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Chapter 4
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HIV-1 RNA Editing, Hypermutation, and Error-Prone Reverse Transcription

Bourara et al. (1) reported that transcripts of the human immunodeficiency virus-type I (HIV-1) were subject to RNA editing in chronically infected cells. They observed multiple guanine-to-adenine (G-to-A) and cytosine-to-uracil (C-to-U) changes in several regions of the HIV-1 RNA; commonly, a G-to-A change in the untranslated leader was present exclusively in spliced HIV-1 messenger RNA (mRNA), but not in the unspliced RNA and the proviral DNA genome. Changes in the viral protein R (vpr) gene were present in spliced and unspliced HIV-1 RNA extracted from the cell, but not in the unspliced RNA genome that is packaged in virion particles. Therefore, Bourara et al. proposed that post-transcriptional mRNA-editing events occur for a subset of viral RNAs. Known editing mechanisms, however, cannot easily explain these changes in the HIV-1 genome. We here propose an alternative mechanistic model based on HIV-1 reverse transcription to explain some of the nucleotide changes.

Bourara et al. argued that the chronically infected cells represent a clonal population with one proviral genome, based on the idea that reinfection of HIV-producing cells is restricted by a mechanism known as superinfection interference. There is convincing evidence, however, that viral interference is not complete in chronically infected cell cultures (2, 3). Thus, reverse transcribed viral genomes are likely to end up in the DNA of a subset of cells, thereby producing a heterogeneous cell population. Also, it is crucial to understand why a chronically infected cell could be established with this cytopathic virus. Viral latency is frequently associated with mutational inactivation of the viral transcription machinery, in particular the essential tat-TAR axis (4, 5).

These mechanisms can result in a very complex population of chronically infected cells. All cells may harbor the original proviral genome that is transcriptionally impaired, but there may be several subsets of cells with additional proviruses that underwent at least one round of reverse transcription. These minority proviruses will not be picked up by Southern blot analysis, but they may largely determine the HIV-1 RNA content of the cell pool. The proviruses can have the typical mutations due to reverse transcription errors, which—because of mutational inactivation of motifs that regulate either splicing (the rev-RRE axis) or RNA packaging (Gag protein and the psi motif)—may not be distributed equally over the spliced versus unspliced RNA and the cellular versus virion HIV-1 RNA. Thus, the chronically infected cell system is too complex to provide evidence for RNA editing based on sequence differences in the viral DNA and RNA.

There is also some experimental evidence that these typical mutations arise by means of reverse transcription. We have accumulated sequence data of spontaneous HIV-1 variants that evolve in long-term tissue culture infections that were started with molecular clones of known sequence. This experimental system reflects a natural infection in that beneficial errors introduced during error-prone reverse transcription will end up in the majority of proviruses by natural selection. These studies focused on the untranslated leader region of the HIV-1 RNA genome. We frequently observed the identical G-to-A change at position 181 that was also reported in the RNA-editing study (Fig. 1A). This residue is immediately upstream of the primer-binding site (PBS) that base-pairs with the tRNA primer for reverse transcription. Remarkably, we found many other G-to-A changes in this region upstream of the PBS, whereas few sequence changes were observed in the regions either further upstream or downstream of the PBS (Fig. 1, A and B).

Because the mutations cluster in the region that is copied first during reverse transcription, we propose that G-to-A mutation is a typical property of the reverse transcriptase (RT) complex that executes the initial stages of reverse transcription. This initial phase of reverse transcription may be hindered by a low deoxycytidine 5′-triphosphate (dCTP) concentration in the virion particle, which can trigger G-T mispairing that is the likely cause of biased G-to-A mutation in the mechanism of hypermutation (6). The subsequent phases of reverse transcription will occur in the cytoplasm of infected cells, and the surplus of deoxyribonucleotide triphosphate (dNTP) building blocks may thus effectively turn off this typical mutational bias. The PBS motif itself is almost invariable, which is not unexpected because this sequence is copied from the tRNAlys3 primer during the process of reverse transcription. The position immediately 3′ of the PBS is highly mutable (720N), because this position corresponds to the extension point after the second strand transfer of reverse transcription. The mechanisms of error-prone initiation of reverse transcription and hypermutation may be very similar, but hypermutation cannot explain the clustering of mutations upstream of the PBS. We also analyzed the sequence context of the residues that undergo the G-to-A change. A preference for mutation of G residues in the NGT trinucleotide sequence is apparent (Fig. 1C), whereas hypermutable G residues are usually in the NGA sequence context (7, 8).

These combined results are consistent with the idea that reverse transcription errors, which are not symmetrically distributed between the strands of the RNA, are a major source of nucleotide changes in HIV-1.
with the hypothesis that reverse transcription, rather than RNA editing, is responsible for the acquired leader mutations. Similarly, the preference for G-to-A and C-to-U changes in the vpr gene is a hallmark of error-prone reverse transcription (Fig. 1B).

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