JOURNAL OF VIROLOGY, Dec. 1989, p. 5475–5477
0022-538X/89/125475-03$02.00/0

Vol. 63, No. 12

Release of Pseudorabies Virus from Infected Cells Is Controlled by Several Viral Functions and Is Modulated by Cellular Components

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Received 30 January 1989/Accepted 21 August 1989

The role of the nonessential glycoproteins gI, gp63, and gIII in the release of pseudorabies virus from different cell lines was investigated. We show that these glycoproteins may have a beneficial or deleterious effect on virus release depending on the type of cell in which the virus is grown. Inactivation of the genes encoding either gI, gp63, or gIII has no detectable effect on virus release from rabbit kidney cells. Inactivation of gI or gp63 strongly promotes virus release from chicken embryo fibroblasts, whereas inactivation of gIII reduces virus release from these cells. A defect in both gI and gIII or in both gp63 and gIII diminishes virus release from rabbit kidney cells but improves release from chicken embyro fibroblasts. We demonstrate that all three nonessential glycoproteins contribute to one specific aspect of viral growth, namely, virus release, and that they affect virus release in conjunction with each other. Furthermore, our results show that the manifestation of the role of each of these viral functions in virus growth may differ in different cell types, i.e., that release is affected by these viral functions in conjunction with some unknown cellular function.

The genome of pseudorabies virus encodes at least seven glycoproteins. Four of these (gIII, gI, gp63, and gX) are nonessential for growth in cell culture (2, 5, 9, 10, 12). Information concerning the functions of these glycoproteins in the interactions of the virus with its host cells is just beginning to emerge.

Several seemingly contradictory reports dealing with the effects of these glycoproteins on virus growth and release have appeared. Glycoprotein gIII has been shown to play a role in adsorption (11); it, as well as glycoprotein gI, may also affect virus release (6, 11, 13). Expression of gI is necessary for the efficient release of pseudorabies virus (Bartha) from rabbit kidney (RK) cells (1, 6) but appears to be deleterious to the growth of another strain of pseudorabies virus [PrV(Ka)] in chicken embryo fibroblasts (CEF) (4). Also, while glycoprotein gIII affects virus release of PrV(Ka) from RK cells only in conjunction with mutations in gI (not alone) (11), a mutant of the Becker strain defective in gIII only appears to be released less effectively from pig kidney (PK15) cells than is the wild-type Becker strain virus (13). Thus, the effect of inactivation of either the gIII or the gI gene on virus release appears to depend either on the virus strain or on the cellular environment in which the virus is grown.

The experiments summarized in this report were performed to gain an understanding of the role glycoproteins gI, gp63, and gIII play in virus release under various growth conditions. To ascertain the effects of these nonessential glycoproteins on virus release, we have compared the release of wild-type PrV(Ka) from CEF and RK cells with that of mutants unable to express either gI, gp63, or gIII as well as mutants unable to express two of these glycoproteins. We show that these glycoproteins may have a deleterious or beneficial effect on virus release depending on the type of cell in which the virus is grown and that, furthermore, a defect in one of these glycoproteins sometimes affects virus release in a detectable manner only in conjunction with a defect in another glycoprotein. Thus, these nonessential viral proteins play a complex role in virus release, and their effect is modulated by cellular components. Although the results obtained with only one of the mutants (gI^-, $gIII^-$, $gp63^-$, $gI^-/gIII^-$, or $gp63^-/gIII^-$) of PrV(Ka) are presented, similar results were also obtained with other, independently isolated mutants of the same strains as well as with mutants of other pseudorabies virus strains (Bartha and Becker). It is clear, therefore, that the differences in the behavior of the wild-type and mutant viruses can be attributed to mutations in the nonessential glycoprotein genes rather than to other adventitious mutations.

The isolation and characterization of the mutants used have been described previously (7). The $gIII^-$ mutants of the Becker strains were obtained from Lynn Enquist. Primary RK cells and CEF were cultivated in Eagle synthetic medium supplemented with 5% dialyzed bovine serum. Virus was titrated by plaque assay in RK or pig kidney cells.

Figure 1 shows one-step growth curves in CEF and in RK cells of PrV(Ka) and of mutants of this virus defective in either gI or gIII or both. The amounts of infectious wild-type virus and of the mutants produced by either CEF or RK cells, as well as the general shape of the virus growth curves, were similar. Whereas the total amounts of infectious PrV(Ka) defective in gI and of wild-type PrV(Ka) that were produced were the same, the virus titer of mutants defective in gIII or in gIII and gI was approximately 10 times lower. (The reduced yield of infectious $gIII^-$ mutants can be ascribed, at least in part, to the poor adsorption of these mutants to their host cells [11].) These results confirm previously published findings (7, 11, 13).

Figure 2 shows the percentage of the total virus produced by the cells that had been released from RK cells and from CEF at various times after infection. Whereas the gI^- mutant, the $gIII^-$ mutant, and the wild-type virus were released similarly from RK cells, the gI^- mutant was released much more readily and the $gIII^-$ mutant was released less readily than was the wild-type virus from CEF. Thus, whereas a defect in either gI or gIII does not affect release from RK cells in a detectable manner, it does significantly

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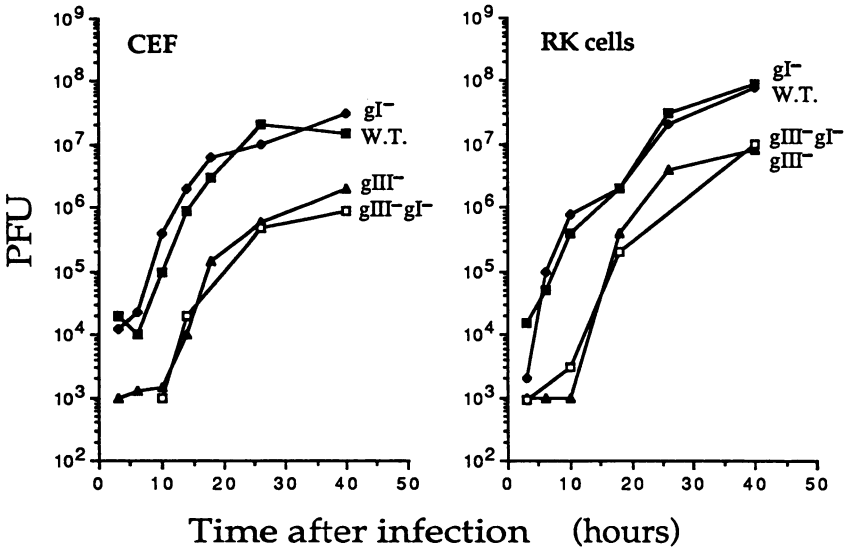

FIG. 1. Replication of wild-type PrV(Ka) (W.T.) and mutants defective in gI and gIII in cultures of RK cells and CEF. Primary RK cells or CEF were grown to confluence on 50-mm petri dishes and infected with 8 PFU of the appropriate mutant per cell in 2 ml of Eagle medium. After a 3-h adsorption period, the cell monolayers were washed extensively to remove unadsorbed virus and further incubated at 37°C in 5 ml of medium. At the indicated times, the cells were scraped into the culture fluid, and the samples were sonicated for 1 min and centrifuged at 7,000 × *g* for 10 min to remove cellular debris. The virus was plaque assayed on Madin-Darby bovine kidney cells.

affect release from CEF. Furthermore, whereas gIII promotes release, gI inhibits release from CEF.

Figure 2 also shows the effects of deleting both gIII and gI on virus release from RK cells or from CEF. While lack of expression of either gIII or gI alone did not affect release of PrV(Ka) from RK cells, the double mutant gIII⁻/gI⁻ was released less efficiently from these cells (confirming our previously published findings [11]). The double mutant gI⁻/gIII⁻ was, however, released more efficiently than was the wild-type virus or the gIII⁻ mutant but less efficiently than was the gI⁻ mutant from CEF.

Results obtained from other experiments similar to the one illustrated in Fig. 2 are summarized in Table 1. The results obtained with the gI⁻, the gIII⁻, or the gIII⁻/gI⁻ mutant of PrV(Ka) were, in general, similar to those illustrated in Fig. 2. Table 1 also shows that gp63 and gI affect virus release similarly. Since glycoproteins gI and gp63 form a complex in the infected cells (14), it is likely that it is this complex that is the functional entity affecting release. The release of viral particles (rather than PFU) from cells infected with the various mutants was also determined (Table 1). The total number of particles yielded by each cell type infected with

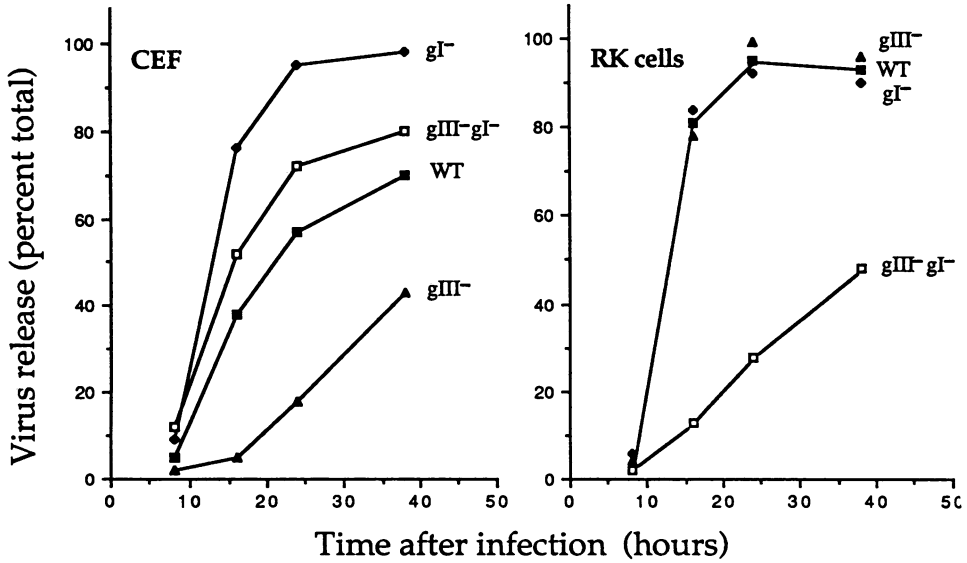

FIG. 2. Release from infected cells of wild-type (WT) virus and of mutants defective in the expression of gI, gIII or gI, and gIII. The experiment was performed as described in the legend to Fig. 1, but at various times after infection, the extracellular virus was obtained by collecting the culture fluids and the intracellular virus was obtained by scraping the cells into fresh medium. The samples were sonicated and centrifuged to remove cellular debris, and the virus was assayed as described in the legend to Fig. 1. The percentage of total virus (extracellular plus intracellular) that was released (extracellular) was calculated.

TABLE 1. Release of different virus mutants or variants from CEF or RK cells

Virus mutant	Phenotype	Release (%) of:			
		Infectious virus[a]		Total particles[b]	
		RK	CEF	RK	CEF
PrV(Ka)	$gIII^+/gp63^+/gI^+$	61	17	58	12
PrV(Ka)gI^-	$gIII^+/gp63^+/gI^-$	61	73	56	52
PrV(Ka)$gp63^-$	$gIII^+/gp63^-/gI^+$	59	73	58	48
PrV(Ka)$gIII^-$	$gIII^-/gp63^+/gI^+$	57	2	53	8
PrV(Ka)$gIII^-/gI^-$	$gIII^-/gp63^+/gI^-$	22	25	38	22
PrV(Ka)$gIII^-/gp63^-$	$gIII^-/gp63^-/gI^+$	20	27	32	24

[a] The results were obtained as described in the legends to Fig. 1 and 2. The number of total PFU released at 15 h postinfection was determined.

[b] RK or CEF cells were infected with the appropriate virus (multiplicity of infection, 8 PFU per cell) and incubated in Eagle medium containing [^{3}H]thymidine (20 μCi/ml). At 18 h postinfection, extracellular virus was obtained from the culture fluid after its clarification by centrifugation at 5,000 × *g* for 5 min. Intracellular virus was obtained from the cells that were scraped from the surfaces of the plates and combined with the pellet obtained from the culture fluid. The virus was purified as described previously (3), and the amount of radioactivity associated with the virus peaks was determined.

the various mutants (as determined by the amount of radioactivity associated with purified populations of viral particles obtained from these cells) was approximately the same (data not shown). The percentage of the total particles formed that were released from cells infected with the different mutants varied, however, and was similar to the relative amounts of PFU that were released from the cells. Thus, total and infectious particles are released to a similar extent from cells infected with the different mutants. As already mentioned, similar results were also obtained with several other independently isolated gI^-, $gp63^-$, $gIII^-$ or $gI^-/gIII^-$, and $gp63^-/gIII^-$ mutants of PrV(Ka) (data not shown). It is clear, therefore, that the effects observed can be attributed to the lack of expression of these nonessential glycoproteins.

The observations reported in this article point to the fact that different viral functions may affect virus release differently in different types of cells. Furthermore, the three nonessential glycoproteins that we have studied affect virus release in conjunction with each other. Thus, not unexpectedly, complex interactions between viral gene products and cellular functions affect the virus growth processes; the apparent role in virus growth of a given viral function may depend on the type of cell in which the role of this function is tested.

Although the three glycoproteins (the functions of which we have studied) are nonessential for growth in vitro, they nevertheless clearly play a role in modulating viral growth. The finding that gI interferes with and that gIII promotes virus release from CEF but that both glycoproteins must be defective to affect release from RK cells is of particular interest because both glycoproteins have been implicated in virus virulence (7, 8). The differential effects of the glycoproteins on the release of virus from different types of cells may be related to the ability of the virus to replicate, spread, and destroy different target cells. In principle, these glycoproteins may therefore contribute significantly to virulence and to the pathogenesis of the virus. Some of our published (7, 8) as well as unpublished results show that they do so.

This investigation was supported by Public Health Service grant AI-10947 from the National Institutes of Health. Thomas C. Mettenleiter was supported by research fellowship Me-854/1-1 from the Deutsche Forschungsgemeinschaft.

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