JOURNAL OF VIROLOGY, June 1997, p. 4717–4727 Vol. 71, No. 6
0022-538X/97/$04.00+0

A Retention Signal Necessary and Sufficient for Golgi Localization Maps to the Cytoplasmic Tail of a *Bunyaviridae* (Uukuniemi Virus) Membrane Glycoprotein

AGNETA M. ANDERSSON, LARS MELIN,† ANDREW BEAN,‡ AND RALF F. PETTERSSON*

Ludwig Institute for Cancer Research, Stockholm Branch, S-17177 Stockholm, Sweden

Received 2 December 1996/Accepted 10 March 1997

Members of the *Bunyaviridae* family mature by a budding process in the Golgi complex. The site of maturation is thought to be largely determined by the accumulation of the two spike glycoproteins, G1 and G2, in this organelle. Here we show that the signal for localizing the Uukuniemi virus (a phlebovirus) spike protein complex to the Golgi complex resides in the cytoplasmic tail of G1. We constructed chimeric proteins in which the ectodomain, transmembrane domain (TMD), and cytoplasmic tail (CT) of Uukuniemi virus G1 were exchanged with the corresponding domains of either vesicular stomatitis virus G protein (VSV G), chicken lysozyme, or CD4, all proteins readily transported to the plasma membrane. The chimeras were expressed in HeLa or BHK-21 cells by using either the T7 RNA polymerase-driven vaccinia virus system or the Semliki Forest virus system. The fate of the chimeric proteins was monitored by indirect immunofluorescence, and their localizations were compared by double labeling with markers specific for the Golgi complex. The results showed that the ectodomain and TMD (including the 10 flanking residues on either side of the membrane) of G1 played no apparent role in targeting chimeric proteins to the Golgi complex. Instead, all chimeras containing the CT of G1 were efficiently targeted to the Golgi complex and colocalized with mannosidase II, a Golgi-specific enzyme. Conversely, replacing the CT of G1 with that from VSV G resulted in the efficient transport of the chimeric protein to the cell surface. Progressive deletions of the G1 tail suggested that the Golgi retention signal maps to a region encompassing approximately residues 10 to 50, counting from the proposed border between the TMD and the tail. Both G1 and G2 were found to be acylated, as shown by incorporation of [^{3}H]palmitate into the viral proteins. By mutational analyses of CD4-G1 chimeras, the sites for palmitylation were mapped to two closely spaced cysteine residues in the G1 tail. Changing either or both of these cysteines to alanine had no effect on the targeting of the chimeric protein to the Golgi complex.

Enveloped viruses acquire their lipoprotein coat by budding though one of several host cellular membranes. The plasma membrane is the budding site for the majority of such viruses (e.g., alpha-, arena-, orthomyxo-, paramyxo-, rhabdo-, and retroviruses). In such cases, virus particles are released directly into the extracellular space following budding. Many viruses, however, bud at internal membranes, such as the endoplasmic reticulum (ER) (e.g., rota- and flaviviruses), inner nuclear membrane (herpesviruses), ER-Golgi intermediate compartment (corona- and poxviruses), and the Golgi complex (*Bunyaviridae* and rubella virus) (36). In these cases, virus particles are released from the infected cells either after cell lysis (e.g., rotaviruses) or after transport of virus-containing vesicles to the cell surface, where the vesicles fuse with the plasma membrane, releasing the virus particles (e.g., coronaviruses bunyaviruses, and rubella virus).

The site of intracellular budding is thought to be largely dependent on the properties of the viral membrane glycoproteins forming the spikes (36). This conclusion is derived from the observation that viral spike proteins usually, but not always (14), accumulate in the budding compartment. However, an active role for the nucleocapsids or other viral components cannot be excluded. Several studies have indicated that one or more of the spike proteins are targeted to and retained in the budding compartment. For correct localization, the spike proteins must contain signals for compartment-specific targeting and retention, similarly to normal compartment-specific cellular proteins. To date, no clearly defined signals have been identified for viral proteins, although the first transmembrane domain (TMD) (22, 53) or a combination of the cytoplasmic tail and the TMDs (2, 21) of the coronavirus M protein, or the TMD of rubella virus E2 (10) protein, has been implicated. The identification of retention signals is important for understanding the mechanisms of virus budding and protein compartmentalization.

Members of the *Bunyaviridae* family have for a long time been known to bud into the Golgi complex. An important determinant for this site of maturation seems to be the accumulation of the two membrane glycoproteins G1 and G2 in this organelle (36, 37). Recent work has shown that G1 and G2 of several *Bunyaviridae* members coexpressed from cloned cDNAs are targeted and retained in the Golgi complex in the absence of other viral components. Only one of the two proteins seems to contain the Golgi-targeting signal, while the other one accumulates in the Golgi complex by binding to the signal-containing one (4, 19, 25, 26, 34, 40).

We are using Uukuniemi (UUK) virus as a model to study the mechanism of budding of *Bunyaviridae* in the Golgi complex. As for all *Bunyaviridae* members, UUK virus has a tripartite, single-stranded, circular, and negative-sense RNA genome (7). The middle-sized segment (M) encodes a precursor (p110) of the glycoproteins G1 and G2 (41, 50). Processing of p110 occurs cotranslationally in the ER (1, 15, 50), followed by

* Corresponding author. Mailing address: Ludwig Institute for Cancer Research, Stockholm Branch, Box 240, S-17177 Stockholm, Sweden. Phone: 468-31 07 01. Fax: 468-33 28 12. E-mail: rpet@licr.ki.se.
† Present address: Perkin Elmer, S-17202 Stockholm, Sweden.
‡ Present address: Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305-5428.

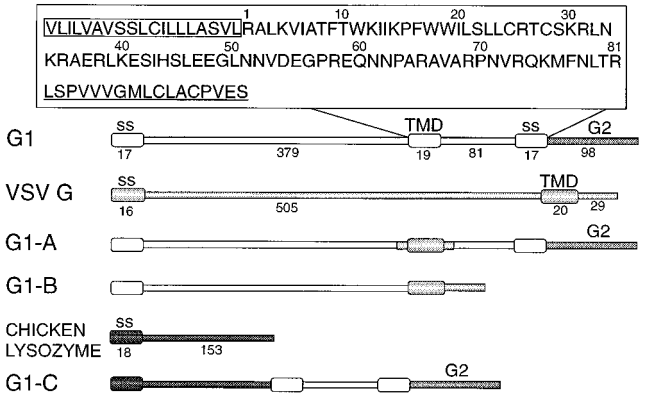

FIG. 1. Schematic representation of the parental and chimeric cDNAs used for expression studies to map the Golgi localization signal in G1. The structures of G1 (open bar) and VSV G (lightly shaded bar) are shown at the top together with the amino acid sequence of the TMD and cytoplasmic tail of G1. The residues are numbered from 1 to 98, starting with the arginine bordering the TMD of G1. The G1 cDNA also encodes 98 residues of the downstream G2 to allow proper processing between G1 and G2 (1). G1-A and G1-B are chimeras between G1 and VSV G. In G1-A, the TMD and 10 flanking residues of G1 have been replaced by the corresponding region from VSV G. In G1-B, the TMD, cytoplasmic tail, and G2 sequences have been replaced by the TMD and tail of VSV G. The chicken lysozyme (dark bar) and the chimera G1-C, containing lysozyme fused with the TMD, cytoplasmic tail, and the G2 sequence, are shown below. The number of amino acid residues of each domain in the parental proteins is indicated below each bar. ss, signal sequence.

N-glycosylation, folding, and heterodimerization of G1 and G2. G1 folds rapidly, while G2 folds very slowly. During these early maturation events in the ER, G1 and G2 are bound to the chaperone grp78/BiP and to protein disulfide isomerase (35). Following a lag period, the G1-G2 complex is transported to the Golgi complex, where the glycans of G1 acquire endoglycosidase H (endo H) resistance, while G2 remains largely endo H sensitive (15). Further transport of G1 and G2 is arrested in the Golgi complex, and nucleoproteins also accumulate in this organelle (16, 17, 18). In virions released from infected cells, G1 and G2 have been reported to exist as homodimers (39). Expression of G1 and G2 from cloned cDNAs has shown that G1 expressed alone is able to exit the ER and travel to the Golgi complex, while G2 expressed on its own is retained in the ER. Coexpression of G2 with G1 encoded by separate cDNAs allows G2 to become exported from the ER and targeted to the Golgi complex (26, 40). From these experiments, we have concluded that G1 contains the necessary information for targeting the G1-G2 complex to the Golgi complex.

In this study, we show that the signal for Golgi-targeting resides in the membrane-proximal half of the 98-residue-long cytoplasmic tail of G1. The evidence for this was derived from expression of chimeric proteins in which different G1 domains were exchanged with corresponding domains of proteins normally transported to the plasma membrane. We also show that both G1 and G2 are palmitylated. Mutational analyses identified two cysteine residues in the cytoplasmic tail of G1 as sites for palmitylation. Abolishment of both palmitylation sites had no effect on the targeting to and retention in the Golgi complex.

MATERIALS AND METHODS

Materials. Enzymes used in the molecular cloning were purchased from either Amersham, Boehringer Mannheim, New England Biolabs, or Promega. Eagle's minimum essential medium (MEM), fetal calf serum, HEPES, L-glutamine, OptiMem, penicillin, streptomycin, tryptose phosphate broth, and Lipofectin were obtained from Life Technologies, Gibco-BRL; a Sequenase kit, version 2.0, was from United States Biochemical; [^{35}S]methionine and [9,10(n)-^{3}H]palmitic acid were from Amersham; bovine serum albumin (BSA), Triton X-100, cycloheximide, iodoacetamide, *p*-phenylenediamine, tetramethyl rhodamine isothiocyanate-conjugated anti-mouse immunoglobulin G (IgG), and fluorescein isothiocyanate-conjugated anti-rabbit IgG were all from Sigma; En^{3}Hance was from Du Pont; a monoclonal antibody against CD4, endo H, and dithiothreitol were from Boehringer Mannheim; the polyclonal antiserum against CD4 was from Intracel; Pansorbin was from Calbiochem; and Trasylol (aprotinin) was from Bayer. Oligonucleotides were synthesized with an Applied Biosystems model 392 synthesizer (Perkin-Elmer).

Cells. HeLa cells were grown in MEM supplemented with 5% fetal calf serum, 2 mM L-glutamine, 100 IU of penicillin/ml, and 100 μg of streptomycin/ml. BHK-21 cells were grown in the same medium supplemented with 5% tryptose phosphate broth.

Construction of recombinant cDNA. The construction of a cDNA encoding G1 has previously been reported (26). Chimeric proteins were constructed by using standard PCR technology, replacing corresponding domains of the G protein of vesicular stomatitis virus (VSV G) (42), chicken lysozyme (28), or CD4 (23) with the cytoplasmic tail and/or the TMD of G1. The cDNAs for VSV G, chicken lysozyme, and CD4 were kindly provided by J. K. Rose (Yale University, New Haven, Conn.) S. Munro (MRC Laboratory, Cambridge, England), and M. Marsh (MRC Laboratory, University College, London, England), respectively. Primers were designed such that no additional amino acids were introduced between the fused domains. In the case of C-terminally truncated constructs, a translational stop codon was introduced at the end of the coding sequence. All cDNA regions cloned from PCR products were completely sequenced by the dideoxy chain-terminating method, using Sequenase.

Chimeric cDNAs containing domains from G1, VSV G, and chicken lysozyme were cloned into the vector pTF7-5X (derived from pTF7-5 [8, 26] and containing an *Xma*I cloning site) under the T7 promoter. All CD4 chimeric cDNAs, which were first cloned into the vector BSSK (Stratagene), were subsequently cloned as *Hin*cII fragments into the *Sma*I site of the vector pSFV1 (20) under the SP6 promoter. The primers used and the details of the PCR and cloning strategies are available at http://www.licr.ki.se.

Virus infection, metabolic labeling, and isolation of virions. Subconfluent BHK-21 cells in plastic dishes were washed with adsorption medium (MEM containing 0.2% BSA, 20 mM HEPES [pH 7.2], 2 mM L-glutamine, 100 IU of penicillin/ml, and 100 μg of streptomycin/ml) and infected with UUK virus at a multiplicity of infection of about 10 PFU/ml. Following a 60-min adsorption period, the virus was aspirated, adsorption medium was added, and incubation continued at 37°C. After 7 h, duplicate dishes were labeled overnight either with 0.1 mCi of [^{35}S]methionine per ml supplemented with 5 mM unlabeled methionine (after the cells were starved for 45 min in methionine-free MEM) or with 0.5 mCi of [9,10(n)-^{3}H]palmitic acid per ml. The medium containing the virions was collected and centrifuged at 11,500 × *g* for 30 min to remove cell debris. The supernatants were diluted in phosphate-buffered saline (PBS) followed by purification over a cushion of 30% sucrose in TNE buffer (50 mM Tris-HCl [pH 7.5], 100 mM NaCl, 1 mM EDTA) at 100,000 × *g* for 45 min at 4°C. The virus pellet was resuspended in PBS and boiled for 3 min in nonreducing electrophoresis sample buffer (final concentrations, 82 mM Tris-HCl [pH 8.8], 0.17% bromophenol blue, 9.8% sucrose, and 3% sodium dodecyl sulfate [SDS]).

Expression of cDNA constructs. Two expression systems were used: the T7 RNA polymerase-driven vaccinia virus (VV) expression system, using VV vTF7-3 for T7 polymerase expression (8), kindly provided by Bernard Moss, or the Semliki Forest virus (SFV) expression system, kindly provided by P. Liljeström (20).

Subconfluent monolayers of HeLa cells were seeded onto coverslips approximately 18 h prior to use. The cells were washed with MEM containing 0.04% BSA, infected with VV vTF7-3 at a multiplicity of infection of about 10 to 20 PFU/ml, and incubated for 45 min at 37°C. After removal of VV and rinsing with OptiMem, the cells were transfected with 0.5 to 1 μg of cDNA by using Lipofectin according to the manufacturer's recommendations.

For the SFV expression system, linearized pSFV1-cDNA was first transcribed in vitro by SP6 RNA polymerase before electroporation of the capped mRNA into trypsinized and PBS-washed BHK-21 cells. The cells were diluted in BHK medium, seeded onto coverslips or plastic dishes, and incubated at 37°C.

Metabolic labeling and pulse-chase. The electroporated and seeded BHK-21 cells were incubated for 5 h, starved for 45 min in methionine-free MEM, and labeled with 0.1 mCi of [^{35}S]methionine per ml for 20 min. Cells were chased for the time periods indicated with an excess of unlabeled L-methionine (5.6 mM) and solubilized with 1% Triton X-100 buffer containing 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA, and 100 IU of aprotinin. For labeling with palmitate, electroporated cells were incubated for 3 h at 37°C, then labeled for 5 h with [^{3}H]palmitic acid at a concentration of 0.5 mCi/ml in BHK medium, and thereafter solubilized in 1% Triton X-100 buffer.

Immunoprecipitation and endo H treatment. Proteins were immunoprecipitated from Triton X-100-solubilized cells as described previously (35) by using a polyclonal antiserum against CD4. Samples to be analyzed by endo H digestion were resuspended in endo H buffer (50 mM sodium acetate [pH 5.5], 0.3% SDS) and boiled for 3 min. After centrifugation at 11,400 × *g* for 10 min, the supernatant was divided into two aliquots. To one of the tubes, 2.5 mU of endo H was added, whereas an equal volume of 50 mM sodium acetate was added to the

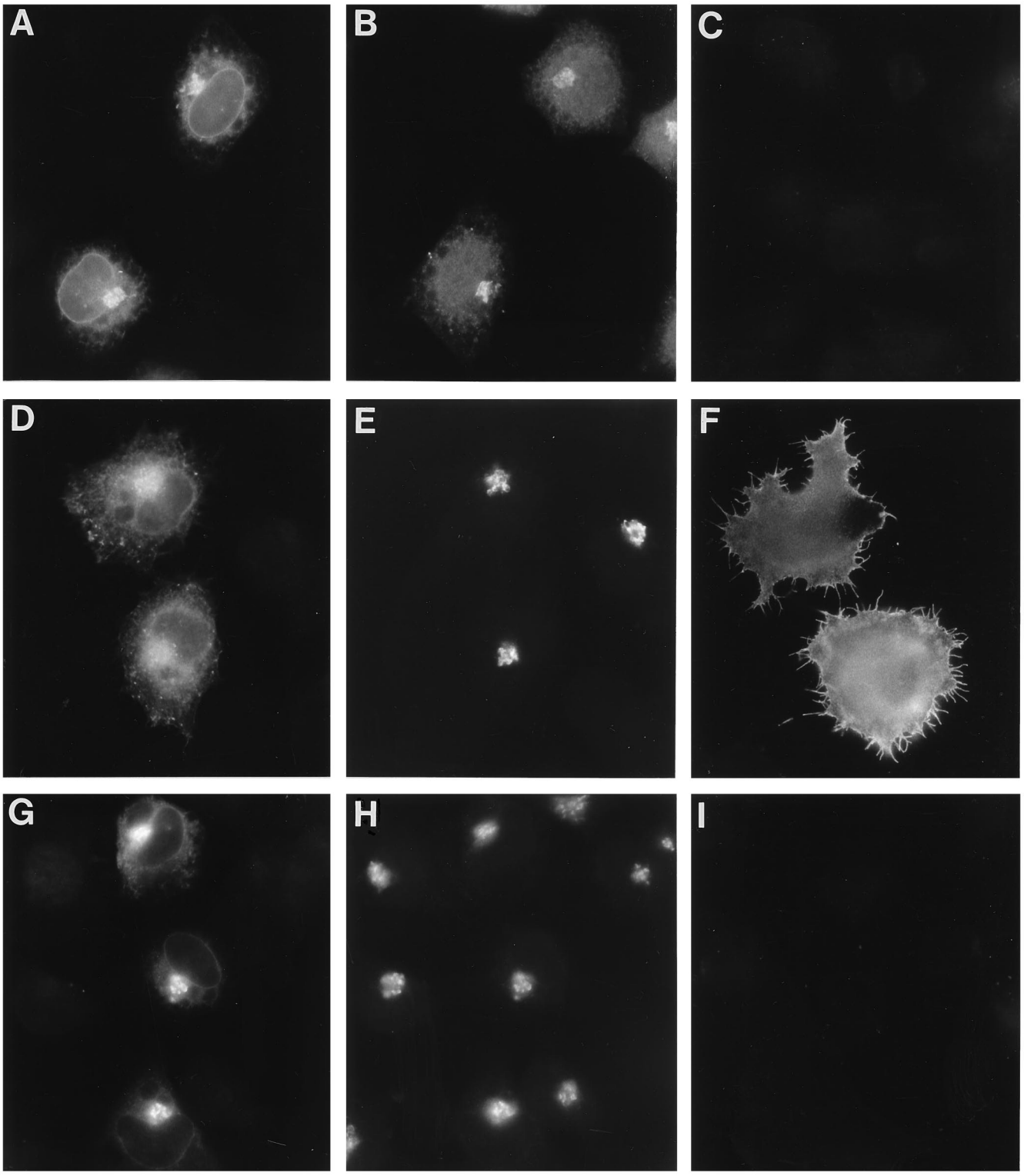

FIG. 2. Localization by indirect immunofluorescence of chimeric proteins expressed in HeLa cells. HeLa cells were infected with vTF7-3 expressing T7 RNA polymerase, followed by transfection of plasmids encoding the chimeric proteins shown in Fig. 1: G1-A (A to C), G1-B (D to F), and G1-C (G to I). Cells were either permeabilized with Triton X-100 (A, B, D, E, G, and H) to stain intracellular proteins or left nonpermeabilized (C, F, and I) to detect surface staining. At 5 h after transfection, cells were treated for 2 h with cycloheximide prior to staining with a monoclonal antibody against G1 (A and C), a polyclonal antiserum against G1 (D and F), a peptide antiserum against the G1 tail (G), or a monoclonal antibody against chicken lysozyme (I). To localize the Golgi complex in cells expressing G1-A, G1-B, and G1-C, cells were double-stained with a polyclonal antiserum against mannosidase II (B) or monoclonal antibody CTR433 (E and H).

other. After incubation for 17 h at 37°C, additional enzyme and buffer were added to the samples. After incubation for a further 4 h, reducing electrophoresis sample buffer (82 mM Tris-HCl [pH 8.8], 0.17% bromophenol blue, 9.8% sucrose, 3% SDS, 16 mM dithiothreitol was added. The samples were boiled for 3 min and cooled, and iodoacetamide was added to a final concentration of 63 mM. The samples were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) as described by Maizel (24) followed by fluorography using En^{3}Hance.

Indirect immunofluorescence microscopy. HeLa or BHK-21 cells grown on coverslips were transfected as described above. After 5 or 6 h, cycloheximide to a final concentration of 0.18 mM was added to inhibit further protein synthesis. The cells were incubated for an additional 2 or 3 h and then washed with cold PBS, fixed with 3% paraformaldehyde in PBS for 15 min at room temperature, washed with PBS, quenched with 10 mM glycine for 20 min at room temperature, washed, and permeabilized with 0.1% Triton X-100 for 30 min at room temperature. The permeabilization step was omitted for surface immunofluorescence staining. Cells were incubated with PBS containing 0.1% BSA and thereafter incubated for 30 min with primary antibodies, washed with PBS-BSA, incubated for 30 min with tetramethyl rhodamine isothiocyanate-conjugated anti-mouse IgG or fluorescein isothiocyanate-conjugated anti-rabbit IgG secondary antibodies, washed, and mounted in 50% glycerol containing 50 mM Tris-HCl (pH 8.0) and 9.2 mM *p*-phenylenediamine. As Golgi markers, monoclonal antibody CTR433 (12) (kindly provided by M. Bornens) or a rabbit polyclonal antiserum against mannosidase II (27) (kindly provided by K. Moremen and M. Farquhar) was used. Additional antibodies used were 6G9, a monoclonal antibody against G1 (54), a polyclonal antiserum against G1 (54), a polyclonal antiserum against a 12-amino-acid-long peptide sequence in the G1 tail (VRQKMFNLTRLS, corresponding to amino acids 72 to 83 in the C terminus of G1) (1), a monoclonal antibody against chicken lysozyme (kindly provided by M. Marsh), a monoclonal antibody against CD4 (Boehringer Mannheim), or a polyclonal antiserum against CD4 (Intracel). Immunofluorescence micrographs were taken by using an Axiophot fluorescence microscope (Zeiss).

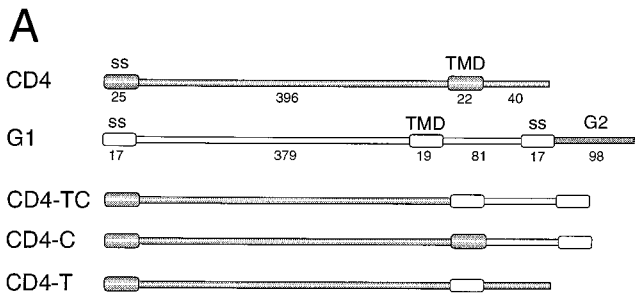

FIG. 3. Localization by indirect immunofluorescence of chimeras between G1 and CD4 expressed in BHK-21 cells. (A) Schematic representation of chimeras between G1 and CD4. The structures of the wild-type CD4 (shaded bar) and G1 (open bar) proteins are shown at the top together with the number of amino acids of the different domains. All chimeras contain the CD4 ectodomain. CD4-TC contains the TMD and cytoplasmic tail of G1, CD4-C contains the cytoplasmic tail of G1, and CD4-T contains only the TMD from G1 and the ectodomain and cytoplasmic tail of CD4. ss, signal sequence. (B) The chimeric proteins were expressed in BHK-21 cells by using the SFV system. In vitro-transcribed mRNAs were electroporated into cells followed by localization of the chimeric proteins by indirect immunofluorescence. At 6 h posttransfection, cells were treated with cycloheximide for 3 h followed by permeabilization with Triton X-100 and staining with a monoclonal antibody to CD4 (a, c, d, f, and g) and a polyclonal antiserum against mannosidase II (manII; b and e). TC, CD4 ectodomain with the TMD and cytoplasmic tail (C) of G1; C, CD4 ectodomain with the cytoplasmic tail of G1; T, CD4 ectodomain and cytoplasmic tail with the G1 TMD.

RESULTS

Exchanging domains between G1 and two exported proteins. A retention signal for Golgi localization could in principle be located anywhere in the UUK virus G1 glycoprotein. We therefore set out to determine whether such a signal is located in the ectodomain, TMD, or cytoplasmic tail of G1. To this, end we first constructed a chimeric protein containing the ectodomain and cytoplasmic tail of G1 and the TMD and 10 flanking residues from the VSV G protein. The latter protein is rapidly and efficiently transported to the cell surface (43). The cytoplasmic tail of G1 also contained 98 N-terminal residues of G2 to allow for correct cleavage downstream of the G2 signal sequence (construct G1-A in Fig. 1) (1). The construct was expressed in HeLa cells by using the T7 RNA polymerase-driven VV system. As shown by indirect immunofluorescence, this chimeric protein was efficiently retained in the Golgi complex (Fig. 2A) and colocalized with mannosidase II, a medial Golgi marker (52) (Fig. 2B). No protein was found on the cell surface of nonpermeabilized cells (Fig. 2C). A reticular staining including the nuclear membrane was also evident. This reflects the inefficiency by which G1 is exported out of the ER in the absence of G2 (26, 35). In this experiment, as in all similar ones described below, the cells were treated with cycloheximide for 2 h before fixation and staining to stop further protein synthesis.

We next expressed a chimera in which the TMD and cytoplasmic tail of G1 were replaced by the corresponding domains from VSV G (construct G1-B in Fig. 1). Intracellularly, this construct displayed an ER-like staining pattern, with some staining also in the Golgi complex (Fig. 2D and E). However, a prominent surface immunofluorescence was also evident (Fig. 2F), suggesting that the Golgi retention signal had been removed.

Finally, we fused the cytoplasmic tail and TMD of G1 with chicken lysozyme, a protein normally secreted from cells. Lysozyme has been used as a reporter in similar experiments for studying retention of resident Golgi glycosyltransferases (28). The G1 domains were able to efficiently retain the reporter protein in the Golgi complex (Fig. 2G and H). No surface staining was observed (Fig. 2I).

Taken together, analyses of expression of these chimeric constructs indicated an important role for the cytoplasmic tail in retaining G1 in the Golgi complex.

Expression of G1-CD4 chimeric proteins. As we have shown before (26, 35) and pointed out above, G1 expressed alone is quite inefficiently exported out of the ER in the absence of G2. As a consequence, the bulk of G1 remains in the ER, hampering the interpretation of results from expressing chimeric proteins containing the G1 ectodomain. We therefore used CD4 as a reporter protein for further studies. CD4 is a monomeric N-glycosylated cell surface marker protein of helper T cells which is efficiently transported to the cell surface (Fig. 3B-g). Chimeric proteins containing the CD4 ectodomain and either the TMD and the cytoplasmic tail of G1 or only the cytoplasmic tail (Fig. 3A) were expressed in BHK-21 cells by using the SFV system (20). Both chimeras were retained in the Golgi complex (Fig. 3B-a and B-d) colocalizing with mannosidase II (Fig. 3B-b and B-e). No surface staining was evident in cells expressing CD4-TC (Fig. 3B-c) or CD-C (see Fig. 4B-b). In contrast, a chimera consisting of the ectodomain and cytoplasmic tail of CD4 and TMD of G1 (Fig. 3A) was not retained intracellularly but was efficiently expressed on the cell surface (Fig. 3B-f). Thus, these results were consistent with those obtained above for chimeras between G1 and VSV G or for lysozyme and indicated the importance of the cytoplasmic tail for Golgi localization.

Effect on the intracellular localization of progressive deletions of the cytoplasmic tail of G1. To further delineate the domain of the G1 tail responsible for Golgi localization, we made progressive deletions from the C terminus of G1. Translational stop codons were introduced in the G1 tail after residues 98 (cleavage site between G1 and G2 [41]), 81, 49, 19, and 4, counting from the proposed border between the TMD and the tail (Fig. 4A). The mutants were expressed in BHK-21 cells by using the SFV system. Removal of the 98 N-terminal residues of G2 had no effect on the Golgi localization (Fig. 4B-a).

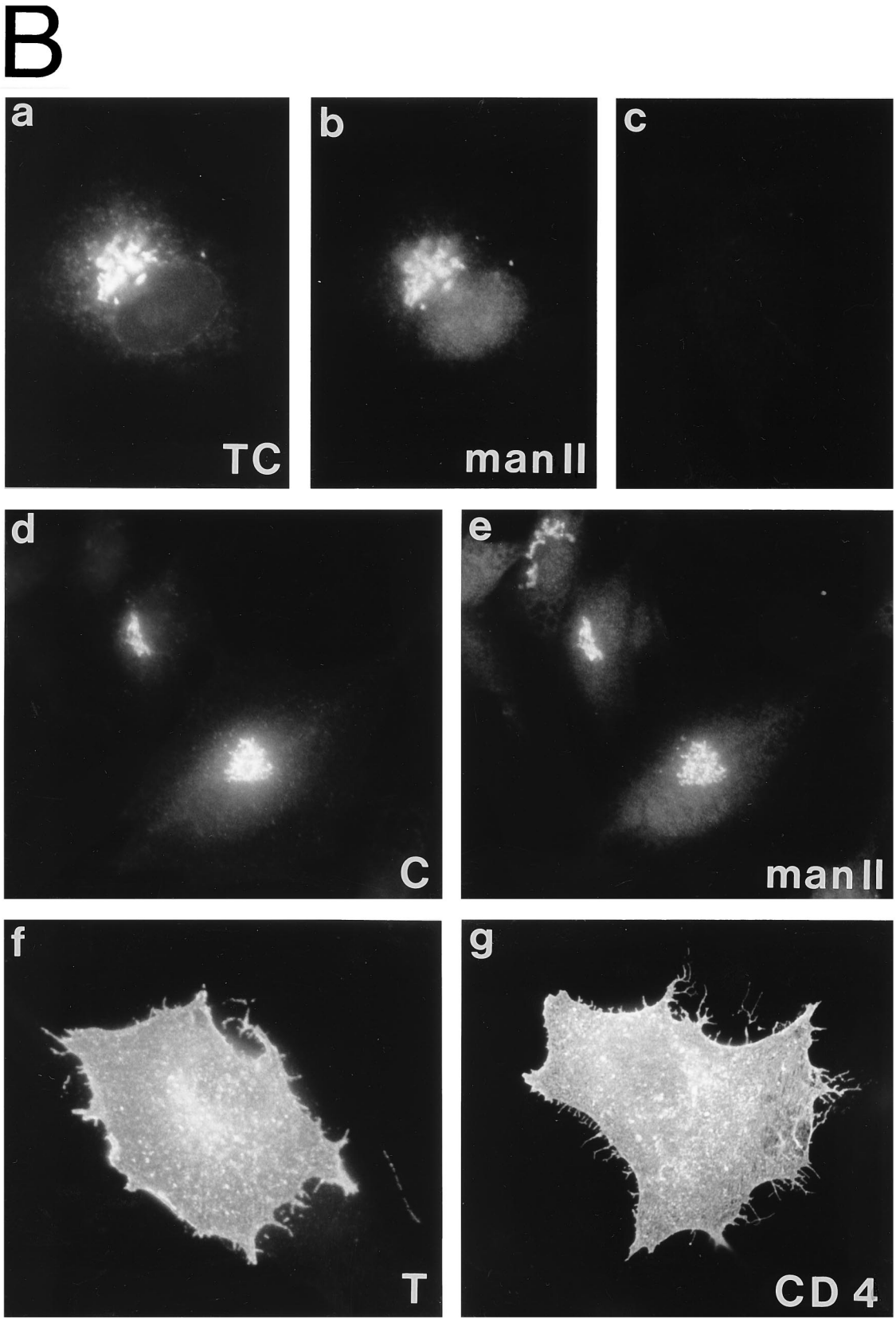

FIG. 3—*Continued.*

We have recently shown that the internal signal sequence of G2 remains covalently linked to the G1 tail (1). Deletion of this 17-residue-long hydrophobic sequence likewise did not affect the Golgi localization (Fig. 4B-c). Deleting a further 32 residues (construct CD4-C_{49}) had a minor effect on Golgi retention, allowing a portion of the reporter protein to leak out to the cell surface (Fig. 4B-e and B-f). Deleting 79 (CD4-C_{19}) residues caused a substantial portion of the chimera to be transported to the plasma membrane (Fig. 4B-h). However, some protein still remained in the Golgi even after 3 h of cycloheximide treatment (Fig. 4B-g). Finally, CD4 with only four residues from the G1 tail was as efficiently expressed on the cell surface as was wild-type CD4 (Fig. 4B-i and B-j). Thus, from these experiments, we conclude that the retention signal is localized approximately between residues 4 and 50.

Tail deletion mutants acquire endo H-resistant glycans. To analyze the efficiency by which the CD4-G1 deletion mutants were transported to the medial Golgi complex, we carried out pulse-chase labeling of transfected cells. The CD4 protein contains two N-linked glycans, and only one of them becomes endo H resistant (46) (Fig. 5, bottom panel). All deletion mutants acquired endo H resistance within 60 to 120 min, with some minor variations in the kinetics between the constructs. In two cases (CD4-C_{19} and -C_4), a small fraction of the molecules remained endo H sensitive even after a 3-h chase. In the case of the mutants with a longer tail (CD4-C, -C_{81}, and -C_{49}) (Fig. 5), the untreated proteins migrated as a doublet or as a smear. The reason for this is unclear, but could be due to a different degree of terminal glycosylation (e.g., sialylation) or acylation (see below).

The cytoplasmic tail of G1 is palmitylated. As shown in Fig. 6A and reported recently (1), both G1 and G2 in virions can be labeled with radioactive palmitate (1). To localize the site(s) for palmitylation, we expressed some of the CD4-G1 tail deletion mutants (CD4-C, -C_{49}, and -C_{19}) in BHK-21 cells in the presence of [^{3}H]palmitic acid or [^{35}S]methionine (controls). Wild-type CD4 served as a positive control, since it is known to be palmitylated at two cysteine residues in the cytoplasmic tail (6). In the chimeric CD4-G1 constructs, the palmitylation sites in CD4 were deleted automatically when the CD4 tail was replaced with that of G1. The labeled proteins were immunoprecipitated and analyzed by SDS-PAGE. Labeling with [^{35}S]methionine showed that all constructs were expressed with roughly equal efficiency (Fig. 6B). Wild-type CD4 and the chimeric CD4-G1 mutants containing either the whole 98-residue G1 tail (CD4-C) or 49 residues (CD4-C_{49}) were readily labeled with [^{3}H]palmitate, while CD4-C_{19} (19 tail residues) was not (Fig. 6C). Thus, we conclude that the cytoplasmic tail of G1 between residues 20 and 98 contains a site(s) for palmitylation.

Mapping of the palmitylation sites in G1. Palmitylation most frequently takes place at cysteine residues via a thioester linkage (see Discussion). We therefore analyzed whether either one of the two cysteine residues at positions 25 and 28 (Fig. 7A) are used as sites for palmitate addition. Since CD4-C_{81} (containing 81 residues from the G1 tail) was found to be palmitylated (Fig. 7C, lane 6), we carried out site-directed mutagenesis on this construct. The cysteine residues at position 25 or/and 28 were changed to alanine, and the mutants were expressed in BHK-21 cells in the presence of either [^{35}S]methionine (Fig. 7B, lanes 2 to 5) or [^{3}H]palmitic acid (Fig. 7C, lanes 6 to 9). As shown in Fig. 7C, both single-site mutants were labeled with [^{3}H]palmitic acid, while the double-site mutant was not. The intensity of the incorporated label in the single-site mutants (lanes 7 and 8) was less than that in the nonmutated CD4-C_{81} protein (lane 6), while all four constructs were labeled equally with [^{35}S]methionine. We conclude that both cysteine residues in the cytoplasmic tail of G1 are used as sites for palmitylation.

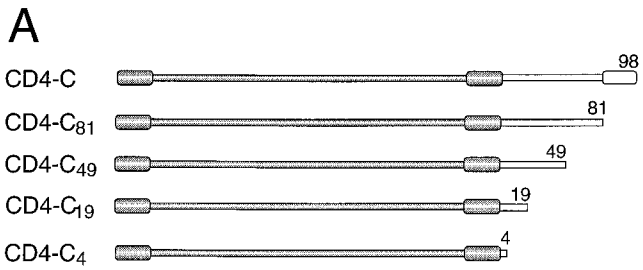

FIG. 4. Expression of C-terminally truncated CD4-G1 tail mutants in BHK-21 cells. (A) Schematic representation of C-terminally truncated CD4-G1 chimeras. The ectodomain and TMD of CD4 were fused to progressive deletions of the G1 cytoplasmic tail by PCR. Translational stop codons were introduced at the cleavage site between G1 and G2 (CD4-C), just before the G2 signal sequence (-C_{81}), and at positions 49 (-C_{49}), 19 (-C_{19}), and 4 (-C_4), counting from the TMD (Fig. 1). (B) The chimeric proteins were expressed in BHK-21 cells by using the SFV system. At 6 h posttransfection, cells were treated with cycloheximide for 3 h. Cells were either fixed and permeabilized with Triton X-100 (a, c, e, g, i, and j) or fixed without permeabilization (b, d, f, and h) prior to staining with a monoclonal antibody against the CD4 ectodomain.

Removal of palmitylation sites does not affect Golgi localization of CD4-G1 chimeras. To study whether palmitylation of the G1 cytoplasmic tail plays any role in targeting CD4-C_{81} to the Golgi complex, we expressed each of the mutants described above in BHK-21 cells. The localization of the chimeric mutant proteins was monitored by indirect immunofluorescence using a monoclonal anti-CD4 antibody. As shown in Fig. 8, all three mutants (C_{81}-C_{25A}, C_{81}-C_{28A}, and C_{81}-$C_{25,28A}$) were targeted to and retained in the Golgi complex (Fig. 8A to C) as efficiently as the nonmutated CD4-C_{81} protein (Fig. 8E). Like the CD4-C_{81} control, the double mutant was not expressed on the cell surface (Fig. 8D and F). Thus, it appears that palmitylation of the G1 tail is not necessary for Golgi localization.

DISCUSSION

Our strategy to determine the basis for Golgi localization of the UUK virus G1 glycoprotein was based on the assumption that a special motif or domain somewhere in G1 harbors a signal for Golgi targeting and retention. By removing such a signal, G1 should be released from the transport block and appear on the cell surface. Conversely, attaching the signal to a reporter protein that is normally transported to the cell surface should result in the retention of the protein in the Golgi. In this study, we have mapped the signal to the cytoplasmic tail of G1 and shown by expression of chimeric proteins that the cytoplasmic tail is both necessary and sufficient for Golgi localization.

Our previous work has shown that the G1 glycoprotein is responsible for keeping the G1-G2 heterodimeric complex in the Golgi complex (26, 40). Similar results have also been obtained for other *Bunyaviridae* members. For all members studied so far, it seems that the Golgi retention signal is present in the protein located N terminally in the glycoprotein precursor. This protein has also been referred to as G_N, while the C-terminally located one has been called G_C (19). The G2 (G_N) glycoprotein of Bunyamwera (19) and La Crosse (4) viruses (members of the *Bunyaviridae* genus) and the G1 (G_N) of Hantaan virus (a hantavirus) (34) and Punta Toro (PT) virus (a phlebovirus) (25) are all retained in the Golgi complex when

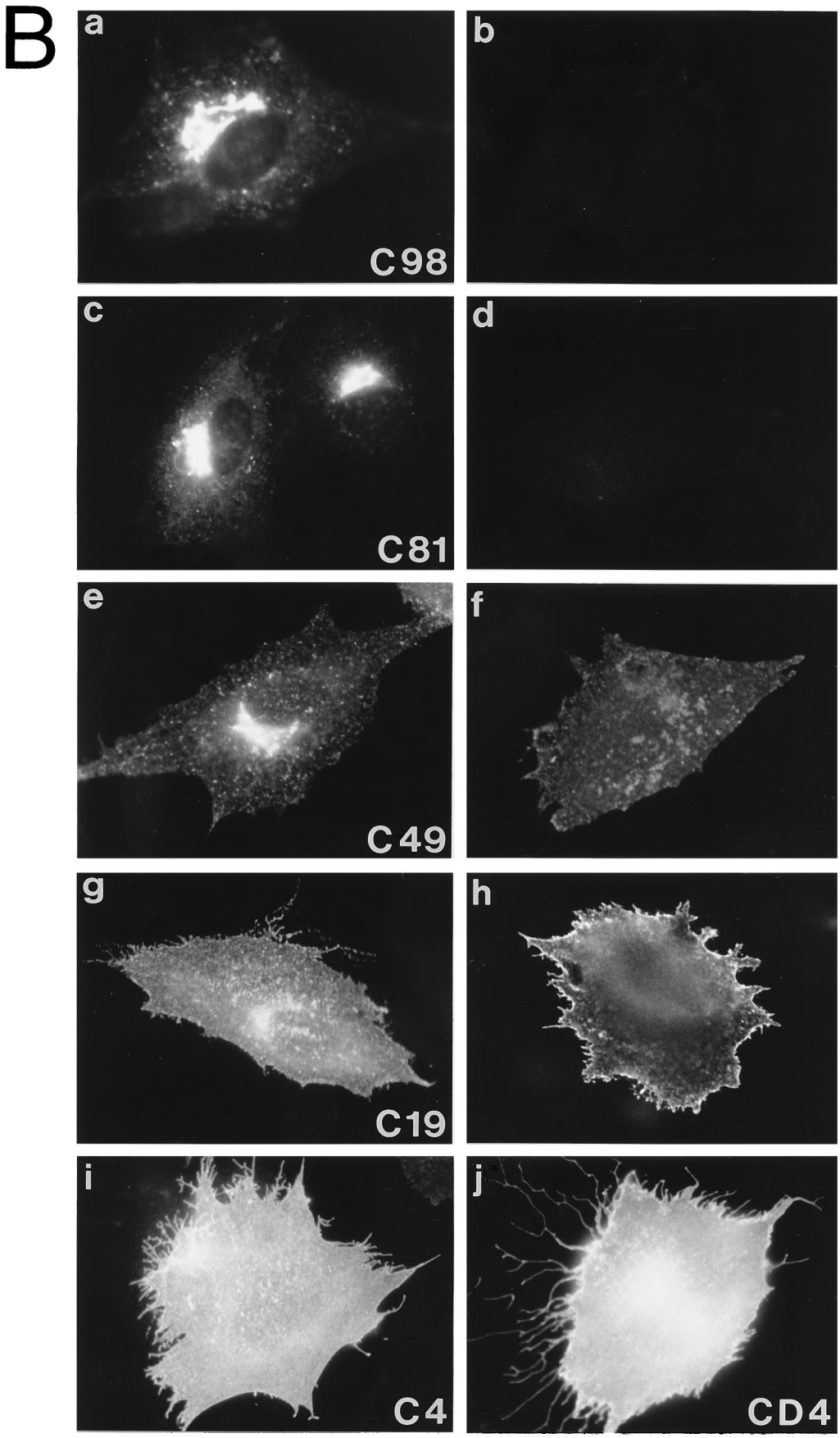

FIG. 4—*Continued.*

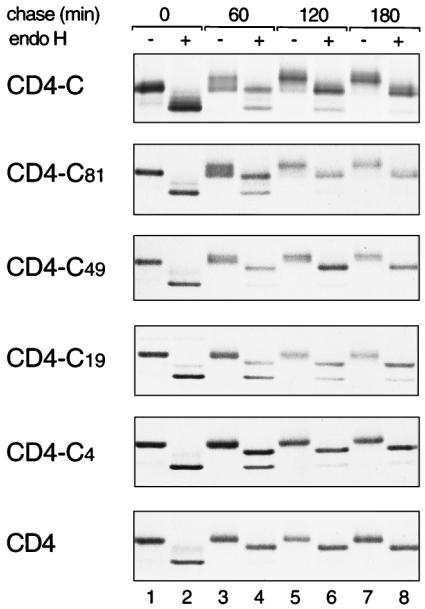

FIG. 5. Kinetics of transport from the ER to or past the medial Golgi complex of C-terminally truncated CD4-G1 tail chimeras. The CD4 and chimeras depicted in Fig. 4A, containing the ectodomain and TM domain of CD4 fused to progressive deletions of the G1 cytoplasmic tail, were expressed in BHK-21 cells by using the SFV system. At 5 h posttransfection, cells were starved in methionine-free medium for 45 min and then pulse-labeled with [^{35}S]methionine for 20 min followed by a chase with an excess of unlabeled methionine for the indicated time periods. Cell lysates were subjected to immunoprecipitation with a polyclonal antiserum against CD4. One half of the precipitates was left untreated and one half was digested with endo H prior to analysis by electrophoresis on an SDS–10 to 15% polyacrylamide gradient gel.

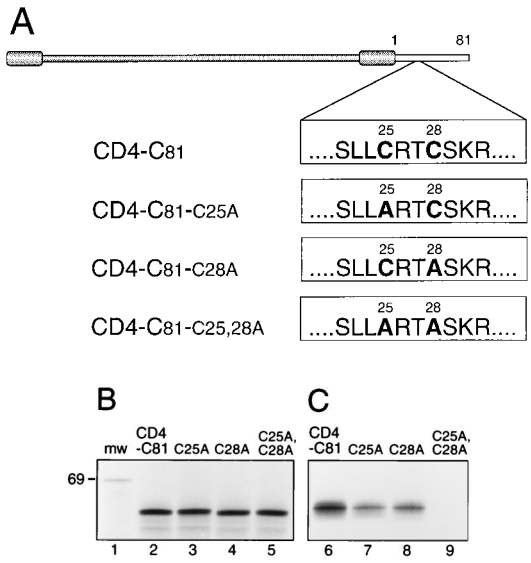

FIG. 7. Identification of the palmitylation sites in the cytoplasmic tail of G1 by site-directed mutagenesis. (A) Chimeric proteins containing the ectodomain and TMD of CD4 fused to the cytoplasmic tail of G1 were subjected to site-directed mutagenesis. The cysteine residues at position 25 (-C_{81}-C25A), position 28 (-C_{81}-C28A), or both positions (-C_{81}-C25,28A) were changed to alanine residues (boldface letters). (B and C) The above-described mutants were expressed in BHK-21 cells by using the SFV system. [^{35}S]methionine-labeled (B, lanes 2 to 5) or [^{3}H]palmitic acid-labeled (C, lanes 6 to 9) proteins were isolated from cell lysates by immunoprecipitation using a polyclonal CD4 antiserum followed by analysis on an SDS–10 to 15% polyacrylamide gradient gel. The position of the 69-kDa marker is shown to the left.

expressed in the absence of G_C. It seems that G_C accumulates in the Golgi complex indirectly by binding to G_N.

By replacing the ectodomain of G1 with either chicken lysozyme (a secreted protein) or the ectodomain of CD4 (a monomeric plasma membrane protein), we could exclude a Golgi retention signal in this domain. Likewise, by replacing the G1 TMD and 10 residues flanking each side of the TMD with the corresponding sequences from VSV G protein, we found that this chimeric protein was still retained in the Golgi complex, indicating no or a minor role of the TMD and flanking residues. This is in striking contrast to the essential role of the TMD and/or lumenal stalk region in retaining resident Golgi glycosyltransferases (all type II membrane proteins) in the Golgi apparatus (see below).

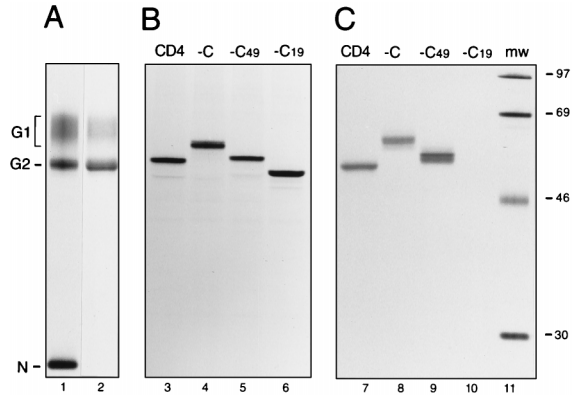

FIG. 6. The cytoplasmic tail of G1 is palmitylated. (A) UUK virus-infected BHK-21 cells were metabolically labeled overnight with either [^{35}S]methionine (lane 1) or [^{3}H]palmitic acid (lane 2), and the purified viruses were analyzed by SDS-PAGE. (B and C) CD4 and three CD4-G1 chimeras with progressive truncations of the G1 cytoplasmic tail (CD4-C, -C_{49}, and -C_{19}) (Fig. 4A) were expressed in BHK-21 cells by using the SFV system. [^{35}S]methionine-labeled (B) or [^{3}H]palmitic acid-labeled (C) proteins were isolated from cell lysates by immunoprecipitation using a polyclonal antiserum against CD4. The immunoprecipitates were analyzed on an SDS–10 to 15% polyacrylamide gradient gel. The positions of molecular weight markers (mw) are shown in kilodaltons to the right. G1 and G2, UUK virus glycoproteins; N, nucleocapsid protein.

Those constructs in which the cytoplasmic tail of G1 was attached to the CD4 reporter protein were targeted to and retained in the Golgi complex, while constructs lacking the G1 tail were expressed on the cell surface. Progressive deletions of the tail, together with the result from the VSV G chimera, tentatively localized the Golgi signal to a region spanning residues 10 to 50, counting from the TMD border toward the C terminus. At this stage, we have not further narrowed down the region essential for retention.

The only *Bunyaviridae* protein for which attempts have been made to map the Golgi retention signal is the G1 protein of PT virus (25). By expressing mutants with progressive deletions from the C terminus, as well as chimeras between G1 and the MCF murine leukemia virus envelope protein, the TMD and the cytoplasmic domain adjacent to the TMD were found to be important. Since we found no role for the TMD of UUK virus G1, the conclusions from the results with PT virus G1 are partly at variance with our results. Although the G1 proteins of UUK and PT viruses (both members of the *Phlebovirus* genus) show a low degree of sequence homology (about 15%) (41), no apparent sequence homology could be found in the proposed TMD or cytoplasmic tail region. The differences in the interpretation of the results may relate to the difficulties in defining exactly the borders between the ectodomain, TMD, and cyto-

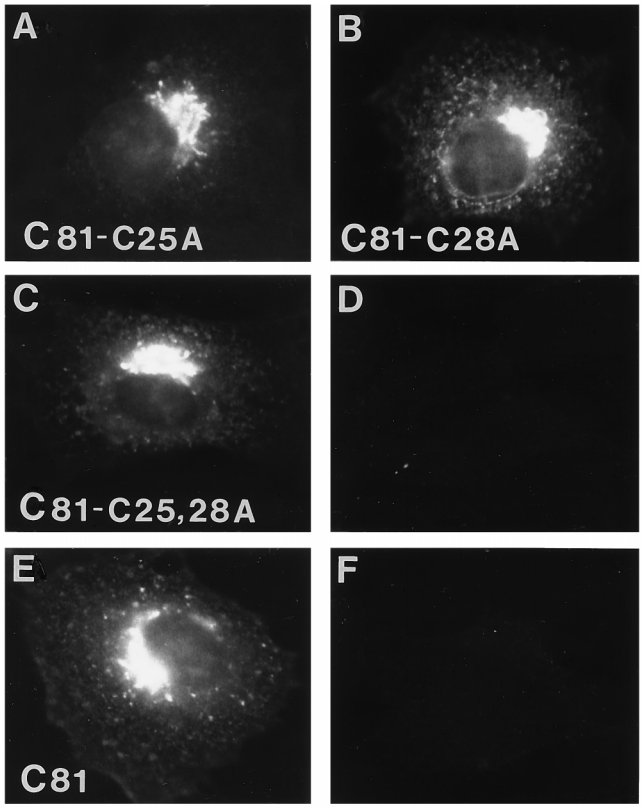

FIG. 8. Removal of palmitylation sites has no affect on the targeting of CD4-G1 tail chimeras to the Golgi complex. The CD4-C_{81} chimeras in which cysteine 25 (A), cysteine 28 (B), or both cysteines (C) (Fig. 7A) were mutated to alanine residues, as well as CD4-C_{81} with both palmitylation sites intact (E), were expressed in BHK-21 cells by using the SFV system. At 6 h posttransfection, cycloheximide was added for 3 h followed by indirect immunofluorescence of permeabilized (A, B, C, and E) or nonpermeabilized (D and F) cells, using a CD4 monoclonal antibody.

plasmic tail of G1 in the two viruses. In the case of UUK virus G1, the TMD has been predicted to consist of a 19-residue-long hydrophobic stretch of amino acids flanked by charged residues (1, 41). However, the sequence upstream from the presumed TMD is also quite hydrophobic, but it is at many points interrupted by charged residues, making it uncertain whether it could span the lipid bilayer. To acquire the correct topology of G1, this upstream region would have to span the membrane twice (1). The proposed length of the cytoplasmic tail of G1 of PT virus is 76 residues (25) (compared to 98 residues for UUK virus G1), assuming that the 15-residue-long internal signal sequence for G2 is not cleaved off similarly to the situation for UUK virus (1). Again, the sequence upstream from the proposed TMD is strikingly hydrophobic. Thus, further analyses of the domain structure and topology of the C-terminal portion of UUK and PT virus G1 proteins are required to allow definite comparison of the sequences specifying Golgi localization.

We identified two cysteine residues in the cytoplasmic tail of G1 as sites for palmitylation. G2 was also labeled with [^{3}H]palmitic acid. Fatty acid acylation usually occurs on cysteine residues located in the cytoplasmic tail or within the TMD of membrane proteins (6, 9, 11, 45, 51, 55). The tail of G2 is only five residues long and does not contain any cysteines (41). The likely site for palmitylation is therefore a cysteine residue within the TMD two residues inward from the TMD-tail border. We found that elimination of palmitylation at either or both sites in the G1 tail had no effect on Golgi retention. At this point, we have not carried out a detailed analysis on the other possible role(s) of fatty acylation of G1. Although the cytoplasmic tails of many viral glycoproteins have been found to be palmitylated, the role of palmitylation remains in most cases unknown. A role in virus formation (11, 56) or fusion activity (30) has been proposed, while other reports have found no role in assembly (13) or biosynthesis (31). It is conceivable that the topology of the UUK virus G1 tail is affected by the presence of palmitates. Thus, it is possible that fatty acylation plays a role in, e.g., virus assembly. Experiments aimed at studying this would require the development of a reverse genetics system for *Bunyaviridae.*

Like members of the *Bunyaviridae* family, coronaviruses and rubella virus also bud into the Golgi complex or the ER-Golgi intermediate compartment. In the case of coronaviruses, the M membrane glycoprotein seems to determine the site of budding. In the avian infectious bronchitis coronavirus, the first (m1) of the three membrane-spanning domains is essential for Golgi retention (22). When the TMD of VSV G is replaced by the infectious bronchitis coronavirus m1 domain, the chimera is retained in the Golgi complex and can be recovered in detergent-insoluble oligomers. Exclusion from transport vesicles due to oligomerization has been proposed as the mechanism for retention (53). In contrast, the Golgi retention signal of the mouse hepatitis coronavirus seems to be composed of two regions: the 22 C-terminal residues of the cytoplasmic tail and the TMDs (2, 21). In the case of rubella virus, the TMD of the E2 membrane glycoprotein has recently been shown to be important for Golgi localization. Again, replacing the TMD of VSV G with that of E2 resulted in the accumulation of the chimeric protein in the Golgi complex (10). The mechanism for retaining the mouse hepatitis coronavirus M protein and rubella virus E2 protein in the Golgi complex has not been elucidated. The conclusion that one can draw from these viral systems is that the domains responsible for targeting a viral protein to the Golgi apparatus are complex and may vary from one protein to another.

The retention signal of resident Golgi glycosyltransferases has been reported to be located mainly in the TMD (28, 29, 48), with some role played also by flanking sequences (5). Two models have been proposed for Golgi retention of these enzymes. According to one, the length (and not the primary sequence per se) of the hydrophobic TMD (15 to 17 residues for Golgi proteins, compared to 20 or more residues for plasma membrane proteins) would result in the segregation of Golgi-specific proteins into cholesterol-poor domains followed by exclusion of the proteins from transport vesicles (3, 28, 29). According to the other model, hetero-oligomerization (kin recognition) between enzymes located in the same cisternae (such as *N*-acetylglucosaminyltransferase I and mannosidase II) would result in hetero-oligomers too large to be included into transport vesicles. Initial results suggested that the TMD played an essential role in this kin recognition (32), but more recent findings with *N*-acetylglucosaminyltransferase I suggest that the stalk region on the lumenal side of the membrane may be exclusively responsible for kin recognition and perhaps also for Golgi retention (33). Interpretation of the results is further complicated by the finding of cell-specific differences in the recognition of Golgi retention signals (49). Thus, the definition of Golgi retention signals and obtaining an understanding of the mechanism by which such signals are capable of retaining proteins in the Golgi have turned out to be very difficult.

At present, the mechanism by which G1 and G2 are retained in the Golgi complex is not known. Our results exclude a role

for the ectodomain of G1 in forming large (transport-incompetent) complexes though lateral interaction of the spikes. Likewise, a role for the TMD comparable to that of glycosyltransferases could also be excluded. Retention could be the result of the cytoplasmic tail interacting with an intercisternal, submembranous Golgi matrix (47). Alternatively, the G1 tail could mediate oligomerization of the spikes, thereby excluding them from transport vesicles. Finally, the tail could contain a retrieval (recycling) signal for keeping the spike complexes in the Golgi region. Such signals have been found in some trans-Golgi network-localized proteins that recycle between the plasma membrane and the Golgi complex (38, 44). In summary, the localization of a Golgi retention signal to the cytoplasmic tail of UUK virus G1 may serve as a useful model to elucidate some aspects of retention of Golgi-specific proteins.

ACKNOWLEDGMENTS

We thank Anita Bergström for excellent technical assistance. We are grateful to Bernard Moss for the VV vTF7-3 and plasmid pTF7-5, Henrik Garoff and Peter Liljeström for plasmid pSFV1, Sean Munro for the lysozyme cDNA, Mark Marsh for the CD4 cDNA, and M. G. Farquhar and K. W. Moreman for antiserum against α-mannosidase II.

REFERENCES

1. **Andersson, A. M., L. Melin, R. Persson, E. Raschperger, L. Wikström, and R. F. Pettersson.** 1997. Processing and membrane topology of the spike proteins G1 and G2 of Uukuniemi virus. J. Virol. **71:**218–225.
2. **Armstrong, J., and S. Patel.** 1991. The Golgi sorting domain of coronavirus E1 protein. J. Cell Sci. **98:**567–575.
3. **Bretscher, M. S., and S. Munro.** 1993. Cholesterol and the Golgi apparatus. Science **261:**1280–1281.
4. **Bupp, K., K. Stillmock, and F. Gonzalez-Scarano.** 1996. Analysis of the intracellular transport properties of recombinant La Crosse virus glycoproteins. Virology **220:**485–490.
5. **Burke, J., J. M. Pettit, H. Schachter, M. Sarkas, and P. A. Gleeson.** 1992. The transmembrane and flanking sequences of β1, 2-N-acetylglucosaminyltransferase specify medial-Golgi localization. J. Biol. Chem. **267:** 24433–24440.
6. **Crise, B., and J. K. Rose.** 1992. Identification of palmitoylation sites on CD4, the human immunodeficiency virus receptor. J. Biol. Chem. **267:**13593–13597.
7. **Elliott, R. M., C. S. Schmaljohn, and M. S. Collett.** 1991. Bunyaviridae genome structure and gene expression. Curr. Top. Microbiol. Immunol. **169:**91–141.
8. **Elroy-Stein, O., T. Fuerst, and B. Moss.** 1989. Cap-independent translation of mRNA conferred by encephalomyocarditis virus 5′ sequence improves the performance of the vaccinia virus/bacteriophage T7 hybrid expression system. Proc. Natl. Acad. Sci. USA **86:**6126–6130.
9. **Funke, C., S. Becker, H. Dartsch, H.-D. Klenk, and E. Mühlberger.** 1995. Acylation of the Marburg virus glycoprotein. Virology **208:**289–297.
10. **Hobman, T. C., L. Woodward, and M. G. Farquhar.** 1995. Targeting of a heterodimeric membrane protein complex to the Golgi complex: rubella virus E2 glycoprotein contains a transmembrane Golgi retention signal. Mol. Biol. Cell **6:**7–20.
11. **Ivanova, L., and M. J. Schlesinger.** 1993. Site-directed mutations in the Sindbis virus E2 glycoprotein identify palmitoylation sites and affect virus budding. J. Virol. **67:**2546–2551.
12. **Jasmin, B. J., J. Cartaud, M. Bornens, and J. P. Changeux.** 1989. Golgi apparatus in chick muscle: changes in its distribution during end plate development and after conversion. Proc. Natl. Acad. Sci. USA **86:**7218–7222.
13. **Jin, H., K. Subbaro, S. Bagai, G. P. Leser, B. R. Murphy, and R. A. Lamb.** 1996. Palmitylation of the influenza virus hemagglutinin (H3) is not essential for virus assembly or infectivity. J. Virol. **70:**1406–1414.
14. **Klumperman, J., J. K. Locker, A. Meijer, M. C. Horzinek, H. J. Geuze, and P. J. M. Rottier.** 1994. Coronavirus M proteins accumulate in the Golgi complex beyond the site of virion budding. J. Virol. **68:**6523–6534.
15. **Kuismanen, E.** 1984. Posttranslational processing of Uukuniemi virus glycoproteins G1 and G2. J. Virol. **51:**806–812.
16. **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.
17. **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.
18. **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.
19. **Lappin, D. F., G. W. Nakitare, J. W. Palfreyman, and R. M. Elliott.** 1994. Localization of Bunyamwera bunyavirus G1 glycoprotein to the Golgi requires association with G2 but not with NSm. J. Gen. Virol. **75:**3441–3451.
20. **Liljeström, P., and H. Garoff.** 1991. A new generation of animal cell expression vectors based on the Semliki Forest virus replicon. Bio/Technology **9:**1356–1361.
21. **Locker, J. K., J. Klumperman, V. Oorschot, M. C. Horzinek, H. J. Geuze, and P. J. M. Rottier.** 1994. The cytoplasmic tail of mouse hepatitis virus M protein is essential but not sufficient for its retention in the Golgi complex. J. Biol. Chem. **269:**28263–28269.
22. **Machamer, C. E., and J. K. Rose.** 1987. A specific transmembrane domain of a coronavirus E1 glycoprotein is required for its retention in the Golgi region. J. Cell Biol. **105:**1205–1214.
23. **Maddon, P. J., D. R. Littman, M. Godfrey, D. E. Maddon, L. Chess, and R. Axel.** 1985. The isolation and nucleotide sequence of a cDNA encoding the T cell surface protein T4: a new member of the immunoglobulin gene family. Cell **33:**153–159.
24. **Maizel, J. V., Jr.** 1971. Polyacrylamide gel electrophoresis of viral proteins. Methods Virol. **5:**179–246.
25. **Matsuoka, Y., S.-Y. Chen, and R. W. Compans.** 1995. A signal for Golgi retention in the Bunyavirus G1 glycoprotein. J. Biol. Chem. **269:**22565–22573.
26. **Melin, L., R. Persson, A. Andersson, A. Bergström, R. Rönnholm, and R. F. Pettersson.** 1995. The membrane glycoprotein G1 of Uukuniemi virus contains a signal for localization to the Golgi complex. Virus Res. **36:**49–66.
27. **Moreman, K. W., O. Touster, and P. W. Robbins.** 1991. Novel purification of the catalytic domain of Golgi α-mannosidase II. Characterization and comparison with the intact enzyme. J. Biol. Chem. **266:**16876–16885.
28. **Munro, S.** 1991. Sequences within and adjacent to the transmembrane segment of α-2,6-sialyltransferase specify Golgi retention. EMBO J. **10:**3577–3588.
29. **Munro, S.** 1995. An investigation of the role of transmembrane domains in Golgi protein retention. EMBO J. **14:**4695–4704.
30. **Naeve, C. W., and D. Williams.** 1990. Fatty acids on the A/Japan/305/57 influenza virus hemagglutinin have a role in membrane fusion. EMBO J. **9:**3857–3866.
31. **Naim, H. Y., B. Amarneh, N. T. Ktistakis, and M. G. Roth.** 1992. Effects of altering palmitylation sites on biosynthesis and function of the influenza virus hemagglutinin. J. Virol. **66:**7585–7588.
32. **Nilsson, T., M. H. Hoe, P. Slusarewicz, C. Rabouille, R. Watson, F. Hunte, G. Watzele, E. G. Berger, and G. Warren.** 1994. Kin recognition between medial Golgi enzymes in HeLa cells. EMBO J. **13:**562–574.
33. **Nilsson, T., C. Rabouille, N. Hui, R. Watson, and G. Warren.** 1996. The role of the membrane-spanning domain and stalk region of N-acetylglucosaminyltransferase I in retention, kin recognition and atructural maintenance of the Golgi apparatus in HeLa cells. J. Cell Sci. **109:**1975–1989.
34. **Pensiero, M. N., and J. Hay.** 1992. The Hantaan virus M-segment glycoproteins G1 and G2 can be expressed independently. J. Virol. **66:**1907–1914.
35. **Persson, R., and R. F. Pettersson.** 1991. Formation and intracellular transport of a heterdimeric viral spike protein complex. J. Cell Biol. **112:**257–266.
36. **Pettersson, R. F.** 1991. Protein localization and viral assembly at intracellular membranes. Curr. Top. Microbiol. Immunol. **170:**67–104.
37. **Pettersson, R. F., and L. Melin.** 1996. Synthesis, assembly, and intracellular transport of *Bunyaviridae* membrane proteins, p. 159–188. *In* R. M. Elliott (ed.), The *Bunyaviridae*. Plenum Press, New York, N.Y.
38. **Ponnambalam, S., C. Rabouille, J. P. Luzio, and T. Nilsson.** 1994. The TGN38 glycoprotein contains two non-overlapping signals that mediate localization of the trans-Golgi network. J. Cell Biol. **125:**253–268.
39. **Rönkä, H., P. Hildén, C.-H. von Bonsdorff, and E. Kuismanen.** 1995. Homodimeric association of the spike glycoproteins G1 and G2 of Uukuniemi virus. Virology **211:**241–250.
40. **Rönnholm, R.** 1992. Localization to the Golgi complex of Uukuniemi virus glycoproteins G1 and G2 expressed from cloned cDNAs. J. Virol. **66:**4525–4531.
41. **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.
42. **Rose, J. K., and C. Gallione.** 1981. Nucleotide sequences of the mRNAs encoding the VSV G and M proteins as determined from cDNA clones containing the complete coding regions. J. Virol. **39:**519–528.
43. **Rose, J. K., and J. E. Bergmann.** 1982. Expression from cloned cDNA of cell-surface secreted forms of the glycoprotein of vesicular stomatitis virus in eucaryotic cells. Cell **30:**753–762.
44. **Schäfer, W., A. Stroh, S. Berghöfer, J. Seiler, M. Vey, M.-L. Kruse, H. F. Kern, H.-D. Klenk, and W. Garten.** 1995. Two independent targeting signals in the cytoplasmic domain determine trans-Golgi network localization and endosomal trafficking of the proprotein convertase furin. EMBO J. **14:**2424–2435.
45. **Schmidt, M. G. F.** 1989. Fatty acylation of proteins. Biochim. Biophys. Acta **988:**411–426.
46. **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.
47. **Slusarewicz, P., T. Nilsson, N. Hui, R. Watson, and G. Warren.** 1994. Isolation of a matrix that binds medial Golgi enzymes. J. Cell Biol. **124:**405–413.
48. **Tang, B. L., S. H. Wong, S. H. Low, and W. Hong.** 1992. The transmembrane domain of N-glucosaminyltransferase I contains a Golgi retention signal. J. Biol. Chem. **267:**10122–10126.
49. **Tang, B. L., S. H. Low, S. H. Wong, and W. Hong.** 1995. Cell type differences in Golgi retention signals for transmembrane proteins. Eur. J. Cell Biol. **66:**365–374.
50. **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.
51. **Veit, M., E. Kretzschmar, K. Kuroda, W. Garten, M. F. G. Schmidt, H.-D. Klenk, and R. Rott.** 1991. Site-specific mutagenesis identifies three cysteine residues in the cytoplasmic tail as acylation sites of influenza virus hemagglutinin. J. Virol. **65:**2491–2500.
52. **Velasco, A., L. Hendricks, K. W. Moreman, D. R. P. Tulsiani, O. Touster, and M. G. Farquhar.** 1993. Cell type-dependent variation of the subcellular distribution of α-mannosidase II. J. Cell Biol. **122:**39–51.
53. **Weisz, O. A., A. M. Swift, and C. E. Machamer.** 1993. Oligomerization of a membrane protein correlates with its retention in the Golgi complex. J. Cell Biol. **122:**1185–1196.
54. **Wikström, L., R. Persson, and R. F. Pettersson.** 1989. Intracellular transport of the G1 and G2 membrane glycoproteins of Uukuniemi virus, p. 33–41. *In* D. Kolakofsky and B. W. J. Mahy (ed.), Genetics and pathogenicity of negative-strand viruses. Elsevier, New York, N.Y.
55. **Yang, C., and R. W. Compans.** 1996. Palmitoylation of the murine leukemia virus envelope glycoprotein transmembrane subunits. Virology **221:**87–97.
56. **Zurcher, T., G. Luo, and P. Palese.** 1994. Mutations at palmitylation sites of the influenza virus hemagglutinin affect virus formation. J. Virol. **68:**5748–5754.