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A *C. elegans* homolog for the UV-hypersensitivity syndrome disease gene *UVSSA*

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ABSTRACT

The transcription-coupled repair pathway (TC-NER) plays a vital role in removing transcription-blocking DNA lesions, particularly UV-induced damage. Clinical symptoms of the two TC-NER-deficiency syndromes, Cockayne syndrome (CS) and UV-hypersensitivity syndrome (UVSS) are dissimilar and the underlying molecular mechanism causing this difference in disease pathology is not yet clearly understood. UV-stimulated scaffold protein A (UVSSA) has been identified recently as a new causal gene for UVSS. Here we describe a functional homolog of the human *UVSSA* gene in the nematode *Caenorhabditis elegans*, *uvs-1* (*UVSSA-like-1*). Mutations in *uvs-1* render the animals hypersensitive to UV-B irradiation and transcription-blocking lesion-inducing illudin-M, similar to mutations in TC-NER deficient mutants. Moreover, we demonstrate that TC-NER factors including UVS-1 are required for the survival of the adult animals after UV-treatment.

1. Introduction

NER is the main repair pathway involved in the repair of bulky DNA lesions that disturb the normal double-helical structure of DNA and is capable of repairing a variety of structurally unrelated lesions. The most important substrates of NER are the ultraviolet (UV) light-induced cyclobutane pyrimidine dimers (CPDs) and pyrimidine 6-4 pyrimidone photoproducts (6-4PPs) [1–3]. Along with the repair of these photoproducts, NER is also involved in the removal of DNA adducts induced by chemotherapeutic drugs like cisplatin [4,5], aromatic hydrocarbons [6], arylamine carcinogens [7], that thermodynamically destabilize the DNA helix [8]. This conserved repair process employs two different mechanisms of DNA damage detection depending on the location of the damage: global genome NER (GG-NER) and transcription-coupled NER (TC-NER). GG-NER scans the entire genome for DNA helix-distorting lesions, whereas, TC-NER is initiated in the transcribed strand of active genes upon the stalling of RNAP II during transcription.

TC-NER deficiencies are associated with two photosensitive syndromes: Cockayne syndrome (CS) and UV-hypersensitivity syndrome (UVSS) [9]. CS is characterized by sun sensitivity, developmental delay, short stature, microcephaly, and severe neurological abnormalities. Most of the CS patients belong to the complementation groups A and B. UVSS patients on the other hand display mild clinical manifestations including sun sensitivity, skin dryness, freckles, pigmentation abnormalities but no developmental or neurological defects [10]. UVSS cells exhibit normal GG-NER but a reduced recovery of RNA synthesis upon UV-B-treatment, similar to TC-NER-deficient cells [11]. The syndrome can be caused by mutations in *CSA* and *CSB* and the recently identified *UVSSA* (previously known as KIAA1530) [11,12]. *UVSSA* (UV-stimulated scaffold protein A) has been implicated in regulating TC-NER and identified as a causal gene for UVSS in recent studies by various approaches: exome sequencing of UVSS patient derived cells, genetic complementation by chromosome transfer, and SILAC-based proteomic approaches [13–15]. Despite these findings the reasons for the clinical differences between the two TC-NER deficiency syndromes have remained unclear.

Abbreviations: bp, base pairs; 6-4 PP, 6-4 photoproduct; cm, centimeter; CPD, cyclobutane pyrimidine dimer; CS, Cockayne syndrome; GG-NER, global-genome nucleotide excision repair; mJ, milli-joule; NER, nucleotide excision repair; NGM, nematode growth medium; nt, nucleotide; ORF, open reading frame; RNAP II, RNA polymerase II; RRS, recovery of RNA synthesis; TC-NER, transcription-coupled nucleotide excision repair; UDS, UV-induced DNA repair synthesis; UTR, un-translated region; UV, ultraviolet; UVSS, UV-hypersensitivity syndrome; XP, xeroderma pigmentosum.

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Dissecting the roles of the homologs of the mammalian NER genes in a simpler model organism could improve the understanding of the molecular mechanisms underlying the two diseases [16]. *C. elegans* is being increasingly used for the characterization of various DNA repair pathways since most of the major mammalian repair pathways, including NER, are conserved at the molecular level in this organism [17,18]. *C. elegans* has a functional NER that repairs UV-induced DNA damage [19,20] with the *CSB* homolog *csb-1* and *XPC* homolog *xpc-1* igniting TC-NER and GG-NER, respectively. Moreover, we recently demonstrated that the ORF *D2013.3* encodes the functional *CSA* homolog in *C. elegans* [21].

In this study, using the OrthoList database [22], which provides a platform for identifying worm-human orthology, we identified the uncharacterized ORF ZK742.2 as a *C. elegans* homolog of *UVSSA*. Consistent with phenotypes observed in UVSS patient cells, *ZK742.2* mutant animals are hypersensitive to UV and the transcription-blocking lesion-inducing drug illudin-M. Similar to *csa-1* and *csb-1* animals, the somatic tissues of *ZK742.2* mutant animals are exquisitely sensitive to UV irradiation during development and adulthood. In contrast to GG-NER-deficient *xpc-1* mutants, however, the germ cells of *ZK742.2* mutants show similar UV resistance as wildtype animals. Furthermore, combined loss of function of ZK742.2 and GG-NER factor *xpc-1* increases UV-sensitivity to the levels of complete NER-deficient *xpa-1* animals. Taken together, our data describe a function of ZK742.2 in the TC-NER pathway and we therefore name this previously uncharacterized gene *uvs-1* for *UVSSA-like-1*.

2. Materials and methods

2.1. Growth conditions

C. elegans strains were cultured under standard conditions at 20 °C on nematode growth media (NGM) plates with *Escherichia coliEscherichia coli* strain OP50 [23]. Strains used were N2 (Bristol; wildtype), *csb-1*(*ok2335*), *xpa-1*(*ok698*), *xpc-1*(*tm3886*), *uvs-1*(*tm6134*), *uvs-1*(*tm6311*), *uvs-1*(*tm6311*); *csb-1*(*ok2335*), *uvs-1*(*tm6311*); *csb-1*(*tm3886*), *uvs-1*(*tm6311*); *xpa-1*(*ok698*). *uvssa-1* mutant worm strains were obtained from National Bioresource Project and other strains from the *Caenorhabditis* Genetics Center.

2.2. Somatic UV-sensitivity assay

Synchronized L1 larvae obtained by hypochlorite treatment were plated on NGM-agar plates and UV-B-irradiated (310 nm) with the indicated doses, using a UV6 lamp (Philips) in a Waldmann UV236B device. A minimum of 500 worms was used for each UV-B-dose, and each treatment was conducted in triplicate.

After UV irradiation, worms were washed off with M9 buffer, concentrated by centrifugation and put on NGM plates with a pregrown OP50 *E. coli* lawn. Plates were incubated at 20 °C for 48 h or 72 h and analyzed by large particle flow cytometry using a Union Biometrica COPAS Biosort system. Larval stages were determined by measuring 'time of flight' (length) and 'laser extinction' (optical density) of individual worms using the Biosort 5291 software and confirmed by manual inspection under a Leica M 165C stereomicroscope.

2.3. Germline UV-sensitivity assay

Staged young adults were treated with indicated UV-doses and allowed to recover for 16 h. Following recovery, timed egg-laying was conducted by transferring three animals per UV-dose to a new NGM plate with OP50 for 3 h. Each treatment was conducted in triplicate. The total number of eggs laid was counted and 48 h later the number of viable eggs was determined. To assess UV-sensitivity, the percentage of eggs that were laid after UV-treatment was compared to untreated and the percentage of viable eggs was determined after hatching.

2.4. Adult-survival after UV-treatment

Synchronized day-1 adult animals obtained by hypochlorite treatment were plated on NGM agar plates with OP50 and UV-treated with the indicated doses. 140 animals were used for each dose and the number of dead animals was scored every day until all the animals were dead. During the assay, the live animals were transferred daily to new plates until the end of the egg-laying period and then onwards every second day.

2.5. Illudin-M sensitivity assay

Synchronized L1 larvae were obtained by hypochlorite treatment and were treated with indicated concentrations of illudin-M in liquid S-basal medium with OP50 for 24 h at 20 °C on a shaking platform. A minimum of 50 animals was used for each illudin-M dose and each treatment was conducted in triplicate. Illudin-M sensitivity was measured by determining the percentage of different larval stages in each treatment. Illudin-M was a kind gift from Prof. Dr. Rainer Schobert (Bayreuth).

2.6. Paraquat survival assay

Synchronized day-1 adult worms obtained by hypochlorite treatment were plated on NGM agar plates with 5 mM methyl viologen dichloride hydrate (Paraquat 856177 Sigma-Aldrich) and OP50. 100 animals were used for each dose and the number of dead animals was scored every day until all the animals were dead.

2.7. $KBrO_3$ sensitivity assay

Synchronized L1 larvae were obtained by hypochlorite treatment and were treated with indicated concentrations of $KBrO_3$ (309087 Sigma-Aldrich) in liquid S-basal medium with OP50 for 24 h at 20 °C on a shaking platform. A minimum of 250 animals

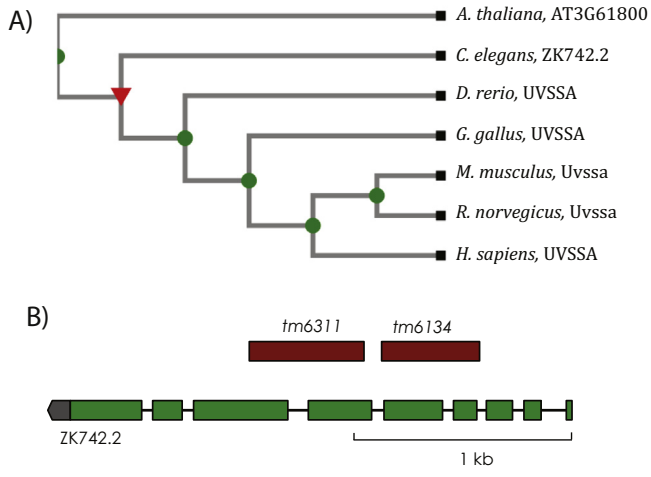

Fig. 1. Identification of the *C. elegans* UVSSA homolog. (A) Phylogenetic tree displaying the evolutionary relationship between UVSSA homologs in different species constructed using the TreeFam database. Uncharacterized *C. elegans* ORF ZK742.2 clusters with the UVSSA proteins. (B) Representation of the genomic architecture of ZK742.2. Green boxes represent exons, black lines represent introns, and untranslated region is in gray. The region deleted in the corresponding alleles is represented in red.