Longevity and resistance to stress correlate with DNA repair capacity in *Caenorhabditis elegans*

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ABSTRACT

DNA repair is an important mechanism by which cells maintain genomic integrity. Decline in DNA repair capacity or defects in repair factors are thought to contribute to premature aging in mammals. The nematode *Caenorhabditis elegans* is a good model for studying longevity and DNA repair because of key advances in understanding the genetics of aging in this organism. Long-lived *C. elegans* mutants have been identified and shown to be resistant to oxidizing agents and UV irradiation, suggesting a genetically determined correlation between DNA repair capacity and life span. In this report, gene-specific DNA repair is compared in wild-type *C. elegans* and stress-resistant *C. elegans* mutants for the first time. DNA repair capacity is higher in long-lived *C. elegans* mutants than in wild-type animals. In addition, RNAi knockdown of the nucleotide excision repair gene *xpa-1* increased sensitivity to UV and reduced the life span of long-lived *C. elegans* mutants. These findings support that DNA repair capacity correlates with longevity in *C. elegans*.

INTRODUCTION

It has been proposed that aging-related disease and dysfunction is caused by cumulative damage in somatic cells and tissues (1,2). DNA is an important target of such age-related cellular damage because DNA encodes all structural and functional cellular components and because somatic cell mutations can have deleterious consequences at the cellular and organismal levels. As the deleterious effects of DNA damage can be prevented by DNA repair, it has been proposed that DNA repair and aging are interrelated.

Defects in some mouse and human DNA repair genes cause a phenotype similar to premature aging, suggesting that lowered capacity for DNA repair might accelerate the process of aging (3–5). For example, mouse mutants with mutations in the Xeroderma Pigmentation Group D (XPD$^{TTD, trichothiodystrophy}$), XPD^{TTD}/XPA, XPF or ERCC1, which are involved in nucleotide excision repair, show many symptoms of premature aging in mice (6–8). Human patients with the rare hereditary disease Cockayne syndrome (CS), caused by mutations in CSA or CSB, have defects in transcription coupled-nucleotide excision repair (TC-NER) and show features of accelerated aging (5,9). In addition, defects in double-strand break (DSB) repair caused by mutations in ATM, p53, Ku80, DNA-PKcs, BRCA1 or RAD50, also cause progeroid phenotypes in mice and humans (5,10). Thus, it is likely that loss of DNA repair capacity and increased DNA damage are major factors in aging-related dysfunction and disease.

Caenorhabditis elegans is an excellent model system for the study of aging, both because of its relatively short life span (11–13) and because of a large number of identified *C. elegans* genes that influence life span (14,15). These include *age-1* and *daf-2*, which are involved in the insulin/IGF-1 pathway (16–19), and whose influence on life span is mediated by *daf-16*, a FOXO family transcription factor (20,21). Long-lived *C. elegans* mutants with defects in the insulin/IGF-1 pathway are resistant to oxidative stress (12,22), high temperature (23), UV radiation (24) and heavy metal stress (25), suggesting that these mutants live longer because of increased resistance to damage induced by multiple forms of stress and possibly due to higher DNA repair capacity. Recently, it has been reported that NER process slows significantly in aging *C. elegans* (26). However, direct evidence showing that DNA repair capacity correlates with stress resistance and/or longevity in *C. elegans* is still lacking.

In this study, gene-specific repair of UV-induced pyrimidine dimers was compared in wild-type *C. elegans* and long-lived *C. elegans* mutants. The results show that DNA repair capacity is higher in long-lived mutants than in wild-type animals. Furthermore, RNAi knockdown of *C. elegans xpa-1* decreased resistance to UV and oxidative stress and reduced life span in long-lived worms.

These findings provide evidence that DNA repair capacity correlates with life span in *C. elegans*.

MATERIALS AND METHODS

Strains, growth and egg preparation

Caenorhabditis elegans strains were maintained and handled on nematode growth media (NGM) plates with *Escherichia coli* OP50 lawn at 20°C. *Caenorhabditis elegans* N2 Bristol was the wild-type hermaphroditic strain and was used as a control. The following mutants from Caenorhabditis Genetics Center (CGC, University of Minnesota, Minneapolis, MN, USA) were used in this study: TJ401 [*age-1(hx546) fer-15(b26)*], TJ1052 [*age-1 (hx546)*], CB1370 [*daf-2(e1370)*], GR1308 [*daf-2(e1370);daf-16(mg54)*], DR26 [*daf-16(m26)*] and RB864 [*xpa-1(ok698)*]. Eggs were prepared by incubating worms in lysis solution (1% NaOCl, 0.5 N NaOH).

Larval development in the presence of paraquat or after UV irradiation

Eggs (20–40) per plate were obtained by incubating two to three egg-laying (gravid) worms. Adult worms were discarded from plates and eggs used for experiments as described subsequently. Eggs were irradiated with UV light and development from eggs to adult worms was monitored on NGM plates. Alternatively, eggs were exposed to various concentrations of paraquat on NGM plates. The developmental stage of each worm was recorded by comparing the length of larva with that of N2 at multiple time points for 2–3 days. Each experiment was repeated 3–5 times.

Life span assays

Life span assays were conducted on NGM agar at 20°C as described previously (23). Briefly, synchronous worms from a large-scale egg-laying procedure were grown on NGM plates with OP50. When larvae reached the L4 stage, 12 worms were transferred onto individual OP50-spotted plates containing 40 μM 5-fluoro-2-deoxyuridine (FUdR). Plates were irradiated with UV. Animals were scored once per day for touch-provoked movement with a platinum wire; animals that failed to respond were considered dead. Each experiment was repeated 3–5 times (N). Mean life span was calculated for each life span assay.

RNA-mediated interference (RNAi) analysis

Worms were treated with RNAi on NGM agar at 20°C as described previously (27). In brief, dsRNA of gene of interest was induced by isopropyl-β-D-thiogalactopyranoside in the RNase-III-deficient *E. coli* HT115 (DE3) strain. Cells were applied to standard NGM/agar media supplemented with IPTG and antibiotics (tetracycline and ampicillin) and incubated overnight. L4 staged hermaphrodite worms were placed onto these NGM plates. Worms from the F2 generation were tested for resistance to stress.

Irradiation, isolation of *C. elegans* genomic DNA and restriction digestion

Worms were grown on NGM plates until ≥80% of animals were L4 or higher stage. The plates were irradiated at the indicated dose using a 15-watt germicidal UV lamp and incubated for the indicated time at 20°C. Worms were rinsed in M9 buffer, harvested in a clinical centrifuge, frozen immediately in liquid nitrogen and stored at –80°C. Genomic DNA was purified with a QIAamp DNA mini kit (QIAGEN GmbH, Germany).

Restriction digestion (100 μl) was performed by incubating genomic DNA (50 μg) in *EcoR* V reaction buffer at 4°C for 5 h before adding *EcoR* V (New England Biolabs, MA, USA) at a concentration of 2 U/μg. Reactions were then incubated at 37°C for 12–16 h. Products of restriction digestion were analyzed on agarose minigels. Restricted DNA was precipitated, washed and resuspended in TE buffer.

T4 endonuclease V treatment and electrophoresis

DNA (1–2 μg) was treated with (+) or without (−) T4 endonuclease V in 10 μl buffer (Trevigen, Frederick, MD, USA) at 37°C for 2 h. DNA was denatured at 50°C for 1 h in buffer containing 1 M glyoxal, 50% dimethyl sulfoxide, 10 mM sodium phosphate (pH 7.0) and 0.5 mM EDTA (28). DNA was subjected to electrophoresis on a 0.7% agarose gel [10 mM sodium phosphate buffer (pH 7.0) and 0.5 mM EDTA] for 16 h at ∼30 V.

Southern blot

After electrophoresis, gels were soaked in alkaline transfer buffer (0.4 N NaOH/1 M NaCl) at room temperature for 20 min. Hybond XL (Amersham Bioscience, GE life sciences, NJ, USA) membrane was also soaked separately in alkaline transfer buffer for 5 min. DNA was transferred to the membrane using a standard Southern blot protocol. After transfer, the membrane was washed with 2× SSPE, dried and cross-linked using Hoefer UV crosslinker (Model UVC-500, Amersham Bioscience, GE life sciences, NJ, USA). The membrane was incubated in 20 mM Tris–HCl (pH 8.0) at 65°C for 30 min and pre-hybridized in Hybrisol (Chemicon, CA, USA) containing denatured calf thymus DNA (100 μg/ml) at 45°C overnight. Fresh Hybrisol was added and incubation continued for 4 h. Radioactive probe was added to the membrane and hybridization was carried out at 45°C overnight. Membranes were washed twice in 2 × SSPE/ 0.1% SDS at room temperature for 15 min, twice in 0.1 × SSPE/0.1% SDS at room temperature for 15 min and once in 0.1× SSPE/0.1% SDS at 60°C for 30 min. Membranes were exposed to Kodak BIOMAX Light and/or MR X-ray film (Eastman Kodak, NY, USA) at −70°C for 1–3 days. DNA probes were labeled with [α-^{32}P]dCTP using the Rediprime II Random Prime Labeling kit (Amersham Bioscience, GE life sciences) and were pre-incubated with Hybond XL membrane at 45°C overnight. The probe was a 4 kb PCR product (Figure 3A) of the *VPS-45* gene (*C. elegans* CGC name,

human vacuolar protein sorting-associated protein 45, located from 974 786 to 970 617 in chromosome X).

Quantification of pyrimidine dimers

The number of CPDs (cyclobutane pyrimidine dimers) in genomic DNA was determined as described previously (29). Autoradiographic signals were quantified by scanning densitometry using Scion Image software (Scion, MD, USA) or with a Fuji Phosphoimager (Fuji Medical Systems, CT, USA). The percentage of CPD-free fragments (the zero class) was calculated from the ratio of the full-length fragment in T4 endo V-treated and -untreated samples. The average number of CPDs per fragment was calculated from the zero class measurements using a Poisson expression. Results from three times experiments were used to calculate mean values ± SD.

RESULTS

Stress resistance and life span of *C. elegans* aging mutants

Almost all long-lived *C. elegans* mutants (also called aging mutants) are resistant to environmental stress, including oxidative stress, heat shock, UV irradiation and exposure to heavy metals (25). This study examines the relationship between resistance to oxidative stress and UV, life span and development in *C. elegans* aging mutants and *daf-16*-related mutants.

Survival of wild-type *C. elegans* and aging mutants was measured in the absence or presence of UV irradiation $(50 \, \mathrm{J/m^2})$. In initial studies, worms were irradiated as adults, when the somatic tissues are quiescent and composed of non-dividing cells. We were able to observe an increased life span for *C. elegans* aging mutants under normal growth conditions (Figure 1A and Table 1) as reported by a previous study (24). The results showed that mean life span of aging mutants (*age-1 fer-15*, *age-1*, *daf-2*) was 26.5–30.5 days after UV irradiation, with an average UV-induced decrease in life span of 16.4%; in contrast, the life span of wild-type *C. elegans* was 13 days in the presence of UV, an average UV-induced decrease in life span of 37.2% (Table 1). Furthermore, the mean life span and the maximum life span of irradiated aging mutants were longer than for unirradiated wild-type worms (Figures 1A–1C; Table 1). These data indicate that *C. elegans* aging mutants are somewhat more resistant to UV and may have greater capacity to repair or tolerate UV-induced damage in somatic cells.

Previous studies report that the phenotype of longer life span and stress resistance conferred by *daf-2* and *age-1* is suppressed by *daf-16* (24,30,31). This result was confirmed here, by measuring the survival of *daf-16* and *daf-2;daf-16* mutants in the presence and absence of UV irradiation. The results showed that *daf-2;daf-16* worms (decrease in life span of 37.1%) were less resistant to UV than *daf-2* mutants with a mean life span of 13 days (decrease in life span of 25.7%) after UV treatment (Figures 1D and 1E; Table 1). However, the UV resistance of *daf-16* and wild-type worms was similar. The slight UV-resistance difference between *daf-16* and *daf-2;daf-16* mutant may be due to hypomorphs of *daf-16* alleles (mg54 in the double
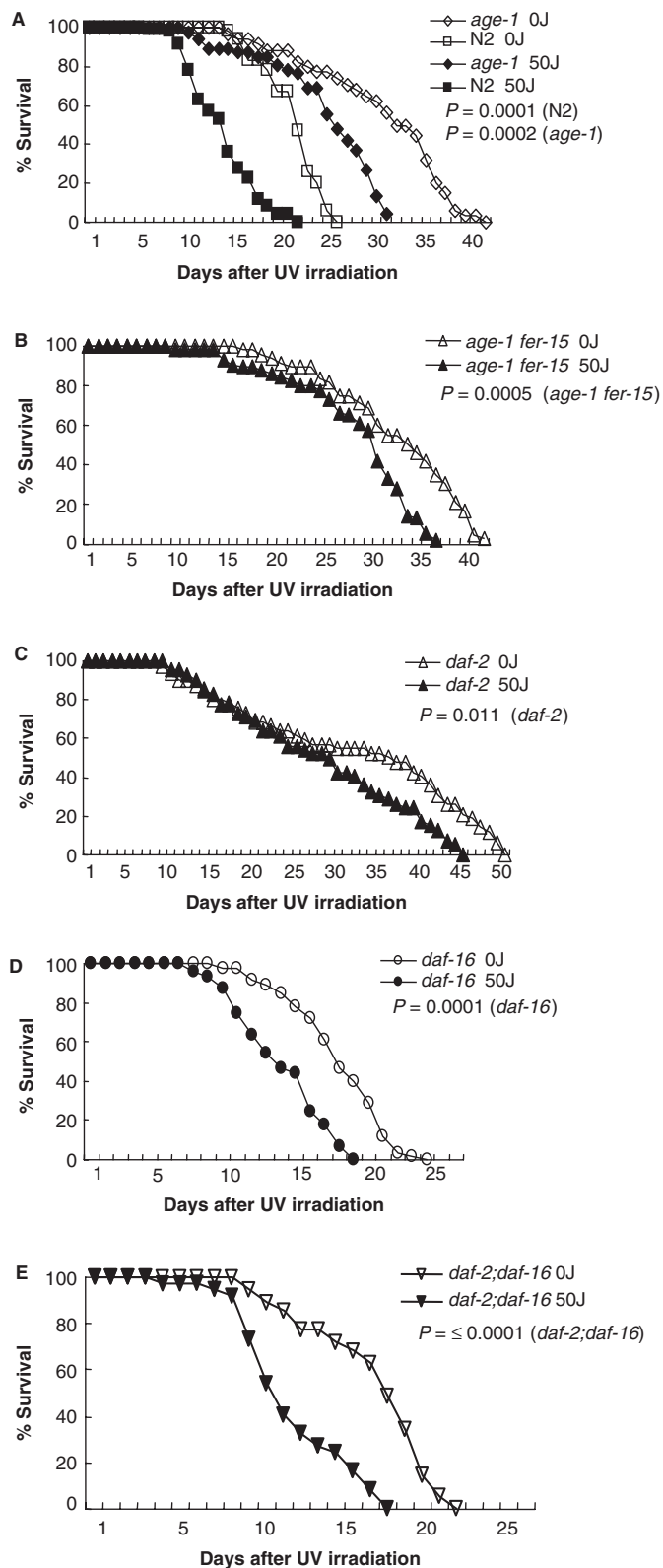

Figure 1. UV sensitivity of adult wild-type *C. elegans* and aging mutants. Survival of adult wild-type worms and *C. elegans* hermaphrodite mutants was measured after exposure to 0 or $50 \, \mathrm{J/m^2}$ UV (see Materials and Methods section). (**A**) *age-1*; (**B**) *age-1 fer-15*; (**C**) *daf-2*; (**D**) *daf-16*; (**E**) *daf-2;daf-16*. Graph represented the mean value of repeated 3–6 times experiments. The *P*-values are for the log rank test.

Table 1. Life span of *C. elegans* strains after UV irradiation

Strains	0 J/m^2			50 J/m^2		
	Mean $\pm$ SD (days)	Maximum (days)	Repeated experiment (total worms)	Mean $\pm$ SD (days)	Maximum (days)	Repeated experiment (total worms)
age-1 (hx546) fer-15(b26)	34.0 $\pm$ 1.4	43	4(48)	30.5 $\pm$ 1.5	38	4(48)
age-1 (hx546)	33.0 $\pm$ 1.6	41	3(36)	26.5 $\pm$ 1.5	31	3(36)
daf-2(e1370)	36.0 $\pm$ 2.2	50	4(48)	29.0 $\pm$ 0.9	45	4(48)
daf-2;daf-16	17.5 $\pm$ 1.1	22	3(36)	11.0 $\pm$ 0.8	18	3(36)
daf-16(m26)	17.5 $\pm$ 0.8	24	6(60)	13.0 $\pm$ 1.3	19	6(60)
Wild-type N2	20.7 $\pm$ 0.8	26	5(60)	13.0 $\pm$ 0.8	21	5(60)

mutant and m26 in the single mutant) employed in two mutants. These data support the observation that UV resistance correlates with adult life span in *C. elegans* aging mutants (24).

UV sensitivity was also tested in proliferatively dividing cells containing eggs and larvae. Eggs laid from *C. elegans* mutants were immediately irradiated and the number of larvae at each stage was counted at multiple post-irradiation time points. In the control (no UV irradiation), >90% of all eggs (i.e. *age-1 fer-15*, *age-1*, *daf-2*, *daf-16*-related and wild-type N2) developed to the L4 stage (Figure 2A and Supplementary data, Table S1). In contrast, ~50% fewer N2 and *daf-16* worms developed to L4 after irradiation at a dose of 25 J/m^2 (Figure 2A and Table S1) For *daf-2;daf-16* double mutants, 20% of irradiated eggs reached L4 and 60% underwent arrest in the L1/L2 stages. When the UV dose was increased to 50 J/m^2, growth arrest increased (Figure 2A and Table S1). However, most *age-1* and *daf-2* eggs developed to L4 after irradiation with 25 J/m^2 and 60% of the eggs reached L4 at a dose of 50 J/m^2 (Figure 2A and Table S1) and many fewer *age-1* and *daf-2* animals arrested in L1-L3 stages than did N2 and *daf-16*-related mutants. Thus, these results show that long-lived mutants have higher resistance to UV radiation in eggs and larvae during development of the worms. Subsequently, this resistance may affect the resistance of adult worms to UV.

Resistance to oxidative stress induced by paraquat was also examined in eggs/larvae of several *C. elegans* strains. Paraquat is a well-characterized genotoxic agent that generates intracellular superoxide. Increasing doses of paraquat (0.2–1.0 mM) caused many N2 eggs to arrest at L1/L2 or L3 stages (Figure 2B and Table S2) and no L4 larvae were detected when exposed to higher doses of paraquat (Figure 2B and Table S2). In contrast, 70% of *age-1* and *daf-2* eggs exposed to 1 mM paraquat developed to L4 (Figure 2B and Table S2) with only a small number of eggs undergoing arrest at L3 at high doses of paraquat (Figure 2B and Table S2). The majority of *daf-16* and *daf-2;daf-16* eggs arrested in L1/L2 stages after exposure to 1 mM paraquat (Figure 2B and Table S2).

While the long-lived mutant strains are slightly more resistant to UV irradiation, their resistance to paraquat is much greater. This undoubtedly reflects the different spectra of damage induced by these two agents.

These results indicate that increased resistance to genotoxic stress during larval stages is likely due to increased genomic maintenance capacity against genotoxic damage and correlates with longer life span in *C. elegans*.

Detection of repair of UV-induced DNA pyrimidine dimers

Figure 1 indicates that life span correlates with resistance to genotoxic stress in several *C. elegans* strains. One possible explanation for these data is that *C. elegans* strains that are more resistant to genotoxic stress have higher DNA repair capacity. This possibility was tested by conducting a gene-specific DNA repair assay that detects repair of UV-induced DNA damage. This assay measures the number of pyrimidine dimers in a target gene (*VPS-45*) using T4 endonuclease V (T4 endo V), making single-strand cleavages with high specificity for DNA pyrimidine dimers (29). In mammalian cells, gene-specific DNA repair occurs preferentially in expressed genes and is more closely correlated with UV survival than overall DNA repair (32). The *VPS-45* gene is a homolog of human vacuolar protein sorting-associated protein 45 and is located from 974 786 to 970 617 on the X chromosome (Figure 3A). *VPS-45* expression has been detected in worms by microarray, SAGE and by cDNA cloning (Wormbase database, www.wormbase.org) and a recent report showed that the *VPS-45* is an essential gene (33).

The gene-specific pyrimidine dimer repair assay was carried out using *EcoR* V restricted *C. elegans* genomic DNA. Control experiments (alkali gel electrophoresis followed by a standard Southern blot protocol) in the absence of T4 endo V showed that restricted genomic DNA was smeared, instead of a distinct band (data not shown). When the restricted genomic DNA was treated with glyoxal/DMSO and subjected to electrophoresis in phosphate buffer (pH 7) (28), the glyoxal/DMSO-treated genomic DNA had a faster electrophoretic mobility than untreated dsDNA (Figure 3B), indicating that the DNA was denatured. Subsequently, pyrimidine dimers were induced in a linear plasmid containing a part of *VPS-45* gene and in purified *C. elegans* genomic DNA using UV doses from 25 to 500 J/m^2. The linear plasmid DNA and EcoR V restricted genomic DNA were treated with T4 endo V, incubated with glyoxal/DMSO, subjected to electrophoresis and analyzed by Southern blot. The results were quantified by a scanning densitometer or
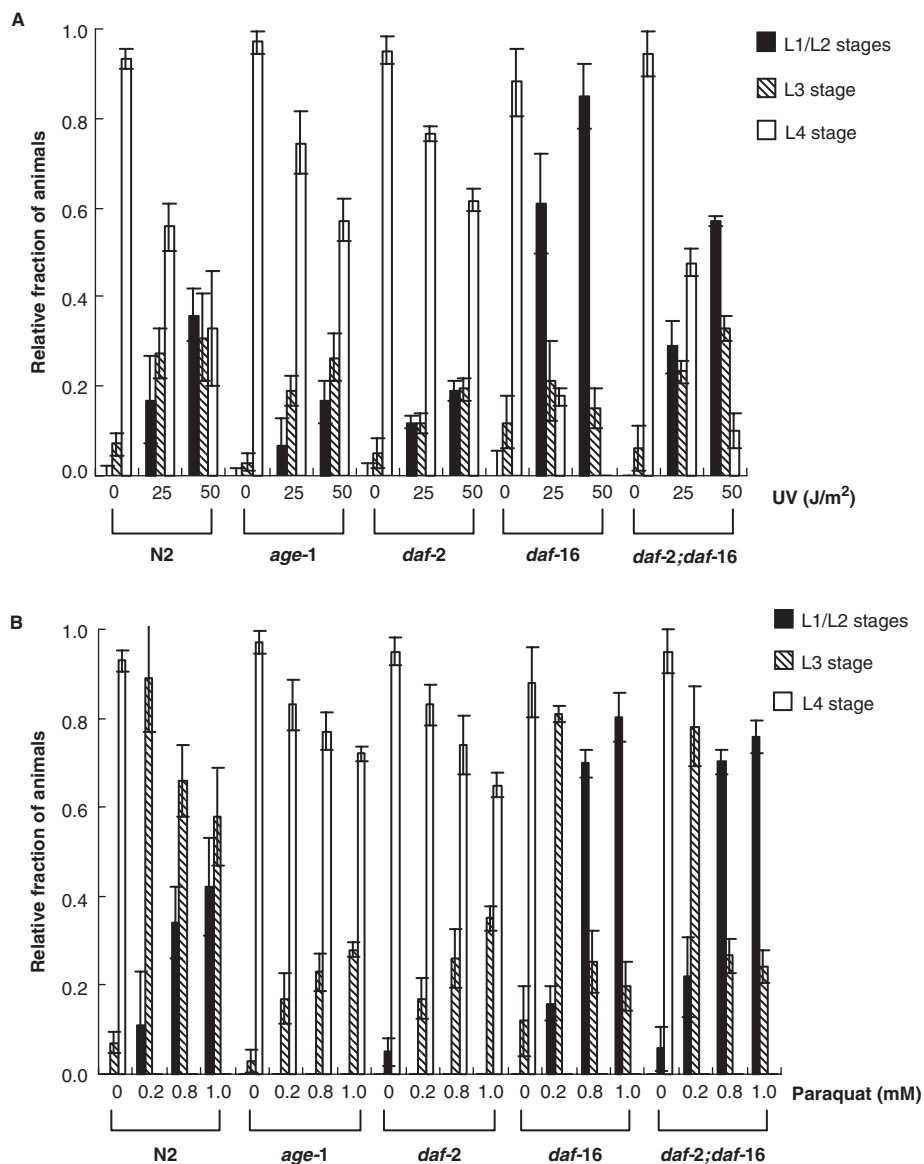
Figure 2. Stress resistance of wild-type *C. elegans* and aging mutants during larval development. Twenty to forty eggs per plate from two to three egg-laying worms were collected and exposed to UV or grown on plates containing paraquat. The developmental stage of each worm on each plate was recorded throughout development. (**A**) Larval development of UV-irradiated eggs. (**B**) Larval development on paraquat-containing plates. Each experiment was repeated 3–4 times.

a Fuji phosphorimager. As expected, the intensities of T4 endo V-treated samples in the linear plasmid (data not shown) and restricted genomic DNA (Figure 3C) were greatly decreased and a dose–response was observed between 0 and $50 \, J/m^2$ as percent dimer formation is already close to 100% at $>50 \, J/m^2$ (Supplementary data, Figure S1). The results indicate that this assay is suitable for detection of UV-induced pyrimidine dimers.

Repair of pyrimidine dimers in wild-type and *C. elegans* aging mutants

DNA repair of *C. elegans* was estimated by quantifying pyrimidine dimers of isolated DNA from UV-irradiated *C. elegans* strains at different time points after

UV-irradiation ($50 \, J/m^2$), providing an estimate of the rate and extent of pyrimidine dimer repair. Percent repair was calculated from the intensity of the full-length restriction fragment at different time points after irradiation as described in Materials and Methods (Figures 4A and 4B). In wild-type N2 worms, percent DNA repair increased from 0 to 8 h after irradiation and then reached a plateau (67% repair) at 12 h. In contrast, the rate and extent of repair was greater for *age-1*, *daf-2* and *age-1 far-15* worms than for wild-type N2 worms (Figure 4B). Percent repair reached 85% at 4 h and ~90% at 12 h after UV irradiation (Figure 4B). These data suggest that *C. elegans* aging mutants have a higher capacity for repair of UV-induced DNA damage than wild-type worms, resulting in less DNA damage for the rest of their lifetimes. As shown
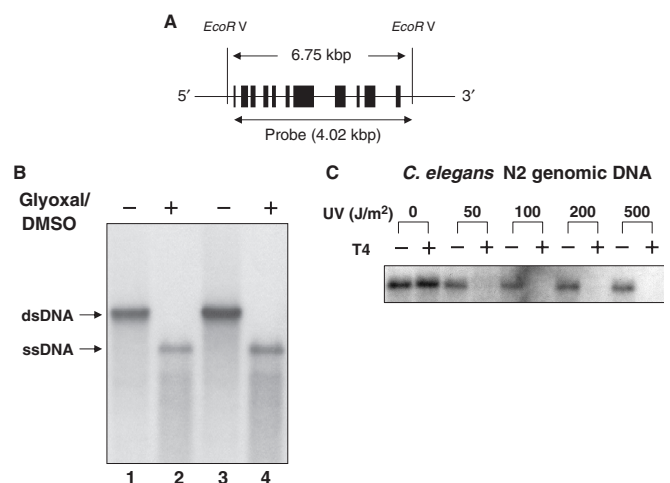
Figure 3. Detection of UV-induced pyrimidine dimers in *VPS-45*. *C. elegans* genomic DNA was digested with *EcoR* V, electrophoresed, transferred to membrane by Southern blot, and hybridized to radio-labeled DNA probe. (**A**) Genomic map of the *VPS-45* gene. "*EcoR* V" indicates location of *EcoR* V restriction sites outside of *VPS-45*. The black boxes indicate exons. The position of the DNA probe used in this study is shown below the gene. (**B**) Southern blot in glyoxal-DMSO buffer. Lanes 1 and 2, 1 μg genomic DNA; lanes 3 and 4, 3 μg genomic DNA. DNA was irradiated with UV light, treated with (+) or without (−) T4 endonuclease, electrophoresed in glyoxal/DMSO, and analyzed by Southern blot. Results are shown for *EcoR* V digested *C. elegans* genomic DNA (**C**).

before for UV resistance and life span, the higher stress-resistance of *C. elegans* aging mutants is *daf-16* dependent (24). The extent of repair of UV-induced DNA damage was similar in *daf-2;daf-16*, wild-type and *daf-16* animals. This result suggests that DAF-16 may regulate DNA repair in aging mutants of *C. elegans*.

Effect of *xpa-1* knockdown (RNAi) on life span of *C. elegans* aging mutants

The above data demonstrate that higher resistance to UV correlates with higher DNA repair capacity and lower levels of UV-induced DNA damage in *C. elegans* aging mutants. Thus, we examined the effect of a mutation in the NER pathway on UV resistance and life span in these *C. elegans* strains. UV-induced pyrimidine dimers are repaired by NER, a DNA repair pathway that is conserved from *E. coli* to humans (34). Although NER is not well characterized in *C. elegans*, the *C. elegans* orthologs of two NER genes, *xpa-1* and *xpf-1*, have been reported and knockout mutants or knockdown worms of these genes are sensitive to UV (27,35,36).

RB864 *xpa-1* mutants (*C. elegans* Gene Knockout Project strain carrying a <1300 bp deletion including *xpa-1* gene region) and *xpa-1(RNAi)* worms were greatly sensitive to UV irradiation (Figure 5A and Table 2). In addition, *xpa-1* had lower capacity to repair UV-induced DNA damage than N2 worms (Figures 5C and 5D), indicating that UV sensitivity is related to the extent of DNA repair in these mutants. When *xpa-1(RNAi);age-1* knockdown worms were generated by feeding *xpa-1* targeted RNAi to *age-1* worms, UV resistance and life
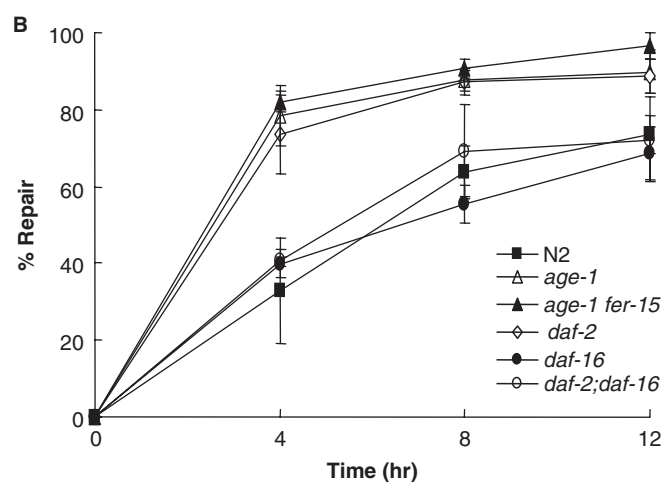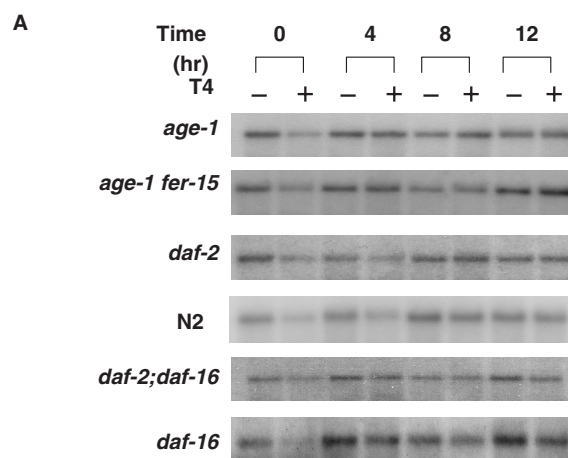

Figure 4. Gene-specific repair in wild-type worms and *C. elegans* mutants. (**A**) Worms were irradiated with $50 \, \text{J/m}^2$ UV and DNA was prepared at various time points (0, 4, 8 and 12 h). Samples were incubated with or without T4 endo V, treated with glyoxal/DMSO, electrophoresed on phosphate agarose gels, transferred to membrane, and hybridized with a probe for *VPS-45*. (**B**) Results shown in (A) were quantified using Scion Image software (NIH Image). The average number of CPDs per fragment was calculated from the zero class measurements using a Poisson expression. Data points are mean ± SD from 3–4 independent experiments.

span decreased significantly. Surprisingly, the mean life span of *xpa-1(RNAi);age-1* knockdown worms was similar to that of wild-type N2 worms (Figure 5B and Table 2). These results suggest that levels of DNA damage and/or DNA repair correlate with life span in *C. elegans*.

Interestingly, the mean life span of unirradiated *xpa-1* (*RNAi);age-1* or *xpa-1(RNAi)* worms is shorter than that of unirradiated *age-1* mutants or L4440(RNAi)N2 worms, respectively (Figures 5A and 5B, Table 2), suggesting that *xpa-1* may influence longevity and/or repair of endogenous DNA damage. This idea was tested by measuring sensitivity of *xpa-1(RNAi);age-1* and *xpa-1(RNAi)*N2 worms to paraquat-induced oxidative damage. The results showed that *xpa-1(RNAi);age-1* worms are more sensitive to paraquat than *age-1* (Figure 6 and Table S3). Although *xpa-1(RNAi)*N2 and N2 worms did not develop to L4
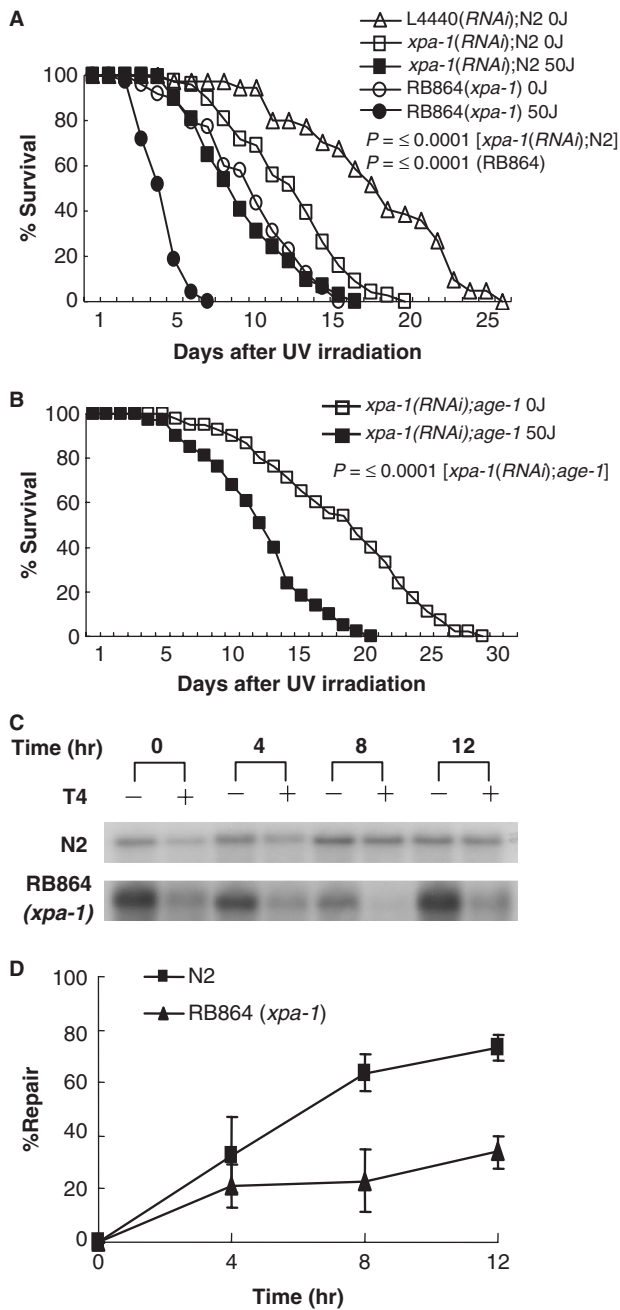
Figure 5. UV sensitivity of *xpa-1* knockdown worms. (**A**) Survival of *xpa-1(RNAi)* worms and RB864(*xpa-1*) worms was measured in the presence of FUdR at 20°C after UV irradiation at 0 or 50 J/m². Each experiment was repeated six times. L4440 is a plasmid vector for RNAi control. (**B**) Survival of *xpa-1(RNAi);age-1* worms was measured after UV irradiation at 0 or 50 J/m². Each experiment was repeated six times. (**C**) Gene-specific repair of UV-induced DNA pyrimidine dimers in RB864(*xpa-1*), performed as described in Figure 4A. (**D**) Quantification of results shown in (**C**). The *P*-values are for the log rank test.

at this concentration of paraquat, a majority of N2 worms underwent developmental arrest at L3 stage. Instead, 50% of *xpa-1(RNAi)*N2 worms were arrested at L1/L2 stages (Table S3). At low concentration of 50 μM paraquat, 60% of N2 worms developed to L4, whereas most of *xpa-1 (RNAi)*N2 worms underwent arrest at L3 (Table S3).

Table 2. Life span of *xpa-1(RNAi)* knockdown strains

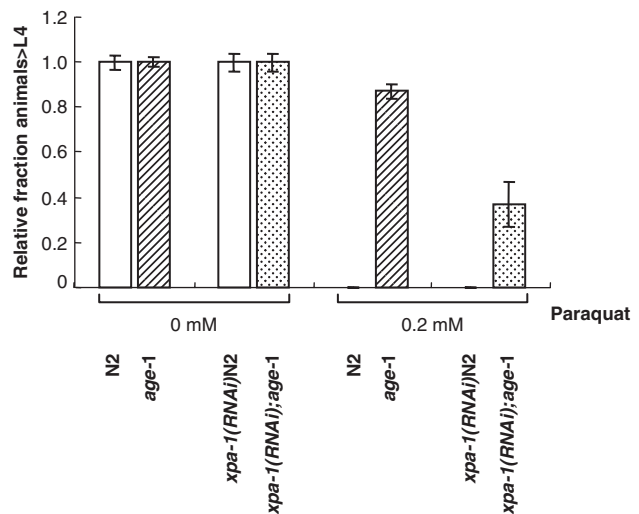|	xpa-1 (RNAi)N2	RB864 (*xpa-1*)	*xpa-1* (RNAi); *age-1*	L4440 (RNAi)N2
0 J/m² Mean life span ± SD (days)	12 ± 0.9	11.5 ± 0.8	18.5 ± 1.8	17.5 ± 3.1
Maximum life span (days)	19	15	28	25
Repeated experiment (total worms)	6(70)	6(70)	6(70)	3(60)
50 J/m² Mean life span ± SD (days)	8 ± 0.8	6.5 ± 0.2	12 ± 0.8	
Maximum life span (days)	15	7	20	
Repeated experiment (total worms)	6(70)	6(70)	6(70)	

Figure 6. Effect of *xpa-1* knockdown on sensitivity to oxidative stress. Eggs were collected from two to three egg-laying worms and grown in the presence or absence of 0.2 mM paraquat. Development of each worm was recorded throughout larval development. Each experiment was repeated four times.

Since there are forms of oxidative DNA damage known to be repaired by NER, the oxidative lesions most relevant to aging are those that can be repaired by NER.

DISCUSSION

This study investigates the relationship between UV resistance, DNA repair capacity and longevity in long-lived mutants of *C. elegans*. The results suggest that an increase in DNA repair capacity correlates with increased stress resistance in these animals and that this may also lead to longer life span.

Mouse and human NER mutants with symptoms of premature aging also show correlation between longevity, aging-related dysfunction and capacity for DNA repair (5,8,37). To further test this relationship, DNA repair capacity needs to be investigated in long-lived animal mutants. Thus, *C. elegans* aging mutants with increased

UV resistance could be an excellent model to explore the relationship between DNA repair capacity and longevity. A wide range of helix distorting DNA lesions, including UV-induced photoproducts and certain oxidative lesions are repaired by NER (38). NER repair capacity has been quantified by gene-specific repair of UV-induced DNA damage (29). This repair assay is a sensitive assay for evaluating the correlation between UV survival and DNA repair and repair in active, essential genes is likely to be more relevant to survival than global genome DNA repair (32). Results present here show that mean life span is longer and DNA repair capacity is higher in UV-irradiated *C. elegans* aging mutants than in UV-irradiated wild-type worms, suggesting that *C. elegans* aging mutants may have higher levels of enzymatic activities involved in repair of UV-induced DNA lesions. Meyer and associates (26) and Hartman *et al.* (39) have also demonstrated that *xpa-1* (formerly known as *rad-3*) are defective for the repair of UV radiation-induced DNA damage. In addition, the kinetics of repair reasonably approximates those observed by the Meyer and Hartman laboratories. However, future studies will be needed to address how DNA repair capacity varies throughout the *C. elegans* genome and its relevance for longevity in other long-lived *C. elegans* mutants such as *clk-1*, *eat-4*, *mev-1* and *gas-1*. It is worth noting that gene-specific repair is lower in hepatocytes from old rats than in hepatocytes from young rats (40). Interestingly, caloric restriction does not alter gene-specific DNA repair in young rats.

Previous studies revealed that *daf-16* decreases UV resistance and shortens life span of long-lived *C. elegans* mutants (20,21). This study confirmed that *daf-2;daf-16* and *daf-16* mutants are less resistant to UV than *daf-2* mutants and that DNA repair capacity of them is lower than that of *daf-2*. However, the precise role of *daf-16* in the response to UV and UV-induced DNA damage is unknown. DAF-16 is a forkhead transcription factor involved in insulin-like signaling, stress resistance and longevity in *C. elegans* (31); thus, it is possible that gene products regulated by *daf-16* directly (or indirectly) regulates DNA repair activity. Additional factors that enhance stress resistance in these strains, heat shock proteins, may be involved in increasing DNA repair activity. In supporting this, there is a growing body of evidence linking HSPs to DNA repair activity. For example, inducible heat shock proteins are required for maintenance for of genomic instability in stress conditions (41). In yeast, HSP90 interacts with Ssl2, the yeast homolog of XPB (42).

Two NER orthologs, *xpa-1* and *xpf-1*, have been reported in *C. elegans* and *RNAi* knockdown of these genes cause sensitivity to UV (27,36). This study showed that *xpa-1(RNAi)* worms and *xpa-1* knockout (RB864) worms have a reduced life span and that *xpa-1* worms have much lower capacity to repair UV-induced DNA damage than wild-type worms (Figure 5A, C and D). In addition, *xpa-1(RNAi);age-1* worms are more sensitive to UV than *age-1* worms and have a mean life span similar to wild-type worms (Figure 5B). This strongly supports the hypothesis that DNA damage and DNA repair play a major role in determining life span in *C. elegans*.

Recent studies also showed that knockdown of the *C. elegans* RecQ orthologs causes sensitivity to DNA damage and a shorter life span (43,44).

The mean life span of unirradiated *xpa-1(RNAi);age-1* worms was significantly lower than for wild-type worms, suggesting higher susceptibility to endogenous DNA damage in the mutant animals. The nature of these endogenous lesions is unknown, but might include lesions caused by oxidative stress, which is implicated as a critical contributing factor in the process of aging. The observation that *xpa-1(RNAi);age-1* worms are more sensitive to paraquat during larval development than *age-1* worms (Figure 6) supports this possibility. Previous studies also show that human *xpa* mutants are sensitive to oxidative damage (45) and that cells from *xpd/xpa* double mutant mice have a reduced life span and are hypersensitive to paraquat (8). However, as paraquat can induce different types of oxidative stress including protein carbonylation, it is hard to rule out other effect such as protein homeostasis on life span.

The importance of endogenous DNA lesion (oxidative damage) for life span has been also reported in *C. elegans*. The finding that superoxide anion levels are higher and SOD activity is lower in short-lived *C. elegans* strains (46,47) also implicates lower oxidative stress and higher antioxidant defense in longevity and aging (48,49). For example, catalase and Cu/Zn superoxide dismutase activity was reported to be higher in *age-1* worms than in wild-type worms (22), and expression of stress–response factors such as *hsp*, *mtl-1*, *gst-1* and *sip-1* were reported to be higher in *daf-2* worms (50–52). Recently, similar observations have been made for *C. elegans mev-1* mutants, in which ROS and nuclear DNA damage are increased (53).

In conclusion, the data presented here demonstrate for the first time that longevity correlates with DNA repair capacity in *C. elegans*. The results strongly support the hypothesis that DNA damage plays a direct role in the process of aging. In addition, this study demonstrates that *C. elegans* has an NER-like DNA repair pathway that repairs UV-induced pyrimidine dimers. However, additional studies are needed to determine the exact relationship between specific factors that influence life span, stress resistance and DNA repair capacity in *C. elegans*.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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REFERENCES

1. Kirkwood,T.B. and Holliday,R. (1975) Commitment to senescence: a model for the finite and infinite growth of diploid and transformed human fibroblasts in culture. *J. Theor. Biol.*, **53**, 481–496.
2. Kirkwood,T.B. (2005) Understanding the odd science of aging. *Cell*, **120**, 437–447.
3. Martin,G.M. (2005) Genetic modulation of senescent phenotypes in Homo sapiens. *Cell*, **120**, 523–532.
4. Karanjawala,Z.E. and Lieber,M.R. (2004) DNA damage and aging. *Mech. Ageing Dev.*, **125**, 405–416.
5. Lombard,D.B., Chua,K.F., Mostoslavsky,R., Franco,S., Gostissa,M. and Alt,F.W. (2005) DNA repair, genome stability, and aging. *Cell*, **120**, 497–512.
6. Dolle,M.E., Snyder,W.K., Gossen,J.A., Lohman,P.H. and Vijg,J. (2000) Distinct spectra of somatic mutations accumulated with age in mouse heart and small intestine. *Proc. Natl Acad. Sci. USA*, **97**, 8403–8408.
7. Niedernhofer,L.J., Garinis,G.A., Raams,A., Lalai,A.S., Robinson,A.R., Appeldoorn,E., Odijk,H., Oostendorp,R., Ahmad,A., van,L.W. *et al.* (2006) A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. *Nature*, **444**, 1038–1043.
8. de Boer,J., Andressoo,J.O., de,W.J., Huijmans,J., Beems,R.B., van,S.H., Weeda,G., van der Horst,G.T., van,L.W., Themmen,A.P. *et al.* (2002) Premature aging in mice deficient in DNA repair and transcription. *Science*, **296**, 1276–1279.
9. Nance,M.A. and Berry,S.A. (1992) Cockayne syndrome: review of 140 cases. *Am. J. Med. Genet.*, **42**, 68–84.
10. Lans,H. and Hoeijmakers,J.H. (2006) Cell biology: ageing nucleus gets out of shape. *Nature*, **440**, 32–34.
11. Johnson,T.E. and Nelson,G.A. (1991) Caenorhabditis elegans: a model system for space biology studies. *Exp. Gerontol.*, **26**, 299–309.
12. Larsen,P.L. (1993) Aging and resistance to oxidative damage in Caenorhabditis elegans. *Proc. Natl Acad. Sci. USA*, **90**, 8905–8909.
13. Lakowski,B. and Hekimi,S. (1996) Determination of life-span in Caenorhabditis elegans by four clock genes. *Science*, **272**, 1010–1013.
14. Hamilton,B., Dong,Y., Shindo,M., Liu,W., Odell,I., Ruvkun,G. and Lee,S.S. (2005) A systematic RNAi screen for longevity genes in C. elegans. *Genes Dev.*, **19**, 1544–1555.
15. Johnson,T.E. (2003) Advantages and disadvantages of Caenorhabditis elegans for aging research. *Exp. Gerontol.*, **38**, 1329–1332.
16. Friedman,D.B. and Johnson,T.E. (1988) A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. *Genetics*, **118**, 75–86.
17. Morris,J.Z., Tissenbaum,H.A. and Ruvkun,G. (1996) A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in Caenorhabditis elegans. *Nature*, **382**, 536–539.
18. Kenyon,C., Chang,J., Gensch,E., Rudner,A. and Tabtiang,R. (1993) A C. elegans mutant that lives twice as long as wild type. *Nature*, **366**, 461–464.
19. Kimura,K.D., Tissenbaum,H.A., Liu,Y. and Ruvkun,G. (1997) daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. *Science*, **277**, 942–946.
20. Lin,K., Dorman,J.B., Rodan,A. and Kenyon,C. (1997) daf-16: an HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans. *Science*, **278**, 1319–1322.
21. Ogg,S., Paradis,S., Gottlieb,S., Patterson,G.I., Lee,L., Tissenbaum,H.A. and Ruvkun,G. (1997) The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. *Nature*, **389**, 994–999.
22. Vanfleteren,J.R. (1993) Oxidative stress and ageing in Caenorhabditis elegans. *Biochem. J.*, **292**(Pt 2), 605–608.
23. Lithgow,G.J., White,T.M., Melov,S. and Johnson,T.E. (1995) Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. *Proc. Natl Acad. Sci. USA*, **92**, 7540–7544.
24. Murakami,S. and Johnson,T.E. (1996) A genetic pathway conferring life extension and resistance to UV stress in Caenorhabditis elegans. *Genetics*, **143**, 1207–1218.
25. Lithgow,G.J. (2000) Stress response and aging in Caenorhabditis elegans. *Results Probl. Cell Differ.*, **29**, 131–148.
26. Meyer,J.N., Boyd,W.A., Azzam,G.A., Haugen,A.C., Freedman,J.H. and Van,H.B. (2007) Decline of nucleotide excision repair capacity in aging Caenorhabditis elegans. *Genome Biol.*, **8**, R70.
27. Park,H.K., Suh,D., Hyun,M., Koo,H.S. and Ahn,B. (2004) A DNA repair gene of Caenorhabditis elegans: a homolog of human XPF. *DNA Repair*, **3**, 1375–1383.
28. Tian,M. and Alt,F.W. (2000) Transcription-induced cleavage of immunoglobulin switch regions by nucleotide excision repair nucleases in vitro. *J. Biol. Chem.*, **275**, 24163–24172.
29. Bohr,V.A., Smith,C.A., Okumoto,D.S. and Hanawalt,P.C. (1985) DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. *Cell*, **40**, 359–369.
30. Henderson,S.T. and Johnson,T.E. (2001) daf-16 integrates developmental and environmental inputs to mediate aging in the nematode Caenorhabditis elegans. *Curr. Biol.*, **11**, 1975–1980.
31. Hsu,A.L., Murphy,C.T. and Kenyon,C. (2003) Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science*, **300**, 1142–1145.
32. Bohr,V.A., Phillips,D.H. and Hanawalt,P.C. (1987) Heterogeneous DNA damage and repair in the mammalian genome. *Cancer Res.*, **47**, 6426–6436.
33. Gengyo-Ando,K., Kuroyanagi,H., Kobayashi,T., Murate,M., Fujimoto,K., Okabe,S. and Mitani,S. (2007) The SM protein VPS-45 is required for RAB-5-dependent endocytic transport in Caenorhabditis elegans. *EMBO Rep.*, **8**, 152–157.
34. Gillet,L.C. and Scharer,O.D. (2006) Molecular mechanisms of mammalian global genome nucleotide excision repair. *Chem. Rev.*, **106**, 253–276.
35. Boulton,S.J., Gartner,A., Reboul,J., Vaglio,P., Dyson,N., Hill,D.E. and Vidal,M. (2002) Combined functional genomic maps of the C. elegans DNA damage response. *Science*, **295**, 127–131.
36. Park,H.K., Yook,J.S., Koo,H.S., Choi,I.S. and Ahn,B. (2002) The Caenorhabditis elegans XPA homolog of human XPA. *Mol. Cells*, **14**, 50–55.
37. Hasty,P. and Vijg,J. (2002) Aging. Genomic priorities in aging. *Science*, **296**, 1250–1251.
38. de Laat,W.L., Jaspers,N.G. and Hoeijmakers,J.H. (1999) Molecular mechanism of nucleotide excision repair. *Genes Dev.*, **13**, 768–785.
39. Hartman,P., Hevelone,J., Dwarakanath,V. and Mitchell,D.L. (1989) Excision repair of UV radiation-induced DNA damage in Caenorhabditis elegans. *Genetics*, **122**, 379–385.
40. Guo,Z., Heydari,A.R., Wu,W., Yang,H., Sabia,M.R. and Richardson,A. (1998) Characterization of gene-specific DNA repair by primary cultures of rat hepatocytes. *J. Cell Physiol.*, **176**, 314–322.
41. Hunt,C.R., Dix,D.J., Sharma,G.G., Pandita,R.K., Gupta,A., Funk,M. and Pandita,T.K. (2004) Genomic instability and enhanced radiosensitivity in Hsp70.1- and Hsp70.3-deficient mice. *Mol. Cell. Biol.*, **24**, 899–911.
42. Flom,G., Weekes,J. and Johnson,J.L. (2005) Novel interaction of the Hsp90 chaperone machine with Ssl2, an essential DNA helicase in Saccharomyces cerevisiae. *Curr. Genet.*, **47**, 368–380.
43. Lee,S.J., Yook,J.S., Han,S.M. and Koo,H.S. (2004) A Werner syndrome protein homolog affects C. elegans development, growth rate, life span and sensitivity to DNA damage by acting at a DNA damage checkpoint. *Development*, **131**, 2565–2575.
44. Grabowski,M.M., Svrzikapa,N. and Tissenbaum,H.A. (2005) Bloom syndrome ortholog HIM-6 maintains genomic stability in C. elegans. *Mech. Ageing Dev.*, **126**, 1314–1321.
45. Hayashi,M., Araki,S., Kohyama,J., Shioda,K. and Fukatsu,R. (2005) Oxidative nucleotide damage and superoxide dismutase

expression in the brains of xeroderma pigmentosum group A and Cockayne syndrome. *Brain Dev.*, **27**, 34–38.

46. Ishii,N., Takahashi,K., Tomita,S., Keino,T., Honda,S., Yoshino,K. and Suzuki,K. (1990) A methyl viologen-sensitive mutant of the nematode Caenorhabditis elegans. *Mutat. Res.*, **237**, 165–171.

47. Senoo-Matsuda,N., Yasuda,K., Tsuda,M., Ohkubo,T., Yoshimura,S., Nakazawa,H., Hartman,P.S. and Ishii,N. (2001) A defect in the cytochrome b large subunit in complex II causes both superoxide anion overproduction and abnormal energy metabolism in Caenorhabditis elegans. *J. Biol. Chem.*, **276**, 41553–41558.

48. Honda,Y. and Honda,S. (2002) Oxidative stress and life span determination in the nematode Caenorhabditis elegans. *Ann. N. Y. Acad. Sci.*, **959**, 466–474.

49. Wallace,D.C. and Melov,S. (1998) Radicals r'aging. *Nat. Genet.*, **19**, 105–106.

50. Halaschek-Wiener,J., Khattra,J.S., McKay,S., Pouzyrev,A., Stott,J.M., Yang,G.S., Holt,R.A., Jones,S.J., Marra,M.A., Brooks-Wilson,A.R. *et al.* (2005) Analysis of long-lived C. elegans daf-2 mutants using serial analysis of gene expression. *Genome Res.*, **15**, 603–615.

51. Murphy,C.T., McCarroll,S.A., Bargmann,C.I., Fraser,A., Kamath,R.S., Ahringer,J., Li,H. and Kenyon,C. (2003) Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. *Nature*, **424**, 277–283.

52. McElwee,J., Bubb,K. and Thomas,J.H. (2003) Transcriptional outputs of the Caenorhabditis elegans forkhead protein DAF-16. *Aging Cell*, **2**, 111–121.

53. Hartman,P., Ponder,R., Lo,H.H. and Ishii,N. (2004) Mitochondrial oxidative stress can lead to nuclear hypermutability. *Mech. Ageing Dev.*, **125**, 417–420.