

Complex between Glycoproteins gI and gp63 of Pseudorabies Virus: Its Effect on Virus Replication

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To ascertain the biological functions of different glycoproteins that are nonessential for pseudorabies virus growth *in vitro*, we have constructed mutants defective in one (or a combination) of these glycoproteins and have examined various aspects of their role in the infective process. We made the following two observations. (i) Glycoproteins gI and gp63 are noncovalently complexed to each other. They are coprecipitated by antisera against either one of these glycoproteins but do not share antigenic determinants: monoclonal antibodies against gp63 do not immunoprecipitate gI from extracts of gp63⁻ mutant-infected cells, and monoclonal antibodies against gI do not immunoprecipitate gp63 from extracts of gI⁻ mutant-infected cells. (ii) Mutants unable to synthesize either gI or gp63 have some common biological characteristics; they have a growth advantage in primary chicken embryo fibroblasts. Furthermore, we have shown previously that in conjunction with glycoprotein gIII, gI and gp63 are necessary for the expression of virulence (T. C. Mettenleiter, C. Schreurs, F. Zuckermann, T. Ben-Porat, and A. S. Kaplan, *J. Virol.* 62, 2712-2717, 1988). These results show that the functional entity affecting virus replication in chicken embryo fibroblasts, as well as affecting virulence, is the complex between gI and gp63. The gI-gp63 complex of pseudorabies virus does not appear to have Fc receptor activity as does its homolog, the gI-gE complex of herpes simplex virus.

Pseudorabies virus (PrV), a herpesvirus of swine, encodes at least four glycoproteins that are not essential for growth in cell culture (2, 12, 18, 21, 22, 26). Primary isolates of PrV, however, do express these glycoproteins; consequently, they must play a role in virus replication, at least *in vivo*. To study the functions of the nonessential glycoproteins of PrV, we have constructed mutants that are defective in the expression of one or a combination of these glycoproteins and have initiated studies dealing with the interactions of the mutants with cells in culture, as well as with the role of these glycoproteins in virus virulence.

We showed previously that both glycoproteins gIII and gI are involved in the release of the virus from rabbit kidney (RK) cells but that each of these glycoproteins affects release from these cells, mainly in conjunction with the other (3, 13, 23). Both glycoproteins gI and gp63 also affect virulence, an effect that is also evident only in conjunction with a defect in glycoprotein gIII (as well as possibly in conjunction with a defect in some other viral function) (14, 15). Furthermore, a defect in gI alone affects virus growth in a cell type-specific fashion; the absence of this glycoprotein confers upon PrV(Ka) a growth advantage in primary chicken embryo fibroblasts (CEF) but not in RK or pig kidney (PK) cells (11). In addition, gIII has been shown to be involved in virus adsorption (23).

In the present communication we show that glycoproteins gI and gp63 are found in the form of a complex within the infected cells and that each affects several aspects of the biology of the virus in the same way. Because mutations in the genes encoding either gI or gp63 have the same effect on at least some growth characteristics of the virus, we conclude that the functional entity that affects these growth characteristics is the complex between gI and gp63.

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MATERIALS AND METHODS

Virus strains and cell cultures. PrV(Ka) is a strain that has been carried in our laboratory for more than 25 years. Herpes simplex virus type 1 (HSV-1), strain H4 (HSV-H4), is a laboratory strain; HSV-T is a primary isolate of HSV-1. RK, PK, and Madin Darby bovine kidney (MDBK) cells, as well as CEF, were cultivated in Eagle synthetic medium supplemented with 5% dialyzed bovine serum. Virus was titrated by plaque assay in RK or PK cells. The deletion or deletion-M13 insertion mutants defective in the gene encoding gI, gp63, or both, used in this study, have been described (13, 14, 15). Figure 1, which summarizes previously published data, is included to facilitate the understanding of the paper.

Biochemicals. [³H]glucosamine (specific activity, 32 Ci/mmol), [^α-³²P]dCTP, and ¹²⁵I-labeled human immunoglobulin G (IgG) (specific activity, 3.8 Ci/μg) were purchased from Du Pont, NEN Research Products. [³⁵S]methionine (specific activity, 800 Ci/mmol) was purchased from Amersham Corp.

Immunoprecipitation. Immunoprecipitation was performed by the method of Kessler (9), as described previously (5). The monoclonal antibodies against gp63 were a gift of P. Desmetre, Rhone-Merieux. The monoclonal antibodies against gp50 were a gift of C. Marchioli, the Upjohn Co.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was performed as described previously (5).

RESULTS

Glycoproteins gI and gp63 form a complex and are coprecipitated. During the course of characterizing mutants that had been rendered defective in various glycoproteins, we observed that when extracts of cells infected with wild-type PrV(Ka) were reacted with monoclonal antibodies specific against either gI or gp63, both proteins were coprecipitated, indicating that they may form a complex. To test whether this is indeed the case, we performed the experiments whose

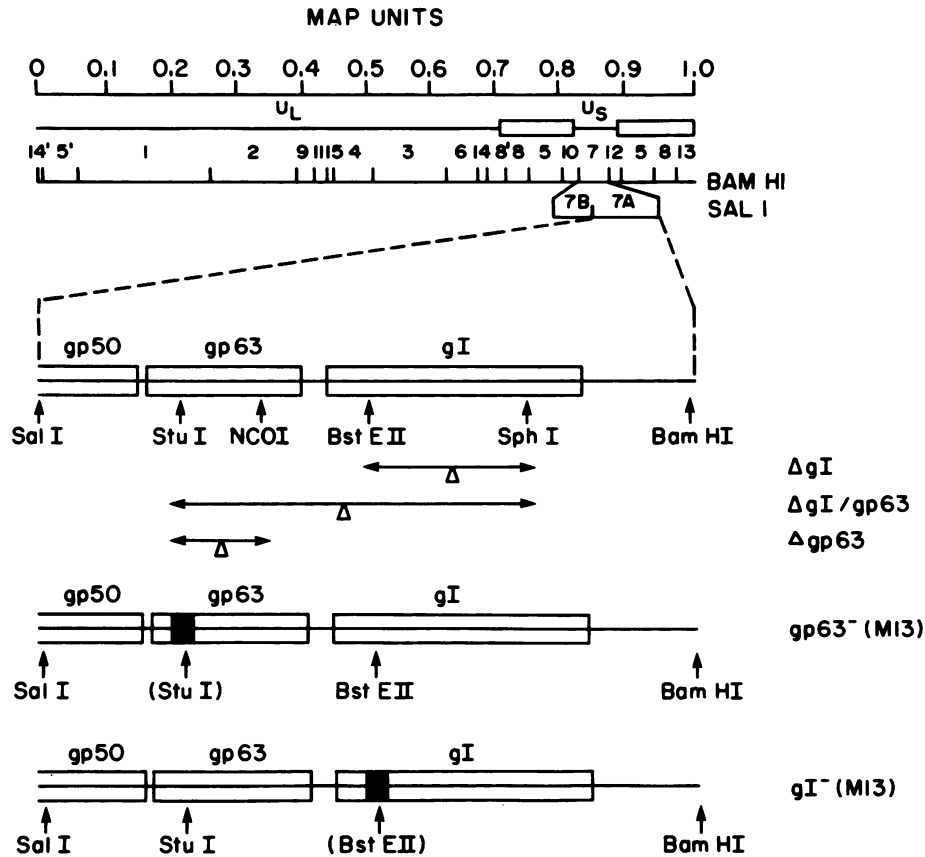


FIG. 1. Maps of PrV mutants with deletions or deletion-insertions in the genes encoding gI and gp63. The top of the figure shows the *Bam*HI restriction map of PrV DNA. The positions of the genes encoding gI, gp63, and part of gp50 are illustrated in line 4. The deletions which span sequences in gI (Δ gI), gI and gp63 (Δ gI/gp63), or gp63 (Δ gp63) are shown in lines 5, 6, and 7, respectively. Lines 8 and 9 illustrate the structures of the region of interest in the deletion-M13 insertion mutants of gp63 and gI, respectively. In these mutants, approximately 200 base pairs flanking the *Stu*I or *Bst*EII sites was removed by digestion with BAL 31 nuclease, and an *Hae*III fragment of M13, approximately 200 base pairs in size, was inserted. Symbol: ■, M13 sequences that have been inserted into these genes.

results are illustrated in Fig. 2 and 3. In these experiments, cells were infected with either PrV(Ka) (Fig. 2), a gI⁻, a gp63⁻, or a gI⁻ gp63⁻ mutant (Fig. 3) and labeled between 3 and 24 h postinfection with either [³H]glucosamine or [³⁵S]methionine. Extracts of the infected cells were then immunoprecipitated with various monoclonal antibodies, and the immunoprecipitates were analyzed by polyacrylamide gel electrophoresis.

Monoclonal antibodies against either gI or gp63 precipitated two protein bands with migration characteristics of gp63 and gI from extracts of wild-type-infected cells (Fig. 2). These two bands appeared whether or not the samples were electrophoresed under reducing conditions (data not shown). Monoclonal antibodies against gp63 precipitated only gp63 from gI⁻ mutant-infected cell extracts, and monoclonal antibodies against gI precipitated only gI from gp63⁻ mutant-infected cell extracts (Fig. 3). Neither of these monoclonal antibodies precipitated any protein from the cell extract infected with the gI⁻ gp63⁻ mutant (Fig. 3).

Two main conclusions can be drawn from these observations. (i) Glycoproteins gp63 and gI are noncovalently complexed with each other. They are coprecipitated by monoclonal antibodies either against gI or against gp63, but do not share common antigenic determinants. Only the appropriate monoclonal antibody precipitated either gp63 or gI from extracts of cells infected with gI⁻ or gp63⁻ mutants. (ii)

Although gI and gp63 form a complex, and although the two genes encoding these glycoproteins are adjacent to each other on the genome, synthesis of one is not dependent on the synthesis of the other.

Effect of gp63 and gI on virus replication. Neither gI nor gp63 is essential for replication in vitro (2, 12, 13, 18). Indeed, the yields of infectious virus and of viral particles obtained from RK and PK cells infected with gI⁻ or gp63⁻ deletion-M13 insertion mutants, with gI⁻ or gp63⁻ deletion mutants, or with wild-type virus are approximately the same (3, 13). Mutations in gI do, however, affect virus growth in a subtle way: gI⁻ mutants of PrV have a small but definite growth advantage over PrV(Ka) in CEF (11). Since gI and gp63 are found in infected cell extracts in the form of a complex, it appeared possible that the two glycoproteins affect virus growth jointly.

To determine whether mutations in the genes encoding gp63 or gI affect virus growth similarly, we infected cells at a low multiplicity of infection (to allow the virus to recycle) with a mixture of wild-type virus and deletion-M13 insertion mutants of either gp63 or gI. At the low multiplicity of infection used (0.01 PFU per cell), much of the virus is produced by cells in which complementation of the mutants by wild-type virus will not take place. The relative growth advantage (or disadvantage) of the mutants over the wild type in certain cell types can then be determined by com-

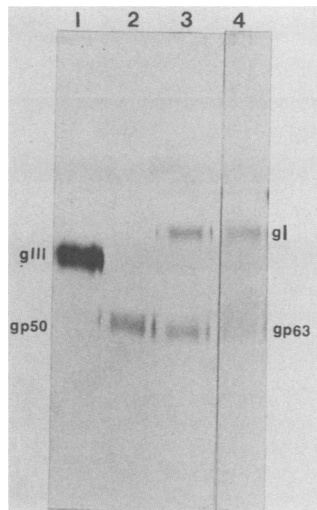


FIG. 2. Immunoprecipitation of glycoproteins from wild-type-infected cells. RK cells were infected with PrV(Ka) (multiplicity of infection, 5 PFU per cell), and the cells were labeled by incubation in Eagle medium containing [3 H]glucosamine (50 μ Ci/ml) between 2 and 24 h postinfection. The cells were harvested, extracts were prepared, and the glycoproteins were immunoprecipitated with a monoclonal antibody against gIII (lane 1), gp50 (lane 2), gI (lane 3), or gp63 (lane 4). The precipitates were subjected to polyacrylamide gel electrophoresis followed by fluorography and autoradiography.

paring the numbers of virions that contain the M13 sequences (within the gene that has been inactivated) with the number of wild-type virions that are present in mixtures of these viruses before and after passage in different cell types.

gp63 and gI deletion-M13 insertion mutants have a selective growth advantage over wild-type virus in CEF (Fig. 4). In the original mixtures of gI or gp63 deletion-M13 insertion mutants and wild type, just a few of about 50 randomly picked plaques contained M13 sequences, i.e., were gp63⁻ or gI⁻ mutants. After five passages in CEF, however, most

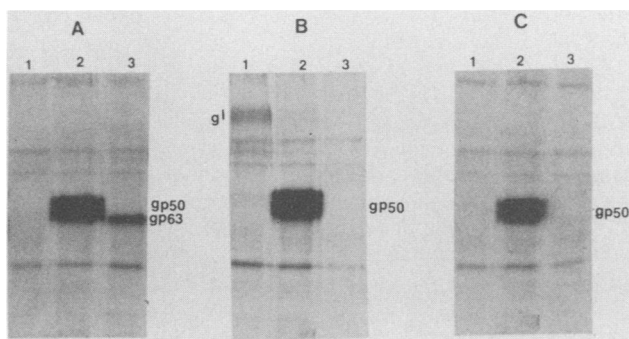


FIG. 3. Immunoprecipitation of glycoproteins from gI⁻, gp63⁻, and gI⁻gp63⁻ deletion mutant-infected cells. RK cells were infected (multiplicity of infection, 5 PFU per cell) with a PrV(Ka)gI⁻ mutant (panel A), a PrV(Ka)gp63⁻ mutant (panel B), or a PrV(Ka)gI⁻gp63⁻ mutant (panel C). The infected cells were incubated between 2 and 24 h postinfection in Eagle medium containing no amino acids except arginine and [35 S]methionine (20 μ Ci/ml). The cells were harvested, extracts were prepared, and the glycoproteins were immunoprecipitated with monoclonal antibodies against gI (lanes 1), gp50 (lanes 2), or gp63 (lanes 3). The precipitates were subjected to polyacrylamide gel electrophoresis followed by fluorography and autoradiography.

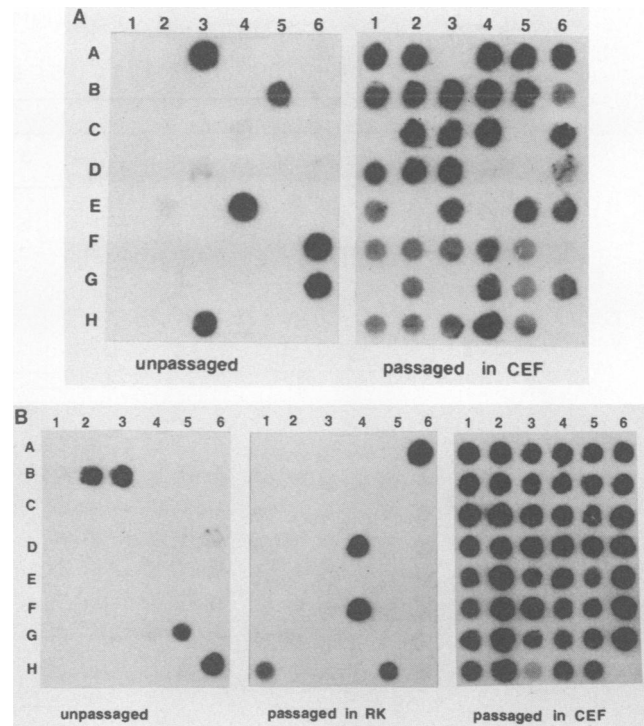


FIG. 4. Effect of passage in cell culture of a mixture of PrV(Ka) and the deletion M13 insertion mutants PrV(Ka)gp63⁻(M13) and PrV(Ka)gI⁻(M13). Wild-type, PrV(Ka), and PrV(Ka)gI⁻(M13) (A) or wild-type, PrV(Ka), and PrV(Ka)gp63⁻(M13) virions (B) were mixed at approximate PFU ratios of 10:1 (wild type to mutant) and passed five times (multiplicity of infection, 0.01 PFU per cell) either in RK cells or in CEF. The populations of virions were plaque assayed, plaques were picked, and the plaque isolates were grown in RK cells in 96-well plates. After virus cytopathic degeneration, a portion of the culture fluid of each was dot blotted onto nitrocellulose filters, hybridized to nick-translated M13 DNA, and exposed. The same blots were also incubated thereafter with nick-translated PrV DNA; all were positive (data not shown).

of the plaques consisted of the mutant virus. gp63⁻ mutants had neither a growth advantage nor a disadvantage in RK cells (Fig. 4B).

A similar experiment was also performed with gI⁻ and gp63⁻ deletion mutants instead of gI⁻ and gp63⁻ deletion-M13 insertion mutants. In this case, the genomes of the mutants were differentiated from those of the wild-type virus by the size of the *Bam*HI-*Sal*I 7A restriction fragment in which deletions in either the gp63 or the gI gene had been introduced (15). A deletion in either the gp63 or the gI gene conferred upon the virus a selective growth advantage in CEF (Fig. 5). This growth advantage was confined to CEF and was not observed when the virus was passaged in any of the other cell types (RK, PK, or MDBK cells). The fact that both gI and gp63 affect the growth of PrV(Ka) similarly in different cell types indicates that the functional entity affecting this characteristic is the complex of gI and gp63.

It is interesting that the virulence of the virus is also affected similarly by deletion of either the gI or gp63 gene (15). Deletion of either one alone has only a slight effect on virulence, but deletion of the gIII gene as well as either the gp63 or the gI gene completely abolishes virulence.

The results dealing with the virulence of the mutants have been published previously (14). However, the similarities between the effects of the deletions in the gI or the gp63 gene

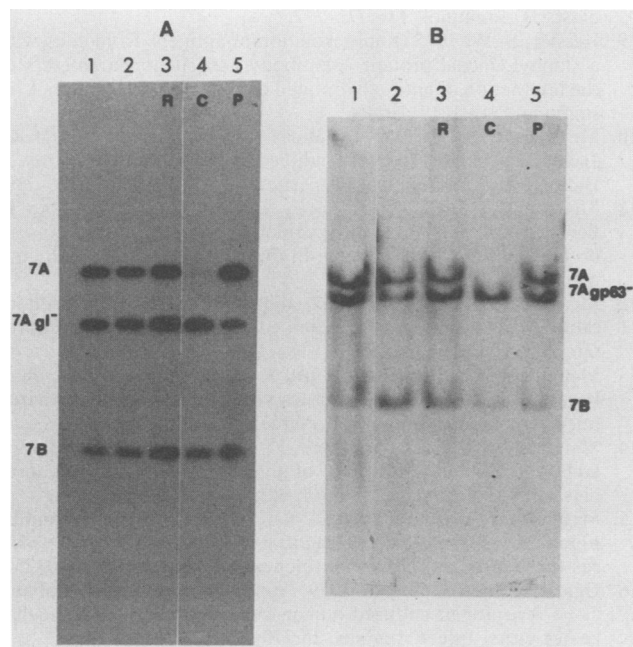


FIG. 5. Effect of passage in cell culture of mixtures of PrV(Ka) and PrV(Ka)gI⁻ or PrV(Ka) and PrV(Ka)gp63⁻ deletion mutants. Wild-type PrV(Ka) and a gI⁻ deletion mutant of PrV(Ka) (panel A) or PrV(Ka) and a gp63⁻ deletion mutant of PrV(Ka) (panel B) were mixed to give approximately equal numbers of PFU. The DNA in the virion mixture was extracted either before or after passage in RK cells or CEFs. Lanes: 1, before further passage in cell culture; 2, after one passage (at low multiplicity, 0.01 PFU per cell) in PK cells; 3, after five passages in RK cells; 4, after five passages in CEFs; 5, after five passages in PK cells. The DNA was digested with *Bam*HI and *Sal*I, electrophoresed, transferred to nitrocellulose paper (24), and probed with nick-translated *Bam*HI fragment 7. *Sal*I cleaves *Bam*HI fragment 7 into two bands, 7A and 7B. The deletions in the gI⁻ or gp63⁻ mutants are located within band 7A.

acquire a new meaning in the context of the complex formed by these two glycoproteins. Table 1 shows the similarities of the effects of deletion of gI or gp63 on virus growth and on virulence.

Infection with PrV does not induce Fc receptor-binding activity. An Fc receptor activity that binds IgG from a variety of species is induced in cells by infection with some herpesviruses (1, 4, 6–10, 16, 17, 20, 25–28). The Fc receptor-binding activity of HSV has been shown to reside in glycoproteins gE and gI (1, 6, 7). HSV glycoprotein gE is a homolog of PrV glycoprotein gI, and HSV glycoprotein gI is a homolog of PrV glycoprotein gp63 (19). It was of interest,

TABLE 1. Similarities between the effects of deletions in the gI or the gp63 genes

Effect	Deletion in ^a :	
	gI	gp63
Growth advantage in CEF ^b	+	+
Growth advantage in RK, PK, or MDBK cells	-	-
Abolition of virulence ^c	-	-
Abolition of virulence in conjunction with deletion in the gIII gene ^c	+	+

^a Symbols: +, positive effect; -, no effect.

^b See Fig. 4 and 5.

^c See Mettenleiter et al. (14, 15).

TABLE 2. Binding of IgG to PrV- and HSV-infected cells^a

Virus	Amt of IgG bound (10 ³ cpm)	
	Expt 1	Expt 2
None (control)	0.9	1.0
HSV-H4	5.6	9.2
HSV-T	8.2	11.2
PrV(Ka)	1.1	0.9
PrV-Phylaxia	0.7	0.7

^a RK cells in 24 wells were infected with virus (multiplicity, 20 PFU per cell) and incubated in Eagle medium without serum for 4 or 6 h postinfection. The medium was then changed to Eagle medium without serum but containing 2 × 10⁵ cpm of human ¹²⁵I-labeled IgG per ml. Three hours later, the medium was removed and the wells were washed six times with Eagle medium without serum. The cells were then scraped into 1 ml of 1 × SSC (0.15 M NaCl plus 0.015 M sodium citrate)-2% sodium dodecyl sulfate, and a portion was counted in a scintillation counter.

therefore, to ascertain whether PrV-infected cells also bind IgG and whether the complex of gI and gp63 is responsible for the binding.

That PrV may not encode an Fc-binding protein was suggested by the observation that no protein is consistently precipitated unspecifically from PrV-infected RK or PK cell extracts by mouse, rabbit, pig, or human serum (T. Ben-Porat, unpublished results) (Fig. 1 and 2). However, to determine unequivocally whether cells infected with PrV bind IgG, we compared the binding of ¹²⁵I-labeled human IgG to PrV- and HSV-infected cells.

In contrast to RK cells infected with HSV-1, RK cells infected with PrV did not bind more IgG than uninfected cells did (Table 2). Thus, despite the sequence homology between gE and gI of HSV with gI and gp63 of PrV, respectively, and the similar behavior of these glycoproteins in terms of complex formation, they differ functionally, since the gE-gI complex of HSV binds IgG but the gI-gp63 complex of PrV does not.

DISCUSSION

The functions of the glycoproteins of PrV in the interactions of the virus with its host cells, in virulence, and in the immune responses of the host are just beginning to be recognized. To ascertain the biological functions of the nonessential glycoproteins, we have constructed mutants defective in one (or a combination) of these glycoproteins and have initiated studies to determine their role in the infective process. We showed previously that although nonessential for growth in vitro, these glycoproteins do affect several aspects of the biology of the virus. Thus, some of these glycoproteins play a role in virulence, in adsorption, and in the release of virus from infected cells. Furthermore, they may affect virus growth in a cell-type-specific fashion (3, 11, 13–15, 23).

The salient findings in this paper may be summarized as follows. (i) Glycoproteins gI and gp63 form a complex; they are coprecipitated by antisera against either one of these glycoproteins. These glycoproteins do not share antigenic determinants, since monoclonal antibodies against gp63 do not immunoprecipitate gI from extracts of gp63⁻ mutant-infected cells and monoclonal antibodies against gI do not immunoprecipitate gp63 from extracts of gI⁻ mutant-infected cells. (ii) Mutants defective in either gI or gp63 share common biological characteristics; mutants defective in either one of these glycoproteins have a selective growth advantage over the wild type in CEF but not in RK, PK, or

MDBK cells. Furthermore, we have shown previously that although mutants defective in either gp63, gI, or gIII are only slightly less virulent (at most fivefold) for 1-day-old chickens than is wild-type virus, the double mutants gIII⁻ gp63⁻ and gIII⁻ gI⁻ are avirulent (14). Thus, gI and gp63 have a similar effect on two different characteristics of PrV: their expression is deleterious for growth in CEF, and, in conjunction with gIII, they are necessary for the expression of virulence.

The similarities of the effects of mutations of the genes encoding gI or gp63 on the growth of the virus in different cell lines, as well as on virulence, indicate that the complex of gI and gp63 is the functionally active component. Both glycoproteins may, however, perform additional functions in the infective process that are not dependent on their forming a joint complex.

As this manuscript was being prepared, a report by Johnson et al. (7) appeared that showed that glycoproteins gE and gI of HSV form a complex and that this complex is responsible for the binding of the IgG Fc receptor. The feature recognized by the viral glycoproteins appears to be shared by IgGs from different mammalian species (25). It is interesting, therefore, that in contrast to the HSV gE-gI complex, the PrV gI-gp63 complex does not exhibit human IgG Fc-binding activity. The HSV-1 and PrV homologous complexes are therefore functionally dissimilar in this respect. It remains to be established whether the gE-gI complex of HSV is involved in virulence and in virus growth in certain cell types as are the gI-gp63 complex of PrV.

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