Sorting Out Mutations in Human T-Cell Leukemia Virus Type 1 Proviruses During *In Vivo* Clonal Expansion

Louis M. Mansky

Retroviruses, like all RNA viruses, have reputations that precede them regarding genetic variability. The lack of proofreading and subsequent high error rates of reverse transcriptase have been linked directly to the tremendous diversity observed in retrovirus populations. Although this association is not straightforward, a fair amount of evidence supports the conclusion that reverse transcriptase plays a major role in generating virus diversity *(1).* Retrovirus variation relies on the mutation rate per replication cycle, the number of replication cycles, the rate of mutation fixation, and the rate of recombination *(2).*

Human T-cell leukemia virus type 1 (HTLV-1), the first pathogenic human retrovirus discovered, is the etiologic agent of an adult T-cell leukemia/lymphoma (ATLL) and of a chronic progressive neuromyelopathy, tropical spastic paraparesis (TSP)/HTLV-1-associated myelopathy (HAM). During the asymptomatic phase of infection, proviral loads are generally lower than they are once TSP/HAM develops, even though proviruses can be abundant in blood mononuclear cells *(3).* HTLV-1 and related viruses in the HTLV/bovine leukemia virus (BLV) genus of the *Retroviridae* family are unusual among retroviruses, in that the observed genetic diversity among isolates is relatively low. It has been shown previously *(4)* that HTLV-1 isolates from Japan have about 97%–99% homology and that isolates from Japan, the Caribbean, and Africa can also share as much as 96%–99% homology. HTLV-1 isolates endemic in different races have been suggested to be of utility in studying the movement of ancient human populations or in anthropologic studies *(5).* However, HTLV-1 isolates from Melanesia would not be as useful as isolates from Japan, the Caribbean, and Africa in anthropologic studies because there is not as much sequence homology to the original Japanese isolate *(6).* This finding suggests that HTLV-1 may have originated in the Pacific Rim region of the world rather than in Africa.

This limited genetic diversity in HTLV-1 populations seems to contradict the type of variation seen with retroviruses (and with other RNA viruses). However, in the case of retroviruses, replication of the proviral DNA occurs with the high-fidelity process of cellular DNA replication during cell proliferation. Along with reverse transcriptase, two other polymerases are involved in replicating the viral nucleic acid, namely, cellular DNA polymerases (primarily DNA polymerase δ) and RNA polymerase II. DNA replication by cellular DNA polymerases is known to be a high-fidelity process and typically is a minor contributor to retrovirus variation. The fidelity of transcription of proviral DNA to RNA by cellular RNA polymerase II has not been determined, and it is not known whether it substantially contributes to retrovirus variation.

Previous studies have revealed that the expansion of HTLV-1-infected cells *(7)* is associated with a clonal expansion of integrated HTLV-1 proviruses *(8).* This clonal expansion was found to occur in both symptomatic and asymptomatic carriers *(9,10).* Although the fidelity of cellular DNA replication is high, the expression of the HTLV-1 Tax protein has been shown to influence cellular DNA repair pathways, the cellular mutation frequency, and the transition from G_1 to S phase *(11,12).* These studies together create a picture that indicates that HTLV-1 replicates primarily as a provirus through continual host cell proliferation and that Tax induces genetic instability of HTLV-1 and the infected cell. To define the basis for genetic instability *(11,12),* one would need to first characterize when mutations in the HTLV-1 genome occur. For the most part, it has been speculated, with little experimental proof, that the mutations observed were due to somatic mutation and not due to errors in reverse transcription.

In this issue of the Journal, Mortreux et al. *(13)* provide evidence that intrapatient genetic variability is largely the result of somatic mutation of HTLV-1 proviral DNA sequences rather than of mutations occurring during reverse transcription. An inverse polymerase chain reaction strategy was used to sort out whether identified mutations occurred during reverse transcription or during cellular DNA replication of the provirus. In particular, the authors focused on mutations in the RU5 region of the long terminal repeat located at the 3′-end of HTLV-1 proviruses as well as in the adjacent flanking cellular sequences. The key to sorting out the observed mutations relies on an understanding of plus- and minus-strand DNA synthesis during the reverse transcription process. Essentially, a mutation that occurs during minus-strand DNA synthesis would lead to a homogeneous population of RU5 sequences. Such mutations were never found, indicating that the mutants identified by these investigators did not occur during minus-strand DNA synthesis.

Separating mutations that occurred during plus-strand DNA synthesis from somatic mutations is a difficult task. A mutation that occurs during plus-strand DNA synthesis (if not repaired before cell division) would result in two populations of infected cells—those with the mutation and those without it. However, the same would be true if the mutation had occurred as a somatic mutation. Mortreux et al. *(13)* put forth two arguments to support their conclusion that these are somatic mutations and not mutations that occurred during plus-strand DNA synthesis.

First, since limited replication of HTLV-1 occurs via infection, Mortreux et al. conclude that the mutation frequency in the RU5 region could be considered to be the result of a single round of reverse transcription. The mutation frequency that they observed (approximately 4.2×10^{-3}) is about 600 and 1000 times higher than that of the *in vivo* forward-mutation rates of HTLV-1 and of BLV, respectively *(14,15).* The HTLV-1 and BLV *in vivo*

Affiliations of author: Department of Molecular Virology, Immunology, and Medical Genetics, Center for Retrovirus Research, The Arthur James Cancer Hospital and Solove Research Institute, and Comprehensive Cancer Center, Ohio State University Medical Center, Columbus.

Correspondence to: Louis M. Mansky, Ph.D., Department of Molecular Virology, Immunology, and Medical Genetics, 2078 Graves Hall, 333 West 10th Ave., Columbus, OH 43210 (e-mail: mansky.3@osu.edu).

See "Note" following "References."

forward-mutation rates were determined in a single round of replication using a mutational target (i.e., lacZα) that was not placed under selection during virus replication *(14,15).* In addition, this value is about 100 times higher than that of the *in vivo* mutation rate of human immunodeficiency virus type 1 (HIV-1) and about 30 times higher than that of the HIV-1 mutation rate when virion incorporation of the DNA repair enzyme uracil DNA glycosylase is prevented *(16,17).* It is a bit of a stretch to compare these mutation rate values to the mutation frequency in RU5, particularly since one would be comparing forward-mutation rates with a neutral target with mutations that occurred under selective pressure. However, the comparison does suggest that these mutations did not arise during reverse transcription.

Second, some of the analyzed RU5 sequences had multiple (i.e., two to four) mutations. Although hypermutants have been observed with both HTLV-1 and HIV-1 *(15–18),* there are limited data to indicate that the existence of hypermutation would support the conclusion that these mutations could occur during one round of plus-strand DNA synthesis of the 379-base-pair RU5 region that was the target for sequencing. Once again, it seems more plausible that these mutations are somatic mutations that arose sequentially over time.

Studies with purified HIV-1 reverse transcriptase have indicated that reverse transcriptase fidelity is 10-fold higher with the use of RNA templates than DNA templates (similar studies have yet to be done in cells) *(19).* Consequently, it is difficult to argue that no mutations created during minus-strand synthesis means no mutations created during plus-strand synthesis (assuming, of course, that HTLV-1 reverse transcriptase behaves similarly on RNA and DNA templates). However, the finding that no mutations were observed that could be due to errors made during minus-strand synthesis indicates that even a relatively small-fold increase in mutation frequency is not likely to account for most of the observed mutations. It is also worth noting that template switching during RNA- and DNA-dependent DNA synthesis for avian retroviruses has been found to be nearly identical *(20).* Given this observation and the observation by Mortreux et al. *(13)* that no mutations occurred during minus-strand synthesis, one can again conclude that somatic mutation is largely responsible for the observed mutations in the HTLV-1 RU5 sequences.

Although it is difficult to assign an origin for all mutations in HTLV-1 proviruses, most mutations observed in HTLV-1 proviruses appear to be due to somatic mutation during cell proliferation and not due to reverse transcription after infection of permissive host cells. This finding further confirms that HTLV-1 replicates more often as an integrated provirus during cell proliferation than as a virus via reverse transcription and that HTLV-1 variation is associated with clonal expansion and with the Tax-associated mutator phenotype. Many questions remain regarding why HTLV-1 and related viruses in the HTLV/BLV genus have chosen such an unusual lifestyle compared with that of other retroviruses. HIV-1, in particular, replicates via reverse transcription at very high rates *(21),* whereas HTLV-1 only on occasion replicates by this route. Further studies will determine the association of HTLV-1 replication as a provirus with the host immune response and with the low risk of development of ATLL in HTLV-1-infected individuals.

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Note

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