JOURNAL OF VIROLOGY, Sept. 1992, p. 5603–5609
0022-538X/92/095603-07$02.00/0

Vol. 66, No. 9

# Identification and Characterization of a Novel Herpes Simplex Virus Glycoprotein, gK, Involved in Cell Fusion

LLOYD HUTCHINSON,[1] KIM GOLDSMITH,[2] DAN SNODDY,[3] HARA GHOSH,[3] FRANK L. GRAHAM,[1,2] AND DAVID C. JOHNSON[1]†*

*Departments of Pathology,[1] Biology,[2] and Biochemistry,[3] Molecular Virology and Immunology Program, McMaster University, Hamilton, Ontario L8N 3Z5, Canada*

Received 11 March 1992/Accepted 29 May 1992

**Antipeptide sera were used to identify a novel glycoprotein encoded by the UL53 gene of herpes simplex virus type 1 (HSV-1). The UL53 gene product is thought to play a central role in regulating membrane fusion because mutations giving rise to the syncytial phenotype, wherein cells are extensively fused, frequently map to this gene. A single 40-kDa protein, designated gK (the ninth HSV-1 glycoprotein to be described), was detected with antipeptide sera in cells infected with both wild-type and syncytial strains of HSV-1 which were labelled with [$^{35}$S]methionine and [$^{35}$S]cysteine or with [$^{3}$H]glucosamine, and this protein was sensitive to treatment of cells with tunicamycin. With all other HSV glycoproteins studied to date, at least two glycosylated species, often differing substantially in electrophoretic mobility, have been observed in infected cells; thus, gK is unusual in this respect. The 40-kDa gK protein was also immunoprecipitated from cells infected with a recombinant adenovirus vector carrying the UL53 gene. Two glycosylated species of 39 and 41 kDa were produced when UL53 mRNA was translated in vitro in the presence of microsomes, and these proteins differed from gK produced in infected cells not only because they possessed different electrophoretic mobilities but also because they were unable to enter gels after being heated. In addition, a 36-kDa protein was detected in extracts from cells infected with HSV-2 with use of these sera.**

Herpes simplex viruses (HSV) are thought to enter cells by fusion of the virion envelope with the plasma membrane (13, 27, 39). By what is likely an analogous process, HSV can also cause fusion of infected cells. Although wild-type HSV causes limited amounts of cell fusion, this phenomenon is more extensive when cells are infected with syncytial mutants which produce large multinucleated cells (18, 40; reviewed in reference 48). By contrast, cells infected with wild-type HSV primarily become rounded as single cells, although a small amount of fusion is observed. Syncytial mutations have been mapped to four genes in the virus genome (reviewed in reference 48), and deletion of the UL20 gene appears to give rise to the syncytial phenotype (1a). Mutations affecting the cytoplasmic domain of glycoprotein B (gB) (4, 10) induce the syncytial phenotype, which, together with the observation that a gB-negative virus cannot enter cells (5), suggests that gB is directly involved in the membrane fusion process, perhaps as the fusion-inducing protein. Two other HSV glycoproteins (gD and gH) are essential for virus entry and affect the extent of cell fusion (12, 31). Evidence has also accumulated that gD acts as a receptor-binding protein (7, 21, 25, 28) and in this capacity may act to promote membrane proximity.

Although some syncytial mutations have been mapped to other regions of the HSV-1 genome, including the UL1 and UL24 genes (20, 33, 36), the majority of syncytial mutations which have been characterized in detail appear to involve the UL53 gene (2, 41, 45). Two independently isolated syncytial strains, MP and syn-20, both possess mutations affecting a single amino acid in the UL53 open reading frame (9, 42), which has homologs in other herpesviruses (36). Although there have been extensive efforts to identify the product of this gene, none to date has been successful (34, 39a, 49). In this study, we used antipeptide sera to identify and characterize a 40-kDa glycosylated protein; this protein, which we have named gK, is the ninth HSV-1 glycoprotein to be described.

**Preparation of antibodies against the UL53 gene product, gK.** The amino acid sequence predicted from DNA sequence analysis of the UL53 gene suggests that the protein is relatively hydrophobic and is predicted to span the lipid membrane three or four times (9, 42). To identify the UL53 gene product, four peptides spanning regions of the molecule predicted to be hydrophilic were synthesized by Bachem Inc. (Torrance, Calif.): UL53-1 (includes residues 31 to 46), UL53-2 (includes residues 66 to 83), UL53-3 (includes residues 273 to 289), and UL53-4 (includes residues 89 to 104). Peptides were coupled to bovine serum albumin or keyhole limpet hemocyanin and injected repeatedly into rabbits. UL53-2–bovine serum albumin and UL53-2–keyhole limpet hemocyanin conjugates were insoluble, and we were unsuccessful in producing antiserum with use of these conjugates. Antipeptide sera were tested by performing immunoprecipitation reactions with extracts from cells labelled with [$^{35}$S]cysteine and [$^{35}$S]methionine ([$^{35}$S]Cys-[$^{35}$S]Met).

Radiolabelling of cells, immunoprecipitations, and gel electrophoresis have been described previously (19) and were performed with the following modifications. Human R-970 cells (44) were infected with HSV-1 (30 PFU per cell) and radiolabelled with [$^{35}$S]Cys-[$^{35}$S]Met (200 μCi of each per ml) from 4.5 to 10.5 h postinfection. Cell extracts were partially precleared by incubation with rabbit anti-gE/gI sera and protein A-Sepharose, and extracts (from approximately $1 \times 10^5$ to $2 \times 10^5$ cells) were mixed with antipeptide serum (20 μl of anti-UL53-1 or 10 μl of anti-UL53-4), which in some

* Corresponding author.
† Currently on sabbatical at Chiron Corp., Room Q3-17, 4560 Horton St., Emeryville, CA 94608-2916.

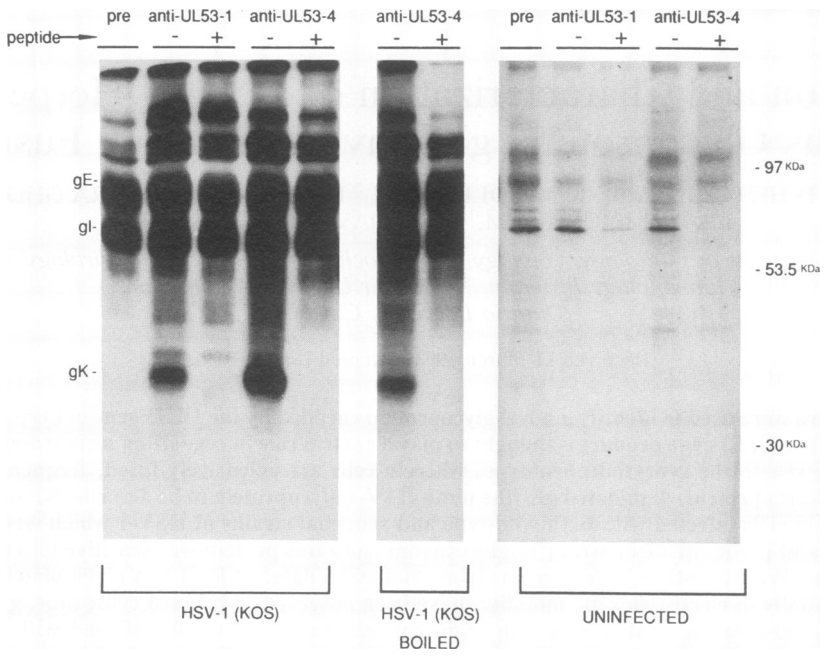


FIG. 1. Recognition by antipeptide sera of gK produced in HSV-1-infected cells. Human R-970 cells were uninfected or were infected with wild-type HSV-1 (KOS). Cells were radiolabelled with [$^{35}$S]Met-[$^{35}$S]Cys, and immunoprecipitations were performed as described in the text. Cell extracts were mixed with pooled preimmune serum (pre), anti-UL53-1 serum preincubated in the presence (+) or absence (−) of peptide UL53-1, or anti-UL53-4 serum preincubated with (+) or without (−) peptide UL53-4. Antigen-antibody complexes were eluted in 50 mM Tris-HCl containing 2% SDS, 2% β-mercaptoethanol, and 15% glycerol at 37°C for 30 min or 100°C (BOILED) for 5 min and analyzed on SDS–12% polyacrylamide gels. Positions of the 40-kDa gK species, HSV IgG Fc receptor proteins gE and gI, and marker proteins of 97, 53.5, and 30 kDa are indicated.

experiments was preincubated with the relevant peptide. Antigen-antibody complexes were washed under stringent conditions (19); and precipitated proteins were eluted with buffer containing 2% sodium dodecyl sulfate (SDS) and 2% β-mercaptoethanol, heated at 37°C for 30 min or 100°C for 5 min, and analyzed by SDS–polyacrylamide (12%) gel electrophoresis (19, 30).

Antisera from animals injected with the UL53-1 or UL53-4 protein-peptide conjugates precipitated a protein of 40 kDa, which was not recognized by pooled serum from preimmune animals (Fig. 1). In other experiments (not shown), anti-UL53-3 antibodies also recognized this protein, although weakly. The 40-kDa protein was initially eluted from protein A-Sepharose beads at 37°C because we had observed that the UL53 gene product translated in vitro aggregated upon boiling (47a), as has been observed with other hydrophobic proteins (46). Subsequently, Ramaswamy and Holland (42a) reported that the UL53 gene product produced in vitro aggregates after boiling. However, when samples of the 40-kDa protein from infected cells were boiled, a substantial fraction of the protein was able to enter polyacrylamide gels (Fig. 1). HSV immunoglobulin G (IgG) Fc receptors, composed of two glycoproteins, gE and gI, were also precipitated by rabbit IgG, as previously observed (22, 23).

To confirm the specificity of the antipeptide antibodies, immunoprecipitations were performed by using uninfected cell extracts and antipeptide sera preincubated with excess quantities of the relevant peptide. The 40-kDa protein was not detected in uninfected cells or when antipeptide sera were preincubated with the relevant peptide (Fig. 1) but was precipitated by anti-UL53-1 preincubated with UL53-4 peptide and anti-UL53-4 sera preincubated with UL53-1 peptide (data not shown). In some experiments with both anti-UL53-1 and anti-UL53-4 antibodies, a small amount of a 38-kDa protein was observed; however, this protein did not disappear when antibodies were preincubated with the relevant peptide (see, for example, Fig. 4), suggesting that it was unrelated to gK. The 40-kDa protein species, which will henceforth be called gK (since the protein is shown below to be glycosylated), was much less abundant than other HSV-1 glycoproteins such as gE and gI (Fig. 1) and gD (see Fig. 3). Increasing the amount of antipeptide sera had little effect on the quantity of gK that was immunoprecipitated (results not shown), suggesting that antibodies to gK were not limiting. However, since antipeptide antibodies may recognize denatured forms of gK, which probably represent only a fraction of the total gK present in infected cells, quantitation of gK by using these antipeptide sera may be inaccurate. The low abundance of gK complicated experiments such as pulse-chase analysis, especially because other, more abundant viral proteins frequently contaminated the immunoprecipitation reaction mixtures. In other experiments, we observed that the addition of phosphonoacetic acid, an inhibitor of HSV DNA replication (35), did not lower gK protein synthesis, while production of gC (a true late gene product) was dramatically reduced, suggesting that gK is the product of an early gene.

**Expression of gK in cells infected with a recombinant adenovirus carrying the gK gene.** To further confirm the specificity of our antipeptide serum and to study the expression of gK outside the context of HSV-infected cells, we expressed gK by using an adenovirus vector, AdgK. An *Nae*I restriction fragment containing the UL53 gene and derived from HSV-1 (KOS) was inserted into the E3 region of adenovirus type 5 (Ad5), using a protocol similar to that previously described (16). Human 293 cells (14) were co-

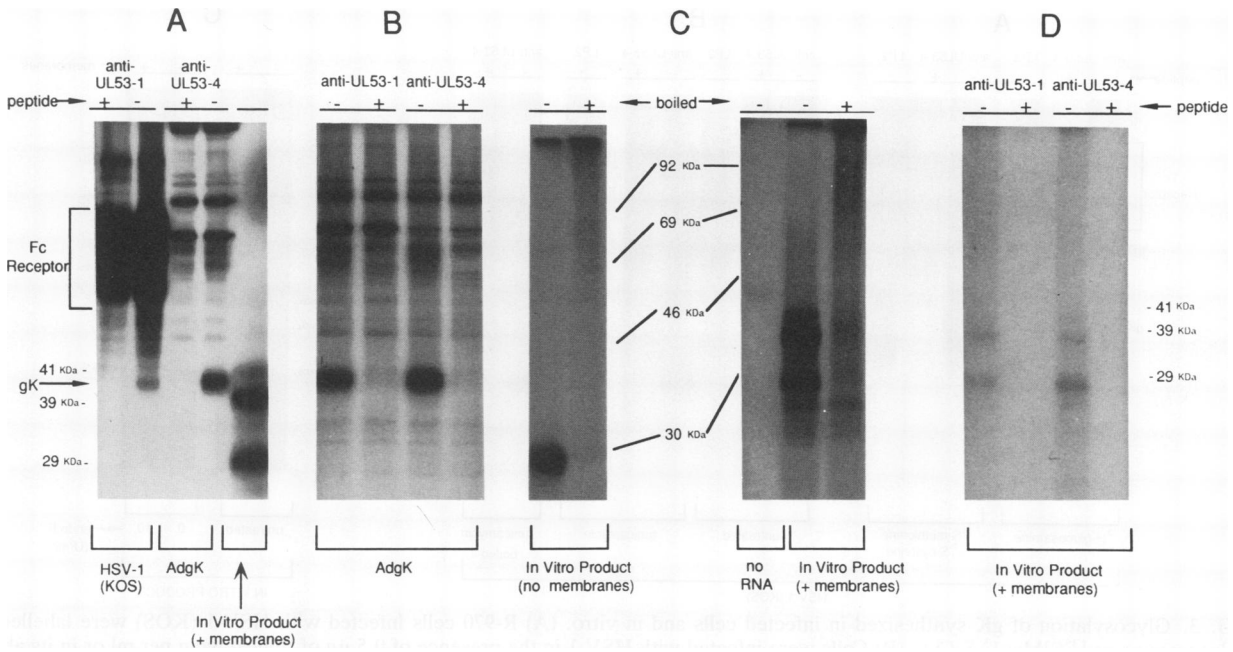


FIG. 2. Recognition by antipeptide sera of gK expressed by an adenovirus vector or translated in vitro. (A and B) R-970 cells were infected with HSV-1 (KOS) or AdgK. Extracts from infected cells were immunoprecipitated by using anti-UL53-1 serum with (+) or without (−) preincubation with UL53-1 peptide or by using anti-UL53-4 serum with (+) or without (−) UL53-4 peptide and proteins eluted at 37°C for 30 min. The electrophoretic mobility of gK from infected cells was compared with that of gK produced in vitro (see below). (C) RNA derived from linearized pGEM4Zsyn DNA was translated in vitro, using a rabbit reticulocyte extract containing [$^{35}$S]Met in the presence (+ membranes) or absence (no membranes) of membranes. In one sample, RNA was not added to the reaction (no RNA). SDS (2%) and β-mercaptoethanol (2%) were added, and the samples were heated at 37°C for 30 min (− boiled) or at 100°C for 5 min (+ boiled) before electrophoresis. (D) Samples translated in vitro with microsomes present were immunoprecipitated with anti-UL53-1 serum or anti-UL53-4 serum preincubated in the presence (+) or absence (−) of the relevant peptide, and then the proteins were eluted at 37°C for 30 min. Positions of the HSV-1 and AdgK 40-kDa species, the in vitro 29-, 39-, and 41-kDa species, HSV IgG Fc receptor proteins, and marker proteins of 92, 69, 46, and 30 kDa are indicated.

transfected with pFG144gK, a plasmid containing the UL53 gene and the right end of Ad5 DNA, and plasmid pFG173, which contains most of Ad5 DNA except for a lethal deletion of viral sequences spanning E3. Plaques appearing after transfection were isolated, and virus progeny was analyzed by restriction analysis of viral DNAs, so that a recombinant virus, designated AdgK, containing the UL53 gene was identified. R970-5 cells (44) were infected with AdgK (30 PFU per cell) and radiolabelled from 24 to 30 h postinfection. Detergent extracts of infected cells were mixed with anti-UL53-1 or anti-UL53-4 antibodies and precipitated with protein A-Sepharose. Both anti-UL53-1 and anti-UL53-4 sera immunoprecipitated, from extracts of AdgK-infected cells, a 40-kDa protein with an electrophoretic mobility identical to that of gK made in HSV-infected cells (Fig. 2A and B). A protein with an apparent molecular size of approximately 80 kDa was specifically precipitated from AdgK-infected cells, and we have preliminary evidence that this is a gK dimer (19a).

**In vitro translation of gK.** To study the properties of gK further, the protein was translated in vitro in the presence or absence of membranes. A vector, pGEM4Zsyn, was constructed by inserting an HSV-1 (KOS) *Nae*I restriction fragment containing the UL53 gene into the *Sma*I site of plasmid pGEM4 (Promega Corp., Madison, Wis.), placing it downstream of an SP6 RNA polymerase promoter. Plasmid DNA was linearized with *Hin*dIII and transcribed with SP6 polymerase (Promega) as described by the supplier. RNA (5 μg/50-μl reaction) was translated in vitro for 1 h at 30°C, using a rabbit reticulocyte extract (Promega) containing 50 μCi of [$^{35}$S]Met. Canine pancreatic microsomal membranes (kindly provided by D. Andrews, McMaster University) were added to some reaction mixtures to process the in vitro-translated proteins (1). After synthesis, the in vitro-translated proteins were immunoprecipitated or analyzed directly on SDS-polyacrylamide gels.

In the absence of membranes, gK translated in vitro had an apparent molecular mass of approximately 29 kDa (Fig. 2C). This result was surprising because DNA sequence data predict that the protein should have a molecular mass of 38 kDa (9). The reason for this difference remains unclear but may reflect the presence of secondary structure if the protein is not fully denatured by SDS at 37°C. Alternatively, the protein may be proteolytically cleaved, as suggested by Ramaswamy and Holland (42a). When UL53 RNA was translated in the presence of dog pancreas membranes, a 29-kDa protein with an apparent molecular mass identical to that of the unprocessed protein was again observed. In addition, two prominent protein species, with apparent molecular masses of 39 and 41 kDa, were detected (Fig. 2C), indicating that posttranslational modifications had occurred. It is possible that the microsomal membranes did not saturate the translated protein so that an unglycosylated 29-kDa species was produced. This hypothesis was supported by the fact that the 29-kDa form was the major species after treatment of the microsomal translation products with endoglycosidase F (Fig. 3C). All three of the protein species

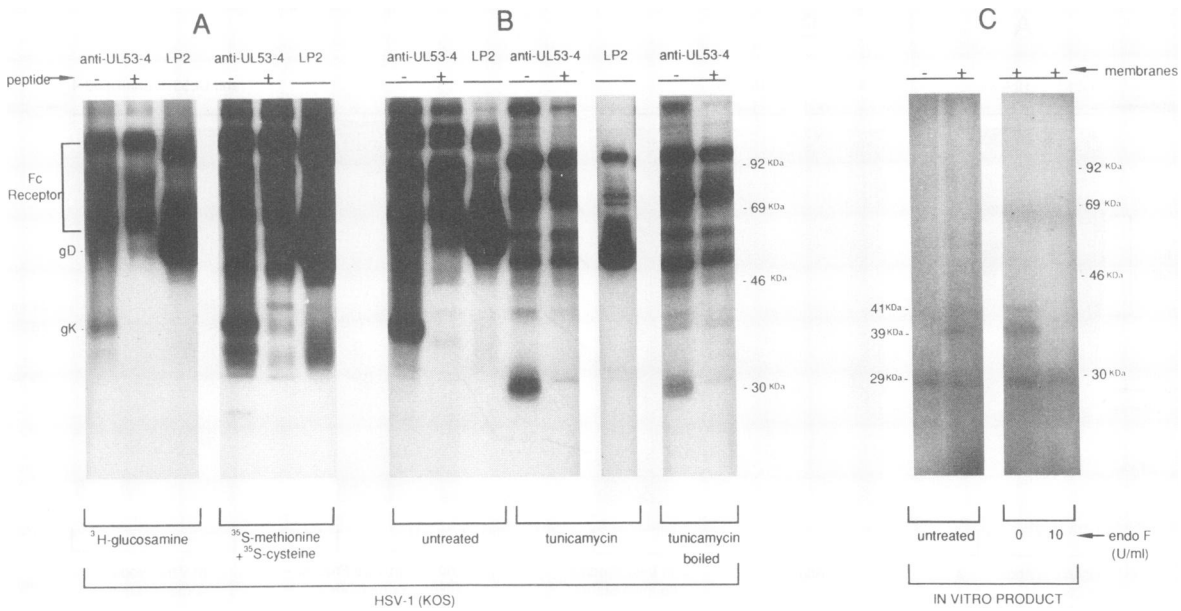


FIG. 3. Glycosylation of gK synthesized in infected cells and in vitro. (A) R-970 cells infected with HSV-1 (KOS) were labelled with [$^3$H]glucosamine or [$^{35}$S]Met-[$^{35}$S]Cys. (B) Cells were infected with HSV-1 in the presence of 0.5 μg of tunicamycin per ml or in its absence (untreated) and labelled with [$^{35}$S]Met-[$^{35}$S]Cys. Cell extracts were immunoprecipitated with monoclonal antibody LP2, specific for gD (37), or anti-UL53-4 serum with (+) or without (−) preincubation with UL53-4 peptide and eluted at 37°C or boiled and electrophoresed in 12% polyacrylamide gels. (C) In vitro translation reactions performed in the presence (+) or absence (−) of microsomes were diluted in 0.1 M $NaPO_4$ buffer (pH 7.5)–0.1% SDS–0.1% β-mercaptoethanol–1.5 mM phenylmethylsulfonyl fluoride and heated at 37°C for 5 min; then octylglucoside (1%) and endoglycosidase F (0 or 10 U/ml) were added, and the samples were incubated for 4 h at 37°C. Positions of the HSV-1 Fc receptor proteins, gD, gK, the 29-, 39-, and 41-kDa in vitro gK species, and marker proteins of 30, 46, 69, and 92 kDa are marked.

translated in the presence of membranes were also recognized by anti-UL53-1 and anti-UL53-4 antibodies (Fig. 2D).

When the 29-kDa protein species produced in the absence of membranes was briefly heated at 100°C, there was a dramatic reduction in the amount of protein detected in the gel and a concomitant increase in the quantity of protein observed at the top of the resolving gel and in the stacking gel (Fig. 2C). Similarly, the 29-, 39-, and 41-kDa species synthesized in the presence of membranes were markedly reduced after samples were boiled. These results suggest that both the unglycosylated and glycosylated forms of gK synthesized in vitro aggregate upon heating. In contrast, gK immunoprecipitated from HSV-infected cells was much less sensitive to heating (Fig. 1). Coupled with the observation that the 39- and 41-kDa species differed in electrophoretic mobility from the 40-kDa gK protein detected in infected cells (Fig. 2A), these results suggest there are major differences in the folding or processing of gK made in infected cells compared with the protein synthesized in vitro.

**Glycosylation of gK.** The gK polypeptide has two potential N-linked glycosylation signals at residues 48 and 58 (9). To ascertain whether gK was glycosylated, HSV-1-infected cells were labelled with [$^3$H]glucosamine (500 μCi/ml) in media containing 0.3 mg glucose per ml or with [$^{35}$S]Met-[$^{35}$S]Cys in media lacking methionine and cysteine from 6.5 to 17 h postinfection. A 40-kDa protein was immunoprecipitated from extracts of cells labelled with [$^3$H]glucosamine, and this protein was not observed when anti-gK antibodies were preincubated with the relevant peptide (Fig. 3A), confirming that gK is glycosylated. A minor 38-kDa species labelled with [$^{35}$S]Met-[$^{35}$S]Cys and migrating slightly faster than the 40-kDa gK was competed for with peptide and was apparently derived from gK by proteolysis because this species was more abundant when protease inhibitors were not used (results not shown).

When HSV-infected cells were treated with tunicamycin to inhibit the attachment of N-linked oligosaccharides, the electrophoretic mobility of gK increased to that observed when the protein was translated in vitro in the absence of membranes (compare Fig. 2C and 3B). Anti-UL53-1 antibodies (which recognize residues 31 to 46) did not recognize gK from tunicamycin-treated cells better than did gK from untreated cells despite the presence of a potential glycosylation site at residue 48. A decrease in the apparent molecular mass of a glycoprotein from 40 to 29 kDa by the removal of one or two N-linked oligosaccharides is relatively unusual but not without precedent (17). Additionally, the 41- and 39-kDa gK species produced in vitro with membranes present were also sensitive to endoglycosidase F (Fig. 3C). We suspect that the 39- and 41-kDa species represent proteins modified with one and two N-linked oligosaccharides, respectively, and that the 40-kDa species observed in infected cells may possess either one or two N-linked oligosaccharides which have been processed to complex oligosaccharides in the Golgi apparatus (29, 32).

**Expression of gK in cells infected with HSV-1 syncytial mutants.** HSV-1 syncytial strains MP (18) and syn-20 (43) both contain substitution mutations at residue 40 in the UL53 gene (9, 42). To examine the expression of gK in cells infected with these mutants and with other virus strains, gK was immunoprecipitated from extracts of radiolabelled cells. We detected gK in cells infected with wild-type HSV-1 strains KOS (47), mP (18), F (11), 17 (3), and PAA$^r$5 (15) as well as with HSV-1 mutants 804, which was reported to contain a syncytial mutation mapping to the UL1 gene (33) (though we have recent data suggesting that the syncytial

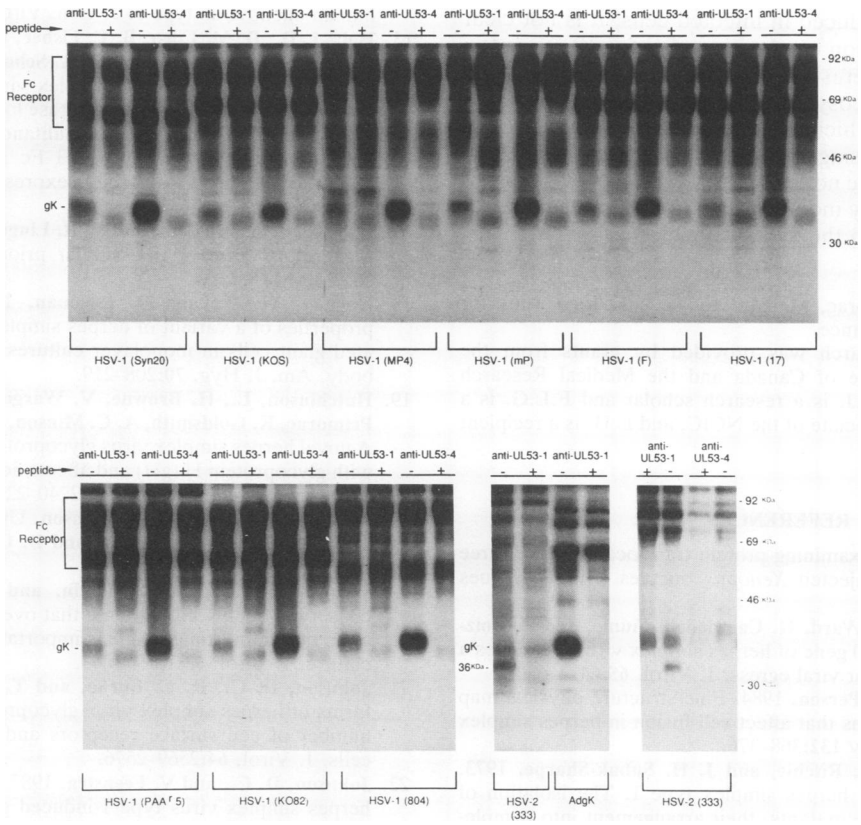


FIG. 4. Expression of gK in cells infected with HSV-1 syncytial mutants. R-970 cells were infected with HSV-1 strain KOS, mP, F, 17, or PAA$^r$5, mutant KO82 (which is unable to express gB), syncytial strain 804, MP-4, or syn-20, HSV-2 strain 333, or AdgK. Infected cells were labelled with [$^{35}$S]Met-[$^{35}$S]Cys, and extracts were immunoprecipitated with anti-UL53-1 serum preincubated in the presence (+) or absence (−) of UL53-1 peptide or with anti-UL53-4 serum preincubated with (+) or without (−) UL53-4 peptide. The precipitated proteins were eluted at 37°C and subjected to electrophoresis on 12% polyacrylamide gels. Positions of HSV-1 40-kDa gK, HSV-2 36-kDa gK, HSV IgG Fc receptor proteins, and marker proteins of 92, 69, 46, and 30 kDa are indicated.

mutation in 804 actually maps to the UL53 gene [44a]), KO82, which is unable to express gB (6), syn-20, and MP (MP4) (Fig. 4). Anti-UL53-1 antibodies recognized the gK produced in syn-20- and MP-4-infected cells even though residue 40, included in the UL53-1 peptide, is altered in these mutant proteins. Furthermore, we detected an HSV-2 gK, having an apparent molecular mass of 36 kDa, in cells infected with HSV-2 strain 333 with use of anti-UL53-1 serum but not anti-UL53-4 serum (Fig. 4). The HSV-1 and HSV-2 UL53 DNA sequences contain nine mismatches in the 16 residues of the HSV-1 UL53-4 peptide, whereas the HSV-1 and HSV-2 UL53-1 peptides are identical (8, 9).

**Conclusions.** We have detected an HSV-1 glycoprotein, designated gK, which is the product of the UL53 gene, known to be involved in HSV-induced membrane fusion (9, 42). Relatively low levels of gK were immunoprecipitated from extracts of infected cells by using antipeptide sera, and gK could be detected in partially purified preparations of HSV-1 virions (results not shown). The protein was also expressed in cells infected with syncytial viruses syn-20 and MP, which carry a mutation in the UL53 gene. It appears that the mutant forms of gK synthesized by these syncytial viruses may be unable to regulate the fusion process. In support of the hypothesis that gK regulates fusion in a negative fashion, we have recently found that transfected cell lines expressing wild-type gK or cell lines infected with AdgK can suppress fusion induced by syn-20 and MP (19b). We also have evidence that gK is essential for HSV-1 replication (19a).

A single electrophoretic form of gK was detected in infected cells, and this protein was modified with N-linked oligosaccharides. This is an unusual observation because all other HSV-1 glycoproteins characterized to date appear as two protein species, an immature form with high-mannose N-linked oligosaccharides and a mature form with processed N-linked oligosaccharides and containing O-linked oligosaccharides (19, 22, 24, 26, 38, 48). Also unusual was the observation that gK made in infected cells was markedly different from gK translated in vitro. Two glycosylated protein species were observed in vitro, and both differed electrophoretically from gK produced in infected cells. Further, the in vitro-synthesized gK was much more sensitive to heat denaturation than was gK produced in infected cells. These observations suggest that gK made in vitro is folded or processed differently from gK synthesized in infected cells.

Recently, Ramaswamy and Holland (42a) reported that the UL53 gene product synthesized in vitro was modified with N-linked oligosaccharides, as judged from the observation that a single 36-kDa protein produced from a full-length form of the UL53 gene and truncated forms of the protein were sensitive to endoglycosidase F. In addition, evidence consistent with the removal of a 30-residue signal sequence was presented. However, in light of our observations that the UL53 gene product produced in vitro differs in several

respects from that produced in infected cells, it is not clear that this latter conclusion can be extended to gK present in infected cells. It is interesting that one of the two potential sites for N-linked glycosylation at residue 48 includes an aspartic acid residue which often reduces the use of the site (29a), and thus only a single site may be utilized in infected cells. In this respect, we note that the 41-kDa species that we observed in vitro, likely modified with two oligosaccharides, was less abundant than the 39-kDa species.

We thank Steve Primorac, Mojgan Hodaie, and John Rudy for excellent technical assistance.

Support for this research was provided by grants from the National Cancer Institute of Canada and the Medical Research Council of Canada. D.C.J. is a research scholar and F.L.G. is a Terry Fox Research Associate of the NCIC, and L.H. is a recipient of an MRC studentship.

## REFERENCES

1. **Andrews, D.** 1989. Examining protein translocation in cell-free systems and microinjected *Xenopus* oocytes. BioTechniques **7**:960–967.
1a. **Baines, J. D., P. L. Ward, G. Campadelli-Fiume, and B. Roizman.** 1991. The UL20 gene of herpes simplex virus 1 encodes a function necessary for viral egress. J. Virol. **65**:6414–6424.
2. **Bond, V. C., and S. Person.** 1984. Fine structure physical map locations of alterations that affect cell fusion in herpes simplex virus type 1. Virology **132**:368–376.
3. **Brown, S. M., D. A. Ritchie, and J. H. Subak-Sharpe.** 1973. Genetic studies with herpes simplex type 1. The isolation of temperature sensitive mutants, their arrangement into complementation groups and recombination analysis leading to a linkage map. J. Gen. Virol. **18**:329–346.
4. **Bzik, D. J., B. A. Fox, N. A. Deluca, and S. Person.** 1984. Nucleotide sequence of a region of the herpes simplex virus type 1 gB glycoprotein gene: mutations affecting rate of virus entry and cell fusion. Virology **137**:185–190.
5. **Cai, W., B. Guand, and S. Person.** 1988. Role of glycoprotein B of herpes simplex virus type 1 in viral entry and cell fusion. J. Virol. **62**:2596–2604.
6. **Cai, W., S. Person, S. C. Warner, J. Zhou, and N. A. DeLuca.** 1987. Linker-insertion nonsense and restriction-site deletion mutants of the gB glycoprotein gene of herpes simplex virus type 1. J. Virol. **61**:714–721.
7. **Campadelli-Fiume, G., M. Arsenakis, F. Farabegoli, and B. Roizman.** 1988. Entry of herpes simplex virus 1 in BJ cells that constitutively express viral glycoprotein D is by endocytosis and results in degradation for the virus. J. Virol. **62**:159–167.
8. **Debroy, C.** 1990. Nucleotide sequence of the herpes simplex virus type 2 syn gene that causes cell fusion. Gene **88**:275–277.
9. **Debroy, C., N. Pederson, and S. Person.** 1985. Nucleotide sequence of herpes simplex virus type 1 gene that causes cell fusion. Virology **145**:36–48.
10. **Deluca, N., D. J. Bzik, V. C. Bond, S. Person, and W. Snipes.** 1982. Nucleotide sequences of herpes simplex virus type 1 (HSV-1) affecting virus entry, cell fusion, and production of glycoprotein gB (VP7). Virology **122**:411–423.
11. **Ejercito, P. M., E. D. Kieff, and B. Roizman.** 1968. Characterization of herpes simplex virus strains differing in their effect on social behaviour of infected cells. J. Gen. Virol. **2**:357–364.
12. **Forrester, A. J., H. Farrell, G. Wilkinson, J. Kaye, N. D. Poynter, and A. C. Minson.** 1991. Construction and properties of a mutant of herpes simplex virus type 1 deleted for glycoprotein H sequences. J. Virol. **66**:341–348.
13. **Fuller, A. O., and P. G. Spear.** 1987. Anti-glycoprotein D antibodies that permit adsorption but block infection by herpes simplex virus 1 prevent virion-cell fusion at the cell surface. Proc. Natl. Acad. Sci. USA **84**:5454–5458.
14. **Graham, F. L., J. Smiley, W. C. Russell, and R. Nairn.** 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. **36**:59–72.
15. **Hall, J. D., D. M. Coen, B. L. Fisher, M. Weisslitz, S. Randall, R. E. Almy, P. T. Gelep, and P. A. Schaffer.** 1984. Generation of genetic diversity in herpes simplex virus: an antimutator phenotype maps to the DNA polymerase locus. Virology **132**:26–37.
16. **Hanke, T., F. L. Graham, V. Lulitanond, and D. C. Johnson.** 1990. Herpes simplex virus IgG Fc receptors induced using recombinant adenovirus vectors expressing glycoproteins E and I. Virology **177**:437–444.
17. **Hay, B., S. B. Prusiner, and V. R. Lingappa.** 1987. Evidence for a secretory form of the cellular prion protein. Biochemistry **26**:8110–8115.
18. **Hoggan, M. D., and B. Roizman.** 1959. The isolation and properties of a variant of herpes simplex producing multinucleated giant cells in monolayer cultures in the presence of antibody. Am. J. Hyg. **70**:208–219.
19. **Hutchinson, L., H. Browne, V. Wargent, N. Davis-Poynter, S. Primorac, K. Goldsmith, A. C. Minson, and D. C. Johnson.** 1992. A novel herpes simplex virus glycoprotein, gL, forms a complex with glycoprotein H (gH) and affects normal folding and surface expression of gH. J. Virol. **66**:2240–2250.
19a. **Hutchinson, L., and D. C. Johnson.** Unpublished data.
19b. **Hutchinson, L., S. Primorac, and D. C. Johnson.** Unpublished data.
20. **Jacobson, J. G., S. L. Martin, and D. M. Coen.** 1989. A conserved open reading frame that overlaps the herpes simplex virus thymidine kinase gene is important for viral growth in cell culture. J. Virol. **63**:1839–1843.
21. **Johnson, D. C., R. L. Burke, and T. Gregory.** 1990. Soluble forms of herpes simplex virus glycoprotein D bind to a limited number of cell surface receptors and inhibit virus entry into cells. J. Virol. **64**:2569–2576.
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. **Johnson, D. C., M. C. Frame, N. W. Ligas, A. M. Cross, and N. D. Stow.** 1988. Herpes simplex virus immunoglobulin G Fc receptor activity depends on a complex of two viral glycoproteins, gE and gI. J. Virol. **62**:1347–1354.
24. **Johnson, D. C., G. Ghosh-Choudhury, J. R. Smiley, L. Fallis, and F. L. Graham.** 1988. Abundant expression of herpes simplex virus glycoprotein gB using an adenovirus vector. Virology **164**:1–14.
25. **Johnson, D. C., and M. W. Ligas.** 1988. Herpes simplex viruses lacking glycoprotein D are unable to inhibit virus penetration: quantitative evidence for virus specific cell surface receptors. J. Virol. **62**:1486–1494.
26. **Johnson, D. C., and P. G. Spear.** 1983. O-linked oligosaccharides are acquired by herpes simplex virus glycoproteins in the Golgi apparatus. Cell **32**:987–997.
27. **Johnson, D. C., M. Wittels, and P. G. Spear.** 1984. Binding to cells of virosomes containing herpes simplex virus type 1 glycoproteins and evidence for fusion. J. Virol. **52**:238–247.
28. **Johnson, R. M., and P. G. Spear.** 1989. Herpes simplex virus glycoprotein D mediates interference with herpes simplex virus infection. J. Virol. **63**:819–827.
29. **Katz, F. N., J. E. Rothman, V. R. Lingappa, G. Blobel, and H. R. Lodish.** 1977. Membrane assembly in vitro: synthesis, glycosylation, and asymmetric insertion of a transmembrane protein. Proc. Natl. Acad. Sci. USA **74**:3278–3282.
29a. **Kornfeld, R., and S. Kornfeld.** 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. **54**:631–640.
30. **Laemmli, U. K.** 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
31. **Ligas, M. W., and D. C. Johnson.** 1988. A herpes simplex virus mutant in which glycoprotein D sequences are replaced by β-galactosidase sequences binds to but is unable to penetrate into cells. J. Virol. **62**:1486–1494.
32. **Lingappa, V. R., A. Devellers-Thiery, and G. Blobel.** 1977. Nascent prehormones are intermediates in the biosynthesis of authentic pituitary growth hormone and prolactin. Proc. Natl.

Acad. Sci. USA **74:**2432–2436.
33. **Little, S. P., and P. A. Schaffer.** 1981. Expression of the syncytial (syn) phenotype in HSV-1, strain KOS: genetic and phenotypic studies of mutants in two syn loci. Virology **112:** 686–697.
34. **MacLean, C. A., S. Efstathiou, M. L. Elliott, F. E. Jamieson, and D. J. McGeoch.** 1991. Investigation of herpes simplex virus type 1 genes encoding multiply inserted membrane proteins. J. Gen. Virol. **72:**897–906.
35. **Mao, J. C.-H., E. E. Robishaw, and L. R. Overby.** 1975. Inhibition of DNA polymerase from herpes simplex virus-infected WI-38 cells by phosphonoacetic acid. J. Virol. **15:**1281–1283.
36. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Donlan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol. **69:**1531–1574.
37. **Minson, A. C., T. C. Hodgman, P. Digard, D. C. Hancock, S. E. Bell, and E. A. Buckmaster.** 1986. An analysis of the biological properties of monoclonal antibodies against glycoprotein D of herpes simplex virus and identification of amino acid substitutions that confer resistance to neutralization. J. Gen. Virol. **67:**1001–1013.
38. **Oloffson, S., J. Blomberg, and E. Lycke.** 1981. O-glycosidic carbohydrate-peptide linkages of herpes simplex virus glycoproteins. Arch. Virol. **70:**321–329.
39. **Para, M. F., R. B. Bauke, and P. G. Spear.** 1980. Immunoglobin G (Fc)-binding receptors on virions of herpes simplex virus type 1 and transfer of these receptors to the cell surface by infection. J. Virol. **34:**512–520.
39a. **Person, S.** Personal communication.
40. **Person, S., R. W. Knowles, G. S. Read, S. C. Warner, and V. C. Bond.** 1976. Kinetics of cell fusion induced by a syncytia-producing mutant of herpes simplex virus type 1. J. Virol. **17:**183–190.
41. **Pogue-Geile, K. L., G. T.-Y. Lee, S. K. Shapira, and P. G. Spear.** 1984. Fine mapping of mutations in the fusion-inducing MP strain of herpes simplex virus type 1. Virology **136:**100–109.
42. **Pogue-Geile, K. L., and P. G. Spear.** 1987. The single base pair substitution responsible for the syn phenotype of herpes simplex virus type 1, strain MP. Virology **157:**67–74.
42a. **Ramaswamy, R., and T. C. Holland.** 1992. In vitro characterization of the HSV-1 UL53 gene product. Virology **186:**579–587.
43. **Read, G. S., S. Person, and P. M. Keller.** 1980. Genetic studies of cell fusion induced by herpes simplex virus type 1. J. Virol. **35:**105–113.
44. **Rhim, J. S., H. Y. Cho, and R. J. Huebner.** 1975. Non-producer human cells induced by murine sarcoma cells. Int. J. Cancer **15:**23–29.
44a. **Roop, C., L. Hutchinson, and D. C. Johnson.** Unpublished data.
45. **Ruyechan, W. T., L. S. Morse, D. M. Knipe, and B. Roizman.** 1979. Molecular genetics of herpes simplex virus. II. Mapping of the major viral glycoproteins and of the genetic loci specifying the social behavior of infected cells. J. Virol. **29:**677–687.
46. **Semenza, J. C., K. G. Hardwick, N. Dean, and H. R. B. Pelham.** 1990. ERD2 a yeast gene required for the receptor-mediated retrieval of luminal ER proteins from the secretory pathway. Cell **61:**1349–1357.
47. **Smith, K. O.** 1964. Relationships between the envelope and the infectivity of herpes simplex virus. Proc. Soc. Exp. Biol. Med. **115:**814–816.
47a. **Snoddy, D., D. C. Johnson, and H. P. Ghosh.** 1990. In vitro expression and characterization of the HSV-1 syn gene product, abstr. 213. Abstr. 15th Int. Herpesvirus Workshop, Washington, D.C.
48. **Spear, P. G.** Membrane fusion induced by herpes simplex virus. *In* J. Bentz (ed.), Viral fusion mechanisms, in press. CRC Press, Boca Raton, Fla.
49. **Spear, P. G.** Personal communication.