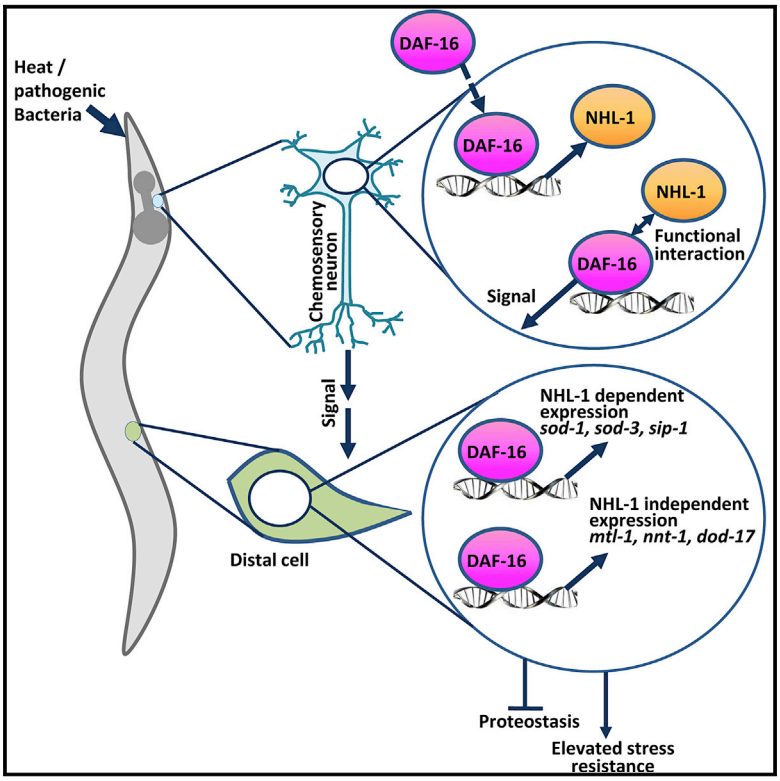
Article

Cell Reports

Differential Regulation of the Heat Shock Factor 1 and DAF-16 by Neuronal *nhl-1* in the Nematode *C. elegans*

Graphical Abstract



Highlights

- *nhl-1* is a DAF-16 cofactor that is required for stress resistance
- Insulin/IGF-1 signaling regulates *nhl-1* expression defining a regulatory circuit
- Neuronal *nhl-1* activates DAF-16, but not HSF-1, in remote tissues
- *nhl-1* knockdown has no effect on lifespan but protects from proteotoxicity

Authors

Yuli Volovik, Lorna Moll, ...,
Michal Bejerano-Sagie, Ehud Cohen

Correspondence

ehudc@ekmd.huji.ac.il

In Brief

The insulin/IGF-1 signaling cascade (IIS) regulates lifespan and stress resistance of *C. elegans*. Volovik et al. now find that NHL-1 acts in neurons to promote stress resistance in distal tissues by activating DAF-16 but not HSF-1. Thus, the activities of these transcription factors are differentially controlled by neurons.

Volovik et al., 2014, Cell Reports *9*, 2192–2205
December 24, 2014 ©2014 The Authors
http://dx.doi.org/10.1016/j.celrep.2014.11.028

OPEN ACCESS
CellPress

Cell Reports
Article

Differential Regulation of the Heat Shock Factor 1 and DAF-16 by Neuronal *nhl-1* in the Nematode *C. elegans*

Yuli Volovik,[1] Lorna Moll,[1] Filipa Carvalhal Marques,[1,2] Moria Maman,[1] Michal Bejerano-Sagie,[1] and Ehud Cohen[1,*]
[1]Department of Biochemistry and Molecular Biology, Institute for Medical Research Israel-Canada (IMRIC), Hebrew University School of Medicine, Jerusalem 91120, Israel
[2]Center of Ophthalmology and Vision Sciences (COCV), Institute for Biomedical Research in Light and Image (IBILI), Faculty of Medicine, University of Coimbra, Coimbra 3000-354, Portugal
*Correspondence: ehudc@ekmd.huji.ac.il
http://dx.doi.org/10.1016/j.celrep.2014.11.028

SUMMARY

In the nematode *Caenorhabditis elegans,* insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS) reduction hyperactivates the transcription factors DAF-16 and heat shock factor 1 (HSF-1), creating long-lived, stress-resistant worms that are protected from proteotoxicity. How DAF-16 executes its distinct functions in response to IIS reduction is largely obscure. Here, we report that NHL-1, a member of the TRIM-NHL protein family, acts in chemosensory neurons to promote stress resistance in distal tissues by DAF-16 activation but is dispensable for the activation of HSF-1. The expression of *nhl-1* is regulated by the IIS, defining a neuronal regulatory circuit that controls the organismal stress response. The knockdown of *nhl-1* protects nematodes that express the Alzheimer-disease-associated Aβ peptide from proteotoxicity but has no effect on lifespan. Our findings indicate that DAF-16- and HSF-1-regulated heat-responsive mechanisms are differentially controlled by neurons and show that one neuronal protein can be involved in the activation of different stress responses in remote tissues.

INTRODUCTION

The ability to maintain homeostasis is critical for organismal function and survival. Organisms have developed complex mechanisms that sense and are activated upon exposure to environmental insults to refold or degrade damaged proteins and restore functional protein homeostasis (proteostasis) (Balch et al., 2008). These mechanisms enable the organism to respond to unfavorable conditions by inducing the transcription of genes that encode protective proteins. Several transcription factors were found to promote stress resistance and maintain proteostasis, including heat shock factor 1 (HSF-1) (Sarge et al., 1993) and members of the forkhead (FOXO) family (reviewed in Partridge and Brüning, 2008).

DAF-16, the sole FOXO transcription factor of the nematode *Caenorhabditis elegans*, is critically required to resist stress conditions, including elevated temperature (Lithgow et al., 1995), oxidative stress (Honda and Honda, 1999), UV radiation (Murakami and Johnson, 1996), and exposure to pathogenic bacteria (Singh and Aballay, 2006). Similarly to HSF-1, DAF-16 activates the expression of chaperones, among them the group of heat shock proteins (hsp's) that play vital roles in stress resistance (Morimoto, 1998) and in the maintenance of proteostasis (Hartl et al., 2011). Members of the superoxide dismutase (sod) family, which are crucial for oxidative stress resistance, are also DAF-16 target genes (Panowski et al., 2007). In addition to their role as stress-resistance regulators, DAF-16 and HSF-1 govern the nematode's lifespan (Hsu et al., 2003; Kenyon et al., 1993; Morley and Morimoto, 2004) and are vital for protection from proteotoxicity (Cohen et al., 2006; Hsu et al., 2003; Morley and Morimoto, 2004).

In the nematode, the activities of HSF-1 and DAF-16 are negatively regulated by the highly conserved, aging-regulating, insulin/insulin-like growth factor (IGF) signaling pathway (IIS). Upon activation, the worm's sole IIS receptor, DAF-2, initiates a signaling cascade that facilitates the phosphorylation of DAF-16 (Henderson and Johnson, 2001; Lee et al., 2001). Phosphorylated DAF-16 is retained in the cytosol and prevented from regulating its target gene networks. To negatively regulate the activity of HSF-1, the IIS downstream kinases phosphorylate the HSF-1-interacting protein DDL-1, thereby controlling HSF-1 cellular localization (Chiang et al., 2012). Accordingly, IIS reduction hyperactivates DAF-16 and HSF-1, elevates stress resistance, protects from proteotoxicity, and extends lifespan.

The correlations between stress resistance, proteostasis, and longevity have suggested that these functions are mechanistically coupled. Although collapsed proteostasis (Ben-Zvi et al., 2009) and protein aggregation (David et al., 2010) are inherent aspects of aging, the functions downstream of the IIS were found to be separable. First, IIS reduction impedes proteotoxicity when it can no longer extend lifespan (Cohen et al., 2010). Second, the deletion of all five *sod* genes of the nematode hypersensitizes the worm to oxidative stress but does not affect lifespan (Van Raamsdonk and Hekimi, 2012). In addition, abolishing the worms' ability to activate the transcriptional

program known as the heat shock response (HSR) sensitizes the animals to heat but has no effect on lifespan (Maman et al., 2013). These studies raise the question of how DAF-16's distinct functions are differentially regulated at the cellular and organismal levels.

At the cellular level, DAF-16 activity relies on different cofactors. For instance, the transcription factor SMK-1 is critically required for the longevity phenotype of *daf-2* mutants and for their elevated resistance to oxidative stress and pathogenic bacteria, but it is dispensable for heat-stress resistance (Wolff et al., 2006).

At the organismal level, DAF-16 activation depends on intertissue communication mechanisms that control longevity, stress resistance, and proteostasis in a non-cell-autonomous fashion. The activation of the HSR of the worm is controlled by thermosensory (Prahlad et al., 2008) and chemosensory neurons (Maman et al., 2013). Similarly, the induction of the unfolded protein response in the endoplasmic reticulum (Taylor and Dillin, 2013) and the innate immune response (Styer et al., 2008) are also regulated by neurons. Interestingly, while DAF-16 regulates subsets of its target genes in a cell-autonomous manner, other genes are controlled by this transcription factor in a non-cell-autonomous fashion (Qi et al., 2012; Zhang et al., 2013).

Collectively, these studies indicate that DAF-16 interacts with various cofactors to differentially regulate its target genes in cell-autonomous and non-cell-autonomous manners. Although many proteins that physically interact with DAF-16 have been identified (Riedel et al., 2013), key aspects of DAF-16's functional network await examination.

Here, we employed the nematode *C. elegans* to search for DAF-16's stress-resistance-mediating partners and discovered that *nhl-1*, a member of the *trim-nhl* gene family that is expressed in chemosensory neurons, is critically required for the expression of DAF-16, stress-responsive target genes in distal tissues. Surprisingly, this protein has no role in the induction of HSF-1-regulated genes upon exposure to heat. The knockdown of *nhl-1* reduced the survival rates of worms that were exposed to heat, oxidative stress, or pathogenic bacteria but had no effect on survival after UV radiation. Furthermore, although *nhl-1* knockdown has no role in the regulation of lifespan, it provides partial protection from proteotoxicity.

This study unveils NHL-1 as a component of a neuronal mechanism that activates DAF-16 in distal tissues and indicates that DAF-16 and HSF-1 are activated in distal tissues by different neuronal mechanisms. Our discoveries strengthen the notions that the ability to respond to stress comes at the expense of proteostasis and that these functions are separable from longevity.

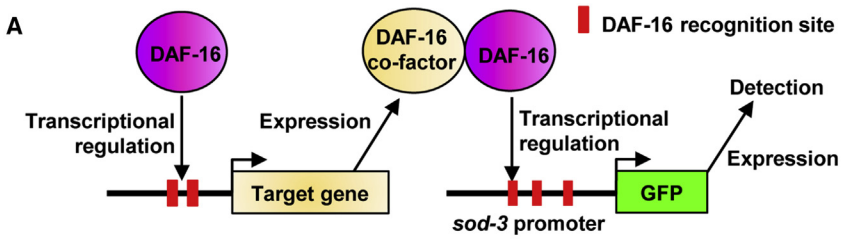
RESULTS

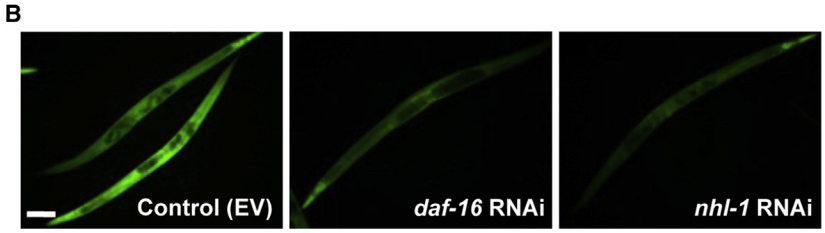
The Identification of *nhl-1* as a Genetic Cofactor of DAF-16

The activity of several signaling pathways is known to be regulated by feedback loops in which a transcription factor controls the expression of genes that encode its own cofactors. Among these, dFOXO, DAF-16's ortholog of the fruit fly *Drosophila melanogaster*, regulates the expression of some of its upstream effectors (Alic et al., 2011). We hypothesized that an analogous regulatory circuit exists in the worm and searched for novel DAF-16 functional cofactors utilizing a dual-step approach (Figure 1A). First, we searched for genes that harbor DAF-16 core recognition sites (TTGTTTa/g c/t or GTAAAc/t) (Furuyama et al., 2000) in their putative promoter regions (Dupuy et al., 2004). Next, we knocked down the expression of these genes by RNAi, asking which genes are required for the transcriptional activity of DAF-16. To address this, we employed *daf-2(e1370)* mutant nematodes that express GFP under the regulation of the *sod-3* promoter (strain CF1580). *sod-3* is a DAF-16 target whose putative promoter contains DAF-16 recognition sites (Figure S1A) (Honda and Honda, 1999) that encodes a mitochondrial Fe/Mn superoxide dismutase 3 (Hunter et al., 1997). Due to their weak *daf-2* allele, CF1580 animals exhibit low IIS activity that results in persistent DAF-16 hyperactivation and constant high GFP expression levels. Accordingly, RNAi-mediated knockdown of DAF-16 or of one of its critical cofactors is expected to lower the intensity of GFP signal in these animals.

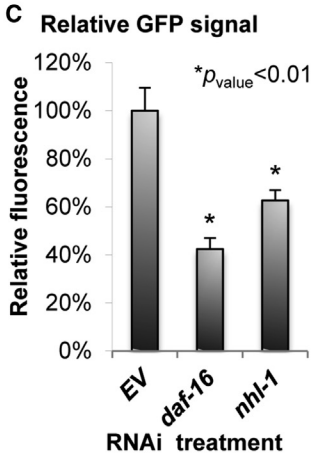
We discovered that the promoter region of the TRIM-NHL family member *nhl-1* contains two core DAF-16 recognition sites and two HSF-1 sites (Figure S1B). Similarly to the knockdown of *daf-16* (Libina et al., 2003), *nhl-1* RNAi-mediated knockdown significantly reduces the GFP signal in different worm tissues compared to worms that were grown on control bacteria that harbor the empty RNAi vector (EV) (Figures 1B, 1C, and S1C). Since *nhl-1* has sequence similarities with other *trim-nhl* family members in the nematode (Loedige and Filipowicz, 2009), we cloned the *nhl-1* 3′ UTR to create a second *nhl-1* RNAi bacterial strain (*nhl-1* 3′ UTR RNAi), which was used to ensure specificity of RNAi-mediated *nhl-1* knockdown. To further establish the observation that *nhl-1* is required for the activity of the *sod-3* promoter and to validate the specificity of this finding, we let CF1580 worms develop on EV bacteria or on bacteria expressing RNAi toward *daf-16*, *nhl-1*, or the *nhl-1* 3′ UTR and performed a western blot (WB) analysis using a GFP antibody. Our results (Figure 1D) confirmed that the knockdown of *nhl-1* reduces the activity of the *sod-3* promoter. We also measured the expression of *sod-3* in *daf-2(e1370)* mutant worms that were cultured on EV bacteria or on bacteria expressing RNAi toward either *daf-16 or nhl-1* by real-time quantitative PCR (qPCR) and found that both RNAi treatments resulted in similar effects of ~85% reduction in *sod-3* expression levels (Figure 1E).

To further explore the roles of NHL-1 as a DAF-16 cofactor, we examined whether the knockdown of *nhl-1* affects the expression of *sod-1*, an additional *sod* family member that is regulated by DAF-16 (Panowski et al., 2007), and *sip-1*, *mtl-1*, *dod-17*, and *nnt-1*, all known DAF-16 targets (Tepper et al., 2013). Using qPCR and *daf-2(e1370)* mutant worms, we found that the knockdown of either *daf-16* or *nhl-1* reduced the expression of *sod-1* by ~60% (Figure 1F). A similar effect was seen when the expression levels of *sip-1* were tested (Figures 1G and S1D). Surprisingly, the knockdown of *nhl-1* by either one of the two RNAi constructs had no significant effect on the expression levels of *mtl-1*, *nnt-1*, and *dod-17* (Figures 1H and S1E–S1G), indicating that *nhl-1* is required for DAF-16's transcriptional regulation of some of its target genes, but not of others.

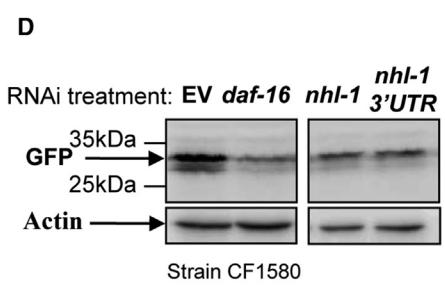




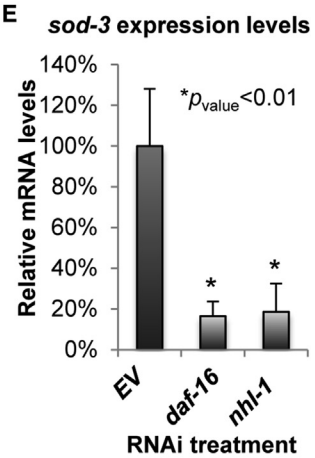
*sod-3*p::GFP in *e1370* background (Strain CF1580)



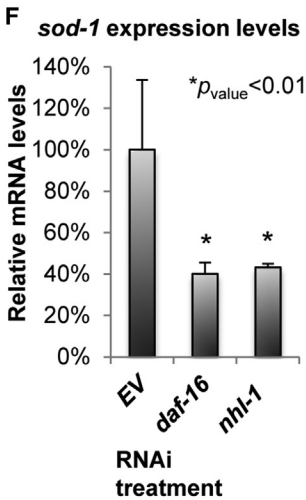
N=3, Strain CF1580

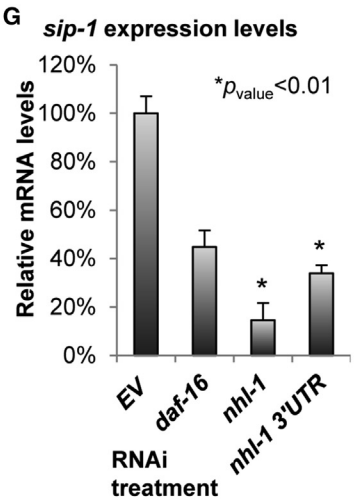


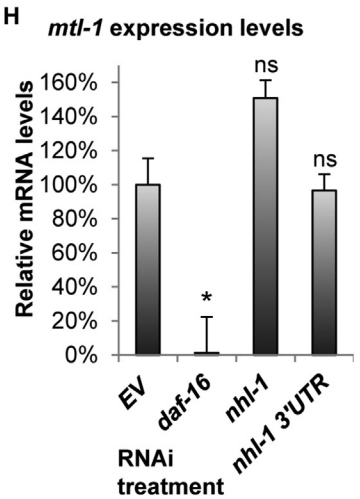
Strain CF1580



N=3, Strain *e1370*







N=3, Strain *e1370*

(legend on next page)

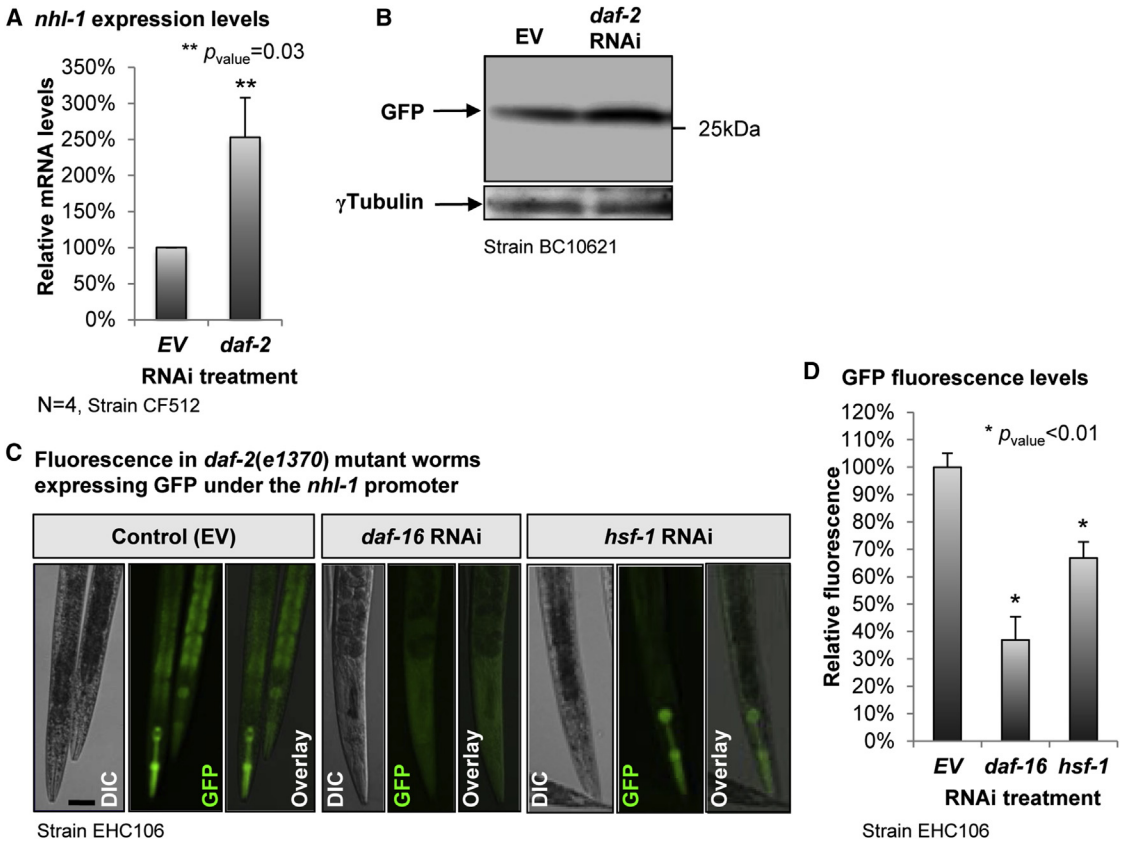


Figure 2. *nhl-1* Is a Transcriptional Target of DAF-16

(A) qPCR experiments revealed that the knockdown of *daf-2* by RNAi increases the expression of *nhl-1* by ~2.5-fold ($p = 0.03$).

(B) *daf-2* RNAi treatment leads to increased GFP levels in worms that express GFP under the regulation of the *nhl-1* promoter (strain BC10621) as evaluated by WB analysis.

(C) The knockdown of *daf-16* by RNAi reduces GFP expression in the pharynx and intestine of *daf-2(e1370)* mutant worms that express GFP under the regulation of the *nhl-1* promoter (strain EHC106). The knockdown of *hsf-1* by RNAi had a partial effect of reducing GFP levels in the intestine, but not in the pharynx (scale bar, 64 μm).

(D) GFP signal quantification in the upper third region of the worms' bodies validated these observations ($p < 0.01$).

Error bars represent SEM.

The IIS Regulates the Expression of *nhl-1*

To test whether DAF-16 regulates the expression of *nhl-1*, we used temperature-sensitive sterile worms (strain CF512) that exhibit natural IIS activity. These nematodes become sterile when exposed to 25°C during development (these conditions do not induce the HSR; Volovik et al., 2012), enabling the examination of *nhl-1* expression in adult worms with no background from embryos. We used qPCR to compare the relative *nhl-1* expression levels in worms that were grown from hatching to day 1 of adulthood on either EV or *daf-2* RNAi bacteria and found that IIS reduction notably increases *nhl-1* expression (Figure 2A). To further test this observation, we used worms that express GFP under the control of the *nhl-1* promoter (strain BC10621). BC10621 worms were left untreated (EV) or treated with *daf-2* RNAi throughout development, and the relative levels of GFP expression were compared by WB. Our results validated that IIS reduction increases the expression of *nhl-1* (Figure 2B), indicating that *nhl-1* is regulated by the IIS.

Figure 1. *nhl-1* Is Required for the Expression of DAF-16 Target Genes

(A) A schematic illustration of the rationale that underlies the search for DAF-16 cofactors.

(B–D) The knockdown of *nhl-1* by RNAi reduces the expression of *sod-3* promoter-driven GFP in *daf-2* mutant worms (CF1580) as visualized by fluorescent microscopy (scale bar, 100 μm) (B), fluorescence intensity quantification (C), and western blot analysis using two different bacterial strains expressing RNAi toward *nhl-1* (Ahringer library) and the *nhl-1* 3′ UTR region (D).

(E–G) qPCR analysis using *daf-2(e1370)* mutant worms that were treated throughout development with either *daf-16* or *nhl-1* RNAi confirmed the key role of *nhl-1* in the expression of *sod-3* (E), *sod-1* (F), and *sip-1* (G).

(H) *nhl-1* RNAi and the *nhl-1* 3′ UTR have no significant effect on the expression levels of *mtl-1*.

Error bars represent SEM.

To examine whether DAF-16 is a transcriptional regulator of *nhl-1*, we crossed BC10621 nematodes with *daf-2(e1370)* mutant worms and obtained animals that have permanently reduced IIS activity and thus overexpress GFP under the regulation of the *nhl-1* promoter (strain EHC106). The worms were grown on EV, *daf-16*, or *hsf-1* RNAi bacteria, and the relative rates of GFP fluorescence were visualized by fluorescent microscopy (Figures 2C and S2). The intensities of GFP signals in the upper region of the animal's body, where it is most prominent, were quantified using image-processing software (Figure 2D). Our results indicated that the knockdown of *daf-16* reduced GFP levels, confirming our prediction that DAF-16 regulates the expression of *nhl-1*. The knockdown of *hsf-1* also resulted in reduced GFP levels, which were foremost visible in the intestine (Figure 2C).

nhl-1 Is Differentially Required for Survival under Stress Conditions

The necessity of *nhl-1* for the expression of stress-resistance-mediating genes (Figure 1) implies that it is required for the worm's survival under stress conditions. The requirement of *nhl-1* for the expression of *sip-1* (Figure 1G), a gene that is needed for heat-stress resistance (Paz-Gómez et al., 2014), strongly suggests that *nhl-1* is needed for the worm to survive in elevated temperatures. To test that, CF512 animals were developed on either EV bacteria or treated with *nhl-1*, *daf-2*, or *daf-16* RNAi and exposed to 35°C, and their rates of survival were recorded after 15 hr. As expected, *daf-2* RNAi-treated worms exhibited an increased survival rate compared to their untreated counterparts (EV) (88% and 52%, respectively), while *daf-16* RNAi treatment reduced the survival rate (12%). Animals that were treated with *nhl-1* RNAi succumbed to heat stress similarly to their *daf-16* RNAi-treated counterparts (14% survival) (Figure 3A). Similar results were obtained when wild-type worms (strain N2) were treated with RNAi toward *daf-16*, *daf-2*, *nhl-1*, or *nhl-1* 3′ UTR (Figure S3A). Worms whose *nhl-1* was deleted (stain VC20) were also found to be more sensitive to heat compared to N2 worms (Figure S3B). Yet, the knockdown of *daf-2* by RNAi increased the survival rates of heat-stressed VC20 animals compared to their untreated counterparts, implying that reducing IIS activity also elevates heat-stress resistance by an *nhl-1*-independent mechanism.

To examine whether *nhl-1* is needed for the elevated heat-stress resistance of *daf-2*(*e1370*) mutant worms, we treated the animals from hatching with RNAi toward *daf-16*, *nhl-1*, or the *nhl-1* 3′ UTR or left them untreated (EV). At day 1 of adulthood, the worms were exposed to 35°C and the rates of survival within the worm groups were recorded 19 hr thereafter. While ~50% of the control animals (EV) survived, the knockdown of either *nhl-1* or *daf-16* had similar effects of significantly reducing the survival rates to less than 15% (Figure S3C), indicating that the knockdown of *nhl-1* also abolishes the elevated heat-stress resistance conferred by reduced IIS. Collectively, these results indicate that *nhl-1* is crucially required for heat-stress resistance.

Next, we sought to explore whether *nhl-1* is also required for oxidative stress resistance. *daf-2(e1370)* mutant worms that were developed on either EV bacteria or *daf-16* or *nhl-1* RNAi were exposed to 0.25 M paraquat and their rates of survival followed over time. While worms of the control group (EV) exhibited 61% survival after 29 hr, the knockdown of *daf-16* or *nhl-1* by RNAi resulted in a significant reduction of survival to 19% and 23%, respectively (Figure 3B; $p < 0.01$).

To assess whether *nhl-1* is required for survival after a sublethal dose of UV radiation, we radiated day 1 adult *daf-2(e1370)* mutant worms that were left untreated (EV) or treated with *daf-16* or *nhl-1* RNAi and recorded their rates of survival daily. While *daf-16* RNAi-treated worms exhibited reduced survival (Figure 3C; mean survival 5.24 ± 1.63 days), *nhl-1* RNAi-treated nematodes and those fed with EV bacteria exhibited similar survival rates (mean survival of 7.92 ± 2.27 and 7.54 ± 2.1 days, respectively), indicating that *nhl-1* has no role in the IIS-mediated protection from UV radiation. This conclusion was supported by the observation that the knockdown of *daf-2* by RNAi promotes extended survival of animals that lack *nhl-1* (strain VC20) after exposure to UV radiation while treatment with *daf-16* RNAi reduces their rate of survival (Figure S3D).

The pathogenic bacteria *Pseudomonas aeruginosa* mitigates the natural resistance of *C. elegans* by activating the IIS (Evans et al., 2008). Accordingly, IIS reduction confers relative resistance to these bacteria in DAF-16- and HSF-1-dependent manners (Singh and Aballay, 2006). This effect is controlled by sensory neurons (Sun et al., 2011). The recent report that resistance to heat stress and pathogenic bacteria are interrelated (Ermolaeva et al., 2013) and the necessity of *nhl-1* for heat-stress resistance led us to anticipate that this gene is also required for the survival of worms fed with *P. aeruginosa.* To examine that, we treated *daf-2(e1370)* mutant worms throughout development with either *daf-16* or *nhl-1* RNAi or left them untreated (EV), transferred them onto plates seeded with *P. aeruginosa*, and followed their rates of survival. *daf-16* RNAi-treated worms (mean survival 10.37 ± 0.83 days) and animals that were treated with *nhl-1* RNAi (mean survival 9.40 ± 1.01 days) exhibited significantly reduced survival compared to the control group (mean survival 13.81 ± 0.86 days; Figures 3D and S3E; $p < 0.01$ for both *daf-16* and *nhl-1* RNAi-treated animals compared to EV worms). Taken together, our results show that *nhl-1* is essential for the innate immune mechanism downstream of the IIS.

Since IIS reduction extends lifespan, we asked whether *nhl-1* is also required for this phenotype. CF512 worms were treated throughout life with RNAi toward *nhl-1*, *daf-2*, or *daf-16* or left untreated (EV), and their survival rates were followed. While *daf-2* RNAi-treated worms were long-lived and their *daf-16* RNAi-treated counterparts exhibited a short lifespan, no difference in lifespan between the control group (EV) and *nhl-1* RNAi-treated animals could be seen (Figures 3E and S3F; Table S1). Similarly, the knockdown of *nhl-1* by RNAi did not shorten the lifespan of long-lived *daf-2(e1370)* mutant animals (Figure 3F; Table S1), indicating that this gene is dispensable for the promotion of longevity by IIS reduction.

Finally, we examined whether the knockdown of *nhl-1* affects IIS-regulated dauer formation and found that, unlike *daf-16*, it has no effect on this feature (Figure S3G).

nhl-1 Is Dispensable for the Induction of HSF-1 Target Genes upon Exposure to Heat

The central role of HSF-1 in the promotion of heat-stress resistance (Sarge et al., 1993) raised the prospect that its activation

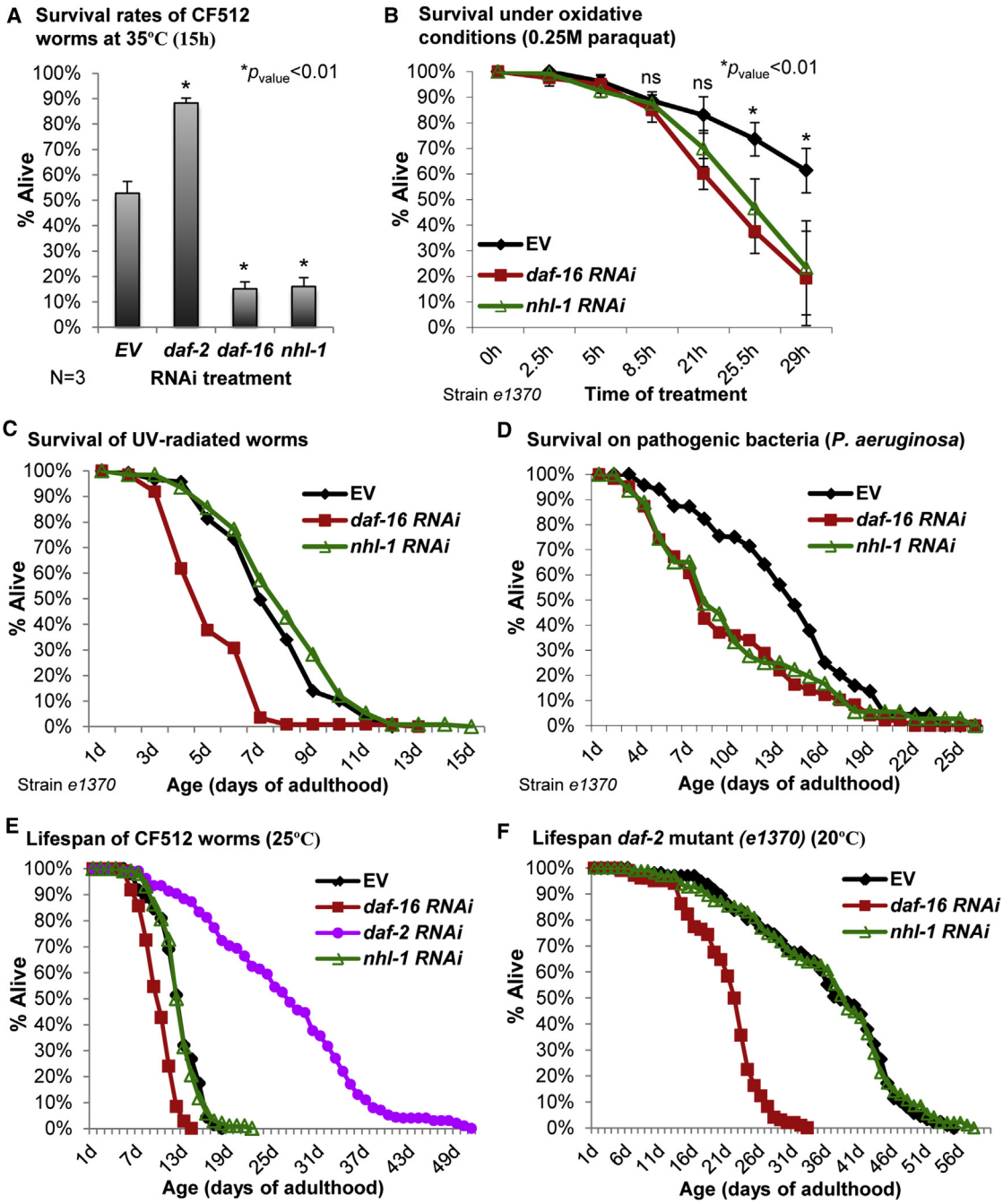


Figure 3. Differential Requirements of *nhl-1* for Survival under Different Stress Conditions

(A) While *daf-2* RNAi-treated CF512 animals exhibited an elevated survival rate after 15 hr of exposure to 35°C compared to the control group (survivals of 88% and 53%, respectively), the knockdown of either *daf-16* or *nhl-1* resulted in reduced survival rates (15% and 16%, respectively).

(B) *daf-2(e1370)* mutant worms treated with either *daf-16* or *nhl-1* RNAi exhibit significantly lower survival rates under long exposure to oxidative stress (0.25 M paraquat) compared to untreated animals ($p < 0.01$).

(C) Unlike *daf-16* RNAi treatment, the knockdown of *nhl-1* had no effect on the survival rates of *daf-2(e1370)* mutant worms that were exposed to sublethal UV radiation.

(D) *daf-2(e1370)* mutant worms that were treated during development with *nhl-1* or *daf-16* RNAi exhibited reduced survival rates (mean survival of 9.4 ± 1.01 days and 10.37 ± 0.83 days, respectively) when grown on pathogenic bacteria (*P. aeruginosa*) compared to untreated animals (mean survival of 13.81 ± 0.86 days, $p < 0.01$).

(E and F) The knockdown of *nhl-1* does not affect the lifespan of CF512 (E) and *daf-2(e1370)* mutant worms (F).

Error bars represent SEM.

upon exposure to heat may also require *nhl-1*. To test this hypothesis, we examined whether the knockdown of *nhl-1* by RNAi prevents the induction of HSF-1-regulated hsps after heat stress. We employed worms that express GFP under the regulation of the *hsp-16.2* promoter (strain CL2070), a well-defined, HSF-1-regulated, small hsp that is primarily expressed in the worm's intestine (Link et al., 1999). CL2070 animals were either left untreated (EV) or treated throughout development with RNAi toward *hsf-1*, *nhl-1*, or *nhl-1* 3′ UTR. At day 1 of adulthood, the worms were exposed to heat shock (33°C for 3 hr) and the levels of GFP were visualized. Unlike the knockdown of *hsf-1*, knocking down the expression of *nhl-1* by either one of the two RNAi bacterial strains had no effect on the activity of the *hsp-16.2* promoter (Figure 4A). WB analysis using a GFP antibody confirmed this observation (Figure 4B). qPCR analysis using primers toward *hsp-16.1*, a close *hsp-16.2* homolog, and heat-stressed CF512 worms that were treated with the RNAi bacterial strains as described above indicated that *nhl-1* also has no role in the regulation of *hsp-16.1* (Figure S4).

To expand our analysis, we utilized nematodes that express GFP under the regulation of *hsp-70* (*C12C8.1*) promoter (Snutch et al., 1988). The worms were treated with RNAi and exposed to heat, as above, and GFP levels were visualized by fluorescent microscopy. Our results (Figure 4C) indicated that the knockdown of *nhl-1* has no effect on the induction of the *hsp-70* promoter after heat shock. Similarly, qPCR using *daf-2(e1370)* mutant worms confirmed that the knockdown of *nhl-1* has no effect on the induction of *hsp-70* in heat-stressed animals (Figure 4D).

The dispensability of *nhl-1* for the induction of HSF-regulated chaperones suggested that the heat sensitivity of worms that were grown on *nhl-1* RNAi emanates from reduced expression of DAF-16 target genes. To test that, we treated *daf-2(e1370)* mutant worms with RNAi toward either *sod-1* or *sod-3*, exposed them for 19 hr to 35°C, and found that these genes are needed for the heat-resistance phenotype of these mutant animals (Figure 4E).

Taken together, our results show that *nhl-1* is unessential for the activation of HSF-1 upon exposure to heat and that the knockdown of this gene reduces heat-stress resistance by mitigating the expression of DAF-16-regulated genes. Our results also imply that activation of DAF-16 and HSF-1 are separately regulated.

The Expression of *nhl-1* in Chemosensory Neurons Confers Its Stress-Resistance Functions

The key roles of neurons in HSR activation (Maman et al., 2013; Prahlad et al., 2008) and in innate immunity (Styer et al., 2008) raised the prospect that NHL-1 executes its counterstress functions in neurons. To examine that, we created worms that express the red fluorescent protein tdTomato under the regulation of the *nhl-1* promoter (strain EHC107). Microscopic visualization of EHC107 worms revealed punctuated red signal in the pharyngeal area (Figures S5A and S5B, arrows), suggesting that *nhl-1* is mainly expressed in neurons. Since the features of different fluorescent proteins used as expression markers may differ, we also sought to determine whether GFP driven by the *nhl-1* promoter is expressed in neurons. By visualizing *daf-2* RNAi-treated BC10621 animals, we identified a prominent GFP signal, indicative of *nhl-1* promoter activity in juxtapharyngeal foci (Figure 5A, arrows), corroborating the theme that the activity of *nhl-1*'s promoter is elevated in neurons.

However, the limited penetrance of RNAi to neurons (Timmons et al., 2001) and the dramatic effect of *nhl-1* RNAi on heat-stress resistance (Figure 3A) raised the question of whether neuronal or nonneuronal *nhl-1* expression regulates stress resistance. To distinguish between these possibilities, we used worms that exhibit an efficient RNAi-mediated gene knockdown in neurons, but not in other cell types (Calixto et al., 2010). In these animals (strain TU3335), the gene *sid-1*, which encodes a channel-forming protein that enables double-stranded RNA (dsRNA) molecules to penetrate cells (Winston et al., 2002), is regulated by the panneuronal promoter *unc-119*.

TU3335 worms were hatched and developed on EV bacteria or on bacteria expressing dsRNA toward *daf-2*, *daf-16*, *hsf-1*, *nhl-1*, or *gcy-8*. *gcy-8* is exclusively expressed in the amphid neurons with finger-like (A*f*D) ciliated endings (AFD) thermosensory neurons, where it is critical for HSR activation in remote tissues and heat-stress resistance (Prahlad et al., 2008). Thus, *gcy-8* RNAi served as a control for gene knockdown in neurons. All the worm groups were exposed to 35°C for 10 hr, and survival rates were recorded. Three independent experiments revealed that worms treated with either *daf-16* or *hsf-1* RNAi and untreated animals exhibited similar rates of survival of ~75%, implying that these transcription factors execute their heat-resistance functions primarily in nonneuronal tissues. Interestingly, the knockdown of *daf-2* did not show any effect of survival, proposing that this receptor also functions in other tissues to confer heat-stress resistance. In contrast, worms treated with either *nhl-1* or *gcy-8* RNAi exhibited increased heat sensitivity, as merely 39% and 28% of the worms survived, respectively (Figure 5B). The analogous effects of *nhl-1* and *gcy-8* RNAi treatments on heat sensitivity proposed that the activity of *nhl-1* in neurons confers heat-stress resistance.

The activity of DAF-16 in neurons was shown to have no effect on the expression level of its target gene, *hsp-12.6*, in other worm tissues (Zhang et al., 2013). We exploited this feature to examine whether NHL-1 is required for DAF-16's transcriptional activity exclusively in neurons by analyzing the relative fluorescence levels in worms that express tdTomato under the control of the *hsp-12.6* promoter (strain EHC102). EHC102 worms were left untreated (EV) or treated with RNAi toward either *daf-16* or *nhl-1* throughout development and exposed to heat shock (33°C, 3 hr), and tdTomato fluorescence levels were visualized and quantified. If NHL-1 affects the activity of DAF-16 exclusively in neurons, one would expect the *hsp-12.6* promoter to exhibit similar activity levels in both untreated and *nhl-1* RNAi-treated animals. However, if NHL-1 serves as DAF-16 cofactor also in nonneuronal tissues, the tdTomato fluorescence levels should be lower in *nhl-1* RNAi-treated worms compared to those of untreated animals. Measuring fluorescence levels of at least 18 animals per treatment, we found that unlike *daf-16*, the knockdown of *nhl-1* by RNAi had no effect on the activity of the *hsp-12.6* promoter (Figures 5C and S5C). qPCR analysis of *hsp-12.6* expression

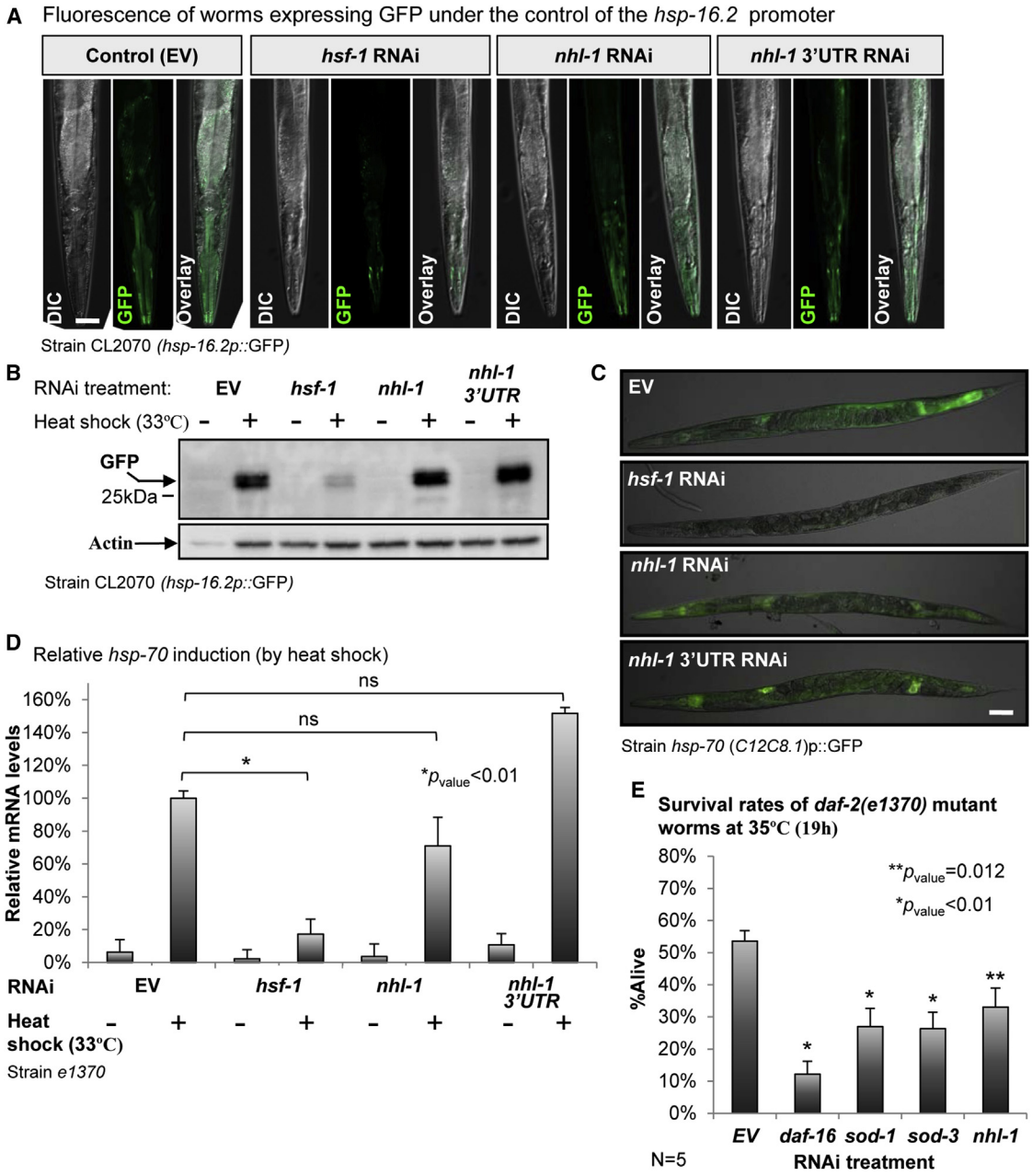


Figure 4. *nhl-1* Is Dispensable for the Induction of HSF-1 Target Heat-Responsive Genes

(A and B) Direct visualization (A) and WB analysis (B) indicated that the expression of GFP is analogously induced by heat (33°C, 3 hr) in untreated CL2070 worms (*hsp-16.2p*::GFP) and in their counterparts that were treated with either one of two *nhl-1* RNAi strains. In contrast, worms that were treated with *hsf-1* RNAi exhibited remarkably reduced GFP levels (scale bar, 25 μm).

(C) *nhl-1* RNAi-treated worms that express GFP under the regulation of the *hsp-70* promoter (*hsp-70* (*C12C8.1*)*p*::GFP) and their untreated counterparts exhibited similar levels of GFP fluorescence after exposure to heat stress (33°C, 3 hr). In contrast, *hsf-1* RNAi treatment resulted in reduced GFP fluorescence (scale bar, 64 μm).

(D) qPCR confirmed that the knockdown of *nhl-1* does not affect the induction of *hsp-70* in heat-stressed *daf-2(e1370)* mutant worms.

(E) The knockdown of *sod-1*, *sod-3*, or *nhl-1* by RNAi mitigates the heat resistance typical of *daf-2(e1370)* mutant worms.

Error bars represent SEM.

levels in heat-stressed CF512 worms that were treated with *daf-16*, *nhl-1*, 3′ UTR *nhl*-1 RNAi or left untreated verified our results (Figure 5D).

We also crossed CF1580 worms with TU3335 animals to obtain nematodes that express GFP under the regulation of the *sod-3* promoter and are hypersensitive to RNAi in neurons. The

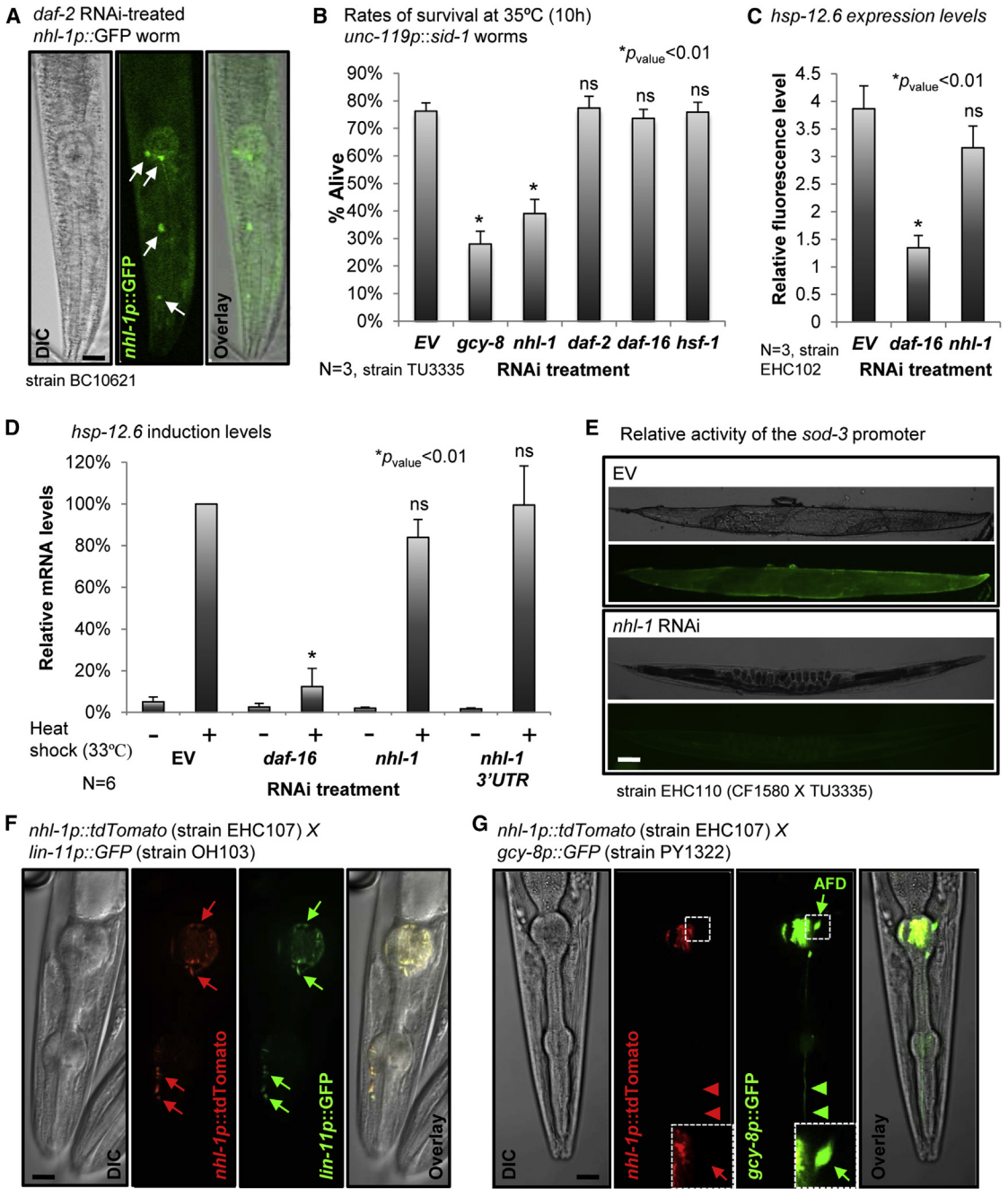


Figure 5. *nhl-1* Executes Its Stress-Resistance-Regulating Functions in Chemosensory Neurons

(A) *daf-2* RNAi-treated BC10621 worms (*nhl-1p*::GFP) exhibit elevated GFP expression in juxtapharyngeal neurons (arrows) (scale bar, 18 μm).

(B) TU3335 worms (*unc-119p*::*sid-1*) that were treated with either *gcy-8* or *nhl-1* RNAi show significantly reduced survival rates (28% and 39%, respectively) after 10 hr of exposure to heat (35°C) compared to untreated worms (76% survival). Identical worms that were treated with RNAi toward *daf-2*, *daf-16*, or *hsf-1* RNAi had survival rates similar to the control group (77%, 74%, 76%, and 76% survival, respectively).

(C) EHC102 worms (*hsp-12.6p*::tdTomato) were treated with either *nhl-1* or *daf-16* RNAi throughout development and exposed to heat (33°C, 3 hr). Unlike *daf-16* RNAi-treated animals, the knockdown of *nhl-1* by RNAi had no significant effect on the level of fluorescence.

(D) CF512 worms were grown on RNAi bacteria toward *daf-16*, *nhl-1*, *nhl-1* 3′ UTR or left untreated (EV). Half of each RNAi-treated worm group was exposed to heat, while the other half was left unstressed. qPCR experiments confirmed that the knockdown of *nhl-1* has no effect on the induction of *hsp-12.6*.

(E) *nhl-1* RNAi-treated EHC110 worms (a cross of CF1580 and TU3335 animals) show lower rate of GFP fluorescence compared to their untreated counterparts (scale bar, 64 μm).

(legend continued on next page)

worms were treated with RNAi toward *nhl-1* or left untreated, and rates of GFP fluorescence in the intestine were compared. Our results (Figure 5E) support the notion that the knockdown of *nhl-1* in neurons reduces the activity of the *sod-3* promoter in somatic tissues.

Collectively, the increased activity of the *nhl-1* promoter in neurons following IIS reduction, the key role of *nhl-1* in heat-shock resistance of TU3335 animals, and the unaffected *hsp-12.6* expression in CF512 nematodes that were treated with *nhl-1* RNAi indicate that NHL-1 serves as DAF-16 cofactor in neurons.

We then sought to identify the specific set of neurons in which *nhl-1* is expressed. EHC107 worms were crossed with animals that express GFP under the regulation of promoters that are active in specific sets of HSR-regulating neurons. First, we used worms that express GFP under the regulation of the *lin-11* promoter (strain OH103), which is active in the head chemosensory neurons ADF and ADL as well as in the AIZ, AVG, and RIC interneurons and is required for the activity of the thermoregulatory neuronal network (Hobert et al., 1998). By visualizing the resulting worms, we discovered that the promoters of *lin-11* and of *nhl-1* are active in the same cells (Figure 5F), indicating that both genes are expressed in chemosensory neurons. In contrast, the expression pattern of *nhl-1* was different than that of the AFD-specific marker *gcy-8* (Figure 5G).

Since the knockdown of neuronal, HSR-activating genes protects model worms from proteotoxicity (Maman et al., 2013; Prahlad and Morimoto, 2011), we predicted that knocking down *nhl-1* would have a similar effect. To test that, we employed worms that express the Alzheimer disease-associated, human Aβ peptide in their body wall muscles (Aβ worms, strain CL2006; Link, 1995) and exhibit progressive paralysis emanating from Aβ aggregation. Aβ worms were treated from hatching with RNAi toward *daf-2* or *nhl-1* or left untreated (EV), and the rates of paralysis were followed daily. Our results (Figures 6A and S6A) show that the knockdown of *nhl-1* ameliorates Aβ toxicity. Three independent paralysis assays confirmed the significance ($p = 0.03$) of this observation (Figure 6B).

To further scrutinize the theme that neuronal *nhl-1* modulates Aβ toxicity in the muscle in a non-cell-autonomous manner, we crossed TU3335 worms with Aβ worms to obtain animals that display RNAi hypersensitivity in neurons and low sensitivity in other tissues and express Aβ in their muscles (strain EHC109). Synchronized worm populations were treated with RNAi toward *daf-2*, *nhl-1*, or the *nhl-1* 3′ UTR, and the rates of paralysis were followed. We found that an efficient knockdown of *nhl-1* in neurons enhances the counterproteotoxic effect to be similar to that of *daf-2* RNAi (Figure 6C).

DISCUSSION

How DAF-16 executes its distinct functions and how they are regulated at the cellular and organismal levels are only partially understood. Here, we searched for DAF-16 functional cofactors and discovered that *nhl-1* is required for the expression of stress-resistance-mediating DAF-16 target genes, members of the *sod* family, in remote tissues. Upon exposure to elevated temperature or pathogenic bacteria (Figure 7I), DAF-16 elevates the expression level of *nhl-1* (II), which in turn participates in the activation of the neuronal signaling mechanism (III) that activates DAF-16 (IV), but not HSF-1 (V), in distal tissues. The activation of DAF-16 confers the induction of a subset of target genes, including members of the *sod* gene family and the chaperone *sip-1*, but not *hsp-12.6*, *mtl-1*, and *nnt-1*. This induction elevates resistance to heat and to pathogenic bacteria (VI) and provides partial protection from proteotoxicity (VII) but has no role in the regulation of lifespan (VIII).

In *C. elegans*, the *nhl* gene family consists of five members: *nhl-1*, *nhl-3*, and *ncl-1*, which are close homologs, and *lin-41* and *nhl-2*, which are more distant family members (Loedige and Filipowicz, 2009). The TRIM-NHL proteins contain RING fingers, zinc fingers, and NHL repeats. The presence of a RING domain suggests that TRIM-NHL proteins act as E3 ubiquitin ligases. Functional assays confirmed that the mammalian TRIM32 exhibits an E3 ubiquitin ligation activity (Kudryashova et al., 2005). However, members of this protein family are involved in additional biological activities. A recent study unveiled that in worms, *nhl-2* is needed for the regulation of gene expression by microRNA (miRNA) (Hammell et al., 2009). In accordance, TRIM32 was also found to be involved in the regulation of miRNA processing in mice through its NHL domain (Schwamborn et al., 2009). Importantly, mutations in TRIM32 are linked to the emergence of the early-onset human hereditary muscle disease limb-girdle muscular dystrophy type 2H (Kudryashova et al., 2005), directly linking the TRIM-NHL family to functional homeostasis in humans.

Together, these studies indicate that TRIM-NHL proteins are involved both in the regulation of gene expression and in proteostasis. However, key aspects of *nhl-1*'s functions as a modulator of stress resistance remain unanswered. Does *nhl-1* direct a negative stress-response regulator for degradation? Does it physically interact with DAF-16? What type of neuronal signaling mechanisms are affected by this gene product?

The Neuronal DAF-16- and HSF-1-Regulating Mechanisms Are Separable

Although neurons were already shown to regulate stress-response mechanisms in remote tissues (Prahlad et al., 2008; Sun et al., 2011), this study unveiled an aspect of neuron-mediated control of stress resistance. We have discovered that knockdown of *nhl-1* by RNAi prevents the induction of stress-resistance-mediating DAF-16 target genes but has no effect on HSF-1-regulated hsp's. This is a particularly interesting finding,

(F) Microscopic visualization of worms that express tdTomato under the regulation of the *nhl-1* promoter (*nhl-1p*::tdTomato) and GFP under the control of *lin-11* promoter (*lin-11p*::GFP) revealed colocalization of the fluorescent signals, indicative of *nhl-1* expression in chemosensory neurons.
(G) No *nhl-1* promoter activity (red) is detected in AFD neurons of worms that express GFP in these cells (regulated by the *gcy-8* promoter) and tdTomato under the control of the *nhl-1* promoter (scale bar, 18 μm).
Error bars represent SEM.

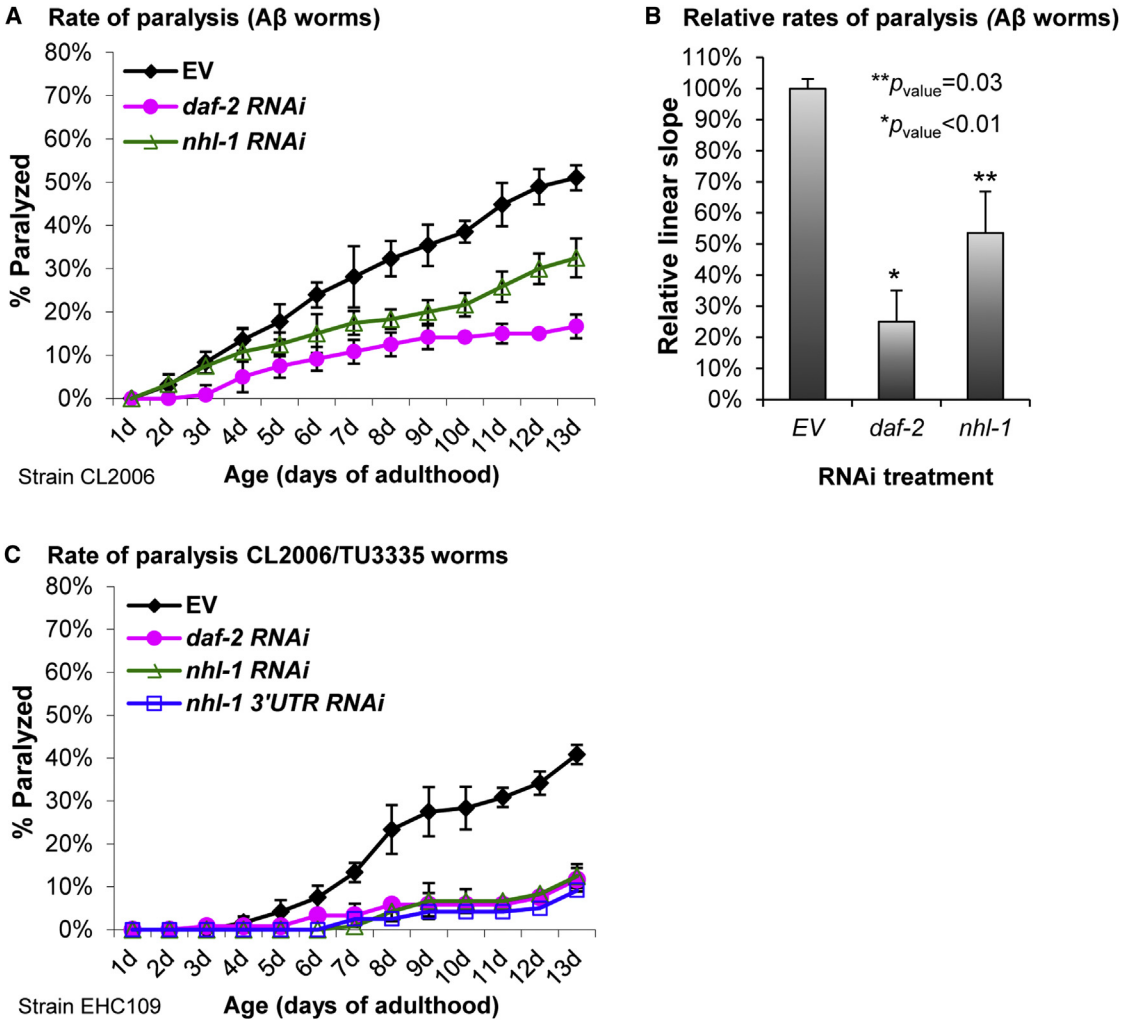


Figure 6. The Knockdown of *nhl-1* Provides Partial Protection from Aβ Proteotoxicity

(A) *nhl-1* RNAi-treated CL2006 worms show a rate of paralysis lower than the control group (EV) but higher than *daf-2* RNAi-treated animals.

(B) The counterproteotoxic effect of *nhl-1* RNAi treatment was confirmed by three independent paralysis assays. Bars represent the relative slopes of the paralysis graphs as in (A) ($p = 0.03$).

(C) The knockdown of *nhl-1* in EHC109 worms (a cross of CL2006 and TU3335 animals) abolished the Aβ-associated paralysis phenotype.

Error bars represent SEM.

as the knockdown of the putative GPCR *gtr-1*, which, similarly to *nhl-1*, is expressed in *lin-11*-expressing neurons, governs the activities of both transcription factors DAF-16 and HSF-1 in distal tissues (Maman et al., 2013). It is important to mention that although *nhl-1* is dispensable for the induction of HSF-1 target genes, the knockdown of *hsf-1* has a small effect on the expression of *nhl-1* (Figure 2D). Our results imply that chemosensory neurons induce a stress response in remote tissues in response to heat by at least two signaling mechanisms. One possibility suggests that *gtr-1* and *nhl-1* play their roles through separate signaling mechanisms. An alternative model proposes that the activation of HSF-1 and DAF-16 is regulated by one mechanism that diverges into two arms. According to this hypothesis, *gtr-1* functions upstream of the splitting point while *nhl-1* acts downstream of this junction in the arm that exclusively regulates DAF-16.

Why is more than one mechanism required for the activation of stress-resistance mechanisms? It is plausible that complex regulatory mechanisms are required to allow the worm to specifically orchestrate the activation of tailored responses to dissimilar challenges.

How do the stress-response-activating neuronal signaling mechanisms differ? It is possible that distinct signals are carried by different types of vesicles. In this regard, it will be important to test whether each of these signaling mechanisms, downstream of *gtr-1* and *nhl-1*, rely on *unc-13*-mediated secretion of small clear vesicles containing neurotransmitters (Madison et al., 2005) or on *unc-31*-dependent secretion of dense core vesicles that mediate the transmission of neuropeptides (Speese et al., 2007). Since proteostasis-maintaining signals secreted by AFD neurons are *unc-31* dependent (Prahlad and Morimoto, 2011), it seems more likely that *nhl-1*-mediated signals are transmitted by neuropeptides.

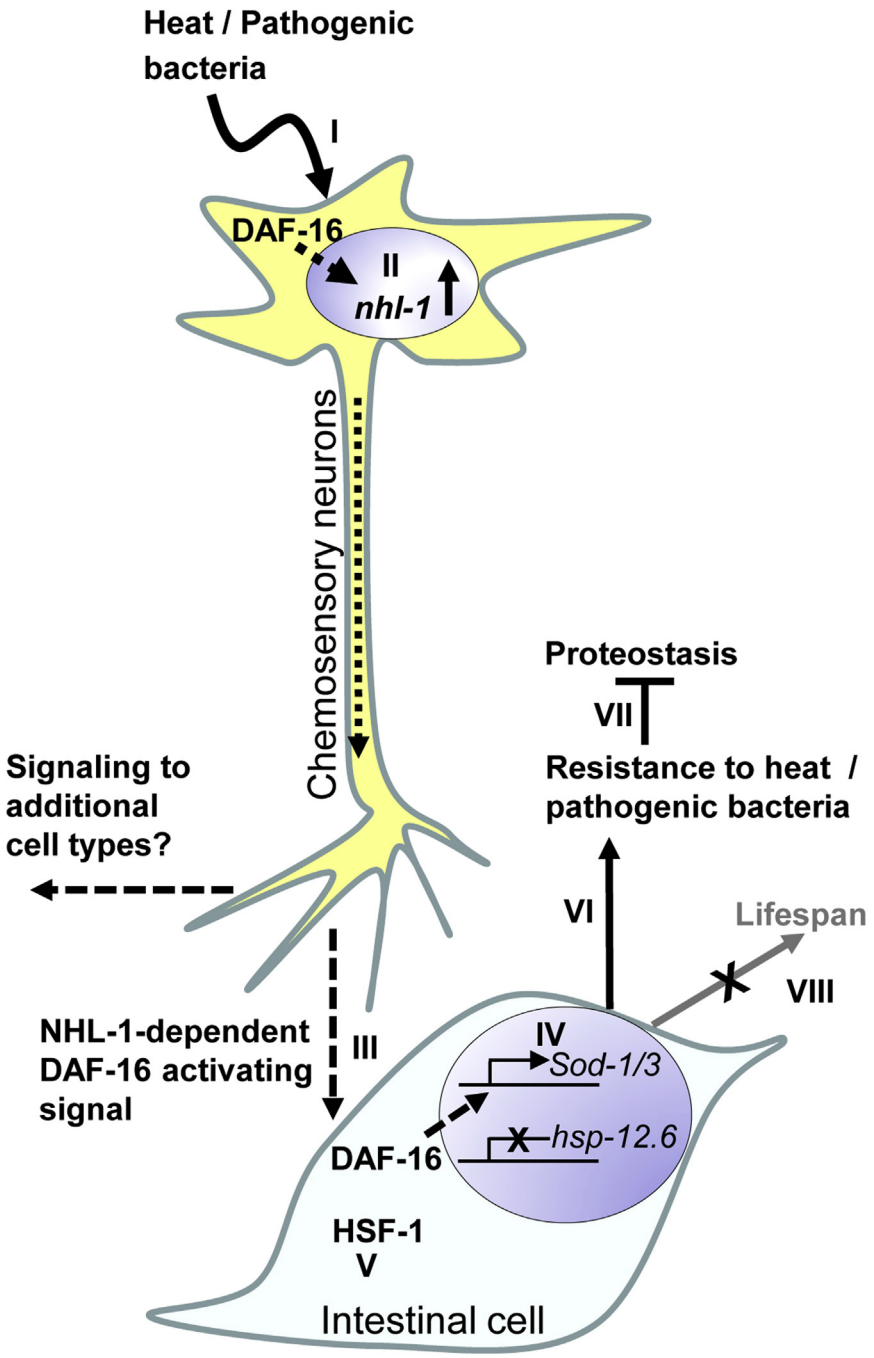


Figure 7. A Model for the Roles of *nhl-1* in the Mediation of Stress Resistance

Upon exposure to heat stress, oxidative conditions, or pathogenic bacteria, DAF-16 enters the nuclei of chemosensory neurons (I) and induces the expression of *nhl-1* (II), among other genes. NHL-1 is involved in the activation of a neuronal signaling cascade that specifically activates DAF-16 (III), but not HSF-1 (IV), in acceptor cells. Activated DAF-16 confers the expression of subsets of its target genes including *sod-3* but excluding *hsp-12.6* (V) that facilitate stress resistance (VI). The worms' ability to resist heat stress comes at the expense of proteostasis (VII). The mechanism downstream of NHL-1 has no role in the regulation of lifespan (VIII).

Different Stress-Response Mechanisms Are Regulated by Chemosensory Neurons

While the knockdown of *gtr-1* does not affect the worm's resistance to *P. aeruginosa* (Maman et al., 2013), *nhl-1 is* needed for survival of worms cultured on these pathogenic bacteria (Figure 3D). This suggests that *nhl-1* is involved in the activation of more than one signaling cascade and infers that chemosensory neurons have a repertoire of signals that can activate different stress-response mechanisms in nonneuronal cells. The ability of specific worm neurons to send more than one type of signal in response to different insults has been described previously. PVD neurons use different mechanisms to respond to low temperature and mechanical stimuli (Chatzigeorgiou et al., 2010). A detailed biochemical analysis will be needed to characterize whether *nhl-1* activates different signaling mechanisms.

The normal lifespan of *nhl-1* RNAi-treated worms, the full longevity phenotype of *daf-2 (e1370)* mutant worms, and the partial protection from proteotoxicity conferred by *nhl-1* RNAi support the idea that stress resistance is separable from longevity and that the ability to respond to heat comes at the expense of proteostasis. Accordingly, it may be possible to develop therapies based on selective manipulations of neuronal signaling mechanisms to postpone the manifestation of proteotoxicity-mediated maladies.

EXPERIMENTAL PROCEDURES

Worm and RNAi Strains

N_2 (wild-type, Bristol), *daf-2(e1370)* mutant worms, TU3335 (*unc-119*p::YFP + *unc-119*p::sid-1 + *mec-6p*::mec-6), CL2006 (*unc-54p::*human $A\beta_{1-42}$), PY1322 (*gcy-8*p::GFP), OH103 (*lin-11*p::GFP), as well as CL2070 *(hsp-16.2p*::GFP) worms were obtained from the Caenorhabditis Genetics Center (Minneapolis, MN). The worms were grown at 20°C. CF512 (*fer-15*(b26)II; *fem-1*(hc17)IV) animals are heat-sensitive sterile and were routinely grown at 15°C. To avoid progeny, CF512 worms were hatched at 20°C and L1 larvae transferred to 25°C for 48 hr and back to 20°C until harvested. To reduce gene expression, we used bacterial strains expressing dsRNA: empty vector (pAD12), *daf-2* (pAD48), *daf-16* (pAD43). *nhl-1*, *hsf-1*, and *gcy-8* dsRNA-expressing bacteria were from an RNAi library (J. Ahringer). *nhl-1* 3′ UTR RNAi was prepared by amplifying the sequence using PCR (forward: 5′-ACA AAG CTT TAT TGA AAC TCG AAA ACA GAA G-3′; reverse: 5′-CCGTTTTTATTTTTAATTTTCGGATCTACCATGAGACTGGCAGT

GGTTTGC-3′) and cloning it into the pL4440 plasmid. RNAi bacteria strains were grown at 37°C in Luria broth with 100 μg/ml ampicillin and then seeded onto nematode growth (NG)-ampicillin plates supplemented with 100 mM isopropyl β-D-1-thiogalactopyranoside (IPTG; 4 mM final concentration).

The Creation of Worm Strains and RNAi Constructs

The following primers were used to amplify the promoter region of *nhl-1* (including restriction sites): forward, 5′-CAT GGATCCTACTTCGGAGAGA TTTCCATTTTC-3′; reverse, 5′-GTG GAGAACTGCTGATAAGGATTGAA-3′. The PCR product was cloned between HindIII and XmaI restriction sites upstream of tdTomato. The plasmid was injected into N2 worms. Selection was performed using fluorescence microscopy.

Microscopy and Signal Quantification

Synchronized worms were washed twice with PBS and snap frozen in liquid nitrogen. Fluorescent images were obtained using a Nikon AZ100 microscope. Quantitative fluorescence analysis was made using ImageJ software. Neural expression patterns were tested using fluorescent microscopy. The worms were washed twice with M9, anesthetized using 18 mM sodium azide (Sigma, #S-2002), and placed on an agar pad. Images were taken using a Zeiss AxioObserver Z1 microscope equipped with an ApoTome 2.

SDS-PAGE and Western Blot Analysis

Twelve thousand CL2070 worms were treated with RNAi bacteria as indicated and homogenized using a Dounce homogenizer. The homogenates were spun for 3 min at 850 × *g* (3,000 rpm in a desktop QIAGEN centrifuge) to sediment debris. The postdebris supernatants were collected, and protein amounts were measured by Bradford reagent (Bio-Rad, #500-0006), supplemented with loading buffer (10% glycerol, 125 mM Tris base, 1% SDS), and boiled for 10 min, and 20 μg total protein was loaded into each well. Proteins were separated on polyacrylamide gels, transferred onto polyvinylidene fluoride membranes, and probed with either GFP antibody (Cell Signaling, cat #2956) or anti-γ-tubulin antibody clone GTU-88 (Sigma, #T-6557). Horseradish peroxidase-conjugated secondary antibodies were used to detect protein signals.

Heat, Oxidative, and Innate Immunity Stress Assays

Synchronized eggs were placed on NG plates seeded with the RNAi bacteria (as indicated) and supplemented with 100 mM IPTG (~4 mM final). The worms were developed and maintained at 20°C. For heat-stress assays, 120 day 1 adult animals were transferred onto fresh plates (12 animals per plate) spotted with RNAi bacteria and exposed to 35°C. Survival rates were recorded after 10, 15, or 19 hr as indicated. To assess resistance to oxidative stress, 90 day 1 adult worms were developed from hatching on the indicated RNAi bacteria, soaked in 0.25 M paraquat (Sigma #36541) in M9. Survival rates were scored after 4 hr of exposure. To evaluate resistance to pathogenic bacteria (innate immunity), eggs of *daf-2(e1370)* worms were placed on plates seeded with the indicated RNAi bacteria, grown to day 1 of adulthood, and transferred onto plates seeded with *P. aeruginosa*. Survival rates were recorded daily.

Statistical Analyses

Statistical significance of the results was performed using the Student's t test using two-tailed distribution and two-sample equal variance. The analyses were done using at least three independent biological repeats of each experiment as indicated. Statistical information of lifespan experiments is presented in Table S1.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.11.028.

AUTHOR CONTRIBUTIONS

E.C. and Y.V. designed and initiated this study and wrote the manuscript. Y.V. performed most of the experimental work, L.M. did WB analyses, F.C.M. performed qPCR experiments, M.M. conducted stress assays, and M.B.S. assisted with cloning procedures.

ACKNOWLEDGMENTS

This study was generously supported by the Israel Science Foundation (EC #671/11) and the European Research Council (ERC) (EC#281010).

Received: July 3, 2014
Revised: October 28, 2014
Accepted: November 19, 2014
Published: December 11, 2014

REFERENCES

Alic, N., Andrews, T.D., Giannakou, M.E., Papatheodorou, I., Slack, C., Hoddinott, M.P., Cochemé, H.M., Schuster, E.F., Thornton, J.M., and Partridge, L. (2011). Genome-wide dFOXO targets and topology of the transcriptomic response to stress and insulin signalling. Mol. Syst. Biol. *7*, 502.

Balch, W.E., Morimoto, R.I., Dillin, A., and Kelly, J.W. (2008). Adapting proteostasis for disease intervention. Science *319*, 916–919.

Ben-Zvi, A., Miller, E.A., and Morimoto, R.I. (2009). Collapse of proteostasis represents an early molecular event in Caenorhabditis elegans aging. Proc. Natl. Acad. Sci. USA *106*, 14914–14919.

Calixto, A., Chelur, D., Topalidou, I., Chen, X., and Chalfie, M. (2010). Enhanced neuronal RNAi in C. elegans using SID-1. Nat. Methods *7*, 554–559.

Chatzigeorgiou, M., Yoo, S., Watson, J.D., Lee, W.H., Spencer, W.C., Kindt, K.S., Hwang, S.W., Miller, D.M., 3rd, Treinin, M., Driscoll, M., and Schafer, W.R. (2010). Specific roles for DEG/ENaC and TRP channels in touch and thermosensation in C. elegans nociceptors. Nat. Neurosci. *13*, 861–868.

Chiang, W.C., Ching, T.T., Lee, H.C., Mousigian, C., and Hsu, A.L. (2012). HSF-1 regulators DDL-1/2 link insulin-like signaling to heat-shock responses and modulation of longevity. Cell *148*, 322–334.

Cohen, E., Bieschke, J., Perciavalle, R.M., Kelly, J.W., and Dillin, A. (2006). Opposing activities protect against age-onset proteotoxicity. Science *313*, 1604–1610.

Cohen, E., Du, D., Joyce, D., Kapernick, E.A., Volovik, Y., Kelly, J.W., and Dillin, A. (2010). Temporal requirements of insulin/IGF-1 signaling for proteotoxicity protection. Aging Cell *9*, 126–134.

David, D.C., Ollikainen, N., Trinidad, J.C., Cary, M.P., Burlingame, A.L., and Kenyon, C. (2010). Widespread protein aggregation as an inherent part of aging in C. elegans. PLoS Biol. *8*, e1000450.

Dupuy, D., Li, Q.R., Deplancke, B., Boxem, M., Hao, T., Lamesch, P., Sequerra, R., Bosak, S., Doucette-Stamm, L., Hope, I.A., et al. (2004). A first version of the Caenorhabditis elegans Promoterome. Genome Res. *14* (10B), 2169–2175.

Ermolaeva, M.A., Segref, A., Dakhovnik, A., Ou, H.L., Schneider, J.I., Utermöhlen, O., Hoppe, T., and Schumacher, B. (2013). DNA damage in germ cells induces an innate immune response that triggers systemic stress resistance. Nature *501*, 416–420.

Evans, E.A., Kawli, T., and Tan, M.W. (2008). Pseudomonas aeruginosa suppresses host immunity by activating the DAF-2 insulin-like signaling pathway in Caenorhabditis elegans. PLoS Pathog. *4*, e1000175.

Furuyama, T., Nakazawa, T., Nakano, I., and Mori, N. (2000). Identification of the differential distribution patterns of mRNAs and consensus binding sequences for mouse DAF-16 homologues. Biochem. J. *349*, 629–634.

Hammell, C.M., Lubin, I., Boag, P.R., Blackwell, T.K., and Ambros, V. (2009). nhl-2 Modulates microRNA activity in Caenorhabditis elegans. Cell *136*, 926–938.

Hartl, F.U., Bracher, A., and Hayer-Hartl, M. (2011). Molecular chaperones in protein folding and proteostasis. Nature *475*, 324–332.

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.

Hobert, O., D'Alberti, T., Liu, Y., and Ruvkun, G. (1998). Control of neural development and function in a thermoregulatory network by the LIM homeobox gene lin-11. J. Neurosci. *18*, 2084–2096.

Honda, Y., and Honda, S. (1999). The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in Caenorhabditis elegans. FASEB J. *13*, 1385–1393.

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.

Hunter, T., Bannister, W.H., and Hunter, G.J. (1997). Cloning, expression, and characterization of two manganese superoxide dismutases from Caenorhabditis elegans. J. Biol. Chem. *272*, 28652–28659.

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.

Kudryashova, E., Kudryashov, D., Kramerova, I., and Spencer, M.J. (2005). Trim32 is a ubiquitin ligase mutated in limb girdle muscular dystrophy type 2H that binds to skeletal muscle myosin and ubiquitinates actin. J. Mol. Biol. *354*, 413–424.

Lee, R.Y., Hench, J., and Ruvkun, G. (2001). Regulation of C. elegans DAF-16 and its human ortholog FKHRL1 by the daf-2 insulin-like signaling pathway. Curr. Biol. *11*, 1950–1957.

Libina, N., Berman, J.R., and Kenyon, C. (2003). Tissue-specific activities of C. elegans DAF-16 in the regulation of lifespan. Cell *115*, 489–502.

Link, C.D. (1995). Expression of human beta-amyloid peptide in transgenic Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *92*, 9368–9372.

Link, C.D., Cypser, J.R., Johnson, C.J., and Johnson, T.E. (1999). Direct observation of stress response in Caenorhabditis elegans using a reporter transgene. Cell Stress Chaperones *4*, 235–242.

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.

Loedige, I., and Filipowicz, W. (2009). TRIM-NHL proteins take on miRNA regulation. Cell *136*, 818–820.

Madison, J.M., Nurrish, S., and Kaplan, J.M. (2005). UNC-13 interaction with syntaxin is required for synaptic transmission. Curr. Biol. *15*, 2236–2242.

Maman, M., Carvalhal Marques, F., Volovik, Y., Dubnikov, T., Bejerano-Sagie, M., and Cohen, E. (2013). A neuronal GPCR is critical for the induction of the heat shock response in the nematode C. elegans. J. Neurosci. *33*, 6102–6111.

Morimoto, R.I. (1998). Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev. *12*, 3788–3796.

Morley, J.F., and Morimoto, R.I. (2004). Regulation of longevity in Caenorhabditis elegans by heat shock factor and molecular chaperones. Mol. Biol. Cell *15*, 657–664.

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.

Panowski, S.H., Wolff, S., Aguilaniu, H., Durieux, J., and Dillin, A. (2007). PHA-4/Foxa mediates diet-restriction-induced longevity of C. elegans. Nature *447*, 550–555.

Partridge, L., and Brüning, J.C. (2008). Forkhead transcription factors and ageing. Oncogene *27*, 2351–2363.

Paz-Gómez, D., Villanueva-Chimal, E., and Navarro, R.E. (2014). The DEAD Box RNA helicase VBH-1 is a new player in the stress response in C. elegans. PLoS ONE *9*, e97924.

Prahlad, V., and Morimoto, R.I. (2011). Neuronal circuitry regulates the response of Caenorhabditis elegans to misfolded proteins. Proc. Natl. Acad. Sci. USA *108*, 14204–14209.

Prahlad, V., Cornelius, T., and Morimoto, R.I. (2008). Regulation of the cellular heat shock response in Caenorhabditis elegans by thermosensory neurons. Science *320*, 811–814.

Qi, W., Huang, X., Neumann-Haefelin, E., Schulze, E., and Baumeister, R. (2012). Cell-nonautonomous signaling of FOXO/DAF-16 to the stem cells of Caenorhabditis elegans. PLoS Genet. *8*, e1002836.

Riedel, C.G., Dowen, R.H., Lourenco, G.F., Kirienko, N.V., Heimbucher, T., West, J.A., Bowman, S.K., Kingston, R.E., Dillin, A., Asara, J.M., and Ruvkun, G. (2013). DAF-16 employs the chromatin remodeller SWI/SNF to promote stress resistance and longevity. Nat. Cell Biol. *15*, 491–501.

Sarge, K.D., Murphy, S.P., and Morimoto, R.I. (1993). Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. Mol. Cell. Biol. *13*, 1392–1407.

Schwamborn, J.C., Berezikov, E., and Knoblich, J.A. (2009). The TRIM-NHL protein TRIM32 activates microRNAs and prevents self-renewal in mouse neural progenitors. Cell *136*, 913–925.

Singh, V., and Aballay, A. (2006). Heat-shock transcription factor (HSF)-1 pathway required for Caenorhabditis elegans immunity. Proc. Natl. Acad. Sci. USA *103*, 13092–13097.

Snutch, T.P., Heschl, M.F., and Baillie, D.L. (1988). The Caenorhabditis elegans hsp70 gene family: a molecular genetic characterization. Gene *64*, 241–255.

Speese, S., Petrie, M., Schuske, K., Ailion, M., Ann, K., Iwasaki, K., Jorgensen, E.M., and Martin, T.F. (2007). UNC-31 (CAPS) is required for dense-core vesicle but not synaptic vesicle exocytosis in Caenorhabditis elegans. J. Neurosci. *27*, 6150–6162.

Styer, K.L., Singh, V., Macosko, E., Steele, S.E., Bargmann, C.I., and Aballay, A. (2008). Innate immunity in Caenorhabditis elegans is regulated by neurons expressing NPR-1/GPCR. Science *322*, 460–464.

Sun, J., Singh, V., Kajino-Sakamoto, R., and Aballay, A. (2011). Neuronal GPCR controls innate immunity by regulating noncanonical unfolded protein response genes. Science *332*, 729–732.

Taylor, R.C., and Dillin, A. (2013). XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. Cell *153*, 1435–1447.

Tepper, R.G., Ashraf, J., Kaletsky, R., Kleemann, G., Murphy, C.T., and Bussemaker, H.J. (2013). PQM-1 complements DAF-16 as a key transcriptional regulator of DAF-2-mediated development and longevity. Cell *154*, 676–690.

Timmons, L., Court, D.L., and Fire, A. (2001). Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in Caenorhabditis elegans. Gene *263*, 103–112.

Van Raamsdonk, J.M., and Hekimi, S. (2012). Superoxide dismutase is dispensable for normal animal lifespan. Proc. Natl. Acad. Sci. USA *109*, 5785–5790.

Volovik, Y., Maman, M., Dubnikov, T., Bejerano-Sagie, M., Joyce, D., Kapernick, E.A., Cohen, E., and Dillin, A. (2012). Temporal requirements of heat shock factor-1 for longevity assurance. Aging Cell *11*, 491–499.

Winston, W.M., Molodowitch, C., and Hunter, C.P. (2002). Systemic RNAi in C. elegans requires the putative transmembrane protein SID-1. Science *295*, 2456–2459.

Wolff, S., Ma, H., Burch, D., Maciel, G.A., Hunter, T., and Dillin, A. (2006). SMK-1, an essential regulator of DAF-16-mediated longevity. Cell *124*, 1039–1053.

Zhang, P., Judy, M., Lee, S.J., and Kenyon, C. (2013). Direct and indirect gene regulation by a life-extending FOXO protein in C. elegans: roles for GATA factors and lipid gene regulators. Cell Metab. *17*, 85–100.