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An efficient deletion mutant packaging system for defective herpes simplex virus vectors: Potential applications to human gene therapy and neuronal physiology

ALFRED I. GELLER*, KHANDAN KEYOMARSI, JOHN BRYAN†, AND ARTHUR B. PARDEE

Division of Cell Growth and Regulation, Dana–Farber Cancer Institute, Boston, MA 02115

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ABSTRACT **We have previously described a defective herpes simplex virus (HSV-1) vector system that permits the introduction of virtually any gene into nonmitotic cells. pHSVlac, the prototype vector, stably expresses *Escherichia coli* β-galactosidase from a constitutive promoter in many human cell lines, in cultured rat neurons from throughout the nervous system, and in cells in the adult rat brain. HSV-1 vectors expressing other genes may prove useful for studying neuronal physiology or performing human gene therapy for neurological diseases, such as Parkinson disease or brain tumors. A HSV-1 temperature-sensitive (ts) mutant, ts K, has been used as helper virus; ts mutants revert to wild type. In contrast, HSV-1 deletion mutants essentially cannot revert to wild type; therefore, use of a deletion mutant as helper virus might permit human gene therapy with HSV-1 vectors. We now report an efficient packaging system for HSV-1 vectors using a deletion mutant, D30EBA, as helper virus; virus is grown on the complementing cell line M64A. pHSVlac virus prepared using the deletion mutant packaging system stably expresses β-galactosidase in cultured rat sympathetic neurons and glia. Both D30EBA and ts K contain a mutation in the *IE3* gene of HSV-1 strain 17 and have the same phenotype; therefore, changing the helper virus from ts K to D30EBA does not alter the host range or other properties of the HSV-1 vector system.**

Defective herpes simplex virus 1 (HSV-1) vectors (1) are the only available method to deliver genes directly into nonmitotic cells, such as neurons and glia, in culture (2, 3) or in the adult mammalian brain (4). The construction of a transgenic animal (5) delivers a gene into every cell in the animal; retrovirus vectors (6) cannot transfect neurons or nonmitotic glia; and fibroblasts, transfected with a retrovirus vector and subsequently transplanted into the adult brain (7), do not deliver genes into neurons or glia and also disrupt the local neuronal circuitry. The prototype HSV-1 vector pHSVlac (refs. 1–3; Fig. 1*A*) contains a transcription unit that places the *Escherichia coli lacZ* gene under the control of the HSV-1 *IE4/5* promoter, a constitutive promoter that functions in most cell types. The *lacZ* gene encodes a β-galactosidase not found in mammalian cells, providing an assay for expression of the transcription unit in pHSVlac. pHSVlac stably expresses β-galactosidase in cultured rat peripheral (2) and central nervous system neurons (3). In addition, pHSVlac expresses β-galactosidase in a wide range of human cells (15). Furthermore, after stereotactic injection of pHSVlac virus into the adult rat brain, β-galactosidase is stably expressed in cells around the injection site and in neurons whose axons project to the injection site (4).

The ability to deliver genes into neurons or glia, in culture or in the adult mammalian brain, has great potential for several fields (1). (*i*) To alter neuronal physiology, genes encoding components of signal-transduction mechanisms or neurotransmitter release could be expressed from HSV-1 vectors (16, 17); for example, since injection of protein kinase C protein into hippocampal neurons mimics some features of long-term potentiation (18), introduction of a protein kinase C gene into neurons might clarify the role of protein kinase C in producing long-term potentiation. (*ii*) HSV-1 vectors might be used to perform human gene therapy (19) for neurological disorders; for example, delivery of the tyrosine hydroxylase gene (20) into neurons in the striatum might be an effective treatment for Parkinson disease (21). Alternatively, brain tumors (22) might be treated with a HSV-1 vector expressing a cytotoxic gene product from a tumor-specific promoter.

pHSVlac has been packaged into HSV-1 particles (13) by using a HSV-1 temperature-sensitive (ts) mutant, HSV-1 strain 17 ts K (23), as helper virus. ts K has several attractive features for a helper virus: ts K contains a mutation in the *IE3* gene, the major regulatory protein of HSV-1; consequently, ts K has an IE phenotype, does not replicate DNA, does not significantly affect cellular physiology, and does not produce progeny virus at 37°C (23). ts mutants revert to wild type. In contrast, HSV-1 deletion mutants (9, 24, 25) essentially do not revert to wild type; therefore, HSV-1 vectors packaged using a deletion mutant as helper virus could be considered for human gene therapy. HSV-1 deletion mutants are isolated by introducing an essential HSV-1 gene into the genome of a fibroblast and then deleting the gene from the virus. The resulting HSV-1 deletion mutant is grown on the complementing cell line.

We now demonstrate that pHSVlac is efficiently packaged into HSV-1 particles using a HSV-1 strain 17 deletion mutant in the *IE3* gene, D30EBA (ref. 9 and Fig. 1*B*; for packaging procedure, see Fig. 1*C*). Southern blot analysis demonstrated that pHSVlac is properly packaged into HSV-1 particles using D30EBA as helper virus, and D30EBA is present in the virus stocks. Furthermore, pHSVlac virus packaged using D30EBA as helper virus expresses β-galactosidase, for at least 1 week, in cultured neurons and glia from rat superior cervical ganglia. In addition, since both ts K (23) and D30EBA (9) contain mutations in the HSV-1 strain 17 *IE3* gene and have the same phenotype, switching the helper virus from ts K to D30EBA should not affect the host range or other properties of the HSV-1 vector system. Thus, the deletion mutant packaging system for HSV-1 vectors renders human gene therapy with HSV-1 vectors a credible proposal.

MATERIALS AND METHODS

Enzymes and Chemicals. Restriction endonucleases were obtained from New England Biolabs. Nytran was obtained

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Abbreviations: HSV-1, herpes simplex virus 1; ts, temperature sensitive; ori, origin of DNA replication; pfu, plaque-forming units; X-Gal, 5-bromo-4-chloro-3-indoyl β-D-galactopyranoside.

*To whom reprint requests should be addressed.
†Present address: Georgetown University School of Medicine, Washington, DC 20007.

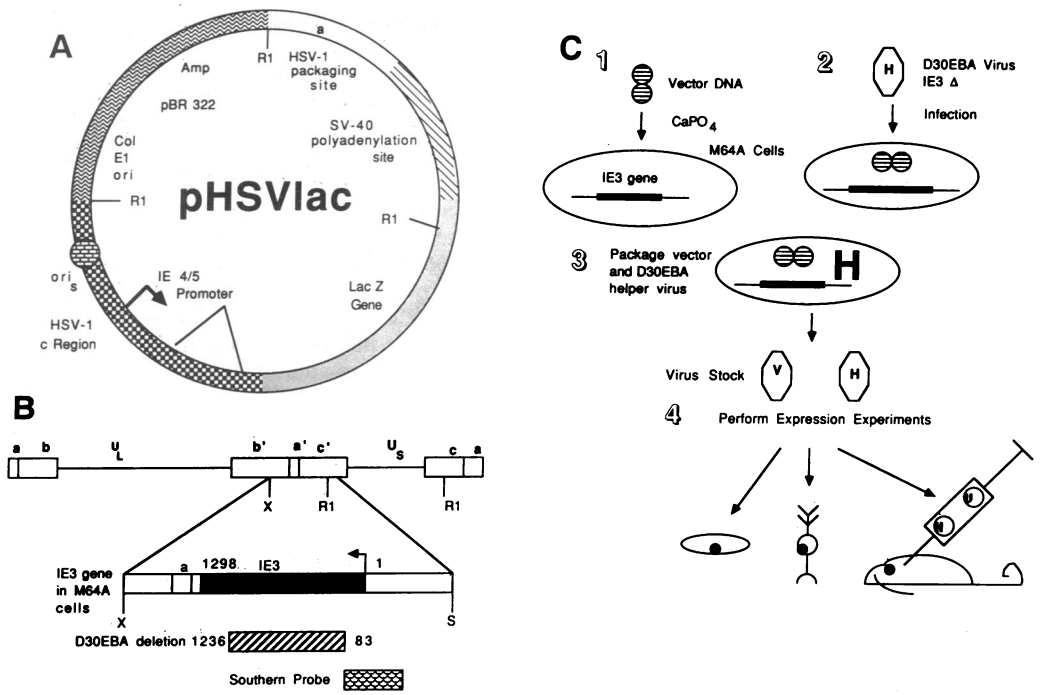

FIG. 1. Structure of pHSVlac (*A*), D30EBA virus and M64A cells (*B*), and the procedure for packaging pHSVlac into HSV-1 particles by using the deletion mutant packaging system (*C*). (*A*) The structure of pHSVlac (1–3). pHSVlac contains three types of genetic elements: (*i*) a transcription unit composed of the HSV-1 *IE* 4/5 promoter (arrow), the intervening sequence after that promoter (triangle), the *lacZ* gene (dotted), and the simian virus 40 early-region polyadenylylation site (diagonal lines); (*ii*) two HSV-1 sequences that permit packaging of pHSVlac DNA into HSV-1 particles, HSV-1 ori_s (small brick-filled circle), and the *a* sequence (open region) that contains the packaging site; and (*iii*) sequences from pBR322 that allow propagation of pHSVlac DNA in *E. coli* (wavy lines). pHSVlac contains three *Eco*RI (RI) fragments: The 4.3-kb fragment containing the HSV-1 *c* region and the *lacZ* gene, the 2.3-kb fragment containing the pBR segment, and the 1.5-kb fragment containing the HSV-1 *a* region. (*B*) The structure of M64A cells (8) and D30EBA virus (9). The top line shows the HSV-1 genome (10). The *IE3* gene, present in two copies in HSV-1, is in the duplicated *c* region. The second line diagrams the HSV-1 DNA in the genome of M64A cells. The fragment is flanked by *Xho* I (X) and *Sma* I (S) sites and contains the *IE3* gene (1298 codons; ref. 11) and the *a* sequence. The third line diagrams the *IE3* deletion (codons 83–1236) in D30EBA virus. The fourth line shows the 659-bp fragment used in Southern blot analysis of D30EBA virus. For Southern blot analysis DNA was digested with *Eco*RI and *Xho* I. Two fragments contain the *IE3* gene and are homologous to the probe (HSV-1 diagram, top line); the 8.5-kb *Eco*RI–*Xho* I fragment spans the joint between the HSV-1 long and short regions and the 5.5-kb *Eco*RI fragment from the short repeat at the right end of HSV-1 (11, 12). In D30EBA virus these fragments are reduced to 5.1 kb (8.5 kb in ts K) and 2.1 kb (5.5 kb in ts K). (*C*) The procedure for packaging (13) pHSVlac into HSV-1 particles using the deletion mutant packaging system. Steps: 1, vector DNA is delivered by calcium phosphate DNA transfection (14) into M64A cells, which contain the *IE3* gene (thick line) in their genome (thin line); 2, the next day these cells are infected with D30EBA (H) virus; 3, the *IE3* gene in M64A cells complements the D30EBA virus, resulting in a productive HSV-1 infection and the resulting virus stock consists of identical HSV-1 particles that contain either vector DNA (V) or D30EBA DNA (H); 4, this virus is used for experiments in cell lines, cultured neurons or glia, or stereotactic injection into the adult rat brain (1).

from Schleicher & Schuell. Tissue culture reagents were obtained from GIBCO. 5-Bromo-4-chloro-3-indoyl β-D-galactopyranoside (X-Gal) was obtained from Boehringer Mannheim.

Cell Culture and Viruses. HSV-1 strain 17 D30EBA virus (9) and the complementing cell line containing the *IE3* gene, M64A (8), were kindly provided by R. Everett (MRC Virology Unit, Glasgow, Scotland). The deletion in D30EBA removes codons 83–1236 of the *IE3* gene, which contains 1298 codons (ref. 11; Fig. 1*B*). M64A cells (ref. 8; Fig. 1*B*) contain the HSV-1 strain 17 *IE3* gene and *a* sequence, nucleotide 844 in the short-repeat region (11) to nucleotide 123,028 in the long-repeat region (12); BHK thymidine kinase-minus cells were transfected with the plasmid p65, which contains the *IE3* gene and the HSV-1 thymidine kinase gene, and M64A cells were isolated by hypoxanthine/adenine/thymidine (HAT) selection, as described (8). CV1 monkey fibroblasts and M64A cells were grown in Dulbecco's modified Eagle's medium with 10% (vol/vol) fetal bovine serum; M64A cells were maintained in HAT medium until just before use. PC12 cells were grown in RPMI 1640 medium containing 10% (vol/vol) horse serum and 5% fetal bovine serum (26). Dissociated neuronal cultures from superior cervical ganglia were prepared from 4-day-old rats (27). Five days after plating, cultures were treated with 40 μM cytosine arabinonucleoside for 24 hr to prevent glial overgrowth. One to 2 weeks later, cultures were infected with pHSVlac virus; at the time of infection, a culture contained 5×10^5 cells and ≈20% of the cells were neurons.

Packaging pHSVlac into HSV-1 Virus Particles by Using a Deletion Mutant Packaging System. pHSVlac was constructed as described (2). pHSVlac was packaged into HSV-1 particles (Fig. 1*C*) by adapting our packaging procedure (13) to M64A cells (8) and D30EBA virus (9): 1.5×10^5 M64A cells were seeded on a 60-mm plate. The next day, the cells were transfected (14) with 0.5 ml of a calcium phosphate coprecipitate containing 1 μg of pHSVlac DNA and 9 μg of salmon sperm DNA. Four hours later, the cells were treated with 15% (vol/vol) glycerol (28). After a 24-hr incubation at 37°C, 8×10^6 plaque-forming units (pfu) of D30EBA virus (9) in 100 μl of medium was added to each plate. After 1 hr at room temperature, an additional 5 ml of medium was added to each plate, and 3 days later virus was harvested. Virus was subsequently passaged at a 1:2 dilution on M64A cells; virus was prepared, passaged, and titered as described (29). pHSVlac virus was titered by determining the number of β-galactosidase-positive PC12 cells 1 day after infection (2). D30EBA virus (9) was titered on M64A cells (8) and rever-

Table 1. pHSVlac virus growth using the deletion mutant packaging system

Passage	pHSVlac titer, infectious particles per ml			
	pHSVlac-1	pHSVlac-2	pHSVlac-3	pHSVlac-4
Transfection	1×10^5	9×10^4	2×10^5	1×10^5
p-1	3×10^6	5×10^6	2×10^6	4×10^6
p-2	1×10^7	6×10^6	5×10^6	8×10^6
p-3	2×10^7	2×10^7	1×10^7	2×10^7

pHSVlac virus stocks were titered on PC12 cells as described (2). In the column labeled passage, transfection refers to the virus stock from the transfection/superinfection used to initiate packaging pHSVlac DNA into HSV-1 particles. Four transfections were performed, pHSVlac-1 through pHSVlac-4. p-1, p-2, and p-3 are the subsequent serial passages of each virus stock.

tants to wild type were detected on CV1 cells (see Tables 1 and 2 for the titers of the virus stocks used in this study).

Infection of Sympathetic Neurons with pHSVlac Virus and Detection of β-Galactosidase Activity. Cultures of sympathetic neurons were infected with 2.5 μl of pHSVlac virus (2×10^7 infectious particles per ml) and incubated for 1 week. Cells were fixed with 1.0% glutaraldehyde for 15 min, washed for three 5-min periods with isotonic phosphate-buffered saline (PBS), and assayed for β-galactosidase activity with X-Gal (30, 31). From 200 to 300 cells were scored under a phase-contrast microscope and the percentage of β-galactosidase-positive cells was calculated.

Analysis of pHSVlac DNA and D30EBA DNA. This procedure has been described in greater detail (2, 3). Total cellular DNA was prepared as described (32). To detect pHSVlac DNA, 5 μg of DNA, or 10 ng of pHSVlac DNA isolated from *E. coli* HB101 as standard, was digested with 12.5 units of *Eco*RI overnight, resolved on 0.7% agarose gels, and transferred to a Nytran membrane (33). Hybridization was performed as described (33); the probe was the 5.9-kilobase (kb) *Eco*RI fragment from the plasmid pCH110 (34) radiolabeled with ^{32}P (35). D30EBA DNA was detected by the same procedure except DNA was digested with *Eco*RI and *Xho* I, and the probe was a 659-base-pair (bp) fragment from the HSV-1 *IE3* gene, nucleotides 1065–1724 (11).

RESULTS

Protocol for Packaging pHSVlac DNA into HSV-1 Particles by Using a HSV-1 Deletion Mutant as Helper Virus. The prototype HSV-1 vector pHSVlac (refs. 1–3; Fig. 1*A*) was packaged into HSV-1 particles using M64A cells (8), which contains the HSV-1 *IE3* gene, and D30EBA virus (9), which contains a deletion in the *IE3* gene (Fig. 1*B*). To package pHSVlac into HSV-1 particles (ref. 13; Fig. 1*C*), M64A cells were transfected with pHSVlac DNA. One day later, these cells were infected with D30EBA virus; the *IE3* gene in M64A cells complements the deletion in the *IE3* gene in D30EBA virus, resulting in a productive HSV-1 infection. Since pHSVlac contains the sequences required to package it into HSV-1 particles (1–3), the progeny virus from this experiment were D30EBA virus and pHSVlac virus.

pHSVlac is maintained in a HSV-1 virus stock due to its growth advantage over the helper virus, no genetic selection is required. pHSVlac contains one HSV-1 origin of DNA replication (ori) in 8.1 kb (1–3), whereas HSV-1 contains three ori in 150 kb or 1 ori in 50 kb (10). Consequently, during serial passage of a virus stock, pHSVlac becomes a larger fraction of the virus particles. To increase the titer of pHSVlac, the virus stock from the initial packaging was passaged three additional times on M64A cells. The titers of pHSVlac, in each of four virus stocks, increased an average of 34-fold during the first passage and 2- to 3-fold in the two subsequent passages (Table 1). We have observed that extended serial passage of pHSVlac virus results in the production of naturally occurring defective-interfering particles (unpublished observation); therefore, these virus stocks were not passaged further. A detailed analysis of the third passage of these four pHSVlac virus stocks was performed; the titer and reversion frequency of the D30EBA virus was determined (Table 2). D30EBA virus grew efficiently in the presence of pHSVlac DNA, and the reversion frequency of D30EBA was 5×10^{-5}, comparable to D30EBA virus alone. By comparison, ts K has an apparent reversion frequency of 2×10^{-3} at 37°C [this includes incomplete penetrance of the ts K allele at 37°C; the true restrictive temperature is 39°C (23)]. The amount of pHSVlac virus was compared with the amount of D30EBA virus in the virus stocks, pHSVlac represented one-half to two-thirds of the virus stock, and the ratio of pHSVlac to ts K was slightly lower, 0.8 (2, 3). Thus, the ratio of pHSVlac to helper virus is similar with ts K and D30EBA. However, the growth of pHSVlac and D30EBA was substantially better than the growth of pHSVlac and ts K; the titer of pHSVlac grown with ts K was 8×10^5 infectious particles of pHSVlac per ml (2, 3) whereas pHSVlac grown with D30EBA gave an average titer of 2×10^7 infectious particles of pHSVlac per ml. In summary, pHSVlac is more efficiently packaged into HSV-1 particles using the deletion mutant D30EBA as helper virus than using ts K.

Analysis of pHSVlac DNA and D30EBA DNA in pHSVlac Virus Stocks. The structure of pHSVlac DNA and D30EBA DNA in HSV-1 particles was determined by Southern blot analysis (33). DNA isolated from the virus stocks (32) was digested with *Eco*RI, displayed on an agarose gel, and a blot was prepared. To detect pHSVlac DNA, the blot was hybridized with the 5.9-kb *Eco*RI fragment from the plasmid pCH110 (34). This fragment contains the pBR sequences and most of the *lacZ* gene, except for 133 bp at the 3′ end (34). pHSVlac contains three *Eco*RI sites, one at each end of the

Table 2. Efficiency of packaging pHSVlac DNA into HSV-1 particles using the deletion mutant packaging system

Virus	HSV-1 titer, pfu		Reversion frequency	pHSVlac titer	pHSVlac/helper
	M64A	CV1			
pHSVlac-1	9×10^6	2×10^2	2×10^{-5}	2×10^7	2.2
pHSVlac-2	2×10^7	7×10^2	4×10^{-5}	2×10^7	1.0
pHSVlac-3	1×10^7	9×10^2	9×10^{-5}	1×10^7	1.0
pHSVlac-4	1×10^7	7×10^2	7×10^{-5}	2×10^7	2.0
D30EBA	8×10^6	1×10^2	1×10^{-5}	—	—

pHSVlac virus was titered on PC12 cells as described (2). D30EBA virus (9) was titered on M64A cells (8) and CV1 cells. All pHSVlac virus stocks are from the third passage, the number after pHSVlac designates which virus stock was used. D30EBA is D30EBA virus grown alone. The reversion frequency is the titer of D30EBA virus on CV1 cells divided by the titer of D30EBA virus on M64A cells. The titer of pHSVlac virus was divided by the titer of D30EBA virus on M64 cells to give the ratio of pHSVlac virus to D30EBA virus (helper).

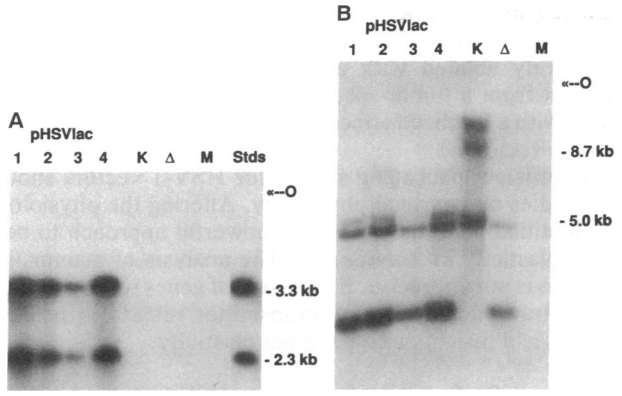

FIG. 2. Southern blot analysis of the structure of pHSVlac DNA (*A*) and D30EBA DNA (*B*) in pHSVlac virus prepared with the deletion mutant packaging system. (*A*) Analysis of pHSVlac DNA. DNA (5 μg) was digested with *Eco*RI and displayed on an agarose gel, and the resulting blot was hybridized with a probe homologous to the pBR segment and the *lacZ* gene, except for the 133 bp at the 3′ end of the *lacZ* gene. The origin of the gel is indicated as O and the sizes are as shown. Lanes 1–4 indicate which virus stock was used; lane Δ is D30EBA DNA alone, lane K is ts K DNA alone, and lane M is uninfected CV1 cell DNA. The standards (Stds) lane contains 10 ng of pHSVlac DNA from *E. coli* digested with *Eco*RI. (*B*) Analysis of the helper virus DNA. The same DNA samples were digested with *Eco*RI and *Xho* I and subjected to Southern blot analysis using a probe from the *IE3* gene (Fig. 1*B*) present in D30EBA. The size standards (not shown) were 8.7 kb and 5.0 kb.

pBR segment and a third in the *lacZ* gene 133 bp from the 3′ end of the fragment (Fig. 1*A*). The 4.3-kb band, which contains most of the transcription unit in pHSVlac, and the 2.3-kb band, which contains the pBR sequences, were present in pHSVlac virus (Fig. 2*A*) but were absent from D30EBA alone, ts K alone, and uninfected cells. The 1.5-kb fragment of pHSVlac contains the 3′ end of *lacZ* gene, the simian virus 40 early region polyadenylylation site, and the HSV-1 *a* sequence; this fragment is not homologous to the probe.

The structure of the helper virus D30EBA was examined. The same DNA samples used to detect pHSVlac DNA were digested with *Eco*RI and *Xho* I and subjected to Southern blot analysis (33) using a probe from the HSV-1 *IE3* gene (Fig. 1*B*); this probe hybridizes to both copies of the *IE3* gene in HSV-1. ts K yielded the expected fragments (Fig. 2*B*) of 8.5 kb, the *Eco*RI–*Xho* I fragment spanning the junction between the short and long regions of HSV-1, and 5.5-kb, the *Eco*RI fragment at the small (rightward) terminus of HSV-1 (Fig. 1*B*; refs. 11 and 12). The band above 8.5 kb at 8.9 kb is probably due to duplication of the 401-bp *a* region. The *a* region can be repeated at the L terminus or at the junction between the long and short regions and the *a* sequence is always present as a single copy at the S terminus of HSV-1 (10). D30EBA (9) contains a 3462-bp deletion in the *IE3* gene, the expected sizes of the fragments are 5.1 kb (8.5 kb in ts K) and 2.1 kb (5.5 kb in ts K). These fragments were present in D30EBA DNA and pHSVlac packaged with D30EBA but were absent from uninfected CV1 cells (Fig. 2*B*). [The faint band just above the 5-kb bands, visible in some lanes, is due to the *IE3* gene in M64A cells (one copy per cell); in a lytic infection, HSV-1 DNA is present at ≈100 copies per cell (10).] Thus, the D30EBA deletion is present in the helper virus. We conclude that pHSVlac DNA was properly and efficiently packaged into HSV-1 particles using the deletion mutant packaging system.

pHSVlac Virus Stably Expresses β-Galactosidase in Cultured Rat Sympathetic Neurons and Glia. The ability of pHSVlac virus, prepared using the deletion mutant packaging system, to stably express β-galactosidase in neurons and glia was determined. Cultured rat sympathetic neurons (27) were infected with pHSVlac virus, and 1 week later an *in situ* assay for β-galactosidase (using X-Gal; refs. 30 and 31) was performed. X-Gal-positive cells that had the morphology of neurons (Fig. 3 *A–D*) and of glia (Fig. 3 *E* and *F*) were observed. Cultures infected with D30EBA alone or mock-infected contained <0.2% X-Gal-positive cells. Approximately 20% of the cells in the cultures were neurons, and ≈10% of the neurons contained β-galactosidase. Since the multiplicity of infection was 0.1, pHSVlac virus efficiently infected neurons. These results are consistent with previous observations that pHSVlac, packaged using ts K, expressed β-galactosidase in both peripheral (2) and central nervous system neurons (3), as demonstrated by colocalization of β-galactosidase-like immunoreactivity and neurofilament-like immunoreactivity.

The β-galactosidase-positive cells could arise from pHSVlac persisting in one cell for a week or from horizontal transmission of pHSVlac from one cell to another. If horizontal transmission occurred, then virtually all the cells in a culture would contain pHSVlac DNA and express β-galac-

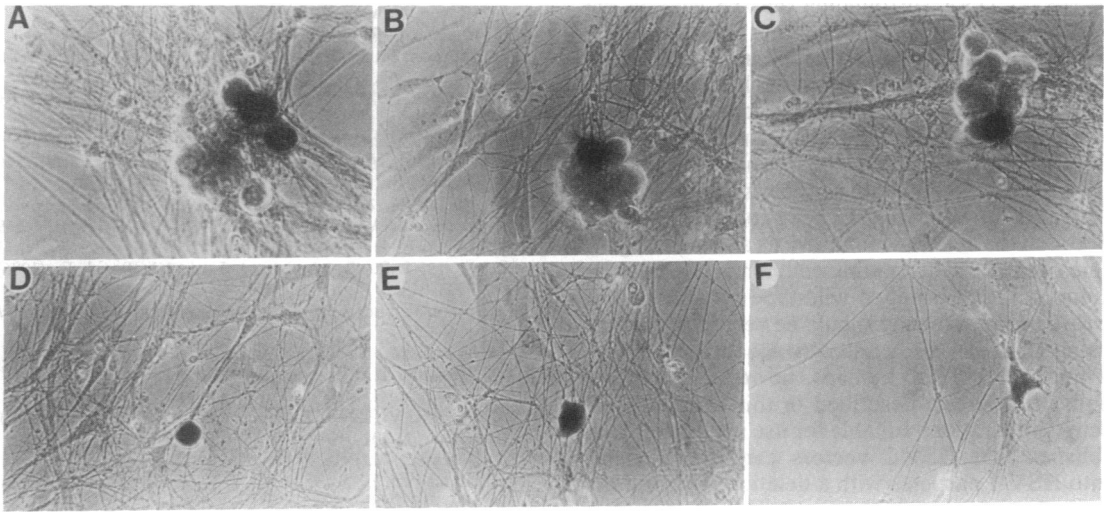

FIG. 3. Stable expression of β-galactosidase in cultured neurons (*A–D*) and glia (*E* and *F*) from superior cervical ganglia 1 week after infection with pHSVlac virus. Cultures of superior cervical ganglia were prepared (27) from newborn rats. Two weeks after preparation the cultures were infected with pHSVlac virus, and 1 week later β-galactosidase activity was detected with X-Gal (30, 31). The length of each photomicrograph represents 230 μm.

tosidase, and both D30EBA and pHSVlac virus would be present in the culture medium. In contrast, ≈90% of the cells were β-galactosidase-negative. In addition, 1 week after infection of three cultures, the culture medium contained <10 infectious particles of pHSVlac per ml and <10 pfu of D30EBA per ml, below detection levels. By comparison, wild-type HSV-1 kills all the cells in a culture in <24 hr. Furthermore, pHSVlac packaged using ts K stably persists in cultured peripheral and central nervous system neurons for at least 2 weeks (2, 3). In conclusion, pHSVlac virus, prepared using the deletion mutant packaging system, efficiently infects and stably expresses β-galactosidase in cultured sympathetic neurons and glia.

DISCUSSION

Until recently, gene transfer techniques were unable to deliver a gene directly into postmitotic cells, such as neurons. Therefore, we developed a HSV-1 vector system to deliver genes into neurons or glia, in culture or in the adult brain (1–4). HSV-1 vectors have been packaged into HSV-1 particles using a ts mutant as helper virus. However, ts K reverts to wild type; the reversion frequency is 2×10^{-3}. Therefore, a packaging system for HSV-1 vectors using a deletion mutant (9) as helper virus was developed. The deletion mutant packaging system is efficient; the concentration of pHSVlac virus per ml is ≈10-fold higher using the deletion mutant packaging system than using ts K as helper virus.

The deletion mutant used as helper virus was D30EBA (9), and the ts mutant used as helper virus was ts K (23), both of which contain mutations in the HSV-1 strain 17 *IE3* gene. Consequently, ts K and D30EBA have the same phenotype under restrictive conditions; they each express the four other *IE* genes, low levels of one or two early genes, and no other HSV-1 genes (9, 23). Therefore, HSV-1 vectors packaged with the two helper viruses should have the same host range and other properties. ts K has little effect on cellular gene expression (36) and latent HSV-1 does not alter electrophysiological properties of neurons (37). Similarly, vectors packaged using D30EBA as helper virus should not effect cellular functions.

D30EBA virus (9) grown on M64A cells (8) has a reversion frequency of 5×10^{-5}; ts K has an apparent reversion frequency at 37°C of 2×10^{-3}. Thus, the reversion frequency of D30EBA is ≈40 times lower than ts K. The revertants of D30EBA presumably arise by homologous recombination between the HSV-1 DNA surrounding the *IE3* gene in the genome of M64A cells and D30EBA virus. M64A cells (8) contain a large amount of HSV-1 DNA flanking the *IE3* gene, including the *a* sequence, which promotes homologous recombination presumably due its repeated sequences. Therefore, cell lines containing only the *IE3* gene might lower the reversion frequency even further, consistent with previous findings with other HSV-1 deletion mutants. Furthermore, isolation of larger deletions in the *IE3* gene could eliminate all homology between the deletion mutant virus and the *IE3* gene in the genome of the complementing cell; since homologous recombination could not produce wild-type virus in such a system, the reversion frequency should be very low. Vectors packaged using D30EBA virus and M64A cells may not be suitable for introduction into humans; however, packaging systems similar to the one described in this report should produce vector preparations suitable for use in humans. This study establishes that HSV-1 vectors can be efficiently packaged into HSV-1 particles with a deletion mutant packaging system.

HSV-1 vectors may prove useful for human gene therapy (19): For example, expression of the tyrosine hydroxylase gene (20) in striatal neurons might be an effective therapy for Parkinson disease (21). Brain tumor cells (22) might be selectively ablated with a vector expressing a cytotoxic product from a tumor-specific promoter. Other neural diseases with a localized affected site could also be treated with HSV-1 vectors.

An efficient packaging system for HSV-1 vectors should aid studies on neuronal physiology. Altering the physiology of identified neurons has been a powerful approach to neuronal plasticity in *Aplysia* (38). The analysis of mammalian systems is not as precise. Expression of genes responsible for signal transduction or neurotransmitter release in neurons might yield insights into neuronal plasticity.

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