Role of host tRNAs and aminoacyl-tRNA synthetases in retroviral replication

Danni Jin and Karin Musier-Forsyth

From the Department of Chemistry and Biochemistry, Center for Retrovirus Research, and Center for RNA Biology, The Ohio State University, Columbus, Ohio 43210

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The lifecycle of retroviruses and retrotransposons includes a reverse transcription step, wherein dsDNA is synthesized from genomic RNA for subsequent insertion into the host genome. Retroviruses and retrotransposons commonly appropriate major components of the host cell translational machinery, including cellular tRNAs, which are exploited as reverse transcription primers. Nonpriming functions of tRNAs have also been proposed, such as in HIV-1 virion assembly, and tRNA-derived fragments may also be involved in retrovirus and retrotransposon replication. Moreover, host cellular proteins regulate retroviral replication by binding to tRNAs and thereby affecting various steps in the viral lifecycle. For example, in some cases, tRNA primer selection is facilitated by cognate aminoacyl-tRNA synthetases (ARSs), which bind tRNAs and ligate them to their corresponding amino acids, but also have many known nontranslational functions. Multi-omic studies have revealed that ARSs interact with both viral proteins and RNAs and potentially regulate retroviral replication. Here, we review the currently known roles of tRNAs and their derivatives in retroviral and retrotransposon replication and shed light on the roles of tRNA-binding proteins such as ARSs in this process.

Retroviruses as a human health concern include HIV type 1 (HIV-1), the causative agent of acquired immune deficiency syndrome (AIDS) (1), and human T-cell leukemia virus type 1 (HTLV-1),2 which causes adult T-cell leukemia/lymphoma and a demyelinating disease named HTLV-associated myelopathy/tropical spastic paraparesis (2). The lifecycle of a typical retrovirus starts with interaction between viral envelope protein (Env) and cellular receptors. This induces membrane fusion and entry of the viral capsid (Fig. 1). The viral genomic RNA (gRNA) is reverse-transcribed into cDNA using a viral reverse transcriptase (RT) and a packaged cellular tRNA as the primer. The viral cDNA enters the nucleus in the form of a pre-integration complex and is inserted into the host genome. The DNA provirus is transcribed by host machinery to produce new gRNAs that are packaged or serve as mRNAs (full-length and spliced variants) that are translated to generate viral proteins. The viral Gag and Gag-Pol polyproteins drive viral assembly at the plasma membrane; during this process, viral gRNA and some host factors, including the tRNA primer, are recruited. The newly budded virion undergoes maturation to become fully infectious (3).

The genome of simple retroviruses, such as murine leukemia virus (MuLV), encodes three polypeptides, Gag, Gag-Pol, and Env, whereas the complex retroviruses, including HIV-1, encode additional regulatory proteins. The Gag structural protein includes three major domains: matrix (MA), capsid (CA), and nucleocapsid (NC) (Fig. 1). During maturation, Gag is cleaved into individual domains by viral protease (PR), a product of the retroviral pol gene, which also encodes enzymatic proteins RT and integrase (IN) (4). Retrotransposons are RT gene-containing mobile genetic elements found in various eukaryotic species. They move from one location in the genome to another via reverse transcription from an RNA intermediate. The mobile nature of retrotransposons is a source of genomic instability as retrotransposition may induce changes in genome structure and gene expression (5). Retrotransposons adopt a similar genomic organization as retroviruses, but unlike retroviruses they do not have an extracellular phase in their replication cycle (6). Long terminal repeat (LTR) retrotransposons contain LTRs similar to a provirus. LTR retrotransposons contain a gag gene that allows the formation of virus-like particles and a pol gene that encodes enzymatic proteins (7).

Retroviruses and retrotransposons exploit various host factors throughout their replication cycles. A hallmark of retroviral replication is reverse transcription, wherein the single-stranded RNA genome is converted into dsDNA. As mentioned above, tRNAs and aminoacyl-tRNA synthetases can interact with viral proteins, including viral proteases and reverse transcriptase, to enhance viral replication. ARSs may also play a role in retroviral replication by binding to tRNAs and affecting various steps in the viral lifecycle. For example, in some cases, tRNA primer selection is facilitated by cognate ARSs, which bind tRNAs and ligate them to their corresponding amino acids, but also have many known nontranslational functions. Multi-omic studies have revealed that ARSs interact with both viral proteins and RNAs and potentially regulate retroviral replication. Here, we review the currently known roles of tRNAs and their derivatives in retroviral and retrotransposon replication and shed light on the roles of tRNA-binding proteins such as ARSs in this process.
above, all retroviruses use specific host tRNAs as the primer for reverse transcription (8). For HIV-1, the identity of the primer is the human tRNA^Lys,3 (9). HTLV-1 uses tRNA^Pro as primer (10), and Rous sarcoma virus (RSV), a retrovirus that causes sarcoma in avian species, uses tRNA^Trp (11). Some retrotransposons also require a tRNA molecule to initiate reverse transcription (12–14).

The enrichment of specific cellular tRNAs in some retroviruses is facilitated by the host machinery. For example, in the case of HIV-1, the cognate ARS, human lysyl-tRNA synthetase (LysRS), mediates tRNA^Lys primer recruitment and packaging (15). Tryptophanyl-tRNA synthetase (TrpRS) has also been identified in RSV virions (16). ARSs are essential components of the cellular translational machinery, as they catalyze the ligation of the correct amino acids onto the 3' end of cognate tRNA molecules. Like LysRS and TrpRS, many tRNA synthetases have evolved nontranslational functions, including roles in transcriptional control (17), translational control (18–20), cell proliferation (21), and the cellular immune response (22, 23).

In this review, we summarize our current understanding of the involvement of tRNAs, tRNA derivatives, and ARSs in the replication cycle of retroviruses and retrotransposons. We also provide an overview of host tRNA-binding proteins that modulate retroviral replication, and the potential functions of ARSs in the retroviral lifecycle suggested by multi-omic studies.

**tRNAs as primers for reverse transcription**

The 5'UTR of retroviruses contains an 18-nucleotide (nt) sequence, termed the primer-binding site (PBS), which is complementary to the 3' acceptor stem and the 5' C arm of a specific host cell tRNA. The sequence of the PBS is important for efficient viral replication and determines the identity of the tRNA primer (24–26). The cellular tRNA that serves as the reverse transcription primer is partially unwound and annealed to the PBS to prime reverse transcription (8). For all retroviruses and most LTR retrotransposons, viral RT extends the tRNA primer to create an RNA–DNA duplex. The RNase H activity of RT degrades most of the genome during the reverse transcription process, leaving the (--) strand DNA with one or more purine-rich RNA fragments known as polypurine tracts annealed to it. The polypurine tracts serve as the primers for the synthesis of (+) strand DNA, resulting in a dsDNA product (27).

The HIV-1 PBS is complementary to human tRNA^Lys,3, which serves as the primer for HIV-1 RT. Efforts have been made to force HIV-1 to use alternative tRNA primers by mutat-
ing the PBS to be complementary to other tRNA species (24, 28). However, the fact that these mutant viruses cannot be stably maintained suggests that additional interactions between the tRNA primer and the viral RNA genome are present (24). Indeed, the U-rich anticodon of tRNA<sub>Lys<sup>3</sup></sub> has been proposed to interact with an A-rich bulge located proximal to the PBS in some HIV-1 isolates (29, 30). In addition, a short sequence upstream of the PBS, termed the primer activation signal, allows additional interaction with the T<sub>ΨC</sub> arm of the tRNA primer (31, 32). A recent cryo-electron microscopy (cryo-EM) study reported the structure of the HIV-1 RT initiation complex. In this structure, the PBS–tRNA<sub>Lys<sup>3</sup></sub> helix is localized in the nucleic acid–binding cleft of an inactive conformation (i.e., open fingers and thumb) of RT, and the nonpriming region of the tRNA folds back and stacks on the PBS to form an elongated helical structure (33). The cryo-EM structure is consistent with previous chemical probing data, in which extensive viral RNA–tRNA interactions beyond the PBS region were not observed at the stage of RT initiation, where the primer is extended by one nucleotide (34, 35). This is in contrast to the additional interactions observed previously in the absence of RT (29, 31).

Retrotransposons adapt diversified tRNA-priming mechanisms. tRNA<sub>Lys<sup>3</sup></sub>-primed reverse transcription is conserved among many LTR retrotransposons, including the copia retrovirus-like particle from <i>Drosophila melanogaster</i> (12), Tp1 and Tp2 from the slime mold <i>Physarum polycephalum</i> (13), and <i>Saccharomyces cerevisiae</i> Ty5 (14). Other LTR retrotransposons such as <i>Schizosaccharomyces pombe</i> TFI undergo tRNA-independent reverse transcription via a self-priming mechanism (36). Most LTR retrotransposons such as <i>S. cerevisiae</i> Ty1 and Ty3 initiate (−)-DNA synthesis from the 3′ end of an intact tRNA (37). In contrast, <i>copia</i> from <i>D. melanogaster</i> and Ty5 from <i>S. cerevisiae</i>, reverse transcription initiation requires a processed 5′ fragment of tRNA<sub>Lys<sup>3</sup></sub> (12, 14).

The annealing of primer tRNA to the retroviral genome is regulated by viral and host proteins. Annealing of tRNA<sub>Lys<sup>3</sup></sub> to the HIV-1 PBS can be facilitated by either the Gag polyprotein or the mature NC protein in vitro (38, 39). Because tRNA is found annealed to gRNA in PR-negative virions, Gag is believed to facilitate the initial placement of the primer onto the PBS (40). It has been shown that the NC domain of Gag is essential for annealing activity in vitro (41). As a nucleic acid chaperone protein, the NC protein facilitates tRNA and gRNA remodeling to form a thermodynamically stable complex (42–44). A multistaged tRNA primer annealing process has been proposed, wherein initial annealing by Gag is followed by more optimized annealing by NC in mature virions (45, 46), a model further supported by in vitro RNA structure–probing analysis (47). Human RNA helicase A, a transcriptional cofactor that unwinds dsRNA and DNA, binds to the 5′ UTR of the HIV-1 genome and has also been proposed to play a role in promoting tRNA primer annealing (48, 49). Together with Gag, RNA helicase A induces a conformational change in the genome, making it favorable for tRNA primer binding.

**Aminoacyl-tRNA synthetases as regulatory factors in RT primer recruitment**

Human tRNA<sup>Lys<sup>3</sup></sub> is selectively packaged into HIV-1 virions, together with another tRNA<sup>Lys<sup>1</sup>–<sup>2</sup></sub> isoacceptor, tRNA<sub>Lys<sup>1</sup>–<sup>2</sup></sub>, that does not serve as a reverse transcription primer (50, 51). This raised the possibility that human LysRS, the only known cellular protein that specifically binds to all tRNA<sup>Lys</sup> isoacceptors, is involved in tRNA<sup>Lys</sup> packaging. Indeed, LysRS is selectively packaged into HIV-1 virions (15), and overexpression of LysRS in cells enhances tRNA<sup>Lys</sup> packaging and viral infectivity (52). The selective packaging of the tRNA primer requires HIV-1 Gag and Gag-Pol (50), and interaction between LysRS and Gag is observed in vitro (53). This interaction has been mapped to the C-terminal domain of the CA region of Gag and the motif 1 dimerization domain of LysRS (54, 55). Cyclic peptides that target the interaction interface have been developed and function to inhibit the protein–protein binding in vitro (56). In a proposed model, a complex containing viral RNA, Gag, and Gag-Pol recruits the LysRS/tRNA<sup>Lys</sup> complex, forming the tRNA<sup>Lys</sup> packaging complex (57). Moreover, tRNA binding to LysRS, but not aminoacylation, is required for tRNA incorporation into the virus (58, 59).

In higher eukaryotes, LysRS is part of a high-molecular weight multi-tRNA synthetase complex (MSC) in the cytoplasm. This complex contains nine ARS activities in eight proteins (aspartyl-tRNA synthetase (AspRS), arginyl-tRNA synthetase (ArgRS), glutaminyl-tRNA synthetase (GlnRS), leucyl-tRNA synthetase (LeuRS), isoleucyl-tRNA synthetase (IleRS), LysRS, methionyl-tRNA synthetase (MetRS), and a bi-functional tRNA synthetase consisting of glutamyl- and prolyl-tRNA synthetases (GluProRS)), and three scaffold proteins known as ARS-interacting multifunctional proteins (AIMPs), AIMP1, AIMP2, and AIMP3 (60). The MSC serves as a reservoir for ARSs with noncanonical functions, as well as facilitating tRNA channeling and translation (61). Interestingly, HIV-1 infection leads to an increased pool of non-MSC-associated ARSs (62).

The source of viral LysRS has been an active area of investigation. In higher eukaryotic cells, LysRS has been reported to be found in the nucleus (63), in mitochondria (64), assembled in the MSC in the cytosol (65), and associated with the plasma membrane (66, 67). Early studies suggested that newly synthesized LysRS is packaged into the virus prior to assembly into the MSC (66, 68). Mitochondrial LysRS has also been proposed to be a source of LysRS used by HIV-1 (69). A recent study suggests that HIV-1 infection induces phosphorylation of LysRS at Ser-207 (62). This phosphorylation event triggers LysRS release from the MSC and nuclear localization (22). Interestingly, the phosphomimetic mutant S207D LysRS is packaged by the virus and maintains full tRNA-binding capability but lacks aminoacylation activity (62). However, phosphoablative mutant S207A LysRS is unable to be packaged, suggesting that phosphorylation at Ser-207 is a requirement for LysRS being packaged by HIV-1 (62). Taken together, a mechanism has been proposed wherein HIV-1 infection liberates LysRS from the MSC by inducing phosphorylation. The released p-LysRS is bound to an uncharged tRNA<sup>Lys</sup> that can serve as an effective
reverse transcription primer. Many open questions regarding the mechanism of tRNA primer packaging and the role of ARSs in this process remain, both in HIV-1 and other retroviral systems. For example, the significance of LysRS nuclear localization in HIV-1–infected cells is unknown. In addition, although an MSC-free fraction of LysRS is observed in HIV-1–infected cells, we cannot rule out the possibility that HIV-1 induces a pool of newly synthesized LysRS that is phosphorylated and thus prevented from associating with the MSC (62). Similar to HIV-1, RSV selectively packages primer tRNATrp, as well as host TrpRS (16), although the viral–host interactions have not been elucidated in detail.

In addition to capturing the tRNA primer through LysRS recruitment, HIV-1 has also evolved a mechanism to displace tRNALys from LysRS for primer annealing. The 5′UTR of the HIV-1 genome contains a tRNA-like element (TLE) that resembles the anticodon loop of tRNALys,3 in both sequence and 3D structure (70–72). The TLE binds LysRS with high affinity and promotes efficient tRNA primer annealing (Fig. 2) (70). This tRNA mimicry by the HIV-1 genome is proposed to compete with tRNA^{lys,3} for specific LysRS binding, and therefore it facilitates the release of the tRNA primer for annealing (72). A divergent sequence but a similar TLE 3D structure is observed in HIV-1 NL4-3 (subtype B) and MAL (subtype A-like) isolates, suggesting that the molecular mimicry is conserved among HIV-1 subtypes (73). A model summarizing HIV-1 tRNA primer packaging and placement is illustrated in Fig. 2.

\section*{tRNA regulates Gag membrane binding}

In addition to NC, which is well known for its viral gRNA binding and chaperone activities, the MA domain of HIV-1 Gag also interacts with RNAs \textit{in vitro} and in cells (74–76). As identified in a cross-linking–immunoprecipitation sequencing (CLIP-seq)-based study, tRNAs comprise over 60% of Gag-bound RNAs (76). Strikingly HIV-1 MA binds almost exclusively to tRNAs, with an apparent preference for tRNA^{Glu},

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{HIV-1 tRNA primer recruitment and packaging. HIV-1 infection induces a pool of free, non-MSC-associated phosphorylated LysRS, which traffics to the nucleus. The role of nuclear LysRS is unknown. The genomic RNA/Gag/Gag-Pol complex recruits the LysRS/tRNA complex. The TLE binds LysRS and releases the tRNA primer for annealing. The cellular location of tRNA primer annealing is unknown but is depicted here in the cytoplasm. The PBS and anti-PBS region of the tRNA are colored in \textit{red}. The TLE and the tRNA region mimicked are colored in \textit{green}.}
\end{figure}
tRNA<sup>lys</sup>, and tRNA<sup>val</sup> isoacceptors. MA binds full-length tRNAs, and the binding sites are in the 5’-half, specifically in the D-loop (76). The MA domain contains a highly basic region essential for regulating Gag relocalization to the plasma membrane by specifically interacting with phosphatidylinositol 4,5-bisphosphate (PI(4,5)P<sub>2</sub>) (77). Using in vitro liposome-binding assays, it was initially reported that RNA binding to MA prevents Gag binding to non-PI(4,5)P<sub>2</sub> membrane lipids (upper pathway). PI(4,5)P<sub>2</sub> outcompetes tRNA for Gag binding (lower pathway). In this model (80), tRNA prevents membrane binding of Gag monomers unassociated with viral gRNA (left). Gag binding to gRNA results in dimers that have a higher probability to overcome the inhibitory effect of tRNA and are capable of membrane binding (middle). Gag molecules binding to non-gRNA are unable to overcome the inhibitory effect of tRNA or associate with the membrane. These models do not address the conformation of Gag, which may interact with the non-gRNAs with both the MA and NC domains simultaneously in a bent conformation (142).

The inhibitory effect of tRNA on Gag-membrane binding was further validated in a minimal system that includes giant unilamellar vesicles, recombinant HIV-1 Gag protein, and purified RNAs (82). In the presence of tRNA, gRNA-bound Gag exhibits a higher likelihood of membrane binding than nonspecific RNA-bound Gag (82). These observations led to another model wherein viral gRNA-bound Gag overcomes the inhibitory effect of tRNA and thereby multimerizes and clusters at the plasma membrane (Fig. 3B) (82).

**Functions of tRNA derivatives in modulating the replication of retroviruses and retroelements**

The development of high-throughput sequencing technologies has led to the discovery of many novel noncoding RNAs (ncRNAs) present in cells and virions. The host RNA packageome of HIV-1 includes a variety of cellular RNAs including...
nonlysyl tRNAs (51). Interestingly, HIV-1 and MuLV selectively package pre-tRNAs containing 3' and 5' extension sequences, although the function of these unprocessed tRNAs in the viral lifecycle is unclear (83, 84).

tRNAs are a source of a class of ncRNAs, known as tRNA-derived fragments, which are increasingly gaining attention. tRNA fragments are involved in regulating basic cellular functions, such as inhibiting mRNA translation (85), and are associated with a variety of human diseases, including pathological stress injuries, cancers, and neurodegenerative diseases (86). Viral infection can also trigger the expression of certain tRNA fragments (87).

tRNA-derived fragments are commonly divided into two categories: tRNA halves (tiRNAs) and shorter tRNA fragments (tRFs). tiRNAs correspond to 30–35-nt 5’-tRNA halves and 40–50-nt 3’-tRNA halves that are produced from specific cleavage by the RNase angiogenin under stress conditions (88). tiRNAs are involved in the stress-induced decrease of global translation (89) and may serve as a biomarker for tissue damage (90). tRFs are smaller and more diverse fragments of tRNA with a typical length of 12–30 nt. Cleavage of mature tRNAs within the D-loop and in/near the T-loop gives rise to 5’ tRFs (tRF-5s) and 3’ CCA tRFs (tRF-3s), respectively. A third class of tRFs, tRF-1s, is derived from tRNA precursors that contain the 3’ trailer sequences but lack the 3’ CCA addition (Fig. 4) (91).

Many tRF-5 molecules have recently been shown to play modulatory roles in the replication of retroelements. For example, 5’ fragments of tRNA<sub>Gly(GCC)</sub> functionally suppress gene expression related to the endogenous retroelement MERVL in mature mouse sperms (92). In addition, tRF-5s that accumulated in Arabidopsis thaliana were shown to direct the cleavage of mRNAs associated with gypsy family transposable elements (93). A recent study suggested tRF-3s also have the potential to control the replication of endogenous retroviruses in mice (94). Two 18- and 22-nt–long 3’ CCA tRFs (Fig. 4, arrows), both containing sequences complementary to the PBS of LTR-retrotransposons, restrict LTR-retrotransposition activity using two distinct mechanisms. While the 22-nt 3’ CCA tRF inhibits gene expression post-transcriptionally by inducing RNAi, the 18-nt 3’ CCA tRF inhibits reverse transcription (94). The 18-nt tRF reduces the accumulation of retroviral RNA intermediates and downstream DNA intermediates of reverse transcription in a PBS-dependent manner but has no effect on the recruitment of the tRNA primer. Thus, it was proposed that the 18-nt tRF interferes with primer annealing by competing with tRNA for PBS binding (94), a novel mechanism of ncRNAs controlling genome stability.

In addition to the reported inhibitory effects of tRFs against retroelements, evidence that tRFs may play a role in the replication of retroviruses has also been presented. Compared with

![Figure 4. tRNA-derived fragments generated from tRNA precursor and mature tRNA. tRF-1s are derived from tRNA precursors and contain the 3’ trailer sequences. Mature tRNAs give rise to tiRNAs and two classes of shorter tRNA fragments, tRF-3s and tRF-5s. tRNA cleavage sites that produce the two 18- and 22-nt tRF species regulating LTR-retrotransposon replication are indicated by arrows.](image)
tRF-5s and tRF-1s, tRF-3s are present in higher abundance in CD4+ T-cells, a natural target of HIV-1 and HTLV-1 (95). One of the most abundant fragments in CD4+ T-cells is tRF-3019, which is derived from the 3’ end of tRNAPro, the primer for HTLV-1 reverse transcription. Interestingly, tRF-3019 is able to prime reverse transcription in vitro and is selectively packaged into HTLV-1 virions in addition to tRNAPro (95). This study raises the possibility that tRFs complementary to the PBS sequence may serve as primers during retrovirus replication. However, to date, there is no direct evidence that retroviruses use tRFs as primers. An 18-nt ncRNA complementary to the PBS, termed PBSncRNA, was discovered in cells latently infected with HIV-1 and in HeLa cells transfected with HIV-1 proviral DNA (96). However, it is believed that PBS ncRNA is produced from an annealed PBS–tRNAArg duplex by dicer processing (96), a source different from that of tRFs expressed in uninfected cells. Thus, this tRNA fragment may be generated by the host cell RNAi machinery and serve to down-regulate HIV-1 gene expression (96).

tRFs may also play regulatory roles independent of the reverse transcription process, although the exact function of these tRFs remains to be explored. For example, nonprimer-derived tRFs, such as a tRNATer-derived 19-nt tRF-3, is highly expressed in transformed cell lines, including those chronically infected with HTLV-1 (97). The HTLV-1 regulatory protein Tax was found to activate RNA polymerase III promoters, raising the possibility that HTLV-1 can actively regulate the expression of tRNAs and tRFs driven by RNA polymerase III (98).

Retroviruses manipulate host cell translation machinery

Although HIV-1 uses the host cell translational machinery to translate viral mRNAs, it is poorly adapted to the codon usage of host cells (99). It has been shown that HIV-1 replication capacity in cell culture can be manipulated by altering codon usage in the viral genome (100). The genomes of HIV-1 and other lentiviruses contain an above-average fraction of A nucleotides (101, 102), which not only affects the rate of viral mRNA translation (103) but also ensures efficient reverse transcription, potentially by maintaining the flexibility of viral RNAs (104). In particular, there is a high frequency of A-ending codons in the protein-coding region of the HIV-1 genome, and these codons are under-represented in the host cell genome (99). HIV-1 early genes (tat, rev, and nef) adapt to the host codon usage better than late genes (gag, pol, env, vif, vpu, and vpr), which contain a higher portion of rare codons (99). However, HTLV-1 does not exhibit differences in codon adaptation between early and late genes (99). Interestingly, tRNAs decoding rare codons are packaged by HIV-1 (51). It is possible that the codon usage in the early HIV-1 genes reflects the tRNA pool present when the host proteins and early HIV-1 proteins are translated, whereas rare tRNAs packaged by HIV-1 reflect the altered tRNA pool for viral mRNA translation and initiation of late gene translation (99). It remains unclear whether this change in tRNA pool is induced directly by viral infection or is a consequence of host cell response.

A link between reverse transcription primer usage and viral translation has been proposed for HIV-1 and MuLV (105). While altering the PBS to be complementary to a tRNA other than tRNAArg does not allow optimal HIV-1 replication (24), the virus has a preference for tRNAArg, tRNALys, tRNAHis, as alternative primers (105). Interestingly, codons for these tRNAs are enriched near the ribosomal frameshift site at the Gag-Pol junction (105). Similarly, MuLV has enriched codons for tRNAPhe, tRNAGly, and tRNAArg at the Gag-Pol junction, and these tRNAs are also favored as alternative primers (105). The observation that primer usage of both HIV-1 and MuLV correlates with the codon distribution at the Gag-Pol junction established a potential link between the primer selection mechanism of these two retroviruses. However, primer selection of HIV-1 is also achieved by selective packaging of LysRS, whereas MuLV, which uses tRNAPro as the primer, does not selectively package ProRS (16).

tRNA-binding proteins as regulators of retroviral replication

The codon usage bias in lentiviral genomes is a potential target of host cell restriction factors. Indeed, the human Schlafen 11 protein was reported to inhibit HIV-1 gene expression via intrinsic properties of the transcripts (106). Human Schlafen proteins are products of interferon-stimulated genes and are involved in various cellular functions, including cell proliferation and innate immunity (107). Of the five human Schlafen isoforms, two (Schlafen 11 and Schlafen 13) were identified as inhibitory factors for HIV-1 (106, 108). Human Schlafen 11, a putative DNA/RNA helicase with regulatory roles in cancer cell lines (109–111), binds to tRNAs and selectively inhibits HIV-1 gene expression in a codon usage–based manner (106). Similarly, equine Schlafen 11 protein restricts the replication of equine infectious anemia virus, another member of the lentivirus genus (112). Interestingly, Schlafen 11 is highly expressed in HIV-1 elite controllers, individuals who have never been exposed to antiretroviral therapy (ART) but naturally suppress HIV-1 viral loads to an undetectable level, compared with noncontrollers and ART-suppressed individuals. This is a unique property of Schlafen 11 among 34 known anti-HIV-1 factors tested (113) and suggests that Schlafen 11 may play an important role in HIV-1 elite control in vivo. While the detailed mechanism of Schlafen inhibition of viral replication remains unknown, a recent study on human Schlafen 13 shed light on the structural basis of Schlafen proteins’ tRNA-binding properties. Schlafen 13 is a mammalian tRNA binding endoribonuclease. The N terminus of human Schlafen 13 is able to cleave tRNAs near the 3’ end in vitro (108). Schlafen 13 also inhibits HIV-1, causing decreased viral protein expression and virion production, and this inhibition depends on the tRNA cleavage activity (108). Thus, Schlafen proteins may inhibit lentivirus replication by sequestering and cleaving primer tRNAs or tRNAs decoding rare codons. Alternatively, Schlafen proteins may indirectly mediate viral replication by generating tRNA fragments that have regulatory effects on the viral lifecycle.

Human P-element–induced wimpy-like (Piwil) 2 protein, also known as Hili protein, is another tRNA-binding factor that exhibits anti-HIV-1 properties (114). Piwil proteins belong to the argonaute family and interact with small Piwi-interacting
RNAs (piRNAs). The resulting complex can target and restrict transposable elements in the germline (115–117). The source of piRNAs includes tRNAs and transposable elements in the germline (115–117). The five ARSs listed exhibit a significant decrease (≥16-fold) in transcript levels. Five other ARSs, AlaRS, IleRS, LysRS, ValRS, and GlnRS, exhibit a moderate decrease (~4-fold) in HIV-1-infected cells.

ARSs are housekeeping proteins, which nevertheless possess a wide variety of alternative functions. Many ARSs respond to stimuli, including viral infection, immune activation, and toxins (18, 23, 129). They play unique regulatory roles in cellular signaling pathways (22, 130), and some ARSs are even secreted and act as cytokines and chemokines (131, 132). The roles that ARSs play in response to viral infection and the immune response make them potential therapeutic targets and diagnostic biomarkers (133). Interestingly, many ARSs have been identified in multi-omic studies focusing on retroviral replication. RNA-binding proteins are highly represented in the interactome of HIV-1 Gag, including all the components of the MSC (134). The MA domain of Gag is a major interaction partner of the MSC, and MA has also been reported to interact with a few non-MSC ARSs (Table 1) (122). HIV-1 infection results in an increased fraction of free, non-MSC–associated synthetases (62). Although the specific viral or host process affecting the assembly state of the MSC is unclear, it is possible that the MA protein of incoming virus or newly synthesized Gag protein interact with the MSC and disrupt its structural stability.

The fate of ARSs released from the MSC is another open question. Based on siRNA screens to identify potential regulatory and inhibitory factors for HIV-1, facilitative roles are proposed for LeuRS and LysRS (see above), and an inhibitory function is proposed for EPRS (134). EPRS has been shown to inhibit the replication of RNA viruses, including vesicular stomatitis virus and influenza A virus by promoting the innate immune response via up-regulation of the mitochondrial antiviral signaling protein (23). The mechanism by which EPRS inhibits the replication of HIV-1 remains unknown. In addition to its positive role in tRNA^Lys^ primer recruitment and packaging, LysRS has also been reported to inhibit HIV-1 PR activity and thereby alter the level of RT (135). IleRS is another synthetase identified in virions (134); however, the significance of this finding is unknown. The observation that mRNA levels of some tRNA synthetases are altered in CD4+ T-cells infected with HIV-1 (Table 1) suggests a delicate arms race between viral infection and expression of these synthetases (128).

Uncovering physical interactions between host proteins and viral RNAs provides potential clues for understanding the roles

| Table 1 | Aminoacyl-tRNA synthetases linked to retrovirus replication |
|---------|------------------------------------------------------------|
| Description | Aminoacyl-tRNA synthetases |
| HIV-1 MA interactome | MetRS*, LysRS*, ArgRS*, GlnRS*, LeuRS*, IleRS*, EPRS*, AspRS*, AsnRS, ThrRS, HisRS |
| HIV-1 Gag interactome | MetRS*, LysRS*, ArgRS*, GlnRS*, LeuRS*, IleRS*, EPRS*, AspRS* |
| siRNA depletion decreases HIV-1 replication | LysRS*, LeuRS* |
| siRNA depletion increases HIV-1 replication | EPRS* |
| Packaged in HIV-1 virion | LysRS*, IleRS* |
| Interacts with HIV-1 5’UTR | ArgRS*, EPRS* |
| Interacts with RSV 5’UTR | LysRS*, SerRS2 |
| Interacts with unspliced full-length HIV-1 RNA | LeuRS* |
| Levels of transcripts decrease upon HIV-1 infection | EPRS*, TyrRS, MetRS*, HisRS, PheRS |
| Levels of transcripts increase upon HIV-1 infection | AsnRS, CysRS |
| Mixed pattern of regulation upon HIV-1 infection | GlyRS |

* MSC–associated tRNA synthetases are shown.
* The five ARSs listed exhibit a significant decrease (≥16-fold) in transcript levels. Five other ARSs, AlaRS, IleRS, LysRS, ValRS, and GlnRS, exhibit a moderate decrease (~4-fold) in HIV-1-infected cells.

Multi-omic studies suggest additional potential roles for aminoacyl-tRNA synthetases in retrovirus replication

Many cellular proteins play facilitative or inhibitory roles in the retroviral lifecycle (121). Identification of new cellular factors involved in retroviral replication remains an active area of research, as a better understanding of virus–host interaction may inform novel therapeutic strategies. In recent years, cellular factors interacting with HIV-1 proteins and RNAs have been identified using proteomic approaches (122–124). Host proteins with potential functions in the viral lifecycle have also been identified using siRNA-based screens (125–127). A transcriptome-wide study of T-cells infected with HIV-1 revealed a series of host transcripts affected by viral infection (128).
of these host factors in viral replication. Using RNA affinity chromatography, human and avian proteins co-isolated with the 5′UTR of HIV-1, RSV, and spleen necrosis virus were identified (123). Interestingly, human EPRS and ArgRS bind to the HIV-1 5′UTR, and avian LysRS and SerRS bind to the 5′UTR of RSV (Table 1). The significance of these findings remains to be explored. In another recent study, a hybridization capture strategy was used to selectively purify unspliced full-length HIV-1 RNA and identify interacting protein partners. Interestingly, LeuRS is among the factors identified, and future studies are required to understand the significance of this finding (124).

Concluding remarks

Retroviral infection is still a global human health concern. Unfortunately, no cure has been developed to combat HIV-1 or HTLV-1. The high mutation rate of retroviruses adds to the difficulty in developing persistent antiviral therapies.

ARSs are housekeeping proteins with a wide variety of non-canonical functions. As discussed in this review, both ARSs and their tRNA substrates are emerging as important players in retroviral replication. Future studies to explore the potential of these host cell factors as therapeutic targets may result in the development of novel anti-retroviral therapies.

Host tRNAs play translational and alternative roles in multiple stages of the retroviral lifecycle, raising the potential of tRNA-binding proteins and tRNA derivatives as regulators for retroviruses. The repertoire of tRNA derivatives in human health is growing. Some tRFs have regulatory functions in the replication cycle of retroelements (95, 96), but the underlying mechanisms remain unclear. More in-depth studies are needed to uncover the physical interactions between tRNA/tRFs and the viral machinery. For example, although tRNAs were identified as interacting with HIV-1 MA (76), whether the interaction is specific is not yet clear. An improved understanding for how tRNA binding affects the structure and function of Gag during retroviral assembly is also needed. Studies on a broader range of retrovirus genera will advance our global understanding of retrovirus–host interactions.

Some caveats of the aforementioned studies and recent advances in the field should also be mentioned. First, tRNA modifications are absent in in vitro–synthesized tRNAs used for the majority of biochemical and structural studies. In addition to potential contributions to the structure and function of tRNA, modifications cause bias in primer extension-based RNA-Seq techniques. A human tRNA microarray has been developed for tRNA quantification and has been applied in tRNA profiling in HIV-1 (31), as well as of specific human tissues and tumor cells, and in identifying the complete interactome of tRNA-binding proteins (136–138). More recently, high-throughput tRNA-specific sequencing techniques have also been developed. Enzymatic removal of tRNA modifications and thermostable group II intron reverse transcriptase are combined to overcome the blocks caused by modifications and structure (139). A hydrolysis-based tRNA sequencing method further allowed detection of tRNA precursors (140). AlkB-facilitated RNA methylation sequencing (ARM-seq), a sequencing method that involves treatment with an Escherichia coli demethylase, led to the discovery of novel tRNA fragments that were previously undetected due to the presence of methylation, as well as the development of a transcriptome-wide modification profile (141). The recent advances in tRNA quantification techniques should facilitate future studies of tRNA involvement in retroviral replication. In addition to more accurate tRNA identification and sequencing methods, advances in techniques for visualizing protein–RNA and RNA–RNA interactions in cells will be necessary for improving our understanding of the localization and timing of events such as tRNA primer annealing in the retroviral lifecycle.

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