glycoproteins expressed from cloned cDNA. Virology **167**:251–260.
16. **Maxwell, I. H., and F. Maxwell.** 1988. Electroporation of mammalian cells with a firefly luciferase expression plasmid: kinetics of transient expression differ markedly among cell types. DNA **7**:557–562.
17. **Pensiero, M. N., G. B. Jennings, C. S. Schmaljohn, and J. Hayn.** 1988. Expression of the Hantaan virus M genome segment by using a vaccinia virus recombinant. J. Virol. **62**:696–702.
18. **Persson, R., and R. F. Pettersson.** 1991. Formation and intracellular transport of a heterodimeric viral spike protein complex. J. Cell Biol. **112**:257–266.
19. **Pesonen, M., E. Kuismanen, and R. F. Pettersson.** 1982. Monosaccharide sequence of protein-bound glycans of Uukuniemi virus. J. Virol. **41**:390–400.
20. **Pettersson, R. F.** 1991. Protein localization and virus assembly at intracellular membranes. Curr. Top. Microbiol. Immunol. **170**:67–106.
21. **Pettersson, R. F., N. Gahmberg, E. Kuismanen, L. Kääriäinen, R. Rönnholm, and J. Saraste.** 1988. Bunyavirus membrane glycoproteins as models for Golgi-specific proteins. Mod. Cell Biol. **6**:65–96.
22. **Pettersson, R. F., M. J. Hewlett, D. Baltimore, and J. M. Coffin.** 1977. The genome of Uukuniemi virus consists of three unique RNA segments. Cell **11**:51–64.
23. **Ranki, M., and R. F. Pettersson.** 1975. Uukuniemi virus contains an RNA polymerase. J. Virol. **16**:1420–1425.
24. **Rose, J. K., and R. W. Doms.** 1988. Regulation of protein export from the endoplasmic reticulum. Annu. Rev. Cell Biol. **4**:257–288.
25. **Rottier, P. J. M., and J. K. Rose.** 1987. Coronavirus E1 glycoprotein expressed from cloned cDNA localizes in the Golgi region. J. Virol. **61**:2042–2045.
26. **Rönnholm, R., and R. F. Pettersson.** 1987. Complete nucleotide sequence of the M RNA segment of Uukuniemi virus encoding the membrane glycoproteins G1 and G2. Virology **160**:191–202.
27. **Ruusala, A., R. Persson, C. S. Schmaljohn, and R. F. Pettersson.** 1992. Coexpression of the membrane glycoproteins G1 and G2 of Hantaan virus is required for targeting to the Golgi complex. Virology **186**:53–64.
28. **Saiki, R., D. Gelfand, S. Stoffel, S. Scharf, R. Higuchi, R. Horn, K. Mullis, and H. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.
29. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
30. **Shin, J., R. L. Dunbrack, Jr., S. Lee, and J. L. Strominger.** 1991. Signals for retention of transmembrane proteins in the endoplasmic reticulum studied with CD4 truncation mutants. Proc. Natl. Acad. Sci. USA **88**:1918–1922.
31. **Stephens, P. E., and M. I. Cockett.** 1989. The construction of a highly efficient and versatile set of mammalian expression vectors. Nucleic Acids Res. **17**:711.
32. **Ulmanen, I., P. Seppälä, and R. F. Pettersson.** 1981. In vitro translation of Uukuniemi virus-specific RNAs: identification of a nonstructural protein and a precursor to the membrane glycoproteins. J. Virol. **37**:72–79.
33. **Wasmoen, T. L., L. T. Kakach, and M. S. Collett.** 1988. Rift Valley fever virus M segment: cellular localization of M segment-encoded proteins. Virology **166**:275–280.
34. **Zoller, M. J., and M. Smith.** 1983. Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors. Methods Enzymol. **100**:468–500.