



# Neuronal circuitry regulates the response of *Caenorhabditis elegans* to misfolded proteins

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The consequence of chronic protein misfolding is the basis of many human diseases. To combat the deleterious effects of accumulated protein damage, all cells possess robust quality-control systems, specifically molecular chaperones and clearance machineries, that sense and respond to protein misfolding. However, for many protein conformational diseases, it is unclear why this quality-control system does not efficiently counter protein aggregation. Previous findings that the heat shock response in *Caenorhabditis elegans* is regulated by thermosensory neurons led us to consider whether neuronal activity could also be responsible for the inadequate response of an organism to chronic protein misfolding. Here we show, in animals expressing polyglutamine expansion proteins and mutant SOD-1<sup>G93A</sup> in intestinal or muscle cells, that the nervous system does indeed control the cellular response to misfolded proteins. Whereas polyglutamine expansion-expressing animals with WT thermosensory neurons readily express protein aggregates, leading to cellular dysfunction without concomitant up-regulation of molecular chaperones, modulation of the nervous system results in chaperone up-regulation that suppresses aggregation and toxicity. The neuronal signal is transmitted through calcium-activated dense core vesicle neurosecretion. Cell-nonautonomous control of chaperone expression by the thermosensory neurons allows *C. elegans* to respond differently to acute stress such as heat shock, and chronic stress caused by the expression of misfolded proteins, suggesting that neuronal signaling determines the course of cellular proteotoxicity.

neuronal control | proteostasis | stress response | Hsp70 | small heat shock proteins

The inability to maintain protein quality control has detrimental effects on cell physiology and function, and is the basis of diseases of protein conformation, including Huntington disease, Parkinson disease, Alzheimer's disease, cancer, and type II diabetes, in which cells accumulate misfolded and aggregated proteins (1, 2). To inhibit aggregation and toxicity, cells and organisms have evolved multiple stress responsive networks that detect and respond to the accumulation of damaged proteins (3–8). Central to these protective measures is the heat shock (HS) response (HSR) and HS transcription factor HSF-1. HSF-1 regulates the inducible expression of HS proteins (HSPs), many of which are molecular chaperones that protect the proteome by enhancing folding, suppressing misfolding, and targeting damaged proteins for degradation (8). The current hypothesis for the induction of chaperones is that elevated levels of misfolded and damaged proteins caused by environmental insults or errors in protein biogenesis titrate the molecular chaperones away from HSF-1 and toward association with misfolded proteins, resulting in the derepression and activation of HSF-1 (9, 10).

Despite the evidence that protein misfolding triggers HSF-1 (9, 10), the up-regulation of chaperones and activation of HSF-1 is infrequently observed in animal models of protein aggregation and tissues from affected humans (11–16). Instead, the protein quality control system appears to be impaired as a result of the titration of chaperones, disruption of proteasomal activity (17–19), modification of HSF-1 and its regulators, or sequestration of important cofactors by aggregation-prone proteins (17–21). However, disruption of proteostasis by other means, such as

genetic knockdown or treatment with small-molecule inhibitors of proteasome or chaperone function, activates HSF-1, increasing the levels of protective HSPs and restoring proteostasis (3, 22). Similarly, the purposeful overexpression of specific chaperones or the expression of constitutively active HSF-1 can inhibit protein aggregation and toxicity in multiple cell and animal models (23–28). Therefore, the absence of a consistent HSR in diseases of protein conformation is unexpected.

Previous studies in the metazoan *Caenorhabditis elegans* have shown that the HSR is regulated by the AFD thermosensory neurons and AIY interneurons (29). Neuronal control provides a means by which the acute response to stress is under organismal control. In this study, we examine whether neuronal activity also regulates the cellular response to chronic expression of misfolded proteins.

## Results

**Thermosensory Neuronal Mutants Suppress Aggregation of Polyglutamine Expansion Proteins Expressed in Intestinal Cells of *C. elegans*.** The role of neuronal signaling on chronic protein misfolding in *C. elegans* was examined by using the *gcy-8* (30) and *ttx-3* (31) loss-of-function mutations that specifically affect the AFD thermosensory neurons and AIY interneurons, respectively. GCY-8 is a guanylyl cyclase expressed only in the two AFD neurons (30), and TTX-3 is an LIM-homeobox transcription factor expressed in the AIY interneurons (31). We monitored misfolding by using the in vivo folding reporter Q44::YFP, which consists of 44 glutamine residues fused to YFP under the control of the intestine-specific *vha-6* promoter (32). Q44::YFP has been shown to aggregate in an age-dependent manner, impairing cellular function (32). Our previous data that *gcy-8* and *ttx-3* mutants are deficient in the HSR (29) led us to expect that mutations in the thermosensory circuitry would exacerbate polyglutamine expansion (polyQ) protein aggregation.

Instead, expression of Q44 in the background of *gcy-8* or *ttx-3* mutations led to a reduction in the onset and extent of polyQ aggregation in intestinal cells (Fig. 1 A–C and Fig. S1A). Whereas 40% of adult day 2 animals expressing Q44::YFP accumulate immobile polyQ aggregates (Materials and Methods), none of the *gcy-8*; Q44 animals, and only 25% of the *ttx-3*; Q44 animals of comparable age, had aggregates (Fig. 1B). This suppression of aggregation persisted on day 3 of adulthood with less than half the *gcy-8*; Q44 and *ttx-3*; Q44 animals with aggregates, and the remaining animals containing fewer than 20 aggregates (Fig. 1 A and B and Fig. S1A), compared with 80% of WT Q44 animals with more than 20 aggregates (Fig. 1 A and B and Fig. S1A). The reduction in polyQ aggregation in the *gcy-8* and *ttx-3* animals was not caused by decreased expression of polyQ mRNA and protein levels relative to WT animals (Fig. S1 B and C).

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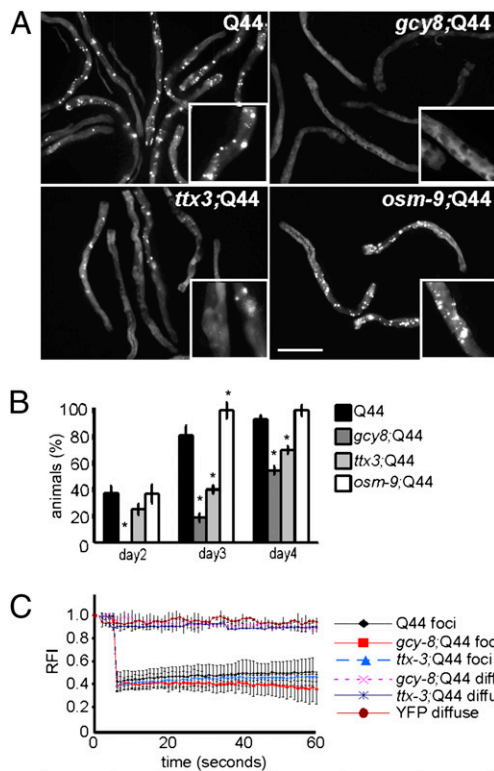
The authors declare no conflict of interest.

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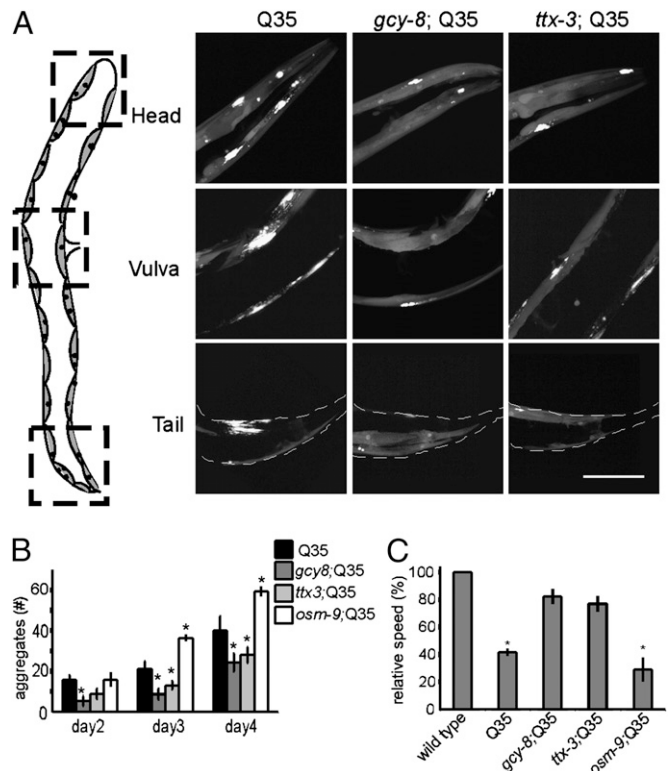


**Fig. 1.** Thermosensory neurons regulate polyQ aggregation in *C. elegans* intestine. (A) Q44 aggregation in the intestine of day 3 adult animals with WT thermosensory neurons (Q44), *gcy-8* mutation (*gcy-8*;Q44), *ttx-3* mutation (*ttx-3*;Q44), and *osm-9* mutation (*osm-9*;Q44). (Scale bar: 100  $\mu$ m.) Inset: Representative areas chosen for photobleaching. (B) Percentage of animals with visible aggregates in Q44, *gcy-8*;Q44, *ttx-3*;Q44, and *osm-9*;Q44 animals on days 2 to 4 of adulthood. Error bars indicate SE (\* $P < 0.01$ ). (C) FRAP measurements on 10 fluorescent aggregates (foci) and 10 to 20 regions that did not contain aggregates (diffuse) in WT Q44, *gcy-8*;Q44, and *ttx-3*;Q44 day 3 adult animals. YFP alone used as a control. Error bars indicate SD.

Fluorescence recovery after photobleaching (FRAP) was used to demonstrate that the aggregates, in all animals, were immobile and not exchangeable with the surrounding diffusible polyQ::YFP (Fig. 1 B and C). In the thermosensory mutant animals that expressed fewer polyQ aggregates, the diffuse polyQ protein in intestinal cells of *gcy-8*; Q44 and *ttx-3*; Q44 animals was soluble, with properties similar to YFP alone (Fig. 1C). Thus, disruption of thermosensory neuronal activity suppressed aggregation and increased soluble polyQ in intestinal cells. This effect was not a consequence of general inhibition of sensory neuronal activity, as disruption of the chemosensory neurons ASH, ADL, and ASE in the *osm-9* mutation (33) (Fig. 1 A and B and Fig. S1A) or *ocr-2* mutation (33) (Fig. S2A–C) did not affect polyQ aggregation.

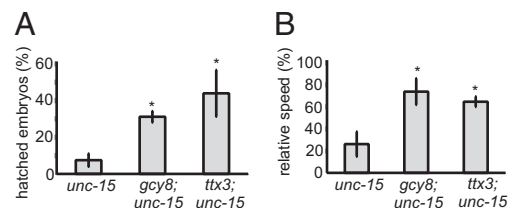
**Thermosensory Neuronal Mutants Suppress Aggregation and Toxicity of Multiple Folding Reporters in *C. elegans*.** These results led us to ask whether the beneficial effects of thermosensory mutations on polyQ aggregation was limited to the intestinal cells or extended to metastable proteins expressed in other tissues of *C. elegans*. To address this, we examined the effect of the *gcy-8* and *ttx-3* mutations on three additional models for protein misfolding corresponding to polyQ aggregation in muscle cells (14, 34), expression of ALS-associated mutant SOD-1<sup>G93A</sup> (35), and an endogenous metastable temperature-sensitive isoform of paramyosin (36).

For each protein, we observed that *gcy-8* and *ttx-3* mutations suppressed misfolding and associated toxicity (Figs. 2 and 3 and



**Fig. 2.** Thermosensory neurons regulate polyQ aggregation in *C. elegans* body wall muscle cells. (A) Collapsed Z-sections of representative regions of the body wall muscle in day 3 adult animals with WT thermosensory neurons (Q35), *gcy-8* mutation (*gcy-8*;Q35), and *ttx-3* mutation (*ttx-3*;Q35). The numbers of aggregates in the head region would be counted as 6, 3, and 2 in the Q35, *gcy-8*;Q35, and *ttx-3*;Q35 respectively. (Scale bar: 20  $\mu$ m.) (B) The average number of aggregates per animal in Q35, *gcy-8*;Q35, *ttx-3*;Q35, and *osm-9*;Q35 as animals age. (C) The average motility of Q35, *gcy-8*;Q35, *ttx-3*;Q35, and *osm-9*;Q35 day 4 animals normalized to WT animals not expressing polyQ. Error bars indicate SE (\* $P < 0.01$ ).

Fig. S3). Q35::YFP expressed in body wall muscle cells exhibits age-dependent aggregation (Fig. 2 A and B), which was markedly suppressed in *gcy-8* and *ttx-3* animals, as determined by morphological criteria (Fig. 2 A and B) and FRAP analysis (Fig. S3A). Suppression of polyQ aggregation in the *gcy-8* and *ttx-3* backgrounds was not caused by decreased expression of Q35 mRNA or protein (Fig. S3 B and C) and was specific to disruption of the thermosensory circuitry, as RNAi-mediated down-regulation of other gene products expressed by the AFD neurons also led to the suppression of aggregation (Fig. S3D), whereas the *osm-9* (Fig. 2B) and *ocr-2* (Fig. S2D) mutations did not.



**Fig. 3.** Thermosensory mutations suppress the toxicity caused by the misfolding of paramyosin. (A) The percentage of embryos that hatched when *unc-15*(ts), *gcy-8*;*unc-15*(ts), and *ttx-3*;*unc-15*(ts) embryos were transferred at the comma stage from 15  $^{\circ}$ C to 25  $^{\circ}$ C. (B) The motility of *unc-15*(ts), *gcy-8*;*unc-15*(ts), and *ttx-3*;*unc-15*(ts) relative to WT animals 2 d after being transferred from 15  $^{\circ}$ C to 25  $^{\circ}$ C. Error bars indicate SE (\* $P < 0.01$ ).

Expression of Q35 in muscle cells causes a 60% reduction in motility of day 4 adult animals (14, 34) relative to age-matched nontransgenic animals (Fig. 2C). However, in *gcy-8* and *ttx-3* animals expressing Q35, the motility rates were restored close to WT levels (Fig. 2C), whereas the *osm-9* mutation showed no effect (Fig. 2C). This protective role of the *gcy-8* and *ttx-3* mutations also extended to another disease-related aggregation prone protein, mutant SOD-1<sup>G93A</sup> (35). Expression of mutant SOD-1<sup>G93A</sup> in the background of a WT thermosensory circuitry causes developmental delays such that only 30% of animals develop past the second larval (L2) stage at 25 °C. In the thermosensory mutant background, nearly all SOD-1<sup>G93A</sup>-expressing animals developed past L2 stage (Fig. S3E).

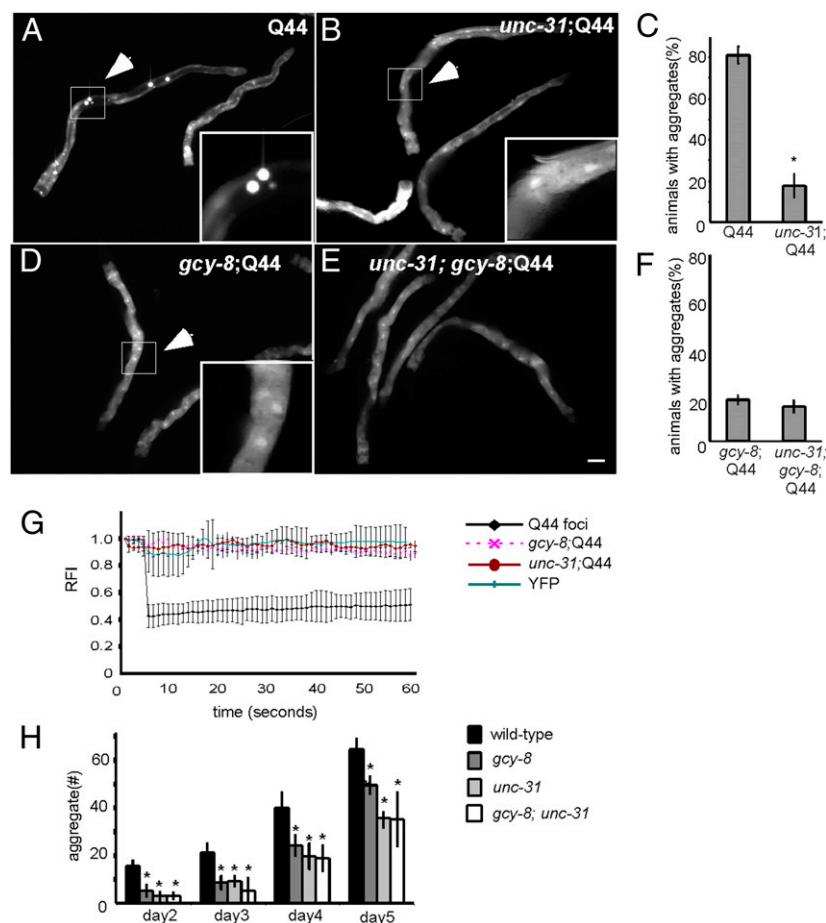
In addition to suppressing the misfolding and toxicity of exogenous aggregation-prone proteins, the thermosensory mutations also suppressed the proteotoxicity associated with misfolding of a metastable temperature-sensitive isoform of paramyosin, *unc-15(ts)* (36). At the restrictive temperature (25 °C), paramyosin (ts) misfolds and aggregates, disrupting muscle structure and function (36). Consequently, only approximately 10% of *unc-15(ts)* embryos develop beyond the comma stage (Fig. 3A) (37), with adult animals showing approximately 75% reduction in motility relative to WT animals (Fig. 3B and Movie S1). In the presence of *gcy-8* and *ttx-3* mutations, the toxicity caused by the misfolding of paramyosin *unc-15(ts)* (Fig. 3) was substantially reduced. Approximately 30% of *gcy-8;unc-15(ts)* and approximately 40% of *ttx-3;unc-15(ts)* embryos hatched (Fig. 3A), and motility of adult *gcy-8;unc-15(ts)* and *ttx-3;unc-15(ts)* animals was restored to near that of WT animals (Fig. 3B and Movie S1).

These results demonstrate that modulation of thermosensory neuronal activity suppressed protein misfolding and related cel-

lular dysfunction as a result of the expression of multiple metastable proteins in separate tissues. This suggests that the protein folding environment was affected globally by cell-nonautonomous control by the thermosensory neuronal circuitry.

**Thermosensory Circuitry Modulates Protein Misfolding Through Dense Core Vesicle-Dependent Neurosecretion.** To investigate how the *gcy-8* mutation influenced aggregation and toxicity, we examined whether the loss of downstream signaling pathways would recapitulate the global suppression of protein misfolding seen in the *gcy-8* animals. The thermosensory mutations not only suppressed aggregation in muscle cells but also affected intestinal tissue that is not innervated, suggesting that neuronal signaling could occur through secreted molecules. Therefore, we tested whether inhibition of one of the major modes of neurosecretion, the dense core vesicle (DCV) release through calcium-activated protein secretion (CAPS) (38, 39), also suppressed polyQ aggregation in intestinal and muscle cells.

Deletion of the single *C. elegans* orthologue of CAPS, *unc-31* (38, 39), and the subsequent loss of DCV-dependent neurosecretion resulted in suppression of Q44::YFP aggregation in the intestine (Fig. 4A–C) and Q35::YFP aggregation in muscle cells (Fig. 4H), and the appearance of soluble protein by FRAP analysis (Fig. 4G). Moreover, polyQ aggregation in the *gcy-8;unc-31* double mutants was indistinguishable from either of the single *gcy-8* or *unc-31* mutants alone (Fig. 4D–F and H), suggesting that *gcy-8* and *unc-31* are in the same genetic pathway. These results showed that the AFD neurons modulate aggregation through suppression of DCV-dependent neurosecretion and suggested that these pathways may exert a net-inhibitory influence on the protein quality-control machinery of nonneuronal cells.



**Fig. 4.** Thermosensory circuitry modulates protein misfolding through DCV-dependent neurosecretion. Q44 aggregation in the intestine of day 3 adult animals with (A) WT thermosensory neurons, (B) *unc-31* mutation, (C) *gcy-8* mutation, and (D) *gcy-8;unc-31* double mutation. Inset: Representative areas chosen for photobleaching. (Scale bar: 100  $\mu$ m.) (E) Percentage of Q44, *unc31;Q44*, *gcy-8;Q44*, and *gcy-8;unc-31;Q44* day 3 adult animals with visible aggregates. (F) FRAP data following photobleaching of areas similar to those indicated in the inset. YFP alone used as a control. (G) The average number of aggregates per animal in Q35, *gcy-8;Q35*, *unc-31;Q35*, and *gcy-8;unc-31;Q35* as animals age. Error bars in E and G indicate SE; error bars in F indicate SD (\* $P < 0.01$ ). (H) The average number of aggregates per animal in wild-type, *gcy-8;Q44*, *unc-31;Q44*, and *gcy-8;unc-31; Q44* animals as animals age.

**Suppression of Proteotoxicity in Thermosensory Mutants Caused by HSF-1–Dependent Up-Regulation of Chaperones.** Protein quality control in *C. elegans* is regulated at the molecular level by HSF-1 (34, 40). Reducing the levels of *hsf-1* by RNAi-induced knockdown restored aggregation in the *gcy-8* animals to that of WT animals (Fig. 5*A* and Fig. S4*B*). Likewise, the suppression of polyQ aggregation in *unc-31* mutant animals was HSF-1–dependent (Fig. S4*C*). RNAi-induced knockdown of DAF-16 did not revert the suppression of polyQ aggregation in the *gcy-8* (Fig. 5*B* and Fig. S4*B*) or *unc-31* animals (Fig. S4*C*), suggesting that the effects of the neuronal mutations on protein aggregation was dependent on HSF-1.

We next examined whether chaperone levels were up-regulated in the thermosensory mutants expressing aggregation-prone proteins. Mutations affecting the thermosensory neurons alone do not alter the basal levels of chaperones (Fig. 5 and Fig. S5). Expression levels of the constitutive HSP70 (*hsp-1*), the inducible HSP70s (C12C8.1 and F44E5.4), the inducible small HSP (*hsp16.2*), and HSP90 (*daf-21*) were similar for *gcy-8* and WT animals (Fig. 5 and Fig. S5). In addition, as seen in other model systems the aggregation of polyQ did not up-regulate chaperone expression (Fig. 5*C* and Fig. S5*A–E*). However, the expression of polyQ in the *gcy-8* animals but not in other chemosensory mutants resulted in the up-regulation of each of these HSP genes (Fig. 5*C* and Fig. S5*E*).

Similar results were obtained with regard to the misfolding of paramyosin. At the permissive temperature of 15 °C, the basal levels of inducible HSP70s (C12C8.1 and F44E5.4) in WT and *gcy-8* animals were similar regardless of whether *unc-15(ts)* was expressed. The misfolding of paramyosin (ts) at the restrictive temperature of 25 °C in animals with WT thermosensory neurons did not cause the up-regulation of *hsp70* (C12C8.1 and F44E5.4) mRNA: chaperone levels in the *unc-15(ts)* animals were similar to animals that expressed the WT isoform of paramyosin (Fig. 5*D* and Fig. S5*F*). In contrast, the misfolding of paramyosin in *gcy-8* mutant animals resulted in up-regulation of the inducible cytoplasmic HSP70s (Fig. 5*D* and Fig. S5*F*).

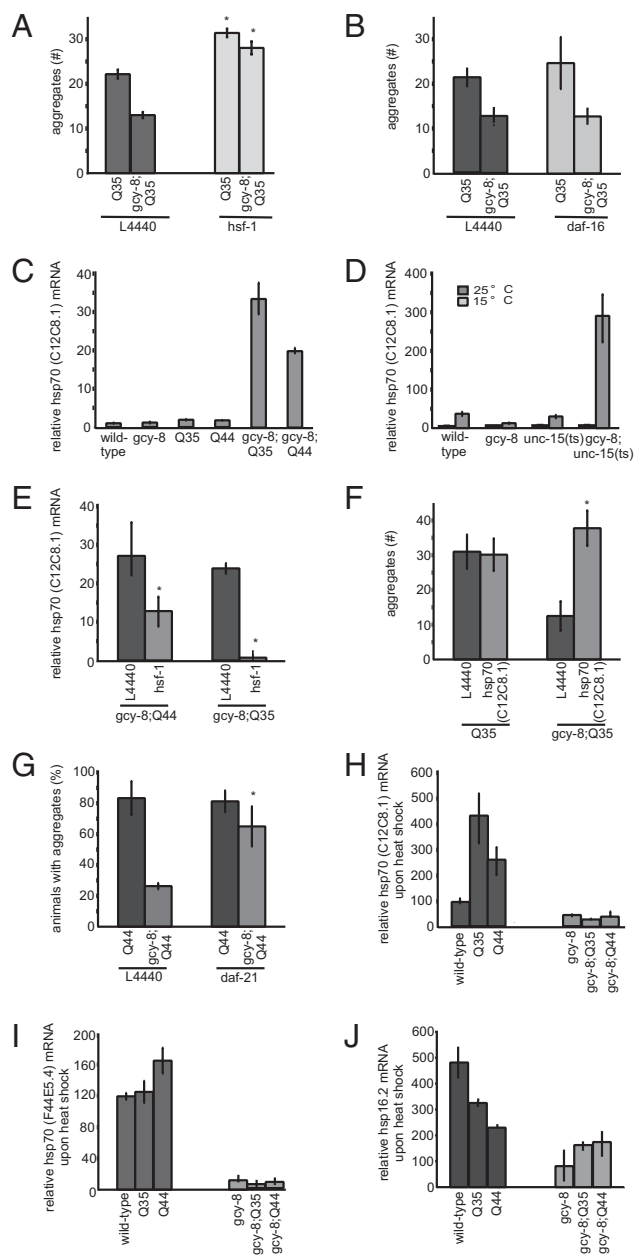
We confirmed that the up-regulation of chaperones in *gcy-8* mutant animals expressing misfolded proteins was dependent on HSF-1 (Fig. 5*E*). Moreover, up-regulation of chaperones was responsible for the suppression of aggregation in the thermosensory mutant animals (Fig. 5*F* and *G*). RNAi-induced knockdown of *hsp70* (C12C8.1) levels in the *gcy-8;Q35* animals prevented the *gcy-8*–dependent rescue of aggregation (Fig. 5*F*). We observed no effect in Q35 animals with WT thermosensory function, presumably as *hsp70* (C12C8.1) was not induced. Similarly, knockdown of *hsp90* (*daf-21*) in the *gcy-8;Q44* animals blocked the suppression of aggregation observed in these animals (Fig. 5*G*).

Thus, the chronic expression of aggregation-prone proteins in *C. elegans* tissues does not result in the up-regulation of chaperones. Disruption of neuronal activity restored HSF-1–dependent chaperone up-regulation upon chronic expression of misfolded proteins, and suppressed aggregation and toxicity.

### Thermosensory Neuronal Function Allows *C. elegans* to Distinguish Between Acute Temperature Stress and Chronic Stress of Protein Misfolding.

The up-regulation of chaperones in thermosensory mutant animals and the rescue of aggregation and toxicity presented a conundrum, as we had previously observed that these mutations in the neuronal circuitry dampened the HSR. We reasoned that this may be because a short, acute HS that causes a rapid and transient change in protein folding dynamics is not recognized in the same manner as a prolonged chronic accumulation of misfolded proteins. The ability of the thermosensory neurons of *C. elegans* to detect and respond to temperature change and regulate proteostasis may allow for distinct organizational responses to these stresses.

As shown previously, aggregation of polyQ in *C. elegans* does not up-regulate chaperones in animals with a WT neuronal circuitry (Fig. 5 and Fig. S5). However, exposure of Q35 and Q44



**Fig. 5.** Thermosensory neurons regulate chaperones expression upon protein misfolding. The average number of aggregates per 3-d adult animals with WT thermosensory neurons or *gcy-8* mutation grown on control bacteria (L4440) and upon RNAi-induced knockdown of (A) *hsf-1* or (B) *daf-16*. (C) mRNA levels of *hsp70* (C12C8.1) in day 3 adult animals quantified by quantitative RT-PCR (qRT-PCR). (D) mRNA levels of *hsp70* (C12C8.1) quantified by qRT-PCR in animals without (15 °C) and with (25 °C) misfolded paramyosin. Values at 15 °C are 0.8 to 1.2, 0.8 to 1.4, 0.6 to 1.0, and 0.6 to 0.7 for WT, *gcy-8*, *unc-15(ts)*, and *gcy-8;unc-15(ts)*, respectively. (E) mRNA levels of *hsp70* (C12C8.1) in 3 d adult *gcy-8;Q35* and *gcy-8;Q44* animals on control bacteria (L4440), and upon RNAi-induced knockdown of *hsf-1*. (F) Number of Q35 aggregates in day 3 adult Q35 and *gcy-8;Q35* animals on control bacteria (L4440) and upon RNAi-induced knockdown of *hsp70* (C12C8.1). (G) Percentage of day 3 adult Q44 animals containing aggregates in Q44 and *gcy-8;Q44* animals on control bacteria (L4440) and upon RNAi-induced knockdown of *hsp90* (*daf-21*). (H) mRNA levels of *hsp70* (C12C8.1), (I) *hsp70* (F44E5.4), and (J) small HSP (*hsp16.2*) quantified by qRT-PCR in day 3 adult animals expressing polyQ proteins following HS. Values in C–E were normalized to WT. Values in H–J were normalized to basal chaperone levels in the absence of HS. Actin was used as an internal control. Error bars in D–F indicate SE (\**P* < 0.01).

animals with WT thermosensory neurons to HS (34 °C for 15 min) induced the expression of HSP genes (Fig. 5 *H–J*), whereas *gcy-8;Q35* and *gcy-8; Q44* animals showed no further increase in chaperone expression beyond basal levels (Fig. 5 *H–J*). This indicates that the expression of aggregation-prone proteins within the cells of *C. elegans* does not compromise the HSR. Rather, a WT thermosensory neuron compromises the response to chronic protein aggregation. To verify that the activity of thermosensory neurons distinguished between acute and chronic stress, we exposed WT and *gcy-8* mutant animals to chronic heat stress. We predicted that *gcy-8* animals, although deficient for chaperone induction upon acute HS, would induce chaperone expression when exposed to the chronic stress. This was indeed the case: even through *gcy-8* animals did not respond to a transient HS (Fig. S6A), chaperone induction was observed upon exposure to repeated or prolonged HS (Fig. S6 B and C).

These data show that the thermosensory neurons inhibit chaperone up-regulation upon chronic stress and the occurrence of misfolded proteins, but permit HSP up-regulation upon acute HS, allowing the animal to respond differently to acute and chronic proteotoxic conditions.

## Discussion

The nervous system of the metazoan *C. elegans* exerts an inhibitory, cell-nonautonomous control over the organismal response to protein misfolding. This inhibitory effect of the AFD neurons on the somatic cell HSR can be relieved by down-regulating the thermosensory neurons or by inhibiting CAPS-mediated DCV neurosecretion. In WT animals, this signal inhibits HSF-1 activity in intestinal and muscle cells even when challenged by the chronic expression of misfolded proteins. We propose that neuronal control of the HSR serves to modulate the organismal response to protein aggregation and to distinguish between acute HS and chronic accumulation of protein damage. Animals with WT thermosensory neurons express basal levels of chaperones despite the chronic accumulation of misfolded proteins, while retaining their ability to respond to acute heat stress. Our results show that mutations in thermosensory neuronal signaling invert this response such that chaperone induction in *gcy-8* mutant animals now occurs when misfolded proteins are chronically expressed, but is dampened in response to acute HS. We hypothesize that thermosensory neurons serve as a homeostatic switch for the control of chaperone expression in *C. elegans*, allowing tissues within the organism to maintain optimal levels of chaperones for normal function and yet respond to transient exposures to environmental stress by up-regulating chaperones (Fig. S7). This would be important because chronic activation of HSF-1 and elevated HSP levels could interfere with growth and cell cycle progression (41, 42) and increase susceptibility to cellular transformation (43). Thus, the tight control over chaperone levels within individual cells would be all the more important for metazoan physiology. Indeed, chaperone levels are not maintained in excess (36), but rather are finely tuned to specific cellular requirements. In support of the role of the AFD neurons as a homeostatic switch over chaperone expression, it was very recently shown that HSF-1 in peripheral tissues modulates AFD thermosensory neuronal activity, providing a means of feedback regulation essential for any homeostatic control system (44).

Our results also show that the AFD thermosensory neurons control protein folding through  $Ca^{2+}$ -dependent DCV secretion. As in other organisms, CAPS-dependent DCV release in *C. elegans* is responsible for peptidergic signaling through the secretion of neuropeptides, insulin-like peptides, and biogenic amines (39). The link between insulin signaling and the HSR is well established: HSF-1 activity in *C. elegans* is essential for lifespan enhancement by modulation of the insulin signaling pathway. Moreover, many of these peptides appear in the pseudocoelom of the animal following neurosecretion, where they would be expected to interact with somatic tissues. Thus, characterization of the molecule(s) released by the DCVs under

physiological conditions, and upon acute heat stress, may help elucidate the signaling pathways by which the AFD neuron exerts its effects on protein folding homeostasis in nonneuronal cells.

The centralized control of HSF-1 activity described here has features in common with the systemic inhibition of acute inflammation in mammals (45, 46). Both the inflammatory response and the HSR are protective under a controlled regimen, but are detrimental if unchecked. In the case of the immune response, the systemic inhibition of acute inflammation by the central nervous system, together with local amplification by the peripheral nervous system, allows the response to be localized to regions of necessity, and subsequently terminated to restore homeostasis (45, 46). A similar hierarchical control of the HSR and HSF-1 activity could be envisioned whereby stress signaling networks between different tissues and the nervous system regulate the organismal response to different stress conditions.

The demonstration in *C. elegans* that the stress of chronic misfolding can be regulated by neuronal cell-nonautonomous control may have broader relevance to proteotoxicity in human disease. Cell-nonautonomous effects of afferent sensory and cortical projections on the toxicity and degeneration of the motor neuron has been described recently in mouse models of human ALS, spinal muscular atrophy, and Huntington disease (47–49). If protein folding homeostasis in diverse tissues of other metazoans is under the regulation of sensory neuronal modalities as we have described for *C. elegans*, this would suggest that another method for treatment of human conformational diseases could involve modulation of neurosensory systems.

## Materials and Methods

***C. elegans* Strains and Growth Conditions.** The *C. elegans* strains and methods of cultivation are listed in *SI Materials and Methods*.

**Scoring of Aggregates.** Aggregates were scored every 24 h post-L4, using a Leica fluorescent stereomicroscope (MZFLIII) with the EYFP filter set (excitation, 510/20; emission, 560/40). Aggregates were recognized visually, based on experience from FRAP regarding which foci did not recover following photobleaching. Aggregates too close to be resolved under the stereomicroscope, or smaller satellite aggregates scattered near a large aggregate were accounted as one. This system was consistently maintained for all strains. The number of aggregates in each worm was scored blind, by independent investigators and yielded very similar results. To quantify Q44 aggregation in the intestine, animals were binned into pools containing 0, 1 to 20, and greater than 20 aggregates. To quantify Q35 aggregation in the body wall muscle cells, all visible aggregates in approximately 100 animals, corresponding to more than three biological samples, were counted over a period of 4 d.

**RNA Extraction and Quantitative RT-PCR.** mRNA was prepared by using TRIzol extraction as previously published and detailed in *SI Materials and Methods*.

**RNAi Experiments.** RNAi experiments were conducted according to standard protocols and the experimental protocol, and RNAi constructs are detailed in *SI Materials and Methods*. RNAi against the AFD genes was conducted in the sensitized strain *rrf-3* strain expressing Q35, which accumulated slightly lower numbers of aggregates.

**Embryonic Lethality Experiments.** Toxicity in the SOD-1<sup>G93A</sup> animals was measured by transferring 10 L4 WT G93A and *gcy-8* G93A larvae from 20 °C to 25 °C, allowing them to lay eggs for 24 to 36 h, counting total embryos, and calculating the percentage of larvae that grew to L3 stage. The experiment was repeated three or four times.

Embryonic lethality in *unc-15* (ts) animals was measured by allowing approximately 10 *unc-15*(ts), *gcy-8;unc-15*(ts), or *ttx-3;unc-15*(ts) animals to lay eggs at 15 °C, and 24 h later, transferring 20 to 50 embryos at the comma cell stage onto fresh plates at 25 °C. The number of larvae that developed into L2 larvae after 24 to 48 h were scored. Experiments were repeated at least three times.

**Motility Assays.** The motility of N2, *gcy-8*, Q35, *gcy-8;Q35*, *ttx-3;Q35*, *unc-15*(ts), *gcy-8;unc-15*(ts), *ttx-3;unc-15*(ts) was assayed upon transferring 10 animals to a new 6-cm plate seeded uniformly with Op50 equilibrated to room temperature. Movies of crawling animals were recorded with a Leica MZ10 microscope and Hamamatsu C10600-10B (Orca-R2) camera by using

SimplePCI software (Leica) at  $2 \times 2$  binning and 5 frames/s, and analyzed by using ImageJ as described in *SI Materials and Methods*. Each sample of 10 animals was measured only once. At least three samples of 10 animals were considered one biological sample, and each report of motility involved three to five biological samples.

**FRAP.** A detailed description of FRAP methodology, the basis for selection of the aggregates, and how many aggregates were analyzed is included in *SI Materials and Methods*.

**SDS/PAGE Gels and Western Blots for Examining Protein Levels.** PolyQ protein expression was measured by boiling 10 adult day 3 animals in SDS sample buffer for 15 min, and conducting a standard Western analysis. Antibodies used are listed in *SI Materials and Methods*. No cleavage of YFP from the polyQ-expressing polypeptide was observed.

**HS.** For acute HS, 10 L4 animals (N2, Q35, Q44, *gcy-8*, *gcy-8*;Q35, *gcy-8*;Q44) were moved onto a fresh plate from a population, allowed to mature into

day 3 adults and heat-shocked in a water bath for 15 min at 34 °C according to previously published methods. We ensured that the polyQ-expressing animals had accumulated aggregates before HS. For prolonged chronic HS, 10 L4 animals were exposed to a constant temperature of 28 °C for 24 h. Repeated acute HSs were delivered by three cycles of exposure to 34 °C for 15 min interspersed by 15 min recovery at 20 °C.

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- Soto C, Estrada LD (2008) Protein misfolding and neurodegeneration. *Arch Neurol* 65:184–189.
- Carrell RW (2005) Cell toxicity and conformational disease. *Trends Cell Biol* 15: 574–580.
- Balch WE, Morimoto RI, Dillin A, Kelly JW (2008) Adapting proteostasis for disease intervention. *Science* 319:916–919.
- Sherman MY, Goldberg AL (2001) Cellular defenses against unfolded proteins: A cell biologist thinks about neurodegenerative diseases. *Neuron* 29:15–32.
- Hartl FU, Hayer-Hartl M (2009) Converging concepts of protein folding in vitro and in vivo. *Nat Struct Mol Biol* 16:574–581.
- Jonikas MC, et al. (2009) Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum. *Science* 323:1693–1697.
- Lindquist S, Craig EA (1988) The heat-shock proteins. *Annu Rev Genet* 22:631–677.
- Akerfelt M, Morimoto RI, Sistonen L (2010) Heat shock factors: Integrators of cell stress, development and lifespan. *Nat Rev Mol Cell Biol* 11:545–555.
- Voellmy R, Boellmann F (2007) Chaperone regulation of the heat shock protein response. *Adv Exp Med Biol* 594:89–99.
- Morimoto RI (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.
- Zourlidou A, et al. (2007) Hsp27 overexpression in the R6/2 mouse model of Huntington's disease: Chronic neurodegeneration does not induce Hsp27 activation. *Hum Mol Genet* 16:1078–1090.
- Hay DG, et al. (2004) Progressive decrease in chaperone protein levels in a mouse model of Huntington's disease and induction of stress proteins as a therapeutic approach. *Hum Mol Genet* 13:1389–1405.
- Muchowski PJ (2002) Protein misfolding, amyloid formation, and neurodegeneration: A critical role for molecular chaperones? *Neuron* 35:9–12.
- Satyal SH, et al. (2000) Polyglutamine aggregates alter protein folding homeostasis in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 97:5750–5755.
- Vlemminckx V, et al. (2002) Upregulation of HSP27 in a transgenic model of ALS. *J Neuropathol Exp Neurol* 61:968–974.
- Ferraiuolo L, et al. (2007) Microarray analysis of the cellular pathways involved in the adaptation to and progression of motor neuron injury in the SOD1 G93A mouse model of familial ALS. *J Neurosci* 27:9201–9219.
- Bence NF, Sampat RM, Kopito RR (2001) Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* 292:1552–1555.
- Stenoien DL, et al. (1999) Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. *Hum Mol Genet* 8:731–741.
- Kim S, Nollen EA, Kitagawa K, Bindokas VP, Morimoto RI (2002) Polyglutamine protein aggregates are dynamic. *Nat Cell Biol* 4:826–831.
- Olzscha H, et al. (2011) Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. *Cell* 144:67–78.
- Tatzelt J, Voellmy R, Welch WJ (1998) Abnormalities in stress proteins in prion diseases. *Cell Mol Neurobiol* 18:721–729.
- Bush KT, Goldberg AL, Nigam SK (1997) Proteasome inhibition leads to a heat-shock response, induction of endoplasmic reticulum chaperones, and thermotolerance. *J Biol Chem* 272:9086–9092.
- Fujimoto M, et al. (2005) Active HSF1 significantly suppresses polyglutamine aggregate formation in cellular and mouse models. *J Biol Chem* 280:34908–34916.
- Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM (2002) Chaperone suppression of alpha-synuclein toxicity in a *Drosophila* model for Parkinson's disease. *Science* 295:865–868.
- Warrick JM, et al. (1999) Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone HSP70. *Nat Genet* 23:425–428.
- Cummings CJ, et al. (2001) Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. *Hum Mol Genet* 10: 1511–1518.
- Fernandez-Funez P, et al. (2000) Identification of genes that modify ataxin-1-induced neurodegeneration. *Nature* 408:101–106.
- Muchowski PJ, et al. (2000) Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proc Natl Acad Sci USA* 97:7841–7846.
- Prahlad V, Cornelius T, Morimoto RI (2008) Regulation of the cellular heat shock response in *Caenorhabditis elegans* by thermosensory neurons. *Science* 320:811–814.
- Inada H, et al. (2006) Identification of guanylyl cyclases that function in thermosensory neurons of *Caenorhabditis elegans*. *Genetics* 172:2239–2252.
- Hobert O, et al. (1997) Regulation of interneuron function in the *C. elegans* thermoregulatory pathway by the *txx-3* LIM homeobox gene. *Neuron* 19:345–357.
- Mohri-Shiomi A, Garsin DA (2008) Insulin signaling and the heat shock response modulate protein homeostasis in the *Caenorhabditis elegans* intestine during infection. *J Biol Chem* 283:194–201.
- Kahn-Kirby AH, Bargmann CI (2006) TRP channels in *C. elegans*. *Annu Rev Physiol* 68: 719–736.
- Morley JF, Brignull HR, Weyers JJ, Morimoto RI (2002) The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 99:10417–10422.
- Gidalevitz T, Krupinski T, Garcia S, Morimoto RI (2009) Destabilizing protein polymorphisms in the genetic background direct phenotypic expression of mutant SOD1 toxicity. *PLoS Genet* 5:e1000399.
- Gidalevitz T, Ben-Zvi A, Ho KH, Brignull HR, Morimoto RI (2006) Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science* 311: 1471–1474.
- Gengyo-Ando K, Kagawa H (1991) Single charge change on the helical surface of the paramyosin rod dramatically disrupts thick filament assembly in *Caenorhabditis elegans*. *J Mol Biol* 219:429–441.
- Hammarlund M, Watanabe S, Schuske K, Jorgensen EM (2008) CAP5 and syntaxin dock dense core vesicles to the plasma membrane in neurons. *J Cell Biol* 180:483–491.
- Speese S, et al. (2007) UNC-31 (CAP5) is required for dense-core vesicle but not synaptic vesicle exocytosis in *Caenorhabditis elegans*. *J Neurosci* 27:6150–6162.
- Morley JF, Morimoto RI (2004) Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones. *Mol Biol Cell* 15:657–664.
- Feder JH, Rossi JM, Solomon J, Solomon N, Lindquist S (1992) The consequences of expressing hsp70 in *Drosophila* cells at normal temperatures. *Genes Dev* 6:1402–1413.
- Akerfelt M, Morimoto RI, Sistonen L (2010) Heat shock factors: Integrators of cell stress, development and lifespan. *Nat Rev Mol Cell Biol* 11:545–555.
- Dai C, Whitesell L, Rogers AB, Lindquist S (2007) Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis. *Cell* 130:1005–1018.
- Sugi T, Nishida Y, Mori I (2011) Regulation of behavioral plasticity by systemic temperature signaling in *Caenorhabditis elegans*. *Nat Neurosci*, 10.1038/nn.2854.
- Sternberg EM (2006) Neural regulation of innate immunity: A coordinated non-specific host response to pathogens. *Nat Rev Immunol* 6:318–328.
- Tracey KJ (2002) The inflammatory reflex. *Nature* 420:853–859.
- Spanpanato J, Gu X, Yang XW, Mody I (2008) Progressive synaptic pathology of motor cortical neurons in a BAC transgenic mouse model of Huntington's disease. *Neuroscience* 157:606–620.
- Mentis GZ, et al. (2011) Early functional impairment of sensory-motor connectivity in a mouse model of spinal muscular atrophy. *Neuron* 69:453–467.
- Ilieva HS, et al. (2008) Mutant dynein (Loa) triggers proprioceptive axon loss that extends survival only in the SOD1 ALS model with highest motor neuron death. *Proc Natl Acad Sci USA* 105:12599–12604.