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Current Topics

"In the Beginning": Initiation of Minus Strand DNA Synthesis in Retroviruses and LTR-Containing Retrotransposons

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ABSTRACT: Sequestering a host-coded tRNA for initiation of minus (−) strand DNA synthesis is central to the reverse transcription cycle of a number of retroviruses and long terminal repeat (LTR) retrotransposons. However, "self-priming" from a hydrolysis product of the viral genome has been observed for the LTR retrotransposon Tf1 and most likely exists for related elements. Furthermore, in contrast to retroviruses, where DNA synthesis is initiated from the 3′-terminus of the cognate tRNA primer, examples are available where nucleotides of the tRNA *anticodon* domain are complementary to the viral primer binding site (PBS), necessitating internal cleavage of the primer to provide the appropriate 3′-OH for DNA synthesis. Thus, although the ensuing steps of reverse transcription are common to these elements, several variations in which the replication primer is used have been exploited. In addition, the PBS of the viral RNA genome can vary in size from an 11 nt sequence, through a bipartite cis-acting element, to 18 contiguous nucleotides complementary to the 3′-end of the replication primer. These diverse tRNA−viral RNA interactions, and their consequences for initiation of (−) strand DNA synthesis, are the subject of this review.

Propagation of retroviruses and long terminal repeat (LTR)[1] retrotransposons requires converting their single-stranded RNA genome into a double-stranded, integration-competent DNA provirus, mediated by the virus-encoded reverse transcriptase (RT) (*1*). Minus (−) strand DNA synthesis is initiated near the 5′-end of the retroelement genome from a primer hybridized to a specific sequence designated the primer binding site (PBS) (*2*). Following almost two decades of research on retroviruses, and supported by data from the *Saccharomyces cerevisiae* LTR transposons Ty1 and Ty3, the prevailing notion was that homology between nucleotides at the 3′-end of the cognate tRNA primer and the PBS of the viral genome established the tRNA−viral RNA duplex from which synthesis is initiated. It was therefore surprising when exceptions to these "rules" were demonstrated for several retrotransposons. Perhaps the best-studied example of non-tRNA priming is Tf1 of *Schizosaccharomyces pombe*, where self-complementarity permits intramolecular base pairing near the 5′-end of the viral genome, after which scission of the duplex RNA provides a short (−) strand primer (*3*). Furthermore, contradictions to the notion that (−) DNA synthesis is always initiated from the 3′-terminus of the cognate tRNA primer are exemplified by the *S. cerevisiae* retrotransposon Ty5 (*4*) and *copia* (an LTR-containing retrotransposon of *Drosophila melanogaster*) (*5*), where nucleotides of the anticodon domain displayed complementarity to the PBS, requiring internal cleavage of the tRNA primer to provide the appropriate 3′-OH for DNA

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[1] Abbreviations: LTR, long terminal repeat; nt, nucleotides; PBS, primer binding site; RNase H, ribonuclease H; RT, reverse transcriptase.

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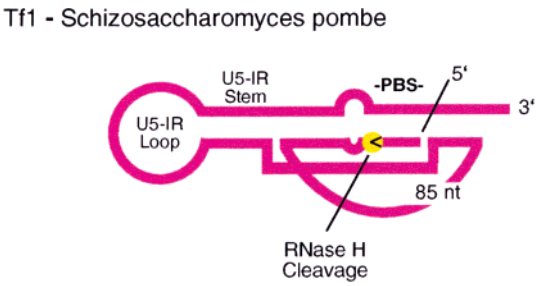

FIGURE 1: tRNA-independent initiation of (−) DNA synthesis in *S. pombe*. The (+) RNA genome is denoted throughout the review in magenta, within which the PBS is indicated. The site of RNase H cleavage 11 nt from the 5′-terminus of the RNA genome, providing the appropriate 3′-OH for DNA synthesis, is depicted in yellow. Note that the substrate for RNase H is duplex RNA, which can be cleaved by this RT-associated function under appropriate conditions (*14*).

synthesis. Several examples of each of these unusual priming events are available, possibly providing important insights into the evolution of the replication primer.

A similar evolution may have occurred in the PBS with respect to its length and structure, ranging from 11 to 18 contiguous nucleotides. Exceptions to an expanded PBS of contiguous nucleotides are again evident in certain *S. cerevisiae* retrotransposons, where the 5′- and 3′-portions of the PBS can be separated by as few as 5 nt in Ty1 (*6*) and as many as several thousand nucleotides in Ty3 (*7*). Regardless of the structural heterogeneity of the RNA duplex constituting the initiation site for (−) strand DNA synthesis, several cis-acting elements on the viral genome have been shown to mediate the efficiency with which this event is catalyzed. First documented in avian retroviruses as a long-range interaction between the tRNA TΨC loop and the viral U5−IR stem (*8*), equivalent control mechanisms have been demonstrated experimentally for the human (*9*) and feline (*10*) immunodeficiency viruses and proposed for several related retroviruses (*11*). Surprisingly, although the *S. pombe* retrotransposon Tf1 does not initiate DNA synthesis from a host-encoded tRNA, analogous long-range interactions mediated by extensive intramolecular base pairing near the initiation site have been invoked (*12*). In bringing these structurally diverse yet functionally similar mechanisms together, this review aims to illustrate that initiation of DNA synthesis in retroviruses and LTR retrotransposons is a complex and multistep process, involving intricate "cross-talk" among the replication primer, viral RNA genome, RT, and additional accessory proteins of the particular element.

Self-Primed (i.e., tRNA-Independent) Reverse Transcription

The notion that reverse transcription in LTR-containing elements need not be primed from a host-encoded tRNA was first suggested by Levin and co-workers (*3*, *13*), who were unable to detect complementarity between the 5′-end of the Tf1 genome and any known host tRNA. In contrast, complementarity between nucleotides at the (+) RNA 5′-terminus and its PBS suggested that formation of a short intramolecular duplex provides the initiation site. This region of the Tf1 genome is illustrated schematically in Figure 1, raising the issue of how the 3′-OH of the primer is created. Potential candidates for endonucleolytic cleavage were either a host-encoded function (e.g., RNase P) or the RNase H domain of the LTR retrotransposon RT. Through an elegant *in vivo* analysis of Tf1 mutants whose RT was altered in either its DNA polymerase or RNase H catalytic center, it was demonstrated that the Tf1-encoded enzyme catalyzes this event *in vivo* (*13*). Although it remains to be established for Tf1 RT, the RNase H domain of retroviral RTs will, under the appropriate conditions, cleave duplex RNA (*14*). Conceivably, this feature of retroviral enzymes might represent a "fossil" of an activity required for self-priming in early LTR retrotransposons.

Another important issue of self-priming concerns the structural or sequence context mediating precise cleavage 11 nt from the Tf1 mRNA 5′-terminus. The 5′-terminus of the (+) RNA genome could provide a signal for binding of the DNA polymerase domain of RT, thereby appropriately positioning its RNase H center over the biologically relevant cleavage site. Alternatively, unpaired nucleotides immediately adjacent to the processing site may fulfill this role. In an analogous context, we recently demonstrated that structural features of the polypurine tract guide both human immunodeficiency virus (HIV) and Ty3 RT to the site of RNase H cleavage necessary to generate the primer for plus strand synthesis (*15*, *16*). Self-priming has been proposed for LTR-containing elements Cft-1 (*Cladosporium fulvum*), Maggy (*Magnaporthe grisea*), Skippy (*Fusarium oxysporum*), Boty (*Botrytis cinerae*), *copia* elements in maize (*17*), the Tf1/sushi group in a variety of vertebrates (*18*), and Fourf in maize (*19*). Because these elements diverged early in the evolution of LTR retrotransposons and well before retroviruses, these findings provide strong evidence that self-priming represents an early form of initiation of reverse transcription in LTR-containing elements.

Priming from an Internal Site of $tRNA_i^{Met}$

Analysis of the early reverse transcription product of *copia* retrovirus-like particles from *Drosophila* provided an unprecedented example of the use of $tRNA_i^{Met}$ in initiating (−) DNA synthesis. Using limited nuclease digestion, Kikuchi *et al.* (*5*) demonstrated that *copia* (−) strand DNA was covalently linked to a 39 nt RNA fragment corresponding to positions 1−39 of $tRNA_i^{Met}$, rather than from the 3′-end of an intact tRNA previously demonstrated for several retroviruses. Furthermore, a region of complementarity between nucleotides immediately adjacent to the 5′-LTR of the retrotransposon (+) RNA genome and positions 25−39 of $tRNA_i^{Met}$ suggested that processing within the anticodon domain is necessary to provide the primer 3′-terminus for initiation of (−) DNA synthesis (Figure 2) (*5*). Although the *Drosophila*-associated activity necessary for $tRNA_i^{Met}$ processing remains to be identified, a series of experiments with the catalytic RNA of RNase P from *Escherichia coli* suggested its *Drosophila* counterpart as an attractive candidate (*20*−*22*). However, to promote correct internal cleavage, an alternative conformation of $tRNA_i^{Met}$ must be invoked, allowing base pairing of nucleotides 40−44 and 65−69, thereby repositioning nt 39 at the base of the resulting stem and accessible to RNase P. The observation that the PBS from a variety of LTR retrotransposons, including *Osser* of *Volvox carteri* (*23*), Tp1 and Tp2 from the slime mold *Physarum polycephalum* (*24*), and most recently Ty5 from

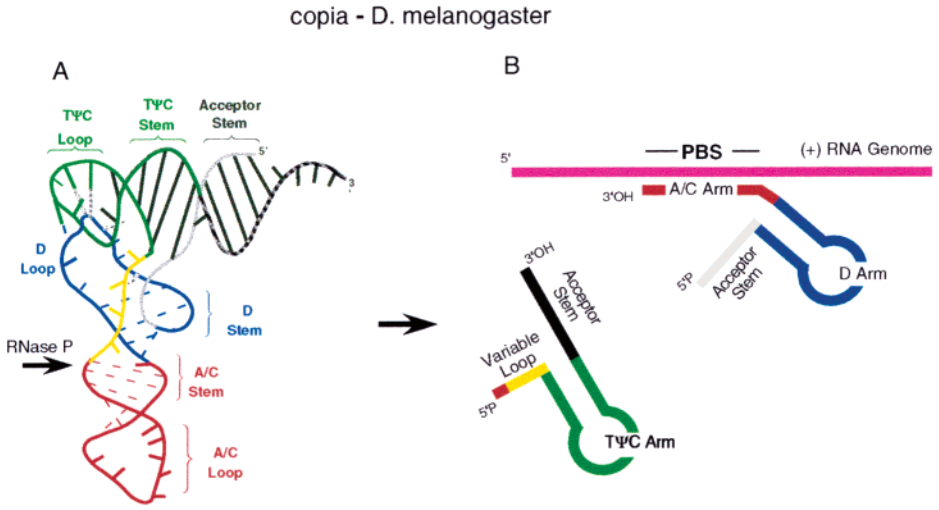

FIGURE 2: Half-tRNA priming of (−) DNA synthesis in *copia*. (A) Schematic representation of $tRNA_i^{Met}$. The L-shaped configuration has been adopted only for illustrative purposes, since an alternative configuration is proposed as the substrate for RNase P. For this and subsequent figures, each domain of the tRNA has been color coded. The site of internal cleavage is indicated. A/C is the anticodon domain. (B) Proposed half-tRNA−viral RNA initiation complex. The color coding follows that of panel A; i.e., following internal cleavage, the anticodon arm of $tRNA_i^{Met}$ (red) is available for hybridization to the PBS. The D arm of this half-tRNA (blue) is depicted as retaining its secondary structure, although this has not been proven experimentally. Additional intermolecular interactions with the viral genome remain to be established.

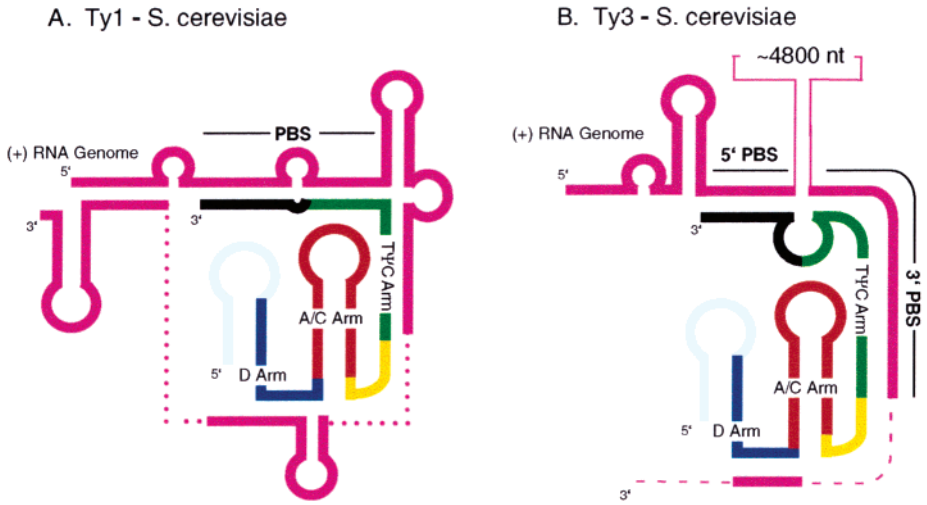

FIGURE 3: Initiation of DNA synthesis from the bipartite PBS of *S. cerevisiae* transposons Ty1 (A) and Ty3 (B). Note that, in addition to an interaction with the PBS, intermolecular interactions with the D and TΨC arms have been proposed to contribute to efficient initiation of DNA synthesis.

S. cerevisiae (*4*), shares complementarity with the anticodon stem−loop region of $tRNA_i^{Met}$ indicates that this process has been conserved among a wide variety of LTR retrotransposons (*25*).

An unresolved issue, however, is the stage at which $tRNA_i^{Met}$ processing occurs. Conceivably, hybridization of the alternately folded tRNA to the PBS could expose the processing site to the appropriate cellular machinery prior to sequestration of the LTR retrotransposon RNA genome into the virus-like particle. An alternative hypothesis would be incorporation of full-length $tRNA_i^{Met}$ into the retrovirus-like particle through an interaction with a *gag-pol* precursor, after which its placement on the PBS induces processing by host functions specifically sequestered by the virus-like particle. Since initiation of (−) DNA synthesis in retroviruses proceeds efficiently from the 3′-terminus of an intact tRNA, the requirement for such an elaborate priming mechanism is not immediately clear. However, the role of RNase P in tRNA processing from larger precursors suggests that the LTR retrotransposon could have exploited a naturally occurring host function and the ability of $tRNA_i^{Met}$ to exist in alternative configurations.

Bipartite PBS Sequences

Although $tRNA_i^{Met}$ is used as the replication primer by *S. cerevisiae* LTR retrotransposons Ty1 and Ty3, these elements differ significantly from Ty5 in that (−) DNA synthesis is initiated from the 3′-terminus of an intact tRNA. However, an intriguing variation to the theme of tRNA primer usage is the nature of the PBS in these elements. For Ty1, an RNA duplex involving 17 nt at the 3′-terminus of the primer can be achieved with the (+) RNA genome, but requires that five template nucleotides in this region form a short loop (Figure 3); i.e., the PBS−$tRNA_i^{Met}$ duplex comprises noncontiguous 10 and 7 bp PBS segments (*6*). An extreme variation of this mechanism is exemplified by Ty3,

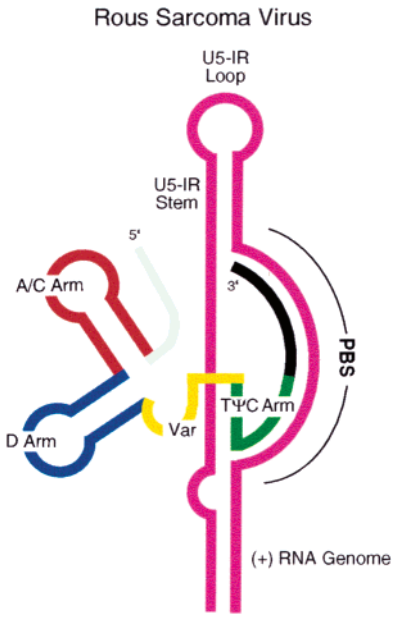

FIGURE 4: RSV initiation complex. In addition to the 18 bp duplex between 3′-terminal nucleotides of $tRNA^{Trp}$ and the PBS, a portion of the TΨC arm forms an intermolecular duplex with sequences of the viral U5−IR stem. In this model, interactions involving the anticodon and D arms have not been depicted.

where equivalent regions of the PBS (designated 5′-PBS and 3′-PBS) are located at the 5′- and 3′-termini of the RNA genome, i.e., separated by almost 4800 nt (*7*). Using a reconstituted *in vitro* system, it was found that an RNA containing only 8 nt of the Ty3 5′-PBS failed to support $tRNA_i^{Met}$ binding and initiation of reverse transcription, but that supplying the 3′-PBS either in cis or in trans complemented this deficiency. These two (−) strand initiation complexes are illustrated in Figure 3. Taken together, these data suggest that tRNA annealing to short PBS sequences adjacent to U5 fails to provide a sufficiently stable complex. Such short regions of PBS complementarity might also result in primer annealing to alternative regions of the RNA genome, an event that would be lethal for replication. Finally, although speculative, the separation of 5′- and 3′-PBS sequences in Ty3 has been proposed as a mechanism of maintaining transposition at a level that is not deleterious to the host (*26*).

tRNA-Primed (−) DNA Synthesis in Retroviruses

Historically, the majority of research on reverse transcription in LTR-containing elements has been conducted with retroviruses, examples of which include Rous sarcoma virus (RSV), murine leukemia virus (MLV), simian immunodeficiency virus (SIV), HIV-1, and HIV-2. For these and all other retroviruses studied to date, a region of 18 nt, comprising the 3′-acceptor stem and extending into the TΨC arm of the cognate tRNA, is complementary to a contiguous stretch of PBS nucleotides. Examples of primer usage in retroviruses include $tRNA^{Trp}$ in RSV, $tRNA^{Pro}$ in MLV, and $tRNA^{Lys,3}$ in HIV-1, HIV-2, SIV, and mouse mammary tumor virus (*1*). However, as illustrated in Figures 4 and 5, extensive intermolecular interactions involving multiple regions of the tRNA primer have been demonstrated to make a significant contribution to the efficiency of reverse transcription. The following section deals with the role of viral RNA sequences, either alone or in concert with the

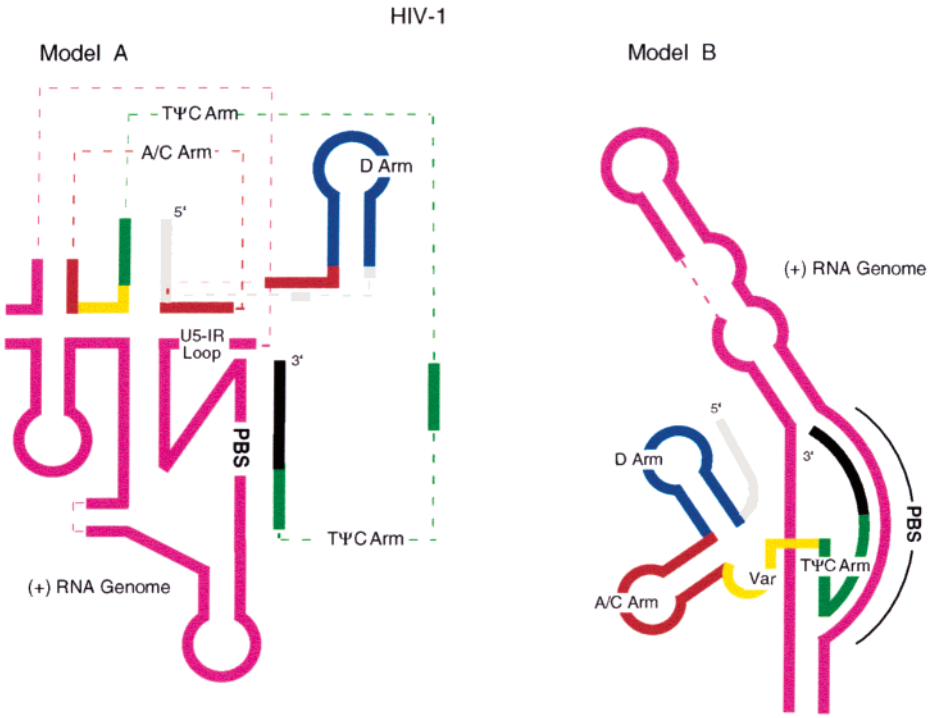

FIGURE 5: Proposed models for the initiation complex of HIV-1. Model A has been proposed from the chemical and enzymatic footprinting data of Isel and co-workers (*9*, *35*) and depicts significant rearrangement of the replication primer ($tRNA^{Lys,3}$) once it is hybridized to the PBS. Of particular note are the intermolecular duplexes assumed by the anticodon and variable arms and a new intramolecular duplex involving the 5′-acceptor stem and TΨC arm. Of the entire tRNA primer, only the D arm is proposed to remain intact. Model B has been proposed recently by Berkhout and co-workers (*46*, *47*, *64*), based on a combination of biochemical and genetic experiments. This model is formally analogous to the RSV model of Figure 4, where the D and anticodon arms are not involved in long-range interactions with the viral genome.

tRNA primer, in controlling initiation of (−) strand DNA synthesis.

Genomic RNA Sequences Mediate Efficient Initiation of DNA Synthesis

As indicated earlier, initiation of Tf1 DNA synthesis in *S. pombe* proceeds via self-priming from an 11 nt RNase H-mediated hydrolysis product of the LTR retrotransposon genome. However, consistent with other LTR-containing retroelements, the early steps of (−) strand synthesis in Tf1 are subject to strict, and somewhat unique, control. Analysis of the Tf1 RNA genome (*27*) suggested a region of secondary structure immediately adjacent to the PBS, designated the U5−IR stem−loop region by analogy with data from avian retroviruses (*11*). The contribution of this structure, comprising a 7 bp stem and a 25 nt loop, to initiation of (−) DNA synthesis has been investigated by Levin and co-workers (*27*). Destabilization of the U5−IR stem by introduction of single-base substitutions was detrimental to Tf1 transposition. However, compensatory mutations restoring base pairing resulted in near-wild-type transposition. Single-base substitutions within the U5−IR loop were well tolerated, suggesting no sequence-specific recognition by the LTR retrotransposon polymerizing machinery. Although reducing the size of the U5−IR loop to 14 nt had no effect, transposition was severely defective when the size of the loop was reduced to 6 nt. Surprisingly, the defects induced by the 6 nt U5−IR loop could be rescued by manipulating the U5−IR stem to increase its stability. A role for the U5−IR loop in preserving the integrity of the U5−IR stem was proposed from these studies, but for what purpose? Further investigation indicated that transposition defects resulting from U5−IR stem−loop alterations manifested themselves in the inability to process the initiation primer from the (+) RNA genome. The U5−IR stem and loop therefore appear to provide a recognition element for Tf1 RT that is critical for accurate positioning of its RNase H catalytic center. Our recent observations on structural features of the (+) strand polypurine tract primer of HIV-1 (*15*) and Ty3 (*16*) that may "guide" RT to the site of RNase H-mediated hydrolysis lend credence to this notion.

A specific advantage in studying tRNA-primed initiation of reverse transcription with *S. cerevisiae* LTR retrotransposons is the capacity for genetic manipulation of the tRNA primer. Chromosomal initiator methionine (IMT) genes, encoding $tRNA_i^{Met}$, can be inactivated and subsequently complemented with a wild-type, plasmid-borne, copy of the gene. Alterations can thereafter be introduced at a number of positions within the tRNA primer to define regions other than the 3′-terminal nucleotides that might contribute to efficient initiation of DNA synthesis. Using this approach, Keeney *et al*. (*28*) demonstrated that additional bases of the $tRNA_i^{Met}$ TΨC arm were critical for efficient (−) DNA synthesis in Ty1, whereas the use of interspecies (*S. pombe* and *Arabidopsis thaliana*) initiator tRNA chimeras pointed to a contribution from D-loop nucleotides. Direct evidence for the involvement of the D arm in long-range interactions with genomic RNA in Ty1 was later provided via both enzymatic footprinting of tRNA−viral RNA complexes (*6*, *29*) and a genetic analysis of Ty1 transposition following $tRNA_i^{Met}$ mutagenesis (*30*). For Ty1 and Ty3, the contribution of nucleotides from the anticodon domain to initiation of reverse transcription was minimal.

The respective complexes for these two LTR transposons are depicted schematically in panels A and B of Figure 3, differing primarily in that the TΨC arm interacts with a region of the Ty3 genome approximately 4800 nt from the 5′-region of the PBS (*7*). However, a recent study by Cristofari *et al.* (*26*) has shown that interactions between the 5′- and 3′-ends of the Ty1 and Ty2 genomes contribute to efficient (−) strand DNA synthesis *in vitro*, suggesting an even closer relationship between these systems. Ty5 appears to be the exception to this model, since (−) strand DNA synthesis is initiated from a site internal to the tRNA primer (*4*), i.e., from a "half-tRNA" that would essentially lack the TΨC arm. This priming mechanism does not necessarily rule out a contribution from the D arm, nor does it exclude the possibility that, despite internal cleavage, the two portions of $tRNA_i^{Met}$ remain base paired in a manner permitting the TΨC arm to contact genomic RNA sequences.

By far the most extensive study of the role of intermolecular tRNA−viral RNA interactions in initiation of reverse transcription has come from work on avian (RSV) and human (HIV-1) retroviruses. Initial genetic studies by Cobrinik *et al*. (*8*) indicated that a deletion within the U5−IR leader stem, a base-paired region immediately 5′ to the PBS, induced a partial replication defect that could be alleviated by introducing compensatory substitutions. Subsequently, Aiyar *et al*. (*31*, *32*) proposed that base pairing between a 7 nt U5 RNA and nucleotides of the $tRNA^{Trp}$ TΨC arm, as outlined schematically in Figure 4, is central to efficient and accurate initiation. Limited nuclease mapping (*33*) indicated that hybridization of $tRNA^{Trp}$ to the PBS induced destabilization of both the U5 leader stem and U5−IR loop, thereby creating new contacts between the U5 stem and the TΨC loop of the primer. Thus, while earlier data from these authors on the role of the U5−IR stem could also be interpreted in terms of their effects on viral RNA encapsidation (*34*), this was unlikely to hold true for distal alterations to the U5 stem.

Although similar structures have been predicted for several related retroviruses (*11*), their physiological significance remains to be established. In particular, extensive *in vitro* and *in vivo* studies with HIV-1 strongly favor an alternative interaction mediated by the anticodon loop of the replication primer. A combination of chemical and enzymatic footprinting (*9*, *35*−*37*) has established for the HIV-1 Mal isolate that the U-rich anticodon loop of $tRNA^{Lys,3}$ forms a stable complex with the A-rich U5−IR loop, mediated in part by two hypermodified bases at positions 34 and 37 of the tRNA. In fact, Figure 5 illustrates that this is only one of several novel rearrangements accompanying hybridization of $tRNA^{Lys,3}$ to the PBS of the HIV-1 genome. The importance of these interactions has been established through (i) identification of HIV-1 RT mutants that fail to extend the tRNA primer but can be rescued by hybridization of tRNA−DNA chimeras to the PBS and 5′-region of the U5−IR stem (*38*) and (ii) genetic manipulation of the PBS and U5−IR loop nucleotides (*39*−*45*).

Despite this wealth of data for HIV-1, an alternative structure for the initiation complex has been proposed by Berkhout and co-workers (*46*, *47*), indicated in Figure 5B, implicating an intermolecular interaction with TΨC and a sequence designated the primer activation signal (PAS) of the U5 stem. In fact, this model bears a striking similarity to earlier proposals derived from work with avian RT (*8*,

31, *32*, *34*, *48*), suggesting that, as DNA synthesis proceeds into the U5−IR stem, alternative structures can be assumed during initiation of reverse transcription. At variance with a biological role for such sequences downstream of the PBS in efficient initiation of DNA synthesis in HIV-1 is the finding that these can be removed without affecting the efficiency of tRNA-primed synthesis *in vitro* (*49*). A logical prediction from these studies would be that retroviruses exploiting a common tRNA primer and whose RTs have a similar quaternary structure would be subject to the same form of control. Although an equivalent mechanism has been suggested for HIV-2 (*50*), the absence of an A-rich U5−IR loop in the feline immunodeficiency virus genome implies either that such stringent control is not imposed or that this is subject to an alternative interaction between the primer and viral genome. Indeed, we recently demonstrated the importance of intermolecular base pairing between nucleotides of the FIV U5−IR loop and the 5′-terminus of the $tRNA^{Lys,3}$ in efficient initiation of (−) strand DNA synthesis (*10*). Hybridization of tRNA 3′-nucleotides to the PBS would make its 5′-terminus available for hybridization to viral U5−IR loop sequences.

Initiation Events Are Kinetically Distinguishable

While elaborate inter- and intramoelcular interactions appear to be the hallmark of the initiation of (−) strand DNA synthesis, there have been only limited studies addressing their consequences on the kinetics of this process. Preliminary studies with HIV-1, comparing the tRNA primer with an oligodeoxynucleotide complementary to the PBS, indicated that the nucleoprotein complex is unstable during tRNA-primed initiation. As a consequence, DNA synthesis during initiation is distributive. However, after addition of a limited number of deoxynucleotides to the tRNA primer, there is a substantial increase in the affinity of RT from the tRNA−viral RNA duplex, and a transition from distributive to processive DNA synthesis. Such a transition is not evident during oligodeoxynucleotide-primed (−) strand synthesis, suggesting structural features of $tRNA^{Lys,3}$, such as hypermodified bases of the anticodon domain, are critical during initiation events (*51*−*53*). Interestingly, this transition from distributive to processive DNA synthesis is very reminiscent of initiation of DNA-dependent RNA synthesis, where the polymerizing machinery goes through cycles of abortive initiation before a stable elongation complex is assumed (*54*−*56*).

In a study involving single-nucleotide addition to a series of tRNA−DNA chimeras designed to simulate initiation events, Lanchy *et al*. (*53*) elegantly demonstrated that, between addition of the sixth and seventh deoxynucleotide to $tRNA^{Lys,3}$, the rate of polymerization increased approximately 3000-fold. This step most likely represents the transition from initiation to elongation and, since an equivalent result was obtained with an oligoribonucleotide primer, may correspond to the junction between duplex RNA (tRNA−PBS RNA) and an RNA−DNA hybrid [(−) DNA−(+) RNA] passing over important structural motifs at the base of the p66 thumb subdomain of HIV-1 RT (*57*, *58*). At a later stage, an increase in the dissociation rate was evident following addition of ∼18 deoxynucleotides to the tRNA primer, corresponding to the same nucleic acid junction leaving the RNase H catalytic center. Dissecting initiation of $tRNA^{Lys,3}$-primed (−) strand DNA synthesis into kinetically distinguishable events has raised the possibility that this step may be therapeutically amenable in the continuing effort to develop more potent anti-HIV agents.

Packaging of the tRNA Primer

Despite the availability of extensive literature on mechanisms underlying tRNA-primed initiation of (−) strand DNA synthesis, an unresolved question surrounds the selectivity with which the tRNA replication primer is packaged. Although complementarity between the tRNA 3′-terminus and PBS nucleotides of the viral genome would appear to be sufficient, as has been shown for MLV (*59*), the absence of $tRNA^{Trp}$ in RSV particles containing RT (*60*) indicates that virus-encoded proteins can also participate. Studies with HIV-1 (*61*) also support the notion that virus-encoded protein factors may mediate selective incorporation of the tRNA replication primer. Cen *et al*. (*62*) have introduced the novel concept that the process might be assisted by additional cellular factors. These authors have demonstrated that lysyl-tRNA synthetase (LysRS), an essential component of the host translational machinery and a protein that is intimately associated with $tRNA^{Lys,3}$, is incorporated into HIV-1 in a complex with either the *gag* or *gag*/*pol* precursor. Thus, a LysRS−tRNA complex may be the original source of virion-associated $tRNA^{Lys,3}$. Interestingly, the form of LysRS found in virions is ∼7 kDa smaller than the cellular counterpart, raising the possibility that a processed form of the synthetase participates in tRNA primer packaging. Consistent with a potential contribution from host tRNA synthetases, a later study (*63*) detected tryptophanyl-tRNA synthetase in RSV, but failed to detect prolyl-tRNA synthetase in MLV, where PBS sequences are sufficient. The involvement of a host function in tRNA incorporation in HIV again opens the possibility of developing antagonists against the truncated form of LysRS, against which the evolution of drug-resistant variants may not be as severe as drugs targeted to viral proteins and enzymes.

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