Determination of the Transmembrane Topology of Herpes Simplex Virus Type 1 Glycoprotein K (gK)*

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Herpes simplex virus type 1 glycprotein K (gK) plays an essential role in viral replication and cell fusion. gK is a very hydrophobic membrane protein that contains a signal sequence and several hydrophobic regions. It has been shown that mutations inducing cell fusion map to two distinct domains of gK, suggesting that these domains are functionally important. To understand the transmembrane topology of gK and the localization of these functional domains, we constructed a set of gK deletion, insertion, and truncation mutants and expressed these by in vitro translation in the presence of microsomal membranes. The transmembrane topology of gK was determined by examination of the post-translational processing and protease sensitivity of the mutant proteins. Our data demonstrate that gK contains three transmembrane domains (amino acids 125–139, 226–239, and 311–325). Another hydrophobic domain (amino acids 241–265), which is relatively less hydrophobic and much longer compared with the transmembrane domains, is located in the extracellular loop. The analysis showed that the domains containing syncytial mutations are both ectodomains. They may interact with each other to form a complex tertiary structure that is critical for the biological function of gK.

Herpes simplex virus type 1 (HSV-1) enters host cells by fusion between the virion envelope and the cell plasma membrane (1). Although many viruses that penetrate by direct fusion also cause syncytia formation (cell fusion), extensive cell fusion is caused by very few wild-type HSV strains. The majority of cells infected by wild-type HSV-1 round up and clump (2). However, mutants of HSV-1 that cause extensive cell fusion have been found. This phenotype has been designated the syncytial (Syn) phenotype (2–6). Mutations that give rise to the Syn phenotype have been mapped to at least four genes in the HSV viral genome: the gK gene (UL53) (7, 8), the gB gene (UL27) (9, 10), the UL24 gene (11, 12) and the UL20 gene (13). A large percentage of Syn mutations have been mapped to the gK gene (1, 7, 8, 14–16). Glycoprotein K has several notable characteristics compared with other HSV-1 glycoproteins. It contains multiple hydrophobic domains and may traverse the plasma membrane several times, giving gK a complex transmembrane structure (8, 16). Also in infected cells, gK is expressed at low levels relative to other glycoproteins of HSV-1 and does not reach the plasma membrane (17, 18). It appears that gK is essential for viral replication since mutant viruses with insertions or deletions in the UL53 open reading frame cannot grow in tissue culture (18, 19). Hutchinson et al. (20) constructed HSV-1 mutant virus FgKb, in which a lacZ gene cassette was inserted downstream of the amino-terminal portion of gK. They found that FgKb was defective in virus egress. Recent studies by Jayachandra et al. (19) on the mutant agK, which lacks the entire gK gene, indicated that gK is required for both capsid envelopment and virus egress.

gK is a highly hydrophobic protein with 338 amino acids. It has characteristics of a membrane protein: a cleavable amino acid NH2-terminal signal sequence, two sites for N-linked glycosylation, and several hydrophobic domains (Fig. 1). Cleavage of the NH2-terminal signal sequence and addition of carbohydrates on asparagine residues at 48 and 58 of the gK amino acid sequence suggest that the NH2-terminal domain of gK is an ectodomain (21, 22). A truncated gK protein containing hydrophobic domain 1 is membrane-bound, indicating that this domain is a transmembrane or membrane-binding domain (22). However, the topology of the remainder of gK is difficult to predict. Eleven Syn mutants from KOS have been found to have mutations mapping to the gK gene (6, 14, 21, 23). Sequence analysis of these mutants indicated that syncytial mutations can occur in two distinct domains of gK: the NH2-terminal ectodomain of gK and the region between hydrophobic domains 2 and 4 (21). These two regions of gK are likely to be functional domains that play a role in regulating cell fusion. Determination of the transmembrane topology of gK, especially the localization of Syn mutation domains, is essential for the understanding of the structure and function of gK.

In this paper, we constructed a set of gK deletion, insertion, and truncation mutants. These were expressed by in vitro translation in the presence of microsomal membranes. The transmembrane topology of gK was determined by examination of their post-translational processing and protease sensitivity. This provided evidence for a gK structure with three transmembrane domains in which the syncytial mutations occur in the gK ectodomains.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Growth Media, and Conditions—E. coli strain INVaF (Invitrogen) and DH5a (Life Technologies) were routinely cultured in Luria broth at 37 °C. These strains were host for the recombinant plasmids used in deletion and truncation mutagenesis experiments. Cells containing recombinant plasmids were selected by addition of ampicillin at a final concentration of 100 μg/ml.

Genetic Manipulations: Plasmid DNA Construction—Wild-type gK was amplified from pBS-KB1N (22) by PCR. The T7 promoter sequence was added to the 5′-primer and BamHI sites were put at the ends of 5′ and 3′ primers (Table I). The plasmid pTagK was constructed by amplifying gK using the primers shown in Table I, followed by cloning of the product in the pCRII TA cloning vector (Invitrogen). The BamHI DNA fragment containing the T7 promoter and the gK open reading frame was inserted downstream of the T7 promoter and TEV protease cleavage site in the pCRII TA cloning vector (Invitrogen).

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In Vitro Transcription—Plasmid DNAs were digested with BamHI, and the fragments containing the T7 promoter and gK open reading frame were gel-purified. An in vitro T7 transcription kit (Mega-script™, Ambion) was used to transcribe the purified DNA fragments. Briefly, each transcription reaction contains 1 μg of DNA template, 1× transcription buffer (Ambion), 7.5 mM of each NTP, 1× T7 RNA polymerase mix, and nuclease-free water in a total volume of 20 μl. The transcription mixture was incubated at 37 °C for 2 h. Then mRNAs were precipitated by 10 μl of LiCl at −70 °C. RNA pellets were washed with 50 μl of 80% ethanol and redissolved in nuclease-free water. RNA transcripts were analyzed by agarose gel electrophoresis before being used for in vitro translation reactions. Some DNA templates were produced directly by PCR. They were gel-purified and transcribed to make RNAs encoding the truncated gK proteins.

In Vitro Translation—RNAs were translated in vitro using a reticulocyte lysate system (Promega). Typically, a 25-μl translational mixture containing 1–2 μg of RNA, 2.5 μl of [35S]methionine, 0.125 mM MgAc2, 12.5 mM KCl, 0.01 mM amino acid mixture (without methionine), and 12.5 μl of rabbit reticulocyte lysate (28). Nuclease-treated microsome membranes (3.5 μl) were added to certain reactions where post-translational processing was needed. After 2 h of incubation at 30 °C, 2 μl of a 10 μg/μl ribonuclease A solution was added to digest RNAs.

Protease K Protection Assay—In protease protection experiments, in vitro translation reactions were divided into several aliquots. These were left untreated or were digested by adding protease K to a final concentration of 2 or 0.5 μg/μl, either with or without addition of Triton X-100 to a final concentration of 1.0%. After 1 h of digestion at 4 °C, proteolysis was stopped by adding excess phenylmethylsulfonyl fluoride (PMSF, final concentration 4 mM). These samples were analyzed on SDS-polyacrylamide gels.

N-Glycosidase Treatment—Endoglycosidase H (Endo H) and peptide N-glycosidase F (PNGase) from New England Biolabs were used for carbohydrate digestion of gK proteins. To test for Endo H sensitivity, samples of translation reactions were denaturated by addition of 0.1 volume of 10× Endo H denaturation buffer, followed by incubation for 30 min at 37 °C. Then, 0.1 volume of 10× Endo H reaction buffer was added to the denaturated sample, followed by incubation with 2 μl of 1,000 units/μl Endo H for 1 h at 37 °C. To test for PNGase sensitivity, samples of translation reactions were first denaturated by addition of 0.1 volume of 10× PNGase denaturation buffer. Then 0.1 volume of 10× PNGase reaction buffer and 0.1 volume of 10% Nonidet P-40 were mixed with the denatured samples. Finally, the mixtures were incubated with 2 μl of 500 units/μl PNGase for 1 h at 37 °C.

Immune precipitation—To immune precipitate MRGSH4-tagged gK proteins, 3–5 μl of in vitro translation reaction was added to 200 μl lysis buffer (% Triton X-100, 1% bovine serum albumin, 10 mM Tris-Cl, pH 8.0, 150 mM NaCl, 0.025% NaN3 freshly supplemented with 1.0 mM PMSF, and 1 mM iodoacetamide) for 1 h at 4 °C. Anti-MRGSH4 antibody (Qiagen) (0.4 μg) was added, and the samples were mixed for 2 h at 4 °C on a rotator. A volume of 25 μl of 50% protein A-Sepharose suspension (Life Technologies, Inc.) was added, and the samples were mixed overnight at 4 °C on a rotator to precipitate antigen-antibody complexes. The beads were washed twice with 1 ml of lysis buffer. Finally, 12.5 μl of 2× ESS loading buffer was used to release proteins from the beads at room temperature.
Hydropathy Analysis of Glycoprotein K—
The gK sequence is shown in Fig. 1B, amino acid sequence of gK. The protein sequence of HSV-1 gK consists of 338 amino acids (Fig. 1B). A strongly hydrophobic region at the NH₂ terminus has been shown to be part of a cleavable signal sequence (22). The four major hydrophobic domains (HD) are indicated. HD₁, HD₂, and HD₃ are relatively short (14–15 amino acids), but are highly hydrophobic. HD₄ meets the criteria for transmembrane domains proposed by Eisenberg et al. (30), but it does not meet those suggested by Kyte and Doolittle (31).

Protease Protection Assay for Analysis of gK Topology—Membrane and secreted proteins translated in vitro in the presence of canine pancreatic microsomal membranes undergo those steps of post-translational processing normally occurring in the endoplasmic reticulum (ER): membrane insertion, signal sequence cleavage, and N-linked glycosylation. Topologically, the interior of the pancreatic micromeres corresponds to the lumen of the ER. The exterior surface of the microsomes is derived from the cytoplasmic side of the ER (27, 32). Protease protection assays have been used to determine the topology of a number of membrane proteins (33–37). Domains protected from protease digestion by microsomes are located in the interior of the microsome or within the microsomal membrane itself and thus are ectodomains or transmembrane domains, respectively. Protease-sensitive domains are cytoplasmic domains that are exposed on the outside of the microsome.

To verify reaction conditions, a well characterized secreted protein, yeast α-mating factor, was studied. Post-translational processing of α-mating factor involves cleavage of its NH₂-terminal signal sequence and addition of N-linked carbohydrate chains (38). After in vitro translation in the presence of microsomes, yeast α-mating factor should be located on the interior of microsomal membranes and should be protected from protease degradation. As shown in Fig. 3A, in the absence of pancreatic microsomes, yeast α-mating factor was synthesized as an 18-kDa protein (Fig. 3A, lane 1). In the presence of microsomes, the majority of this protein was processed to a form with an apparent molecular mass of 28 kDa (Fig. 3A, lane 2). A partially glycosylated form of 25 kDa was also observed, as well as a small amount of the unprocessed 18-kDa form. Treatment of in vitro translated and microsomally processed α-mating factor with proteinase K in the presence of Triton X-100 resulted in complete degradation of the protein (Fig. 3A, lane 3). This was inhibited by the protease inhibitor PMSF (Fig. 3A, lane 4). When the microsomes were treated with proteinase K alone at the concentration of 2 or 0.5 µg/µl, the 28-kDa and 25-kDa forms of α-mating factor were protected, but the 18-kDa form was completely degraded (Fig. 3A, lanes 5 and 6). Protection of the 28-kDa and 25-kDa forms by the microsomal membranes indicated that these forms were on the interior of the microsomes, whereas the unprocessed 18-kDa form remained outside the microsomes. In summary, the microsomes protected α-mating factor proteins that were translocated into the microsomal vesicles.

To use protease protection analysis to determine gK topology, the BamHI DNA fragment from pgKwt containing the T7 promoter and the gK open reading frame (Fig. 2B) was used as a template for in vitro transcription. Transcripts of wild-type gK were translated in vitro in the presence or absence of microsomal membranes. Microsomal processed samples were aliquoted, and proteinase K and Triton X-100 were added as indicated in Fig. 3B. The samples were run on a 15.5% SDS-PAGE gel. Without post-translational processing, gK migrated with an apparent molecular mass of 28 kDa (Fig. 3B, lane 1). This is considerably smaller than the predicted molecular mass of unpurified gK (38 kDa). This is due to incomplete denaturation of gK in these samples, which have not been heated. Previous studies have shown that heating of in vitro translated gK, even in SDS sample buffer, leads to complete aggregation of the protein (22). A few bands larger than 28 kDa were observed in the absence of microsomes (Fig. 3B, lane 1). These
appear to be ubiquitin-conjugated gK proteins. The bands form a ladder with approximately 8-kDa spacing, consistent with addition of multiple ubiquitins (molecular mass 8.5 kDa). Rabbit reticulocyte lysates are known to be able to carry out ubiquitination reactions (39, 40). Unprocessed gK protein translated in the absence of microsomes is misfolded, and this abnormal protein is apparently recognized by ubiquitinylating enzymes to add ubiquitin to one or more of the six lysines in gK. In the presence of microsomes, wild-type gK protein was mostly processed to a form with an apparent molecular mass of 38 kDa. A partially glycosylated form of approximately 35 kDa was also observed, as well as a small amount of the unprocessed 28-kDa form (Fig. 3B, lane 2). Ramaswamy and Holland (22) showed that 38-kDa and 35-kDa forms have undergone addition of N-linked carbohydrates and signal sequence cleavage. Treatment of in vitro translated and microsomally processed gK with proteinase K in the presence of Triton X-100 resulted in complete degradation of all forms of gK (Fig. 3B, lane 5). When microsomally processed gK samples were digested with proteinase K alone at concentrations of 2 or 0.5 mg/ml, protected bands of 26 and 23 kDa were observed (Fig. 3B, lanes 3 and 4). Since the NH₂-terminal domain of gK was expected to be an ectodomain (22), this domain should be protected from protease digestion. This was verified by treating samples of microsomally processed, proteinase K-treated gK with endoglycosidases. After PNGase and Endo H treatment, the 26- and 23-kDa forms of gK were reduced in apparent size to 22 and 19 kDa, respectively (data not shown). Since the N-glycosylation sites are located in the NH₂-terminal domain of gK, this confirmed that the NH₂-terminal domain is an ectodomain.

To study the transmembrane topology of gK, truncated DNA templates were made either by cleavage at restriction sites within the gK open reading frame or by primer-mediated PCR mutagenesis (Fig. 2C). After in vitro translation of RNAs transcribed from these DNA templates, the proteins were separated and analyzed by SDS-PAGE. To analyze the function of hydrophobic domain 1 of gK, this domain was deleted by PCR mutagenesis to produce gKΔ1. Truncated forms of wild-type gK and gKΔ1 lacking hydrophobic domains 2, 3, and 4 were produced by two methods. In the first method, gK and gKΔ1 templates were digested with BstEII prior to transcription and
transmembrane domain. In contrast, gKΔ123 also appeared to be unaffected by proteinase K treatment (Fig. 5B, lanes 8 and 9), but due to the small size of the domain COOH-terminal to hydrophobic domain 4 (13 amino acids), it could not be determined whether this domain was degraded.

Analysis of the COOH-terminal Domain of gK by Immunoprecipitation—To study the function of hydrophobic domain 4, two additional gK mutants were made by PCR mutagenesis. The MRGSH4 epitope was added to the COOH terminus of KR281 to generate gKΔ1,2RGSH4 and to gKΔ1,2 to generate gKΔ1,2RGSH6. gKΔ1,2RGSH4 contains hydrophobic domain 3 only, and gKΔ1,2RGSH6 has hydrophobic domains 3 and 4 (Fig. 2C). gKΔ1,2RGSH4 and gKΔ1,2RGSH6 were translated in vitro in the presence or absence of microsomal membranes, as indicated in Fig. 6. After translation, proteinase K digestion of microsomally processed gK, the 34-kDa form of hydrophobic domain 1 and hydrophobic domain 2 (amino acids 226–239), has hydrophobic domain 3 only and was truncated after residue 310 of wild-type gK (arginine). KR256, lacking hydrophobic domains 1–3, was terminated after residue 310 of wild-type gK (arginine). gKΔ123 contains hydrophobic domain 4 but lacks hydrophobic domains 1–3 (Fig. 2C). After in vitro translation and proteinase K treatment, the processed forms of KR270 were reduced in apparent size from 34 and 30 kDa to 31 and 27 kDa (Fig. 5, lanes 2 and 3 of panels A and B). This decrease in mass was consistent with digestion of most of the peptide from the COOH-terminal side of HD4 (46 amino acids). In contrast, no reduction in size of KR281 was observed (Fig. 5A, lanes 6 and 7). These results indicate that hydrophobic domain 2 is a transmembrane domain, but that hydrophobic domain 3 is not. KR256, which lacks all hydrophobic domains, was completely protected from proteinase K degradation (Fig. 5B, lanes 5 and 6). This is consistent with secretion into the interior of the microsomes. gKΔ123 also appeared to be unaffected by proteinase K treatment (Fig. 5B, lanes 8 and 9), but due to the small size of the domain COOH-terminal to hydrophobic domain 4 (13 amino acids), it could not be determined whether this domain was degraded.

To determine the function of hydrophobic domains 2 and 3, three additional gK mutants (KR256, KR270, and KR281) were produced by PCR mutagenesis. To eliminate the effects of hydrophobic domain 4, PCR was used to produce transcription templates truncated upstream of this domain. These truncated open reading frames were terminated with stop codons. The mutant KR270, lacking hydrophobic domain 1 (amino acids 125–139) and hydrophobic domain 3 (amino acids 241–265), contains hydrophobic domain 2 only and terminates after residue 310 of wild-type gK (arginine). KR281, lacking hydrophobic domain 1 and hydrophobic domain 4 (amino acids 226–239), has hydrophobic domain 3 only and was truncated after residue 310 of wild-type gK (arginine).

The proteins produced were designated gKwtB2 and gKΔ1B2 (Fig. 2C). Since we were concerned that proteins translated from mRNA lacking stop codons might be processed abnormally, we also constructed gK and gKΔ1 templates with stop codons added at the BstEI site (gKwtB2S and gKΔ1B2S) (Fig. 2C). As shown in Fig. 4A, both gKwtB2 and gKwtB2S were synthesized as 22-kDa proteins in the absence of pancreatic microsomes (Fig. 4A, lanes 1 and 5). In the presence of microsomes, the majority of this protein was processed to a form with an apparent molecular mass of 28 kDa. A partially glycosylated form of approximately 26 kDa was also observed, as well as a small amount of the unprocessed 22-kDa form (Fig. 4A, lanes 2 and 6). When the microsomally processed samples were subjected to proteinase K digestion, the apparent molecular masses of these fully and partially glycosylated forms were decreased by 3 kDa. This decrease in mass was consistent with cleavage within the 66-amino acid cytoplasmic tail of gKwtB2/2S. On the other hand, the small amount of unprocessed 22-kDa form was completely degraded (Fig. 4A, lanes 3 and 7). In contrast to the case for gKwtB2/2S, the microsomally processed 27-kDa and 24-kDa forms of gKΔ1B2/2S were unaffected by proteinase K treatment, but the unprocessed 20-kDa form was degraded (Fig. 4B, lanes 3 and 7). This suggests that in the absence of HD1, the gKΔ1B2/2S proteins were released into the interior of the microsomes, indicating that HD1 is a transmembrane domain.

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Analysis of the COOH-terminal Domain of gK by Immunoprecipitation—To study the function of hydrophobic domain 4, two additional gK mutants were made by PCR mutagenesis. The MRGSH4 epitope was added to the COOH terminus of KR281 to generate gKΔ1,2RGSH4 and to gKΔ1,2 to generate gKΔ1,2RGSH6. gKΔ1,2RGSH4 contains hydrophobic domain 3 only, and gKΔ1,2RGSH6 has hydrophobic domains 3 and 4 (Fig. 2C). gKΔ1,2RGSH4 and gKΔ1,2RGSH6 were translated in vitro in the presence or absence of microsomal membranes, as indicated in Fig. 6. After translation, proteinase K digestion of microsomally processed gK, the 34-kDa form of...
and gK have noted that the transmembrane domains of proteins are relatively short (14–15 amino acids). Bretscher et al. (41) have demonstrated, the transmembrane domains of gK are likely to be sequestered to intracellular membranes.

As we have demonstrated, the transmembrane domains of gK is likely to be sequestered to intracellular membranes. Our results also suggest that gK is localized to intracellular membranes, such as the endoplasmic reticulum, are typically about 15 residues in length, compared with transmembrane domain lengths of 20–25 residues for plasma membrane proteins. The shorter intracellular transmembrane domains are also richer in the bulky residue phenylalanine (13.7% versus 5.2%) (41). The first two transmembrane domains of gK each contain two phenylalanines, for an average phenylalanine content of 13.8%. Taken together, these data suggest that gK is likely to be an ER protein. This is consistent with the reported role of gK in envelopment and virion maturation (19, 20).

If gK is localized to intracellular membranes, its role in cell fusion must be indirect. It is possible that gK may influence cell fusion by regulating the conformation or interactions of other HSV glycoproteins, specifically those involved in cell fusion (i.e. gB, gD, and gH:gL). Precedent for control of the conformation of a viral fusion protein by another viral membrane protein exists; fowl plague virus hemagglutinin assumes an abnormal conformation unless coexpressed with the fowl plague virus M2 protein (42), although the mechanism of action of M2 (transmembrane proton transport) is likely to be different from that of gK. It is possible that gK acts as an HSV-specific chaperone-like protein. Cellular chaperonins exist in both the cytoplasm and lumen of the endoplasmic reticulum and assist in the folding of nascent proteins (43). Both membrane-bound and soluble chaperonins have been found in the ER lumen (44, 45). The fact that gK Syn mutations map to the gK ectodomains suggests that it may act on the ectodomains of other HSV glycoproteins. It is not clear at this time whether the gK function affecting cell fusion is directly related to its roles in virion envelopment and egress or is a separate function.

Homologs of HSV-1 gK have been reported in a number of other members of the alphaherpesvirinae subfamily, including HSV-2, bovine herpesvirus 1, galldil herpesvirus 1, pseudorabiesvirus, equine herpesvirus 1, Marek's disease virus, and varicella zoster virus (46–52). Alignment of these proteins using the CLUSTALW program (53) shows substantial similarity among them. Structural features conserved among the gK homologs of these viruses include the amino-terminal signal sequence, the presence of N-linked glycosylation sites in the amino-terminal domains, and of the 13 cysteine residues found in HSV-1 gK. Of the conserved cysteines, all but one are located in gK ectodomains, suggesting that they may participate in disulfide bridges important to the structure of the gK ectodomains. In addition, these proteins contain hydrophobic

Fig. 6. Analysis of hydrophobic domain 4 of gK. gK\(1\),2RGS\(H\)4 and gK\(1\),2RGS\(H\)6 were translated in vitro in the presence or absence of microsomal membranes as indicated. After translation, proteinase K and Triton X-100 were added as indicated. Samples were immunoprecipitated by anti-RGS\(H\)4 IgG1 and analyzed by 15.5% SDS-PAGE. Protein bands immunoprecipitated by antibody are indicated by dots.

gK\(1\),2RGS\(H\)4 was immunoprecipitated, whereas the 36-kDa form of gK\(1\),2RGS\(H\)6 was not (Fig. 6, lanes 3 and 7). This indicated that microsomally processed gK\(1\),2RGS\(H\)4 was completely protected by the microsomes, and that the RGS\(H\)4 epitope of gK\(1\),2RGS\(H\)6 remained outside the microsomes. Together, this suggested that hydrophobic domain 4 is a transmembrane domain.

Transmembrane Topology of Glycoprotein K—These studies indicated that gK is a membrane-bound glycoprotein with three transmembrane domains (amino acids 125–139, 226–239, and 311–325). These correspond to hydrophobic domains 1, 2, and 4, which had been identified by hydrophobicity analysis. Hydrophobic domain 3 (amino acids 241–265) is located in the extracellular loop. Therefore, gK has two ectodomains and two cytoplasmic domains. The amino-terminal ectodomain (amino acids 31–124) contains two N-linked glycosylation sites (N48, N58). The entire domain between residues 141 and 225 is located in the cytoplasm and does not traverse the membrane. The COOH terminus (amino acids 326–338) is also a cytoplasmic domain. The topological arrangement of gK based on the data presented in this study is shown in Fig. 7.

DISCUSSION

We have determined the transmembrane topology of gK by examination of the post-translational processing and protease sensitivity of mutant forms of gK. In this study, we identified hydrophobic domains 1, 2, and 4 as transmembrane domains. Therefore, all known Syn mutations in gK are located in either the amino-terminal ectodomain or the ectodomain between hydrophobic domains 1, 2, and 4, which had been identified by hydrophobicity analysis. Hydrophobic domain 3 (amino acids 241–265) is located in the extracellular loop. Therefore, gK has two ectodomains and two cytoplasmic domains. The amino-terminal ectodomain (amino acids 31–124) contains two N-linked glycosylation sites (N48, N58). The entire domain between residues 141 and 225 is located in the cytoplasm and does not traverse the membrane. The COOH terminus (amino acids 326–338) is also a cytoplasmic domain. The topological arrangement of gK based on the data presented in this study is shown in Fig. 7.

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Fig. 7. The proposed transmembrane topology of gK. gK has been shown to have three transmembrane domains. These correspond to hydrophobic domains 1, 2, and 4 and are represented by closed boxes. Hydrophobic domain 3 is indicated by a grey box. The amino-terminal ectodomain contains two N-linked glycosylation sites (Asn\(^{48}\), Asn\(^{58}\%). The amino-terminal region and the ectodomain loop may interact each other to form a complex tertiary structure that may be involved in the regulation of viral glycoprotein-induced cell fusion.

2 C. Mo and T. C. Holland, unpublished data.
domains similar in structure and position to those of HSV-1 gK, indicating similar transmembrane structure.

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