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Article

The role of *xpa-1* and *him-1* in UV protection of *Caenorhabditis elegans*

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SUMMARY

***Caenorhabditis elegans xpa-1* and *him-1* are orthologs of human *XPA* (xeroderma pigmentosum group A) and human *SMC1A* (Structural maintenance of chromosomes 1A), respectively. Mutations in the *XPA* are correlated with Xeroderma pigmentosum, a condition that induces hypersensitivity to ultraviolet (UV) radiation. Alternatively, *SMC1A* mutations may lead to Cornelia de Lange Syndrome, a multi-organ disorder that makes patients more sensitive to UV-induced DNA damage. Both *C. elegans* genes have been found to be involved in protection against UV radiation, but their combined effects have not been tested when they are both knocked down. We hypothesized that because these genes are involved in separate pathways, the simultaneous knockdown of both of these genes using RNA interference (RNAi) in *C. elegans* will cause them to become more sensitive to UV radiation than either of them knocked down individually. UV protection was measured via the percent survival of *C. elegans* post 365 nm and $5.4x10^{-19}$ joules of UV radiation. The double xpa-1/him-1 RNAi knockdown showed a significantly reduced percent survival after 15 and 30 minutes of UV radiation relative to wild-type and *xpa-1* and *him-1* single knockdowns. These measurements were consistent with our hypothesis and demonstrated that *xpa-1* and *him-1* genes play distinct roles in resistance against UV stress in *C. elegans*. This result raises the possibility that the *xpa-1/him-1* double knockdown could be useful as an animal model for studying the human disease Xeroderma pigmentosum and Cornelia de Lange Syndrome.**

INTRODUCTION

Caenorhabditis elegans is a free-living nematode commonly used as an animal model because of its small size, short life cycle, and easy maintenance in a laboratory setting (1). Many *C. elegans* genes have orthologs in humans, making *C. elegans* an excellent model for studying human diseases (2). Similar to humans, *C. elegans* have genes that resist ultraviolet (UV) radiation rays. These rays are agents that cause different cytotoxic and mutagenic DNA lesions, and there are multiple types of UV rays. Ultraviolet A (UVA) rays are the weakest and most common group of ultraviolet radiation rays and vary in wavelength between 315 nm and 400 nm. Ultraviolet B (UVB) rays range from 280 nm to 315 nm and produce harmful effects on habitats. Ultraviolet C (UVC) rays are the strongest group of UV rays and have wavelengths less than 280 nm (3). All UV radiation damages DNA in *C. elegans* and reduces their lifespan (4). Specifically, UV radiation causes the formation of intrastrand thymine dimers and distorts the DNA paired structure, leading to errors in DNA replication and transcription (5).

Both *xpa-1* and *him-1* play a role in UV protection in *C. elegans*, although their combined effects have not been studied (6). *Xpa-1* is an ortholog of human *XPA* (xeroderma pigmentosum group A) that is involved in repairing damaged DNA, regulating lifespan, and responding to UV radiation in *C. elegans*. The gene product binds to damaged DNA and is involved in the nucleotide excision repair (NER) pathway (7). The human ortholog (*XPA*) is a zinc finger protein that acts as a scaffold to assemble the NER complex (8). Mutations in *XPA* can cause a genetic disorder called Xeroderma pigmentosum, which decreases a patient's ability to repair UV-damaged DNA and predisposes them to skin cancer (9). Patients with Xeroderma pigmentosum must avoid direct sunlight by wearing sunscreen or adopting other sun-avoidance methods, such as wearing protective clothing (10). *Him-1* is an ortholog of human *SMC1A* and is involved in chromosome segregation in *C. elegans*, responding to UV radiation, and binding DNA (11). *SMC1A* is involved in chromosome cohesion, and mutations in *SMC1A* are associated with Cornelia de Lange Syndrome (12, 13). Patients with Cornelia de Lange Syndrome experience stagnated body development and moderate to severe intellectual disability similar to autism spectrum disorder (14).

RNA interference (RNAi) technology is employed to knock down the expression of specific genes and determine their phenotypes. This potent, rapid, and simple gene regulatory mechanism uses an enzyme called Dicer to cut double-stranded RNA (dsRNA) into microRNA (miRNA), which later binds to Argonaute proteins to specifically silence genes by limiting transcription or degrading mRNA (15). Combinatorial RNAi can be used to knock down several genes at once to characterize their loss-of-function phenotypes (16). RNAi can be induced in *C. elegans* by delivering dsRNA through microinjection, feeding, and soaking in dsRNA solution (17). We chose the feeding method since it is a relatively simple protocol where the dsRNA is transcribed in *Escherichia coli* and ingested by *C. elegans* (17). We hypothesized that the double *xpa-1/him-1* knockdown organisms will be more sensitive to UV radiation than either of the single knockdowns.

RESULTS

In this study, we sought to determine the effects of single and double *xpa-1* and *him-1* RNAi knockdowns on the response to UV stress in *C. elegans*. UV radiation is

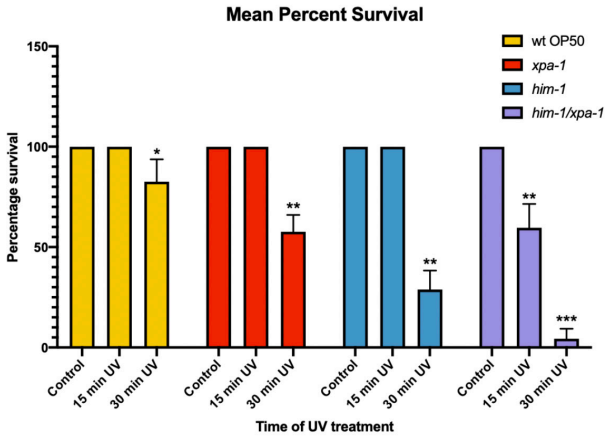

Figure 1: UV survival assay with *C. elegans*, cultured on *E. coli* OP50, *xpa-1*, *him-1*, and *xpa-1/him-1* RNAi *E. coli*. Control worms were not exposed to UV. The percent survival was calculated 2 days post-exposure to UV as the ratio of live to total worms. Data is shown as mean ± SD for each the of three experiments. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

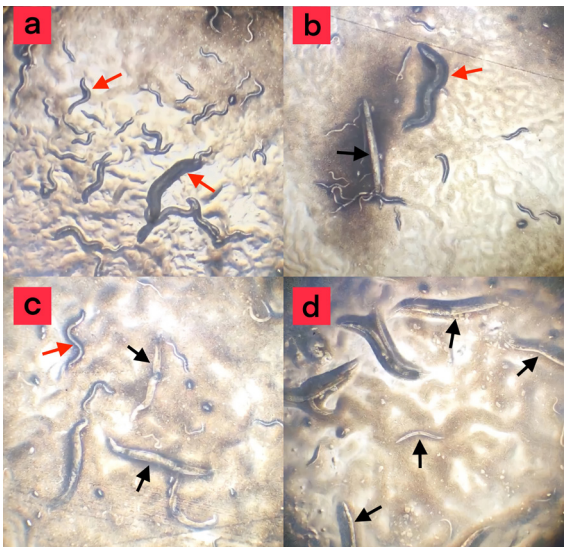

Figure 2: Representative images from the survival assay of *C. elegans* strains post 30 minutes of UV treatment. a) wild-type worms b) *xpa-1* RNAi worms c) him-1 RNAi worms d) *xpa-1/him-1* RNAi worms. The black arrows indicate dead worms, and the red arrows indicate live worms.

known for damaging DNA and reducing the lifespan of organisms, including *C. elegans*. Therefore, we exposed *C. elegans* knockdowns for different lengths of time to 365 nm UV radiation and measured their survival relative to wild-type worms. Worms that did not respond to the poke test and had straight body shapes were considered dead. The negative control plates consisted of wild-type worms, *xpa-1* knockdown, *him-1* knockdown, and *xpa-1/him-1* double knockdown that were not exposed to UV and grown under identical conditions.

All negative controls showed 100% survival of *C. elegans* **(Table 1 and Figure 1)**. Most wild-type worms, even after exposure to 30 minutes of UV radiation, retained their response to the poke test and their curved body shape **(Table 1, Figures 1 and 2a)**. In contrast, almost all worms were unresponsive to the poke test and had straight, rigid bodies in the double *xpa-1/him-1* knockdown **(Figures 1 and 2d)**. The single knockdown worms had intermediate survival percentages as shown by their response to the poke test and body shapes **(Table 1, Figures 1, 2b, and 2c)**. More specifically, for the double *xpa-1/him-1* RNAi knockdown, 59.6% of the worms survived compared to the 100% worm survival in wild-type (*t*-test, n = 3, $p = 0.004$) after 15 minutes of UV treatment **(Table 1, Figure 1)**. At 30 minutes of treatment, survival of the double knockdown reduced to 4.35% relative to 82.58% survival in the wild-type worms (*t*-test, n = 3, $p < 0.001$) **(Table 1 and Figure 1)**. Knockdown of *xpa-1* and *him-1* individually did not affect the survival of worms after 15 minutes of UV but did show a significant difference at 30 minutes of UV treatment **(Table 1 and Figure 1)**. Only 28.89% of *him-1* worms (*t*-test, n = 3, $p = 0.003$) and 57.64% of *xpa-1* worms survived after 30 minutes of UV exposure (*t*-test, n = 3, $p = 0.036$) relative to 82.58% survival in wild-

	OP50			*xpa-1*		
	Control	**15 minutes**	**30 minutes**	**Control**	**15 minutes**	**30 minutes**
1	100%	100%	95.00%	100%	100%	57.98%
2	100%	100%	79.16%	100%	100%	49.10%
3	100%	100%	73.57%	100%	100%	65.85%
Mean ± SD	100% ± 0%	100% ± 0%	82.58% ± 11.12%	100% ± 0%	100% ± 0%	57.64% ± 8.38%
	him-1			***xpa-1/him-1***		
	Control	**15 minutes**	**30 minutes**	**Control**	**15 minutes**	**30 minutes**
1	100%	100%	30.77%	100%	71.96%	0.00%
2	100%	100%	37.21%	100%	58.60%	3.23%
3	100%	100%	18.69%	100%	48.24%	9.81%
Mean ± SD	100% ± 0%	100% ± 0%	28.89 ± 9.40%	100% ± 0%	59.60 ± 11.89%	4.35 ± 5.00%

Table 1. Percent survival of wild-type, *xpa-1*, *him-1*, and *xpa-1/him-1* knockdown *C. elegans*. *E. coli* OP50 is a wild-type strain used as a bacterial food in the growth of *C. elegans*. Data are shown as means of three different replicates with corresponding standard deviation values. Control worms were not irradiated.

type worms, suggesting that both genes have important roles in UV protection in *C. elegans*.

DISCUSSION

Xpa-1 and Him-1 have both been implicated in different mechanisms of DNA repair and chromosome segregation in response to UV radiation in *C. elegans*, but their combined effects have not been investigated previously. Our results show that the knockdown of both *xpa-1* and *him-1* makes the worms significantly more susceptible to death by UV radiation compared to either wild-type, *xpa-1*, and *him-1* single RNAi knockdowns (Table 1 and Figure 1). This may suggest that both genes exert protective effects via distinct mechanisms in *C. elegans*. Neither of the single or double knockdowns exhibited reduced survival in the absence of UV radiation, suggesting that the knockdowns themselves do not affect the lifespan of *C. elegans* in our assay. By targeting two separate pathways, we were able to create a UV hypersensitive *C. elegans* knockdown that may be used as an animal model for studying different agents that provide UV protection and UV-induced human diseases like Xeroderma pigmentosum.

One limitation of our study was that only a few time points after UV treatment were used to determine the percent survival of worms due to time constraints. Additionally, our experiments lacked an assay, such as a qPCR, to verify that the RNAi knockdown of *xpa-1* and *him-1* was indeed successful. In a future experiment, we would like to use age-matched *C. elegans* as well as XO males to prevent reproduction during the experiment. We would also like to conduct more trials to more reliably estimate the variability of the results.

MATERIALS AND METHODS

Worm and Bacterial strains

E. coli OP50 is a strain conventionally used as a bacterial food in the growth of *C. elegans* on agar plates in the lab (18). Wild-type *C. elegans* strains and *E. coli* OP50 were procured from Carolina Biological. *E. coli* HT115 (DE3) with plasmids L4440 containing *xpa-1* miRNA sequences and *E. coli* HT115 (DE3) with plasmids L4440 containing him-1 miRNA sequences were procured from Source BioScience. The experiment was performed in triplicate, and each trial consisted of wild-type *C. elegans* on a plate of *E. coli* OP50, *E. coli* with the **xpa-1** RNAi construct, *E. coli* with the *him-1* RNAi construct, and *E. coli* with both *xpa-1* and *him-1* RNAi constructs (Ahringer). The *xpa-1* insert sequence was generated using the following primers: sjj_K07G5.2_F: 5' TTGTCAAATCGAGACCTCAAAAT, and sjj_K07G5.2_R: 5' TACTAGTCTTCGTGTAGCCCGTC. The *him-1* insert sequence was generated using the following primers: sjj_F28B3.7_F:5' GTTTCCACAAACGAATTGAAGAG, and sjj_F28B3.7_R:5' GCAACAAAATATGCTGACATTGA. The number of RNAi-treated worms ranged from 428 to 700 per Petri dish.

Culture conditions

C. elegans were grown on standard 60-mm Petri dishes prepared with Nematode Growth Medium (NGM) agar from US Biological Life Sciences according to packaging directions. LB broth was prepared in Sigma-Aldrich broth powder according to package instructions. *E. coli* OP50 and HT115 (DE3) with plasmids L4440 containing *xpa-1* and *him-1* miRNA sequences were cultured overnight at 37 °C in 3 mL of LB broth, and the cultures with RNAi constructs also contained 50 µg/mL of ampicillin (Carolina Biological). After culturing, 200 µl of each *E. coli* liquid culture were spread on the NGM agar ampicillin plates and incubated at 37 °C overnight. For combinatorial RNAi, 100 µl of *xpa-1 E. coli* culture and 100 µl of *him-1 E. coli* culture were spread on a single plate. Each type of *E. coli* was grown for 24 hours overnight before *C. elegans* were added to the plate.

UV radiation assay

Adult *C. elegans* were transferred onto the Petri dishes and incubated at 20 °C for 2 days to induce RNAi (17). After 48 hours, the wild-type, *xpa-1, him-1,* and *xpa-1/him-1* RNAi worms were irradiated with UVA light for either 15 minutes or 30 minutes. Control plates from each category were not exposed to UV. The plates were placed under a UVP Inc. Transilluminator (Model TM-36) with $5.4x10^{-19}$ Joules, 365 nm, 115 volts, 60 Hz, and 1.2 Amperes.

Percent survival measurements

Percent survival was calculated as a ratio of the number of live worms to total (live + dead) worms in each plate. The worms were visualized with a Swift SW380T compound microscope under 40x magnification. Worm survival was measured 2 days after 15 or 30 minutes of UV treatment; worms that did not respond to being poked with a sterilized worm pick were considered dead (19). The shape and texture of the worms also verified their death as dead worms were immobile and had straight body shapes while live worms were mobile and had curved body shapes (20). After death, *C. elegans* were autoclaved and disposed of into regular trash. The data was shown as the mean of 3 replicates with standard deviations. The *p*-value for statistical significance was calculated using the unpaired student *t*-test in GraphPad Prism software. The values were considered statistically significant when $p < 0.05$.

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