

MUTATIONS THAT REDUCE THE FIDELITY OF RETROTRANSPOSITION

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The accuracy with which retroviruses replicate their genomes is subject to both the accuracy of the host's transcription and the accuracy of the retroviral reverse transcriptases. We identified mutations in both these processes that reduce the fidelity of retrotransposition. We have alleles of both *RPB1* and *RPB2* in yeast that result in error prone RNA polymerase II. RNA polymerase from these mutants have elevated misincorporation and/or transcriptional slippage. To identify mutations that reduce the fidelity of HIV-1 RT, we use a hybrid element composed of the yeast retrotransposon Ty1 in which the reverse transcriptase has been replaced by HIV-1 RT. Propagation of these hybrid elements is dependent on the HIV-1 RT and RN activities. They are sensitive to NNRTIs unless they carry resistant HIV-1 RT variants. We developed assays that allow us to monitor the accuracy of retrotransposition and have identified mutants of HIV-1 RT that increase the error rate. Biochemical characterization of one of these mutants confirms a higher misincorporation frequency. It also reduces the ability of HIV-1 to replicate. Such fidelity mutants may make it possible to understand how HIV balances its need to accurately copy its genome with its need to generate variants that are drug resistant or evade host immune responses.