Transfer RNA modifications and DNA editing in HIV-1 reverse transcription

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Abstract

Reverse transcription is a central step in HIV-1 replication that represents a typical case of interplay between viral and cellular factors. HIV-1 diverts a cellular tRNA, $tRNA^{Lys}_3$, to prime reverse transcription. The post-transcriptional modifications of $tRNA^{Lys}_3$ are crucial for completion of reverse transcription. In some HIV-1 isolates, they are required for efficient initiation of (-) strand DNA synthesis, and in all strains, methylation of A58 is required to allow productive strand transfer during (+) strand DNA synthesis. On the other hand, some human cell types have evolved an innate antiretroviral mechanism by promoting extensive deamination of the (-) strand DNA during reverse transcription. In the absence of viral defence, this hyper-editing induces DNA degradation and lethal mutagenesis of the viral DNA. However, Vif, one of the HIV-1 "accessory" proteins, is able to inhibit DNA deamination by preventing incorporation of the editing enzymes APOBEC3G and APOBEC3F into the viral particles.

1 Introduction

Retroviruses are diploid, with two identical copies of the positive strand viral RNA packaged within the viral particle (Paillart et al. 2004). Upon cell infection, the viral core, composed of a shell of capsid (CA) proteins surrounding the dense assembly of viral RNA and nucleocapsid protein (NC), is disassembled. The viral genome then undergoes an elaborate retrotranscription process involving several strand transfers, as schematised in Figure 1. As a result of this intricate mechanism, the single stranded genomic RNA is copied into a doubled stranded DNA molecule that is actually longer than the RNA template, with long terminal repeats (LTR) duplicated at each end. Within this process, there are several steps involving enzymatic modifications of the nucleotides within the various polynucleotide partners, leading either to modified nucleotides or to editing processes. The cellular tRNA that serves as a primer contains modified ribonucleotides that are important at several steps of the replicative cycle, and the genomic DNA can undergo deamination, a restriction mechanism developed by host cells and against which the virus has evolved a defence mechanism. During the infective cycle, the virus has to cope with these modifications that would otherwise seriously interfere with

Topics in Current Genetics
H. Grosjean (Ed.): Fine-Tuning of RNA Functions by Modification and Editing
DOI 10.1007/b106366 / Published online: 7 January 2005
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its replication. In order to overcome these difficulties, HIV-1 has evolved elaborate strategies that either neutralize the modification process or allow to circumvent its negative effect. In some cases, the cellular nucleotide modifications are even used by the virus to its advantage, either as landmarks for reverse transcription or as mediators of ancillary stabilizing interactions. Three viral factors are at the centre of the corresponding processes: the reverse transcriptase (RT), the nucleocapsid protein (NC) and the accessory protein Vif. The first two proteins interact directly with the modified polynucleotides, whereas Vif blocks the cellular modifying activity. The aim of this review is to provide a detailed perspective of how the virus bypasses the inhibitory effects of cellular nucleotide modifications and editing.

2 tRNA modification and HIV-1 reverse transcription

The successful completion of viral replication is strictly dependent upon a host RNA molecule, the primer tRNA. Indeed, retroviral reverse transcriptases (RT), as most DNA polymerases, are dependent upon a primer molecule to initiate synthesis of a DNA strand. The 5' region of the viral genome contains a specific sequence, the primer binding site (PBS), which is complementary to the 3' end of a given host cytoplasmic tRNA that is recruited and annealed onto the viral genome (reviewed in Marquet et al. 1995; Le Grice 2003; Kleiman et al. 2004) (Fig. 1). For instance, all known mammalian lentiviruses use host $tRNA^{Lys}_3$ as a primer. This RNA-RNA duplex is recognised by RT, which elongates the (-) DNA strand, thus, producing a chimeric tRNA-DNA strand (Fig. 1, Steps 1 and 2). As reverse transcription proceeds, the template RNA is progressively hydrolysed by the RNase H activity of RT, except in polypurine tract regions (PPT), which are used in turn by RT as primers for the synthesis of the (+) DNA strand (Fig. 1, Step 4). After being used for initiation of reverse transcription, the primer tRNA, which is still attached to the end of the minus DNA strand, will then serve as template for the synthesis of the DNA counterpart of the PBS sequence (Fig. 1, Step 5). The very early steps of reverse transcription might actually take place within the virion, and the primer tRNA is recruited and packaged within the budding viral particle and placed onto the PBS before the infection of a new cell takes place (Huang et al. 1997).

2.1 Function of the modified nucleotides of tRNA

The transfer RNA of all living organisms contain a number of nucleotides which are post-transcriptionally modified, either at the level of the sugar or of the base. Within the context of cellular translation of mRNA, the function of these modifications is mainly twofold: (i) Some modifications contribute significantly to the folding of the molecule, mostly by allowing additional stabilising interactions within the 2D or 3D fold, or sometimes by preventing the formation of competing