

255

Mutations in the HIV-1 -1 frameshift signal: affecting frameshift, viral infectivity and replication

Dominic Dulude¹, Yamina Berchiche^{1,2}, Léa Brakier-Gingras¹
& Nikolaus Heveker^{1,2}.

¹Département de Biochimie, Université de Montréal, et

²Centre de Recherche, Hôpital Ste-Justine,
Montréal, Québec, Canada, H3T 1J4.

lea.brakier.gingras@umontreal.ca

Phone : 514 343 6316; Fax : 514 343 2210.

nikolaus.heveker@recherche-ste-justine.qc.ca

Phone : 514 345 4931 ext. 4190; Fax : 514 345 4801.

Background

The Gag-Pol polyprotein of the human immunodeficiency virus type-1 (HIV-1), which is the precursor of the virus enzymatic activities, is produced via a programmed -1 translational frameshift.

Rationale

We introduced mutations within the slippery sequence of the viral genome, where the frameshift occurs, or in the frameshift stimulatory signal, a downstream irregular stem-loop that controls the frameshift efficiency and we investigated the effect of these mutations on the virus replication.

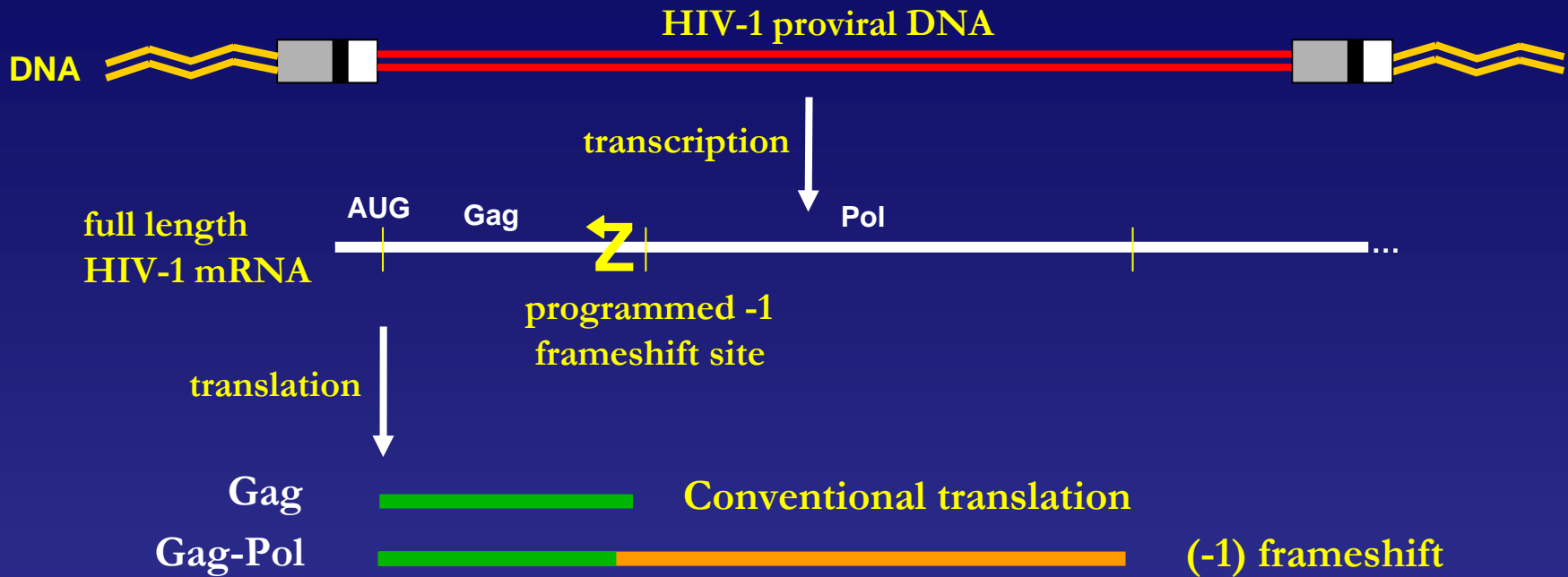
Methodology and Results

All the mutations that were introduced decreased the frameshift efficiency in a range extending from about 20 to 95% of the wild-type efficiency. The level of Gag-Pol incorporated in the VLPs decreased proportionally to the decrease in the frameshift efficiency but the release of the VLPs was not affected. Single-round infectivity assays with a β -galactosidase reporter revealed a range in the infectivity of the mutants, which varies from less than 1 to about 50% of the wild-type. Long term replication kinetics showed a delay in the replication of the mutants that correlates with the decrease observed in single-round assays. Mutants for which the frameshift efficiency is higher than 60% of the wild-type were not significantly altered in replication, whereas mutants with frameshifting efficiency lower than 50% of the wild-type have replication kinetics that are severely delayed.

Conclusion

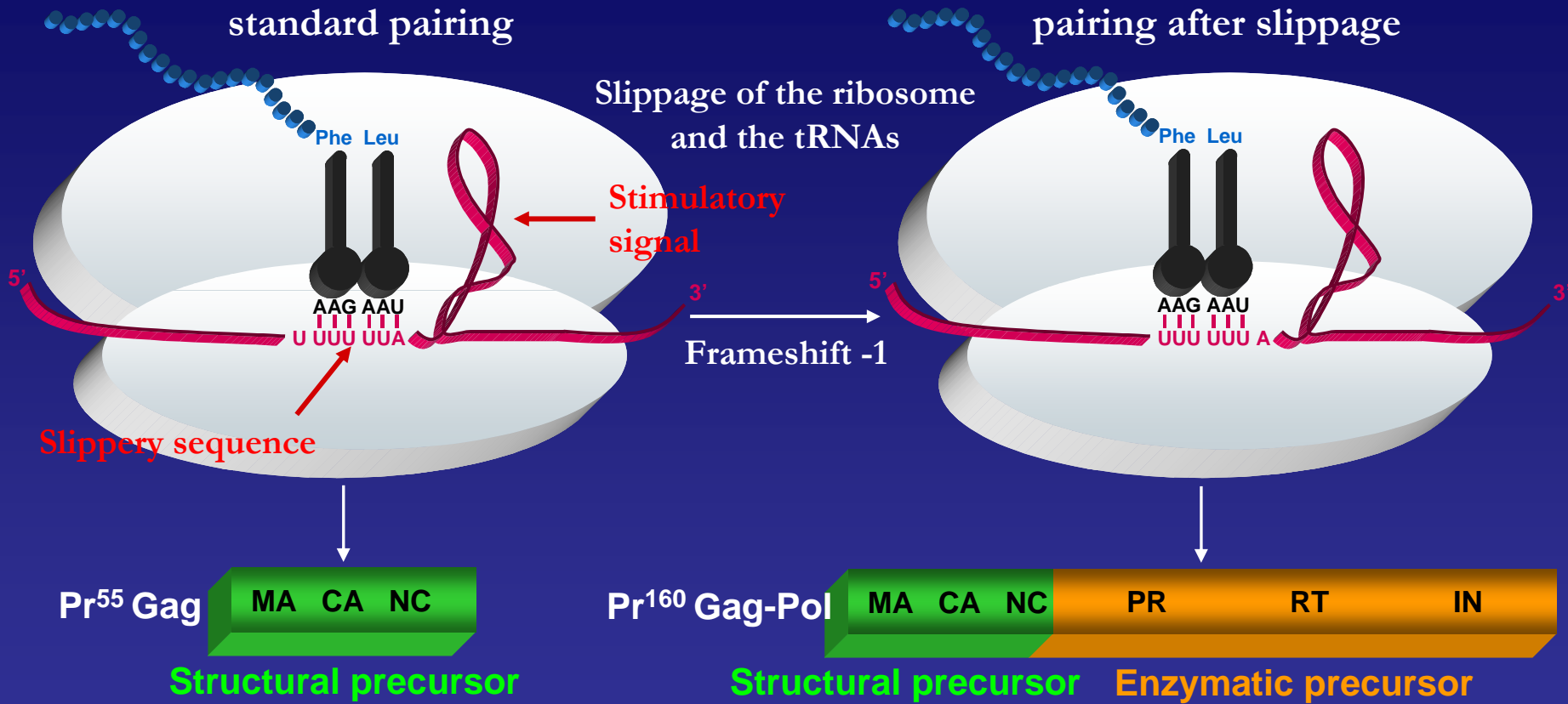
The spectrum of replication observed in the long term replication kinetics suggests that a given level of Gag-Pol is needed for the virus to achieve near-wild-type level of replication. Mutants with a delayed replication produce an amount of viral enzymes below a required threshold.

Synthesis of HIV-1 Gag-Pol requires a programmed -1 frameshift



- Gag and Pol, which are the precursors of the viral structural and enzymatic proteins, respectively, are produced from the same mRNA.
- Whereas Gag is produced by conventional translation, Pol is produced as a Gag-Pol fusion protein by a programmed -1 ribosomal frameshift.
- Gag and Gag-Pol are synthesized in a 20 to 1 ratio.

Mechanism of the programmed -1 ribosomal frameshift

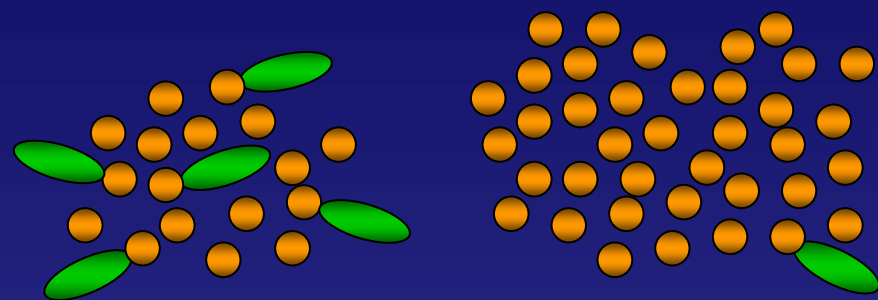
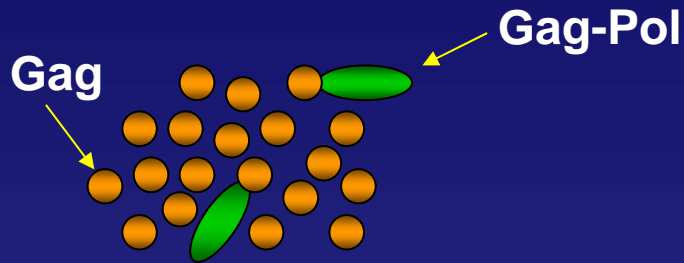


Stimulatory signal: specific RNA secondary structure that controls the efficiency of frameshifting, and therefore controls the Gag to Gag-Pol ratio that is critical for the assembly of infectious particles.

The Gag to Gag-Pol ratio is critical for an efficient viral replication

Normal frameshift efficiency

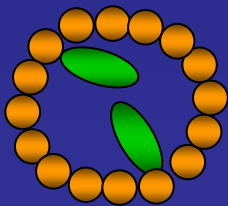
Altered frameshift efficiency



Normal ratio
Gag-Pol to Gag (1:20)

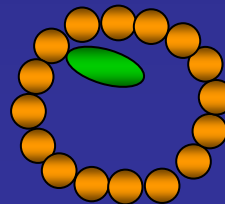
Higher ratio
(more Gag-Pol)

Lower ratio
(less Gag-Pol)

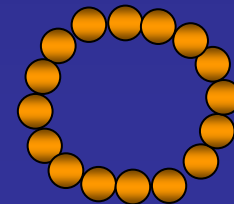


Infectious viral particles

No or abnormal viral particles released



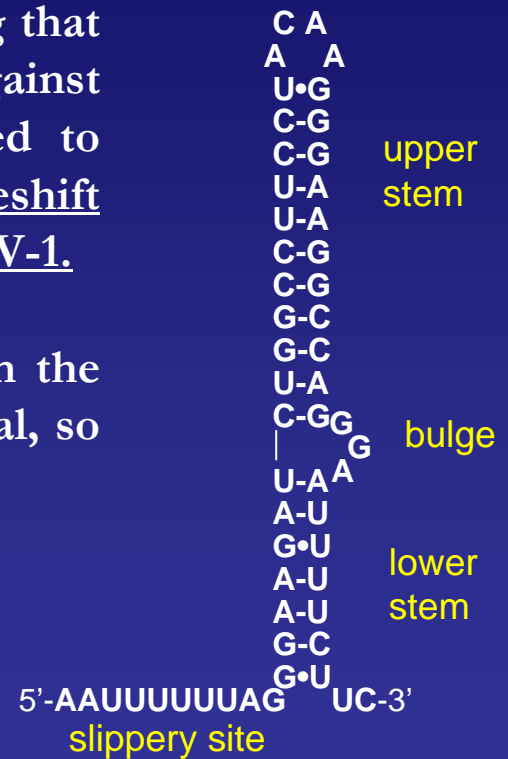
Abnormal viral particles released



Aim of the project

Several studies have shown the importance of the Gag-Pol to Gag ratio or the infectivity of retroviruses. Any drug that could interfere with this ratio could be valuable against AIDS. In order to validate such target, we decided to investigate whether small variations of the frameshift efficiency can affect the assembly and infectivity of HIV-1.

To achieve this goal, we introduced mutations within the slippery sequence and the frameshift stimulatory signal, so as to alter the frameshift efficiency.

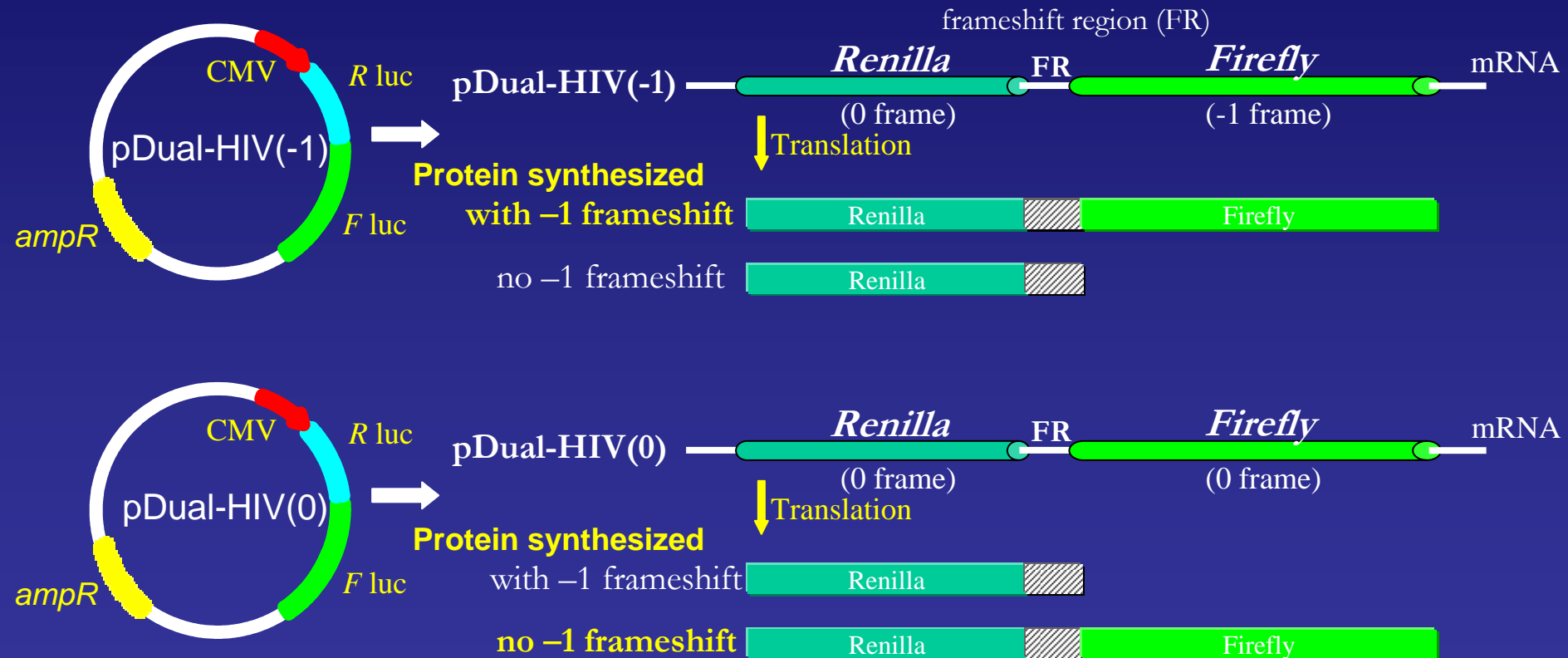


HIV-1 frameshift stimulatory signal

Experimental approaches I

For the frameshifting assays in 293FT, the HIV-1 frameshift region is inserted between the Renilla luciferase (*R luc*) and Firefly luciferase (*F luc*) of a dual luciferase reporter plasmid. In pDual-HIV(-1), the insertion is such that only ribosomes that make a -1 frameshift produce a Renilla-Firefly fusion protein.

In pDual-HIV(0), the in-frame control plasmid, an adenine was inserted immediately after the slippery site such that ribosomes that do not frameshift produce a Renilla-Firefly fusion protein.



$$\text{Frameshifting efficiency} : \frac{[\text{Firefly luciferase} / \text{Renilla luciferase}]_{\text{pDual-HIV(-1)}}}{[\text{Firefly luciferase} / \text{Renilla luciferase}]_{\text{pDual-HIV(0)}}} \times 100$$

VLPs assessments

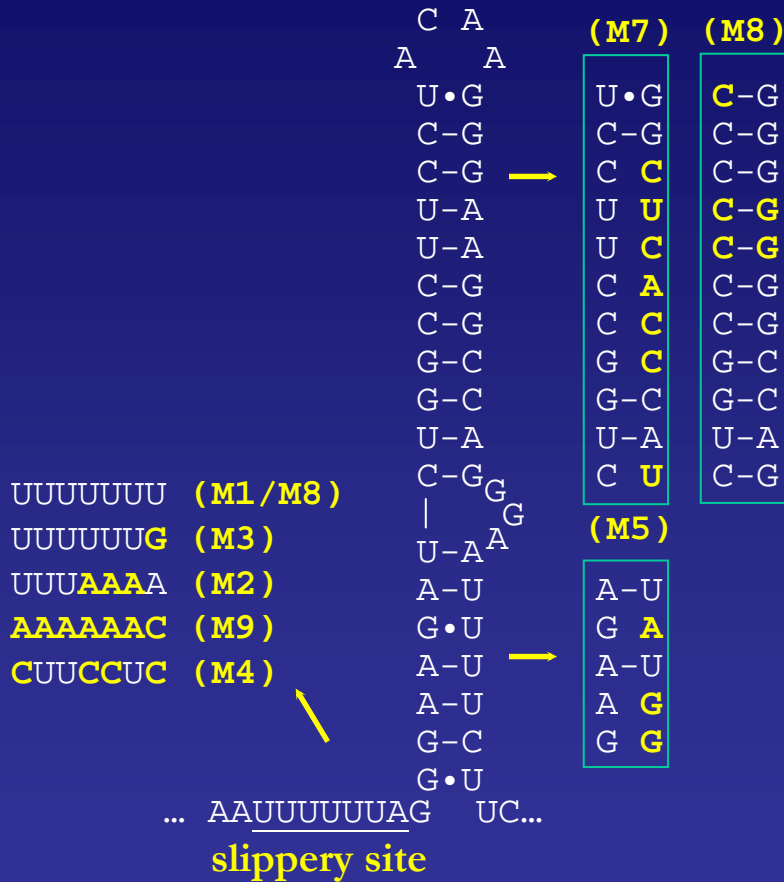
- We use the plasmid pSVGagPol-rre, which contains the full-length *gag-pol* gene of HIV-1 under control of a SV40 promoter so as to produce HIV virus-like particles (VLPs).
- The effects of mutations in the frameshift region on viral assembly and release are measured by assessing the production of VLPs from COS7 cells co-transfected with pSVGagPol-rre and a second vector coding for HIV-1 Rev protein.
- Quantification of Gag (from p24 Elisa) and Gag-Pol (from reverse transcriptase enzymatic assay; RT) can assess the integrity of the released VLPs.

Infectivity assays

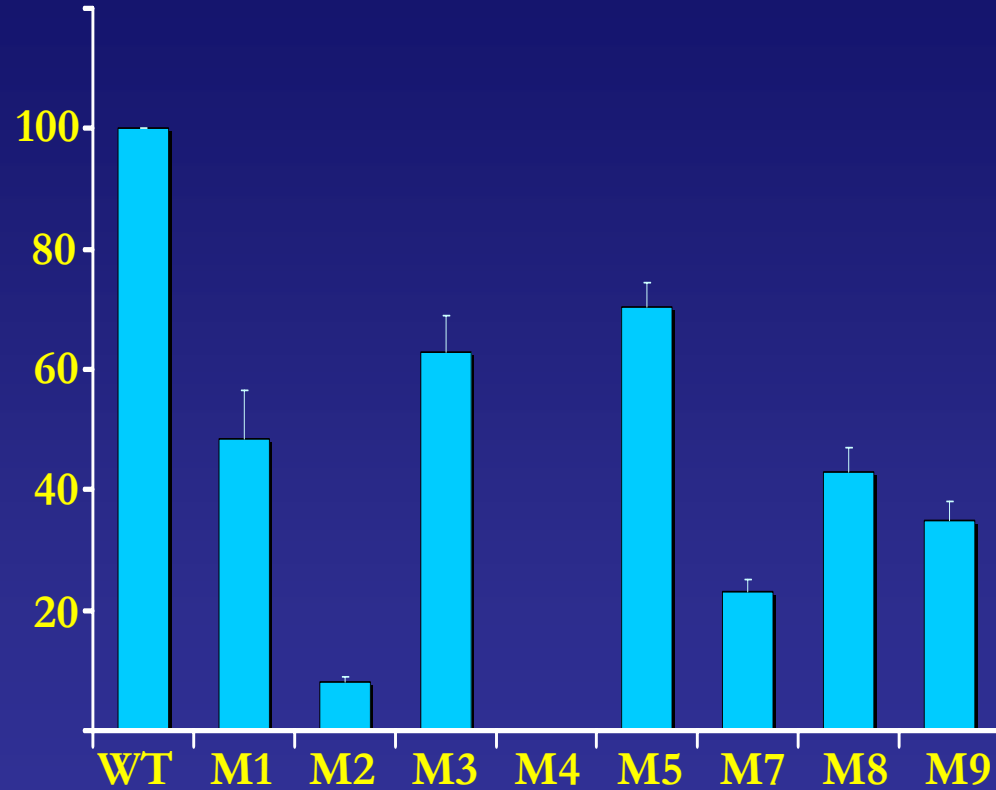
- The infectivity of the frameshift mutants is assessed in single round infectivity assays, using an indicator cell line (HeLaP4) that carries the beta-galactosidase gene under the control of the HIV LTR promoter.
- Replication kinetics of HIV-1 and mutants with an altered frameshift efficiency are assessed using CEM T-cells infected with p24 normalized amounts of wild-type HIV-1 and mutants viruses. Viral replication was monitored in regular intervals by quantification of the p24 concentration in the culture supernatant with a Elisa p24 assay.
- The viral particles for this assay are produced from HeLa cells transfected with a proviral clone (LAV; group M, subtype B) containing either the wild-type or a frameshift mutant.

Results I Effect of mutations on the HIV-1 frameshifting efficiency

Mutants of the HIV-1 frameshift region

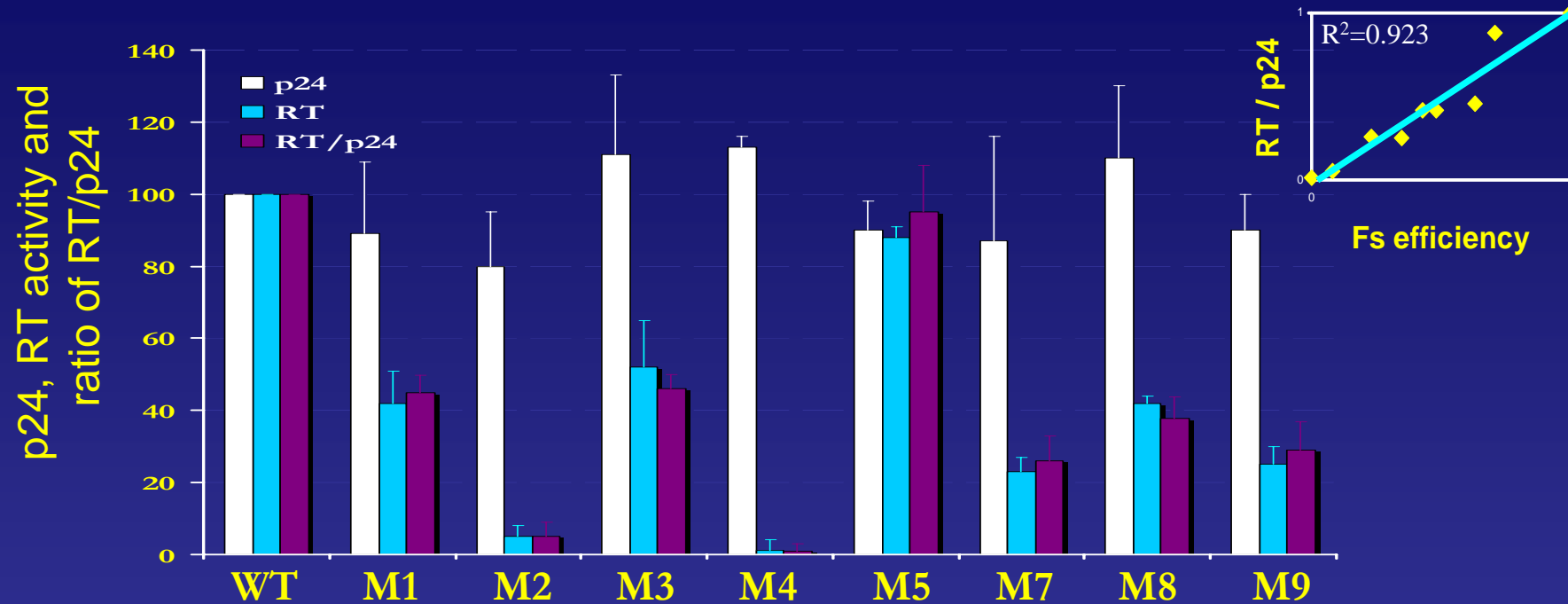


Frameshifting efficiency of the frameshift mutants relative to WT



- Mutations introduced within the frameshift region altered the frameshifting efficiency in a range from about 0.1 to 80% of the wild-type.

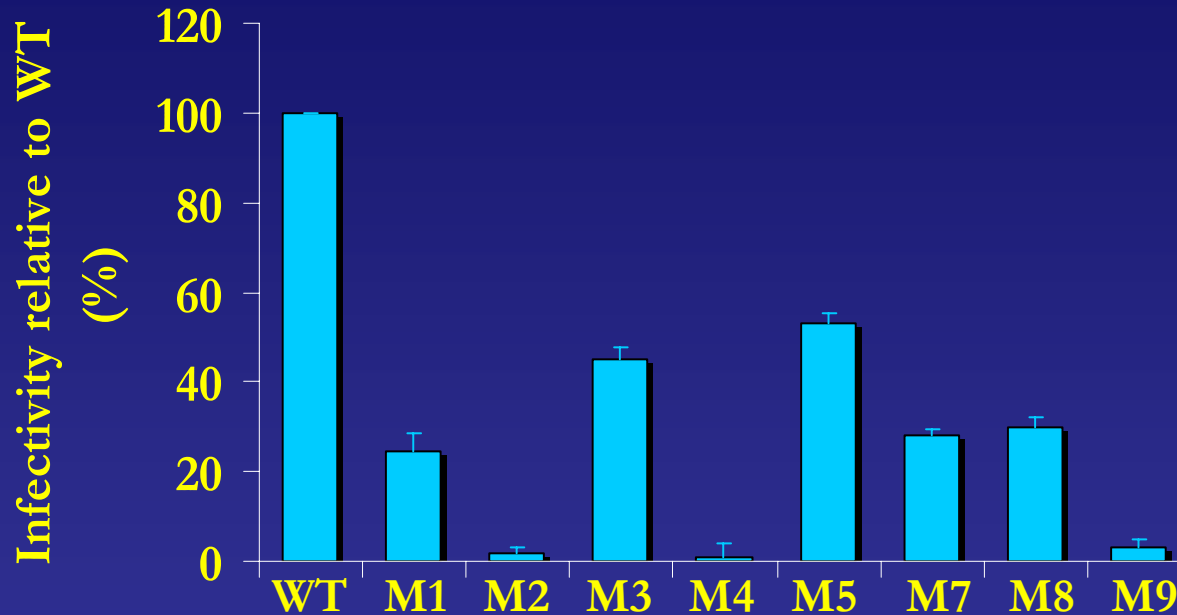
Effect of changes in frameshift efficiency on the release of VLPs, on the RT content of the released VLPs, and on the ratio of Gag-Pol to Gag in these VLPs



VLPs studies reveal that :

- no mutation within the frameshift region altered the release of p24
- there was a range of changes in the amount of RT (Gag-Pol) incorporated in the released VLPs
- the ratio of Gag-Pol to Gag incorporated in the VLPs paralleled the changes in frameshift efficiency ($R^2=0.923$)

Infectivity of HIV-1 and derivatives mutated in the frameshift region assessed by single-round infectivity assays using HeLa-P4 cells.

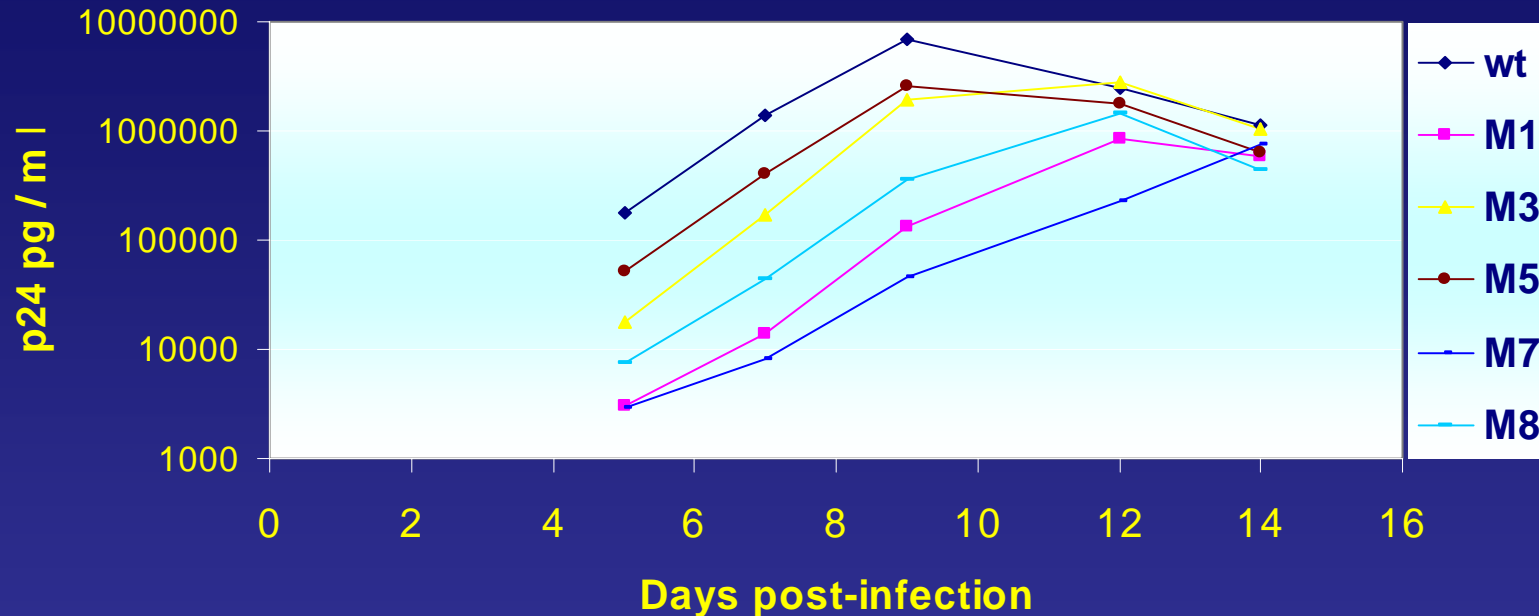


Single-round infectivity assays reveal that :

- all mutations decreased the infectivity compared to wild-type
- the decrease in infectivity was amplified compared to the perturbation of the frameshifting event.

Results IV

Replication kinetics of HIV-1 mutants with an altered frameshift efficiency



- Mutants M3, M5 for which the frameshift efficiency was equal or higher than 60% of the wild-type did not significantly differ from wild-type. For these mutants the replication pattern was delayed by less than two days compared to wild-type.
- Mutants M1, M2, M4, M7, M8 and M9 for which the frameshift efficiency was about 50% or lower than the wild-type were at least 30 fold less infectious and replicate at least twice slower. Mutants M2, M4 and M9 replicate below the level of detection in long term kinetics assays.

Summary and perspectives

- Our studies suggest that minor changes in the frameshifting efficiency can significantly reduce viral infectivity.
- The decrease of infectivity with mutations that decrease frameshifting is related to the lower level of enzymes (Gag-Pol) incorporated in the virions.
- In addition, the incorporation of tRNA^{Lys} into viruses or the correct genomic RNA dimerization, two processes driven by Gag-Pol, could also be affected in mutants with a significant decrease in Gag-Pol.
- The delay observed in long term kinetics with mutants altered in frameshifting efficiency suggests that the HIV-1 frameshifting event is a target of choice, and drugs that decrease frameshifting by at least 50% could delay propagation of HIV-1 and have a strong potential benefit when used in combination with traditional drugs.

Supported by CIHR