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# The virion-associated Gag–Pol is decreased in chimeric Moloney murine leukemia viruses in which the readthrough region is replaced by the frameshift region of the human immunodeficiency virus type 1

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**Abstract**

The human immunodeficiency virus type 1 (HIV-1) requires a programmed −1 translational frameshift event to synthesize the precursor of its enzymes, Gag–Pol, when ribosomes from the infected cells translate the full-length viral messenger RNA. Translation of the same RNA according to conventional translational rules produces Gag, the precursor of the structural proteins of the virus. The efficiency of the frameshift controls the ratio of Gag–Pol to Gag, which is critical for viral infectivity. The Moloney murine leukemia virus (MoMuLV) uses a different strategy, the programmed readthrough of a stop codon, to synthesize Gag–Pol. In this study, we investigated whether different forms of the HIV-1 frameshift region can functionally replace the readthrough signal in MoMuLV. Chimeric proviral DNAs were obtained by inserting into the MoMuLV genome the HIV-1 frameshift region encompassing the slippery sequence where the frameshift occurs, followed by the frameshift stimulatory signal. The inserted signal was either a simple stem-loop, previously considered as the stimulatory signal, or a longer bulged helix, now shown to be the complete stimulatory signal, or a mutated version of the complete signal with a three-nucleotide deletion. Although the three chimeric viruses can propagate essentially as the wild-type virus in NIH 3T3 cells, single-round infectivity assays revealed that the infectivity of the chimeric virions is about three to fivefold lower than that of the wild-type virions, depending upon the nature of the frameshift signal. It was also observed that the Gag–Pol to Gag ratio was decreased about two to threefold in chimeric virions. Comparison of the readthrough efficiency of MoMuLV to the HIV-1 frameshift efficiency, by monitoring the expression of a luciferase reporter in cultured cells, revealed that the frameshift efficiencies were only 30–60% of the readthrough efficiency. Altogether, these observations indicate that replacement of the readthrough region of MoMuLV with the frameshift region of HIV-1 results in virions that are replication competent, although less infectious than wild-type MoMuLV. This type of chimera could provide an interesting tool for in vivo studies of novel drugs targeted against the HIV-1 frameshift event.


*Keywords:* HIV-1; Murine leukemia virus; Ribosomal frameshift; Gag–Pol/Gag ratio; Readthrough

## Introduction

The acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus type 1 (HIV-1), has killed over 21 million people since its appearance (Los Alamos National Laboratory, 2002). The current therapy, based on the use of inhibitors of viral enzymes, is hampered by the emergence of resistant variants to these drugs (Coakley et al., 2000; Isel et al., 2001; LaBonte et al., 2003; Prabu-Jeyabalan et al., 2003). Novel antiviral drugs that target other steps of the viral replication cycle, such as viral entry, were recently developed (Chantry, 2004; Clapham and McKnight, 2002; Lee and Rossi, 2004; Matthews et al., 2004). However, the virus also develops a resistance to these drugs (Miller and Hazuda, 2004). Promising results were obtained in cultured cells with the

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RNA interference strategy (Berkhout, 2004; Lee and Rossi, 2004), but escape mutants were also isolated with this approach (Boden et al., 2003). It is therefore imperative to investigate other potential viral targets.

The programmed translational frameshift event used by HIV-1 to produce Gag–Pol, the precursor of its enzymes (Jacks et al., 1988), is one potential target worth investigating. Gag, the precursor of the viral structural proteins, and Gag–Pol are both translated from the full-length viral mRNA but are encoded in two different reading frames. Gag is synthesized according to conventional translation rules whereas Gag–Pol synthesis requires a −1 ribosomal frameshift during the translation of the messenger. This strategy allows the virus to maintain a specific Gag–Pol to Gag ratio, which is critical for particle assembly, RNA dimerization, viral replication and infectivity (Hung et al., 1998; Karacostas et al., 1993; Park and Morrow, 1992; Shehu-Xhilaga et al., 2001). The synthesis of Gag–Pol depends upon two *cis*-acting elements, the slippery sequence, where the shift occurs and a specific downstream secondary structure, which acts as a frameshift stimulatory signal (reviewed in Brierley, 1995; Brierley and Pennell, 2001). This secondary structure was long assumed to be a simple 11-bp stem-loop, the classical stimulatory signal (Jacks et al., 1988; Kang, 1998). However, Dulude et al. (2002) showed that the stimulatory signal is more complex and consists in a longer stem-loop where the upper part corresponds to the classical or short stem-loop signal and the lower part results from pairing the spacer region following the slippery sequence and preceding the short stem-loop with a complementary segment downstream of this stem-loop. A three-purine bulge separates the two parts of the helix. This bulged helix, which we name the long frameshift stimulatory signal, stimulates the frameshift efficiency twice as much as the short signal.

It will be interesting to develop novel anti-HIV-1 agents that perturb the frameshift efficiency and, consequently, the Gag–Pol to Gag ratio in HIV-1. However, assays with primates that are traditionally used for investigating anti-HIV drugs in vivo are very expensive and, most often, only small numbers of animals can be used, which limits the number of compounds that can be tested. It is therefore important to develop models using small mammals to assess in vivo the effects of potential anti-frameshift agents. We thus decided to construct a chimera of a murine retrovirus, the Moloney murine leukemia virus (MoMuLV), in which the frameshift region of HIV-1 directs the synthesis of the Gag–Pol polyprotein from MoMuLV. MoMuLV is a simple C-type murine retrovirus that induces T-cell lymphomas in susceptible mice after a latency of several months (reviewed in Gardner, 1978). In this well-characterized virus (Goff, 1984; Jones et al., 1989; Shinnick et al., 1981; Tsichlis, 1987), the *gag* and *pol* genes are in the same reading frame and the Gag–Pol polyprotein is expressed by a translational readthrough of the UAG stop codon at the end of the *gag* coding sequence. The UAG stop codon is followed at a distance of 8 nucleotides by a pseudoknot structure that acts as a readthrough stimulatory signal (Alam et al., 1999; Felsenstein and Goff, 1988, 1992; Feng et al., 1992; Wills et al., 1991, 1994; Yoshinaka et al., 1985). In a previous study, we constructed a chimeric MoMuLV in which we inserted the short frameshift region of HIV-1. The construct was such that the synthesis of Gag–Pol depends upon a −1 frameshift rather than the readthrough signal normally used by MoMuLV (Brunelle et al., 2003). When NIH 3T3 cells were transfected with this chimeric proviral DNA, the mutated virus propagated about as well as the wild-type virus. However, the conditions of propagation in transfected NIH 3T3 cells could fail to detect small changes in infectivity that can affect viral propagation in vivo. In the present study, we constructed a chimeric MoMuLV containing the long frameshift region of HIV-1 and another chimera containing a mutant of this long signal with a three-nucleotide deletion. We then assessed the function of the HIV-1 frameshift signal in the three chimeras by measuring the capacity of the viral particles produced with the chimeric proviral DNA to propagate in cultured cells and by monitoring their infectivity with a one-cycle infectivity assay. We also determined the ratio of Gag–Pol to Gag in the chimeric virions. In parallel, we compared the readthrough efficiency of the MoMuLV to the frameshift efficiency of HIV-1 using a reporter gene, the firefly luciferase, whose expression depended upon the readthrough region of MoMuLV or the short, the long and the long mutated frameshift region of HIV-1. Altogether, the results obtained show that all the chimeric virions are replication competent, but less infectious than the wild-type virus, a defect that can be directly correlated to a decrease in the Gag–Pol to Gag ratio, consistent with the lower efficiency of the frameshift compared to the readthrough.

## Results

### *Description of the chimeras used in the study*

In this study, we assessed the infectivity of a chimeric murine retrovirus derived from MoMuLV for which the synthesis of Gag–Pol depends upon the HIV-1 frameshift region instead of the region responsible for the readthrough of a stop codon. We had previously constructed such a chimeric provirus, pGMofs-short, which contains the complete proviral DNA of MoMuLV with the short frameshift region of HIV-1 group M subtype B inserted between the *gag* and *pol* genes (Brunelle et al., 2003). Here, we introduced the long frameshift region of HIV-1 between the MoMuLV *gag* and *pol* genes, generating pGMofs-long. We also investigated a third chimera, pGMofs-B*, which contains the frameshift region of a natural isolate of HIV group M subtype B, B*, where the frameshift stimulatory signal is mutated into a helix interrupted by an internal loop, consequently to a three-