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Cellular RNA helicases and HIV-1: Insights from genome-wide, proteomic, and molecular studies

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Abstract

RNA helicases are ubiquitous in plants and animals and function in many cellular processes. Retroviruses, such as human immunodeficiency virus (HIV-1), encode no RNA helicases in their genomes and utilize host cellular RNA helicases at various stages of their life cycle. Here, we briefly summarize the roles RNA helicases play in HIV-1 replication that have been identified recently, in part, through genome-wide screenings, proteomics, and molecular studies. Some of these helicases augment virus propagation while others apparently participate in antiviral defenses against viral replication.

Keywords

RNA helicases; Human Immunodeficiency virus type 1 (HIV-1); DEAD-Box domain; antiviral

RNA helicases are enzymes that catalyze the unwinding of duplexed RNA by employing the energy from nucleotide triphosphate hydrolysis (Rocak and Linder, 2004). All identified RNA helicases contain a highly conserved NTP-binding domain and signature helicase domains (Tanner and Linder, 2001). Based on genome sequencing, the human genome encodes at least 85 RNA helicases. These RNA helicases are involved in various aspects of RNA metabolism, including nuclear transcription, pre-mRNA processing/splicing, mRNA transportation, translation, RNA stability, RNA maturation, and ribosome biogenesis (Anantharaman et al., 2002). Many have been characterized and have demonstrated roles in RNA metabolism, RNA-induced silencing or host innate responses (Table 1). These roles are carried out in the context of large RNPs, including the spliceosome (e.g. UAP56), the eukaryotic translation initiation complex (e.g. eIF4A), termination machinery (e.g. Upf1) and RNA-induced silencing complex (e.g. Dicer). An activity common to these RNPs is the progressive remodeling of the RNA-protein components, which contributes to RNA quality control.

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RNA helicases exhibit both ATP-dependent and ATP-independent activities (see Linder and Jankowsky, 2011). For RNA strand separation, ATP binding without hydrolysis is sufficient (Linder and Jankowsky, 2011; and references therein). Progression to an ATP hydrolysis step catalyzes fast release of the RNA substrate. In addition, RNA helicases function to nucleate large RNA–protein complexes. Recent structural and mechanistic results have demonstrated that ATP binding at the central helicase core triggers structural clamping by the flanking domains (Dufu et al., 2010). In combination, structural, biophysical and molecular biology studies are beginning to characterize these conformational changes. The corollary roles played by cofactors at the flanking domains are on the horizon. Recent advances have identified cofactors by mass spectrometry on complexes isolated by immunoprecipitation of RNA helicases and/or their RNA substrates (Sharma and Boris-Lawrie, 2012).

Cellular RNA helicases catalyze RNA-RNA or RNA-protein rearrangements (e.g. UAP56 or eIF4A) and assist as conformational scaffold for alternative RNA-protein or protein-protein interactions fueled by ATP binding, but independently of ATP hydrolysis (e.g. Dicer, RIG-I). Their roles in RNA metabolism span nuclear transcription, pre-mRNA processing/splicing, mRNA transport, translation, and turnover (Anantharaman et al., 2002; and reviewed in Linder and Jankowsky, 2011).

Classification of RNA helicases

All helicases (DNA and RNA) are classified into one of five different superfamilies (SF1–5), based on sequence conservation in their helicase core (Linder, 2006) (Figure 1). As shown in Figure 1, SF1 RNA helicases are designated Upf1-like. Upf1 is a component of the RNP that recognizes premature termination codons and triggers nonsense mediated decay. MOV10 is another SF1 member. SF2 encompasses most other RNA helicases, which are of cellular (DExD/H group) or viral origin (Figure 1). The central helicase core of SF1 and SF2 proteins consist of two domains (1 and 2) that resemble domain the bacterial recombinase A protein. Domain 1 and 2 are comprised of characteristic sequence motifs located at conserved positions (Figure 2). (Rocak and Linder, 2004, Jankowsky and Jankowsky, 2000; Linder and Jankowsky, 2011; Gorbalenya and Koonin, 1993; Jankowsky and Bowers, 2006; Singleton et al., 2007) (Figure 1). The characteristic amino acids of motif II (Figure 2) are used to designate SF members as DEAD, DEAH, RIG-I-like DExH or viral DExH proteins (Figure 1). The structural conformation of domains 1 and 2 is an equally prominent feature of SF2. Domain 1 and 2 fold to form a cleft that juxtaposes the ATP-binding and RNA binding sites (reviewed in Linder Jankowsky, 2011).

The central helicase core is the diagnostic feature of the RNA helicase superfamily and is conserved across the 3 kingdoms of life and encoded in some viruses. These proteins (typically designated DDX or DHX) significantly diverge in domains that flank their central helicase core. The flanking domains supply a plethora of regulatory and accessory properties that convey a broad functional scope to this protein superfamily. Helicases of SF1 and SF2 do not form toroidal hexameric ring-like structures, and their helicase core consists of two almost identical RecA-like domains arranged in tandem (Jankowsky and Jankowsky, 2000; Linder and Jankowsky, 2011). As mentioned above, to date, most of the known eukaryotic RNA helicases belong to SF2 and only a few belong to SF1 (Jankowsky, 2011; Rocak and Linder, 2004) (Figure 1). Members of SF3–5 are comprised of helicases that form toroidal hexameric ring-like structures, and their helicase core consists of one RecA-like domain. Helicases within SF3–5 are predominantly of bacterial or viral origin (Abdelhaleem, 2004).

The central helicase core has a high degree of structural similarity and is composed of two distinct, but redundant domains that are arranged in tandem (Figure 2). These domains share

nine conserved sequence motifs: motifs Q, I, Ia, Ib, II, and III in domain 1; and motifs IV, V and VI in domain 2 (motif Q is only present in DEAD-box helicases) (Hanada and Hickson, 2007; Linder et al., 2001) (Figure 2). Genetic, biochemical and structural analyses have been used to assign distinct functions to each motif. Of the nine motifs, the functions of motif I (Walker A) and II (Walker B) are best characterized and have been shown to be necessary for NTP binding and hydrolysis. All SF1 and SF2 RNA helicases contain Walker A and Walker B motifs, and typically five to seven different flanking conserved motifs.

SF1 and SF2 are sub-divided into distinct, well-defined, families and groups based on the consensus sequence in conserved motifs and comparative structural and functional properties (Fuller-Pace, 2006). As shown in Figure 1, SF1 is divided into three families. Two families are DNA helicases, and the other is the Upf1-like RNA helicases which contain MOV10. Three of the SF2 RNA helicase families share eight conserved motifs (motifs I, Ia, Ib, II, III, IV, V and VI) and are collectively referred to as the DExD/H group of RNA helicases: 1) DEAD box (for the conserved amino acid residues Asp-Glu-Ala-Asp), 2) DEAH box (for the conserved amino acid residues Asp-Glu-Ala-His), and 3) Ski2 (Superkiller-like 2)-like (Figure 1)(Gorbalenya and Koonin, 1993; Jankowsky and Bowers, 2006; Singleton et al., 2007). Members within each sub-family share sequence conservation and mechanistic features, such as choice of NTP bound to stimulate hydrolysis and the polarity of unwinding duplexes. The RNA helicases identified from genome-wide screenings, proteomics and molecular studies are predominantly SF2 family members; an exception is the SF1 member MOV10.

Modular structure of RNA helicases

While the central helicase core of RNA helicases is highly conserved, it is surrounded by variable N- and C- terminal domains. In most helicases, the flanking domains are larger in size than the helicase domain and are not well conserved within or between helicase families. Since RNA helicases are usually found within large RNP complexes, it is thought that the specificity of binding to target RNAs is conferred by the interactions of the flanking domains of the helicase with other proteins or the target RNA (Fairman-Williams et al., 2010). In addition, RNA helicases may function as RNase to displace proteins from structured or unstructured RNA in an ATP-driven reaction (Linder and Jankowsky, 2011). Other functions of the N- and C-terminal domains may include enzymatic activities (nuclease function), oligomerization, and sensing of pathogen-associated molecular patterns (Bleichert and Baserga, 2007).

Virus-encoded RNA helicases facilitate replication and infectivity of progeny virions

Eight cell-encoded RNA helicases have documented roles in the replication cycle of RNA or DNA viruses (reviewed in Ranji and Boris-Lawrie 2010). Extensive mutagenesis studies have noted that viral helicase functions are biologically required for the replication of many types of viruses including vaccinia virus, poliovirus, alphaviruses, bomomosaic virus, nidoviruses, and flaviviruses (see citations in Jeang and Yedavalli, 2006). Here, we summarize briefly, three types of virus-encoded RNA helicases have been described to be important in the expression of viral RNA and morphogenesis of the infectious virion.

The nonstructural protein 3 (NS3) of hepatitis C virus (HCV), yellow fever virus and dengue virus exhibits an SF1-like central helicase core (reviewed in Frick, 2007). Based on data from HCV, NS3 helicase is an essential viral protein and participates in viral RNA metabolism and activity of the viral RNP. The NS3 ATP-dependent unwinding activity facilitates viral RNA synthesis. In addition, NS3 nucleates assembly of viral proteins and

yet-to-be defined cellular cofactors, thereby promoting infectious virus production independently of the role in HCV RNA replication.

Three poxviruses encode a homologous nucleoside triphosphate phosphohydrolase II (NPH-II): vaccinia, variola and fowlpox virus. NPH-II contains a DEVH ATP binding domain and DUF and exhibits homology with RHA/DHX9. NPH-II participates in early viral DNA transcription. The ATP binding and RNA unwinding activity of NPH-II are involved and were postulated to resolve R loop formation behind the elongating RNA polymerase, which otherwise may result in genomic instability. NPH-11 is incorporated into the vaccinia virion and is an essential viral gene (Gross and Shuman, 1998).

The first reported plant virus RNA helicase is cylindrical inclusion protein (CI) from the potyvirus plum pox virus, tobacco-mosaic virus and lilly-mottle virus (Fernandez et al., 1997). Plum pox virus CI contains a DECH nucleotide binding domain and exhibits NTP-dependent helicase activity that is necessary for viral replication. CI nucleates viral components promoting the spread of the virus from cell to cell. In sum, NS3, NDH-II and CI each display roles in viral RNA synthesis and virus particle morphogenesis.

Evidence for the involvement of many RNA helicases in HIV-1 replication

HIV-1 is an example of a virus that does not encode an RNA helicase. Nonetheless, cDNA microarray analyses have found that HIV-1 infection changes the expression profile of several cellular RNA helicases including DHX9, DDX11, DDX18, DDX21 and DDX24 (Krishnan and Zeichner, 2004; van 't Wout et al., 2003), suggesting an interplay between cellular RNA helicases and HIV-1 replication. A case for a functionally important interaction of HIV-1 with cellular RNA helicases is further made by recent genome-wide screening studies. Over the past three years, four siRNA/shRNA-based genome-wide screenings for HIV-1 dependency factors have been performed (Brass et al., 2008; König et al., 2008; Yeung et al., 2009; Zhou et al., 2008a). Three of these studies employed siRNA-mediated transient knockdown of mRNAs in adherent HeLa/293T cells, while a fourth study employed shRNA-mediated durable knockdown of mRNAs in suspension Jurkat T cells (Yeung et al., 2009). It is noteworthy that siRNA-HeLa/293T cell results revealed the importance of at least 12 discrete RNA helicases for HIV-1 replication (Table 1). Aside from DDX3X, which was previously identified as being important for HIV-1 Rev function in the export of unspliced viral RNA (Yedavalli et al., 2004), no other RNA helicase was identified more than once in any of the genome-wide knockdown studies (Table 2). Because cell culture and assay conditions are different between the three siRNA-HeLa/293T cell studies, the lack of overlap in the identified RNA helicases could mean that different helicases may provide context dependent redundant functions. Adding complexity to the interpretation that RNA helicases provide pivotal viral replication function(s) is the absence of identification of any RNA helicase in the genome-wide shRNA-Jurkat knockdown study (Yeung et al., 2009). This result could mean that the long term down-regulation of RNA helicases is generally cytotoxic in Jurkat suspension cells (although not in adherent cells); if this is the case, then the contributions of individual helicases to HIV-1 replication in suspension cells cannot be studied with this approach.

The involvement of RNA helicases in the HIV-1 lifecycle is additionally suggested by three recent mass spectrometric-based proteomic studies. Krogan and colleagues (Jager et al., 2011) reported that Gag complexed with DHX9, DDX18, DDX21, and DDX24. They also found that DDX20 interacted with Vpr, and DDX6 interacted with Env gp120 (Jager et al., 2011). In a separate proteomic analysis, Gerace and coworkers (Naji et al., 2011) studied the role of RNA helicases in Rev/RRE (Rev-responsive element) interaction and found the involvement of DDX1, DDX3, DDX5, DHX9, DDX17, DDX24, DHX36 and DDX47.

Moreover, DDX24 was found to be required for the packaging of HIV-1 RNA through interaction with Rev protein, whereas the over-expression of DHX30 has been reported to restrict the packaging of HIV-1 RNA (Ma et al., 2008; Zhou et al., 2008b). It should be pointed out that while the Krogan study, which measured direct protein-protein interaction, did not detect any Rev-interacting cellular RNA helicases (Jager et al., 2011), the Gerace study (Naji et al., 2011) was performed in a different manner by including the HIV-1 RRE-RNA in their mass spectrometry-proteomic readouts. Thus, the latter would reflect not just protein-protein interaction, but also interactions between proteins that are bridged by RNAs. In a third study, Kula et al. recently showed that several known RNA helicases are involved in various steps of HIV-1 RNA expression using affinity purification of viral transcripts via flag-MS2, coupled with mass spectrometry (Kula et al., 2011). Factors such as DDX3X, BAT1, UPF1 and the UPF-1 like helicase-MOV10 were characterized as regulators of HIV-1 RNA metabolism.

There was wide divergence in the results from the above three proteomic and four genome-wide screening studies, suggesting that the precise cellular roles and significance of RNA helicases for HIV-1 replication and pathogenesis will require further characterization. It will be important, for future understanding, to design experiments which can segregate helicases that serve direct, although perhaps overlapping and redundant, roles in HIV-1 replication from those that might participate indirectly in the HIV-1 life cycle. Nevertheless, the convergence of evidence suggests that many cellular RNA helicases do contribute to regulating various steps in HIV-1 replication.

Below, we discuss briefly a few selected examples of helicases that have been molecularly characterized for their contributions to HIV-1 infection (Figure 3).

RNA helicases that contribute positively to HIV-1 replication

A) DHX9/RNA helicase A (RHA)

RHA plays an active role in transcription by bridging the CREB-binding protein (CBP) and RNA polymerase II (Nakajima et al., 1997). Conflicting evidence has been published for a possible role for RHA in HIV-1 RNA synthesis and nuclear export. The discrepancy is related to differences in experimental approaches. For instance, conclusions in favor of RHA stimulating HIV-1 RNA synthesis and export involved measurements of reporter protein without RNA analysis or employed the over expression of RHA (Li et al., 1999; Tang et al., 1999). More recently, RHA was shown to interact with the HIV-1 5'-leader and facilitate polyribosome association. RHA downregulation reduced the synthesis of HIV-1 proteins, but did not affect HIV-1 RNA abundance or nuclear export. The translational efficiency of the HIV-1 transcripts was downregulated, unless rescued by exogenous expression of epitope-tagged RHA. Rescue was not observed by RHA mutated in the ATP binding site (K417R). In aggregate, the results indicate RHA is an HIV-1 RNA binding protein that facilitates translation (Bolinger et al., 2010).

Other activities reported for RHA include roles in reverse transcription in target cells (Roy et al., 2006) and evidence that RHA associates with HIV-1 Gag and was packaged into HIV-1 particles in a viral RNA-dependent manner (Roy et al., 2006). Recent analysis of RHA in virions measured 2 RHA molecules per virion. The observed direct stoichiometry proportional to the dimeric virion RNA further suggests that RHA is assembled into virions in complex with viral RNA (Sharma and Boris-Lawrie, 2012). The downregulation of RHA by siRNA in virus producer cells generated virions with diminished infectivity on primary lymphocytes (Bolinger et al., 2010), and RHA-deficient virions exhibited reduced reverse transcription (Roy et al., 2006) that has been attributed to a role by RHA in promoting tRNA(3)(Lys) annealing to viral genomic RNA (Xing et al., 2011) or to an inherent defect in

viral RNP. One speculation is that RHA is important during virion morphogenesis in shaping the Gag-virion RNP (Bolinger et al., 2010). The above findings appear to be compatible with two possible explanations. First, it is conceivable that RHA participates in the formation of infectious virus particles either by shaping the Gag–RNA interaction during viral particle assembly or during budding. Failure of RHA to properly restructure viral RNP (ribonucleoprotein complex) could explain the observed reduced infectivity. Second, HIV-1 particles that do not contain RHA have reduced virion-endogenous reverse transcriptase activity. In this respect, it may be that RHA assists HIV-1 reverse transcriptase to copy RNA more efficiently by unwinding the RNA secondary structure or by promoting the interaction of viral RNA with the nucleocapsid protein in order to assemble a better reverse transcription complex.

B) DDX3 and DDX1

There is evidence that HIV-1 RNA export from the nucleus into the cytoplasm by CRM1-Rev/RRE utilizes the ATP-dependent co-factor DDX3 (Yedavalli et al., 2004). DDX3 is a nucleocytoplasmic shuttling protein that binds CRM1 and localizes to nuclear pores. Experimentally, abolishing the cell's DDX3 activity suppressed Rev/RRE function in the export of unspliced and partially spliced HIV-1 RNAs, supporting a role for DDX3 in the CRM1 RNA export pathway, analogous to the postulated role for Dbp5, which has been shown to be involved in the nuclear export of yeast mRNA and unspliced Rous sarcoma virus RNA (Hodge et al., 2011; LeBlanc et al., 2007). Others have also reported that DDX3 enhances internal ribosomal entry site (IRES)-mediated translation of HIV-1 RNA (Liu et al., 2011). In addition, DDX3 was demonstrated to be involved in the epithelial-mesenchymal-like transformation of cancer cell and activation of DDX3 can promote growth, proliferation and neoplastic transformation of breast epithelial cells (Botlagunta et al., 2008). Like DDX3, a second RNA helicase DDX1 has also been found in virus infected cells to influence HIV-1 replication (Fang et al., 2004; Edgcomb et al., 2012). Whether DDX3 and DDX1 and other related helicases serve complementary and/or redundant functions needs further clarification.

C) RH116 and Werner syndrome helicase

RH116 is a 116 kDa interferon-induced helicase with two caspase recruitment domains (CARDs). The overexpression of RH116 increased the levels of unspliced and singly spliced, but not multiply spliced, HIV-1 RNA, suggesting a role of RH116 in a post-transcriptional gene expression step (Cocude et al., 2003). Although early work identified RH116 as a positive factor for HIV-1 replication (Cocude et al., 2003), a highly related cellular protein, MDA5, has been ascribed antiviral function(s). RH116 and MDA5 exhibit 99.5% sequence identity. Comparative examination of the roles of RH116 and MDA5 is warranted.

The SF2 member Werner syndrome helicase has been shown to promote HIV-1 transcriptional activation. Werner syndrome helicase has been identified to interact with the HIV-1 Tat protein and acts to recruit the transcription factors PCAF (p300/CREB-binding protein-associated factor) and p-TEFb (positive transcription elongation factor b) to the HIV-1 LTR in promoting its transcriptional activation (Sharma et al., 2007).

D) Up-frameshift protein 1 (UPF1)

UPF1, an ATP-dependent RNA helicase of the SF1 superfamily, has been characterized as an essential factor for nonsense-mediated mRNA decay (NMD) (Applequist et al., 1997; Bhattacharya et al., 2000). Recent studies have demonstrated that UPF1 is also involved in DNA repair, telomere homeostasis and replication-dependent histone mRNA decay (Hogg and Goff, 2010; Imamachi et al., 2012). Furthermore, UPF1 was revealed to be a component

of the HIV-1 RNP, which consists of HIV-1 genomic RNA, Gag protein and the host protein Staufen1 (STAU1) (Ajamian et al., 2008). Knockdown of UPF1 resulted in a decrease of HIV-1 RNA and Gag expression. On the other hand, the over-expression of UPF1 increased the levels of Gag protein and HIV-1 genomic RNA. The effect of UPF1 on HIV-1 expression is correlated with its ATPase activity (Ajamian et al., 2008).

RNA helicases that negatively regulate HIV-1 replication

A) miRNA/RISC –associated RNA helicases

Dicer is a ribonuclease with a *bona fide* DEAD-Box RNA helicase domain (Forstemann et al., 2005; Gregory et al., 2005). This DEAD-Box RNA helicase has two potential functions which include the unwinding of the miRNA duplex and the remodeling of the conformation of the miRNA/miRISC (miRNA-induced silencing complex) complex (Tang, 2005). It has been shown that human Dicer works in concert with three other proteins: Argonaute 2 protein (Ago2), HIV-1 transactivation responsive element (TAR) RNA-binding protein (TRBP), and the dsRNA-binding protein PACT (Forstemann et al., 2005; Gregory et al., 2005; Lee et al., 2006). HIV-1 infection has been shown to impact the activity of Dicer (Bennasser and Jeang, 2006) as well as change the profile of miRNAs in virus-infected cells and individuals (Triboulet et al., 2007; Yeung et al., 2005; Houzet et al., 2008; Hayes et al., 2011; Witwer et al., 2012). There is evidence that cellular miRNAs provide a first layer of antiviral defense to check viral replication in host cells (Hariharan et al., 2005; Huang et al., 2007; Kumar, 2007; Kumar and Jeang, 2008; Chable-Bessia et al., 2009; Jeang, 2012).

Besides Dicer there are additional RNA helicases that are constituents of miRISC. One of these helicases is the RCK/p54 DEAD box helicase. RCK/p54 is an effector molecule in miRISC that represses translation of targeted mRNAs (Chu and Rana, 2006). Additionally, there are reports that RHA interacts with RISC in human cells, suggesting a role for RHA in miRNA-loading into the RISC (Robb and Rana, 2007). Moreover, several other RNA helicases have also recently been identified that associate with TRBP, including DDX17, DDX54 and DDX5 (Chi et al., 2011). Hence, RNA helicases may be important for the activities of miRNAs and in the interaction of miRNAs with viral mRNAs.

B) RIG-I, MDA5 and innate sensing of HIV-1

The DExD/H RNA helicases retinoic acid inducible gene-1 (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) are cytoplasmic RNA sensors that activate type I interferon (IFN)-dependent innate immunity (Kang et al., 2002; Yoneyama et al., 2004). Transfection of *in vitro* transcribed HIV-1 RNA into cells induces type 1 IFN response in a RIG-I dependent manner (Berg et al., 2012). However, it was curiously shown that HIV-1 infection inhibited RIG-I-mediated antiviral signaling (Solis et al., 2011) and that MDA5, but not RIG-I, is important in sensing dsRNA after simian immunodeficiency virus (SIV) infection (Co et al., 2011). These findings suggest that the independent or complementary roles for RIG-I and/or MDA5 in sensing HIV-1 need further study. Recently, it was reported that DDX3 interacts with the CARD domain of IFN- β promoter stimulator-1 IPS-1 (Oshiumi et al., 2010), a mitochondrial adaptor molecule that mediates innate immune signaling. Separately, the Vaccinia virus protein K7 has been found to potently inhibit IFN- β promoter activation, and DDX3 was identified as a target of K7 (Schroder et al., 2008). The latter raises a possibility of DDX3's involvement in an antiviral signaling pathway leading to type 1 IFN induction. As mentioned above, DDX3 was first reported as shuttling protein that assisted with the export of HIV-1 RNA. The new results raise the possibility that DDX3 could also contribute to innate sensing and response to viral infections.

C) MOV10

P body-associated protein moloney leukemia virus 10 homolog (MOV10) is the human homolog of the *Arabidopsis thaliana* RNA amplification factor gene SDE3 and the *Drosophila melanogaster* RISC-maturation factor gene Armitage (aimi) (Haussecker et al., 2008). Mov10 is a putative RNA helicase that was previously reported to belong to the DExD superfamily and was recently clarified to belong to the SF1 Upf-1-like group of helicases. MOV10 inhibits HIV-1 replication at multiple stages (Burdick et al., 2010). The overexpression of MOV10 in infected cells resulted in the reduction of HIV-1 Gag protein, virus production, and infectivity. Silencing of MOV10 expression in a human T cell line enhanced HIV-1 replication (Wang et al., 2010), and it was recently found that MOV10 can be packaged into HIV-1 virions by binding to the nucleocapsid region of Gag and accordingly inhibit viral replication at a postentry step (Abudu et al., 2012). A further study revealed that mutation of cysteine and histidine residues between positions 188–202 significantly compromised MOV10's anti-HIV-1 activity (Abudu et al., 2012). MOV10 is also an Ago2-associated protein, suggesting a possible role in regulating miRNA-mRNA interaction (Meister et al., 2005). Additionally, MOV10 can interact with telomerase protein and single-/ double- stranded telomere DNA (Nakano et al., 2009), and may be essential for silencing retrotransposons in the mouse male germline (Frost et al., 2010). A new study has noted that while over-expressed MOV10 acts to restrict retroviral infection, cell endogenous MOV10 appears physiologically not to have this activity and is apparently functional only in the regulation of retrotransposons (Arjan-Odedra et al., 2012). These investigators also found in their experiments that MOV10 does not play a role in miRNA- or siRNA- mediated mRNA silencing (Arjan-Odedra et al., 2012); however, they did not explore the previously reported requirement of MOV10 for piRNA-mediated silencing of retrotransposons (Frost et al., 2010).

RNA helicase inhibitors as new potential anti-HIV-1 drugs

This review summarizes in brief several RNA helicases that may impact HIV-1 replication in human cells. Since the first reports of AIDS in 1981, small molecule therapeutic compounds have been developed to target viral proteins such as reverse transcriptase, protease, and integrase (reviewed in Wainberg and Jeang, 2008). Due to the high mutation rate of the HIV-1 genome, a consequence of targeting viral proteins is the selection for the rapid emergence of mutated proteins that confer drug-resistance. The increased prevalence of drug-resistant HIV-1s highlights an urgent need to develop new treatment approaches.

One way to circumvent the mutability of the HIV-1 genome is to target cellular factors used by the virus for replication. Among hundreds of host cellular co-factors required by HIV-1 for its replication, the human DDX3 could be a suitable target for antiviral treatment since it was shown in multiple independent reports to be important for HIV-1 replication. Indeed, there is an effort to develop anti-DDX3 small molecules. Two studies have characterized small molecule inhibitors targeted to ATPase activity of DDX3 to inhibit HIV-1 replication (Maga et al., 2008; Yedavalli et al., 2008). Thus, a biological screening of known NTPase/ helicase inhibitors identified ring-expanded nucleosides (REN) as potential DDX3 inhibitors (Yedavalli et al., 2008). A recent study also found that N, N'-diarylurea derivatives can interfere with RNA binding by the helicase domain of DDX3 and can thereby reduce HIV-1 replication in peripheral blood mononuclear cells (PBMCs) (Radi et al., 2012) (Figure 4). These studies raise the possibility that RNA helicase inhibitors could be applied toward anti-retroviral therapies.

Concluding remarks

In conclusion, this review highlights the diverse roles of several RNA helicases in HIV-1 infection. Some helicases are positive regulators of HIV-1 replication, and they represent potential therapeutic targets. The screening and development of specific RNA helicase inhibitors have been conducted and have led to discoveries of new treatments for viral infections (Kwong et al., 2005). Future advances on this front promise to impact the translation of anti-helicase compounds into tangible anti-retroviral drugs.

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Highlights

- We review RNA helicases that affect HIV-1 replication.
- Potential drugs against RNA helicase for HIV-1 therapy are discussed.
- RNA helicases are used in diverse cellular functions

Figure 1. Classification of helicases into superfamilies and families

All DNA and RNA helicases are classified into five superfamilies (SF) 1–5 based on sequence conservation in the helicase core. SF1 and SF2 are sub-divided into distinct families and groups based on the conservation in the helicase core. RNA helicase families are shown in black and DNA helicase families are shown in gray. SF1 is divided into three families. SF2 is divided into five families each of DNA and RNA helicases. Three of the five RNA helicase families, DEAD box (for the conserved amino acid residues Asp-Glu-Ala-Asp), DEAH (for the conserved amino acid residues Asp-Glu-Ala-His)/RHA-like and Ski2-like, are collectively referred to as the DExD/H group of RNA helicases. The viral DExH family is divided into three groups: nucleoside triphosphate phosphohydrolase-II (NPH-II)-like helicases of *Poxviridae*, nonstructural protein 3 (NS3)-like helicases of *Flaviviridae*, and cytoplasmic inclusion (CI)-like helicases of *Potyviridae*. SF3 contains DNA helicases found in viruses. SF4 and SF5 are DNA helicases of bacterial origin.

DHX9/RHA

Figure 2. Organization of the conserved sequence motifs of SF1 and SF2 RNA helicases
 (I) the helicase core is composed of two distinct but highly similar domains (Domain 1 and 2) arranged in tandem. These two domains contain nine conserved sequence motifs. Motifs I (Walker A) and II (Walker B) are present in all SF1 and SF2 RNA helicases and function in NTP binding and hydrolysis. Motifs Ia, Ib, IV and V bind the substrate RNA. Motifs III and VI promote coupling of NTP binding and hydrolysis to the helicase activity. Motif Q is only present in DEAD-box RNA helicases and acts as a sensor of bound ATP and, thus, regulates ATP binding and hydrolysis. The N- and C- terminal residues flanking the helicase core domain typically encode other conserved domains that modulate cofactor interactions, substrate specificity and catalytic activity. (II) Example of domain structure of SF2 member DHX9/RNA helicase A (RHA). The indicated domains include: two N-terminal double-stranded RNA binding domains (dsRBD 1 and 2), two domains of the helicase core – DEAD-like helicase superfamily ATP binding domain (DExDc) and helicase superfamily C-terminal domain associated with DExH/D box proteins (HELICc), and the C-terminal helicase-associated domain (HA2), domain of unknown function (DUF), and arginine and glycine-rich (RG-rich) carboxyl terminal residues. The numbers indicate amino acid residues.

Figure 3. Host RNA helicases involved in HIV-1 replication

The figure is intended to provide simplified illustrative examples; several of the illustrated activities remain debated and need additional experimental verification. RHA and MOV10 have been suggested to participate in HIV-1 reverse transcription and virion assembly, although these activities need further clarification. Reverse transcription occurs in the cytoplasm, and the reverse transcribed cDNA is then imported into the nucleus for integration. In the nucleus, RHA and RH116 have been reported to regulate HIV-1 transcription. DDX3, DDX1 and DDX17 interaction with Rev/RRE have been implicated in the export of unspliced/partially spliced HIV-1 RNAs from the nucleus into the cytoplasm via the CRM1 pathway. DDX24, DDX18 and DDX21, and RHA have been reported to regulate HIV-1 mRNA maturation and translation. The figure is modified after Jeang and Yedavalli, 2006; and Ranji and Boris-Lawrie, 2010; and readers are encouraged to consult these reviews for additional details.

Figure 4. DDX3 as a drug target

Small molecule inhibitors, such as FE15 and REN, are designed to target ATPase activity and compete with ATP binding. N, N'-diarylurea derivatives inhibit RNA binding of helicase domain.

Table 1

RNA helicases are ubiquitously involved in RNA biology

Examples of SF1 and SF2 proteins involved in fundamental cellular process		
RNA metabolism	RNA-induced silencing	Innate response
DBP5, DDX5/p68, RHA/DHX9, DDX17, DDX 19, DDX20, DDX25, DDX39, UPF1, UAP56	DICER; TRBP, RHA/DHX9, DDX5, DDX6, DDX17, DDX54, Mov10	RIG-I/DDX58, MDA5, DDX3, RHA/DHX9

Table 2

RNA helicases identified in screens as candidate cofactors in the HIV-1 life cycle

Assay	RNA helicases	Cell lines	References
RNAi screening	DDX3X, DDX10, DDX33, DDX53, DDX55	HeLa(CD4+)	(Brass et al., 2008)
	DHX15, DDX23, DDX26	HEK293	(Konig et al., 2008)
	DDX3X, DDX49, DDX50, DHX58, DDX60L	HeLa(CD4+)	(Zhou et al., 2008a)
Mass spectrometry	DDX6, DDX49, DDX18, DDX21, DDX24, DDX20, DHX9/RHA	HEK293/Jurkat	(Jager et al., 2011)
	DDX1, DDX3, DDX5, DDX17, DDX24, DDX47 DHX9, DHX36	HeLa(CD4+)	(Naji et al., 2011)
	DDX3X, BAT1, MOV10, USF1	U2OS	(Kula et al., 2011)