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Mini ways to stop a virus: microRNAs and HIV-1 replication

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Abstract

Cellular restriction of HIV-1 replication has traditionally been thought of as protein mediated: APOBEC3G hypermutates HIV-1 genomic RNA, but is counteracted by Vif; Tetherin inhibits the release of budding virions but is counteracted by Vpu. In recent years, new evidence suggesting that miRNAs and other components of the miRNA pathway act as HIV-1 restriction factors has come to light, along with the identification of strategies that HIV-1 employs to surmount these host obstacles. In this article, we summarize and discuss the literature to date regarding the complex relationship between HIV-1 and miRNA-mediated inhibition.

Keywords

HIV-1; HIV restriction factors; latency; miRNA

miRNAs

RNA silencing in eukaryotes is mediated by three classes of small noncoding RNAs: miRNAs, siRNAs and Piwi-interacting RNAs (piRNAs) [1]. While piRNAs mostly act in germ-line cells to silence transposons, and endogenous mammalian siRNAs have only been identified in oocytes and stem cells [2], miRNAs are ubiquitously expressed and have been shown to be powerful regulators of post-transcriptional gene expression in virtually every pathway examined thus far [3–6]. Mammalian miRNAs (~21–23 nt) have been estimated to target anywhere between 30 and 90% of all protein-coding genes [7,8], with over 1000 human miRNAs having been identified to date [9]. The biogenesis of human miRNAs most commonly begins with Pol II-mediated transcription from the introns of both coding and noncoding transcriptional units, although some miRNAs are encoded by independent genes or in exonic regions [1,10], and some miRNAs are Pol III transcribed [11]. The primary, or pri-miRNA, transcript is typically several kilobases in length and forms a hairpin structure, which is then bound by the DiGeorge syndrome critical region gene 8 complex, also known as the Microprocessor. Drosha, an RNase III enzyme, cleaves the pri-miRNA stem to generate an approximately 70-nt stem-loop precursor miRNA with an approximately 2-nt overhang at the 3' end. Some precursor miRNAs are generated by the direct splicing and

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debranching of very short introns, called mirtrons, thereby bypassing Drosha cleavage entirely [12].

The precursor miRNA is exported from the nucleus via exportin 5, where it is then further processed by Dicer, another RNase III family member. Dicer cleavage excises the terminal loop of the hairpin structure, producing an approximately 22-nt RNA duplex. Noncanonical miRNA duplexes can also be generated by Dicer-mediated cleavage of tRNAs and small nucleolar RNAs [13–15], which may also function in post-transcriptional gene silencing. While the biogenesis of endogenous siRNAs (~21 nt) requires Dicer-dependent cleavage of either long dsRNA templates derived from the transcription of transposable elements and annealing of complementary transcripts or ssRNA transcripts encoding long hairpins, Drosha processing is not involved. piRNAs (~24–32 nt) are mostly derived from intergenic repetitive elements and do not require processing by either Drosha or Dicer [1].

Maximal Dicer activity requires the association of trans-acting responsive (TAR) RNA-binding protein (TRBP) and PACT, and all three proteins serve as subunits of the miRNA-induced silencing complex (miRISC). The core enzyme of miRISC is one of the Argonaute (Ago) proteins; there are two Ago subfamilies, the Ago subfamily, comprising Ago1–4, and the Piwi subfamily members, which mediate piRNA silencing [1]. Ago subfamily proteins bind both the miRNA and its target mRNA [16], and evidence suggests that while the four human Ago proteins are somewhat redundant and interchangeable [17], argonaute-2 (Ago2) may be a more powerful and universal mediator of miRNA-based gene silencing [18,19]. Whereas imperfect sequence complementarity between a mature miRNA and its target mRNA results in translational inhibition, perfect base-pairing results in mRNA cleavage by Ago2, the only Ago protein that possesses slicer activity, or the ability to induce RNAi via endonucleolytic cleavage of perfectly complementary RNA strands [20,21]. miRISC also includes GW182 proteins, which are also essential for silencing [22]. Following its cytoplasmic processing, the miRNA:miRNA* duplex is loaded onto the Ago protein [1]. Usually, owing to the lower thermodynamic stability of the first few bases at the 5' end [23,24], the guide strand of the miRNA duplex is selected for incorporation into the mature miRISC, while the passenger strand (miRNA*) is released and degraded. Occasionally, miRNA* strands are incorporated into miRISC, where they can also mediate translational inhibition [25,26].

Much of the mechanism by which miRNA-loaded RISC represses mRNA translation is still unclear, although further insights may soon be forthcoming in this field of active research. However, numerous studies have clearly demonstrated the crucial importance of the miRNA seed sequence, nucleotides 2–8, in determining mRNA target specificity [27,28]. Unlike canonical siRNAs, miRNAs typically bind to their targets with imperfect sequence complementarity, and as a consequence, it has been estimated that a single miRNA may regulate upwards of 100 different transcripts [29,30]. miRNA binding sites are most commonly found in the 3'UTR of mRNAs, although there are known examples of functional miRNA silencing sites located in gene-coding regions [31,32], and sites are often well conserved, indicating selective pressure to retain miRNA binding ability [7,33]. Computational methods for predicting miRNA targets rely heavily on seed sequence complementarity and 3'UTR binding site conservation [34], and despite vast improvements over recent years, they still have high false-positive rates. Initial confirmation of algorithm predictions commonly involves the following methods: assessing decreases in protein level following miRNA overexpression (typically by western blotting) and assaying a miRNA's ability to decrease expression of a reporter gene cloned upstream of putative miRNA binding sites.

While there is still much that is unknown, there are currently several proposed mechanisms of miRNA-mediated post-transcriptional gene repression, each backed by various lines of supporting (and also contradicting) evidence: first, repression of translation initiation (inhibiting formation of the ribosome initiation complex or inhibiting cap recognition); second, repressing post-initiation of translation (inhibiting elongation); and third, deadenylation of the poly(A) tail, leading to decapping and mRNA decay [35]. While it was originally thought that translational repression occurs in the absence of any significant effects on mRNA levels, it is now understood that miRNAs also mediate decreases in target transcript levels [36,37], and in fact, it has been suggested that mRNA destabilization, not translational suppression, is the predominant driving force behind miRNA-mediated decreases in protein levels [38]. To further complicate things, it has recently been discovered that some miRNAs can also mediate an increase in the translation of their target transcripts [39–41].

It has been observed that components of miRISC, including Ago2, GW182 proteins and miRNAs, localize to sites associated with both mRNA decay and storage, such as P-bodies, stress granules and multivesicular bodies [35,42]. Vesicles secreted from multivesicular bodies, or late endosomes, have been found to contain miRNAs, and it is unclear whether this is simply a result of miRISC turnover and elimination, or a means of paracrine signaling between cells. miRNA delivery can also take place upon cell contact, as it has been shown that miRNAs can be transferred between T and B cells, while retaining functional silencing ability in the recipient cells [43]. Again, the *in vivo* relevance of this exchange is unknown.

Antiviral action of small RNAs

Plants, fungi and *Caenorhabditis elegans* all generate antiviral siRNAs by cleaving long dsRNA intermediates produced during viral infection [44]. These siRNAs then target and silence the expression of transcripts that are complementary to the parent sequence, and expression of antiviral siRNAs can be amplified by cellular RNA-dependent RNA polymerases not found in insects or vertebrates [45]. Not surprisingly, many viruses have evolved suppressors of silencing, or proteins that interfere with cellular siRNA processing or function, in order to counteract host defenses [46]. Although RNAi-mediated antiviral immunity has been thought to be replaced by the evolution of the interferon response in mammalian cells [47], it was recently demonstrated, using a broad range of mammalian cell types and viruses, that virus-derived siRNAs are also produced upon infection [48], suggesting that the RNAi pathway may still contribute to host defense in mammals.

Notably, it has been found that the cellular miRNA pathway in mammals can also exert powerful effects on virus replication, and in turn, some viruses have been found to encode their own miRNAs. For instance, miR-32 restricts replication of primate foamy virus type 1 in human cells [49], while liver-specific miR-122 increases replication of HCV [50] and is an attractive target for pharmacological intervention that has already proven successful in a chimpanzee model [51]. All herpesviruses examined to date encode viral miRNAs [52], and Kaposi's sarcoma-associated herpesvirus miRNA miR-K9* has recently been shown to modulate expression of RTA, a viral protein considered to be the master regulator of the switch between lytic and latent infection [53]. As these examples demonstrate, the interplay between viruses and miRNAs is quite varied and comprises a relatively new but quite fascinating area of research.

Does HIV-1 encode viral miRNAs?

As miRNAs are powerful tools for the down-regulation of cellular proteins, many speculated as to whether viruses would be found to express their own miRNAs as yet another means to create a favorable milieu for replication, or as a way to persist in the latent state. Indeed, 25

virally encoded miRNAs have since been identified in Epstein–Barr virus, 13 in Kaposi's sarcoma-associated herpesvirus and 11 in human cytomegalovirus [9]. Among other functions, these miRNAs contribute to regulating viral gene expression, counteracting host antiviral defenses and modulating proteins involved in viral latency [54,55]. However, identification of miRNAs encoded by RNA viruses has proved elusive [56,57]; while a few HIV-derived miRNAs have been identified (Table 1), they are somewhat controversial in nature.

The TAR element of the HIV-1 genome initially attracted interest as a potential viral miRNA candidate as its hairpin structure resembles endogenous targets of Dicer cleavage. Two groups independently demonstrated that recombinant Dicer cleavage of *in vitro* transcribed TAR generated an approximately miRNA-sized product, while northern blotting detected the presence of the TAR-derived miRNA in primary lymphocytes and CD4⁺ T cells infected with HIV-1 [58,59]. Furthermore, knockdown of Dicer decreased detection of the TAR miRNA in infected cells. Northern blotting of MT-4 T cells chronically infected with the HIV-1 IIIB strain also identified approximately 25 nt species derived from the viral gene *Nef*, which appear to downregulate *Nef* expression and decrease long terminal repeat (LTR)-driven transcription [60,61]. By contrast, initial efforts employing sequencing of cDNA libraries cloned from small RNAs failed to detect convincing HIV-derived miRNA candidates in either infected CD4⁺ HeLa cells [56], the latently infected ACH-2 cell line [62] or ACH-2 cells activated with phorbol myristate acetate (PMA) [63]. However, later pyrosequencing of NL4.3-infected MT-4 T cells detected single copies of TAR- and *Nef*-derived miRNA [64], indicating that previous efforts may have been unsuccessful owing to low copy number; in fact, only approximately 0.5% of all small miRNAs detected were HIV-derived small RNAs, mostly present in single copies. An additional miRNA derived from the Rev-response element was also identified in this study, as was a novel cellular miRNA produced by cleavage of a duplex formed between tRNA(lys3) and the HIV-1 primer-binding site (PBS). While cellular tRNAs can be processed into short ncRNAs (~18 nt) in uninfected cells [14], expression of PBS ncRNA, as it was termed, increased dramatically upon virus induction with PMA in latently infected U1 cells, and PBSncRNA was immunoprecipitated with overexpressed Ago2 in pNL4.3-transfected cells. AntagomiRs to PBSncRNA increased HIV-1 replication, indicating a possible role for this virus-dependent cellular miRNA in defending against infection. The pyrosequencing effort also confirmed a viral siRNA, previously detected by the same group via northern blotting, which derives from a perfectly matched 19-bp duplex in the *Env* gene [65]. Overexpression of the vsiRNA significantly decreased *Env* RNA expression in HeLa cells transfected with pNL4.3.

While evidence exists to suggest that HIV-1 does encode legitimate Dicer templates, acknowledgement of these viral miRNAs is not universal [57], and overall it appears unlikely that HIV relies heavily on viral miRNA-mediated downregulation of cellular or viral targets as a replication strategy *in vivo*. Estimates of copy numbers in NL4.3-infected MT-4 T cells made using real-time quantitative PCR analysis and small U6 RNA as a benchmark calculated roughly 3×10^3 copies of TAR miRNA, 61 copies of *Nef*-derived miRNA and 19 of the Rev-response element-derived miRNA per cell [64]. It has been estimated that 100 copies or more of a miRNA species are required to mediate noticeable repression [66], indicating that the TAR miRNA may be the most significant of the HIV-derived miRNAs. The first report identifying the miRNA also detailed the ability of transfected TAR RNA, which would presumably be processed into TAR miRNA, to recruit HDAC-1 to the viral LTR [58]. Histone deacetylation is associated with transcriptional silencing, suggesting that TAR miRNA silences transcription from the viral LTR and, thus, may play a role in the maintenance of viral latency. Further work by the same group suggested an additional role in resisting apoptosis, as transfection of an antagomiR against

the TAR miRNA rendered a HIV-infected HeLa cell line (HLM-1) slightly more susceptible to apoptosis induced by serum starvation, and transfection of wild-type but not mutant TAR RNA decreased levels of the proapoptotic proteins ERCC1 and IER3 [67]. While HIV may intentionally risk encoding miRNAs in genomic RNA copies subject to destruction upon Dicer processing, the low number of HIV miRNAs that have thus far been identified and their generally low copy number both suggest that it is more likely that highly conserved secondary structures, such as the stem-loop structure of TAR, evolved out of necessity, and, as a side effect, included susceptibility to cleavage by Dicer. The low copy number of most HIV-derived miRNAs identified may also reflect limited accessibility to miRNA processing proteins such as Drosha and Dicer due to interactions of viral RNAs with both viral and other cellular proteins.

Does Tat suppress antiviral RNAi mechanisms?

A topic studied with even more controversy than HIV-derived miRNAs is the proposed suppressor of RNA silencing (SRS) activity of the viral protein Tat. Infection by all RNA viruses except retroviruses results in the production of long dsRNAs at some point in the life cycle, which, in invertebrates and plants, are cleaved by Dicer into siRNAs that target their complementary sequences in genomic RNA for degradation [52]. This innate antiviral response is often encountered by viral proteins with SRS activity, for example, the p19 protein of tomato bushy stunt virus, which binds to siRNAs and prevents their incorporation into RISC. Mammalian cells, by contrast, recognize long dsRNA via pattern-recognition receptors like TLR3, which then induce the interferon response via signaling cascades [68]. Interferon-responsive proteins include the OAS/RNase L family members, which tag and degrade dsRNAs, and PKR, which leads to a global arrest of translation. Many viruses are known to subvert the interferon response; for instance, influenza A viruses encode the NS1 protein, which decreases production of IFN- β and inhibits the functions of OAS and PKR [69]. Given the efficacy of the interferon response, it is unclear whether mammalian cells also utilize RNAi strategies in antiviral defense, and whether animal viruses encode proteins with SRS function to counteract this. Evidence for SRS in animal retrovirus proteins has been controversial, although the Tas protein of primate foamy virus type 1 has been suggested to repress miRNA- and RNAi-induced silencing [49]. The first report to propound Tat's potential SRS activity demonstrated that Tat, in a dose-dependent manner, suppressed the inhibitory activity of shRNAs targeting luciferase expression, which was normalized to a co-transfected β -galactosidase plasmid [65]. Tat's ability to repress shRNA silencing of a luciferase plasmid was subsequently confirmed by an independent group, and was shown to be independent of its transactivation activity [70]. However, a repeat of this assay by another independent group failed to show any SRS activity of Tat after normalizing firefly luciferase expression to a co-transfected control plasmid expressing renilla luciferase, indicating that Tat expression nonspecifically increased expression from both plasmids [62]. Furthermore, levels of two cellular miRNAs were unaffected by Tat expression at physiological levels, indicating that Tat was not inhibiting Dicer function, as had been suggested [65]. In a more recent study, miR-30 was co-transfected with Tat and a luciferase reporter construct carrying eight copies of a miR-30 binding site. Tat suppressed the miR-30-mediated inhibition of luciferase expression without affecting the processing of pre-miR-30 into the mature form or luciferase mRNA levels [71], indicating that Tat does possess some SRS activity in this context. Furthermore, the p19 protein of tomato bushy stunt virus, a known suppressor of RNAi, could substitute for a mutant Tat that lacks the purported SRS activity but retains transcriptional transactivation ability. The mutant virus is inhibited for Gag translation, presumably because it has lost the ability to counteract RNAi mechanisms, possibly cellular miRNAs that target Gag, as translation of Gag was increased after Dicer knockdown. P19 restored Gag protein translation without affecting Gag mRNA levels or global translation. Considering the contradicting reports regarding Tat's SRS activity, further work will be

necessary to determine if Tat is a *bona fide* SRS protein counteracting a cellular RNAi-mediated immune response against HIV-1, as it is unclear how Tat is able to differentially affect repression by miR-30 and luciferase shRNA, and it seems more likely that evolutionary pressure would lead to escape mutations relieving targeting by cellular miRNAs. Furthermore, it is unclear whether Tat acts as an SRS protein under physiological conditions, and if the few HIV-derived miRNAs that have been demonstrated to negatively affect virus replication are present in a high enough copy number to select for SRS activity.

The TRBP was initially identified by (and named for) its high affinity for TAR RNA [72], although it was later discovered to be a co-factor of Dicer and a component of RISC [73,74]. Processing of miRNA is impaired in the absence of TRBP, and silencing activity induced by miRNA or siRNA is also inhibited. Given the high affinity of TRBP for TAR RNA, it was hypothesized that TAR RNA transcribed during infection may sequester TRBP from Dicer, thereby reducing RNAi activity [75]. Overexpression of *in vitro* transcribed TAR RNA did inhibit Dicer activity, which could be rescued by overexpressing exogenous TRBP. However, knockdown of TRBP decreased viral replication, suggesting that TRBP has other, more important, functions in viral replication than its putative role in suppressing RNAi [76]. For instance, TRBP can increase LTR-driven expression by inhibiting PKR [77]. Whether the physiological levels of TAR RNA expressed during infection substantially contribute to decreased miRNA activity has yet to be elucidated, as has the question of whether TAR RNA is more efficiently processed into TAR miRNA via recruitment of Dicer by TRBP binding.

Cellular miRNAs target cellular co-factors of HIV-1

Whether or not HIV-1 encodes miRNAs that suppress cellular genes or whether mammalian cells process viral-encoded miRNAs and siRNAs to wield against HIV-1, it has been established that Dicer or Drosha knockdown dramatically increases the kinetics of viral replication from HIV-1-infected donor peripheral blood mononuclear cells (PBMCs) [78], suggesting that cellular miRNAs have an overall negative effect on HIV-1. Viral production from the latently infected U1 cell line was also increased after Drosha and Dicer knockdown, additionally suggesting a role for miRNAs in the maintenance of viral latency. miRNA profiling of pNL4.3-transfected HeLa cells [79], NL4.3-infected Jurkat cells [78] and PBMCs from HIV-infected patients [80] suggested that viral replication results in reproducible changes in the miRNA profile, suggesting that the virus actively downregulates cellular miRNAs targeting HIV-1 RNA itself or cellular co-factors of replication (Figure 1), while upregulated miRNAs may be controlling expression of viral restriction factors.

PCAF, a histone acetyltransferase previously shown to be a Tat co-factor [81], was the first host protein found to be targeted by miRNAs that are downregulated following HIV-1 infection [78]. NL4.3 infection of Jurkat cells was found to decrease expression of the miR-17/92 polycistronic cluster, and miR-17/92 overexpression reduced HIV-1 production from HIV-1-infected donor PBMCs, while miR-17/92 antagomiRs induced the opposite effect. Expression of PCAF lacking its 3'UTR (and presumably not targeted for miRNA repression) rescued the inhibitory effects of miR-17/92 on HIV-1.

As successful HIV-1 replication is highly dependent on the physiological status of its target cells, it is also possible that alterations in the miRNA profile, as resting CD4⁺ T cells are activated and monocytes differentiate into macrophages, play a key role in determining new-found viral permissivity. Expression profiling comparing resting and activated CD4⁺ T cells or monocytes and macrophages demonstrates that the expression level of certain miRNAs are characteristically distinct between the two cell types [82,83; Chiang and Rice, Unpublished Data]. Therefore, as opposed to active up- or down-regulation by HIV-1

infection, changes in miRNAs that affect HIV-1 replication might also be indirectly caused by cell activation or differentiation. Our laboratory has demonstrated that miR-198 restricts protein expression of Cyclin T1, the Tat co-factor necessary for RNA polymerase II elongation of viral transcripts [84] in monocytes [83], which are nonpermissive for viral replication. Upon differentiation into macrophages, miR-198 levels are strongly downregulated, correlating with increased Cyclin T1 expression and permissivity for HIV-1 replication. Overexpression of miR-198 inhibited viral replication in a monocytic cell line activated with PMA. A similar mechanism appears to exist in resting CD4⁺ T cells, in which miR-27b inhibits Cyclin T1 protein expression but is downregulated after activation; miR-27b can also inhibit HIV-1 replication when overexpressed [Chiang K & Rice AP, Unpublished Data]

Cellular miRNAs directly target HIV-1 RNA

While resting CD4⁺ T cells do not support HIV-1 replication, they comprise the major reservoir of latently infected cells *in vivo* [85,86]. It is possible to detect the production of viral transcripts encoding singly and multiply spliced viral genes in latently infected resting CD4⁺ T cells isolated from HIV-positive donors on effective HAART regimens who have undetectable viral loads [87,88]. Translational repression of such transcripts by miRNAs presented a potential mechanism for the maintenance of HIV-1 latency. Cellular miRNAs miR-28, miR-125b, miR-150, miR-223 and miR-382 were computationally predicted to target the 3'UTR region of HIV-1 mRNA, which is found on all viral transcripts excluding *Nef*, and were shown to be expressed in primary resting CD4⁺ T cells but downregulated upon activation [82]. Whereas transfection of various proviral plasmids into resting CD4⁺ T cells resulted in some viral mRNA expression but very low levels of viral replication, co-transfection of antagomiRs to these five miRNAs significantly increased replication of all strains tested without increasing viral mRNA levels or activating the resting cells. Furthermore, transfection of all five antagomiRs increased virus production from resting CD4⁺ T cells isolated from HIV-1-infected and HAART-experienced donors who were aviremic, suggesting that inhibition of the five miRNAs was sufficient to activate latent viruses. Although it might be expected that HIV-1 could rapidly mutate its sequence to avoid miRNA restriction and latency, it was found that the predicted binding sites of these five miRNAs were relatively well conserved across various HIV-1 strains. Another report correlated the high expression of these five miRNAs with the inability of monocytes to support viral replication, and the contrasting permissivity of macrophages [89]. It was shown that, paralleling the situation in resting and activated CD4⁺ T cells, the miR-NAs were all downregulated upon macrophage differentiation. Combined transfection of the five antagomiRs increased HIV-1 replication in primary monocytes.

There exists a considerable amount of evidence indicating that the majority of viral mRNAs produced during latency are unlikely templates for translation, owing to abortive transcriptional elongation [87,90], nuclear retention of multiply spliced transcripts [91] and the fact that roughly only one in ten latently infected cells is capable of producing infectious virus upon re-activation [92]. Given these considerations, it is likely that the five miR-NAs examined in the aforementioned studies may also target cellular co-factors involved in viral replication and latency, as opposed to solely repressing the translation of HIV-1 mRNAs present in latently infected cells. Nonetheless, the ability of miRNA inhibition to activate latent viruses *ex vivo* shows promise for the development of novel therapeutic approaches to eradicate the latent reservoir in HIV-infected patients.

Two groups have published reports on the ability of miR-29a to repress HIV-1 replication. A putative miR-29a-binding site in the viral *Nef* gene was first identified via computational predictions using several algorithms [93], and was found to be well conserved across clades

(excluding group O). A luciferase reporter construct containing the purported site in *Nef* was repressed in a miR-29a-dependent fashion, while overexpression of miR-29a inhibited replication of NL4.3 in Jurkat cells by approximately threefold [94]. Another group showed that miR-29a was predicted to target a well-conserved site in the 3'UTR of HIV-1 mRNA [95]. Transfection of miR-29a antagomiRs increased viral production, while VSV-G pseudotyped NL4.3-luc (R-E-) virus containing a mutated miR-29a binding site showed increased single-round infectivity in the H9 T-cell line. Ago2 immunoprecipitation indicated that overexpression of miR-29a can target HIV-1 mRNA to RISC, whereas association of viral mRNA derived from the mutant virus is decreased to approximately a third of that of the wild-type. HIV-1 mRNA was also demonstrated to immunoprecipitate with P-body proteins in a manner partially dependent on miR-29a targeting. As there is evidence that miRNA-mediated translational repression involves the targeting of mRNA to P-bodies [96–98], which are cytoplasmic foci involved in mRNA degradation and sequestration [99], the authors used siRNA knockdown of key proteins to disrupt P-body formation and noted an increase in reporter virus production. Very similar results were also found by an independent group [100].

Effect of proteins involved in miRNA-mediated repression on HIV-1

Various P-body-associated proteins have been shown to affect HIV-1 replication. Cellular restriction factors APOBEC3G (A3G) and APOBEC3F (A3F) had been suggested to target viral mRNAs to P-bodies, leading to inhibition of their translation [101]. To differentiate between increased HIV-1 replication following P-body disruption caused by alleviation of A3G- or miRNA-mediated inhibition, P-body proteins RCK (also known as p54 or DDX6) and LSM-1 were knocked down with siRNA, and cells were then co-transfected with pNL4.3 lacking the *Vif* gene, which is known to counteract A3G activity, and wild-type or mutant A3G with no antiviral activity [100]. Depletion of P-body proteins enhanced HIV-1 replication regardless of the presence of A3G, while A3G-mediated restriction was not affected by P-body disruption. Since this suggested that antiviral activity mediated by miRNAs but not A3G required P-bodies, and it was known that miR-NAs contribute to HIV-1 latency [78,82], PBMCs from HIV-1 infected donors on suppressive HAART were transfected with siRNA against RCK and were examined for viral reactivation from latency. RCK knockdown stimulated virus production from these cells to a similar magnitude as Drosha knockdown, indicating that disruption of localized foci of miRNA-mediated inhibition can reactivate latent virus.

Recently, another protein localized to P-bodies, Moloney leukemia virus 10 homolog (MOV10), has been demonstrated to inhibit HIV-1 replication [102,103]. The gene's identification began with the screening of mice that did not develop viremia despite carrying murine leukemia virus provirus in their germline [104]; it was later found that proviral integration had disrupted a protein-coding gene that was named *MOV10* [105]. The protein was found to be a putative SF-1 RNA helicase [106], and was shown to associate with human Ago1 and Ago2 and undergo incorporation into RISC. *MOV10* is the homolog of the Arabidopsis gene *SDE3*, which is necessary for the post-transcriptional gene-silencing activity involved in plant viral defense [107]. SiRNA knockdown decreased the silencing of miRNA-responsive reporter constructs [108,109], and was also found to inhibit hepatitis D virus replication, likely via involvement in the cellular RNA-mediated transcription of the viral genome [110]. MOV10 also associates with A3G and A3F within P-bodies [111–113], although the functional significance of this interaction is unknown. MOV10 overexpression in producer cells dramatically decreased HIV-1 infectivity, while overexpression in target cells had little effect on infectivity [102,103,114]; the infectivity of other lentiviruses and the gammaretrovirus murine leukemia virus was likewise affected by MOV10 overexpression in producer cells [114]. However, MOV10 silencing in producer 293T cells led to conflicting

results: approximately twofold decreased infectivity of virus [114], approximately threefold increased infectivity [102] or no effect [103]. All three groups found that MOV10 was packaged into virions and seemed to inhibit the production of late reverse transcripts upon infection of target cells. However, as viral RNA production in producer cells and incorporation into virions appeared to be unaffected, further elucidation of the mechanism behind MOV10 restriction of HIV-1 should prove quite interesting, and may address the question of whether MOV10's involvement in the miRNA pathway is relevant to its antiviral effects.

Dicer protein levels have been shown to be substantially lower in monocytes than in resting CD4⁺ T cells and undergo a modest increase upon macrophage differentiation [58]. It was recently demonstrated that pNL4.3 transfection of monocytes stimulated with PMA repressed the upregulation of Dicer protein typically seen upon differentiation in U937 cells and in primary monocytes isolated from a single blood donor [115]. These results suggest that HIV-1 may actively suppress Dicer expression in macrophages, although further work encompassing more donors will be needed to confirm these initial observations.

Conclusion

HIV-1 infection interacts with the cellular miRNA pathway via four general mechanisms: first, virus-encoded miRNAs have been identified, of which the miRNA derived from Dicer processing of TAR RNA appears to be the most abundant, while the rest are present in very low copy numbers. Second, the viral transactivator protein Tat has been suggested to possess SRS activity, as a way to subvert cellular or viral miRNA-mediated silencing of HIV-1 gene expression. Both of these mechanisms are somewhat controversial, as it is uncertain if the HIV-encoded miRNAs have significant effects on viral replication, and contradicting evidence has been presented regarding Tat's ability to interfere with miRNA gene repression. Given that knockdown of Drosha or Dicer, the miRNA processing enzymes, significantly increases replication, it can be generalized that the cellular miRNA pathway usually exerts negative effects on HIV-1 replication. Third, miRNAs that can decrease HIV-1 replication by directly targeting sequences in HIV-1 genomic RNA have been identified, along with miRNAs that can modulate expression of essential viral co-factors, and it has been suggested that some of these miRNAs may contribute to the maintenance of HIV-1 latency. Finally, proteins involved in the miRNA pathway may also act as HIV-1 restriction factors.

Future perspective

While a handful of cellular miRNAs have been found to possess anti-HIV-1 activity to date, either by inhibiting the expression of essential HIV-1 co-factors or by directly suppressing the expression of viral genes, given their promiscuity in targeting and the number of miRNAs expressed in the relevant cell types, we predict that many more associations still await discovery. The application of newer methods, such as high-throughput sequencing of RNAs isolated by cross-linking immunoprecipitation using antibodies against miRISC components [116], may be highly useful in defining the set of mRNAs targeted by miRNAs in CD4⁺ T cells and macrophages, and in identifying changes following infection. Furthermore, immunoprecipitation of miRISC may be used to delineate the numerous mRNAs that a single miRNA targets, allowing investigators to determine the effects of artificially altering miRNA expression on other pathways besides those involved in viral replication. Identifying these off-target effects becomes especially important in the context of miRNA-based therapeutics. Since the application of an antagomiR cocktail to latently infected CD4⁺ T cells re-activated virus [82], such an approach may be feasible *in vivo* as a first step in eradicating the latently infected reservoir. Treatment of chimpanzees with an

antagomiR of miR-122 reduced HCV viral load without apparent side effects [51], and, in general, miRNA delivery faces relatively fewer technological hurdles owing to their small size and nonantigenic nature. While HIV-1 infection, CD4⁺ T-cell activation and macrophage differentiation all alter the miRNA expression profile, the majority of reports that have identified anti-HIV miRNAs responsive to these conditions have not investigated the regulatory mechanisms behind shifts in miRNA expression. Further elucidation of the interplay between the miRNA pathway and HIV-1 may lead to a better understanding of the many mechanisms that the virus uses during infection, and additional therapeutic targets.

Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol.* 2009; 10(2):126–139. [PubMed: 19165215]
2. Okamura K, Lai EC. Endogenous small interfering RNAs in animals. *Nat Rev Mol Cell Biol.* 2008; 9(9):673–678. [PubMed: 18719707]
3. O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol.* 2010; 10(2):111–122. [PubMed: 20098459]
4. Croce CM. Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genet.* 2009; 10(10):704–714. [PubMed: 19763153]
5. Inui M, Martello G, Piccolo S. MicroRNA control of signal transduction. *Nat Rev Mol Cell Biol.* 2010; 11(4):252–263. [PubMed: 20216554]
6. Gangaraju VK, Lin H. MicroRNAs: key regulators of stem cells. *Nat Rev Mol Cell Biol.* 2009; 10(2):116–111. [PubMed: 19165214]
7. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* 2009; 19(1):92–105. [PubMed: 18955434]
8. Miranda KC, Huynh T, Tay Y, et al. A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. *Cell.* 2006; 126(6):1203–1217. [PubMed: 16990141]
9. Griffiths-Jones S, Saini HK, Van Dongen S, Enright AJ. miRBase: tools for microRNA genomics. *Nucleic Acids Res.* 2008; 36(Database issue):D154–D158. [PubMed: 17991681]
10. Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. *Cell.* 2009; 136(4):642–655. [PubMed: 19239886]
11. Borchert GM, Lanier W, Davidson BL. RNA polymerase III transcribes human microRNAs. *Nat Struct Mol Biol.* 2006; 13(12):1097–1101. [PubMed: 17099701]
12. Berezikov E, Chung WJ, Willis J, Cuppen E, Lai EC. Mammalian mirtron genes. *Mol Cell.* 2007; 28(2):328–336. [PubMed: 17964270]
13. Cole C, Sobala A, Lu C, et al. Filtering of deep sequencing data reveals the existence of abundant Dicer-dependent small RNAs derived from tRNAs. *RNA.* 2009; 15(12):2147–2160. [PubMed: 19850906]
14. Haussecker D, Huang Y, Lau A, Parameswaran P, Fire AZ, Kay MA. Human tRNA-derived small RNAs in the global regulation of RNA silencing. *RNA.* 2010; 16(4):673–695. [PubMed: 20181738]
15. Ender C, Krek A, Friedlander MR, et al. A human snoRNA with microRNA-like functions. *Mol Cell.* 2008; 32(4):519–528. [PubMed: 19026782]
16. Wang Y, Juranek S, Li H, Sheng G, Tuschl T, Patel DJ. Structure of an argonaute silencing complex with a seed-containing guide DNA and target RNA duplex. *Nature.* 2008; 456(7224):921–926. [PubMed: 19092929]

17. Landthaler M, Gaidatzis D, Rothballer A, et al. Molecular characterization of human Argonaute-containing ribonucleoprotein complexes and their bound target mRNAs. *RNA*. 2008; 14(12):80–96.
18. Azuma-Mukai A, Oguri H, Mituyama T, et al. Characterization of endogenous human Argonautes and their miRNA partners in RNA silencing. *Proc Natl Acad Sci USA*. 2008; 105(23):7964–7969. [PubMed: 18524951]
19. Schmitter D, Filkowski J, Sewer A, et al. Effects of Dicer and Argonaute down-regulation on mRNA levels in human HEK293 cells. *Nucleic Acids Res*. 2006; 34(17):4801–4815. [PubMed: 16971455]
20. Liu J, Carmell MA, Rivas FV, et al. Argonaute2 is the catalytic engine of mammalian RNAi. *Science*. 2004; 305(5689):1437–1441. [PubMed: 15284456]
21. Meister G, Landthaler M, Patkaniowska A, Dorsett Y, Teng G, Tuschl T. Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol Cell*. 2004; 15(2):185–197. [PubMed: 15260970]
22. Liu J, Rivas FV, Wohlschlegel J, Yates Jr 3rd, Parker R, Hannon GJ. A role for the P-body component GW182 in microRNA function. *Nat Cell Biol*. 2005; 7(12):1261–1266. [PubMed: 16284623]
23. Schwarz DS, Hutvagner G, Du T, Xu Z, Aronin N, Zamore PD. Asymmetry in the assembly of the RNAi enzyme complex. *Cell*. 2003; 115(2):199–208. [PubMed: 14567917]
24. Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. *Cell*. 2003; 115(2):209–216. [PubMed: 14567918]
25. Ro S, Park C, Young D, Sanders KM, Yan W. Tissue-dependent paired expression of miRNAs. *Nucleic Acids Res*. 2007; 35(17):5944–5953. [PubMed: 17726050]
26. Chiang HR, Schoenfeld LW, Ruby JG, et al. Mammalian microRNAs: experimental evaluation of novel and previously annotated genes. *Genes Dev*. 2010; 24(10):992–1009. [PubMed: 20413612]
27. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. *Cell*. 2003; 115(7):787–798. [PubMed: 14697198]
28. Doench JG, Sharp PA. Specificity of microRNA target selection in translational repression. *Genes Dev*. 2004; 18(5):504–511. [PubMed: 15014042]
29. Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. *Nature*. 2008; 455(7209):64–71. [PubMed: 18668037]
30. Selbach M, Schwanhauser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. *Nature*. 2008; 455(7209):58–63. [PubMed: 18668040]
31. Duursma AM, Kedde M, Schrier M, Le Sage C, Agami R. miR-148 targets human DNMT3b protein coding region. *RNA*. 2008; 14(5):872–877. [PubMed: 18367714]
32. Forman JJ, Legesse-Miller A, Collier HA. A search for conserved sequences in coding regions reveals that the let-7 microRNA targets Dicer within its coding sequence. *Proc Natl Acad Sci USA*. 2008; 105(39):14879–14884. [PubMed: 18812516]
33. Xie X, Lu J, Kulbokas EJ, et al. Systematic discovery of regulatory motifs in human promoters and 3' UTRs by comparison of several mammals. *Nature*. 2005; 434(7031):338–345. [PubMed: 15735639]
34. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009; 136(2):215–233. [PubMed: 19167326]
35. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem*. 2010; 79:351–379. [PubMed: 20533884]
36. Lim LP, Lau NC, Garrett-Engle P, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature*. 2005; 433(7027):769–773. [PubMed: 15685193]
37. Hendrickson DG, Hogan DJ, McCullough HL, et al. Concordant regulation of translation and mRNA abundance for hundreds of targets of a human microRNA. *PLoS Biol*. 2009; 7(11):E1000238. [PubMed: 19901979]
38. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature*. 2010; 466(7308):835–840. [PubMed: 20703300]

39. Orom UA, Nielsen FC, Lund AH. MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. *Mol Cell*. 2008; 30(4):460–471. [PubMed: 18498749]
40. Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. *Science*. 2007; 318(5858):1931–1934. [PubMed: 18048652]
41. Henke JI, Goergen D, Zheng J, et al. microRNA-122 stimulates translation of hepatitis C virus RNA. *EMBO J*. 2008; 27(24):3300–3310. [PubMed: 19020517]
42. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet*. 2010; 11(9):597–610. [PubMed: 20661255]
43. Rechavi O, Erlich Y, Amram H, et al. Cell contact-dependent acquisition of cellular and viral nonautonomously encoded small RNAs. *Genes Dev*. 2009; 23(16):1971–1979. [PubMed: 19684116]
44. Ding SW, Voinnet O. Antiviral immunity directed by small RNAs. *Cell*. 2007; 130(3):413–426. [PubMed: 17693253]
45. Mlotshwa S, Pruss GJ, Vance V. Small RNAs in viral infection and host defense. *Trends Plant Sci*. 2008; 13(7):375–382. [PubMed: 18550416]
46. Voinnet O. Origin, biogenesis, and activity of plant microRNAs. *Cell*. 2009; 136(4):669–687. [PubMed: 19239888]
47. Voinnet O. Induction and suppression of RNA silencing: insights from viral infections. *Nat Rev Genet*. 2005; 6(3):206–220. [PubMed: 15703763]
48. Parameswaran P, Sklan E, Wilkins C, et al. Six RNA viruses and forty-one hosts: viral small RNAs and modulation of small RNA repertoires in vertebrate and invertebrate systems. *PLoS Pathog*. 2010; 6(2):E1000764. [PubMed: 20169186]
49. Lecellier CH, Dunoyer P, Arar K, et al. A cellular microRNA mediates antiviral defense in human cells. *Science*. 2005; 308(5721):557–560. [PubMed: 15845854]
50. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science*. 2005; 309(5740):1577–1581. [PubMed: 16141076]
51. Lanford RE, Hildebrandt-Eriksen ES, Petri A, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science*. 2010; 327(5962):198–201. [PubMed: 19965718]
52. Umbach JL, Cullen BR. The role of RNAi and microRNAs in animal virus replication and antiviral immunity. *Genes Dev*. 2009; 23(10):1151–1164. [PubMed: 19451215]
53. Bellare P, Ganem D. Regulation of KSHV lytic switch protein expression by a virus-encoded microRNA: an evolutionary adaptation that fine-tunes lytic reactivation. *Cell Host Microbe*. 2009; 6(6):570–575. [PubMed: 20006845]
54. Cullen BR. Viral and cellular messenger RNA targets of viral microRNAs. *Nature*. 2009; 457(7228):421–424. [PubMed: 19158788]
55. Ghosh Z, Mallick B, Chakrabarti J. Cellular versus viral microRNAs in host-virus interaction. *Nucleic Acids Res*. 2009; 37(4):1035–1048. [PubMed: 19095692]
56. Pfeffer S, Sewer A, Lagos-Quintana M, et al. Identification of microRNAs of the herpesvirus family. *Nat Methods*. 2005; 2(4):269–276. [PubMed: 15782219]
57. Cullen BR. Five questions about viruses and microRNAs. *PLoS Pathog*. 2010; 6(2):E1000787. [PubMed: 20195463]
58. Klase Z, Kale P, Winograd R, et al. HIV-1 TAR element is processed by Dicer to yield a viral micro-RNA involved in chromatin remodeling of the viral LTR. *BMC Mol Biol*. 2007; 8:63. [PubMed: 17663774]
59. Ouellet, DI; Plante, I.; Landry, P., et al. Identification of functional microRNAs released through asymmetrical processing of HIV-1 TAR element. *Nucleic Acids Res*. 2008; 36(7):2353–2365. [PubMed: 18299284]
60. Omoto S, Ito M, Tsutsumi Y, et al. HIV-1 nef suppression by virally encoded microRNA. *Retrovirology*. 2004; 1:44. [PubMed: 15601474]
61. Omoto S, Fujii YR. Regulation of human immunodeficiency virus 1 transcription by nef microRNA. *J Gen Virol*. 2005; 86(Pt 3):751–755. [PubMed: 15722536]

62. Lin J, Cullen BR. Analysis of the interaction of primate retroviruses with the human RNA interference machinery. *J Virol.* 2007; 81(22):12218–12226. [PubMed: 17855543]
63. Landgraf P, Rusu M, Sheridan R, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell.* 2007; 129(7):1401–1414. [PubMed: 17604727]
64. Yeung ML, Bennasser Y, Watashi K, Le SY, Houzet L, Jeang KT. Pyrosequencing of small non-coding RNAs in HIV-1 infected cells: evidence for the processing of a viral-cellular double-stranded RNA hybrid. *Nucleic Acids Res.* 2009; 37(19):6575–6586. Cloning of small RNAs isolated from NL4.3-infected MT-4 cells followed by pyrosequencing was used to identify HIV-derived miRNAs and siRNAs. Verified the presence of previously reported TAR miRNA and a few others, mostly in low copy number. [PubMed: 19729508]
65. Bennasser Y, Le SY, Benkirane M, Jeang KT. Evidence that HIV-1 encodes an siRNA and a suppressor of RNA silencing. *Immunity.* 2005; 22(5):607–619. [PubMed: 15894278]
66. Brown BD, Gentner B, Cantore A, et al. Endogenous microRNA can be broadly exploited to regulate transgene expression according to tissue, lineage and differentiation state. *Nat Biotechnol.* 2007; 25(12):1457–1467. [PubMed: 18026085]
67. Klase Z, Winograd R, Davis J, et al. HIV-1 TAR miRNA protects against apoptosis by altering cellular gene expression. *Retrovirology.* 2009; 6:18. [PubMed: 19220914]
68. Bowie AG, Unterholzner L. Viral evasion and subversion of pattern-recognition receptor signalling. *Nat Rev Immunol.* 2008; 8(12):911–922. [PubMed: 18989317]
69. Hale BG, Randall RE, Ortin J, Jackson D. The multifunctional NS1 protein of influenza A viruses. *J Gen Virol.* 2008; 89(Pt 10):2359–2376. [PubMed: 18796704]
70. Haasnoot J, De Vries W, Geutjes EJ, Prins M, De Haan P, Berkhout B. The Ebola virus VP35 protein is a suppressor of RNA silencing. *PLoS Pathog.* 2007; 3(6):E86. [PubMed: 17590081]
71. Qian S, Zhong X, Yu L, Ding B, De Haan P, Boris-Lawrie K. HIV-1 Tat RNA silencing suppressor activity is conserved across kingdoms and counteracts translational repression of HIV-1. *Proc Natl Acad Sci USA.* 2009; 106(2):605–610. [PubMed: 19122141]
72. Gatignol A, Buckler-White A, Berkhout B, Jeang KT. Characterization of a human TAR RNA-binding protein that activates the HIV-1 LTR. *Science.* 1991; 251(5001):1597–600. [PubMed: 2011739]
73. Chendrimada TP, Gregory RI, Kumaraswamy E, et al. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature.* 2005; 436(7051):740–744. [PubMed: 15973356]
74. Haase AD, Jaskiewicz L, Zhang H, et al. TRBP, a regulator of cellular PKR and HIV-1 virus expression, interacts with Dicer and functions in RNA silencing. *EMBO Rep.* 2005; 6(10):961–967. [PubMed: 16142218]
75. Bennasser Y, Yeung ML, Jeang KT. HIV-1 TAR RNA subverts RNA interference in transfected cells through sequestration of TAR RNA-binding protein, TRBP. *J Biol Chem.* 2006; 281(38):27674–27678. [PubMed: 16887810]
76. Christensen HS, Daher A, Soye KJ, et al. Small interfering RNAs against the TAR RNA binding protein, TRBP, a Dicer cofactor, inhibit human immunodeficiency virus type 1 long terminal repeat expression and viral production. *J Virol.* 2007; 81(10):5121–5131. [PubMed: 17360756]
77. Daher A, Longuet M, Dorin D, et al. Two dimerization domains in the trans-activation response RNA-binding protein (TRBP) individually reverse the protein kinase R inhibition of HIV-1 long terminal repeat expression. *J Biol Chem.* 2001; 276(36):33899–33905. [PubMed: 11438532]
78. Triboulet R, Mari B, Lin YL, et al. Suppression of microRNA-silencing pathway by HIV-1 during virus replication. *Science.* 2007; 315(5818):1579–1582. Demonstrated that Dicer and Drosha knockdown increases viral replication in HIV-infected patient peripheral blood mononuclear cells, and that HIV-1 suppresses miR-17/92 expression. Mir-17/92 was found to target PCAF, a Tat co-factor. [PubMed: 17322031]
79. Yeung ML, Bennasser Y, Myers TG, Jiang G, Benkirane M, Jeang KT. Changes in microRNA expression profiles in HIV-1-transfected human cells. *Retrovirology.* 2005; 2:81. [PubMed: 16381609]

80. Houzet L, Yeung ML, De Lame V, Desai D, Smith SM, Jeang KT. MicroRNA profile changes in human immunodeficiency virus type 1 (HIV-1) seropositive individuals. *Retrovirology*. 2008; 5:118. [PubMed: 19114009]
81. Kiernan RE, Vanhulle C, Schiltz L, et al. HIV-1 tat transcriptional activity is regulated by acetylation. *EMBO J*. 1999; 18(21):6106–6118. [PubMed: 10545121]
82. Huang J, Wang F, Argyris E, et al. Cellular microRNAs contribute to HIV-1 latency in resting primary CD4⁺ T lymphocytes. *Nat Med*. 2007; 13(10):1241–1247. Transfection of antagomiRs against five miRNAs targeting the 3'UTR of HIV-1 RNAs re-activated latently infected CD4⁺ T cells isolated from patients with undetectable viral loads. [PubMed: 17906637]
83. Sung TL, Rice AP. miR-198 inhibits HIV-1 gene expression and replication in monocytes and its mechanism of action appears to involve repression of cyclin T1. *PLoS Pathog*. 2009; 5(1):E1000263. Discusses that miR-198 represses Tat co-factor Cyclin T1 in monocytes, but is downregulated upon macrophage differentiation when cells become permissive for HIV-1 replication. [PubMed: 19148268]
84. Rice AP, Herrmann CH. Regulation of TAK/P-TEFb in CD4⁺ T lymphocytes and macrophages. *Curr HIV Res*. 2003; 1(4):395–404. [PubMed: 15049426]
85. Coiras M, Lopez-Huertas MR, Perez-Olmeda M, Alcamí J. Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. *Nat Rev Microbiol*. 2009; 7(11):798–812. [PubMed: 19834480]
86. Han Y, Wind-Rotolo M, Yang HC, Siliciano JD, Siliciano RF. Experimental approaches to the study of HIV-1 latency. *Nat Rev Microbiol*. 2007; 5(2):95–106. [PubMed: 17224919]
87. Lassen KG, Bailey JR, Siliciano RF. Analysis of human immunodeficiency virus type 1 transcriptional elongation in resting CD4⁺ T cells *in vivo*. *J Virol*. 2004; 78(17):9105–9114. [PubMed: 15308706]
88. Chun TW, Justement JS, Lempicki RA, et al. Gene expression and viral production in latently infected, resting CD4⁺ T cells in viremic versus aviremic HIV-infected individuals. *Proc Natl Acad Sci USA*. 2003; 100(4):1908–1913. [PubMed: 12552096]
89. Wang X, Ye L, Hou W, et al. Cellular microRNA expression correlates with susceptibility of monocytes/macrophages to HIV-1 infection. *Blood*. 2009; 113(3):671–674. [PubMed: 19015395]
90. Adams M, Sharmeen L, Kimpton J, et al. Cellular latency in human immunodeficiency virus-infected individuals with high CD4 levels can be detected by the presence of promoter-proximal transcripts. *Proc Natl Acad Sci USA*. 1994; 91(9):3862–3866. [PubMed: 8171003]
91. Lassen KG, Ramyar KX, Bailey JR, Zhou Y, Siliciano RF. Nuclear retention of multiply spliced HIV-1 RNA in resting CD4⁺ T cells. *PLoS Pathog*. 2006; 2(7):E68. [PubMed: 16839202]
92. Chun TW, Carruth L, Finzi D, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature*. 1997; 387(6629):183–188. [PubMed: 9144289]
93. Hariharan M, Scaria V, Pillai B, Brahmachari SK. Targets for human encoded microRNAs in HIV genes. *Biochem Biophys Res Commun*. 2005; 337(4):1214–1218. [PubMed: 16236258]
94. Ahluwalia JK, Khan SZ, Soni K, et al. Human cellular microRNA hsa-miR-29a interferes with viral nef protein expression and HIV-1 replication. *Retrovirology*. 2008; 5:117. [PubMed: 19102781]
95. Nathans R, Chu CY, Serquina AK, Lu CC, Cao H, Rana TM. Cellular microRNA and P bodies modulate host-HIV-1 interactions. *Mol Cell*. 2009; 34(6):696–709. Discusses that miR-29a decreases HIV-1 replication by targeting a site in the 3'UTR of HIV-1 RNA, and also enhances association of viral RNA with P-bodies. [PubMed: 19560422]
96. Liu J, Valencia-Sanchez MA, Hannon GJ, Parker R. MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat Cell Biol*. 2005; 7(7):719–723. [PubMed: 15937477]
97. Sen GL, Blau HM. Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. *Nat Cell Biol*. 2005; 7(6):633–636. [PubMed: 15908945]
98. Pillai RS, Bhattacharyya SN, Artus CG, et al. Inhibition of translational initiation by Let-7 MicroRNA in human cells. *Science*. 2005; 309(5740):1573–1576. [PubMed: 16081698]
99. Beckham CJ, Parker R. P bodies, stress granules, and viral life cycles. *Cell Host Microbe*. 2008; 3(4):206–212. [PubMed: 18407064]

100. Chable-Bessia C, Meziane O, Latreille D, et al. Suppression of HIV-1 replication by microRNA effectors. *Retrovirology*. 2009; 6:26. [PubMed: 19272132]
101. Wichroski MJ, Robb GB, Rana TM. Human retroviral host restriction factors APOBEC3G and APOBEC3F localize to mRNA processing bodies. *PLoS Pathog*. 2006; 2(5):E41. [PubMed: 16699599]
102. Wang X, Han Y, Dang Y, et al. Moloney leukemia virus 10 (MOV10) protein inhibits retrovirus replication. *J Biol Chem*. 285(19):14346–14355. Along with [103] and [114], this paper demonstrated reduced HIV-1 infectivity upon overexpression of P-body protein and Ago binding partner MOV10. [PubMed: 20215113]
103. Burdick R, Smith JL, Chaipan C, et al. P body-associated protein MOV10 inhibits HIV-1 replication at multiple stages. *J Virol*. 84(19):10241–10210.3. [PubMed: 20668078]
104. Schnieke A, Stuhlmann H, Harbers K, Chumakov I, Jaenisch R. Endogenous Moloney leukemia virus in nonviremic Mov substrains of mice carries defects in the proviral genome. *J Virol*. 1983; 45(2):505–513. [PubMed: 6834466]
105. Mooslehner K, Muller U, Karls U, Hamann L, Harbers K. Structure and expression of a gene encoding a putative GTP-binding protein identified by provirus integration in a transgenic mouse strain. *Mol Cell Biol*. 1991; 11(2):886–893. [PubMed: 1899287]
106. Fairman-Williams ME, Guenther UP, Jankowsky E. SF1 and SF2 helicases: family matters. *Curr Opin Struct Biol*. 20(3):313–324. [PubMed: 20456941]
107. Dalmay T, Horsefield R, Braunstein TH, Baulcombe DC. SDE3 encodes an RNA helicase required for post-transcriptional gene silencing in Arabidopsis. *EMBO J*. 2001; 20(8):2069–2078. [PubMed: 11296239]
108. Meister G, Landthaler M, Peters L, et al. Identification of novel argonaute-associated proteins. *Curr Biol*. 2005; 15(23):2149–2155. [PubMed: 16289642]
109. Chendrimada TP, Finn KJ, Ji X, et al. MicroRNA silencing through RISC recruitment of eIF6. *Nature*. 2007; 447(7146):823–828. [PubMed: 17507929]
110. Haussecker D, Cao D, Huang Y, Parameswaran P, Fire AZ, Kay MA. Capped small RNAs and MOV10 in human hepatitis delta virus replication. *Nat Struct Mol Biol*. 2008; 15(7):714–721. [PubMed: 18552826]
111. Gallois-Montbrun S, Kramer B, Swanson CM, et al. Antiviral protein APOBEC3G localizes to ribonucleoprotein complexes found in P bodies and stress granules. *J Virol*. 2007; 81(5):2165–2178. [PubMed: 17166910]
112. Gallois-Montbrun S, Holmes RK, Swanson CM, et al. Comparison of cellular ribonucleoprotein complexes associated with the APOBEC3F and APOBEC3G antiviral proteins. *J Virol*. 2008; 82(11):5636–5642. [PubMed: 18367521]
113. Kozak SL, Marin M, Rose KM, Bystrom C, Kabat D. The anti-HIV-1 editing enzyme APOBEC3G binds HIV-1 RNA and messenger RNAs that shuttle between polysomes and stress granules. *J Biol Chem*. 2006; 281(39):29105–29119. [PubMed: 16887808]
114. Furtak V, Mulky A, Rawlings SA, et al. Perturbation of the P-body component MOV10 inhibits HIV-1 infectivity. *PLoS ONE*. 2010; 5(2):E9081. [PubMed: 20140200]
115. Coley W, Van Duyne R, Carpio L, et al. Absence of DICER in monocytes and its regulation by HIV-1. *J Biol Chem*. 2010; 285(42):31930–31943. [PubMed: 20584909]
116. Chi SW, Zang JB, Mele A, Darnell RB. Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. *Nature*. 2009; 460(7254):479–486. [PubMed: 19536157]

Figure 1. Mechanisms of cellular miRNA-mediated inhibition of HIV-1 replication

Transcription of miRNA coding regions generates primary miRNAs (pri-miRNAs), which form stem-loop structures due to imperfect complementarity. Pre-miRNA is generated when the Droscha-DGCR8 complex cleaves at the hairpin stem of the pri-miRNA. The pre-miRNA is exported to the cytoplasm and cleaved near the terminal loop by Dicer, in complex with TRBP and one of the Argonaute proteins. The miRNA:miRNA* duplex is then loaded onto Ago, where it is unwound, followed by preferential incorporation of the guide strand and release and degradation of the miRNA* strand. The miRISC also includes, among many other proteins, Dicer and the GW182 proteins, which are necessary for gene repression. Recognition of target mRNA is mediated by sequence complementarity, which is typically imperfect, with the miRNA; binding sites are typically found in the 3'UTR. miR-198 and miR-17/92 target Cyclin T1 mRNA and PCAF mRNA, respectively, for translational inhibition, which results in decreased viral transcription, as both Cyclin T1 and PCAF are co-factors of the viral transactivation protein Tat. Nascent viral transcripts form a stem-loop structure (TAR RNA) that is bound by Tat, which then recruits Cyclin T1 and Cdk9 (the P-TEFb complex). These are necessary for the processivity of Pol II during transcriptional elongation, while PCAF is a histone acetyltransferase that acetylates Tat, increasing its transactivation activity. Cellular miRNAs, such as miR-28, miR-29a-c, miR-125b, miR-150, miR-223 and miR-382 can also target HIV-1 RNA directly, via sites mostly located in the 3'UTR, which leads to decreased viral gene expression. Furthermore, miR-29a has been shown to enhance viral RNA association with P-bodies, cytoplasmic foci with high concentrations of proteins involved in translational repression and mRNA decapping, deadenylation and degradation. Sequestration of transcripts targeted by miRNAs in P-bodies presumably results in translational repression or mRNA degradation, as disruption of P-bodies increases viral production. RISC proteins Ago and GW182 also localize to P-bodies, where they associate with the Dcp1-Dcp2 mRNA decapping complex, the Ccr4-Not1 deadenylation complex, Rck, a decapping activator, APOBEC3G and 3F (A3G, A3F), the HIV-1 restriction factors and MOV10, a putative RNA helicase that is also incorporated into HIV-1 virions and inhibits infectivity. Ago2: Argonaute-2; DGCR8: DiGeorge syndrome critical region gene 8; pri-miRNAs: Primary miRNA; pre-miRNA: Precursor miRNA; TRBP: TAR RNA-binding protein; RISC: RNA-induced silencing complex.

Table 1

Virus-encoded and cellular ncRNAs affecting HIV-1 replication.

Name	Location/cellular viral duplex/target	Notes	Ref.
<i>Viral miRNAs</i>			
TAR miRNA	TAR RNA	Recruits HDAC-1 to LTR; antiapoptotic activity	[58,67]
miR-N367	<i>Nef</i>	Decreases Nef expression	[60,61]
<i>Viral siRNAs</i>			
RRE ncRNA	<i>Env</i> : Rev-response element	Many species with the same core sequence but different termini were found; unknown function	[64]
vsRNA/vsiRNA*	<i>Env</i> : Rev-response element	Decreases <i>Env</i> mRNA	[64,65]
<i>Cellular ncRNAs derived from pairing with viral RNA</i>			
PBSncRNA	tRNA(lys3): PBS	Processed from tRNA(lys3) following binding to the HIV-1 PBS; antagomiR treatment increases viral replication	[64]
<i>Cellular miRNAs targeting cellular co-factors</i>			
miR-17/92	PCAF	Expression is suppressed by HIV-1; PCAF is a co-factor of Tat	[78]
miR-198	Cyclin T1	Represses Cyclin T1, a co-factor of Tat, in monocytes, and is downregulated upon macrophage differentiation	[83]
<i>Cellular miRNAs targeting HIV-1</i>			
miR-28, miR-125b, miR-150, miR-223, miR-382	miR-28: 5' and 3' LTR miR-125b, miR-150: <i>Env</i> miR-382, 223: 3' LTR	Combined antagomiRs re-activate latent virus from resting CD4 ⁺ T cells; individual antagomiRs have modest effects on reporter virus expression, better when combined	[82]
miR-29a	<i>Nef</i> /3' LTR	Increases association of viral RNA with P-bodies; miR-29b and miR-29c also target the same region and can repress single-round replication	[95]

LTR: Long terminal repeat; PBS: Primer-binding site.