Translesion synthesis DNA polymerases promote error-free replication through the minor-groove DNA adduct 3-deaza-3-methyladenine

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N3-Methyladenine (3-MeA) is formed in DNA by reaction with S-adenosylmethionine, the reactive methyl donor, and by reaction with alkylating agents. 3-MeA protrudes into the DNA minor groove and strongly blocks synthesis by replicative DNA polymerases (Pols). However, the mechanisms for replicating through this lesion in human cells remain unidentified. Here we analyzed the roles of translesion synthesis (TLS) Pols in the replication of 3-MeA-damaged DNA in human cells. Because 3-MeA has a short half-life in vitro, we used the stable 3-deaza analog, 3-deaza-3-methyladenine (3-dMeA), which blocks the DNA minor groove similarly to 3-MeA. We found that replication through the 3-dMeA adduct is mediated via three different pathways, dependent upon Pols/Polɛ, Polθ, and Polλ. As inferred from biochemical studies, in the Polɛ/Polɛ pathway, Polɛ inserts a nucleotide (nt) opposite 3-dMeA and Polλ extends synthesis from the inserted nt. In the Polθ pathway, Polθ carries out both the insertion and extension steps of TLS opposite 3-dMeA, and in the Polλ pathway, Polλ extends synthesis following nt insertion by an as yet unidentified Pol. Steady-state kinetic analyses indicated that Polɛ and Polθ insert the correct nt opposite 3-dMeA with a much reduced catalytic efficiency and that both Pols exhibit a high propensity for inserting a wrong nt opposite this adduct. However, despite their low fidelity of synthesis opposite 3-dMeA, TLS opposite this lesion replicates DNA in a highly error-free manner in human cells. We discuss the implications of these observations for TLS mechanisms in human cells.

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2. The abbreviations used are: 3-MeA, N3-methyladenine; 3-dMeA, 3-deaza-3-methyladenine; Pol, polymerase; TLS, translesion synthesis; nt, nucleotide; Kan, kanamycin.

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Results

Genetic control of replication through the 3-dMeA lesion in human cells

3-dMeA was incorporated in the lacZ target sequence in the leading strand (Fig. 1A) and the schematic of siRNA depletions and the various steps for the analyses of TLS frequency and for mutation analyses is shown in Fig. 1B. In the plasmid system, TLS through the lesion generates Kan$^+$ blue colonies and the frequency of Kan$^+$ blue colonies among the total Kan$^-$ colonies gives a very reliable and highly repeatable estimate of TLS frequency.

To identify the TLS pols required for replicating through the 3-dMeA lesion, we examined the effects of siRNA depletion of various TLS Pols, including Pols $\eta$, $\iota$, $\kappa$, $\theta$, and $\zeta$. For all the TLS Pols analyzed, we ascertained that siRNA treatment led to a highly efficient depletion of the intended protein similar to that shown previously (10, 11). The TLS data we obtained from independent TLS assays in human cells are highly reproducible as evidenced from the high repeatability of data for TLS opposite UV-induced lesions, cis-syn TT dimer, and the (6–4)-TT photoproduct reported in different studies (10, 12, 13).

TLS opposite 3-dMeA in normal human fibroblasts treated with control (NC) siRNA occurs with a frequency of $\sim$46% (Table 1), and the TLS frequency remains the same in Pol$\eta$-depleted cells indicating that Pol$\eta$ is not required. By contrast, TLS frequency is reduced to $\sim$27% in Pol$\iota$-, Pol$\kappa$-, or Pol$\zeta$-depleted cells. To determine whether Pols $\iota$ and $\kappa$ function together or independently, we examined the effects of their simultaneous depletion on TLS frequency. Our observation that their simultaneous depletion causes no further reduction in TLS frequency than that conferred by their individual depletion indicates that they function together in mediating replication through the 3-dMeA lesion. Depletion of Pol$\theta$ or the Rev3 catalytic or Rev7 accessory subunit of Pol$\zeta$ also led to a reduction in TLS frequency to $\sim$25%. To determine whether Pol$\theta$ and Pol$\zeta$ function together in one TLS pathway or whether they constitute independent pathways, we exam-
Table 1
Effects of siRNA knockdowns of TLS polymerases on the replicative bypass of 3-dMeA carried on the leading strand template in human fibroblasts

| siRNA   | No. Kan* colonies | No. blue colonies among Kan* | TLS   |
|---------|-------------------|------------------------------|-------|
| NC      | 446               | 206                          | 46.2  |
| Polθ    | 358               | 161                          | 45.0  |
| Polκ    | 326               | 90                           | 27.6  |
| Polκ + Polθ | 268             | 72                           | 26.9  |
| Polθ + Polκ | 385             | 94                           | 24.4  |
| Polκ + Polκ | 415             | 105                          | 25.3  |
| Polκ + Rev3 | 427             | 108                          | 25.3  |
| Rev3    | 305               | 78                           | 25.6  |
| Polθ + Rev3 | 405             | 52                           | 12.8  |
| Polκ + Polθ | 306             | 43                           | 14.1  |
| Polκ + Polκ | 348             | 47                           | 13.5  |
| Polκ + Rev3 | 350             | 48                           | 13.7  |
| Polκ + Rev7 | 350             | 48                           | 13.7  |
| Polκ + Rev3 | 408             | 52                           | 12.7  |

Table 2
Effect of siRNA knockdowns of Rev1 in combination with other TLS Pols on the replicative bypass of 3-dMeA carried on the leading strand template in human fibroblasts

| siRNA   | No. Kan* colonies | No. blue colonies among Kan* | TLS   |
|---------|-------------------|------------------------------|-------|
| NC      | 446               | 206                          | 46.2  |
| Rev1    | 408               | 96                           | 23.5  |
| Rev1 + Polκ | 371             | 90                           | 24.3  |
| Rev1 + Polθ | 415             | 95                           | 22.9  |
| Rev1 + Polκ | 402             | 51                           | 12.7  |
| Rev1 + Rev3 | 390             | 48                           | 12.3  |

Figure 2. TLS pathways for replicating through 3-dMeA in human cells. Replication through 3-dMeA occurs via 3 different Pol/Polκ-, Polθ, and Polκ-dependent pathways and all three pathways mediate error-free TLS through the lesion. The roles of Pols in inserting a nt opposite 3-dMeA and in extension of synthesis from the inserted nt are indicated.

Figure 3. Error-free replication through 3-dMeA in human cells
Sequence analyses of TLS products from cells treated with control siRNA revealed no mutational events, and we also found no evidence of mutational events among the TLS products obtained from Polκ-, Rev1-, Polθ-, or Polκ-depleted cells (Table 3). Thus, TLS opposite 3-dMeA occurs in a highly error-free manner in human cells.

Non-catalytic role of Rev1 in the Polκ/Polκ pathway
Rev1 specifically incorporates a C opposite template G (14). In the Rev1 active site, G does not form a base pair with the incoming C; instead, template G is pushed out into a solvent-filled cavity and a conserved Arg residue in Rev1 then pairs with the incoming C (15, 16). Thus, Rev1 DNA polymerase activity is specifically adapted to insert a C opposite N2-dG DNA adducts and would play no role in TLS opposite 3-dMeA. However, in mammalian cells, Rev1 also plays an indispensable role as a scaffolding component of Y-family Pols η, κ, and θ (10). Hence, even though the Rev1 catalytic activity would play no role in TLS opposite 3-dMeA, its scaffolding role would be required for TLS mediated by the Polκ/Polκ pathway but not for TLS mediated by the Polθ or Polκ pathways. To confirm this, we examined the effects of co-depletion of Rev1 with Polκ, Polθ, or Polκ. As shown in Table 2, Rev1 depletion reduces the TLS frequency to ~23%, and this frequency remained the same upon co-depletion of Rev1 with Polκ or Polθ. By contrast, simultaneous depletion of Rev1 with Polθ or Rev3 reduced TLS frequency to ~12%. The epistasis of Rev1 with Polκ or Polκ and the reduction in TLS frequency upon co-depletion of Rev1 with Polκ or Polκκ is consistent with the requirement of Rev1 for Polκ/Polκ-dependent TLS but not for TLS mediated by Polθ or Polκ.

Error-free replication through 3-dMeA in human cells
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Catalytic efficiency and fidelity of TLS Pols for DNA synthesis opposite 3-dMeA
Poll primarly inserts a T opposite 3-dMeA, and in the presence of 4 dNTPs, it inserts a T opposite the lesion but does not extend synthesis from the inserted nt (Fig. 3). Using steady-state kinetic analyses, we determined the catalytic efficiency and fidelity of Polκ for nt insertion opposite undamaged A versus 3-dMeA. As shown in Table 4, Polκ inserts a T nt opposite an undamaged A with over 1000-fold higher catalytic efficiency than an incorrect nt. However, compared with the incorporation of a T opposite undamaged A, T is incorporated opposite 3-dMeA with an ~8-fold lower efficiency. Moreover, compared with the efficiency for T incorporation opposite 3-dMeA, the efficiency for incorporating the incorrect nt A or G opposite 3-dMeA is reduced by only ~25–30-fold, and the efficiency for incorporating a C is reduced by ~90-fold. Thus, the catalytic efficiency of Polκ for incorporating the correct nt opposite 3-dMeA is reduced and the efficiency for incorporating an incorrect nt is enhanced. As determined by steady-state kinetic analyses, Polκ extends synthesis from the 3-dMeA:T base pair by incorporating dATP opposite the next 5’ template base with
an ~10-fold reduced efficiency compared with that for the undamaged A:T base pair (Table 5).

Polθ primarily incorporates a T opposite 3-dMeA; however, it incorporates an A also, albeit not as well, and in the presence of 4 dNTPs, Polθ replicates DNA through the 3-dMeA lesion (Fig. 4). Steady-state kinetic analyses indicate that opposite undamaged A, Polθ incorporates a T with an over 10^3 higher catalytic efficiency than any of the incorrect nts; however, compared with that opposite undamaged A, the catalytic efficiency of Polθ for T incorporation opposite 3-dMeA is reduced almost 100-fold (Table 6). Moreover, compared with the efficiency for T incorporation opposite 3-dMeA, Polθ incorporates an A opposite this lesion with only an ~10-fold reduction in efficiency, a G with an ~30-fold reduction in efficiency, and a C with an ~120-fold reduction in efficiency (Table 6). Thus, the catalytic efficiency and fidelity of Polθ for incorporating the correct nt opposite 3-dMeA is greatly reduced. The efficiency of Polθ for incorporating dATP opposite the next 5’T template base is reduced ~20-fold compared with the efficiency for extending synthesis from the undamaged A:T base pair (Table 7).

Polζ is a proficient extender of synthesis from nts incorporated opposite DNA lesions by another TLS Pol (17–21). We find that the efficiency of Polζ for extension of synthesis from the 3-dMeA:T base pair is reduced ~30-fold compared with that for extending the undamaged A:T base pair (Table 8). Because Polζ exhibits poor efficiency for inserting nts opposite DNA lesions, we presume that another as yet unidentified TLS Pol inserts nts opposite 3-dMeA from which Polζ then extends synthesis.

### Discussion

**A major role of TLS in promoting replication through the 3-dMeA lesion**

Our results indicating that TLS in normal human fibroblasts accounts for almost 50% lesion bypass strongly suggest that TLS provides a primary means for replicating through this DNA lesion. Because the 3-dMeA lesion could have been removed from the plasmid by the 3-alkyladenine DNA glycosylase (22), or by nucleotide excision repair, and because removal of the lesion by these repair processes will generate Kan⁺ white colonies, the proportion of Kan⁺ blue colonies, indicative of TLS, will be correspondingly reduced. Thus, the TLS frequency in excision repair proficient cells underestimates the frequency with which TLS actually occurs. The high frequency of TLS in repair proficient cells supports the premise that TLS provides the primary mechanism for replicating through the 3-dMeA lesion and that alternative lesion bypass mechanisms such as filling-in of the gap opposite the lesion site by template switching play a much less significant and subsidiary role. TLS also plays a prominent role in promoting replication through other DNA lesions such as UV induced cis-syn TT dimer (12) and (6–4)-TT photoproduct (13), thymine glycol (11, 23) generated by the reaction of oxygen-free radicals with thymine; and N1-methyladenine (24) generated by reaction with naturally occurring methyl halides and by reaction with environmental methylating agents.

### Action mechanisms of TLS Pols in DNA synthesis opposite 3-dMeA

The ability of Polζ to push the purine template into a syn conformation and to form a Hoogsteen base pair with the incoming pyrimidine nt provides a mechanism by which Polζ could insert a T opposite 3-dMeA (7, 8), and the ability of Polε to accommodate the 3-dMeA:T base pair at the primer terminus (9) would allow it to extend synthesis from this base pair. In the absence of structural information, it is unclear whether Polθ pushes the 3-dMeA lesion into a syn conformation for forming a Hoogsteen base pair with the incoming dTTP and then it extends synthesis from the 3-dMeA:T Hoogsteen base pair; or whether the minor groove disruption by 3-dMeA presents no steric hindrance to the Polθ active site; consequently, it forms a 3-dMeA:T Watson-Crick (W-C) base pair and then extends synthesis from the W-C base pair. For the Polζ-dependent pathway, we expect Polζ to extend synthesis from the T nt inserted opposite 3-dMeA by a DNA polymerase whose identity remains to be determined. However, the possibility that human Polζ can insert a nt opposite 3-dMeA as well as extend synthesis from the inserted nt cannot be entirely excluded at this point.

### Highly error-free replication through the 3-dMeA lesion

Sequence analysis of TLS products has indicated that all three pathways dependent upon Polζ/Polκ, Polθ, and Polε, respectively, conduct TLS opposite 3-dMeA in an error-free manner. In striking contrast to the lack of mutational TLS products in human cells, in vitro biochemical studies have indicated that TLS Pols synthesize DNA opposite 3-dMeA with a...
the Michaelis-Menten equation. Apparent incorporation rate was plotted against dNTP concentration and the data were fit to the Michaelis-Menten equation. Apparent $k_{\text{cat}}$ and $K_m$ values were obtained from the fit and used to calculate the efficiency of deoxynucleotide incorporation ($k_{\text{cat}}/K_m$).

#### Table 4

**Steady-state kinetic analyses of nucleotide incorporation opposite undamaged A or 3-dMeA by human Pol**

Pol (0.02–0.2 nM) was incubated with primer:template DNA substrate (10 nM) and increasing concentrations of dNTPs for 10 min at 37 °C. The nucleotide incorporation rate was plotted against dNTP concentration and the data were fit to the Michaelis-Menten equation. Apparent $K_m$ and $k_{\text{cat}}$ values were obtained from the fit and used to calculate the efficiency of deoxynucleotide incorporation ($k_{\text{cat}}/K_m$).

| Template nucleotide | Incoming nucleotide | $k_{\text{cat}}$ | $K_m$ | $k_{\text{cat}}/K_m$ | Catalytic efficiency relative to T |
|---------------------|---------------------|-----------------|-------|---------------------|-----------------------------------|
| A                   | T                   | 10.7 ± 0.6      | 5.3 ± 0.9 | 2                   | 1 × 10^{-3} (↓ 1000 ×)            |
| A                   | A                   | 0.54 ± 0.02     | 220 ± 38.4 | 0.002               | 7 × 10^{-4} (↓ 1428 ×)            |
| G                   | G                   | 0.48 ± 0.04     | 332 ± 70  | 0.0014              |                                   |
| C                   | ND                  |                 | ND      |                     |                                   |
| 3-dMeA              | T                   | 3.2 ± 0.09      | 12.4 ± 1.2 | 0.25                | 1                                |
| A                   | A                   | 1.5 ± 0.22      | 192 ± 96  | 0.0078              | 3.1 × 10^{-2} (↓ 32 ×)            |
| G                   | G                   | 0.62 ± 0.02     | 59 ± 12.0 | 0.01                | 4 × 10^{-2} (↓ 25 ×)             |
| C                   | C                   | 0.37 ± 0.02     | 131.6 ± 29 | 0.0028              | 1.1 × 10^{-4} (↓ 89 ×)           |

#### Table 5

**Kinetic parameters of extension reaction catalyzed by human Pol**

Pol (0.02–0.07 nM) was incubated with primer:template DNA substrate (10 nM) and dNTPs for 5–10 min at 37 °C. The catalytic efficiency for inserting the incorrect nt opposite undamaged A with a 1000-fold or even higher catalytic efficiency than an incorrect nt, it incorporates a T opposite 3-dMeA to a greatly reduced efficiency compared with that opposite undamaged A, and the catalytic efficiency of Polθ for inserting an incorrect nt opposite 3-dMeA is increased. The striking discrepancy between the low fidelity of DNA synthesis by Polθ opposite 3-dMeA observed in *in vitro* biochemical studies versus the high fidelity of TLS opposite this lesion observed in human cells strongly suggests that the fidelity of TLS Pols is tightly regulated during replication through this DNA lesion in human cells.

#### Low fidelity

Thus, whereas Pol incorporates a T opposite undamaged A with a 1000-fold or even higher catalytic efficiency than an incorrect nt, it incorporates a T opposite 3-dMeA with a reduced efficiency than opposite undamaged A and the catalytic efficiency for inserting the incorrect nt opposite 3-dMeA is enhanced. For Polθ, the proficiency for incorporating a T opposite 3-dMeA is greatly reduced compared with that opposite undamaged A, and the catalytic efficiency of Polθ for inserting an incorrect nt opposite 3-dMeA is increased. The striking discrepancy between the low fidelity of DNA synthesis by Pol or Polθ opposite 3-dMeA observed in *in vitro* biochemical studies versus the high fidelity of TLS opposite this lesion observed in human cells strongly suggests that the fidelity of TLS Pols is tightly regulated during replication through this DNA lesion in human cells.

#### Experimental procedures

**Construction of plasmid vectors containing 3-dMeA**

Since the half-life of 3-MeA in *in vitro* has been established to be between 12 and 24 h (3), that precludes the construction of oligos containing this DNA adduct. To circumvent this problem, a stable 3-deaza analog of the nucleoside 3’-methyl-2’-deoxyadenosine was incorporated into oligos as 3-dMeA. 3-dMeA projects into the DNA minor groove and blocks synthesis by replicative Pols. Hence, for all the genetic and biochemical studies, we used 3-dMeA lesion-containing DNAs.

3-Deaza-3-methyldeoxyadenosine oligonucleotides were synthesized on a Model 8909 Expedite DNA synthesizer using standard DNA synthesis chemistry. The 3-deaza-3-methyl-deoxyadenosine was incorporated using an offline coupling mode for incorporation of the 3-deaza-3-Me-dA-cyanoethyl phosphoramidite (purchased from Berry and Associates, Inc., Dexter, MI 48130). The oligos were deprotected using standard concentrated ammonia deprotection, and purified and analyzed by reverse phase HPLC on a Beckman System Gold
Table 6
Steady-state kinetic analyses of nucleotide incorporation opposite undamaged A or 3-dMeA by human Polθ
Polθ (1 nM) was incubated with primer/template DNA substrate (10 nM) and increasing concentration of dNTPs for 10 min, at 37 °C. The nucleotide incorporation rate was plotted against dNTP concentration and the data were fit to the Michaelis-Menten equation. Apparent $k_{cat}$ values were obtained from the fit and used to calculate the efficiency of deoxynucleotide incorporation ($k_{cat}/K_{m}$).

| Template nucleotide | Incoming nucleotide | $k_{cat}$ min$^{-1}$ | $K_{m}$ μM | $k_{cat}/K_{m}$ | Catalytic efficiency relative to T
|---------------------|---------------------|---------------------|--------------|-----------------|---------------------------------|
| A                   | T                   | 0.35 ± 0.02         | 0.0078 ± 0.0018 | 45.2           | 1.24 × 10$^{-4}$ (4113 ×)      |
| G                   | A                   | 0.62 ± 0.06         | 52.6 ± 10.8   | 0.011          | 1.5 × 10$^{-4}$ (6464 ×)       |
| C                   | G                   | 0.59 ± 0.05         | 79.5 ± 15.6   | 0.007          | 4.8 × 10$^{-4}$ (2056 ×)       |
| 3-dMeA              | T                   | 0.79 ± 0.05         | 35.5 ± 5.5    | 0.022          | 8.3 × 10$^{-5}$ (120 ×)        |

Table 7
Kinetic parameters of extension reaction catalyzed by human Polθ from A:T or 3-dMeA:T base pair
Polθ (1 nM) was incubated with primer/template DNA substrate (10 nM) and increasing concentration of dNTPs for 10 min, at 37 °C. The nucleotide incorporation rate was plotted against dNTP concentration and the data were fit to the Michaelis-Menten equation. Apparent $K_{m}$ and $k_{cat}$ values were obtained from the fit and used to calculate the efficiency of deoxynucleotide incorporation ($k_{cat}/K_{m}$).

| Primer/template base pair | dNTP added | $k_{cat}$ min$^{-1}$ | $K_{m}$ μM | $k_{cat}/K_{m}$ | Relative catalytic efficiency
|---------------------------|------------|---------------------|-------------|-----------------|---------------------------------|
| A/T                       | dATP       | 0.84 ± 0.02         | 0.10 ± 0.009 | 8.4             | 1                              |
| 3-dMeA/T                  | dATP       | 0.55 ± 0.3          | 1.3 ± 0.18   | 0.42            | 20 1                           |

Table 8
Kinetic parameters of extension reaction catalyzed by yeast Polξ from A:T or 3-dMeA:T base pair
Polξ (0.2 nM) was incubated with primer/template DNA substrate (10 nM) and increasing concentration of dNTPs for 10 min, at 37 °C. The nucleotide incorporation rate was plotted against dNTP concentration and the data were fit to the Michaelis-Menten equation. Apparent $K_{m}$ and $k_{cat}$ values were obtained from the fit and used to calculate the efficiency of deoxynucleotide incorporation ($k_{cat}/K_{m}$).

| Primer/template base pair | dNTP added | $k_{cat}$ min$^{-1}$ | $K_{m}$ μM | $k_{cat}/K_{m}$ | Relative catalytic efficiency
|---------------------------|------------|---------------------|-------------|-----------------|---------------------------------|
| A/T                       | dATP       | 0.5 ± 0.012         | 0.04 ± 0.005 | 12.5            | 1                              |
| 3-dMeA/T                  | dATP       | 0.7 ± 0.07          | 1.7 ± 0.64   | 0.41            | 33 1                           |

HPLC. Oligonucleotides were additionally analyzed and confirmed by MALDI-MS on a Bruker Autoflex MALDI mass spectrophotometer.

The in-frame target sequence of the lacZ’ gene containing 3-dMeA is shown in Fig. 1A. Because the lacZ’ sequence in the 3-dMeA-containing DNA strand is in-frame, it encodes functional β-galactosidase (β-gal); the opposite DNA strand harbors an Spel restriction site containing a +1 frameshift, which makes it non-functional for β-gal. The 3-dMeA-containing strand carries the kanamycin gene (Kan'), whereas the other DNA strand has the kan’ gene (Fig. 1A). The detailed methods for the construction of lesion-containing SV40-based duplex plasmids have been published previously (12, 13).

Assays for translesion synthesis and mutation analyses of TLS products in human cells

The schematic of TLS assay is shown in Fig. 1B and the detailed methods for TLS assays have been published previously (12, 13). Briefly, human fibroblast GM637 cells were transfected with the particular siRNA and after 48 h of incubation, the target vector DNA and siRNA (second transfection) were co-transfected. After 30 h of incubation, plasmid DNA was transfected into Escherichia coli XL1-Blue super competent cells (Stratagene) and cells plated on LB/kan plates containing isopropyl 1-thio-β-D-galactopyranoside (GenDEPOT) and 100 μg/ml of X-gal (GenDEPOT). TLS frequency is determined from the number of blue colonies among total colonies growing on LB/Kan plates and mutation frequencies and mutational changes were analyzed by DNA sequencing.

DNA polymerase assays

DNA substrates consisted of a radiolabeled oligonucleotide primer annealed to a 75-nt oligonucleotide DNA template by DNA polymerase assays and it contained an undamaged A or 3-dMeA at the underlined position. For examining the incorporation of dATP, dTTP, dCTP, or dGTP nucleotides individually, or of all 4 dNTPs, a 44-mer primer 5'-GTT TTC CGT GAC TGG GAA AAC-3' was annealed to the above 75-mer template. The standard DNA polymerase reaction (5 μl) contained 25 mM Tris-HCl (pH 7.5), 5 mM MgCl$_2$, 1 mM dithiothreitol, 100 μg/ml of BSA, 10% glycerol, 10 mM DNA substrate, and 0.2 nM Pol or 1 nM Polθ. For nucleotide incorporation assays, 50 or 25 μM dATP, dTTP, dCTP, or dGTP (Roche Biochemicals) were used for Polξ and Polθ, respectively, and for examining synthe-
Steady-state kinetic analysis

Steady-state kinetic analyses for deoxynucleotide incorporation were performed as described (25). Gel band intensities of the substrate and products of the deoxynucleotide incorporation reactions were quantified using a PhosphorImager and ImageQuant software (Molecular Dynamics). The observed rate of deoxynucleotide incorporation, $v_{obs}$, was determined by dividing the amount of product formed by the reaction time and protein concentration. The $v_{obs}$ was graphed as a function of the deoxynucleotide concentration, and the data were fit to the Michaelis-Menten equation describing a hyperbola: $v_{obs} = \left(\frac{k_{cat}[E]}{K_m} \times [dNTP])/(K_m + [dNTP])\right)$. From the best fit curve, the apparent $K_m$ and $k_{cat}$ steady-state kinetics parameter were obtained for the incorporation of dATP, dGTP, dCTP, and dTTP and the efficiencies of the nucleotide incorporation ($k_{cat}/K_m$) were determined.

Author contributions—J. H. Y. performed and analyzed the experiments on the genetic control of TLS and mutagenicity. J. R. C. performed the biochemical experiments and analyzed the data. J. P. contributed to the genetic experiments, S. P. and L. P. designed and coordinated the study and wrote the paper. All authors reviewed the results and approved the final version of the manuscript.

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