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ity (the Exo function). Although some replicative DNA polymerases have separate Pol and Exo subunits, the catalytic centers for the two gp43 activities reside in separate structural modules of the same polypeptide (15). It is clear that the biological role of the Exo function is to erase errors committed by the Pol function, but it is much less clear how the Pol function makes (or avoids) errors in the first place and how the enzyme recruits the Exo function to reverse such errors. Some insights have been obtained from biochemical studies of T4 gp43 and of the polymerase I family (A family) T7 DNA polymerase, which, like gp43, bears separate Pol and Exo modules in the same polypeptide (19–21). Kinetic assays with these enzymes indicate that the fidelity of the Pol function is achieved through two transactions that precede proofreading and that occur at or near the Pol catalytic center (20). The first step is an accurate selection of an incoming dNTP at the single dNTP-binding site, and the second step is a slow primer extension from a mispaired base at the primer terminus. The base selection step provides a large contribution to fidelity and appears to depend both on base pair geometry and on the hydrogen bonding potential of the incoming dNTP (22–25). The primer extension step depends particularly strongly on the hydrogen bonding potential of the 3′-terminal nucleotide (26) and may provide the signal for transferring the primer terminus to the Exo catalytic site for proofreading (20). Both structural and biochemical evidence suggest that the switch from primer extension to proofreading involves a conformational transformation in the enzyme from a “closed” (or Pol) mode to an open (or Exo) mode. This transition includes fraying the primer end to allow its appropriate positioning relative to the Exo catalytic center (17, 27).

Recently, a cluster of amino acid residues at the juncture of the Palm and Fingers domains of RB69 gp43 was implicated in dNTP binding (28). One of these residues, Tyr567, was proposed to play a role in interactions with the base component of the incoming dNTP during the alignment of the nucleotide for nucleotidyl transfer. We show here that substitutions at this residue can dramatically increase replication errors while exhibiting only small effects on total DNA synthesis and viable phage production. An RB69 gp43 with the Y567A substitution is highly mutagenic in vivo while exhibiting normal 3′-exonuclease activity in vitro. Thus, the mutator activity of Y567A-gp43 is not caused by a proofreading-exonuclease defect. An RB69 Y567A-gp43 mutant that is also defective in the Exo function (through the introduction of a D222A/D327A double substitution) does not support viable phage production, although it does support 50–70% of the normal amount of DNA synthesis. Combining Y567A with the proofreading defect increases the mutation rate only modestly over the increase from a “closed” (or Pol) mode to an open (or Exo) mode. This transition includes fraying the primer end to allow its appropriate positioning relative to the Exo catalytic center (17, 27).

Three T4 rII mutants (30) were used for reversion tests. 

### Methods

#### Growth, Screening, and Assay Conditions—Cells for genetic experiments were grown in LB broth in a rotary shaker water bath at 37 °C. Plates were incubated overnight at 37 °C. T4 am43 stocks (with or without rII mutations) were grown on BB cells carrying the desired version of pRB.43. QA1 cells were used to assay am43 rI− revertants. B40 su II cells were used to assay am43 stocks with or without rII mutations. BB cells carrying pRB.43 (wild type) were used to screen for mutant plaques displaying the rII phenotype.

#### Cloning—Plasmid CW19R carries a wild type RB69 gene 43 (designated pRB.43 Pol Exo) under control of the T7 +10 promoter of cloning vector pSP72 (Promega) (11, 18). Mutant derivatives of pCW19-borne gene 43 were constructed by site-directed mutagenesis and confirmed by sequencing and are designated pRB.43 followed by one of the Pol Exo annotations described below. Plasmid pCW107 (pRB.43 Pol1174 Exo) expresses gp43 Pol1174 Exo, plasmid pSNG14-1 (pRB.43 Pol1174 Exo) expresses gp43 Y567S, plasmid pSNG3–1 (pRB.43 Pol1174 Exo) expresses gp43 Y567T, plasmid pCW50R (pRB.43 Pol Exo) expresses exonuclease-deficient gp43 D222A/D327A, and plasmid pSNG3–1 (pRB.43 Pol1174 Exo) expresses gp43 Y567A D222A/D327A, where "CW" plasmids were constructed by C.-C. Wang and "SNG" plasmids were constructed by S. Ng.

#### Phage T4 DNA Synthesis and Progeny Production in Vivo—For the experiments to be described later in Fig. 1A, cultures of E. coli BB cells carrying a pRB.43 plasmid were grown at 30 °C with vigorous aeration to 3 × 108 cells/ml in M9SB medium containing ampicillin at 20 μg/ml. Then 0.2 ml of culture was added to 0.1 ml of fresh medium containing 6 × 106 plaque-forming units of T4 43am phage (multiplicity of infection = 10), and aeration was continued at 30 °C. After 22 min, [3H]thymididine was added (5 μCi/ml) at a specific activity of 10 μCi/μg dT. At 15 min (37 min postinfection), further [3H]thymidine labeling was slowed in an ice bath and trichloroacetic acid-precipitable [3H] counts were determined. Only T4 DNA is synthesized after the first few moments of infection.

To measure burst sizes, E. coli BB was grown to 3 × 108 cells/ml at 30 °C in M9SB medium. At 0 min, 1-ml samples were infected with 107 plaque-forming units of T4 43am and the wild type added to 10 μl of M9SB. At 12 min the cultures were diluted 104-fold in M9SB and
aerated at 30 °C, and infective centers were assayed. Under these conditions, cell lysis begins at about 42 min. Cell lysis was completed by the addition of chloroform to the diluted infected cultures at 60 min. To measure T4-induced DNA synthesis in E. coli Napi cells carrying prbR43 (shown later in Fig. 1B), the cells were grown at 35 °C with 10 μg/ml of ampicillin in LB medium containing 20 μg/ml of [3H]thymidine at 20 μCi/μg dT. Samples (0.1 ml) were withdrawn at various times thereafter to determine thymidine-5′-triphosphate-precipitable counts. The corrected culture to justify using the expression μS = ln(N/N0), where μS is the mutation rate of the rI gene per replication, f is the observed mutation frequency, N is the final population size, and N0 is the initial population size, or, for N0 ≪ NμS, the expression μS = ln(N/N0) (37, 38). The genomic mutation rate μr = μS/(168,987 base genomes/294 rI pairs), where C is the ratio of all mutations to detected mutations (the inverse of the efficiency of mutation detection). We assume that all non-BPS mutations are detected but that only chain-terminating mutations are efficiently detected among BPSs, synonymous mutations and many missense mutations remaining undetected (37). If we let B be the fraction of mutations that are BPSs and D = the correction factor for the fraction of all BPSs detected, then C = (1 − B) + BB. The Pol EcoR1 spectrum contained 47 BPSs of which 9 progeny chain-terminating codons. Because the 47 nucleotide sequence is about two-thirds AT, about 0.073 of random BPSs will produce a chain-terminating mutation (37). Therefore, D = 9/(47 × 0.073) = 2.6. Although only approximate, this value is lower than those encountered in most of a variety of other systems (37), demonstrating that the rI system detects many missense mutations.

In Vivo Veritas

Neutralizing rI Mutants—Mutant plaques were resuspended in 40 μl of water, and the rI region was directly amplified by PCR and sequenced. The upstream primer was 5′-GTAAAGCCCTGGCTACG-3′ and the downstream primer was 5′-CCTAAGTATTCACCGCCTTT-3′ for both PCR and sequencing. The PCR consisted of 30 cycles of 1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C, with a final extension time of 10 min at 72 °C using Taq large fragment polymerase (Display System Biosciences, Gaithersburg, MD). PCR products were purified with the PCR purification kit (Qiagen). Sequencing was performed with an ABI Prism 377 automatic sequencer using the dRhodamine terminator cycle sequencing kit (PE Applied Biosystem). Each mutation was identified by sequencing in both directions.

Determining Kinetic Parameters—Steady-state kinetic parameters for both misinsertion and missense substitution were determined as described (28). For missense assays, 13-mers were 5′-3′-labeled by standard procedures, annealed with the complementary 20-mers, and used as substrates. Three sets of 13/20-mers were designed for misinsertion assays, where the base in bold type is the template base pair to the correct or incorrect incoming dNTP: P/T-1 for T/T misinsertion, 5′-CGCAGCCCTTG-3′/3′-GGCTGGTCCGAACTGCGG-5′; P-2 for T/G misinsertion, 5′-CGCAGCCCTTG-3′/3′-GGCTGGTCCGAACTGCGG-5′; and P-3 for C/G misinsertion, 5′-CGCAGCCCTTG-3′/3′-GGCTGGTCCGAACTGCGG-5′. For mispair extension assays, the 13/20-mer primer terminus and its cognate template base are in bold type: P-4, 5′-CCGACCAGCCGAA-3′/3′-GGCTGGTCCGAACTGCGG-5′; P-5, 5′-CCGACCAGCCGAA-3′/3′-GGCTGGTCCGAACTGCGG-5′; and P-6, 5′-CCGACCAGCCGAA-3′/3′-GGCTGGTCCGAACTGCGG-5′. RESULTS

Effects of Substitutions at RB69 gp43 Tyr567—The tyrosine at position 567 of RB69 gp43 (Tyr567 in T4 gp43) is highly conserved in Region III of B family DNA polymerases (7, 11). Nevertheless, we found that replacing this residue with alanine or several other amino acids affects replication reactions only weakly. T4 am43 was used to infect E. coli BB cells carrying a plasmid expressing one or another allele of RB69 gene 43, and both DNA synthesis and phage growth were monitored (Fig. 1, A and B). The EcoR1 defect had little effect on DNA synthesis and phage growth. The Y567A substitution slightly reduced the rate of DNA synthesis in vivo (by ~20%), whereas phage growth was hardly affected. The Y567S, Y567T, and Y567V substitutions reduced DNA synthesis moderately (by 30–40%) and phage yield somewhat less (Fig. 1A).

In contrast to alanine, serine, threonine, and valine substitutions at Tyr567, alanine substitutions at conserved residues located close to Tyr567 in the crystal structure sharply reduce DNA synthesis and/or phage production, e.g. N564A (Fig. 1, A and C) and others (28). Unexpectedly, the conservative substi-
tution Y567F failed to support DNA replication. In addition, gp43 PolY567F Exo2 exhibits very weak activity in an M13 gap filling assay (results not shown). The poor catalytic efficiency of Y567F-gp43 was confirmed both by repeating its construction and by reverting the mutated gene at the 567 site (results not shown). Tyr 567 is unusual among highly conserved RB69 gp43 residues tested to date (results not shown) in tolerating nonconservative amino acid substitutions while not tolerating a conservative substitution.

The Pol Y567A Exo2 construct reduced DNA synthesis by a moderate 40% but reduced phage growth sharply. Unlike the other gp43 constructs listed in Fig. 1, RB69 PolY567A Exo2 also inhibited the growth of wild type T4. This dominant lethality has two possible explanations, both probably operating here. First, dominant lethality can be exhibited by RB69 gp43 mutants that are deficient in polymerase activity but retain the capacity to repress the translation of the gene 43 mRNA transcribed by the infecting wild type T4 particles (3, 11). This PolY567A Exo2 enzyme can indeed repress heterologous translation (results not shown). Second, as we show later, the PolY567A Exo2 enzyme has such low fidelity that most of the genomes it synthesizes carry numerous mutations.

When we used denaturing gel electrophoresis to examine the size distributions of 3H-labeled DNA synthesized in vivo, no notable differences were observed among the four genotypes used in this study (PolT4, PolY567A, Exo1, or Exo2) (Fig. 1C). In particular, the PolY567A Exo2 enzyme appears to accumulate no more single-stranded DNA of reduced size than does the Pol1 Exo1 enzyme. We therefore presume that DNA is packaged in phage progeny with similar efficiency in all four infections.

Reversion Tests—Measuring reversion of specific T4 rII mutations can quickly reveal changes in mutation rates along particular mutational pathways. To this end, we used rII mutants that revert by +1 frameshifts in a run of five A-T base pairs or by base pair substitutions at either a G-C base pair or an ochre codon (TAA/ATT). The results are summarized in Table I. When Tyr567 was replaced by serine, threonine, or alanine, the result was weak mutator activity for frameshift mutations in an A-T run and strong mutator activity for BPSs at both a G-C and an A-T site. The polymerases with serine or threonine substitutions appear to be slightly more prone to frameshift mutator activity than the polymerase with an alanine substitution. In contrast to these Pol effects, a defect in exonuclease function strongly promotes all three mutational
pathways. Note that the values in Table I are frequencies and not rates; under these experimental conditions, values for rates are roughly an order of magnitude smaller than for frequencies, but relative rates are similar to relative frequencies.

Forward Mutation Tests—We used forward mutation in the T4 rI gene to determine the mutational spectrum generated by the RB69 gp43 mutator mutants. In contrast to reversion tests, which tend to display high sensitivity, forward mutation tests provide generality and, when augmented by sequencing, provide detailed information about mutability at specific sites. Forward mutation tests can also reveal classes of mutations that are not detected in reversion tests.

For the polymerases that supported high levels of T4 DNA synthesis and phage production, it was straightforward to measure r mutant frequencies (discussed later) and to collect mutants of independent origin for sequencing. For gp43 PolY567A Exo1, which failed to support the production of viable phage, we designed a procedure in which T4 infection was supported competitively by T4 gp43 Pol1 and RB69 gp34 PolY567A Exo1 (see “Experimental Procedures”). The results of an infection supported by this mixture of gp43s appear in Table II. The average number of viable progeny per infected cell fell about 90-fold, whereas the frequency of r mutants among the progeny rose by 60-fold. Thus, although the ratio of DNA synthesis conducted by the two competing gp43s is unknown, the DNA in the large majority of the mutated rI regions must have been synthesized by the double-mutator gp43. We believe that these rI regions are embedded in genomes that were mostly synthesized by Pol1 Exo1 polymerase, the mutated rI regions then finding their way into otherwise little-mutated genomes by recombination; T4 has a high frequency of recombination, about 1% per 150 base pairs. An alternative but less attractive possibility is that the mutated rI regions were introduced by brief intervals of synthesis by the double-mutator enzyme. Because these r mutants arose during a single round of infection, they are all presumed to be of independent origin.

Mutational Classes—Table III lists the numbers of mutations of different kinds arising in Pol1 Exo1, Pol1 Exo2, PolY567A Exo1, and PolY567A Exo2 backgrounds. Complex mutations (closely spaced multiple BPSs and/or frameshift mutations) (5) were excluded from further analysis here because they are rarely produced by our mutator polymerases.

The Pol1 Exo1 mutational distribution contains a majority of BPSs, a characteristic of most collections of spontaneous mutations in diverse wild type organisms studied in vivo; the exceptions involve distributions in genes that harbor extraordinarily strong frameshift mutation hot spots or organisms experiencing outbursts of transposon mobility. The Pol1 Exo1 distribution contains roughly twice as many transversions as transitions, whereas the Pol1 Exo2 spectrum displays the reverse ratio. Small additions and small deletions (frameshift mutations) are equally frequent in the Pol1 Exo1 spectrum, whereas small additions predominate in the Pol1 Exo2 spectrum. Therefore, gp43 proofreading appears to operate roughly four times more efficiently to repair transition mispairs than transversion mispairs and three or four times more efficiently to repair +1 than −1 frameshift mutations. (Remember that T4 mutations are not subject to mismatch repair.) These results are in good agreement with the reversion tests (Table I).

The PolY567A Exo1 distribution consists almost exclusively of BPSs with a substantial bias in favor of transitions. This distribution is consistent with the reversion tests (Table I).

When three different substitutions at Tyr567 in the Exo1 background were compared in the forward mutation test, their mutational patterns (Table IV) and their spectra (not shown) were qualitatively similar, although quantitative differences were discernable. In reversion tests, we observed more frameshift mutations with PolY567S and PolY567T than with PolY567A (Table I). This tendency is repeated in the forward mutation tests (5% for PolY567S combined versus 1% for PolY567A), PolY567S and PolY567T also seemed to produce a higher ratio of transitions to transversions than did PolY567A tests (66:3 for PolY567S combined versus 68:10 for PolY567A). Codon usage patterns and critical amino acids can bias the recovery of missense mutations but probably not sufficiently to determine the observed ratios of various kinds of BPSs.

In all of the distributions, G:C → A:T transitions outnumbered A:T → G:C transitions. G:C → T:A mutations predominated among the transversions, whereas G:C → C:G transversions were completely absent. The predominance of G:C → A:T transitions and G:C → T:A transversions is consistent with the A:T-rich nature of the T4 genome. The rarity of G:C → C:G transversions suggests that these polymerases form C:G mispairs much less readily or extend such mispairs less efficiently than they do G:A, C:T, and other transversion-generating mispairs. Almost all frameshift mutations in these spectra arise within short repeats of single base pairs.

Mutational Spectra—Mutational spectra reveal widely different intrinsic mutabilities at different sites. Highly mutable sites are often called hot spots, but this designation is arbitrary because sites typically display a smooth gradient of mutabilities rather than discrete steps, at least within the resolving power of almost all spectra. Hot spots are of considerable interest because they identify genetically unstable sequences. They may also interfere with the analysis of error proclivities
whereas positions 248 and 250 each contain two G→C transitions. It contains 23% of all the mutations in the spectrum, z→transitions. The hot spot at position 203 produces C→G. 

The hot spot at position 247 is specific for G→C. 

The PolY567A Exo− spectrum tends to reinforce the conclusion drawn from the mutation distribution (Table III) that the PolY567A and the Exo− mutator activities do not interact multiplicatively. The exceptions to a simple spectral mixture noted above highlight the complexity of this interaction.

**Sequence Determinants of Genetic Instability**—Mutationally warm and hot sites and regions constitute DNA sequences that constrain polymerase fidelity and that therefore may provide insights into fidelity mechanisms. The hot spot at rI position 247 is imbedded in a generally hypermutable sequence, 5′-TGCGC-3′, that is similar to another hypermutable sequence, 5′-TGGCAA-3′, previously described in the T4 rII gene (44). In both cases the central G is a transition hot spot, whereas the adjacent C mutates moderately often and the first G only slightly more than average. (The complement of this sequence, TTGCCA, is located at position 26–31 but does not contribute mutations to any of our spectra; however, a transition at the first C of this sequence would produce a Ser→Val replacement, which might well go undetected.) The hot spot at rI position 203 is imbedded in yet another generally hypermutable sequence, 5′-CCCGTG-3′, where the third C is the most mutable, sometimes producing both transitions and transversions, and the T is a little less mutable and produces transversions. The hypermutable region around position 203 begins with three G-C base pairs, the only run of three G base pairs. Such differential stability might modulate the melting of the adjacent C of this sequence, the sum of CCC and GGG expected from a random distribution of bases is 3.5, so perhaps the frequency of these runs has been reduced by mutation pressure. The entire T4 genome has A-T=109278 and G-C=59619 and is unequivocally depleted of such runs (expected=1857, observed=1239), and this deficit also appears in the observed infrequent use in T4 of the codons CCX, XXC, GXX, and XGG.

Further inspection of all six rI spectra reveals a strong association between hypermutability and GG (or CC) dinucleotides. We then examined four other spontaneous spectra available for T4 (all produced by T4 gp43) (36, 45) and found an identical association. Both the central GG/CC motif and sites to its left and right frequently display increased mutability, and this motif accounts for almost all sites of hypermutability observed in T4 in vivo. The increased mutability of G-C-rich regions may in part reflect a previously suggested role for the increased stability of G-C base pairs compared with A-T base pairs. Such differential stability might modulate the melting of an adjacent mispair prior to partitioning from the Pol site to the Exo site (21, 46). Indeed, the contribution of GG and CC regions to mutability is higher in the two Exo− spectra than in the Pol− spectrum (Table III). The PolY567A Exo− spectrum should resemble the Pol− spectrum, whereas the PolY567A Exo− spectrum tends to reinforce the conclusion drawn from the mutation distribution (Table III) that the Pol− and the Exo− mutator activities do not interact multiplicatively. The exceptions to a simple spectral mixture noted above highlight the complexity of this interaction.

**Mutation Rates**—An experimentally determined mutation frequency f (such as total mutants per total phages) can be

by diluting out other kinds of mutations. Most spontaneous frameshift hot spots are now interpreted as being the result of slippage within repeated bases or short sequences during DNA replication. Repeats of six or more base pairs form very strong frameshift hot spots, on the other hand, remain uncharacterized ways that can vary with local DNA sequence up to a dozen (25) probably modulate error frequencies in still uncharacterized polymerase and both the primer template and the incoming dNTP misincorporation. Frameshift hot spots in phage T4 (39, 40) and account, for example, for half of all rII mutations (41); fortunately, the rI reporter gene lacks repeats of more than five base pairs. Spontaneous base substitution hot spots, on the other hand, remain largely unexplained. The many contacts between a DNA polymerase and both the primer template and the incoming dNTP (25) probably modulate error frequencies in still uncharacterized ways that can vary with local DNA sequence up to a dozen bases away (42, 43).

Fig. 2 shows the mutational spectra obtained with Pol+ Exo−, Pol+ Exo−, PolY567A Exo−, and PolY567A Exo− gp43s. As expected, frameshift mutations cluster within AAAAA, TTTTT, AAAA, and TTTT, and most are simple additions or deletions of single bases.

The Pol+ Exo− spectrum exhibits no pronounced hot spots but does display numerous sites of intermediate mutability. The PolY567A Exo− spectrum displays two hot spots, each imbedded within a small region of generally increased mutability. The hot spot at position 247 is specific for G→A transition. It contains 23% of all the mutations in the spectrum, whereas positions 248 and 250 each contain two G→A transitions. The hot spot at position 203 produces C→G transitions and C→A transitions about equally often, and together they account for about 19% of all the mutations in this spectrum.

The PolY567A Exo− and PolY567A Exo− spectra are very similar to the PolY567A Exo− spectrum and are therefore not shown here. Their ratios of transitions to transversions, which are somewhat higher than those of the PolY567A Exo− spectrum (Table IV), are reflected in the sharply reduced numbers of G-C→T-A transversions at position 203 and the mildly increased numbers of transitions at positions 203 and 205.

At sites of multiple occurrences, the PolY567A Exo− spectrum much more often resembled the PolY567A Exo− than the Pol+ Exo− spectrum. However, the PolY567A Exo− mutations at positions 2, 3, 77, 109, 110, 131–135, and 154 are predicted by neither the Pol+ Exo− nor the PolY567A Exo− spectrum. If the PolY567A and Exo− mutators acted sequentially and independently, then the PolY567A Exo− spectrum should resemble the predominant PolY567A mutational input rather than the Pol+ mutational input seen in the Pol+ Exo− spectrum. Thus, the PolY567A Exo− spectrum tends to reinforce the conclusion drawn from the mutation distribution (Table III) that the PolY567A and the Exo− mutator activities do not interact multiplicatively. The exceptions to a simple spectral mixture noted above highlight the complexity of this interaction.

**TABLE III**

| Change | Number of mutations |
|--------|---------------------|
| G→A    | 8                   |
| C→T    | 4                   |
| A→G    | 0                   |
| T→C    | 3                   |
| A→C    | 0                   |
| T→G    | 1                   |
| A→T    | 0                   |
| T→A    | 0                   |
| G→T    | 11                  |
| C→A    | 20                  |
| Transitions | 15          |
| Transversions | 32         |
| Complex | 17                 |
| +1      | 4                   |
| -1      | 5                   |
| -2      | 2                   |
| Large ≤ | 5                   |

**TABLE IV**

| Polymerase | Number of mutations | Ts | Tv | Fs | HS 203 | HS 247 |
|------------|---------------------|----|----|----|--------|--------|
| Y567A      | 79                  | 68 | 10 | 1  | 15     | 18     |
| Y567S      | 36                  | 32 | 2  | 2  | 9      | 6      |
| Y567T      | 37                  | 34 | 2  | 1  | 6      | 7      |
Mutations, the Poisson distribution predicts that be random. Such is the case (Fig. 3). For randomly distributed tant, and the distributions of these distances should appear to

many base pairs away from each other in any particular mu-

to the coding strand.

P

mutations and M

mate the PolY567A Exo

ficed to provide a mutational spectrum, also sufficed to esti-

However, the growth conditions described above, which suf-

dated among $r_I$ genes, then most multiple mutations should lie

the condition $N_f \approx 1$ is not met here, we must calculate the

the average $f$ values are then converted to rates using $f = \ln(\mu N)$. (In the case of PolY567A $Exo^-$, we assumed that the average number of progeny phage was the same, 182, for both infections described in Table II but that most of the phages in the mixed polymerase infection carried lethal mutations. This assump-

on is justified by the previously described vigor of the polym-

erase in vivo where nearly normal amount of DNAs of normal

sizes are synthesized by PolY567A $Exo^-$. Here, $N = 182$ instead

of the total number of particles in the stock. However, because

the condition $N_0 \ll 1/\mu$ is not met here, we must calculate the

rate using $\mu = \ln(N/N_0)$. The multiplicity of infection was about 10, but not all particles can participate under our exper-

mental conditions. We therefore used the full estimated range $N_0 = 1–8.$ Note, however, that these multiples values are

overestimates for at least two reasons. One is that all of the

synonymous mutations and some of the missense mutations

among the multiples have an

phenotype as singles and

therefore engender false multiples in the sense of our argu-

ment. Another is that some of the multiples may have arisen by

recombination between singles. These confounding issues can

be roughly factored out by determining the ratio of the multi-

ple rates to the median rate for the Pol $^+$ $Exo^-$ and Pol$^{Y567A}$ $Exo^-$ polymerases, averaging them (to obtain $5.32$), and then

dividing the multiples rate for Pol$^{Y567A}$ $Exo^-$ (0.0391–0.652) by

this average value to obtain the corrected median value

FIG. 2. Mutational spectra characteristic of different RB69 gene 43 alleles. The sequence is that of the wild type $r_I$ strand complementary to the coding strand. $P^+$, Pol $^+$; $E^+$, $Exo^+$; $P^{00}$, Pol$^{Y567A}$; $E^-$, $Exo^-$. Transitions are entered above the wild type bases, and transversions are entered below. Additions ($\uparrow$) appear above the underlined short repeats and are almost all of single bases. Deletions ($\downarrow$) appear below the underlined short

repeats and are almost all of single bases. Complex mutations and large additions and deletions are omitted because they appeared almost exclusively in the Pol $^+$ $Exo^+$ spectrum and only once among the 268 mutations in the other spectra; they will be described in a subsequent report. The four top displays (above the horizontal line) are the first halves of the four different spectra, and each second half appears below the dividing line and position numbers.

converted into a mutation rate $\mu$ (such as mutations per chro-

mosome replication) provided that the topology of replication is

known (such as semiconservative DNA replication) and the

growth parameters of the population have been measured

(most often the final population size $N$). The conditions re-

quired to apply the expression $\mu = f\ln(\mu N)$ are well met for the
gp43 constructs studied here except for gp43 Pol$^{Y567A}$ $Exo^-$. However, the growth conditions described above, which suffi-
ced to provide a mutational spectrum, also sufficed to esti-

mate the Pol$^{Y567A}$ $Exo^-$ mutation rate. Because of the high

rates characteristic of these mutants, mutants sometimes contain

multiple mutations. If the mutations are randomly distrib-

uted among $r_I$ genes, then most multiple mutations should lie

many base pairs away from each other in any particular mu-
tant, and the distributions of these distances should appear to

be random. Such is the case (Fig. 3). For randomly distributed

mutations, the Poisson distribution predicts that $M_{r_I} = M - 1 - f(1/\varepsilon - 1)$, where $M_{r_I}$ is the number of mutants with $\geq 2$ $r_I$
multiplications and $M$ is the total number of $r_I$ mutants. Thus, $f$ can be estimated solely from the numbers of multiple and single
mutations among sequenced mutants and can then be com-

bined with an estimate of $N$ to obtain the mutation rates; this

is the method of multiples. The numbers of single and multiple
mutations for the relevant RB69 gp43 variants are shown in
Table V. Several $r_I$ mutation rates were then calculated in two

ways. The method of the median was simply the median $r_I$
mutation rate calculated using $\mu = f\ln(\mu N)$ and was used to

obtain the Pol $^+$ $Exo^-$ and Pol$^{Y567A}$ $Exo^-$ rates. The method of

multiples was used to obtain values of $f$ for all three gp43s. These $f$ values were then converted to rates using $\mu = f\ln(\mu N)$. 
The mutator activity of the Pol is served in the Pol$^+$ Exo$^-$ spectrum.) The mutator averaged to obtain the third value, which was then applied to the bottom multiples value to obtain the bottom (underlined) median value. Where complex mutations are always close together, most pairs of reversion tests, all of the mutant polymerases display strong reversion tests, all of the mutant polymerases display strong.

**Kinetic Parameters in Vitro**—Using highly purified, nuclease-free Exo$^-$ gp43, we measured steady-state kinetic constants for Pol$^+$ and Pol$^{Y567A}$ for the incorporation and extension of one correct base pair and various mispairs. Using measured values of $k_{cat}$ and $K_{m(app)}$, we calculated the steady-state catalytic efficiency $k_{cat}/K_{m}$, a discrimination factor against mispair formation by a particular gp43, and a mutator factor or antimutator factor for gp43 Pol$^{Y567A}$ versus gp43 Pol$^+$. The kinetic parameters for mispair formation are presented in Table VII. Although these results are preliminary, the trends are unequivocal. The catalytic efficiency with Pol$^{Y567A}$ Exo$^-$ for the correct G-C base pair is about 0.2 of the efficiency with Pol$^+$ Exo$^-$ This difference is somewhat smaller than the relative rate of total DNA synthesis in vivo for these two genotypes (about 0.7; Fig. 1). However, the in vivo value of 0.2 varies depending on the sequence context of the measured site. In vitro, gp43 Pol$^+$ Exo$^-$ discriminates strongly against all tested mispairs but more strongly against transversion mispairs than against transition mispairs (a result concordant with the higher in vivo frequency of transitions than transversions seen in Table III). Conversely, the Pol$^{Y567A}$ Exo$^-$ mutator factor is stronger for a transition mispair than for any of three transversion mispairs. Discrimination against C-C and G-G are particularly strong in gp43 Pol$^+$ Exo$^-$ and remain strong in gp43 Pol$^{Y567A}$ Exo$^-$ (with no change at all in the case of C-C), a result concordant with the absence of G-C → C-G transversions.

### Table V

**Distributions of multiple rI mutations produced by mutant RB69 gp43s**

| Polymerase | Number of stocks | $rI \times 10^5$ | Rel. $rI$ | $g$ |
|------------|------------------|-----------------|-----------|-----|
| Pol$^+$ Exo$^-$ | 19 | 0.43 | 1 | 0.0048 |
| Pol$^+$ Exo$^-$ | 7 | 220 | 510 | 2.8 |
| Pol$^{Y567A}$ Exo$^+$ | 7 | 210 | 490 | 3.1 |
| Pol$^{Y567S}$ Exo$^+$ | 5 | 240 | 560 | 3.5 |
| Pol$^{Y567S}$ Exo$^-$ | 6 | 290 | 670 | 4.2 |
| Pol$^{Y567A}$ Exo $^+$ | 1 | 740–1200 | 1700–2900 | 11–18 |

### Table VI

**Forward mutation rates produced by mutant RB69 gp43s**

| Polymerase | Number of stocks | $rI \times 10^5$ | Rel. $rI$ | $g$ |
|------------|------------------|-----------------|-----------|-----|
| Pol$^+$ Exo$^-$ | 19 | 0.43 | 1 | 0.0048 |
| Pol$^+$ Exo$^-$ | 7 | 220 | 510 | 2.8 |
| Pol$^{Y567A}$ Exo$^+$ | 7 | 210 | 490 | 3.1 |
| Pol$^{Y567S}$ Exo$^+$ | 5 | 240 | 560 | 3.5 |
| Pol$^{Y567S}$ Exo$^-$ | 6 | 290 | 670 | 4.2 |
| Pol$^{Y567A}$ Exo $^+$ | 1 | 740–1200 | 1700–2900 | 11–18 |

---

**Pol$^+$ Exo$^-$**

Pol$^+$ Exo$^-$ is the rate relative to the Pol$^+$ Exo$^-$ value, and $g$ is the genomic rate per replication.

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**Pol$^{Y567A}$ Exo$^+$**

Pol$^{Y567A}$ Exo$^+$ is the rate relative to the Pol$^{Y567A}$ Exo$^+$ value.

---

**Pol$^{Y567S}$ Exo$^+$**

Pol$^{Y567S}$ Exo$^+$ is the rate relative to the Pol$^{Y567S}$ Exo$^+$ value.

---

**Pol$^{Y567S}$ Exo$^-$**

Pol$^{Y567S}$ Exo$^-$ is the rate relative to the Pol$^{Y567S}$ Exo$^-$ value.

---

**Pol$^{Y567A}$ Exo $^+$**

Pol$^{Y567A}$ Exo $^+$ is the rate relative to the Pol$^{Y567A}$ Exo $^+$ value.

---

**Pol$^{Y567A}$ Exo $^-$**

Pol$^{Y567A}$ Exo $^-$ is the rate relative to the Pol$^{Y567A}$ Exo $^-$ value.

---

**Pol$^+$ Exo$^-$**

Pol$^+$ Exo$^-$ is the rate relative to the Pol$^+$ Exo$^-$ value.

---

**Pol$^{Y567A}$ Exo$^+$**

Pol$^{Y567A}$ Exo$^+$ is the rate relative to the Pol$^{Y567A}$ Exo$^+$ value.

---

**Pol$^{Y567S}$ Exo$^+$**

Pol$^{Y567S}$ Exo$^+$ is the rate relative to the Pol$^{Y567S}$ Exo$^+$ value.

---

**Pol$^{Y567S}$ Exo$^-$**

Pol$^{Y567S}$ Exo$^-$ is the rate relative to the Pol$^{Y567S}$ Exo$^-$ value.

---

**Pol$^{Y567A}$ Exo $^+$**

Pol$^{Y567A}$ Exo $^+$ is the rate relative to the Pol$^{Y567A}$ Exo $^+$ value.

---

**Pol$^{Y567A}$ Exo $^-$**

Pol$^{Y567A}$ Exo $^-$ is the rate relative to the Pol$^{Y567A}$ Exo $^-$ value.
in all these spectra (Table III). For all four mispairs, the mutator factor is influenced more strongly by a change in $k_{cat}$ than in $K_m$. Thus, studies both in vivo (Table III) and in vitro (Table VIII) demonstrate that PolY567A is a strong BPS mutator with a preference for generating transition mutations.

The kinetic parameters for mispair extension are presented in Table VIII. The catalytic efficiencies for extending a normal base pair are similar ($2.2$ versus $3.1$) for these two gp43s. Both a transition mispair and a transversion mispair were extended very inefficiently, in some cases beyond the limits of measurement. Perhaps surprisingly, the PolY567A enzyme extended both the transition and the transversion mispairs less efficiently than did the Pol$^+$ enzyme, with the $K_m$ contribution outweighing the $k_{cat}$ contribution. A somewhat similar result was reported for the Y766S variant of the Klenow fragment of E. coli DNA polymerase I (49), where catalytic efficiencies rather than discrimination factors revealed a misinsertion mutator factor of about 130, although in this case most of the difference was contributed by the $K_m$ term. We note in closing that a mispair might extend poorly either because of its inappropriate geometry within the Pol site (25) or if it resided for a long time in the inactivated Exo site.

**DISCUSSION**

This report describes the first detailed analysis in vivo of determinants of the fidelity of DNA synthesis in a B family DNA polymerase. One determinant is the proofreading exonuclease (Exo) function, which was studied using a bimutational DNA polymerase. One determinant is the proofreading exonuclease (Exo) function, which was studied using a bimutational DNA polymerase. One determinant is the proofreading exonuclease (Exo) function, which was studied using a bimutational DNA polymerase. One determinant is the proofreading exonuclease (Exo) function, which was studied using a bimutational DNA polymerase.

**The Proofreading Contribution to Fidelity**—Although T4 Exo$^+$ mutations often impair polymerase activity (47), we were fortunate to observe only a small effect of the D222A/D327A combination on polymerase activity either in vivo (Fig. 1) or in vitro (28). The Exo$^+$ form produces a strong mutator effect, increasing both the $r_I$ mutant frequency (Table III) and rate (Table VI) and the total $r$ frequency (18) about 500-fold. In reversion tests, the Exo$^+$ state increases mutation frequencies by 500- to 2600-fold (Table I) and rates by similar factors. The standard DNA microbial genomic mutation rate is 0.0034/replication (50). For phage RB69, whose genome size is very close to that of T4 (34), this genomic rate corresponds to an average rate per base pair of $2 \times 10^{-6}$. If proofreading contributes a fidelity factor of about 1/500 = $2 \times 10^{-3}$ to this rate, then the average fidelity of DNA synthesis itself must be $10^{-7}$-base pair. Applying the standard genomic rate to E. coli and dividing by genome size gives an average rate/base pair of $7.3 \times 10^{-15}$. Extending fidelity factors for base substitution (51) to all kinds of mutations gives a synthesis fidelity of $0.9 \times 10^{-5}$, a proofreading factor of $1.7 \times 10^{-2}$, and a mismatch repair factor of $5 \times 10^{-3}$-base pair. Thus, RB69 achieves its spontaneous mutation rate starting with almost exactly the same accuracy of DNA synthesis as achieved by E. coli but attains the remaining balance in a proofreading step that is about 8-fold stronger than in E. coli. E. coli uses additional powerful DNA mismatch repair systems to achieve the standard rate. However, we should point out that these computations do not take into account any coupling that may occur between the several determinants of fidelity.

In E. coli, proofreading discriminates about 4-fold more strongly against transversions than against transitions, whereas mismatch repair discriminates about 14-fold more against transitions than against transversions (51). Transition mismatches are more easily extended and are less efficiently proofread than transversion mismatches by most polymerases (21, 25). Because RB69, like T4, almost certainly lacks mismatch repair, we might expect to find a reversed proofreading balance, and we do. The transition rate increases about 1370-fold (from $0.43 \times 10^{-5}$ to $15 \times 10^{-5}$) in Pol$^+$ Exo$^+$ to $220 \times 10^{-5}$ to $39/77$ in Pol$^+$ Exo$^-$, whereas the transversion rate increases only about 310-fold (calculated similarly). The corresponding factors for frameshifts arising predominantly in runs are roughly 1800-fold for +1 mutations and 530-fold for −1 mutations.

The absence of complex mutations and large additions and deletions from the Pol$^+$ Exo$^-$ distribution may mean either that these arise at intrinsically low frequencies and are not proofread efficiently or that they are generated by an aberration of proofreading in the first place. The latter, for instance, might occur by the removal of a correct base followed by mis-aligned reannealing of the primer terminus to a distant complementary template sequence.

**A Polymerase Contribution to Fidelity**—We chose to examine the role in accuracy of RB69 gp43 residue Tyr$^{567}$ because this residue is unequivocally close to the Pol active site (10, 15) and because kinetic parameters for the incorporation of correct bases were almost unaffected in a PolY567A Exo$^-$ mutant (28). It therefore seemed likely that if Tyr$^{567}$ interacts with the incoming dNTP, it does so with the base rather than with the phosphate or deoxyribose. As it turns out, modifications at this residue produce either a robust polymerase with sharply reduced fidelity (Y567A/7S/T) or a moribund polymerase (Y567F) whose fidelity could not be measured.

RB69 gp43 Tyr$^{567}$ may be related functionally (even if not strictly structurally) to either or both of two A family E. coli Klenow Pol site residues, Tyr$^{766}$ (15) and Phe$^{765}$ (14), that are crucial for accuracy. Because substitutions at gp43 Tyr$^{567}$ increase BPS mutagenesis far more than frameshift mutagenesis, Tyr$^{567}$ is involved more deeply in the fidelity of base selection than in preventing slippage errors. This result is consistent with the properties of Klenow fragment Y766S and Y766A, which are strong BPS mutators promoting especially T-G and G-T mispairs in vitro but also generating deletions of two or more bases (49, 52). The mutator activities of RB69 gp43 Y567A, Y567S, and Y567T are similar, although Y567S and Y567T are slightly more prone to transition and frameshift mutagenesis than is Y567A (Tables IV and VI). Understanding

**TABLE VIII**

| T | F | gp43$^+$ | $k_{cat}$ | $k_{on(app)}$ | CE$^c$ | DF$^d$ | AMF$^e$ |
|---|---|---------|----------|-------------|------|------|-------|
| G · C | Pol$^+$ | 0.37 | 0.17 | 2.2 |
| PolY567A | 0.62 | 0.20 | 3.1 |
| T · G | Pol$^+$ | 0.027 | 250 | 1.1 $\times 10^{-4}$ | 20,000 |
| PolY567A | 0.01 | >2000 | <5 $\times 10^{-5}$ | >620,000 >31 |
| T · C | Pol$^+$ | 0.037 | 500 | 7.4 $\times 10^{-5}$ | 29,000 |
| PolY567A | 0.01 | >9000 | <5 $\times 10^{-6}$ | >620,000 >21 |

$^a$ T, template base; P, primer terminus base.
$^b$ All gp43 are Exo$^-$ Pol$^+$ PolY567A.
$^c$ CE, catalytic efficiency ($k_{on(app)}/K_{m}^{app}$).
$^d$ DF, discrimination factor (CE for G · C in Pol$^+$)/CE for mispair in Pol$^+$) or CE for G · C in PolY567A)/CE for mispair in PolY567A.
$^e$ AMF, antimitator factor (DF for PolY567A)/DF for Pol$^+$.)

Steady-state kinetic parameters for mispair extension by Exo$^-$ Pol$^+$ and Exo$^-$ PolY567A.
the similar effects of these three substitutions and the unanticipated near lethality of the Y567F substitution must await further structural information.

Genomic Mutation Rates—The genomic mutation rates of the mutators are instructive. The rate for each of the single mutators is about 3–4. T4 can survive with this high rate at least long enough to grow into a population of roughly 10^9 phages because of several relieving factors: more than half of the genome is comprised of genes whose function is not required for survival under laboratory conditions, the fraction of BPBs is at least 75% among the mutators (and many of these are relatively innocuous), and selection against deleterious mutations occurs during the growth of a stock. However, the genomic rate for the double mutator is roughly 15, rendering it unable to propagate. Despite the uncertainty in this last value, it is clearly greater than 3, a value that does permit propagation. Despite the uncertainty in this last value, it is also crucial to gain structural information on gp43 in the closed configuration complexed with a primer template and an incoming dNTP. The logical later extension is a structural comparison of correct versus incorrect dNTPs, extending eventually to a comparative structural analysis of mutationally cold and mutationally hot sequences.

Pol-Exo Coupling—Depictions of the accuracy of replicative DNA synthesis usually attribute multiplicative effects to fidelity factors for insertion, proofreading, and mismatch repair. In E. coli, mutationally dissecting the latter two revealed them to be coupled; specifically, a double knockout displayed mutator strength not much greater than did a knockout of proofreading alone, because the large number of input mutations that were not proofread quickly saturated mismatch repair (53). In phage T4, both the deleterious effects of Exo defects upon polymerase activity (47) and the properties of numerous mutants that seem to affect partitioning between the Pol and Exo site (13) imply coupling between the Exo and Pol sites. In no polymerase, however, had the interaction between Pol and Exo mutants been examined previously. After overcoming the complications of error catastrophe, we observed that Pol^Y567A Exo^- interact only a little more strongly than additively. First, the double-mutator spectrum is an approximate mixture of the component mutator activities in Pol^Y567A Exo^- multiplied, in which case the genomic mutation rate would be roughly 1800.

In a structural sense, coupling means that the partitioning of mispairs between the Pol and Exo sites is determined by more than simple melting and diffusion, and in particular it means that local changes in molecular architecture can affect partitioning strongly. Our results might be explained in several ways: 1) Most DNA polymerases extend mispaired primer termini far more slowly than correctly paired primer termini (25), favoring partitioning to the Exo site. If gp43 Tyr^567A replaces replacement strongly promoted mismatch extension, residence time in the Exo site could be diminished sufficiently to render the Pol^Y567A Exo^- enzyme functionally Exo^- in vitro (Sanchez, M. T., and Drake, J. W. 1994). However, the Pol^Y567A Exo^- and Pol^Y567A Exo^- mutational distributions and spectra would then be very similar, which they are not, and direct estimates of mispair extension would reveal a large increase, whereas they reveal a substantial decrease (Table VIII). 2) Because Tyr^567A replacements reduce mismatch extension, the DNA might simply dissociate from the enzyme, and the defective primer terminus might then be unable to reassoc-
Dull, T. J., and Noller, H. F. (1981) *J. Mol. Biol.* **149**, 337–376

Benzer, S. (1961) *Proc. Natl. Acad. Sci. U. S. A.* **47**, 403–415

Conkling, M. A., Koch, R. E., and Drake, J. W. (1980) *J. Mol. Biol.* **143**, 303–315

Sugino, A., and Drake, J. W. (1984) *J. Mol. Biol.* **176**, 239–249

Conkling, M. A., Koch, R. E., and Drake, J. W. (1980) *J. Mol. Biol.* **143**, 303–315

Sugino, A., and Drake, J. W. (1984) *J. Mol. Biol.* **176**, 239–249

Bessman, M. J., and Reha-Krantz, L. J. (1977) *J. Mol. Biol.* **116**, 115–123

Reha-Krantz, L. J., Stocki, S., Nonay, R. L., Dimayuga, E., Goodrich, L. D., Konigsberg, W. H., and Spicer, E. K. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 2417–2421

Frey, M. W., Nossal, N. G., Capson, T. L., and Benkovic, S. J. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 2579–2583

Carroll, S. S., Cowart, M., and Benkovic, S. J. (1991) *Biochemistry* **30**, 804–813

Drake, J. W., Charlesworth, B., Charlesworth, D., and Crow, J. F. (1998) *Genetics* **146**, 1667–1686

Schaaper, R. M. (1993) *J. Biol. Chem.* **268**, 23762–23765

Bell, J. B., Eckert, K. A., Joyce, C. M., and Kunkel, T. A. (1997) *J. Biol. Chem.* **272**, 7345–7351

Schaaper, R. M., and Radman, M. (1989) *EMBO J.* **8**, 3511–3516
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