JOURNAL OF VIROLOGY, May 1989, p. 2374–2378
0022-538X/89/052374-05$02.00/0

Vol. 63, No. 5

Formation of Infectious Hybrid Virions with Gibbon Ape Leukemia Virus and Human T-Cell Leukemia Virus Retroviral Envelope Glycoproteins and the *gag* and *pol* Proteins of Moloney Murine Leukemia Virus

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Received 19 October 1988/Accepted 11 January 1989

The gibbon ape leukemia virus, SEATO strain, and human T-cell leukemia virus type I envelope glycoproteins can be functionally assembled with a Moloney murine leukemia virus core into infectious particles. The envelope-host cell receptor interaction is the major determinant of the host cell specificity for these hybrid virions.

Recently it has been demonstrated that the envelope region of a murine type C amphotropic retrovirus, 4070, could be directly substituted for the highly related ecotropic Moloney murine leukemia virus (MoMuLV) envelope region in a MoMuLV packaging-deficient retroviral expression plasmid (3, 10). This plasmid, when transfected into NIH 3T3 cells with a packagable MoMuLV-based genome (8), gives rise to virions with an amphotropic envelope glycoprotein and ecotropic MoMuLV *gag* and *pol* proteins. The resultant virions can infect cells susceptible to infection by murine amphotropic retroviruses (e.g., primate, canine, feline, and chicken cells), a host range considerably more diverse than the host range of ecotropic Moloney murine retrovirions (6, 12). In addition, NB tropism is determined by the *gag* region of the murine retrovirus. 4070 is N-tropic and cannot infect BALB/c cells, whereas MoMuLV is NB-tropic and can therefore infect a wider variety of murine cells, including BALB/c. Recombinant virions containing the defective genome retain the *gag*-coding region of MoMuLV. The 4070-MoMuLV virions, therefore, have a broader host range than do either of the parental viruses; they can infect N and B types of murine cells as well as cells infectable by amphotropic virions.

(C.W. is a doctoral candidate at George Washington University, and this work represents partial fulfillment of dissertation requirements.)

The successful generation of 4070-MoMuLV recombinant virions demonstrated that it is possible to assemble envelope glycoproteins from a murine amphotropic virus with the viral core proteins of a highly related murine ecotropic virus together with a MoMuLV-based recombinant genome into composite virions. We were interested in determining whether the envelope proteins derived from nonmurine retroviruses, such as the gibbon ape leukemia virus (GaLV), SEATO strain, the human T-cell leukemia virus type I (HTLV-I), or the human immunodeficiency virus type 1 (HIV-1) could also interact with MoMuLV nucleoids or viral cores to produce infectious retroviral particles. This necessitated the construction, however, of expression vectors for functional envelope proteins of GaLV and HTLV-I. We therefore designed the appropriate retroviral envelope expression plasmids and a plasmid which could express MoMuLV *gag* and *pol* but not *env* proteins, transfected the plasmids into appropriate host cells, and determined the competence and host range of the resultant virions.

Several retroviral expression plasmids were constructed to determine whether the murine retroviral *gag* and *pol* proteins could be assembled with a heterologous primate retroviral (GaLV SEATO, HTLV-I, or HIV-1) envelope glycoprotein into a functional hybrid virion encapsidating a murine recombinant RNA genome. The plasmid designated MOV-*env* minus, derived from the plasmid pMOV-CD (Fig. 1), was designed to express the MoMuLV *gag* and *pol* gene products (Fig. 2A). The murine, HTLV-I, and GaLV envelope glycoproteins were encoded by the MOV-MOV *env* (Fig. 2B), MOV HTLV-I *env* (Fig. 2D), and MOV GaLV SEATO *env* (Fig. 2C) plasmids, respectively. The third plasmid component of the system, the defective recombinant MoMuLV genome designated F-EVX (Fig. 2E) and SPFX (Fig. 2F), contains 5′ and 3′ MoMuLV long terminal repeats (LTRs), part of the *gag* coding region, the packaging signal, 5′ and 3′ splice signals, and the mammalian antibiotic G418^r, or *neo*, gene. These two plasmids differ from each other in that the *neo* transcript in the SPFX vector initiates from an internal simian virus 40 promoter, whereas the *neo* transcript in the F-EVX plasmid initiates from the MoMuLV promoter located within the 5′ LTR. The replication-defective viral RNA genomes derived from the F-EVX or SPFX plasmids were then tested for their ability to be packaged into recombinant viral particles containing MoMuLV-derived *gag* and *pol* viral proteins and either MoMuLV, HTLV-I, or GaLV-derived envelope glycoproteins.

The MOV-*env* minus plasmid, along with either the SPFX or F-EVX defective genome and one of the envelope expression plasmids (MOV-*env*, GaLV SEATO *env*, or HTLV-I *env* plasmid), was cotransfected onto NIH 3T3 cells (Fig. 3). At 48 h later, the cell supernatant containing the presumptive viral particles was filtered and added to target cells. The values presented in Table 1 represent mean CFU/10 ml of supernatant as determined by counting the number of G418-resistant colonies generated by 1 to 10 ml of transfected cell supernatant. When the supernatant from NIH 3T3 cells transfected with only the F-EVX or SPFX plasmid and the MOV-*env* minus plasmid (in the absence of an envelope

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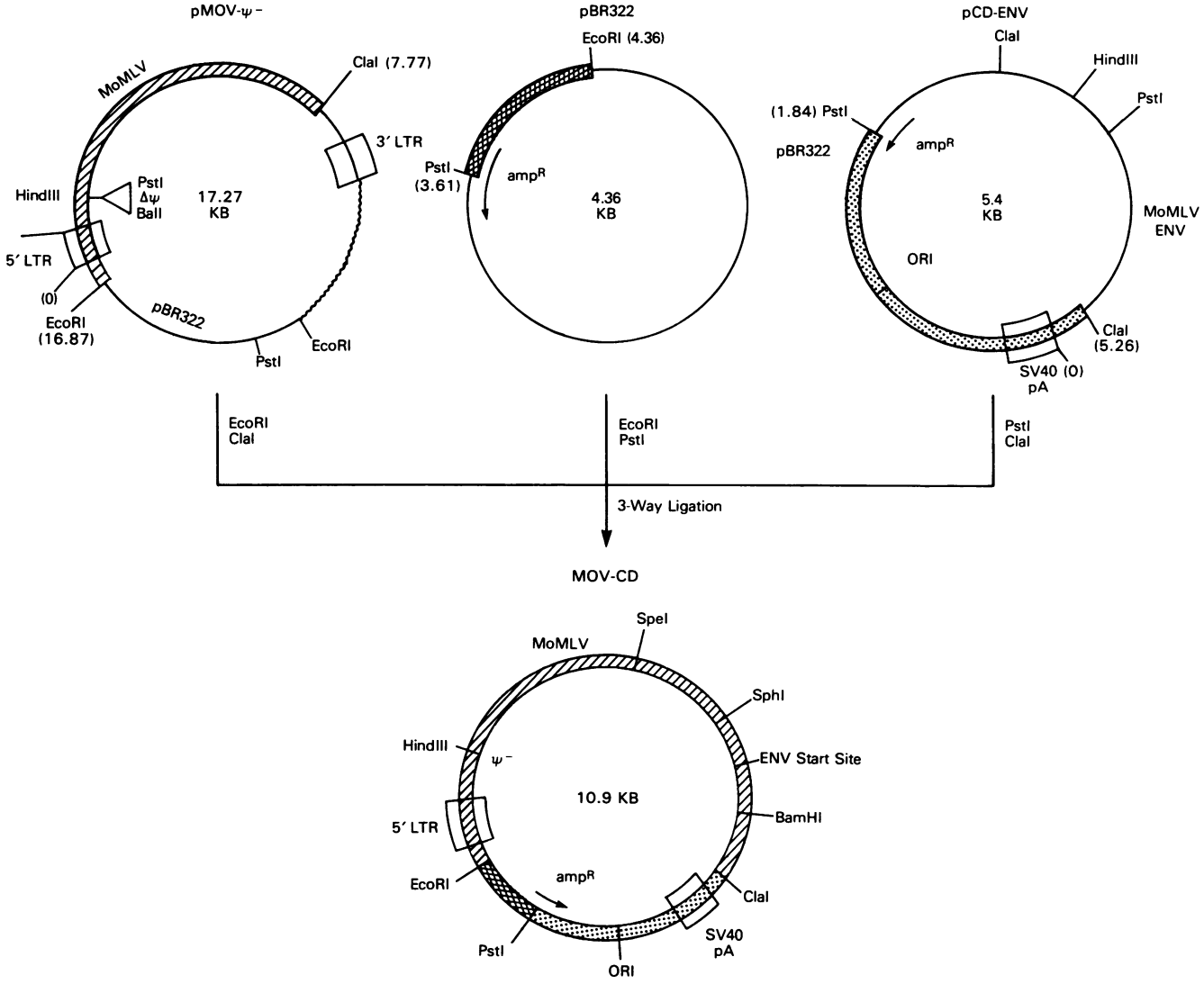

FIG. 1. Plasmid construction scheme for the derivation of the plasmid pMOV-CD. pMOV-CD was generated by a three-way ligation of (i) an 8.2-kilobase (kb) *Eco*RI-*Cla*I fragment from the pMOV-ψ⁻ plasmid, kindly provided by R. Mulligan, Whitehead Institute, Cambridge, Mass.; (ii) a 0.75-kb *Eco*RI-*Pst*I fragment from pBR322; and (iii) a 2-kb *Pst*I-*Cla*I fragment from the pCDenv plasmid. The product of the ligation of these three fragments, the plasmid pMOV-CD, contains a MoMuLV 5′ LTR and *gag*, *pol*, and *env* coding regions, but lacks the ψ site and the 3′ LTR, which is replaced with a simian virus 40 polyadenylation site. The regions of the pMOV-CD plasmid that derive from pCD-ENV ( ), pMOV-ψ⁻ ( ), and pBR322 ( ) are shown.

expression plasmid) was assayed in a similar manner for the ability to transform target cells, no G418-resistant colonies were observed with any of the cell types tested. The lack of virion formation in the absence of an envelope expression plasmid indicates that NIH 3T3 cells do not express any endogenous murine *env* gene products which can complement the MoMuLV cores and form infectious virus in this type of assay system.

As determined by the transformation of rat-2 and rat XC cells to G418 resistance, GaLV SEATO-MoMuLV hybrid particles were successfully generated at titers approximately equivalent to those obtained with the purely MoMuLV particles (Table 1). Cell types infectable by the hybrid GaLV SEATO-MoMuLV virions correlated exactly with the previously reported in vitro host range of wild-type GaLV SEATO. The wild-type GaLV retrovirus is able to infect rat, bat, mink, bovine, and human cells, but, unlike MoMuLV, is not able to infect mouse cells (7). NIH 3T3 cells are resistant to infection by GaLV SEATO enveloped virions containing either the SPFX or F-EVX genome. Since both the MoMuLV F-EVX and MoMuLV SPFX virions can infect NIH 3T3 (Table 1), the inability of the GaLV-MoMuLV retrovirions to infect NIH 3T3 cells is most probably caused by the absence of the appropriate functional virus receptors on these cells. Conversely, the successful infection of mink cells with all of the primate hybrid virions, but not the MoMuLV recombinant virions, supports and extends earlier observations that resistance to infection in these cells is due to the absence of the appropriate receptor for MoMuLV on mink cells and can be circumvented by pseudotyping the MoMuLV RNA genome with a xenotropic mouse envelope (1).

HTLV-I MoMuLV hybrid virions were generated in a

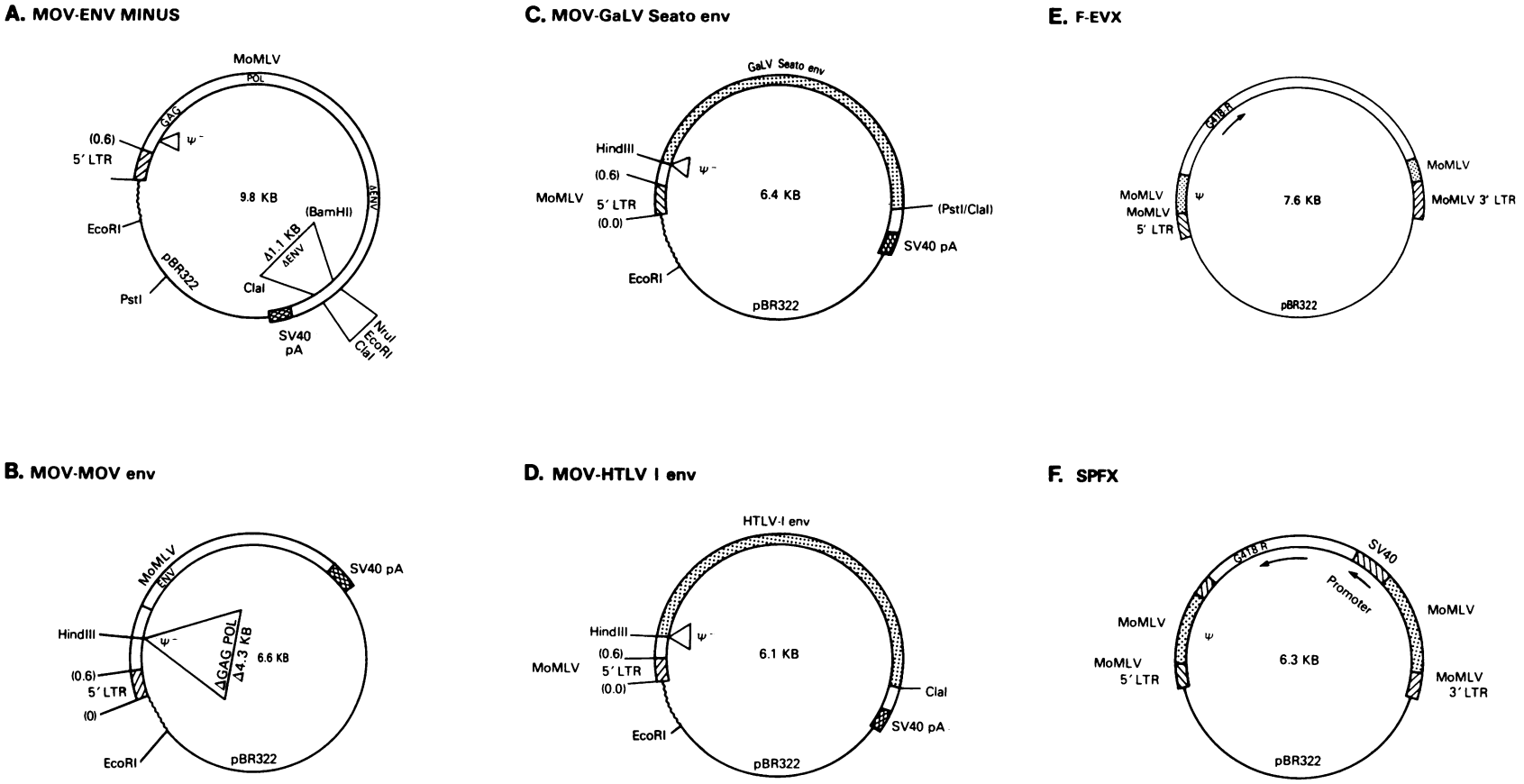

FIG. 2. Diagrammatic representation of the expression plasmids used in this study. The MOV-*env* minus plasmid (A) was derived from the pMOV-CD by a series of cloning steps. First, a 2.5-kb *Sph*I-*Cla*I fragment was excised from pMOV-CD and subcloned into pBR322. A synthetic linker, containing the restriction enzyme sites *Cla*I, *Eco*RI, *Nru*I, and *Bam*HI, was substituted for the 1.4-kb *Bam*HI-*Cla*I fragment. The truncated 1.1-kb *Sph*I-*Cla*I fragment was then isolated from the linker-modified pBR-MOV-CD subclone and inserted into a *Sph*I-*Cla*I-digested pMOV-CD plasmid to generate the MOV-*env* minus plasmid. The MOV-MOVenv plasmid (B) was constructed by deleting the *Hin*dIII fragments. The GaLV envelope in the MOV-GaLV SEATO *env* plasmid (C) was derived from a GaLV SEATO genomic clone (pGAS II [5], provided by C. D. Trainor, Northwestern University, Evanston, Ill.). The *Hin*dIII-*Pst*I (filled in) 3.0-kb envelope fragment isolated from pGAS II was ligated to the 3.4-kb *Cla*I-*Hin*dIII fragment from pMOV-CD (after filling in the *Cla*I site) to generate the MOV-GaLV SEATO *env* plasmid. The pCH-1 plasmid, a plasmid genomic subclone of the HTLV-I proviral bacteriophage clone λCH-1 (13), was the source of the HTLV-I envelope gene used to generate the MOV–HTLV-I *env* plasmid (D). The 6.7-kb *Hin*dIII-*Cla*I fragment of pMOV-CD was replaced with the 2.25-kb *Hin*dIII-*Cla*I fragment from pCH-1 to generate the MOV–HTLV-I *env* plasmid. The SPFX and F-EVX plasmids (E and F, respectively) were kindly provided by M. Feinberg, Massachusetts Institute of Technology, Cambridge.

TABLE 1. Transformation of cells to G418^r following infection with hybrid virions

Target cell origin	Amt of following virus recovered (CFU)[a]:					
	MoMuLV		GaLV SEATO		HTLV-I	
	F-EVX	SPFX	F-EVX	SPFX	F-EVX	SPFX
Rat fibroblast (rat-2)	1.1×10^4	1.3×10^4	8.1×10^3	ND[b]	0	ND
Rat epithelial (rat-XC)	7.7×10^4	5.8×10^4	3.4×10^4	ND	ND	ND
Human osteogenic sarcoma (HOS)	0	ND	1.7×10^3	80	5	50
Mink lung (CCL 65)	0	0	2.2×10^3	ND	70	100
Bat lung (CCL 88)	0	ND	5.6×10^2	ND	0	0
Bovine kidney (MDCK)	0	0	2.5×10^4	ND	ND	ND
Mouse mesenchymal (NIH 3T3)	8.5×10^3	6.4×10^3	0	0	0	0

[a] Numerical values are average CFU of recombinant virus recovered from the supernatant of a 10-cm plate of transfected NIH 3T3 cells transiently expressing hybrid virions.
[b] ND, Not done.

similar manner but appeared to form infectious particles less efficiently than MoMuLV or GaLV-MoMuLV hybrid particles did (Table 1). The HTLV-I hybrid virions were able to infect human and mink cells, but not bat, mouse, or rat cells, a target cell specificity consistent with the in vitro host range demonstrated for HTLV-I (11). The apparent titers of HTLV-I–MoMuLV virions (1 to 10 CFU/ml) were substantially lower than the titers achieved with either MoMuLV or GaLV-MoMuLV recombinant virions. This result may reflect low levels of HTLV-I *env* gene expression, or, alternatively, inefficient hybrid viral assembly, lability of the hybrid virion, and/or inefficient uptake of the hybrid virion by target cells.

An HIV-1 envelope expression plasmid (containing the HIV-1 envelope-coding region derived from the pHXB2D [4] plasmid clone, the MoMuLV 5′ LTR, and a simian virus 40 polyadenylation signal) was assayed in a similar manner to determine whether the HIV-1 envelope could complement MoMuLV viral cores (data not shown). No infectious virions were detected after the appropriate period of selection, as determined by transformation of the target cells to G418 (for H9 T cells) or mycophenolic acid (for CD4-positive HeLa cells) resistance.

We describe here a method for generating phenotypically mixed retroviral particles which contain the core proteins of MoMuLV and the envelope proteins of heterologous retroviruses. The method involves a three-plasmid system, with the MoMuLV core proteins expressed on one plasmid and the envelope proteins of another virus expressed on a second plasmid. The third plasmid contains a selectable marker gene and the packaging region of MoMuLV. Virions are generated transiently following a three-way cotransfection onto NIH 3T3 cells. GaLV-MoMuLV pseudotyped recombinant virions are generated at titers equivalent to those obtained by using the fully homologous three MoMuLV plasmids, whereas the apparent titers of HTLV-I–MoMuLV hybrid virions are 10- to 100-fold lower than those observed with MoMuLV. No infectious HIV-1–MoMuLV virions were detectable by using similar methods to both synthesize virions and assess viral titers.

The efficient generation of infectious GaLV-MoMuLV hybrid virions via complementation of MoMuLV *gag* and

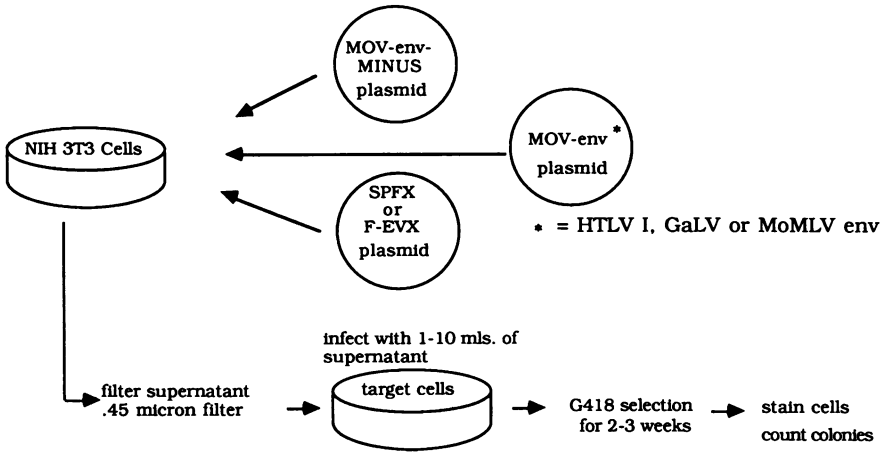

FIG. 3. Schematic representation of the protocol used to assay the infectivity and approximate titer of recombinant virions. NIH 3T3 cells were seeded at 5×10^5 cells per 10-cm plate. The next day, the MOV-*env* minus, the MoLV, GaLV SEATO, or HTLV-I envelope plasmids, and either SPFX or F-EVX plasmid DNA was precipitated and transfected onto NIH 3T3 fibroblasts as described previously (2). At 48 h later, target cells were infected with 1 to 10 ml of transfected cell supernatant in the presence of 3 μg of Polybrene per ml. At 24 h after infection, target cells were split and seeded onto four plates at a density of approximately 10^5 cells per 10-cm dish. The target cells were then assayed for infection by selection for 2 to 3 weeks in media containing various concentrations of the antibiotic G418 (250 μg/ml for HOS cells, 400 μg/ml for NIH 3T3 and bat cells, 450 μg for rat-2 cells, 600 μg/ml for rat XC cells, 650 μg/ml for mink lung cells, and 800 μg/ml for bovine kidney cells). At the end of the period of selection, cell colonies were fixed with methanol, stained with methylene blue-carbol fuchsia, and counted to determine the virus titer.

pol proteins with GaLV envelope proteins represents a novel and simple way of constructing infectious recombinant retrovirions which contain exclusively MoMuLV virion core components but which exhibit a host range specified by a heterologous envelope. The development of stable GaLV-MoMuLV packaging cell lines is presently in progress. Such helper lines will facilitate the generation of high-titer virus with a GaLV host range.

Chimeric GaLV SEATO-MoMuLV and HTLV-I–MoMuLV envelope genes should prove useful for dissecting both the cellular and the viral determinants critical for the specificity of MoMuLV, GaLV, or HTLV-I retrovirus-target cell receptor interactions. The use of chimeric envelope proteins could lead to the development of hybrid virion packaging lines capable of generating retroviruses with host ranges distinct from those of any naturally occurring retrovirus. Finally, this recombinant heterologous complementation system provides a safe means of generating recombinant retroviral particles. The elimination of several necessary viral sequences and the dissociation of components required for productive viral infection onto three different plasmids make the recombinational events required for the generation of hybrid wild-type viral particles extremely unlikely (9).

We thank N. Deacon for sharing unpublished GaLV sequence information, D. Rausch for scientific assistance and helpful discussions, K. Koller for a critical review of the manuscript, and M. J. Brownstein for his support and insightful comments.

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