Host Cell-Specific Growth Advantage of Pseudorabies Virus with a Deletion in the Genome Sequences Encoding a Structural Glycoprotein

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Several attenuated strains of pseudorabies virus contain genomes that carry a deletion in their short unique (U_S) component. The sizes of the deletions are different in the various attenuated strains; the deletions may include part of one of the inverted repeats as well as part of the U_S region of the genome. In most cases, the deletion includes the gene encoding the glycoprotein gI. The attenuated strains with a deletion in their S component have a common history of having been cultivated in chicken embryo fibroblasts (CEF). We show here that passage of wild-type virus in CEF promotes the emergence of populations of virions with a deletion in their S component. The emergence of these mutants is the result of their growth advantage over the wild type and is related to the lack of expression of gI, as shown by the following. (i) The Norden strain (which has a deletion in the U_S) was marker rescued to restore an intact U_S . The nonrescued Norden strain had a growth advantage over the rescued Norden strain in CEF. (ii) Passage of wild-type (gI^+) virus in CEF but not in rabbit kidney or pig kidney cells resulted invariably in the emergence of virions whose genomes had a deletion in the S component. (iii) Passage of a gI^- mutant in CEF did not result in the emergence of such virions. The emergence of virions with a deletion in their S component thus appears to be linked to gI expression. We conclude that gI is deleterious to the growth of pseudorabies virus in CEF and that this effect is cell type specific.

The restriction endonuclease digestion patterns of the genomes of different isolates of pseudorabies virus (PrV) vary greatly (1, 10, 11, 21, 26). Many of the changes in the migration rates of specific restriction fragments of PrV DNA are the result of reiterations and deletions (6; unpublished results). The biological significance of these changes is largely unknown. In principle, deletions or reiterations of sequences in a variant viral genome may, under certain growth conditions, confer upon the virion useful biological functions, leading to the dominance in the replication of the variant virus over the parental virus. Indeed, we have shown previously that some modifications of the standard structure of the PrV genome may provide the virus with an improved ability to perpetuate itself under certain environmental conditions (2, 6, 18, 40).

Deletions in the short unique (U_S) component of the genome are present in the genomes of several viable variants of PrV (7, 17, 22, 27). Interestingly, several attenuated strains of PrV consist of homogeneous populations of virions with genomes that carry such deletions. The experiments described in this report were designed to uncover the basis for the evolution of the virion populations carrying these deletions and to investigate the possibility that, under certain conditions, those deletions confer a growth advantage upon the virus.

Our results show the following. (i) Genomes with a deletion in the S component accumulate readily upon passage of wild-type virus strains in cultures of chicken embryo fibroblasts (CEF) but more rarely in other cell lines. (ii) Virions carrying this deletion have a growth advantage over the nondeleted virions in CEF cells. (iii) The growth advantage

of virions with genomes that carry a deletion in the S component is related to the elimination of the gene encoding glycoprotein gI; genomes with a deletion in the S component are enriched for during growth in CEF because the expression of gI appears to interfere with optimum growth of the virus in these cells.

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; its origin is uncertain (15). PrV90 is a recent field isolate. The Norden and Bartha avirulent vaccine strains were received from P. S. Paul; the origins of these strains have been described previously (29). The Tatarov (MK25) strain was obtained from A. L. J. Gielkens. The attenuated derivatives of the Buk strains were independently isolated after parallel passage of the Buk 120 strain in CEF (34-36, 41-45). These strains were gifts from R. Skoda and A. Zuffa. Buk Z300 is an avirulent strain isolated by A. Zuffa from a virus population of Buk strain after it was passaged 300 times in CEF. Buk Z900 is a virus strain that lost its virulence for swine after 900 passages in CEF. Buk Z920 is a plaque isolate derived from Z900; in contrast to Z900, it has lost its virulence for rabbits. Buk SK624 is a virus strain isolated in the laboratory of R. Skoda that lost its virulence for piglets after 624 passages in CEF. The SK900 and Norden strains were derived by further independent passage of SK624 in CEF. Both were further attenuated.

Rabbit kidney (RK) cells, pig kidney (PK) cells, and CEF were cultivated in Eagle synthetic medium supplemented with 3% dialyzed bovine serum. Virus was titrated by plaque assay in RK or PK cells.

Isolation of deletion-M13 insertion mutants in glycoprotein gI. The detailed method used to construct mutants with deletions and insertions in the gI-coding sequences will be

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described elsewhere (Mettenleiter et al., manuscript in preparation). In brief, fragment *Bam*HI/*Sal*I 7A of the PrV genome, which encompasses the gene encoding gI (22, 28), was cloned into pBR325. The plasmid was cleaved at a unique *Bst*EII site present approximately 350 nucleotides downstream from the 5' end of the gI-coding sequences, and approximately 100 nucleotides from each end were removed by digestion of the linearized plasmid with the exonuclease *Bal* 31. The DNA was dephosphorylated with bacterial alkaline phosphatase, and a *Hae*III fragment of M13 which includes the multiple cloning site was introduced.

Radiochemicals and enzymes. [α - ^{32} P]dCTP was purchased from New England Nuclear Corp. Restriction enzymes were purchased from Bethesda Research Laboratories, Inc.

Purification of virions and extraction of DNA. Virions were purified as described previously (5). Sodium sarkosinate (final concentration, 2%) was added to the samples, which were heated (60°C for 15 min) and digested with nuclease-free pronase (1 mg/ml) for 2 h. The DNA was then extracted four times with phenol-chloroform-isoamyl alcohol (50:48:2) and either dialyzed against buffer (0.01 M Tris, 0.001 M EDTA, pH 7.6) or alcohol precipitated.

Restriction enzyme digestion and gel electrophoresis of DNA fragments. Digestion and agarose gel electrophoresis of viral DNA were carried out as described previously (4). Filter strips to which restriction fragments of PrV DNA were fixed were prepared by the method of Southern (37).

Nick translation of cloned PrV DNA restriction fragments. PrV DNA restriction fragments, cloned in pBR325 as described previously (16), were nick translated by the method of Rigby et al. (31).

Immunoprecipitation. Immunoprecipitation was performed as described previously (13).

RESULTS

Mapping deletions in the S component of attenuated strains. During the course of studies designed to elucidate the genetic basis for the virulence of PrV, we examined the characteristics of several independently isolated vaccine strains of PrV and mapped their genomes by the Southern technique (37), as has been described previously for the Bartha and Norden vaccine strains (17). Various changes were observed in the structure of the genomes of the vaccine strains. Of particular interest was the finding that a majority had a deletion in the S component of their genomes similar to the deletion previously observed in the Norden and Bartha vaccine strains (12, 17).

Figure 1 shows the *Kpn*I digestion patterns of the DNA of the various PrV strains. *Kpn*I fragment I, which includes part of the U_S (Fig. 2), is larger in the genomes of wild-type virions (Fig. 1, lanes 7 and 8) than of the attenuated strains (*Kpn*I fragment I is indicated in each case [O]). Five of the seven attenuated strains analyzed (lanes 1, 3, 4, 5, and 11) have relatively large deletions in *Kpn*I fragment I; the two other attenuated strains (lanes 2 and 6) have smaller deletions in *Kpn*I fragment I. In all of these strains, the normal-sized *Kpn*I fragment I disappeared and a smaller fragment appeared. That the smaller fragment was indeed a deleted form of *Kpn*I fragment I was shown conclusively by hybridization with cloned *Kpn*I fragment I of wild-type virus (data not shown).

Of the more than 200 plaque isolates of PrV(Ka) that were analyzed during the course of other experiments, 2 plaque isolates also had a large deletion in *Kpn*I fragment I. One of these plaque isolates was obtained from a virus stock that

had been passaged several times in CEF (Fig. 1, lane 10); the other was obtained from a population containing defective interfering particles that had been passaged at high multiplicity in RK cells (lane 9). Some restriction enzyme polymorphism involving *Kpn*I fragment I was also sometimes observed in primary isolates of PrV. However, large deletions in this fragment, similar to those observed in some of the attenuated strains, were not a characteristic of primary isolates of PrV (data not shown).

Most of the U_S region has been sequenced, and detailed restriction maps of that region of the genome are available (27, 28). The availability of these restriction maps allowed us to map the deletions in some of the vaccine strains in detail. The results (Fig. 2) revealed the following. (i) The sizes of the deletions, and consequently probably the sequences that border the deletions, were different in the different virus variants, indicating that the deletions did not result from specific recombinational events. (ii) In most cases, the deletions included the sequences encoding gI. Indeed, gI was not synthesized by cells infected with any of the variant strains carrying the large deletion in the S component (Table 1).

The deletion in strain Buk Z920 exhibits some interesting features. In this strain, part of one of the inverted repeats bracketing the U_S and part of the U_S including the gene encoding gI have been deleted. Some of the sequences that are normally part of the inverted repeats were thereby converted to unique sequences. Furthermore, reiterations of some sequences of the U_S were also observed in the genome of this strain.

The mapping of the various virus variants (Fig. 2) revealed that a large part of the U_S of PrV was nonessential for growth in vitro. In addition, part of the sequences of the inverted repeats did not need to be diploid to allow virus growth in vitro. These findings are similar to those previously reported for herpes simplex virus; mutants of both herpes simplex virus types 1 and 2 have been described in which parts of the U_S and of one of the inverted repeats have been deleted (8, 9, 14, 20, 30, 39).

Repeated passage of PrV in CEF results in the emergence of populations of virions with a deletion in the S component. The

FIG. 1. *Kpn*I restriction patterns of the DNA of various strains of PrV. DNA was extracted from purified virions and digested with *Kpn*I. The digests were electrophoresed, and the gels were stained with ethidium bromide. Lanes: 1, Buk Z300; 2, Buk Z900; 3, Buk Z920; 4, SK71; 5, Norden; 6, Tatarov; 7, PrV(90); 8, PrV(Ka); 9, PrV(Ka)B8p5; 10, PrV(Ka)16T; 11, Bartha. *Kpn*I fragment I and its deleted forms are indicated (O).

FIG. 2. Maps of the deletions in the S component of the genomes of some PrV variants. The top line indicates the region of the genome that includes the deletions observed in the various variants. In lines 2, 3, 4, 5, and 6, the regions that are deleted from each of the indicated variants are blank. In the bottom part of the figure, the *Bam*HI and *Kpn*I restriction maps of PrV DNA are shown; the region of the genome of interest is expanded, and the positions of the genes for gp50, gp63, and gI are indicated.

vaccine strains that have a deletion in their S component have a common history of being either repeatedly passaged in CEF (to attenuate them) or grown in CEF. We therefore considered the possibility that growth in CEF might promote the emergence of virions with genomes carrying a deletion in the S component. To determine whether this was the case, four different isolates of wild-type PrV were passaged re-

peatedly at low multiplicity (0.01 PFU per cell) in either CEF, PK, or RK cells. The virion populations produced by these cells after different passage levels were isolated; the DNA was purified and digested with restriction enzymes and analyzed by the Southern technique (37). Figure 3 shows the *Kpn*I digestion patterns of the DNA obtained from one PrV strain [PrV(Ka)] passaged in CEF. As the virus was pas-

TABLE 1. Reactivity of monoclonal antibodies against viral glycoproteins with extracts of cells infected with different virus variants^a

Virus strain	cpm ($\times 10^3$) precipitated with the indicated monoclonal antibody				
	gII		gI		
	M5	M3	M133	M138	M156
Buk Z300	151 (+) ^b	128 (+)	0 (-)	0 (-)	0 (-)
Buk Z900	162 (+)	163 (+)	98 (+)	110 (+)	102 (+)
Buk Z920	155 (+)	115 (+)	0 (-)	0 (-)	0 (-)
Buk SK900	165 (+)	155 (+)	0 (-)	0 (-)	0 (-)
Norden	176 (+)	145 (+)	0 (-)	0 (-)	0 (-)
Norden rescued	162 (+)	136 (+)	87 (+)	83 (+)	110 (+)
Tatarov	127 (+)	121 (+)	122 (+)	0 (-)	121 (+)
PrV(90)	190 (+)	166 (+)	183 (+)	164 (+)	145 (+)
PrV(Ka)	165 (+)	153 (+)	108 (+)	131 (+)	128 (+)
PrB8p5	195 (+)	201 (+)	0 (-)	0 (-)	0 (-)
Pr16T	175 (+)	183 (+)	0 (-)	0 (-)	0 (-)
Bartha	227 (+)	241 (+)	0 (-)	0 (-)	0 (-)
PrV(Ka)gI ⁻ (M13)	197 (+)	229 (+)	0 (-)	0 (-)	ND ^c

^a [³H]glucosamine-labeled cell extracts obtained from cultures infected with the different virus strains were immunoprecipitated by the indicated monoclonal antibodies as described in Materials and Methods. In all cases, the background ($<10^4$ cpm) was subtracted. The amount of radioactivity precipitated was determined.

^b +, Reactivity of the monoclonal antibody; -, lack of reactivity.

^c ND, Not determined.

FIG. 3. *KpnI* restriction patterns of the DNA of virions obtained after different numbers of passages in CEF cells. Strain PrV(Ka) was passaged repeatedly at low multiplicity (0.01 PFU per cell) in CEF cells. After the indicated number of passages, virions were purified, and the DNA was extracted and digested with *KpnI*. The digests were electrophoresed and stained with ethidium bromide.

saged, *KpnI* fragment I became less abundant and a new fragment, *KpnI* fragment I*, appeared. The hybridization pattern of the *KpnI* digests to nick-translated *BamHI* fragment 7 of wild-type DNA is shown in Fig. 4. The sequences in *BamHI* fragment 7 normally hybridize to *KpnI* fragments I and J (fragment N has run off the gel; see Fig. 3 for restriction maps). After passage of the virus in CEF, fragment J remained unmodified but fragment I became under-represented; instead a deleted version of this fragment, I*, appeared. A similar analysis of the DNA in these populations of virions after cleavage with *BamHI* or *Sall* revealed that part of the U_S , as well as part of the inverted repeat, had been deleted (data not shown). The deletion in this population of virions after passage in CEF was similar to that found in the Norden strain (Fig. 2).

The results in Fig. 4 and those obtained with other restriction enzymes (data not shown) revealed that several deleted forms of *KpnI* fragment I were present in the population of virions at early levels of passage in CEF but that one of the fragments became predominant after further passage. For example, after 10 passages in CEF, fragment I became underrepresented and fragment I* was present in approximately the same amount as some other aberrantly migrating fragments hybridizing to *BamHI* fragment 7. Fragment I* became predominant by passage 30, but some of the minor deleted forms of fragment I were still present, indicating that the population was not completely homogeneous. These results showed that genomes with several different deleted forms of *KpnI* fragment I were formed upon passage of the virus in CEF, but either virions with genomes with one of these deleted forms outgrow the virions carrying the others upon further passage in CEF or the process of the deletion of sequences from the S component occurs in several stages. The experiments described below will show that the former is the case.

Passage of three other strains (primary isolates) in CEF also resulted in the emergence of virion populations with a deletion in the U_S . In all cases, the sizes of the predominant deletion in the different virus populations varied somewhat, but the sequences encoding gI were always deleted (data not

shown). On the other hand, after 50 passages of the four virus strains in RK cells, the emergence of virus populations with a deletion in the S component was observed in only one of the strains, and, of the four virus strains that were passaged 50 times in PK cells, none became enriched for genomes with a deletion in the U_S . Thus, whereas passage in CEF appears to result invariably in the appearance of virions with genomes that have a deletion in the S component, passage in RK or PK cells does so far more rarely.

Growth advantage of virions with a deletion in the S component. The following interpretation is compatible with the results described above: genomes with a deletion in the S component arise spontaneously, and virions with genomes that carry this deletion have a growth advantage in CEF.

To assess whether this interpretation is correct and whether the deletion in the S component contributes to a growth advantage of PrV in CEF, we determined whether restoration of an intact S component to the Norden vaccine strain (which has a deletion in the S component; Fig. 2) would change its ability to replicate in CEF. A Norden strain variant with an intact S component was obtained by cotransfecting cells with the DNA of the Norden strain and with *PstI* fragment I of the PrV(Ka) genome, a fragment that encompasses the region deleted from the Norden genome (17, 19). The progeny was plaque assayed, and plaques were analyzed by hybridization and Southern analysis for the presence of sequences in the S component normally deleted from the Norden strain. An isolate (rescued Norden) with an intact S component was identified (Fig. 5). (The Norden strain has a deletion in the S component which includes parts of *BamHI* fragment 7 and fragment 12 including the *BamHI* site between them. As a consequence, a fusion fragment 7 + 12 composed of parts of each of these fragments is formed.) As expected, in contrast to the parental Norden strain, the rescued Norden isolate expressed glycoprotein gI (Table 1).

PK cells and CEF were infected with a 10:1 mixture of rescued Norden and parental Norden virus. This mixed virus

FIG. 4. Hybridization patterns of *KpnI* restriction digests of DNA obtained after different numbers of passages in CEF cells. Restriction digests similar to those described in the legend to Fig. 3 were electrophoresed, transferred to nitrocellulose filters, and hybridized to nick-translated *BamHI* fragment 7 (see map in Fig. 2). p, Passage.

FIG. 5. Restriction digests of Norden DNA and rescued Norden DNA. Norden virus was marker rescued to restore to it an intact U_S , as described in the text. DNA preparations of PrV(Ka) (lane A), Norden (lane B), and rescued Norden (lane C) were digested with *Bam*HI or *Kpn*I, electrophoresed, and stained with ethidium bromide. The positions of *Bam*HI fragments 7, 7 + 12, and 12 and *Kpn*I fragments I and I* are indicated (O).

population was passaged at low multiplicity nine times in either CEF or PK cells. Because the cells were infected at low multiplicity (0.01 PFU per cell), much of the virus yielded by the cultures was produced by cells in which complementation between the two virus variants had not taken place. Figure 6 shows the hybridization patterns of *Bam*HI digests of the DNA obtained from these virus populations to nick-translated *Bam*HI fragment 7. In the original virus mixture, the fragment characteristic of the Norden rescued strain (*Bam*HI fragment 7) was, as expected, approximately 10 times more abundant than that of the fragment characteristic of the Norden strain (fragment 7 + 12). The relative abundance of the two fragments did not change detectably after passage in PK cells. However, after passage in CEF, the fragment characteristic of the Norden strain (*Bam*HI fragment 7 + 12) increased in abundance relative to that of the fragment characteristic of the rescued Norden strain (*Bam*HI fragment 7), and the two fragments were present in almost equal amounts. Thus, the parental Norden strain (with a deletion in its S component) had become enriched for in the virion population after passage in CEF. Therefore, it appears that the deletion of the sequences in the S component confer upon the virus a growth advantage in CEF, i.e., the presence of an intact S component is deleterious to virus growth. This effect was not detectable in PK cells.

Although the presence of some sequences in the S component of the genome appears to be deleterious to virus growth in CEF, this effect is subtle. No difference in the final yield of infectious virus or in the kinetics of replication of the virus was observed when the Norden strain and the Norden rescued variants were grown individually in either CEF or PK cells (data not shown). The selective growth advantage of the Norden strain over the rescued Norden variant in CEF was detected only after passage at low multiplicity of a mixture of the two virus variants in cultures of CEF.

Passage in CEF of virions that do not express gI does not result in the emergence of populations of virions with a deletion in the S component of their genomes. The results described above show that virions with a deletion in the S component appear to have a growth advantage in CEF over virions with an intact S component. Furthermore, although several different populations of virions that have been passaged in CEF may have different deletions in their S component, they all lack the sequences encoding gI and, consequently, fail to synthesize gI (Fig. 2 and Table 1). We considered the possibility, therefore, that the expression of gI may be deleterious to growth of PrV in CEF.

To determine whether this is indeed the case, we ascertained whether populations of mutants of PrV(Ka), which do not express gI, would become enriched for virions with genomes with a deleted S component after passage in CEF as is the case for wild-type virus. To this end, the parental virus and a gI⁻ mutant [PrV(Ka)gI⁻(M13)] in which sequences of phage M13 had been inserted near the 5' end of the gI gene and which does not express gI (Table 1) were passaged in CEF. After 19 passages, the virions produced by the cells were purified, and the DNA was extracted and analyzed for deletions in the S component. In two independently passaged populations of wild-type virus, deletions in the S component of the genome were observed (Fig. 7). In the DNA of one population (wild type, passage 19a), *Kpn*I fragment I had become undetectable and a smaller fragment, *Kpn*I fragment I*, appeared. Fragment I* hybridized only weakly to the sequences of *Bam*HI fragment 7 that were used as a probe because a large part of the sequences of *Kpn*I fragment I that normally hybridize to *Bam*HI fragment 7 had been deleted. The other population of PrV(Ka) passaged in CEF (Fig. 7; wild type, passage 19b) contained a mixture of genomes; some had retained an intact *Kpn*I fragment I, whereas others had a deletion in *Kpn*I fragment I. The deletion in *Kpn*I fragment I was smaller in the genomes of the passage 19b population than it was in that of the passage 19a population, but in both cases it included the sequences encoding gI. This was determined by hybridization of the Southern blots to sequences derived specifically from the gI gene (data not shown). Interestingly, each population was relatively homogeneous with respect to the type of deletion in the S component it had acquired.

FIG. 6. Hybridization pattern of restriction digests of the DNA of mixtures of Norden and rescued Norden after passage in CEF or PK cells. Rescued Norden and Norden virions were mixed at a ratio of 10:1 and were then passaged at low multiplicity (0.01 PFU per cell) nine times in either CEF or PK cells. Virions were purified; their DNA was extracted, digested with *Bam*HI, electrophoresed, transferred to nitrocellulose filters, and probed with nick-translated *Bam*HI fragment 7 (see map in Fig. 2). p, Passage.

FIG. 7. Hybridization patterns of *KpnI* restriction digests of the wild type and a *gI*⁻ mutant DNA after passage in CEF. Wild-type PrV(Ka) (W.T.) or a *gI*⁻ mutant of this virus [*gI*⁻(M13)] were passaged at low multiplicity (0.01 PFU per cell) in CEF. After 1 or 19 passages, the virions were purified; their DNA was extracted, digested with *KpnI*, and electrophoresed. The DNA was transferred to nitrocellulose filters and probed with nick-translated *Bam*HI fragment 7. Passages a and b indicate two independent parallel passages.

In the *gI*⁻ mutant [PrV(Ka)*gI*⁻(M13)], sequences of M13 that include the multiple cloning site were inserted into the coding sequences of *gI*. Consequently, digestion of the mutant DNA with *KpnI* cleaved *KpnI* fragment I, giving rise to two fragments, Ia and Ib. In both parallel passages of PrV(Ka)*gI*⁻(M13) in CEF, no change in the sizes of either Ia or Ib was observed (Fig. 7). Furthermore, a loss of the sequences encoding *gI* was also not observed (data not shown). We conclude from these results that in the *gI*⁻ mutant-infected cells, in contrast to wild type-infected cells, either viruses with genomes that have a deletion in the S component were not formed or such viruses did not have a selective advantage and did not become enriched for upon passage of the *gI*⁻ mutant in CEF.

In the *gI*⁻ mutant used in this study, approximately 200 base pairs of the *gI* gene had been replaced by 257 base pairs of M13 DNA; no other alterations of the genome had been introduced (see Materials and Methods). The recombinational events which would lead to the emergence of the genomes with a deletion in the S component were therefore possible. Because genomes with a deletion in the S component did not emerge upon passage in CEF of *gI*⁻ mutants, it appears that in the absence of expression of *gI* genomes with a deletion in their S component are not enriched for in the virus population. The selection upon passage of wild-type virus in CEF for virions with genomes with a deletion in their S component appears, therefore, to be linked to the elimination of the *gI* gene from these genomes. We conclude that expression of *gI* is deleterious to the growth of PrV in CEF.

DISCUSSION

The studies described in this report were based on the observation that some independently isolated vaccine strains of PrV have a deletion in the S component of their genomes. Because all of these strains had been passaged (or grown) in CEF, we considered the possibility that growth of the virus in CEF may select for virions with genomes carrying this deletion. Our results indicate that, indeed, selection for mutants with a deletion in the S component occurs invari-

ably during growth of the wild-type PrV in CEF (Fig. 3, 4, and 7) but occurs less readily in RK or PK cells. The emergence of populations of virions with the deletion in the S component appears therefore to depend upon the cell type in which the virus is passaged. A host cell-specific accumulation of a deletion mutant of HSV-1 has also been reported (33).

It is likely that the deletions in the S component of the genome upon passage of PrV in CEF arise because of instabilities in the virus genome, i.e., are the result of recombinational events. The recombinational events generating the deletions in the S component do not appear to be sequence specific because several deleted forms of the genome appeared initially upon passage of the virus in CEF, one becoming predominant (probably because it has some selective advantage) upon additional passages (Fig. 4). This can also be deduced from the finding that different attenuated strains and different wild-type strains passaged in CEF acquire different size deletions in their S component (Fig. 1 and 2; data not shown), indicating that the recombinational events leading to these deletions can occur at several different loci of the viral genome.

PrV grows poorly in CEF and becomes adapted only after repeated passage in these cells. Several changes in the wild-type virus genome are observed after this adaptation process has taken place. (i) Sequences normally present at the left end of the L component become translocated next to the internal inverted repeat, and consequently the L component becomes invertible (18). (ii) In many cases some of the sequences of the U_L normally adjacent to the internal inverted repeat are deleted (18). (iii) Sequences from the S component of the genome, including the sequence encoding *gI*, are deleted (this paper). Each of the three modifications mentioned above can occur independently of one another, and virions that have experienced only one of these changes can be isolated at early levels of passage in CEF (unpublished results).

Although populations of wild-type virions acquired a deletion in their S component upon passage in CEF, a characteristic that confers upon them a growth advantage in those cells, a population of *gI*⁻ mutants did not become enriched for genomes with such a deletion after being passaged similarly in CEF. Because the *gI*⁻ mutant possessed all of the sequences that border the deletions that normally appear after passage of wild-type virus in CEF, all the recombinational events leading to the emergence of the deleted genomes were possible. The lack of enrichment for genomes with a deletion in the S component, after passage of *gI*⁻ mutants in CEF, indicates therefore that deletion of part of the S component does not have the same selective advantage in CEF for *gI*⁻ virions as it does for *gI*⁺ virions. We conclude that the selective advantage of virions with genomes carrying a deletion in their S component resides in the lack of synthesis of *gI* by cells infected with these virions and that *gI* is deleterious to growth of PrV in CEF. An accumulation of virions that express a modified (possibly nonfunctional) *gI* glycoprotein has also been observed after passage in bovine cells (23). The molecular basis for the selective growth advantage of *gI*⁻ mutants in certain cell types is unknown at present.

The PrV genome encodes at least eight different glycoproteins; four of these, including *gI*, are nonessential for growth in vitro (22, 27, 32, 38). We have studied in some detail the function of *gI*. This glycoprotein exerts a subtle effect on virus growth in cell culture. Inactivation of *gI*, in conjunction with a defect in at least one other viral function, inhibits the

release of the virus from RK cells but not from PK cells (3, 24). We show here that the expression of gI is, on the other hand, deleterious to growth of wild-type PrV in CEF. It appears, therefore, that the effect of gI on virus growth in cell culture is cell type specific and is modulated by the expression of other viral functions. The cell type-specific effect exhibited by the gI glycoprotein indicates that it may play an important role in cell tropism and in the pathogenesis of the virus. Indeed, gI mutants have been shown to have reduced levels of virulence for both chickens and swine (25).

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