Identification of a Simian Immunodeficiency Virus Reverse Transcriptase Variant with Enhanced Replicational Fidelity in the Late Stage of Viral Infection*

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Genomic hypermutation of human and simian immunodeficiency viruses (HIV and SIV) enables these viruses to adapt and escape from various types of antiviral selection by altering the molecular properties of viral gene products. In this study, we examined whether the biochemical and catalytic properties of SIV DNA polymerases (reverse transcriptases; RT) can change during the course of viral infection. For this test, we analyzed RTs obtained from two SIV clones, SIVMNE CL8 and SIVMNE 170. SIVMNE 170 was isolated during the late symptomatic phase of infection with the parental strain, SIVMNE CL8. We found these two RTs have identical DNA polymerase specific activities and kinetics with three different DNA and RNA templates. In addition, the processivity of these two SIV RT proteins were also similar. However, as demonstrated by a misincorporation assay, the SIVMNE 170 RT showed much higher fidelity than SIVMNE CL8. The fidelity difference between these two SIV RTs was also confirmed by a steady state kinetic fidelity assay. These findings suggest that the fidelity of lentiviral RTs may change during the course of viral infection, possibly in response to alterations of host anti-viral immune capability. In addition, our sequence analysis of these two RT genes proposes possible structural strategies that the virus may employ to alter RT fidelity.

Evolution of infectious parasitic organisms is driven by host defense mechanisms that generate various types of anti-parasite selective pressures during the course of infection. Microorganisms that undergo escape from host selection systems are equipped with their own defense devices that can antagonize the anti-parasite mechanisms of the infected hosts. In the case of lentiviruses such as human and simian immunodeficiency viruses (HIV and SIV), genomic hypervariability has been considered to be an evolutionary tool that allows the viruses to escape from highly developed host anti-viral immune responses (1–3).

Alterations in the biochemical and structural properties of virally encoded proteins have consistently been observed during the course of lentiviral infection (4, 5). Genomic hypermutation of lentiviruses underlies these molecular and functional shifts of viral gene products. For example, hypermutation at the variable (V) regions of lentiviral envelope proteins enables the virus to alter Env-related virological properties (i.e. cell tropism, infectivity, and cytopathicity) (6, 7). These molecular alterations of env genes allow for adaptation and selection of the viral population, leading to viral escape from the host anti-viral immune system. However, even with constant molecular diversification of viral envelope protein, it is obvious that fundamental biological functions of the envelope protein must remain intact (8). Sequence variation has also been observed in the pol gene, which encodes the viral DNA polymerase (reverse transcriptase; RT) (9). It is also obvious that the basic enzymatic activities of RT, such as DNA polymerase and RNase H activities, should remain active enough to support viral replication. However, it is not clear whether other biochemical properties of lentiviral RTs such as replicational fidelity remain constant in the face of sequence variations during the course of viral infection.

Lentiviral RTs are the most error prone DNA polymerases known to be involved in DNA replication (10, 11), and RT infidelity is thought to be the major driving force behind the generation of new viral variants, which are essential for viral escape from host immune pressure (10, 12). In fact the level of host immune capability gradually but progressively decreases during the course of lentiviral infection, ultimately declining to a level that results in clinically apparent immune deficiency (i.e. AIDS) and in overwhelming opportunistic infections. Therefore, it is a logical assumption that the viral capability to mutate, evolve, and escape may also vary, particularly as host anti-viral selective pressure becomes diminished during the late stages of viral infection. Therefore, if RT infidelity is a source of viral hypermutagenesis, it is possible that the level of RT fidelity may change when the virus grows under conditions of reduced or no anti-viral selective pressure (i.e. the symptomatic phase of infection). Even though several RT mutants with slightly increased fidelity have been isolated following the anti-viral chemotherapy (13, 14), RT mutants with greatly enhanced fidelity have not been isolated from the natural lentiviral population. Therefore, whether RT fidelity changes during the course of viral infection remains unanswered.

In this study, we examined the enzymatic properties of SIV RTs obtained from two representative viruses (SIVMNE CL8 and SIVMNE 170). Alterations in the biochemical and catalytic properties of SIVMNE CL8 and SIVMNE 170 RTs were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™ EBI Data Bank with accession number(s) M32741 (SIVMNE CL8), AF361745 (SIVMNE 170), and M32682 (SIVmac239). To whom correspondence should be addressed: 601 Elmwood Ave., Box 672, Dept. of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY 14627. Tel.: 716-275-6916; Fax: 716-473-9573; E-mail: baek_kim@urmc.rochester.edu.

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† The abbreviations used are: RT, reverse transcriptase; T/P, template/primer.
and SIVMNE 170) that were isolated from the same animal at two different stages of infection (15–17). SIVMNE 170 is a molecular clone of SIVMNE that was isolated from a pig-tailed macaque during the late symptomatic phase of infection with the parental SIVMNE CL8 virus (17, 18). These two clones have been used as representative clones to investigate alterations in various viral phenotypes (i.e. co-receptor use, cytopathicity, and pathogenicity) during lentiviral infection (15–19).

In this report, we present evidence that SIVMNE 170 RT has much higher fidelity than the RT of initial SIVMNE CL8. This finding suggests that RT fidelity can change during the course of viral infection. Presumably, this fidelity change could be due to a viral response to alterations in host immune capability. Possible molecular and structural strategies that the virus may employ to alter its RT fidelity are also discussed.

**EXPERIMENTAL PROCEDURES**

**Plasmids and Strains—**Escherichia coli NM522 (Strategene, La Jolla, CA) was used for construction of plasmids, and BL21 (Novagen, Madison, WI) for overexpression of SIVMNE RT proteins. pBK90 and 125 clones of BL21 were transformed with double stranded (20 ng per reaction) SIVMNE CL8 and 170 RT genes, fused at the N-terminus to a 3′ histidine residues, respectively. The RT genes were derived from pSIVMNE170 and pSIVMNECL8 (15, 16, 18). pSIVmac239-5′ was obtained from Dr. Stephen Dinhurst (University of Rochester).

**Cloning of RT Genes from SIVMNE CL8 and 170 Viral DNAs—**For the expression plasmids for SIVMNE CL8 and 170 RTs, RT genes were amplified by Pfu DNA polymerase (CLONTECH, San Francisco, CA) from pSIVMNECL8 and pSIVMNE170 by using SRT SD F forward (5′-TTCGAGTCGTGATGCCATGC-3′) and the expression plasmids for SIVMNE CL8 and 170 RT genes were derived from pSIVMNE170 and pSIVMNECL8 (15–19).

**Sequencing of Cloned SIVMNE CL8 and 170 RT Genes—**The 396-nucleotide fragment of N-T regions were used for DNA sequencing. Two independent clones of each SIV RT gene were analyzed for both sequencing and biochemical analysis.

**Extension of Mismatched Primers—**To measure RTs capability to extend mismatch primer, we used two different mismatched primers annealed to either the RNA or DNA template used in the misincorporation assay. 16-mer G/T mismatched primer (5′-AACCGGGCGCGAATTGCTGCGGATGCGCGCCG-3′) to a 17-mer (5′-CCGGCGCGGACATTCGCGGCTGCTG-3′) was used to determine misinsertion frequencies. Reaction mixtures (20 μl) contained 10 μM template/primer, 10 to 40 μM SIVMNE RT proteins as specified in the figure legends, 3 or 4 dNTPs (25 μM each), [3H]TTP (10 μM, 1000 CPM, 25 μCi/μl). The incorporation of [3H]TP was linear up to 15 min incubation time under this condition. The protocol for this assay was previously described (22).

**Misincorporation Assay with DNA or RNA Templates—**Procedures were modified from those of Presten et al. (11). The DNA template-primer was prepared by annealing a 40-mer 5′-GAAGCUGUCGGACGAATATTGCTGCGGATGCGGGCGG-3′ (polypolymerase RNA template-primer is a 3,569-nucleotide long MS2 phage (Roche Molecular Biochemicals) annealed to 20-mer DNA primer (5′-GGCTTAGAATCAGCGGGAATTCGGCGCG-3′, Roche Molecular Biochemicals, Branchburg, NJ). Reaction mixtures (20 μl) contained 2 μM SIVMNE RT protein, dNTPs (100 μM each), [3H]TTP (10 μM, 1000 CPM), 25 μM Tris-HCl (pH 8.0), 100 mM KCl, 2 mM dithiothreitol, 5 mM MgCl2, 0.1 mg/ml bovine serum albumin and either gapped salmon sperm DNA (5 μg), polyA(VdG)oligo(dT) (1 μg), or heterologous RNA annealed 17-mer DNA (1 ng), incubated at 37 °C for 5 min, followed by addition of 400 μl of 10% trichloroacetic acid. The incorporation of [3H]TTP was linear up to 15 min incubation time under this condition.

**RT Fidelity Alteration during SIV Infection**
amplified from pSIVmac239–5 pressing SIVmac239 RT was constructed using SIVmac239 RT genes sequencing system (see above). 184 mutations created in pBK90 and pBK125 were identified by ABI and of pBK90 and pBK125 digested with M and I, respectively. Met-184 mutations (bold) and I mutations (underlined) were constructed by polymerase chain reaction-based site-directed mutagenesis. For N-T RT polymerase chain reaction, two different mutagenesis reverse primers (5′-TTTTTTTTTTTCTAGCTACTAAGTGTCATCTAC-3′; for M184V (SIVMNE CL8 RT) or pBK125 (SIVMNE 170 RT) by Pfu DNA polymerase. A C-terminal 243-amino acid long fragment (residue 190–433) of SIV RTs were amplified with two primers, SacI SIV RT primer (5′-TTTTTTTTTTCTAGCTACGGACAGAAGACCTGGAAA-3′) encoding (+)-strand sequences of the YMDD region and SacI (underlined), and SpeI SIV RT primer (5′-CTTACTAGTGGGTGTTGTTGTTGTTG-3′) encoding (−)-strand sequence of SpeI region (RT amino acids 426–433). These two amplified N-T and C-T fragments were double-digested by NdeI and SacI, respectively. These two digested fragments of SIVMNE CL8 and 170 RTs were then inserted into 3.5-kilobase backbone DNAs of pBK90 and pBK125 digested with NdeI and SpeI, respectively. Met-184 mutations created in pBK90 and pBK125 were identified by ABI sequencing system (see above).

Construction and Purification of SIVmac239 RT—A plasmid expressing SIVmac239 RT was constructed using SIVmac239 RT genes amplified from pSIVmac239–5′ by using the SRT SD F and SRT C-T R primers that were used for construction of pBK90 and pBK125 (see above). SIVmac239 RT protein was purified by using the same protocol as one used for SIVMNE RT proteins (see above).

Processivity Assay—Reaction condition for measuring processivity requires a single round of primer extension (25, 26). RT proteins were preincubated with 20 nM poly(rA) (average size = 260 nucleotides, Amersham Pharmacia Biotech, Piscataway, NJ) annealed to the 32P-labeled 20-mer oligo(dT) (Amersham Pharmacia Biotech, Piscataway, NJ) for 3 min at 20 °C. The concentrations of wild-type RTs and M184I RTs of SIVMNE CL8 and 170 were 10 and 25 nM, respectively. The extension reactions were initiated by adding the trap mixture containing dNTPs (0.5 mM with final concentration 5 mM MgCl2), molar excess of cold poly(rA)/oligo(dT) (20 μM) and heparin (10 μg/20 μl). The extension reactions were terminated with 4 μl of stop solution after 3 min incubation at 37 °C. Two control reactions were performed as described (25, 26), confirming the single round of primer extension under this reaction condition. In the +Trap control reaction, the trap mixture was added during preincubation of RT and T/P, which prevents RT from extending the primer. In the −Trap reaction, the T/P preincubated with RT was extended by only dNTP in the absence of the trap molecules, which allows multiple round of primer extension. The terminated processivity reaction and control reactions were analyzed by 8% polyacrylamide-urea gel after 3 min heat inactivation.

RESULTS

DNA Polymerase Activities of Purified SIVMNE RT Proteins—To test whether the DNA polymerase activity of lentiviral RT can change during the course of viral infection, we purified RT proteins from two SIV representative clones, SIVMNE CL8 (early asymptomatic phase) and SIVMNE 170 (late symptomatic phase) using a bacterial overexpression system. We determined the DNA polymerase specific activities of these two purified SIVMNE RT proteins with a heteropolymeric DNA template and two homo- and heteropolymeric RNA templates. As shown in Table I, these two SIV RT proteins showed very similar levels of DNA- and RNA-dependent DNA polymerase activities with both heterogenous and homogenous DNA and RNA templates. The similarity of DNA polymerase activity between these two SIV RT proteins was also confirmed by the RT assay, using a gel-based primer extension reaction (see below and Fig. 1, lanes containing all dNTPs), and steady state kinetic measurement (Km and kcat for correct dNTPs, dCTP, and dGTP; Fig. 3 and Table II). We purified RTs from two independent clones of each SIVMNE strain. Purified RT proteins from two independent clones of each SIVMNE also showed similar specific activities with both DNA and RNA templates. This result shows that the DNA polymerase catalytic activities of these two SIVMNE RTs, isolated in different stages of viral infection, are largely identical.

Misincorporation Assay with Matched Primer—Next, we examined the fidelity of SIVMNE CL8 and 170 RT proteins using the misincorporation assay. This is a primer extension assay that monitors both misinsertion and mismatch extension of a primer. We tested both dNTPs primer annealed to 40-mer RNA (A) or DNA (B) template. The 32P-labeled 17-mer primer (“S” primer) annealed to 40-mer RNA (A) or DNA (B) template was extended by the same concentrations (10 nM for RNA template reactions; 40 nM for DNA template reactions) of SIVMNE CL8 and 170 RT proteins at 37 °C for 5 min. The extension reactions were performed in the presence of all 4, or only 3 complementary dNTPs (minus dATP and minus dCTP for RNA template reaction; minus dATP and minus TTP for DNA template reaction). The reactions were analyzed by 14% polyacrylamide-urea denaturing gel. The sequence of the extended part of the primer is shown. F, fully extended products; S, un-extended primer; “*”, stop sites.
RT Fidelity Alteration during SIV Infection

**TABLE II**

Misinsertion fidelity \( f_{\text{ins}} \) of SIVMNE CL8 and 170 RTs in G to A viral mutation events

| RTs   | Template | Correct dNTPs | Incorrect dNTPs | \( f_{\text{ins}} \) | Fold changea |
|-------|----------|--------------|-----------------|----------------------|--------------|
| CL8   | RNA\(^{c}\) | 60 \( nM \) \( k_{\text{cat}} \) 2.67 | 110 \( \mu M \) \( k_{\text{cat}} \) 0.13 | 2.7 \( \times \) 10\(^{-5} \) | \( \times 1 \) |
| 170   | RNA      | 62 \( nM \) \( k_{\text{cat}} \) 2.48 | 215 \( \mu M \) \( k_{\text{cat}} \) 0.03 | 3.5 \( \times \) 10\(^{-5} \) | \( \times 7.7 \) |
| CL8   | DNA\(^{d}\) | 76 \( nM \) \( k_{\text{cat}} \) 0.59 | 205 \( \mu M \) \( k_{\text{cat}} \) 0.06 | 3.8 \( \times \) 10\(^{-5} \) | \( \times 1 \) |
| 170   | DNA      | 80 \( nM \) \( k_{\text{cat}} \) 0.69 | ND\(^{e}\) \( <0.006' \) | 0.034 \( \times \) 10\(^{-5} \) | \( \times 11 \) |

\(^{a}\) Misinsertion fidelity, \( f_{\text{ins}} = (k_{\text{cat}}/K_{m}) \) with incorrect dNTP/\( k_{\text{cat}}/K_{m} \) with correct dNTP.

\(^{b}\) Fold difference = \( f_{\text{ins}} \) of CL8 RT/\( f_{\text{ins}} \) of 170.

\(^{c}\) First DNA strand synthesis with correct dCTP and incorrect TTP.

\(^{d}\) Second DNA strand synthesis with correct dGTP and incorrect dATP.

\(^{e}\) \( K_{m} \) and \( k_{\text{cat}} \) values of 170 RT for incorrect (dATP) could not be determined due to little misinsertion (<5% of the primer) even at the highest 170 RT protein concentration. The minimum \( f_{\text{ins}} \) difference was calculated by the minimum \( k_{\text{cat}} \) difference (10-fold difference) and similar \( K_{m} \) value with CL8 RT.

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**FIG. 2.** Extension of matched (A) and two mismatched (B and C) primers by SIVMNE 170 and CL8 RT proteins. The \(^{32}\)P-labeled 15-mer matched (“S”) primer (A) and 16-mer G/T mismatch (“MP”) primer (B) annealed to 40-mer RNA template (A) were extended by two quantities (4 \( \times \) and 1 \( \times \), 10 and 2.5 \( \times \)) of SIVMNE CL8 and 170 RT proteins at 37 °C for 5 min. The \(^{32}\)P-labeled 19-mer C/A mismatched primer (C) annealed to 40-mer DNA template (B) was also extended by two quantities (4 \( \times \) and 1 \( \times \), 40 and 10 \( \times \)) of two SIV RT proteins. The extension reactions were performed in the presence of all 4 dNTPs. The reactions were analyzed by 14% polyacrylamide-urea denaturing gel. The sequence of the extended part of the primer is shown.

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**FIG. 3.** A steady state kinetic fidelity assay for C to T mutation in the first DNA strand synthesis. The \(^{32}\)P-labeled 15-mer C primer (arrow) annealed to the 38-mer RNA template was extended in the presence of different concentrations of dCTP (correct) or TTP (incorrect) by SIVMNE CL8 (A) or 170 (B) RTs. The concentrations of SIVMNE CL8 and 170 RT proteins for correct and incorrect dNTPs were 20 and 250 \( nM \), respectively. Different dNTP concentrations were used in order to obtain values within the linear range. The concentrations of dCTP used were 6, 16, 32, 64, 125, 250, 500, and 1000 \( nM \), and the concentrations of TTP used were 6.25, 12.5, 25, 50, 100, 200, 400, and 800 \( \mu M \). These concentrations were accounted for in calculating kinetic values. These data were used to calculate \( K_{m} \) and \( k_{\text{cat}} \) values which were used to determine misinsertion fidelity \( f_{\text{ins}} \) (Table II). Arrow, un-extended primer.

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In the misincorporation assay shown in Fig. 1, extension of primer beyond the stop sites measured the capability of the RT proteins to misinsert and then to extend the mismatched primer. Next, we examined the capability of SIVMNE 170 and CL8 RT proteins only to extend the mismatched primer (2nd step of mutation synthesis). In this study, we used two different mismatched primers (MP) annealed to either RNA or DNA template. One T/P contains a G/T mismatch at the 3' end of the primer annealed to the RNA template. The other T/P contains a C/A mismatch at the 3' end of the primer annealed to the DNA template. These C/T (1st DNA strand synthesis) and C/A (2nd DNA strand synthesis) mismatched T/Ps represent the replication intermediates that can be synthesized during the process of G to A mutation, which is the predominant lentiviral mutation (27). We tested whether SIVMNE 170 RT (high fidelity) has a reduced capability to extend these two mismatched...
TPs for creating the G to A mutation. The primer extension reactions were performed with all dNTPs, which allowed the extension of the matched and mismatched primers to the end of the templates. As seen in Fig. 2A, in reactions with the matched primer, both SIVMNE 170 and CL8 RT proteins showed the same level of total primer extension (or remaining unextended primer), indicating that the total RT activities of these RT proteins added in this assay were the same. However, SIVMNE 170 RT protein showed a much lower level of primer extension from both the G/T (Fig. 2B) and C/A (Fig. 2C) mismatched primers than SIVMNE CL8 RT protein. These results suggest that the SIVMNE 170 RT has a reduced capability to extend the mismatched primer, compared with SIVMNE CL8 RT.

Kinetic Measurements for Misinsertion Fidelity of Two SIVMNE RTs—Next, we employed a steady state kinetic assay for measuring misinsertion fidelity (f_{ins}) SIVMNE CL8 and 170 RT proteins. The f_{ins} value is calculated by two kinetic parameters, k_{cat} and K_{m}, which are indicative of the capability of DNA polymerases to incorporate dNTPs (correct or incorrect). Since the G to A mutation is the most dominant mutation in lentiviral mutagenesis (27), we measured misinsertion fidelity for the C (correct) to T (incorrect) mutation using an RNA template (1st DNA strand synthesis: Fig. 2, Table I) and the G (correct) to A (incorrect) mutation using a DNA template (2nd DNA strand synthesis: Table I). The k_{cat} and K_{m} values of two SIV RT proteins with correct versus incorrect nucleotides were measured by a gel-based analysis and Hanes-Woolf equation (24). As seen in Table II, and consistent with the misinsertion assay data (Figs. 1 and 2), SIVMNE 170 RT shows 8 and 11 times higher misinsertion fidelity than SIVMNE CL8 RT in the G to A viral mutation events.

Effect of Met-184 Mutations on Fidelity of SIVMNE RT Proteins—3TC-resistant RT mutations, M184V and M184I, have been reported to be high fidelity RTs in HIV, even though the M184V mutant showed only a very slight increase (∼2 times) (28) of fidelity, compared with M184I (4 times) (28). Treatment of SIV with 3TC also results in the development of M184 mutations (29). We tested whether M184I high fidelity mutations affect the fidelity of SIVMNE CL8 and 170 wild type RTs by using the misinsertion assay. As seen in Fig. 4B (minus dATP), the M184I mutant of SIVMNE CL8 showed a reduced level of misinsertion compared with wild type SIVMNE CL8 RT, indicating that M184I enhances the fidelity of both SIV RT proteins. However, the M184V mutant of SIVMNE CL8 showed a wild type (high) level of misinsertion. The M184I mutation also exerted similar effects on both SIVMNE RTs in misinsertion assays with different deleted dNTPs (−dGTP, −dTMP, and −dCTP; data not shown). These findings suggest that the fidelity of both SIVMNE CL8 and 170 RTs can be enhanced by the M184I mutation.

Misinsertion Assay with SIVMNE170 and SIVmac239 RT Proteins—We compared the fidelity of SIVMNE 170 RT and SIVmac239 RT by using the misinsertion assay. Two concentrations (4× and 1×) of RT proteins were used in this assay. As shown in Fig. 5, SIVMNE 170 RT showed less extension of the primer beyond the stop sites (Fig. 5F for −T), indicating that SIVMNE 170 RT has higher fidelity than SIVmac239. Similar differences between SIVMNE 170 and SIVmac239 RTs in primer extension assays were also observed in reactions with other kinds of biased dNTP pools (data not shown). This result shows that SIVmac239 RT, like SIVMNE CL8 RT, has low replicational fidelity.

Processivity Assay with SIVMNE RT Proteins—We examined the processivity of the two SIVMNE RTs by using poly(rA) (template/oligo(dT) (primer). The processivity assay requires a single round of primer extension, which can be established by using trap molecules (heparin and molar excess of cold T/P). As shown in two trap controls (Fig. 6), primer extension was inhibited by trap molecules which were added at the beginning of the reaction (+Trap control), whereas primer extension was made in multiple rounds in the absence of the trap molecule, as shown in synthesis of long products (−Trap control). As shown in the processivity reaction (Proc) where the RT molecules pre-bound to T/P can extend the primer only once, both SIVMNE CL8 and 170 RTs showed identical processivity. It has been shown that M184I mutation reduces processivity of HIV-1 RT (30). As shown in Fig. 6, M184I mutants of both SIVMNE CL8 and 170 RTs also showed reduced processivity, suggesting that the processivity of both SIV RTs was affected by the M184I mutation.

Sequence Comparison of SIVMNE CL8 and 170 RT—We determined the sequence of the SIVMNE 170 RT gene between residues 1 and 396 (this corresponds to the DNA polymerase domain of RT). By sequence comparison of SIVMNE CL8 and 170 RT genes, we found that six residues in this region have sequence variations between these two SIVMNE RT genes (Fig. 7). Residues affected are 73, 148, 173, 211, 303, and 332 (Fig. 7). We also compared the sequences of these two SIVMNE RT genes with that of the SIVmac239 RT gene (31). As shown above, both SIVmac239 and SIVMNE CL8 RTs have low fidelity.
ity, compared with SIVMNE 170 RT. Among the 6 residues showing variations between SIVMNE CL8 and 170 RTs, four residues (148, 173, 211, and 303) of SIVMNE 170 RT also show variations from SIVmac239 RT. The location of these residues of SIVMNE RT was examined by using a structural model of HIV-1 RT solved by Huang et al. (32). Interestingly, none of the six residues, which differ between SIVMNE 170 RT and CL8 RT (or SIVmac239 RT), directly interact with RT substrate molecules (template, primer and incoming dNTPs). Furthermore, none of the six putative SIVMNE 170 fidelity mutations overlap with previously defined HIV-1 RT fidelity mutations (Fig. 7).

DISCUSSION

Genomic hypervariability of lentiviruses constantly shifts molecular and functional properties of viral gene products during the course of viral infection. For example, functional modifications of the lentiviral envelope gene result in temporal, but essential, alterations in viral infectivity, cellular tropism, cytopathicity, and in vivo pathogenesis (escape from host immune). In this study, we examined whether biochemical properties of lentiviral RTs, DNA polymerase activities, fidelity, and processivity, can also be altered during the course of viral infection. For this test, we have analyzed SIV RT proteins derived from two cloned SIV strains, SIVMNE CL8 and SIVMNE 170, that are representative clones for asymptomatic (early) and symptomatic (late) phases of viral infection, respectively (15–18). SIVMNE 170 clone was obtained from the symptomatic (late) phase of infection of a macaque initially inoculated with SIVMNE CL8 (the parent strain). These two representative clones have been used previously to understand alterations in various viral phenotypes (i.e. co-receptor use, replication kinetics, cytopathicity, and pathogenicity) during lentiviral pathogenesis (15–19).

For biochemical analysis of these two SIV RTs, we purified these RT proteins from a bacterial overexpression system. DNA polymerase assays with three different DNA and RNA templates revealed that these two SIVMNE RTs contain almost identical DNA- and RNA-dependent DNA polymerase specific activities. These two proteins also showed identical processivity (Fig. 6). These two controls show that there was only single round of primer extension in the processivity assay. All reactions were analyzed by 8% polyacrylamide denaturing gel electrophoresis. L, 100 base pair ladder.
fidelity with respect to the generation of the predominant G to A mutation (27). Since the insertion fidelities of SIVMNE 170 RT for both RNA- and DNA-dependent DNA polymerase activities were higher than those of SIVMNE CL8 RT, it is likely that SIVMNE 170 may produce less mutations in both 1st and 2nd strand syntheses of viral replication.

3TC resistant RT mutations, M184V and M184I, were initially reported to be high fidelity HIV-1 RTs, even though later biochemical studies showed that the overall mutation rate of M184V is very similar to that of wild type. A recent in vivo study also showed that M184V mutant HIV-1 did not show an altered mutation rate, compared with wild type virus (34). In contrast to M184V, the overall mutation rate of the M184I mutation is slightly higher (~3 times) than wild type. As shown in our misincorporation assay with SIVMNE CL8 wild type and SIVMNE 170 RT residues (148, 173, 211, and 303) were also different between the SIVMNE 170 and CL8 RT. Four of these SIVMNE 170 RT residues (148, 173, 211, and 303) were also different between SIVMNE CL8 and SIVmac239 RT. Indeed, the M184I mutation was able to enhance fidelity of both SIVMNE 170 and SIVMNE CL8 RT. Since the M184V mutation can further alter mutation rate, compared with wild type virus (34). In contrast to M184V, the overall mutation rate of the M184I mutation is slightly higher (~3 times) than wild type. As shown in our misincorporation assay with SIVMNE CL8 wild type and SIVMNE 170 RT residues (148, 173, 211, and 303) were also different between the SIVMNE 170 and CL8 RT. Four of these SIVMNE 170 RT residues (148, 173, 211, and 303) were also different between SIVMNE CL8 and SIVmac239 RT. Indeed, the M184I mutation was able to enhance fidelity of both SIVMNE 170 and SIVMNE CL8 RT. Since the M184V mutation can further alter mutation rate, compared with wild type virus (34). In contrast to M184V, the overall mutation rate of the M184I mutation is slightly higher (~3 times) than wild type. As shown in our misincorporation assay with SIVMNE CL8 wild type and SIVMNE 170 RT residues (148, 173, 211, and 303) were also different between the SIVMNE 170 and CL8 RT. Four of these SIVMNE 170 RT residues (148, 173, 211, and 303) were also different between SIVMNE CL8 and SIVmac239 RT. Indeed, the M184I mutation was able to enhance fidelity of both SIVMNE 170 and SIVMNE CL8 RT. Since the M184V mutation can further alter mutation rate, compared with wild type virus (34). In contrast to M184V, the overall mutation rate of the M184I mutation is slightly higher (~3 times) than wild type. As shown in our misincorporation assay with SIVMNE CL8 wild type and SIVMNE 170 RT residues (148, 173, 211, and 303) were also different between the SIVMNE 170 and CL8 RT. Four of these SIVMNE 170 RT residues (148, 173, 211, and 303) were also different between SIVMNE CL8 and SIVmac239 RT. Indeed, the M184I mutation was able to enhance fidelity of both SIVMNE 170 and SIVMNE CL8 RT. Since the M184V mutation can further alter mutation rate, compared with wild type virus (34). In contrast to M184V, the overall mutation rate of the M184I mutation is slightly higher (~3 times) than wild type. As shown in our misincorporation assay with SIVMNE CL8 wild type and SIVMNE 170 RT residues (148, 173, 211, and 303) were also different between the SIVMNE 170 and CL8 RT. Four of these SIVMNE 170 RT residues (148, 173, 211, and 303) were also different between SIVMNE CL8 and SIVmac239 RT. Indeed, the M184I mutation was able to enhance fidelity of both SIVMNE 170 and SIVMNE CL8 RT.
RT Fidelity Alteration during SIV Infection

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