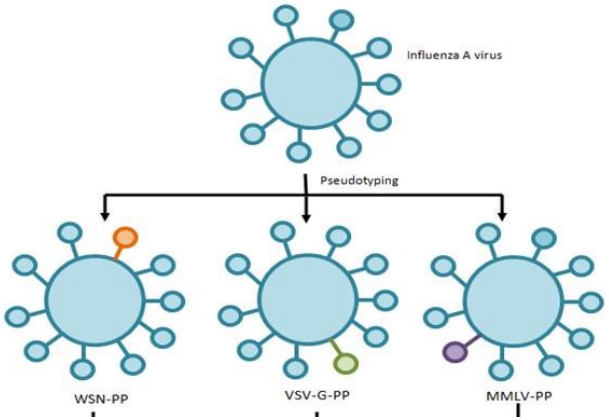
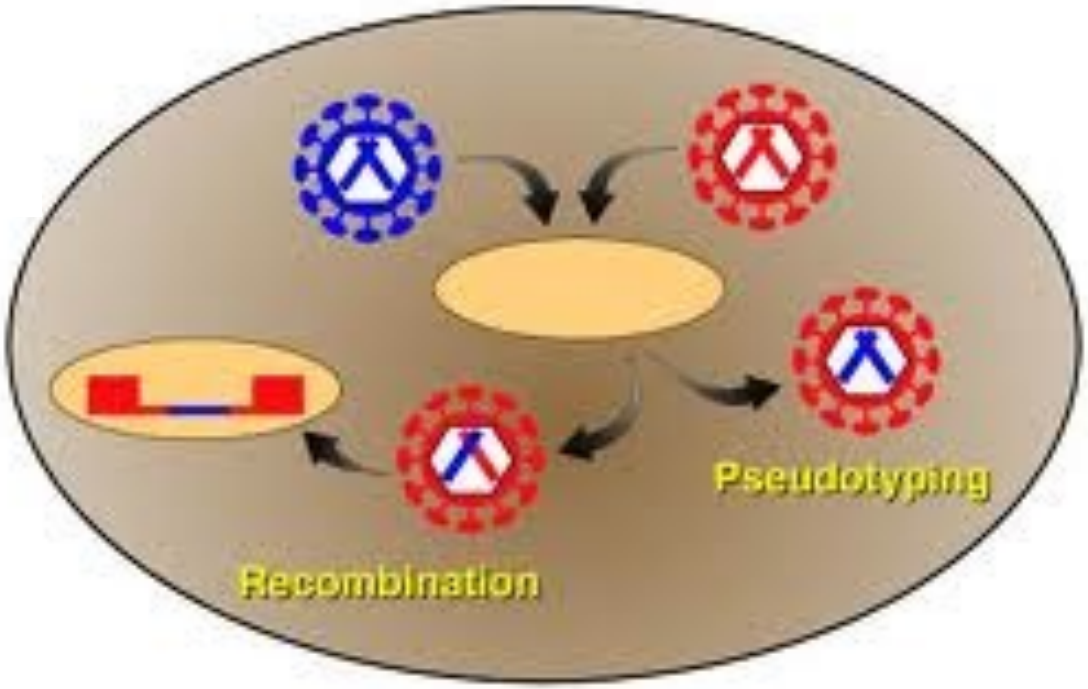
Lentivirus

These viruses acquired their *lenti* (in Latin meaning *slow*) etymological prefix due to the protracted incubation period elapsing between the initial infection and the onset of the disease, usually lasting for months or even years.

Ecotropic pseudotyped virus can only infect mouse or rat cells
Amphotropic virus can infect most mammalian cells
Pantropic virus (VSVG pseudotyped) virus can infect cells of any species

the pseudotyping story: changing the virus specificity of infection





Pseudotyping

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High-Efficiency Gene Transfer into CD34$^+$ Cells with a Human Immunodeficiency Virus Type 1-Based Retroviral Vector Pseudotyped with Vesicular Stomatitis Virus Envelope Glycoprotein G

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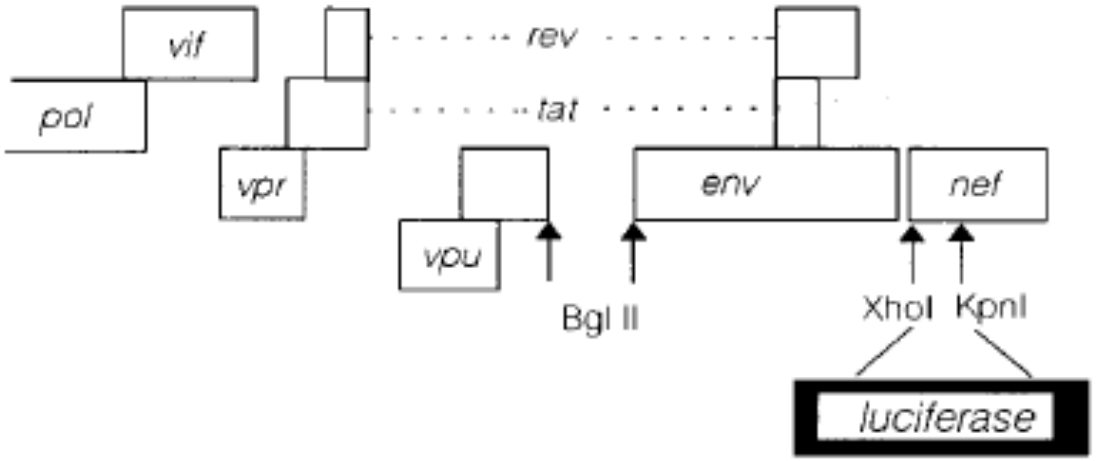

FIG. 1. Schematic representation of the 3′ portion of the envelope-defective HIV-1_{NL4-3}-based retroviral vector containing a luciferase expression marker (HIV-NL4-3 Luc). This HIV-1-based vector was generated by substituting *nef* gene sequences of the HIV-1_{NL4-3} genome with the firefly luciferase gene and deleting the envelope gene sequences located between two *Bgl*II restriction endonuclease sites as previously described (4, 25).

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Altering the Tropism of Lentiviral Vectors through Pseudotyping

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VSV-G Pseudotyping

Vantaggi:

- **Sembra interagire con recettori ubuquitari (amphotropic envelope protein)**
- **Elevata stabilità delle particelle virali**

Svantaggi

- **VSV-G è tossica se espressa costitutivamente (espressione inducibile/transiente)**
- **È inattivata dal siero**

To date, VSV-G remains the most popular GP for pseudotyping HIV-1-based vectors as well as other lentiviral vectors including vectors based on HIV-2 [Poeschla, 1998a] and on simian immunodeficiency virus (SIV) [Nakajima, 2000; Negre, 2000; Schnell, 2000]. Vectors based on nonprimate lentiviruses include feline immunodeficiency virus (FIV) [Poeschla, 1998b], equine infectious anemia virus (EIAV) [Mitrophanous, 1999], bovine immunodeficiency virus (BIV) [Berkowitz, 2001a], Jembrana disease virus (JDV) [Metharom, 2000], visna virus (VV) [Berkowitz, 2001b], and caprine arthritis encephalitis virus (CAEV) [Mselli-Lakhal, 2000] (Table 1).

Lentiviral vector assembly

one of the first papers describing the HIV-1-based lentivaril vector for transduction of cell lines and primary cells

In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector.
Naldini L, Blömer U, Gallay P, Ory D, Mulligan R, Gage FH, Verma IM, Trono D. Science. 1996 Apr 12;272(5259):263-7.

A retroviral vector system based on the human immunodeficiency virus (HIV) was developed that, in contrast to a murine leukemia virus-based counterpart, **transduced heterologous sequences into HeLa cells and rat fibroblasts blocked in the cell cycle, as well as into human primary macrophages**. Additionally, the HIV vector could mediate stable in vivo gene transfer into terminally differentiated neurons. The ability of HIV-based viral vectors to deliver genes in vivo into nondividing cells could increase the applicability of retroviral vectors in human gene therapy.

The rationale

Vectors based on onco-retroviruses, including Moloney murine leukemia virus (Mo-MLV) depend on cell proliferation for completion of their life cycle.

Breakdown of the nuclear envelope that accompanies mitosis appears to be essential for the nuclear import of the viral preintegration complex and to allow its integration into the genome of the host cell.

In contrast, **lentiviruses,** including HIV type 1 (HIV-1)**, differ fundamentally from onco-retroviruses in that they are relatively independent of cell division for completion of their replicative cycle.**
A number of experimental observation show that HIV-1 is able to infect quiescent cells *in vitro and* that cell activation and/or differentiation, rather than cell division, are required, in some instances, for optimal replication of this virus

Experimental design

•Replication-defective HIV type 1 (HIV-1) vectors carrying neomycin phosphotransferase (neo) or mouse heat stable antigen (HAS) replacing the HIV-1 sequences encoding gp160.
•Pseudotyped with ecotropic or the amphotropic Mo-MLV envelope proteins or the vesicular stomatitis virus G protein.
•Virus production after single or double transfections of either human 293T or monkey COS-7 cells.
•Titers of up to 10^7 colony-forming units per milliliter. 10-to 20-fold concentration of the pseudotyped particles by a simple ultrafiltration procedure.
•These vectors along with Mo-MLV-based vectors were used to transduce
•primary human skin fibroblasts and human peripheral blood CD341 cells.

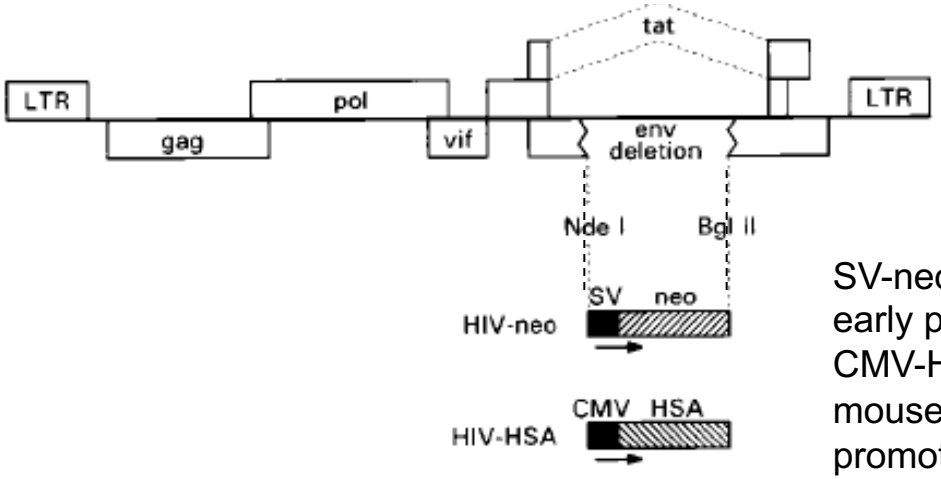
Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 15266–15271, December 1996
Genetics

Transduction of nondividing cells using pseudotyped defective high-titer HIV type 1 particles

(gene therapy/quiescent cells/human skin fibroblasts/CD34+ cells/cell cycle)

JAKOB REISER*†, GEORGE HARMISON‡, STEPHANIE KLUEPFEL-STAHL*, ROSCOE O. BRADY*, STEFAN KARLSSON*, AND MANFRED SCHUBERT‡

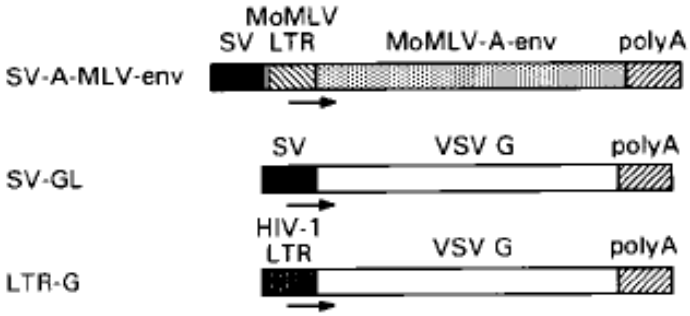
A



The vector was HXB2 molecular clone, encodes truncated forms of *Vpr, Vpu, and Nef.* *A 1.2-kb* deletion in the *env coding region was introduced, leaving the rev responsive element and the tat and rev exons intact*

SV-neo: expression cassette consisting of the SV40 early promoter driving the bacterial neo gene
CMV-HAS (Heat Stable Antigen): citomegalovirus mouse reporter gene under the CMV (cytomegalovirus) promoter.

B



Different Env constructs

FIG. 1. Recombinant plasmids for production of HIV-1 pseudotypes. (*A*) Recombinant HIV vectors. (*B*) Structure of Env expression vectors.

Virus titer

The efficiency of transfection was assessed indirectly by measuring the levels of extracellular p24 in the supernatants of the cells. Extracellular p24 levels were measured using a RETRO-TEK ELISA kit (Cellular Products)

The yield of infectious virus particles was determined by measuring the efficiency of transduction of HOS cells, human medulloblastoma (TE671) cells, and mouse 3T3 cells and scored through the formation of G418-resistant colonies.

La produzione virale dipende dalla presenza della proteina envelope; si ottengono alti titoli con la VSVG

Table 1. Generation of HIV-neo pseudotypes

Cells transfected	Virus envelope	p24, ng/ml	Virus titer on cells,* cfu/ml		
			3T3	HOS	TE671
293T	None	242	$<10^1$	$<10^1$	$<10^1$
	Mo-MLV-A	278	6.1×10^6	6.0×10^6	7.8×10^6
	VSV-G (SV-GL)	44	1.2×10^5	1.1×10^6	8.0×10^5
	VSV-G (LTR-G)	95	2.3×10^7	3.1×10^7	3.1×10^7
BOSC-23	Mo-MLV-E	278	5.3×10^6	NA	NA
BING	Mo-MLV-A	266	1.7×10^5	NA	NA
COS-7	Mo-MLV-A	27.5	5.8×10^6	4.2×10^6	3.7×10^6
	VSV-G (SV-GL)	7.9	2.0×10^6	3.0×10^5	6.0×10^5
	VSV-G (LTR-G)	6.7	2.2×10^6	2.0×10^6	4.0×10^6

NA, not available; cfu, colony-forming units.

Infected cells were trypsinized 3 days after transduction and cultured for 10–14 days in medium with G418. Colonies were stained using 0.2% crystal violet in 20% ethanol. Data from one representative experiment of several are shown.

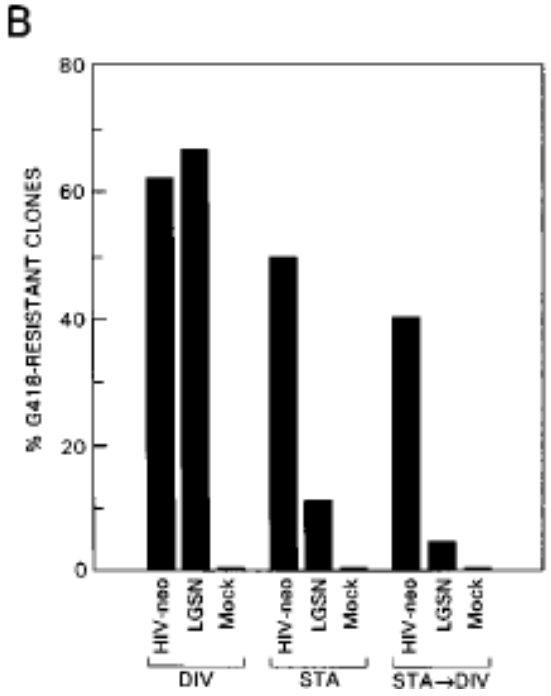
Alti titoli si ottengono sia con MoLV- A che VSV-G dopo ultrafiltrazione

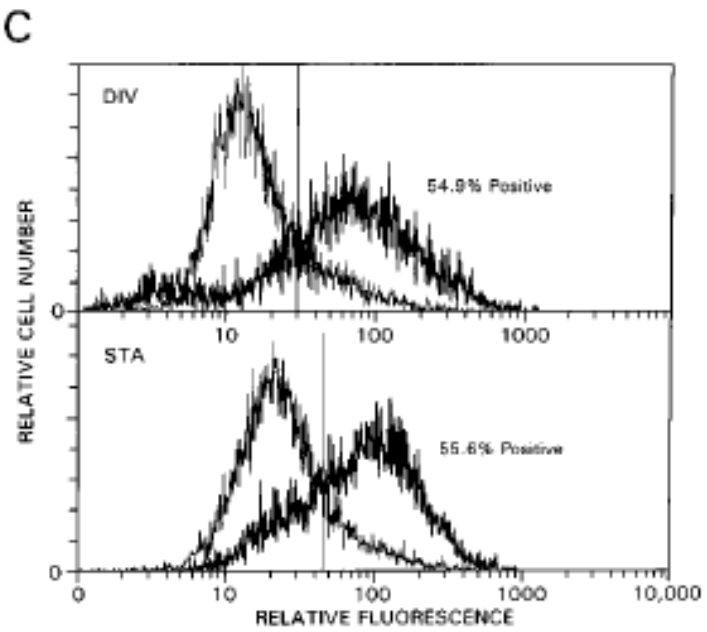
Table 2. Concentration of virus stocks by ultrafiltration

Virus envelope	Initial titer, cfu/ml	Final titer, cfu/ml	Volume change	Titer increase	% recovery
Experiment 1					
Mo-MLV-A	2.0×10^6	2.8×10^7	14-fold	14.2-fold	100
VSV-G (SV-GL)	1.8×10^5	1.5×10^6	14-fold	8.5-fold	60
Experiment 2					
Mo-MLV-A	1.0×10^7	6.7×10^7	10-fold	6.7-fold	66
VSV-G (LTR-G)	1.0×10^7	1.0×10^8	12-fold	10-fold	83

In experiment 1, 293T cells in 82-mm dishes were transfected with 22 μg of pHIV-neo along with 21 μg of pSV-A-MLV env or 10.5 μg of pSV-GL. Sixty-five hours after transfection, the culture supernatants were collected and filtered through a 0.45-μm filter. In experiment 2, transfection was carried out in 6-well dishes with 5 μg of pHIV-neo and 5 μg of the Env-encoding plasmid per well. Ten-milliliter aliquots of the supernatants were concentrated by using a Centriprep 100 concentrator. Virus titers were determined by measuring the formation of G418-resistant colonies using TE671 cells (experiment 1) or HOS cells (experiment 2).

VSV-G pseudotyped HIV-vector transduces dividing and stationary cells





B) Relative transduction efficiency of dividing (DIV) and stationary (STA) fibroblasts. G418R clones (%) were counted 2 weeks after selection.

C) FACS analysis of dividing and stationary HSF (primary human skin fibroblasts) transduced with pseudotyped HIV-vector.

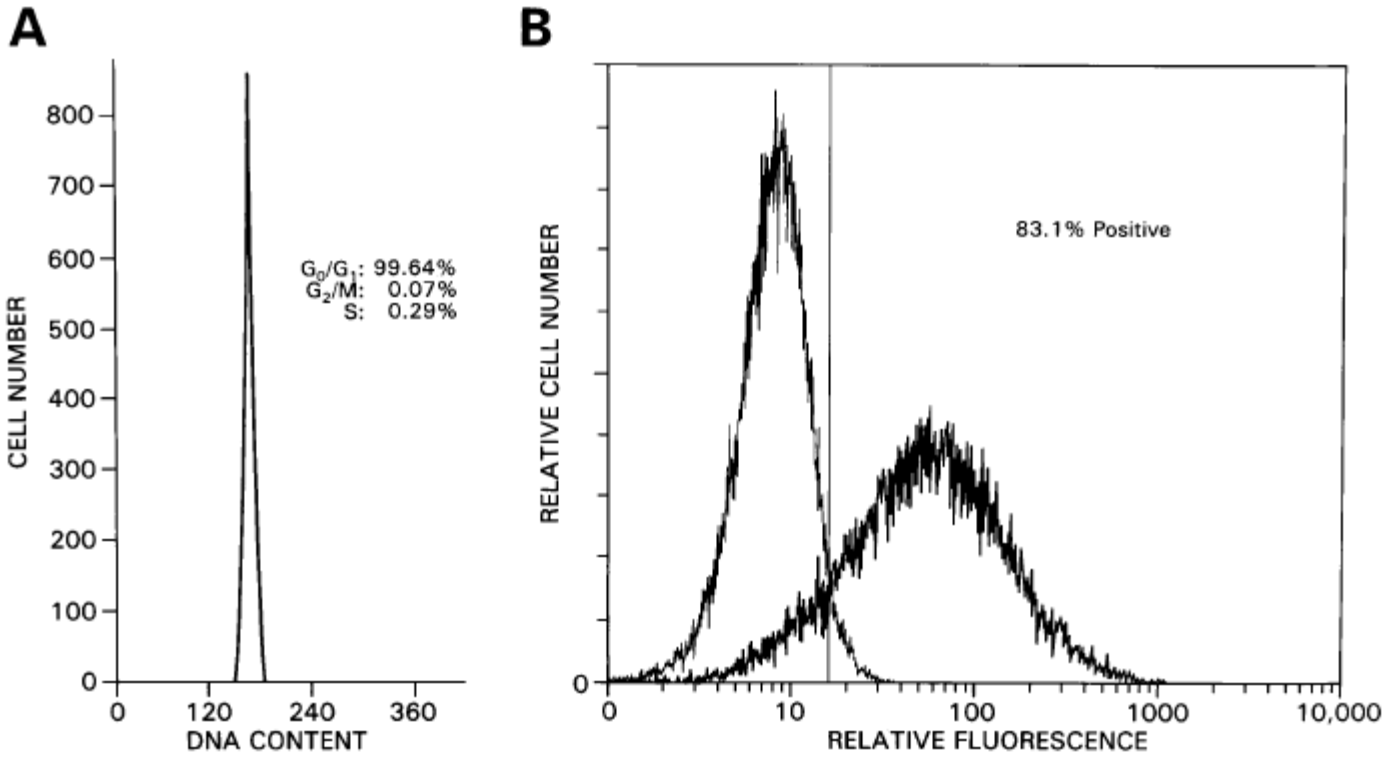
HIV-neo pseudotyped with VSV-G

LGSN - Mo-MLV vector

Transduction of nondividing human CD34+ cells with pseudotyped HIV-1 based lenti vector

Cell cycle analysis of peripheral blood CD34+ cells

FACS analysis. CD34+ cells (4 x 10^5) infected and processed for FACS analysis 71 hr later



Transduction of nondividing human CD34+ cells. (A) Cell cycle analysis of peripheral blood CD34+ cells. (B) FACS analysis. CD34+ cells (4 x 10^5) were infected and processed for FACS analysis 71 hr later. Dead cells were gated away after propidium iodide staining. Thick line: infected cells; thin line: mock infected cells.

Reiser J, Harmison G, Kluepfel-Stahl S, Brady RO, Karlsson S, Schubert M. Transduction of nondividing cells using pseudotyped defective high-titer HIV type 1 particles. Proc Natl Acad Sci U S A. 1996 Dec 24;93(26):15266-71. doi: 10.1073/pnas.93.26.15266. PMID: 8986799; PMCID: PMC26392.