Synthesis of DNA by Human Immunodeficiency Virus Reverse Transcriptase Is Preferentially Blocked at Template Oligo(deoxyadenosine) Tracts*

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The genome of human immunodeficiency virus (HIV) and especially the envelope gene are mutated with unusually high frequency during in vivo replication. Recent studies indicate that HIV reverse transcriptase (RT) is unusually prone and that the number of generated mutations is disproportionately high within repetitive base sequences. To study the ability of recombinant and wild-type HIV RT to traverse specific homo-oligomeric stretches, we used bacteriophage M13 DNA templates that contain different oligo(purine) and oligo(pyrimidine) inserted tracts. The progress of HIV RT along these templates was potently inhibited from further progression only at a (dA)16 insert. Comparison with other polymerases indicates that the almost complete blockage of polymerization beyond an oligo(dA) insert is unique to HIV RT and Moloney murine leukemia virus RT, which has high sequence homology with HIV RT. The extent of termination of HIV RT at the oligo(dA) run is not affected by alterations in the concentration of KCl, Mg2+, dNTP, or by a decrease in pH. Obstruction of HIV RT opposite the oligo(dA) insert is not alleviated by moving the primer position further upstream from the oligo(dA) insert. Lastly, HIV RT purified directly from virions is also specifically arrested at an oligo(dA) tract. Competition experiments indicate that the concentration of active HIV RT in the presence of M13(dA)16 DNA is similar to that observed in the presence of M13(dG)16 DNA. In addition, preincubation of M13(dA)16 DNA with HIV RT does not subsequently inhibit avian myeloblastosis virus RT from successfully traversing the (dA)16 insert. Therefore, it appears that the blockage of chain elongation of HIV RT at the (dA)16 insert is not the result of trapping the enzyme at this site.

Human Immunodeficiency Virus (HIV)* has been proposed as the causative agent of acquired immunodeficiency syndrome (Fauci, 1988). HIV is a retrovirus that contains a diploid single-stranded RNA genome. Upon infection of susceptible cells, the viral RNA is copied by its reverse transcriptase (RT) into a noncoding strand DNA, and this in turn is replicated by HIV RT into double-stranded DNA. Because of its high mutation frequency, the viral genome is up to 104-fold higher than in most other genomes (Coffin, 1986; Myers et al., 1989). In all cases, sequence variability is found to be extremely high, especially within the envelope gene. The calculated mutation rate during replication of the HIV genome is up to 104-fold higher than for most other genomes (Coffin, 1986; Wabl et al., 1985). Since HIV RT is a likely candidate responsible for this exceptionally high rate of mutation, the mechanics of catalysis of DNA replication by this polymerase have become the subject of extensive study. The processivity and fidelity of replication of synthetic and natural DNA and RNA templates by purified HIV RT have been examined by several authors (Huber et al., 1989; Preston et al., 1988; Roberts et al., 1988; Takeuchi et al., 1988; Bebenek et al., 1989). The absence of a 3′,5′-exonuclease activity in retroviral RTs confers on this class of enzymes a fidelity that is consistently lower than that of polymerases that contain such proofreading nuclease. Furthermore, HIV RT is found to be exceptionally error prone even among RTs, and its level of fidelity is up to 10-fold lower than that of avian myeloblastosis and murine leukemia virus RTs (Roberts et al., 1988). Even with a poly(rA) template that allows HIV RT its highest processivity, fidelity remains 3-fold lower than that of other retroviral RTs (Takeuchi et al., 1988). It was also observed that errors produced by HIV RT are nonrandomly distributed along wild-type DNA templates and that several mutational hotspots are located within homo-oligomeric sequences that inhibit the advance of HIV RT (Bebenek et al., 1989). It is evident, therefore, that factors additional to the lack of a 3′,5′-exonuclease may contribute to the decreased fidelity of HIV RT and that template nucleotide sequence might play a role in the determination of the level of accuracy of DNA.
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In an effort to understand better the effect of template base sequence on the processivity of DNA polymerases, we have recently examined the polymerase blocking ability of different oligo(purine)- and oligo(pyrimidine)-inserted stretches within recombinant M13 DNA templates (Weisman-Shomer et al., 1989). Using these templates we now report that HIV RT is preferentially inhibited from traversing beyond an oligo(dA) stretch within M13 heteropolymeric DNA.

EXPERIMENTAL PROCEDURES

Materials

Enzymes—HIV RT, purified from an overproducer strain of E. coli containing a plasmid carrying the coding region for the reverse transcriptase of HIV-1, was a generous gift of Dr. S. Wilson (National Institutes of Health, Bethesda, MD). Bacteriophage T4 DNA polymerase was the product of New England Biolabs (Beverly, MA). HIV reverse transcriptase (HIV RT) was a generous gift of Dr. S. Wilson (NIH-NCI). Virion HIV RT was purified as described previously (Beverly, MA).

Chemicals—Sephadex G-50 and G-100 were purchased from Pharmacia LKB Biotechnology Inc. Dithiothreitol and bromphenol blue were purchased from Sigma. Polyacrylamide was provided by Fisher Scientific. Urea was from Research Organics (Cleveland, OH). Deionized formamide and xylene cyanole were purchased from J. T. Baker Chemical Co.

Methods

DNA Polymerase Assays and Analysis of Product DNA—Both "-20" and "-40" M13 primers were 5' end labeled in a total volume of 10 μl using [γ-32P]ATP and T4 polynucleotide kinase as described by Maniatis et al. (1982). The labeling reaction was terminated by the addition of EDTA to a final concentration of 10 mM, and the mixture was heated at 65 °C for 15 min to inactivate the kinase. The volume was adjusted to 100 μl, and the primer was purified by centrifugation through a 1-ml Sephadex G-50 minicolumn equilibrated with dH2O (Maniatis et al., 1982). The end-labeled primers were annealed at a 2.4:1 molar ratio of primer to unmodified single-stranded DNA. M13 or AMV reverse transcriptase was added to the mixture at a molar excess of 37:1 and 11:1, respectively (unless otherwise indicated), over the DNA template. Incubation was at 37 °C for periods of time as indicated. Reactions were terminated by rapid cooling to 4 °C and by the addition of 85 μl of 10 mM EDTA. Extended DNA products were purified from free primers and salts by centrifugation through a 1-ml Sephadex G-100 minicolumn equilibrated with dH2O, and the mixtures were dried in a SpeedVac concentrator. The desiccated samples were resuspended in 3-6 μl of 95% formamide, 20 mM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanole, and the DNA products were resolved by electrophoresis through 15% polyacrylamide, 8 M urea gels. Gels were fixed in 10% acetic acid, 10% methanol, transferred to Whatman 3MM paper, and dried. To visualize the primer extension bands, the dried gels were exposed to Kodak XAR-5 film at room temperature or at -70 °C. Amounts and size distribution of the products of the primer extension reactions were quantified by cutting each radioactive lane into sectors and counting by Cerenkov radiation.

RESULTS

HIV RT Is Specifically Blocked at an Oligo(dA) Stretch within the Recombinant M13 DNA Template—The ability of HIV reverse transcriptase to traverse homo-oligomeric or alternating purine or pyrimidine stretches in DNA was assessed by use of recombinant M13 DNA templates. Shown in Fig. 1 are patterns of in vitro primer extension by HIV RT over unmodified single-stranded M13mp2 DNA and over recombinants of this template that contain, at the same position, inserts of (dG)ₙ, (dT)ₙ, (dA)ₙ, (dC)ₙ, or (dC-T)ₙ.

![Figure 1](http://example.com/image1.png)

FIG. 1. Pause sites of recombinant HIV RT along unmodified M13mp2 and recombinant M13 DNA templates. Unmodified M13mp2 and six recombinant M13 DNA templates were annealed to a 5'-52P-labeled 17-mer universal primer that is positioned 5 bases upstream to the EcoRI site of homopolymer insertion (see "Methods"). Each primer-template was extended at 37 °C for 5 min by recombinant HIV RT at a molar excess to template of 3:1 and 11:1, respectively.
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(dA-G)$_{16}$). On unmodified M13mp2 DNA, polymerization by HIV RT proceeds with a few strong pause sites that are evident only at lower enzyme to template ratio so that the predominant products are polynucleotides of 30–1000 nucleotides or greater in length. With increasing enzyme concentration, most pause sites are not visualized, and there is a consequent increase in the length of the newly synthesized products (Fig. 1). Insertion of some homopyrimidines or alternating pyrimidine stretches into the M13 DNA template lead to the introduction of new pause sites. Whereas pause sites of HIV RT are similar for unmodified M13mp2 DNA and for the M13(dG)$_{16}$, M13(dC)$_{16}$, and M13(dA-G)$_{16}$ template, new arrest sites are evident along M13(dT)$_{16}$ and M13(dC-T)$_{16}$ DNA (Fig. 1). However, each of these base runs is traversed by the enzyme, and products of increasing length accumulate as the molar ratio of enzyme to template is elevated. It appears, however, that HIV RT encounters a major difficulty in traversing the (dA)$_{16}$ tract, and even at 1:1 molar ratios of enzyme to template, synthesis terminates in the middle of the oligo(dA) tract. Longer exposure of the autoradiogram fails to reveal products extended beyond the (dA)$_{16}$ stretch as evident for all the other inserts (data not shown), and only after extended periods of incubation is there limited traversal of the oligo(dA) tract by HIV RT (see Fig. 2 below). To examine whether pausing at an oligo(dA) tract is distinctive to HIV RT, a similar analysis was carried out using the recombinant form of reverse transcriptase obtained from AMV. Pause sites of AMV RT along the various templates are similarly located to those of HIV RT. However, none of the recombinant M13 DNA templates, including M13(dA)$_{16}$, blocks AMV RT completely, and even at the lowest enzyme to template molar ratio of 3:7:1, AMV RT synthesizes products of 300–1000 nucleotides or greater in length (results not shown). A similar ability of the large fragment of E. coli DNA polymerase I and of calf thymus DNA polymerase α to traverse the oligo(dA) tract within M13 DNA was reported recently (Weisman-Shomer et al., 1989). MMLV RT does exhibit a strong stop within the oligo(dA) tract similar to HIV RT (results not shown) and has also been reported to have strong sequence homology with HIV RT (Johnson et al., 1996). Hence, HIV RT appears to be distinguishable among DNA polymerases, with the exception of MMLV RT, by its almost complete inability to copy DNA past a template stretch of oligo(dA).

Kinetics of Traversal of the Oligo(dA) Tract—The kinetics of traversal by HIV and AMV reverse transcriptases of the (dA)$_{16}$ stretch within recombinant M13 DNA is presented in Fig. 2. The autoradiographs in Fig. 2A is a comparison of the abilities of the two reverse transcriptases to copy the recombinant M13(dA)$_{16}$ template and unmodified M13mp2 DNA. Equal amounts of activity of HIV and AMV RTs, representing molar ratios of enzyme to template of 37 and 11, respectively (see "Methods"), were added to each reaction. As judged by its greater difficulty in accumulating product DNA molecules that are extended beyond the (dA)$_{16}$ insert, HIV RT traverses this stretch less efficiently than AMV RT. As shown in Fig. 2A, in the course of incubation at 37 °C, HIV RT is blocked within the (dA)$_{16}$ insert pausing primarily opposite (dA) residues 5–11 and 9–16 after 5 and 120 min at 37 °C, respectively. Some product extension by HIV RT beyond the (dA)$_{16}$ insert is observed only after 60 min of incubation with slightly darker bands evident after 120 min of incubation. With AMV RT, pausing occurs within the (dA)$_{16}$ insert opposite nucleotides 3–16. However, the (dA)$_{16}$ tract does not prevent traversal by AMV RT, and high molecular size products are already evident after 3 min at 37 °C and accumulate progressively with time (Fig. 2A). Fig. 2B is a plot of a similar kinetics experiment in which individual lanes were excised from the gel, and the distribution of radioactivity along each lane was quantified by Cerenkov radiation counting. The presented results indicate that products of HIV RT-catalyzed primer extension are accumulated mainly opposite the (dA)$_{16}$ sequence even after 120 min of incubation. Only by 60 and 120 min, respectively, 25 and 40% of the radioactivity appear in products extended past the insert (Fig. 2B).

Blocking of HIV RT at the (dA)$_{16}$ Tract Is Also Blocked at the (dA)$_{16}$ Tract—To examine whether, in addition to recombinant HIV RT, the (dA)$_{16}$ run constitutes a barrier to virion RT, the enzyme was purified directly from virions, and its ability to extend the "-40" primer over M13mp2 and M13(dA)$_{16}$ DNA templates was assayed. As evident in Fig. 3, even after 5 min of copying of M13mp2 DNA, the lowest concentration of enzyme catalyzed the accumulation of high molecular size products. By contrast, even after 60 min at 37 °C the highest concentrations of virion HIV RT produced very modest extension beyond the (dA)$_{16}$ insert on the M13(dA)$_{16}$ template. Hence, the virion and recombinant HIV RTs appear to be inhibited similarly from traversing beyond the (dA)$_{16}$ run within M13 DNA.

Blocking of HIV RT at the (dA)$_{16}$ Tract Is Not Alleviated by Varying the Conditions of the Polymerization Reaction—To examine whether the observed difficulty of HIV RT to extend the primer beyond the (dA)$_{16}$ insert is a result of specific reaction conditions, the major components in the assay were varied systematically. As seen in Fig. 4, HIV RT fails to extend the primer beyond the (dA)$_{16}$ insert in the presence of 20 or 70 mM KCl. In the absence of added KCl, synthesis proceeds one or two nucleotides further downstream within the (dA)$_{16}$ insert but does not extend the primer beyond it. By clear contrast, although the higher KCl concentration decreases to some extent the ability of HIV RT to form high molecular size products with M13mp2 and M13(dG)$_{16}$ DNA, both templates are copied extensively in the presence of 0–70 mM KCl (Fig. 4). The pattern of HIV RT-specific pausing at the (dA)$_{16}$ tract does not change significantly in the presence of 2 or 8 mM Mg$^{2+}$, and neither is it altered upon lowering the pH of the reaction mixture from 8.2 to 7.0 (Fig. 4). The effect of altering the dNTP concentration was examined using both AMV RT and HIV RT (Fig. 5). Not surprisingly, lowering the concentration of each dNTP from 0.1 to 0.0027 mM decreases the ability of both enzymes to traverse the insert. However, whereas HIV RT fails to traverse the (dA)$_{16}$ tract at all concentrations of dNTPs and at all incubation periods, AMV RT progressively catalyzes the formation of products well beyond the insert at all concentrations of dNTPs except at 2.0 mM (Fig. 5). The decreased processivity of both AMV and HIV RTs in the presence of each dNTP at 2.0 mM might conceivably be due to the titration of magnesium.

HIV RT Does Not Remain Bound to M13(dA)$_{16}$ DNA, nor Does It Inhibit Subsequent Template Extension by AMV RT—To study the mechanism of pausing opposite the (dA)$_{16}$ homooligomeric sequence, we preincubated HIV RT with unlabeled
Fig. 2. Kinetics of primer extension by recombinant HIV RT and AMV RT along M13(dA)$_{16}$ and unmodified M13mp2 DNA templates. A, each template was annealed to 5'-$^{32}$P-labeled 17-mer universal primer as described under "Methods." Primer extension reaction mixtures at a final volume of 100 µl were prepared for each of the two primed M13 templates. One 6X174 unit of activity of recombinant HIV RT or of AMV RT (see "Methods") was added for each reaction time point. The mixtures were incubated at 37 °C, and 15-µl aliquots were removed at the indicated time points. Shown are autoradiographs of the electrophoretically separated DNA products with positions of the primer and (dA)$_{16}$ insert within the M13(dA)$_{16}$ template indicated. B, products of the extension of 5'-$^{32}$P-labeled primer over M13(dA)$_{16}$ were synthesized and resolved electrophoretically as in A. Individual lanes were excised from the gel, and the distribution of radioactivity along each lane was quantified by Cerenkov radiation counting. ○, primer and first five nucleotide extension products; △, primer extension products opposite (dA)$_{16}$ insert area; O, primer extension products downstream of the (dA)$_{16}$ insert.

M13(dA)$_{16}$ or M13(dG)$_{16}$ DNA and then challenged with labeled M13mp2 DNA. In this series of experiments, HIV RT was preincubated for 5 min at 37 °C with increasing concentrations of unlabeled primed M13(dA)$_{16}$ or M13(dG)$_{16}$ DNA. Subsequently, M13mp2 DNA annealed to $^{32}$P-labeled primer was added to each enzyme-DNA mixture, and incubation was continued for an additional 30 min at 37 °C. Molar ratios of the second radioactively labeled M13mp2 DNA to the initial nonlabeled recombinant templates were 1:1, 1:10, 1:50, 1:100, and 1:500. As evident from results shown in Fig. 6, primer extension of the second M13mp2 DNA is inhibited by preincubation with the same concentration of the second radioactively labeled M13mp2 DNA to the initial nonlabeled recombinant templates. However, it is equally affected by preincubation with the same concentration of M13(dA)$_{16}$, which HIV RT cannot fully traverse or with M13(dG)$_{16}$, which HIV RT successfully traverses. Therefore, regardless of with which recombinant template HIV RT was preincubated, there is a roughly equal amount of free enzyme available at each template concentration to copy the added M13mp2 DNA template. These results indicate similar association-dissociation kinetics of the enzyme on both recombinant templates and therefore argue against irreversible binding by the polymerase at the insert.

Further evidence that synthesis by HIV RT does not inactivate the template-primer complex for further extension is demonstrated in Fig. 7. M13(dA)$_{16}$ DNA was annealed to $^{32}$P-labeled primer and preincubated with HIV RT for 15 min at 37 °C. Incubation was continued with AMV RT for 10, 30, or 60 min. Incubation of M13(dA)$_{16}$ with HIV RT alone did not result in extension of the template beyond the (dA)$_{16}$ insert (Fig. 7, panel A). Subsequent addition of AMV RT resulted
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**FIG. 3.** Kinetics of primer extension by purified virion HIV RT. M13(dA)$_{16}$ and unmodified M13mp2 DNA templates were annealed to 5'-32P-labeled 17-mer primer positioned 25 bases upstream ("-40" primer) to the EcoRI site of the (dA)$_{16}$ insert. Primer extension reaction mixtures at a final volume of 100 µl were prepared for each of the two primed M13 templates. Purified virion RT was added to each reaction mixture at a molar excess to template of approximately 1:1; 2:56:1; 3:111:1. Reactions were incubated at 37 °C under standard assay conditions, and 15-µl aliquots were removed at the indicated times. Shown are autoradiographs of the electrophoretically separated DNA products with positions of the primer and (dA)$_{16}$ insert within the M13(dA)$_{16}$ template indicated.

in primer extension beyond the (dA)$_{16}$ insert (Fig. 7, panel C), and the pattern is the same as that seen by incubation with AMV RT alone (Fig. 7, panel B). A linear increase in amount of extended product is observed in the presence of an increased concentration of AMV RT and/or longer times of incubation (results not shown). These results argue against irreversible template-primer alteration by HIV RT such as incorrect terminal nucleotide insertions or some other form of tertiary DNA complex unable to be extended by any polymerase.

**DISCUSSION**

The most likely sources for the high rate of mutation within the HIV genome are errors in copying by the viral reverse transcriptase. Analyses of the copying of natural DNA templates (Bebenek et al., 1989; Preston et al., 1988; Roberts et al., 1988) and synthetic poly(rA) (Takeuchi et al., 1988) by purified HIV RT indicate that this polymerase exhibits the lowest fidelity among retroviral reverse transcriptases so far analyzed. In addition, primer extension experiments have indicated that synthesis by HIV RT is highly distributive on both natural and synthetic templates, i.e. the enzyme dissociates from the template after very few nucleotide addition steps. This lack of processivity has been correlated with the generation of mutational hotspots primarily within repetitive sequences (Bebenek et al., 1989). In this study, we addressed directly the question of pausing by HIV RT in copying stretches of single or alternating purines and pyrimidines inserted within a natural DNA template. Wild-type and recombinant M13 genomes containing inserts of repetitive tracts of each of the four bases or alternating purines (d(A-G)) or pyrimidines (d(C-T)) were copied in vitro by purified HIV RT. The ability of both recombinant and viral RT to traverse the oligonucleotide inserts was determined by gel electrophoresis.

With all templates tested in this study, HIV RT produces more pause sites overall than AMV RT. Most conspicuously, however, the (dA)$_{16}$ insert within M13(dA)$_{16}$ DNA presents a unique obstacle to synthesis by both HIV and AMV reverse transcriptases; yet AMV RT is able to traverse the oligo(dA) tract and to synthesize DNA past this insert (Figs. 2 and 5). Also, the large (Klenow) fragment of E. coli polymerase I and calf thymus DNA polymerase a were shown recently to have no difficulty in traversing the (dA)$_{16}$ insert in recombinant M13 DNA (Weisman-Shomer et al., 1989). By clear contrast, HIV RT traverses this block poorly even when it is added at a large excess over the template and synthesizes for extended periods (Figs. 1–3). Further, as shown recently for other pause sites of HIV RT (Bebenek et al., 1989), virion and recombinant form RT are indistinguishably blocked at the oligo(dA)
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Fig. 5. Effect of altered dNTPs concentration on pause sites of recombinant HIV RT and AMV RT along M13(dA)16. M13(dA)16 DNA template was annealed to 5'-32P-labeled 17-mer universal primer, and two primer extension reaction mixtures at a final volume of 50 µl were prepared for each examined concentration of dNTPs. One φX174 unit of activity of recombinant HIV RT or of AMV RT (see "Methods") was added for each reaction time point, the mixtures were incubated at 37 °C, and 15-µl aliquots were removed at the indicated times. Shown are autoradiographs of the electrophoretically separated DNA products with positions of the primer and (dA)16 insert indicated.

Fig. 6. Pause sites of recombinant HIV RT along M13mp2 DNA in the presence of excess M13(dA)16 or M13(dG)16 DNA. M13mp2 DNA was annealed to 5'-32P-labeled universal primer, and M13(dA)16 and M13(dG)16 DNA were annealed to unlabeled universal primer as described under "Methods." Equal amounts of recombinant HIV RT were preincubated at 37 °C for 5 min with different concentrations of unlabelled primed M13(dA)16 or M13(dG)16 DNA. Thereafter, 1.4 × 10⁻¹ pmol of radioactively labeled M13mp2 DNA was added to each reaction mixture, and incubation at 37 °C was continued for an additional period of 30 min. Molar ratios of HIV RT to the increasing concentrations of unlabelled recombinant templates were 10:1, 1:1, 1:5, 1:10, and 1:50, and the molar ratio of HIV RT to radioactively labeled M13mp2 DNA template was 10:1 in all reactions. The first three lanes (from the left) show the distribution of primer extension products from reactions that contained a single indicated radioactively labeled template and no competitor. A designates reaction mixtures containing an indicated molar excess of unlabeled M13(dA)16 DNA over radioactively labeled M13mp2 DNA; G designates reactions containing an indicated molar excess of unlabeled M13(dG)16 DNA.

There are at least three possible explanations for the accumulation of newly synthesized DNA opposite the oligo(dA)16 stretches. (i) HIV reverse transcriptase could dissociate from the template more frequently when copying over oligo(dA)16 stretches, causing increased termination of DNA synthesis at these sites. (ii) HIV reverse transcriptase could bind to these sites with a lower affinity than other sites on the template, causing increased pausing of the enzyme. (iii) HIV reverse transcriptase could bind to these sites with a higher affinity than other sites on the template, causing increased pausing of the enzyme.
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FIG. 7. Effect of subsequent addition of AMV RT to primer extension of M13(dA)16 by HIV RT. M13(dA)16 DNA was annealed to 5'-32P-labeled 17-mer universal primer, and each primed template was extended at 37 °C for the indicated time periods as described under "Methods." Panels A and B are control primer extension reactions containing 1.6 X 174 units of HIV RT or AMV RT for each of the indicated time points. In panel C, 1.6 X 174 unit of activity of recombinant HIV RT (for each of the indicated time points) was preincubated with the M13(dA)16 template at 37 °C for 15 min. Equal units of AMV RT were then added to the reaction mixture and 15-μl aliquots removed at the indicated time points. Shown are autoradiographs of the electrophoretically separated DNA products with positions of the primer and (dA)16 insert within the M13(dA)16 template indicated (dN)16.

The enzyme could become bound irreversibly to the oligo(dA)16 tract, causing further elongation to be blocked.

3) HIV RT could remain bound to these stretches and catalyze chain elongation at a decreased rate. To distinguish among these possibilities, template challenge experiments were performed. The results of these experiments eliminate at least one of the above possibilities. The degree of extension of labeled primer along M13mp2 DNA by HIV RT was roughly the same whether the enzyme was preincubated with increasing molar excess of unlabeled M13(dA)16 or M13(dG)16 DNA (Fig. 6). If HIV RT were to bind irreversibly to the (dA)16 insert, creating a physical enzyme-template barrier, a decreased extent of copying of M13mp2 DNA due to decreased availability of free enzyme would be apparent.

These experiments also indicate that the kinetics of association-dissociation of HIV RT from the template appear to be independent of the nature of the insert. If the enzyme were to dissociate more frequently from the (dA)16 insert, an increased extent of copying of M13mp2 DNA should be apparent in mixtures exposed to competing M13(dA)16 DNA because of greater availability of free enzyme relative to mixtures exposed to M13(dG)16 DNA. Hence, association and dissociation of HIV RT from the template, as reflected in the competition assay, appear to be unaffected by the initial template. The results of these experiments therefore favor a decreased rate of chain elongation at each nucleotide addition step opposite oligo(dA)16 stretches, without increased enzyme-template dissociation. However, one cannot unambiguously eliminate the possibility of increased dissociation of the enzyme from the template opposite the oligo(dA)16 stretches.

The observed sharp increase in pausing of HIV RT opposite oligoadenyl tract could be ascribed to physical constraints imposed by template secondary structure and/or surrounding the (dA)16 insert. However, with the exception of MMLV RT, this strong stoppage is not seen with several other polymerases. Hence, without the formation of a complex with a specific polymerase or reverse transcriptase, template secondary structure may not play a significant role in this phenomenon.

Reverse transcriptases, overall, have higher error rates than other DNA polymerases (Steinhauer and Holland, 1987; Temin, 1988). This lack of fidelity is commonly attributed to the lack of an associated proofreading exonuclease. However, the average in vitro error rate of HIV RT on wild-type templates is 10-fold higher than that of other reverse transcriptases (Preston et al., 1988; Roberts et al., 1988). This high degree of infidelity of HIV RT correlates with the high level of genomic variability in all isolates of HIV so far studied (Hahn et al., 1986; Fisher et al., 1988; Saag et al., 1988; Myers et al., 1989). In addition, the HIV envelope (env) gene exhibits significantly increased variability relative to the rest of the genome (Coffin, 1986; Willey et al., 1986; Starcich et al., 1986; Modrow et al., 1987). Although it is speculated that the increased mutation rate within the env gene is required in order for the virus to evade the host's immune system, the mechanism of this augmented hypervariability has not yet been elucidated.

We have observed strong pausing of HIV reverse transcriptase opposite homo-oligomeric (dA) stretches in vitro, which has also been associated with increased errors (Bebenek et al., 1988). Takeuchi et al. (1988) demonstrated that in copying a poly(rA) template HIV RT exhibits the lowest fidelity among several tested reverse transcriptases. A computer analysis comparing all reported genomic sequences of HIV isolates (Myers et al., 1989) indicates that the amount and variability of oligo(A) tracts (≥5 rA) within the viral env gene is significantly higher than all other comparable repetitive sequences in all other regions of the viral genome (data not shown). If HIV RT also copies homo-oligomeric (rA) stretches with decreased fidelity in vivo, this could possibly account for the increased variation of these stretches within the env gene of the HIV genomic isolates.

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Synthesis of DNA by human immunodeficiency virus reverse transcriptase is preferentially blocked at template oligo(deoxyadenosine) tracts.

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