Chronic low-dose ultraviolet-induced mutagenesis in nucleotide excision repair-deficient cells

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ABSTRACT

UV radiation induces two major types of DNA lesions, cyclobutane pyrimidine dimers (CPDs) and 6-4 pyrimidine–pyrimidine photoproducts, which are both primarily repaired by nucleotide excision repair (NER). Here, we investigated how chronic low-dose UV (CLUV)-induced mutagenesis occurs in rad14Δ NER-deficient yeast cells, which lack the yeast orthologue of human xeroderma pigmentosum A (XPA). The results show that rad14Δ cells have a marked increase in CLUV-induced mutations, most of which are C→T transitions in the template strand for transcription. Unexpectedly, many of the CLUV-induced C→T mutations in rad14Δ cells are dependent on translesion synthesis (TLS) DNA polymerase η, encoded by RAD30, despite its previously established role in error-free TLS. Furthermore, we demonstrate that deamination of cytosine-containing CPDs contributes to CLUV-induced mutagenesis. Taken together, these results uncover a novel role for Polη in the induction of C→T transitions through deamination of cytosine-containing CPDs in CLUV-exposed NER-deficient cells. More generally, our data suggest that Polη can act as both an error-free and a mutagenic DNA polymerase, depending on whether the NER pathway is available to efficiently repair damaged templates.

INTRODUCTION

Cellular DNA is continuously exposed to DNA damaging agents, which presents a challenge to genome integrity and cellular survival. One component of sunlight is UV irradiation, which is a primary environmental cause of DNA damage. UV light induces two major photoproducts in DNA, cis–syn cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs), both of which are primarily repaired by the nucleotide excision repair (NER) pathway in Escherichia coli, yeast and human cells (1). Persistent CPDs and 6-4PPs block DNA synthesis by the replicative DNA polymerases, but they can be bypassed by a mechanism known as DNA damage tolerance (also known as post-replication repair). In Saccharomyces cerevisiae, post-replicative repair of persistent UV-induced DNA lesions involves the translesion synthesis (TLS) of DNA polymerases (see ahead in the text) or template switching mediated by Rad5-Mms2-Ubc13 (2–4).

Three TLS polymerases have been identified in S. cerevisiae: Polη, Polζ and Rev1 (5–7). Polζ is a heterodimer composed of the Rev3 catalytic subunit and the Rev7 accessory subunit, encoded by REV3 and REV7 genes, respectively. It belongs to the B family of DNA polymerases, which includes the accurate replicative DNA polymerases Pol α, δ and ε. Unlike other B family polymerases, Polζ displays highly mutagenic properties in vitro because of its lack of 3′-to-5′ exonuclease activity. Consistent with this, deletion of REV3 or REV7 leads to a strong reduction in spontaneous and induced mutation rate (8–10).

The Rev1 polymerase is a protein with deoxycytidyl transferase activity that transfers dCMP to 3′ DNA termini (11,12). This activity seems to have little or no role on survival and mutagenesis in response to UV and methyl methanesulfonate (MMS) damage, although other non-catalytic domains of Rev1 have been implicated in the recognition and/or the recruitment of TLS polymerases to sites of DNA lesions (7,13,14). Recently, it was reported that the catalytic activity of Rev1 may be critical when cells are exposed to specific types of DNA damage, such as 4-nitroquinoline-1-oxide (15).

Polη, encoded by RAD30, is a member of the Y family of DNA polymerases and is highly conserved in eukaryotes (5). Compared with other TLS polymerases, Polη demonstrates higher replication fidelity during the
bypass of UV lesions under most experimental conditions in yeast and human cells (16–19). Consistent with this, Pol η mutants display a higher mutation rate than the isogenic wild-type strain (10,20–23). Nevertheless, Pol η replicates undamaged DNA with low-fidelity (24), and under some conditions, yeast Pol η seems to play a role in UV-induced mutagenesis (10,25,26) and chemical-induced mutagenesis (27).

In previous studies, numerous experiments performed so far to study about the cellular responses to UV irradiation has adopted high-dose UV (i.e. 1–500 J/m²) within relatively short irradiation time (second- to minute-time scale). These acute conditions are rare cases in the real environmental situations. But rather, organisms are exposed intermittently to low-doses of UV for a long time. Therefore, understanding the cellular response to CLUV exposure is an important complementary approach alongside the more traditional approaches to help clarify the biological significance of specific DNA damage response pathways. Our previous studies demonstrated that NER-defective rad14 yeast cells, which lack the yeast orthologue of human xeroderma pigmentosum A (XPA), lose viability following acute high-dose UV irradiation, but grow in the presence of CLUV, despite the observation that CPDs continued to accumulate with increasing CLUV exposure (28). To examine this more closely, this study analyses the mutations contributing to canavanine resistance in CLUV-exposed rad14 yeast cells. Our results showed that rad14 yeast cells exhibited a marked increase in CLUV-induced mutation rate in CAN1, and that a large fraction of the CLUV-induced mutations consisted of C→T transitions in the transcribed strand. Unexpectedly, a lower-rate of CLUV-induced mutagenesis was observed in rad14 rad30 cells than in rad14 cells, indicating that DNA pol η plays a mutagenic role in the bypass of CLUV-induced DNA lesions. These and other data presented here support a model in which DNA polymerase η promotes transcription-coupled C→T transitions through deamination at cytosine-containing CPDs.

MATERIALS AND METHODS

Strains and plasmids

All yeast strains used in these experiments were derived from BY4741 (MATα leu2Δ0 met15Δ0 ura3Δ0 his3Δ1) and are listed in Table 1. All double and triple mutants were constructed by standard genetic procedures (29). For Pol η overexpression, wild-type RAD30 and rad30Δ155A coding regions amplified by PCR were cloned into the galactose-inducible vector, p415GAL1 (30), producing pGRad30 and pGRad30Δ155A, respectively. The SacI-KpnI fragments of pGRad30 and pGRad30Δ155A were cloned separately into the integration vector, pAUR101 (TAKARA). The resulting plasmids were linearized at a unique StuI site within the AUR1 sequence of pAUR101 and then introduced into the AUR1 locus. The DNA sequences of recombinant plasmids were confirmed by sequencing the appropriate regions.

Table 1. Saccharomyces cerevisiae strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>References</th>
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<tbody>
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<td>BY4741</td>
<td>MATα leu2Δ0, met15Δ0, ura3Δ0, his3Δ1</td>
<td>ATCC</td>
</tr>
<tr>
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<td>(28)</td>
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<td>rad30Δ::HIS3</td>
<td>This study</td>
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<tr>
<td>NHY033</td>
<td>rev3Δ::KanMX</td>
<td>This study</td>
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<td>NHY034</td>
<td>rad1Δ::LUE2, rev3Δ::KanMX</td>
<td>This study</td>
</tr>
<tr>
<td>NHY097</td>
<td>rad30Δ::HIS3, rad1Δ::LUE2</td>
<td>This study</td>
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<tr>
<td>NHY098</td>
<td>rad1Δ::LUE2, rev3Δ::KanMX, rad30Δ::HIS3</td>
<td>This study</td>
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<tr>
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<td>This study</td>
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<td>rad1Δ::LUE2, ung1Δ::KanMX</td>
<td>This study</td>
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<tr>
<td>NHY086</td>
<td>rev3Δ::KanMX, rad30Δ::HIS3</td>
<td>This study</td>
</tr>
<tr>
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<td>rad1Δ::KanMX, aur1C::pGRad30</td>
<td>This study</td>
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<tr>
<td>NHY101</td>
<td>rad1Δ::KanMX, aur1C::pGRad30-Δ155A</td>
<td>This study</td>
</tr>
</tbody>
</table>

Media and growth conditions

Cells were grown in yeast extract-peptone-dextrose (YPD) medium containing 0.01% adenine sulfate (YPAD) at 30°C. Canavanine-resistant mutants were selected on synthetic complete medium lacking arginine and containing 60 mg canavanine/l (SC+CAN). For Pol η overexpression, cells grown in YP-Raffinose (2% Raffinose) were collected, resuspended in the same volume of YP-Galactose (1% Galactose + 1% Raffinose) and incubated for 4 hours to induce Pol η expression. Cells were then incubated under CLUV conditions.

CLUV exposure and mutagenesis

CLUV exposure was carried out as described previously (28,31). Cells were incubated with horizontal shaking at 30°C under continuous exposure to UV irradiation (254 nm) at a dose of ≈0.12 J/m²/min. To determine the frequency of CanR mutations, five independent colonies grown on YPAD plates for two days were scraped, suspended in the same volume of YP-Galactose (1% Galactose + 1% Raffinose) and incubated for 4 hours to induce CanR mutation sites were determined by PCR amplification of the appropriate regions.

Sequence analysis of CAN1 allele

CanR mutation sites were determined by PCR amplification of the CAN1 gene followed by DNA sequence analysis. Genomic DNA was prepared (Promega) and the CAN1 gene was amplified by PCR using primers CAN1F1 (5'-TATTTCCAGCTTCTTACG-3') and CAN1R1 (5'-GGTGTACTTATGAGGTGAAGAA-3'). PCR products were then sequenced using three primers: CAN1F2 (5'-TATTTGCTTTTCTTGGGCA-3'), CAN1F3 (5'-TTGAGAACTCTGTTGACAA-3') and CAN1R3 (5'-CA
RESULTS

Forward mutagenesis assay for CLUV-induced canavanine resistance

Our initial studies showed that CLUV does not impair the growth of wild-type or rad14Δ cells (Figure 1A). To examine the effect of CLUV on genome stability, we measured mutation frequencies at the CAN1 locus. Any mutation that inactivates the arginine permease encoded by CAN1 confers canavanine resistance (CanR). A small number of CanR mutants were detected in CLUV-exposed wild-type cells (Figure 1B), implying that CLUV-induced mutagenesis might promote evolutionary fitness. However, in NER-deficient rad14Δ cells, CLUV induced a much higher level of dose-dependent CanR mutations (Figure 1B), such that the mutation frequency of rad14Δ cells was approximately 25-fold higher than wild-type cells after 16 hours of irradiation. This infers that NER plays a critical role in the maintenance of genome stability by repairing CLUV-induced mutagenic lesions.

Polζ- and Polη-dependent mutagenesis in CLUV-irradiated cells

Previous studies showed that DNA polymerase ζ (Polζ, encoded by REV3 and REV7) promotes mutagenic bypass of DNA lesions in yeast cells exposed to acute high-dose UV, whereas DNA polymerase η (Polη, encoded by RAD30) promotes relatively error-free bypass under identical conditions (8,20). Here, we confirmed that inactivation of Polζ blocks acute UV-induced mutagenesis, whereas inactivation of Polη significantly enhances it (Supplemental Figure S1A). Similarly, the CLUV-induced mutation frequencies were substantially higher in rad30Δ cells and lower in rev3Δ cells than in wild-type cells (Supplemental Figure S1B). We then examined the potential roles of Polζ and Polη in CLUV-induced mutagenesis in rad14Δ cells. As shown in Figure 2A, deletion of REV3 or RAD30 only slightly impaired the growth of rad14Δ cell in the presence of CLUV. Notably, the CanR mutation frequency after 16 hours CLUV was significantly lower in rad14Δ rev3Δ (325 × 10⁻⁷ ± 28 × 10⁻⁷) and rad14Δ rad30Δ (305 × 10⁻⁷ ± 30 × 10⁻⁷) than in rad14Δ cells (635 × 10⁻⁷ ± 46 × 10⁻⁷) (Figure 2B and C), although it was still significantly higher than in wild-type cells (35 × 10⁻⁷ ± 4 × 10⁻⁷). These results suggest that both Polζ and Polη promote CLUV-induced mutagenesis in rad14Δ cells. Consistent with this, rad14Δ rev3Δ rad30Δ triple mutants, which grew slowly in the presence of CLUV (Figure 2A), completely suppressed CLUV-induced mutagenesis (Figure 2C). This result supports the idea that Polζ and Polη contribute to CLUV-induced mutagenesis in rad14Δ cells.

To test whether Polη promotes mutagenic bypass of other DNA lesions in rad14Δ cells, wild-type, rad14Δ, rad30Δ and rev3Δ single and double-mutant cells were treated with MMS, and the CanR mutation frequency was determined. As reported previously, rev3Δ cells were more sensitive to MMS than rad30Δ or wild-type cells (Figure 3A). In addition, MMS-induced mutagenesis was completely suppressed in rev3Δ cells, but it was higher in
chromosomal site under the control of the GAL1 promoter. Immunoblot analysis confirmed that a high level of PolZ expression was induced by galactose in cells carrying an inducible ectopic PolZ (Figure 4A). The CLUV-induced mutation frequency was significantly higher in rad14Δ cells overexpressing PolZ than in rad14Δ cells (Figure 4B) (P < 0.01), which is consistent with our data showing that the deletion of RAD30 inhibited CLUV-induced mutagenesis in rad14Δ cells. Importantly, the CLUV-induced mutation frequency was lower in rad14Δ cells overexpressing PolZD155A, an allele that lacks polymerase activity because of a mutation in the active site residue D155, than in rad14Δ cells (Figure 4B) (P < 0.01). The mutation frequency in PolZD155A overexpressing rad14Δ cells was similar to the mutation frequency in rad14Δ rad30Δ cells, suggesting that the polymerase function of PolZ is required for CLUV-induced mutagenesis in rad14Δ cells.

Characterization of CLUV-induced CanR mutations

To further investigate the role of PolZ in CLUV-induced mutagenesis, we overexpressed PolZ in rad14Δ cells and measured the CAN1 mutation frequency after exposure to CLUV. For this purpose, the RAD30 gene was integrated into the AUR1 locus and expressed from its single copy chromosomal site under the control of the GAL1 promoter. Immunoblot analysis confirmed that a high level of PolZ expression was induced by galactose in cells carrying an inducible ectopic PolZ (Figure 4A). The CLUV-induced mutation frequency was significantly higher in rad14Δ cells overexpressing PolZ than in rad14Δ cells (Figure 4B) (P < 0.01), which is consistent with our data showing that the deletion of RAD30 inhibited CLUV-induced mutagenesis in rad14Δ cells. Importantly, the CLUV-induced mutation frequency was lower in rad14Δ cells overexpressing PolZD155A, an allele that lacks polymerase activity because of a mutation in the active site residue D155, than in rad14Δ cells (Figure 4B) (P < 0.01). The mutation frequency in PolZD155A overexpressing rad14Δ cells was similar to the mutation frequency in rad14Δ rad30Δ cells, suggesting that the polymerase function of PolZ is required for CLUV-induced mutagenesis in rad14Δ cells.

Overexpression of PolZ in CLUV-irradiated cells

To further investigate the role of PolZ in CLUV-induced mutagenesis, we overexpressed PolZ in rad14Δ cells and measured the CAN1 mutation frequency after exposure to CLUV. For this purpose, the RAD30 gene was integrated into the AUR1 locus and expressed from its single copy chromosomal site under the control of the GAL1 promoter. Immunoblot analysis confirmed that a high level of PolZ expression was induced by galactose in cells carrying an inducible ectopic PolZ (Figure 4A). The CLUV-induced mutation frequency was significantly higher in rad14Δ cells overexpressing PolZ than in rad14Δ cells (Figure 4B) (P < 0.01), which is consistent with our data showing that the deletion of RAD30 inhibited CLUV-induced mutagenesis in rad14Δ cells. Importantly, the CLUV-induced mutation frequency was lower in rad14Δ cells overexpressing PolZD155A, an allele that lacks polymerase activity because of a mutation in the active site residue D155, than in rad14Δ cells (Figure 4B) (P < 0.01). The mutation frequency in PolZD155A overexpressing rad14Δ cells was similar to the mutation frequency in rad14Δ rad30Δ cells, suggesting that the polymerase function of PolZ is required for CLUV-induced mutagenesis in rad14Δ cells.

Characterization of CLUV-induced CanR mutations

To understand the molecular mechanisms underlying CLUV-induced mutagenesis, the CLUV-induced mutation spectrum in CAN1 was analysed in rad14Δ, rad14Δ rev3Δ, rad14Δ rad30Δ and rad14Δ rev3Δ rad30Δ cells. The CanR DNA sequence was determined, and the CanR mutations were tabulated (Table 2). Most CanR mutations in rad14Δ cells, either spontaneous or CLUV-induced, were single base substitutions. In addition, CLUV preferentially induced mutations at pyrimidine sites, such that the fraction of base substitutions at tandem pyrimidines increased significantly (91% with CLUV vs. 76% without CLUV) (data not shown). The proportion of mutations at...
dipyrimidine sites in unirradiated rad14A cells is close to the proportion of those sites (77%) in the CAN1 gene, suggesting a stochastic mechanism.

The distribution of CLUV-induced single base substitutions conferring canavanine resistance in different strains is presented graphically in Figure 5A and Supplemental Figure S2. The majority of CLUV-induced mutations in rad14A cells were C→T (G/C to A/T) transitions (86%), which were present at much lower levels in unirradiated cells (26%). C→T transitions were also predominant in CLUV-exposed rad14A rad30A, rad14A rev3Δ and rad14A rev3Δ rad30A mutants (Figure 5A). Notably, in CLUV-exposed rad14A rev3Δ cells, in which Polη is the primary TLS polymerase, all of the mutations were C→T transitions (Figure 5A), 70% of which occurred in the context of a 5′-TC-3′ dinucleotide (TC + TCC + TCT; underlined nucleotide is the mutated site) (Table 3). The proportion of these changes was significantly higher in rad14A rev3Δ cells (70%) than in rad14A cells (30%) (FET, P = 0.0001). In contrast, no significant difference was observed in the rad14A and rad14A rev3Δ cells (FET = 0.3). Figure 5B compares the CLUV-induced mutation frequency for each type of base substitution mutation in rad14A, rad14A rev3Δ and rad14A rad30A cells. The C→T mutation frequency (468 × 10⁻⁷) was significantly higher (160-fold) in CLUV-irradiated than in unirradiated rad14A cells (3 × 10⁻⁷), but this rate was 30–65% lower in rad14A rev3Δ (325 × 10⁻⁷) and rad14A rad30A (159 × 10⁻⁷) cells. Furthermore, CLUV-induced C→T transitions were nearly completely suppressed in rad14A rev3Δ rad30A cells (Figure 5B). These results demonstrate that C→T transitions are most prominent feature of CLUV-induced mutagenesis. Most bypass events caused by Polη seem to involve misincorporation of an A opposite the 3′C in a 5′-TC dinucleotide, leading to a C→T transition in the dipyrimidine site.

It was previously shown that C→T transitions are frequently the result of UV-induced lesions in the transcribed strand (TS) (33). Consistent with this observation, we found that CLUV-induced C→T transitions in CAN1 in rad14A cells occurred preferentially in the transcribed strand (TS 38; non-TS 4) (Figure 5C). Interestingly, although a similar pattern was observed in rad14A rev3Δ cells (TS 43; non-TS 7), a much smaller preference for C→T transitions in the transcribed strand was observed in rad14A rad30A cells (TS 16; non-TS 10) (FET, P = 0.006), as compared with those in rad14A cells (Figure 5C). These results suggest that CLUV

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Table 2. DNA sequence changes in CanR mutants induced by CLUV

<table>
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<th>Types of mutations</th>
<th>No UV</th>
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<tr>
<td></td>
<td>rad14Δ</td>
<td>rad14Δ</td>
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<tr>
<td>Single:</td>
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<tr>
<td>Base substitution (BS)</td>
<td>47 (92%)</td>
<td>49 (86%)</td>
</tr>
<tr>
<td>Deletion 1 nt</td>
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<tr>
<td>Insertion 1 nt</td>
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<td>2 (4%)</td>
</tr>
<tr>
<td>Tandem double:</td>
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<td></td>
</tr>
<tr>
<td>CC-&gt;GG-&gt;TT•AA</td>
<td></td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Other BS</td>
<td></td>
<td>1 (2%)</td>
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<tr>
<td>Non-tandem double:</td>
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<td></td>
</tr>
<tr>
<td>BS</td>
<td></td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Multiple:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insertion</td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Repeats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deletions</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Total mutants</td>
<td>51 (100%)</td>
<td>57 (100%)</td>
</tr>
</tbody>
</table>
preferentially induces C→T transitions in the template strand by a mechanism involving PolZ.

Effect of PR on CLUV-induced mutagenesis

CPDs are responsible for the majority of UV-induced mutations in many organisms because they occur much more frequently than 6-4PPs and because they are repaired more slowly than 6-4PPs by NER (1,6,34,35). In *S. cerevisiae*, the Phr1 photolyase catalyzes light-dependent PR of CPDs but does not photoreactivate 6-4PPs (36). To assess the relative effects of CPDs and 6-4PPs in our experimental system, PR-proficient and PR-deficient *rad14*Δ cells were exposed to acute UV with or without exposure to UV-A (365–385 nm) light. As shown in Figure 6A, *rad14*Δ and *rad14*Δ*phr1*Δ mutants were extremely sensitive to acute UV irradiation (254 nm) at a dose of 5 J/m². However, exposure to UV-A light for 1 hour immediately following acute UV irradiation fully suppressed the UV sensitivity of *rad14*Δ cells (PR-competent) but not *rad14*Δ*phr1*Δ cells (PR-deficient) (Figure 6A). Thus, Phr1-dependent reversal of CPDs facilitates survival of acute UV-exposed NER-deficient cells.

To assess the relative contribution of CPDs and 6-4PPs to CLUV (254 nm)-induced mutagenesis, *CAN1* mutation frequency was measured in cells exposed to CLUV for 16 hours with or without UV-A irradiation to facilitate PR. PR in the absence of CLUV had no or little effect on *CAN1* mutation frequency in *rad14*Δ cells or *rad14*Δ lacking Polζ or Polη (Figure 6B). *CAN1* mutation frequencies were reduced in CLUV exposed PR-competent *rad14*Δ cells, but not in PR-defective *rad14*Δ*phr1*Δ cells after inducing PR by UV-A irradiation (Figure 6B). CLUV had no significant effect on growth of either *rad14*Δ or *rad14*Δ*phr1*Δ cells (data not shown). These results indicate that CPDs, which are rapidly reversed by PR, contribute significantly to CLUV-induced mutagenesis in *rad14*Δ cells, although a small fraction of CLUV-induced mutagenesis still occurs...
at 6-4PPs. In addition, although PR in the absence of CLUV had no or little effect on CAN1 mutation frequency in rad14Δ cells or rad14Δ lacking Polζ or Polη (Figure 6B), CLUV-induced mutagenesis was higher in rad14Δ rad30Δ and lower in rad14Δ rev3Δ than in rad14Δ cells in the presence of PR (Figure 6B). Taken together, these results suggest that Polη specifically contributes to the mutagenic bypass of CPDs.

Deamination of cytosine-containing CPDs contributes to CLUV-induced mutagenesis

The above experiments show that CLUV irradiation generates a large number of C→T transitions in rad14Δ cells. One possible explanation for this specificity is that deamination of cytosine to uracil in nascent CPDs leads to the mis-incorporation of an A (instead of G) during TLS in NER-deficient cells, whereas efficient repair of CPDs could circumvent these events in NER-proficient cells. As PR converts CPDs into the original sequences, we predicted that PR on uracil-containing CPDs would be mutagenic in NER-deficient cells lacking uracil DNA glycosylase (Ung1, encoded by UNG1) (Figure 6C). To confirm the presence of uracil-containing CPDs in CLUV-exposed rad14Δ cells, UNG1-deficient cells were exposed to CLUV + PR. CLUV had no significant effect on growth of either rad14Δ or rad14Δ ung1Δ cells, irrespective of the presence of PR (data not shown). Although there was no significant difference in mutation frequency between CLUV-exposed rad14Δ and rad14Δ ung1Δ cells, an 8-hour period of PR by UV-A irradiation before the cessation of CLUV treatment significantly stimulated CLUV-induced mutagenesis in rad14Δ ung1Δ cells, as compared with those in rad14Δ cells (P < 0.01). These results are consistent with the hypothesis that deamination of cytosine-containing CPDs contributes to CLUV-induced mutagenesis in NER-deficient cells.

DISCUSSION

UV-induced CPDs and 6-4PPs are thought to be mutagenic when they are not removed by NER, as they block replication fork progression and are subsequently
bypassed by error-prone TLS. Previous studies demonstrated preferential induction of C→T transitions in *E. coli*, yeast and human cells exposed to UV and in human skin cancer cells, whereas C→T transitions are relatively rare in internal malignancies in humans (37–39). Thus, the molecular mechanism leading to C→T transitions in long-term sun-exposed human skin and other UV-exposed cells is of great interest.

This study demonstrates that CLUV irradiation does not affect the CanR mutation frequency in wild-type cells, but greatly induces it in NER deficient rad14Δ cells, indicating a role for the NER pathway in the maintenance of genome stability under CLUV conditions. Notably, CLUV-induced mutagenesis in rad14Δ cells is partially suppressed in rad14Δ rev3Δ and rad14Δ rad30Δ cells, and completely suppressed in rad14Δ rev3Δ rad30Δ cells, suggesting that Polζ- and Polη-mediated translesion synthesis contribute to mutagenic bypass of CLUV-induced DNA lesions. Consistent with these findings, overexpression of Polη in rad14Δ cells significantly enhanced CLUV-induced mutagenesis. Overexpression of PolηD155A, a mutant lacking polymerase activity, partially suppressed CLUV-induced mutagenesis, possibly through a dominant-negative effect in which the mutant Polη binds to a stalled replication fork at the site of UV damage and inhibits the binding and/or activity of wild-type Polη. A previous study found that overproduction of PolηD155A was mutagenic in the polymerase ε exonuclease or the mismatch repair deficient strains (40). These apparent differences between their and our results may be because of the two different experimental systems, one is for spontaneous mutagenesis in exonucleolytic proofreading or DNA mismatch repair deficient background and the other is for CLUV-induced mutagenesis in NER deficient background. In addition, PR of CPDs by UV-A irradiation strongly inhibited CLUV-induced mutagenesis in rad14Δ rev3Δ cells, but not in rad14Δ rad30Δ cells. This is consistent with previous study based on UV-induced mutagenesis, which have found that Polζ is responsible for virtually all damage-induced mutagenesis at 6-4PPs and that Polη promotes error-free bypass of 6-4PPs (10). Our study also revealed that all of the CLUV-induced CAN1 mutations in rad14Δ rev3Δ cells, in which Polη is the primary TLS polymerase, were C→T transitions, mostly occurring at the 3' C in a TC dinucleotide 5'T (TC+TCC+TCT). In contrast, a different distribution of base substitutions was observed in rad14Δ rad30Δ cells (Table 3). These data demonstrate that Polη specifically promotes C→T transitions under CLUV conditions.

Previous studies showed that deamination of a cytosine CPD is approximately 10,000-fold faster than deamination of an undamaged cytosine, and it is significantly faster in the context of single-stranded DNA (ssDNA) than in double-stranded DNA (41–43). Therefore, it is possible that CLUV irradiation in NER-deficient cells generates ssDNA at sites of transcription-blocking lesions that provide time for deamination of cytosine CPDs at the transcribed strand. This suggests that deamination of persistent transcription-blocking CPDs might lead to preferential induction of C→T transitions in the transcribed strand. Consistent with this hypothesis, C→T transitions

Figure 7. A model summarizing Polη-dependent mutagenesis in CLUV-exposed NER-deficient cells. In NER-deficient cells, persistent CPDs deaminate spontaneously to uracil-containing CPDs, especially at stalled transcription complexes. The resulting uracil-containing CPDs cause the replication fork stall, and can be then bypassed by Polη-dependent ‘correct’ incorporation of A opposite the U in an uracil-containing CPDs.
were predominantly observed in the transcribed strand in CLUV-exposed rad14A cells. Furthermore, deletion of UNG1 caused an increase in CLUV-induced mutations when cells were treated with PR, implying the accumulation of uracil in CLUV-exposed rad14A cells. We can only speculate as to the CLUV-induced C→T transitions. One possibility is that C→T transitions may result from correct bypass by Polζ of deaminated CPDs as proposed previously (10,23,44). Based on these findings and implications, we propose a mechanism by which Pol ζ could promote C→T transitions through TLS at uracil-containing CPDs in NER-deficient cells (Figure 7). In NER-deficient cells, persistent CPDs increase spontaneous deamination of cytosine-containing CPDs, especially at stalled transcription complexes. As these lesions cannot be bypassed by the replicative polymerases, they have a potential to block the progression of the replication fork. In that case, Pol ζ may promote C→T transitions through the ‘correct’ bypassing of uracil-containing CPDs. This model is consistent with the observations that human Pol ζ incorporates AA opposite TU- and TT-CPDs with the same efficiency (45). Previous in vivo studies in yeast showed that Pol ζ accurately bypasses TC- and CC-containing CPDs, incorporating G opposite C (22). Although this seems to be inconsistent with the high-incidence of Pol ζ-dependent C→T transitions observed in this study, it might be explained by the frequent deamination of cytosine-containing CPDs in CLUV-exposed rad14A cells. It should be noted that although the frequency of C→T (G/C to A/T) transitions is lower in rad14A rad50A than in rad14A cells, the frequencies of G/C to T/A and A/T to T/A transversions are substantially higher (Figure 5B). Thus, Pol ζ still plays a role in error-free bypass of some CLUV-induced lesions in NER-deficient cells.

In conclusion, this study demonstrates that Pol ζ and Pol ζζ play critical roles in CLUV-induced mutagenesis in NER-deficient yeast cells. This is particularly striking because of the well-established role of Pol ζ in error-free bypass of CPDs. We also showed that C→T transitions occur preferentially in the transcribed strand by a mechanism that involves Pol ζ. Moreover, we provide in vivo evidence that deamination of cytosine-containing CPDs leads to CLUV-induced mutagenesis in NER-deficient cells. Thus, these results uncover a novel role for Pol ζ in the induction of transcription-coupled base substitutions, the need for which becomes evident in the absence of NER. A similar mechanism may occur in CLUV-exposed NER-competent mammalian cells, where CPDs are repaired more slowly than in NER-competent yeast.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online: Supplementary Figures 1 and 2.

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REFERENCES
has a thymine dimer bypass DNA polymerase activity. *EMBO J.*, 19, 3491–3501.