DDX17 promotes the production of infectious HIV-1 particles through modulating viral RNA packaging and translation frameshift

René-Pierre Lorgeoux a,c, Qinghua Pan a, Yann Le Duff a,b, Chen Liang a,b,c,*

a Lady Davis Institute-Jewish General Hospital, Montreal, QC, Canada H3T 1E2
b Departments of Medicine and Microbiology & Immunology, McGill University, Montreal, QC, Canada H3A 2B4

Abstract
RNA helicases are a large family of proteins that rearrange RNA structures and remodel ribonucleic protein complexes using energy derived from hydrolysis of nucleotide triphosphates. They have been shown to participate in every step of RNA metabolism. In the past decade, an increasing number of helicases were shown to promote or inhibit the replication of different viruses, including human immunodeficiency virus type 1. Among these helicases, the DEAD-box RNA helicase DDX17 was recently reported to modulate HIV-1 RNA stability and export. In this study, we further show that the helicase activity of DDX17 is required for the production of infectious HIV-1 particles. Overexpression of the DDX17 mutant DQAD in HEK293 cells reduces the amount of packaged viral genomic RNA and diminishes HIV-1 Gag-Pol frameshift. Altogether, these data demonstrate that DDX17 promotes the production of HIV-1 infectious particles by modulating HIV-1 RNA metabolism.

Introduction
RNA helicases are a large family of proteins that are involved in virtually all the steps of RNA metabolism (Cordin et al., 2006; Linder and Jankowsky, 2011). They utilize the energy derived from nucleotide triphosphates (NTPs) hydrolysis to unwind nucleic acids and remodel ribonucleic protein (RNP) complexes (Tanner and Linder, 2001). RNA helicases are classified into five super families (SF1 to SF5) on the basis of their conserved motifs. They are prevalent in eukaryotes, prokaryotes and viruses (Kwong et al., 2005; Singleton et al., 2007). The SF2 has the most members, and includes the DEAD-box helicases that harbor the Asp-Glu-Ala-Asp signature motif (Linder et al., 1989). In humans, malfunction of helicases causes various types of cancers (Clark et al., 2008; Fuller-Pace and Moore, 2011; Rezazadeh, 2012).

Viruses need helicases to successfully replicate their nucleic acid genome. Some viruses carry their own helicase, such as Hepatitis C virus that encodes the NS3 protein (Kanai et al., 1995; Kim et al., 1995), while others need to hijack cellular ones to assist viral replication. Human immunodeficiency virus type 1 (HIV-1), like all retroviruses, does not encode a viral helicase. Over the past decade, many cellular RNA helicases have been reported to promote or inhibit HIV-1 replication at distinct steps. These include RNA helicase A (RHA), Moloney leukemia virus 10 (MOV10), XPB/XPD, Ku70/Ku80, Werner syndrome (WRN) helicase, DDX1, DDX3, DDX17, DDX24, Upf1 and DHX30 (reviewed in (Jeang and Yedavalli, 2006; Lorgeoux et al., 2012; Ranji and Boris-Lawrie, 2010; Steinier and Klostermeier, 2012)). RHA promotes HIV-1 reverse transcription, transcription and translation (Bolinger et al., 2010; Fujii et al., 2001; Roy et al., 2006; Xing et al., 2011). MOV10 inhibits viral reverse transcription (Burdick et al., 2010; Furtak et al., 2010). DDX1, DDX3 and DDX17 promote Rev-dependent HIV-1 RNA export (Fang et al., 2004; Naji et al., 2012; Yedavalli et al., 2004). In addition to its role in HIV-1 RNA export, DDX3 also enhances HIV-1 translation (Geissler et al., 2012; Lai et al., 2010, 2008; Soto-Riffo et al., 2012, 2013). Upf1 and DDX17 are involved in HIV-1 RNA degradation (Ajaman et al., 2008; Zhu et al., 2011). DDX24 promotes HIV-1 RNA packaging (Ma et al., 2008), while DHX30 was reported to inhibit this step (Zhou et al., 2008). Recently, SLFN11 was shown to inhibit HIV-1 translation in a codon-usage-dependent manner (Li et al., 2012), adding a new layer of viral RNA regulation by cellular helicases.

DDX17, also known as RH70, has two isoforms, p72 and p82, as a result of alternative translation of its messenger RNA (mRNA) (Uhlmann-Schiffer et al., 2002). DDX17 can form heterodimers with its paralog DDX5 (Ogilvie et al., 2003) and is involved in multiple aspects of RNA metabolism. Through interaction with other cellular factors, including HDAC1 (Wilson et al., 2004), estrogen receptor alpha (Wortham et al., 2009) and U1snRNP...
DDX17 regulates gene transcription and alternative splicing. Its dysfunction can lead to cancer development (Dardenne et al., 2012; Honig et al., 2002; Shin et al., 2007). Recently, DDX17 was reported to act as a co-factor of the zinc finger antiviral protein ZAP (Chen et al., 2008), which is involved in the degradation of the multiply spliced HIV-1 mRNA (Zhu et al., 2011). Another study by Naji et al., (2012) showed that DDX17 associates with HIV-1 Rev protein, and promotes the nuclear export of HIV-1 transcripts. We now further show that DDX17 affects the production of infectious HIV-1 particles. Knockdown and over expression of DDX17 change the ratios of unspliced vs spliced HIV-1 RNA. Furthermore, over expression of the DDX17 DQAD mutant leads to a dramatic decrease in the amount of packaged viral RNA. Moreover, DDX17 is required for efficient Gag processing through its effect on HIV-1 Gag-Pol frameshift. Altogether, our results demonstrate new roles for DDX17 in promoting the production of infectious HIV-1 particles through regulating HIV-1 Gag-Pol frameshift and genomic RNA packaging.

Materials and methods

Plasmid DNA, viruses and antibodies

The cDNA clone of DDX17 gene was purchased from ATCC (MGC-2030). Short and long isoforms of DDX17 cDNA were amplified by PCR and a Flag tag was added at the N terminus using primer pairs DDX17-SS/DDX17-A, DDX17-5L/DDX17-A, respectively (Table 1). The PCR products were digested with BamHI and MluI and inserted into the pRetroX-Tight-Pur retroviral vector (Clontech) to create DNA plasmids Tet-DDX17S and Tet-DDX17L that are expressed in response to doxycyclin. DDX17 mutants were generated by site-directed mutagenesis using primers DQAD-S and DQAD-A (Table 1). DDX5 cDNA clone was kindly provided by Dr Stahl (Rosser et al., 2000). The infectious HIV-1 proviral DNA clone NL4-3 was obtained from the NIH AIDS Research and Reference Reagent Program. Tat plasmid was kindly provided by Dr. Gatignol (Gatignol et al., 1991). The frameshift luciferase reporter constructs FS (0) and FS (-1) were previously described (Greentzmann et al., 1998) and provided by Dr. Brakier-Gingras. HIV-1 viruses were generated by transfecting HEK293T cells with the proviral DNA clone NL4-3, using lipofectamine2000 (Invitrogen) according to the manufacturer’s instruction.

Cell culture and transfections

TZM-bl, HeLa and HEK293 cells were grown at 37 °C with 5% CO2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). Plasmids and short interfering RNA (siRNA) were transfected into cells using lipofectamine2000 according to the manufacturer's protocol.

Knockdown of DDX5 and DDX17 in HeLa cells

siRNA oligonucleotides targeting DDX17 (nt 1694–1713) were purchased from Qiagen (5′-AAT ACA CCT ATG GTC AAC GCA-3′) (Cat. no. 1027423). siRNAs oligonucleotides targeting DDX5 and the DDX5-DDX17 subfamily were previously described (Jalal et al., 2007). Control siRNA was purchased from Qiagen (Cat. No. 1027281), siRNA targeting DDX17 (nt 1525–1545) was purchased from Sigma (5′-CCC AAT CTG ATG CAT GAT-3′). Control shRNA was also purchased form SIGMA (Cat. No. SHC016). HeLa cells (2 × 105 per well) were seeded in 6-well plates 1 day before siRNA transfection. DDX5 and DDX17 were either knocked down individually, or simultaneously. 10 nM of each siRNA was used in transfection. After two sequential siRNA transfections, the cells were transfected with HIV-1 NL4-3 DNA. Cells and supernatants were harvested 40 h post HIV-1 NL4-3 transfection. Protein levels were analyzed by Western blotting, virus production was measured by infecting the TZM-bl indicator cells.

DDX17 overexpression in HEK293 cells

HEK293 cells (5 × 105 per well) were seeded in 6-well plates 1 day before transfection. 500 ng of DDX17 DNA constructs were co-transfected with 100 ng Tet-ON plasmid and 100 ng HIV-1 NL4-3. Cells were washed in 1 × phosphate buffered saline the next day and fresh DMEM containing 100 ng/mL doxycyclin was added to induce DDX17 expression. Cells and supernatants were harvested 40 h post HIV-1 NL4-3 transfection. Protein levels were analyzed by Western blotting and virus production was measured by infecting TZM-bl indicator cells.

Viral RNA analysis

Levels of viral RNA in cells were assessed by Northern blots as previously described (Roy et al., 2006; Zhou et al., 2008) (and RT-PCR followed by Southern blot, described below). Briefly, transfected cells were washed with 1 × phosphate buffered saline and lysed in Trizol (Invitrogen). RNA was extracted according to the manufacturer’s instructions. Northern blot analysis was performed as previously described (Zhou et al., 2008). Briefly, RNA was subjected to electrophoresis on a 1% denaturing agarose gel. RNA was transferred to nylon membranes (GE Healthcare UK Limited) and incubated with [α-32P] labeled HIV-1 DNA. Membranes were washed with 1 × saline-sodium citrate (SSC) buffer containing 0.1% SDS and viral RNA signals were visualized by exposure to X-ray films.

Levels of virion-associated viral RNA and total viral RNA were determined by RT-PCR followed by Southern blots. RNA was first
extracted from viruses using Trizol-LS (Invitrogen), followed by DNase I (Amp Grade) digestion at room temperature for 15 min to remove any contaminating plasmid DNA. DNase I (Amp Grade) was inactivated at 65 °C for 10 min. The effectiveness of DNase I (Amp Grade) treatment was tested by amplifying the treated RNA samples by PCR with the primers FP and FL (Table 1). This primer pair amplifies HIV-1 cDNA sequence spanning nucleotides 665 to 1729. Reverse primers RP1 and RP2 were used for the amplification of singly spliced and multiply spliced HIV-1 transcripts, respectively, and were previously described (Zhu et al., 2011). RNA equivalent to 25 ng of p24 was reverse transcribed and amplified with the primers FP and FL or RP1 or RP2, using the Titan One Tube RT-PCR System (Roche) according to the manufacturer's instruction and 20 PCR cycles (Reverse transcription at 50 °C for 1 h, followed by reverse transcriptase inactivation at 94 °C for 5 min; PCR: denaturation at 95 °C for 30 s, annealing at 45 °C for 30 s and elongation at 68 °C for 2 min). RT-PCR products were subjected to electrophoresis on a 1% agarose gel (0.5 × Tris Borate EDTA) for 2 h at 60 V. The gel was treated in 0.2 M HCl for 13 min, NaOH 0.5 M for 30 min, NaCl 0.5 M (pH 7.5) for 30 min and double-distilled H2O for 10 min. The DNA was transferred to a nylon Hybond-N membrane (GE Healthcare UK Limited) with 10 × SSC overnight. The next day, the membrane was washed in 6 × SSC for 5 min at room temperature and RNA was fixed by UV cross-linking. Next, the membrane was incubated with 10 ml pre-hybridization buffer (Ambion) at 42 °C for 2 h followed by overnight hybridization at 42 °C with a [α-32P] labeled HIV-1 probe. The next day, the membrane was washed with 1 × SSC, 0.1% SDS at room temperature for 15 min. Signals were quantified using a phosphoscreen and the Phosphoinager (Amersham).

Virus production assays

The amount of infectious viruses in culture supernatants was determined by infecting the TZM-bl cells. Cells were seeded in 24-well plate at a density of 4 × 104 cells per well 1 day before infection. 50 μL of supernatant from transfected cells were used to infect TZM-bl cells. Forty-eight hours after infection, cells were lysed in 1 × passive lysis buffer (Promega), and levels of firefly luciferase activity in the cell lysates were measured using the Luciferase Assay kit and the Glomax luminometer (Promega). Virus production was determined by measuring viral reverse transcriptase (RT) activity in the supernatants (Lu et al., 2011).

Results

Knockdown of DDX17 but not DD5X reduces HIV-1 production and infectivity

DDX17 was recently identified as a co-factor of ZAP that causes the degradation of HIV-1 multiple spliced mRNAs (Chen et al., 2008). DDX17 was also reported to associate with Rev and promote the export of incompletely spliced HIV-1 RNAs from the nucleus to the cytoplasm (Naji et al., 2012). To determine the effect of DDX17 on HIV-1 production, we silenced DDX17 in HeLa cells using siRNAs, followed by transfection of HIV-1 NL4-3 DNA (Fig. 1A). We observed a two-fold decrease in the amount of viral particles upon DDX17 knockdown (Fig. 1B), which correlates with a diminution of the viral p24 levels in the supernatant (Fig. 1C). Furthermore, quantification of infectious viruses by infecting TZM-bl indicator cells revealed a four-fold decrease (Fig. 1D). Therefore, the infectivity of viral particles is decreased by two-fold. Together with the work from other studies, our data suggest that DDX17 affects the amount and the quality of HIV-1 particles made. When DD5X, a paralog of DDX17, was silenced, we observed a slight increase in the amount of HIV-1 virions and no change in the infectivity of HIV-1 particles (Fig. 1D). Interestingly, silencing DD5X moderately increased DDX17 expression (including both the p72 and p82 isoforms) (Fig. 1A). This suggests that cells compensate for the loss of DD5X by increasing DDX17 expression, as reported by others (Shin et al., 2007). Along this line, over expression of DD5X or DD17 also moderately diminished the level of DDX17 or DD5X (Fig. S1), which further indicates the mutual effects on their expressions. Knockdown of both DD5X and DDX17 severely diminished cell viability, thus was not tested on HIV-1 production (data not shown). In order to confirm the observed inhibitory effect of DDX17 knockdown on HIV-1 production, we utilized an shRNA to deplete endogenous DDX17 in HeLa cells. A profound decrease in the generation of infectious HIV-1 particles was again observed (Fig. 1E). Collectively, we conclude that DDX17 is required for HIV-1 production.

HIV-1 production is inhibited by DDX17 mutant carrying the mutated DEAD box motif

As a typical RNA helicase, DDX17 carries seven conserved motifs including the DEAD box that is critical for helicase activity (Fig. 2A). To determine whether the helicase activity of DDX17 is required for its effect on HIV-1 production, we mutated the DEAD motif to DQAD in the context of both the long (p82) and short (p72) forms of DDX17 (Fig. 2A). We then transfected HEK293 cells with various DDX17 constructs and the HIV-1 proviral DNA NL4-3 (Fig. 2B), and analyzed viral particles released in the supernatant. DDX17 DQAD (L) mutant expression led to a 3.5-fold decrease in the amount of viral particles as being determined by measuring viral reverse transcriptase activity in the supernatants (Fig. 2C). We further determined the levels of infectious HIV-1 particles by infecting the TZM-bl indicator cells, and observed that the DQAD (L) mutant caused a 10-fold reduction (Fig. 2E). Considering the 3.5-fold decrease in the viral RT level (Fig. 2C) and the diminution in p24 found in the supernatant (Fig. 2D), we conclude that DQAD (L) decreases the infectivity of HIV-1 particles by three-fold. For the DQAD (S) mutant, we observed a 50% decrease in the viral RT (Fig. 2C) and a 70% decrease in virus production (Fig. 2E). We also ectopically expressed DDX17 and its DQAD mutants in HeLa cells and observed a more than five-fold reduction in the production of infectious HIV-1 particles as a result of DQAD expression (Fig. S2). Taken together, these results suggest that the DQAD mutant of DDX17 impairs the production of infectious HIV-1 particles. Considering the existence of endogenous DDX17 when DQAD mutant is ectopically expressed, we conclude that this mutant acts in a dominant negative fashion to prevent the endogenous DDX17 from promoting HIV-1 production.

DDX17 expression increases HIV-1 genomic RNA packaging

To understand how the DDX17 DQAD mutant reduces the production of infectious HIV-1 particles, we first measured the levels of viral RNA associated with HIV-1 particles. We thus transfected 293 cells with the DDX17 and HIV-1 NL4-3 DNA clones. Viral particles from culture supernatants were harvested 2 days post transfection and amounts of particles were determined by ELISA against HIV-1 p24. Viral RNA was amplified by RT-PCR and further quantified in Southern blotting (Fig. 3). We observed that the short isoform of wild-type DDX17 over expression led to a 40% increase in the amount of RNA that is packaged into HIV-1 particles. In contrast, the DDX17 DQAD mutants resulted in a dramatic 5- to 10-fold decrease in the amount of packaged HIV-1 genomic RNA (gRNA). The stronger inhibition of HIV-1 RNA packaging by the longer DQAD mutant may result from its higher expression level than the shorter DQAD mutant. Alternatively, the
extra 79 N-terminal amino acids in the DDX17 long isoform (p82), which are absent in DDX17 short forms (p72) may be involved in preventing HIV-1 RNA packaging. These results suggest that the DQAD mutant impedes HIV-1 RNA packaging and thus reduces virus infectivity.

The DDX17 DQAD mutant disturbs the balance of the unspliced vs spliced HIV-1 RNA pools

A decrease in the amount of packaged HIV-1 RNA can be the result of multiple causes, including defects in viral RNA expression.

Fig. 1. Effect of DDX5 and DDX17 knockdown on HIV-1 production. (A) HeLa cells (1.5 x 10^5) were transfected with 0.1 μg HIV-1 NL4-3 DNA and 20 pmol/mL siRNAs directed against DDX17 or DDX5. Levels of endogenous DDX5 and DDX17 were determined by Western blotting. (B) Culture supernatants were harvested 48 h post transfection and the amounts of virus particles were determined by measuring viral reverse transcriptase activity. (C) Levels of viral particles in the culture supernatants were analyzed by Western blotting with anti-HIV-1 p24 antibody. (D) Levels of infectious HIV-1 particles in the supernatants were determined by infecting the TZM-bl indicator cells. ** represents a P-value < 0.001, n=6. The P value was calculated with reference to the control siRNA data. p82, DDX17 L (long) isoform; p72, DDX17 S (short) isoform; p68, DDX5.
in the cell. We first tested whether DDX17 affects HIV-1 Long Terminal Repeat (LTR) promoter activity by transfecting DDX17 DNA and Tat into the TZM-bl indicator cells that contain the HIV-1 LTR-luciferase reporter (Fig. 4A). We observed no detectable effect of DDX17 on Tat-dependent HIV-1 transcription. Transfection of 40 ng HIV-1 Tat showed a further 10-fold increase in luciferase activity, attesting that the assay was not saturated (data not shown).

Recently, DDX17 was reported to act as a cofactor of ZAP and contribute to the degradation of multiply spliced HIV-1 RNA (Zhu et al., 2011). In addition, no noticeable effect was seen on the levels of full length viral RNA upon silencing of ZAP (Zhu et al., 2011). In another recent study, Naji et al. (2012) reported that DDX17 silencing led to a decrease in the levels of both multiply spliced and unspliced HIV-1 RNAs. Naji et al. (2012) also reported that DDX17 associates with the viral protein Rev to promote the export of RRE-containing viral RNAs. To further determine the effect of DDX17 on HIV-1 RNA expression, we transfected HEK293 cells with DDX17 plasmids and HIV-1 NL4-3, and examined HIV-1 RNA levels by Northern blot (Fig. 4C). We found that overexpression of wild-type DDX17 led to a slight increase in HIV-1 full length RNA while over expression of DDX17 DQAD mutants induced an increase in HIV-1 multiply spliced mRNA species and a decrease in HIV-1 full length RNA (Fig. 4C). To confirm this observation, we silenced DDX17 and DDX5 in HeLa cells and analyzed HIV-1 RNA by Northern blot (Fig. 4B). We observed that silencing DDX17 led to an increase in HIV-1 spliced transcripts and a decrease in the viral full length RNA (Fig. 4B). Interestingly, DDX5 knockdown displayed an opposite effect, as we observed an increase in HIV-1 full length RNA and a decrease in HIV-1 RNA spliced forms (Fig. 4B). Consistent with the effect of DDX17 knockdown on viral particles shown in Fig. 2C, this pattern can be explained by the fact that the cells compensate for the loss of DDX5 by upregulating the expression of DDX17.
To distinguish the different spliced mRNA species and to quantify their relative amounts, we analyzed viral transcripts by RT-PCR followed by Southern blotting as described in (Zhu et al., 2011). We observed that over expression of DDX17 decreased in full length RNA (Fig. 4D and E), which results in 6.5-fold increase for the multiply spliced RNAs, and up to a 50% decrease in the full length RNA upon DDX17 silencing or DDX17 DQAD over expression. The results suggest that DDX17 affects the balance of spliced and unspliced HIV-1 RNA populations.

**DDX17 modulates HIV-1 Gag processing**

Expression of DDX17 DQAD mutant in HEK293 cells decreases the amount of viral p24 in the supernatant (Fig. 1D). This defect may result from diminished HIV-1 protein expression in cells. To test this, we transfected HEK293 cells with DDX17 constructs and HIV-1 NL4-3 DNA and analyzed viral protein expression by Western blot (Fig. 5A). We observed that expression of DDX17 DQAD decreased the amount of viral p24. However, the level of Pr55\textsuperscript{Gag} was not affected, suggesting that the DDX17 mutant likely inhibits Gag processing rather than impeding Gag production (Fig. 5A). Similarly, DDX17 knockdown in HeLa cells reduced the amount of viral p24 but not Pr55\textsuperscript{Gag}, suggesting the same defect in Gag cleavage (Fig. 5B).

HIV-1 genome encodes two polyproteins Pr\textsubscript{160}\textsuperscript{Gag-Pol} and gp\textsubscript{160}\textsuperscript{env}. While gp\textsubscript{160}\textsuperscript{env} precursor is cleaved in gp120 and gp41 by the cellular protease Furin (Hallenberger et al., 1992), the Gag-Pol polyprotein is self-cleaved by the viral protease (PR) (Darke et al., 1988). HIV-1 Pol gene encodes the three viral enzymes, including protease (PR), reverse transcriptase (RT) and integrase (IN) among which PR is responsible for the processing of Pr\textsubscript{160}\textsuperscript{Gag-Pol} and Pr\textsubscript{55}\textsuperscript{Gag} precursors. In order to assess the effect of DDX17 on total Gag expression, we transfected HEK293 cells with DDX17 DNA and treated the cells with 100 nM duranavir, an HIV-1 PR inhibitor, and assessed the levels of Pr\textsubscript{160}\textsuperscript{Gag-Pol} and Pr\textsubscript{55}\textsuperscript{Gag} in the cell lysate (Fig. 5C). For wild-type DDX17, DRV treatment led to the accumulation of Pr\textsubscript{160}\textsuperscript{Gag-Pol}, as a result of HIV-1 PR-dependent cleavage inhibition. No overall changes were observed for total Gag levels upon wild-type DDX17 expression (Fig. 5C). Interestingly, overexpression of DDX17 mutants led to a slight decrease in the amount of Pr\textsubscript{160}\textsuperscript{Gag-Pol} but no significant effect on Pr\textsubscript{55}\textsuperscript{Gag} expression (Fig. 5C). In addition, as seen in Fig. 5A, p24 levels were dramatically diminished upon the expression of both DQAD (S) and DQAD (L) mutants. Our results suggest that the DDX17 DQAD mutant compromises Gag processing, and the decrease in the overall amount of Gag-Pol products suggests a possible defect in HIV-1 frameshift activity.

**Effect of DDX17 on Gag-Pol frameshift**

To regulate the ratio of Gag and Gag-Pol expression, HIV-1 has evolved a slippery RNA sequence that causes -1 frameshift, which allows producing HIV-1 enzymes (Jacks et al., 1988; Vaishnav and Wong-Staal, 1991). Therefore, a decrease in HIV-1 frameshift efficiency implies a defect in the production of viral enzymes, including HIV-1 PR that is responsible for Gag cleavage, thus reduces virus particle infectivity. To test this hypothesis, we used two dual-luciferase reporter plasmids FS (0) and FS (−1) previously described by Grentzmann to measure HIV-1 Gag-Pol frameshift (Grentzmann et al., 1998) (Fig. 6A). We transfected HEK293 cells with DDX17 DNA with either FS (0) or FS (−1) reporter and quantified luciferase expression levels. We observed that over expression of DDX17 DQAD mutant led to up to 40% decrease in frameshift efficiency (Fig. 6B). No significant effect was observed for the wild type DDX17. These results suggest that the helicase activity of DDX17 is involved in maintaining the proper ratio of Gag/Pol gene expression required for optimal infectivity.

**Discussion**

In this study, we found that DDX17 promotes HIV-1 particle infectivity by modulating HIV-1 RNA packaging and Gag-Pol frameshift. Viral RNA analysis also demonstrates that the over expression of the helicase negative DDX17 DQAD mutant increases the multiply spliced/unspliced HIV-1 RNA ratio by up to six-fold (Fig. 4F). Recently, Chen et al., (2008) reported that the zinc finger antiviral protein ZAP interacts with DDX17 that acts as a cofactor to potentiate the activity of ZAP in inhibiting the replication of a few viruses, including murine leukemia virus. The same group also showed that ZAP targets HIV-1 multiply spliced mRNA to exosomes for degradation (Zhu et al., 2011). The crystal structure of the N-terminal domain of ZAP reveals the RNA binding region that is likely involved in HIV-1 RNA recognition (Chen et al., 2012). In addition to its role in ZAP-mediated degradation of multiply spliced HIV-1 mRNA, DDX17 also has ZAP-independent functions in HIV-1 replication. Naji et al. (2012) found that DDX17 interacts with HIV-1 Rev and that DDX17 silencing reduced the amount of viral transcripts and inhibited the export of both unspliced and spliced HIV-1 mRNAs to the cytoplasm.

In our study, we also found that DDX17 silencing led to a decrease in full length HIV-1 RNA (Fig. 4C). Interestingly, this decrease in full length RNA correlates with an increase in multiply spliced viral transcripts (Fig. 4). We propose that several mechanisms may account for the accumulation of multiply spliced HIV-1 RNA upon DDX17 silencing or DDX17 DQAD over expression. The first mechanism is simply the inefficient ZAP-mediated degradation of HIV-1 multiply spliced RNA (Zhu et al., 2011). A second
mechanism involves excessive splicing due to sequestration of HIV-1 RNA in the nucleus, which leads to decreased amount of unspliced viral mRNA and increased amount of multiply spliced HIV-1 mRNAs. Lastly, DDX17 may directly modulate HIV-1 RNA splicing, as previously reported for DDX17 to affect alternative splicing of cellular genes (Honig et al., 2002). HIV-1 splicing is a highly regulated process, which involves stem-loops regulatory elements called splicing silencers and splicing enhancers and generates more than 30 HIV-1 RNA species (Stoltzfus, 2009). During splicing, RNA is remodeled in order to expose splicing silencers and splicing enhancer stem-loops that act as docking sites for distinct members of the spliceosome machinery, thereby influencing exon definition (Chen and Manley, 2009; Stoltzfus, 2009). One hypothesis would be the involvement of DDX17 in remodeling HIV-1 RNA structure, leading to a change in exon definition, resulting in an increase of full length and a decrease in spliced HIV-1 RNAs, as seen in Fig. 4. Generally, moderate effects were seen for the over expression of wild type DDX17, which can be due to the fact that endogenous levels are sufficiently high to promote the production of infectious HIV-1 particles.

We found that expression of DDX17 mutants induce a dramatic decrease in the amount of packaged gRNA (Fig. 3), which leads to
the production of less infectious viral particles. Considering that the amount of full-length HIV-1 RNA decreased by only two-fold in the cells for DQAD (L), we conclude that the packaging of gRNA was diminished by five-fold. Similarly, we observed a 1.5 fold decrease in full length HIV-1 RNA for DQAD (S), but a 3.5-fold diminution in packaged gRNA (Fig. 3). This data suggests that the helicase activity of DDX17 is necessary to promote HIV-1 gRNA packaging. HIV-1 RNA packaging is a complicated process that involves interactions of cellular and viral proteins with specific viral RNA motifs. HIV-1 5′UTR contains a number of stem-loop (SL) structures that act as regulatory sites for multiple processes, including splicing and packaging. Being a RNA helicase, it is possible that DDX17 remodels the viral RNP complex within the nucleus, therefore modifying its composition through the recruitment of various factors and changes the fate of the viral RNA. We do not lose sight of the possibility that perturbing the levels of DDX17 in cells may modulate cellular processes such as RNA transcription and splicing which in turn affects the process of HIV-1 packaging.

Interestingly, although no noticeable change in the level of Pr55Gag was observed, over expression of DDX17 DQAD mutants in HEK293 cells and knockdown of DDX17 in HeLa cells lead to a dramatic decrease in viral p24 levels, which suggests that DDX17 DQAD mutants inhibit Gag processing. This effect on Gag processing correlated with a slight diminution of Pr160Gag-Pol (Fig. 5C), suggesting that the helicase activity of DDX17 is required for frameshift to occur and for producing optimal ratios of Gag vs Gag-Pol. Indeed, results of experiments with the frameshift reporter constructs showed that DDX17 DQAD (L) led to a significant 40% decrease in frameshift efficiency (Fig. 6B), which is expected to result in less production of Pr160Gag-Pol. The decrease in Pr160Gag-Pol synthesis is expected to reduce viral protease concentration in the progeny virus particles and consequently, diminish viral Gag processing.

Competing interests

The authors declare they have no competing interests.

Acknowledgments

We thank Vicky Cheng and Zhenlong Liu for technical assistance in this study, Drs Hans Stahl, Anne Gatignol and Léa Brakier-Gingras for providing valuable reagents. This work was supported by funding from the Canadian Institutes of Health Research.
Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.virol.2013.05.026.

References


HIV-1 infected T cells. J. Virol. 79 (22), 41205–41210.


Li, R.-P., Lorgeoux et al. / Virology 443 (2013) 384–392

Ribosome frameshifting in HIV-1 gag-pol expression. Nucleic Acids Res. 21 (1), 160–166.

Li, R.-P., Lorgeoux et al. / Virology 443 (2013) 384–392

Regulation of cell growth, and is associated with improved infectivity of human immunodeficiency virus type 1 RNA. Nature 491 (7422), 125–128.


