JOURNAL OF VIROLOGY, May 1991, p. 2220–2224
0022-538X/91/052220-05$02.00/0

Vol. 65, No. 5

Construction and Properties of Retrovirus Packaging Cells Based on Gibbon Ape Leukemia Virus

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Received 20 November 1990/Accepted 28 January 1991

We have constructed hybrid retrovirus packaging cell lines that express the gibbon ape leukemia virus *env* and the Moloney murine leukemia virus *gag-pol* proteins. These cells were used to produce a retrovirus vector at over 10^6 CFU/ml, with a host range that included rat, hamster, bovine, cat, dog, monkey, and human cells. The *gag-pol* and *env* expression plasmids were separately transfected to reduce the potential for helper virus production, which was not observed. The NIH 3T3 mouse cells from which the packaging lines were made are not infectable by gibbon ape leukemia virus; thus, the generation and spread of possible recombinant viruses in the packaging cells is greatly reduced. These simian virus-based packaging cells extend the host range of currently available murine and avian packaging cells and should be useful for efficient gene transfer into higher mammals.

Retroviral vectors have proven useful in a variety of gene transfer applications. A key feature of their utility is the availability of retrovirus packaging cells that allow production of retroviral vectors in the absence of helper virus and thus prevent spread of the vector in infected cells. Retrovirus packaging cells based on ecotropic and amphotropic murine retroviruses, avian leukosis virus, and spleen necrosis virus have been constructed (15). However, not all cell types can be efficiently infected by using the available packaging cell lines, and cells from several species of experimental importance, notably bovine and hamster cells, are not easily infected. The range of cells that are infectable is determined primarily by the envelope protein of the virus and the presence of appropriate receptors for this protein on the surface of infected cells. For example, viruses that infect human cells can be separated into eight groups based on the use of different receptors for cell entry (28).

Gibbon ape leukemia virus (GaLV) uses an internalization receptor that is different from those of the available packaging cell lines. The same receptor is used by simian sarcoma-associated virus (28), which is apparently a substrain of GaLV (5), and feline leukemia virus type B (28). The human receptor for GaLV has recently been cloned and shows a wide cell type and species distribution (22). Indeed, GaLV can infect many mammalian species (13), with the notable exception of mouse cells. We have constructed retrovirus packaging cells expressing the Moloney murine leukemia virus (MoMLV) *gag-pol* proteins and the GaLV *env* protein that can produce high-titer retroviral vectors with an extended host range that includes hamster and bovine cells.

MATERIALS AND METHODS

Cell culture. The cell lines used included NIH 3T3 TK^- mouse fibroblasts (29), *Mus dunii* tail fibroblasts (4), PA317 amphotropic retrovirus packaging cells (17; ATCC CRL 9078), NRK normal rat kidney cells (6), 208F $HPRT^-$ Fischer rat fibroblasts (25), CHO Chinese hamster ovary cells (11), CCC-81 cat cells transformed with Moloney murine sarcoma virus (7), Cf2Th canine thymus cells (21), CTAC canine carcinoma cells (12), Vero African green monkey kidney cells (ATCC CCL 81), HeLa human cervical carcinoma cells (9), LNSV simian virus 40 (SV40)-transformed $HPRT^-$ Lesch-Nyhan skin fibroblasts (2), MDBK bovine kidney cells (ATCC CCL 22), and primary bovine aortic endothelial cells (BAEC; Cell Systems Corp., Kirkland, Wash.). Cells were grown in Dulbecco modified Eagle medium with high glucose (4.5 g/liter) supplemented with 10% fetal bovine serum (Hyclone, Logan, Utah) except for the CHO cells, which were grown in Ham's F12 medium with 5% fetal bovine serum, and the BAEC, which were grown in CS complete endothelial cell medium (15% fetal bovine serum/RPMI formulation; Cell Systems Corp.) with 50 μg of heparin-binding growth factor I/heparin (HBGF-I/H; Cell Systems Corp.) per ml. Cells were free of mycoplasma as determined by fluorescence microscopy after fixation and staining of the cells with the DNA stain Hoechst 33258 (26). The PG13 and PG13/LN c8 cell lines generated in this study are available from the American Type Culture Collection (ATCC CRL 10,686 and 10,685, respectively).

Virus production and assay. Virus was harvested from confluent dishes of cells by replacing the cell culture medium with fresh medium (4 ml per 6-cm dish or 10 ml per 10-cm dish) and harvesting the conditioned medium 12 to 24 h later. The medium was centrifuged at 3,000 × *g* for 5 min to pellet cells and debris, and the virus was used immediately or stored at −70°C. For virus assay, target cells were seeded at 5×10^5 per 60-mm dish on day 1. On day 2, the medium was changed to medium containing Polybrene (4 μg/ml) and various amounts of test virus were added. The heparin present in the culture medium used for BAEC inhibited virus infection, so for infection of BAEC the cells were washed two times with medium without HBGF-I/H, and the cells were infected in the presence of recombinant basic fibroblast growth factor (Imcera Bioproducts Inc., Terre Haute, Ind.) and 4 μg of Polybrene per ml. Heparin is required for activity of HBGF-I, but is not required for recombinant basic fibroblast growth factor activity. On day 3, the cells were

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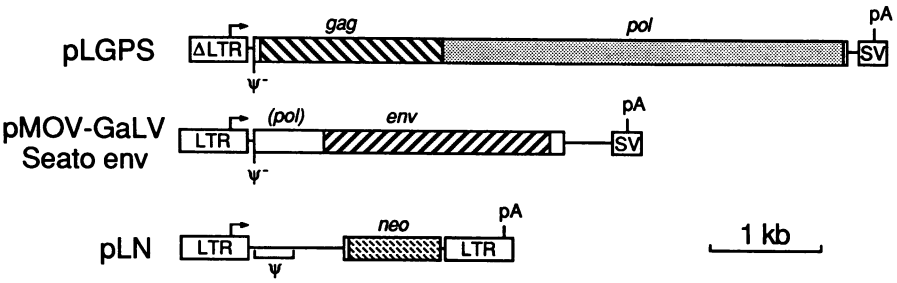

FIG. 1. Expression constructs for MoMLV *gag-pol* and GaLV *env* proteins. The construct used for expression of MoMLV *gag* and *pol* proteins, pLGPS, consists of a 5'-truncated MoMLV long terminal repeat (LTR) promoter (MoMLV bases −351 [*Sau*3AI] to 214 [*Bal*I]) without the adjacent retroviral packaging signal, the MoMLV *gag-pol* coding region (MoMLV bases 563 [*Pst*I] to 5873 [*Sca*I]), MoMLV sequences from 7676 (*Cla*I) to 7774 (end of the *env* gene), and the SV40 early polyadenylation signal (SV40 bases 2770 [*Bcl*I] to 2533 [*Bam*HI]). These sequences were all cloned into the *Bam*HI site of a modified version of the poison-sequence-minus pBR322 derivative pML (14) called pMLCN. pMLCN was made from pML by destroying the single *Cla*I site in the plasmid and removing the two *Nae*I fragments from the *tet* gene in the plasmid. The construct used for expression of the GaLV *env* protein, called pMOV-GaLV Seato env, has been previously described (30). It consists of an MoMLV LTR promoter without the adjacent retroviral packaging signal, the GaLV *env* coding region isolated as a 2.8-kb *Hin*dIII-*Pst*I fragment from the GaLV Seato genomic clone pGAS II (8), and the SV40 late polyadenylation signal, all cloned into a pBR322 plasmid backbone. The retroviral vector pLN has been described (19). Arrows indicate promoters, pA indicates a polyadenylation signal, shaded areas indicate coding regions, ΔLTR indicates the 5'-deleted MoMLV LTR, ψ^- indicates the position of the packaging signal deletion, *neo* indicates the neomycin phosphotransferase gene, and (*pol*) indicates a residual portion of the *pol* gene of GaLV.

trypsinized and seeded at 1:10 to 1:500 dilutions into 6-cm dishes in medium containing G418 at the following concentrations (active): 100 μg/ml for BAEC; 500 μg/ml for NRK and CCC-81; 750 μg/ml for NIH 3T3 TK$^-$, 208F, CHO, Vero, CTAC, Cf2Th, MDBK, and *M. dunii*; and 1,000 μg/ml for HeLa. Colonies were stained and counted on days 8 to 10. Virus titer (CFU per milliliter) was calculated by dividing the number of colonies by the volume (milliliters) of virus used for infection and multiplying by the dilution factor used after trypsinization of the infected cells prior to selection. For more rapid screening of many samples, the assay was simplified to plating 10^5 cells per 6-cm dish, infecting the next day, and adding G418 directly to the cells 1 day after infection. This simplified assay indicates a titer about fivefold lower than that indicated by the standard assay but can still be used to determine relative titer. Helper virus was measured by using the S$^+$L$^-$ assay as previously described (18).

RESULTS

Construction of a cell line expressing MoMLV *gag* and *pol* proteins. The MoMLV *gag-pol* expression construct depicted in Fig. 1 was introduced into NIH 3T3 TK$^-$ cells by cotransfection using the herpes simplex virus thymidine kinase gene as a selectable marker, as previously described (17). The ratio of selectable marker plasmid to *gag-pol* expression plasmid was 1:20 or 1:100. Seventeen TK$^+$ clones were isolated in HAT medium (30 μM hypoxathine, 1 μM amethopterin, 20 μM thymidine) and analyzed for production of reverse transcriptase as a measure of *gag-pol* protein production. Although these cells do not make *env* proteins, cells that make *gag* and *pol* proteins can still produce defective virions containing reverse transcriptase. Reverse transcriptase production from six of the best clones was compared with production in the parental cells (NIH 3T3 TK$^-$), the amphotropic packaging cell line PA317 (17), and PA317 cells that produce the LNL6 retroviral vector (PA317/LNL6 c8 [3]; Table 1). Clone 91-22 produced at least four times more reverse transcriptase than any of the other clones or the amphotropic packaging cells, and this clone was used in further experiments.

Addition of the GaLV *env* expression plasmid. The GaLV *env* expression plasmid was introduced into cells expressing MoMLV *gag-pol* by cotransfection (17) with either a mutant methotrexate-resistant dihydrofolate reductase gene (*dhfr**) contained in plasmid pFR400 (27) or a hygromycin phosphotransferase (*hpt*) gene contained in plasmid pSV2Δ13-hyg (gift from Paul Berg). The ratio of selectable marker plasmid to *env* expression plasmid was 1:20 or 1:100. Cell colonies containing the genes were selected in medium containing 100 nM methotrexate and dialyzed fetal bovine serum or 0.4 mg of hygromycin per ml, respectively, and were isolated by using cloning rings. The reason that we generated cell lines with either marker was so that a GaLV packaging cell line would be available for use with any dominant marker, i.e., would not be restricted by the presence of the same marker already in the cells. Clone designations all begin with PG (packaging cells having a GaLV pseudotype), followed by a number from 1 to 49 for clones transfected with *dhfr** or from 50 to 99 for clones transfected with *hpt*.

About 20 clones made with each marker were tested for packaging function by measuring transient virus production 2 days after transfection with the retrovirus vector plasmid pLN (Fig. 1) as described previously (19). Virus was assayed by using HeLa cells as targets for infection. The LN vector

TABLE 1. Reverse transcriptase production by cells transfected with the MoMLV *gag-pol* expression construct[a]

gag-pol-transfected clone(s)	Reverse transcriptase (cpm)	Cell line	Reverse transcriptase (cpm)
2-2	1,730	NIH 3T3 TK$^-$	19
2-4	910	PA317	2,240
2-7	2,510	PA317/LNL6 c8	2,800
91-22	10,900		
91-23	1,870		
92-24	1,150		
Pooled clones	290		

[a] Medium was harvested from confluent cultures of the indicated cells and assayed for reverse transcriptase as described previously (10), with the modification that the results were quantitated by scintillation counting.

TABLE 2. Titer of vector produced by clonal PG13 and PG53 cell lines infected with the LN vector[a]

PG13 clone	Vector titer (CFU/ml)	PG53 clone	Vector titer (CFU/ml)
2	1×10^5	1	1×10^4
3	2×10^5	2	1×10^4
4	2×10^5	3	5×10^4
5	1×10^5	4	3×10^4
7	4×10^5	7	1×10^5
8	6×10^5	9	3×10^4
9	2×10^5	10	1×10^5
10	1×10^5		
11	1×10^5		
12	1×10^5		

[a] Virus was harvested from confluent dishes of PG13/LN and PG53/LN clones and was used to infect HeLa cells plated the day before at 10^5 per 6-cm dish. Fresh medium containing G418 was added to the HeLa cells on the day after infection, and resultant colonies were stained and counted 7 days after infection.

expresses neomycin phosphotransferase (*neo*), and selection was performed in 1 mg of G418 per ml (active). We also transfected PA317 amphotropic packaging cells in each experiment as a control. Packaging function in the clones varied from undetectable to 10 times better than that of the PA317 cells in the same experiment (data not shown). Of the clones cotransfected with the *dhfr** gene, clone PG13 produced the highest-titer virus; of the clones cotransfected with the *hpt* gene, clone PG53 produced the highest-titer virus. All of the clones were helper virus free (<1 focus-forming units per ml) as measured by the S^+L^- assay.

Vector production by GaLV packaging cells. LN virus produced by PA317 amphotropic packaging cells was used to infect PG13 and PG53 cells, and $G418^r$ clones were isolated. Ten PG13 and seven PG53 clones were assayed for production of the vector by using HeLa cells as recipients (Table 2). All clones tested produced virus, and the range of virus titers for each cell line was fairly narrow, indicating that most cells in each line have equivalent packaging function. The best clone was PG13/LN c8, which produced the LN vector at a titer of 6×10^5 CFU/ml. The PG53 clones produced virus at 5- to 10-fold-lower titer than the PG13 cells, the best producing the LN vector at a titer of 10^5 CFU/ml. All of the clones were helper virus free (<1 focus-forming units per ml) as measured by the S^+L^- assay.

Host range of GaLV packaging cells. Virus produced by PG13/LN c8 cells was assayed for its ability to infect cells from a range of mammalian species (Table 3). For comparison, amphotropic virus from PA317/LN c11 cells was also assayed. GaLV pseudotype virus was unable to infect mouse cells, as expected on the basis of previous work (30). Occasional colonies were observed on NIH 3T3 cells, but the rate of infection was 10^6-fold lower than when we used amphotropic virus, which infects mouse cells efficiently. GaLV pseudotype virus was able to infect all of the other cell types assayed, including rat, hamster, bovine, cat, dog, monkey, and human cells. Of interest is the ability of GaLV pseudotype virus to infect bovine and hamster cells, since in general the murine retroviruses infect these cells poorly if at all. An exception was the BAEC bovine cell strain, which was infected equally well with amphotropic or GaLV pseudotype virus. On average, the titers of GaLV and amphotropic viruses were similar, indicating comparable efficiencies of virus production by PG13 and PA317 packaging cells.

GaLV is similar in host range to murine xenotropic viruses, which in general do not infect mouse cells but infect cells from many other mammalian species. However, the mouse xenotropic virus NZB is capable of infecting cells from mice of the species *M. dunii* (Table 4), while GaLV pseudotype vectors cannot (Table 3). The hybrid amphotropic virus AM-MLV (18), which is composed of the MoMLV *gag-pol* and 4070A amphotropic virus *env* genes, can infect *M. dunii* cells (Table 4), showing that the inability of the GaLV pseudotype virus to infect these cells is due to the GaLV *env* protein and not the MoMLV *gag-pol* proteins present in the GaLV hybrid virions. In addition, NZB xenotropic virus is unable to efficiently infect CHO hamster cells (Table 4). These results show that the host range of GaLV can be distinguished from that of the NZB xenotropic virus.

TABLE 3. Host range of LN vector produced by using GaLV packaging cells in comparison with amphotropic packaging cells[a]

Target cells	Vector titer (CFU/ml)	
	PG13/LN c8[b]	PA317/LN c11[b]
Mouse (NIH 3T3 TK^-)	10	2×10^7
Mouse (*M. dunii*)	<5	4×10^6
Rat (NRK)	4×10^5	3×10^6
Rat (208F)	2×10^5	2×10^6
Hamster (CHO)[c]	$500–10^5$	<5–200
Bovine (MDBK)	5×10^4	<20
Bovine (BAEC)	1×10^5	1×10^5
Cat (CCC-81)	1×10^6	2×10^6
Dog (CTAC)	2×10^6	1×10^6
Dog (Cf2Th)	3×10^6	2×10^6
Monkey (Vero)	1×10^5	2×10^5
Human (HeLa)	2×10^6	7×10^5

[a] Target cells were plated the day before infection at 5×10^5 per 6-cm dish and were split 1:10 to 1:500 into G418 the day after infection, and colonies were counted after 5 to 7 days in selective medium as described in Materials and Methods.

[b] Cell lines used to produce LN virus.

[c] We found a large variation in the infectability of CHO cells from different sources. Those that were more infectable with virus from PG13 cells were also more infectable with virus from PA317 cells.

DISCUSSION

We have been able to make packaging cell lines based on GaLV that can produce retroviral vectors at high titer, $>10^6$ CFU/ml. The virions produced are hybrids between the *gag-pol* proteins of MoMLV and the *env* protein of GaLV. These results support earlier work showing that virions containing MoMLV *gag-pol* could be made as efficiently by

TABLE 4. Ability of mouse NZB xenotropic virus to infect *M. dunii* cells[a]

Target cells	Vector titer (CFU/ml)	
	Amphotropic	Xenotropic
NIH 3T3 TK^- (mouse)	5×10^7	<50
M. dunii (mouse)	4×10^7	2×10^7
208F (rat)	1×10^7	1×10^7
LNSV (human)	1×10^6	1×10^6
CHO (hamster)	ND	<5

[a] Virus was produced by using *M. dunii* cells containing the neovirus N2 (1) and either amphotropic (AM-MLV) (18) or xenotropic (NZB) (23) helper virus. Target cells were plated at 5×10^5, infected the next day, and trypsinized and seeded into G418 the day after infection. Colonies were counted after 5 days of selection. ND, Not done.

using the *env* gene from GaLV or from MoMLV in a transient transfection assay (30). Results presented here and previously (30) show that these hybrid GaLV pseudotype virions are able to infect rat, hamster, mink, bat, bovine, cat, dog, monkey, and human cells. Previous experiments using wild-type GaLV suggest that the hybrid virions should also infect rabbit cells (13). The host range of the GaLV packaging cells extends the range of cells infectable by helper-free retroviral vectors to hamster and bovine cells. In particular, CHO cells have been extensively used for genetic analyses, and the GaLV packaging line now allows infection of these cells. The generation of transgenic cows by microinjection of DNA into zygotes has not been reported, presumably because of technical difficulties (24), but the development of GaLV packaging cells may allow infection of bovine embryos as an alternative gene transfer technique.

Ecotropic and amphotropic packaging cells express receptors for the virus produced by these cells and can be infected by virus of the same pseudotype, albeit at lower frequency (20). Thus, a retroviral vector can spread at a slow rate in ecotropic and amphotropic packaging cells. If the vector is prone to rearrangement, the packaging cell line can harbor rearranged viruses as well as the original vector, even if the cells are grown from a single infected clone. In contrast, the GaLV-based packaging cells that we have constructed were made in mouse cells that do not express the receptor for GaLV. Thus, vector spread in the packaging cells should be very low, and the potential for generation of recombinant vectors, which often are transmitted with higher efficiency than the parental vector (16), is much reduced.

We have not detected the production of helper virus in any of the GaLV packaging cell lines or derivatives containing retroviral vectors by using the S^+L^- assay, which measures rescue of a transforming virus from CCC-81 cat cells by its ability to induce morphologically transformed foci of NRK cells. We have shown that both CCC-81 and NRK cells are infectable by GaLV pseudotype virus (Table 3); thus, the S^+L^- assay should be capable of detecting potential helper virus production. Generation of helper virus would require three recombination events between the vector and the two independently transfected expression plasmids for the viral proteins, which we expect to be a very rare event. In addition, while there is a region of overlap between the MoMLV *gag-pol* and GaLV *env* expression plasmids in the *pol* region (Fig. 1), these sequences are only 59% identical and thus are probably not good substrates for homologous recombination. As discussed in the previous paragraph, virus spread in the GaLV packaging cells is not expected because of the absence of the GaLV receptor in these cells; thus, the potential for generating recombinant helper viruses is also reduced.

One of the reasons for developing packaging cells based on GaLV was the possibility that virus from such lines would be better able to infect cells from higher mammals than the currently available packaging lines based on amphotropic murine virus, which would be important for developing gene therapy techniques for humans. For example, while gene transfer into hematopoietic stem cells of the mouse has been convincingly demonstrated by using packaging cells based on murine viruses, the same is not true of higher mammals, including monkeys and dogs. While we do observe slightly higher infection efficiencies by using GaLV pseudotype virus in dog and human cells (Table 3), more experiments are needed to determine whether GaLV pseudotype virus will allow better infection of cells from higher mammals that are difficult to infect, such as hematopoietic stem cells.

ACKNOWLEDGMENTS

We thank John Medina and Steven Schwartz for assistance with the culture of BAEC, Carol Jones for providing CHO-K1 cells, and J. Kimpton for technical assistance in constructing the pLGPS-containing cell lines.

This work was supported by grants AI07995 (J.V.G.) and HL36444 (A.D.M.) from the National Institutes of Health and by a postdoctoral fellowship (C.M.L.) from Genetic Therapy, Inc., Gaithersburg, Md.

REFERENCES

1. **Armentano, D., S. F. Yu, P. W. Kantoff, T. von Ruden, W. F. Anderson, and E. Gilboa.** 1987. Effect of internal viral sequences on the utility of retroviral vectors. J. Virol. **61**:1647–1650.
2. **Bakay, B., W. L. Nyhan, C. M. Croce, and H. Koprowski.** 1975. Reversion in expression of hypoxanthine-guanine phosphotransferase following cell hybridization. J. Cell Sci. **17**:567–578.
3. **Bender, M. A., T. D. Palmer, R. E. Gelinas, and A. D. Miller.** 1987. Evidence that the packaging signal of Moloney murine leukemia virus extends into the *gag* region. J. Virol. **61**:1639–1646.
4. **Chattopadhyay, S. K., M. R. Lander, S. Gupta, E. Rands, and D. R. Lowy.** 1981. Origin of mink cytopathic focus-forming (MCF) viruses: comparison with ecotropic and xenotropic murine leukemia virus genomes. Virology **113**:465–483.
5. **Delassus, S., P. Sonigo, and S. Wain-Hobson.** 1989. Genetic organization of gibbon ape leukemia virus. Virology **173**:205–213.
6. **Duc-Ngugen, H., E. N. Rosenblum, and R. F. Zeigel.** 1966. Persistent infection of a rat kidney cell line with Rauscher murine leukemia virus. J. Bacteriol. **92**:1133–1140.
7. **Fischinger, P. J., S. Nomura, and D. P. Bolognesi.** 1975. A novel murine oncornavirus with dual eco- and xenotropic properties. Proc. Natl. Acad. Sci. USA **72**:5150–5155.
8. **Gelmann, E. P., C. D. Trainor, F. Wong-Staal, and M. S. Reitz.** 1982. Molecular cloning of circular unintegrated DNA of two types of the SEATO strain of gibbon ape leukemia virus. J. Virol. **44**:269–275.
9. **Gey, G. O., W. D. Coffman, and M. T. Kubicek.** 1952. Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. Cancer Res. **12**:264.
10. **Goff, S. P., P. Traktman, and D. Baltimore.** 1981. Isolation and properties of Moloney murine leukemia virus mutants: use of a rapid assay for release of virion reverse transcriptase. J. Virol. **38**:239–248.
11. **Kao, F. T., and T. T. Puck.** 1968. Genetics of somatic mammalian cells. VII. Induction and isolation of nutritional mutants in Chinese hamster cells. Proc. Natl. Acad. Sci. USA **60**:1275–1281.
12. **Kasza, L.** 1964. Establishment and characterization of canine thyroid adenocarcinoma and canine melanoma cell lines. Am. J. Vet. Res. **25**:1178–1185.
13. **Lieber, M. M., C. J. Sherr, G. J. Todaro, R. E. Benveniste, R. Callahan, and H. G. Coon.** 1975. Isolation from the Asian mouse *Mus caroli* of an endogenous type C virus related to infectious primate type C viruses. Proc. Natl. Acad. Sci. USA **72**:2315–2319.
14. **Lusky, M., and M. Botchan.** 1981. Inhibition of SV40 replication in simian cells by specific pBR322 sequences. Nature (London) **293**:79–81.
15. **Miller, A. D.** 1990. Retrovirus packaging cells. Hum. Gene Ther. **1**:5–14.
16. **Miller, A. D., M. A. Bender, E. A. S. Harris, M. Kaleko, and R. E. Gelinas.** 1988. Design of retroviral vectors for transfer and expression of the human β-globin gene. J. Virol. **62**:4337–4345.
17. **Miller, A. D., and C. Buttimore.** 1986. Redesign of retrovirus packaging cell lines to avoid recombination leading to helper virus production. Mol. Cell. Biol. **6**:2895–2902.
18. **Miller, A. D., M. F. Law, and I. M. Verma.** 1985. Generation of helper-free amphotropic retroviruses that transduce a dominant-acting methotrexate-resistant dihydrofolate reductase gene. Mol. Cell. Biol. **5**:431–437.
19. **Miller, A. D., and G. J. Rosman.** 1989. Improved retroviral

vectors for gene transfer and expression. BioTechniques **7**:980–990.

20. **Miller, A. D., D. R. Trauber, and C. Buttimore.** 1986. Factors involved in the production of helper virus-free retrovirus vectors. Somatic Cell Mol. Genet. **12**:175–183.
21. **Nelson-Rees, W. A., R. B. Owens, P. Arnstein, and A. J. Kniazeff.** 1976. Source, alterations, characteristics and use of a new dog cell line (Cf2Th). In Vitro **12**:665–669.
22. **O'Hara, B., S. V. Johann, H. P. Klinger, D. G. Blair, H. Rubinson, K. J. Dunn, P. Sass, S. M. Vitek, and T. Robins.** 1990. Characterization of a human gene conferring sensitivity to infection by gibbon ape leukemia virus. Cell Growth Differ. **1**:119–127.
23. **O'Neill, R. R., C. E. Buckler, T. S. Theodore, M. A. Martin, and R. Repaske.** 1985. Envelope and long terminal repeat sequences of a cloned infectious NZB xenotropic murine leukemia virus. J. Virol. **53**:100–106.
24. **Pursel, V. G., C. A. Pinkert, K. F. Miller, D. J. Bolt, R. G. Campbell, R. D. Palmiter, R. L. Brinster, and R. E. Hammer.** 1989. Genetic engineering of livestock. Science **244**:1281–1288.
25. **Quade, K.** 1979. Transformation of mammalian cells by avian myelocytomatosis virus and avian erythroblastosis virus. Virology **98**:461–465.
26. **Russell, W. C., C. Newman, and D. H. Williamson.** 1975. A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses. Nature (London) **253**:461–462.
27. **Simonsen, C. C., and A. D. Levinson.** 1983. Isolation and expression of an altered mouse dihydrofolate reductase cDNA. Proc. Natl. Acad. Sci. USA **80**:2495–2499.
28. **Sommerfelt, M. A., and R. A. Weiss.** 1990. Receptor interference groups of 20 retroviruses plating on human cells. Virology **176**:58–69.
29. **Wei, C. M., M. Gibson, P. G. Spear, and E. M. Scolnick.** 1981. Construction and isolation of a transmissible retrovirus containing the *src* gene of Harvey murine sarcoma virus and the thymidine kinase gene of herpes simplex virus type 1. J. Virol. **39**:935–944.
30. **Wilson, C., M. S. Reitz, H. Okayama, and M. V. Eiden.** 1989. 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. J. Virol. **63**:2374–2378.