DNA polymerase $\lambda$ promotes error-free replication through Watson–Crick impairing N1-methyl-deoxyadenosine adduct in conjunction with DNA polymerase $\zeta$

In a previous study, we showed that replication through the N1-methyl-deoxyadenosine (1-MeA) adduct in human cells is mediated via three different Pol$\lambda$/Pol$\theta$, Pol$\eta$, and Pol$\zeta$-dependent pathways. Based on biochemical studies with these Pols, in the Pol$\lambda$/Pol$\theta$ pathway, we inferred a role for Pol$\lambda$ in the insertion of a nucleotide (nt) opposite 1-MeA and of Pol$\theta$ in extension of synthesis from the inserted nt; in the Pol$\eta$ pathway, we inferred that this Pol alone would replicate through 1-MeA; in the Pol$\zeta$ pathway, however, the Pol required for inserting an nt opposite 1-MeA had remained unidentified.

In this study, we provide biochemical and genetic evidence for a role for Pol$\lambda$ in inserting the correct nt T opposite 1-MeA, from which Pol$\lambda$ would extend synthesis. The high proficiency of purified Pol$\lambda$ for inserting a T opposite 1-MeA implicates a role for Pol$\lambda$—which normally uses W-C base pairing for DNA synthesis—in accommodating 1-MeA in a syn confirmation and forming a Hoogsteen base pair with T. The potential of Pol$\lambda$ to replicate through DNA lesions by Hoogsteen base pairing adds another novel aspect to Pol$\lambda$'s role in translesion synthesis in addition to its role as a scaffolding component of Pol$\zeta$. We discuss how the action mechanisms of Pol$\lambda$ and Pol$\lambda$ could be restrained to inserting a T opposite 1-MeA and extending synthesis thereafter, respectively.

Translesion synthesis (TLS) DNA polymerases (Pols) exhibit a high specificity for replicating through different types of DNA lesions. Whereas replication through certain DNA lesions can be performed by just one Pol, such as by Pol$\eta$ opposite cyclobutane pyrimidine dimers (CPDs) (1–6), replication through a vast array of DNA lesions requires the sequential action of two Pols, wherein one Pol inserts a nucleotide (nt) opposite the DNA lesion and another Pol extends synthesis from the inserted nt. Biochemical and structural studies with yeast Pol$\lambda$ have provided strong evidence for its role in extending synthesis from nts inserted opposite DNA lesions by other TLS Pols (7–10), and genetic evidence accrued from TLS studies opposite a number of DNA lesions in human cells aligns with such a Pol$\zeta$ role (5, 6, 11, 12).

In yeast or cancer cells, Rev1 functions as a scaffolding component of Pol$\zeta$ and TLS by Rev1-Pol$\zeta$ operates in a highly error-prone manner (13–18). In normal human cells, however, Rev1 functions as an indispensable scaffolding component of the Y-family Pols $\eta$, $\iota$, and $\kappa$; and TLS studies oppose a number of DNA lesions have indicated that Rev1-dependent TLS by Y-family Pols operates in a much more error-free manner in human cells than indicated from the fidelity of the purified Pols (11, 19–21). Furthermore, in a recent study we provided evidence for an indispensable role of Pol$\lambda$ as a scaffolding component of Pol$\zeta$ and from TLS studies opposite a number of DNA lesions, we inferred that Pol$\lambda$-dependent TLS by Pol$\lambda$ operates in a predominantly error-free manner in human cells (22). In that study we analyzed Pol$\lambda$'s role in TLS opposite the UV lesions CPDs and (6–4) pyrimidine- pyrimidine photoproducts (6–4) PPs, the oxidative DNA lesion thymine glycol (Tg), and the 1,N6-ethenodeoxyadenosine (eda) lesion—formed in DNA through interaction with aldehydes derived from lipid peroxidation. In TLS opposite CPD, Tg, and eda, Pol$\lambda$ extends synthesis from the nt inserted opposite the lesion site by another DNA Pol; and although Pol$\lambda$ is indispensable for Pol$\zeta$'s role in TLS opposite these DNA lesions, its DNA polymerase activity is not required. Thus, for TLS opposite these DNA lesions, only Pol$\lambda$’s scaffolding activity is required (22). For TLS opposite (6–4) PPs, however, Pol$\lambda$’s polymerase activity is also required, and Pol$\lambda$ promotes error-free replication through this lesion in human and mouse cells (22). Since (6–4) TT PP induces a large structural distortion in DNA and since it impairs the ability of the 3'T to form a normal Watson–Crick (W-C) base pair with the correct nt (23–26), it remains unclear how Pol$\lambda$, which uses W-C base pairing for normal DNA synthesis, manages error-free TLS opposite this DNA lesion.

N1-methyl-deoxyadenosine (1-MeA) is repaired by direct demethylation, primarily by the ABH2 enzyme in human cells (27). The evidence that 1-MeA residues accumulate over time in the genomic DNA of the livers from ABH2 null mice has indicated that endogenous DNA methylation contributes to their formation (27). In human cells, TLS through the 1-MeA adduct is mediated via three independent pathways in which
Polι and Polθ function in one pathway and Polς η and ζ function in the other two pathways, respectively (28). TLS by all three pathways operates in a predominantly error-free manner in human cells. For the Polι/Polθ pathway, following nt insertion by Polι opposite 1-MeA by forming a Hoogsteen base pair with the T residue (29), Polθ would extend synthesis, whereas in the Polς pathway, Polς would perform both the steps of TLS (28). Our evidence for the requirement of Polλ as an indispensable scaffolding component of Polζ strongly suggested that it would be required for Polζ-dependent TLS opposite 1-MeA; further, it raised the possibility that Polλ may insert the correct nt opposite 1-MeA from which Polζ could extend synthesis.

Here we provide genetic and biochemical evidence for the role of Polλ in conjunction with Polκ in mediating error-free replication through 1-MeA by inserting the correct nt opposite it. We discuss how by adopting Hoogsteen base pairing as a mechanism for inserting the correct nt opposite 1-MeA, Polλ could promote error-free replication through this adduct.

**Results**

**Requirement of Polλ for TLS opposite 1-MeA in conjunction with Polκ**

In our previous analyses of the genetic control of TLS opposite 1-MeA in human cells, we identified the involvement of three independent Polι/Polθ, Polς, and Polζ pathways (28). In the Polζ pathway, however, the identity of the Pol that could insert an nt opposite 1-MeA had remained unknown. To determine whether Polλ functions together with Polκ, we analyzed the effects of siRNA depletion of Polλ alone and in combination with depletion of other TLS Pols on TLS frequency opposite 1-MeA carried on the leading strand template in the duplex plasmid in which bidirectional replication initiates from an origin of replication (28).

As shown in **Table 1**, TLS in normal human fibroblasts (HFs) treated with control (NC) siRNA occurs with a frequency of ~63%. In Polς depleted cells, TLS frequency is reduced to ~53% and depletion of Polι, Polθ, Rev3, or Polλ reduced TLS frequency to 40 to 46%. Our evidence that codepletion of Polλ with Polς, Polι, or Polθ reduces TLS frequency nearly to ~27% indicated a role for Polλ in a TLS pathway independent of Polζ or Polι/Polθ pathways, and our observation that TLS frequency remains the same in cells codepleted for Polλ and Rev3 (~42%) as in cells depleted for either Pol alone implicated a role for Polλ in TLS in conjunction with Polζ.

To provide further evidence for the role of Polλ in TLS with Polκ, we analyzed the effects or Polλ depletion alone and in combination with the depletion of other TLS Pols in XPV HFs (Table 2). In control siRNA-treated XPV HFs, TLS opposite 1-MeA occurs with a frequency of ~47% and as expected from the role of Polι/Polθ and Polλ/Polζ in Polς-independent pathways, TLS frequency is reduced to ~30% in XPV HFs depleted for Polι, Polθ, Rev3, or Polλ (Table 2). Our results that TLS frequency is reduced to ~5% in XPV HFs codepleted for Polλ either with Polι or with Polθ and that TLS frequency remains nearly the same (~29%) in XPV HFs codepleted for Polλ and Rev3 as in cells depleted for either Pol alone add further support for the role of Polλ in TLS opposite 1-MeA together with Polζ and independent of Polι and Polθ. Altogether from TLS analyses in WT HFs and XPV HFs, we conclude that TLS through 1-MeA operates via three independent Polι/Polθ, Polς, and Polλ/Polζ pathways (Fig. 1).

**Requirement of Polλ’s polymerase activity for TLS opposite 1-MeA**

To determine if Polλ’s polymerase activity was required for TLS opposite 1-MeA, we analyzed the effects of the D427A, D429A mutations, which inactivate this activity. For these studies, we stably expressed siRNA-resistant wild-type human Polλ or the D427A, D429A catalytic mutant Polλ in WT HFs. As shown in **Table 3**, TLS opposite 1-MeA in Polλ-depleted HFs harboring the vector plasmid occurs with a frequency of ~45%, and the frequency rises to ~64% in cells expressing WT Polλ. Our results that TLS frequency is reduced to the same level (~45%) in cells expressing the D427A, D429A catalytic mutant as in cells harboring the vector plasmid establish the requirement of Polλ’s polymerase activity for TLS through 1-MeA in human cells. Additionally, we confirmed the requirement of Polλ’s polymerase activity for TLS through this adduct in Polλ<sup>−/−</sup> MEFs (Table 4).

**Polλ’s BRCT domain is not required for TLS opposite 1-MeA**

Polλ is a 575 residue polypeptide that contains an N-terminal BRCT domain. We have shown previously that N-terminally deleted Polλ comprised of residues 245 to 575, which lacks the BRCT domain and the proline-rich region, physically interacts with the Rev7 subunit of Polι and that this N-terminally deleted Polλ supports TLS through (6–4) TT photoproduct in human cells (22). Our results that expression of (245–575) Polλ in HFs supports WT levels of TLS (Table 3) confirm that the N-terminal BRCT domain and the adjoining proline-rich region are also not required for Polλ’s role in TLS through 1-MeA in HFs (Table 3); additionally, we confirmed these results in Polλ<sup>−/−</sup> MEFs (Table 4).
Table 2

| siRNA          | Number of $Kan^{+}$ colonies | Number of blue colonies among $Kan^{+}$ colonies | TLS (%) |
|----------------|-----------------------------|-----------------------------------------------|--------|
| NC             | 396                         | 180                                           | 45.5   |
| Pol$\lambda$   | 410                         | 124                                           | 30.2   |
| Pol$\beta$     | 395                         | 110                                           | 27.8   |
| Rev3           | 426                         | 129                                           | 30.3   |
| Pol$\omega$    | 502                         | 145                                           | 28.9   |
| Pol$\lambda$ + Pol$\lambda$ | 230 | 11                                           | 4.8    |
| Pol$\beta$ + Pol$\lambda$ | 426 | 23                                           | 5.4    |
| Rev3 + Pol$\lambda$ | 472 | 136                                          | 28.8   |

**Purified Pol$\lambda$ conducts error-free TLS through 1-MeA**

The requirement of Pol$\lambda$'s polymerase activity for TLS through the 1-MeA adduct in human cells suggested that Pol$\lambda$ would insert an nt opposite 1-MeA from which Pol$\lambda$ would extend synthesis. Hence, we examined purified Pol$\lambda$ for its ability to insert dATP, dTTP, dGTP, or dCTP opposite 1-MeA and to synthesize DNA through the adduct in the presence of all four dNTPs. As shown in Figure 2, Pol$\lambda$ replicates through the undamaged template residue A by inserting a T. Opposite 1-MeA also Pol$\lambda$ inserts a T and then extends synthesis similar to that on undamaged DNA.

The high proficiency of Pol$\lambda$ for inserting the correct nt opposite 1-MeA stands in sharp contrast to the error-proneness of purified Pol$\beta$ or Pol$\gamma$ opposite this adduct (28). Thus, in addition to the insertion of a T, Pol$\lambda$ insert an A or a C opposite 1-MeA, and steady-state kinetic analyses have indicated that it does so with only an ~100-fold lower catalytic efficiency than for the insertion of correct T (29). Likewise, compared with the catalytic efficiency for the insertion of a T, Pol$\lambda$ inserts an A or a G opposite 1-MeA with only an ~40-fold reduction in catalytic efficiency, and it inserts a C opposite 1-MeA with only an ~100-fold reduction in catalytic efficiency (Table 5). Nevertheless, in spite of their error-proneness in vitro, both these Pols conduct predominantly error-free TLS through 1-MeA in human cells (28).


![Figure 1](image_url)

**Figure 1. TLS pathways for replication through 1-MeA.** In the Pol$\lambda$/Pol$\beta$ pathway, following nt insertion by Pol$\beta$ by Hoogsteen base pairing opposite 1-MeA, Pol$\beta$ would extend synthesis; in the Pol$\lambda$/Pol$\beta$ pathway, this Pol$\lambda$ would act alone at both the steps of TLS. TLS by Pol$\beta$ and Pol$\lambda$ requires the non-catalytic and scaffolding role of Rev1. In the Pol$\lambda$/Pol$\lambda$/Pol$\gamma$ pathway, following the insertion of T opposite 1-MeA by Pol$\lambda$, Pol$\gamma$ would extend synthesis. In this pathway, Pol$\lambda$'s scaffolding role would be additionally required for assembly with Pol$\gamma$. **TLS through 1-MeA by DNA polymerase $\lambda$**

The ability of purified Pol$\lambda$ for replicating through the 1-MeA adduct with nearly the same proficiency as for replicating undamaged DNA raised the question of whether Pol$\lambda$’s polymerase activity was required for TLS through this adduct in human cells. To examine this, we expressed full-length WT Rev3, or the D2781A, D2783A mutant Rev3, defective in its polymerase activity, in HFs. In Rev3-depleted HFs harboring the vector plasmid, TLS opposite 1-MeA occurs with a frequency of ~42% and TLS frequency rises to ~66% in cells expressing WT Rev3. Our results that in cells expressing the D2781A, D2783A mutant Rev3, TLS is reduced to the same level (~44%) as in cells harboring the vector control (Table 3) confirm that the Rev3 DNA polymerase activity is, in fact, required for TLS through 1-MeA in human cells.

**Requirement of noncatalytic role of Rev1 for TLS opposite 1-MeA in conjunction with Pol$\lambda$ and Pol$\gamma$**

In previous studies opposite a number of DNA lesions, we have provided evidence for a scaffolding role of Rev1 in TLS by Y-family Pols (11, 19–21). To confirm that Rev1 plays a similar role in TLS by Pol$\lambda$ and Pol$\gamma$ opposite 1-MeA, we analyzed the epistatic relationship of Rev1 with these Pols. Our results that TLS occurs at the same frequency in Rev1-deleted HFs (~30%) as in cells deleted for Rev1 together with Pol$\gamma$ or with Pol$\lambda$ and that TLS frequency is reduced to ~6% in cells depleted for Rev1 together with Rev3 or with Pol$\lambda$ (Table 6).

**Table 3**

| Vector expressing | Number of $Kan^{+}$ colonies | Number of blue colonies among $Kan^{+}$ colonies | TLS (%) |
|-------------------|-------------------------------|-----------------------------------------------|--------|
| No Pol$\lambda$ (control) | Pol$\lambda$ | 372                                          | 166    | 44.6 |
| WT (1–575) Pol$\lambda$ | Pol$\lambda$ | 326                                          | 208    | 63.8 |
| D427A, D429A Pol$\lambda$ (245–575) | Pol$\lambda$ | 306                                          | 138    | 45.1 |
| No Rev3 (control) Rev3 | Rev3 | 334                                          | 216    | 64.7 |
| WT Rev3 | Rev3 | 294                                          | 124    | 42.2 |
| D2781A, D2783A Rev3 | Rev3 | 409                                          | 180    | 44.0 |
| No Rev1 (control) Rev1 | Rev1 | 354                                          | 106    | 29.9 |
| WT Rev1 | Rev1 | 484                                          | 302    | 62.4 |
| D570A, E571A Rev1 | Rev1 | 248                                          | 158    | 63.7 |

**Table 4**

| Vector expressing | Number of $Kan^{+}$ colonies | Number of blue colonies among $Kan^{+}$ colonies | TLS (%) |
|-------------------|-------------------------------|-----------------------------------------------|--------|
| No Pol$\lambda$ (control) | Pol$\lambda$ | 340                                          | 146    | 42.9 |
| WT Pol$\lambda$ | Pol$\lambda$ | 318                                          | 194    | 61.0 |
| D427A, D429A Pol$\lambda$ (245–575) | Pol$\lambda$ | 375                                          | 153    | 40.8 |
| D570A, E571A Pol$\lambda$ | Pol$\lambda$ | 276                                          | 170    | 61.6 |

J. Biol. Chem. (2021) 297(1) 100868
Discussion

Hoogsteen base pairing as a mechanism for nt insertion opposite 1-MeA by Polλ

Since the addition of a methyl group to the N1 atom of deoxyadenosine disrupts W-C base pairing (29), the insertion of T opposite 1-MeA by Polλ could occur only if the adduct is accommodated in a syn conformation in its active site and the adduct forms a Hoogsteen base pair with T. Thus Polλ active site, which normally accommodates template residues in an anti conformation and forms a W-C base pair with the incoming nt (30), would stabilize 1-MeA in a syn conformation. Such an ability of Polλ active site to accommodate a W-C impairing DNA lesion in a syn conformation would add another novel aspect to Polλ’s function in TLS—in addition to its role as a scaffolding component of Polζ for TLS in human cells.

Modulation of the action mechanism of Polλ and Polζ for TLS through 1-MeA

Even though purified Polλ inserts a T opposite 1-MeA and extends synthesis, Polζ’s polymerase activity is still required for replication through 1-MeA in human cells. The requirement of both the Polλ and Polζ polymerase activities strongly suggests that their polymerase activities are restrained to act at the nt insertion or the extension step of TLS in human cells. We presume that in the multiprotein ensemble of Polλ-Polζ, the action mechanism of the two Pols is restrained such that Polλ’s action is limited to inserting a T opposite 1-MeA and Polζ functions at the extension step. The decipherment of the action mechanism of these Pols in human cells would require the identification of the components of the Polλ-Polζ multiprotein ensemble and biochemical analyses of Polλ’s and Polζ’s role in TLS opposite 1-MeA in the Polλ-Polζ ensemble.

Role of Rev1 in the formation of multiprotein ensembles of Y-family Pols

Our evidence that similar to the requirement of Rev1 as a scaffolding component of Y-family Pols for TLS opposite CPDs, (6–4) PPs, 3-methyl deoxyadenosine, dA, and other DNA lesions, Rev1’s scaffolding role is required for TLS opposite 1-MeA by Polλ and Polζ suggests that Rev1 would effect the assembly of these Y-family Pols with the other protein components and that the fidelity of Polλ and Polζ for TLS opposite 1-MeA would be elevated in the respective multiprotein ensemble thus formed.

Hoogsteen base pairing by Polλ for TLS opposite 1-MeA and modulation of its fidelity in human cells

Polλ differs from other Y-family Pols in that its active site accommodates a template purine A or G in a syn conformation, which then forms a Hoogsteen base pair with the incoming nt (31–33). This allows Polλ to accommodate DNA lesions, such as dA, which impair W-C base pairing, in a syn conformation and to form a Hoogsteen base pair with the incoming nt (9). However, biochemical studies with Polλ have indicated that it can insert not only the correct nt T opposite dA but also the incorrect nt C with only a few-fold reduction in catalytic efficiency, and structure studies have shown that dA in Polλ active site adopts a syn conformation and that it Hoogsteen base pairs with the incoming dTTP or dCTP (9). Nevertheless, in spite of the penchant of Polλ for inserting a C opposite dA, Polλ conducts error-free TLS opposite this adduct in human cells (11). Similar to dA, 1-MeA is accommodated in a syn conformation in Polλ’s active site, and it forms a Hoogsteen base pair with T (29); however, even though purified Polλ misincorporates an A or a C at a frequency of ~10⁻² (29), it promotes error-free TLS through 1-MeA in human cells. The adoption of entirely error-free TLS opposite dA and of predominantly error-free TLS opposite 1-MeA in human cells could be explained if Polλ’s error-
proneness is annulled in the multiprotein ensemble that the scaffolding role of Rev1 would assemble.

**Hoogsteen base pairing by Polη for nt insertion opposite 1-MeA**

Polη uses W-C base pairing for replicating undamaged DNA (34) and for replicating through the two covalently linked pyrimidines of a CPD (2, 4, 35–37). And Polη can replicate through both the guanines of a cisplatin GG intrastrand cross-link with the incoming dCTP (38, 39). Hence, the hallmark of Polη has been its ability to accommodate two template residues in its active site and to form a W-C base pair with the incoming nt. The proficiency of purified Polη for incorporating a T opposite 1-MeA and the genetic evidence that Polη replicates through this adduct in human cells strongly suggest that Polη accommodates 1-MeA in its active site in a syn conformation, which then Hoogsteen base pairs with the correct nt T or with incorrect nts. We presume that the intrinsic error-proneness of Polη for TLS through 1-MeA is attenuated in the Polη multiprotein ensemble.

**Experimental procedures**

**Construction of plasmid vectors containing 1-MeA**

The heteroduplex vectors containing 1-MeA on the leading or the lagging strand template were constructed as described previously (28).

**Cell lines and cell culture**

Normal human fibroblasts (Coriell Institute Cell Repository, GM00637), XPV fibroblasts (Coriell Institute Cell Repository, GM03617), Polλ−/− MEFs, and big blue mouse embryonic fibroblasts (Agilent) were grown in DMEM medium (GenDEPOT) containing 10% fetal bovine serum (GenDEPOT) and 1% antibiotic-antimycotic (GenDEPOT). Cells were grown on plastic culture dishes at 37 °C in a humidified incubator with 5% CO₂.

**Translesion synthesis assays in HFs and Polλ−/− MEFs**

Steady-state kinetic analyses for deoxynucleotide incorporation (kcat/Km).

**DNA polymerase assays with Polλ**

The 75-mer oligonucleotide contained the sequence 5’ AGC AAG TCA CCA ATG TCT AAG AGT TCG TAT AAT GCC TAC ACT GGA GTA CCG GAG CAT GTG GAC TGG GAA AAC-3’, and it harbored an undamaged A or a 1-MeA at the underlined position. For examining the incorporation of dATP, dTTP, dCTP, or dGTP nucleotides opposite undamaged A or 1-MeA at the underlined position. For examining the incorporation of dATP, dTTP, dCTP, or dGTP nucleotides containing 10% fetal bovine serum (GenDEPOT) and 1% antibiotic-antimycotic (GenDEPOT). Cells were grown on plastic culture dishes at 37 °C in a humidified incubator with 5% CO₂.

**Steady-state kinetic analyses**

Steady-state kinetic analyses for deoxynucleotide incorporation opposite undamaged A or 1-MeA by human Polη containing 10% fetal bovine serum (GenDEPOT) and 1% antibiotic-antimycotic (GenDEPOT). Cells were grown on plastic culture dishes at 37 °C in a humidified incubator with 5% CO₂.

**Data availability**

All relevant data are contained within the article.

**Acknowledgments**—This work was supported by grants from the National Institutes of Health (R01 GM126087). The content is...
TLS through 1-MeA by DNA polymerase λ

solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author contributions—J.-H. Y., J. R. C., and S. P. formal analysis; J.-Y. Y., D. B., J. R. C., and S. P. investigation; J.-Y. Y. methodology; J.-H. Y., D. B., J. R. C., S. P., and L. P. writing-review and editing; S. P. and L. P. conceptualization; S. P. and L. P. supervision; S. P. validation; S. P. writing-original draft; S. P. and L. P. project administration; L. P. funding acquisition.

Conflict of interest—The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations—The abbreviations used are: 1-MeA, N1-methyl-deoxyadenosine; (6–4) PPs, (6–4) pyrimidine-pyrimidone photoproducts; CPD, cyclobutane pyrimidine dimer; NC, negative control; nt, nucleotide; Pol, DNA polymerase; TLS, translesion synthesis; W-C, Watson-Crick.

References
1. Johnson, R. E., Kondratchik, C. M., Prakash, S., and Prakash, L. (1999) hRAD30 mutations in the variant form of xeroderma pigmentosum. Science 285, 263–265
2. Johnson, R. E., Prakash, S., and Prakash, L. (1999) Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, Polh. Science 283, 1001–1004
3. Johnson, R. E., Washington, M. T., Prakash, S., and Prakash, L. (2000) Fidelity of human DNA polymerase η. J. Biol. Chem. 275, 7447–7450
4. Masutani, C., Kusumoto, R., Yamada, A., Dohmae, N., Yokoi, M., Yuasa, M., Araki, M., Iwai, S., Takio, K., and Hanaoka, F. (1999) The XPY (xeroderma pigmentosum variant) gene encodes human DNA polymerase η. Nature 399, 700–704
5. Yoon, J. H., McArthur, M. J., Park, I., Basu, D., Wakamiya, M., Prakash, L., and Prakash, S. (2019) Error-prone replication through UV lesions by DNA polymerase theta protects against skin cancers. Cell 176, 1295–1309
6. Yoon, J.-H., Prakash, L., and Prakash, S. (2009) Highly error-free role of DNA polymerase η in the replicative bypass of UV induced pyrimidine dimers in mouse and human cells. Proc. Natl. Acad. Sci. U. S. A. 106, 18219–18224
7. Johnson, R. E., Haracska, L., Prakash, S., and Prakash, L. (2001) Role of DNA polymerase η in the bypass of a (6–4) TT photoproduct. Mol. Cell. Biol. 21, 3558–3563
8. Johnson, R. E., Washington, M. T., Haracska, L., Prakash, S., and Prakash, L. (2000) Eukaryotic polymerases ι and η act sequentially to bypass DNA lesions. Nature 406, 1015–1019
9. Nair, D. T., Johnson, R. E., Prakash, L., Prakash, S., and Aggarwal, A. K. (2006) Hoogsteen base pair formation promotes synthesis opposite the 1,N6-etheno-deoxyadenosine lesion by human DNA polymerase iota. Nat. Struct. Mol. Biol. 13, 619–625
10. Malik, R., Kopylov, M., Gomez-Llorente, Y., Jain, R., Johnson, R. E., Prakash, L., Prakash, S., and Haracska, L. (2020) Structure and mechanism of B-family DNA polymerase ζ specialized for translesion DNA synthesis. Nat. Struct. Mol. Biol. 27, 913–924
11. Yoon, J. H., Johnson, R. E., Prakash, L., and Prakash, S. (2019) DNA polymerase theta accomplishes translesion synthesis opposite 1,N(6)-ethenodeoxyadenosine with a remarkably high fidelity in human cells. Genes Dev. 33, 282–287
12. Yoon, J.-H., Bhatia, G., Prakash, S., and Prakash, L. (2010) Error-free replicative bypass of thymine glycol by the combined action of DNA polymerases η and ζ in human cells. Proc. Natl. Acad. Sci. U. S. A. 107, 14116–14122
13. Acharya, N., Johnson, R. E., Prakash, S., and Prakash, L. (2006) Complex formation with Rev1 enhances the proficiency of yeast DNA polymerase ζ for mismatch extension and for extension opposite from DNA lesions. Mol. Cell. Biol. 26, 9555–9563
14. Baynton, K., Bresson-Roy, A., and Fuchs, R. P. P. (1998) Analysis of damage tolerance pathways in Saccharomyces cerevisiae: A requirement for Rev3 DNA polymerase in translesion synthesis. Mol. Cell. Biol. 18, 960–966
15. Doles, J., Oliver, T. G., Cameron, E. R., Hsu, G., Jacks, T., Walker, G. C., and Hemmann, M. T. (2010) Suppression of Rev3, the catalytic subunit of Polh, sensitizes drug-resistant lung tumors to chemotherapy. Proc. Natl. Acad. Sci. U. S. A. 107, 20786–20791
16. Gibbs, P. E. M., McDonald, J., Woodgate, R., and Lawrence, C. W. (2005) The relative roles in vivo of Saccharomyces cerevisiae Polh, Polη, Rev1 protein and Pol32 in the bypass and mutation induction of an abasic site, T–T (6–4) photodadduct and T–T cis-syn cyclobutane dimer. Genetics 169, 575–582
17. Nelson, J. R., Gibbs, P. E. M., Nowicka, A. M., Hinkle, D. C., and Lawrence, C. W. (2000) Evidence for a second function for Saccharomyces cerevisiae Rev1p. Mol. Microbiol. 37, 549–554
18. Xie, K., Doles, J., Hemmann, M. T., and Walker, G. C. (2010) Error-prone translesion synthesis mediated acquires chemoresistance. Proc. Natl. Acad. Sci. U. S. A. 107, 20792–20797
19. Yoon, J. H., Hodge, R. P., Hackfeld, L. C., Park, I., Roy Choudhury, J., Prakash, S., and Prakash, L. (2018) Genetic control of predominantly error-free replication through an acrolein-derived minor-groove DNA adduct. J. Biol. Chem. 293, 2949–2958
20. Yoon, J. H., Park, J., Conde, J., Wakamiya, M., Prakash, L., and Prakash, S. (2015) Rev1 promotes replication through UV lesions in conjunction with DNA polymerases ι, η and θ but not DNA polymerase ζ. Genes Dev. 29, 2588–2662
21. Yoon, J. H., Roy Choudhury, J., Park, J., Prakash, S., and Prakash, L. (2017) Translesion synthesis DNA polymerases promote error-free replication through the minor-groove DNA adduct 3-deaza-3-methyladenine. J. Biol. Chem. 292, 18682–18688
22. Yoon, J. H., Basu, D., Sellamuthu, K., Johnson, R. E., Prakash, S., and Prakash, L. (2021) A novel role of DNA polymerase lambda in translesion synthesis in conjunction with DNA polymerase zeta. Life Sci. Alliance 4, e20200900
23. Kemmink, J., Boelens, R., Koning, T., van der Marel, G. A., van Boom, J. H., and Kaptein, R. (1987) 3H NMR study of the exchangeable protons of the duplex d(GCGTTGCG).d(CGCAACGC) containing a thymine photodimer. Nucleic Acids Res. 15, 4645–4653
24. Kim, J.-K., and Choi, B.-S. (1995) The solution structure of DNA duplex-decamer containing the (6–4) photoproduct of thymidyl(3’–5’)thymidine by NMR and relaxation matrix refinement. Eur. J. Biochem. 228, 849–854
25. Kim, J.-K., Patel, D., and Choi, B.-S. (1995) Contrasting structural impacts induced by cis-syn cyclobutane dimer and (6–4) adduct in DNA duplex decamers: Implication in mutagenesis and repair activity. Photochem. Photobiol. 62, 44–50
26. Lee, J.-H., Hwang, G.-S., and Choi, B.-S. (1999) Solution structure of a DNA decamer duplex containing the stable 3’ T G base pair of the pyrimidine(6–4)pyrimidone photoproduct [(6–4) adduct]: Implications for the highly specific 3’ T – C transition of the (6–4) adduct. Proc. Natl. Acad. Sci. U. S. A. 96, 6632–6636
27. Ringvold, I., Nordstrand, L. M., Vagbo, C. B., Talstad, V., Reite, K., Aas, P. A., Lauritzen, K. H., Liabakk, N. B., Bjork, A., Doughty, R. W., Falnes, P. O., Krokan, H. E., and K lungland, A. (2006) Repair defective mice reveal mABH2 as the primary oxidative demethylase for repairing 5mC lesions in DNA. EMBO J. 25, 2189–2198
28. Conde, J., Yoon, J. H., Roy Choudhury, J., Prakash, L., and Prakash, S. (2015) Genetic control of replication through N1-methyladenine in human cells. J. Biol. Chem. 290, 29794–29800
29. Jain, R., Choudhury, J. R., Buku, A., Johnson, R. E., Prakash, L., Prakash, S., and Aggarwal, A. K. (2017) Mechanism of error-free DNA synthesis across N1-methyl-deoxyadenosine by human DNA polymerase- iota. Sci. Rep. 7, 43904
30. Garcia-Diaz, M., Bebenek, K., Krahn, J. M., Kunkel, T. A., and Pedersen, L. C. (2005) A closed conformation for the Pol lambda catalytic cycle. Nat. Struct. Mol. Biol. 12, 97–98
31. Nair, D. T., Johnson, R. E., Prakash, L., Prakash, S., and Aggarwal, A. K. (2005) Human DNA polymerase ι incorporates dCTP opposite template G via a G.C+ Hoogsteen base pair. *Structure* **13**, 1569–1577

32. Nair, D. T., Johnson, R. E., Prakash, S., Prakash, L., and Aggarwal, A. K. (2004) Replication by human DNA polymerase ι occurs via Hoogsteen base-pairing. *Nature* **430**, 377–380

33. Nair, D. T., Johnson, R. E., Prakash, S., Prakash, L., and Aggarwal, A. K. (2006) An incoming nucleotide imposes an anti to syn conformational change on the templating purine in the human DNA polymerase-ι active site. *Structure* **14**, 749–755

34. Washington, M. T., Helquist, S. A., Kool, E. T., Prakash, L., and Prakash, S. (2003) Requirement of Watson-Crick hydrogen bonding for DNA synthesis by yeast DNA polymerase η. *Mol. Cell. Biol.* **23**, 5107–5112

35. Biertumpfel, C., Zhao, Y., Kondo, Y., Ramon-Maiques, S., Gregory, M., Lee, J. Y., Masutani, C., Lehmann, A. R., Hanaoka, F., and Yang, W. (2010) Structure and mechanism of human DNA polymerase η. *Nature* **465**, 1044–1048

36. Johnson, R. E., Prakash, L., and Prakash, S. (2005) Distinct mechanisms of cis-syn thymine dimer bypass by Dpo4 and DNA polymerase η. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 12359–12364

37. Silverstein, T. D., Johnson, R. E., Jain, R., Prakash, L., Prakash, S., and Aggarwal, A. K. (2010) Structural basis for the suppression of skin cancers by DNA polymerase eta. *Nature* **465**, 1039–1043

38. Ummat, A., Rechkoblit, O., Jain, R., Roy Choudhury, J., Johnson, R. E., Silverstein, T. D., Buku, A., Lone, S., Prakash, L., Prakash, S., and Aggarwal, A. K. (2012) Structural basis for cisplatin DNA damage tolerance by human polymerase eta during cancer chemotherapy. *Nat. Struct. Mol. Biol.* **19**, 628–632

39. Zhao, Y., Biertumpfel, C., Gregory, M. T., Hua, Y. J., Hanaoka, F., and Yang, W. (2012) Structural basis of human DNA polymerase eta-mediated chemoresistance to cisplatin. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 7269–7274

40. Yoon, J.-H., Prakash, L., and Prakash, S. (2010) Error-free replicative bypass of (6–4) photoproducts by DNA polymerase ζ in mouse and human cells. *Genes Dev.* **24**, 123–128

41. Johnson, R. E., Prakash, L., and Prakash, S. (2006) Yeast and human translesion DNA synthesis polymerases: Expression, purification, and biochemical characterization. *Methods Enzymol.* **408**, 390–407