# New Developments in Lentiviral Vectors
# Nouveaux Développements des Vecteurs Lentiviraux

Jacques Mallet
CNRS UMR 7091 UNIVERSITE PARIS 6
Hôpital de la Pitié Salpêtrière, Paris France

HIV and other lentiviral vectors are able to transduce both dividing and non-dividing cells through their ability to cross the nuclear membrane

Lentiviral vectors derived from HIV have been developed and tested on a wide range of cell types, both *ex vivo* and *in vivo*. These vectors, which are able to carry about 8kb of exogenous DNA can transduce organs including the CNS and hematopoietic cells. Most importantly, they are able to transduce post-mitotic cells and sustain long-term expression of the transgene *in vivo*. Their easy high-titer stock production, their high transduction efficiency for most eukaryotic cells and the possibility to pseudotype their envelope to modify their infection specificity make them powerful tools for gene transfer.

The process for producing lentiviral relies on transcomplementation of the structural genes and the envelope needed for encapsidation of the recombinant genome. To improve biosafety, the viral transcomplementing genome nedds to be deficient in the psi encapsidation signal, both 5' and 3' LTRs and most of the accessory genes of the wild-type virus genome. The only viral elements that may remain in the vector plasmid (which contains the transgene expression cassette) are: the LTRs, the psi encapsidation signal, the central flap sequence and the RRE (Rev Responsive Element). The promoter and the transgene are inserted downstream from the RRE sequence. The recombinant particles are obtained by transitory co-transfection of the vector plasmid and the two plasmids carrying the essential structural genes in 293T cells.

In collaboration with Pierre Charneau (Institut Pasteur, Paris) we have studied the effect of the viral sequence between the central polypurine tract (cPPT) and the central termination sequence (CTS) on the efficiency of lentiviral transduction *ex vivo* and *in vivo* in the CNS. This sequence is conserved in all lentiviruses and determines a retrotranscription mechanism different from that of oncoretroviruses. The final product of lentiviral retrotranscription is a linear DNA with a central structure presenting a strand overlap of one hundred base pairs, called the central flap (or triplex). This central flap determines a specific mechanism for nuclear import of the proviral preintegration complex. Most interestingly, we have shown in all cells tested that the presence of the central *flap* sequence resulted in a transduction efficiency two to five times higher than that of vectors without a central triplex.

We will more specifically discuss several aspects of lentiviral vectors pertaining first to the regulation of the transgenes with a particular attention to RNA interference. Then, we will present results and strategies which aim to resolve the problem of the potential mutagenesis of lentiviral vectors.

**Regulation by small inducer molecules.**

The use of gene transfer as a therapeutic tool requires the development of vectors that permit the control of the expression of the therapeutic gene by administration of small inducer molecules. The treatment could then be adapted to the needs of the patient and, should complications arise, the therapy could be stopped or interrupted. Optimized vectors that only need to be applied to

the specific patient in minimal amounts would contribute further to the safety of gene transfer for human therapy. Therefore, we constructed and characterized a single optimized lentivirus vector allowing doxycycline-regulated expression of transgenes. Transgene expression from this vector can be regulated *in vitro* and *in vivo* over two orders of magnitude by administration and withdrawal of doxycycline. Thus, the vector may be useful for many applications in gene therapy research.

**RNA interference**

RNA interference (RNAi) mediated by expression of short hairpin RNAs (shRNAs) is a powerful tool for efficiently suppressing the expression of target genes. The approach allows studies of the function of individual genes and may also be applied to human therapy. However, in many instances regulation of RNAi by administration of a small inducer molecule will be required. To date, the development of appropriate regulatory systems has been hampered by the few possibilities for modification within RNA polymerase III promoters capable of driving efficient expression of shRNAs. We have developed an inducible minimal RNA polymerase III promoter that is activated by a novel recombinant transactivator in the presence of doxycycline. The recombinant transactivator and the engineered promoter together form a system permitting regulation of RNAi by Dox-induced expression of shRNAs. Regulated RNAi was mediated by one single lentiviral vector and allowed inhibition of p53 and green fluorescent protein (GFP) in wild type HEK 293T cells and in a HEK 293T derived cell line thereby stably expressing the GFP transgene, respectively. RNA interference was induced in a dose- and time-dependent manner by administration of Dox, silenced the expression of both target genes by 90% and was in particular reversible after withdrawal of Dox.

**Solving the problem of potential insertional mutagenesis of lentiviral vectors:**

- **Expression of a transgene via a non-integrative lentiviral vector**

Classical oncoretrovirus and lentivirus based vectors used for gene transfer integrate into the host genome and allow a long term expression of a therapeutic protein. But this random integration induces a risk of insertional mutagenesis, a phenomenon that is known to have occurred in one SCID X clinical trial. Although the insertional mutagenesis risk is theoritically very low, it does represent a real concern when using, for gene therapy, vectors that integrate pseudo-randomly in the human genome. Developing a lentiviral-based vector that allows a targeted integration of the transgene or does not integrate at all would thus provide an important step toward the development of fully safe vectors for gene therapy.

We have introduced mutations into the integrase gene affecting the catalytic activity of the enzyme. This mutated integrase gene has been introduced in the transcomplementation plasmid in place of the wild-type integrase gene. With this plasmid it is possible to produce lentiviral vectors (derived from HIV-1) containing a mutated IN (mIN vectors).

These vectors have been tested and compared to integrating lentiviral vectors. The mIN vectors are able to efficiently transduce dividing cell lines and primary cells. However, the expression of the transgene is abolished following the division of the transduced cells, as opposed to what happens with integrating vectors. This expected result suggests that the mIN vectors are able to transduce cells but that they remain in an episomal form (1LTR and 2LTR circles) that is lost by dilution through cell divisions. In non-dividing cells (primary cells and *in vivo*), the expression of the transgene is stable (longest time-point tested: 6 months). These vectors represent a step

forward clinically despite that the fact that the expression level of the transgene is lower (3 to 5 folds) when using these vectors compared to the commonly used integrative lentiviral vectors.

- **Development of novel lentiviral vectors whose integration is directed to a selected locus.**

Strategies that allow the site-targeted integration of a transgene by directing the integration of the vector genome into a "neutral" locus could be used for safe gene therapy. Moreover, such strategies would allow the generation of targeted *null* mutations in any cell type (*in vitro* or *in vivo*) and would thus open the way to non-reversible somatic and germinal mutagenesis.

Such objectives could be met through the generation of lentiviral vectors for which the integrase specificity will be modified Or through the use of unidirectional site-specific recombinases. These strategies will be discussed.