

Defective Herpes Simplex Virus Type 1 Vectors Harboring *gag*, *pol*, and *env* Genes Can Be Used To Rescue Defective Retrovirus Vectors

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A retroviral packaging transcription unit was constructed in which the Moloney murine leukemia virus (MoMLV) *gag-pol* and *env* genes are expressed under the control of herpesvirus regulatory sequences. This transcription unit, lacking long terminal repeats, primer binding sites, and most of the retrovirus packaging signal but retaining both retroviral donor and acceptor splice sites, was cloned into a herpes simplex virus type 1 (HSV-1) amplicon plasmid, and amplicon vectors (the *gag-pol-env* [GPE] vectors) were generated by using a defective HSV-1 vector as helper virus. The GPE vector population was used to infect human TE671 cells (ATCC CRL 8805), harboring a *lacZ* provirus (TE-*lac2* cells), and supernatants of infected cells were collected and filtered at different times after infection. These supernatants were found to contain infectious ecotropic *lacZ* retroviral particles, as shown both by reverse transcription-PCR and by their ability to transduce a β -galactosidase activity to murine NIH 3T3 cells but not to human TE671 cells. The titer of retroviral vectors released by GPE vector-infected TE-*lac2* cells increased with the dose of infectious amplicon particles. Retrovirus vector production was inhibited by superinfection with helper virus, indicating that helper virus coinfection negatively interfered with retrovirus production. Induction of retrovirus vectors by GPE vectors was neutralized by anti-HSV-1 but not by anti-MoMLV antiserum, while transduction of β -galactosidase activity to NIH 3T3 cells by supernatants of GPE vector-infected TE-*lac2* cells was neutralized by anti-MoMLV antiserum. These results demonstrate that HSV-1 GPE amplicon vectors can rescue defective *lacZ* retrovirus vectors and suggest that they could be used as a sort of launching ramp to fire defective retrovirus vectors from within virtually any *in vitro* or *in vivo* cell type containing defective retroviral vectors.

Among the different available gene delivery systems, retrovirus vectors offer the possibility of efficiently integrating relatively limited transgenic sequences into the cell's chromosomes, whereas herpesvirus vectors can transduce large amounts of foreign genetic sequences, which remain episomic. In this work we generated a novel chimeric vector system which combines the advantages of both such viral vector systems.

Two types of viral vectors have been constructed with herpes simplex virus type 1 (HSV-1) as the backbone, recombinant vectors and amplicon vectors (22). In recombinant vectors, the transgene of interest is inserted directly into the genome of the virus. Amplicon vectors consist of a plasmid containing HSV-1 origin of replication and packaging sequences as well as the transgenic transcription unit. Because the amplicon does not encode any further viral sequences, it can only be grown in the presence of *trans*-acting functions provided by helper virus. In the presence of helper virus, amplicon DNA is amplified into a head-to-tail concatemer which is then packaged into defective HSV-1 particles, up to the genome size. Amplicon vector preparations used for infections thus consist of the helper particles plus the amplicon particles containing the transgene (37).

Standard methods to generate retroviral vectors currently use producer cell lines expressing both the *gag-pol* and *env* genes of murine leukemia virus (MLV) (the packaging com-

ponent) and a defective retroviral vector genome expressing the transgenic protein (the vector component) (25, 26). These stable producer cell lines, however, have several major drawbacks: vector titers generated by them are generally too low to allow an efficient *in vivo* gene transfer, they may evolve to produce recombinant retroviral particles, and they are most suitable to provide vectors *in vitro*. Alternative improvements to overcome some of these limitations have been described previously, all of them based on the use of transient retroviral vector production systems (4, 8, 12, 21, 27, 36, 38, 40). Although these systems differ in several respects, a common shared feature is that the retroviral packaging and vector components are introduced into cells by transfection procedures, thus limiting the efficiency of these new strategies.

The introduction of both the packaging and vector components into cells by infection, using viral particles of a heterologous virus system, might help to solve some of the limitations imposed by the low efficiency of transient transfection. For example, the packaging and/or the vector components of the retrovirus vector could be integrated, either separately or simultaneously, into the backbone of a large DNA virus and introduced into target cells through viral infection. Both retroviral components could in this manner enter cells at high multiplicities and be strongly expressed from the DNA virus backbone, by using the high efficiency of viral transcriptional activation. This could in turn lead to the production of high titers of retrovirus particles, both *in vitro* and *in vivo*, including production by cells normally nonpermissive for retrovirus infection.

Whether such a strategy would work is the question addressed by this study. Such an approach could fail because of

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negative interferences which could inhibit the synthesis or release of retroviral vectors. Therefore, in order to study whether retrovirus vectors could be generated through infection of cells by a DNA virus-derived vector system we have generated a defective HSV-1 amplicon vector (37) expressing Moloney MLV (MoMLV) *gag-pol* and *env* (ecotropic) proteins, modified in such a way as to be expressed as an immediate-early gene during HSV-1 infection. This packaging amplicon vector was subsequently used to infect human TE-lac2 cells (39), expressing a defective MoMLV vector encoding β -galactosidase. Results presented in this study show that infection of TE-lac2 cells with amplicons expressing the packaging component of MoMLV allowed the rescue of infectious ecotropic retroviral vectors, thus demonstrating that our theoretical approach is practically feasible.

Construction and functional assays of the GPE transcription units. Plasmid pCRIP (6), bearing a packaging-deficient retroviral transcription unit encoding MoMLV *gag*, *pol*, and *env* genes, was used to derive three different *gag-pol-env* (GPE) transcription units driven by heterologous regulatory sequences. Firstly, the simian virus 40 polyadenylation signal of pCRIP was replaced by an *MscI/PvuII* fragment from pAGO (5), encompassing the thymidine kinase (TK)-encoding gene polyadenylation signal from HSV-1 (24). A *BglII* restriction site was introduced into that plasmid, 35 nucleotides upstream of the splicing donor site of MoMLV (34), by PCR-mediated mutagenesis. The *BglII* site was then used to clone either (i) a *PvuII/BglII* fragment from pAGO, containing the TK promoter from HSV-1, giving transcription unit TK-GPE, or (ii) a PCR-amplified *DraIII/BamHI* DNA fragment, carrying the IE3 gene promoter from HSV-1, giving transcription unit IE3-GPE. Finally, a *NotI/PstI* fragment from pA-SF1 (23), containing the human cytomegalovirus major immediate-early enhancer/promoter (IE-HCMV), was cloned into the *NotI/MluI* sites of TK-GPE, giving transcription unit CMV-GPE (Fig. 1A). Compared to the parental MoMLV genome, these GPE transcription units lacked primer binding sites and polypurine tracts, both long terminal repeats (LTRs), and the packaging sequence E. This design of this defective-retrovirus genome was adopted to abolish or minimize its packaging, reverse transcription (RT), and integration. Each transcription unit was then cloned into a plasmid containing one HSV-1 origin of replication (*ori-S*) and one HSV-1 packaging signal ("a") (23). The resulting amplicon plasmids were called pA-TK-GPE, pA-IE3-GPE, and pA-CMV-GPE.

The three amplicon plasmids, as well as the parental pCRIP plasmid, were tested in a transient-complementation assay for their ability to mobilize a *lacZ* replication-defective retroviral vector from human TE-lac2 cells (39), which harbor the MFGnslacZ retroviral vector (11). About 5 μ g of each plasmid was transfected into TE-lac2 cells by the calcium phosphate method (28). The transfected cell supernatants were filtrated and used to infect murine NIH 3T3 cells the next day. Infected cells were stained for β -galactosidase activity with X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) 48 h later (32). Plasmids pCRIP and pA-CMV-GPE efficiently rescued the *lacZ* retroviral vectors (releasing 2.1×10^3 and 0.9×10^3 *lacZ* infectious units [iu] per ml, respectively). Plasmid pA-IE3-GPE rescued *lacZ* vectors to a somewhat lesser extent, and pA-TK-GPE rescued only very low levels of *lacZ* retroviral vectors. These results were expected, since the LTR, HCMV, and IE3 promoters can be expressed constitutively while the TK promoter has a very low activity in the absence of HSV-1 transactivators ICP0 and ICP4 (9). Thus, the chimeric transcription units, placed under the control of herpesvirus constitutive promoters, are expressed and give rise to the synthesis of

retroviral packaging elements, resulting in the rescue of the *lacZ* replication-defective retroviral vectors. Plasmid pA-CMV-GPE (Fig. 1B) was used to generate infectious amplicon vectors.

Generation of defective GPE amplicon vectors. Five micrograms of pA-CMV-GPE (Fig. 1B) was transfected into M64A cells (7) expressing the HSV-1 IE3 gene. Transfected cells were superinfected the next day by the IE3⁻ HSV-1 D30EBA (29) as a helper virus, at 1 PFU per cell, as previously described (15). When total cytopathic effect was observed, cells were frozen and thawed three times to release the infectious virus. Titers of the helper virus in viral stocks were determined by plaque assays, performed on M64A cells, as already described (3). To estimate the amount of amplicon virions, we used the comparative procedure already described by Bergold et al. (1) and Hong et al. (17). Briefly, the relative amounts of amplicon vector DNA were determined by blot hybridization (2), in comparison with the helper virus DNA, by using a ³²P-labelled probe corresponding to cloned *ori-S* sequences, which recognize both helper and amplicon vector DNAs. Titers of the defective amplicon vectors were then estimated by the helper virus titers as well as the ratios of the amplicon DNA to the helper virus DNA (amplicon-to-helper ratios). As a control, the same procedure was performed with the amplicon vector pA-SF1 (23), which carries the *lacZ* gene and can be titrated independently by staining β -galactosidase-positive cells with X-Gal.

The vector population was called pA-CMV-GPE/D30EBA (referred to as the GPE amplicon). GPE amplicon stocks were serially passaged onto fresh M64A cells by using a 1:2 dilution in order to increase the vector-to-helper ratio (37). For each passage, total DNA was extracted (31) from an aliquot of infected cells, digested with *KpnI*, and analyzed by Southern blotting (2) by using as the probe a DNA fragment digested from pCRIP (6) that was labelled with [α -³²P]dCTP (Amersham) by the random-priming method (10) using a kit from Amersham. Figure 2 shows that the amplicon DNA did not undergo major genetic rearrangements during passages, a fact that was further confirmed by digesting the DNA with other restriction enzymes (data not shown). Figure 2 also shows that the amount of amplicon vector DNA increased from passage 1 (P1) to P5 and decreased somewhat in later passages. Since the titer of the helper virus remained relatively constant during the passages (ranging from 2×10^7 to 5×10^7 PFU/ml), we conclude that P5 had the highest vector-to-helper ratio. GPE amplicons from P5 were thus used for most further studies. We estimate that P5 contains 2.1×10^7 PFU of helper virus particles/ml and has a helper-to-vector ratio of about 5:1.

Rescue of retroviral vectors by infection of TE-lac2 cells. To study whether GPE amplicons could rescue *lacZ* retroviral vectors, approximately 10^6 TE-lac2 cells were infected with 5 μ l of GPE amplicons from P5, corresponding to a multiplicity of infection (MOI) of 0.1 for the helper particles and of about 0.02 for the amplicon particles. Cell culture supernatants were collected 48 h later, filtered with a 0.2- μ m-pore-size filter (Gelman Sciences), and centrifuged at 1,000 rpm (Heraeus Megafuge) to eliminate cell debris. To check the assembly and release of *lacZ* retroviral particles, filtrated supernatants were then ultracentrifuged at 35,000 rpm (Sorvall RC 5C Plus) for 1 h at 4°C to pellet retrovirus particles, and pellets were analyzed by RT-PCR using oligonucleotides specific for the *lacZ* retroviral vector. The expected fragment was detected by RT-PCR, but not by PCR, from pelleted supernatants taken from GPE-infected TE-lac2 cells (data not shown). This fragment was found to be indistinguishable from both the RT-PCR band generated from total RNA extracted from noninfected TE-lac2

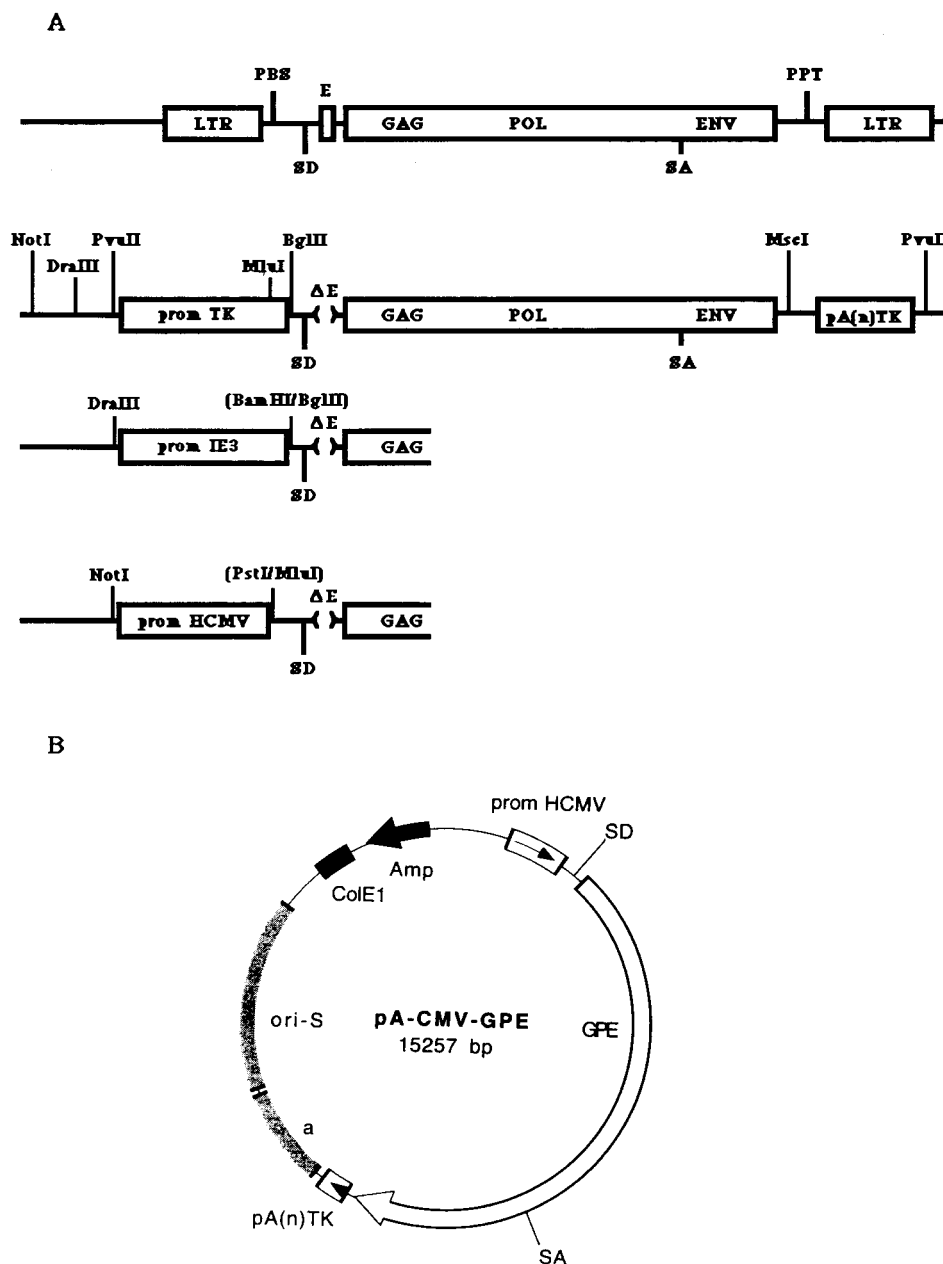


FIG. 1. Schematic structures of the transcription units used to construct the GPE amplicons. (A) The 5' LTR of MoMLV (first line) was replaced by either the HSV-1 TK promoter (prom TK), the HSV-1 IE3 promoter (prom IE3), or the major immediate-early promoter of human cytomegalovirus (prom HCMV), to give TK-GPE, IE3-GPE, and CMV-GPE transcription units, respectively (second to fourth lines, respectively). In these three plasmids, the 3' LTR of MoMLV is replaced by poly(A) sequences derived from HSV-1 TK gene [pA(n)TK]. These constructs also lack the primer binding site (PBS), most of the packaging signal (E), and the polypurine tract (PPT) of MoMLV. (B) Genetic structure of pA-CMV-GPE, an amplicon plasmid containing the CMV-GPE transcription unit, used to construct the GPE amplicon vector. ColE1 and Amp are bacterial sequences enabling replication and selection of plasmids. *NotI*, *DraIII*, *PvuII*, *MluI*, *BglIII*, and *MscI* indicate the cloning sites used to obtain the constructs shown. SD and SA, splice donor and acceptor sites, respectively; ΔE , deletion of the encapsidation sequence; TK and IE3, HSV-1 TK and IE3-encoding genes; HCMV, human cytomegalovirus major immediate-early gene; ori-S and a, origin of replication and cleavage/packaging signal of HSV-1, respectively.

cells and the PCR band generated from a plasmid harboring the *lacZ* retrovirus vector (11). No DNA fragments could be detected after RT-PCR with supernatants taken from noninfected TE-lac2 cells or from TE-lac2 cells infected with D30EBA helper virus alone. Thus, the *lacZ*-defective retrovirus genome expressed by TE-lac2 cells could be packaged and released only after infection of these cells with the GPE amplicons.

To check whether the retroviral particles detected by RT-PCR could transduce β -galactosidase activity to susceptible cells and also to estimate the amount of infectious vectors present in the supernatants, aliquots of filtrated supernatants of GPE amplicon-infected TE-lac2 cells were overlaid on either murine NIH 3T3 or human TE671 (ATCC CRL 8805) target cells in the presence of Polybrene (Sigma) and β -galactosidase expression was checked 48 h later. NIH 3T3 cells, but

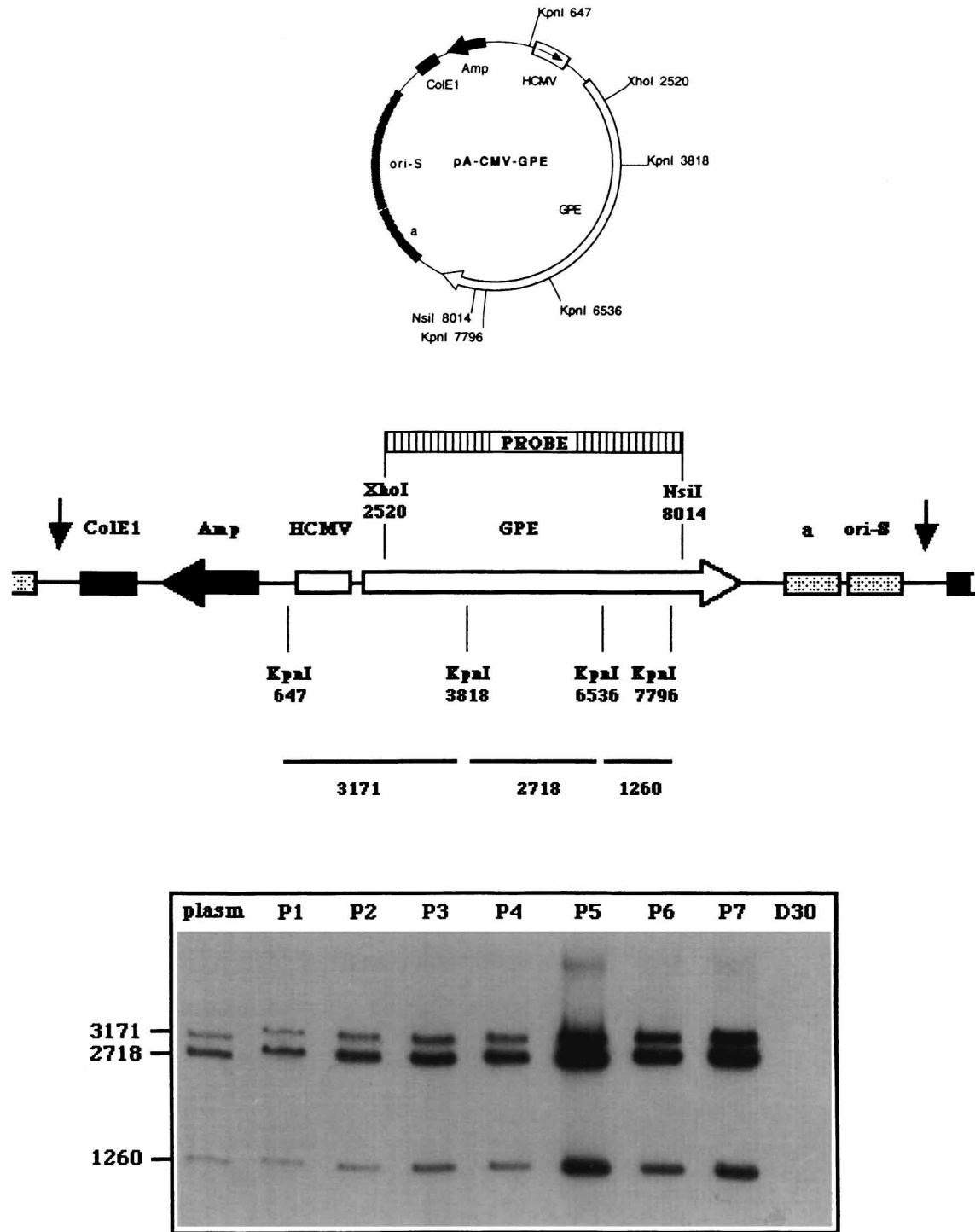


FIG. 2. The top part of the figure shows details of the schematic structure of pA-CMV-GPE. The bottom part of the figure shows the Southern blot analysis of different passages of the GPE amplicon vectors. M64A cells were infected at 1 PFU/cell with each of the different passages of GPE amplicon vectors (P1 to P7) or with the helper D30EBA virus alone (D30). The next day, total DNA was extracted and digested with *KpnI*, and the resulting fragments were analyzed by electrophoresis and transferred to nylon membranes. Hybridizations were performed with a labelled *XhoI/NsiI* fragment, specific for the GPE retrovirus sequences, as a probe. Control *KpnI*-digested pA-CMV-GPE plasmid (lane plasm) is also shown. The positions of relevant restriction sites in either the pA-CMV-GPE plasmid or the vector concatemer are indicated. Vertical arrows above the linear diagram indicate arbitrary limits of reiterated sequences in the vector genome.

not TE671 cells, displayed *lacZ* colonies (data not shown), indicating that the infecting supernatants contained ecotropic *lacZ* retroviral vectors. No *lacZ* colonies appeared in NIH 3T3 cells infected with control supernatants taken from nonin-

fecting TE-lac2 cells or from TE-lac2 cells infected with D30EBA helper virus alone. The average titer of *lacZ* retrovirus vectors released by TE-lac2 cells infected with 5 μ l of P5 amplicons (containing approximately 2×10^4 GPE vector par-

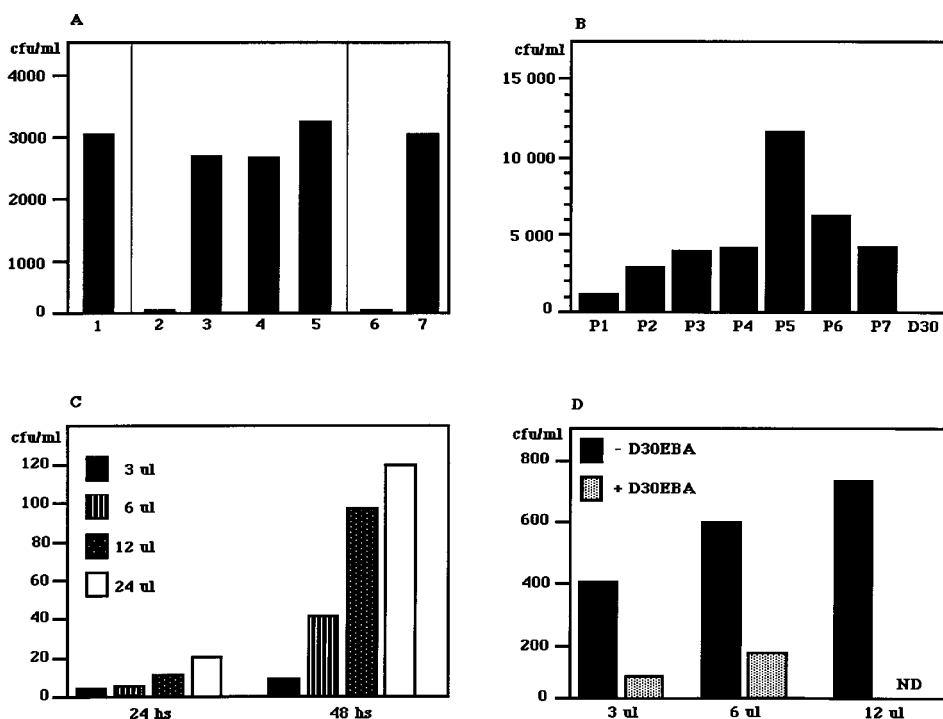


FIG. 3. (A) NIH 3T3 cells were infected with supernatants taken from GPE amplicon-infected TE-lac2 cells. The GPE amplicon population was treated with rabbit anti-HSV-1 neutralizing antibodies (bar 2), with rabbit nonimmune antibodies (bar 3), with goat antibodies specific for MoMLV (bar 4), or with goat antibodies specific for RD114 virus (bar 5) before infecting TE-lac2 cells. The supernatants taken from the GPE amplicon-infected TE-lac2 cells were treated with antibodies specific for MoMLV (bar 6) or for RD114 (bar 7) before infecting NIH 3T3 cells. No antibodies were added at any step of the protocol for the result shown by bar 1. (B) NIH 3T3 cells were infected with supernatants from TE-lac2 cells that had been infected with 5 μ l of each of the different passages of the GPE amplicon vectors (bars P1 to P7) or with D30EBA helper virus alone (bar D30). β -Galactosidase activity was revealed 2 days postinfection by using X-Gal. (C) NIH 3T3 cells were infected with supernatants from TE-lac2 cells that had been infected with increasing amounts of a GPE amplicon vector population that had been diluted 50-fold. β -Galactosidase activity was revealed at either 1 or 2 days postinfection. (D) NIH 3T3 cells were infected with supernatants from TE-lac2 cells that had been infected with increasing amounts of GPE amplicon vectors in the presence (+ D30EBA) or absence (- D30EBA) of extra D30EBA helper virus (1 PFU/cell). ND, not done.

ticles) in four independent experiments was 1.2×10^4 iu/ml (standard error, 2.5×10^3), corresponding to 3×10^4 total iu per infected culture.

Two neutralization experiments were performed to further explore this novel vector-generating system. In the first one, designed to assess whether the rescue of *lacZ* retrovirus particles from TE-lac2 cells was exclusively induced by the HSV-1 amplicon vector (and not by naked amplicon DNA or by retrovirus transfer of GPE functions), GPE amplicons were treated with antibodies directed against either HSV-1 or MoMLV envelope glycoproteins before infecting TE-lac2 cells. As expected, anti-HSV-1 antibodies (B-114; DAKO) could block the generation of *lacZ* retrovirus particles if added before the infection of TE-lac2 cells, while nonimmune antibodies (Z-182; DAKO) or neutralizing antibodies specific for MoMLV or RD114 gp70-SU protein (Quality Biotech Inc.) did not block the generation of retrovirus particles at this step (Fig. 3A, bars 2 to 5).

In the second neutralization assay, designed to confirm that the *lacZ* activity transduced to NIH 3T3 cells by the supernatants of GPE amplicon-infected TE-lac2 cells resulted from ecotropic retroviral vectors, the supernatants were treated either with antibodies against the ecotropic envelope glycoprotein or with antibodies against RD114 retrovirus, which belongs to a distinct receptor group (35). Only the antibodies specific to the ecotropic envelope glycoprotein showed neutralization activity when added before infection of NIH 3T3 cells (Fig. 3A, lanes 6 and 7).

GPE amplicon-infected TE-lac2 cells were found to release

the *lacZ* retroviral vector particles for at least 3 days. To know whether the supernatants of the GPE amplicon-infected TE-lac2 cells were devoid of replication-competent retroviral particles that might have arisen from recombination events, aliquots of these supernatants were overlaid on 3T3-LacZ cells (39), which express a defective *lacZ* retroviral vector. The supernatants of infected 3T3-LacZ cells were tested after 3 days for the presence of retroviral vectors able to transduce β -galactosidase expression to NIH 3T3 cells. No rescue of *lacZ* vectors could be observed, suggesting that the retrovirus vectors produced by the GPE amplicon infection were devoid of replication-competent retroviruses.

Our results thus clearly demonstrate that it is possible to rescue a retrovirus vector by infecting cells with a defective HSV-1 amplicon vector as the source of *gag*, *pol*, and *env* genes. This implies that all steps required to give rise to retrovirus particles (transcription, splicing, translation, posttranslational modifications, assembly, packaging, and viral egress) have correctly occurred by using the gene products encoded by the herpesvirus vector. Although it has already been reported that other species of herpesvirus genomes may carry inserted retrovirus sequences (18, 19) and though other viruses have been found to express functional *gag-pol* proteins (14, 30), to our knowledge this report is the first to demonstrate the ability of defective HSV-1 vectors to allow such a complex morphogenetic process involving the synthesis and functional assembly of all proteins and nucleic acid required for the generation of infectious particles of a different virus family. These results

illustrate the usefulness and very low cytotoxicity of amplicon particles.

HSV-1 helper particles can inhibit retrovirus rescue. To further explore the relationship between the efficacy of *lacZ* retrovirus vector mobilization and the input dose of GPE amplicon vector, similar aliquots (5 μ l) of GPE amplicons from P1 to P7 were used to infect TE-lac2 cells. Results shown in Fig. 3B indicate that (i) in all cases, infection of TE-lac2 cells resulted in the synthesis and release of infectious *lacZ* retrovirus vectors and (ii) the higher the proportion of amplicon vector in the virus population was (as shown in Fig. 2), the higher the rescue of the *lacZ* retrovirus vector was, reaching a titer of more than 10^4 iu/ml for P5 and showing a good correlation with GPE amplicon input dose. On the other hand, we further noticed that as the MOI of the helper component of the GPE amplicon became close to 1 PFU/cell, the amount of retroviral vector released by TE-lac2 cells decreased. This suggested that HSV-1 helper virus could negatively interfere with retrovirus production. To further explore this observation, two experiments were done. Firstly, different populations of GPE amplicons were diluted 50-fold in order to decrease their helper virus titers to less than 10^6 PFU/ml and increasing input doses of them were used to infect TE-lac2 cells. This resulted in low but increasing amounts of retrovirus vector production, as illustrated in the example shown in Fig. 3C. Secondly, the helper-to-vector ratio of a particular GPE amplicon dilution was enriched by adding extra helper particles (MOI, 1 PFU/cell) to the vector population and was tested in the *lacZ* retroviral vector mobilization assay in comparison with the initial population (without the additional helper particles). As shown in Fig. 3D, increasing the relative amount of helper particles resulted in inhibition of retrovirus production. Taken together, these experiments indicate (i) a dose-dependent effect of the amplicon vectors on the synthesis and release of retroviral vectors at low input doses and (ii) a negative interference exerted by the helper virus at higher input doses. These results strongly suggest that *lacZ* retrovirus vector mobilization can only be achieved in those TE-lac2 cells that are singly infected with amplicon particles.

Thus, both the ratio of amplicon vector to helper particles and the absolute amount of helper particles in the virus populations are critical factors affecting the generation of retrovirus particles. In agreement with these observations, recent studies reported that the expression or targeting of a transgenic protein encoded by an amplicon genome can differ in cells that were coinfecting with both vector and helper particles and in cells that were infected with vector particles alone (23). Furthermore, we have recently demonstrated that in coinfecting cells, HSV-1 can affect expression from a transgenic promoter carried by an adenovirus vector genome (33). Several HSV-1 functions are likely to interfere with the generation of retroviral particles in the present case. For example, the nonessential virus host shutoff (VHS) protein, which is incorporated into HSV-1 virions, can degrade polysome-associated mRNAs of infected cells (20). This may result in a decrease in the amount of packageable RNA of the *lacZ* retroviral vector, in a dose-dependent manner, as a function of the input HSV-1 particles of both amplicon and helper species. Alternatively, expression of proteins encoded by the helper virus genome is likely to impair retrovirus assembly or protein expression from the amplicon genome. For example, ICP27, a factor expressed by the D30EBA helper virus, binds to the spliceosome complex (16) and might thus interfere with the formation of spliced *env* mRNAs encoded by the amplicon vector in those cells which are coinfecting by both types of HSV-1 particles.

In the context of the present study, TE-lac2 cells were in-

fecting with GPE amplicons at low multiplicities to avoid such negative interferences. Under our experimental conditions, when 10^6 TE-lac2 cells were inoculated at 0.1 PFU of helper virus particles/cell with P5, no more than 2×10^4 cells actually became infected with amplicon particles, and some of them were probably coinfecting with helper particles (23), as predicted by the Poisson distribution. The total amount of retroviral vectors released under these conditions averaged 10^4 iu per culture, suggesting that the ratio of retroviral vectors produced to single-amplicon-infected cells is close to 1:1. Current clonal cell lines produce similar ratios ($\sim 10^6$ iu/ml is released by $\sim 10^6$ clonal cells). Thus, in the absence of coinfection with helper particles, the GPE vector particle-infected TE-lac2 cells seem as efficient as clonal cell lines in producing retrovirus vectors. Furthermore, our data predict that higher titers of retroviral particles might be obtained once viral preparations become either substantially enriched in helper-free amplicon vectors (13) or generated by HSV-1 helper viruses defective in all immediate-early functions and/or in structural toxic functions.

The system described in this work also provides a new approach to exploring the interactions between gene products of human herpesviruses and retroviruses. Furthermore, this model might be useful for studying the consequences of possible integrations of human retrovirus sequences into human herpesvirus backbones, as already observed with avian viruses (18).

From a more pragmatic point of view, a GPE amplicon vector might be used to remobilize in vivo a retroviral vector integrated in a population of cells, thereby allowing its limited spreading in neighboring cells. This may help to better infect particular cell types that are more or less refractory to retrovirus transduction in vitro, like hematopoietic stem cells. Finally, it may become an alternative strategy to generate therapeutic retroviral vectors in vivo after inoculation of patients with amplicon vectors carrying both the packaging and the vector components of a retrovirus vector bearing the therapeutic transgene.

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