Lentiviral RNAs can use different mechanisms for translation initiation

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Abstract

The full-length genomic RNA of lentiviruses can be translated to produce proteins and incorporated as genomic RNA in the viral particle. Interestingly, both functions are driven by the genomic 5′-UTR (5′-untranslated region), which harbours structural RNA motifs for the replication cycle of the virus. Recent work has shown that this RNA architecture also functions as an IRES (internal ribosome entry site) in HIV-1 and -2, and in SIV (simian immunodeficiency virus). In addition, the IRES extends to the *gag* coding region for all these viruses and this leads to the synthesis of shorter isoforms of the Gag polyprotein from downstream initiation codons. In the present study, we have investigated how different members of the lentivirus family (namely HIV-1 and -2, and SIV) can initiate protein synthesis by distinct mechanisms. For this, we have used the competitive reticulocyte lysate that we have recently described. Our results show that HIV-1 is able to drive the synthesis of the Gag polyprotein both by a classical cap-dependent mechanism and an IRES, whereas HIV-2 and SIV appear to use exclusively an IRES mechanism.

Introduction

Lentiviruses are complex retroviruses, which code for the canonical retroviral proteins (Gag, Pol and Env), but also for accessory genes that are not incorporated into the viral particle but are necessary for the regulation of the replication cycle. The genomic RNA is capped and polyadenylated and codes for the Gag and Gag/Pol proteins. In lentiviruses, the same genomic RNA molecule is first translated and then encapsidated [1,2]. However, both processes are mutually exclusive and the elements that regulate the transition from one function to the other are yet to be found. Previously, it has been shown that the Gag polyprotein that is produced from translation of the full-length RNA interacts with its cognate mRNA through the encapsidation signals located within the 5′-UTR (5′-untranslated region) [3]. The 5′-UTR of the lentiviral genomic RNA is long and contains several structural elements necessary for the replication cycle such as the TAR (transactivation response) domain (for transcription), the primer binding site (for reverse transcription) and the encapsidation signals (for genomic RNA packaging). These structural signals could potentially interfere with translation and affect the production of the Gag polyprotein by inhibiting ribosomal scanning from the cap [4]. In the present paper, we have compared the mechanism of translation of three different lentiviruses [HIV-1, HIV-2 and SIV (simian immunodeficiency virus)] using an *in vitro* competitive reticulocyte lysate and several cap-dependent protein synthesis inhibitors.

Key words: eukaryotic initiation factor 4G (eIF4G), Gag, internal ribosome entry site (IRES), lentivirus, translation, 5′-untranslated region (5′-UTR).
Abbreviations used: eIF, eukaryotic initiation factor; FMDV, foot-and-mouth disease virus; IRES, internal ribosome entry site; SIV, simian immunodeficiency virus; 5′-UTR, 5′-untranslated region.
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Results

Translation of the Gag polyprotein: cap-dependent compared with IRES (internal ribosome entry site)-driven recruitment of the ribosomes

The study of lentiviral translation has led to the characterization of IRES in HIV-1 [5,6], HIV-2 [7] and SIV [8,9] to control Gag production from the full-length RNA. These IRESs can be located not only within the *gag* coding region (for all these lentiviruses) but also in the 5′-UTR (for HIV-1 and SIV), and their activity has been shown to be modulated by the cellular environment, such as the arrest of cells at G_2/M (in the case of HIV-1) or stress caused by infection [5]. The presence of IRESs driving translation of the full-length RNA would allow the bypass of the structural elements located in the 5′-UTR. However, the contribution of IRES to cap-mediated initiation of the *gag* reading frame has not been clearly defined yet. Thus, in an attempt to further investigate translational mechanisms used by lentiviral genomic RNAs, we performed an *in vitro* translation assay in a competitive reticulocyte lysate (Figure 1). This cell-free extract that contains endogenous mRNAs (mainly coding for the β-globin and lipoxygenase) was shown to faithfully recapitulate the synergy between the cap and the poly(A) tail [10]. Cap-dependent translation was then blocked by two different means: first, addition of high amounts of cap analogue that compete with capped mRNAs for the recruitment of eIF (eukaryotic initiation factor) 4E, thus impeding translation initiation from the capped end; secondly, cleavage of the initiation factor eIF4G by the addition of the virally encoded protease L [from the FMDV (foot-and-mouth disease virus)], which impairs cap-dependent but stimulates IRES-dependent translation in the

Figure 1 | Translation of capped and polyadenylated full-length RNAs of HIV-1 (A), HIV-2 (B) and SIV (C) in the untreated rabbit reticulocyte lysate after the addition of 0, 125 or 250 mM cap analogue

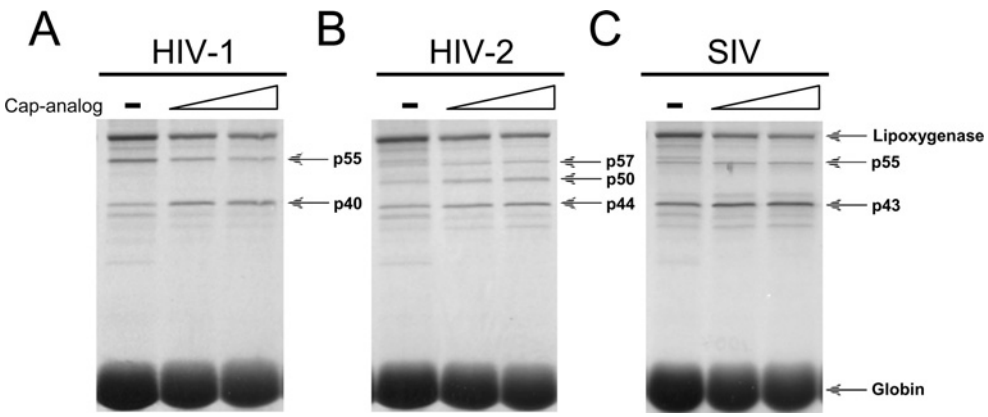
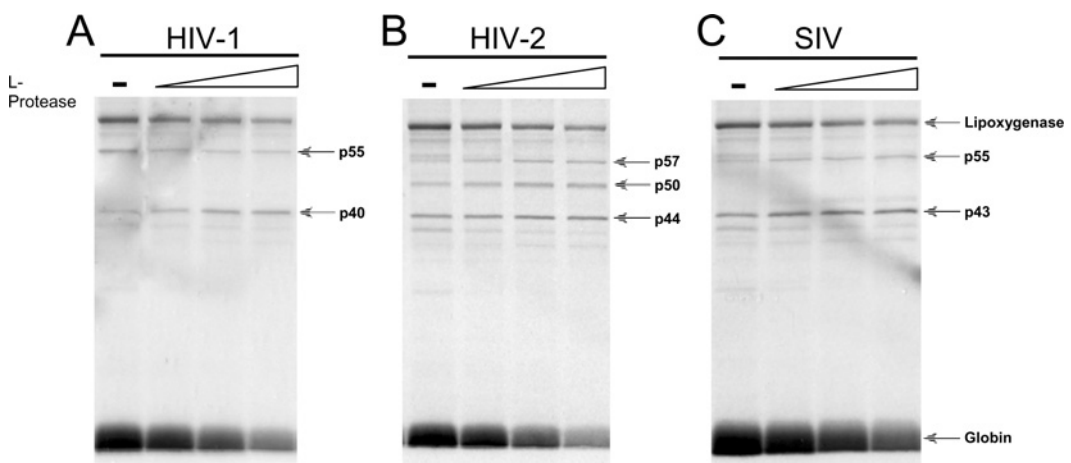


Figure 2 | Translation of capped and polyadenylated full-length RNAs of HIV-1 (A), HIV-2 (B) and SIV (C) in the untreated rabbit reticulocyte lysate treated with increasing amounts of L-protease



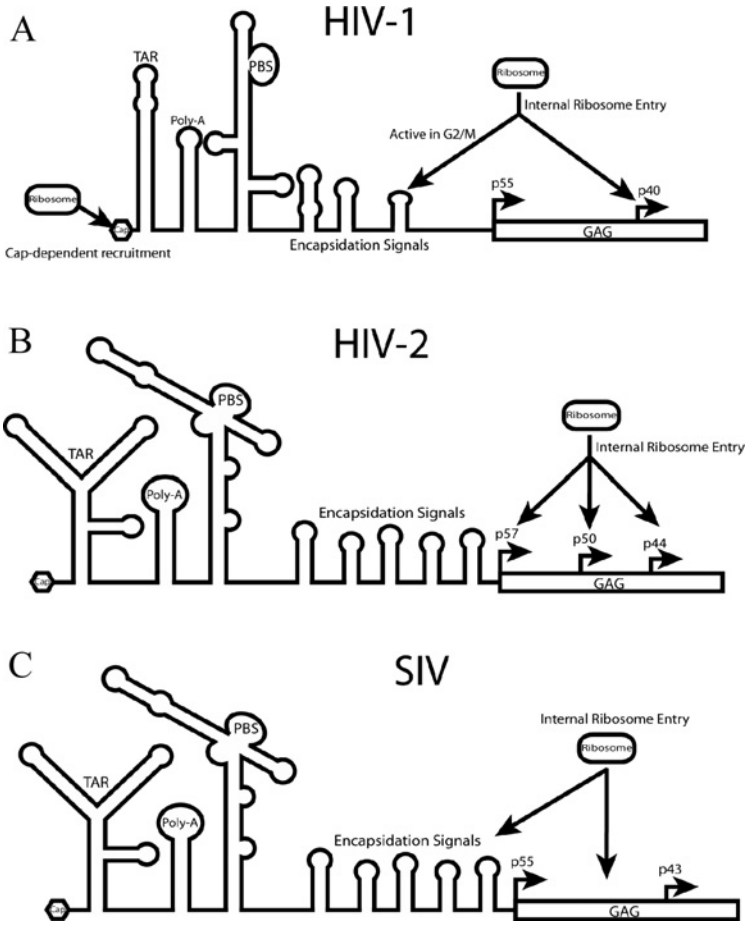
competitive lysate. Translation of the endogenous mRNAs present in the cell-free extract (i.e. lipoxygenase and globin mRNAs, Figures 1 and 2) was used as an internal control. It is noteworthy that the degree of inhibition of translation of these endogenous mRNAs was much greater when the L protease was used (Figure 2) than with cap analogue (Figure 1). This may reflect the fact that the viral enzyme targets at least two initiation factors (eIF4G and PABP), whereas the cap analogue only interferes with eIF4E function.

As shown in Figure 1, addition of cap analogue led to an inhibition of translation from the authentic AUG *gag* codon for HIV-1 (p55) (Figure 1A), but did not affect production of the largest Gag isoforms of HIV-2 (p57) and SIV (p55) (Figures 1B and 1C). This result suggests that synthesis of the full-length Gag polyprotein from HIV-1 relies mostly on a cap-dependent mechanism *in vitro*. This is in contrast with HIV-2 and SIV that predominately use a cap-independent mechanism of recruitment of the ribosomes. Furthermore, the level of inhibition upon addition of cap analogue varies, with HIV-1 (Figure 1A) being strongly affected (80 % of inhibition of p55 production after quantification of the bands on a Fuji FLA 5100 PhosphoImager). Taken together, these results suggest that HIV-1 uses a mixture of cap- and IRES-dependent translation to produce full-length Gag, whereas both HIV-2 and SIV seem to rely exclusively on IRES-driven initiation.

To further investigate this hypothesis, we have studied *in vitro* translation of these lentiviruses under conditions when the initiation factor eIF4G was proteolysed. This was performed by using the L-protease from the FMDV. Such a treatment was shown to severely inhibit cap-dependent translation and stimulate IRES-driven protein synthesis [11]. Addition of the enzyme to the reticulocyte lysate resulted in a drastic inhibition of endogenous globin translation as a consequence of eIF4G cleavage (Figure 2). Under these experimental conditions, production of the HIV-1 Gag polyprotein was diminished upon cleavage of eIF4G, confirming the use of a cap-dependent mechanism (Figure 2A). As expected, translation

Figure 3 | Different translation strategies developed by lentiviruses

(**A**) For HIV-1, synthesis of full-length Gag polyprotein (p55) can occur both by a cap-dependent and an IRES mechanism. (**B**, **C**) For HIV-2 and SIV, production of full-length Gag (p57 for HIV-2 and p55 for SIV) depends exclusively on an IRES element. Translation of alternative shorter Gag isoforms occurs exclusively from an IRES located within the coding region (**A**–**C**).



of the HIV-2 and SIV full-length RNAs was stimulated by the addition of the L-protease, confirming that a cap-independent mechanism was at play (Figures 2B and 2C).

Translational control of shorter Gag isoforms and their role in the replication cycle

A conserved feature of lentivirus translation is the production of shorter Gag isoforms that are translated from initiation codons located within the Gag coding region [6–8,12]. The number of Gag isoforms varies from 1 [for HIV-1 (p40) and SIV (p43)] to 2 [for HIV-2 (p50 and p44)] (see Figure 1). The precise function of these proteins has not been totally elucidated, but their deletion affects HIV-1 and SIV replication [6,13]. Interestingly, in the case of HIV-2, the shorter isoforms have been shown to be incorporated within the viral particle [7], suggesting that they could contribute to the packaging of the genomic RNA.

Results presented in this paper show that production of these short isoforms from HIV-1 and -2, and SIV is not inhibited, and rather stimulated by, any of the cap-dependent inhibitors used [cap analogue (Figure 1) or L-protease (Figure 2)]. This strongly suggests that production of these Gag isoforms occurs exclusively from the IRES located within the Gag coding region downstream of the authentic initiation codon of *gag*. In fact, further investigation on HIV-2 has recently revealed the presence of three independent IRESs entirely located in the *gag* coding region [14].

Discussion

Translation of the lentiviral genomic RNA occurs in the cytoplasm of the host-infected cell and is often accompanied by modification of the physiological conditions due to stress induced by viral infection and/or the expression of some viral proteins such as Vpr that has the ability to arrest the host cell cycle at the G_2/M transition phase [15]. It should also be noted that both HIV-1 and -2 proteases were shown to impact cellular translation by cleaving the initiation factor

eIF4G and PABP [poly(A)-binding protein] [16,17]. Thus it seems more likely that lentiviral genomic RNAs have the ability to switch from different mechanisms of translation initiation depending on the cellular environment. Results presented in the present article show that a mixture of cap-dependent and IRES-driven translation is used by most members of the lentiviral family to produce the full-length Gag polyprotein. Interestingly, synthesis of the alternative isoforms appears to be exclusively the result of ribosomal entry from IRESs located in the coding region. A schematic cartoon summarizing these results is presented in Figure 3. On one side, HIV-1 was shown to be capable of using a 5′-cap-dependent scanning mechanism together with internal ribosome entry (Figures 3A). On the other side, we find the closely related HIV-2 and SIV that seem to exclusively use an IRES mechanism to produce the full-length Gag and the shorter isoforms (Figures 3B and 3C).

Although the molecular determinants that control the switch between cap-dependent and IRES translation remain unknown, the Gag polyprotein may be involved in this process. Recent data have shown that a low concentration of Gag stimulates protein synthesis from its cognate RNA, whereas it has an inhibitory effect at a high dose [18]. Interestingly, for HIV-2, addition of recombinant Gag protein to a rabbit reticulocyte lysate inhibits production of the full-length protein but stimulates synthesis of the shorter isoforms [14]. Thus it is tempting to speculate that the binding of newly synthesized Gag protein to RNA signals within the 5′-UTR may result in a progressive blockage of scanning ribosomes and may trigger the switch to the use of an IRES mechanism. Future work will be needed to characterize all the molecular determinants involved in the control of lentivirus translation.

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