Deficiency of the *Caenorhabditis elegans* DNA Polymerase η Homologue Increases Sensitivity to UV Radiation during Germ-line Development

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ABSTRACT. Defects in the human *XPV/POLH* gene result in the variant form of the disease xeroderma pigmentosum (XP-V). The gene encodes DNA polymerase η (Pol η), which catalyzes translesion synthesis (TLS) past UV-induced cyclobutane pyrimidine dimers (CPDs) and other lesions. To further understand the roles of Pol η in multicellular organisms, we analyzed phenotypes caused by suppression of *Caenorhabditis elegans* *POLH* (*Ce-POLH*) by RNA interference (RNAi). F1 and F2 progeny from worms treated by *Ce-POLH*-specific RNAi grew normally, but F1 eggs laid by worms treated by RNAi against *Ce-POLD*, which encodes Pol δ did not hatch. These results suggest that Pol δ but not Pol η is essential for *C. elegans* embryogenesis. Pol η -targeted embryos UV-irradiated after egg laying were only moderately sensitive. In contrast, Pol η -targeted embryos UV-irradiated prior to egg laying exhibited severe sensitivity, indicating that Pol η contributes significantly to damage tolerance in *C. elegans* in early embryogenesis but only modestly at later stages. As early embryogenesis is characterized by high levels of DNA replication, Pol η may confer UV resistance in *C. elegans*, perhaps by catalyzing TLS in early embryogenesis.

Key words: *C. elegans*/DNA polymerase η /RNAi/embryogenesis

Introduction

DNA can be damaged by a wide range of physical and chemical agents, both endogenous and environmental. To maintain the integrity of the genetic material, cells possess multiple pathways to repair various types of DNA damage (Friedberg *et al.*, 1995a). Nucleotide excision repair (NER) is the main pathway involved in the removal of cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts produced by UV radiation, although CPDs are repaired fairly slowly by NER. Unrepaired damage can interfere with basic processes such as DNA replication and transcription and

lead to mutations and cell death (Friedberg *et al.*, 1995b).

In eukaryotes, another major pathway that circumvents such threats involves DNA polymerases that catalyze translesion synthesis (TLS) past DNA damage. Most TLS polymerases belong to the recently discovered Y-family (Ohmori *et al.*, 2001). After UV irradiation, cultured cells from XP-V patients are defective in DNA replication and exhibit an increased mutation frequency with an altered spectrum (Lehmann *et al.*, 1975; Maher *et al.*, 1976; Wang *et al.*, 1991, 1993; Waters *et al.*, 1993; Sary *et al.*, 2003). The *XPV* gene encodes human pol η (Masutani *et al.*, 1999a; Johnson *et al.*, 1999) which bypasses CPDs by incorporating correct nucleotides (Masutani *et al.*, 1999b, 2000; Johnson *et al.*, 2000; Washington *et al.*, 2001; Kusumoto *et al.*, 2004; McCulloch *et al.*, 2004), indicating that Pol η is involved in the relatively accurate TLS pathway for bypass of UV-induced lesions *in vivo* and *in vitro*. In contrast, other TLS polymerases, such as Pol ι (Tisser *et al.*, 2000a, 2000b; Frank *et al.*, 2001), Pol κ (Gerlach *et al.*, 1999; Ogi *et al.*, 1999; Ohashi *et al.*, 2000a, 2000b), Rev1 (Nelson *et al.*, 1996a; Lin *et al.*, 1999a; Gibbs *et al.*, 2000) and Rev3 with

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Abbreviations: *Ce*, *Caenorhabditis elegans*; CPD, cyclobutane pyrimidine dimer; IPTG, isopropyl-1-thio- β -D-galactoside; NER, nucleotide excision repair; NGM, nematode growth medium; Pol, DNA polymerase; RNAi, RNA interference; TLS, translesion synthesis; XP, xeroderma pigmentosum.

Rev7 (Pol ζ) (Lin *et al.*, 1999b; Nelson *et al.*, 1996b; Gibbs *et al.*, 1998), are mutagenic polymerases for bypass of UV-induced lesions.

In XP-V patients, unrepaired UV-induced lesions are more likely to be bypassed by the mutagenic pathway, because the relatively accurate pathway is inactivated. As a result, XP-V patients are sensitive to sunlight and exhibit a concomitant increase in the incidence of cancer. However, the regulation of the TLS polymerases is still unclear (Kannouche and Stry, 2003; Fischhaber and Friedberg, 2005). The nematode *C. elegans* also has TLS polymerases such as Pol η , Polk, Rev1 (McDonald *et al.*, 2001; Ohmori *et al.*, 2001) and Rev3 (Lawrence, 2002), making this model multicellular organism an effective tool to explore DNA repair pathways relevant to TLS *in vivo*. However, at present little detailed information is available about *C. elegans* Y-family polymerases.

Here, we examined the sensitivity of *C. elegans* to UV radiation at various stages of embryogenesis using a feeding RNAi method. We show that suppression of *Ce-POLH* in early embryogenesis and gametogenesis stages confers hypersensitivity to UV radiation but that suppression at later stages confers only modest sensitivity. Our data suggest a possible role for *Ce-Pol η in damage tolerance during embryogenesis and gametogenesis.*

Materials and Methods

C. elegans culture

Wild type N2 worms were grown on nematode growth medium (NGM) plates seeded with *E. coli* OP50 at 20°C (Sulston and Hodgkin, 1988).

Cloning of the *Ce-POLH* and *Ce-POLD* genes

To prepare the full-length coding region of F53A3.2, a DNA fragment containing the complete cDNA sequence was amplified by PCR from a *C. elegans* embryonic cDNA library, kindly provided by Dr. Hideyuki Okano (Department of Physiology; Keio University School of Medicine), using the following primers, *Ce-POLH*-1: 5'-GGT TAC TAG TAT GAA AAG AGT GAT CAG CCT CAT-3' and *Ce-POLH*-2: 5'-GGT TCC CGG GTT AGG GTT TCT TCT TTT TGA AAA-3', containing nucleotides 26349–26327 and 21212–21234 of the F53A3 cosmid clone (accession number: AF025460), respectively. PCR was performed as follows: initial denaturing at 94°C for 1 min, followed by six cycles of 94°C for 30 s, 51°C for 30 s, and 72°C for 1.5 min, in which the annealing temperature was decreased 1°C every two cycles until it reached 49°C; followed by 24 cycles of 94°C for 30 s, 47°C for 30 s, and 72°C for 1.5 min; with a final elongation at 72°C for 10 min. PCR products were loaded onto a 1% agarose gel and purified using the QIAEX II gel extraction kit (QIAGEN) according to the manufacturer's instructions. *Ce-POLD* fragment was amplified

by PCR using the following primers, *Ce-POLD*-F: 5'-GGT AAC TAG TAT GAC CTC CAA GCG TCC TG-3' and *Ce-POLD*-R: 5'-CGA TCC CGG GTG GCA GTG ATA GAA CTT TAG CAC G-3', containing nucleotides 18897–18915 and 20269–20246 of the F10C2 cosmid clone (accession number: Z81497), respectively. PCR was performed as follows: initial denaturing at 94°C for 1 min, followed by six cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 1.5 min, in which the annealing temperature was decreased 1°C every two cycles until it reached 52°C; followed by 24 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1.5 min; with a final elongation at 72°C for 10 min. PCR products were digested with *Spe* I and *Sma* I and cloned into pBlueScript SK (Stratagen). The cloned products were sequenced on both strands with a BigDye terminator cycle sequencing kit (Applied Biosystems). Multiple sequence alignments were compiled using Clustal W <www.ebi.ac.uk/clustalw/>.

Amplification and analysis of predicted exon 7 of *Ce-POLH*

Adult hermaphrodites were collected, suspended in 100 μ l disruption buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8.0 and 0.02% lysozyme), quickly frozen at –80°C for 10 min, and incubated at 37°C for 3 h. Total RNA was extracted using the Total RNA Isolation kit (Macherey-Nagel, Inc.) according to the manufacturer's instructions, and then detected by RT-PCR using the following primers, *Ce-POLH*-3: 5'-AAA GCG GCG ATT TAC GAT G-3', and *Ce-POLH*-4: 5'-TTT TGG TGT TGG ATT CGG C-3', corresponding to nucleotides 22460–22442 and 22311–22329 of the F53A3 cosmid clone, respectively. The same primers were used to amplify fragments from cDNA clone derived from *C. elegans* embryonic cDNA libraries. Amplified fragments were electrophoresed on a 15% acrylamide gel and visualized under UV light.

Bacteria-mediated RNAi of *Ce-POLH*

The vector L4440 (pPD129.36), which contains two convergent T7 RNA polymerase promoters in opposite orientation, and the bacterial strain HT115 (DE3) (W3110, *rnc14:: Δ Tn10*), which has an IPTG-inducible T7 RNA polymerase gene and is deficient in RNase III, were kindly provided by Dr. A. Fire (Carnegie Inst., Washington). RNAi induced by the feeding method was performed as described (Kamath *et al.*, 2000; Timmons *et al.*, 2001). Briefly, the plasmids described above were digested with *Spe* I and *Sma* I. Similarly digested PCR products were inserted between the two T7 RNA polymerase promoters of L4440 (pPD129.36) and the resulting plasmids were transformed into HT115 (DE3) cells. IPTG (1 mM) was added to transformed cells at log-phase and these were spread onto NGM agar plates containing 1 mM IPTG and 50 μ g/ml ampicillin, which were incubated for 24 h at 20°C and then used as RNAi plates. As a control, HT115 (DE3) cells transformed by the L4440 vector were plated. Worms at the young adult stage were placed on these plates and transferred to fresh RNAi plates after 12 h feeding. To investigate the effects of *Ce*-

POLH RNAi after UV irradiation, F1 eggs laid over the next 5 h and young adult F1 worms were irradiated with a germicidal lamp (254 nm) at doses of 0, 5, 10, 25, 50 J/m². UV sensitivity was examined for the hatching abilities of the UV irradiated F1 eggs and F2 eggs laid by UV irradiated young adults. Hatching rates were scored 36 h after eggs were laid.

RT-PCR analysis of *Ce-POLH* expression following RNAi

The absence of *Ce-POLH* transcripts following RNAi was examined by RT-PCR using the primers *Ce-POLH*-5: 5'-CGA ACT GCC GAG AAT CTG G-3' and *Ce-POLH*-6: 5'-GCG TGT CAA AAT TGT ACA GAA GTA AT-3', corresponding to nucleotides 23889–23871 and 21184–21209 of the F53A3 cosmid clone, respectively. As a control, *ACT-1* transcripts were examined using the primers *Ce-ACT-1*-F: 5'-CGT GGT TAC TCT TTC ACC ACC ACC GCT G-3' and *Ce-ACT-1*-R: 5'-CAT TTA GAA GCA CTT GCG GTG AAC GAT GG-3', corresponding to nucleotides 29624–29651 and 30221–30193 of the T04C12 cosmid clone (accession number: Z81584), respectively. RT-PCR was performed as described (Hashmi *et al.*, 2002). RNAi-treated adult hermaphrodites were collected, suspended in 100 µl disruption buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8.0 and 0.02% lysozyme), quickly frozen at –80°C for 10 min, and then incubated at 37°C for 3 h. RNA was extracted using the Total RNA Isolation kit (Macherey-Nagel, Inc.), and RT-PCR was carried out using the SuperScript One-Step RT-PCR System (Invitrogen) according to the manufacturer's instructions. Each 50-µl reaction mixture was split into two tubes into which either *Ce-POLH* or *Ce-ACT-1*-specific primers were added. After 30–40 cycles of amplification, the RT-PCR products were separated on 1% agarose gels.

Results

Sequence analysis of *C. elegans* *POLH* and Polη protein

Information provided by the genomic sequencing consortium indicated that *C. elegans* has three Y-family DNA polymerases, Polη, Polk and Rev1. Polη is encoded by the gene F53A3.2 and based on the genomic sequence was predicted to be composed of eight exons. This sequence was used to obtain *Ce-POLH* cDNA by PCR of embryonic cDNA libraries with primers that included the predicted start (*Ce-POLH*-1) and stop codons (*Ce-POLH*-2). Sequencing of the cDNA clone thus obtained (accession number: AB244413) revealed that it has a 57-bp deletion in predicted exon 7 with respect to the genomic sequence (Fig. 1B). To confirm whether *Ce-POLH* mRNA lacks these nucleotides, we performed RT-PCR with total RNA isolated from adult worms and primers flanking the deletion (*Ce-POLH*-3 and *Ce-POLH*-4). As shown in Fig. 1C, RT-PCR (lane 1) gave rise only to a 93-bp PCR product which lacks

the 57-bp sequence found in genomic DNA, as did PCR (lane 2) of the cDNA clone obtained from the *C. elegans* embryonic cDNA library. The corresponding amino acids encoded by the 57-bp sequence are also absent in cDNA sequences of human and *Drosophila melanogaster* Polη proteins. In fact, this region includes a splice site consensus sequence. Taking these results together, it is likely that the *Ce-POLH* gene consists of nine exons (Fig. 1A) and encodes a protein of 584 amino acids which shares 30% and 26% identity with the human and *Drosophila melanogaster* Polη proteins, respectively (Fig. 1D).

Effects of RNAi of *Ce-POLH* on *C. elegans*

To investigate the role of Polη in *C. elegans*, *Ce-POLH* gene expression was suppressed by feeding worms with *E. coli* strains that contain plasmid vectors designed for the production of dsRNA by the bidirectional transcription of inserts (Timmons and Fire, 1998). Worms at the young adult stage were fed on *E. coli* producing dsRNA specific to *Ce-POLH* or control *E. coli* transformed by the vector alone. F1 embryos from these RNAi-treated worms were also fed on RNAi plates. To confirm the absence of *Ce-POLH* transcripts following RNAi treatment, RT-PCR using specific primers for *Ce-POLH* and for a control, *ACT-1*, which encodes the body wall actin gene, was carried out with total RNA prepared from RNAi-treated F1 adult worms. Fig. 2 shows that *POLH* mRNA is depleted in Polη-targeted adults (lane 3) as compared with control worms (lane 1), while *ACT-1* mRNA is expressed in both control and treated animals (lanes 2 and 4). F1 and F2 progeny from worms which were fed *E. coli* producing *Ce-POLH*-dsRNA hatched and grew normally, but eggs laid by worms which were fed *E. coli* producing *Ce-POLD*-dsRNA did not hatch (Table I). These results suggest that the replicative DNA polymerase, Polδ, is essential for *C. elegans* embryogenesis, as expected, but that the TLS polymerase Polη is not.

Effects of RNAi on *C. elegans* UV sensitivity

The UV sensitivity of RNAi-treated worms was determined by monitoring the hatching rates of F2 embryos. The exper-

Table I. HATCHING RATES OF UN-IRRADIATED EGGS

RNAi	F1		F2	
	eggs laid	hatched (%)	laid	hatched (%)
vector	345	98.8	1793	98.8
RNAi- <i>POLH</i>	378	96.0	1639	96.5
RNAi- <i>POLD</i>	367	0	—	—

F1 eggs laid by worms that were fed *E. coli* producing *Ce-POLD*-dsRNA showed 100% embryonic lethality. F1 and F2 eggs from worms which were fed *E. coli* producing *Ce-POLH*-dsRNA developed normally as well as control eggs.

Fig. 1.

imental scheme is shown in Fig. 3A. When previously laid eggs were UV-irradiated, Polη-targeted embryos were only modestly sensitive compared with untargeted embryos (Fig. 3B). In contrast, Polη-targeted F2 eggs laid 0–8 h after irradiation were clearly sensitive (Fig. 3C). In this case, eggs were irradiated while they were at the 32-blastomere stage and at the diakinesis-diplotene stages of oogenesis (Takanami *et al.*, 2003). These results suggest that Polη significantly contributes to damage tolerance in *C. elegans* in early embryogenesis but less so in later stages. As early embryogenesis is characterized by high levels of replication, TLS by Polη may play an important role at this time. Interestingly, eggs laid 8–24 h after irradiation, which were exposed at the pachytene stage, also clearly showed UV sensitivity (Fig. 3D). To examine the contribution of Polη to damage tolerance in specific stages during embryogenesis in more detail, eggs at various developmental stages were irradiated and hatching rates were determined. The

progression of gametogenesis and embryogenesis in *C. elegans* is conventionally classified into six periods (Fig. 4A) (Takanami *et al.*, 2000). In control eggs, radiation sensitivity changed slowly throughout development, such that later-stage embryos were more sensitive than younger embryos (Fig. 4B). Periods II and III were the most UV-sensitive stages of *Ce-POLH* RNAi-treated eggs, but period I embryos were also somewhat sensitive (Fig. 4B and 4C). This result suggests that the genomic stability of fertilized eggs in early embryogenesis may depend on an accurate pathway using TLS to rapidly bypass DNA damage.

Discussion

In this paper, we examined the roles of the *C. elegans* *XPV*/*POLH* homologue by the RNAi method. The embryos laid by worms treated by RNAi (*Ce-POLH*) were not significantly different from those of control worms, and larvae did not show any defects and further development into adults was normal. This result suggests that *Ce-Polη* is not essential under normal growth conditions. On the other hand, we showed that the down-regulation of Polη in *C. elegans* results in an increased sensitivity to UV radiation by several development and differentiation processes, including meiosis and embryogenesis. It has been reported that wild-type *C. elegans* embryos are significantly more resistant to the effects of UV radiation on DNA synthesis than are those of other organisms (Hartman and Nelson, 1998). For example, the UV dose required to reduce DNA synthesis by 50% in wild type *C. elegans* is 200 J/m², a dose that in mammalian cells results in the rapid and permanent cessation of DNA synthesis (Hartman *et al.*, 1991). NER is the main pathway involved in the removal of CPDs and (6-4) photoproducts induced by UV irradiation. However, in mammalian cultured cells, removal of (6-4) photoproducts is very fast, but CPD repair is much slower (Friedberg *et al.*, 1995b). In contrast, the kinetics of repair of CPDs and (6-4) photoproducts in *C. elegans* from embryos to adults were similar to, if not slower than, that of CPDs in cultured human cells (Hartman *et al.*, 1989). These data suggest that UV resistance in *C. elegans* embryos may depend on TLS, which rapidly bypasses DNA damage, in addition to the removal of damage by the NER system. Recently, it was reported that RNAi of the *C. elegans* homologues of human *XPA* (Park

Fig. 2. RT-PCR of total RNA isolated from F1 adult worms treated by RNAi (*Ce-POLH* or control vector). PCR primers were specific to the endogenous *Ce-POLH* gene (*Ce-POLH*-5 and *Ce-POLH*-6) or to an internal control gene (*Ce-ACT-1*-F and *Ce-ACT-1*-R). Lanes 2 and 4, control *Ce-ACT-1* gene mRNA. Lanes 1 and 3, *Ce-POLH* mRNA. M, 1 kb DNA size marker. The figure is a negative image of an ethidium bromide-stained agarose gel.

Fig. 1. Gene and domain structure of *Ce-POLH*. (A) Genomic map of linkage group III (LG III) in relation to F53A3.2 and the intron/exon structure of the *Ce-POLH* gene. The gene F53A3.2, which is located between the *pqn-41* and *rps-22* loci, consists of nine exons, instead of the eight exons previously assumed. Primer positions (arrow heads) used for RT-PCR and product sizes are indicated below the exon schematic. (B) Nucleotide sequence of exon 7 of the *Ce-POLH* gene. The 57 bases surrounded by red box are deleted in the cDNA clone derived from the cDNA library. (C) RT-PCR with total RNA from adult worms and PCR of the cDNA clone derived from the cDNA library. M, ϕ X174 *Hae*III DNA size marker. The figure is the negative image of an ethidium bromide-stained agarose gel. (D) A comparison of *Ce-Polη* (accession number: AB244413) with the human Polη (AB024313) and the *Drosophila melanogaster* Polη (AB049433) proteins. Identical and similar residues are shaded in dark and light gray, respectively. Palm (red), fingers (blue), thumb (green), and little finger (purple) domains are marked with boxes. The asterisk indicates the position of the 19 a.a. (57 bp) deletion.

Fig. 3. Effects of *Ce-POLH* RNAi on the UV sensitivity of hatching. (A) Schematic of RNAi experiments. Young adults were placed on RNAi plates that contained bacteria producing *Ce-PolH*-dsRNA. After 12 h feeding, the worms were transferred to fresh RNAi plates. F1 eggs laid over the next 5 h were used for experiments. Young F1 adults were transferred to fresh RNAi plates, and the hatching rates of F2 eggs irradiated with UV 0–5 h after laying, 0–8 h before laying, and 8–24 h before laying were determined. (B) F2 eggs laid over the course of 5 h by F1 worms were UV-irradiated and hatching rates were scored. (C) Young F1 adults were UV-irradiated, and the hatching rates of F2 eggs laid up to 8 h after irradiation were scored. (D) The hatching rates of F2 eggs laid during 8–24 h after irradiation were scored. Over 100 eggs were tested for each UV dosage in a single set of experiments, and each experiment was repeated three times. Error bars indicate standard deviations. Closed circles: control worms; closed triangles: *Ce-POLH* RNAi-treated worms.

et al., 2002) and human *XPF* (Park *et al.*, 2004), which participate in the NER pathway, increases the UV sensitivity of *C. elegans* embryos. It was also shown that down-regulation of *CSB*, which plays an important role in a NER sub-pathway, transcription-coupled excision repair, inhibits germ cell proliferation and enhances apoptosis after UV irradiation (Lee *et al.*, 2002). However, embryos from worms in which *Ce-POLH* expression is suppressed are much more sensitive to UV irradiation than are embryos in which *XPA* (Park *et al.*, 2002), *XPF* (Park *et al.*, 2004) or *CSB* (Lee *et al.*, 2002) is suppressed. For example, the hatching rate of eggs from *Ce-POLH* RNAi worms irradiated with UV (50 J/m²) was 61–64% lower than that of eggs from control worms (Fig. 3C and D), whereas the hatching efficiency of

eggs from worms in which *XPA* (Park *et al.*, 2002), *XPF* (Park *et al.*, 2004) or *CSB* (Lee *et al.*, 2002) was suppressed was only about 15% lower at the same UV dose. In rat male germ cells, intact early spermatocytes exhibit no or low NER activity, although cell extracts from early spermatocytes show high levels of incision activity. In contrast to somatic cells, little is known regarding the inhibition of the mutagenic effects of DNA damage in germ cells (Jansen *et al.*, 2001). Rapidly proliferating germ cells and embryonic cells may be more dependent on Pol η -mediated TLS than on NER in response to UV-induced DNA damage.

For various embryonic stages, we examined the effects of *Ce-POLH* RNAi on sensitivity to UV at a dose of 10 J/m² (Fig. 4B). The most UV-sensitive stages were periods II and

Fig. 4. UV sensitivities at various embryonic stages. (A) Schematic of gametogenesis and early embryogenesis in a young gravid hermaphrodite. Gametogenesis and embryogenesis are conventionally classified into six periods as described in (Takanami *et al.*, 2000). The eggs in period I (5 h after laying) contain ~100–550 nuclei and are at the stage from gastrulation to early morphogenesis. The eggs in period II (–0.5 h to 0 h after laying, with time 0 corresponding to the laying of the first egg) are at the early cleavage stage. The eggs in period III (–4 h to –0.5 h after laying) are at the stage from full grown oocytes and to fertilization and early cleavage. The eggs in period IV (–8 h to –4 h after laying) are at the oocyte to late pachytene stage. The eggs in period V (–16 h to –8 h after laying) and VI (–24 h to –16 h after laying) are at the pachytene nucleus stage. (B) UV sensitivity of eggs during periods I–VI. The survival of eggs laid during periods I–VI by a *Ce-POLH* RNAi hermaphrodite following UV irradiation of 10 J/m². Closed circles, eggs from control worms; closed triangles, eggs from *Ce-POLH* RNAi worms. Independent triplicate experiments were carried out and the averages of scores from periods I–VI were 22, 3, 15, 18, 46 and 49 eggs per adult worm. Error bars indicate standard deviations. (C) Relative UV sensitivity of eggs during periods I–VI. The hatchability for eggs from *Ce-POLH* RNAi worms in Fig. 4B is expressed with respect to that for eggs from control worms in Fig. 4B as 100%.

III, which include zygote formation, early cleavage, and establishment of embryonic axes, and the determination of somatic and germ-line founder cell fates after fertilization (Wood, 1988). In early *C. elegans* embryos, it was reported that the correct execution and timing of cell division is essential for proper cell fate patterning (Encalada *et al.*, 2000). Conversely, embryos in period I, which covers the stage from gastrulation to early morphogenesis (Wood, 1988), were only moderately sensitive compared with controls (Fig. 3A, 4B and 4C), suggesting that Polη only modestly contributes to damage tolerance at this stage. Differences in these developmental stages may reflect distinct pathways that circumvent the effects of DNA damage. It was reported that *Ce-rdh-51*, the *RAD51* homologue of *C. elegans*, contributes to damage tolerance after X-ray irradiation

most largely in meiotic pachytene nuclei (Takanami *et al.*, 2000). Although stage-specific activities of NER have not been elucidated, it may be possible that alternative DNA repair pathways express in a stage specific manner.

In summary, our observations suggest that *Ce-Polη* contributes to damage tolerance against UV irradiation to ensure the successful completion of embryogenesis, thus providing important insights into its role in DNA damage tolerance in germ and embryonic cells. *C. elegans* treated by RNAi is a good model system to investigate DNA repair in germ and embryonic cells. A drawback of this model system is that UV irradiation does not induce tumors in worms, regardless of the level of *Ce-POLH* expression. To further understand the role of Polη, we are studying *XPV*-deficient mice in detail.

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