

Host RNA Packaging by Retroviruses: A Newly Synthesized Story

Matthew J. Eckwahl,^a Alice Telesnitsky,^b Sandra L. Wolin^{a,c}

Department of Cell Biology, Yale School of Medicine, New Haven, Connecticut, USA^a; Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan, USA^b; Department of Molecular Biophysics and Biochemistry, Yale School of Medicine, New Haven, Connecticut, USA^c

ABSTRACT A fascinating aspect of retroviruses is their tendency to nonrandomly incorporate host cell RNAs into virions. In addition to the specific tRNAs that prime reverse transcription, all examined retroviruses selectively package multiple host cell noncoding RNAs (ncRNAs). Many of these ncRNAs appear to be encapsidated shortly after synthesis, before assembling with their normal protein partners. Remarkably, although some packaged ncRNAs, such as pre-tRNAs and the spliceosomal U6 small nuclear RNA (snRNA), were believed to reside exclusively within mammalian nuclei, it was demonstrated recently that the model retrovirus murine leukemia virus (MLV) packages these ncRNAs from a novel pathway in which unneeded nascent ncRNAs are exported to the cytoplasm for degradation. The finding that retroviruses package forms of ncRNAs that are rare in cells suggests several hypotheses for how these RNAs could assist retrovirus assembly and infectivity. Moreover, recent experiments in several laboratories have identified additional ways in which cellular ncRNAs may contribute to the retrovirus life cycle. This review focuses on the ncRNAs that are packaged by retroviruses and the ways in which both encapsidated ncRNAs and other cellular ncRNAs may contribute to retrovirus replication.

Retroviruses are ribonucleoprotein (RNP) particles that contain both viral and cellular RNAs. Each infectious particle contains two complete copies of the viral genomic RNA (gRNA), an 8-to-10-kb RNA that is packaged in an RNA dimer (1). Retroviral particles also contain numerous host cell-derived RNAs that collectively comprise up to half the total virion RNA by mass (2). Because most of these RNAs are noncoding RNAs (ncRNAs) of 300 nucleotides (nt) or less, cellular RNAs vastly outnumber the gRNA on a molar basis. The best characterized of these RNAs, in terms of both its mechanism of encapsidation and its role in retrovirus replication, is the specific tRNA used to prime reverse transcription of the gRNA (3) (Fig. 1). All examined retroviruses also package a 300-nt ncRNA called 7SL RNA. This ncRNA, which was discovered because of its abundance in avian and murine retroviruses (4–9), functions in cells as part of a signal recognition particle (SRP), an RNP important for cotranslational targeting of nascent secretory and membrane proteins to the endoplasmic reticulum (10). Consistent with selective packaging, 7SL is enriched 250-fold more in HIV virions than actin mRNA (9). Other ncRNAs that are packaged by one or more retroviruses include 5S rRNA, the spliceosomal U6 small nuclear RNA (snRNA), cytoplasmic ncRNAs called Y RNAs, and some nonprimer tRNAs (8,11–15). In contrast, although mRNAs are detectable in virion populations and mRNAs have been suggested to substitute biochemically for gRNA when none is available, no specific mRNA is a major virion component (8, 16, 17).

Although the role of the primer tRNA is well established, the ways in which other host ncRNAs contribute to the retrovirus life cycle are far less studied. In recent years, there has been new interest in this topic, most likely due to the discovery of numerous ncRNAs in cells and the realization that these RNAs play critical roles in an enormous variety of cellular processes (18). Also, the application of new tools, such as whole-transcriptome sequencing and *in vivo* cross-linking, has allowed both transcriptome-wide views of the RNAs bound by the retrovirus structural protein Gag during virus assembly and comprehensive analyses of the RNAs packaged into virions. These experiments have resulted in the identification of new

roles for host tRNAs (19) in HIV-1 replication and in the discovery that retroviruses selectively encapsidate newly made ncRNAs (17). Here, we emphasize recent advances in our understanding of the types of ncRNAs that are packaged by retroviruses, the mechanisms by which these RNAs are selected, and their proposed and possible roles in retrovirus replication.

THE RETROVIRUS LIFE CYCLE

Retroviruses interface with cellular RNAs and RNPs at numerous points in their life cycle (Fig. 1). For all orthoretroviruses, after fusion with the plasma membrane and entry of the viral capsid into the cytoplasm, the gRNA is reverse transcribed using a packaged host tRNA as a primer. The reverse-transcribed cDNA, in the form of a “preintegration complex,” then accesses the nucleus and integrates into the host genome (3). Transcription of this provirus yields mRNAs that are translated on cellular ribosomes to produce the structural polyprotein Gag, the GagPol polyprotein that also encodes reverse transcriptase, integrase and the viral protease, and the envelope protein Env (3). For murine leukemia virus (MLV), unspliced full-length gRNA encodes Gag and Gag-Pol (which is produced by readthrough of the Gag termination codon) (20), while splicing generates Env mRNA. For human immunodeficiency virus type 1 (HIV-1), Gag-Pol is produced by ribosome frameshifting (21). HIV-1, a complex retrovirus, performs these basic replication steps and also encodes numerous accessory proteins whose mRNAs are generated by alternative splicing (22).

Virus assembly is largely driven by the Gag polyprotein (Fig. 1B), which mediates specific recruitment of gRNA and is the

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Address correspondence to Sandra L. Wolin, sandra.wolin@yale.edu.

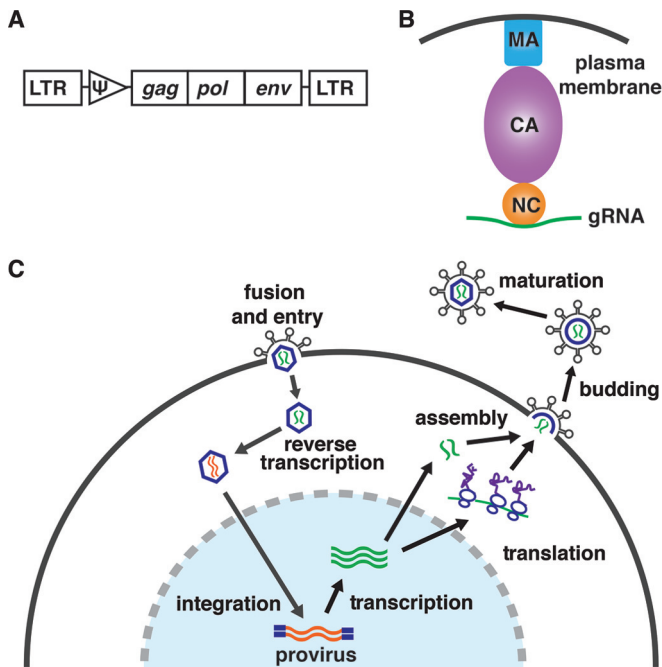


FIG 1 (A) Retrovirus genome organization. Although retroviruses vary in terms of their complexity, all share the same basic genomic structure (*gag-pol-env*). The Ψ sequence is required for packaging the viral genome into the capsid. The long terminal repeats (LTRs) contain signals for retroviral gene expression. (B) Schematic of the Gag polyprotein. The structural polyprotein Gag contains three major domains: (i) MA (Matrix), which is important for the association of Gag with the plasma membrane; (ii) CA (capsid), which forms the protein core of the virus through intermolecular interactions with adjacent CA domains; and (iii) NC (nucleocapsid), which binds the viral genomic RNA (gRNA). Upon virus release, the Gag polyprotein is matured by a protease (usually encoded with *pol*) to yield MA, CA, and NC proteins. (C) The retrovirus life cycle. Infection begins when mature virus particles bind to cell surface receptors through the Env protein, resulting in membrane fusion. After entering the cytoplasm, the viral gRNA is copied into DNA, accesses the nucleus, and integrates into the chromosomes of infected host cells. Afterward, the integrated provirus undergoes transcription, producing mRNAs that are translated into viral proteins and unspliced gRNAs, which assemble to form new virus particles.

major structural component of virus particles (3). Indeed, expression of *gag* alone is sufficient to drive assembly of virus-like particles (VLPs) that bud from the plasma membrane. An early step in assembly is Gag dimerization, a step that is promoted by RNA binding (23). Since Gag has been demonstrated to both form dimers and interact with gRNA in the cytoplasm, prior to arriving at the plasma membrane (24), gRNA may or may not be the RNA that normally carries out this function. However, interactions between Gag and some form of RNA are necessary for HIV-1 assembly both in reconstituted reactions and during virus replication (25), and since assembly of VLPs remains efficient when gRNA packaging is prevented or when gRNA is absent, host cell RNAs can fulfill this function. Upon arrival at the plasma membrane, a large number of Gag molecules polymerize around the Gag-RNA complex to nucleate virus assembly, with maturation of the virus core occurring after particle release. Since RNase treatment disrupts MLV core particles (26), but not HIV-1 cores (27), RNAs may scaffold interactions between individual Gag molecules in some retroviruses.

tRNAs PRIME REVERSE TRANSCRIPTION AND MAY HAVE OTHER ROLES

The primer tRNA is currently the only example of a host ncRNA that is known to be required for retrovirus replication. Each retrovirus packages a specific tRNA that serves to prime reverse transcription of its gRNA (28). The 3' end of this tRNA anneals to two complementary sites near the 5' end of gRNA, a process that requires the chaperone activity of the nucleocapsid (NC) domain of the Gag polyprotein (29, 30). The primer tRNAs for HIV (tRNA-Lys3/UUU), MLV (tRNA-Pro), and Rous sarcoma virus (RSV) (tRNA-Trp-CCA) are all packaged in excess of gRNA, at approximately 4 to 10 molecules per virion (31–34). Despite the sequence complementarity, packaging of these tRNAs does not require gRNA (35, 36). Specificities in tRNA packaging appear to be multifaceted and may differ among retroviruses. A requirement for interactions with the reverse transcriptase domain of the GagPol polyprotein has been reported (33, 37). Also, some primer tRNAs, such as HIV-1 tRNA-Lys3/UUU, are packaged in complex with their respective tRNA synthetases (28, 38). This has been best studied in HIV-1, where packaging of this tRNA includes specific interactions between the capsid (CA) domain of Gag and the lysyl tRNA synthetase (39).

Cellular tRNAs may also contribute to retrovirus replication in other ways. For example, tRNAs may shield the membrane-binding surface of the matrix (MA) domain from inappropriate interactions with intracellular membranes prior to arrival of the HIV-1 Gag polyprotein at the plasma membrane (19). *In vivo* cross-linking revealed that MA binds specific tRNAs in cytosol, an association that is reduced in membrane fractions and largely absent in virions (19). Using mutagenesis, tRNA binding was found to involve a basic region in MA that contributes to association of Gag with the plasma membrane by binding acidic phospholipids unique to this membrane (40, 41). Experiments performed in extracts had shown previously that RNA binding to this region prevents MA from interacting with phospholipids present in other membranes, such as those of the endoplasmic reticulum (42, 43). The cross-linking data indicate that host tRNAs may be the RNAs that bind MA *in vivo* to increase the specificity with which Gag is targeted to the plasma membrane (19).

Cellular tRNAs have also been proposed to assist HIV-1 nuclear import. Since HIV infects nondividing cells, the reverse-transcribed DNA, expressed in the form of a nucleoprotein called the preintegration complex, must enter the nucleus, most likely via the nuclear pores. Although the mechanism(s) by which HIV-1 accesses nuclei is not well understood (see reference 44), tRNAs lacking the 3' terminal CCA were found to promote nuclear import of HIV preintegration complexes in permeabilized cells (13). In yeast, aberrant cytoplasmic tRNAs, such as those with unprocessed ends or lacking certain nucleotide modifications, undergo nuclear reimport as part of a quality control pathway (45). A similar “retrograde transport” pathway occurs in mammalian cells (46), raising the possibility that HIV piggybacks onto this pathway to enter nuclei.

Although the proposed role for tRNAs in shielding HIV-1 MA during virus assembly and in assisting HIV-1 nuclear import may not require encapsidation, some nonprimer tRNAs are selectively packaged into virions. Because HIV-1 recruits primer tRNA-Lys3 in the form of a tRNA-synthetase complex, with interactions between Gag and synthetase contributing to recruitment, other

lysine tRNA isoacceptors are also packaged (reviewed in reference 39). Additionally, tRNA-Asn-GUU and tRNA-Ile-UAU are enriched in HIV-1 virions produced in human HEK293T cells relative to their concentrations in cells (15). Remarkably, tRNA-Asn-GUU is nearly as abundant in virions as the tRNA-Lys isoacceptors (15). Although tRNA-Ile-UAU is rare in cells, it is enriched more than 7-fold in virions relative to its cellular concentration (15). While the role(s) of these tRNAs in HIV-1 replication is unknown, the A-rich HIV genome is characterized by many suboptimal codons, resulting in inefficient translation of most viral proteins. In this regard, it is intriguing that the AUA codon decoded by tRNA-Ile-UAU, while rare in cellular mRNA, is the most common isoleucine codon in the HIV-1 genome (15).

Notably, HIV infection alters the composition of cellular tRNA pools to enhance translation of its own mRNAs, a replication strategy counteracted by the interferon-induced protein schlafen 11 (47). Specifically, in cells depleted for schlafen 11, the levels of many tRNAs, including rare tRNAs such as tRNA-Ile-UAU, increase during HIV infection. In these cells, both translation of the Gag polyprotein and virus production are strongly enhanced (47). Since tRNA-Asn-GUU and tRNA-Ile-UAU are among the most upregulated tRNAs in the absence of schlafen 11 and since both are present in virions as mature, CCA-containing tRNAs (15), it is possible that their presence is related, in an as-yet-unknown way, to the manipulation of host tRNA pools by HIV-1.

SPECIFIC NONCODING RNAs ARE ALSO ENRICHED IN VIRIONS

In addition to tRNAs, several host ncRNAs are abundant virion components. The best characterized, 7SL RNA, is packaged by all studied retroviruses, including RSV, MLV, HIV-1, and avian myeloblastosis virus (4–9). On a molar basis, 7SL is among the most abundant encapsidated RNAs, with 6 to 8 copies per MLV virion and 14 to 26 copies per HIV-1 virion (9, 48). Other ncRNAs packaged in stoichiometric quantities by one or more retroviruses include two Y RNAs, each of which is present in 4 to 5 copies per MLV virion (49), U6 snRNA, which is present in 1 copy per RSV virion (12), and the cytoplasmic-vault ncRNA, which is present in 1 copy per MLV virion (17). Other ncRNAs, such as the U1 and U2 spliceosomal snRNAs, appear to be packaged in substoichiometric quantities in all examined retroviruses (8, 12, 17).

In contrast to ncRNAs, mRNAs are not major virion components. Sequencing of the RNAs within MLV virions revealed that most reads that mapped to the mouse genome were derived either from ncRNAs (40%) or from VL30 elements (36%), a class of endogenous retroviruses that are well packaged by MLV (17, 50). Only 6% of reads derived from mRNAs (17). Moreover, in contrast to the selectivity with which specific ncRNAs are packaged (see below), most mRNAs are present in MLV virions in proportion to their cellular levels, although a small number are enriched relative to their cellular concentrations (16). Additionally, examination of actin mRNA revealed that it was less selectively packaged than the viral spliced Env mRNA, an RNA that does not undergo selective encapsidation (8).

NASCENT NONCODING RNAs ARE SELECTIVELY PACKAGED

Many ncRNAs appear to be packaged shortly after synthesis, before assembling into mature RNPs. For example, HIV virions do not contain detectable SRP54 (9, 51), the final SRP subunit to bind 7SL RNA (10). Similarly, SRP19, the subunit whose binding to

7SL precedes SRP54 association (10), was not detected in MLV virions (17). Consistent with the hypothesis that newly made 7SL RNA is recruited into virions in competition with its assembly into SRP, overexpression of SRP19 reduces 7SL packaging by HIV-1 (48, 52, 53). Similarly, Ro60 and TEPI, the major protein partners of Y and vault RNAs, respectively, are not detected in MLV virions (17, 49). Moreover, although Y RNAs are unstable in cells lacking Ro60 (54, 55), packaging of these RNAs into MLV virions is not strongly affected in these cells (49), suggesting that virus recruitment occurs prior to Ro60 binding. However, because none of these RNAs undergo significant 5' or 3' processing as part of their biogenesis, the evidence that they are packaged as nascent RNAs is indirect.

We recently obtained direct evidence that some ncRNAs are indeed packaged as nascent RNAs (17). In these experiments, we used high-throughput sequencing to characterize the MLV “RNA packageome,” using highly purified virus as starting material. In addition to expected ncRNAs such as 7SL RNA and Y RNAs, we identified numerous precursors to specific tRNAs, snRNAs, and small nucleolar RNAs (snoRNAs). For example, many tRNA-derived sequencing reads contained the 5' leaders, introns, and/or 3' trailer sequences that are absent from mature tRNAs. We also obtained reads derived from 3' extended snRNAs, as well as 5' and 3' extended small nucleolar RNAs (snoRNAs). Using Northern blotting, we demonstrated that the precursors were far more enriched in virions, relative to their cellular levels, than their respective mature RNAs (17). For example, although intron-containing pre-tRNA-Ile-UAU is rare in cells, this pre-tRNA is enriched 65-fold more in MLV virions than the mature tRNA. Similarly, 3' extended pre-U2 snRNAs, which are present at very low levels in cells, are enriched more than 100-fold more in MLV virions than the far more abundant mature U2 snRNAs (17).

AT LEAST SOME NASCENT RNAs ARE PACKAGED FROM THE CYTOPLASM

The finding that retroviruses package newly synthesized RNAs is surprising when one considers that retroviruses assemble at the plasma membrane and raises the issue as to where in the cell the initial contact with Gag occurs. Most steps in SRP assembly occur in nucleoli (56–58), and pre-tRNAs, pre-snoRNAs, and U6 snRNA were reported to reside entirely within nuclei (59). However, while RSV Gag enters the nucleus to recruit its gRNA (60, 61), MLV and HIV-1 assembly takes place in the cytoplasm and it is controversial whether their Gag proteins normally traffic through nuclei (62). Although “nuclear ncRNAs” such as nascent 7SL RNA, U6 snRNA, and pre-tRNAs could potentially access the cytoplasm by base pairing with gRNA, packaging of 7SL, U6, and pre-tRNAs by MLV and packaging of 7SL by HIV-1 all remain efficient in mutant viruses that fail to package gRNA (9, 16, 17).

Remarkably, packaging of at least some “nuclear ncRNAs” occurs in the cytoplasm. First, by examining U2 snRNA precursors, which are normally exported to the cytoplasm and then reimported into nuclei (63), we found that packaging of pre-U2 snRNAs by MLV increased when nuclear import was blocked, as expected if encapsidation was a cytoplasmic event (17). Second, by depleting specific export receptors, we found that packaging of pre-tRNAs and U6 snRNA by MLV required Exportin-5, a nuclear export receptor known to transport pre-miRNAs and several other small ncRNAs (64, 65).

Subsequent experiments indicated that MLV packages both U6

snRNA and pre-tRNAs from a previously undetected decay pathway. Specifically, we found that aberrant forms of U6 snRNA and pre-tRNAs accumulated in cells and virions when specific cellular ribonucleases were depleted (17). Cell fractionation revealed that at least some aberrant forms of U6 were cytosolic (17). Together with the finding that packaging of 7SL is reduced when SRP19 is overexpressed (48), our results indicate that some newly synthesized ncRNAs are recruited in the cytoplasm, in competition with their normal biogenesis and decay pathways.

HOW ARE NASCENT NONCODING RNAs SELECTED FOR PACKAGING?

In contrast to gRNA recruitment, in which one or two zinc knuckles (C-X₂-C-X₄-H-X₄-C, where C represents cysteine, H represents histidine, and X represents any amino acid) in the Gag NC domain interact with specific sequence and structural elements within the gRNA packaging signal (called psi or Ψ) (23, 66–68), relatively little is known of the mechanisms by which ncRNAs are selected for encapsidation. Nonetheless, a few principles have emerged. First, although it was proposed that cellular ncRNAs may be recruited into virions through base pairing with gRNA (12, 69), most ncRNAs continue to be encapsidated when gRNA packaging is prevented (9, 14, 16, 17, 51, 70). Second, unlike mature tRNAs, where sequences in Pol have been implicated in packaging (15, 33, 36, 37), 7SL packaging remains efficient in HIV-1 VLPs containing only Gag (9, 53, 70). Finally, although mutation of the NC zinc knuckles strongly reduces gRNA packaging, packaging of 7SL and U6 RNAs is unaffected (14, 51, 70, 71). Thus, the requirements for packaging 7SL and U6 differ from those for gRNAs and mature tRNAs.

To date, only 7SL packaging has been studied in detail. Remarkably, 7SL is incorporated into minimal virus-like particles formed from mutant Gag proteins that lack all RNA-binding domains (72). Specifically, although RNA binding by NC promotes Gag oligomerization, this role can be bypassed when NC is replaced with a leucine zipper (73), a module known to form protein-protein interactions (74). Although the VLPs formed from the chimeric Gag proteins are greatly reduced in RNA (75, 76), Northern blotting revealed a 7SL fragment (72). Since this fragment is not detected in cells, 7SL may be recruited into virions as the full-length RNA and may subsequently undergo degradation to the shorter RNA form in the absence of NC (72). Since this same fragment is found in leucine zipper-containing VLPs that lack MA and part of CA, none of the characterized RNA-binding domains of HIV-1 Gag are required for 7SL encapsidation (72). This implies either that unknown RNA-binding determinants reside in the retained portions of Gag or that 7SL recruitment is mediated by host RNA-binding proteins that are incorporated into minimal VLPs.

Studies of the *cis*-acting sequences required for 7SL packaging have identified two mechanisms for uptake. 7SL consists of two domains (Alu and S) separated by a long double-stranded RNA linker (10). Consistent with a saturable pathway requiring specific interactions, truncated 7SL RNAs containing the Alu domain competed with endogenous full-length 7SL for HIV-1 packaging (77). The Alu domain contains the binding site for an SRP9/14 heterodimer (10), and an intact SRP9/14 binding site was required for the competitive recruitment (77). Mutational analyses revealed that a specific helix within the Alu domain was also important (77). These findings were incorporated into a model in which

SRP9/14 binding, which occurs early in 7SL biogenesis, transiently produces a structure recognized by Gag (77). Consistent with this model, although both SRP19 and SRP54 are absent from virions (9, 51), small amounts of SRP14 are present (17). Complicating this picture is the finding that Alu domain derivatives with a mutated SRP9/14 binding site, and also S domain derivatives, are packaged by HIV-1 but do not compete for encapsidation with endogenous 7SL (77). Together, these results support the idea of the existence of both an uptake pathway that requires specific interactions (either direct or indirect) between 7SL RNA and Gag and a second, less-specific “additive” pathway that can also be used to encapsidate host ncRNAs.

Little is known as to how other ncRNAs are recruited into virions. Given the large number of encapsidated ncRNAs, at least some are likely recruited through interactions that lack sequence specificity. For example, since nascent RNAs may contain protein-free regions, Gag could select these RNAs through contacts to their backbones or to any accessible 5′ or 3′ ends. Consistent with the idea that recruitment of some ncRNAs occurs through non-sequence-specific interactions, levels of both 5S rRNA and Y3 RNAs increase in HIV virions depleted of 7SL (52).

FUNCTIONAL IMPLICATIONS OF NASCENT RNA PACKAGING

How might newly synthesized ncRNAs contribute to the retrovirus life cycle? Although it has long been known that RNA binding enhances Gag oligomerization (78), the identity of the RNA(s) that promotes oligomerization *in vivo* is unknown. Since HIV-1 Gag forms dimers and small oligomers in the cytoplasm and since it also binds some gRNA in this compartment, it was proposed that gRNA binding initiates oligomerization (24). However, cross-linking experiments revealed that the majority of RNAs contacted by Gag in the cytoplasm consist of host cell mRNAs and ncRNAs (19). Indeed, both MLV assembly and HIV-1 assembly remain efficient under conditions where gRNA synthesis is inhibited or its packaging is prevented (79, 80). Since levels of some mRNAs in virions increase when gRNA packaging is prevented (16), one possibility is that long RNAs, such as mRNAs, substitute for gRNA in seeding Gag oligomerization. However, since a variety of short and long RNAs are efficient in nucleating Gag assembly *in vitro* and since oligonucleotides as short as 15 to 16 nt suffice (78, 81–83), long RNAs may not be necessary.

Since newly synthesized RNAs that have not assembled with their normal protein partners could contain sufficient protein-free RNA to bind Gag, these RNAs may be ideally suited to nucleate virus assembly. In this respect, newly made ncRNAs likely differ from their counterparts in mature RNPs, which often contain only short stretches of accessible RNA. For example, although naked 7SL is accessible to chemical probes *in vitro*, the RNA is largely inaccessible when assembled into SRP (84). Similarly, most mRNAs are coated with mRNA-binding proteins in cells (85). While less is known of the cellular proteins that interact with gRNA in the cytoplasm, only short regions of gRNA, consisting primarily of sequences in the 5′ end and the Rev response element (RRE), were major sites of Gag cross-linking in cells, suggesting that other gRNA regions may not be accessible (19). Thus, one possibility is that newly synthesized ncRNAs, because they are less compactly folded and/or relatively protein poor, are the RNAs that normally promote Gag assembly *in vivo*. In this scenario, a wide variety of newly synthesized ncRNAs could function to enhance virus assembly.

The packaged ncRNAs may also contribute to the cellular defense against retroviruses. Specifically, host ncRNAs have been implicated in packaging of APOBEC3G (A3G), a cytidine deaminase that it is a potent inhibitor of HIV-1 replication (86). In the absence of the HIV-1 Vif protein, A3G is incorporated into virions. Upon infection, A3G inhibits reverse transcription and deaminates cytidines in the newly made cDNA (87). For this antiviral effect, A3G must be recruited into virions, a process that requires both the Gag NC domain and RNA (70, 88, 89). Attempts to identify the specific RNA required have been controversial, as gRNA (51) and 7SL RNA (48) were each reported to be required for packaging, while other laboratories found that A3G continues to be packaged when either or both RNAs are depleted or absent (52, 53, 88). Most recently, *in vivo* cross-linking revealed that A3G is bound to both gRNA and host RNAs in virions (53). Moreover, when Gag proteins lacking NC were fused to a variety of RNA-binding modules, many different RNAs were able to mediate A3G packaging (53). *In vitro*, at least 10 nt of single-stranded RNA is required for formation of complexes between A3G and NC (90). If, *in vivo*, A3G binds those RNAs that contain 10 or more nucleotides of accessible single-stranded RNA, newly synthesized ncRNAs that have not assembled with their protein partners could be the RNAs that normally mediate packaging.

Finally, packaged host ncRNAs could impact retrovirus replication by interfacing with innate immune sensors during infection. The major cytoplasmic RNA sensors are the endosomal Toll-like receptors (TLRs) that recognize endocytosed viruses and the cytosolic retinoid acid-inducible gene (RIG)-like receptors (RLRs). Both MLV and HIV-1 activate Toll-like receptor 7 (TLR7); however, whether this occurs during productive infection or represents endocytosis of defective virus is unknown (91, 92). Although viral gRNA was assumed to be responsible for the observed TLR7 activation (91, 92), those studies did not examine contributions from host RNAs. Notably, TLR7 recognizes single-stranded uridine-rich ligands that lack nucleotide modifications (93, 94) and *in vitro*-made transcripts of some packaged RNAs, such as U1 and U6 snRNAs, are robust TLR7 ligands (94). Although mature spliceosomal U1, U2, U4, and U5 snRNAs contain numerous nucleotide modifications, the modifications are added after nuclear reimport (95) and thus should be absent from the packaged RNAs. Similarly, newly made U6 snRNAs that are exported to the cytoplasm for degradation may lack modifications found in the mature RNA. In cytosol, incoming gRNA and packaged ncRNAs may be shielded from innate immune sensors by the viral capsid (96, 97). However, whether ncRNAs reside within or outside virion cores has not been determined, and in viruses carrying defective capsids (98), or under conditions where retroviruses undergo rapid cytoplasmic uncoating, such as during Trim5 α restriction (99), packaged ncRNAs could potentially interact with cytosolic sensors such as RIG-I. Indeed, since many packaged ncRNAs, such as 7SL, Y RNAs, and pre-tRNAs, are RNA polymerase III transcripts, they should contain the 5' triphosphates that enhance RIG-I recognition.

CONCLUSIONS AND PERSPECTIVES

Although there is now much evidence that retroviruses package nascent host ncRNAs (17, 48, 49), the challenge is to determine the extent to which these and other cellular ncRNAs assist virus replication. In principle, common features of these RNAs, such as accessible single-stranded regions or 5' triphosphates, could allow

many different RNAs to collectively function in a single process, such as promoting Gag oligomerization or interfacing with innate immune sensors. Those ncRNAs present in multiple copies per virion, such as 7SL, vault, or Y RNAs, could also have specific roles. Although HIV-1 assembly and infectivity were unchanged when 7SL levels were reduced by 90% (48, 53), 7SL could contribute to infection of specific cell types or assist replication during some types of environmental stress. Additionally, as exemplified by the finding that host tRNAs shield Gag from inappropriate interactions with phospholipids prior to its arrival at the plasma membrane (19), some roles of host ncRNAs may not require packaging into virions. Given the large number of cellular functions now known to involve ncRNAs, some of which could not have been imagined prior to their discovery, we consider it likely that novel roles for host ncRNAs in the retrovirus life cycle remain to be uncovered. Additionally, knowledge of the features of ncRNA sequence and structure that confer encapsidation could potentially be useful for laboratory manipulations of retrovirus content and for designing RNA therapeutics that, upon packaging, interfere with retrovirus replication.

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