JOURNAL OF VIROLOGY, Nov. 1997, p. 8397–8404 Vol. 71, No. 11
0022-538X/97/$04.00+0

Structure-Function Analysis of the gE-gI Complex of Feline Herpesvirus: Mapping of gI Domains Required for gE-gI Interaction, Intracellular Transport, and Cell-to-Cell Spread

J. D. F. MIJNES, B. C. H. LUTTERS, A. C. VLOT, E. VAN ANKEN, M. C. HORZINEK, P. J. M. ROTTIER, AND R. J. DE GROOT*

Virology Unit, Department of Infectious Diseases and Immunology, Veterinary Faculty, Utrecht University, 3584 CL Utrecht, The Netherlands

Received 23 June 1997/Accepted 12 August 1997

Alphaherpesvirus glycoproteins gE and gI form a noncovalently associated hetero-oligomeric complex, which is involved in cell-to-cell spread. In the absence of gI, feline herpesvirus (FHV) gE is transport incompetent and fully retained in the endoplasmic reticulum. Here, we assess the effect of progressive C-terminal truncations of FHV gI on the biosynthesis, intracellular transport, and function of the gE-gI complex. The truncated gI proteins were coexpressed with gE in the vaccinia virus-based vTF7-3 expression system. The results were corroborated and extended by studying FHV recombinants expressing truncated gI derivatives. The following conclusions can be drawn. (i) Deletion of the cytoplasmic tail, the transmembrane region plus the C-terminal half of the ectodomain of gI, does not affect intracellular transport of gE. Apparently, the N-terminal 166 residues of gI constitute a domain involved in gE-gI interaction. (ii) A region mediating stable association with gE is located within the N-terminal 93 residues of gI. (iii) The cytoplasmic domain of gI is not essential for gE-gI-mediated cell-to-cell transmission of FHV, as judged from plaque morphology. Deletion of the cytoplasmic tail of gI reduced plaque size by only 35%. (iv) Recombinants expressing the N-terminal 166 residues of gI display a small-plaque phenotype but produce larger plaques than recombinants with a disrupted gI gene. Thus, a complex consisting of gE and the N-terminal half of the gI ectodomain may retain residual biological activity. The implications of these findings for gE-gI interaction and function are discussed.

The *Alphaherpesvirinae* subfamily, a group of large enveloped DNA viruses, comprises several important pathogens of mammals and birds (for a review, see reference 35). Among these are herpes simplex virus and varicella-zoster virus affecting humans, pseudorabies virus affecting swine, bovine herpesvirus 1 affecting cattle, and feline herpesvirus (FHV), the causative agent of feline rhinotracheitis (for a review, see reference 33). The viral genome is linear, 130 to 150 kb in length, and contains more than 75 genes, a number of which encode glycoproteins. Several glycoprotein species have been shown to be essential for the infection of cultured cells. For example, gB, gH, and gL are involved in entry and mediate pH-independent fusion of the viral envelope with the plasma membrane of the target cell (for a review, see reference 39).

Membrane glycoproteins gE and gI are commonly regarded as "dispensable," as their genes can be deleted from the viral genome with little or no effect on replication in vitro (3, 12, 28, 32, 40, 45). However, they do play an important role in vivo. Mutant viruses lacking gE and/or gI are attenuated and, both in the natural and in heterologous hosts, produce smaller primary lesions, cause milder clinical signs, and exhibit decreased neurovirulence compared to the wild-type virus (8, 9, 12, 13, 16, 23, 24, 31, 34, 40, 41, 43, 45).

gE and gI form a noncovalently associated hetero-oligomeric complex (22, 30, 43, 44, 47, 49). Although its precise function is unknown, there is ample evidence that gE-gI promotes cell-to-cell transmission. Typically, viruses deficient in either glycoprotein display a small-plaque phenotype (3, 12, 21, 28, 30, 32, 40, 48) and are impaired in virus-induced cell-cell fusion (3, 11–13, 48). The mechanism of cell-to-cell spread is by no means understood at the molecular level, but this mode of infection differs in several respects from entry via the extracellular route. It apparently entails the transfer of virus across cell junctions in a manner resistant to neutralizing antibodies (12). Moreover, viruses deficient in either gE or gI bind to and enter the target cell with an efficiency equal to and with kinetics similar to those of the parental wild-type virus (3, 12, 48).

We have recently identified the genes for gE and gI of FHV and characterized their expression products both in infected cells and in the vaccinia virus vTF7-3 expression system (30). FHV gE and gI show all the characteristics of class I membrane proteins. In accordance with findings made for other alphaherpesviruses (43, 44, 47), the FHV proteins become N-glycosylated and oligomerize shortly after synthesis in the endoplasmic reticulum (ER) (30). The resulting gE-gI complex is then transported through the Golgi apparatus to the plasma membrane, concomitantly acquiring extensive posttranslational modifications, including O-glycosylation. In the absence of gE, gI is also transported to the plasma membrane, albeit inefficiently. Transport of FHV gE, however, is dependent on the presence of gI. gE, when expressed in the absence of gI, is fully retained in the ER. Similarly, in cells infected with a gI-deficient recombinant FHV only the endoglycosidase H-sensitive 83-kDa ER species is produced and maturation of gE does not occur (30). Here, we have studied gE-gI interaction in further detail. By C-terminal deletion mutagenesis of gI, we show that the N-terminal half of the gI ectodomain is sufficient

* Corresponding author. Phone: 31-30-2532460. Fax: 31-30-2536723. E-mail: R.Groot@vetmic.dgk.ruu.nl.

TABLE 1. Oligonucleotide primers

Primer	Nucleotide sequence (5′ to 3′)	Gene	Position[a]	Polarity
370	atacttaatatgtcgtcgatagcc	gI	−9–15	+
547	tatgaattcttaggatgtgtttatggaaag	gI	340–357	−
617	atattaatcagctttgttattatgg	gI	438–456	−
698	taggatcctaatatatttataaacaaata	gI	1153–1173	+
699	taacgcgttaatatatttataaacaaataaaat	gI	1153–1177	+
700	tgaatagtagaacttgatga	gE	46–65	−

[a] Numerical position on the genome of FHV B927 as counted from the initiation codon of the gE or gI gene.

to induce gE maturation, thus defining a gI domain involved in gE-gI complex formation. The effect of C-terminal deletions in gI on cell-to-cell transmission is discussed.

MATERIALS AND METHODS

Cells, viruses, antisera, and plasmids. Cells were maintained in Dulbecco's modified Eagle's medium (Gibco BRL, Life Technologies, Inc.) supplemented with 10% fetal calf serum and 100 IU of penicillin and 100 μg of streptomycin per ml (DMEM–10% FCS). FHV strain B927 (19) was obtained from D. A. Harbour and propagated in Crandell feline kidney (CRFK) cells (American Type Culture Collection). Recombinant vaccinia virus vTF7-3, expressing the bacteriophage T7 RNA polymerase (18), was obtained from B. Moss and propagated in RK-13 cells. Transient expression experiments were performed in OST7-1 cells (15). The monospecific rabbit antisera against FHV gE (Ra-αgE) and gI (Ra-αgI), the polyclonal cat antiserum against FHV (Cat-αFHV), and plasmids pBS-gE and pBS-gI have all been described previously (30).

Recombinant DNA techniques. Recombinant DNA techniques were performed according to the methods of Sambrook et al. (37) and Ausubel (2). Sequence analysis was performed with the T7 sequencing kit (Pharmacia Biotech). PCR was performed as described previously (36) with the thermostable DNA polymerase of *Thermus aquaticus* (Promega) according to the instructions of the manufacturer.

Deletion mutagenesis of pBS-gI. Plasmid pBS-gI was constructed by inserting the PCR-amplified FHV gI gene into *Eco*RV-digested pBluescript SK$^-$ (pBS-SK$^-$) downstream of the T7 promoter. pBS-gIΔM and -ΔB were made by cutting pBS-gI with either *Mlu*I (position 497) or *Bam*HI (position 926), respectively, and with *Xba*I at a site downstream of the gI gene within the polylinker region of pBS-SK$^-$. The 3′ recessive ends were filled in by using the large fragment of DNA polymerase I and then joined by blunt-end ligation. pBS-gIΔXb was created by directly ligating the *Xba*I site at position 1122 of the gI gene to the *Xba*I site in the polylinker region. pBS-gIΔC was made by ligating the *Cla*I site at position 635 of the gI gene to the *Cla*I site in the polylinker region. pBS-gIΔN and pBS-gIΔP were constructed by cutting pBS-gI with either *Nde*I (position 601) or *Pml*I (position 277), respectively, and with *Sma*I at a site in the polylinker region. Prior to ligation, the 3′ recessive end generated by *Nde*I was made blunt ended by using the large fragment of DNA polymerase I. pBS-gIΔXh was constructed by fusing the blunted *Xho*I site at position 206 to the blunted *Bam*HI site in the polylinker region. Finally, pBS-gI119 and pBS-gI152 were constructed by amplifying nucleotides −9 through 357 and −9 through 456 of the gI gene, respectively, by PCR with oligonucleotide primers 370 and 547 or 617 (Table 1). The PCR products were cloned into *Eco*RV-digested pBS-SK$^-$. As a result of the deletions and the nucleotide changes made, termination codons were created immediately downstream of each of the truncated gI genes. A sequence analysis of the relevant regions of each construct confirmed that no inadvertent changes had been introduced during cloning or during PCR amplification.

Transfection of vTF7-3-infected cells and metabolic labeling. Subconfluent monolayers of OST7-1 cells grown in 35-mm-diameter dishes were washed once with DMEM and infected with vaccinia virus vTF7-3 at a multiplicity of infection of 3 in DMEM at 37°C. One hour postinfection (p.i.), the cells were washed with DMEM and transfected with plasmid DNA as follows. A transfection mixture consisting of 2 to 5 μg of plasmid DNA, 500 μl of DMEM, and 10 μl of Lipofectin (Gibco BRL, Life Technologies, Inc.) was added to the monolayers. After a 5-min incubation at room temperature, 500 μl of DMEM was added and incubation was continued at 37°C. At 2 h p.i., the incubation temperature was lowered to 32°C. From 4 to 5 h p.i., the cells were incubated with 1 ml of minimum essential medium with Earle's salts, lacking cysteine and methionine (Gibco BRL, Life Technologies, Inc.). Then, 100 μCi of Redivue PRO-MIX [^{35}S] cell labeling mixture (^{35}S[Met+Cys]; Amersham) was added to the culture medium and the incubation was continued for 1 h. The cells were harvested either immediately or after a 2-h chase with DMEM–10% FCS containing 5 mM (each) L-methionine and L-cysteine.

Metabolic labeling of FHV-infected cells. Subconfluent monolayers of CRFK cells in 35-mm-diameter dishes were washed once with DMEM and infected with either wild-type FHV strain B927 or recombinant FHV at a multiplicity of infection of 5 at 37°C. At 1 h p.i., the culture supernatant was replaced by DMEM–10% FCS and the incubation was continued. Metabolic labeling was done as described for vTF7-3-infected cells, except that the methionine and cysteine depletion procedure and subsequent labeling procedures were performed 2 h later and at 37°C.

RIPA and SDS-PAGE. Metabolically labeled cells were washed once with ice-cold phosphate-buffered saline (PBS) and then lysed on ice in 600 μl of lysis buffer (20 mM Tris-Cl [pH 7.5], 1 mM EDTA, 100 mM NaCl, 1% Triton X-100) containing 1 μg of pepstatin A, 40 μg of aprotinin, and 1 μg of leupeptin per ml. Nuclei and cell debris were pelleted for 1 min at 10,000 × *g* and 4°C. Of the supernatant, 200 μl was mixed with 1 ml of Detergent Mix (50 mM Tris-Cl [pH 8.0], 62.5 mM EDTA, 0.4% sodium deoxycholate, 1% Nonidet P-40), sodium dodecyl sulfate (SDS) was added to a final concentration of 0.25% unless indicated otherwise, and incubation was performed for 15 min on ice. Subsequently, the antisera were added (Cat-αFHV and Ra-αgE: 3 μl; Ra-αgI: 5 μl) and the mixtures were incubated for 16 h at 4°C. Immune complexes were collected with 50 μl of a 10% (wt/vol) suspension of formalin-fixed *Staphylococcus aureus* cells (Pansorbin; Calbiochem) in Detergent Mix. After a 30-min incubation at 4°C, they were washed three times with radioimmunoprecipitation assay (RIPA) buffer (10 mM Tris-Cl [pH 7.4], 150 mM NaCl, 0.1% SDS, 1% sodium deoxycholate, 1% Nonidet P-40). Treatment of immunoprecipitated proteins with endoglycosidase H (Endo-H; Boehringer Mannheim) was performed according to the method of Machamer et al. (26). Finally, the proteins were taken up in 30 μl of Laemmli sample buffer containing 5% β-mercaptoethanol, heated for 5 min at 95°C, and analyzed in SDS-polyacrylamide gel electrophoresis (PAGE) gels.

Construction of FHV recombinants. FHVΔgI-LZ has been described previously (30). In this mutant, the gI gene has been disrupted by replacing nucleotides 203 to 923 with an expression cassette consisting of the LacZ gene under the control of the encephalomyocarditis virus (EMCV) internal ribosomal-entry site (IRES). To restore the gI locus of FHVΔgI-LZ or to introduce a gI gene truncated either at nucleotide position 498 (gIΔM) or 927 (gIΔB), we constructed the transfer vectors pUS1, pUS1-gIΔM, and pUS1-gIΔB. pUS1 contains a 7-kb *Eco*RV-*Bam*HI fragment spanning the genes for gD, gI, gE, US8.5, and US9 and approximately 2 kb of the terminal repeat sequence (38, 46).

pUS1-gIΔM was constructed by replacing the sequences between the *Mlu*I site in the gI gene and the *Xma*I site upstream of the gE gene by an *Mlu*I-*Xma*I-digested PCR product which had been generated by amplifying the gE-gI intergenic region with oligonucleotide primers 699 and 700 (Table 1). This product contained a newly created *Mlu*I site at its 5′ end immediately followed by the authentic termination codon of the gI gene. pUS1-gIΔB was constructed similarly, i.e., by replacing the sequences between the *Bam*HI site in the gI gene and the *Xma*I site with a *Bam*HI-*Xma*I-digested PCR product which had been generated with oligonucleotide primers 698 and 700 (Table 1). A sequence analysis of the relevant regions of pUS1-gIΔM and pUS1-gIΔB showed that these plasmids had the desired structure and that no inadvertent mutations had been introduced during the cloning procedures.

To generate recombinant viruses FHV-gI*rev*, FHV-gIΔM, and FHV-gIΔB, 10^6 CRFK cells, seeded in 35-mm-diameter dishes, were subjected to cotransfection with approximately ± 50 ng of FHVΔgI-LZ DNA and 1 to 2 μg of transfer vector DNA. The tissue culture supernatants were harvested 7 days after transfection, and plaque assays were performed. Recombinant viruses that had lost the EMCV IRES-LacZ expression cassette were identified by in situ staining of plaques with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) as a substrate and were plaque purified three times prior to the preparation of virus stocks. In each case, proper insertion of the genes was confirmed by Southern blot analysis.

Plaque assays and immunohistochemistry. Confluent monolayers of CRFK cells grown in 35-mm-diameter dishes were washed with DMEM and infected with 50 to 100 PFU of either wild-type FHV B927 or recombinant FHV at 37°C. At 1 h p.i., the cells were washed three times with PBS and a solid-phase overlay of 2 ml of DMEM–10% FCS containing 1.5% agar was applied. Incubation was continued at 37°C. At 72 h p.i., the cells were fixed with 1 ml of 9% paraformaldehyde for 1 h at room temperature. Subsequently, the overlay was removed and the cells were washed with PBS–10 mM glycine. The cells were permeabilized with 1% Triton X-100 and then incubated with Cat-αFHV, diluted 1:500 in PBS–0.5% Tween 80–5% FCS. Bound antibodies were detected by peroxidase staining with horseradish peroxidase-conjugated goat anti-cat immunoglobulin G (Cappel), diluted 1:500, as a conjugate and 3-amino-9-ethylcarbazole as a substrate. To determine the average plaque size, 25 randomly chosen plaques were measured along the *x* and *y* axes at a 20-fold magnification. The average plaque size in millimeters squared was then calculated from the mean radius (*r*) by using the term πr^2.

RESULTS

A gI derivative truncated at residue 166 induces intracellular transport of gE. To identify domains in gI required for the formation and intracellular transport of the gE-gI hetero-oligomer, we constructed a set of expression plasmids encoding gI derivatives with progressive C-terminal deletions (Fig. 1). The

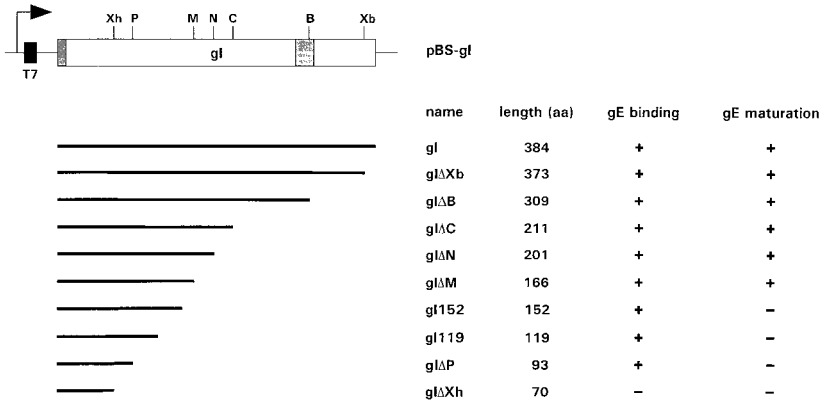

FIG. 1. Construction of a set of expression plasmids encoding gI derivatives with progressive C-terminal deletions. The upper panel shows a schematic representation of the gI gene as cloned in plasmid pBS-gI. The gene is represented by an open box. The hatched regions indicate the nucleotide sequences coding for the N-terminal signal peptide and the transmembrane region. Restriction sites are abbreviated as follows: Xh, *Xho*I; P, *Pml*I, M, *Mlu*I; N, *Nde*I; C, *Cla*I; B, *Bam*HI; Xb, *Xba*I. The bacteriophage T7 RNA polymerase promoter is represented by a black box. The arrow indicates the direction of transcription. The lower panel shows the structures of the various gI deletion constructs and the sizes of the encoded polypeptides, which include the predicted cleavable N-terminal signal sequence. Also summarized are the effects of the gI mutations on the binding to and the maturation of gE. aa, amino acids.

truncated gI genes were tested for proper expression in the vaccinia virus-based vTF7-3 system (18). vTF7-3-infected OST7-1 cells were transfected with plasmid DNA at 1 h p.i. and labeled with ^{35}S[Met+Cys] from 5 to 6 h p.i. Cell lysates were subjected to immunoprecipitation with an antiserum directed against residues 20 to 36 of gI (Ra-αgI), and the immunoprecipitates were analyzed in SDS–15% PAGE gels. In the case of gIΔXb, -ΔB, and -ΔP single products of the anticipated sizes of 65, 58, and 7.5 kDa, respectively, were found (Fig. 2a). The detection of the 6-kDa gIΔXh protein was somewhat unexpected. pBS-gIΔXh encodes a protein of this size, but this product contains no Met or Cys residues other than the initiator Met at its N terminus. It would thus appear that the gIΔXh protein seen in Fig. 2a still retained its signal peptide. Perhaps, because of its small size, gIΔXh becomes trapped in the translocon and is not released into the lumen of the ER (10). Expression of gIΔM and gI-152 yielded four products each. Treatment with Endo-H revealed that these represent differentially glycosylated protein species (Fig. 2b and data not shown). Potential N-glycosylation sites are present in gI at amino acid positions 44, 65, and 117. Apparently, the 17- and 15-kDa products of gIΔM and gI-152, respectively, represent the nonglycosylated protein backbones, whereas the Endo-H-sensitive larger products carry either one, two, or three N-linked glycans. As illustrated for gIΔM (Fig. 2b), the gI derivatives did not acquire Endo-H resistance following a 2-h chase and, like the membrane-anchored intact gI, were not efficiently transported from the ER in the absence of gE.

Each of the truncated gI proteins was coexpressed with gE. Metabolic labeling was performed for 1 h, followed by a 2-h chase to allow for complex formation and intracellular transport. RIPA was performed now with a gE-specific rabbit anti-

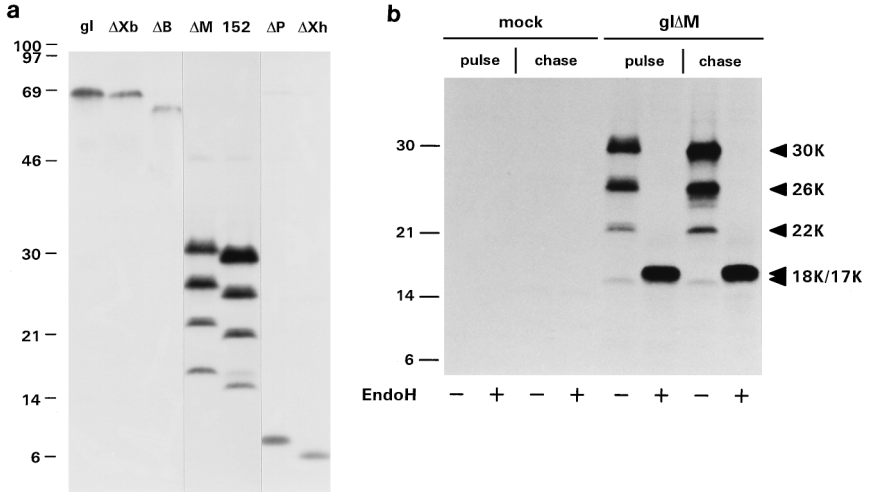

FIG. 2. Biosynthesis of C-terminally truncated gI derivatives as studied by heterologous expression. (a) Monolayers of OST7-1 cells were infected with vaccinia virus vTF7-3 and transfected with plasmid DNA encoding either full-length gI (gI) or the truncated gI derivatives, gIΔXb, -ΔB, -ΔM, -152, -ΔP, and -ΔXh. Cells were metabolically labeled with ^{35}S[Met+Cys] from 5 to 6 h p.i., and cell lysates were subjected to RIPA with the gI-specific antiserum, Ra-αgI. (b) Biochemical analysis of gIΔM. vTF7-3-infected OST7-1 cells were mock transfected or transfected with pBS-gIΔM and were metabolically labeled as described above. The cells were harvested either immediately after labeling (pulse) or after a 2-h chase (chase). gIΔM, immunoprecipitated with Ra-αgI, was either treated with Endo-H (+) or mock treated (−). Note that the Endo-H-deglycosylated 18-kDa form of gIΔM still carries *N*-acetylglucosamine moieties and thus migrates slightly slower than the unglycosylated 17-kDa form. The proteins were separated in SDS–15% PAGE gels. Molecular sizes are in kilodaltons (K).

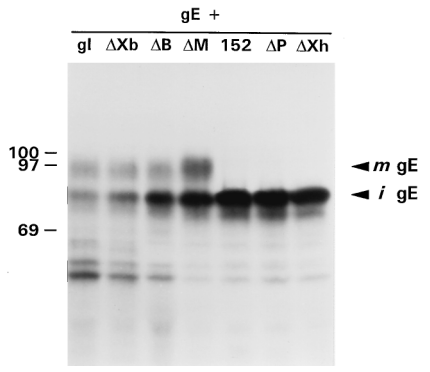

FIG. 3. Coexpression of gE and C-terminally truncated gI derivatives in vTF7-3-infected cells. vTF7-3-infected OST7-1 cells were transfected with plasmid pBS-gE and each of the pBS-gI derivatives listed in Fig. 1. The cells were metabolically labeled from 5 to 6 h p.i., followed by a 2-h chase. Cell lysates were subjected to RIPA with the gE-specific rabbit antiserum, Ra-αgE. Samples were analyzed in SDS–7.5% PAGE gels. Only the results obtained for gI and gIΔXb, -ΔB, -ΔM, -152, -ΔP, and -ΔXh are shown. Arrowheads indicate the immature 83-kDa ER form (*i* gE) and the Endo-H-resistant, mature 95-kDa gE species (*m* gE). Molecular sizes are in kilodaltons.

serum (Ra-αgE), and the precipitated proteins were analyzed in SDS–7.5% PAGE gels. Maturation of gE, i.e., the conversion of the Endo-H-sensitive 83-kDa species into the Endo-H-resistant 95-kDa form, was interpreted to indicate the formation of a gE-gI hetero-oligomer and its subsequent transport from the ER to the Golgi apparatus (Fig. 3; summarized in Fig. 1) (30). The deletion of the C-terminal 11 residues of gI, as in gIΔXb, the complete cytoplasmic tail, as in gIΔB, or even the transmembrane region plus up to one-third of the ectodomain, as in gIΔC and gIΔN (data not shown), did not affect intracellular transport of gE. In fact, the N-terminal 166 amino acid residues of gI, represented by gIΔM, proved to be sufficient to induce gE maturation (Fig. 3). However, maturation of gE no longer occurred in the presence of gI derivatives truncated at residue 152 (gI-152; Fig. 3) or at positions closer to the N terminus.

gIΔP, a gI derivative truncated at amino acid residue 93, binds to gE but does not induce its release from the ER. One obvious explanation for the failure of gI-152 and the smaller gI derivatives to induce gE maturation was that these truncated proteins were no longer able to bind to gE. However, it was also possible that binding to gE did occur but that the resulting hetero-oligomers were recognized as misfolded by the ER quality control system and therefore were retained in the ER (14). Indeed, in a RIPA performed on lysates of cells coexpressing gI-152 and gE, a 28-kDa protein was coprecipitated with the 83-kDa gE species (Fig. 4a, left lane). This product corresponded to the fully glycosylated gI-152 species. Analysis of the samples in SDS–15% PAGE gels revealed coprecipitation also of the three smaller gI-152 species (data not shown). In the 7.5% PAGE gels these products were not resolved but rather migrated in the dye front. Consistent with these findings, in RIPAs with the gI-specific antiserum, the 83-kDa gE species was coprecipitated with gI-152 as well as with gI-119 and gIΔP. However, gE was not coprecipitated with gIΔXh (Fig. 1 and 4a). gIΔP contains cysteine residues at positions 79 and 91. Theoretically, the coprecipitation of gE could have resulted from illegitimate intermolecular disulfide bonding. However, gE was also coprecipitated with gIΔPC$_0$, a gIΔP derivative in which both Cys residues had been replaced by Ser residues through PCR mutagenesis (Fig. 4a, right lane). Finally, to exclude the possibility that the coprecipitation merely resulted from aspecific binding to the antibodies or from an artifactual gE-gI interaction occurring subsequent to cell lysis, gE and gI-152 were expressed separately after which cell lysates were mixed and subjected to RIPA. As shown in Fig. 4b, the anti-gI serum only detected gI-152 products, whereas the anti-gE serum only detected gE. These results indicate that a gI derivative truncated at residue 93 can still bind to gE but that the resulting complex does not leave the ER.

Biochemical analysis of FHV recombinants expressing gIΔB and gIΔM. To corroborate the observations made for the heterologous vTF7-3 expression system, we constructed FHV recombinants expressing gIΔB and gIΔM. To this end, we made use of FHVΔgI-LZ, an FHV strain B927 recombinant in which residues 203 through 923 of the gI gene had been replaced by an EMCV IRES-LacZ expression cassette (Fig. 5a) (30). The disrupted gI locus of FHVΔgI-LZ was restored by homologous recombination with transfer vectors carrying either the intact gI gene or truncated genes encoding gIΔB or gIΔM. The genomic structures of the resulting FHV recombinant viruses, FHV-gI*rev*, FHV-gIΔB and FHV-gIΔM, are depicted schematically in Fig. 5a.

To test for expression of the gI mutants and for gE maturation, CRFK cells were infected and metabolic labeling was performed with ^{35}S[Met+Cys] from 7 to 8 h p.i.. Cells were harvested either immediately or after a 2-h chase, and cell lysates were subjected to RIPA with the gI- or gE-specific antisera. Cells infected with FHVΔgI-LZ were taken along as a negative control. As shown in Fig. 5b, the reintroduced gI genes were properly expressed and in all cases products of the anticipated size were found (for comparison, see Fig. 2a). In cells expressing gIΔM, each of the three N-glycosylated species was detected, but the biglycosylated form was most abundant. Maturation of gE occurred in cells infected with FHV-gI*rev*, -gIΔB, and -gIΔM, but not in cells infected with FHVΔgI-LZ (Fig. 5c). In cells infected with FHV-gI*rev* or -gIΔB, the immature gE species was converted into the 95-kDa mature form.

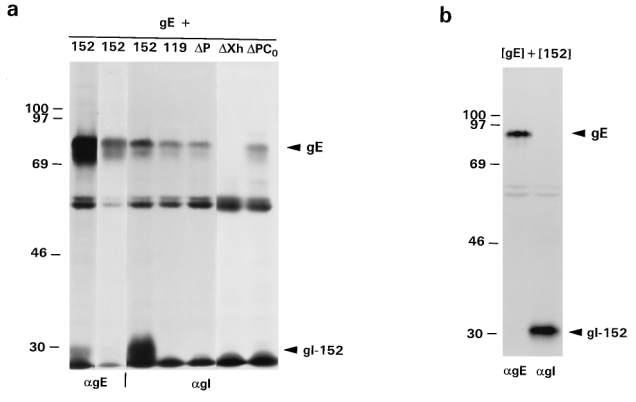

FIG. 4. Coprecipitation of gE and C-terminally truncated gI derivatives. (a) gE was coexpressed with gI-152, gI-119, gIΔP, gIΔXh, and gIΔPC$_0$ in vTF7-3-infected OST7-1 cells. The cells were metabolically labeled from 5 to 6 h p.i., followed by a 2-h chase. The cell lysates were subjected to RIPA with either Ra-αgE (αgE) or Ra-αgI (αgI). The samples were analyzed in an SDS–7.5% PAGE gel. Arrowheads indicate the immature, 83-kDa ER form of gE and the triglycosylated species of gI-152, migrating immediately above the dye front. The leftmost lane is an overexposure of the adjacent lane to show the coprecipitation of gI-152 with gE in a RIPA with Ra-αgE. The products of 59 and 55 kDa represent vaccinia virus products nonspecifically binding to the immunosorbent (30). (b) vTF7-3-infected cells, transfected with either pBS-gE or pBS-gI-152, were metabolically labeled from 5 to 6 h p.i. Cells were lysed in 0.5 times the volume used in standard assays, and the lysates were mixed 1:1 and subsequently subjected to RIPA with Ra-αgE and Ra-αgI. The samples were analyzed in an SDS–10% PAGE gel. Molecular sizes are in kilodaltons.

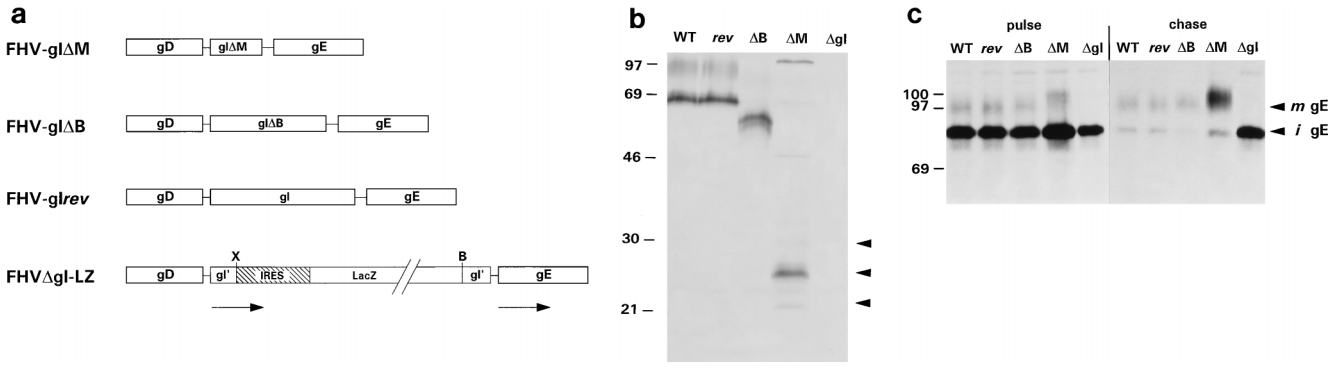

FIG. 5. Biochemical analysis of FHV recombinants expressing C-terminally truncated gI derivatives. (a) Schematic representation of the gI loci in the unique short regions of FHV-gIΔM, FHV-gIΔB, FHV-gI*rev*, and FHVΔgI-LZ. The genes for gD, gI, gE, and β-galactosidase (LacZ) are depicted as open boxes. The hatched box represents the IRES of EMCV. Arrows indicate the direction of transcription. X and B indicate *Xho*I and *Bam*HI restriction sites, respectively. (b) Expression of truncated gI proteins in FHV-infected cells. Monolayers of CRFK cells were infected with either FHV strain B927 (WT), FHV-gI*rev* (*rev*), FHV-gIΔM (ΔM), FHV-gIΔB (ΔB), or FHVΔgI-LZ (ΔgI). The cells were metabolically labeled from 7 to 8 h p.i. and lysed as described in Materials and Methods. To dissociate gE-gI complexes, SDS was added to the lysates to a final concentration of 1.5% and the lysates were incubated for 5 min at 95°C prior to RIPA with Ra-αgI. The samples were analyzed in an SDS–15% PAGE gel. Arrowheads indicate the mono-, bi-, and tri-N-glycosylated forms of gIΔM. The 80- to 100-kDa smears observed in lanes WT and *rev* represent mature gI species. Previously, we failed to detect mature gI in FHV-infected cells (30). However, Ra-αgI does recognize mature gI; because of the heterogenous posttranslational modifications, the detection of this product is obscured in SDS–7.5% PAGE gels. (c) Maturation of gE in FHV-infected cells expressing truncated gI proteins. CRFK cells were infected and metabolically labeled as described above. After the labeling, the cells were harvested either immediately (pulse) or after a 2-h chase (chase). Cell lysates were subjected to RIPA with Ra-αgE. The samples were analyzed in an SDS–7.5% PAGE gel. Arrowheads indicate the immature (*i* gE) and mature (*m* gE) forms of gE. Molecular sizes are in kilodaltons.

Mature gE, immunoprecipitated from lysates of FHV-gIΔM-infected cells, produced a more diffuse band in SDS–7.5% PAGE gels (Fig. 5c). Apparently, the gE-gIΔM complex is transport competent, but gE acquires more extensive posttranslational modifications.

Effect of C-terminal gI deletions on plaque size. FHVΔgI-LZ exhibits a small-plaque phenotype (30). To determine whether this defect was restored in the revertant FHV-gI*rev* and to study the plaque morphologies of FHV-gIΔM and -gIΔB, plaque assays were performed in CRFK cells. Plaques were visualized immunohistochemically with an FHV-specific feline hyperimmune serum (Fig. 6a). The plaque sizes (in millimeters squared) were measured and compared to those of wild-type FHV strain B927 and recombinant FHVΔgI-LZ (Fig. 6b). FHV-gI*rev* produced plaques indistinguishable from those of wild-type strain B927. FHV-gIΔB produced plaques much larger than those of FHVΔgI-LZ, but about 35% smaller than those of wild-type FHV. In contrast, FHV-gIΔM produced small plaques, which were only about 25% the size of those of wild-type FHV. On average, however, the plaques of FHVgIΔM were larger than those of FHVΔgI-LZ.

DISCUSSION

We have previously reported that, in the absence of gI, FHV gE is transport incompetent and fully retained in the ER (30). Here, we have exploited this feature to study gE-gI interaction in more detail and have assessed the effects of C-terminal deletions in gI on the biosynthesis, intracellular transport, and function of the gE-gI complex. Results, obtained by vTF7-3-driven expression of cloned genes, were corroborated and extended by studying FHV recombinants expressing truncated gI derivatives. Our data suggest that the N terminus of gI represents the business end of the molecule.

Comparative amino acid sequence analysis revealed that most of the sequence conservation among alphaherpesvirus gI proteins is in the N-terminal half of the ectodomain (1, 27). We now show that this region of gI comprises a domain intimately involved in gE-gI interaction. This conclusion is based upon the observation that FHV gI derivative gIΔM, which is truncated at residue 166, can assemble into a transport-competent complex with gE. Protein folding and oligomerization in the ER are facilitated and controlled by foldases and molecular chaperones. The latter proteins associate transiently with folding and assembly intermediates and, more permanently, with misfolded products or incomplete subunits of oligomeric complexes. In general, only properly folded proteins and fully assembled oligomers are allowed to proceed to the Golgi complex and beyond (14, 20, 42). Apparently, the interaction with gIΔM is sufficient to release gE from its chaperone(s); i.e., the C-terminal half of the gI ectodomain, the transmembrane anchor, and the cytoplasmic tail are not required for this process. How the dissociation of gE is brought about is not known. gIΔM may directly compete with chaperones for gE binding. Alternatively, the interaction with gIΔM may cause an essential conformational change in gE.

Complex formation per se is not sufficient to trigger intracellular transport. The gI-152 product, which is only 14 residues shorter than gIΔM, and even gIΔP, which is truncated at residue 93, still associate with gE, as demonstrated by coimmunoprecipitation. However, the resulting complexes are retained in the ER. Our findings confirm and extend observations by Basu et al. (5); on the basis of deletion and linker insertion mutagenesis of herpes simplex virus gI, these authors suggested that a region comprising residues 43 to 192 (corresponding to residues 38 to 233 of FHV gI) complexes with gE. Under the assumption that the overall protein structure of gI has been conserved during alphaherpesvirus divergence, our findings would suggest that a region mediating stable association with gE is located between residues 38 and 93. It is not known which regions of gE bind to gI. For HSV gE, a region comprising residues 183 to 288 (corresponding to residues 153 to 255 of FHV gE) has been implicated in the gE-gI interaction (4). It remains to be determined whether this part of gE, which is highly conserved (1), binds to the N-terminal domain of gI.

Truncation of gI at residue 166 does not interfere with complex formation and subsequent intracellular transport, but it does have an effect on the posttranslational modification of gE. In cells expressing gIΔM, a mature gE species of 95 to 100 kDa was found instead of the typical 95-kDa form (Fig. 3 and 5c).

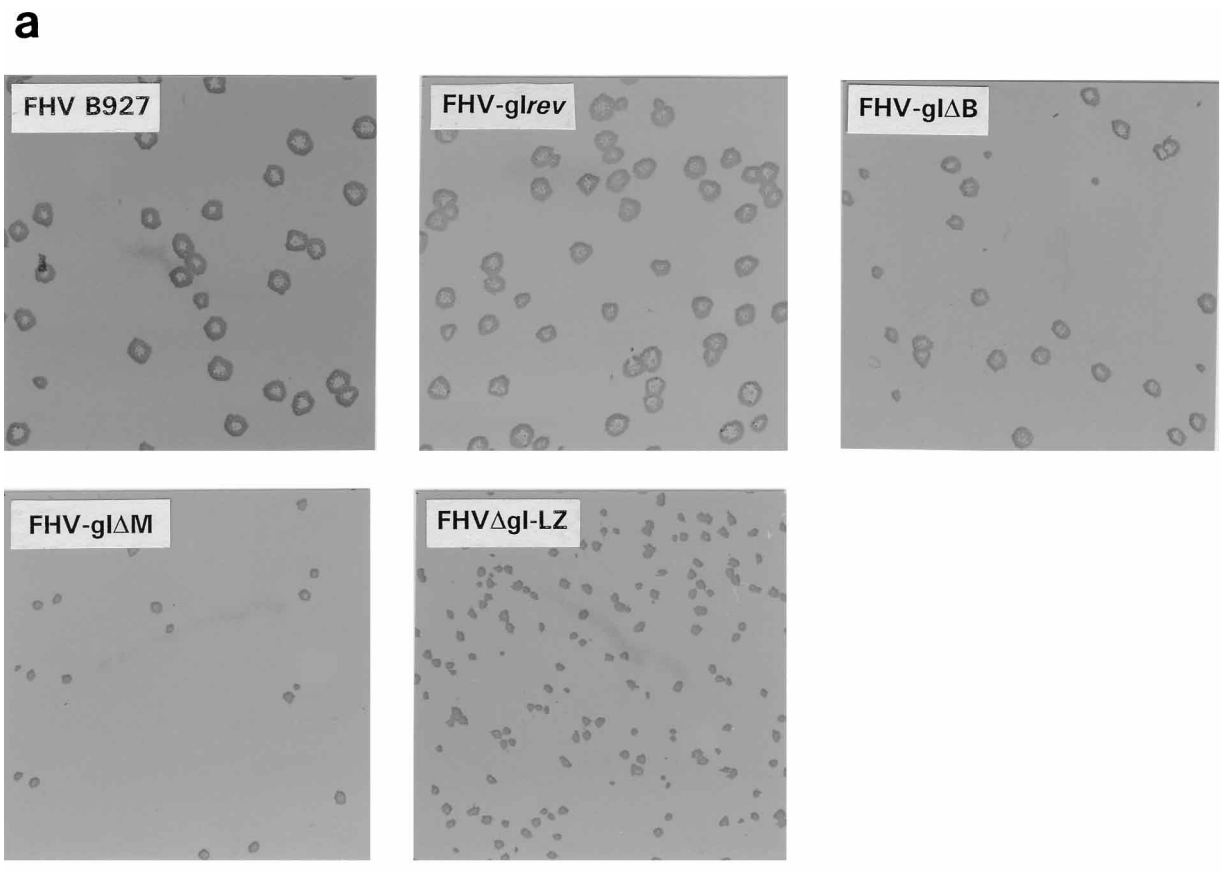

FIG. 6. Plaque morphology of FHV recombinants expressing C-terminally truncated gI species. (a) Monolayers of CRFK cells, grown in 35-mm-diameter dishes were infected with 50 to 100 PFU. One hour p.i., a solid-phase overlay was applied, and incubation was continued for 72 h at 37°C. The monolayers were paraformaldehyde fixed, the overlay was removed, and the plaques were visualized immunohistochemically. Magnification, 2.3. (b) Quantification of plaque size. For each recombinant, 25 randomly chosen plaques were measured along the *x* and *y* axes at a 20-fold magnification to determine the mean diameter. The average plaque size in millimeters squared was then calculated from the mean radius (*r*) by using the term πr^2. The histogram shows the plaque sizes of the FHV mutants relative to that of parental FHV strain B927 (WT). Standard deviations are indicated by bars. The experiment was performed three times in two feline cell lines (CRFK and IRC5 cells), yielding essentially identical results. The results of a typical experiment are presented.

Apparently gE, when in a complex with gIΔM, acquires additional posttranslational modifications. Experiments in which gE was treated with peptide:*N*-glycosidase F indicate that the increase in apparent molecular weight is caused by a more elaborate decoration of N-glycans (29). Presumably, the N-glycans involved are inaccessible to further processing in the authentic gE-gI complex. These findings are not unprecedented. Aberrant posttranslational processing has also been seen for varicella-zoster virus gE and gI when they are expressed separately (47) and for FHV gI produced in the absence of gE (30).

Several lines of evidence suggest that gE-gI is critically involved in cell-to-cell transmission (3, 11–13, 39, 48). Mutant viruses deficient in gE and/or gI exhibit a small-plaque phenotype, yet are reportedly similar to the wild type with regard to kinetics of entry, growth rate, and yield of extracellular virus (3, 12, 28, 29, 48). Recombinant FHVΔgI-LZ, in which the gI gene has been disrupted by the insertion of an EMCV IRES-LacZ expression cassette, produces plaques only 15% the size of those of the parental virus. In revertant FHV-gI*rev*, plaque morphology was fully restored to that of wild-type FHV. Plaque morphology was also restored in an FHVΔgI-LZ derivative which had been complemented allotopically by inserting a functional gI gene into the thymidine kinase locus (29). These observations provide formal evidence that the small-plaque phenotype of FHVΔgI-LZ is caused by the disruption of the gI gene and not by "nearest neighbor effects," such as down-regulation of gD expression (46). Moreover, these findings provided a basis to test the effects of C-terminal deletions in gI on cell-to-cell transmission as measured by plaque size. Like the gI proteins of other alphaherpesviruses,

FHV gI possesses a relatively large cytoplasmic tail, 73 residues in length. However, this domain can be deleted—as in FHV-gIΔB—without much effect on plaque size. We therefore conclude that the cytoplasmic tail of gI is not essential for gE-gI-mediated cell-to-cell spread, at least not under our in vitro conditions. In contrast, the deletion of the C-terminal 218 residues, as in gIΔM, resulted in a dramatic decrease in plaque size. The most obvious explanation for these findings is that gI sequences downstream of residue 166, for example, the transmembrane region, are required for efficient cell-to-cell spread. Theoretically, the loss in function may also have resulted from the altered posttranslational modifications of gE. It is of note, however, that the plaques produced by FHV-gIΔM were consistently larger than those produced by gI-deficient FHVΔgI-LZ. Interestingly, this difference was even more pronounced when gIΔM was expressed allotopically. From these results, it would appear that the gE-gIΔM complex retains residual biological activity and, thus, that membrane anchoring of gI is not essential for function. These findings and their implications will be described in more detail elsewhere (29).

The gE-gI complex is a virulence factor and as such plays an important role in alphaherpesvirus pathogenesis. Although for some alphaherpesviruses gE-gI may be involved in immune evasion by acting as an Fc receptor (4–7, 17, 22, 25), its main function appears to be to promote cell-to-cell spread. The molecular mechanism of this process remains to be resolved. As has been speculated (3, 12), gE-gI may be involved in interactions at the plasma membrane and, by binding to a specific ligand in the plasma membrane of adjacent cells, may facilitate viral transmission via the contact route. Further studies on the biosynthesis of gE-gI may provide additional clues as to its function and may perhaps even lead to the development of new antiherpetic drugs.

ACKNOWLEDGMENTS

We thank D. Harbour and B. Moss for providing the virus stocks of FHV strain B927 and vTF7-3, respectively, and Antoine de Vries for stimulating discussions and for critical reading of the manuscript.

J. D. F. Mijnes was supported by Rhône Mérieux, Lyon, France. The research of R. J. de Groot was made possible by a fellowship of the Royal Netherlands Academy for Sciences and Arts.

REFERENCES

1. **Audonnet, J.-C., J. Winslow, G. Allen, and E. Paoletti.** 1990. Equine herpesvirus type 1 unique short fragment encodes glycoproteins with homology to herpes simplex virus type 1 gD, gI and gE. J. Gen. Virol. **71:**2969–2978.
2. **Ausubel, F. M.** 1989. Current protocols in molecular biology. Greene Publishing Associates and Wiley-Interscience, New York, N.Y.
3. **Balan, P., N. Davis-Poynter, S. Bell, H. Atkinson, H. Browne, and T. Minson.** 1994. An analysis of the in vitro and in vivo phenotypes of mutants of herpes simplex virus type 1 lacking glycoproteins gG, gE, gI or the putative gJ. J. Gen. Virol. **75:**1245–1258.
4. **Basu, S., G. Dubin, M. Basu, V. Nguyen, and H. M. Friedman.** 1995. Characterization of regions of herpes simplex virus type 1 glycoprotein E involved in binding the Fc domain of monomeric IgG and in forming a complex with glycoprotein I. J. Immunol. **154:**260–267.
5. **Basu, S., G. Dubin, T. Nagashunmugam, M. Basu, L. T. Goldstein, L. Wang, B. Weeks, and H. M. Friedman.** 1997. Mapping regions of herpes simplex virus type 1 glycoprotein I required for formation of the viral Fc receptor for monomeric IgG. J. Immunol. **158:**209–215.
6. **Baucke, R. B., and P. G. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. V. Identification of an Fc-binding glycoprotein. J. Virol. **32:**779–789.
7. **Bell, S., M. Cranage, L. Borysiewicz, and T. Minson.** 1990. Induction of immunoglobulin G Fc receptors by recombinant vaccinia viruses expressing glycoproteins E and I of herpes simplex virus type 1. J. Virol. **64:**2181–2186.
8. **Card, J. P., M. E. Whealy, A. K. Robbins, and L. W. Enquist.** 1992. Pseudorabies virus envelope glycoprotein gI influences both neurotropism and virulence during infection of the rat visual system. J. Virol. **66:**3032–3041.
9. **Card, J. P., M. E. Whealy, A. K. Robbins, R. Y. Moore, and L. W. Enquist.** 1991. Two alpha-herpesvirus strains are transported differentially in the rodent visual system. Neuron **6:**957–969.
10. **Crowley, K. S., S. Liao, V. E. Worrell, G. D. Reinhardt, and A. E. Johnson.** 1994. Secretory proteins move through the endoplasmic reticulum membrane via an aqueous, gated pore. Cell **78:**461–471.
11. **Davis-Poynter, N., S. Bell, T. Minson, and H. Browne.** 1994. Analysis of the contributions of herpes simplex virus type 1 membrane proteins to the induction of cell-cell fusion. J. Virol. **68:**7586–7590.
12. **Dingwell, K. S., C. R. Brunetti, R. L. Hendricks, Q. Tang, M. Tang, A. J. Rainbow, and D. C. Johnson.** 1994. Herpes simplex virus glycoproteins E and I facilitate cell-to-cell spread in vivo and across junctions of cultured cells. J. Virol. **68:**834–845.
13. **Dingwell, K. S., L. C. Doering, and D. C. Johnson.** 1995. Glycoproteins E and I facilitate neuron-to-neuron spread of herpes simplex virus. J. Virol. **69:**7087–7098.
14. **Doms, R. W., R. A. Lamb, J. K. Rose, and A. Helenius.** 1993. Folding and assembly of viral membrane proteins. Virology **193:**545–562.
15. **Elroy-Stein, O., and B. Moss.** 1990. Cytoplasmic expression system based on constitutive synthesis of bacteriophage T7 RNA polymerase in mammalian cells. Proc. Natl. Acad. Sci. USA **87:**6743–6747.
16. **Enquist, L. W., J. Dubin, M. E. Whealy, and J. P. Card.** 1994. Complementation analysis of pseudorabies virus gE and gI mutants in retinal ganglion cell neurotropism. J. Virol. **68:**5275–5279.
17. **Frank, I., and H. M. Friedman.** 1989. A novel function of the herpes simplex virus type 1 Fc receptor: participation in bipolar bridging of antiviral immunoglobulin G. J. Virol. **63:**4479–4488.
18. **Fuerst, T. R., E. G. Niles, F. W. Studier, and B. Moss.** 1986. Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase. Proc. Natl. Acad. Sci. USA **83:**8122–8126.
19. **Gaskell, R. M., and R. C. Povey.** 1979. The dose response of cats to experimental infection with feline viral rhinotracheitis virus. J. Comp. Pathol. **89:**179–191.
20. **Hurtley, S. M., and A. Helenius.** 1989. Protein oligomerization in the endoplasmic reticulum. Annu. Rev. Cell Biol. **5:**277–307.
21. **Jacobs, L., H. J. Rziha, T. G. Kimman, A. L. J. Gielkens, and J. T. Van Oirschot.** 1993. Deleting valine-125 and cysteine-126 in glycoprotein gI of pseudorabies virus strain NIA-3 decreases plaque size and reduces virulence in mice. Arch. Virol. **131:**251–264.
22. **Johnson, D. C., and V. Feenstra.** 1987. Identification of a novel herpes simplex virus type 1-induced glycoprotein which complexes with gE and binds immunoglobulin. J. Virol. **61:**2208–2216.
23. **Kritas, S. K., M. B. Pensaert, and T. C. Mettenleiter.** 1994. Invasion and spread of single glycoprotein deleted mutants of Aujeszki's disease virus (ADV) in the trigeminal nervous pathway of pigs after intranasal inoculation. Vet. Microbiol. **40:**323–334.
24. **Kritas, S. K., M. B. Pensaert, and T. C. Mettenleiter.** 1994. Role of envelope glycoproteins gI, gp63 and gIII in the invasion and spread of Aujeszky's disease virus in the olfactory nervous pathway of the pig. J. Gen. Virol. **75:**2319–2327.
25. **Litwin, V., W. Jackson, and C. Grose.** 1992. Receptor properties of two varicella-zoster virus glycoproteins, gpI and gpIV, homologous to herpes simplex virus gE and gI. J. Virol. **66:**3643–3651.
26. **Machamer, C. E., S. A. Mentone, J. K. Rose, and M. G. Farquhar.** 1990. The E1 glycoprotein of an avian coronavirus is targeted to the cis Golgi complex. Proc. Natl. Acad. Sci. USA **87:**6944–6948.
27. **McGeoch, D. J.** 1990. Evolutionary relationships of virion glycoprotein genes in the S regions of alphaherpesvirus genomes. J. Gen. Virol. **71:**2361–2367.
28. **Mettenleiter, T. C., C. Schreurs, F. Zuckermann, and T. Ben-Porat.** 1987. Role of pseudorabies virus glycoprotein gI in virus release from infected cells. J. Virol. **61:**2764–2769.
29. **Mijnes, J. D. F., and R. J. De Groot.** Unpublished observations.
30. **Mijnes, J. D. F., L. M. van der Horst, E. van Anken, M. C. Horzinek, P. J. M. Rottier, and R. J. de Groot.** 1996. Biosynthesis of glycoproteins E and I of feline herpesvirus: gE-gI interaction is required for intracellular transport. J. Virol. **70:**5466–5475.
31. **Mulder, W. A., L. Jacobs, J. Priem, G. L. Kok, F. Wagenaar, T. G. Kimman, and J. M. Pol.** 1994. Glycoprotein gE-negative pseudorabies virus has a reduced capability to infect second- and third-order neurons of the olfactory and trigeminal routes in the porcine central nervous system. J. Gen. Virol. **75:**3095–3106.
32. **Neidhardt, H., C. H. Schröder, and H. C. Kaerner.** 1987. Herpes simplex virus type 1 glycoprotein E is not indispensable for viral infectivity. J. Virol. **61:**600–603.
33. **Povey, R. C.** 1979. A review of feline viral rhinotracheitis (feline herpesvirus 1 infection). Comp. Immunol. Microbiol. Infect. Dis. **2:**373–387.
34. **Rajcani, J., U. Herget, and H. C. Kaerner.** 1990. Spread of herpes simplex virus (HSV) strains SC16, ANG, ANGpath and its glyC minus and glyE minus mutants in DBA-2 mice. Acta Virol. **34:**305–320.
35. **Roizman, B.** 1996. Herpesviridae, p. 2221–2230. *In* B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), Fields virology, 3rd ed, vol. 2. Lippincott-Raven, Philadelphia, Pa.
36. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplifica-

tion of DNA with a thermostable DNA polymerase. Science **239:**487–491.
37. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **Spätz, S. J., P. A. Rota, and R. K. Maes.** 1994. Identification of the feline herpesvirus type 1 (FHV-1) genes encoding glycoproteins G, D, I and E: expression of FHV-1 glycoprotein D in vaccinia and raccoon poxviruses. J. Gen. Virol. **75:**1235–1244.
39. **Spear, P. G.** 1993. Membrane fusion induced by herpes simplex virus, p. 201–232. *In* J. Bentz (ed.), Viral fusion mechanisms. CRC Press, Inc., Boca Raton, Fla.
40. **Sussman, M. D., R. K. Maes, J. M. Kruger, S. J. Spätz, and P. J. Venta.** 1995. A feline herpesvirus-1 recombinant with a deletion in the genes for glycoproteins gI and gE is effective as a vaccine for feline rhinotracheitis. Virology **214:**12–20.
41. **Van Engelenburg, F. A. C., M. J. Kaashoek, F. A. M. Rijsewijk, L. van den Burg, A. Moerman, A. L. J. Gielkens, and J. T. van Oirschot.** 1994. A glycoprotein E deletion mutant of bovine herpesvirus 1 is avirulent in calves. J. Gen. Virol. **75:**2311–2318.
42. **Wei, J., and L. M. Hendershot.** 1996. Protein folding and assembly in the endoplasmic reticulum. Exper. Suppl. (Basel) **77:**41–55.
43. **Whealy, M. E., J. P. Card, A. K. Robbins, J. R. Dubin, H. J. Rziha, and L. W. Enquist.** 1993. Specific pseudorabies virus infection of the rat visual system requires both gI and gp63 glycoproteins. J. Virol. **67:**3786–3797.
44. **Whitbeck, J. C., A. C. Knapp, L. W. Enquist, W. C. Lawrence, and L. J. Bello.** 1996. Synthesis, processing, and oligomerization of bovine herpesvirus 1 gE and gI membrane proteins. J. Virol. **70:**7878–7884.
45. **Willemse, M. J., W. S. K. Chalmers, and P. J. A. Sondermeijer.** 1996. *In vivo* properties of a feline herpesvirus type 1 mutant carrying a lacZ insertion at the gI locus of the unique short segment. Vaccine **14:**1–5.
46. **Willemse, M. J., I. G. Strijdveen, S. H. van Schooneveld, M. C. van den Berg, and P. J. Sondermeijer.** 1995. Transcriptional analysis of the short segment of the feline herpesvirus type 1 genome and insertional mutagenesis of a unique reading frame. Virology **208:**704–711.
47. **Yao, Z., W. Jackson, B. Forghani, and C. Grose.** 1993. Varicella-zoster virus glycoprotein gpI/gpIV receptor: expression, complex formation, and antigenicity within the vaccinia virus-T7 RNA polymerase transfection system. J. Virol. **67:**305–314.
48. **Zsak, L., F. Zuckermann, N. Sugg, and T. Ben-Porat.** 1992. Glycoprotein gI of pseudorabies virus promotes cell fusion and virus spread via direct cell-to-cell transmission. J. Virol. **66:**2316–2325.
49. **Zuckermann, F. A., T. C. Mettenleiter, C. Schreurs, N. Sugg, and T. Ben-Porat.** 1988. Complex between glycoproteins gI and gp63 of pseudorabies virus: its effect on virus replication. J. Virol. **62:**4622–4626.