Potential Mechanism for Sustained Antiretroviral Efficacy of AZT-3TC Combination Therapy

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Combinations of antiretroviral drugs that prevent or delay the appearance of drug-resistant human immunodeficiency virus–type 1 (HIV-1) mutants are urgently required. Mutants resistant to 3′-azidothymidine (AZT, zidovudine) became phenotypically sensitive in vitro by mutation of residue 184 of viral reverse transcriptase to valine, which also induced resistance to (−)2′-deoxy-3′-thiacytidine (3TC). Furthermore, AZT-3TC coresistance was not observed during extensive in vitro selection with both drugs. In vivo AZT-3TC combination therapy resulted in a markedly greater decrease in serum HIV-1 RNA concentrations than treatment with AZT alone, even though valine-184 mutants rapidly emerged. Most samples assessed from the combination group remained AZT sensitive at 24 weeks of therapy, consistent with in vitro mutation studies.

Current treatment of HIV-1 infection with single antiretroviral drugs is associated with only a limited duration of benefit. Among the likely causes of this limited benefit is the emergence of drug-resistant strains during monotherapy (*1*). Drug combinations that reduce HIV-1 replication more effectively and delay the onset of drug resistance are therefore urgently required. The criteria used to select suitable drug combinations include lack of cross-resistance, nonoverlapping toxicity profiles, and in vitro synergy. Additionally, it might be possible to exploit resistance "suppressor" mutations to prevent or delay the development of coresistance among inhibitors (*2*). This phenomenon has now been explored by studies of the combination of AZT and 3TC. Both these drugs are nucleoside analogs whose triphosphate derivatives inhibit HIV-1 reverse transcriptase (RT); resistance is mediated by mutation of this viral enzyme (*1*, *2*). AZT-resistant HIV-1 strains develop within 6 to 12 months of therapy in individuals with advanced disease, although they emerge more slowly during treatment of earlier disease (*3*, *4*). HIV-1 gradually becomes resistant to AZT by the stepwise accumulation of four out of five specific mutations in RT (at codons 41, 67, 70, 215, or 219) (*5*), with certain mutational combinations more common than others in clinical isolates (for example, Leu^{41}-Tyr^{215}, Leu^{41}-Asn^{67}-Arg^{70}-Tyr^{215}, and Asn^{67}-Arg^{70}-Phe^{215}-Gln^{219}). In contrast to AZT, monotherapy with 3TC results in the rapid appearance of highly resistant virus (*6*). In vitro selection experiments and site-directed mutagenesis have demonstrated that a >500-fold increase in 3TC resistance is conferred by a single mutation at codon 184 in RT, resulting in the substitution of Val or Ile for Met (*7*, *8*). The same mutations appear in vivo during 3TC therapy, although Val^{184} mutant viruses are more common than Ile^{184} mutants (*6*).

To clarify the significance of these mutations, we performed in vitro growth competition experiments with pure virus populations containing either Val^{184} or Ile^{184} in RT (*9*). Serial passage of equal mixtures of both viruses, in the presence or absence of inhibitors, resulted in the outgrowth of Val^{184} virus (Fig. 1). In the absence of inhibitor, ~95% of virus contained Val^{184} by passage 8 (Fig. 1A). In the presence of 10 μM 3TC or 10 μM of the related inhibitor 5-fluoro-3′-thiacytidine (FTC), 95% of the viral population contained Val^{184} by passages 5 and 3, respectively (Fig. 1A). Thus, Val^{184} mutant virus showed a greater growth competence than the Ile^{184} mutant, in addition to conferring higher 3TC and FTC resistance (*10*). It is thus surprising that selection and outgrowth of Ile^{184} mutant virus occurs at all. However, this might be attributable to the inherently higher frequency of G → A mutation than A → G transition in HIV-1 (*11*). Mutation of G → A creates an Ile codon at position 184 (ATG → ATA), whereas mutation of A → G is necessary for a Val codon (ATG → GTG). Growth competition experiments with mixtures of Val^{184} and wild-type virus (in the absence of inhibitor) showed that the Val^{184} mutation conferred only a slight growth disadvantage on the virus in vitro compared to wild type (at passage 11, ~30% of virus was Val^{184}) (Fig. 1B).

In addition to conferring 3TC resistance, the codon 184 mutation suppresses AZT resistance in specific mutational backgrounds (*8*). Analysis of additional mutant viruses constructed by site-directed mutagenesis extended these observations (Fig. 2). Specifically, HIV-1 containing different combinations of the five established AZT-resistance mutations showed increased sensitivity to AZT in the presence of the Val^{184} mutation. Introduction of another RT amino acid substitution (Trp^{210}) often associated with AZT-resistant genotypes in clinical isolates (*12*) had little effect on AZT resistance or suppression of resistance by Val^{184} (Fig. 2). Thus, in all genetic backgrounds assessed, Val^{184} induced a phenotypic suppressive effect on AZT resistance.

To determine the relative ease by which HIV-1 could become coresistant to AZT and 3TC, we performed a series of in vitro passage experiments (*13*). Limited passage of wild-type virus (HXB2-D) in the presence of both inhibitors resulted in the rapid appearance of Val^{184} and Ile^{184} mutants, with no changes in genotypic AZT resistance (*14*). We next passaged an AZT-resistant cloned isolate containing Leu^{41} and Tyr^{215} (*5*) (in the HXB2-D background) in the presence of either 3TC alone or both inhibitors. By the second passage in 3TC alone, codon 184 mutations

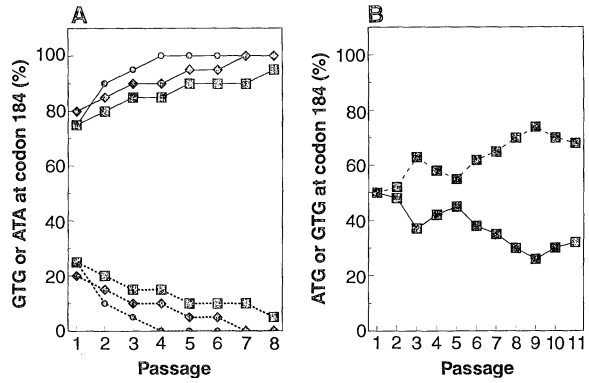

Fig. 1. Growth competition experiments with mutant and wild-type HIV-1 strains. Virus strains prepared by site-directed mutagenesis of the RT gene (HXB2-D clone) (*8*) were mixed 50:50 and used to infect MT-4 cells (*9*). After each round of infection, progeny virus recovered in the culture supernatants was used to infect fresh MT-4 cells at an MOI of <0.1 PFU per cell. DNA extracted from infected cells after each passage was used as the target for PCR to amplify a region of the HIV-1 RT gene that included codon 184. The resulting fragments were subjected to automated DNA sequence analysis and the proportion of ATG (Met), GTG (Val), and ATA (Ile) at RT codon 184 determined (*19*, *26*). (**A**) Growth competition between mixtures of HXB2 184V and HXB2 184I. The proportion of Val^{184} (solid lines) or Ile^{184} (dashed lines) at each passage is shown for cultures containing no inhibitor (■), 10 μM 3TC (◆), or 10 μM FTC (●). (**B**) Growth competition between mixtures of HXB2 184V and wild-type HXB2-D. The proportions of Val^{184} (solid line) and wild-type Met^{184} (dashed line) are shown at each passage in the absence of inhibitor.

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appeared and the AZT-resistance mutations persisted (*15*). Virus recovered from the third passage was 3TC resistant and phenotypically sensitive to AZT (*15*). Passage of the same virus in the presence of a constant concentration of AZT and increasing concentrations of 3TC resulted in marked suppression of viral growth. After extensive passage, the AZT resistance mutations persisted and codon 184 remained wild type (Table 1). Virus recovered from the sixth passage remained phenotypically AZT resistant and 3TC sensitive. By passage 10, codon 184 had mutated to Val, resulting in 3TC resistance and AZT sensitivity, indicating that suppression of AZT resistance can occur under these conditions. Although the rapid development of AZT and 3TC coresistance in this genetic background did not occur, the eventual emergence of coresistant virus in vitro or in vivo is not precluded.

Combination therapy with AZT-3TC appears attractive in view of the suppression of AZT resistance by RT Val^{184} and difficulty in selecting AZT-3TC coresistant virus. To assess the virological impact of this combination, we studied blinded clinical samples from 50 of 129 patients from multiple study sites randomized in the phase II-III European NUCB3001 trial (*16*). The combination of AZT and 3TC was compared to AZT alone in antiretroviral-naïve HIV-1–infected individuals. Significant and sustained increases in $CD4^+$ cell number were observed with AZT-3TC relative to AZT alone during 48 weeks (*16*). The amount of HIV-1 RNA in serum samples collected longitudinally from ~25 individuals in each treatment group was determined with a quantitative polymerase chain reaction (PCR) assay (*17, 18*). A marked difference in the extents of the decreases in viral RNA copies per milliliter of serum was apparent between the groups during a 24-week assessment period (Fig. 3A). The maximum mean log decrease in RNA copies was 1.8 in the combination group versus 0.7 in individuals treated with AZT alone. An increase in viral load was apparent in both groups after 24 weeks of therapy; however, a mean log decrease of 1.2 was maintained in the combination group, whereas only a 0.2 log decrease was apparent with

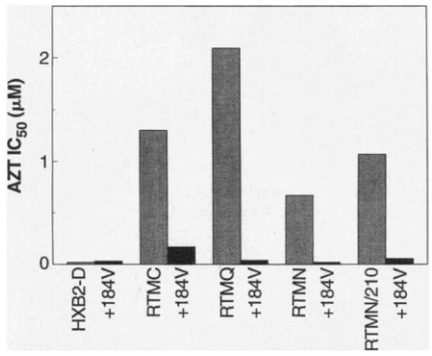

Fig. 2. Suppression of AZT resistance by RT Val^{184}. Mutant strains were constructed by site-directed mutagenesis to convert wild-type RT codon 184 (Met) to the 3TC-resistance mutation Val^{184} (*5, 8*), and were assessed for AZT susceptibility by plaque assay in HeLa-$CD4^+$ cells (*3, 21*). AZT susceptibility is expressed as the IC_{50} value (μM) of each virus, and each value represents the average of two or three separate determinations. The drug-resistance mutations in each strain are as follows: HXB2-D, wild type; RTMC, Asn^{67}, Arg^{70}, Phe^{215}, and Gln^{219}; RTMQ, Leu^{41}, Asn^{67}, Arg^{70}, and Tyr^{215}; RTMN, Leu^{41}, and Tyr^{215}; and RTMN/210, Leu^{41}, Trp^{210}, and Tyr^{215}. The addition to each virus of Val^{184} is indicated by +184V. Inhibitor susceptibility data for HIVRTMC and HIVRTMN have been described previously (*8*).

Table 1. Passage of AZT-resistant HIV-1 in the presence of AZT and 3TC. The AZT-resistant strain HIVRTMN (Leu^{41} and Tyr^{215}) (*5*) was passaged in MT-4 cells (*25*) as described (*13*). During each passage, the culture medium was supplemented with AZT (5 μM) and 3TC (0.1 to 100 μM) as indicated. Culture time indicates the time at which cell-free virus was harvested from the medium. Virus from certain passages was titrated and assessed for susceptibility to AZT and 3TC in the HeLa-$CD4^+$ cell plaque assay (*3, 21*). The IC_{50} values for AZT and 3TC for the parental virus (HIVRTMN) were 0.7 and 0.42 μM, respectively. DNA sequence analysis was performed on PCR-amplified RT fragments from infected-cell DNA (*19, 26*).

Passage	Inhibitor concentration (μM)		Culture time (days)	RT codon			Virus IC_{50} (μM)	
	AZT	3TC		41	215	184	AZT	3TC
1	5	0.1	7	Leu	Tyr	Met		
2	5	0.5	5					
3	5	0.5	6					
4	5	2	6	Leu	Tyr	Met		
5	5	5	13	Leu	Tyr	Met		
6	5	10	11	Leu	Tyr	Met	0.96	0.88
7	5	20	8	Leu	Tyr	Met		
8	5	20	8					
9	5	50	10	Leu	Tyr	Met	1.36	0.76
10	5	100	12	Leu	Tyr	Val	0.01	168

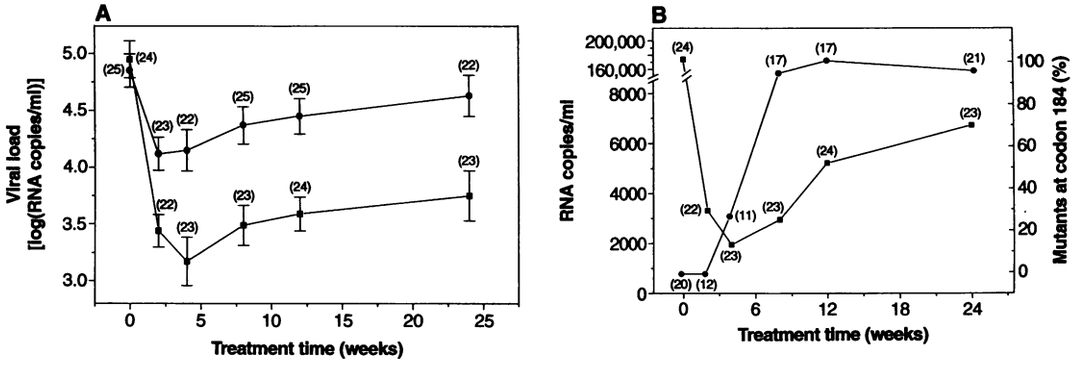

Fig. 3. Changes in HIV-1 viral load and RT codon 184 during AZT-3TC combination therapy. (**A**) RNA was extracted from serum obtained from individuals treated with AZT alone (●) or AZT and 3TC (■), and the number of HIV-1 RNA copies was determined by quantitative RT-PCR (*18*). Values are the mean (±SE) log (RNA copies per milliliter of serum). (**B**) The change in HIV-1 RNA copies per milliliter of serum for the AZT-3TC combination therapy group (■), and the emergence of codon 184 mutations in RT (●). RNA copies per milliliter are the median values for each time point. The status of RT codon 184 was determined by DNA sequence analysis of RT fragments after RT-PCR of serum RNA samples (*19, 26*). All mutants were Val^{184}, with Ile^{184} not detected. The genotype of some samples could not be determined because of the low RNA copy number in samples between weeks 2 and 12, resulting in insufficient PCR products for sequencing. Numbers in parentheses indicate the numbers of individuals from whom samples were evaluated (either for viral load or sequence analysis).

AZT alone at this time (Fig. 3A).

To investigate the mutational changes that occurred in vivo during this treatment period, we sequenced PCR-amplified DNA fragments obtained from reverse-transcribed HIV-1 RNA in serum (*19*). The Val^{184} mutation developed rapidly in the combination group. Evidence of this mutation was first detected in week-4 samples, and by week 8 virtually all (95%) isolates contained Val^{184}, indicating 3TC resistance (Fig. 3B). The small increase in viral load in the combination group coincided with the detection of Val^{184} mutants (Fig. 3B), suggesting that this increase was attributable to outgrowth of 3TC-resistant virus.

During the 24-week period of blinded treatment, relatively few AZT-resistance mutations were detected in either treatment group. Most resistance mutations in the monotherapy group were at codon 70, which suggests that this mutation alone was sufficient to cause the viral load rebound apparent during AZT therapy. However, at week 24, significantly more of the combination group samples remained wild type at the AZT-resistance codons (75%, compared with 31% for AZT alone; $P = 0.006$ by chi-square test). All samples but one from the AZT-3TC group contained Val^{184} at week 24, whereas no samples from the AZT monotherapy arm had this mutation. It was possible to determine phenotypic sensitivity by constructing recombinant virus strains from PCR-derived DNA fragments and an RT-deleted proviral HIV-1 clone (*20, 21*). None of the recombinant viruses obtained from the combination group showed significant AZT resistance but all were highly 3TC-resistant (Table 2). These results were attributable either to a lack of AZT-resistance mutations or the presence of Val^{184}, which suppressed AZT resistance [for example, A474197 contained Tyr^{215} and Val^{184} and showed an AZT IC_{50} of 0.05 μM, lower than that expected for Tyr^{215} alone (*5*)]. Thus, the sustained in vivo antiviral effects of AZT-3TC combination therapy may be attributable to interactions in HIV-1 RT between Val^{184} and AZT-resistance mutations. In the NUCB3001 trial, no AZT-resistant strains were initially present and resistance reversal therefore could not occur. However, after the Val^{184} mutation appeared during combination therapy, there appeared to be constraints on the subsequent development of AZT resistance. The fact that high-level 3TC resistance rapidly occurred suggests that continued viral suppression was attributable to AZT in the combination. Cessation of AZT at this time might result in a rapid increase in viral load, because this virus was highly 3TC-resistant (Table 2).

The combination of AZT and 3TC is the most efficacious pair of drugs tried to date with respect to the magnitude and duration of changes in $CD4^+$ cell number and viral load. A recent study of antiretroviral-naïve individuals that compared the combinations of AZT with ddI (2′,3′-dideoxyinosine) or ddC (2′,3′-dideoxycytidine) showed that neither combination reduced the rate at which AZT resistance developed, although ddI or ddC resistance was apparent infrequently (*22*). However, changes in $CD4^+$ cell number and viral load were less marked and sustained with these combinations than with AZT and 3TC.

Significant effects of AZT-3TC combination therapy on $CD4^+$ cell number were also observed in AZT-pretreated individuals (NUCB3002 trial) (*23*). It is expected that virological analysis of samples from this trial will confirm the mechanism of sustained viral suppression seen in NUCB3001. Reversal of AZT resistance may be evident, because there will be preexisting AZT-resistant strains. An alternative possibility is that changes in viral populations will occur with the simultaneous appearance of 3TC-resistant virus and disappearance of AZT-resistant strains. The frequency of AZT-3TC coresistance can also be investigated in this cohort. In summary, our results demonstrate that in vitro mutational data, particularly resistance suppression, can be used as a basis for selecting potentially efficacious drug combinations. In view of recent insights into the dynamics of HIV-1 replication in vivo (*24*), the marked effect of AZT-3TC combination therapy on viral load may translate into greater clinical benefit than previously achieved.

Table 2. Susceptibility of recombinant viruses to AZT and 3TC. RNA from serum samples from individuals treated with AZT and 3TC for 24 weeks was subjected to RT-PCR and used to construct recombinant HIV-1 strains (*20, 21*). The AZT and 3TC susceptibility of these viruses was determined by the HeLa-$CD4^+$ cell plaque assay (*3, 21*). RT amino acid residues that influence AZT or 3TC sensitivity are also shown [dashes indicate the wild-type (HXB2-D) residue]. These residues were deduced by DNA sequence analysis of the recombinant virus strains and concurred with data derived from direct sequencing of RT-PCR products from patient serum (*19, 26*).

Sample	RT codon						Virus IC_{50} (μM)	
	41	67	70	215	219	184	AZT	3TC
HXB2-D	Met	Asp	Lys	Thr	Lys	Met	0.01	0.25
A473371	–	–	–	–	–	Val	0.02	153
A659598	Leu	–	–	–	–	Val	0.01	>200
A474197	–	–	–	Tyr	–	Val	0.05	175
A679860	–	–	–	–	–	Val	0.02	>200
A339943	–	–	–	–	–	Val	0.02	119
A474181	–	–	–	–	–	Val	0.01	>200
A670695	–	–	–	–	–	Val	0.02	119
A314599	–	–	–	–	–	Val	0.01	>200
A660074	–	–	–	–	–	Val	0.03	>200
A506001	Leu	–	Lys/Arg	Thr/Tyr	–	Val	0.03	186
A384865	–	–	–	–	–	Val	0.02	>200

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9. MT-4 cells (*25*) were infected with either pure populations or 50:50 mixtures (based on HeLa-$CD4^+$ cell plaque titer) of HXB2 184V and HXB2 184I [prepared by site-directed mutagenesis (*8*)] at a multiplicity of infection (MOI) of <0.1 plaque-forming unit (PFU) per cell. Cells were incubated at 37°C in RPMI 1640 medium supplemented with 10% fetal bovine serum, polybrene (2 μg/ml), and antibiotics (RPMI/10), in the absence or presence of 10 μM 3TC or 10 μM FTC. Progeny virus was serially passaged in MT-4 cells and the proportion of Val^{184} (GTG) and Ile^{184} (ATA) viral mutants at each passage was determined by automated DNA sequence analysis of PCR products obtained from infected cell DNA (*19, 26*). The pure virus populations remained genetically stable throughout the entire culture period (eight passages).
10. In drug susceptibility assays (*3*), the 50% inhibitory concentration (IC_{50}) values for 3TC and HXB2 184V and HXB2 184I were >200 and 125 μM, respectively. The IC_{50} values for FTC with HXB2 184V and HXB2 184I were >200 and 50 μM, respectively.
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13. MT-4 cells (*25*) were infected with HIV-1 [wild-type HXB2-D; or the AZT-resistant mutant HIVRTMN (*5*), based on this stain] at an MOI of 0.1 PFU per cell [virus titer in HeLa-$CD4^+$ cells (*21*)]. Cells were incubated at 37°C in RPMI/10 in the presence of various

concentrations of inhibitors. Progeny virus was harvested from cell-free supernatants when the maximum cytopathic effect was observed, and was subsequently used to infect fresh MT-4 cells for further rounds (passages) of selection.
14. Wild-type virus was serially passaged in MT-4 cells (*25*) in the presence of 10 μM 3TC and increasing concentrations of AZT (*13*). During the first three passages (0.05 to 0.5 μM AZT), DNA sequence analysis (*19, 26*) of infected-cell DNA revealed mixtures of ATG (Met), GTG (Val), and ATA (Ile) at RT codon 184. By passage 4 (2 μM AZT), codon 184 was completely GTG (Val). During this time, RT codons 41, 67, 70, 215, and 219 remained wild type.
15. The AZT-resistant virus HIVRTMN (Leu^{41} and Tyr^{215} in RT) (*5*) was used to infect MT-4 cells (*25*) and passaged three times in the presence of 3TC (15, 50, and 100 μM). Rapid virus growth occurred during the second and third passages, and a high titer of virus was recovered after 3 days of culture in each instance. DNA sequence analysis (*19, 26*) revealed mixtures of ATG (Met), GTG (Val), and ATA (Ile) in infected-cell DNA samples from passages 2 and 3. The 3TC IC_{50} value increased from 0.2 μM (parental virus) to 200 μM (passage-3 virus), whereas the AZT IC_{50} value decreased from 0.7 μM (parental virus) to 0.08 μM (passage-3 virus).
16. C. Katlama and The European Lamivudine HIV Working Group, paper presented at the 2nd International Congress on Drug Therapy in HIV Infection, Glasgow, Scotland, 20 November 1994.
17. HIV-1–specific RNA copy number was determined as described (*18*) with a prototype quantitative PCR assay developed by Roche Molecular Systems. Briefly, RNA was extracted from 200 μl of serum and the equivalent of 50 μl was subjected to RT-PCR amplification with *rTth* DNA polymerase in the presence of an internal RNA standard. The relative amounts of the internal standard and HIV-1–specific PCR products were quantitated after 28 cycles of PCR with a microtiter-format enzyme-linked immunosorbent–like assay. The lower limit of detection of this assay is ~10 RNA copies (200 copies per milliliter), with a linear dynamic range of at least 4 log units and a coefficient of variation of <25% (*18*).
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19. Nested RT-PCR was performed with 0.25 μg of oligonucleotide primers A-35 (5′-TTGGTTGCACTTTAAATTTTCCCATTAGTCCTATT-3′) and NE1-35 (5′-CCCACTAACTTCTGTATGTCATTGACAGTCCAGCT-3′) in the first round of PCR, and primers Comb2 (5′-CTGTACCAGTAAAATTAAAGCCAGG-3′) and biotinylated primer Comb3 (5′-ATAGGCTGTACTGTCCATTTATCAGG-3′) in the second round, in a Perkin Elmer 9600 thermal cycler. RNA was extracted from serum as described (*17*) and 25-μl portions were reverse transcribed in a total volume of 50 μl containing 200 U of Superscript II RT (Life-Technologies), 13% (w/v) glycerol, 10 mM dithiothreitol, 75 mM KCl, 50 mM tris (pH 8.3), 3 mM $MgCl_2$, 200 μM deoxynucleoside triphosphates (dNTPs), and primer NE1-35. This RT reaction was separated from a lower PCR buffer containing 50 mM tris (pH 8.3), 25 mM KCl, 1.5 mM $MgCl_2$, 10 μg of bovine serum albumin, 200 μM dNTPs, primer A-35, and 5 U of *Taq* DNA polymerase by a layer of wax (Ampliwax). After incubation for 45 min at 45°C, the temperature was increased to 95°C for 20 s. Five cycles of denaturation at 95°C (20 s), annealing at 55°C (10 s), and extension at 72°C (60 s) were performed, followed by 30 additional PCR cycles of denaturation at 90°C (10 s), annealing at 55°C (10 s), and extension at 72°C (60 s for the first cycle, increasing by 5 s with each additional cycle). Portions (1 to 4 μl) of the PCR products were carried over to a second-round PCR, performed in a buffer containing 50 mM tris (pH 8.3), 25 mM KCl, 2.5 mM $MgCl_2$, 10% (w/v) glycerol, 10 μg of bovine serum albumin, 200 μM dNTPs, and 2.5 U of *Taq* DNA polymerase for 35 cycles of denaturation at 95°C (10 s), annealing at 60°C (10 s), and extension at 72°C (30 s). DNA sequencing was performed on an ABI 373 automated sequencer with standard T7 DNA polymerase sequencing procedures (*26*).
20. Recombinant viruses were constructed with a previously described system (*21*); however, the HIV proviral RT-deleted clone pHIVΔRTBstEII was replaced by a partially RT-deleted clone (pHIVΔBst1107I, with a 578–base pair deletion spanning codons 40 to 231 in RT). In addition, PCR products used in the recombination experiments were from the oligonucleotide primer pair Comb2 and Comb3 (*19*).
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Excision of Deoxyribose Phosphate Residues by DNA Polymerase β During DNA Repair

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Eukaryotic DNA polymerase β (pol β) can catalyze DNA synthesis during base excision DNA repair. It is shown here that pol β also catalyzes release of 5′-terminal deoxyribose phosphate (dRP) residues from incised apurinic-apyrimidinic sites, which are common intermediate products in base excision repair. The catalytic domain for this activity resides within an amino-terminal 8-kilodalton fragment of pol β, which comprises a distinct structural domain of the enzyme. Magnesium is required for the release of dRP from double-stranded DNA but not from a single-stranded oligonucleotide. Analysis of the released products indicates that the excision reaction occurs by β-elimination rather than hydrolysis.

Pol β, one of the four known nuclear DNA polymerases, consists of a single polypeptide (39 to 45 kD in vertebrates) that is highly conserved among higher eukaryotes. Pol β has been implicated in DNA repair on the basis of three observations: It is expressed at relatively constant concentrations throughout the cell cycle (*1*), its concentrations increase after treatment of cells with certain DNA-damaging agents (*2*), and it can fill small gaps and nicks in DNA (*3*). Studies with in vitro repair systems indicate that pol β catalyzes DNA synthesis during base excision repair (*4*–*6*).

Base excision repair is a major pathway for correction of modified bases. According to one proposed model (*7*), modified bases are repaired by five sequential reactions: (i) removal of a modified base by a specific DNA *N*-glycosylase to leave an apurinic-apyrimidinic (AP) site, a common intermediate product; (ii) incision of the AP site at its 5′ side by a class II AP endonuclease; (iii) excision of the 5′-terminal dRP to leave a single-nucleotide gap; (iv) DNA synthesis by DNA polymerase to fill the gap; and (v) sealing by DNA ligase. In the case of higher eukaryotes, characterization of in vitro repair reactions including pol β supports this model (*5*), although there is an alternative pathway for AP site repair that uses proliferating cell nuclear antigen and pol δ for the excision and DNA synthesis reactions (*6*). In the pol β–dependent base excision repair, pol β had been thought to act only to fill a single-nucleotide gap. Although some distinct activities such as deoxyribonuclease V and adenosine triphosphatase are known to interact with pol β (*8*), no intrinsic activities other than DNA polymerization have been attributed to pol β.

In a reconstituted system with proteins derived from *Xenopus laevis* ovaries, AP sites are efficiently repaired by AP endonuclease, pol β, and a partially purified fraction, BE-1B (*6*, *9*). For further analysis of pol β–dependent AP site repair, we used rat pol β that had been purified after overexpression in bacteria and bacteriophage T4–encoded DNA ligase as substitutes for *X. laevis* pol β and the BE-1B fraction, respectively. AP sites were successfully repaired in reactions containing these two proteins and *X. laevis* AP endonuclease (Fig. 1, A and B), which indicates that at least one of the three proteins is responsible for excision of the 5′-terminal dRP.

In prokaryotes, two proteins—Fpg and RecJ—have been reported to possess dRP excision activity. The Fpg protein has a DNA *N*-glycosylase activity specific for

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