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Commentary

Error-prone retrotransposition: Rime of the ancient mutators

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In 1964, Howard Temin (1) first proposed that genetic information might actually flow in reverse (i.e. from RNA to DNA) in some organisms. The subsequent codiscovery of RNA-dependent DNA polymerases (reverse transcriptases) by Temin and Mizutani (2) and Baltimore (3) solidified this revolutionary idea and provided a key to the identification of a large group of evolutionarily related mobile genetic elements that encode their own reverse transcriptases and replicate through RNA intermediates (DNA → RNA → DNA; 4–6). These "retroelements" exist across broad phylogenetic boundaries (bacteria, algae, plants, fungi, insects, birds, fish, and mammals) and include bacterial episomal elements (msDNA), mitochondrial retroplasmids and group II introns, interspersed repeated sequences [long terminal repeat (LTR) retrotransposons and non-LTR long interspersed nuclear elements (LINE)-like elements], and replicating viruses (retroviruses, hepadnaviruses, and caulimoviruses). Comparisons of genome organizations, replication mechanisms, and reverse transcriptase sequences and functions clearly point to evolutionary relationships among retroelements (6–9). Although the temporal order of retroelement evolution is controversial (6), it has been suggested that retroelements were evolutionary links between ancient RNA and DNA worlds (10).

A hallmark of retroelements is their genetic heterogeneity. This was discovered first (11) and is best documented (12–15) for retroviruses that have been shown to exist as complex mixtures of genetically heterogeneous virions ("quasispecies") that are ever-changing. Studies of nonviral retrotransposons and mitochondrial retroplasmids suggest that genetic variation is also a property of other retroelements (16–23; GenBank data base).

The frequency of genetic variants within a population of retroelements may be influenced by many factors, including mutation rate, the number of retrotransposition cycles, selection, population size, competition, and random sampling (24). The distinction between mutation frequency (i.e., the proportion of mutants in a population at any given time) and mutation rate (i.e., the number of *de novo* mutations arising per nucleotide per cycle of replication) is very important if one is to understand underlying mechanisms of genetic variation (25). Recent studies using recombinant and clonally purified retroviruses have permitted the quantitation of mutation rates after a single cycle of replication in the absence of strong selection (reviewed in ref. 15). These studies show that retroviruses, like other RNA viruses, mutate at very high rates (0.05–1 mutation per genome per replication cycle), resulting in base substitutions, frameshifts, genetic rearrangements, and hypermutations. Although it has long been suspected that other retroelements also mutate at high rates (16, 17), technical difficulties have precluded a rigorous quantitation of these rates.

Fidelity of Ty1 Retrotransposition

Taking advantage of yeast genetics and a clever expression/selection system developed by Jef Boeke at Johns Hopkins University School of Medicine, the group of Abram Gabriel at Rutgers University (26) has now determined the rate of mutation of the yeast LTR retrotransposon Ty1 after a single cycle of retrotransposition. The technical difficulties associated with studies of Ty1 mutation (polymorphisms, variable expression, and multiple endogenous copies) were circumvented by examining retrotransposition of a recombinant Ty1 genome under control of the *GAL1* promoter in yeast harboring an allele (*spt3*) that represses expression of endogenous Ty1 LTR promoters (16, 27). The use of recombinant *GAL*-Ty1 in *spt3* yeast permitted high levels of expression of a defined parental sequence without the confounding effects from coexpression of chromosomal (LTR-driven) Ty1 elements. Thus, in this system essentially all Ty1 progeny arise from a single parental genome. The *GAL1*-Ty1 *spt3* host cells are also deleted for their chromosomal *HIS3* gene (*his3Δ200*) and carry a promoterless, but otherwise fully functional, *HIS3* allele on a target plasmid. In this somewhat complex genetic scheme, Ty1 insertions that occur directly upstream of the promoterless *HIS3* allele result in expression of this allele from the Ty1 LTR promoter and are detected by selection for histidine prototrophy. Additional cycles of retrotransposition do not occur, because the *GAL1* promoter does not transpose with the rest of the Ty1 genome, and the newly formed LTR-Ty1 genomes are not expressed in *spt3* yeast. Thus, this is a very powerful experimental system that permits the study of genetically defined Ty1 genomes during a single cycle of retrotransposition.

Reporter gene systems have been used to score mutation rates in retroviruses (15). However, such systems have not yet been developed for Ty1. Thus, to detect mutations, Gabriel *et al.* (26) used brute-force sequencing. Although this approach is labor-intensive and lacks sensitivity, it has the benefit of examining mutations arising in natural Ty1 sequences with minimum selection bias. This, as it turns out, was very important, because the hotspots for mutation in Ty1 occurred at specific regions of the Ty1 genome that would not have been detected using a foreign reporter gene (see below). After sequencing the entire genomes of 29 independent Ty1 transposition events (173,000 total bases), 13 base substitutions were detected. This corresponds to a mutation rate of 2.5×10^{-5} per nucleotide per replication cycle, or about 1 mutation for every 40,000 nucleotides polymerized. When expressed on a per-genome basis, mutations are introduced at an average rate of 0.15 per Ty1 genome per replication cycle. At this rate, 1 out of every 6–7 retrotranspositions results in a Ty1 element with a new genomic sequence.

Error-Prone Nature of Retrotransposition

Although the mutation rate of Ty1 is high compared with that of cellular DNA (10^{-8}–10^{-11} mutations per nucleotide per cycle; ref. 28), it is comparable to that of retroviruses and more distantly related RNA viruses and RNA bacteriophage (Table 1). It is becoming increasingly clear that RNA viruses in general mutate at high rates (24, 29). This "mutator" phenotype was first observed for phage Qβ replicating in *E. coli* (30) and has since been demonstrated for a broad range of eukaryotic RNA viruses including poliovirus, vesicular stomatitis

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Table 1. Base-substitution mutation rates of plus-strand RNA viruses and retroelements

Virus	Mutation rate, mutations per nucleotide per replication cycle*
RNA bacteriophage	
Qβ	10^{-4}
RNA viruses	
Vesicular stomatitis	10^{-3}–10^{-4}
Polio	10^{-3}–10^{-6}
Influenza A	10^{-5}
Retroelements	
LTR retrotransposons:	
Retroviruses	10^{-4}–10^{-6}
Ty1	10^{-5}
Non-LTR retrotransposons	ND
Group II introns	ND
Retroplasmids	ND
msDNA	ND

*The data for Ty1 are from Gabriel *et al.* (26). Mutation rates for RNA viruses and retroviruses are summarized in recent reviews (15, 24, 29). Rates for non-LTR retrotransposons, group II introns, mitochondrial retroplasmids, and bacterial msDNA retroelements have not been determined (ND).

virus, influenza virus, and several retroviruses (summarized in refs. 24, 29). These viruses are thought to benefit from a mutator phenotype (14, 24), as it allows them to rapidly adapt to changing selective forces in the host environment (e.g., immunity, drugs, and cell tropism). It is also thought that the progression of some diseases (e.g., AIDS) may, in part, be mediated by the adaptive nature of the causative virus (25, 31).

The Gabriel and Boeke data (26) show that endogenous Ty1 elements are also spontaneous mutators. Thus, error-prone replication is not exclusive to infectious viruses. This is interesting in that there is no obvious benefit for an endogenous retrotransposon in a unicellular organism to mutate at high rates in the absence of an immune system or other highly adaptable replication antagonist. Yeast and other unicellular organisms do experience environmental changes that can alter cellular metabolism and may impose some level of selection on retrotransposition within the cell. Thus, mutator retrotransposons might be more adaptable to such environmental changes. In some circumstances, competition for intracellular molecules may also favor mutators (32).

Another view is that there is no benefit to (and thus no selection for) retrotransposons that replicate with high fidelity. Retrotransposons may mutate at high rates simply because there is no selection to do otherwise. In fact, there may be selection against the acquisition of high-fidelity replication mechanisms (increased nucleotide discrimination, exonucleolytic proofreading, and postreplication mismatch repair) because of the energetic costs exacted by these accessory biochemical systems (33) and/or because genes coding for these systems may not be readily accommodated within the retrotransposon genome. Organisms throughout evolution tend to acquire high-fidelity replication machinery only as needed to avoid error catastrophe and in proportion to the complexity and lengths of their genomes (34). Thus, there is probably selection against high-fidelity replication in organisms that do no require it (33). This is likely the case, at least in part, for Ty1. Although selection for a mutator phenotype cannot be formally excluded, it seems more plausible that this phenotype in Ty1 is a vestige of ancestral RNA viruses and that the acquisition of antimutator systems is either neutral or selected against.

Sources of Mutation

The high mutation rates of retroviruses and Ty1 presumably reflect the error-prone nature of their life cycles (15). These genetic elements retrotranspose (DNA → RNA → DNA) by similar mechanisms (5, 6, 35, 36), thus suggesting common sources of mutation. It appears that the extracellular phase of retrovirus replication contributes little to the overall rate of mutation, because both types of retroelements mutate at similar rates. There are several likely sources of retroelement mutation: (*i*) reverse transcriptase errors, (*ii*) RNA polymerase II errors, (*iii*) RNA editing, and (*iv*) spontaneous chemical "decay" of RNA or DNA (15).

The evidence implicating reverse transcriptases in retroviral mutagenesis is indirect but compelling (15, 37–39). Reverse transcriptases lack 3′→5′ exonucleolytic proofreading activity and must rely exclusively on their polymerase domains to "read" each template base properly and to select correct dNTPs for incorporation into the nascent retroelement DNA strands. Thus, unlike cellular replicative polymerases, which are associated with proofreading functions, reverse transcriptases have no means of correcting errors that arise during polymerization and are therefore error prone. Estimates of *in vitro* error rates of purified retroviral reverse transcriptases range from one error in every 10^2 to one error in every 10^7 nucleotides polymerized, depending on the source of the reverse transcriptase, the sequence of the template, and the type of error (15, 37–39). Clearly, retroviral reverse transcriptases are sufficiently error-prone to account for the mutations arising during retrotransposition. Given the homologies among retroelement reverse transcriptases (6–8) and their general lack of exonucleolytic proofreading, it is likely that reverse transcriptase infidelity is also a source of mutation for Ty1 and probably all retroelements. It should be noted that the error-prone nature of reverse transcriptases is not unique. RNA-dependent RNA polymerases from other, presumably ancestral, viruses are also error-prone (40) as are essentially all DNA polymerases that lack exonucleolytic proofreading (41).

This trend of error-proneness in polymerases lacking exonucleolytic proofreading points to another potential and frequently overlooked source of retroelement mutation: cellular RNA polymerase. All retroelements require the synthesis of genomic RNA as part of their retrotransposition cycle. This synthesis is catalyzed by cellular RNA polymerases (often RNA polymerase II). Studies, primarily in prokaryotes, show that RNA synthesis is also relatively error-prone, introducing about one error in every 10^4–10^5 nucleotides polymerized (42, 43). Thus, RNA polymerases and reverse transcriptases are about equally error-prone, and both, therefore, are predicted to contribute significantly to retroelement mutagenesis.

Other potential sources of mutation include RNA editing and template modification by chemical decay (15). Although the latter has not been explored, RNA editing by the cellular enzyme dsRAD (double-strand RNA adenosine deaminase) is the likely cause of a specific class of A→G hypermutations in retroviruses (44, 45). All retroelements could potentially hypermutate in a similar way, with rates depending on substrate accessibility in the host cell and on the levels of dsRAD (or other RNA editing enzyme).

Mutation by Blunt-End Nucleotide Addition

Gabriel *et al.* (26) made the striking observation that Ty1 mutations are nonrandom. Because their system for scoring mutations is minimally influenced by selection, this nonrandom pattern most likely reflects hotspots for replication errors. One-third of the mutants (3 of 10) had mutations within seven bases of the primer binding site (PBS), where reverse transcriptase initiates minus-strand DNA synthesis from a cellular tRNA primer (Fig. 1). A fourth mutant had a mutation ≈90

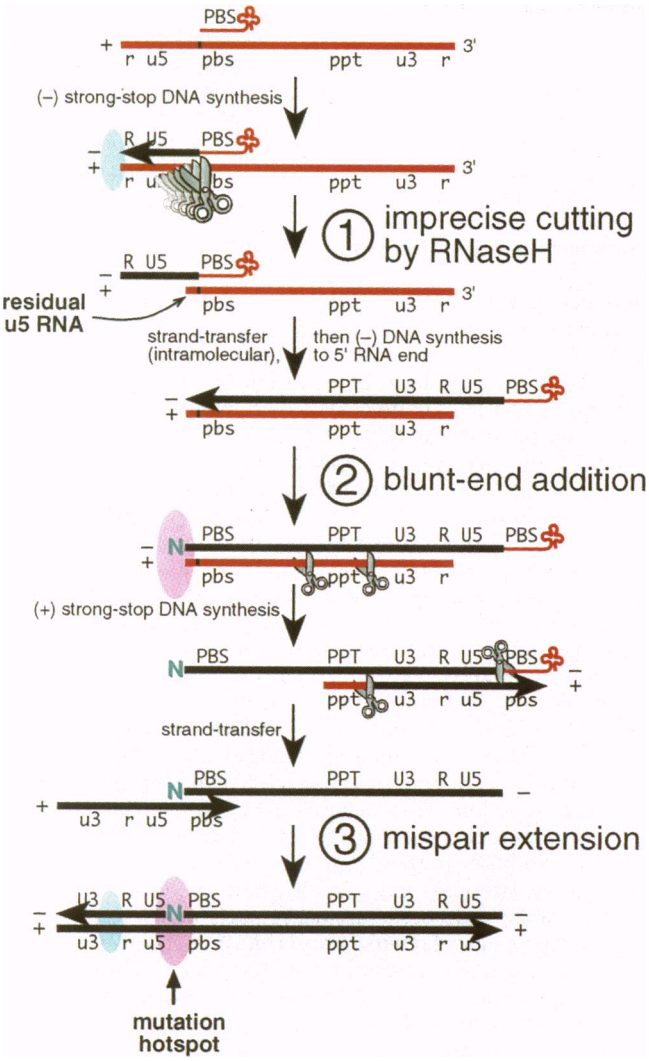

FIG. 1. Mechanism of mutation by blunt-end addition of nucleotides. The accepted model for conversion of RNA to double-stranded DNA in LTR retroelements is shown emphasizing three steps proposed to result in US/PBS junctional mutations: step 1, imprecise cutting by RNaseH leaving residual u5 RNA bases at the 5′-RNA end; step 2, blunt-end addition of one or more extra nucleotides after polymerization of minus-strand DNA to the 5′-RNA template end; and step 3, extension of the mispairs formed by these extra nucleotides after second strand-transfer. Mechanisms of LTR retroelement DNA synthesis are described elsewhere (5, 6, 35, 36). RNA is indicated by red; DNA is indicated by black. Genomic R, U5, PBS, PPT, and U3 regions are indicated, with lower- and uppercase corresponding to plus- and minus-strand, respectively. The u5/pbs junction in the genomic RNA is shown by a short vertical line. The blurred scissors represent imprecise RNaseH cutting at the u5/pbs junction. Other obligatory RNaseH cleavage sites are shown by unblurred scissors; secondary sites for initiation of plus-strand DNA synthesis are not shown (36). For simplicity, intramolecular strand-transfer products are represented as linear molecules rather than circular forms. N, extra nucleotide(s) added at blunt end; rose-colored ovals, hotspot for Ty1 blunt-end addition and mutation at U5/PBS junction; and light blue ovals, site of blunt-end addition and mutation at U3/R junction.

bases from the PBS, immediately upstream of the initiation site for genomic Ty1 RNA synthesis (i.e., between the "U3" and "R" regions of the Ty1 5′-LTR sequence). All of these mutations were base substitutions and all occurred in the 5′-LTR but not the 3′-LTR of the nascent Ty1 DNA.

As pointed out by the authors, this nonrandom pattern suggests a very specific mechanism of mutation involving three imprecise but apparently common enzymatic steps (Fig. 1). The first is imprecise RNaseH cleavage of genomic plus-strand RNA after minus-strand DNA synthesis initiated from the tRNA primer (Fig. 1, step 1). It is well documented *in vitro* that retroviral RNasetts inherently cleave at multiple sites and that cleavage is often incomplete, leaving short stretches of uncut RNA·DNA duplexes (36, 46). Thus, it is not surprising that the homologous Ty1 RNaseH would exhibit a similar pattern of cleavage *in vivo*. The result of this imprecision is a heterogeneous population of 5′-RNA ends. While the majority of RNAs are expected to terminate precisely at the 5′-junction of the pbs (i.e., the canonical cleavage site in current models of LTR retroelement replication; ref. 36), imprecise cleavage will result in slightly longer plus-strand RNA intermediates with a few residual u5 ribonucleotides 5′ to the pbs. Recent studies provide direct evidence for such variable-length RNA intermediates during Ty1 retrotransposition (E. Mules and A. Gabriel, personal communication).

The creation of heterogeneous 5′-RNA ends localizes the next error-prone step (i.e., blunt-end nucleotide addition) to the region immediately adjacent to the tRNA primer binding site (Fig. 1, step 2). This next step assumes that the first strand-transfer event, which is a normal part of LTR retroelement replication (36), is intramolecular. After this first strand-transfer, reverse transcription proceeds the length of the RNA genome and eventually reaches the heterogeneous 5′-template ends generated by imprecise RNaseH cleavage. Recent studies of retroviral reverse transcriptases show that, once polymerization reaches the 5′-end of a template, 3′-overhanging nucleotides are frequently added to the growing DNA strand (47–51). This blunt-end addition of one to four extra nucleotides occurs processively (i.e., without dissociation of reverse transcriptase from the nascent DNA) and at high rates (30–50% of *in vitro* products contain extra nucleotides at their ends; ref. 51). All four nucleotides are added with similar efficiencies when reactions are conducted in the presence of all four dNTPs (ref. 48; P. Patel and B.P., unpublished data). The ability to catalyze blunt-end nucleotide addition is not limited to reverse transcriptases and is seen with several other DNA polymerases (all lacking 3′→5′ exonucleolytic proofreading activity; refs. 47, 48, and 52) and at least one RNA polymerase (53). Assuming that Ty1 reverse transcriptase catalyzes blunt-end addition with a similar high efficiency, the resultant Ty1 minus-strand replication intermediates will have heterogeneous 3′-ends that terminate at different locations (as determined by the original sites of RNaseH cleavage) and that contain one or more apparently "nontemplated" terminal nucleotides (resulting from blunt-end addition catalyzed by reverse transcriptase).

The last imprecise enzymatic step (i.e., mispair extension; Fig. 1, step 3) occurs after the second strand-transfer event required for normal LTR retroelement replication. This strand transfer forms DNA·DNA partial duplexes with recessed 3′-ends that serve as primers for completion of double-stranded DNA synthesis. As a result of blunt-end nucleotide addition in the previous step, the minus-strand 3′-end (at a variable distance from the U5/PBS junction) contains one or more nontemplated nucleotides that are unlikely to hybridize precisely with the plus-strand DNA template. To complete DNA synthesis and reconstitute the Ty1 5′-LTR, these mispaired 3′-primer termini must be extended by reverse transcriptase. Again, based on studies of retroviral reverse transcriptases (54–58), it is predicted that Ty1 reverse transcriptase will extend these mispaired primer termini with reasonably high efficiency. This will seal the mispairs into the final DNA products, which are then integrated into the yeast genome (or target plasmid in the system of Gabriel *et al.*; ref. 26) to complete the retrotransposition cycle. Presumably the resultant heteroduplexes are resolved to homoduplexes via the action of cellular mismatch repair systems (59). In the absence of strand discrimination, this mismatch repair will resolve half

of the mispairs to the new mutant sequences and half back to wild-type. Thus, the frequency of this class of Ty1 mutations may be underestimated by 50%.

This mechanism, although complex, accounts for the class (base substitutions), location (U5/PBS junctions in 5′-LTRs only), and high rate of these mutations observed in Ty1. Each of the biochemical steps (incomplete RNaseH cleavage, blunt-end addition, and mispair extension) are catalyzed with high frequency by reverse transcriptases *in vitro* and thus seem likely to occur at high rates during retrotransposition in the cell. The mutation observed at the Ty1 U3/R junction can also be accounted for by a similar mechanism, if one assumes that the first strand-transfer event is intermolecular and that blunt-end nucleotide addition occurs opposite the 5′-end of an uncleaved genomic RNA template with an intact 5′-terminal r region. A similar sequence of events could explain the high rate of reverse transcriptase errors immediately adjacent to primers on RNA templates *in vitro* (60).

Alternative mutation mechanisms involving simple misincorporation by reverse transcriptase or "dislocation" mutagenesis (39) are also possible but seem less likely. The rates of nucleotide misincorporation by reverse transcriptases *in vitro* (10^{-3}–10^{-7} mutations per nucleotide polymerized) are not high enough to account for the rates of U5/PBS and U3/R junctional mutations observed in Ty1 (5 mutations localized to 4 nucleotide positions in 29 independent retrotranspositions $\approx 2 \times 10^{-2}$ mutations per nucleotide polymerized). Some reverse transcriptases do catalyze dislocation mutations at these high rates *in vitro* (61, 62). However, the Ty1 mutations at U5/PBS and U3/R do not occur at homopolymeric repeats and thus lack a hallmark of dislocation mutagenesis, which involves transient primer·template slippage stabilized by short runs of like nucleotides (39). Interestingly, three other Ty1 mutations observed by Gabriel *et al.* (26) were in homopolymeric runs elsewhere in the genome, and two of these precisely match the pattern of dislocation mutagenesis. Thus, dislocation mutagenesis appears to occur in Ty1, but probably not at the U5/PBS and U3/R hotspots.

"Nontemplated" Nucleotide Addition as a Common Event in Retrotransposition

DNA polymerization in the absence of a conventional template appears to be common among retroelements. Extra nucleotides of unknown origin are often observed in retroviruses at circle junctions (see ref. 51 and references therein), deletion junctions (63), and U3/R junctions (P. O'Neil, H. Yu, P. Dougherty, and B.P., unpublished data) and at recombination junctions mediated by reverse transcriptase from the human non-LTR retrotransposon L1 (20). Similar apparently nontemplated additions also occur with high frequency at recombination junctions in *Neurospora* mitochrondrial retroplasmids (19) and at primer initiation junctions in specific constructs of the *Bombyx mori* non-LTR retroelement R2 (21). Thus, extra nucleotide additions appear to be introduced by reverse transcriptases from LTR and non-LTR retrotransposons as well as simple (and possibly more primitive) mitochondrial retroplasmids.

The extra nucleotides observed in these cases are referred to as nontemplated, because there is no obvious template guiding these additions. However, blunt-end addition *in vitro* can be templated by discontinuous oligonucleotides that align noncovalently at the 5′-ends of templates in conventional primer·template substrates (ref. 48; P. Patel and B.P., unpublished data). This mechanism of blunt-end addition using discontinuous templates appears similar to that used by telomerases during reverse transcription at chromosome ends (64). Interestingly, a subclass of *Drosophila* retrotransposons frequently transpose to broken chromosome ends and appear to use these ends as discontinuous primers to initiate reverse transcription (65–67). Thus, all reverse transcriptases may share a common mechanism for catalyzing extra nucleotide additions by using discontinuous oligonucleotides as guide templates. These guide templates can be quite short (e.g., mononucleotides; ref. 48; P. Patel and B.P., unpublished data) and may be derived from a variety of sources such as RNaseH cleavage products, specific RNAs (as with telomerases), and/or normal intermediates in cellular nucleic acid metabolism and turnover.

Summary and Perspectives

Retroelements comprise a family of genetic elements that use reverse transcriptase to propagate through RNA intermediates. Their unique mode of replication suggests that retroelements occupy a position in evolution bridging ancient RNA and DNA worlds. The first identified members of this family were the retroviruses that, like other RNA viruses, exist as complex mixtures or quasispecies that have the capacity to rapidly adapt to their environment via mutation. The paper by Gabriel, Boeke, and colleagues (26) now shows that this mutator phenotype is also associated with a closely related endogenous LTR retrotransposon, Ty1. Thus, high mutation rates are not unique to viruses that spread extracellularly.

De novo mutations are generated by molecular steps in retrotransposition that are inherently imprecise. Reverse transcriptase infidelity likely contributes significantly to the mutator phenotype. Errors introduced by cellular transcription and RNA editing apparati, although largely unexplored, may also contribute to mutagenesis. Gabriel *et al.* (26) noted that Ty1 mutations are nonrandom, occurring preferentially at the U5/PBS junction. This suggests a mechanism involving sequential imprecise steps of RNaseH cleavage, blunt-end nucleotide addition (either nontemplated or templated by discontinuous strands), and mispair extension. This mechanism appears to be widespread among retroelements, reflecting an inherent property of reverse transcriptases to catalyze addition of extra nucleotides after reaching template 5′-ends.

Perhaps equally notable is that all of the Ty1 mutations were base substitutions. This is unlike retroviruses, which accrue frameshift mutations and genetic rearrangements (deletions, insertions, and complex genomic changes) at rates equal to or exceeding those for base substitution mutations (15, 63). Although this may, in part, reflect random sampling errors (26), the data suggest that frameshifts and genetic rearrangements in Ty1 are infrequent relative to mutations mediated by blunt-end addition. Studies in retroviruses have focused on scoring mutations that inactivate nonviral reporter genes and have not looked at genomic sites predicted to mutate via blunt-end addition. Thus, the apparent differences between Ty1 and retroviral mutation spectra may reflect biases imposed by experimental design. In support of this, recent studies of human immunodeficiency virus (HIV) reveal a high frequency of mutations that are likely mediated by blunt-end addition (P. O'Neil, H. Yu, P. Dougherty, and B.P., unpublished results), and there is also evidence for high rates of at least one class of genetic rearrangements in Ty1 (17). Additional studies are required to more extensively examine blunt-end addition mutations in retroviruses and frameshifting and genetic rearrangements in Ty1.

Finally, although it is now evident that members from two different LTR retrotransposon subgroups (Ty1-*copia* and retroviruses) are spontaneous mutators, the mutation rates of other retroelements (non-LTR LINE-like retrotransposons, group II introns, retroplasmids, bacterial retroelements, and pararetroviruses) have not been rigorously determined. This will require the development of replication systems starting with genetically homogeneous retroelements that can be replicated for a single cycle under conditions of minimal selection. Recombinant systems that might be adapted for this purpose are already available for many retroelements (68–74). Given the evolutionary relationships among error-prone reverse

transcriptases and RNA-dependent RNA polymerases, it seems likely that all retroelements mutate at high rates and together comprise a large group of ancient mutators.

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1. Temin, H. M. (1964) *Natl. Cancer Inst. Monogr.* **17,** 557–570.
2. Temin, H. M. & Mizutani, S. (1970) *Nature (London)* **226,** 1211–1213.
3. Baltimore, D. (1970) *Nature (London)* **226,** 1209–1211.
4. Boeke, J. D. (1988) in *RNA Genetics: Retroviruses, Viroids, and RNA Recombination*, eds. Domingo, E., Holland, J. J. & Ahlquist, P. (CRC, Boca Raton), Vol. 2, pp. 59–103.
5. Gabriel, A. & Boeke, J. D. (1993) in *Reverse Transcriptase*, eds. Skalka, A. M. & Goff, S. P. (Cold Spring Harbor Lab. Press, Plainview, New York), pp. 275–328.
6. Eickbush, T. H. (1994) in *The Evolutionary Biology of Viruses*, ed. Morse, S. S. (Raven, New York), pp. 121–157.
7. Doolittle, R. F., Feng, D.-F., Johnson, M. S. & McClure, M. A. (1989) *Q. Rev. Biol.* **64,** 1–30.
8. McClure, M. A. (1993) in *Reverse Transcriptase*, eds. Skalka, A. M. & Goff, S. P. (Cold Spring Harbor Lab. Press, Plainview, New York), pp. 425–444.
9. Singer, M. F. (1995) *J. Biol. Chem.* **270,** 24623–24626.
10. Cech, T. R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4360–4363.
11. Temin, H. M. (1960) *Virology* **10,** 182–197.
12. Katz, R. A. & Skalka, A. M. (1990) *Annu. Rev. Genet.* **24,** 409–445.
13. Wain-Hobson, S. (1992) *Curr. Top. Microbiol. Immunol.* **176,** 181–193.
14. Eigen, M. (1993) *Sci. Am.* **269,** 42–49.
15. Preston, B. D. & Dougherty, J. P. (1996) *Trends Microbiol.* **4,** 16–21.
16. Boeke, J. D., Garfinkel, D. J., Styles, C. A. & Fink, G. R. (1985) *Cell* **40,** 491–500.
17. Xu, H. & Boeke, J. D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8553–8557.
18. Boeke, J. D., Eichinger, D., Castrillon, D. & Fink, G. R. (1988) *Mol. Cell. Biol.* **8,** 1432–1442.
19. Chiang, C. C., Kennell, J. C., Wanner, L. A. & Lambowitz, A. M. (1994) *Mol. Cell. Biol.* **14,** 6419–6432.
20. Dombroski, B. A., Feng, Q., Mathias, S. L., Sassaman, D. M., Scott, A. F., Kazazian, H. H., Jr., & Boeke, J. D. (1994) *Mol. Cell. Biol.* **14,** 4485–4492.
21. Luan, D. D. & Eickbush, T. H. (1995) *Mol. Cell. Biol.* **15,** 3882–3891.
22. Casacuberta, J. M., Vernhettes, S. & Grandbastien, M. A. (1995) *EMBO J.* **14,** 2670–2678.
23. Furano, A. V. & Usdin, K. (1995) *J. Biol. Chem.* **270,** 25301–25304.
24. Domingo, E. & Holland, J. J. (1994) in *The Evolutionary Biology of Viruses*, ed. Morse, S. S. (Raven, New York), pp. 161–184.
25. Coffin, J. M. (1995) *Science* **267,** 483–489.
26. Gabriel, A., Willems, M., Mules, E. H. & Boeke, J. D. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 7767–7771.
27. Boeke, J. D., Styles, C. A. & Fink, G. R. (1986) *Mol. Cell. Biol.* **6,** 3575–3581.
28. Drake, J. W. (1991) *Annu. Rev. Genet.* **25,** 125–146.
29. Drake, J. W. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 4171–4175.
30. Batschelet, E., Domingo, E. & Weissmann, C. (1976) *Gene* **1,** 27–32.
31. Nowak, M. A. & McMichael, A. J. (1995) *Sci. Am.* **273,** 58–65.
32. Roux, L., Simon, A. E. & Holland, J. J. (1991) *Adv. Virus Res.* **40,** 181–211.
33. Drake, J. W. (1993) *J. Mol. Biol.* **229,** 8–13.
34. Drake, J. W. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7160–7164.
35. Boeke, J. D. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington, DC), pp. 335–374.
36. Katz, R. A. & Skalka, A. M. (1994) *Annu. Rev. Biochem.* **63,** 133–173.
37. Preston, B. D. & Garvey, N. (1992) *Pharma. Technol.* **16,** 34–51.
38. Williams, K. J. & Loeb, L. A. (1992) *Curr. Top. Microbiol. Immunol.* **176,** 165–180.
39. Bebenek, K. & Kunkel, T. A. (1993) in *Reverse Transcriptase*, eds. Skalka, A. M. & Goff, S. P. (Cold Spring Harbor Lab. Press, Plainview, New York), pp. 85–102.
40. Ward, C. D., Stokes, M. A. M. & Flanegan, J. B. (1989) *J. Virol.* **62,** 558–562.
41. Loeb, L. A. & Kunkel, T. A. (1982) *Annu. Rev. Biochem.* **52,** 429–457.
42. Anderson, R. P. & Menninger, J. R. (1986) in *Accuracy in Molecular Processes: Its Control and Relevance to Living Systems*, eds. Kirkwood, T. B. L., Rosenberger, R. F. & Galas, D. J. (Chapman and Hall, New York), pp. 159–189.
43. de Mercoyrol, L., Corda, Y., Job, C. & Job, D. (1992) *Eur. J. Biochem.* **206,** 49–58.
44. Felder, M. P., Laugier, D., Yatsula, B., Dezelee, P., Calothy, G. & Marx, M. (1994) *J. Virol.* **68,** 4759–4767.
45. Hajjar, A. M. & Linial, M. L. (1995) *J. Virol.* **69,** 5878–5882.
46. Champoux, J. J. (1993) in *Reverse Transcriptase*, eds. Skalka, A. M. & Goff, S. P. (Cold Spring Harbor Lab. Press, Plainview, New York), pp. 103–117.
47. Clark, J. M. (1988) *Nucleic Acids Res.* **16,** 9677–9686.
48. Clark, J. M. (1991) *Gene* **104,** 75–80.
49. Peliska, J. A. & Benkovic, S. J. (1992) *Science* **258,** 1112–1118.
50. Peliska, J. A., Galasubramanian, S., Giedroc, D. P. & Benkovic, S. J. (1994) *Biochemistry* **33,** 13817–13823.
51. Patel, P. H. & Preston, B. D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 549–553.
52. Clark, J. M., Joyce, C. M. & Beardsley, G. P. (1987) *J. Mol. Biol.* **198,** 123–127.
53. Milligan, J. F. & Uhlenbeck, O. C. (1989) *Methods Enzymol.* **180,** 51–62.
54. Perrino, F. W., Preston, B. D., Sandell, L. L. & Loeb, L. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8343–8347.
55. Mendelman, L. V., Petruska, J. & Goodman, M. F. (1990) *J. Biol. Chem.* **265,** 2338–2346.
56. Creighton, S., Huang, M.-M., Cai, H., Arnheim, N. & Goodman, M. F. (1992) *J. Biol. Chem.* **267,** 2633–2639.
57. Pulsinelli, G. A. & Temin, H. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 9490–9494.
58. Zinnen, S., Hsieh, J. C. & Modrich, P. (1994) *J. Biol. Chem.* **269,** 24195–24202.
59. Modrich, P. (1991) *Annu. Rev. Genet.* **25,** 229–253.
60. Boyer, J. C., Bebenek, K. & Kunkel, T. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6919–6923.
61. Bebenek, K., Abbotts, J., Roberts, J. D., Wilson, S. H. & Kunkel, T. A. (1989) *J. Biol. Chem.* **264,** 16948–16956.
62. Roberts, J. D., Preston, B. D., Johnston, L. A., Soni, A., Loeb, L. A. & Kunkel, T. A. (1989) *Mol. Cell. Biol.* **9,** 469–476.
63. Parthasarathi, S., Varela-Echavarría, V., Ron, Y., Preston, B. D. & Dougherty, J. P. (1995) *J. Virol.* **69,** 7991–8000.
64. Blackburn, E. H. (1993) in *Reverse Transcriptase*, eds. Skalka, A. M. & Goff, S. P. (Cold Spring Harbor Lab. Press, Plainview, New York), pp. 411–424.
65. Biessmann, H., Champion, L. E., M., O. H., Ikenaga, K., Kasravi, B. & Mason, J. M. (1992) *EMBO J.* **11,** 4459–4469.
66. Levis, R. W., Ganesan, R., Houtchens, K., Tolar, L. A. & Sheen, F. M. (1993) *Cell* **75,** 1083–1093.
67. Sheen, F. M. & Levis, R. W. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12510–12514.
68. Chalker, D. L. & Sandmeyer, S. B. (1992) *Genes Dev.* **6,** 117–128.
69. Evans, J. P. & Palmiter, R. D. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8792–8795.
70. Jensen, S. & Heidmann, T. (1991) *EMBO J.* **10,** 1927–1937.
71. Kinsey, J. A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9384–9387.
72. Levin, H. L. & Boeke, J. D. (1992) *EMBO J.* **11,** 1145–1153.
73. Pelisson, A., Finnegan, D. J. & Bucheton, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4907–4910.
74. Shimamoto, T., Inouye, S. & Inouye, M. (1995) *J. Biol. Chem.* **270,** 581–588.