Three models describing frameshift mutations are “classical” Streisinger slippage, proposed for repetitive DNA, and “misincorporation misalignment” and “dNTP-stabilized misalignment,” proposed for non-repetitive DNA. We distinguish between models using prereplication state fluorescence kinetics to visualize transiently misaligned DNA intermediates and nucleotide incorporation products formed by DNA polymerases adept at making small frameshift mutations in vivo. Human polymerase (pol) μ catalyzes Streisinger slippage exclusively in repetitive DNA, requiring as little as a dinucleotide repeat. Escherichia coli pol IV uses dNTP-stabilized misalignment in identical repetitive DNA sequences, revealing that pol μ and pol IV use different mechanisms in repetitive DNA to achieve the same mutational end point. In non-repeat sequences, pol μ switches to dNTP-stabilized misalignment. pol β generates −1 frameshifts in “long” repeats and base substitutions in “short” repeats. Thus, two polymerases can use two different frameshift mechanisms on identical sequences, whereas one polymerase can alternate between frameshift mechanisms to process different sequences.

Pre-replication state kinetic studies of DNA polymerase fidelity have been focused on base substitution mutagenesis mechanisms (1, 2). Simple frameshift mechanisms have not yet been addressed despite the destructive biological consequences of having one or a few bases deleted or added. Small frameshifts, predominantly one-base deletions, are made on undamaged DNA by human DNA pol1 μ, pol λ, pol β (to a lesser extent) (3–7), and Escherichia coli pol IV (called simply “pol IV” throughout) (8–10). Three models have been proposed to explain −1 frameshifts, namely the classical Streisinger model (11), direct misincorporation misalignment (3, 12), and dNTP-stabilized misalignment (13, 14) (Fig. 1).

Streisinger slippage results in simple deletions by displacement, i.e. the “looping out” of one or more bases as a primer strand slides along a run of reiterated template bases during replication (Fig. 1). Misincorporation misalignment occurs when DNA polymerase initially forms a mismatched base pair at the 3′-primer end that subsequently realigns by pairing with a complementary downstream template base prior to undergoing further extension (Fig. 1). Alternatively, DNA misalignment could occur as the first step followed by the “correct” incorporation of an incoming dNTP opposite a complementary downstream template base, a process referred to as dNTP-stabilized misalignment (Fig. 1), which has been observed in the crystal structure of the pol IV homolog Sulfolobus solfataricus Dpo4 in ternary complex with DNA and an incoming nucleotide (15). The bottom line is that all three processes can follow different paths to arrive at the same mutational end point, a −1 deletion. Determining precise frameshifting mechanisms for individual DNA polymerases during replication and repair is an essential step toward understanding the basic principles of mutagenesis.

In this study we perform pre-replication state fluorescence kinetics analysis with three polymerases known to generate small deletions, using the fluorescent base analog 2-aminopurine (2AP) to visualize frameshift intermediates and nucleotide incorporation products as they are occurring during real time catalysis. We report the first pre-replication state observation of two different slippage mechanisms within the same polymerase, pol μ. The data further reveal that pol μ and pol IV use different deletion mechanisms on identical repetitive sequences to achieve the same mutational end point, whereas pol β performs a mix of deletions and base substitutions in similar repetitive sequences of different lengths. We derive a minimal kinetic model to account for pol μ-catalyzed slippage in both reiterated and non-reiterated DNA to explain how end processing in a k light chain VJ recombination (16, 17) would be aided by the multipotent activities of DNA pol μ.

**EXPERIMENTAL PROCEDURES**

**Expression and Purification of Native Human pol μ and E. coli pol IV—**pol μ cDNA was subcloned into vector pET41b (Novagen) and expressed in strain BL21(DE3) RIL Codon Plus (Stratagene). Cells were grown at 30 °C in Luria-Bertani medium supplemented with kanamycin (5 μg/ml) and chloramphenicol (30 μg/ml) to an A590 of 0.7 and induced with isopropyl-1-thio-β-D-galactopyranoside (1 mM) for an additional 3 h. Cells were resuspended in 50 mM Tris-Cl (pH 7.5), 1 mM NaCl, 2 mM DTT, and 10% sucrose and lysed with lysozyme (2 mg/ml) while stirring for 1 h at 4 °C. Soluble protein was recovered by centrifugation (12,000 rpm in a GSA rotor at 4 °C) and kept at 4 °C for the remaining purification steps. Ammonium sulfate was added to 40% saturation, and pellets were resuspended in PC buffer (50 mM Tris-Cl (pH 7.5), 10% glycerol, 1 mM EDTA, and 2 mM DTT) supplemented with 500 mM NaCl and dialyzed against the same buffer overnight. The sample was diluted in PC buffer to 150 mM NaCl and immediately loaded onto a Whatman P-11 phosphocellulose column, washed with 20 column volumes of PC buffer supplemented with 150 mM NaCl and eluted with a 150–500 mM NaCl gradient (~10 column volumes). pol μ-containing fractions were loaded onto a Superdex 200 column (Amersham Biosciences) in PC buffer plus 250 mM NaCl. The clearest fractions were applied to a Heparin Hi-Trap column (Amersham Biosciences), washed with 20 column volumes of PC buffer supplemented

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1 The abbreviations used are: pol, DNA polymerase; p/t, primer-template; 2AP, 2-aminopurine; DTT, dithiothreitol.
with 250 mM NaCl, and eluted with a gradient of 250 mM to 1 M NaCl (>15 column volumes). Finally, the clearest fraction of pol μ was loaded onto a Superdex 75 column (Amersham Biosciences) equilibrated in PC buffer plus 250 mM NaCl. The eluted fractions were aliquoted and stored at −80 °C until used.

DNA Substrates and Reaction Conditions—Oligonucleotides were synthesized on an Applied Biosystems 392 DNA/RNA synthesizer and purified on denaturing 16% polyacrylamide gels. 2-Aminopurine phosphoramidite and a 5′-C6 amino linker phosphoramidite (used in rhodamine X labeling; described previously) (18) were purchased from Glen Research. Primers for quenched-flow and gel assay experiments were labeled with [32P]dATP (ICN) using T4 polynucleotide kinase (United States Biochemical Corp.). Primers and templates were annealed at a 1:3 primer/template ratio by heating to 95 °C for 3 min and slow cooling to room temperature. Ultra-pure deoxynucleoside triphosphates were purchased from Amersham Biosciences and Sigma. Reaction buffer with p/t DNA (100 nM) in one syringe. The reaction was initiated by mixing equal volumes with a second syringe filled with 400 nM p/t DNA, and either 1 mg/ml 32P-labeled p/t DNA (100 nM) and pol μ (1.6 μM), with syringe 2 containing either dTTP or dGTP (800 μM). Reactions were quenched with EDTA (0.5 mM) at various time points. Reaction products were loaded onto 16% PAGE, analyzed on a Storm PhosphorImager (Amersham Biosciences), and the data were plotted using Sigmaplot 8.0. Reactions using pol IV contained final concentrations of 200 nM p/t, 2 μM pol IV, and 2 mM dNTP. The Kd of dGTP for pol μ was measured by varying the concentration of dGTP. The rate (kobs) at each dGTP concentration was plotted and fit to a rectangular hyperbola (kobs = kpol[dNTP]/(Kd + [dNTP])).

Pre-steady State 2AP Real Time Fluorescence Analysis—Real time fluorescence analysis of nucleotide incorporation was performed using a *180 stopped-flow instrument from Applied Photophysics. The p/t DNA containing 2AP was excited at 312 nm with a xenon-mercury lamp, and emission was monitored using a 360-nm cutoff filter. Reactions were initiated by combining a syringe filled with 400 nM p/t DNA and 1.6 μM pol μ with a syringe containing either 800 μM dTTP or dGTP. Final concentrations were 200 nM p/t DNA, 800 nM pol μ, and 400 μM dNTP. Reactions using pol IV contained final concentrations of 200 nM p/t DNA, 2 μM pol IV, and 2 mM dNTP. The change in 2AP fluorescence as a function of time was fit to either a single or a double exponential equation to obtain reaction rates.

Results

pol μ Frameshifts Occur by a Streisinger Slippage Mechanism in Reiterative DNA—2AP, the base analog of A, forms Watson-Crick base pairs with T (19). When excited at 310 nm, 2AP emits a fluorescent signal at 370 nm, enabling the detection of distortions in local DNA structure by a change in fluorescence intensity (20–23). The changes in 2AP fluorescence intensity accompanying slippage events during replication are a decrease in fluorescence when T is incorporated opposite 2AP directly or when 2AP slips opposite a 3′-primer T and an increase in fluorescence when 2AP “grips” out of the helical plane (Fig. 1). By placing 2AP adjacent to a homopolymer run of A residues in the p/t DNA such that the analog can easily re-align along the primer end, concurrent changes in the amplitude and direction of 2AP fluorescence intensity that occur during slippage can thereby reveal the mechanisms at work in the different polymerases (Fig. 1).
To establish a reference fluorescence profile for incorporation by pol μ on a five-base repeat sequence without a frameshift, we monitored the addition of dTTP forming a T-2AP base pair. This resulted in a biphasic quench in fluorescence fitting to a double exponential with rates of 14 ± 2.5 s⁻¹ and 0.7 ± 0.15 s⁻¹ (Fig. 2a, black curve). The second phase of the reaction represents the rate-limiting step for chemistry, which disappears (Fig. 2a, gray curve) when deoxy-T is placed at the 3’-primer end. Further verification of the chemistry rate was obtained by pre-steady state quenched-flow analysis using single turnover reactions that yielded a rate of 0.68 ± 0.09 s⁻¹ (data not shown), closely matching the rate observed via fluorescence (0.7 ± 0.15 s⁻¹).

The kinetics for incorporating the “next correct” nucleotide G opposite C is measured by omitting dTTP from the reaction (Fig. 2b, black curve). A biphasic fluorescence quench is observed with rates of 15 ± 3 s⁻¹ and 1.5 ± 0.35 s⁻¹. The slower rate disappears using the deoxy-T-terminated primer (Fig. 2b, gray curve) and corresponds to the rate-limiting step for chemistry measured by single turnover experiments using quenched-flow (1.2 ± 0.27 s⁻¹) (Fig. 2c). The direct incorporation of T opposite 2AP or the slipped incorporation of G opposite C occurs at similar rates of -1 s⁻¹ under these conditions.

The biphasic fluorescence quench during the incorporation of G holds true even when the p/t is modified to contain just a dinucleotide repeat (Fig. 3a, black curve). To confirm that the incorporation of G on these repeat sequences is occurring opposite C on a transiently misaligned template and not directly opposite 2AP, we replaced the template C just downstream of 2AP with T (Fig. 3a, gray trace). If the slower fluorescence quench results from direct misincorporation of G opposite 2AP and not slippage, then the chemistry quench should not be governed by the presence of either C or T downstream but should yield essentially similar profiles when copying 3⁰APT⁵ or 3⁰APT⁻⁵ templates. Fig. 3a shows that this is not the case. When C is replaced with T in the template, incorporation of dGTP fails to occur, as indicated by a lack of a change in the fluorescence signal (Fig. 3a, gray trace) and confirmed by the absence of product formation using quenched flow on the 3⁰APT⁻⁵ template (data not shown). The data suggest the incoming dGTP directly forms a correct base pair with the downstream template C immediately before chemistry and that the 2AP is concurrently “slipping” under the primer end T, possibly forcing a more upstream base out of the helical plane in the duplex region of the primer-template. Frame-shift events where the template base has “slipped” under the primer were described by Streisinger (11), and this mechanism appears to be at work in pol μ, even in the case of just a dinucleotide repeat. The likelihood of misinserting G opposite 2AP is extremely small because of the gross instability of G opposite C on a transiently misaligned template and not directly opposite 2AP, we replaced the template C just downstream of 2AP with T (Fig. 3a, gray trace). If the slower fluorescence quench results from direct misincorporation of G opposite 2AP and not slippage, then the chemistry quench should not be governed by the presence of either C or T downstream but should yield essentially similar profiles when copying 3⁰APT⁵ or 3⁰APT⁻⁵ templates. Fig. 3a shows that this is not the case. When C is replaced with T in the template, incorporation of dGTP fails to occur, as indicated by a lack of a change in the fluorescence signal (Fig. 3a, gray trace) and confirmed by the absence of product formation using quenched flow on the 3⁰APT⁻⁵ template (data not shown). 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The pre-chemistry quench could be attributable to either of two causes. One possible explanation is that rapid dNTP binding quenches 2AP through increased template stacking (26, 27) as the substrates line up with Mg²⁺ in preparation for catalysis in the active site (21, 28). Supporting this possibility is our observation that the pre-chemistry quench becomes significantly greater as the concentration of dGTP increases (data not shown). Alternatively, the concomitant closure of the pol μ active site upon dNTP/Mg²⁺ binding might also account for the initial quench. A similar pre-chemistry 2AP quench was observed previously using pol β (29, 30) and was attributed specifically to concomitant closure of the active site upon dNTP/Mg²⁺ binding based on the crystal structure of the binary versus ternary complex (31). A crystal structure for pol μ will likely be necessary to help distinguish between active site closure and increased 2AP stacking as the cause for the initial pre-chemistry quench, although the two explanations are not mutually exclusive.
**pol** μ Uses dNTP-stabilized Misalignment in Non-reiterative DNA—To verify that 2AP movement under the T at the primer end is the cause of the quench observed during dGTP incorporation (Fig. 2b, five-base repeat; Fig. 3a, dinucleotide repeat), we used a p/t DNA sequence containing an A at the 3′-primer end rather than a T, eliminating the potential for base pairing between the 3′-terminal base of the primer with 2AP in the template (Fig. 3b). If 2AP cannot slip under the primer, then the only means to achieve chemistry with dGTP would be to force 2AP out of the template plane to accommodate the G-C pair in a dNTP-stabilized misalignment mode (Fig. 1), causing the disruption of base stacking and an increase in fluorescence. Using the modified p/t sequence, dGTP no longer triggers a quench but rather an increase in fluorescence (Fig. 3b). 2AP is “skipped” by slow displacement in the active site (0.0025 ± 0.001 s⁻¹; Fig. 3b), yielding the predicted fluorescence increase that matches a reduction in the rate of chemistry to 0.0031 ± 0.001 s⁻¹ as measured by quenched-flow (Fig. 3c). Bond formation thus may not occur until 2AP moves out of the way for proper G-C base pair formation. In the non-reiterative sequence, pol μ is limited to making frameshift mutations via dNTP-stabilized misalignment. We believe that these experiments provide the first “dynamic” (i.e. real time) evidence that a single polymerase (pol μ) can use two kinetically distinct mechanisms to achieve the same mutational (--1 deletion) end point.

Direct Observation of dNTP-stabilized Misalignment on Reiterative p/t DNA Using E. coli pol IV—The crystal structure of *S. solfataricus* Dpo4 suggests that a dinB (pol IV) homolog favors dNTP-stabilized misalignment as a primary means to produce frameshifts (15). A steady state fluorescence kinetics analysis (32) provides independent support that dNTP-stabilized misalignment is the predominant frameshift mechanism for *E. coli* pol IV. We have measured the slipped-mispaired intermediates in the pol IV frameshift pathway for direct comparison with pol μ on an identical p/t DNA (Fig. 4). A rapid quench in fluorescence fitting to a double exponential at rates of 6.4 ± 2.2 s⁻¹ and 0.31 ± 0.089 s⁻¹ is observed for a correct T-2AP base pair in a three-base repeat p/t DNA sequence (Fig. 4a).

In the non-slippage T-2AP reaction, the faster rate of quench for pol IV (6.4 ± 2.2 s⁻¹) closely matches the rate-limiting step for the chemistry obtained by quenched-flow (6.9 ± 0.55 s⁻¹, Fig. 4c). In contrast, incorporation of dGTP by pol IV causes an increase in fluorescence on p/t DNA of varying degrees of reiteration (Fig. 4b) but in the opposite direction of the fluorescence change seen for Streisinger slippage on the same p/t (Fig. 3). A rapid increase in fluorescence is observed for pol IV (Fig. 4b), indicating that 2AP unstacks and is skipped, allowing dGTP to align with the next template base C via dNTP-stabilized misalignment as the Dpo4 crystal structure predicts (15). The fluorescence increase with pol IV and dGTP is abolished when the downstream template C is replaced with T (data not shown) and also with a dideoxy-T on the primer end (data not shown).

The rate of dNTP-stabilized misalignment with pol IV changes as a function of the number of reiterative A residues in the template (Fig. 4b), with a run of five A residues upstream of 2AP causing the fastest rise in signal at 0.065 ± 0.019 s⁻¹, a run of three A residues at 0.036 ± 0.018 s⁻¹, and a single A residue upstream of 2AP causing little change at all. The rate-limiting chemical step measured by quenched-flow is accordingly also reduced in the run of five-A-residue (0.069 ± 0.011 s⁻¹) and the run of three-A-residue (0.033 ± 0.0053 s⁻¹) sequences (Fig. 4d). When G has been incorporated in identical sequences by pol IV (skip equals an increase in fluorescence) and is compared directly to pol μ (slip equals a decrease in fluorescence), it is clear that these two enzymes are using different mechanisms to generate the same frameshifts on repetitive DNA.

**Terminal Nucleotidyl Transferase Activity Does Not Contribute to the Streisinger Slippage Mechanism**—pol μ contains a...
potent nucleotidyl transferase activity reported to be more active on duplex p/t DNA than on single-stranded DNA (7). In quenched flow studies, we have found that the apparent rate of transferase activity on a single-stranded primer sequence is 10-fold faster at 6.8 \pm 0.5 \text{s}^{-1} (data not shown) than the observed rate for single T addition in the polymerase mode on duplex DNA of 0.7 \pm 0.15 \text{s}^{-1} (Fig. 2a).

Could the robust transferase activity observed on the single-stranded primers be contributing to the Streisinger slippage process? Transient melting of the reiterative primer end could generate a short single-stranded region on which a G could be added in the transferase mode. If this extended primer rapidly anneals to a realigned template, it could cause a quench in fluorescence. To test this hypothesis, we performed quenched-flow assays using radiolabeled primer in the absence of template to determine whether the rate of chemistry for the dGTP addition in transferase mode could account for the observed Streisinger slippage rates of 1.5 \pm 0.35 \text{s}^{-1} and 1.2 \pm 0.27 \text{s}^{-1} seen in Fig. 2a, b and c, respectively. Our results show that the transferase-based addition of dGTP onto the primer end in the single-stranded form is considerably slower (0.004 \pm 0.002 \text{s}^{-1}; data not shown), and does not appear to contribute to Streisinger slippage. In the transferase mode, pol \(\mu\) strongly favors the addition of T over G on single-stranded DNA with T at the 3'-end. The contribution of pol \(\mu\) transferase activity to the observed dNTP-stabilized misalignment on non-reiterative p/t DNA cannot, however, be unequivocally excluded, but is not likely considering the presence of a template in the active site of the enzyme.

pol \(\mu\) Makes Frameshifts More Readily in Reiterative Sequences Than Does pol \(\beta\) — Although we were able to elicit Streisinger frameshifts by pol \(\mu\) using only dGTP in the reactions, it was important to determine whether such slippage events are possible in a less stringent system where discrimination between all four nucleotides is necessary. To accomplish this, we turned to an assay in which we allowed replication by pol \(\mu\) to proceed from a \(\beta\)-P-labeled version of the p/t DNA for 30 min with all four dNTPs present and followed with restriction digestion using BsrDI (Fig. 5, a–c). The six-base recognition site of BsrDI is located immediately downstream of the first template C, and the enzyme cuts opposite the 5'-side of template 2AP. If the template 2AP slipped under the primer during extension, the BsrDI recognition site would be shifted one base closer to the primer end and thus cut opposite the 5'-side of 2AP. Fig. 5, a–c show the results of this experiment when performed on three different lengths of reiterative p/t DNA.

Remarkably, with only a simple dinucleotide (Fig. 5a), pol \(\mu\) converts at least 25% of cleavable extension products into frameshift mutations as indicated by an increase in the band

**Fig. 4.** Pre-steady state kinetics of E. coli DNA pol IV-catalyzed, dNTP-stabilized misalignment. a, stopped-flow fluorescence assay for incorporation of dTTP opposite 2AP (denoted Ap in the graph) in a three-base repeat sequence. The curve was fit to a double exponential decay with a rapid rate of 6.4 \pm 2.2 \text{s}^{-1} followed by a slower rate of 0.31 \pm 0.089 \text{s}^{-1}. b, fluorescence studies for dGTP-mediated slippage of 2AP adjacent to five, three, or one reiterated A base(s). 2AP slippage by pol IV was observed in both five-A (black) and three-A (dark gray) DNAs with rate constants of 0.065 \pm 0.019 \text{s}^{-1} and 0.036 \pm 0.018 \text{s}^{-1}, respectively. On the other hand, 2AP slippage was not observed in one-A DNA (light gray). c, rapid-quench assay of dTTP insertion opposite 2AP. The resulting data fit to a single exponential equation with a rate of 6.9 \pm 0.55 \text{s}^{-1}, comparable with that observed in Fig. 4a. d, rapid quench rates for 2AP slippage in p/t DNA containing runs of five A and three A residues were found to be 0.069 \pm 0.011 \text{s}^{-1} and 0.033 \pm 0.0053 \text{s}^{-1}, respectively, and correspond to those observed in Fig. 4b using fluorescence.
of all cleaved extension products as the result of a slippage process for pol in the cleavage bands (Fig. 6). Expansion of individual off- and on-rates (k).

right panel remain the result of non-slipped replication events (H11022) of cleavage products, revealing a slippage event for pol same high rate (Kd).

polymerase modes on duplex DNA, it was not possible to isolate a

Minimum Kinetic Models and Intermediate Steps Depicting pol-Catalyzed −1 Deletion Pathways—To complete the characterization of pol-catalyzed −1 frameshifts, we performed additional measurements to determine the Kd for DNA, including individual off- and on-rates (koff and kon) of the polymerase, the Kd for dNTPs, and the rate of pyrophosphorolysis (kpyp). Fig. 6a shows the binding of pol μ to p/t DNA (five-base repeat; see sketch in Fig. 2a) as a function of polymerase concentration with the data fit to a quadratic equation yielding a Kd for DNA of 92 ± 14 nM. Fig. 6b shows the results of measuring koff for pol μ from 5′-rhodamine labeled p/t DNA. The same off rate was observed using either an unlabeled p/t trap with a koff of 9.0 ± 1.2 s⁻¹ (Fig. 6b, gray line) or a heparin trap with a koff of 9.5 ± 1.6 s⁻¹ (Fig. 6b, black line). Using the kinetically measured koff and Kd for DNA, an estimated kon was calculated to be 1 × 10⁸ M⁻¹ s⁻¹ (koff/Kd).

To determine the Kd for dGTP during a Streisinger slippage event, the rate-limiting chemical step for nucleotide incorporation was monitored using pre-steady state quenched-flow measurements at various concentrations of dGTP. Fig. 6c shows the hyperbolic plot of the rates of incorporation using dGTP as a function of nucleotide concentration, revealing a value of 58 ± 7.9 μM for the Kd of dGTP and a maximum polymerization rate (kpol) of 2 ± 0.7 s⁻¹. Because of the propensity of pol μ to use dTTP in both transferase and polymerase modes on duplex DNA, it was not possible to isolate a Kd value for dTTP polymerization alone on the duplex reiterative substrate (data not shown).

Pyrophosphorolysis, the reverse reaction of polymerization (shown in Fig. 6d), was measurable using only the highest soluble concentration of pyrophosphate (2 mM) and occurred so slowly (2.3 × 10⁻³ s⁻¹) that only steady state measurements could be made. A summary of the entire slippage reaction mechanism for pol μ is summarized for both the favored Streisinger and the less efficient dNTP-stabilized misalignment modes (Fig. 7).
DISCUSSION

In this study we have addressed the mechanism by which pol μ/H9262 and pol IV rearrange p/t DNA to cause frameshifts. Combining fluorescent studies in a stopped-flow instrument with the power of quenched-flow and gel assay experiments using 32P-labeled p/t DNA, we have captured and identified the major steps in the slippage processes of pol μ/H9262 and pol IV, as well as the minimal mechanism of slippage for pol μ/H9262. This study marks the first pre-steady analysis of DNA polymerase frameshift mechanisms.

pol μ Nucleotide Incorporation Dynamics Reveal a Putative Conformational Change and Chemical Step—Correct incorporation of both dTTP opposite 2AP and dGTP opposite the downstream C in the reiterative p/t DNA resulted in a biphasic quench. The rapid phase using the normal deoxynucleotide- and dideoxynucleotide-terminated p/t DNA are dependent upon nucleotide binding, and the slower phase of fluorescence quench represents the rate-limiting chemistry step (Fig. 2).

The crystal structure of the closest homologue to pol μ, TdT, revealed that it appears to be locked in a conformation equivalent to the closed form of pol β (33). It is not known if pol μ undergoes large scale conformational shifts between open and closed forms like pol β (31) or instead is locked in the closed form like TdT, but our data suggest that pol μ might undergo some form of closure in the active site prior to chemistry.

pol μ and pol IV Use Different Slippage Mechanisms in Repetitive DNA—Previous studies suggested that pol μ and pol IV could both misalign p/t DNA, causing frameshifts (5, 34). A pre-steady state comparison of pol μ/H9262 and pol IV using 2AP allowed us to visually distinguish the mechanisms at work that produce frameshifts in the active sites of these two enzymes. We demonstrate that, whereas pol μ re-aligns repetitive sequences to "slip" the template under the 3' -primer end to cause a quench in 2AP fluorescence (Fig. 2a), pol IV on the other hand prefers to "skip" the first template base, moving it out of the way in its active site to accommodate the next correct nucleotide pair through dNTP-stabilized misalignment, resulting in 2AP fluorescence increase (Fig. 4b).
FIG. 7. Minimum kinetic schemes accounting for pol μ-catalyzed -1 deletion mutations. Models summarizing steps in the mechanism of pol μ Streisinger-based frameshift mutation (top) and dNTP-stabilized misalignment (bottom) are shown. During Streisinger slippage (top) the initial association of enzyme (E) to the DNA is limited by the second order binding constant \(1 \times 10^5 \text{ M}^{-1} \text{s}^{-1}\) and the \(K_c\) for DNA of \(92 \pm 14\) nM. The enzyme-DNA complex can also dissociate at 9 s\(^{-1}\). Binding of the second substrate, dGTP coupled with a magnesium ion, occurs at a rate of 15 \(\pm 3\) s\(^{-1}\) and is limited by the binding constant \(K_c\) for dGTP on this substrate of 58 \(\pm 7\) μM. Binding of the dGTP causes a concurrent slippage of the p/t DNA into a misaligned conformation that is rapidly fixed into position by chemistry occurring at a maximum rate of 2 \(\pm 0.7\) s\(^{-1}\). The reverse reaction for polymerization occurs very slowly at a rate of 2.3 \(\times 10^{-2}\) s\(^{-1}\), driving pol μ in the forward direction to another round of nucleoside addition. DNA pol μ dNTP-stabilized misalignment (bottom) is identical to the Streisinger model above, except the chemistry rate is effectively reduced by the insertion of a slow misalignment step (0.003 s\(^{-1}\)) in which 2AP is displaced from the helical plane prior to chemistry.

incoming nucleotide with the downstream template base in a manner similar to the observed ternary complex for S. solfataricus Dpo4 (15). We found that pol μ also can make frame- shifts by dNTP-stabilized misalignment on non-reiterative p/t DNA sequences, albeit with dramatically reduced efficiency compared with the Streisinger mode of slippage (Fig. 3). We have also shown that pol β, another member of the family X polymerases, does not slip as readily as its counterpart, pol μ, in the same reiterative sequences (Fig. 5), probably a reflection of their evolution toward distinct cellular purposes.

In vivo, pol μ appears to be involved in a rearrangement of light chain immunoglobulin genes (17, 35) likely mediated by its interactions with non-homologous end-joining proteins Ku and DNA-PK (16). The ability of pol μ to slip efficiently in a simple dinucleotide run and use microhomologies to insert the next base would be extremely beneficial to such a system, enabling two partially complementary broken ends to come together that might otherwise fail to do so. Perhaps as pol μ assists in VJ recombination (17) a variety of complicated junctions are encountered, requiring it to call upon all of its functions (transferase, polymerase, and microhomology searching) to help process the DNA intermediates properly.

It is important that cells limit frameshift-prone polymerases from wreaking havoc throughout their chromosomes. E. coli has evolved a solution to this problem by limiting the access of pol IV to DNA through the SOS regulon, which up-regulates its expression only during times of extreme stress (36). Human pol μ may be constrained from working on DNA by the requirement of additional cofactors such as the non-homologous end-joining proteins. Through the comparative analysis of polymerases from humans and E. coli, we have attempted to provide a global perspective on deletion mutagenesis, visualizing two unique frameshift mechanisms in real time and proving the old saying that “there is more than one way to skin a cat.”

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