No false start for novel pseudotyped vectors

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Pseudotyped vectors can be used to introduce genes into cells or to study the entry process of the virus from which the outer shell of the recombinant virus is derived. Recently, several novel pseudotyped retroviruses and lentiviruses have been constructed. Virus vectors pseudotyped with an alphavirus glycoprotein hold special promise. The increasing diversity of the available pseudotyped vectors offers expanded opportunities for gene transfer to specific cells.

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Abbreviations

AAV	adeno-associated virus
ASLV-A	avian sarcoma and leukosis virus subgroup A
FIV	feline immunodeficiency virus
HBV	hepatitis B virus
HIV-1	human immunodeficiency virus type 1
HSV-1	herpes simplex virus type 1
LCMV	lymphocytic choriomeningitis virus
Mo-MuLV	Moloney murine leukemia virus
RRV	Ross River virus
VSV-G	vesicular stomatitis virus G protein

Introduction

Pseudotyping, in its original sense, means that one or more of the structural proteins of a virus particle are not encoded by the nucleic acid carried by the virus. Using this broad definition, pseudotyped viruses include any recombinant viral gene transduction system that is dependent for genome packaging upon helper proteins expressed from defective genomes in the viral producer cells or upon infection by a helper virus. The common current usage in the gene-transfer field dictates that a pseudotyped virus is one in which the outer shell (the envelope glycoproteins of an enveloped virus or the capsid proteins of a nonenveloped virus) originates from a virus that differs from the source of the genome and the genome replication apparatus. In the case of enveloped viruses, the mechanism of genome packaging normally demands that the core (i.e. matrix, capsid, and/or nucleocapsid) proteins come from the same virus as the genome (Figure 1).

This review focuses mainly on pseudotyped viral vectors in which the genome and outer shell come from considerably different viruses (not pseudotypes between different murine leukemia viruses or between the various adeno-associated virus (AAV) serotypes, for example), although some description of recent results obtained with the latter type of pseudotyped viruses is presented. The potential role of pseudotype formation in the evolution of viruses is not explored here; an article on this issue with regard to insect viruses has recently been published [1•].

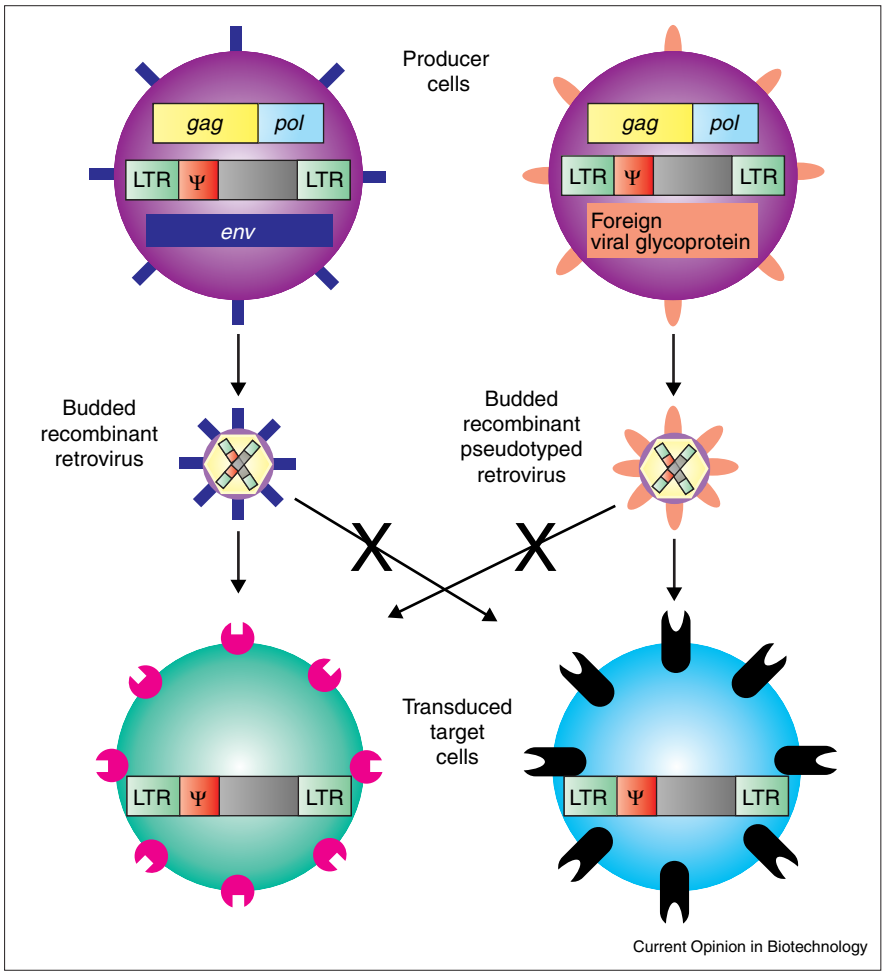
Why pseudotype?

Pseudotyped vectors have several experimental and clinical applications (Box 1). Firstly, the outer shell of a virus, through its interaction with cellular receptor molecules, plays a major role in determining the tropism of a virus, specifically at the entry step. Pseudotyping a viral vector can thereby provide it with an expanded ensemble of target cells or can restrict it to specific cells that are the object of experimental or therapeutic interest. Secondly, a pseudotyped vector can have an altered stability and/or interaction with the host immune system that increases its efficacy. One example is pseudotyped viral vectors that can be produced and/or concentrated to higher transduction titers than the viral vector with its native outer shell. Thirdly, comparison of transduction by a pseudotyped vector and by a vector bearing a native shell can be used to identify processes that are peculiar to entry mediated by the native proteins. Finally, the entry process of the virus from which the outer shell of the pseudotyped virus is derived can be illuminated.

The construction of novel recombinant pseudotyped retroviral vectors containing the glycoproteins of the Ross River virus (RRV; an alphavirus) illustrates several of these applications [2••]. Transduction by the RRV-glycoprotein-pseudotyped retrovirus was comparable to transduction by the commonly utilized vesicular stomatitis virus G protein (VSV-G) pseudotyped viruses. The retrovirus pseudotyped with RRV glycoprotein can transduce human and nonhuman cells from a number of tissues and can be efficiently concentrated. Stable cells producing RRV-pseudotyped retrovirus can be produced, and no pseudotransduction (transient transfer of the protein product of the gene that is carried by the vector in the absence of stable vector integration) is observed. The RRV-pseudotyped retroviruses thus possess several advantages over the widely used VSV-G-pseudotyped viruses (Box 2). Transduction by the RRV-glycoprotein-pseudotyped virus could be specifically inhibited by antibodies that recognized the RRV E2 glycoprotein. Entry of the pseudotyped virus was also demonstrated to occur through acidified endosomes confirming most of the earlier data on alphaviral entry and helping to resolve a longstanding controversy.

Novel recombinant pseudotyped retroviral and lentiviral vectors bearing the glycoprotein of lymphocytic choriomeningitis virus (LCMV; an arenavirus) have some similar advantages to those of the RRV-glycoprotein-pseudotyped viruses. Pseudotyping by LCMV glycoproteins was first demonstrated through the recovery of transducing virus after LCMV infection of a Moloney murine leukemia virus (Mo-MuLV) packaging cell line [3]. The original attempts to produce pseudotypes containing LCMV glycoprotein from transfected glycoprotein cDNAs were unsuccessful,

Figure 1



Schematic representation of the production of pseudotyped retroviruses and transduction by them. Producer cells contain three DNA segments. One contains the *gag* and *pol* genes that encode the retroviral core structural proteins and replication enzymes, respectively. The second contains the viral long terminal repeats (LTRs), which possess the signals for transcription and integration of the viral genome, the Ψ RNA packaging signal, which promotes the specific encapsidation of the transcribed RNA into recombinant particles, and the gene that is transduced (gray shading). Not shown, but included in practice, are the small regions required for reverse transcription. In the cell depicted on the left, the third DNA encodes the retroviral envelope (Env) protein. In the cell on the right, a membrane-fusion-promoting envelope glycoprotein from a foreign virus is encoded. Recombinant virus is produced that carries the recombinant genome (green, red and gray bar) surrounded by the Gag core (yellow) and bearing either retroviral Env proteins (left, blue) or the foreign viral glycoproteins (right, orange). The recombinant retrovirus with the Env proteins on its surface can transduce the green cell with the magenta receptors, but not the blue cell. The pseudotyped retrovirus has an altered tropism and can transduce the blue cell with the black receptors but not the green cell.

presumably due to the lack of cell-surface expression of the LCMV glycoprotein. Cloning of a new LCMV glycoprotein cDNA from the virus yielded a construct that led to cell-surface expression of the glycoprotein [4] and pseudotype formation [5••]. A general lesson is that failure to generate a pseudotype with a particular expression construct for a glycoprotein from a given virus does not mean that pseudotype formation with glycoprotein from that virus is impossible. There might be a defect in the expression construct. In addition, glycoproteins from variant strains of a virus could have different capacities to form successful pseudotypes. One additional point concerns the confirmation of the widely reported stability advantage during concentration of some pseudotypes producing nonretroviral glycoproteins over those producing murine leukemia virus (MuLV) envelope proteins. The lability of the MuLV envelope proteins is the consequence of a thiol-disulfide exchange reaction that leads to the dissociation of its surface and transmembrane subunits [6,7]. It is possible that concentration of virus producing MuLV envelope proteins in medium at mildly acidic pH could prevent MuLV glycoprotein dissociation and the consequent reduction in transduction titers [7,8].

An important issue is that the subcellular distribution of a given glycoprotein might not be optimal for pseudotype formation. Glycoprotein expression at a particular membrane (e.g. plasma membrane, Golgi apparatus, endoplasmic reticulum) or site within a membrane (e.g. lipid rafts) might be critical for efficient pseudotype formation [9]. In the case of the RRV and LCMV glycoproteins, cell-surface expression was demonstrated (through acidic pH-dependent syncytia formation and flow cytometry, respectively). Conversely hepatitis B virus (HBV) assembles at post-endoplasmic reticulum/pre-Golgi membranes; however, cell-surface expression of the HBV large antigen can be obtained. Retroviral pseudotypes bearing the HBV surface antigens, which transduced solely human primary hepatocytes, could be produced, although with fairly low transduction titers [10]. It was demonstrated that the HBV large surface antigen was sufficient for the production of transducing viruses, but that the small surface antigen increased large surface antigen expression at the cell membrane and transduction titers.

A recent study of MuLV pseudotyped with envelope proteins of visna virus (a lentivirus) confirms the previously

Box 1

Advantages of pseudotyped viruses for the study of viral entry.

1. Study of the pseudotyped vector can have safety advantages.
2. The pseudotyped virus can be experimentally manipulated with greater ease. Mutations in genes in the outer shell can be introduced and Tested more readily in the pseudotyped virus. The entry process of the pseudotyped virus might be more easily quantified.
3. Identification of cellular receptors or attachment factors for the virus is facilitated. For the purpose of this review the distinction is that Attachment factors allow for accumulation of the virus on the cell surface so that they can bind to the receptors that actually facilitate Viral entry.
4. Examination of the function of the shell proteins in a foreign context allows the experimenter to isolate the entry process from the other steps in viral replication.
5. Chemical and immunological reagents that specifically inhibit entry can be screened.

recognized efficacy of removing bulky cytoplasmic domains of glycoproteins for successful pseudotype formation [11•]. Conversely, lentivirus vectors pseudotyped with C-terminal truncated envelope proteins from avian sarcoma and leukosis virus subgroup A (ASLV-A; a retrovirus) have been reported [12], which make possible the efficient transduction of nondividing cells expressing a transgenic ASLV-A receptor (pseudotyped retroviruses generally transduce only dividing cells, whereas pseudotyped lentiviruses, such as the human immunodeficiency virus type 1 [HIV-1], are capable of transducing nondividing cells). An article on the construction of Mo-MuLV vectors pseudotyped with spleen necrosis virus envelope proteins with corrected glycoprotein cytoplasmic domain sequences has been published [13].

The list of enveloped viruses that can be pseudotyped is not restricted to the retroviruses. A vesicular stomatitis pseudotype bearing hepatitis C virus envelope glycoproteins may have utility for the study of hepatitis C virus entry [14], although this conclusion has been challenged [15]. Herpes simplex virus type 1 (HSV-1) vectors producing VSV-G and lacking the otherwise essential HSV-1 glycoprotein gB were capable of gene transfer, although at reduced transduction titers [16]. These examples demonstrate that there are many opportunities for moving beyond the limited set of pseudotyped viruses previously available.

Tropism determination

Numerous recent publications address cellular transduction by pseudotyped lentiviruses *in vivo*. One example is the transduction of cynomolgus monkey retinal cells by VSV-G-pseudotyped feline immunodeficiency virus (FIV) [17]. Another study demonstrated that equine infectious anemia virus pseudotyped with the rabies G protein underwent retrograde axonal transport to distal neurons, whereas virus pseudotyped with the VSV-G protein did not [18]. Recently it has been shown that when RRV-glycoprotein-pseudotyped FIV is injected intravenously into mice, this pseudotyped vector transduces mouse hepatocytes *in vivo* far more efficiently than VSV-G-pseudotyped FIV (Figure 2) and with no detectable liver toxicity [19••]. By contrast, injection of the VSV-G-pseudotyped FIV resulted in a substantial increase in liver enzyme release into the serum. The effectiveness of the RRV-glycoprotein-pseudotyped FIV may result from enhanced stability in the bloodstream. In other experiments it was found that RRV-glycoprotein-pseudotyped FIV preferentially transduced neuroglial cells after injection into the brain, whereas VSV-G-pseudotyped FIV targeted predominantly neurons.

Pseudotyping of recombinant genomes from one AAV serotype with the capsids of other AAV serotypes has gained popularity recently. In one example, the dependence of entry upon heparan-sulfate-bearing cellular proteoglycans as well as transduction of several tissues by the various pseudotypes was compared [20,21].

Engineering targeted-pseudotyped viruses

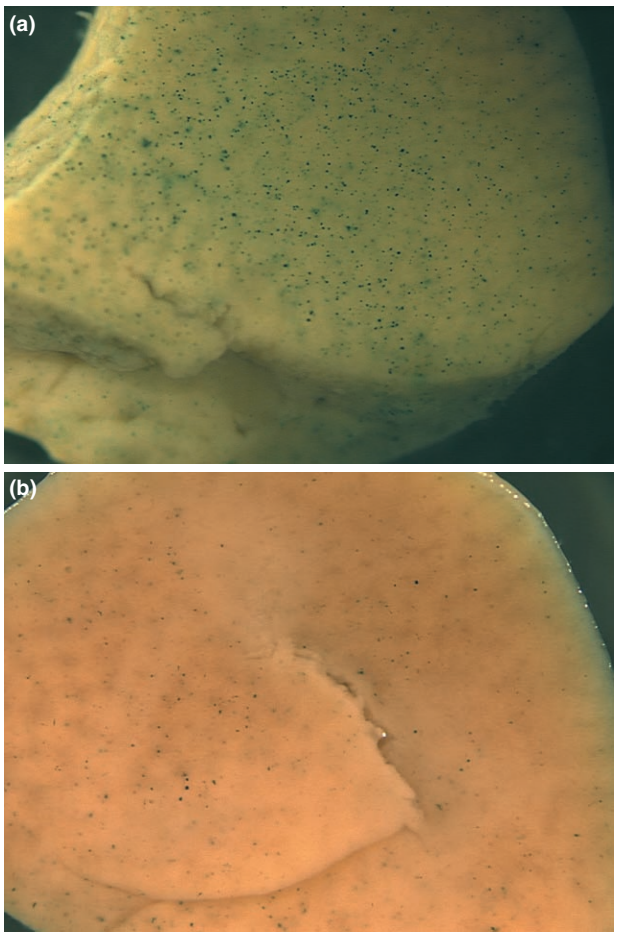
Targeting of specific cells through the introduction of ligands onto the shells of viral vectors that thereby bind to proteins expressed on particular cells has been a long-time goal of gene transfer researchers. Unfortunately, this field is a microcosm of the field of gene therapy in which early high-profile studies of questionable validity [22] convinced some people that the problem had been solved and discredited the discipline in the minds of others. Most of the careful studies utilizing modified enveloped viruses have not met with success, although in some systems alterations in tropism have been achieved. It appears that very

Box 2

Shortcomings of VSV-G-pseudotyped retroviruses and lentiviruses.

1. Cells constitutively expressing VSV-G protein cannot be constructed [35,36]. Cells with inducible VSV-G expression frequently have Detectable levels of protein produced, even when expression is not induced, and there appears to be selection for lower levels of production
   With passage of the cells.
2. Pseudotransduction (i.e. the transient transfer of the protein product of the gene that is carried by the pseudotyped virus in the absence of Stable integration of the vector) by concentrated vector preparations has been observed [37,38].
3. VSV-G-pseudotyped viruses are neutralized by human serum [39].
4. Toxicity of the viral preparations to primary cells and *in vivo* has often been observed, although it is not commonly reported in the literature.

Figure 2



FIV pseudotyped with RRV glycoprotein transduces the liver more efficiently than FIV pseudotyped with VSV-G. Mice were intravenously injected via the tail vein with equivalent transduction units of **(a)** RRV/FIV vector or **(b)** VSV-G/FIV vector carrying the *E. coli* β-galactosidase gene. Three weeks later, the mice were sacrificed and the liver was stained with X-gal.

few of these approaches have been applied successfully to target gene transfer *in vivo* [23•], although some success with the targeting of modified naked viruses has been achieved [24]. Sometimes the best results are achieved through 'negative' or 'inverse' targeting where tropism for a given tissue is achieved by reducing the transduction of a competing tissue in the organism [25]. Recent publications appear to offer direction for future efforts. Pseudotyped retrovirus bearing Mo-MuLV envelope proteins into which small peptide ligands for human cell-surface proteins had been inserted were capable of transducing human cells, albeit with low efficiency, whereas those bearing the wild-type Mo-MuLV envelope proteins were not capable of transducing human cells [26•,27]. The issue still remains whether the entry into human cells is due to the usage of the cell-surface protein as a novel receptor or merely as an attachment factor (the distinction is described in Box 1). In the latter case it could be that interaction with the human cell-surface protein facilitates binding to the human Mo-MuLV receptor homologue, which normally has low affinity for the Mo-MuLV envelope proteins.

A different approach relies on the modular nature of the alphavirus glycoprotein complex, which consists of a trimer of a heterodimer of two polypeptides (E2 and E1), each of which possesses its own membrane-spanning domain. E2 is responsible for receptor binding, whereas E1 promotes membrane fusion. It had previously been shown that the tropism of Sindbis virus (an alphavirus) could be altered by inserting the IgG-binding domains of protein A (a cell wall protein of *Staphylococcus aureus* that binds the Fc portion of IgG) into the E2 glycoprotein and incubating the virus with antibodies against a cell-surface protein [28]. Infection could be targeted to cells expressing the antigen recognized by the antibodies. It was found that this redirection of alphaviral entry could be combined with the favorable properties of pseudotyped virus vectors containing alphavirus (RRV) glycoprotein (noted above). High-efficiency transduction by viruses bearing the modified Sindbis glycoproteins (expressed from genes constructed anew — presumably because of the lack of availability of the original published modified Sindbis genes, an example of another phenomenon that plagues the field) in the presence of specific antibodies could be targeted to cell-surface antigen-expressing cells [29•]. Pseudotyped viruses producing modified alphaviral glycoproteins could prove to be the vectors of choice for targeted gene delivery.

Receptor identification

Pseudotyped retroviruses producing a viral glycoprotein and carrying a selectable marker or drug resistance gene can be used to identify cellular receptors. One requirement is a cell line that is not susceptible to entry by the pseudotyped virus. For example, if hamster cells are not susceptible to entry, then panels of radiation hybrids between hamster cells and cells from a species that is susceptible to entry (the hybrids contain stably inherited fragments of the DNA from that species) can be screened for those that can be transduced by the pseudotyped virus. This allows functional mapping and eventually cloning of the receptor genes. Alternatively, cDNA libraries from susceptible cells can be transfected into nonsusceptible cells; those now containing the receptor genes can be transduced by the pseudotyped virus and selected. The Jaagsiekte sheep retrovirus receptor was identified by the radiation hybrid technique [30,31••]. The expression screening approach was used to identify a protein involved in the cellular entry of Marburg and Ebola viruses, although the exact role of this protein is unclear [32] (see also Update). It is likely that pseudotyped retroviruses will prove to be the probes of choice for examination of factors required for entry by a large variety of enveloped viruses.

Investigation of the cell biology of viral entry

Pseudotyped viruses offer several advantages for the study of the process of entry of the virus from which the outer

shell is derived (Box 1). As noted above, viruses pseudotyped with RRV glycoproteins were instrumental in confirming the low-pH dependence of alphaviral entry. HIV-1 particles produced in cells expressing the Nef protein are more infectious than those produced in cells lacking Nef [33]. It has been shown that transduction by HIV-1 pseudotyped with the glycoproteins of two viruses (VSV and Ebola virus) that enter cells in a low pH-dependent manner are not affected by Nef expression in the producer cell, whereas transduction by HIV-1 pseudotyped with the amphotropic MuLV envelope protein, which is capable of promoting membrane fusion at the cell surface at neutral pH, is increased when the virus is produced in cells expressing Nef [34]. Although it is clear that the path of ingress of the pseudotyped virus affects the dependence of transduction upon Nef, it cannot necessarily be concluded at this time that it is pH-dependence alone that determines Nef dependence. Pseudotyping experiments were also used to demonstrate that there is an effect of ubiquitination of capsid proteins upon transduction by AAV particles bearing either type 2 or type 5 capsids [20].

Nomenclature

Many of us are guilty of a lack of specificity in our descriptions of the components of pseudotyped viruses. The VSV-G protein that is commonly utilized is from the Indiana strain (no, this is not a plug for my current home state) and should be so indicated. Ecotropic or amphotropic MuLV is an insufficient description. There are numerous ecotropic MuLVs (e.g. Moloney, Friend, AKV); the amphotropic MuLV envelope protein is probably derived from 4070A. Investigators should give as complete a description of the viral source of the glycoproteins and cores as possible.

Conclusions

Pseudotyped viruses are more than interesting laboratory artifacts. In the past decade they have assumed their place at the forefront of gene transfer and therapy applications. Their increasing diversity seems to offer the greatest opportunities for successful transduction of specific cell types both *in vitro* and *in vivo*. They are also becoming more widely appreciated as tools for the investigation of viral entry pathways. Their integration into the laboratory and clinic offers many opportunities and challenges in the near future.

Update

Recent data obtained in experiments utilizing lentiviruses pseudotyped with the Ebola virus glycoprotein have shown that, although Jurkat cells are normally not transduced by such recombinant viruses, Jurkat cells expressing either of the lectins DC-SIGN (dendritic cell-specific ICAM-3 grabbing nonintegrin) or L-SIGN are susceptible to transduction [40]. These results bring into question the significance of the previously identified receptor in Ebola virus entry. In addition, the receptors for the human endogenous retrovirus type W (HERV-W) were identified through the use of HIV pseudotyped with the HERV-W envelope protein [41].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Pearson MN, Rohrmann GF: **Transfer, incorporation, and substitution of envelope fusion proteins among members of the Baculoviridae, Orthomyxoviridae, and Metaviridae (insect retrovirus) families.** *J Virol* 2002, **76**:5301-5304.
A minireview of insect viral glycoproteins and their transfer between virus families. Viral evolution through recombination and consequent natural pseudotyping.

2. •• Sharkey CM, North CL, Kuhn RJ, Sanders DA: **Ross River virus glycoprotein-pseudotyped retroviruses and stable cell lines for their production.** *J Virol* 2001, **75**:2653-2659.
First report of the production of and transduction by recombinant alphavirus-glycoprotein pseudotyped retroviruses. Favorable properties as well as stable producer cells are described. Pseudotyped viruses used to probe cell biology of alphavirus entry.

3. Miletic H, Bruns M, Tsiakas K, Vogt B, Rezai R, Baum C, Kuhlke K, Cosset FL, Ostertag W, Lother H *et al.*: **Retroviral vectors pseudotyped with lymphocytic choriomeningitis virus.** *J Virol* 1999, **73**:6114-6116.

4. Beyer WR, Miletic H, Ostertag W, von Laer D: **Recombinant expression of lymphocytic choriomeningitis virus strain WE glycoproteins: a single amino acid makes the difference.** *J Virol* 2001, **75**:1061-1064.

5. •• Beyer WR, Westphal M, Ostertag W, von Laer D: **Oncoretrovirus and lentivirus vectors pseudotyped with lymphocytic choriomeningitis virus glycoprotein: generation, concentration, and broad host range.** *J Virol* 2002, **76**:1488-1495.
Description of advantageous properties of recombinant arenavirus glycoprotein-pseudotyped retroviruses.

6. Pinter A, Kopelman R, Li Z, Kayman SC, Sanders DA: **Localization of the labile disulfide bond between SU and TM of the murine leukemia virus envelope protein complex to a highly conserved CWLC motif in SU that resembles the active site sequence of thiol-disulfide exchange enzymes.** *J Virol* 1997, **71**:8073-8077.

7. Sanders DA: **Sulfhydryl involvement in fusion mechanisms**. In *Fusion of Biological Membranes and Related Problems*. Edited by Hilderson H, Fuller S. New York: Kluwer Academic/Plenum Publishers; 2000:483-514.

8. Opstelten DJ, Wallin M, Garoff H: **Moloney murine leukemia virus envelope protein subunits, gp70 and Pr15E, form a stable disulfide-linked complex.** *J Virol* 1998, **72**:6537-6545.

9. Pickl WF, Pimentel-Muinos FX, Seed B: **Lipid rafts and pseudotyping.** *J Virol* 2001, **75**:7175-7183.

10. Sung VM, Lai MM: **Murine retroviral pseudotype virus containing hepatitis B virus large and small surface antigens confers specific tropism for primary human hepatocytes: a potential liver-specific targeting system.** *J Virol* 2002, **76**:912-917.

11. • Bruett L, Clements JE: **Functional murine leukemia virus vectors pseudotyped with the visna virus envelope show expanded visna virus cell tropism.** *J Virol* 2001, **75**:11464-11473.
Evaluation of glycoprotein cytoplasmic domain properties that are advantageous for pseudotype production.

12. Lewis BC, Chinnasamy N, Morgan RA, Varmus HE: **Development of an avian leukosis-sarcoma virus subgroup A pseudotyped lentiviral vector.** *J Virol* 2001, **75**:9339-9344.

13. Engelstadter M, Buchholz CJ, Bobkova M, Steidl S, Merget-Millitzer H, Willemsen RA, Stitz J, Cichutek K: **Targeted gene transfer to lymphocytes using murine leukaemia virus vectors pseudotyped with spleen necrosis virus envelope proteins.** *Gene Ther* 2001, **8**:1202-1206.

14. Matsuura Y, Tani H, Suzuki K, Kimura-Someya T, Suzuki R, Aizaki H, Ishii K, Moriishi K, Robison CS, Whitt MA *et al.*: **Characterization of pseudotype VSV possessing HCV envelope proteins.** *Virology* 2001, **286**:263-275.

15. Buonocore L, Blight KJ, Rice CM, Rose JK: **Characterization of vesicular stomatitis virus recombinants that express and incorporate high levels of hepatitis C virus glycoproteins.** *J Virol* 2002, **76**:6865-6872.

16. Tang J, Yang T, Ghosh HP, Geller AI: **Helper virus-free HSV-1 vectors packaged both in the presence of VSV G protein and in the absence of HSV-1 glycoprotein B support gene transfer into neurons in the rat striatum.** *J Neurovirol* 2001, **7**:548-555.

17. Lotery AJ, Derksen TA, Russell SR, Mullins RF, Sauter S, Affatigato LM, Stone EM, Davidson BL: **Gene transfer to the nonhuman primate retina with recombinant feline immunodeficiency virus vectors.** *Hum Gene Ther* 2002, **13**:689-696.

18. Mazarakis ND, Azzouz M, Rohll JB, Ellard FM, Wilkes FJ, Olsen AL, Carter EE, Barber RD, Baban DF, Kingsman SM *et al.*: **Rabies virus glycoprotein pseudotyping of lentiviral vectors enables retrograde axonal transport and access to the nervous system after peripheral delivery.** *Hum Mol Genet* 2001, **10**:2109-2121.

19. •• Kang Y, Stein CS, Heth JA, Sinn PL, Penisten AK, Staber PD, Ratliff KL, Shen H, Barker KC, Martins I *et al.*: ***In vivo* gene transfer using a nonprimate lentiviral vector pseudotyped with Ross River Virus glycoproteins.** *J Virol* 2002, **76**:9378-9388.
First report of *in vivo* application of recombinant alphavirus-glycoprotein pseudotyped lentiviruses for gene transfer. Efficient transduction of liver and brain glial cells observed with no apparent toxicity.

20. Yan Z, Zak R, Luxton GW, Ritchie TC, Bantel-Schaal U, Engelhardt JF: **Ubiquitination of both adeno-associated virus type 2 and 5 capsid proteins affects the transduction efficiency of recombinant vectors.** *J Virol* 2002, **76**:2043-2053.

21. Rabinowitz JE, Rolling F, Li C, Conrath H, Xiao W, Xiao X, Samulski RJ: **Cross-packaging of a single adeno-associated virus (AAV) type 2 vector genome into multiple AAV serotypes enables transduction with broad specificity.** *J Virol* 2002, **76**:791-801.

22. Kasahara N, Dozy AM, Kan YW: **Tissue-specific targeting of retroviral vectors through ligand-receptor interactions.** *Science* 1994, **266**:1373-1376.

23. • Lavillette D, Russell SJ, Cosset FL: **Retargeting gene delivery using surface-engineered retroviral vector particles.** *Curr Opin Biotechnol* 2001, **12**:461-466.
Very good review appropriately focusing mainly, although not exclusively, on the authors' efforts in the field.

24. Reynolds PN, Nicklin SA, Kaliberova L, Boatman BG, Grizzle WE, Balyasnikova IV, Baker AH, Danilov SM, Curiel DT: **Combined transductional and transcriptional targeting improves the specificity of transgene expression *in vivo.*** *Nat Biotechnol* 2001, **19**:838-842.

25. Peng KW, Pham L, Ye H, Zufferey R, Trono D, Cosset FL, Russell SJ: **Organ distribution of gene expression after intravenous infusion of targeted and untargeted lentiviral vectors.** *Gene Ther* 2001, **8**:1456-1463.

26. • Gollan TJ, Green MR: **Redirecting retroviral tropism by insertion of short, nondisruptive peptide ligands into envelope.** *J Virol* 2002, **76**:3558-3563.
This and the accompanying article examine the effects of inserting short peptide ligands at various places in the Mo-MuLV Env upon transduction tropism. Promising results were obtained.

27. Gollan TJ, Green MR: **Selective targeting and inducible destruction of human cancer cells by retroviruses with envelope proteins bearing short peptide ligands.** *J Virol* 2002, **76**:3564-3569.

28. Ohno K, Sawai K, Iijima Y, Levin B, Meruelo D: **Cell-specific targeting of Sindbis virus vectors displaying IgG-binding domains of protein A.** *Nat Biotechnol* 1997, **15**:763-767.

29. • Morizono K, Bristol G, Xie YM, Kung SK, Chen IS: **Antibody-directed targeting of retroviral vectors via cell surface antigens.** *J Virol* 2001, **75**:8016-8020.
Utilization of virus pseudotyped with modified alphaviral glycoproteins containing the IgG-binding domains of protein A for targeting cells expressing particular cell-surface antigens. The report provides support for the proposition that these glycoproteins have important advantages as frameworks for the outer shells of targeted retroviruses.

30. Rai SK, DeMartini JC, Miller AD: **Retrovirus vectors bearing jaagsiekte sheep retrovirus Env transduce human cells by using a new receptor localized to chromosome 3p21.3.** *J Virol* 2000, **74**:4698-4704.

31. •• Rai SK, Duh FM, Vigdorovich V, Danilkovitch-Miagkova A, Lerman MI, Miller AD: **Candidate tumor suppressor HYAL2 is a glycosylphosphatidylinositol (GPI)-anchored cell-surface receptor for jaagsiekte sheep retrovirus, the envelope protein of which mediates oncogenic transformation.** *Proc Natl Acad Sci USA* 2001, **98**:4443-4448.
Identification of receptor through use of pseudotyped retrovirus. Pseudotyped virus also has potential for use in gene transduction of the lung.

32. Chan SY, Empig CJ, Welte FJ, Speck RF, Schmaljohn A, Kreisberg JF, Goldsmith MA: **Folate receptor-alpha is a cofactor for cellular entry by Marburg and Ebola viruses.** *Cell* 2001, **106**:117-126.

33. Chowers MY, Spina CA, Kwoh TJ, Fitch NJ, Richman DD, Guatelli JC: **Optimal infectivity *in vitro* of human immunodeficiency virus type 1 requires an intact nef gene.** *J Virol* 1994, **68**:2906-2914.

34. Chazal N, Singer G, Aiken C, Hammarskjold ML, Rekosh D: **Human immunodeficiency virus type 1 particles pseudotyped with envelope proteins that fuse at low pH no longer require Nef for optimal infectivity.** *J Virol* 2001, **75**:4014-4018.

35. Burns JC, Friedmann T, Driever W, Burrascano M, Yee JK: **Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells.** *Proc Natl Acad Sci USA* 1993, **90**:8033-8037.

36. Ory DS, Neugeboren BA, Mulligan RC: **A stable human-derived packaging cell line for production of high titer retrovirus/vesicular stomatitis virus G pseudotypes.** *Proc Natl Acad Sci USA* 1996, **93**:11400-11406.

37. Liu ML, Winther BL, Kay MA: **Pseudotransduction of hepatocytes by using concentrated pseudotyped vesicular stomatitis virus G glycoprotein (VSV-G)-Moloney murine leukemia virus-derived retrovirus vectors: comparison of VSV-G and amphotropic vectors for hepatic gene transfer.** *J Virol* 1996, **70**:2497-2502.

38. Gallardo HF, Tan C, Ory D, Sadelain M: **Recombinant retroviruses pseudotyped with the vesicular stomatitis virus G glycoprotein mediate both stable gene transfer and pseudotransduction in human peripheral blood lymphocytes.** *Blood* 1997, **90**:952-957.

39. DePolo NJ, Reed JD, Sheridan PL, Townsend K, Sauter SL, Jolly DJ, Dubensky TW Jr.: **VSV-G pseudotyped lentiviral vector particles produced in human cells are inactivated by human serum.** *Mol Ther* 2000, **2**:218-222.

40. Alvarez CP, Lasala F, Carrillo J, Muniz O, Corbi AL, Delgado R: **C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in *cis* and in *trans*.** *J Virol* 2002, **76**:6841-6844.

41. Lavillette D, Marin M, Ruggieri A, Mallet F, Cosset FL, Kabat D: **The envelope glycoprotein of human endogenous retrovirus type W uses a divergent family of amino acid transporters/cell surface receptors.** *J Virol* 2002, **76**:6442-6452.