

FIDELITY OF HIV-1 REVERSE TRANSCRIPTION: RT VARIANTS WITH INCREASED LEVELS OF MUTATION

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HIV-1 is an effective pathogen due in part to its ability to generate sufficient genetic diversity in response to environmental challenges while maintaining efficient replication. Advantageous mutations introduced during replication make it possible for HIV-1 to evade host defense mechanisms, avoid immune responses and become resistant to antiviral drugs. Mutational diversity may be generated during transcription by cellular RNA polymerase or during first and second strand cDNA synthesis by HIV-1 reverse transcriptase (RT). The portion of diversity due to errors introduced by RNA polymerase is unknown. Given the fact that HIV-1 RT lacks a conventional proof-reading activity and replicates nucleic acids with low fidelity, it has been assumed that most diversity is generated during reverse transcription. To identify error-prone HIV-1 RT variants, we extensively mutated HIV-1 RT and carried out a genetic screen for fidelity variants using a coupled reverse transcription/fidelity assay. In this system, RT activity is monitored with a reverse transcription indicator gene while fidelity is measured by reversion of a second selectable gene.

RT variants with both increased and decreased fidelity were identified. Amino acid changes associated with decreased fidelity cluster in the fingers region (V60, A62 & F77), an area previously implicated in nucleotide selectivity and the b7/b8 loop of the p51 (T131 & I132). These variants caused increased mutation/decreased fidelity in genetic reversion assays and F77S showed significant levels of base misincorporation in multiple biochemical assays. Consistent with this observation, F77S severely compromised viral replication. F77S and T131I show altered mutational spectra and base substitution specificities in a single cycle replication assay where *lacZ*- α is embedded in the viral genome and used to score mutation. These fidelity variants expand the repertoire of enzyme:nucleic acid interactions that determine the quality of replication and suggest that determinants far removed from nucleotide binding and catalysis impact fidelity. Some of these variants may prove useful in distinguishing the contributions of RT and RNA polymerase to HIV-1 diversity.