SUPPORTING INFORMATION

Kinetic Analysis of the Bypass of a Bulky DNA Lesion Catalyzed by Human Y-family DNA Polymerases

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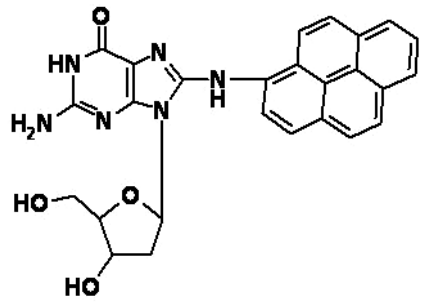
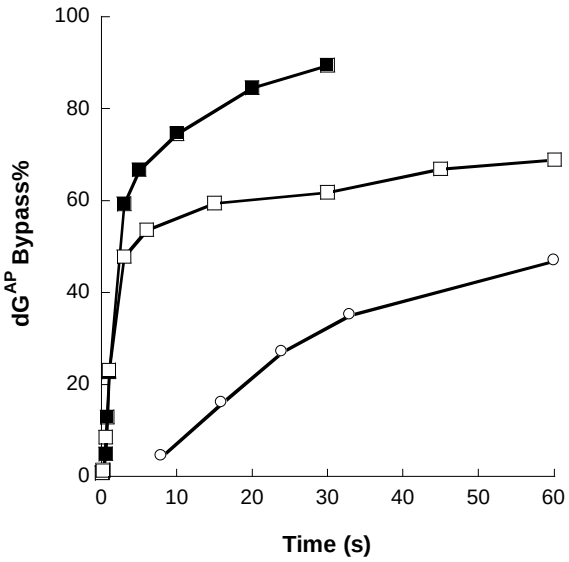
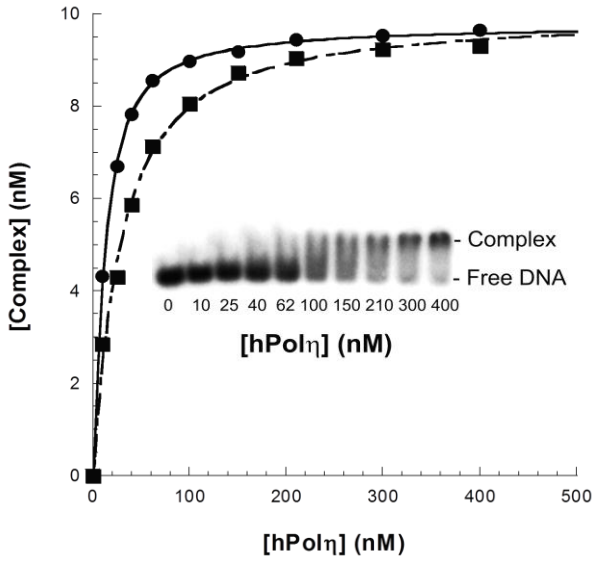


N-(deoxyguanosin-8-yl)-1-aminopyrene
(dG^{AP})

Supplementary Figure 1. Chemical structure of dG^{AP}.



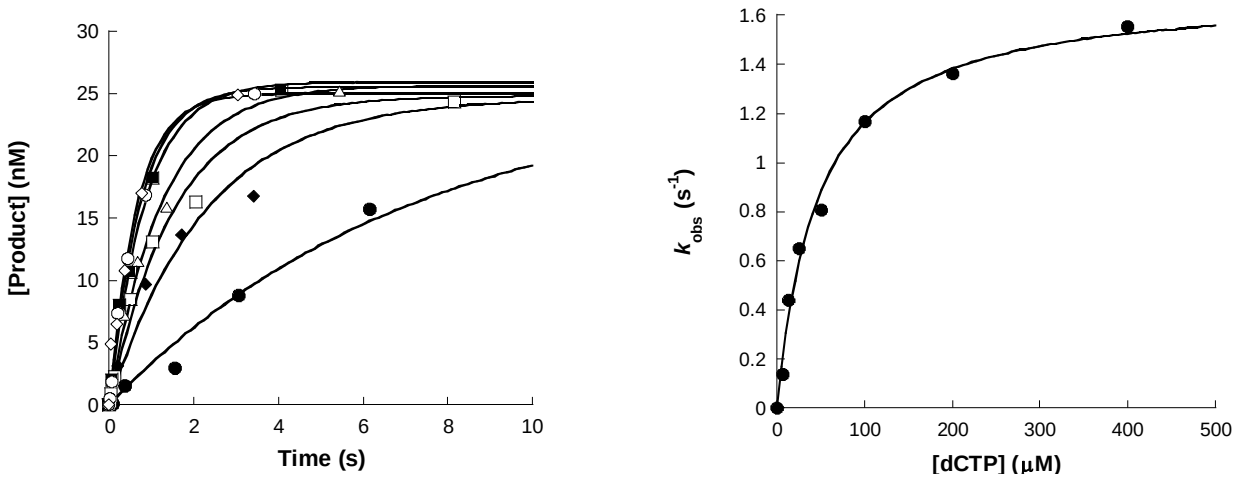
Supplementary Figure 2. The percentage of dG^{AP} bypass catalyzed by human Y-family DNA polymerases. The quantification of gel images for the running start assays (Figure 1) is for hPolη (■), hPolκ (□), and hPolι (○).



Supplementary Figure 3. EMSA for the binding of hPolη to 5′-[^{32}P]-labeled DNA. The inset is the gel image for the binding of hPolη to 20-mer/26-mer-dG^{AP}. The plots for 20-mer/26-mer (■) and 20-mer/26-mer-dG^{AP} (●) were fit to Equation 2 to yield $K_{d,\,DNA}$ of 23 ± 2 nM for 20-mer/26-mer and 7.9 ± 0.3 nM for 20-mer/26-mer-dG^{AP}.

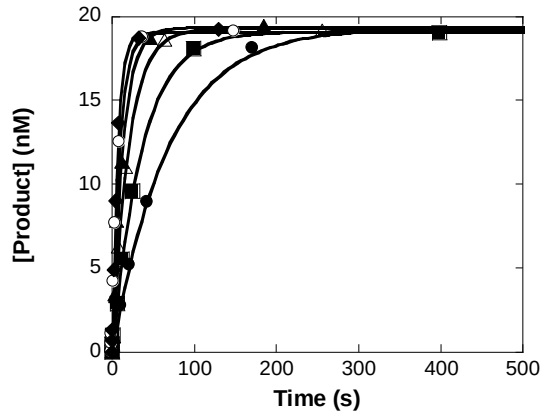
A.

B

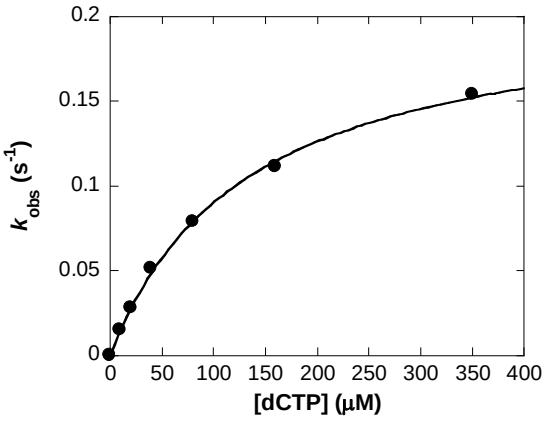


Supplementary Figure 4. Single-turnover kinetics of correct dCTP incorporation onto 20-mer/26-mer catalyzed by hPolκ. (A) A preincubated solution of hPolκ (300 nM) and radiolabeled 20-mer/26-mer (30 nM) at 37 °C were rapidly mixed with increasing concentrations of dCTP (6.25 μM, ●; 12.5 μM, ♦; 25 μM, □; 50 μM, Δ; 100 μM, ■; 200 μM, ○; 400 μM, ◊) for various time intervals. Each time course corresponding to each dCTP concentration was fit to a single exponential curve to obtain k_{obs}. (B) Plot of calculated k_{obs} as a function of dCTP concentration, which was fit to a hyperbola curve to $K_{d,\,dCTP}$ (46 ± 6 μM) and k_p (1.7 ± 0.1 s^{-1}).

A.

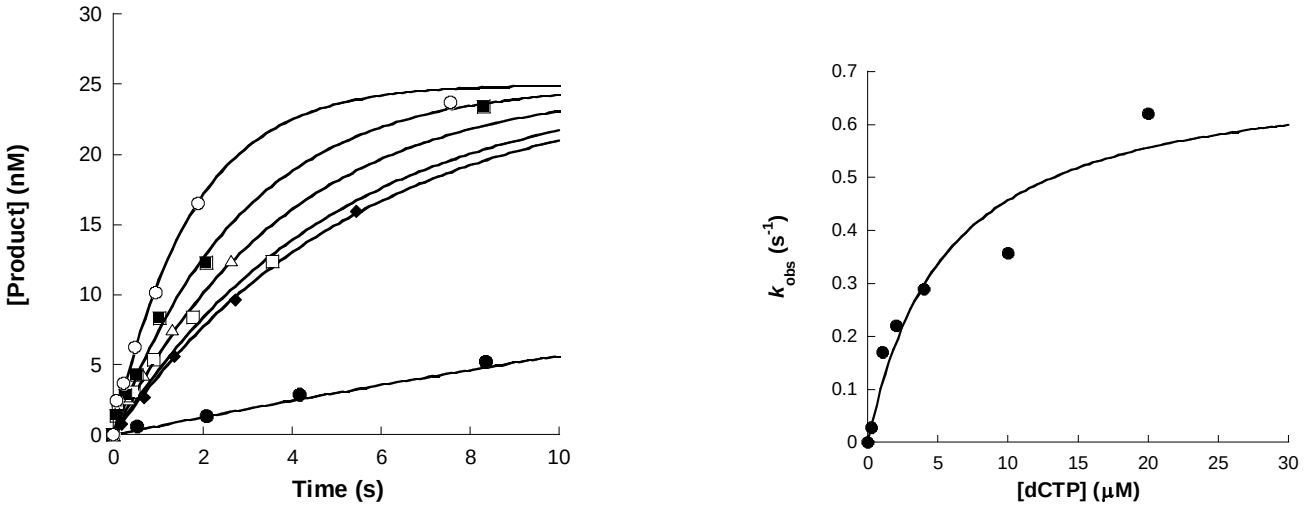


B.

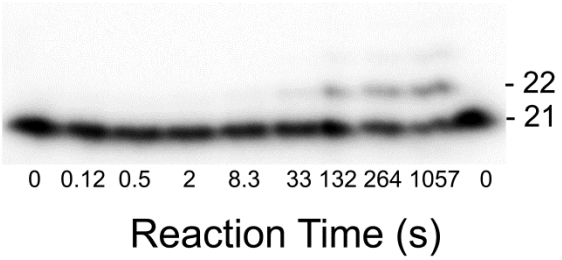


Supplementary Figure 5. Single-turnover kinetics of correct dCTP incorporation onto 20-mer/26-mer catalyzed by hPolι. (A) A preincubated solution of hPolι (130 nM) and radiolabeled 20-mer/26-mer (20 nM) at 37 °C were rapidly mixed with increasing concentrations of dCTP (10 μM, ●; 20 μM, ■; 40 μM, Δ; 80 μM, ▲; 160 μM, ○; 350 μM, ♦) for various time intervals. Each time course corresponding to each dCTP concentration was fit to a single exponential curve to obtain k_{obs}. (B) Plot of calculated k_{obs} as a function of dCTP concentration, which was fit to a hyperbola curve to $K_{d,\,dCTP}$ (133 ± 8 μM) and k_p (0.21 ± 0.01 s^{-1}).

A. B.



Supplementary Figure 6. Single-turnover kinetics of correct dCTP incorporation onto 20-mer/26-mer catalyzed by hRev1. (A) A preincubated solution of hRev1 (120 nM) and radiolabeled 20-mer/26-mer (30 nM) at 37 °C were rapidly mixed with increasing concentrations of dCTP (0.25 μM, ●; 1 μM, ♦; 2 μM, □; 4 μM, Δ; 10 μM, ■; 20 μM, ○) for various time intervals. Each time course corresponding to each dCTP concentration was fit to a single exponential curve to obtain k_{obs}. (B) Plot of calculated k_{obs} as a function of dCTP concentration, which was fit to a hyperbola curve to $K_{d,\,dCTP}$ (5.4 ± 1.2 μM) and k_p (0.78 ± 0.07 s^{-1}).



Supplementary Figure 7. Effectiveness of a DNA trap. A preincubated solution of hPolη (130 nM), unlabeled DNA trap D-1 21/41-mer (5 μM, Table 1), and 5′-radiolabeled 21-mer/26-mer (20 nM) was mixed with a solution dGTP (0.8 mM) for various times before being quenched with EDTA (0.37 M). The gel image revealed negligible nucleotide incorporation within 33 s.

Supplementary Table 1. Kinetic parameters of nucleotide incorporation onto undamaged DNA catalyzed by hPolη.

dNTP	$K_{d,\ dNTP}$ (μM)	k_p (s^{-1})	$k_p/K_{d,\ dNTP}$ ($\mu M^{-1}s^{-1}$)	Fidelity[a]
Template dG (20-mer/26-mer)				
dCTP	85 ± 11	48 ± 2	5.6×10^{-1}	-
dATP	350 ± 36	$(9.5 \pm 0.4)\times10^{-1}$	2.7×10^{-3}	4.8×10^{-3}
dGTP	37 ± 8	$(4.0 \pm 0.2)\times10^{-2}$	1.1×10^{-3}	2.0×10^{-3}
dTTP	494 ± 70	3.3 ± 0.2	6.6×10^{-3}	1.2×10^{-2}
Template dC (21-mer/26-mer)				
dGTP	46 ± 8	39 ± 2	8.4×10^{-1}	-
dATP	242 ± 25	3.4 ± 0.1	1.4×10^{-2}	1.7×10^{-2}
dCTP	119 ± 21	2.2 ± 0.1	1.8×10^{-2}	2.1×10^{-2}
dTTP	712 ± 73	3.3 ± 0.2	4.6×10^{-3}	5.4×10^{-3}

[a]Calculated as $(k_p/K_{d,\ dNTP})_{incorrect}/[(k_p/K_{d,\ dNTP})_{correct} + (k_p/K_{d,\ dNTP})_{incorrect}]$.

All given errors were derived from data fitting.

Supplementary Table 2. Kinetic parameters of nucleotide incorporation onto undamaged DNA catalyzed by hPolκ.

dNTP	$K_{d,\ dNTP}$ (μM)	k_p (s^{-1})	$k_p/K_{d,\ dNTP}$ ($\mu M^{-1}s^{-1}$)	Fidelity[a]
Template dG (20-mer/26-mer)				
dCTP	46 ± 6	1.7 ± 0.1	3.7×10^{-2}	-
dATP	539 ± 71	$(3.2 \pm 0.2)\times10^{-1}$	6.0×10^{-4}	1.6×10^{-2}
dGTP	388 ± 29	$(4.1 \pm 0.1)\times10^{-1}$	1.1×10^{-3}	2.9×10^{-2}
dTTP	693 ± 66	$(5.5 \pm 0.3)\times10^{-1}$	8.0×10^{-4}	2.1×10^{-2}
Template dC (21-mer/26-mer)				
dGTP	87 ± 9	$(9.9 \pm 0.3)\times10^{-1}$	1.1×10^{-2}	-
dATP	596 ± 121	$(2.4 \pm 0.2)\times10^{-2}$	4.0×10^{-5}	3.6×10^{-3}
dCTP	1343 ± 474	$(9.1 \pm 2.0)\times10^{-2}$	6.8×10^{-5}	6.1×10^{-3}
dTTP	645 ± 84	$(2.5 \pm 0.2)\times10^{-2}$	3.9×10^{-5}	3.5×10^{-3}

[a]Calculated as $(k_p/K_{d,\ dNTP})_{incorrect}/[(k_p/K_{d,\ dNTP})_{correct} + (k_p/K_{d,\ dNTP})_{incorrect}]$.

All given errors were derived from data fitting.

Supplementary Table 3. Kinetic parameters of nucleotide incorporation onto undamaged DNA catalyzed by hPolι.

dNTP	$K_{d,\ dNTP}$ (μM)	k_p (s^{-1})	$k_p/K_{d,\ dNTP}$ ($\mu M^{-1}s^{-1}$)	Fidelity[a]
Template dG (20-mer/26-mer)				
dCTP	133 ± 8	$(2.1 \pm 0.1)\times10^{-1}$	1.6×10^{-3}	-
dATP	667 ± 105	$(1.0 \pm 0.1)\times10^{-2}$	1.5×10^{-5}	9.3×10^{-3}
dGTP	323 ± 25	$(5.3 \pm 0.2)\times10^{-3}$	1.6×10^{-5}	9.9×10^{-3}
dTTP	447 ± 35	$(9.1 \pm 0.3)\times10^{-2}$	2.0×10^{-4}	1.1×10^{-1}
Template dC (21-mer/26-mer)				
dGTP	117 ± 7	$(19 \pm 0.4)\times10^{-2}$	1.6×10^{-3}	-
dATP	ND	ND	-	-
dCTP	ND	ND	-	-
dTTP	783 ± 44	$(13 \pm 0.4)\times10^{-2}$	1.7×10^{-4}	9.6×10^{-2}

[a]Calculated as $(k_p/K_{d,\ dNTP})_{incorrect}/[(k_p/K_{d,\ dNTP})_{correct} + (k_p/K_{d,\ dNTP})_{incorrect}]$.

ND denoted 'not determined'.

All given errors were derived from data fitting.

Supplementary Table 4. Kinetic parameters of nucleotide incorporation onto undamaged DNA catalyzed by hRev1.

dNTP	$K_{d,\ dNTP}$ (μM)	k_p (s^{-1})	$k_p/K_{d,\ dNTP}$ ($\mu M^{-1}s^{-1}$)	Fidelity[a]
Template dG (20-mer/26-mer)				
dCTP	5.4 ± 1.2	$(7.8 \pm 0.7)\times10^{-1}$	1.4×10^{-1}	-
dTTP	80 ± 14	$(2.1 \pm 0.1)\times10^{-1}$	2.6×10^{-2}	1.6×10^{-1}
dATP	99 ± 17	$(70 \pm 0.4)\times10^{-4}$	7.1×10^{-5}	5.1×10^{-4}
dGTP	256 ± 14	$(14 \pm 0.3)\times10^{-1}$	5.3×10^{-3}	3.6×10^{-2}

[a]Calculated as $(k_p/K_{d,\ dNTP})_{incorrect}/[(k_p/K_{d,\ dNTP})_{correct} + (k_p/K_{d,\ dNTP})_{incorrect}]$.

All given errors were derived from data fitting.

Supplementary Table 5. Biphasic kinetic parameters of correct nucleotide incorporations catalyzed by hPolη.

DNA Substrate (Primer/Template)	**A_1 (nM)**	**k_1 (s^{-1})**	**A_2 (nM)**	**k_2 (s^{-1})**
21-mer/26-mer	7.8 ± 0.3 (39%)[a]	72 ± 4.9	9.5 ± 0.3 (47%)	4.4 ± 0.5
21-mer/26-mer-dG^{AP}	2.9 ± 0.1 (14%)	11 ± 1.2	1.1 ± 0.2 (5.3%)	0.2 ± 0.02

[a]Calculated as (reaction amplitude/20 nM)×100.

All given errors were derived from data fitting.