Regulation of HIV-1 reverse transcription
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Summary
SUMMARY

The replication cycle of HIV-1 and other retroviruses is characterized by reverse transcription of the viral RNA genome into a double stranded DNA that subsequently integrates into the host cell genome. In chapter 1 an overview is presented of the HIV-1 replication cycle, and the mechanism of reverse transcription is discussed. The focus is on the initiation phase of this complex process, in which the tRNA primer is annealed onto the viral RNA template and extended by the viral RT enzyme.

Chapters 2 and 3 describe studies to analyze the role of the U5-PBS hairpin in virus replication. We introduced mutations to specifically stabilize or destabilize this hairpin structure. Note that RNA secondary structure probing experiments performed in chapter 6 suggest an alternative folding of this domain to form the U5-top hairpin. Mutations that destabilize both the U5-PBS hairpin and the alternative U5-top hairpin affect virus replication and the correct placement of the tRNA primer onto the PBS. Stabilization of the U5-PBS hairpin severely reduces virus replication and inhibits tRNA annealing onto the PBS, which is partially occluded by the U5-PBS hairpin. Fast replicating revertant viruses were selected with additional mutations that reduce the stability of the modified U5-PBS hairpin, and that may also restore folding of the alternative U5-top hairpin. It remains possible that both RNA conformations play a role in specific stages of the viral life cycle. For instance, there is some phylogenetic support for the existence of the U5-PBS hairpin (2,3), and alternative RNA conformations have been reported to exist for other domains of the HIV-1 leader RNA (4,10).

Over the years many “forced evolution” experiments were performed in our laboratory, including the one already mentioned with the stabilized U5-PBS hairpin mutant. The cumulative sequence data of these virus evolution experiments demonstrate that G-to-A mutations are introduced most frequently into the viral genome. These mutations probably reflect G-dT mispairing during minus-strand synthesis in the initial phase of reverse transcription (19). A recent report claimed that a novel RNA editing mechanism is responsible for several G-to-A changes in the viral RNA genome (5). In chapter 4 we argue that it is more likely that these changes are introduced by error-prone reverse transcription.

In chapters 5 to 8 we study the role of the extended U5-leader stem in virus replication and reverse transcription by a detailed mutational analysis. In chapter 5 we demonstrate that stabilization of the U5-leader stem reduces virus replication and interferes with both initiation and elongation of reverse transcription. In chapter 6 we identify a novel sequence motif in the upstream U5 region that is critical for tRNA<sup>lys</sup>-mediated initiation of reverse transcription in vitro. This motif
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does not affect tRNA annealing onto the PBS and is not required in DNA-primed control experiments. We propose that this motif interacts with the T\(\text{\textasciitilde}\text{C}\) arm of tRNA\(_{\text{lys}}\) to activate initiation of reverse transcription. This U5-motif was termed Primer Activation Signal (PAS). Interestingly, this element is masked in the viral transcript through base-pairing in the U5-leader stem, and reverse transcription can be activated by exposure of the PAS through mutation of the "opposing" leader sequence. Furthermore, stabilization of the U5-leader stem was found to inhibit reverse transcription (chapter 5). The presence of the PAS enhancer element in a repressive RNA structure may provide a mechanism for positive and negative regulation of HIV-1 reverse transcription. In chapter 7 we verified that the PAS is also important for efficient reverse transcription in vivo, that is in virus-infected cells. With the help of revertant viruses, we demonstrate that the PAS interaction is required for efficient initiation and elongation of reverse transcription. The combined results indicate that the additional interaction between the tRNA primer and the viral RNA template through the PAS-antiPAS interaction is needed to assemble an initiation-competent and processive reverse transcription complex. In chapter 8 we provide further evidence for a direct base-pairing interaction between the PAS motif in the viral RNA and the antiPAS sequence in the tRNA\(_{\text{lys}}\) molecule. We show that the efficiency of initiation of reverse transcription can be modulated by PAS mutations that strengthen or weaken the interaction with tRNA\(_{\text{lys}}\). It has proven very difficult to change the identity of the tRNA primer for HIV-1 reverse transcription by alteration of the PBS sequence (8,14,20). Using in \textit{vitro} reverse transcription assays, we demonstrate that the identity of the priming tRNA species can be switched by simultaneous alteration of the PBS and PAS motifs to accommodate a new tRNA primer. These combined results indicate that the PAS-antiPAS interaction is important for tRNA primer selection and efficient reverse transcription.

A similar interaction between a U5 motif in the genome of the avian Rous sarcoma virus (RSV) and the T\(\text{\textasciitilde}\text{C}\) arm of its tRNA\(_{\text{trp}}\) primer has been demonstrated previously to stimulate initiation of reverse transcription (1,13), and a similar vRNA-tRNA interaction was also proposed for HIV-2 (3,9). Extensive phylogenetic analysis of different retrovirus genera indicates that a PAS-like element is present in the U5 region of all retroviral genomes (chapter 8). Thus, the PAS-antiPAS interaction appears to be conserved in evolution, despite diversity in the tRNA species that is used by different retroviruses. These results suggest that the process of reverse transcription is regulated by a common mechanism in all retroviruses. Several other interactions between the HIV-1 RNA and tRNA\(_{\text{lys}}\) have been proposed previously, which are based mainly on biochemical probing studies (11,12). The putative interaction between the A-rich loop (A168-A171) in the viral RNA and the U-rich anticodon loop of the tRNA\(_{\text{lys}}\) primer (U33-U36) has been studied extensively. However, this interaction appears to be specific for HIV, since
other members of the lentivirus genus that also utilize tRNA$^{lys3}$ do not possess an "A-rich loop" in their genome. This indicates that the "A-loop" interaction is not a general property of retroviruses. The HIV-1 mutants that were used in our studies were not designed to test all of these interactions, and further research is needed to determine whether a series of specific vRNA-tRNA interactions take place during reverse transcription. Multiple structural rearrangements of the viral RNA-tRNA complex may occur during the initiation and elongation phase of reverse transcription.

It appears that multiple levels of specificity restrict aberrant reverse transcription from non-self tRNA primers. The self-tRNA primer is not only selectively packaged into virions (15,16), but it is also specifically recognized and used for reverse transcription by the corresponding viral RT enzyme (6,7,17). The additional PAS-antiPAS interaction may further increase the specificity of reverse transcription. This interaction is required for efficient initiation of reverse transcription, and may thus restrict priming events from non-self primers. The PAS enhancer motif is occluded by base-pairing in the U5-leader stem of the HIV-1 genome, and mutation of the "opposing" leader sequence stimulates reverse transcription. This indicates that the initiation step is actively suppressed by RNA secondary structure, which may provide a mechanism for the temporal regulation of reverse transcription. This mechanism may preclude premature reverse transcription in the virus-producing cell, such that the viral RNA genome is only copied upon infection of a new host cell. Although binding of tRNA$^{lys3}$ to the PBS may occur relatively early, e.g. in the virus-producing cells, activation of the primer will require a structural rearrangement of the vRNA-tRNA complex to establish the PAS-antiPAS interaction. This conformational change may be facilitated by the viral nucleocapsid protein (NC), which acts as an RNA chaperone (18). Because NC is only released from the Gag precursor protein during virus maturation, this will ensure the proper timing for initiation of reverse transcription. Recent studies describe another conformational switch in the HIV-1 leader RNA that is mediated by NC (10). The RNA dimerization signal is masked by RNA secondary structure in the viral template, but can be exposed by the addition of NC. Thus, RNA secondary structure in the HIV-1 leader RNA may provide a more general mechanism to mask "late" replication signals that are involved in e.g. RNA dimerization, packaging and reverse transcription. Conformational changes or "RNA switches" may expose these signals and ensure the precise timing and coordination of these processes that are critical for retroviral replication.
REFERENCES


