[Frontiers in Bioscience 4, d481-496, June 1, 1999]

LENTIVIRAL VECTORS AND GENE THERAPY

Ekaterina Klimatcheva, Joseph D. Rosenblatt and Vicente Planelles

Departments of Medicine and Microbiology & Immunology, University of Rochester Cancer Center

TABLE OF CONTENTS

1. ABSTRACT

Gene therapy is a novel method under investigation for the treatment of genetic, metabolic and neurologic diseases, cancer and AIDS. The primary goal of gene therapy is to deliver a specific gene to a pre-determined target cell, and to direct expression of such a gene in a manner which will result in a therapeutic effect. Retroviral vectors have the ability to integrate in the host cell DNA irreversibly and therefore, are suitable vectors for permanent genetic modification of cells. Retrovirus-mediated gene transfer has been limited, however, by the inability of onco-retroviruses to productively infect non-dividing cells. Lentiviruses are unique among retroviruses because of their ability to infect target cells independently of their proliferation status. This chapter presents an up-to-date description of available lentiviral vectors, including vector design, applications to disease treatment and safety considerations. In addition, general aspects of the biology of lentiviruses with relevance to vector development will be discussed.

2. INTRODUCTION

Gene therapy is a promising novel approach to the treatment of a variety of disorders including infectious, genetic and neurologic diseases and cancer (1-5). It employs various methods to deliver foreign genes into somatic cells with the ultimate goal of incorporation and stable expression of the gene of interest. Methods used for gene therapy are categorized into non-viral (6) and viral. Non-viral methods of gene delivery include cationic and cholesterol-containing liposomes, peptide-lipid vectors, activated dendrimers (branched DNA-binding carbohydrates), bacteria, artificial chromosomes and artificial viruses (liposomes with viral components).

2.1. Virus-mediated gene therapy systems

The viral vectors used for gene transfer include retroviruses, adenoviruses, adeno-associated viruses, herpes simplex virus and flaviviruses (7, 8). Each specific vector group has its own limitations for use in human gene therapy. For instance, the use of adenoviruses is hampered

Table 1. Description of principal genetic features of HIV-1-based lentiviral vectors

Molecular determinant	Function	Role in lentiviral vector	References
I. *Cis*-acting			
LTRs	Contain sequences required for viral gene expression; reverse transcription; and integration	Essential	90, 97, 101
PBS	Required for the initiation of minus-strand synthesis	Essential	
Ψ	Required for encapsidation of the genomic transfer RNA	Essential	90, 97, 101
RRE	Interacts with Rev. Required for processing and transport of viral RNAs	Beneficial	134-137
PPT	Required for priming of the plus-strand synthesis	Essential	7
att sites	Required for viral DNA integration	Essential	7
II.*Trans*-acting			
gag / pol	Encodes structural proteins and enzymes required for viral replication	Essential	
env	Encodes envelope glycoprotein	Essential	84
vif	Affects assembly of the virions and infectivity in certain cell types	Dispensable	42, 138-142
vpu	Downregulates CD4 and MHC class I molecules; stimulates release of virions;	Dispensable	42, 43, 55, 56
vpr	Allows infection of non-dividing cells; increases production of the viral progeny. Induces cell cycle arrest and apoptosis	Dispensable	42, 51, 53, 143
tat	Necessary for high level expression from the viral LTR	Dispensable	144
rev	Necessary for expression of unspliced and singly-spliced mRNAs *in vivo*	Beneficial	134-137, 145
nef	Required for high viral burden; down-regulation of CD4	Dispensable	42, 58, 59, 146

by their induction of immune responses in the host and their inability to stably integrate into the host genome. The lack of the stable integration has also been a problem when using herpes simplex virus-based vectors. Adeno-associated viruses, although capable of integration into genomic DNA, depend on "helper" viruses (e.g. adenovirus or herpes simplex virus) for productive infection (9). The major drawback of the commonly used onco-retroviral vectors is their inability to transduce non-dividing cells (10).

The family *Retroviridae* comprises a large group of viruses that infect primarily vertebrates, although infection of other animals has been described in a few instances (11). A unifying feature of these viruses is that replication involves the process of conversion of the viral RNA genome into double-stranded DNA, hence the designation "retro" (backward) viruses. Members of the *Retroviridae* family are classified into three subfamilies: (i) *oncovirinae*, which includes the oncogenic retroviruses and closely related non-oncogenic viruses; (ii) *spumavirinae*, whose members establish persistent infections in the absence of clinical disease and cause a characteristic cytopathic effect *in vitro*; and (iii) *lentivirinae*, a group of viruses that are associated with slow, progressive diseases affecting the immune system (7).

Lentiviruses include a variety of primate (e.g. human immunodeficiency viruses [HIV-1 and 2], and simian immunodeficiency viruses [SIV]) and non-primate viruses (e.g. maedi-visna virus [MVV], feline immunodeficiency virus [FIV], equine infectious anemia virus [EIAV], caprine arthrithis encephalitis virus [CAEV] and bovine immunodeficiency virus [BIV]) viruses. The ability to integrate into the genome of non-dividing cells (12-14) makes lentiviruses particularly attractive in human gene therapy. Examples of non-dividing cells which are potential targets for gene therapy include hepatocytes, neurons, hematopoietic stem cells, myocytes and macrophages. Lentiviruses which are currently used as the basis for development of gene therapy vectors are HIV-1 and 2, SIV, FIV, EIAV and CAEV.

3. THE BIOLOGY OF LENTIVIRAL INFECTION

The biology of lentiviral infection has been reviewed extensively elsewhere (7, 15, 16). This part of the chapter will provide an overview of the organization of lentiviral genome and life cycle with the emphasis on those aspects which are relevant to gene therapy.

3.1. Molecular aspects of lentiviral infection

Lentiviruses possess a complex genome. In addition to the structural genes (*gag*, *pol* and *env*) common to all retroviruses, lentiviruses also contain a plethora of regulatory and accessory genes involved in modulation of viral gene expression, assembly of viral particles and structural and functional alterations in the infected cell (table 1).

Replication, integration and packaging of lentiviruses are mediated, in part, by *cis*-acting RNA or DNA sequences, which do not encode proteins (table 1). Most of such *cis*-acting elements are essential in the design of lentiviral vectors and are usually included in the transfer element (i.e., the part of a retroviral or lentiviral vector which will integrate in the host cell genome and which encodes the gene of interest).

The *trans*-acting viral elements encode three groups of proteins: structural, regulatory and accessory. Lentiviral vectors are defective for replication and are, therefore, unable to produce progeny. Thus, only the early steps (attachment, entry, reverse transcription and integration) of the lentivirus life cycle must be maintained in a lentiviral vector. Since early steps do not depend on viral protein synthesis, most *trans*-acting genes can be excluded so that the transfer vector only encodes the gene(s) of interest. Elimination of the *trans*-acting genes renders the transfer vector replication-defective. *Trans*-acting genes are provided *in trans* by a packaging plasmid.

3.1.1. Structural genes of HIV-1

HIV-1 encodes three structural genes: *gag*, *pol* and *env*. The product of *gag* is translated from unspliced mRNA as a precursor protein and cleaved into the following protein subunits: matrix (MA), essential for virion assembly and infection of non-dividing cells (17, 18); capsid (CA), which forms the hydrophobic core of the virion and is essential for virion assembly and maturation; nucleocapsid (NC), which coats viral RNA stochiometrically and remains tightly associated with viral RNA in virions; and several additional polypeptides of small size and unknown function, such as p1, p2 and p6.

The *pol* gene encodes three enzymes required for viral replication: protease (PRO), reverse transcriptase (RT) and integrase (IN). PRO cleaves Gag and Gag-Pol polyproteins which are essential for the maturation of the virion. RT contains three enzymatic activities: RNA-dependent DNA polymerase, RNAase H and DNA-dependent DNA polymerase.

In addition, the *pol* genes of certain non-primate lentiviruses (FIV, CAEV, MVV, EIAV) encode deoxyuridine triphosphatase (dUTPase), an enzyme that decreases intracellular concentration of dUTP and thus limits misincorporation of deoxy-uracil into viral DNA (19-22). It is thought that the presence of dUTPase in these viruses decreases the mutation rate by reducing incorporation of deoxy-uracil into DNA during reverse transcription (19).

The *env* gene is essential for viral binding and entry into the host cells. It encodes the precursor glycoprotein, gp160, which is cleaved into a surface moiety, gp120 (SU), and a transmembrane moiety, gp41 (TM). The surface glycoprotein is required for binding to cellular receptors, whereas the transmembrane glycoprotein is responsible for the fusion with the cellular membrane.

The regulatory genes *tat* and *rev* encode transactivator proteins essential for viral replication. The *tat* gene is present in all lentiviruses except FIV (23). The *rev* gene is present in all lentiviruses. Doubly spliced viral mRNAs encoding Rev, Tat and Nef proteins are the first ones to be synthesized *de novo* following viral integration. Once synthesized, Rev and Tat augment production of viral mRNAs.

Rev contains a nuclear export signal (NES) and allows nuclear export of unspliced and singly spliced mRNAs that encode viral structural proteins. In the absence of Rev the only mRNAs detected in the infected cells are doubly-spliced ones. Two interactions of Rev are required for attaining this function. First Rev interacts with the Rev responsive Element (RRE) overlapping the *env* coding sequence. Second, Rev interacts with the nuclear pore proteins, nucleoporins. Rev, therefore, acts as a shuttle between the nucleus and cytoplasm, mediating the nuclear export of the RRE-containing viral mRNAs.

Since it is absolutely essential for viral replication, Rev has been a target for a number of anti-HIV gene therapeutic strategies (24, 25) including protein- and RNA-based strategies (26-28)). In HIV-1-based vectors, the presence of *rev* is essential unless its function is accomplished by an alternative genetic element. Thus, Rev/RRE can be substituted with the *cis*-acting 219-nucleotide constitutive transport element (CTE) from the Mason-Pfizer monkey virus (MPMV) that has been shown to allow Rev-independent HIV-1 replication (29).

The viral protein Tat upregulates viral transcription at the level of elongation via interaction with the Tat activation region (TAR) located at the 5' end of all viral mRNAs. In addition to interacting with TAR, the binding to the cyclin-dependent kinase (Cdk)-Cyclin T complex has also been shown to be required for the activity of Tat (30). The potential gene therapy approaches involving Tat include trans-dominant negative mutants (31, 32) and TAR decoys (28).

In gene therapy vectors, requirement for Tat can be overcome by including a strong constitutive promoter, such as the human cytomegalovirus immediate early (HCMV-IE) enhancer/promoter into the transfer vector (33, 34).

The accessory genes include *vif*, *vpr*, *vpu* and *nef*. These genes were named accessory because they are nonessential for virus replication in cell culture (35).

Vif (virion infectivity factor) has been identified in all lentiviruses except EIAV. Vif is not essential for HIV-1 replication in permissive cells such as HeLa-CD4 or SupT1 (36, 37). However, it is necessary for production of infectious virions by cells that are natural targets for infection, including CD4-positive T-lymphocytes, macrophages, and H9 cells (38-40). A recent study has suggested that nonpermissive cells contain an endogenous inhibitor of HIV-1 production that is overcome by the virus-encoded Vif protein (41). The potential role of Vif in an HIV-1-derived vector was directly examined and it was concluded that *vif* is dispensable (42, 43). It may be inferred that the vector producing cells utilized in these studies (42, 43) may have a "permissive" phenotype with respect to Vif, and that the presence of Vif in a replication-defective vector may not be necessary because target cells will not produce viral progeny.

Vpr (viral protein R) is a virion-associated protein present only in primate lentiviruses. The first function of Vpr to be documented was its ability to act as a weak transcriptional transactivator of the viral LTR (44, 45). In addition, HIV-1 Vpr participates in viral infection of non-dividing cells (12, 46-48). Vpr has also been shown to cause cell cycle block in G2 and apoptosis (49-54). Despite its role in the transduction of non-dividing cells, *vpr* is not required in an HIV-1 packaging construct (42, 43), due largely to the existence of the other mechanisms (MA and IN) allowing the nuclear transport of pre-integration complexes.

The *vpu* gene encodes a cytoplasmic viral protein and present exclusively in HIV-1. It promotes degradation of CD4 in the endoplasmic reticulum of target cells. Degradation of CD4 allows transport of Env to the cell surface and its incorporation into virions. Vpu is able to stimulate the release of viral particles from certain types of cells including T-lymphocytes, HeLa cells and colonic carcinoma SW480 cells (55, 56). Another function of Vpu is to downregulate the expression of MHC I molecules on the surface of infected cells (57). This prevents recognition by cytotoxic T-cells. Despite its multiple functions, *vpu* is a dispensable gene in vector system as its exclusion from the vector does not not seem to influence the properties of the *vpu*-negative viral particles (42, 43).

The *nef* gene is only found in primate lentiviruses and it is essential for viral infectivity *in vivo*, but not *in vitro* (58). Nef reduces interactions between Env and intracellular CD4 by inducing internalization and degradation of CD4 (59). Nef also downregulates cell surface expression of MHC I molecules and protects infected cells from killing by cytotoxic T-lymphocytes (60). Nef was also shown to enhance the infectivity of viral particles, independently of the effects on CD4 (61, 62). This Nef-dependent enhancement of infectivity occurs at the level of proviral DNA synthesis, early in the infectious cycle (63-65). However, later studies demonstrated that the dependence on Nef for achieving optimal infectivity in a vector system could be overcome by the use of VSV-G glycoprotein-pseudotypes (66). This observation suggested that Nef only increases infectivity of virions entering the cells by direct fusion with the cellular membrane, but not via receptor-mediated endocytosis. These results are in agreement with other studies which directly demonstrate that Nef is entirely dispensable in lentiviral vectors when pseudotyed with VSV-G (42, 43).

Other open reading frames found in the genomes of lentiviruses include *orf2* of FIV, *S2 orf* of EIAV and *tmx, vpy* and *vpw* of BIV. *Orf2* is a transcriptional trans-activator needed for replication of FIV in primary T-lymphocytes (23). The function of *S2 orf* is still unknown. *S2 orf* is not an essential gene, however, since it is not required for viral infectivity or replication (67). The functions of the *tmx, vpy* and *vpw* genes of BIV are not known. It has been speculated that they encode proteins which are analogues of Nef, Vpr/Vpx and Vpu of other lentiviruses (68).

3.1.2. Molecular determinants of infection of non-dividing cells

Among retroviruses, the ability to integrate into the genome of non-dividing cells is unique to lentiviruses. Three molecular elements appear to determine infection of non-dividing cells by HIV-1: MA, IN and Vpr (12, 17, 18, 69). These proteins utilize the cellular nuclear import machinery to target the pre-integration complex (PIC) to the nucleus.

The cellular nuclear import system consists of the cytosolic proteins, importin-α and importin-β and nuclear pore proteins called nucleoporins. Importin-α carries a binding site for nuclear localization sequences (NLS). When NLS-containing proteins bind to importin-α, conformational changes occur that permit interaction with importin-β. Importin-β then targets the complex to the nuclear pore by binding it to the nucleoporins (70).

Both MA and IN carry conventional NLSs and utilize the importin-α/β pathway for nuclear transport. Vpr, on the other hand, does not contain a canonical NLS and acts as an importin-β analogue (71) interacting with both importin-α and nucleoporins. Moreover, the interaction of Vpr with importin-α increases the affinity of importin-α for the NLSs of MA and IN and, therefore, enhances nuclear import of the PIC into non-dividing cells.

In addition to participating in the nuclear transport of PICs, Vpr was shown to induce cell cycle arrest in G2 and apoptosis (See section 3.1.1 above). From a theoretical standpoint, an ideal lentiviral vector would encode the former function of Vpr (nuclear transport of PICs) but not the latter (cell cycle arrest and apoptosis). This was possible in a SIV-based packaging system due to the segregation of the function into *vpx* (nuclear transport) and *vpr* (cell cycle arrest). As predicted, a vector expressing SIVmac *vpx*, but not SIV mac *vpr* was fully infectious in non-dividing cells (72). In practice, however, it has been proposed that Vpr may be entirely dispensable in a lentiviral vector without dramatically affecting infectivity of non-dividing cells (42, 43).

3.2. Life cycle of lentiviruses

The lentiviral life cycle is similar for all the members of the *Retroviridae* family and consists of the steps outlined below (sections 3.2.1. to 3.2.5.).

3.2.1. Attachment and entry

The interaction of the lentivirus with the target cell occurs via binding of the viral envelope glycoprotein to a specific receptor on the cell membrane, which defines the cellular target for the virus (viral tropism). Primate lentiviruses infect helper T-lymphocytes, macrophages, microglial, dendritic and Langerhans cells by interacting with CD4 and one of several chemokine receptors, most frequently CCR5 or CXCR4 (73-76). FIV utilizes feline homologues of the CD9 receptor (77) and CXCR4 (78) to infect CD4+ and CD8+ T lymphocytes, B-lymphocytes, platelets, monocytes, astrocytes, microglia and dendritic cells.

Once bound to the cellular receptor, the viral membrane undergoes fusion with the cellular membrane (79). After virion-bound matrix and capsid proteins disassemble and the nucleoprotein complex is delivered into the cells, reverse transcription begins in the cytoplasm.

One property common to most retroviruses is the ability to form pseudotypes, retroviral particles which incorporate a heterologous envelope glycoprotein (80-82). The envelope glycoprotein G of the vesicular stomatitis virus (VSV-G) is commonly used to create such pseudotypes as it provides two major advantages to the development of the gene delivery vectors. First, entry of VSV into the target cell occurs via binding to ubiquitous phospholipid components of the cell membrane: phosphatidylinositol, phosphatidylserine and GM3 ganglioside (83). This mode of entry provides VSV (and most retroviruses pseudotyped with VSV-G) a broad host range which includes non-mammalian cells derived from fish, *Xenopus*, mosquito, and *Lepidoptera* (84).

Second, the VSV envelope forms highly stable viral particles. Owing to this increased stability, efficient concentration of the vectors can be achieved by ultracentrifugation (84, 85). Concentration of vectors leads to increased titers by about 2 orders in magnitude.

The first VSV-G pseudotyped retroviral vector in which the only element from VSV was the glycoprotein G was described by Burns and coworkers (84). This vector contained the core and genome of the murine leukemia virus. When concentrated by ultracentrifugation, this vector exhibited titers of approximately 10^9 infectious units per milliliter (IU/ml), a titer which would be difficult to achieve when using the homologous (MuLV) envelope glycoprotein.

3.2.2. Reverse transcription

Reverse transcription leads to the synthesis of double-stranded, linear DNA from a single-stranded RNA template using cellular tRNA as a primer. The enzyme that catalyzes this step in viral replication, reverse transcriptase, has low fidelity due to the lack of proofreading ability and therefore, is partly responsible for the high variability of the viral genome.

3.2.3. Integration

Once synthesis of the linear viral DNA is complete, viral integrase performs specific cleavages at the 5' and 3' termini and catalyzes integration into the host genome. Integration is essential for retroviral gene expression (86, 87) and allows the provirus to become a permanent genetic element in the host.

Accessibility of different regions of the genome for avian leukosis virus (ALV) was directly studied by Withers-Ward and collaborators (88). This study concluded that there were no preferences for integration within the genome of avian cells, although local differences in DNA structure (as evidenced by DNAse I susceptibility) may affect the integration specificity. Integration for other retroviruses is expected to follow a similar pattern, although this remains to be directly tested (88). Attempts to manipulate the site of retroviral integration have achieved a certain degree of success (89) but this strategy will need further development.

3.2.4. Transcription and viral protein synthesis

Early transcription from the provirus results in the production of doubly spliced mRNAs encoding Rev, Tat and Nef. Translation of mRNA leads to accumulation of Tat and Rev. Together, Tat and Rev induce shift to "late" transcription mode whereby unspliced and singly spliced RNA species are primarily produced. Unspliced and singly spliced mRNAs encode structural genes necessary for the production of viral progeny.

3.2.5. Virion assembly and release

The viral RNA and structural proteins are packaged into viral particles and released by budding at the plasma membrane. After the polyproteins, Gag and Gag/Pol, are cleaved by the viral protease, mature particles become fully infectious. In currently available lentiviral vectors, *gag/pol* are not present in the transfer construct, but are provided *in trans* by a packaging construct (72, 90). Thus, once the transfer vector is integrated in the host cell, its inability to direct production of *gag/pol* ensures that there will be no subsequent viral progeny.

4. LENTIVIRAL VECTORS

4.1. Applications of lentiviral vectors

The applicability of a safe lentiviral vector in human disease is broad because (1) the host range of lentiviruses can be virtually unlimited when using vesicular stomatitis G glycoprotein (VSV-G) to produce envelope pseudotypes; (2) many relevant targets for gene therapy are non-dividing cells (neurons, hepatic cells, hematopoietic stem cells, myocytes); and (3) the stability of the transgene is potentially indefinite due to chromosomal integration.

Lentiviral vectors offer potential for treatment of a wide variety of syndromes (table 2), including genetic/metabolic deficiencies, viral infection and cancer. Inherited genetic defects such as adenosine deaminase deficiency, familial hypercholesterolemia, cystic fibrosis, mucopolysaccharidosis type VII, types I and II diabetes, classical phenylketonuria and Gaucher disease may be overcome by lentiviral vector-mediated gene therapy because they constitute single-gene deficiencies for which the involved genes are known. In fact, an HIV-1-based vector was constructed that successfully delivered the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) into human undifferentiated bronchial epithelium cells derived from the CF patient implanted into nude mice. Expression of CFTR resulted in functional correction of the CF defect after maturation of the airway epithelium (91).

Certain types of cancer may benefit from the use of lentiviral vectors. Hypoxia and lack of vascularization lead to the generation of tumor cells which exhibit limited

Table 2. Disorders and lentiviral vectors, targets and tools for gene therapy.

Disorder	Target cells	Lentiviral vectors that transduce target cells	References
I. Inherited			
Cystic fibrosis	Airway epithelia	Human cells: HIV-1-based	91
Retinitis pigmentosa	Retinal photoreceptor cells	Human cells: HIV-1-based	147
Hematopoietic disorders: sickle cell anemia, ß-thalassemia; lysosomal storage disorders, mucopolysaccharidoses; severe combined immunodeficiency syndrome	Progenitors for red blood cells; macrophages; lymphocytes	Human cells: HIV-1-based, HIV-1/HIV-2-based, HIV-2-based, HIV-1/SIV-based, FIV-based	72, 85, 124, 128, 131, 148, 149
Gaucher's disease	Bone marrow cells, macrophages	Human cells:HIV-1/SIV-based; HIV-1/HIV-2-based; FIV-based	72, 128, 131
Familial hypercholesterolaemia	Liver cells	Rat cells: HIV-1-based	102
II. Acquired			
HIV infection	Mostly T-lymphocytes and macrophages	Human cells: HIV-1/SIV-based; HIV-1/HIV-2-based; FIV-based	72, 128, 131
Neurodegenerative (Parkinson's, Alzheimer's diseases)	Brain tissue, neurons, glial cells	Rat tissue/cells: HIV-1-based Mouse cells: HIV-1/SIV-based Human cells: FIV-based	72, 90, 104, 131
Cardiovascular	Endothelial cell, cardiac myocytes	Human cells: HIV-1-based	104
Cancer	Cancer cells in various tissues (e.g. liver, brain)	Various vectors (see above)	

or no proliferation. Partly because of the lack of growth, these cells are highly resistant to genotropic agents. A lentiviral vector may prove to be a useful vehicle for delivery of a "lethal" gene (such as herpes virus thymidine kinase) to quiescent tumor cells.

Viral diseases may also constitute appropriate targets for lentiviral gene delivery. In particular, a number of gene therapy approaches have been proposed for the treatment of HIV infection (see section 4.3.1.4.). For some of these strategies, phase I studies have recently begun in humans. Preliminary studies have dealt with defective murine oncoviruses for delivery of anti-sense RNAs, ribozymes and trans-dominant proteins against HIV replication.

4.2. Elements of a prototypical lentiviral vector

Contemporary lentiviral vectors are produced via a transient expression system that consists of three genetic elements: transfer construct, packaging construct and Env expression construct (72, 84, 90). The transfer construct contains retroviral *cis*-acting elements and the gene(s) of interest. The packaging construct directs expression of viral structural proteins, except for the envelope. Proteins expressed by the packaging construct (Gag/Pol, predominantly) form the capsid and polymerase components, and recognize specific *cis*-acting sequences in the retroviral RNA genome and its reverse-transcribed DNA products (92). This recognition leads to reverse transcription and integration. The envelope construct typically contains a heterologous envelope (e.g. vesicular stomatitis virus glycoprotein G [VSV-G]). The three expression constructs are maintained in the form of bacterial plasmids and can be transfected into mammalian cells to produce replication-defective virus stocks (72, 90).

An example of such a prototypical lentiviral vector (figure 1) was described by Naldini and collaborators (90). In this vector system, the transfer construct contained a reporter gene (either β-galactosidase or luciferase) under control of an internal CMV promoter and the *cis*-acting sequences of HIV-1 including LTRs, splice signals, about 350 base pairs of the *gag* gene and the RRE. The packaging construct contained all the viral proteins (except Env and Vpu) whose expression was driven by the HCMV promoter. All the *cis*-acting sequences required for packaging (Ψ), reverse transcription (PBS, PPU) and integration (*att*) were deleted from the packaging construct. In addition, the 5'LTR was substituted by the HCMV-IE promoter, and the 3' LTR was substituted by a polyadenylation signal from the insulin gene.

4.3. HIV-1- based vectors

4.3.1. Early lentiviral vectors derived from HIV-1

Early HIV-1-based vectors (93, 94) were constructed for studies of HIV-1 infectivity rather than gene therapy. These vectors were produced by co-transfection of two expression plasmids: an envelope-defective HIV-1 genome expressing guanine phosphoribosyltransferase (*gpt*) in place of *env* and a plasmid that expressed the envelope glycoprotein. In addition to expression plasmids encoding HIV-1 *env*, Page *et al.* and Landau *et al.* used heterologous Env proteins including amphotropic and ecotropic MoMLV, and human T-cell leukemia virus Type I (HTLV-I) Env glycoproteins. The resulting virions possessed an expanded cell tropism

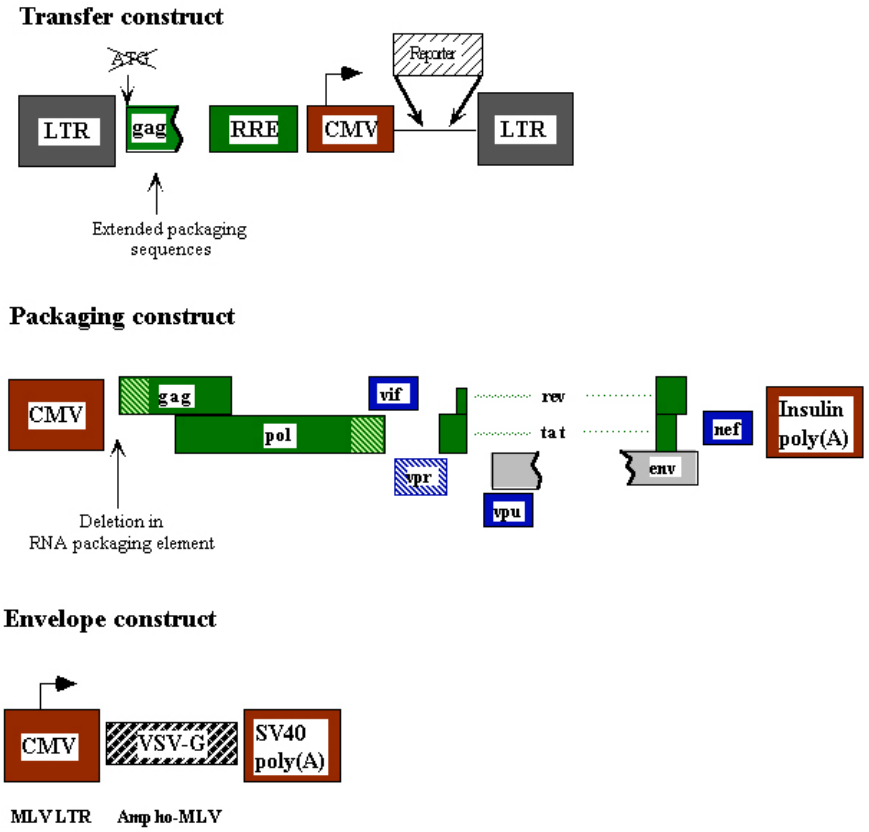

Figure 1. Elements of a prototypical lentiviral vector. A typical lentiviral vector consists of three genetic elements, as shown. Green and blue boxes represent essential and accessory genes, respectively. Hatched areas represent determinants of infection of a non-dividing cell. CMV, cytomegalovirus immediate early promoter; RRE, Rev responsive element. Adapted, with permission, from reference 90.

and host range, as they were able to infect a wide variety of cells including CD4+ and CD4- human cells and murine cells. The viral titers yielded by such systems were less than $2x10^5$ IU/ml. Subsequent studies (95, 96) used similar two-plasmid systems to derive the HIV-1-based vectors which were used to study envelope dependence of the viral hostrange and biological consequences of HIV-1 integration. These vectors were produced by the cotransfection of the *env*-defective HIV-1 bearing the firefly luciferase reporter gene in place of *nef* with a vector expressing the envelope glycoproteins of various HIV-1 strains or amphotropic MLV envelope. The infectivity of the resulting vectors was tested in a variety of cells of primate (e.g. human and simian) and non-primate origin (e.g. murine), and their tropism correlated with that of the virus that gave rise to the *env* gene.

Buchschacher and Panganiban (97) separated the viral genome into two constructs: a packaging construct and a transfer construct. The packaging construct expressed all the proteins, except Env. Env would be required *in trans* for production of infectious viral particles. The transfer construct contained a reporter gene and the *cis*-acting sequences required for packaging, reverse transcription, integration and expression of viral genes. Inclusion of about 700 bp located at the 5' end of *gag* into the transfer construct increased the transduction efficiency of the viral particles. These vectors had low titers of about 10^2 IU/ml.

The use of low-titer vectors for gene therapy can be problematic because of the low transduction efficiency in certain cell types such as hematopoietic cells (98, 99). By using the vesicular stomatitis virus envelope glycoprotein G (VSV-G), vector stocks can be concentrated to titers of about 10^7 IU/ml (85, 100).

4.3.2. First generation of HIV-1-based vectors for gene therapy

Knowledge about the roles of *cis* and *trans*-acting elements of HIV-1 led to the first generation of HIV-1-based vectors intended as a gene delivery vehicles (101). Parolin and coworkers developed a packaging system which segregated viral structural genes into two different plasmids. One of the packaging plasmids expressed *gag*, *pol*, *tat* and *vif* under the control of the HCMV-IE promoter. The second packaging construct expressed *env* and *rev*. In both plasmids, the packaging signal (region between the 5' splice donor site and the initiation codon of *gag*) was deleted and the 3' LTRs were replaced with the polyadenylation sequence from simian virus 40. The transfer constructs contained *cis*-acting sequences

required for efficient transduction and encoded the neomycin phosphotransferase (*neo*^{R}) reporter gene under control of ecotropic MLV LTR. These vectors had titers of approximately $10^{2.2}$ IU/ml. Despite such low titers, these studies provided the conceptual basis for the subsequent development of more efficient vector systems.

Naldini *et al.* (90) described a similar vector system that also utilized three constructs: transfer, packaging and envelope. These vectors yielded titers of about 10^5 IU/ml when pseudotyped with the MLV envelope and about $5X10^5$ IU/ml when pseudotyped with VSV-G. VSV-G pseudotyped HIV-1 vectors successfully transduced growth-arrested HeLa cells and rat 208F fibroblasts and were able to mediate stable *in vivo* transfer into adult rat neurons. However, significantly lower transduction efficiencies were observed when same cells were transduced with MLV pseudotyped HIV-1 vectors. Kafri *et al.* (102) constructed a subsequent vector lacking Vpr and Vif proteins, which efficiently delivered the green fluorescent protein (GFP) gene *in vivo* into rat liver and muscle. Long-term expression of the transgene was observed in the transduced tissues (more than 5 months in the liver and more than 8 weeks in the muscle). No immune response was elicited by the transduced cells, thus making subsequent administration of vector possible.

4.3.3. Role of accessory genes in transduction by HIV-1-derived vectors.

Pathogenesis by lentiviruses is largely attributed to the action of the accessory genes. Thus several groups tested the possibility that accessory genes may be eliminated from lentiviral vectors (42, 103, 104). This was accomplished by creating various packaging constructs with deletions in all accessory genes. Infectious titers and the ability to transduce non-dividing cells were then measured.

High-titer ($2x10^6$ IU/ml) retroviral particles deleted in all accessory genes (*vif, vpu, vpr, nef*) were produced from a system developed by Zufferey *et al.* (42). These vectors were able to mediate efficient *in vitro* transduction into dividing and growth-arrested 293T cells and *in vivo* delivery into adult rat neurons. Although the presence of Vpr was dispensable for transduction of neurons, about 50% reduction was observed when using Vpr(-) vector to transduce macrophages. No significant differences in vector properties were attributable to *nef*, *vif* or *vpr*.

Other groups (103, 104) used multiply attenuated vectors to transduce human hematopoietic stem cells, cardiac myocytes and post-mitotic rat cerebellar neurons. In agreement with the studies by Zufferey *et al.* (42), these studies concluded that viral accessory genes (*vpr*, *vif*, *vpu* and *nef*) are dispensable for efficient gene delivery into non-dividing cells.

4.3.4. Elimination of Tat and Rev functions.

Using a conventional three-plasmid expression system Dull and co-workers (34) created a vector whose packaging construct was reduced to the *gag*, *pol* and *rev* genes. A defect in *tat* function was created by introducing two mutations into the *tat* gene: mutation of the translational initiation codon and insertion of a linker producing a translational stop at codon 46. The requirement for t*at* was overcome by replacing the U3 region of the 5' LTR with the HCMV enhancer/promoter. Surprisingly, the *tat*-deficient phenotype did not affect either the titer or transduction efficiency of the viral particles. These vectors had a titer of about $5x10^6$ IU/ml and efficiently transduced rat neurons *in vivo*. Furthermore, vectors with improved biosafety features (34) were constructed by deleting the *rev* gene from the packaging construct and providing Rev *in trans* on a separate expression plasmid. Co-transfection of the *tat*-free transfer construct, VSV-G construct, a plasmid expressing *gag-pol* and a plasmid expressing *rev*, yielded high-titer viral particles, demonstrating the efficiency of such a split-genome packaging system.

Gasmi and co-workers (105) chose a similar approach to create a safe gene therapy vector: they deleted *rev* from the packaging construct thus reducing it to *gag*, *pol* and *tat*. The requirement for Rev, an essential player in HIV-1 replication, was bypassed by replacing RRE in the packaging construct with the constitutive transport element (CTE) of Mason-Pfizer monkey virus (MPMV). Although the CTE-containing vector allowed Rev/RRE-independent expression of *gag*, its titer on 293T cells was 10-fold lower than that of an RRE-containing vector. This finding indicated that CTE could only partially substitute for the function of Rev/RRE, and that vectors could be generated most efficiently if Rev and RRE were present in the packaging construct.

4.3.5. Conditionally replicating HIV-1-based vectors

The use of Tat and Rev may allow construction of conditionally replicating or regulated gene therapy vectors. A vector was constructed by Buchschacher and Panganiban (97) where the reporter gene was placed upstream of the major 5' splice donor and, therefore, expressed directly from the LTR in a Tat-dependent fashion. Such a vector was able to propagate in HeLa-T4 cells expressing Tat, but not in parental HeLa T4 cells. It was speculated that Tat-dependent vectors can be used in the future to carry anti-HIV genes and, therefore, to treat individuals previously infected with HIV.

Conditionally replicating vectors (crHIV) capable of inhibiting HIV-1 infection were constructed by Dropulic *et al.* (106). These vectors contained deletions in all of the viral genes and encoded a U5-specific hammerhead ribozyme that selectively cleaved HIV-1 RNA. The U5 region of crHIV RNA was rendered ribozyme-resistant by substituting critical oligonucleotides at the cleavage site. Such vectors were only able to replicate in the presence of live HIV-1 and, when co-transfected into Jurkat cells, inhibited viral replication.

4.3.6. HIV-1 packaging cell lines

The lentiviral vectors described above were produced by transient transfection of multiple plasmids. Although relatively high titers of RCR-free virus can be

produced by this method, transient transfection systems have certain drawbacks. First, the infectious titer of the transiently produced vectors is subject to high batch-to-batch variability. Second, the volume of vector particles produced by this method is limited. Thus, stable packaging cell lines have been constructed for large-scale production of replication-defective vectors. These cell lines provide *in trans* all viral proteins required for assembly of viral particles.

In the HIV-1 packaging cell line designed by Corbeau *et al.* (107), the major packaging site, including 37 nucleotides between the major splice donor site and the beginning of *gag* was deleted from HIV-1 genome. The resulting packaging plasmid (Ψ) was co-transfected with a plasmid containing the neo^R gene into HeLa cells and, after selection with G418, a resistant clone named Ψ422 was isolated. Although, the Ψ422 cell line was able to synthesize HIV-1 proteins, virions produced by these cells were noninfectious due to the inability of the genomic RNA to be packaged. To assess gene transfer, Ψ422 cells were transfected with an HIV-1-based transfer vector (101). This vector contained sequences responsible for packaging including the first 653 nucleotides of *gag* and the RRE. Viral particles produced in this manner were free of RCR and were capable of transducing CD4+ HeLa-T4 cells, but the titer was relatively low (approximately $5x10^4$ IU/ml).

A potential problem with the type of packaging cell line described by Corbeau *et al.* (107) is that a single recombination event between the HIV-1 sequences in the packaging cells and the transfer vector could lead to the production of the RCR. To overcome this problem, a split genome approach was developed by Srinivasakumar *et al.* (108). To create the packaging cell line, CMT3-COS were stably transduced with five separate plasmids. One of the plasmids expressed *gag-pol* and *vif* and the remaining plasmids expressed one of the other genes, including *env*, *nef*, *tat* or *rev*. The 5' sequences required for packaging were deleted from each plasmid. When transfected with a transfer vector, the packaging cells produced helper-free viral particles able to infect HeLa-CD4 cells with titers of up to 10^4 IU/ml.

In addition to providing desired safety features, split genome systems allowed an assessment of the effects of individual viral proteins on vector titer by introducing one gene at a time. Thus, in the vector system developed by Srinivasakumar and coworkers, the presence of either Tat or Nef in the packaging cells increased the viral titers about 5- to 10 fold, whereas the presence of Rev was absolutely essential for vector production (108). To circumvent the requirement for Rev and RRE, the constitutive transport element from MPMV was introduced into packaging plasmids replacing RRE. In addition, *rev* was eliminated from the producer cells. Contrary to the results reported by Gasmi *et al.* (105), the titers of the vectors produced by the above Rev-independent packaging cells were similar to those obtained using a Rev-dependent system.

The constitutive expression of HIV-1 proteins (e.g. Vpr and protease) is highly cytotoxic. To overcome this problem, inducible packaging cell lines were developed (109-111). These packaging cell lines produce viral proteins only after the removal of tetracycline from the culture medium and yield low-titer (0.6-2 $x10^3$ IU/ml), RCR- free viral particles upon transfection with an HIV-1 transfer vector.

Packaging cell lines have one common drawback: they produce low vector titers when compared to transiently transfected producer cells. To address this problem, Kafri and co-workers (111) developed a tetracycline-inducible HIV-1 packaging cell line that produced VSV-G pseudotyped vectors. In this system, the VSV-G cDNA and an HIV-1 packaging cassette, which included all HIV-1 genes except *env*, were cloned into tetracycline-inducible vectors and stably transfected into the SODk0 cell line. The resulting HIV-1 vectors displayed a titer of greater than 10^6 IU/ml and could be concentrated by ultracentrifugation to 10^9 IU/ml. Moreover, these vectors were capable of transducing growth-arrested HeLa cells, human embryonic fibroblasts, and adult rat neurons *in vivo*.

4.4. Safety issues regarding design of lentiviral vectors

The most urgent issue regarding the safety of lentiviral vectors is the potential for recombination leading to replication-competent retrovirus (RCR), also referred to as "helper". Generation of helper virus in preparations of replication-defective vectors has been documented in numerous instances involving oncoviruses (112-116). In later generations of vectors in which viral protein-coding regions were split in the packaging cells, the frequency of recombination leading to helper was decreased, but not eliminated (117). Helper virus has the potential for inducing pathogenesis as demonstrated by studies in which monkeys were infused with transduced bone marrow cells after ablation of endogenous marrow with gamma irradiation (112-114). In these studies, helper virus gave rise to lymphoma in monkeys.

Contemporary lentiviral vectors were made virtually helper-free by segregating vector components into three plasmids (see section 4.2.).

Another important safety issue involves the activation of the cellular proto-oncogenes by the inserted provirus (7). The genetic elements that can cause such insertional activation include: promoter, enhancer and the poly(A) site of the virus. For example, leukemia viruses (e.g. ALV) have been shown to induce lymphomas via the activation of the *c-myc* expression by the promoter of the provirus (118, 119). Theoretically, insertion of HIV-1 LTR's might lead to transcriptional activation of heterologous genes. However, this has not been yet demonstrated. To address the issue of insertional activation, self-inactivating (SIN) vectors were developed (120-122). The same principles were applied to recent lentiviral vectors (33, 123). For example, self-inactivating vector described by Miyoshi *et al.* (33) was constructed by deleting 133 bp in the U3 region of the 3' LTR including the TATA box and the binding sites for Sp1 and NF-kB. This deletion not only minimizes expression of vector RNA, but it may also be useful

in preventing the insertional activation of cellular proto-oncogenes by the integrated provirus.

4.5. Vectors based on lentiviruses other than HIV-1

The use of HIV-1-based vectors in human gene therapy faces a number of obstacles due mainly to the fact that HIV-1 is a deadly pathogen. Thus, the possibility of the appearance of RCR in preparations of HIV-1-derived vectors, though remote, has spurred efforts to develop vectors based on other lentiviruses.

4.5.1. HIV-2-based vectors

HIV-2-derived vectors have potential advantages over HIV-1-derived ones such as decreased pathogenicity and segregation of the genes responsible for infection of non-dividing cells (*vpx*) and growth arrest (*vpr*). *Cis*-acting regions of HIV-2 responsible for genomic encapsidation were defined by Poeschla *et al.* (124) who found that deletion of 61 nucleotides from the region between the major 5` splice donor and the *gag* initiation codon prevented HIV-2 packaging and replication, but not expression of viral genes. The packaging plasmid was derived from the apathogenic, HIV-$2_{kr}$, virus by deleting the 61-nucleotide fragment and replacing 3` LTR with the bovine growth hormone polyadenylation signal. Transient co-expression of the packaging construct with VSV-G and a transfer vector bearing lacZ yielded titers of 10^6 IU/ml before concentration and 10^8 IU/ml after concentration. These viral particles were able to efficiently transduce human T and monocytoid cell lines, growth-arrested HeLa cells, human macrophages, NTN2 neurons and human hematopoietic progenitor cells.

Moreover, Poeschla *et al.* used their packaging plasmid to develop an HIV-2 packaging cell line, which was capable of stable expression of HIV-2 structural proteins, but did not produce replication-competent virus (125).

4.5.2. RNA pseudotypes

Retroviral particles which package a heterologous genomic RNA are referred to as RNA pseudotypes (126, 127). RNA pseudotype vectors have the potential advantage or decreased risk for homologous recombination between the packaging and the transfer vectors.

Corbeau and coworkers (128) used the HIV-1 packaging cell line Ψ422 (see section 4.3.6.) and HIV-2 transfer vector. This HIV-1/HIV-2-based vector exhibited titers of 10^4 IU/ml and was capable of transducing non-dividing cells such as primary human macrophages.

An HIV-1/SIV-derived vector was recently described by our laboratory (72). It employs elements from HIV-1 and SIVmac1A11. SIVmacA11 is an infectious molecular clone which is apathogenic in rhesus macaques (129, 130). *In vitro*, SIVmac1A11 infects human and rhesus peripheral blood lymphocytes and monocyte-derived macrophages. A vector in which the packaging construct is based on SIVmac1A11 retains the features of other lentiviral systems (i.e. integration and infection of non-dividing cells) yet it may constitute a safe alternative to HIV-1-derived systems (72).

The RNA-pseudotype between HIV-1 and SIVmac1A11 was produced by transient co-transfection of three vectors. The packaging construct was derived from SIVmac1A11 lacking packaging sequences and expressing all SIV genes, except *env* and *vpr*. The transfer construct was derived from HIV-1, including *cis*-acting sequences required for packaging, reverse transcription and integration. The envelope plasmid encoded the VSV-G glycoprotein. These vectors were free of RCR, displayed titers of $2.3x10^6$ IU/ml and were able to transduce growth arrested HeLa cells, human T-lymphocytes, fetal mouse neurons and human peripheral blood macrophages. In contrast, these non-dividing cells were resistant to infection by the murine leukemia-based vector, LNCX-GFP.

4.5.3. FIV-based vectors

Because FIV is a non-primate lentivirus, it is thought that its pathogenic potential in humans is low. Furthermore, FIV does not productively infect human or other non-feline cells. To explore the possibility of creating an FIV-based vector with extended tropism which could be used for human gene therapy, Poeschla and coworkers (131) used a three-plasmid expression system. The packaging plasmid was constructed by replacing the 5` LTR with the HCMV promoter and the 3` LTR by the bovine growth hormone polyadenylation signal. In addition, 875 nucleotides from the *env* gene were deleted. In the transfer construct, the U3 element within 5` LTR was replaced by fusing the HCMV promoter to the R repeat. A frameshift mutation was introduced into the *gag* gene and either *lacZ* or *GFP* reporter genes driven by the HCMV promoter were inserted. When co-transfected with VSV-G expression plasmid, viral particles were produced that exhibited titers of 10^5 IU/ml and were capable of transducing primary human macrophages and postmitotic NTN2 neurons. Most importantly, this work revealed that the 5` U3 region of FIV limits the productive phase of FIV replication in human cells and that the substitution of this region with the heterologous promoter (HCMV) allows the expression of FIV proteins in human cells.

4.5.4. Other potential lentiviral vectors

Other non-primate lentiviruses are under investigation for potential use as gene delivery vehicles. For example, replication-defective vectors based on Caprine Arthritis Encephalitis Virus (CAEV) were constructed by Mselli-Lakhal *et al.* (132). The CAEV-derived vectors were only tested on goat cells lines and had a low transduction efficiency (3-4 logs lower than that of the parental CAEV). Other potential candidates for use in human gene therapy include equine infectious anemia virus (6) and bovine immunodeficiency virus.

5. PERSPECTIVE

Since the inception of the Human Genome Project in 1990, considerable progress has been made in our understanding of human genetics (133). More disease-related genes are being constantly identified and

characterized widening the range of applications for human gene therapy.

This growing body of knowledge requires development of new and improved gene delivery vectors. Development of "helper" detection assays for lentiviral vectors will be necessary prior to testing in humans. A standard assay for detection of potential replication-competent virus generated in defective lentiviral systems does not exist at the moment. It will be crucial to the safety of novel gene therapy vectors to develop rapid and sensitive assays which will detect such recombinants in vector preparations. We envision that this may be accomplished by the use of indicator cells which show apparent cytopathic effects (syncytium formation) and/or express inducible reporter genes under the control of a lentivirus promoter. Development of a helper assay should be a concerted effort among many groups who specialize in the production and use of lentiviral vectors, so that detection conditions can be standardized.

The use of lentiviral vectors in humans will also require investigators to develop a large-scale production methods which are currently unavailable. Once these obstacles are overcome, the potential for modifications of the human genome using lentiviral vectors is virtually unlimited.

6. REFERENCES

1. Dranoff G.: Cancer gene therapy: connecting basic research with clinical inquiry. *J Clin Oncol*. 16, 2548-2556 (1998)
2. Blomer U., Naldini L., Verma I.M., Trono D. & Gage F.H.: Applications of gene therapy to the CNS. *Hum Mol Genet*. 5 Spec No, 1397-1404 (1996)
3. Bunnell B.A. & Morgan R.A.: Gene therapy for infectious diseases. *Clin Microbiol Rev*. 11, 42-56 (1998)
4. Karlsson S.: Treatment of genetic defects in hematopoietic cell function by gene transfer. *Blood*. 78, 2481-2492 (1991)
5. Whartenby K.A., Abraham G.N., Calabresi P.A., Abboud C.N., Calabresi P., Marrogi A. & Freeman S.M.: Gene-modified cells for the treatment of cancer. *Pharmacol Ther*. 66, 175-190 (1995)
6. Mitchell P.: Vector problems still thwart gene-therapy promise. *Lancet*. 351, 346 (1998)
7. Coffin J.M., Hughes S.H. & Varmus H.E.: Retroviruses. Cold Spring Harbor Laboratory Press (1997)
8. Hermens W.T. & Verhaagen J.: Viral vectors, tools for gene transfer in the nervous system. *Prog Neurobiol*. 55, 399-432 (1998)
9. Hoggan M.D., Blacklow N.R. & Rowe W.P.: Studies of small DNA viruses found in various adenovirus preparations: physical, biological, and immunological characteristics. *Proc Natl Acad Sci U S A*. 55, 1467-1474 (1966)
10. Harel J., Rassart E. & Jolicoeur P.: Cell cycle dependence of synthesis of unintegrated viral DNA in mouse cells newly infected with murine leukemia virus. *Virology*. 110, 202-207 (1981)
11. Coffin J.M.: *Retroviridae*: the viruses and their replication, In: Fields Virology Fields B.N., Knippe, D.M., Howley, P.M., vol. 2. Lippincott-Raven, Philadelphia 1767-1847 (1996)
12. Bukrinsky M.I. & Haffar O.K.: HIV-1 nuclear import: in search of a leader. *Front Biosci*. 2, 578-587 (1997)
13. Narayan O., Wolinsky J.S., Clements J.E., Strandberg J.D., Griffin D.E. & Cork L.C.: Slow virus replication: the role of macrophages in the persistence and expression of visna viruses of sheep and goats. *J Gen Virol*. 59, 345-356 (1982)
14. Trowbridge R.S., Lehmann J., Torchio C. & Brophy P.: Visna virus synthesized in absence of host-cell division and DNA synthesis. *Microbios*. 29, 71-80 (1980)
15. Joag Sanjay V., Stephens E.B. & Narayan O.: Lentiviruses, Third edition ed, vol. 2. Lippincott-Raven Publishers, Philadelphia (1996)
16. Frankel A.D. & Young J.A.: HIV-1: fifteen proteins and an RNA. *Annu Rev Biochem*. 67, 1-25 (1998)
17. Gallay P., Stitt V., Mundy C., Oettinger M. & Trono D.: Role of the karyopherin pathway in human immunodeficiency virus type 1 nuclear import. *J Virol*. 70, 1027-1032 (1996)
18. Gallay P., Swingler S., Song J., Bushman F. & Trono D.: HIV nuclear import is governed by the phosphotyrosine-mediated binding of matrix to the core domain of integrase. *Cell*. 83, 569-576 (1995)
19. Lerner D.L., Wagaman P.C., Phillips T.R., Prospero-Garcia O., Henriksen S.J., Fox H.S., Bloom F.E. & Elder J.H.: Increased mutation frequency of feline immunodeficiency virus lacking functional deoxyuridine-triphosphatase. *Proc Natl Acad Sci U S A*. 92, 7480-7484 (1995)
20. Elder J.H., Lerner D.L., Hasselkus-Light C.S., Fontenot D.J., Hunter E., Luciw P.A., Montelaro R.C. & Phillips T.R.: Distinct subsets of retroviruses encode dUTPase. *J Virol*. 66, 1791-1794 (1992)
21. Koppe B., Menendez-Arias L. & Oroszlan S.: Expression and purification of the mouse mammary tumor virus gag-pro transframe protein p30 and characterization of its dUTPase activity. *J Virol*. 68, 2313-2319 (1994)
22. Threadgill D.S., Steagall W.K., Flaherty M.T., Fuller F.J., Perry S.T., Rushlow K.E., Le Grice S.F. & Payne S.L.: Characterization of equine infectious anemia virus dUTPase: growth properties of a dUTPase-deficient mutant. *J Virol*. 67, 2592-2600 (1993)
23. Elder J.H., Dean G.A., Hoover E.A., Hoxie J.A., Malim M.H., Mathes L., Neil J.C., North T.W., Sparger E., Tompkins M.B., Tompkins W.A., Yamamoto J., Yuhki N., Pedersen N.C. & Miller R.H.: Lessons from the cat: feline immunodeficiency virus as a tool to develop intervention strategies against human immunodeficiency virus type 1. *AIDS Res Hum Retroviruses*. 14, 797-801 (1998)
24. Heguy A.: Inhibition of the HIV Rev transactivator : A new target for therapeutic intervention. *Front Biosci*. 2, d283-297 (1997)
25. Steffy K. & Wong-Staal F.: Genetic regulation of human immunodeficiency virus. *Microbiol Rev*. 55, 193-205 (1991)
26. Liu J., Woffendin C., Yang Z.Y. & Nabel G.J.: Regulated expression of a dominant negative form of Rev

improves resistance to HIV replication in T cells. *Gene Ther*. 1, 32-37 (1994)
27. Zhou C., Bahner I.C., Larson G.P., Zaia J.A., Rossi J.J. & Kohn E.B.: Inhibition of HIV-1 in human T-lymphocytes by retrovirally transduced anti-tat and rev hammerhead ribozymes. *Gene*. 149, 33-39 (1994)
28. Corbeau P. & Wong-Staal F.: Anti-HIV effects of HIV vectors. *Virology*. 243, 268-274 (1998)
29. Bray M., Prasad S., Dubay J.W., Hunter E., Jeang K.T., Rekosh D. & Hammarskjold M.L.: A small element from the Mason-Pfizer monkey virus genome makes human immunodeficiency virus type 1 expression and replication Rev- independent. *Proc Natl Acad Sci U S A*. 91, 1256-1260 (1994)
30. Wei P., Garber M.E., Fang S.M., Fischer W.H. & Jones K.A.: A novel CDK9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA. *Cell*. 92, 451-462 (1998)
31. Pearson L., Garcia J., Wu F., Modesti N., Nelson J. & Gaynor R.: A transdominant tat mutant that inhibits tat-induced gene expression from the human immunodeficiency virus long terminal repeat. *Proc Natl Acad Sci U S A*. 87, 5079-5083 (1990)
32. Modesti N., Garcia J., Debouck C., Peterlin M. & Gaynor R.: Trans-dominant Tat mutants with alterations in the basic domain inhibit HIV-1 gene expression. *New Biol*. 3, 759-768 (1991)
33. Miyoshi H., Blomer U., Takahashi M., Gage F.H. & Verma I.M.: Development of a self-inactivating lentivirus vector. *J Virol*. 72, 8150-8157 (1998)
34. Dull T., Zufferey R., Kelly M., Mandel R.J., Nguyen M., Trono D. & Naldini L.: A third-generation lentivirus vector with a conditional packaging system. *J Virol*. 72, 8463-8471 (1998)
35. Luciw P.A. Human immunodeficiency viruses and their replication, In: *Fields Virology* Fields B.N., Knippe, D.M., Howley, P.M., vol. 2. Lippincott-Raven, Philadelphia 1881-1975 (1996)
36. Sakai H., Shibata R., Sakuragi J., Sakuragi S., Kawamura M. & Adachi A.: Cell-dependent requirement of human immunodeficiency virus type 1 Vif protein for maturation of virus particles. *J Virol*. 67, 1663-1666 (1993)
37. Gabuzda D.H., Lawrence K., Langhoff E., Terwilliger E., Dorfman T., Haseltine W.A. & Sodroski J.: Role of vif in replication of human immunodeficiency virus type 1 in CD4+ T lymphocytes. *J Virol*. 66, 6489-6495 (1992)
38. Blanc D., Patience C., Schulz T.F., Weiss R. & Spire B.: Transcomplementation of VIF- HIV-1 mutants in CEM cells suggests that VIF affects late steps of the viral life cycle. *Virology*. 193, 186-192 (1993)
39. Fouchier R.A., Simon J.H., Jaffe A.B. & Malim M.H.: Human immunodeficiency virus type 1 Vif does not influence expression or virion incorporation of gag-, pol-, and env-encoded proteins. *J Virol*. 70, 8263-8269 (1996)
40. von Schwedler U., Song J., Aiken C. & Trono D.: Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. *J Virol*. 67, 4945-4955 (1993)
41. Madani N. & Kabat D.: An endogenous inhibitor of human immunodeficiency virus in human lymphocytes is overcome by the viral Vif protein. *J Virol*. 72, 10251-10255 (1998)
42. Zufferey R., Nagy D., Mandel R.J., Naldini L. & Trono D.: Multiply attenuated lentiviral vector achieves efficient gene delivery in vivo. *Nat Biotechnol*. 15, 871-875 (1997)
43. Kim V.N., Mitrophanous K., Kingsman S.M. & Kingsman A.J.: Minimal requirement for a lentivirus vector based on human immunodeficiency virus type 1. *J Virol*. 72, 811-816 (1998)
44. Ogawa K., Shibata R., Kiyomasu T., Higuchi I., Kishida Y., Ishimoto A. & Adachi A.: Mutational analysis of the human immunodeficiency virus vpr open reading frame. *J Virol*. 63, 4110-4114 (1989)
45. Cohen E.A., Terwilliger E.F., Jalinoos Y., Proulx J., Sodroski J.G. & Haseltine W.A.: Identification of HIV-1 vpr product and function. *J Acquir Immune Defic Syndr*. 3, 11-18 (1990)
46. Heinzinger N.K., Bukinsky M.I., Haggerty S.A., Ragland A.M., Kewalramani V., Lee M.A., Gendelman H.E., Ratner L., Stevenson M. & Emerman M.: The Vpr protein of human immunodeficiency virus type 1 influences nuclear localization of viral nucleic acids in nondividing host cells. *Proc Natl Acad Sci U S A*. 91, 7311-7315 (1994)
47. Balotta C., Lusso P., Crowley R., Gallo R.C. & Franchini G.: Antisense phosphorothioate oligodeoxynucleotides targeted to the vpr gene inhibit human immunodeficiency virus type 1 replication in primary human macrophages. *J Virol*. 67, 4409-4414 (1993)
48. Connor R.I., Chen B.K., Choe S. & Landau N.R.: Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. *Virology*. 206, 935-944 (1995)
49. He J., Choe S., Walker R., Di Marzio P., Morgan D.O. & Landau N.R.: Human immunodeficiency virus type 1 viral protein R (Vpr) arrests cells in the G2 phase of the cell cycle by inhibiting p34cdc2 activity. *J Virol*. 69, 6705-6711 (1995)
50. Jowett J.B., Planelles V., Poon B., Shah N.P., Chen M.L. & Chen I.S.: The human immunodeficiency virus type 1 vpr gene arrests infected T cells in the G2 + M phase of the cell cycle. *J Virol*. 69, 6304-6313 (1995)
51. Poon B., Grovit-Ferbas K., Stewart S.A. & Chen I.S.Y.: Cell cycle arrest by Vpr in HIV-1 virions and insensitivity to antiretroviral agents. *Science*. 281, 266-269 (1998)
52. Rogel M.E., Wu L.I. & Emerman M.: The human immunodeficiency virus type 1 vpr gene prevents cell proliferation during chronic infection. *J Virol*. 69, 882-888 (1995)
53. Planelles V., Jowett J.B.M., Li Q.X., Xie Y., Hahn B. & Chen I.S.Y.: Vpr-induced cell cycle arrest is conserved among primate lentiviruses. *J Virol*. 70, 2516-2524 (1996)
54. Stewart S.A., Poon B., Jowett J.B. & Chen I.S.: Human immunodeficiency virus type 1 Vpr induces apoptosis following cell cycle arrest. *J Virol*. 71, 5579-5592 (1997)
55. Klimkait T., Strebel K., Hoggan M.D., Martin M.A. & Orenstein J.M.: The human immunodeficiency virus type 1-specific protein vpu is required for efficient virus maturation and release. *J Virol*. 64, 621-629 (1990)
56. Gottlinger H.G., Dorfman T., Cohen E.A. & Haseltine W.A.: Vpu protein of human immunodeficiency virus type

1 enhances the release of capsids produced by gag gene constructs of widely divergent retroviruses. *Proc Natl Acad Sci U S A*. 90, 7381-7385 (1993)
57. Kerkau T., Bacik I., Bennink J.R., Yewdell J.W., Hunig T., Schimpl A. & Schubert U.: The human immunodeficiency virus type 1 (HIV-1) Vpu protein interferes with an early step in the biosynthesis of major histocompatibility complex (MHC) class I molecules. *J Exp Med*. 185, 1295-1305 (1997)
58. Kestler H.W., Ringler D.J., Panicaly D.L., Sehgal P.K., Daniel M.D. & Desrosiers R.C.: Importance of the *nef* gene for maintenance of high virus load and for development of AIDS. *Cell*. 65, 651-662 (1991)
59. Piguet V., Gu F., Foti M., Demaurex N., Gruenberg J., Carpentier J.L. & Trono D.: Nef-induced CD4 degradation: a diacidic-based motif in Nef functions as a lysosomal targeting signal through the binding of beta-COP in endosomes. *Cell*. 97, 63-73 (1999)
60. Collins K.L., Chen B.K., Kalams S.A., Walker B.D. & Baltimore D.: HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature*. 391, 397-401 (1998)
61. Miller M.D., Warmerdam M.T., Page K.A., Feinberg M.B. & Greene W.C.: Expression of the human immunodeficiency virus type 1 (HIV-1) nef gene during HIV-1 production increases progeny particle infectivity independently of gp160 or viral entry. *J Virol*. 69, 579-584 (1995)
62. Goldsmith M.A., Warmerdam M.T., Atchison R.E., Miller M.D. & Greene W.C.: Dissociation of the CD4 downregulation and viral infectivity enhancement functions of human immunodeficiency virus type 1 Nef. *J Virol*. 69, 4112-4121 (1995)
63. Chowers M.Y., Pandori M.W., Spina C.A., Richman D.D. & Guatelli J.C.: The growth advantage conferred by HIV-1 nef is determined at the level of viral DNA formation and is independent of CD4 downregulation. *Virology*. 212, 451-457 (1995)
64. Aiken C. & Trono D.: Nef stimulates human immunodeficiency virus type 1 proviral DNA synthesis. *J Virol*. 69, 5048-5056 (1995)
65. Schwartz O., Marechal V., Danos O. & Heard J.M.: Human immunodeficiency virus type 1 Nef increases the efficiency of reverse transcription in the infected cell. *J Virol*. 69, 4053-4059 (1995)
66. Aiken C.: Pseudotyping human immunodeficiency virus type 1 (HIV-1) by the glycoprotein of vesicular stomatitis virus targets HIV-1 entry to an endocytic pathway and suppresses both the requirement for Nef and the sensitivity to cyclosporin A. *J Virol*. 71, 5871-5877 (1997)
67. Li F., Puffer B.A. & Montelaro R.C.: The S2 gene of equine infectious anemia virus is dispensable for viral replication in vitro. *J Virol*. 72, 8344-8348 (1998)
68. Gonda M.A., Luther D.G., Fong S.E. & Tobin G.J.: Bovine immunodeficiency virus: molecular biology and virus-host interactions. *Virus Res*. 32, 155-181 (1994)
69. Gallay P., Hope T., Chin D. & Trono D.: HIV-1 infection of nondividing cells through the recognition of integrase by the importin/karyopherin pathway. *Proc Natl Acad Sci U S A*. 94, 9825-9830 (1997)
70. Gorlich D. & Mattaj I.W.: Nucleocytoplasmic transport. *Science*. 271, 1513-1518 (1996)
71. Vodicka M.A., Koepp D.M., Silver P.A. & Emerman M.: HIV-1 Vpr interacts with the nuclear transport pathway to promote macrophage infection. *Genes Dev*. 12, 175-185 (1998)
72. White S.M., Renda M., Nam N.Y., Klimatcheva E., Zhu Y., Fisk J., Halterman M., Rimel B.J., Federoff H., Pandya S., Rosenblatt J.D. & Planelles V.: Lentivirus vectors using human and simian immunodeficiency virus elements. *J Virol*. 73, 2832-2840 (1999)
73. Zaitseva M., Blauvelt A., Lee S., Lapham C.K., Klaus-Kovtun V., Mostowski H., Manischewitz J. & Golding H.: Expression and function of CCR5 and CXCR4 on human Langerhans cells and macrophages: implications for HIV primary infection. *Nat Med*. 3, 1369-1375 (1997)
74. Lowe J., MacLennan K.A., Powe D.G., Pound J.D. & Palmer J.B.: Microglial cells in human brain have phenotypic characteristics related to possible function as dendritic antigen presenting cells. *J Pathol*. 159, 143-149 (1989)
75. Bell J.E.: The neuropathology of adult HIV infection. *Rev Neurol (Paris)*. 154, 816-829 (1998)
76. Albright A.V., Shieh J.T., Itoh T., Lee B., Pleasure D., O'Connor M.J., Doms R.W. & Gonzalez-Scarano F.: Microglia express CCR5, CXCR4, and CCR3, but of these, CCR5 is the principal coreceptor for human immunodeficiency virus type 1 dementia isolates. *J Virol*. 73, 205-213 (1999)
77. Willett B.J., Hosie M.J., Jarrett O. & Neil J.C.: Identification of a putative cellular receptor for feline immunodeficiency virus as the feline homologue of CD9. *Immunology*. 81, 228-233 (1994)
78. Willett B.J., Picard L., Hosie M.J., Turner J.D., Adema K. & Clapham P.R.: Shared usage of the chemokine receptor CXCR4 by the feline and human immunodeficiency viruses. *J Virol*. 71, 6407-6415 (1997)
79. Maddon P.J., McDougal J.S., Clapham P.R., Dalgleish A.G., Jamal S., Weiss R.A. & Axel R.: HIV infection does not require endocytosis of its receptor, CD4. *Cell*. 54, 865-874 (1988)
80. Schnitzer T.J., Weiss R.A. & Zavada J.: Pseudotypes of vesicular stomatitis virus with the envelope properties of mammalian and primate retroviruses. *J Virol*. 23, 449-454 (1977)
81. Witte O.N. & Baltimore D.: Mechanism of formation of pseudotypes between vesicular stomatitis virus and murine leukemia virus. *Cell*. 11, 505-511 (1977)
82. Kang C.Y. & Lambright P.: Pseudotypes of vesicular stomatitis virus with the mixed coat of reticuloendotheliosis virus and vesicular stomatitis virus. *J Virol*. 21, 1252-1255 (1977)
83. Mastromarino P., Conti C., Goldoni P., Hauttecoeur B. & Orsi N.: Characterization of membrane components of the erythrocyte involved in vesicular stomatitis virus attachment and fusion at acidic pH. *J Gen Virol*. 68, 2359-2369 (1987)
84. Burns J.C., Friedmann T., Driever W., Burrascano M. & Yee J.K.: 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 U S A*. 90, 8033-8037 (1993)
85. Akkina R.K., Walton R.M., Chen M.L., Li Q.X., Planelles V. & Chen I.S.: 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. *J Virol*. 70, 2581-2585 (1996)
86. Sakai H., Kawamura M., Sakuragi J., Sakuragi S., Shibata R., Ishimoto A., Ono N., Ueda S. & Adachi A.: Integration is essential for efficient gene expression of human immunodeficiency virus type 1. *J Virol*. 67, 1169-1174 (1993)
87. Masuda T., Planelles V., Krogstad P. & Chen I.: Genetic analysis of human immunodeficiency virus type 1 integrase and the U3 att site: unusual phenotype of mutants in the zinc finger-like domain. *J Virol*. 69, 6687-6696 (1995)
88. Withers-Ward E.S., Kitamura Y., Barnes J.P. & Coffin J.M.: Distribution of targets for avian retrovirus DNA integration in vivo. *Genes Dev*. 8, 1473-1487 (1994)
89. Bushman F.D. & Miller M.D.: Tethering human immunodeficiency virus type 1 preintegration complexes to target DNA promotes integration at nearby sites. *J Virol*. 71, 458-464 (1997)
90. Naldini L., Blomer U., Gallay P., Ory D., Mulligan R., Gage F.H., Verma I.M. & Trono D.: In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science*. 272, 263-267 (1996)
91. Goldman M.J., Lee P.S., Yang J.S. & Wilson J.M.: Lentiviral vectors for gene therapy of cystic fibrosis. *Hum Gene Ther*. 8, 2261-2268 (1997)
92. Miller N. & Whelan J.: Progress in transcriptionally targeted and regulatable vectors for genetic therapy. *Hum Gene Ther*. 8, 803-815 (1997)
93. Page K.A., Landau N.R. & Littman D.R.: Construction and use of a human immunodeficiency virus vector for analysis of virus infectivity. *J Virol*. 64, 5270-5276 (1990)
94. Landau N.R., Page K.A. & Littman D.R.: Pseudotyping with human T-cell leukemia virus type I broadens the human immunodeficiency virus host range. *J Virol*. 65, 162-169 (1991)
95. Camerini D., Planelles V. & Chen I.: CD26 antigen and HIV fusion? *Science*. 264, 1160-1161 (1994)
96. Planelles V., Bachelerie F., Jowett J.B.M., Haislip A., Xie Y., Banooni P., Masuda T. & Chen I.S.Y.: Fate of the human immunodeficiency virus type 1 provirus in infected cells: a role for vpr. *J Virol*. 69, 5883-5889 (1995)
97. Buchschacher G.L., Jr. & Panganiban A.T.: Human immunodeficiency virus vectors for inducible expression of foreign genes. *J Virol*. 66, 2731-2739 (1992)
98. Hock R.A. & Miller A.D.: Retrovirus-mediated transfer and expression of drug resistance genes in human haematopoietic progenitor cells. *Nature*. 320, 275-277 (1986)
99. Hogge D.E. & Humphries R.K.: Gene transfer to primary normal and malignant human hemopoietic progenitors using recombinant retroviruses. *Blood*. 69, 611-617 (1987)
100. Reiser J., Harmison G., Kluepfel-Stahl S., Brady R.O., Karlsson S. & Schubert M.: Transduction of nondividing cells using pseudotyped defective high- titer HIV type 1 particles. *Proc Natl Acad Sci U S A*. 93, 15266-15271 (1996)
101. Parolin C., Dorfman T., Palu G., Gottlinger H. & Sodroski J.: Analysis in human immunodeficiency virus type 1 vectors of cis-acting sequences that affect gene transfer into human lymphocytes. *J Virol*. 68, 3888-3895 (1994)
102. Kafri T., Blomer U., Peterson D.A., Gage F.H. & Verma I.M.: Sustained expression of genes delivered directly into liver and muscle by lentiviral vectors. *Nat Genet*. 17, 314-317 (1997)
103. Uchida N., Sutton R.E., Friera A.M., He D., Reitsma M.J., Chang W.C., Veres G., Scollay R. & Weissman I.L.: HIV, but not murine leukemia virus, vectors mediate high efficiency gene transfer into freshly isolated G0/G1 human hematopoietic stem cells. *Proc Natl Acad Sci U S A*. 95, 11939-11944 (1998)
104. Mochizuki H., Schwartz J.P., Tanaka K., Brady R.O. & Reiser J.: High-titer human immunodeficiency virus type 1-based vector systems for gene delivery into nondividing cells. *J Virol*. 72, 8873-8883 (1998)
105. Gasmi M., Glynn J., Jin M.J., Jolly D.J., Yee J.K. & Chen S.T.: Requirements for efficient production and transduction of human immunodeficiency virus type 1-based vectors. *J Virol*. 73, 1828-1834 (1999)
106. Dropulic B., Hermankova M. & Pitha P.M.: A conditionally replicating HIV-1 vector interferes with wild-type HIV- 1 replication and spread. *Proc Natl Acad Sci U S A*. 93, 11103-11108 (1996)
107. Corbeau P., Kraus G. & Wong-Staal F.: Efficient gene transfer by a human immunodeficiency virus type 1 (HIV- 1)-derived vector utilizing a stable HIV packaging cell line. *Proc Natl Acad Sci U S A*. 93, 14070-14075 (1996)
108. Srinivasakumar N., Chazal N., Helga-Maria C., Prasad S., Hammarskjold M.L. & Rekosh D.: The effect of viral regulatory protein expression on gene delivery by human immunodeficiency virus type 1 vectors produced in stable packaging cell lines. *J Virol*. 71, 5841-5848 (1997)
109. Yu H., Rabson A.B., Kaul M., Ron Y. & Dougherty J.P.: Inducible human immunodeficiency virus type 1 packaging cell lines. *J Virol*. 70, 4530-4537 (1996)
110. Kaul M., Yu H., Ron Y. & Dougherty J.P.: Regulated lentiviral packaging cell line devoid of most viral cis-acting sequences. *Virology*. 249, 167-174 (1998)
111. Kafri T., van Praag H., Ouyang L., Gage F.H. & Verma I.M.: A packaging cell line for lentivirus vectors. *J Virol*. 73, 576-584 (1999)
112. Purcell D.F., Broscius C.M., Vanin E.F., Buckler C.E., Nienhuis A.W. & Martin M.A.: An array of murine leukemia virus-related elements is transmitted and expressed in a primate recipient of retroviral gene transfer. *J Virol*. 70, 887-897 (1996)
113. Donahue R.E., Kessler S.W., Bodine D., McDonagh K., Dunbar C., Goodman S., Agricola B., Byrne E., Raffeld M., Moen R., Bacher J., Zsebo K.M. & Nienhuis A.W.: Helper virus induced T cell lymphoma in nonhuman primates after retroviral mediated gene transfer. J Exp Med. 176, 1125-1135 (1992)
114. Vanin E.F., Kaloss M., Broscius C. & Nienhuis A.W.: Characterization of replication-competent retroviruses from nonhuman primates with virus-induced T-cell lymphomas

and observations regarding the mechanism of oncogenesis. *J Virol*. 68, 4241-4250 (1994)
115. Markowitz D., Goff S. & Bank A.: Construction and use of a safe and efficient amphotropic packaging cell line. *Virology*. 167, 400-406 (1988)
116. Chong H. & Vile R.G.: Replication-competent retrovirus produced by a 'split-function' third generation amphotropic packaging cell line. *Gene Ther*. 3, 624-629 (1996)
117. Otto E., Jones-Trower A., Vanin E.F., Stambaugh K., Mueller S.N., Anderson W.F. & McGarrity G.J.: Characterization of a replication-competent retrovirus resulting from recombination of packaging and vector sequences. *Hum Gene Ther*. 5, 567-575 (1994)
118. Fung Y.K., Fadly A.M., Crittenden L.B. & Kung H.J.: On the mechanism of retrovirus-induced avian lymphoid leukosis: deletion and integration of the proviruses. *Proc Natl Acad Sci U S A*. 78, 3418-3422 (1981)
119. Westaway D., Payne G. & Varmus H.E.: Proviral deletions and oncogene base-substitutions in insertionally mutagenized c-myc alleles may contribute to the progression of avian bursal tumors. *Proc Natl Acad Sci U S A*. 81, 843-847 (1984)
120. Dougherty J.P. & Temin H.M.: A promoterless retroviral vector indicates that there are sequences in U3 required for 3' RNA processing. *Proc Natl Acad Sci U S A*. 84, 1197-1201 (1987)
121. Yu S.F., von Ruden T., Kantoff P.W., Garber C., Seiberg M., Ruther U., Anderson W.F., Wagner E.F. & Gilboa E.: Self-inactivating retroviral vectors designed for transfer of whole genes into mammalian cells. *Proc Natl Acad Sci U S A*. 83, 3194-3198 (1986)
122. Miller A.D. Development and application of retroviral vectors, In: *Retroviruses* Coffin J.M. and Hughes S.H. & Varmus H.E., vol. . Cold Spring Harbor Laboratory Press, Cold Spring Harbor 437-473 (1997)
123. Zufferey R., Dull T., Mandel R.J., Bukovsky A., Quiroz D., Naldini L. & Trono D.: Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. *J Virol*. 72, 9873-9880 (1998)
124. Poeschla E., Gilbert J., Li X., Huang S., Ho A. & Wong-Staal F.: Identification of a human immunodeficiency virus type 2 (HIV-2) encapsidation determinant and transduction of nondividing human cells by HIV-2-based lentivirus vectors. *J Virol*. 72, 6527-6536 (1998)
125. Poeschla E., Corbeau P. & Wong-Staal F.: Development of HIV vectors for anti-HIV gene therapy. *Proc Natl Acad Sci U S A*. 93, 11395-11399 (1996)
126. Rizvi T.A. & Panganiban A.T.: Simian immunodeficiency virus RNA is efficiently encapsidated by human immunodeficiency virus type 1 particles. *J Virol*. 67, 2681-2688 (1993)
127. Embretson J.E. & Temin H.M.: Lack of competition results in efficient packaging of heterologous murine retroviral RNAs and reticuloendotheliosis virus encapsidation- minus RNAs by the reticuloendotheliosis virus helper cell line. *J Virol*. 61, 2675-2683 (1987)
128. Corbeau P., Kraus G. & Wong-Staal F.: Transduction of human macrophages using a stable HIV-1/HIV-2-derived gene delivery system. *Gene Ther*. 5, 99-104 (1998)
129. Marthas M.L., Sutjipto S., Higgins J., Lohman B., Torten J., Luciw P.A., Marx P.A. & Pedersen N.C.: Immunization with a live, attenuated simian immunodeficiency virus (SIV) prevents early disease but not infection in rhesus macaques challenged with pathogenic SIV. *J Virol*. 64, 3694-3700 (1990)
130. Marthas M.L., Ramos R.A., Lohman B.L., Van Rompay K.K., Unger R.E., Miller C.J., Banapour B., Pedersen N.C. & Luciw P.A.: Viral determinants of simian immunodeficiency virus (SIV) virulence in rhesus macaques assessed by using attenuated and pathogenic molecular clones of SIVmac. *J Virol*. 67, 6047-6055 (1993)
131. Poeschla E.M., Wong-Staal F. & Looney D.J.: Efficient transduction of nondividing human cells by feline immunodeficiency virus lentiviral vectors. *Nat Med*. 4, 354-357 (1998)
132. Mselli-Lakhal L., Favier C., Da Silva Teixeira M.F., Chettab K., Legras C., Ronfort C., Verdier G., Mornex J.F. & Chebloune Y.: Defective RNA packaging is responsible for low transduction efficiency of CAEV-based vectors. *Arch Virol*. 143, 681-695 (1998)
133. Guyer M.S. & Collins F.S.: How is the Human Genome Project doing, and what have we learned so far? *Proc Natl Acad Sci U S A*. 92, 10841-10848 (1995)
134. Berchtold S., Hornung U. & Aepinus C.: The activation domain of simian immunodeficiency virus SIVmac239 Rev protein is structurally and functionally analogous to the HIV-1 Rev activation domain. *Virology*. 211, 285-289 (1995)
135. Dillon P.J., Nelbock P., Perkins A. & Rosen C.A.: Function of the human immunodeficiency virus types 1 and 2 Rev proteins is dependent on their ability to interact with a structured region present in env gene mRNA. *J Virol*. 64, 4428-4437 (1990)
136. Le S.Y., Malim M.H., Cullen B.R. & Maizel J.V.: A highly conserved RNA folding region coincident with the Rev response element of primate immunodeficiency viruses. *Nucleic Acids Res*. 18, 1613-1623 (1990)
137. Olsen H.S., Beidas S., Dillon P., Rosen C.A. & Cochrane A.W.: Mutational analysis of the HIV-1 Rev protein and its target sequence, the Rev responsive element. *J Acquir Immune Defic Syndr*. 4, 558-567 (1991)
138. Chen J., Ido E., Jin M., Kuwata T., Igarashi T., Mizuno A., Koyanagi Y. & Hayami M.: Replication of human immunodeficiency virus type 1 (HIV-1), simian immunodeficiency virus strain mac (SIVmac) and chimeric HIV-1/SIVmac viruses having env genes derived from macrophage-tropic viruses: an indication of different mechanisms of macrophage-tropism in human and monkey cells. *J Gen Virol*. 79, 741-745 (1998)
139. Liu H., Wu X., Newman M., Shaw G.M., Hahn B.H. & Kappes J.C.: The Vif protein of human and simian immunodeficiency viruses is packaged into virions and associates with viral core structures. *J Virol*. 69, 7630-7638 (1995)
140. Park I.W., Myrick K. & Sodroski J.: Effects of vif mutations on cell-free infectivity and replication of simian immunodeficiency virus. *J Acquir Immune Defic Syndr*. 7, 1228-1236 (1994)
141. Zou J.X. & Luciw P.A.: The requirement for Vif of SIVmac is cell-type dependent. *J Gen Virol*. 77, 427-434 (1996)

142. Yamamoto Y., Saito Y., Iida S., Asano J., Sone S. & Adachi A.: Functional analysis of vif genes derived from various primate immunodeficiency viruses. *Virus Genes*. 14, 195-200 (1997)
143. Fletcher T.M., Brichacek B., Sharova N., Newman M.A., Stivahtis G., Sharp P.M., Emerman M., Hahn B.H. & Stevenson M.: Nuclear import and cell cycle arrest functions of the HIV-1 Vpr protein are encoded by two separate genes in HIV-2/SIV(SM). *Embo J*. 15, 6155-6165 (1996)
144. Shibata R., Sakai H., Ogawa K., Ishimoto A. & Adachi A.: Comparative studies on tat mutants of three primate lentiviruses. *Arch Virol*. 114, 243-250 (1990)
145. Viglianti G.A., Sharma P.L. & Mullins J.I.: Simian immunodeficiency virus displays complex patterns of RNA splicing. *J Virol*. 64, 4207-4216 (1990)
146. Jamieson B.D., Aldrovandi G.M., Planelles V., Jowett J.B., Gao L., Bloch L.M., Chen I.S. & Zack J.A.: Requirement of human immunodeficiency virus type 1 nef for in vivo replication and pathogenicity. *J Virol*. 68, 3478-3485 (1994)
147. Miyoshi H., Takahashi M., Gage F.H. & Verma I.M.: Stable and efficient gene transfer into the retina using an HIV-based lentiviral vector. *Proc Natl Acad Sci U S A*. 94, 10319-10323 (1997)
148. Sutton R.E., Wu H.T., Rigg R., Bohnlein E. & Brown P.O.: Human immunodeficiency virus type 1 vectors efficiently transduce human hematopoietic stem cells. *J Virol*. 72, 5781-5788 (1998)
149. Johnston J. & Power C.: Productive infection of human peripheral blood mononuclear cells by feline immunodeficiency virus: implications for vector development. *J Virol*. 73, 2491-2498 (1999)

Key words: HIV, Lentivirus, Vector, Gene therapy, SIV, FIV, Retrovirus.

Send correspondence to: Vicente Planelles, PhD, Departments of Medicine and Microbiology & Immunology, University of Rochester Cancer Center, 601 Elmwood Avenue, Box 704, Rochester, NY 14642, Tel: 716-273-4474, Fax:716-273-1042, E-mail: vicente_planelles@urmc.rocheste

Received 4/28/99 Accepted 5/5/99