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Understanding the role of DAF-16 mediated pathway in *Caenorhabditis elegans* during UV-A mediated photoaging process

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ABSTRACT

Even though Sun is the major source of energy to all living beings in the universe, continuous and prolonged exposure to sunlight will lead to detrimental effects. Human skin will undergo extrinsic aging, known as photoaging upon prolonged exposure to sunlight which is characterized by wrinkles, dryness, loss of elasticity, and so on. The model nematode *Caenorhabditis elegans* which is widely used in aging studies, could be used to study photoaging also. Transcription factor DAF-16, which regulates longevity, stress resistance and many other physiological events, mediates the photoaging mechanism in *C. elegans*. Elevation in extracellular ROS and altered expression of SGK-1 indicates the role of DAF-16 during UV-A exposure. Further, the role of *daf-2*, the receptor gene and *lys*-7, an effector gene of DAF-16 were characterized through mutant based studies. The long lived *daf-2* mutants upon UV-A exposure showed reduction in lifespan, but the upregulation of *daf-16* allowed the other molecular mechanisms like healthspan, antimicrobial and stress resistance to be active. In the case of *lys*-7 mutants, the lifespan was reduced and all other molecular mechanisms were also downregulated. However, the *daf-16* mutants showed no change in lifespan irrespective of UV-A exposure. This signifies the role of DAF-16 during UV-A mediated photoaging in *C. elegans*. The present study helps in understanding the role of *daf-16* in UV-A mediated stress response which will be of considerable importance in the field of pharmacy in designing targets for specific agents against photoaging.

1. Introduction

Aging is a natural process experienced by every living individual from birth. Aging can happen both intrinsically and extrinsically, wherein, the former is the chronological process and the latter is due to external stress (McDaniel et al., 2015). The chronological aging is a constant process which cannot be altered and can be determined by decreased amount of fibroblast and collagen, which will lead to wrinkles and other damages in aged adults (McDaniel et al., 2015). On the other hand, exposure to ultraviolet radiations irradiated by sun induces photoaging, which accounts for almost 90% of extrinsic aging and can be determined by the extent of sun tan and other cascade of effects along with the damages seen in intrinsic aging. Among the various forms of UV radiations, UV-A and UV-B only can cross the ozone barrier and reach earth and cause photoaging (Hung et al., 2015).

According to World Health Organization (WHO), there is a growing incidence of UV-A mediated photoaging which is affecting humankind. Normally, when UV-A (400 – 315 nm) directly contacts the human skin, it causes oxidative stress which leads to the formation of reactive oxygen species (ROS) resulting in damage of tissues (Yan et al., 2016). A recent study proved that continuous exposure of retinal cells to UV-A increased the production of ROS significantly along with inducing apoptosis (Tringali, Sampaolese, & Clementi, 2016). Several antioxidants (Yan et al., 2016) and flavonoids (Patwardhan & Bhatt, 2016) are known to reduce the level of ROS thereby maintaining tissue integrity. In spite of these developments, the mode of action of UV-A inside the host and the stress responsive actions elucidated by the host have not been completely elucidated.

A suitable model system with simple neuronal network will be helpful to study the molecular mechanism behind UV-A mediated photoaging inside the host. The soil nematode *Caenorhabditis elegans* is one of the most widely used laboratory model to study aging (Copes et al., 2015) with different events like neuronal activity (Delaney, Chen, Graniel, Dumas, & Hu, 2017), heat shock (Kumsta, Chang, Schmalz, & Hansen, 2017; Prithika, Deepa, & Balamurugan, 2016), innate immune system (Yunger, Safra, Levi-Ferber, Haviv-Chesner, & Henis-Korenblit,

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2017) and reproduction (Wan et al., 2017) which can be directly correlated to aging studies (Akhoon, Pandey, Tiwari, & Pandey, 2016). Many age related, neurodegenerative diseases like Alzheimer's (Yang et al., 2017), Huntington's (Machiela, Dues, Senchuk, & Van Raamsdonk, 2017) and dementia (Akinola, 2016) were studied using this versatile model. Different stress markers including ROS can alter the stress response and immune system, which can also be studied using *C. elegans* (Kamaladevi, Ganguli, Kumar, & Balamurugan, 2013; Miranda-Vizuete & Veal, 2016). Added to it, *C. elegans* were also helpful to screen and analyze many chemicals and natural extracts which could be used as potential anti-aging compounds (Liu et al., 2016; Shen et al., 2017; Wan et al., 2014). Our previous study have demonstrated that *C. elegans* gets affected by the UV-A mediated photoaging process (Prasanth, Santoshram, Bhaskar, & Balamurugan, 2016).

The evolutionary conserved longevity mechanism in *C. elegans* is mediated by DAF-16, mTOR and other signaling pathways (Lapierre & Hansen, 2012). The insulin like signaling pathway, otherwise known as DAF-16 dependent pathway is one of the most widely studied to mediate aging in the nematode (Kenyon, 2010). The pathway comprises of *daf-2* and *age-1* which negatively regulate lifespan and *daf-16,* which positively regulate lifespan (Kenyon, Chang, Gensch, Rudner, & Tabtiang, 1993). It has been observed that mutation of *daf-2* or *age-1* could extend the lifespan of the nematode to many folds (Ayyadevara, Alla, Thaden, & Shmookler Reis, 2008; Kenyon et al., 1993). These mutations lead to dephosphorylation of DAF-16 followed by its nuclear accumulation where it regulates the transcription of a cascade of genes essential for longevity, fat metabolism, stress resistance, pathogenic resistance and so on (Ihara, Uno, Miyatake, Honjoh, & Nishida, 2017; Tullet, 2015). Transcription of more than hundreds of genes in *daf-2* mutants have been affected in a DAF-16 dependent manner (Kenyon, 2010). The stress responsive pathway JNK (Marudhupandiyan & Balamurugan, 2016; Oh et al., 2005), growth mediating AMPK mediated pathway (Greer et al., 2007) and innate immune regulating p38 MAPK pathway (Troemel et al., 2006) are all related to *daf-16* either directly or indirectly to induce lifespan and delay aging (Hesp, Smant, & Kammenga, 2015).

In this study, we try to explore and understand the role of DAF-16 in UV-A mediated photoaging process in a better way using mutants of specific genes dependent on DAF-16. A mutant of *daf-2,* which when activated will phosphorylate *daf-16* thereby preventing its nuclear accumulation, was used along with a mutant of *lys*-7, an effector gene which could get activated only after the nuclear accumulation of *daf-16*. These mutants highlighted the importance of *daf-16* during UV-A mediated stress response which was further confirmed using *daf-16* mutants.

2. Materials and methods

2.1. Nematodes, reagents, and equipments

The *C. elegans* strains used in this study, wild-type (Bristol N2), *daf-2* mutant (CB1370), *lys*-7 mutant (RB1285) and *daf-16* mutant (CF1330) were procured from *Caenorhabditis* Genetics Center, University of Minnesota, USA. The uracil auxotroph, *E. coli* OP50 was used as standard food source for *C. elegans*. All strains were grown in Nematode Growth Medium at 20 °C as described earlier (Brenner, 1974). All experiments were done in triplicates with age synchronized young adult worms. Synchronization was done by bleaching of gravid adults (Sivamaruthi, Prasanth, & Balamurugan, 2015).

Both the wild type and mutant strains of *C. elegans* were exposed to UV-A at 365 nm for 2, 4 and 6 h using a UV transilluminator. All other chemicals and reagents used were purchased from Sigma-Aldrich (St. Louis, MO, USA) and HiMedia Laboratories (Mumbai, India).

2.2. Measurement of extracellular ROS using DCF

ROS measurement was done as previously described by Kamaladevi et al. (2013). Briefly, *C. elegans* exposed to UV-A for 2, 4 and 6 h were incubated with 5 µg/ml of DCFH-DA for 10 min. After incubation, excess of the reagent was washed away using PBS. Worms were further transferred in a drop of sodium azide on 2% agar pads. Fluorescent images of worms were captured using Nikon Eclipse Ti-S, Japan.

2.3. Lifespan assay

Lifespan assay was carried out in liquid conditions as described (Sivamaruthi et al., 2015) with some modifications. Known number of UV-A exposed (mutant) worms were maintained with M9 buffer along with laboratory food source in 24 well microtitre plate for liquid assay. The worms were monitored routinely every 24 h till their death. Fresh media was replenished on every alternate day(s) to neglect false positive data due to the presence of young ones. Worms were considered as dead when they did not respond to a gentle tap or touch with a platinum wire pick. Nematodes unexposed to UV-A were considered as control.

2.4. Total RNA isolation and qPCR analysis

Synchronized populations of young adult worms were collected from *E. coli* OP50 lawns using M9 buffer and washed several times to make it devoid of bacteria. Worms were then exposed to UV-A for 2, 4 and 6 h. Worms unexposed to UV-A were kept as control. After exposure, they were washed once and treated with TRIzol reagent (RNA X Press reagent, Sigma) for isolating total RNA. The isolated RNA were reverse-transcribed using oligodT primer and MultiScribe™ Reverse Transcriptase (Applied Biosystems) enzyme. After first-strand synthesis, qPCR was done to analyze the expression pattern of candidate genes that regulate lifespan, healthspan and other pathological aspects using gene-specific primers. The expression data was normalized using the unexposed control values and internal control actin and then represented as upregulated or downregulated. The sequences of the primers are given in Table 1.

2.5. Western blot analysis

To monitor the changes at protein level, total proteins were isolated from the UV-A exposed samples by sonication method. 60 µg of each protein sample (exposed and control) was boiled to break the complex protein structure, which was further separated in 12% SDS-PAGE. The proteins were transferred to a PVDF membrane at a constant voltage (15 V) for 3 h. Immunodetection was performed by using specific antibodies against candidate (SGK-1) protein. The antibodies used in the present study include rabbit polyclonal antibody raised against SGK-1 (Santa Cruz Biotechnology, Inc.) at 1:1000 dilution and monoclonal anti-actin purified mouse immunoglobulin (Sigma-Aldrich) at 1:1000 dilution, followed by exposure to corresponding secondary antibody for

Table 1
List of primers.

Gene name	Forward primer	Reverse primer
utx-1	GCAGAACACCAGCTCATCAG	ATCAACGCCATTCTTCTCGC
daf-2	TCGAGCTCTTCCTACGGTGT	CATCTTGTCCACCACGTGTC
age-1	ATAGAGCTCCACGGCACTTT	TGTCAAGCACGTTTTCTTCG
egl-8	CGTATCGTTGCGCTTCTCA	AGTAGTGACACAGCGGTTG
egl-30	TCAGAAAGGCGGAAGTGGAT	GGTTCTCGTTGTCACACTCG
skn-1	ATCCATTCGGTAGAGGACCA	GGCGCTACTGTCGATTTCTC
clec-60	TGTCTGCATTCTTCCAGTCG	CCCATACCCAGACACCTTTG
clec-87	AATTCGTGTTCAAGCCAAGG	AGCCAGTTGATTTTGGTTGG
lys-7	TTGCAGTACTCTGCCATTCG	GCACAATAACCCGCTTGTTT

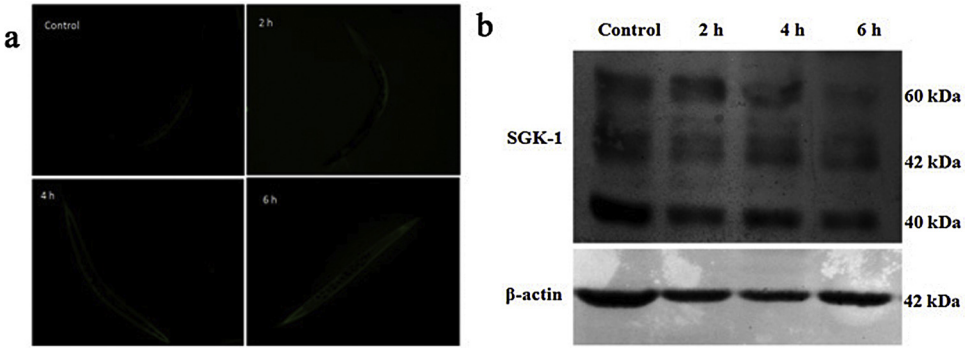

Fig. 1. DAF-16 gets differentially regulated during UV-A exposure **a** Fluroscent microscopic images of wild type nematodes exposed to UV-A and then treated with DCFH-DA to determine the level of extracellular ROS. The images indicate that as the time of exposure increases, the level of ROS in the nematode also increases **b** Western blot analysis of SGK-1. The 3 subunits (40, 42 and 60 kDa) of SGK-1 were found to be downregulated during the course of exposure. β-actin has been used as internal control.

4 h. The membrane was developed by transferring to 1X Alkaline Phosphatase (AP) buffer containing substrate NBT and 5-bromo-4-chloro-3-indolyphosphate until intense bands were observed in the membrane (Durai, Singh, Kundu, & Balamurugan, 2014).

3. Results

3.1. UV-A induced generation of ROS in C. elegans

The level of extracellular ROS production in the wild type worms was monitored in order to understand the severity of stress infused to the nematode. Continuous exposure to UV-A induced stress response inside *C. elegans*. The increased level of fluorescence clearly indicates that UV-A exposure has triggered oxidative stress to the nematodes (Fig. 1a).

3.2. SGK-1 alters its regulation during UV-A exposure

The expression of SGK-1 was analyzed in wild type nematodes in order to confirm the role of DAF-16 in UV-A mediated photoaging process. The 3 subunits of SGK-1 were observed to be downregulated which was analyzed by Image J analysis software. Subsequent analysis suggests the downregulation of DAF-16 during UV-A exposure (Fig. 1b). To further validate the role of this transcription factor, mutants of an upstream regulator *daf-2* and a downstream effector *lys*-7 were analyzed after exposing to UV-A.

3.3. LYS-7 mutants are susceptible to UV-A

Our previous study stated that UV-A reduced the lifespan of *C. elegans* which is dependent on DAF-16. The lifespan assay followed by qPCR analysis performed with *daf-2* mutants confirmed that they were susceptible to the radiations (Prasanth et al., 2016). In the present study, the lifespan of *lys*-7 mutants were analyzed after exposing to UV-A for 2, 4 and 6 h and it was observed that the lifespan were reduced to 180, 168 and 156 h respectively when compared to unexposed controls (Fig. 2a).

3.4. DAF-16 mediated pathway shows differential regulation in lys-7 mutants

Our previous study has shown that both in wild type and *daf-2* mutants, the level of *daf-16* were downregulated during the course of exposure which lead to the decreased lifespan (Prasanth et al., 2016). Even though the regulation of *daf-16* was high as compared to control at any given time point, the UV-A radiations had reduced the characteristic feature of extended survival of *daf-2* mutants (Prasanth et al., 2016). In the present study using *lys*-7 mutants, the regulation of candidate genes of the DAF-16 mediated pathway (*utx-1*, *daf-2* and *age-1*) were analyzed. Even though *utx-1* was slightly downregulated and *daf-2* was almost similar to control during the course of exposure, *age-1* got

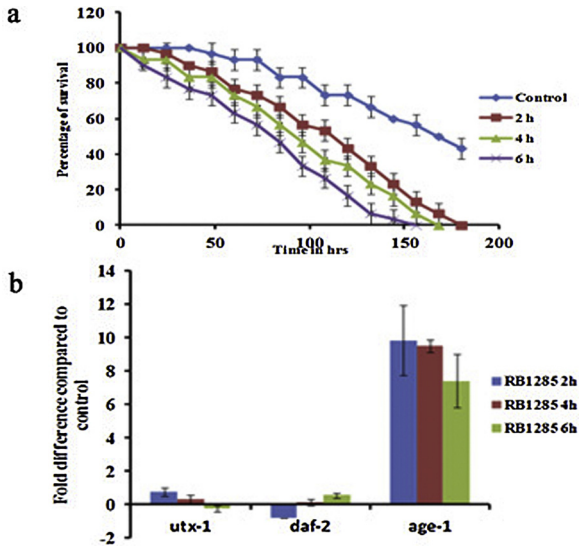

Fig. 2. LYS-7 mutants got differentially regulated **a** Mutants of *lys*-7 when exposed to UV-A for 2, 4 and 6 h survived upto 180, 168 and 156 h respectively wherein the unexposed control worms showed survival of 40% at 180 h. **b** qPCR analysis of *utx-1, daf-2* and *age-1* were done in *lys*-7 mutants exposed to UV-A for 2, 4 and 6 h. Regulation of *utx-1* was found to be downregulated and *daf-2* was upregulated during the course of exposure whereas *age-1* was upregulated at all the time points.

upregulated which would have aided to the reduced survival of the worms (Fig. 2b).

3.5. DAG pathway remained less affected in daf-2 mutants

The candidate genes of the healthspan regulating DAG pathway, *egl-8* and *egl-30*, which are necessary for the normal locomotion and other physiological functions were analyzed for their fold expression through qPCR. In the case of *daf-2* mutants, both the genes were upregulated as compared to control, wherein *egl-8* was downregulated during the course of exposure whereas *egl-30* remained almost constant (Fig. 3a). In the case of *lys*-7 mutants, both the genes showed upregulation initially (2 h exposure) but they were downregulated during the course of exposure (Fig. 3b).

3.6. Antimicrobial and anti-aging genes were differentially regulated

In another experiment, the expression levels of selective candidate genes responsible for antimicrobial and anti-aging properties were analyzed. The stress regulating gene, *skn-1* was found to be upregulated almost constantly in *daf-2* mutants, whereas in *lys*-7 mutants, it was upregulated initially (2 h exposure) and then downregulated during the course of UV-A exposure. However, at any given time, the expression was higher than control in both mutants (Fig. 4a and b).

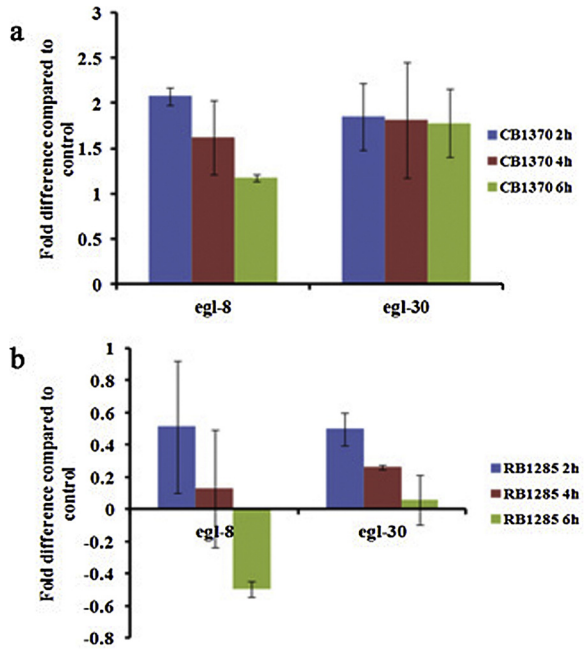

Fig. 3. DAG pathway genes indication healthspan showed differential regulation **a** In *daf-2* mutants exposed to UV-A, *egl-8* showed downregulation during the course of exposure whereas *egl-30* remained constant. **b** In *lys*-7 mutants exposed to UV-A, both *egl-8* and *egl-30* got downregulated during the course of exposure, wherein *egl-8* showed lesser regulation than control at 6 h exposure.

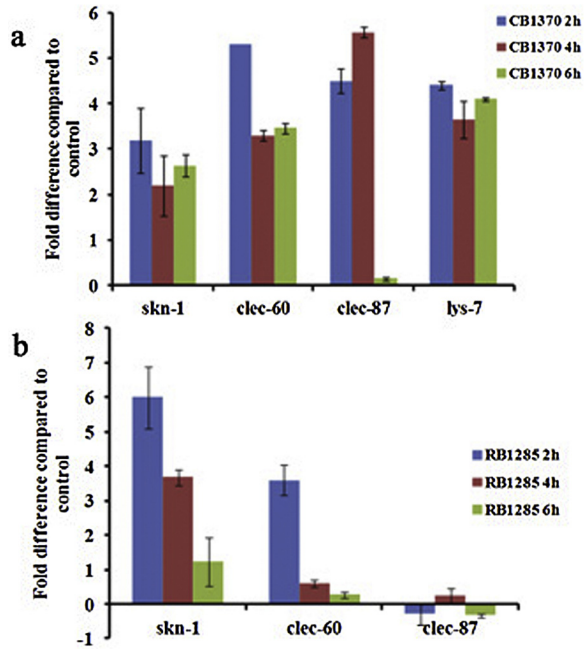

Fig. 4. Antimicrobial and stress regulating genes showed differential regulation **a** In *daf-2* mutants exposed to UV-A, stress regulating gene *skn-1* showed altered regulation, even though it was upregulated throughout the exposure time. Similarly, *lys*-7 was found to be upregulated irrelevant of the time of exposure. Antimicrobial genes, *clec-60* showed upregulation at 2 h exposure, which was subsided during the later hours and *clec-87* showed upregulation at 2 and 4 h which was subsided at 6 h exposure. **b** In *lys*-7 mutants, stress regulating gene *skn-1* showed downregulation during the course of exposure, even though it was upregulated throughout the exposure time. Antimicrobial genes, *clec-60* showed downregulation during the course of exposure, whereas *clec-87* showed altered regulation as it was upregulated only at 4 h exposure.

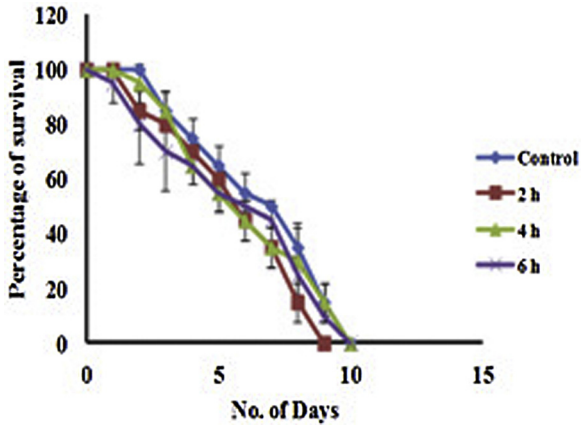

Fig. 5. DAF-16 mutants were not affected by UV-A exposure. The mutants when exposed to UV-A for 2, 4 and 6 h respectively survived upto 9, 10 and 10 days respectively while the control worms also survived upto 10 days.

Simultaneously, the expression levels of antimicrobial genes, *clec-60* and *clec-87* were also analyzed. In *daf-2* mutants, both the genes showed upregulation at the initial time of exposure which got reduced during the course of exposure (Fig. 4a). Similarly, the expression of *lys*-7 was found to be upregulated during exposure (Fig. 4a) in *daf-2* mutants. On the other hand, in *lys*-7 mutants, it was observed that the expression of *clec-60* was downregulated whereas *clec-87* showed altered expression (Fig. 4b).

3.7. DAF-16 mutants did not alter their lifespan against UV-A exposure

The *daf-16* mutants were exposed to UV-A and analyzed for their changes in lifespan. It was observed that the mutant nematodes exposed to UV-A for 2 h survived for 9 days, whereas those exposed for 4 and 6 h survived for 10 days. The unexposed control mutant nematodes also survived for 10 days (Fig. 5). This indicates that UV-A exposure has not made any changes in *daf-16* mutants suggesting the importance of the gene in identifying and eliciting a response during UV-A exposure.

4. Discussion

The ultraviolet radiations emitted by the sun which reach the earth mainly consist of UV-A and UV-B where the former comprises of about 90% (Scharffetter-Kochanek et al., 2000). Exposure to these radiations leads to a series of events ranging from premature skin aging (Jallad, 2017), oxidative stress (Larroque-Cardoso et al., 2015), which may even lead to skin cancer, in humans. The model nematode *C. elegans* could be used to understand the molecular mechanism involved during UV-A exposure. Our previous study suggests that UV-A can cause a decrease in the lifespan and healthspan of the nematode which is dependent on DAF-16 (Prasanth et al., 2016). In the present study, the role of DAF-16 was further analyzed to shed more light into the molecular mechanism taking place inside the nematode during UV-A exposure.

Since UV-A are known to increase the expression of ROS, the expression of extracellular ROS in the wild type nematodes was analyzed after UV-A exposure. The elevated expression of ROS provides a direct indication that oxidative stress has occurred inside the host system (Fig. 1a). Previous reports suggest that ROS plays a vital role in regulating lifespan through DAF-16 (Abergel, Livshits, Shaked, Chatterjee, & Gross, 2017; Rathor, Pant, Awasthi, Mani, & Pandey, 2017) which was parallel to our previous finding that UV-A can invoke changes in DAF-16 mediated pathway (Prasanth et al., 2016). The expression of SGK-1, an activator of DAF-16 (Prithika et al., 2016) was analyzed through western blot analysis to further confirm the involvement of DAF-16 during UV-A exposure. The expression levels were found to be

downregulated which suggest that DAF-16 appeared to be differentially regulated (Fig. 1b). To further validate our findings, we analyzed the effect of UV-A in *daf-2* and *lys*-7 mutants, which are upstream regulator and downstream effector of DAF-16 respectively.

DAF-16, which encodes the FOXO transcription factor, regulates the transcription of a wide variety of genes having differential functions, ranging from stress resistance, dauer formation, longevity, fat metabolism, pathogenic resistance, etc. (Tullet, 2015) which makes it as a master regulator (Hesp et al., 2015). Several functional foods like cranberry were observed to mediate DAF-16 induced mechanism for lifespan extension and stress resistance (Guha et al., 2013; Scerbak, Vayndorf, Hernandez, McGill, & Taylor, 2018). Mutation or downregulation of receptor gene *daf-2* and its downstream player *age-1* normally de-phosphorylate *daf-16* thereby allowing its entry into the nucleus which will eventually lead to lifespan extension and stress resistance (Kenyon et al., 1993; Tullet, 2015). In our previous study, the *daf-2* mutants when exposed to UV-A had reduced survival during the course of exposure. However, the expression of *daf-16* was found to be upregulated in *daf-2* mutants, which aided in the survival of worms exposed to 2, 4 and 6 h to 40, 35 and 30 days respectively (Prasanth et al., 2016).

In addition, *daf-2* mutants are known for their extended healthspan and stress resistance (Ding et al., 2017; Prithika et al., 2016). UV-A radiations invoke oxidative stress response inside the living system (Ohba et al., 2016). In this regard, we analyzed the expression of *skn-1*, which is a stress responsive gene (Blackwell, Steinbaugh, Hourihan, Ewald, & Isik, 2015; Wang, Ma, Li, & Cui, 2016; Wu et al., 2016) along with *egl-8* and *egl-30* which indicates the healthspan (Govorunova et al., 2010; Prasanth et al., 2016). It was observed that, all the three genes were upregulated during UV-A exposure, wherein *skn-1* (Fig. 4a) and *egl-8* (Fig. 3a) got slightly downregulated during the later hour (4 and 6 h) of exposure and *egl-30* remained constant during the course of exposure (Fig. 3a). This indicates that even during UV-A exposure, the *daf-2* mutants were able to maintain their characteristic feature of extending healthspan and stress resistance (Ding et al., 2017; Prithika et al., 2016) which is dependent on DAF-16 (Kenyon, 2010).

Earlier reports suggested that *daf-2* mutants are resistant to bacterial pathogenesis (Evans, Kawli, & Tan, 2008; Garsin et al., 2003), which could be mediated by the increased expression of *lys*-7 (Zhang, Judy, Lee, & Kenyon, 2013). In the present study also, the expression of *lys*-7 was higher in *daf-2* mutants irrelevant of the time of exposure (Fig. 4a). Parallel to this, the expression of antimicrobial genes, *clec-60* and *clec-87* which are well known to identify an invading pathogen and to elicit the innate immune response (Sivamaruthi & Balamurugan, 2014; Sivamaruthi et al., 2015) were also analyzed. It was observed that both the genes were upregulated during the initial hour of exposure. The expression of *clec-60* was reduced during the later hours but that of *clec-87* was downregulated during the later hour of exposure (Fig. 4a). The higher fold expression of *clec-60* in *daf-2* mutants could be directly correlated to the enhanced pathogenic resistance of the mutants (Evans et al., 2008; Garsin et al., 2003). However, continuous exposure to UV-A made the nematodes more susceptible to the radiations, which appears to be the cause of its reduced survival.

C. elegans is known to have an innate immune system which has different molecules like lysozymes and lectins which will act as first line of defense against any kind of external stimuli (Kong, Tan, & Nathan, 2014). Among the different lysozymes present in *C. elegans*, *lys*-7 is one of the predominant player to act, which is mediated by DAF-16 pathway (Portal-Celhay, Bradley, & Blaser, 2012). Previous reports suggests the suppression of *lys*-7 during the infection of *S.* Typhi (Sivamaruthi & Balamurugan, 2014), *S. aureus* (JebaMercy, Prithika, Lavanya, Sekar, & Balamurugan, 2015) and *P. aeruginosa* (Evans et al., 2008; Vigneshkumar, Pandian, & Balamurugan, 2012) which highlights the importance of this player during infection. Mutants of *lys*-7 had reduced survival during *E. coli* and *Salmonella* infection, even though the bacterial load present in the intestine was similar to that of wild type (Portal-Celhay et al., 2012). This suggests that, *lys*-7 is important to provide host immunity than reducing the bacterial load. In the present study, the expression of upstream regulators of DAF-16, mainly *utx-1, daf-2* and *age-1* was analyzed in the mutant. It was observed that *utx-1* was downregulated slightly and *daf-2* fold expression was almost similar to control. However, the expression of *age-1* was found to be upregulated which would have eventually lead to the reduced survival of *lys*-7 mutants (Fig. 2b). It was observed that these mutants survived only upto 180, 168 and 156 h when exposed to UV-A for 2, 4 and 6 h respectively (Fig. 2a).

Similar to that of *daf-2* mutants, the other molecular players responsible for healthspan, stress and pathogenic resistance were analyzed in *lys*-7 mutants. The healthspan regulating genes, *egl-8* and *egl-30* (Fig. 3b) along with the stress associated gene *skn-1* (Fig. 4b) were found to be downregulated during the course of exposure. The higher level of expression of *skn-1* during the initial hours of exposure indicates the activation of stress associated mechanism mediated by DAF-16 (Blackwell et al., 2015; Wang et al., 2016; Wu et al., 2016). Parallel to this, the antimicrobial genes, *clec-60* and *clec-87* were also analyzed wherein the former got downregulated during the course of exposure after initial upregulation and the latter remained downregulated except for 4 h exposure (Fig. 4b). The *lys*-7 mutants are known for its immunocompromised condition (Portal-Celhay et al., 2012) and the exposure to UV-A radiation has reduced its survival as the mutants were not able to withstand the harmful radiations.

The *daf-2* mutants which are known to have increased expression of *daf-16* have a characteristic feature of enhanced lifespan, healthspan, stress resistance and antimicrobial gene expression (Kenyon, 2010). During UV-A exposure also, these cognitive functions were observed to be upregulated initially, with the help of DAF-16, but as the time of exposure was further extended, those functions were observed to be slowly subsided. This was indicated by the fold expression of *egl-8, egl-30, skn-1, clec-60* and *clec-87* (Fig. 6a). On the other hand, the mutant of *lys*-7, showed reduced expression in all the above mentioned cognitive functions which could be because of the reduced level of DAF-16 in the *lys*-7 mutant nematode exposed to UV-A (Fig. 6b). Finally, *daf-16* mutants were exposed to UV-A to understand the changes taking place inside the mutants, which could validate our findings. These mutants showed no difference in their lifespan irrelevant of UV-A exposure (Fig. 5). This underlines the importance of *daf-16* in initiating and combating specific response against UV-A mediated stress. DAF-16, which is very important to mediate stress resistance and lifespan of the nematode (Kenyon, 2010; Kurino, Furuhashi, Sudoh, & Sakamoto, 2017) turns out to be an important player inside the nematode in combating UV-A mediated stress response.

5. Conclusion

This study underlines the importance of DAF-16 in combating UV-A stress response. Mutations in *daf-2* are known to double the lifespan when compared to wild type nematodes. Lyzozyme gene, *lys*-7 is known for its antimicrobial activity and aids in the immunity of the nematode. Mutants of both the genes when exposed to UV-A showed reduced survival when compared to unexposed controls. This indicates the role of DAF-16 in combating the harmful radiations. The expression of extracellular ROS and the expression of SGK-1 support this view. Moreover, increased expression of DAF-16 dependent healthspan, antimicrobial and stress regulating genes in *daf-2* mutants and decreased expression of the same genes in *lys*-7 mutants pinpoints the importance of DAF-16. Mutants of *daf-16* showed no changes in the survival rate irrelevant of exposure validates the need of DAF-16 in combating UV-A mediated stress response. Further, any implications in the field of therapeutics for the efficient treatment of UV-A mediated photoaging could be done via DAF-16.

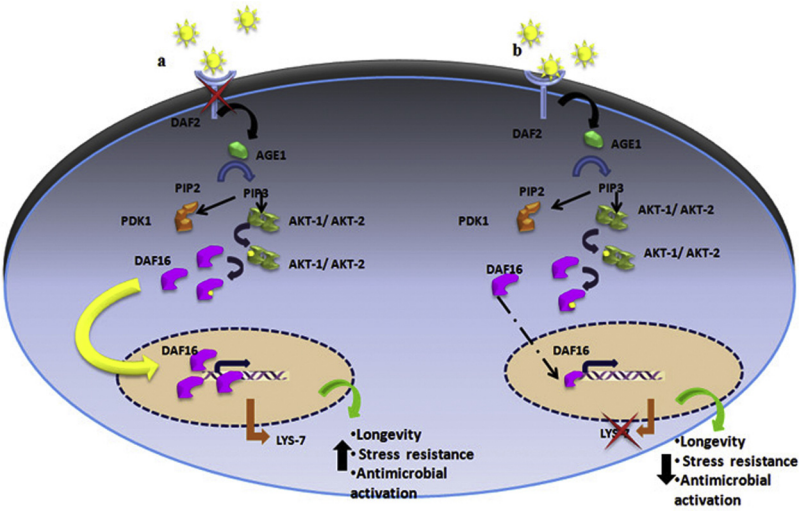

Fig. 6. Schematic representation of IIS pathway during UV-A exposure **a** In *daf-2* mutants, the regulation of *age-1* and other downstream players will not take place which will help in the nuclear localization of *daf-16*. This helps in the increased regulation of healthspan, antimicrobial and stress resistance genes **b** In *lys-7* mutants, *daf-2* and *age-1* were upregulated which phosphorylates *daf-16* which reduces its nuclear localization. This leads to the decreased regulation of healthspan, antimicrobial and stress resistance genes.

Conflict of interest

The authors state that they have no conflict of interest.

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