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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 the *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

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bypass of UV lesions under most experimental conditions in yeast and human cells (16–19). Consistent with this, Polη mutants display a higher mutation rate than the isogenic wild-type strain (10,20–23). Nevertheless, Polη replicates undamaged DNA with low-fidelity (24), and under some conditions, yeast Polη 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/m^2) 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 *rad14Δ* 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 *rad14Δ* yeast cells. Our results showed that *rad14Δ* 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 *rad14Δ rad30Δ* cells than in *rad14Δ* cells, indicating that DNA polη 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 (*MATa leu2Δ0 met15Δ0 ura3Δ0 his3Δ1*) and are listed in Table 1. All double and triple mutants were constructed by standard genetic procedures (29). For Polη-overexpression, wild-type *RAD30* and $rad30^{D155A}$ coding regions amplified by PCR were cloned into the galactose-inducible vector, p415GAL1 (30), producing pGRad30 and $pGRad30^{D155A}$, respectively. The *Sac*I-*Kpn*I fragments of pGRad30 and $pGRad30^{D155A}$ were cloned separately into the integration vector, pAUR101 (TAKARA). The resulting plasmids were linearized at a unique *Stu*I 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

Strain	Genotype	References
BY4741	MATa *leu2Δ0, met15Δ0, ura3Δ0, his3Δ1*	ATCC
NHY017	BY4741 *rad14Δ::KanMX*	(28)
NHY046	BY4741 *rad30Δ::HIS3*	This study
NHY033	BY4741 *rev3Δ::KanMX*	This study
NHY034	BY4741 *rad14Δ::LUE2, rev3Δ::KanMX*	This study
NHY097	BY4741 *rad14Δ::LUE2, rad30Δ::HIS3*	This study
NHY098	BY4741 *rad14Δ::LUE2, rev3Δ::KanMX, rad30Δ::HIS3*	This study
NHY085	BY4741 *rad14Δ::LUE2, phr1Δ::KanMX*	This study
NHY072	BY4741 *rad14Δ::LUE2, ung1Δ::KanMX*	This study
NHY086	BY4741 *rev3Δ::KanMX, rad30Δ::HIS3*	This study
NHY100	BY4741 *rad14Δ::KanMX, aur1C::pGRad30*	This study
NHY101	BY4741 *rad14Δ::KanMX, aur1C::pGRad30-D155A*	This study

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 Polη 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 Polη 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/m^2/min. To determine the frequency of Can^R mutations, five independent colonies grown on YPAD plates for two days were scraped, suspended in YPAD medium and grown under CLUV irradiation. Cells from each culture were washed and spread onto plates at an appropriate dilution to determine the total cell number on YPAD plates, and the number of Can^R mutants on SC + CAN plates. Colonies arising on YPAD and SC + CAN plates were counted after 3 or 4 days of growth at 30°C. The mutation frequency was determined from the number of Can^R colonies per milliliter divided by the number of viable cells per milliliter, and the average was determined for at least three independent sets of experiments.

Sequence analysis of *CAN1* allele

Can^R 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′-TATTTCAGAGTTCTTCAG-3′) and CAN1R1 (5′-GGTGTACTTATGAGGGTGAGAA-3′). PCR products were then sequenced using three primers: CAN1F2 (5′-TATTGGTTTTCTTGGGCA-3′), CAN1F3 (5′-TTGAGAACTCTGGTACAA) and CAN1R3 (5′-CA

CCGTAATATTTGACAG). The significance of the differences between the percentages was assessed by a two-tailed Fisher extract test (FET).

Preparation of yeast extracts and western blotting

Protein extracts were prepared from 2×10^7 logarithmically growing cells using the TCA method, as described previously (32). Proteins were analysed by SDS-PAGE, transferred to polyvinylidene fluoride (PVDF) membrane, and probed with anti-Polη monoclonal antibody (Santa Cruz).

Photoreactivation (PR)

For spot assays, logarithmically growing cells were harvested and resuspended in water. Ten-fold serial dilutions of cultures of the indicated mutants were spotted onto YPAD plates and exposed to 254 nm of UVC light ($5\,J/m^2$). Immediately after UVC exposure, plates were irradiated for 1 hour in the presence of UVA light (UV-A lamp; 365–385 nm, main peak 360 nm; FL-6BLB-A, Toshiba co. Ltd.) and then incubated in the dark for 3 days. The dose rate of UVA was determined using an UVX-radiometer fixed with UVX-36 long-range sensor (UVP co. Ltd.) and was adjusted $23\,J/m^2$/min. For UV- and PR-induced mutagenesis, cells were exposed to dual UV lights (254 nm and 360 nm) for the indicated times, and mutation frequencies were determined as described above. PR treatment of the *rad14Δ ung1Δ* mutant was initiated 8 hours after the initiation of 16 hours CLUV irradiation period (i.e. PR treatment was given for the past 8 hours of the 16 hours CLUV irradiation period) to avoid the PR of cytosine dimers before deamination.

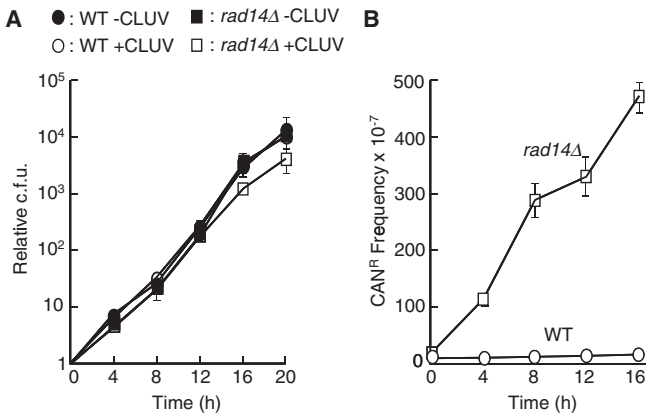

Figure 1. CLUV-induced mutation frequency in *rad14Δ* cells. (**A**) The plating efficiency of wild type (WT) (circle) and *rad14Δ* (square) strains in the absence (closed symbol) or presence (open symbol) of CLUV irradiation. Asynchronous log phase cells were grown in rich media under CLUV irradiation at 30°C, and samples were taken every 4 hours to determine plating efficiency. Cell number is represented as relative colony forming units (=1 at time 0). (**B**) CLUV-induced mutation frequencies at *CAN1* in WT (circle) and *rad14Δ* (square) cells. Data points are the average of at least three independent experiments. Error bars indicate the standard error for each data point.

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 (Can^R). A small number of Can^R 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 Can^R 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 Can^R mutation frequency after 16 hours CLUV was significantly lower in *rad14Δ rev3Δ* ($325 \times 10^{-7} \pm 28 \times 10^{-7}$) and *rad14Δ rad30Δ* ($305 \times 10^{-7} \pm 30 \times 10^{-7}$) than in *rad14Δ* cells ($635 \times 10^{-7} \pm 46 \times 10^{-7}$) (Figure 2B and C), although it was still significantly higher than in wild-type cells ($35 \times 10^{-7} \pm 4 \times 10^{-7}$). 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 Can^R 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

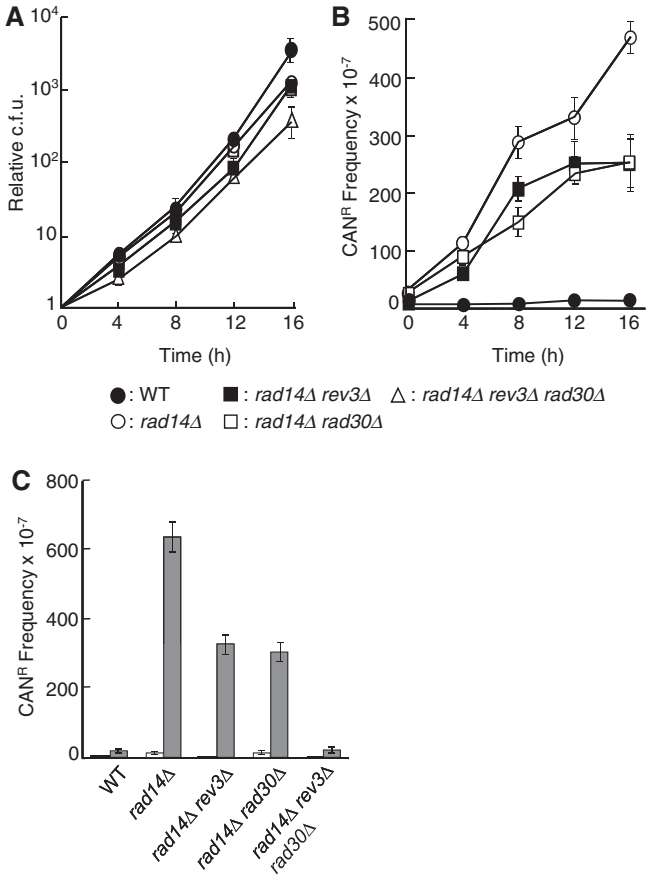

Figure 2. Role of Polζ and Polη in CLUV-induced mutagenesis in NER-deficient cells. (**A**) The plating efficiency of WT (closed circle) and *rad14Δ* (open circle), *rad14Δ rev3Δ* (closed square), *rad14Δ rad30Δ* (open square) and *rad14Δ rev3Δ rad30Δ* (open triangle) cells under CLUV conditions. (**B**) *CAN1* mutation frequencies as a function of length (time) of CLUV irradiation. Symbols are as in (**A**). (**C**) *CAN1* mutation frequencies for the indicated strains after 16 hours without (open bars) or with (gray bars) CLUV. (**A–C**) Data points are the mean of at least three independent experiments. Error bars indicate the standard error for each data point.

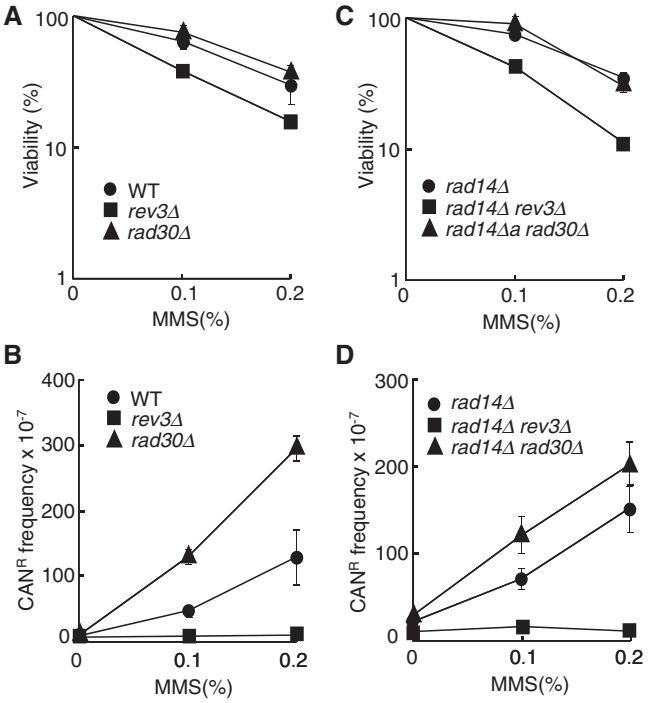

Figure 3. MMS sensitivity and mutability of NER-proficient- and deficient strains. Cells were treated with MMS at the indicated concentrations for 1 hour. Samples were withdrawn and plated on YPAD and SC + CAN medium to determine viability (**A** and **C**) and mutation frequency (**B** and **D**), respectively. The data represent averages of three independent experiments and error bars represent standard error for each data point.

rad30Δ cells than in wild-type cells (Figure 3B), indicating that Polη promotes error-free recovery from MMS-induced DNA damage. Deletion of *RAD14* had no effect on the MMS sensitivity or the MMS-induced mutation frequency in *rev3Δ*, *rad30Δ* or wild-type cells (Figure 3C and D). Thus, these data suggest that Polη is specifically involved in mutagenic bypass of CLUV-induced DNA lesions in *rad14Δ* cells.

Overexpression of Polη in CLUV-irradiated cells

To further investigate the role of Polη in CLUV-induced mutagenesis, we overexpressed Polη 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 Polη expression was induced by galactose in cells carrying an inducible ectopic Polη (Figure 4A). The CLUV-induced mutation frequency was significantly higher in *rad14Δ* cells overexpressing Polη 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 Polη^{D155A}, 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 Polη^{D155A} overexpressing *rad14Δ* cells was similar to the mutation frequency in *rad14Δ rad30Δ* cells, suggesting that the polymerase function of Polη is required for CLUV-induced mutagenesis in *rad14Δ* cells.

Characterization of CLUV-induced Can^R 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 Can^R DNA sequence was determined, and the number and type of base substitutions and other types of mutations present in >50 independent Can^R clones per strain were tabulated (Table 2). Most Can^R mutations in *rad14Δ* cells, either spontaneous or CLUV-induced, were single base substitutions. In addition, CLUV preferentially induced mutations at dipyrimidine 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 *rad14Δ* 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 *rad14Δ* cells were C→T (G/C to A/T) transitions (86%),

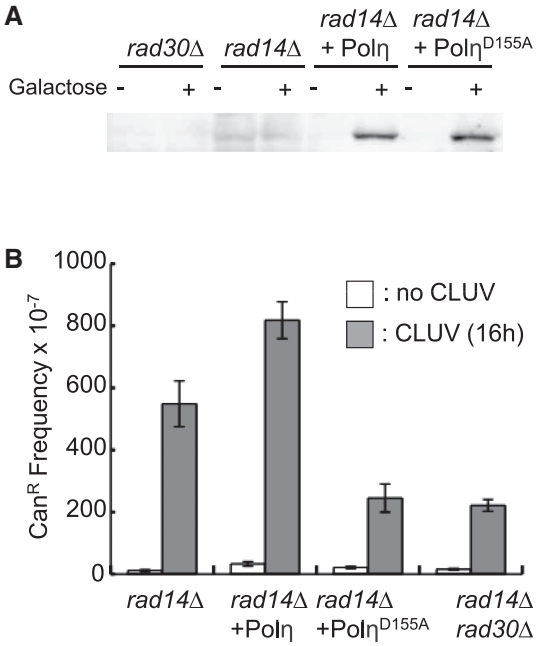

Figure 4. Effect of Polη overexpression on *CAN1* mutation frequency in CLUV-exposed *rad14Δ* cells. (**A**) The indicated strains were grown for 4 hours in liquid YP raffinose with or without galactose as described in Materials and Methods. Protein extracts were prepared and analysed by 10% SDS-PAGE followed by western blotting, using an anti-Polη antibody. (**B**) Cells were grown for 16 hours in the presence of CLUV. Bar graph shows the *CAN1* mutation frequency of the indicated strains. Data points are the average of at least three independent experiments. Error bars indicate the standard error for each data point.

which were present at much lower levels in unirradiated cells (26%). C→T transitions were also predominant in CLUV-exposed *rad14Δ rad30Δ, rad14Δ rev3Δ* and *rad14Δ rev3Δ rad30Δ* mutants (Figure 5A). Notably, in CLUV-exposed *rad14Δ 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 *rad14Δ rev3Δ* cells (70%) than in *rad14Δ* cells (30%) (FET, $P = 0.0001$). In contrast, no significant difference was observed in the *rad14Δ* and *rad14Δ rev3Δ* cells (FET = 0.3). Figure 5B compares the CLUV-induced mutation frequency for each type of base substitution mutation in *rad14Δ*, *rad14Δ rev3Δ* and *rad14Δ rad30Δ* cells. The C→T mutation frequency (468×10^{-7}) was significantly higher (160-fold) in CLUV-irradiated than in unirradiated *rad14Δ* cells (3×10^{-7}), but this rate was 30–65% lower in *rad14Δ rev3Δ* (325×10^{-7}) and *rad14Δ rad30Δ* (159×10^{-7}) cells. Furthermore, CLUV-induced C→T transitions were nearly completely suppressed in *rad14Δ rev3Δ rad30Δ* 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 *rad14Δ* cells occurred preferentially in the transcribed strand (TS 38: non-TS 4) (Figure 5C). Interestingly, although a similar pattern was observed in *rad14Δ rev3Δ* cells (TS 43: non-TS 7), a much smaller preference for C→T transitions in the transcribed strand was observed in *rad14Δ rad30Δ* cells (TS 16: non-TS 10) (FET, $P = 0.006$), as compared with those in *rad14Δ* cells (Figure 5C). These results suggest that CLUV

Table 2. DNA sequence changes in Can^R mutants induced by CLUV

Types of mutations	No UV	CLUV (16 hours)			
	rad14Δ	*rad14Δ*	*rad14Δ rev3Δ*	*rad14Δ rad30Δ*	Triple mutant
Single:					
Base substitution (BS)	47 (92%)	49 (86%)	50 (100%)	41 (82%)	43 (86%)
Deletion 1 nt	3 (6%)				2 (4%)
Insertion 1 nt		2 (4%)		2 (4%)	1 (2%)
Tandem double:					
CC•GG->TT•AA		2 (4%)		7 (14%)	
Other BS		1 (2%)			
Non-tandem double:					
BS	–	2 (4%)			
Multiple:					
Insertion		1 (2%)			
Repeats					3 (6%)
Deletions	1 (2%)				1 (2%)
Total mutants	51 (100%)	57 (100%)	50 (100%)	50 (100%)	50 (100%)

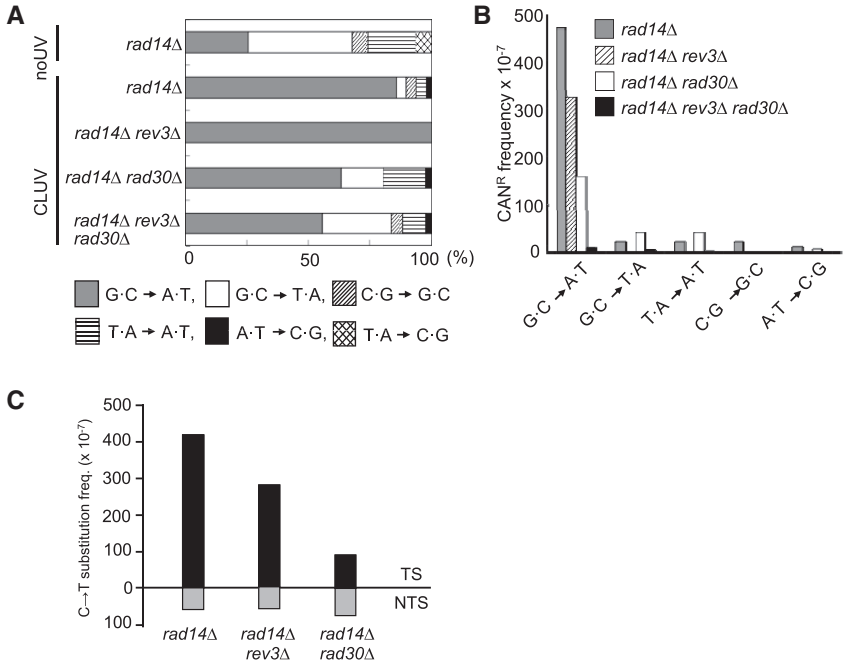

Figure 5. Distribution of base substitutions in CLUV–irradiated cells. (**A**) Proportion of the indicated CLUV-induced or spontaneous base substitutions in NER-deficient cells. Can^R DNA sequence was determined in at least 50 independent canavanine-resistant clones for each strain. (**B**) Frequency of the indicated single-base substitutions in the indicated strains. (**C**) Distribution of C→T transitions in the TS and non-transcribed strands (NTS) of the *CAN1* gene in CLUV-exposed *rad14Δ*, *rad14Δ rev3Δ* and *rad14Δ rad30Δ* cells.

Table 3. Site specificity of base substitutions

Site	Amount of mutations in strains					
	rad14Δ		*rad14Δ rev3Δ*		*rad14Δ rad30Δ*	
	No.(%)	$f \times 10^{-7}$	No.(%)	$f \times 10^{-7}$	No.(%)	$f \times 10^{-7}$
G C->A T:						
5'-CC-3'	9 (18)	100	8 (16)	52	1 (2)	6
5'-CC-3'	5 (10)	56	3 (6)	20	9 (22)	55
5'-TC-3'	6 (12)	67	19 (38)	124	5 (12)	31
5'-CCC-3'	13 (27)	145	4 (8)	26	6 (15)	37
5'-TCC-3'	8 (16)	89	14 (28)	91	2 (5)	12
5'-TCT-3'	1 (2)	11	2 (4)	13	1 (2)	6
5'-CCT-3'	–		–		1 (2)	6
5'-ACA-3'	–		–		1 (2)	6
A T->T A:	–		–			
5'-TT-3'					1 (2)	6
5'-TT-3'	1 (2)	11	–		5 (12)	30
5'-ATA-3'	–		–		1 (2)	6
Others:						
5'-CC-3'	1 (2)	11	–		2 (5)	12
5'-TC-3'	–		–		4 (10)	24
5'-TCC-3'	1 (2)	11	–		–	
5'-TT-3'	1 (2)	11	–		–	
5'-ACA-3'	3 (6)		–		1 (2)	6
5'-CAC-3'	–		–		1 (2)	6
Total	49 (100)	546	50 (100)	325	41 (100)	250

preferentially induces C→T transitions in the template strand by a mechanism involving Polη.

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 *rad14Δ* cells were exposed to acute UV with or without exposure to UV-A (365–385 nm) light. As shown in Figure 6A, *rad14Δ* and *rad14Δ phr1Δ* mutants were extremely sensitive to acute UV irradiation (254 nm) at a dose of 5 J/m^2. However, exposure to UV-A light for 1 hour immediately following acute UV irradiation fully suppressed the UV sensitivity of *rad14Δ* cells (PR-competent) but not *rad14Δ phr1Δ* 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 *rad14Δ* cells or *rad14Δ* lacking Polζ or Polη (Figure 6B). *CAN1* mutation frequencies were reduced in CLUV exposed PR-competent *rad14Δ* cells, but not in PR-defective *rad14Δ phr1Δ* cells after inducing PR by UV-A irradiation (Figure 6B). CLUV had no significant effect on growth of either *rad14Δ* or *rad14Δ phr1Δ* cells (data not shown). These results indicate that CPDs, which are rapidly reversed by PR, contribute significantly to CLUV-induced mutagenesis in *rad14Δ* cells, although a small fraction of CLUV-induced mutagenesis still occurs

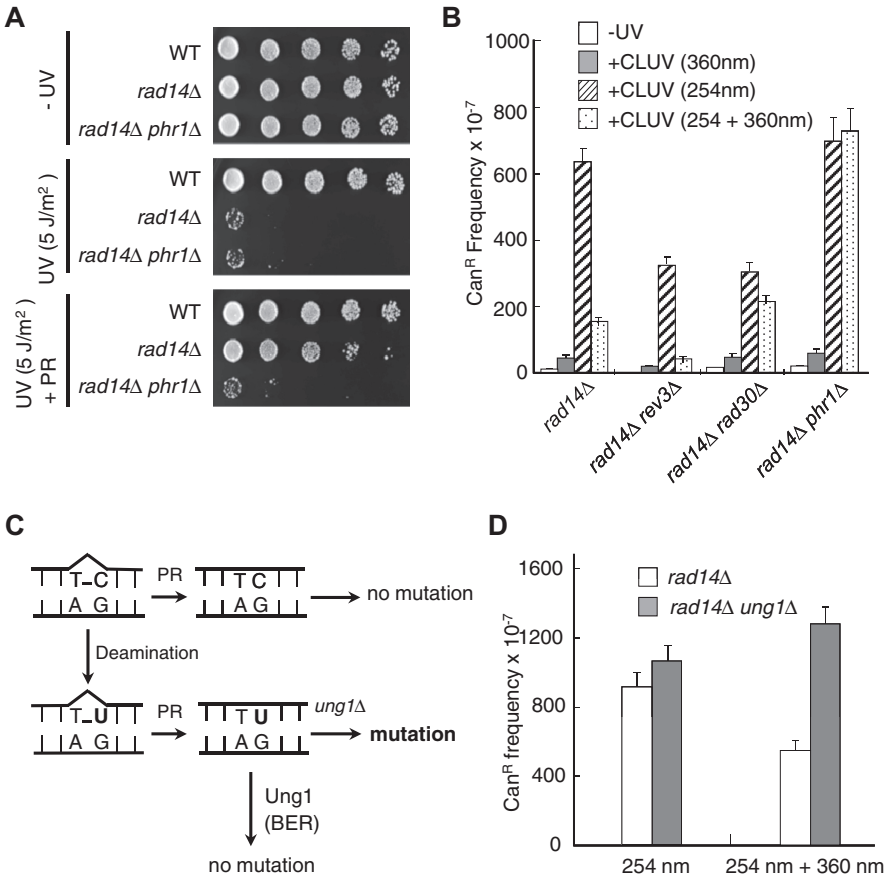

Figure 6. The effects of PR on the survival and mutation frequencies in NER-deficient cells. (**A**) Ten-fold serial dilutions of the indicated yeast strains were spotted onto YPD plates. For each plate: top, no UV treatment; middle, UVC irradiation (5 J/m^2); bottom, long-term UVC irradiation (5 J/m^2) plus UVA (23 J/m^2/min) for 1 hour. (**B**) *CAN1* mutation frequencies in the indicated strains after 16 hours incubation. Cells were continuously exposed to 360 nm, 254-nm and 360-nm + 254-nmUV irradiation or treated with no UV irradiation as a control sample. (**C**) Experimental design: NER-deficient cells containing unrepaired UV photoproducts were exposed to 360-nm UV irradiation to allow PR of CPDs. Uracil–containing CPDs are mutagenic after PR in *ung1Δ* cells, but not in *UNG1* cells. The difference in mutation frequency in *ung1Δ and UNG1* cells provides an estimate of the frequency of deamination of cytosine-containing CPDs. (**D**) CLUV-induced mutation frequency in *rad14Δ* (open bars) and *rad14Δ ung1Δ* (gray bars) in the presence or absence of 360-nm UV irradiation. The cells were irradiated with 360-nm light during the past 8 hours of a 16-hour CLUV (254 nm) irradiation.

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 Can^R 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 $Polη^{D155A}$, 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 $Polη^{D155A}$ 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

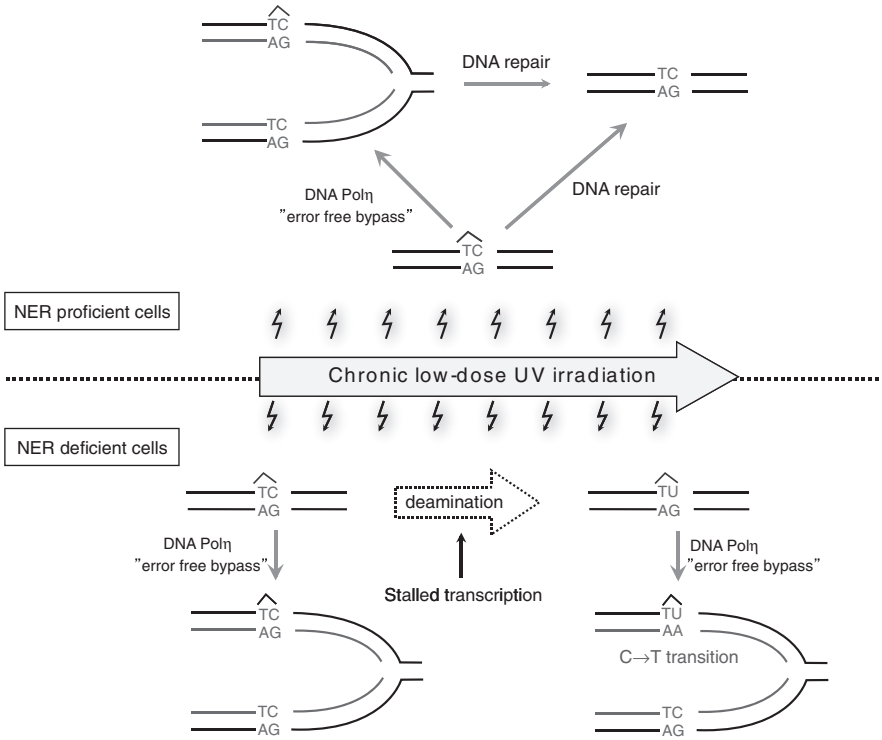

Figure 7. A model summarizing Polη-depnedent mutagenesis in CLUV-exposed NER-deficient cells. In NER-deficient cells, persistent CPDs deaminate spontaneously to uracil-containing CPDs, especially at stalled transcription complexes. The resulting uracil-containing CPDs cause the replication fork stall, 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 *rad14Δ* 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 *rad14Δ* 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 Polη of deaminated CPDs as proposed previously (10,23,44). Based on these findings and implications, we propose a mechanism by which Polη 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, Polη may promote C→T transitions through the 'correct' bypassing of uracil-containing CPDs. This model is consistent with the observations that human Polη incorporates AA opposite TU- and TT-CPDs with the same efficiency (45). Previous *in vivo* studies in yeast showed that Polη accurately bypasses TC- and CC-containing CPDs, incorporating G opposite C (22). Although this seems to be inconsistent with the high-incidence of Polη-dependent C→T transitions observed in this study, it might be explained by the frequent deamination of cytosine-containing CPDs in CLUV-exposed *rad14Δ* cells. It should be noted that although the frequency of C→T (G/C to A/T) transitions is lower in *rad14Δ rad30Δ* than in *rad14Δ* cells, the frequencies of G/C to T/A and A/T to T/A transversions are substantially higher (Figure 5B). Thus, Polη still plays a role in error-free bypass of some CLUV-induced lesions in NER-deficient cells.

In conclusion, this study demonstrates that Polη and Polζ play critical roles in CLUV-induced mutagenesis in NER-deficient yeast cells. This is particularly striking because of the well-established role of Polη in error-free bypass of CPDs. We also showed that C→T transitions occur preferentially in the transcribed strand by a mechanism that involves Polη. 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 Polη 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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