

Table 1: Estimates of Rate and Dissociation Constants Describing Pol  $\delta$ -Catalyzed Reactions

	Pol $\delta 4$	Pol $\delta 3$	$\delta 4/\delta 3^a$
$k_{\text{cat}}^b$	$0.24 \pm 0.08 \text{ s}^{-1}$	$0.18 \pm 0.03 \text{ s}^{-1}$	1.3
$k_{\text{pol}}^c$	$87 \pm 5.7 \text{ s}^{-1}$	$19 \pm 2.9 \text{ s}^{-1}$	4.6
$K_{\text{dNTP}}^c$	$3.6 \pm 0.8 \mu\text{M}$	$3.2 \pm 1.6 \mu\text{M}$	1.0
$K_{\text{DNA}}^d$	$34 \pm 5.4 \text{ nM}$	$35 \pm 6.5 \text{ nM}$	1.0
$k_{\text{pol}}(\text{mismatch})^e$	$7.6 \pm 2.4 \text{ s}^{-1}$	$2.5 \pm 0.6 \text{ s}^{-1}$	3.0
$k_{\text{exo}}^f$	$0.8 \pm 0.2 \text{ s}^{-1}$	$1.9 \pm 0.4 \text{ s}^{-1}$	0.42
$k_{\text{pol-exo}}^g$	$0.003 \pm 0.0004 \text{ s}^{-1}$	$0.026 \pm 0.003 \text{ s}^{-1}$	0.12
$k_{\text{pol-exo}}(\text{mismatch})^h$	$0.062 \pm 0.006 \text{ s}^{-1}$	$0.29 \pm 0.03 \text{ s}^{-1}$	0.21
$k_{\text{pol-exo}}:k_{\text{pol}}^i$	1:29000	1:730	40
$k_{\text{pol-exo}}(\text{mismatch}):k_{\text{pol}}^i$	1:123	1:8.6	14

<sup>a</sup>Calculated by dividing the value observed with Pol  $\delta 4$  by the value observed with Pol  $\delta 3$ . <sup>b</sup>Steady-state initial velocities of incorporation performed at various dNTP concentrations were fit to the Michaelis–Menten equation (eq 3) to determine a  $V_{\text{max}}$  (Figure 3H). The  $V_{\text{max}}/E_{\text{t}}$  relationship (active sites, Figure 3G) was used to determine a steady-state  $k_{\text{cat}}$ . Uncertainties are standard errors from three independent reaction sets. <sup>c</sup>Values were obtained by fitting eqs 1 and 2 to time courses obtained at various dNTP concentrations with Pol  $\delta 4^{\text{exo-}}$  or Pol  $\delta 3^{\text{exo-}}$  (Figure 3F). Errors are standard errors from nonlinear regression. <sup>d</sup>Values represent the average  $K_{\text{DNA}}$  obtained when eq 4 is used to fit three independent titrations with DNA (Figure 2B). Errors are standard deviations from three separate fits. <sup>e</sup>Observed first-order rate constant describing the burst phase for extension of a mismatched primer (Figure 1C). Errors are standard errors from nonlinear regression. <sup>f</sup>Observed first-order rate constant describing the degradation of single-stranded DNA (Figure 4A). Errors are standard errors from nonlinear regression. <sup>g</sup>Observed first-order rate constant describing the degradation of matched duplex DNA (Figure 4B). Errors are standard errors from nonlinear regression. <sup>h</sup>Observed first-order rate constants describing the degradation of mismatched duplex DNA (Figure 4C). Errors are standard errors from nonlinear regression. <sup>i</sup>The ratios indicate the probabilities for excision against extension of a primer terminus; alterations in the ratios when a mismatch terminus is present reflect the proofreading efficiency (37, 44, 45).

37. Reha-Krantz, L. J. (1998) Regulation of DNA polymerase exonucleolytic proofreading activity: Studies of bacteriophage T4 “anti-mutator” DNA polymerases. *Genetics* 148, 1551–1557.
44. Wu, P., Nossal, N., and Benkovic, S. J. (1998) Kinetic characterization of a bacteriophage T4 anti-mutator DNA polymerase. *Biochemistry* 37, 14748–14755.
45. Nick McElhinny, S. A., Stith, C. M., Burgers, P. M., and Kunkel, T. A. (2007) Inefficient proofreading and biased error rates during inaccurate DNA synthesis by a mutant derivative of *Saccharomyces cerevisiae* DNA polymerase  $\delta$ . *J. Biol. Chem.* 282, 2324–2332.