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Heat shock factor 1 prevents the reduction in thrashing due to heat shock in *Caenorhabditis elegans*

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ABSTRACT

Heat shock factor 1 (HSF-1) is activated by heat stress and induces the expression of heat shock proteins. However, the role of HSF-1 in thermotolerance remains unclear. We previously reported that heat stress reversibly reduces thrashing movement in *Caenorhabditis elegans*. In this study, we analyzed the function of HSF-1 on thermotolerance by monitoring thrashing movement. *hsf-1* RNAi suppressed the restoration of thrashing reduced by heat stress. In contrast, *hsf-1* knockdown cancelled prevention of movement reduction in insulin/IGF-1-like growth factor 1 receptor (*daf-2*) mutant, but didn't suppress thrashing restoration in *daf-2* mutant. In addition, *hsf-1* RNAi accelerated the reduction of thrashing in heat-shocked wild-type *C. elegans*. And, *daf-16* KO didn't accelerate the reduction of thrashing by heat stress. Taken together, these results suggest that HSF-1 prevents the reduction of thrashing caused by heat shock.

1. Introduction

We previously investigated thermotolerance in *Caenorhabditis elegans* by analyzing thrashing movement as an index and found that: (1) heat stress reduced thrashing, (2) lack of *daf-16*, a homolog of FoxO, failed to restore thrashing movement reduced by heat stress, and (3) knockout of *daf-2*, a homolog of insulin/IGF receptor, promoted restoration of the movement reduced by heat stress in a *daf-16*-dependent manner. However, *daf-2* mutants retained the movement immediately after heat stress in a *daf-16*-independent manner. Therefore, it was suggested that DAF-16 is not the only inducer of thermotolerance and that some other factors prevent the reduction of thrashing movement during heat stress. One protein that was of interest in this phenomenon was heat shock factor (HSF).

HSF responds to heat stress and, as part of the heat-shock responses, promotes the transcription of heat shock proteins (HSPs), which act as molecular chaperones [2,3]. Thus, HSF is thought to be a key factor in thermotolerance.

HSF-1, one of HSF in *C. elegans*, is associated with longevity [4–6] and is known to inhibit paralysis or aggregation caused by proteotoxic diseases [4,7–9]. It is also involved in the regulation of innate immunity in *C. elegans* [10]. HSF-1 is negatively regulated by the insulin-like signaling pathway [4–6]; is activated by knockout (KO) of *daf-2*; and is necessary for lifespan extension, the prevention of proteotoxic disease [4], and promotion of innate immunity [10] in *daf-2* mutants. Exposure to low levels of heat stress for a few hours promotes innate immunity [10] and thermotolerance [11–13] in dependent on HSF-1.

However, roles of HSF-1 on thermotolerance are still remained unclear. In fact, the survival time for *C. elegans* under heat stress is not affected by *hsf-1* RNAi or KO [12]. In addition, *daf-2* knockdown by RNA interference extends survival under heat stress in independent of HSF-1 [12], although lifespan extension in response to *daf-2* KO depends on HSF-1 [4–6].

In this study, we used thrashing as an index of thermotolerance and investigated the roles of HSF-1 and HSPs in this process in *C. elegans*. We report a novel function for HSF-1.

2. Materials and methods

2.1. Strains and culture

WT *C. elegans* Bristol N_2, *daf-2* (*e1370*), and *daf-16* (*mgDf50*) were provided by the Caenorhabditis Genetics Center (CGC). Each strain was cultured on nematode growth medium (NGM) agar plates seeded with *Escherichia coli* OP50 as previously described [14].

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2.2. NaClO treatment

To synchronize the growth of *C. elegans*, adult worms were treated with a 10:1 NaClO:10 N NaOH solution (NaClO: Haiter, KAO, Tokyo, Japan; NaOH: WAKO, Osaka, Japan). The eggs were incubated in S-basal media (0.1 M NaCl (Kanto Chemical, Tokyo, Japan), 50 mM potassium phosphate buffer [pH 6.0]) at 20 °C until hatching.

2.3. Preparation of cDNA

Adult worms were washed with S-basal media followed by double-distilled water to remove *E. coli*. RNA was purified from whole-cell extracts of worms using RNAiso PLUS (Takara, Shiga, Japan) and was treated with DNase I (Takara) to prevent contamination of genomic DNA. cDNA was synthesized using Moloney murine leukemia virus (MML-V) reverse transcriptase (Takara), PrimeScript® RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara) or PrimeScript® RT Master Mix (Perfect Real Time) (Takara).

2.4. Feeding RNAi

Plasmid DNA L4440 (Fire Laboratory) containing *hsf-1* cDNA (primers used to obtain the cDNA are shown in Table 1) was transfected into *E. coli* HT115 treated with 50 mM $CaCl_2$ (WAKO). The transfected cells were then treated with isopropyl-β-D-thiogalactopyranoside (IPTG; WAKO) to induce dsRNA expression. After treatment, the transfected cells were seeded onto NGM plates for RNAi [15,16]. Age-synchronized L1 larvae were cultured for 3 days at 20 °C on NGM plates seeded with *E. coli* OP50. After 3 days, worms were transferred onto RNAi plates and cultured for 24 h at 20 °C.

2.5. Determination of restoration of thrashing after exposure to heat stress

Age-synchronized L1 larvae were transferred onto a plate and cultured for 4 days at 20 °C. After 4 days, adult worms were transferred onto NGM plate (*E. coli* (−)) and cultured at 20 °C or 35 °C for 4 h. After heat stress, the worms were transferred onto a new plate and cultured for 0–24 h, and then ten worms were picked and moved into S-basal at random to count the movement for 15 s. These methods have been previously described [1].

2.6. Determination of inhibition of thrashing

Adult worms were transferred onto NGM plates (*E. coli* (−)) and cultured at 35 °C for 1 h. Then, the plates were incubated at room temperature for 10 min after which the worms were observed for thrashing for 15 s.

2.7. RT-PCR

cDNA was amplified using an ABI-2720 Thermal Cycler (Applied Biosystems, CA, USA) and Taq DNA Polymerase (Ampliqon, Herlev, Denmark). The cycling conditions were as follows: 94 °C/5 min, (94 °C/30 s, 55 or 57 °C/30 s, 72 °C/30 s) × 21–25, and 72 °C/7 min *gpd-1* was used as the internal control. Primers are shown in Table 1.

2.8. Quantitative RT-PCR

cDNA was amplified using Thermal Cycler Dice® Real Time System Lite (Takara) and Thunderbird SYBR qPCR Mix (TOYOBO, Osaka, Japan) according to the manufacturer's instructions. *actin* was used as the internal control. cDNA samples for quantitative RT-PCR were prepared as previously described [1]. Primers are shown in Table 1.

2.9. Statistical analysis

Statistical analyses were performed using the analysis software SPSS (IBM, NY, USA). Statistical significance for the values plotted on the graph were analyzed using the t-test and Games–Howell test with statistical differences represented by $^{*}p < 0.05$ and $^{**}p < 0.005$.

3. Results

3.1. HSF-1 induces thermotolerance via HSPs expression

Our previous result showed that thrashing movement of *C. elegans* was reduced by heat stress (35 °C for 4 h) and recovered after culturing at 20 °C [1]. First, to determine the role of HSF-1 in the change of thrashing movement, we applied heat stress to worms treated with *hsf-1* RNAi. We observed that *hsf-1* RNAi interferes with the sequential restoration of thrashing after heat stress (Fig. 1A). To know whether RNAi specifically interfere the mRNA expression of *hsf-1*, we analyzed mRNA expression of *hsf-1*. Based on the result of RT-PCR, *hsf-1* mRNA expression is suppressed specifically by *hsf-1* RNAi (Fig. 1B) and *daf-16* level wasn't knocked down by *hsf-1* RNAi (Fig. 1B). We next analyzed expression of *hsp-16.2* and *hsp-70*. It is known that mRNA expression of these genes is positively regulated by HSF-1, and is inducible by heat stress and *daf-2* KO [6]. It was found that heat stress increases the expression of both genes temporarily (Fig. 2A and B). These results suggest that HSF-1 induces thermotolerance and that HSP expression may trigger the thermotolerance.

Table 1
Primer list.

Gene	Sense (5′–3′)	Antisense (5′–3′)
qRT-PCR		
actin [21]	TCGGTATGGGACAGAAGGAC	CATCCCAGTTGGTGACGATA
hsp-16.2	TGTTGGTGCAGTTGCTTCGAATC	TTCTCTTCGACGATTGCCTGTTG
hsp-70	ACCCTTCGTTGGATGGAACG	GCATCCGGAACCTGATTGGGC
RT-PCR, RNAi		
hsf-1	CATGAATTCTGATAATGCGTGTTCCG	CATGAATTCATATTGCTGTTGGCGAGC
daf-16 [22]	CATGGATCCATCCAGATGCAAAGCCAG	CATGGATCCGTATGCTGTGCAGCTACA
gpd-1 [23]	ATGTCGAAGGCCAACGTC	GTTTTGTCCAGCACCGCG

qPCR was performed using a Thermal Cycler Dice® Real Time System Lite with the default cycling conditions (95 °C/30 s, [95 °C/5 s, 60 °C/30 s] × 40). *actin* was used as the internal control. PCR was performed using an ABI-2720, and the cycling conditions were as follows: 94 °C/5 min, (94 °C/30 s, 55 or 57 °C/30 s, 72 °C/30 s) × 21–25, and 72 °C/7 min *gpd-1* was used as the internal control.

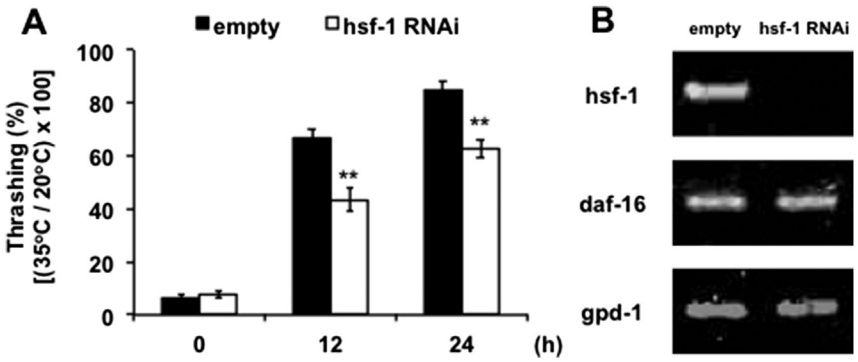

Fig. 1. The role of HSF-1 in restoring thrashing in *C. elegans* exposed to heat stress. (A) Worms treated with *hsf-1* siRNA were transferred to *E. coli*-free NGM plate and cultured for 4 h at 20 °C or 35 °C. After 4 h, worms were transferred onto fresh NGM RNAi media plates and cultured for 0–24 h. After 0, 12, or 24 h, ten worms were observed for thrashing for 15 s. The graph shows the ratio of the number of thrashing activity of heat treated worms divided by that of heat-untreated worms. Three independent experiments were performed and these data were combined for making a graph. Statistical significance was analyzed with t-test. Mean ± SE, $^{*}p < 0.05$, $^{**}p < 0.005$. Three independent trials were conducted and showed similar result. (B) RNA was extracted from *C. elegans* treated with siRNA, and cDNA was synthesized. Expression of *hsf-1*, *daf-16*, and *gpd-1* were detected by RT-PCR. Two independent trials were conducted and showed similar result.

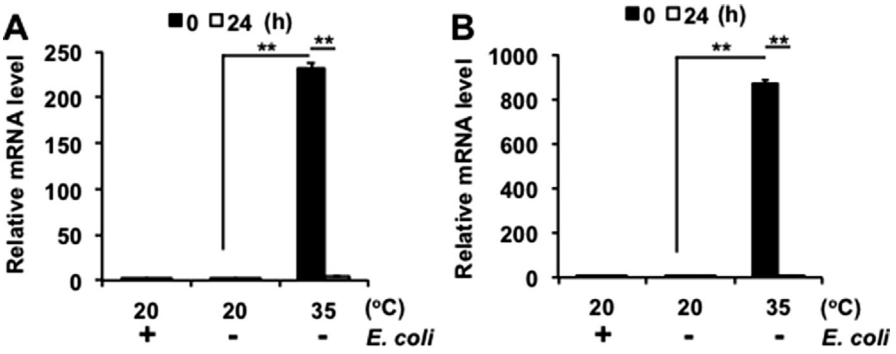

Fig. 2. Expression of genes downstream of HSF-1. Worms were transferred to NGM plates seeded with OP50 (+) or *E. coli*-free NGM plates (−) and cultured for 4 h at 20 °C or 35 °C. After incubation in each condition, worms were transferred to NGM plates seeded with OP50 and cultured for 24 h at 20 °C. Then RNA was extracted, and cDNA was synthesized. Expression of *hsp-16.2* (A) and *hsp-70* (B) were detected by quantitative RT-PCR. Each cDNA sample was amplified in three wells. Statistical significance was analyzed with Games–Howell test. Mean ± SE, $^{*}p < 0.05$, $^{**}p < 0.005$. Two independent trials were conducted.

3.2. Activated HSF-1 prevents heat stress-induced reduction of thrashing

It has been reported that the insulin/IGF-1-like signaling pathway negatively regulates HSF-1 activity [4–6]. In addition, *daf-2* mutant promotes restoration of thrashing movement reduced by heat stress in a *daf-16*-dependent manner, and retains the movement immediately after heat stress in a *daf-16*-independent manner [1]. Then, to analyze the physiological relation of *hsf-1* to *daf-2* mutant phenotype, we applied RNAi of *hsf-1* to *daf-2* mutants. As a result, *daf-2* mutants treated with *hsf-1* RNAi could not retain the movement under heat stress (Fig. 3). Furthermore, thrashing activity was restored to the level that is close to that observed in *daf-2* mutants treated with empty vector control (Fig. 3). These results indicate that activated HSF-1 prevents thrashing reduction by heat stress but does not accelerate restoration.

3.3. HSF-1 prevents thrashing reduction due to heat stress

Although the previous results showed that HSF-1 was not involved in restoration of thrashing in heat-stressed *daf-2* mutants (Fig. 3), *hsf-1* RNAi inhibited restoration of thrashing in WT worms (Fig. 1A). In addition, *hsf-1* RNAi suppressed the prevention of thrashing in *daf-2* mutants (Fig. 3). Therefore, we hypothesized that the extent of restoration is associated with the extent of reduction in thrashing under heat stress. We applied heat stress for 1 h to worms treated with *hsf-1* siRNA. As a result, thrashing activity of worms treated with *hsf-1* RNAi was lower than that of worms treated with control vector (Fig. 4A). This result shows that *hsf-1* RNAi accelerates the reduction in thrashing due to heat stress.

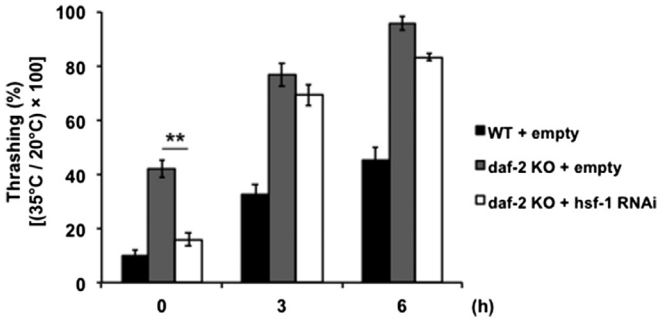

Fig. 3. The role of HSF-1 in the reduction of thrashing by heat stress in *daf-2* mutants. *daf-2* mutant worms treated with *hsf-1* RNAi were transferred to *E. coli*-free NGM plates and cultured for 4 h at 20 °C or 35 °C. After 4 h, the worms were transferred onto new NGM RNAi media plates and cultured for 0–6 h. After 0, 3, or 6 h, ten worms were observed for thrashing for 15 s. The graph shows the ratio of the number of thrashing activity of heat treated worms divided by that of heat-untreated worms. Three independent experiments were performed and these data were combined for making a graph. Statistical significance was analyzed with Games–Howell test. Mean ± SE, $^{*}p < 0.05$, $^{**}p < 0.005$.

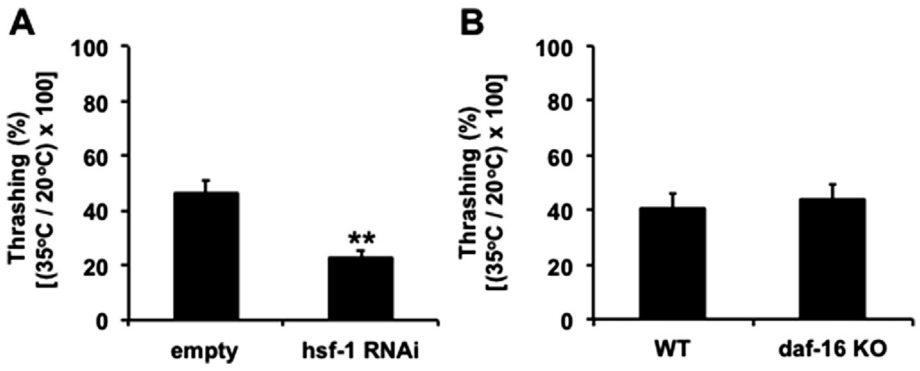

Fig. 4. The roles of HSF-1 and DAF-16 in the prevention of thrashing reduction by heat stress. (A) Worms treated with *hsf-1* RNAi were transferred to *E. coli*-free NGM plates and cultured for 1 h at 20 °C or 35 °C. The plates were incubated for 10 min at room temperature, and ten worms were observed for thrashing for 15 s. The graph shows the ratio of the number of thrashing activity of heat treated worms divided by that of heat-untreated worms. Three independent experiments were performed and these data were combined for making a graph. Statistical significance was analyzed with t-test. Mean ± SE, $^{*}p < 0.05$, $^{**}p < 0.005$. (B) Adult worms were transferred onto *E. coli*-free NGM plates and cultured for 1 h at 20 °C or 35 °C. The plates were incubated for 10 min at room temperature, and ten worms were observed for thrashing for 15 s. The graph shows the ratio of the number of thrashing activity of heat treated worms divided by that of heat-untreated worms. Three independent experiments were performed and these data were combined for making a graph. Statistical significance was analyzed with t-test. Mean ± SE, $^{*}p < 0.05$, $^{**}p < 0.005$.

Furthermore, thrashing activity wasn't changed between WT and *daf-16* mutant after heat shock for 1 h (Fig. 4B). Therefore, it was suggested that HSF-1 prevents the reduction of thrashing caused by heat stress.

4. Discussion

The results in this study indicate that HSF-1 prevents the reduction of thrashing caused by heat stress (Figs. 3 and 4A). This is the first finding which was obtained through analyzing the physiological function of HSF-1 on thermotolerance in viewpoint of prevention.

Previous studies have reported that the movement reduction caused by aging is accelerated by *unc-15* KO, the gene that encodes paramyosin, in *C. elegans,* and that this movement reduction is induced by the mislocalization of paramyosin [17], which is caused by protein misfolding [18]. In addition, it was shown that paramyosin misfolds at 25 °C in the *unc-15* mutant [18], that *hsf-1* RNAi exacerbates the reduction in movement seen in *unc-15* mutant [17], and that overexpression of *hsf-1* prevents mislocalization of paramyosin in the *unc-15* mutant [17]. Therefore, we speculated that HSF-1 prevents the reduction of thrashing caused by heat stress by preventing protein misfolding or denaturation. Consequently, HSP, whose expression is positively regulated by HSF-1, may be important for preventing the reduction of movement caused by heat stress.

We found that heat stress increased the expression of *hsp-16.2* and *hsp-70* (Fig. 2A and B) [4–6]. And, these mRNA are increased by heat stress or *daf-2* KO in dependent on HSF-1 [6]. A previous study showed that HSP-70 family could prevent the aggregation of denatured protein [3]. In addition, it was shown that overexpression of HSP-70 prevents protein denaturation and promotes the refolding of denatured protein [19]. Paralysis caused by accumulation of β-amyloid is promoted by *hsf-1* RNAi in *C. elegans* [7,8], whereas overexpression of *hsp-16.2* prevents aggregation of β-amyloid and subsequent paralysis [20]. In addition, it was reported HSP-16.1 increased by HSF-1 prevents cell death resulting from Ca^{2+} leakage from the golgi to the cytosol through prevention the denaturation of PMR-1, a Ca^{2+} golgi channel [13]. Therefore, it is expected that HSF-1 and HSPs may prevent damage from heat stress.

hsf-1 RNAi did not suppress the restoration of thrashing activity reduced by heat stress in *daf-2* mutants (Fig. 3). This result indicates that HSF-1 is not associated with the restoration of thrashing movement. In previous study, we indicated that DAF-16 is a key factor for the restoration of thrashing after heat stress [1]. However, *daf-16* KO didn't affect the reduction of thrashing under heat stress (Fig. 4B). Therefore, it was suggested that HSF-1 and DAF-16 induced thermotolerance *via* different mechanisms.

However, *hsf-1* RNAi inhibited the restoration of thrashing after heat stress (Fig. 1A). This may be because *hsf-1* RNAi exacerbates damage caused by heat stress. In fact, *hsf-1* RNAi accelerates the reduction of thrashing under heat stress in WT (Fig. 4A). Therefore, it is likely that damage aggravated by *hsf-1* interferes with the restoration of thrashing after heat stress.

In the previous study, heat stress significantly decreased the expression of *daf-28* and *ins-7*, which encode DAF-2 agonists [1]. In addition, HSF-1 activity was negatively regulated by the insulin/IGF-1-like signaling pathway [4–6]. Moreover, *hsf-1* RNAi suppressed the prevention of the heat stress-induced reduction in thrashing observed in *daf-2* mutants (Fig. 3). Therefore, it was expected that HSF-1 is activated by heat stress *via* inactivation of the insulin/IGF-1-like signaling pathway.

Above all, it was suggested that HSF-1 is activated by heat stress *via* inactivation of insulin/IGF-1-like signaling pathway and prevents movement disorder induced by heat stress. And it is expected that HSF-1 can prevent damage accumulation of heat stress.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Transparency document

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