DNA Polymerase V Allows Bypass of Toxic Guanine Oxidation Products in Vivo

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Reactive oxygen and nitrogen radicals produced during metabolic processes, such as respiration and inflammation, combine with DNA to form many lesions primarily at guanine sites. Understanding the roles of the polymerases responsible for the processing of these products to mutations could illuminate molecular mechanisms that correlate oxidative stress with cancer. Using M13 viral genomes engineered to contain single DNA lesions and Escherichia coli strains with specific polymerase (pol) knockouts, we show that pol V is required for efficient bypass of structurally diverse, highly mutagenic guanine oxidation products in vivo. We also find that pol IV participates in the bypass of two spiroiminodihydantoin lesions. Furthermore, we report that one lesion, 5-guanidino-4-nitroimidazole, is a substrate for multiple SOS polymerases, whereby pol II is necessary for error-free replication and pol V for error-prone replication past this lesion. The results spotlight a major role for pol V and minor roles for pol II and pol IV in the mechanism of guanine oxidation mutagenesis.

The genome is continually damaged by spontaneously generated and environmental chemical agents. Many of the lesions formed in DNA strongly inhibit replicative polymerases. If repair of these lesions fails or is not fast enough, cells can biochemically adapt to “tolerate” the toxic DNA lesions, as evidenced by replication of the damaged DNA (1). Human cells possess at least 15 DNA polymerases, including 5 that are specifically involved in the replication of damaged DNA. Four of these enzymes, pol η, pol ι, pol κ, and REV1, belong to the Y family of DNA polymerases, whereas pol ζ belongs to the B family (2). Escherichia coli possess three polymerases, pol II, pol IV, and pol V, whose expression levels are up-regulated in response to DNA damage. Extensive research on the structure and function of these polymerases and their homologues suggests that these polymerases allow replication to progress in the presence of DNA lesions that are strongly inhibitory to the replicative polymerase, pol III (1). The presence of the three SOS-inducible polymerases allows E. coli to be used as a model system for DNA replication past damage products.

pol II (pol B) (3) is a B family DNA polymerase and has 3′ → 5′-exonuclease activity. Consequently, this enzyme replicates normal DNA with high fidelity and has an error rate less of than 10⁻⁶ (4). pol II participates in error-free replication restart (5, 6) and can also carry out TLS past abasic sites (3, 7), 3,N⁴-ethenocytosine (8), and N-(deoxyguanosin-8-yl)-2-acetylaminofluorene (9). pol IV (DinB) (10) and pol V (UmuD²,C) (11) are members of the Y family of DNA polymerases, which have less sterically restrictive active sites than normal replicative polymerases (12) and lack exonuclease activity (1). The error rates of these polymerases are much higher than the other E. coli polymerases and are on the order of 10⁻⁴ to 10⁻⁵ for pol IV (13) and 10⁻³ to 10⁻⁴ for pol V (13, 14) in vitro. pol IV does not appear to profoundly affect the chromosomal mutation rate but functions in adaptive mutagenesis by inducing −1 frameshifts (15). pol IV can also participate in TLS past several lesions, including a tetrahydrofuran abasic site, N²-benzo(a)pyrene-dG, and N²-furfuryl-dG (16–19). pol V is the major TLS polymerase (20) and has been shown to bypass lesions such as abasic sites, UV photoproducts of DNA, and bulky adducts (13, 19, 21–23) and also is involved in untargeted mutagenesis (14).

Because guanine (G) possesses the lowest redox potential of the four DNA nucleobases (24), agents such as peroxynitrite (ONOO⁻) preferentially oxidize G as compared with the other natural nucleobases (25). ONOO⁻ is a powerful oxidizing and nitrating agent (26) that forms from the diffusion-limited reaction of the endogenous radicals nitric oxide (‘NO) and superoxide (O²⁻) (27). Two types of inflammatory cells secrete these radicals during the immune response, namely activated macrophages (28) and neutrophils (29). Previous studies in our laboratory have shown that several DNA lesions derived from ONOO⁻ oxidation of G are substrates for the SOS response system in E. coli (30, 31). These findings strongly suggest involvement of the E. coli polymerases II, IV, and/or V and prompted us to investigate the roles of these polymerases in the bypass of G oxidation products. We present here a systematic examination of the effects of the SOS-inducible DNA polymer-
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ases on the bypass and mutagenicity of 7,8-dihydro-8-oxoguanine (8-oxoG), guanidinohydrantoin (Gh), spiroiminodihydan- 

tin (Sp) (an in vivo product of DNA oxidation (32)), oxaluric acid (Oa), urea (Ua), and 5-guanidino-4-nitromidazole (NI), which are formed either by oxidation of G or 8-oxoG as shown in Fig. 1 (reviewed in Ref. 33). The results of these experiments serve to examine the mechanisms at the molecular level for how ONOO− may cause genotoxicity and mutagenesis.

EXPERIMENTAL PROCEDURES

DNA sequences (5′-GAA GAC CTX GGC GTC C-3′, where X is G or a base analogue) containing 8-oxoG, Gh, Sp1, Sp2, Oa, NI, or a tetrahydrofuran abasic site were prepared as described previously (34–37). Sp1 and Sp2 refer to the faster and slower eluting peaks, respectively, during purification by anion-exchange high pressure liquid chromatography (see Supplemental Material) (38). The oligodeoxynucleotide containing Oa was prepared by hydrolysis of the Oa-containing oligodeoxynucleotide (30). Each oligonucleotide was ligated into a single-stranded bacteriophage M13mp7L2 vector as described (39). Details are published as Supplemental Material.

The E. coli strains used were AB1157 [F− thr−1 araC14 leuB6(Am) Δ(ppt-proA)62 lacY1 tsx−33 supE44(As) galK2(oc) hisG4(oc) rfbD1 mtl-1 argE3(oc) thi-1], pol II (AB1157 but polBΔ1::Ω Sm-Sp), pol IV− (AB1157 but ΔdinBW2::cat), GW8017 (AB1157 but umuCDC595::cat), and pol II−/pol IV−/pol V− (AB1157 but polBΔ1::Ω Sm-Sp dinB umuCDC595::cat). All strains were provided by G. Walker (Massachusetts Institute of Technology). The pol II− strain was created by P1 transduction (40) of the polBΔ1 allele from E. coli ME101 (41). For the pol IV− strain, P1 transduction was used to transfer the ΔdinBW2::cat allele (18) to E. coli AB1157. The pol II−/pol IV−/pol V− strain was prepared by first removing the antibiotic resistance cassette from the ΔdinBW2::cat allele in the pol IV− strain using the procedure of Datsenko and Wanner (42) followed by transferring the polBΔ1 and umuCDC595::cat alleles into the resultant ΔdinB strain by P1 transduction.

All strains were characterized by PCR and DNA sequencing of the gene regions of interest. E. coli genomes were isolated from 1 ml of an overnight LB culture grown in the presence of 25 μg/ml chloramphenicol (pol IV− and GW8017 strains), 70 μg/ml spectinomycin (pol II− strain), or both chloramphenicol and spectinomycin (pol II−/pol IV−/pol V− strain). PCR amplification of the E. coli genomic DNA was carried out using Taq DNA polymerase (Invitrogen). The 50-μl PCR mixture for wild-type polB, dinB, and umuCDC gene regions and ΔdinB and ΔumuDC gene regions included 25 mM KCl, 10 mM Tris-HCl (pH 8.3), 3.5 mM MgCl2, 2% Me2SO, 0.5 μM each primer, 0.05 mM each dNTP, 0.05 unit/μl Taq, and 2 μg of genomic DNA. The amplification cycle consisted of denaturation at 94 °C for 0.5 min, annealing at 56 °C for 0.5 min, and extension at 72 °C for 2 min. After 30 cycles, samples were incubated at 72 °C for 10 min and stored at 4 °C until further use. The primers 5′-CTG GAA CGA AGT GTA TTA CGG GTT TCG-3′ and 5′-TAT GGT ACT GCA TGG TAT CCT CG-3′ were used for amplification of the polB and polBΔ1 regions. The primers 5′-CAT GGG GAT AAA GTG GTG CAG-3′ and 5′-CTG GCA CTG AAG AGA TAT CCT GCT GCG-3′ were used for amplification of the dinB region. The primers 5′-CCG GTG GAG ATA AGA GAA CGC-3′ and 5′-TAC CTG ATT GTC GCA GTG CTG G-3′ were used for amplification of the umuDC region. The PCR products were analyzed by 1% agarose gel electrophoresis in 1× TBE buffer. To 5 μl of each PCR product, solution was added at 5 μl of H2O and 2 μl of 6× Ficoll loading buffer (25% Ficoll), and 10 μl were loaded into each well. The gel was run at 130 V for 1:40 h and stained with 1× SYBR Gold (Molecular Probes) in 1× TBE for 1 h.

DNA sequencing was performed using the following primers. For strains containing wild-type polB, 5′-TAT GAT AGC AGC GCA GCC AAT GCC-3′, 5′-CGA TCA TCC GCA CTT TCT GAA TTG-3′, 5′-CAA TCA GAA AGT TGC GCA TGA TCG-3′, and 5′-TAT GGT ACT GCA TGG TAT CCT CG-3′ were used as primers for DNA sequencing. For strains containing polBΔ1, 5′-CTG GAA CGA AGT GTA TTA CGG GTT TCG-3′ was used in place of 5′-TAT GCA TGC AGC GCA AAT GCC-3′. For strains containing wild-type dinB and ΔdinB, the same primers used for PCR were used for DNA sequencing. For strains containing wild-type umuCDC and ΔumuDC, 5′-GAA AGT TGC AAC CTC TTA CGT GCC-3′ and the same primers used for PCR were used for DNA sequencing. DNA sequencing was performed by the Massachusetts Institute of Technology Biopolymers Laboratory.

Genotoxicity and mutation analyses were performed using the competitive replication of adduct bypass (31, 39) and restriction endonuclease and postlabeling analysis of mutation frequency (43) methods. Details are published as Supplemental Material. The bypass and point mutation data are reported as the average of three independent experiments, and the frame-shift mutation data are reported as the average of two independent experiments. DecisionSite 8.1 (Spotfire, Inc.) was used to present the bypass and point mutation data in heat map format. In the bar graphs of figures, error bars represent a 95% confidence interval of the mean.

RESULTS

To evaluate the role of the SOS-inducible E. coli polymerases in TLS past G oxidation products, M13 viral genomes containing single 8-oxoG, Oa, Gh, Sp1, Sp2, Ua, or NI lesions were constructed and then replicated in wild-type, pol II-deficient, pol IV-deficient, pol V-deficient, and pol II/pol IV/pol V-deficient E. coli strains under uninduced and SOS-induced conditions for a total of 10 cellular experimental systems (Fig. 1). The translesion bypass efficiencies and mutational properties of the lesions were determined using the recently developed competitive replication of adduct bypass (31, 39) and restriction endonuclease and postlabeling analysis of mutation frequency methods (43), which precisely and accurately report the average
values for thousands of TLS events. The resultant data set consists of over 1500 data points and therefore represents a very comprehensive study of the bypass and mutational properties of G oxidation products. Fig. 2 summarizes the lesion bypass and mutagenesis data in a single heat map, which serves as a convenient framework for viewing the main findings of the work that are described in detail below.

**Translesion Bypass Efficiency**—In the uninduced wild-type strain, Gh, Sp1, Sp2, Ua, and NI exhibited poor bypass efficiencies but were excellent substrates for the SOS system (Fig. 3). As a control demonstrating a blocking lesion and showing induction of the SOS system, the bypass efficiency of a tetrahydrofurane synthetic abasic site was determined and found to increase from 1.0 to 19%, or about 20-fold. The bypass efficiency of Gh increased about 3-fold from 19 to 65%; Sp1 increased about 4-fold from 16 to 69%; Sp2 increased about 6-fold from 8.2 to 50%; Ua increased about 6-fold from 5.2 to 34%; and NI increased about 8-fold from 4.5 to 38%. These results and our earlier studies (30, 31) rank the relative SOS responsiveness of eight structurally diverse DNA lesions. The bypass efficiencies of 8-oxoG and Oa were identical within experimental error to G (100%) and did not differ among the 10 replicative environments evaluated (Fig. 3 and supplemental Figs. S1–S3).

With the exception of 8-oxoG and Oa, which were not blocks to replication, deletion of pol V produced dramatic changes in the bypass efficiencies of the lesions (with and without SOS induction) (Fig. 3). The bypass efficiencies were markedly lower in pol V-deficient cells. Importantly, the bypass efficiencies for the blocking lesions failed to increase upon induction of the SOS system in the absence of pol V. These results indicate a pivotal role for pol V in the bypass of these oxidative lesions under both uninduced and SOS-induced conditions. In the pol V-deficient strain, Gh was bypassed with an efficiency of about 10% regardless of SOS status. The bypass efficiencies of the Sp diastereomers decreased to less than 1% and of Ua and NI to about 2% upon removal of pol V. Results from the pol II/pol IV/pol V-deficient strain (supplemental Fig. S1) mirrored those from the pol V-deficient strain. Taken together, these results demonstrate that pol V is essential for the SOS-dependent bypass of these G oxidation products. In the case of Gh, removal
TABLE 1

Contribution of pol II and pol IV to translesion synthesis past Sp1 and Sp2 in vivo

| Lesion | Polymerase knocked out | Bypass efficiency (%) | SOS− | SOS+ |
|--------|------------------------|-----------------------|------|------|
| Sp1    | None                   | 15.9 ± 2.1            | 69.1 ± 4.9 |
|        | pol II                 | 8.2 ± 2.2             | 68.4 ± 16.3 |
|        | pol IV                 | 10.4 ± 1.0            | 59.8 ± 0.9 |
| Sp2    | None                   | 8.2 ± 2.3             | 50.1 ± 8.5 |
|        | pol II                 | 6.8 ± 2.7             | 61.3 ± 19.8 |
|        | pol IV                 | 5.5 ± 1.9             | 68.0 ± 13.7 |

Of all three SOS polymerases caused only about a 50% decrease in bypass efficiency when the SOS system was not induced as compared with wild-type cells, suggesting involvement of an SOS-independent polymerase (pol I or pol III).

Deletion of pol II or pol IV had no statistically significant effect on the bypass efficiencies of Gh, Sp2, Ua, or NI but halved the bypass efficiency of Sp1 from 16 to 8.2% in uninduced pol II cells (Table 1 and supplemental Fig. S2) and from 16 to 10% and 69 to 60% in uninduced and SOS-induced pol IV-deficient cells, respectively (Table 1 and supplemental Fig. S3). These results suggest a moderate involvement of pol II and pol IV in the bypass of Sp1.

**Mutation Type and Frequency**—The mutational signature of each lesion was determined using the restriction endonuclease and post-labeling analysis of mutation frequency method (43). This assay allows both frameshift and point mutations to be quantified.

**Frameshift Mutations**—In both uninduced and SOS-induced wild-type cells, frameshift mutations were negligible for all lesions (less than 1%; data not shown). Frameshifts (−1 and −2) on the order of several percent were observed upon replication of Sp1, Sp2, and NI in the pol V-deficient and the pol II/pol IV/pol V-deficient strains. A previous study suggested pol V suppresses frameshift mutagenesis in favor of base substitutions (44). NI also induced about 2% −1 frameshifts in the pol II- and pol IV-deficient strains. Further details are published as Supplemental Material. In assessing the significance of the observed frameshift mutations, it is important to consider that these mutations were generated under conditions of very low lesion bypass.

**Point Mutations**—In wild-type uninduced cells, the 8-oxoG hyperoxidation products Gh, Sp1, Sp2, Oa, and Ua were almost completely miscoding as shown in Fig. 4, respectively; however, the mutational signatures differed within this group as observed previously (30, 38). Throughout this section, the percents refer to the percentage of total bypass events that are mutagenic: Gh bypass induced 37% G → T and 59% G → C mutations (Fig. 4A), whereas Sp1 caused 83% G → T and 15% G → C mutations (Fig. 4B), and Sp2 caused 43% G → T and 54% G → C mutations (Fig. 4C). Oa generated 99% G → T mutations (Fig. 4D), and Ua produced 55% G → T, 35% G → C, and 9% G → A mutations (Fig. 4E). In comparison, replication of 8-oxoG was only 2% mutagenic, and the mutations were essentially all G → T (supplemental Fig. S4). NI was 64% mutagenic and induced 30% G → T, 18% G → C, and 16% G → A mutations (Fig. 4F). Induction of the SOS system had a slight to negligible effect on the mutational signatures of Gh, Sp1, Oa, and Ua. This observation could indicate pol V-dependent bypass either in uninduced conditions or in an SOS-induced subpopulation that exists in the absence of external induction. Interestingly, the mutational signature of Sp2 shifted from 43 to 58% G → T mutations and from 54 to 39% G → C mutations (Fig. 4C). The mutation frequency of NI decreased to 48%, and the mutational signature shifted to fewer G → A mutations (9%) and G → C mutations (5%) (Fig. 4F).

The most striking effect in mutational signatures was observed upon removal of pol V. Again, for the well bypassed lesions 8-oxoG (supplemental Fig. S4) and Oa (Fig. 4D), small effects were observed. The mutational signature of Gh, although unchanged among uninduced strains, shifted from 45% G → T and 50% G → C in SOS-induced wild-type cells to 35% G → T and 64% G → C mutations in SOS-induced pol V-deficient cells (Fig. 4A). This result suggests a preference of pol V to insert A opposite the lesion, thereby inducing G → T mutations. The data further suggest pol V inserts T opposite the lesion to a small extent because removal of pol V eliminates the G → A mutations. Also, because the bypass efficiency of Gh is unchanged at about 10% in the pol V-deficient strain versus the pol II/pol IV/pol V-deficient strain (supplemental Fig. S3), the data indicate a relatively significant contribution to mutational signature by a fourth polymerase, either pol I or pol III. Deletion of pol V had a small effect on the mutagenicity of Sp1 bypass, decreasing it from 99% in uninduced and SOS-induced pol V-proficient cells to 93 and 94% in uninduced and SOS-induced pol V-deficient cells, respectively, while still inducing principally G → T mutations (Fig. 4B). Large differences in the mutational signature of Sp2 were observed upon elimination of pol V (Fig. 4C). In both uninduced and SOS-induced wild-type cells, the overall mutation frequency decreased from 99 to 86% when compared with pol V-deficient cells, and the mutational signature shifted from similar amounts of G → T and G → C mutations to mostly G → T mutations. These data suggest pol V preferentially inserts G opposite Sp2 and that insertion of A opposite the lesion results from a different polymerase activity. With the exception of pol V, the participation of any polymerase, including those not induced by the SOS system, in the genesis of the large amount of G → T mutations cannot be excluded. A large shift in mutational signature was observed for Ua upon deletion of pol V (Fig. 4E). Although substantial amounts of G → A, G → T, and G → C mutations were observed in wild-type cells, G → T transitions comprised nearly all the mutations in the pol V-deficient strains and were greater than 90%. These data show that pol V preferentially inserts both T and G opposite Ua and that pol V is not responsible for the majority of G → T mutations. Removal of pol V caused a large decrease in the amount of G → T mutations induced by NI with a corresponding decrease in mutation frequency. In uninduced cells, the overall mutation frequency of NI decreased from 64% in the wild-type strain to 40% in the pol V-deficient strain and to 51% in the pol II/pol IV/pol V-deficient strain (Fig. 4F). In SOS-induced cells, the mutation frequency decreased from 48 to 21% when pol V was removed and decreased slightly to 45% when all three SOS-inducible polymerases were removed. Concurrently, the amount of G → T mutations induced in pol V-deficient cells relative to wild-type cells decreased from 30 to 7% in uninduced
FIGURE 4. Point mutation data. A, coding properties of Gh. B, coding properties of Sp1. C, coding properties of Sp2. D, coding properties of Oa. E, coding properties of Ua. F, coding properties of NI.
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cells and from 35% to 4% in SOS-induced cells, indicating involvement of pol V in generating G → T mutations from NI. Results from the pol II/pol IV/pol V-deficient strain resembled those from the pol V single knock-out strain. Together, the mutation data for these lesions indicate that it is the nature of the oxidized base that largely influences which nucleotide pol V incorporates opposite the lesion.

In general, elimination of pol II or pol IV had minor effects on mutational signature as compared with elimination of pol V. The effects were especially small on the two well bypassed lesions included in this study, 8-oxoG (supplemental Fig. S4) and Oa (Fig. 4D). Replication of Sp2 in SOS-induced pol IV-deficient cells reduced the amount of G → T mutations from 57 to 49% and increased the amount of G → C mutations from 38 to 48% as compared with SOS-induced pol II-deficient cells indicating a possible role for pol IV in the insertion of nucleotides opposite Sp2 (Fig. 4C). These differences for Sp2 may also apply in comparison with SOS-induced wild-type cells, but the variance inherent in the bypass assay precluded this determination. In uninduced cells, deletion of pol II increased the amount of G → T mutations induced by Ua from 55 to 69% and decreased the G → C mutations from 35 to 22%. Similarly, deletion of pol IV increased the G → T mutations from 55 to 64% and decreased the G → C mutations from 35 to 28% (Fig. 4E). pol II and pol IV thus affect the mutational signature of Ua in uninduced cells; however, induction of the SOS system eliminated these effects, which were presumably overwhelmed by the increased levels of the more influential pol V. Although deletion of pol II or pol IV caused an increase in the amount of the dominant mutation induced by Sp2 and Ua, an intriguing exception was noted with NI, which had a very different mutational signature in the absence of pol II (Fig. 4F). Relative to wild-type cells, NI was overall more mutagenic, and the amount of G → T mutations induced increased from 40 to 45% in uninduced cells and from 35 to 62% in SOS-induced cells. Because the elimination of pol II had the effect of increasing the overall mutation frequency from 64 to 81% in uninduced cells and from 48 to 82% in SOS-induced cells, these results suggest pol II participates in an error-free replication mechanism of NI.

**DISCUSSION**

8-OxoG and NI (45) are two initial products formed from oxidation of G. 8-OxoG, a commonly used biomarker of oxidative stress, is even more readily oxidized than G (46), and oxidation of this lesion produces a variety of DNA hyperoxidation products (33), including Gh, Sp1, Sp2 (47), cyanuric acid (48), Oa (37), Ua (30), 2-aminomimidazolone, and oxazalone (49). These hyperoxidation products were shown previously to be more potently mutagenic in vivo as determined by M13 virally based mutagenesis studies (30, 31, 34, 38). To date, Sp and 8-oxoG have been detected in vivo (32, 50). Given the abundance of 8-oxoG found within the cell (on the order of one lesion per 10^6 bases (51)) and its propensity for oxidation, it seems plausible that other 8-oxoG products also may exist in vivo. Additionally, 8-nitroguanine forms directly from G and is used as a biomarker of inflammation (52). Given that 8-nitroguanine and NI form in double-stranded DNA in approximately equal proportions in vitro and appear to arise mechanistically from the same neutral G radical precursor (53), NI may form in vivo under the conditions that also generate 8-nitroguanine.

To explore potential mechanisms of carcinogenesis as a result of inflammation, we have examined the role of the SOS-inducible E. coli polymerases in the bypass of DNA lesions generated by ONOO\textsuperscript− oxidation of DNA. Fig. 2 summarizes our results, which indicate that for poorly bypassed lesions, pol V was responsible for the vast majority of TLS and was essential for SOS-dependent bypass of the oxidation products in this study. Furthermore, the results show that pol V clearly bypassed Gh, Sp1, Sp2, Ua, and NI in an error-prone manner and that the mutagenicity of NI, in comparison with the wild-type results, increased upon removal of pol II and decreased upon the removal of pol V. The bypass results from wild-type, pol V-deficient, and pol II/pol IV/pol V-deficient cells demonstrated that pol I and/or pol III can bypass Gh, Ua, and NI to a small extent and Sp1 and Sp2 to a negligible extent (but still measurable in terms of determining mutation frequency). The data also indicated a role for pol II and pol IV in the bypass of Sp1 (Table 1). In the case of pol IV knock-out, a decrease in bypass efficiency is observed both in the absence and presence of SOS induction indicating a relatively significant contribution of this polymerase to the TLS past Sp1. In contrast, elimination of pol II decreased the bypass efficiency under uninduced conditions, but no effect from this polymerase was observed upon induction of the SOS system indicating that the increased levels of pol IV and pol V are sufficient to overcome the deficiency created by the lack of pol II.

The Sp diastereomers also exhibited vastly different mutational signatures and responded differently to the knock-out of pol V. The stereochemical difference between Sp1 and Sp2 is the logical explanation for the observed differences in bypass efficiency and mutational signature. Although two groups recently reported assignment of the absolute stereochemistry of the Sp diastereomers, they arrived at opposite conclusions leaving the true stereochemical assignment as an issue that awaits resolution (54, 55). Jia et al. (56) performed a computational study of duplexes containing the Sp diastereomers. The authors noted that the orientation in the duplex of one isomer is opposite that of the other isomer and that the duplex containing the R isomer is always lower in energy than the duplex containing the S isomer. Furthermore, both isomers possess a hydrogen bonding face similar to thymine, whereas the R isomer forms a more stable base pair with G than the S isomer. These computational results suggest a precedent for differences in bypass efficiency and coding specificity between Sp1 and Sp2 based on stereochemistry. Indeed, in this study Sp2 showed more of a propensity to pair with G than did Sp1, suggesting an assignment of S and R for Sp1 and Sp2, respectively.

Fundamentally, the bypass of a DNA lesion consists of two parts as follows: insertion of a nucleotide opposite the lesion and extension of the nascent strand (57). Experiments with Klenow fragment suggest that the extension step of TLS is rate-limiting for Gh (35, 58, 59), Ua (60), and NI (61). Thus, it is possible that the requirement of pol V for bypass of these lesions reflects significant participation in extension rather than insertion. However, pol V undoubtedly had a significant role in the insertion step for Sp2, Ua, and NI based on the large
Inactivation of pol V causes a large increase in the amount of A inserted opposite the lesion and a significant decrease in the amount of C incorporated opposite the lesion, while causing no decrease in the bypass efficiency. This result suggests pol II is involved principally during the insertion step of TLS and preferably inserts C opposite the lesion, which leads to no mutation. Inactivation of pol V causes a large increase in the amount of C inserted opposite NI and a significant decrease in the amount of A inserted opposite the lesion. Accordingly, this result demonstrates that pol V preferentially inserts A opposite the lesion leading to G → T mutations. Given that similar amounts of C and A are incorporated opposite NI in wild-type cells, and the relative amounts change dramatically and with similar magnitude in the absence of pol II or pol V, these results suggest a similar contribution of both pol II and pol V in the nucleotide insertion step of NI bypass. Coupled with the strong dependence of NI bypass efficiency only on pol V, the results suggest pol V is required for extension of the nascent strand after incorporation of a nucleotide opposite NI by either pol II or pol V (Fig. 5).

Separation of polymerase roles has been proposed to occur for TLS past DNA lesions in eukaryotic systems. For pyrimidine-pyrimidone (6–4) photoproducts, abasic sites, thymine glycol, and N2-acetylimidofluorene adducted G, a nucleotide is inserted opposite the lesion by a specific polymerase, such as pol η, pol ι, REV1, or pol δ, whereas a separate polymerase, such as pol ζ, performs the extension step (2). Although pol V has no human homologue, recent work suggests this protein shares considerable structural similarity and substrate specificity with pol η (62), which therefore may be able to bypass the lesions discussed here. Pol η has been shown both to insert and extend opposite at least one G oxidation product, 8-oxoG, with similar efficiency to G and does so in an error-free fashion (63). Future studies using purified bypass polymerases should allow more precise roles to be defined for these proteins in the bypass of DNA oxidation damage. Ultimately, in vivo experiments using mammalian cells and the G oxidation products described in this work will be necessary to understand the mechanisms by which mutations may be induced by these lesions in humans.

Oxidative stress is ubiquitous in aerobic organisms and is implicated as a cause of many human diseases, particularly cancer (64–66). One origin of oxidative stress is inflammation, and a clear correlation exists between inflammation and cancer risk (67). Because immune system cells use reactive oxygen and nitrogen species to damage foreign biological matter, there is a need to understand how collateral damage of host tissues may result in both toxicity and gene damage. Equally important is the fact that invading organisms mount a robust defense against immune system cells (68), and the SOS polymerases are likely critical parts of that network. Whereas polymerases are induced in the invaders as a countermeasure to DNA damage, the human homologues and orthologues of the SOS polymerases may be an important link between DNA oxidation and malignant transformation because they can directly mutate the genome by synthesizing past normally replication-inhibitory lesions. Should high rates of replication error among these lesions also be found to occur in mammalian cells, it may prove therapeutic to inhibit the activity of the offending DNA polymerase. In fact, recent work shows that elimination of pol ζ dramatically increases the sensitivity of cells to killing by molecules that generate NO (69). Inhibiting the bypass of oxidative DNA lesions could allow more time for repair processes such as base excision repair or recombination to occur. In the absence of effective repair mechanisms, cell death may occur given the high toxicity exhibited by these lesions. Either outcome could reduce the mutagenic and potentially carcinogenic effects of these DNA damage products.

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