A PQM-1-Mediated Response Triggers Transcellular Chaperone Signaling and Regulates Organismal Proteostasis

Highlights
- POM-1 is activated in the neurons or gut to induce TCS-mediated hsp-90 expression
- Neuron-induced TCS is mediated via PQM-1/CLEC-41 signaling
- Intestine-induced TCS is mediated via PQM-1/ASP-12 signaling
- TCS via PQM-1 is required for proteostasis and reduces amyloid beta misfolding

In Brief
O’Brien et al. find that the GATA transcription factor PQM-1 functions as a mediator of transcellular chaperone signaling (TCS). Depending on the sender tissue, PQM-1 activates a neuron-specific or intestine-specific TCS route that triggers hsp-90 expression in remote tissues. TCS-mediated hsp-90 induction reduces amyloid beta oligomerization and toxicity.

Data and Software Availability
GSE108005
A PQM-1-Mediated Response Triggers Transcellular Chaperone Signaling and Regulates Organismal Proteostasis

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SUMMARY

In metazoans, tissues experiencing proteotoxic stress induce “transcellular chaperone signaling” (TCS) that activates molecular chaperones, such as hsp-90, in distal tissues. How this form of inter-tissue communication is mediated to upregulate systemic chaperone expression and whether it can be utilized to protect against protein misfolding diseases remain open questions. Using C. elegans, we identified key components of a systemic stress signaling pathway that links the innate immune response with proteostasis maintenance. We show that mild perturbation of proteostasis in the neurons or the intestine activates TCS via the GATA zinc-finger transcription factor PQM-1. PQM-1 coordinates neuron-activated TCS via the innate immunity-associated transmembrane protein CLEC-41, whereas intestine-activated TCS depends on the aspartic protease ASP-12. Both TCS pathways can induce hsp-90 in muscle cells and facilitate amelioration of Aβ3-42-associated toxicity. This may have powerful implications for the treatment of diseases related to proteostasis dysfunction.

INTRODUCTION

All living systems are confronted with a broad range of environmental insults and physiological changes that can result in cellular stress and macromolecular damage (Morimoto et al., 1997). To re-establish protein homeostasis or proteostasis, each cell has the ability to rapidly activate stress response pathways. These include the heat shock factor-1 (HSF-1)-mediated heat shock response (HSR), the unfolded protein response (UPR), and the insulin-like-signaling pathway (ILS) mediated by the FOXO/DAF-16 transcription factor (Gardner et al., 2013; Hsu et al., 2003; Morimoto et al., 1997). Stress response pathways upregulate cell-autonomous modifiers of the proteostasis network (PN), such as molecular chaperones, that protect protein conformation during stress, refold misfolded proteins, and target irreversibly misfolded proteins to the degradation machinery (Akerfelt et al., 2010; Morimoto et al., 1997).

In addition to stress response cues that act locally and within the cell, multicellular organisms also require intercellular stress response pathways that allow for coordinated activation of protective PN components across tissues to preserve proteostasis in the entire organism. In C. elegans, this is achieved by activation of the HSR via the thermo-sensory neuronal circuitry in response to heat stress (Prahлад et al., 2008). In response to stressors of intracellular origin, such as perturbed mitochondrial function, DNA damage, and altered chaperone expression in specific tissues, a systemic stress signaling response is activated that promotes PN components in cells distinct from the stressed sender cell (Chikka et al., 2016; Durieux et al., 2011; Ermolaeva et al., 2013; van Oosten-Hawle and Morimoto, 2014; Prahland and Morimoto, 2011; Taylor et al., 2014; Taylor and Dillin, 2013). Transcellular regulation of protective stress responses is not unique to the metazoan system of C. elegans and has been observed in other model systems, including vertebrates (Mahadevan et al., 2011; Owusu-Ansah et al., 2013; Williams et al., 2014). Understanding how the PN coordinates the activation of protective components in a systemic manner is fundamentally important.

Transcellular chaperone signaling (TCS) can be activated by overexpression of the essential molecular chaperone heat shock protein 90 (HSP-90) in specific tissues. This generates a form of tissue-specific proteostatic imbalance that induces non-cell-autonomous hsp-90 expression and suppresses misfolding of metastable clients in remote tissues (van Oosten-Hawle et al., 2013). In this study, we investigated the central question of how non-cell-autonomous regulation of hsp-90 chaperone expression is achieved and whether it can be utilized to protect from the toxic consequences of aggregation-prone human disease proteins. Which signaling cues are activated in the “sender” cell to initiate TCS-mediated protective mechanisms in a distal tissue affected by protein misfolding disease?

We identified key components of TCS that are, depending on the sender tissue, differentially regulated by the transcription factor PQM-1. When initiated from the neurons, PQM-1 orchestrates TCS via the innate immunity-associated transmembrane protein CLEC-41, whereas TCS initiated from the intestine depends on the aspartic protease ASP-12. Both
TCS pathways mediate “transcellular” hsp-90 induction in the muscle and protect against muscle-expressed amyloid protein misfolding.

RESULTS

Increased Neuron- and Intestine-Specific Expression of HSP-90 Activates TCS and Suppresses Aβ3-42 Toxicity in Muscle Cells

Activation of TCS in neurons, intestine or muscle cells by expressing elevated HSP-90::mCherry protein in these tissues (henceforth called HSP-90::RFPneuro, HSP-90::RFPtr or HSP-90::RFPmuscle) leads to the non-cell-autonomous induction of a transcriptional hsp-90 reporter fused to GFP (P_{hsp-90::GFP}) (Figure 1A). In HSP-90::RFPneuro, this corresponded to a 30% increase of HSP-90::RFP protein present in the neurons relative to whole-animal endogenous HSP-90 levels (Figures S1A and S1B). This resulted in a 2.5-fold induction of the transcriptional Phsp-90::GFP reporter resulting in 5-fold increased expression of GFP mRNA and a 2-fold induction of hsp-90 mRNA levels (Figure 1D). Induction of Phsp-90::GFP transcriptional activity and hsp-90 mRNA levels was significantly higher in HSP-90::RFPneuro (Figures 1A–1D), despite the fact that the expression level of HSP-90::RFP protein in

Figure 1. Increased Expression of HSP-90 in the Neurons, Intestine, or Bodywall Muscle Activates TCS and Protects against Aβ3-42 Protein Toxicity in Muscle

(A–D) P_{hsp-90::GFP} expression in control animals (i and ii), HSP-90::RFPneuro (iii and iv), HSP-90::RFPtr (v and vi), or HSP-90::RFPmuscle (vii and viii) (A). Scale bar, 50 μm. GFP and hsp-90 mRNA levels in HSP-90::RFPneuro (B), HSP-90::RFPtr (C), and HSP-90::RFPmuscle (D) relative to control animals expressing P_{hsp-90::GFP}. (B–D) Bar graphs represent the mean of three independent experiments. Error bars represent ± SEM. *p < 0.05, ***p < 0.001; ns, not significant.

(E) Paralysis assays of C. elegans expressing Aβ3-42 in the bodywall muscle (CL2006) compared to wild-type (N2) or HSP-90::RFPneuro, Aβ3-42, HSP-90::RFPtr, Aβ3-42, or HSP-90::RFPmuscle, Aβ3-42. Paralysis data represent SEM of 3 biological replicates (n = 100 animals). Statistical significance was determined by Wilcoxon matched-pairs signed-rank test. *p < 0.05.

(F) Western blot analysis of amyloid beta monomeric and oligomeric amyloid beta expression in day 3 adult control animals (mAβ3-42), HSP-90::RFPneuro, mAβ3-42, HSP-90::RFPtr, mAβ3-42, or HSP-90::RFPmuscle, mAβ3-42. Data are expressed as mean density of the indicated band based from three independent experiments. Error bars represent SEM. One-way ANOVA; *p < 0.05.

See also Figure S1.
Figure 2. The Zinc-Finger Transcription Factor PQM-1 Mediates TCS in C. elegans
(A) Ab initio motif discovery with HOMER identifies a significantly enriched motif (GATAA) in the promoters of 27 of the 34 genes upregulated in HSP-90::GFP compared to control (N2).
(B–E) Collapsed z stack images of young adult C. elegans expressing the Phsp-90::GFP reporter in control background (B), HSP-90::RFPneuro (C), HSP-90::RFPint (D), and HSP-90::RFPmuscle (E) compared with expression of the Phsp-90::GFP reporter in a pqm-1(ko) mutant background. Expression of Phsp-90::GFP in a pqm-1(ko) mutant background (Biv), HSP-90::RFPneuro; pqm-1 (ko) (Civ), HSP-90::RFPint; pqm-1(ko) (Dv), or HSP-90::RFPmuscle (Ev). 20× magnification of the

(legend continued on next page)
**Neuron-Specific and Intestine-Specific Overexpression of HSP-90 Suppresses Aβ_{3-42} Toxicity in Muscle Cells via TCS**

In *C. elegans*, expression of human Aβ_{3-42} in the bodywall muscle leads to age-dependent aggregation and toxicity of the protein, resulting in decreased motility and paralysis (Link, 1995; McColl et al., 2012). Amyloid beta (Aβ)–associated toxicity can be suppressed by PN components (Cohen et al., 2009; Morley et al., 2002), and depends on hsp-90 expression (Brehme et al., 2014). We therefore asked whether the TCS-mediated upregulation of hsp-90 in the bodywall muscle in HSP-90::RFPneuro and HSP-90::RFPnt would suppress the toxic consequences of human Alzheimer’s Aβ protein misfolding.

We used a *C. elegans* model for Alzheimer’s disease, which expressed the cytotoxic human beta amyloid protein (mAβ_{3-42}) in the *C. elegans* bodywall muscle (Link, 1995), leading to increased paralysis with age (Figure 1E). Importantly, overexpression of HSP-90::GFP in muscle, intestine, or neurons alone had no effect on motility throughout aging compared to control (N2) animals (Figure S1D). HSP-90::RFPmuscle completely abrogated mAβ_{3-42}-associated paralysis resulting in animals that behaved similar to control animals (Figure 1E). HSP-90::RFPneuro significantly suppressed Aβ_{3-42}-dependent muscle dysfunction, with only 25% of HSP-90::RFPneuro;mAβ_{3-42} animals being paralyzed at day 8 of adulthood compared to 45% same age mAβ_{3-42} control animals (Figure 1E). Surprisingly, increased expression of HSP-90::RFPnt led to an equal level of suppression of mAβ_{3-42}-associated toxicity, with 26% of animals being paralyzed at day 8 of adulthood (Figure 1E).

Intrigued by this observation, we determined whether suppression of paralysis in all three HSP-90 overexpression strains was associated with a decrease of toxic Aβ_{3-42} conformational variants. HSP-90::RFPmuscle diminished the levels of Aβ mono- and oligomeric species, consistent with the strongly reduced paralysis in this strain (Figures 1E and 1F), which is likely a cell-autonomous consequence of HSP-90::RFPmuscle overexpression. In HSP-90::RFPneuro and HSP-90::RFPnt, accumulation of monomer and smaller oligomeric species (<17 kDa) is clearly present; however, the accumulation of higher oligomeric species (26 kDa) was reduced to 60% in HSP-90::RFPneuro and to 85% in HSP-90::RFPnt, compared with a 58% reduction in HSP-90::RFPmuscle (Figure 1G). Thus, the significant reduction of higher oligomeric species (26 kDa) in all three HSP-90 overexpression strains likely contributes to the suppression of Aβ toxicity.

**The Transcription Factor PQM-1 Mediates TCS and Triggers Innate Immune Gene Expression**

To investigate the underlying mechanism that mediates TCS upon HSP-90 overexpression, we performed RNA sequencing (RNA-seq) analyses using HSP-90::GFPneuro animals that express a 46% increase of HSP-90::GFP (Figures S1A and S1B). RNA-seq analysis identified 225 genes differentially expressed in HSP-90::GFPneuro relative to N2 control animals (Table S1) of which 34 genes (excluding 5 pseudogenes) were significantly upregulated >1.5-fold (p-adj < 0.05) (Table S2). Among these, the gene ontology (GO) term associated with innate immune responses was overrepresented (19 out of 34 genes) (Table S2). Confirmation of gene expression levels by qRT-PCR in HSP-90::GFPneuro revealed generally lower levels of expression (Figure S1C), indicating that a common transcriptional program is induced upon neuronal HSP-90 overexpression. We performed hypergeometric optimization of motif enrichment (HOMER) analysis on the upregulated genes which identified a motif with the consensus AGATAACA or TGTTATCT enriched in the promoter regions in 27 of the 34 upregulated genes in HSP-90::GFPneuro (p = 1 × 10^{-8}, found in 28% of targets vs. 0.16% of background) (Figure 2A). The motif resembled the GATA-like DAE (DAF-16-associated element) which has been previously identified as the binding site for the C2H2 zinc-finger transcription factor PQM-1 (Tepper et al., 2013). Consistent with our finding, a previous PQM-1 chromatin immunoprecipitation sequencing (ChiP-seq) analysis confirms binding of PQM-1 to 16 promoters of genes identified in our dataset (see Table S2) (Niu et al., 2011).

To determine whether PQM-1 is involved in TCS, we used a *pqm-1(ok485)* knockout mutant (*pqm-1(KO)*), which contains a deletion mutation to remove exons 2 to 6, effectively depleting expression of *pqm-1* transcripts (Figure S1E) (Dowen anterior (head) region (Br, Bv, Cil, Cv, Dil, Dv, El, and Ev). Differential interference contrast (DIC) Nomarski, GFP, RFP overlay images. Tissues showing P_{hsp-90};GFP expression are indicated with a white arrow. bwrm, bodywall muscle; exc, excretory cell; exc canal, excretory canal; ph, pharynx; and int, intestine. (F–I) Quantification of GFP fluorescence intensity in *C. elegans* expressing P_{hsp-90};GFP in the *pqm-1(KO)* mutant compared to control background (F). HSP-90::RFPneuro;*pqm-1(KO)* (G), or HSP-90::RFPnt;*pqm-1(KO)* (H), or HSP-90::RFPmuscle;*pqm-1(KO)* (I) relative to control. (J–M) GFP and hsp-90 mRNA levels are reduced in HSP-90::GFPneuro;P_{hsp-90};GFP;*pqm-1(KO)* (K), HSP-90::GFPneuro;P_{hsp-90};GFP;*pqm-1(KO)* (L), and HSP-90::GFPmuscle;P_{hsp-90};GFP;*pqm-1(KO)* (M), but not in P_{hsp-90};GFP;*pqm-1(KO)* (J) relative to the P_{hsp-90};GFP control strain. Scale bar, 50 μm (B–E). Bar graphs represent the mean of three independent experiments (F–M). Error bars represent ± SEM. *p < 0.05, ns, not significant. See also Figure S2. **
Depletion of pqm-1 in C. elegans expressing HSP-90::RFP<sup>neuro</sup>;P<sub>hsp-90::GFP</sub> reduced GFP fluorescence intensity to 40% (Figures 2C, 2G, and S2B) and to 90% in HSP-90::RFP<sup>neuro</sup>;P<sub>hsp-90::GFP</sub> (Figures 2D, 2H, and S2C), whereas depletion of pqm-1 in HSP-90::RFP<sup>muscle</sup>; P<sub>hsp-90::GFP</sub> reduced GFP fluorescence to 75% (Figures 2E, 2I, and S2D), suggesting that pqm-1 mediates TCS. However, absence of pqm-1 in the P<sub>hsp-90::GFP</sub> control strain did not affect GFP fluorescence intensity (Figures 2B, 2F, and S2A).

At the level of individual tissues, pqm-1(KO) in HSP-90::RFP<sup>neuro</sup> and HSP-90::RFP<sup>muscle</sup> decreased P<sub>hsp-90::GFP</sub> expression in excretory cell and canal, pharynx, and bodywall muscle (Figures 2C and 2D, respectively), whereas in HSP-90::RFP<sup>muscle</sup> this led to a visibly decreased expression of the reporter in pharynx and intestine (Figure 2E). Pqm-1(KO) in all three TCS-activated strains resulted in a general reduction of hsp-90 mRNA levels (Figures 2K, 2L, and 2M), reducing global hsp-90 expression back to the basal levels of the control strain (Figure 2J). The pqm-1(KO) mutation did not affect hsp-90 or GFP mRNA expression levels in the control strain (Figure 2J), suggesting that pqm-1 does not regulate hsp-90 expression under normal growth conditions. Consistently, knockdown of pqm-1 by RNAi in the control strain (Figures S2E and S2I), HSP-90::RFP<sup>neuro</sup>;P<sub>hsp-90::GFP</sub> (Figures S2F and S2J), in HSP-90::RFP<sup>neuro</sup>;P<sub>hsp-90::GFP</sub> (Figures S2G and S2K) or HSP-90::RFP<sup>muscle</sup>;P<sub>hsp-90::GFP</sub> (Figures S2H and S2L) phenocopied the effect of the pqm-1(KO) mutation on P<sub>hsp-90::GFP</sub> reporter and global hsp-90 mRNA levels.

RNAi-mediated knockdown of hsf-1 or daf-16 had no effect on GFP fluorescence intensity of the reporter or GFP and hsp-90 mRNA levels in all three TCS-activated strains, whereas hsp-90 expression in the control strain was dependent on hsf-1 or daf-16 (Figure S3), confirming that neither DAF-16 nor HSF-1 are involved in TCS (van Oosten-Hawle et al., 2013).

The motif enrichment analysis also identified the Foxa transcription factor PHA-4 (ranked at position 13, p = 1e-5), previously shown to be required for TCS in C. elegans (van Oosten-Hawle et al., 2013). However, the PHA-4 consensus sequence was found in the promoter of only one putative target gene (Y41C4A.11) (Table S2). To investigate whether pqm-1 and pha-4 could act in the same genetic pathway to regulate TCS-induced hsp-90 expression, we knocked down pha-4 by RNAi in addition to pqm-1(KO), which further reduced GFP and hsp-90 mRNA levels in all three TCS-activated strains, indicating that pqm-1 and pha-4 may control TCS via two distinct pathways.

As pqm-1 mediates TCS in response to tissue-specific HSP-90 overexpression, we sought to determine whether this was also the case when hsp-90 is constitutively knocked down (KD) via tissue-specific hsp-90 hairpin RNAi in the neurons (hsp-90<sup>neuro</sup>(KD)) or intestine (hsp-90<sup>int</sup>(KD)), which leads to systemic upregulation of hsp-70 (C12C8.1) (Figure S4A) (van Oosten-Hawle et al., 2013). However, the absence of pqm-1 had no effect on hsp-70 mRNA expression in hsp-90<sup>neuro</sup>(KD) and hsp-90<sup>int</sup>(KD) (Figure S4A), and pqm-1 mRNA levels were not affected by tissue-specific knockdown of hsp-90 (Figure S4B). Thus, PQM-1 mediates TCS specifically in response to tissue-specific HSP-90 overexpression in the neurons or intestine, but not in response to reduced expression of hsp-90 in the same tissues.

PQM-1 Is Required for Stress-Induced hsp-90 Expression and Heat Stress Resistance

PQM-1 complements DAF-16/FOXO to regulate longevity in C. elegans (Tepper et al., 2013). It is, however, unclear whether PQM-1 is involved in the regulation of cell stress responses or the maintenance of proteostasis in general.

We measured mRNA levels of a range of heat-inducible chaperones, including stress-inducible hsp-70 (C12C8.1), hsp-90, the small Hsp hsp-16.2, and constitutively expressed Hsp70 (hsp-1), by qRT-PCR after a 1-hr heat shock (HS) at 35°C in pqm-1(KO) mutants (Figures 3A, 3B, S4C, and S4D). While induction of all four chaperones were significantly reduced in the hsf-1(sy441) mutant that cannot induce a proper HSR (Hajducronin et al., 2004), heat-inducible mRNA accumulation of hsp-70, hsp-1, and hsp-16.2 was comparable between pqm-1(KO) and wild-type (WT) animals (Figures 3A, S4C, and S4D, respectively), as well as between the hsf-1(sy441);pqm-1(KO) mutant and hsf-1(sy441) (Figures 3A, S4C, and S4D), indicating that stress induction of these chaperones is dependent on functional hsf-1, but not pqm-1. Interestingly, both the pqm-1(KO) mutant and the hsf-1(sy441);pqm-1(KO) mutant showed significant decreases in heat-inducible hsp-90 mRNA compared to controls (Figure 3B). After HS treatment, hsp-90 was weakly induced in the hsf-1(sy441) mutant, albeit starting at a 50% lower basal expression level (Figure 3B). However, hsp-90 mRNA induction after HS remained at its basal expression level in the pqm-1(KO) and hsf-1(sy441);pqm-1(KO) mutants (Figure 3B), with HSP-90 protein expression levels equally reduced in the hsf-1(sy441) and pqm-1(KO) compared to control animals (Figures S4E and S4F). This indicates that heat-inducible hsp-90 expression is dependent on the presence of pqm-1, while basal hsp-90 expression depends on functional hsf-1.

Next, we investigated whether loss of pqm-1(KO) has any consequences for HS survival of C. elegans. While 45% of control animals survived a 6-hr heat shock, only 20% of pqm-1(KO) mutants survived, comparable to animals treated with hsf-1 RNAi (Figure 3C). RNAi-mediated knockdown of hsf-1 in the pqm-1(KO) mutant further amplified this effect, with less than 5% of pqm-1(KO) animals treated with hsf-1 RNAi surviving HS (Figure 3C). Similar results were obtained for pqm-1(KO) animals crossed into the genetic background of hsf-1(sy441) mutants (Figure S4G), indicating that pqm-1 regulates heat stress survival in a pathway independent or complementary to hsf-1. These results show that although pqm-1(KO) mutants are capable of inducing hsp-70 and hsp-16.2 mRNA after HS, the inability of pqm-1(KO) mutants to induce protective levels of hsp-90 is detrimental for survival.

pqm-1 Is Required for Proteostasis Maintenance

To assess whether pqm-1 is required for the age-associated decline of proteostasis, we used C. elegans expressing Q35::YFP in the bodywall muscle (Morley et al., 2002). Q35::pqm-1(KO) animals accumulated an average of 70 aggregates per animal by day 4 of adulthood, whereas Q35::YFP control animals accumulated 55 aggregates (Figures 3D and 3E). At day 8 of...
Our finding that PQM-1-dependent genes are upregulated in HSP-90::RFPneuro in the neurons indicates that the transcriptional activity of PQM-1 must also be increased. Consistent with previous reports, we found PQM-1::GFAP::FLAG protein nuclearly localized in intestinal cells throughout all larval stages (L1 to L4) in WT background, reflecting its active transcriptional role during development (Dowen et al., 2016; Tepper et al., 2013) (Figures 4B1 and 4Bv). PQM-1 remained localized to the nucleus, signaling increased transcriptional activity, whereas no nuclear distribution of PQM-1 is observed in HSP-90::RFPmuscle (Figure 4B, iii and vi) or WT animals (Figures 4Avi and 4Axii). Quantification revealed that 25% of PQM-1::GFAP::FLAG remained in intestinal nuclei in HSP-90::RFPneuro and 20% in HSP-90::RFPint, compared to only 5% nuclear PQM-1 in the control strain and 10% (ns) in HSP-90::RFPmuscle (Figure 4C). However, PQM-1::GFAP::FLAG protein levels remained constant across WT and TCS-activated strains (Figure 4D). Therefore, PQM-1 nuclear localization and presumably transcriptional activity is extended into reproductive adulthood through neuron- or intestine-specific overexpression of HSP-90.

Because pqm-1 is required for resistance to the pathogenic bacteria Pseudomonas aeruginosa (Shapira et al., 2006), we asked whether the TCS-activated strains exhibiting increased toxicity (Figure 3F). Combined these results show that PQM-1 is a regulatory component of the proteostasis network that is required for stress survival and maintenance of proteostasis throughout aging.

**Nuclear Localization of PQM-1 Is Increased in the Intestine of HSP-90::RFPneuro**

adulthood, 75% of Q35;pqm-1(KO) animals were paralyzed compared to 50% of control animals, indicating increased toxicity (Figure 3F). Combined these results show that PQM-1 is a regulatory component of the proteostasis network that is required for stress survival and maintenance of proteostasis throughout aging.

**Figure 3. PQM-1 Regulates Heat-Inducible Expression of hsp-90 and Is Involved in Proteostasis**

(A and B) mRNA levels of heat-inducible hsp-70 (C12C8.1) (A) or hsp-90 (B) before and after heat shock (1 hr at 35°C) in young adult wild-type, pqm-1(KO) mutant, hsf-1(y441) mutant, and pqm-1(KO);hsf-1(y441) double-mutant animals relative to heat-shocked control animals (wild-type, N2). (C) thermo-sensitivity of L4 animals (n = 20; five biological replicates) with indicated genotypes during control (EV) or hsf-1 RNAi after exposure to a 35°C heat shock for 4, 5, or 6 hr. Survival was measured after a recovery period of 16 hr at 20°C. (D) Q35::YFP aggregation is enhanced in pqm-1(KO) mutants. Quantification of accumulated Q35 foci in age-synchronized day 4 adults of pqm-1(KO) mutants and control animals. *p < 0.05. (E) Day 4 adult C. elegans expressing Q35::YFP in the bodywall muscle of control animals (i and iii) and pqm-1(KO) mutants (ii and iv), 20x magnification of anterior (head) region of control and pqm-1(KO) mutants (ii and iv). Scale bar, 50 μm. (F) Q35-associated toxicity is increased in pqm-1(KO) mutants. Paralysis was measured in age-synchronized pqm-1(KO) mutants expressing Q35::YFP in the bodywall muscle (Q35) compared with Q35 control animals at the indicated time points (100 animals per biological replicate, N = 3). *p < 0.05; **p < 0.01; paired t test. Error bars represent ± SEM.

*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 (A–C). Bar graphs represent combined mean values of five independent experiments. Error bars represent ± SEM. See also Figures S3 and S4.
nuclear PQM-1 localization were more resistant. Only HSP-90::RFP\textsuperscript{nuero} showed increased survival to *P. aeruginosa* (Figure S4H), consistent with the higher measured PQM-1 nuclear localization in this strain (Figures 4B and 4C). Depletion of *pwm-1* in HSP-90::RFP\textsuperscript{nuero}, but not in control WT animals, reversed this effect (Figure S4H). Although PQM-1 nuclear localization is also induced in HSP-90::RFP\textsuperscript{int}, it is lower when compared to HSP-90::RFP\textsuperscript{nuero} (Figures 4B and 4C) and may not be sufficient for an effective innate immune response.

**Neuron- and Intestine-Specific HSP-90 Overexpression Triggers PQM-1 Transcriptional Activity in the Same Tissue to Regulate Systemic TCS**

PQM-1 is expressed in the intestine and neuronal cells (Reece-Hoyes et al., 2007; Tepper et al., 2013), although we could not detect PQM-1::GFP::FLAG expression by fluorescence microscopy in *C. elegans* neurons. We therefore wanted to determine whether PQM-1 is required in the neurons or the intestine of *C. elegans* to regulate systemic TCS.

**Figure 4. PQM-1::GFP::FLAG Remains Nuclearly Localized in the Intestine of Young Adult *C. elegans* Expressing HSP-90::RFP\textsuperscript{nuero} or HSP-90::RFP\textsuperscript{int}**

(A) Expression of PQM-1::GFP::FLAG (strain OP201) during larval stages L1 (i and vii), L2 (ii and vii), L3 (iii and xi), and L4 (iv and x). PQM-1::GFP nuclear localization becomes diffuse in early pre-reproductive adults (66 hr after hatching at 20°C) (vi and x) and disappears in day 1 adults (66 hr after hatching) (v and xii). Scale bar, 20 μm (i–xii). Scale bar, 50 μm (v–vi). Yellow arrows denote eggs present in the day 1 adult.

(B) PQM-1::GFP::FLAG localizes to intestinal nuclei in TCS-activated strains HSP-90::RFP\textsuperscript{nuero} (i and iv), HSP-90::RFP\textsuperscript{int} (ii and v), and HSP-90::RFP\textsuperscript{muscle} (iii and vi). Scale bar, 50 μm. Yellow arrows indicate eggs.

(C) PQM-1::GFP::FLAG in HSP-90::RFP\textsuperscript{nuero}, HSP-90::RFP\textsuperscript{int}, or HSP-90::RFP\textsuperscript{muscle} was scored for nuclear and cytosolic localization and compared to the control strain (n > 20 per strain). *p < 0.05; *p < 0.01; Kruskal-Wallis test. Error bars represent ± SEM.

(D) Western blot analysis of PQM-1::GFP::FLAG in TCS-activated strains compared to control animals using an anti-FLAG antibody. Tubulin was used as a loading control.

HSP90::RFP\textsuperscript{nuero} animals for TCS-mediated hsp-90 induction in other tissues. Moreover, we considered the possibility that PQM-1 is activated in the bodywall muscle, where *Phsp-90::GFP* expression is largely induced as a consequence of TCS (Figures 2B–2D). We therefore generated *C. elegans* strains in which the effects of RNAi are restricted to neurons (HSP-90::RFP\textsuperscript{nuero};Phsp-90::GFP, sid-1(pk3321);unc-119p::SID-1) (Calixto et al., 2010), or intestine (HSP-90::RFP\textsuperscript{int};Phsp-90::GFP, sid-1(pk3321);unc-54p::SID-1).

To confirm that tissue-specific RNAi was indeed restricted to neurons, intestine, or muscle, we exposed the aforementioned *C. elegans* strains to hsp-90 RNAi and measured tissue-specific HSP-90::RFP fluorescence intensity (Figure S5). For example, HSP-90::RFP fluorescence intensity in the neurons was reduced to 25% by systemic hsp-90 RNAi (Figures S5A and S5B) and to 30% by neuron-specific hsp-90 RNAi (Figures S5Ca and S5Cb), but had no effect upon intestine-specific or muscle-specific hsp-90 RNAi (Figures S5Da and S5Db and Figures S5Ea and SSEb, respectively). Consistently, hsp-90 RNAi in the RNAi-resistant control strain HSP-90::RFP\textsuperscript{nuero};Phsp-90::GFP, sid-1(pk3321) did not reduce HSP-90::RFP expression (Figures S5Ba and S5Bb). Similar results showing the tissue specificity of this RNAi approach were also obtained in HSP-90::RFP\textsuperscript{int} and HSP-90::RFP\textsuperscript{muscle} strains (see Figure S5).
Neuron-specific pqm-1 RNAi in HSP-90::RFP\textsuperscript{neuro} effectively reduced GFP fluorescence intensity of the P\textsubscript{hsp-90}::GFP reporter compared to animals treated with control RNAi (Figure 5A), corresponding to a 40% reduction of both GFP and hsp-90 mRNA levels (Figure 5D). Interestingly, intestine-specific knockdown of pqm-1 by RNAi in HSP-90::RFP\textsuperscript{euro} had no effect on P\textsubscript{hsp-90}::GFP reporter expression (Figure 5B) or GFP and hsp-90 mRNA levels (Figure 5E). These results suggest that PQM-1 is required in the neurons, but not the intestine, of HSP-90::RFP\textsuperscript{euro} to mediate TCS-induced hsp-90 expression in distal tissues. The higher distribution of P\textsubscript{QPM-1} to intestinal nuclei in this strain (Figures 4B and 4C) may be a consequence of TCS but is not required for TCS-mediated hsp-90 expression.

Intestine-specific pqm-1 RNAi in HSP-90::RFP\textsuperscript{int} reduced GFP fluorescence intensity of the P\textsubscript{hsp-90}::GFP reporter (Figure 5H) which corresponded to a 50% decrease of GFP and hsp-90 mRNA expression relative to control animals (Figure 5K). However, neuron-specific pqm-1 RNAi in the same strain left P\textsubscript{hsp-90}::GFP reporter expression unchanged (Figure 5G) and had no effect on GFP or hsp-90 mRNA expression levels (Figure 5J). Combined, these results indicate that PQM-1 may be activated in the same tissue that overexpresses HSP-90::RFP to induce TCS, but does not require PQM-1 to be expressed in other tissues for TCS.

Interestingly, muscle-specific pqm-1 RNAi had no effect on TCS-mediated hsp-90 expression in HSP-90::RFP\textsuperscript{euro} (Figures 5C and 5F), HSP-90::RFP\textsuperscript{int} (Figures 5I and 5L) or HSP-90::RFP\textsuperscript{muscle} (Figures 5O and 5P). This indicates that neurons and intestine are key tissues for TCS and suggests that PQM-1 indirectly regulates hsp-90 expression in the muscle.

PQM-1 Indirectly Regulates TCS-Induced hsp-90 Expression in Muscle via Innate Immune Factors clec-41 and asp-12

Next, we investigated whether PQM-1 directly regulates TCS-induced hsp-90 expression by binding to a putative PQM-1 binding motif 119-129 bp upstream of the TSS in the hsp-90 promoter. Therefore we generated a transcriptional reporter containing the native hsp-90 promoter region upstream of GFP, P\textsubscript{hsp-90(WT)}::GFP, or lacking the putative PQM-1 binding motif sequence, P\textsubscript{hsp-90(del)}::GFP (Figure S6A). P\textsubscript{hsp-90(del)}::GFP reporter animals did however not show a significant change in GFP intensity relative to the native P\textsubscript{hsp-90(WT)}::GFP reporter (Figures S6B and S6D). Although both the native or modified P\textsubscript{hsp-90}::GFP reporter exhibited a significant upregulation of P\textsubscript{hsp-90}::GFP expression in the bodywall muscle and pharyngeal tissues when crossed into HSP-90::RFP\textsuperscript{euro} (Figure S6C), this TCS-mediated hsp-90 induction was similar between the native and modified reporter constructs (Figures S6D, S6E, and S6G). Thus, deletion of the putative PQM-1 binding motif in the hsp-90 promoter had no effect on TCS-mediated hsp-90 expression in distal tissues, corroborating the idea that PQM-1 indirectly controls hsp-90 expression possibly via a pqm-1-regulated effector.

To examine this possibility further, we performed an RNAi mini-screen of the 16 genes identified in our RNA-seq dataset that contain a PQM-1 binding motif in their promoters (Niu et al., 2011) (Table S2). We identified three hits, namely F21F8.4 (asp-12), B0365.6 (clec-41), and B0285.9 (ckb-2) that upon RNAi-mediated knockdown reduced GFP fluorescence by more than 50% in HSP-90::RFP\textsuperscript{euro}P\textsubscript{hsp-90}::GFP (Figure 6A and Figure S7A). mRNA expression of all three genes are induced in HSP-90::RFP\textsuperscript{euro}P\textsubscript{hsp-90}::GFP (Figure 6B) and RNAI-mediated knockdown of pqm-1 reduced mRNA expression of all three genes back to control levels (Figure 6B), confirming the requirement for pqm-1 to regulate their expression. Systemic ckb-2, asp-12, or clec-41 RNAI in HSP-90::RFP\textsuperscript{euro} reduced P\textsubscript{hsp-90}::GFP (Figure S7A), as well as GFP and hsp-90 mRNA expression relative to control RNAI, indicating their involvement in TCS-mediated hsp-90 induction (Figure 6D).

PQM-1 Orchestrates Neuron-Activated TCS via PQM-1/CLEC-41 and Intestine-Activated TCS via PQM-1/ASP-12 Signaling

HSP-90::RFP\textsuperscript{euro} requires PQM-1 in the neurons to trigger TCS; therefore, we tested whether neuronal expression of ckb-2, asp-12, or clec-41 is required in the neurons for TCS-mediated hsp-90 induction in the muscle. Strikingly, neuron-specific RNAI of only one of the gene hits, clec-41, reduced TCS-induced hsp-90 expression (Figure 6E). At the level of individual tissues this corresponded to reduced expression of the P\textsubscript{hsp-90}::GFP reporter in bodywall muscle and pharynx, indicating the requirement for clec-41 expression in the neurons to mediate TCS-induced expression of hsp-90 in muscle (Figures 6F and S7C). Simultaneous clec-41 and pqm-1 RNAI did not lead to further reduction of TCS activity (Figure 6D), suggesting that pqm-1

Figure 5. PQM-1 Is Required in the Neurons in HSP-90::RFP\textsuperscript{euro} and in the Intestine in HSP-90::RFP\textsuperscript{int} to Induce TCS

(A–C) Expression of the P\textsubscript{hsp-90}::GFP transcriptional reporter in HSP-90::RFP\textsuperscript{euro} during tissue-specific control or pqm-1 RNAI in neurons (A), intestine (B), or bodywall muscle (C).

(D–F) Transcript levels of GFP and hsp-90 in HSP-90::RFP\textsuperscript{euro}P\textsubscript{hsp-90}::GFP during neuron-specific (D), intestine-specific (E), and muscle-specific (F) control (EV) or pqm-1 RNAI. \( p < 0.05 \), ns, non-significant. Bar graphs represent combined mean values of three independent experiments. Error bars represent ± SEM.

(G–I) Expression of the P\textsubscript{hsp-90}::GFP transcriptional reporter in HSP-90::RFP\textsuperscript{int} adults during tissue-specific control RNAI (EV) or pqm-1 RNAI in neurons (G), intestine (H), or bodywall muscle (I).

(J–L) Transcript levels of GFP and hsp-90 during neuron-specific (J), intestine-specific (K), or muscle-specific (L) control (EV) or pqm-1 RNAI in HSP-90::RFP\textsuperscript{int}P\textsubscript{hsp-90}::GFP.

(M–O) Expression of the P\textsubscript{hsp-90}::GFP transcriptional reporter in HSP-90::RFP\textsuperscript{muscle} adults during tissue-specific control RNAI (EV) or pqm-1 RNAI in neurons (M), intestine (N), or bodywall muscle (O).

(P–R) Transcript levels of GFP and hsp-90 mRNA in HSP-90::RFP\textsuperscript{muscle}P\textsubscript{hsp-90}::GFP during neuron-specific (P), intestine-specific (O), or muscle-specific (R) control (EV) or pqm-1 RNAI. \( p < 0.05 \), ns, non-significant. Bar graphs represent combined mean values of three independent experiments. Error bars represent ± SEM (A–C, G–I, and M–O). Scale bar, 50 \( \mu \text{m} \).

See also Figures S5 and S6.
Figure 6. *pam-1*-Regulated Genes Are Required for TCS-Induced hsp-90 Expression

(A) Quantification of P_{hsp-90}::GFP fluorescence intensity in HSP-90::RFP^neuro;P_{hsp-90}::GFP animals during RNAi-mediated knockdown of indicated 16 genes relative to control (EV) RNAi. Scored hits (clec-41, asp-12, and ckb-2) show less than 50% GFP fluorescence intensity.

(legend continued on next page)
and clec-41 function in the same signaling pathway to regulate TCS-mediated hsp-90 induction.

Because HSP-90::RFP\textsuperscript{neuro} induces TCS in a pqm-1-dependent manner, we sought to determine whether ckb-2, asp-12 or clec-41 could also play a role in TCS-mediated hsp-90 expression in HSP-90::RFP\textsuperscript{neuro}. Indeed, both ckb-2 and asp-12 expression were upregulated (Figure 6C). Systemic ckb-2 or asp-12 RNAi significantly reduced GFP mRNA expression in HSP-90::RFP\textsuperscript{neuro}.\textsuperscript{hsp-90::GFP}, with asp-12 RNAi leading to a significant 50% decrease of global hsp-90 mRNA expression relative to control RNAi (Figures 6G and S7D). Intestine-specific ckb-2 RNAi was ineffective, but intestine-specific asp-12 RNAi reduced global hsp-90 levels to 75% (Figure 6H), and reduced \(P_{\text{asp-90::GFP}}\) reporter expression in muscle cells (Figures 6I and S7E). Simultaneous asp-12 and pqm-1 RNAi led to no further reduction of hsp-90 or GFP mRNA expression levels, confirming that asp-12 and pqm-1 likely function in the same intestine-activated TCS pathway (Figure 6K). Importantly, the neuron-induced TCS route was unaffected by intestine-specific clec-41 or asp-12 RNAi (Figure S7J), whereas intestine-activated TCS was unaffected by neuron-specific clec-41 or asp-12 RNAi (Figure S7J), demonstrating that both TCS routes function independent of each other and are differentially regulated.

**Neuron-Induced TCS Promotes Non-cell-autonomous hsp-90 Expression through Glutamate-Mediated Neurotransmission**

We have previously shown that TCS-mediated hsp-90 expression is controlled independently of dense-core vesicle unc-31- or small core vesicle unc-13-mediated neuro-secretion (van Oosten-Hawle et al., 2013), yet neuron-induced TCS regulates hsp-90 expression in distal tissues via neurally activated PQM-1/CLEC-41 signaling. One explanation for this discrepancy is that upon depletion of unc-31-mediated dense core vesicle neuro-secretion the unc-13-mediated small core vesicle route is still active and so compensates for loss of the other and vice versa. Depletion of unc-31 had no effect on TCS-mediated hsp-90 or GFP mRNA expression (Figures S7F–S7H) (van Oosten-Hawle et al., 2013), and additional RNAi-mediated knockdown of unc-13 in the neurons of these strains did not alter global hsp-90 or GFP mRNA expression (Figures S7F–S7H). Nevertheless, a signal needs to be transmitted from neurons to distal tissues in HSP-90::RFP\textsuperscript{neuro}. We therefore tested neurotransmitter-mediated pathways previously identified to be required for systemic stress signaling in *C. elegans*, such as serotonergic and octopaminergic signaling (Sun et al., 2012; Tatum et al., 2015b) for their involvement in TCS. HSP-90::RFP\textsuperscript{neuro} animals deficient for serotonergic signaling via deletion of the metabotropic serotonin receptor *ser-1*, exhibited increased global GFP and hsp-90 mRNA expression (Figure 6L), similar to HSP-90::RFP\textsuperscript{neuro} animals deficient for octopaminergic signaling, via depletion of the G-protein-coupled receptor *oct-1* (Figure 6L). Thus, serotonergic and octopaminergic signaling are both involved in TCS; however, they may function as suppressors rather than facilitators.

The innate immune peptide CLEC-41 is a predicted plasma membrane-associated protein containing two beta-barrel forming domains known as “CUB domains,” which are found almost exclusively in extracellular and plasma membrane-associated proteins (Bork and Beckmann, 1993) and enriched in the neurons and intestine (Reece-Hoyes et al., 2007; Spencer et al., 2011). Interestingly, other neuronally expressed CUB domain proteins in *C. elegans*, e.g., *sol-1*, are known to participate in the gating mechanism for ions to pass through AMPA-type glutamate receptors (Zheng et al., 2006). We therefore investigated the role of glutamatergic signaling for TCS and used a *glr-1* mutant that expresses a non-functional version of the ionotropic AMPA-type glutamate receptor (Rongo et al., 1998) in HSP-90::RFP\textsuperscript{neuro}. This decreased GFP and global hsp-90 mRNA expression by 25% relative to the control (Figure 6L), suggesting that glutamatergic signaling is indeed a neuronal signaling route required for TCS.

**TCS via Neuronal PQM-1/CLEC-41 Signaling and Intestinal PQM-1/ASP-12 Signaling Protects against maβ2-Associated Toxicity**

Neuron-induced and intestine-induced TCS mediate hsp-90 induction in the muscle, thereby alleviating muscle-expressed...
mAβ3,42-associated toxicity (Figure 1E). Remarkably, pqm-1 or clec-41 RNAi in the TCS-activated HSP-90::RFPneuro,mAβ3,42 strain exacerbated paralysis (Figures 7A and 7B, respectively) reversing the protective effect of neuron-induced TCS. While 23% of HSP-90::RFPneuro,mAβ3,42 animals grown on control RNAi were paralyzed at day 8 of adulthood, pqm-1 RNAI in HSP-90::RFPneuro,mAβ3,42 abolished the TCS-mediated protective effect by increasing the amount of paralyzed HSP-90::RFPneuro,mAβ3,42 animals to 38% (Figure 7A), comparable to mAβ3,42 control animals (Figure 7A). Likewise, pha-4 RNAI reversed the protective effect of HSP-90::RFPneuro on amyloid toxicity in the bodywall muscle (Figure S7K). Clec-41 RNAI resulted in 50% of HSP-90::RFPneuro,mAβ3,42 animals being paralyzed at day 8 (Figure 7B), reducing the TCS-mediated protection against amyloid toxicity. However, pqm-1 or clec-41 RNAI in mAβ3,42 control animals alone did not further increase the fraction of paralyzed mAβ3,42 animals (Figures 7A and 7B), indicating that the protective effect via PQM-1/CLEC-41 signaling was specific to neuronal activation of TCS. Strikingly, pqm-1 and asp-12 RNAI during intestine-induced TCS led to an even more severely exacerbated paralysis of HSP-90::RFPint,mAβ3,42 compared to the mAβ3,42 control strain (Figures 7C and 7D, respectively). In summary, our data show that neuron-induced TCS mediates suppression of amyloid protein-associated toxicity via hsp-90 induction in the muscle dependent on neuronal PQM-1/CLEC-41 signaling, while the same effect via intestine-induced TCS is dependent on PQM-1/ASC-12 signaling.

**DISCUSSION**

Our study identified key components of signaling cues that regulate transcellular activation of hsp-90 chaperone expression in C. elegans. We identified PQM-1 as a mediator of TCS that differentially orchestrates the induction of innate immune peptides to promote hsp-90 expression in distal tissues. Activation of TCS in...
the neurons requires the plasma-membrane-associated innate immune peptide CLEC-41 in the neurons (Figure 7E), while activation of TCS in the intestine depends on the aspartic protease ASP-12 to induce hsp-90 expression in distal tissues (Figure 7F). Both TCS cues—the neuron-activated PQM-1/CLEC-41 signaling cue and the intestine-activated PQM-1/ASP-12 signaling pathway—induce hsp-90 in muscle cells to ameliorate Aβ_{3-42}-associated toxicity and reduce the accumulation of toxic oligomeric amyloid species.

The PQM-1-dependent TCS network appears to require PQM-1 activity in the stressed sender tissue and not the responding tissue (Figures 7E and 7F). While hsp-90 is ubiquitously expressed in C. elegans (Casanueva et al., 2012; Mendenhall et al., 2015; van Oosten-Hawle et al., 2013), expression of pqm-1 overlaps with hsp-90 only in neuronal and intestinal tissue (Reece-Hoyes et al., 2007; Spencer et al., 2011). Moreover, pqm-1 does not appear to be required in the muscle to directly regulate hsp-90 expression. Further evidence arguing against the possibility of PQM-1 directly regulating hsp-90 expression, is provided via deletion of the putative PQM-1 GATA binding site in the hsp-90 promoter. However, before completely excluding this possibility, a more detailed promoter analysis that includes >1000 bp of the hsp-90 promoter or ChIP-seq experiments will be needed to characterize PQM-1’s role in the induction of stress-induced hsp-90 expression. To this end, our data suggest that PQM-1 regulates hsp-90 expression via an indirect mechanism that involves pqm-1 regulated innate immune genes asp-12 and clec-41.

While neither ASP-12 nor CLEC-41 act as the “transcellular chaperone activating signal” itself, as neither are predicted secreted peptides (Table S2), both could facilitate the transmission of a “signal” to other tissues. As a CUB domain containing protein expressed in neurons (Zheng et al., 1999), CLEC-41 could be a factor that facilitates neuron-activated TCS via glutamatergic signaling. Future work should define the neuron-specific function for CLEC-41 in TCS in more detail.

Many known PQM-1 target genes are associated with the innate immune response (Niu et al., 2011; Shapira et al., 2006; Templeman et al., 2018) and are also identified in our TCS-induced dataset. While our results indicate a clear requirement for pqm-1 for P. aeruginosa resistance during neuronal activation of TCS, this is not the case in the pqm-1(KO) mutant alone (Figure S4H) and contrary to a previous report (Shapira et al., 2006). This discrepancy may however stem from differences in strain backgrounds used (pqm-1(KO) versus pqm-1 RNAi in a glp-4 (bn2);nrf-3 mutant) and different assay temperatures (20°C vs. 25°C). It may be that an increased temperature (25°C) poses an additional challenge that affects pqm-1-dependent survival on pathogenic bacteria.

Our discovery of PQM-1 as an important component of the PN reveals that metazoans make use of non-canonical stress transcription factors that function complementary to HSF-1 and DAF-16 to control proteostasis. For example, HSP-90 chaperone expression can be regulated by the myogenic transcription factor HLH-1 during C. elegans muscle development (Bar-Lavan et al., 2016) and by PHA-4 during TCS (van Oosten-Hawle et al., 2013).

Moreover, C. elegans utilizes multiple regulatory pathways to specify the type of stress-response activated in remote cells. For example, DAF-16-mediated stress-resistance is separated from HSF-1-mediated activity through a distinct chemosensory neuronal circuit (Volovik et al., 2014), whereas HSF-1 uses a thermo-sensory circuit to promote non-cell-autonomous expression of HSPs (Douglas et al., 2015; Prahlad and Morimoto, 2011; Prahlad et al., 2008). Likewise, activation of UPR pathways in response to bacterial infection utilizes octopaminergic signaling (Sun et al., 2012).

Similar to the differential regulatory cues initiated via HSF-1 or DAF-16, stress conditions that activate TCS also utilize more than one regulatory mechanism. TCS-mediated hsp-90 expression via PHA-4 is regulated differently from the PQM-1-dependent signaling routes described here. Moreover, neuronal activation of TCS triggers a PQM-1-dependent signaling route that relies on CLEC-41 and glutamatergic signaling, whereas activation of TCS from the intestine requires ASP-12 as a key transcellular signaling node.

In neuron-activated TCS, global hsp-90 expression is induced 2.5-fold, whereas hsp-90 expression is induced 1.75-fold in HSP-90::RFP(neuro), which is lower compared to our previous study using the same strain (van Oosten-Hawle et al., 2013). It is possible that this measured discrepancy is due to suppressor mutations that have accumulated in HSP-90::RFP(neuro). Despite this difference in the level of hsp-90 induction, both HSP-90::RFP(neuro) and HSP-90::RFP(neuro) induce TCS, which results in a substantial and similar suppression of the toxic effects of Aβ expression in the muscle in both strains (Figures 1E–1G), comparable to the previously observed suppression of metastable myosin misfolding in muscle (van Oosten-Hawle et al., 2013). Although monomer and smaller oligomeric Aβ species (< 17 kDa) are unaffected by TCS-induced hsp-90 expression in HSP-90::RFP(neuro) and HSP-90::RFP(neuro), both significantly reduce the accumulation of higher Aβ oligomers (>26 kDa). Thus HSP-90 may be involved in the assembly of Aβ oligomers, as has been suggested in vitro (Evans et al., 2006), and this may be comparable to the suppressive effect of HSP-16.2 overexpression in the Aβ_{3-42} C. elegans model (Fonte et al., 2008). In this light, it is also interesting to note that increased expression of the HSP90 co-chaperone STI1 protects against the toxic effects of Aβ expression in neuronal cells and a mouse model of Alzheimer’s disease (Ostapchenko et al., 2013). Further work would be required to understand how HSP-90 reduces formation of Aβ oligomers in vitro and in vivo. Moreover, it would be interesting to determine whether modulation of the neuronal PQM-1/CLEC-41 or the intestinal PQM-1/ASP-12 pathway, via genetic activation or small molecules could provide an avenue for the treatment of protein misfolding diseases.

EXPERIMENTAL PROCEDURES

C. elegans Growth Conditions

C. elegans were grown on nematode growth media (NGM) plates seeded with the E. coli OP50-1 strain and cultured by standard methods (Brenner, 1974). Strains were obtained from the Caenorhabditis Genetic Center ( CGC ). For a detailed description of C. elegans strains generated in this study, please refer to Supplemental Experimental Procedures.
RNAi Experiments

For RNAi-mediated knockdown of indicated genes, 50 synchronized L1 animals were placed on E. coli strain HT115(DE3), which was transformed with the appropriate RNAi vector (J. Ahring, University of Cambridge, Cambridge, UK), as described previously (van Oosten-Hawle et al., 2013). For RNAi-mediated knockdown of indicated genes, 50 synchronized L1 animals were placed on ice for 30 s. RNA was then purified using the Zymo-prep RNA Mini Isolation kit (Zymo Research, Cambridge Biosciences). qRT-PCR was essentially performed as described previously (van Oosten-Hawle et al., 2013).

Statistical Analysis

All experiments were repeated at least three times (3 biological replicates). p values were calculated using Student’s t test, * denotes p < 0.05, ** denotes p < 0.01, and *** denotes p < 0.001; (ns) denotes non-significant. To compare two independent populations (analysis by simulation), we calculated p values using the Wilcoxon Mann-Whitney rank-sum test. Prism (v.7) software was used for all statistical analysis.

Detailed protocols for all the methods are provided in the Supplemental Experimental Procedures.

DATA AND SOFTWARE AVAILABILITY

The accession number for the full RNA-seq dataset reported in this study is GEO: GSE108005.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and two tables and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.05.093.

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AUTHOR CONTRIBUTIONS

Conceptualization, D.O. and P.v.O.-H.; Methodology, D.O., L.M.J., S.G., J.M., R.A., C.E.S., M.S.V., D.R.W., and P.v.O.-H.; Investigation, D.O., L.M.J., S.G., J.M., R.A., C.E.S., M.S.V., D.R.W., and P.v.O.-H.; Writing – Original Draft, D.O. and P.v.O.-H.; Writing – Review and Editing, Project Administration, Visualization, Supervision, and Funding Acquisition, P.v.O.-H.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

A PQM-1-Mediated Response Triggers Transcellular Chaperone Signaling and Regulates Organismal Proteostasis

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Nematode strains

Strains RB711 (pqm-1(ok435)), OP201(wgls201 [pqm-1::TY1::EGFP::3xFLAG(92C12)+unc-119(+)]), N2 Bristol, PS3351 (hsf-1(sy441)), TU3401 (uas69[pCFJ90 (myo-2p::mCherry+unc-119p::SID-1)]), NL3231 (sid-1(pk3231)), KP4 (glr-1(n2461)), CX13079 (ocr-1(ok3710)), DA1814 (ser-1(ok345)), and CL2006 (dvl2 [pL12(unc-54/human Abeta peptide minigene)+RFP]) were obtained from the Caenorhabditis Genetics Center. The Q35 Huntington’s Disease model was a gift from Dr. Richard I. Morimoto (AM167 [rmls156[unc-54p::Q35::YFP]].

C. elegans strains used for tissue-specific overexpression of HSP-90 (DAF-21) were as described in (van Oosten-Hawle et al., 2013), either overexpressing HSP-90::GFP in the neurons (AM778 [rmls314[F25B3.3p::DAF-21::GFP]; pCFJ90[myo-2p::RFP]) or HSP-90::RFP in the neurons HSP-90::RFP (AM987 [rmls345[F25B3.3p::DAF-21::RFP]]; or the intestine HSP-90::RFP (AM986 [rmls346[vha-6p::DAF-21::RFP]); or the bodywall muscle HSP-90::RFP (AM988 [rmls347[unc-54p::DAF-21::RFP]]).

The hsp-90 reporter strain (AM799 [rmls317[DAF-21::GFP], pCeh361]) was used as a read-out for transcriptional activity of hsp-90 in the different tissues. Strains overexpressing HSP-90::RFP in the neurons (HSP-90::RFP) and intestine HSP-90::RFP or the bodywall muscle HSP-90::RFP were crossed into the hsp-90 reporter strain resulting in (AM1011 [rmls345[F25B3.3p::DAF-21::RFP], rmIs317[Pdaf-21::GFP], pCeh361]) and AM1010 (rmls346[vha-6p::DAF-21::RFP], rmIs317[Pdaf-21::GFP], pCeh361]) or AM1012 (rmls345[unc-54p::DAF-21::RFP], rmIs317[Pdaf-21::GFP], pCeh361]) respectively as described previously (van Oosten-Hawle et al., 2013).

The Aβ(1-42) expressing strain (CL2006) was crossed into the genetic background of strains overexpressing HSP-90::RFP in the neurons, intestine or bodywall muscle, resulting in strains PVH85 (rmls345[F25B3.3p::DAF-21::RFP], dvls2), PVH127 (rmls346[vha-6p::DAF-21::RFP], dvls2), and PVH50 (AM988 [rmls347[unc-54p::DAF-21::RFP], dvls2], respectively.

The pqm-1 (ok435) knockout mutant (RB711) was crossed into strains AM799, AM1011 and AM1010 and AM1012 to result in strains PVH72 (rmls317[daF-21::GFP], pCeh361, pqm-1(ok435)), PVH68 (rmls346[vha-6p::DAF-21::RFP]; rmls317[Pdaf-21::GFP], pCeh361); pqm-1(ok435)) and PVH55 (rmls345[F25B3.3p::DAF-21::RFP]; rmls317[Pdaf-21::GFP], pCeh361), pqm-1(ok435)) and PVH128 (rmls346[unc-54p::DAF-21::RFP]; rmls317[Pdaf-21::GFP], pCeh361); pqm-1(ok435)) respectively. The pqm-1(ok435) mutant was also crossed into AM167 to result in PVH45 (rmls156[unc-54p::Q35::YFP; pqm-1(ok435)], and crossed into the hsf-1(sy441) mutant to result in PVH46 (hsf-1(sy441); pqm-1(ok435)).

To test which neuronal signalling pathway might be involved via neuron-induced TCS, strain AM1011 was crossed into the genetic background of the glr-1(n2461) mutant KP4, or ser-1(ok435) mutant DA1814 or ocr-1(ok371) mutant CX13079 resulting in strains PVH135 (rmls345[F25B3.3p::DAF-21::RFP]; rmls317[Pdaf-21::GFP], pCeh361]; glr-1(n2461)); PVH136 (rmls345[F25B3.3p::DAF-21::RFP]; rmls317[Pdaf-21::GFP], pCeh361]; ser-1(ok435)) or PVH138 (rmls345[F25B3.3p::DAF-21::RFP]; rmls317[Pdaf-21::GFP], pCeh361]; ocr-1(ok371)).

Strains overexpressing HSP-90::RFP in the neurons (AM987) or the intestine (AM986) or the muscle (AM988) were crossed with OP201 to result in strains PVH58 (rmls345[F25B3.3p::DAF-21::RFP]; wgls201) and PVH64 (rmls346[vha-6p::DAF-21::RFP]; wgls201) and PVH145 rmls346[unc-54p::DAF-21::RFP]; wgls201).

RNAi insensitive strains and strains sensitive to RNAi in specific tissues created in this study were: PVH26 (rmls345[F25B3.3p::DAF-21::RFP]; rmls317[Pdaf-21::GFP, pCeh361]; sid-1(pk3231)); PVH14 (rmls346[vha-6p::DAF-21::RFP]; rmls317[Pdaf-21::GFP, pCeh361]; sid-1(pk3231)) and PVH16 (rmls345[F25B3.3p::DAF-21::RFP]; rmls317[Pdaf-21::GFP, pCeh361]; sid-1(pk3231); uls69); PVH17 (rmls345[F25B3.3p::DAF-21::RFP]; rmls317[Pdaf-21::GFP, pCeh361]; sid-1(pk3231); pccIs04[pCFJ90 (myo-2p::mCherry+vha-6p::SID-1)]; PVH19 (rmls346[vha-6p::DAF-21::RFP]; rmls317[Pdaf-21::GFP, pCeh361]; sid-1(pk3231) uls69); PVH20 (rmls346[vha-6p::DAF-21::RFP]; rmls317[Pdaf-21::GFP, pCeh361]; sid-1(pk3231); pccIs04); PVH18 (rmls345[F25B3.3p::DAF-21::RFP]; rmls317[Pdaf-21::GFP, pCeh361]; sid-1(pk3231) uls69); PVH21 (rmls346[vha-6p::DAF-21::RFP]; rmls317[Pdaf-21::GFP, pCeh361]; pccIs05[pCFJ90 (myo-2p::SID-1::unc-54 3'UTR +myo-2::RFP)]; sid-1 (pk3231)); PVH22 (rmls346[vha-6p::DAF-21::RFP]; rmls317[Pdaf-21::GFP, pCeh361]; pccIs05[pCFJ90 (myo-2p::SID-1::unc-54 3'UTR +myo-2::RFP)]; sid-1 (pk3231)); PVH19 (rmls346[vha-6p::DAF-21::RFP]; rmls317[Pdaf-21::GFP, pCeh361]; sid-1(pk3231)); PVH129
To deplete neurotransmitter and neuropeptide signalling simultaneously, strains PVH16, PVH19 and PVH29 allowing for neuron-specific RNAi, were crossed into the genetic background of \textit{unc-31(e928)} mutants (strain CB928), resulting in strains PVH148, PVH149 and PVH150 respectively.

**Generation of hsp-90 (daf-21) transcriptional GFP reporters containing or deleted for the putative PQM-1 binding motif**

The \textit{hsp-90} promoter region (1,128 bp upstream of the start codon) was amplified and cloned, using \textit{MscI} and \textit{BamHI} restriction sites, into a pPD95.75 vector which had been modified to contain \textit{Chr-unc-119} (Marvin, 2015). A modified version of the promoter (purchased from Eurofins, UK) containing a 10 bp deletion which corresponds to the predicted PQM-1 binding site (119-129 upstream of the start codon), was also cloned into the same vector. DNA sequencing was performed (GATC Biotech, UK) prior to \textit{C. elegans} transformation. Clones were linearised with \textit{EagI} and microprojectile bombardments were performed using the Bio-Rad PDS-1000/He with Hepta adapter as previously described (Dupuy et al., 2004). Five stable strains were isolated for each construct and three strains for each construct were crossed into AM987 (\textit{HSP-90::RFPneuro}). GFP expression was assessed in five hermaphrodites on the first day of adulthood for each strain using an inverted Zeiss LSM 510 META Axiovert 200M and Image Pro Analyser 7.0 (Media Cybernetics).

**RNA-Seq Analysis**

The experiment was performed with three biological replicates of wild type and \textit{HSP-90::GFP\textsubscript{neuro}}, grown at 20°C. Eggs were synchronized to L1 larvae overnight in M9 and 1000 larvae were grown to L4 on NGM seeded with OP50. Animals were collected and washed three times with M9 media to remove bacteria. Worms were then snap frozen in liquid nitrogen. RNA was extracted using TriZol and then purified using the RNeasy mini kit (QIAGEN). RNA quality was measured using an Agilent Technologies 2100 Bioanalyzer. All samples had an RNA integrity number of > 9.5. cDNA libraries were prepared from 5 µgs of total RNA using the TruSeq RNA Sample Preparation v2 kit (Illumina). 50 cycle single-end sequencing was performed on an Illumina HiSeq 2000 by the IGSB Sequencing Core (Institute for Genomics and Systems Biology, University of Chicago, U.S.A). RNA-Seq analysis was performed by ContigExpress (contigexpress.com). Read quality on the raw sequencing data was performed with FastQC. Reads were aligned to the \textit{C. elegans} genome (version WS220). Reference sequences were indexed using Bowtie2 (version 2.0.0-beta7) and the raw reads were mapped using Tophat (version 2.0.4). Cufflinks (version 2.0.2) was used for read quantification and differential expression analysis between wild type and the \textit{C. elegans} strain overexpressing \textit{HSP-90::GFP} in the neurons (\textit{HSP-90::GFP\textsubscript{neuro}}). Differentially expressed genes were identified with the p-adj cut-off 0.05.

**Motif Discovery**

\textit{Ab initio} and known motif discovery was performed using HOMER (Hypergeometric Optimization of Motif EnRichment) (Benner et al., 2017). Target sequences were defined from 1000 bases upstream of the transcription start site for all up-regulated genes in \textit{HSP-90::GFP\textsubscript{neuro}}. Statistically enriched motifs were identified, with the PQM-1 motif significantly enriched in overall up-regulated genes in \textit{HSP-90::GFP\textsubscript{neuro}} ($P = 1e-9$).

**Gene Ontology, signal peptide analysis and target compartment prediction analysis**

Entries of the 34 significantly upregulated genes (Table S2) were manually curated for Gene Ontology classification on wormbase (www.wormbase.org). Putatively secreted peptides were analysed for the presence of signal peptides using SignalIP (www.cbs.dtu.dk/services/SignalIP) as described in Petersen et al. (Petersen et al., 2011) and for target compartments using WoLF PSORT (https://wolfsort.hgc.jp) as described in Horton et al. (Horton et al., 2007).
Quantitative RT-PCR primers used

cdc-42 forward
5’-TGTCGGTAAAACTTGTCCTCGT-3’
cdc-42 reverse
5’-ATCCTAATGTGATGGCTCGC-3’
hsp-90 forward
5’-GACCAGAAACCCAGACGATATC-3’
hsp-90 reverse
5’-GAAGAGCAGGAATCAAGTTG-3’
GFP forward
5’-CCACATTGGTCCTTCTTGGAGTTT-3’
GFP reverse
5’-ATAGTTCATCCATGCCATGTA-3’

hsp-70 (C12C8.1) forward
5’-CTACATGCAAAGGGATTGGA-3’
hsp-70 (C12C8.1) reverse
5’-GGCGTAGTCTTGCTCCCTC-3’

hsp-1 forward
5’-AAGCCGCTTAAAAATGTCG-3’
hsp-1 reverse
5’-GGCCAATCCTTCCAAATCTTCTG-3’

hsp-16.2 forward
5’-TGCATTAGATCTTCTTGAGAGA-3’
hsp-16.2 reverse
5’-TGGTTGAACGTGACCGTTGA-3’
pqm-1 forward
5’-TGCAGGAGGTATCGGACAAGG-3’
pqm-1 reverse
5’-TCATTGATAATTTGATAAGCCATCTT-3’
clec-41 forward
5’-GATCAATGTGTTCTTGTCTGGA-3’
clec-41 reverse
5’-TCCACATTCAACAAGGAGGTATC-3’
ckb-2 forward
5’-CCTGGAATCAAATGATGAGA-3’
ckb-2 reverse
5’-ATGGTGAAACGGTTTTTGAGC-3’
asp-12 forward
5’-CGGAGATGGGACATTTTGAG-3’
**asp-12 reverse**

5’-ACCACAGTTGCCGAGCAC-3’

### Microscopy and Fluorescence Image Quantification

*C. elegans* was imaged using a Zeiss LSM880 confocal microscope through a 10x 1.0 or a 20x 1.0 numerical aperture objective with a 488 nm line for excitation of GFP and 587 nm line for excitation of mCherry (RFP). For imaging, age-synchronised L4 animals or Day 1 adult animals (L4 + 24 hours) were anesthesised using 5 mM Levamisole solution in M9 buffer and mounted on 2% agar pads. Quantification of *hsp-90p::GFP* or *HSP-90::RFP* fluorescence intensity was performed using ImageJ software of three biological replicate images (n > 15). Fluorescence intensity was calculated as pixels per unit area, and background fluorescence subtracted. Intensities were normalised to untreated controls.

### Western Blot Analysis and Quantification of Protein levels

For Western Blot analysis, cell extracts were prepared of 10,000 age-synchronised animals grown on 10 cm NGM plates at a population density of 1000 worms per plate. Young adult animals were harvested into a worm pellet of 200 ul and flash frozen in liquid nitrogen. The frozen pellet was ground with an Eppendorf pestle and re-suspended in Worm Lysis Buffer (10 mM Tris pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0.5% NP-40; 1 mM PMSF), supplemented with EDTA-free protease inhibitor cocktail tablet (Complete Mini, EDTA-free, Roche). The cell extract was prepared by centrifugation at 10,000 g for 5 minutes at 4°C and protein concentration was determined using the Bio Rad protein assay kit (Bradford assay). Cell extracts were mixed with 5x SDS sample buffer and boiled for 5 min. 25 ug total protein was loaded onto a 10 % SDS-PAGE and western blot analysis was performed as described previously (van Oosten-Hawle et al., 2013). To detect *C. elegans* endogenous HSP-90 or the GFP or RFP-tagged version, a poly clonal anti-*C.e. HSP-90* antibody raised in rabbit was used (van Oosten-Hawle et al., 2013). A monoclonal mouse anti-tubulin antibody (Sigma) was used to detect tubulin as a loading control. HRP-conjugated anti-mouse or anti-rabbit antibodies were used as secondary antibodies and ECL reagent (Thermo Fisher Scientific) was used for detection. A monoclonal anti-FLAG antibody (Sigma) was used to detect PQM-1::GFP::FLAG. The gel analysis tool of ImageJ software was used to quantify HSP-90::GFP or HSP-90::RFP relative to endogenous HSP-90 levels and normalised to tubulin.

### Western Blotting of Aβ species

The Aβ species of *C. elegans* expressing Aβ(1-42) in the bodywall muscle (CL2006) or in strains overexpressing HSP-90 in the muscle (PVH50), intestine (PVH127) or neurons (PVH85) was identified by immunoblotting using a 16% Tris-Tricine gel containing 6M urea, and the standard Western blotting protocol, except that the 0.2 um nitrocellulose membranes were boiled in PBS buffer for 5 min after the transfer. 200 nematodes per strain were collected at Day 3 of adulthood into ice-cold M9 buffer, washed in M9 and flash frozen in liquid nitrogen. Nematodes were lysed by grinding using the following lysis buffer: 62 mM Tris-HCl pH 6.8, 5% b-mercaptoethanol, 10% glycerol, 2% SDS, and 1x protease inhibitor cocktail tablet (Roche). 50 ug of total protein for each sample was loaded onto the 16% Tris-Tricine gel. Amyloid monomeric and oligomeric species were detected with 6E10 monoclonal antibody (1:500, Absolute Antibody). Tubulin (anti-tubulin antibody; Sigma) was used as a loading control.

### *P. aeruginosa* (PA14) survival assays

*P. aeruginosa* cultures were grown in LB medium at 37°C overnight. Bacterial lawns used for *C. elegans* killing assays were prepared by spreading 5 ul of an overnight culture of the bacterial strain on modified NGM agar plates (50 mM NaCl, 0.35% peptone) in 3 cm diameter plates. Plates were incubated 24h at 25°C before seeding with L4 *C. elegans* grown on OP50 plates. The killing assays were performed at 20°C and animals were scored and transferred each day to fresh plates. Animals were considered dead when they failed to respond to the touch-nose-response.
**Heat Shock experiments**

Synchronised populations of 30 *C. elegans* animals were grown at 20°C and heat-shocked for 1 hour in a water bath equilibrated at 35°C and allowed to recover for 1 hour at 20°C before they were collected for quantitative RT-PCR, as previously described (van Oosten-Hawle et al., 2013). Each heat shock experiment and following qRT-PCR was performed in triplicate.

**Thermotolerance assays**

Synchronised populations of 100 L4-staged animals were placed on a NGM-Agar plate seeded with *OP50-1 E. coli* and incubated in a water bath equilibrated at 35°C for indicated time points. 4 samples, each consisting of 25-30 L4 animals were used for one time point. After collection of the plates at the indicated time points, animals were allowed to recover for 16 hours at 20°C before scoring for touch-induced movement and pharyngeal pumping. Each experiment was repeated five times.

**Paralysis assays**

An age-synchronised population of 100 animals per strain was scored by monitoring their movement via the touch-nose response, using a platinum wire. Animals were transferred to fresh OP50-1 plates or indicated RNAi plates every day.

**Quantification of Q35::YFP aggregates**

Age-synchronised *C. elegans* (n > 30) expressing *unc-54p::Q35::YFP (mQ35)* were imaged using a Leica MZ10F fluorescent stereoscope with a YFP filter and the number of aggregates was counted each day of adulthood (L4 = Day 0). Aggregates defined as fluorescent foci were brighter and clearly distinguishable from background YFP fluorescence (Morley et al., 2002).

**Subcellular localisation of PQM-1::GFP::FLAG**

Each strain was age-synchronised via egg laying and > 20 animals were imaged at a confocal fluorescent microscope (LSM880) at 20x and scored blindly for clearly visible nuclear or cytosolic localisation of GFP. Statistical analysis was performed using a Kruskal-Wallis test.

**SUPPLEMENTAL TABLES**

Table S1. Differentially expressed genes in *C. elegans* overexpressing HSP-90::GFP in the neurons (*HSP-90::GFPneuro*) relative to control animals (N2), at normal growth conditions (20°C). Related to Figure 2 and Figure S1.

Table S2. Genes upregulated > 1.5-fold in *C. elegans* overexpressing HSP-90::GFP in the neurons (*HSP-90::GFPneuro*). Pseudogenes are indicated in red. Y=Yes; N=No. Related to Figure 2 and Figure S1.
Figure S1. Analysis of *C. elegans* strains overexpressing HSP-90 protein in the neurons; Related to Figure 1 and 2.

(A) Western blot analysis of Day 1 adult *HSP-90::GFP*<sup>neuro</sup>, *HSP-90::RFP*<sup>neuro</sup>, *HSP-90::RFP*<sup>int</sup> or *HSP-90::RFP*<sup>muscle</sup> using an anti-*C. elegans* HSP-90 antibody. (B) Quantification of total HSP-90 protein levels, corresponding to the sum of endogenous HSP-90 and transgenic *HSP-90::GFP/RFP* expressed in *HSP-90::GFP*<sup>neuro</sup>, *HSP-90::RFP*<sup>neuro</sup> and *HSP-90::RFP*<sup>int</sup> compared to control (N2). HSP-90::GFP/RFP protein levels are normalised to the loading control (tubulin) and relative to endogenous HSP-90. (C) Heat-map of gene expression levels of genes induced > 1.5-fold in the RNA-seq analysis of *HSP-90::GFP*<sup>neuro</sup> relative to control (N2). Analysis of expression levels of the 34 induced genes in *HSP-90::GFP*<sup>neuro</sup> by qRT-PCR compared to *HSP-90::GFP*<sup>neuro</sup> (RNA-Seq) revealed a medium-high correlation following Pearson correlation analysis ($r = 0.55$, $P = 0.001$; two-tailed t-test). qRT-PCR analysis of expression levels in *HSP-90::RFP*<sup>neuro</sup> compared to the RNA-Seq analysis of *HSP-90::GFP*<sup>neuro</sup> showed a medium correlation ($r = 0.4$; $P < 0.02$; two tailed t-test), and a high correlation between qRT-PCR analysis of induced genes between *HSP-90::RFP*<sup>neuro</sup> and *HSP-90::GFP*<sup>neuro</sup> ($r = 0.62$, $P = 0.00008$). (D) Paralysis assays of *HSP-90::RFP*<sup>neuro</sup>, *HSP-90::RFP*<sup>int</sup> or *HSP-90::RFP*<sup>muscle</sup> compared to wild type (N2) animals. (E) *pqm-1* transcript levels in wild type control animals (N2) and the *pqm-1*(ok435) deletion mutant.
Figure S2. Depletion of pqm-1 reduces TCS-induced hsp-90 expression in distal tissues; Related to Figure 2.

Depletion of pqm-1 reduces TCS-induced hsp-90 expression in distal tissues. P<sub>hsp-90<sup>°</sup></sub>:GFP expression (A i, iii, v) in control animals or (A ii, iv, vi) crossed into a pqm-1 knockout (k.o.) mutant (pqm-1(ok435)); (B i, iii, v) in HSP-90::RP<sub>muscle</sub> or (B ii, iv, vi) HSP-90::RP<sub>muscle</sub>;pqm-1(k.o.) and (C i, iii, v) in HSP-90::RP<sub>int</sub> or (C ii, iv, vi) HSP-90::RP<sub>int</sub>;pqm-1(k.o.), or in (D i, iii, v) HSP-90::RP<sub>neuro</sub> or (D ii, iv, vi) HSP-90::RP<sub>neuro</sub>;pqm-1(k.o.). (A, B, C, D iii and iv) 20x magnification of (A iii) tail region or (A iv; B, C, D iii and iv) head region. Scale bar, 50 μm. (E-H) P<sub>hsp-90<sup>°</sup></sub>:GFP expression in (E, i) wild type animals treated with empty vector (EV) control RNAi or (E, iv) with pqm-1 RNAi. P<sub>hsp-90<sup>°</sup></sub>:GFP expression (F, i) in HSP-90::RP<sub>muscle</sub> during EV control RNAi or (F, iv) pqm-1 RNAi; (G, i) P<sub>hsp-90<sup>°</sup></sub>:GFP expression in HSP-90::RP<sub>muscle</sub> during EV RNAi or (G, iv) pqm-1 RNAi. (H, i) P<sub>hsp-90<sup>°</sup></sub>:GFP expression in HSP-90::RP<sub>neuro</sub> during EV RNAi or (H, iv) pqm-1 RNAi.

(E, F, G, H, ii and iv) 20x magnification of the anterior (head) region of (E, ii) wild type. (F, ii) HSP-90::RP<sub>muscle</sub> and (G, ii) HSP-90::RP<sub>int</sub> and (H, ii) HSP-90::RP<sub>neuro</sub> animals expressing the P<sub>hsp-90<sup>°</sup></sub>:GFP reporter during EV control RNAi and (E, v) wild type, (F, v) HSP-90::RP<sub>muscle</sub> and (G, v) HSP-90::RP<sub>int</sub> and (H, v) HSP-90::RP<sub>neuro</sub> expressing the P<sub>hsp-90<sup>°</sup></sub>:GFP reporter treated with pqm-1 RNAi. (E-H, iii) Differential interference contrast (DIC) Normarski images. Scale bar, 50 μm. (I - L) GFP and hsp-90 transcript levels in C. elegans expressing the P<sub>hsp-90<sup>°</sup></sub>:GFP reporter during pqm-1 RNAi, relative to control (EV) RNAi in (I) wild type animals expressing the P<sub>hsp-90<sup>°</sup></sub>:GFP reporter (J) in HSP-90::RP<sub>muscle</sub> and (K) in HSP-90::RP<sub>neuro</sub> P<sub>hsp-90<sup>°</sup></sub>:GFP or (L) in HSP-90::RP<sub>int</sub> P<sub>hsp-90<sup>°</sup></sub>:GFP.
Figure S3; Related to Figure 3 and Figure 4

**A** P<sub>hsp-90::GFP</sub> reporter in (A, i) wild type animals expressing the P<sub>hsp-90::GFP</sub> reporter (J) in HSP-90::RFP<sup>muscle</sup> control strain but not in P<sub>hsp-90::GFP</sub> animals. (I-F) Bar graphs represent the combined results of three independent experiments. Error bars represent +/- SEM. *P < 0.05; **P < 0.01; (n.s.) non-significant.
Figure S4. PQM-1 is required for heat-inducible expression of hsp-90 but not heat- or TCS-induced expression of hsp-70; Related to Figure 3 and 4.

(A and B) TCS-induced hsp-70 (C12C8.1) levels are not affected in tissue-specific hsp-90 hairpin RNAi strains depleted for pqm-1 (hs-p90neuro (k.d.) and hsp-90int (k.d.)). (A) hsp-70 mRNA levels are induced in (hs-p90neuro (k.d.) and hsp-90int (k.d.) compared to the control strain (sid-1(pk3321)) and unaffected when crossed into the pqm-1(k.o.) mutant background. (B) pqm-1 mRNA expression levels in sid-1(pk3321);pqm-1(k.o.) mutants, hsp-90neuro (k.d.) and hsp-90int (k.d.) strains compared to N2 animals. No pqm-1 mRNA transcripts were measured in sid-1(pk3321);pqm-1(k.o.) mutants, hsp-90neuro (k.d.);pqm-1(k.o.) or hsp-90int(k.d.);pqm-1(k.o.) strains harbouring the pqm-1(ok435) deletion mutation.

Total mRNA accumulation of (C) hsp-1 (constitutive hsp70) and (D) heat-inducible hsp16.2 before (20°C) and after heat shock (HS; 1h at 35°C), in Day 1 adult wild type, pqm-1(k.o.) mutant, hsf-1(sy441) mutant, and pqm-1(k.o.);hsf-1(sy441) double mutant relative to heat shocked wild type animals. (A - D) Bar graphs represent combined means of three biological replicate experiments. Error bars represent +/- SEM. *P < 0.05; **P < 0.01; n.s. = not significant.

(E) Western blot analysis of HSP-90 protein expression at 20°C (+), immediately after a 1h 35°C HS (+), and after a 1h recovery period at 20°C in young adult control animals, hsf-1(sy441) mutant, pqm-1(k.o.) mutant and pqm-1(k.o.);hsf-1(sy441) double mutants. (F) Quantification of HSP-90 levels. HSP-90 levels are normalised to the loading control (tubulin) and relative to HSP-90 levels in control animals at 20°C. Bar graphs represent combined mean values of three independent experiments. Error bars represent +/- SEM.

(G) Thermo-sensitivity of L4 animals (n=100) with indicated genotypes after exposure to 35°C heat stress for 4 hours or 6 hours. Survival was measured after a recovery period of 16 hours at 20°C. *P < 0.05, **P < 0.01. Bar graphs represent combined mean values of four independent experiments. Error bars represent +/- SEM. (H) HSP-90::RFP<sup>const</sup> require pqm-1 for resistance to pathogenic challenge by Pseudomonas aeruginosa. HSP-90::RFP<sup>const</sup> or HSP-90::RFP<sup>muscle</sup> as well as the pqm-1(k.o.) mutant show similar survival curves as control animals (N2, wild type). Wilcoxon matched pairs-signed rank test; *P < 0.05.
Figure S5; Related to Figure 5

Ab system RNAi

Ba HSP-90::RFP fluorescence

C a HSP-90::RFP fluorescence

Da HSP-90::RFP fluorescence

Ea HSP-90::RFP fluorescence

Fb system RNAi

Gb RNAi insensitive

Hb RNAi insensitive

Jb muscle-specific RNAi

Ka system RNAi

La HSP-90::RFP fluorescence

Ma HSP-90::RFP fluorescence

Na HSP-90::RFP fluorescence

Ob muscle-specific RNAi

Kb system RNAi

Lb RNAi insensitive

Mb RNAi insensitive

Nb RNAi insensitive
Figure S5. Analysis of systemic and tissue-specific hsp-90 RNAi in HSP-90::RFP\textsubscript{neuro}, HSP-90::RFP\textsubscript{int} and HSP-90::RFP\textsubscript{muscle}; Related to Figure 5.

(Aa) HSP-90::RFP\textsubscript{neuro} animals treated with (Aa, i and ii) control (EV) RNAi or (Aa, iii and iv) hsp-90 RNAi.

(Ba) RNAi-resistant HSP-90::RFP\textsubscript{neuro};P\textsubscript{hsp-90\textsubscript{neuro}}::GFP; sid-1(pk3321) animals treated with (Ba, i and ii) control (EV) RNAi or (Ba, iii and iv) hsp-90 RNAi. (Ca) HSP-90::RFP expression in the neurons during neuron-specific (Ca, i, ii and iii) control (EV) RNAi or (Ca, iv, v, vi) hsp-90 RNAi in HSP-90::RFP\textsubscript{neuro};P\textsubscript{hsp-90\textsubscript{neuro}}::GFP;sid-1(pk3321);unc-119p::SID-1 animals. (Ca, ii and v) 20x magnification of the posterior region. The posterior region was chosen to avoid interference with the pharyngeal P\textsubscript{myo-2}::mCherry co-injection marker. (Da) HSP-90::RFP\textsubscript{int};P\textsubscript{hsp-90\textsubscript{int}}::GFP;sid-1(pk3321);vha-6p::SID-1 animals treated with (Da, i and ii) control (EV) RNAi or (Da, iii and iv) hsp-90 RNAi. (Da, ii and v) 20x magnification of the posterior region. (Ca, Da, Ea) Yellow arrow indicates a P\textsubscript{myo-2}::mCherry co-injection marker. (Fa) HSP-90::RFP\textsubscript{int} animals treated with (Fa, i and ii) control (EV) RNAi or (Fa, iii and iv) hsp-90 RNAi. (Ga) RNAi-resistant HSP-90::RFP\textsubscript{int};P\textsubscript{hsp-90\textsubscript{int}}::GFP; sid-1(pk3321) animals treated with (Ga, i and ii) control (EV) or (Ga, iii and iv) hsp-90 RNAi. (Ha) HSP-90::RFP expression in HSP-90::RFP\textsubscript{int};P\textsubscript{hsp-90\textsubscript{int}}::GFP;sid-1(pk3321);unc-119p::SID-1 animals, allowing neuron-specific (Ha, i and ii) control RNAi or (Ha, iii and iv) hsp-90 RNAi.

(IA) HSP-90::RFP\textsubscript{int};P\textsubscript{hsp-90\textsubscript{int}}::GFP; sid-1(pk3321);vha-6p::SID-1 animals allowing intestine-specific (IA, i and ii) control (EV) RNAi or (IA, iii and iv) hsp-90 RNAi. (Ja) HSP-90::RFP\textsubscript{int};P\textsubscript{hsp-90\textsubscript{int}}::GFP; sid-1(pk3321);myo-3p::SID-1 animals allowing muscle-specific (Ja, i and ii) control (EV) RNAi or (Ja, iii and iv) hsp-90 RNAi.

(Ka) HSP-90::RFP\textsubscript{int} animals treated with (Ka, i and ii) systemic control (EV) RNAi or (Ka, iii and iv) hsp-90 RNAi. (La) RNAi-resistant HSP-90::RFP\textsubscript{int};P\textsubscript{hsp-90\textsubscript{int}}::GFP; sid-1(pk3321) animals treated with (La, i and ii) control (EV) or (La, iii and iv) hsp-90 RNAi. (Ma) HSP-90::RFP\textsubscript{int};P\textsubscript{hsp-90\textsubscript{int}}::GFP; sid-1(pk3321);unc-119p::SID-1 animals, allowing neuron-specific (Ma, i and ii) control RNAi or (Ma, iii and iv) hsp-90 RNAi.

(NA) HSP-90::RFP\textsubscript{int};P\textsubscript{hsp-90\textsubscript{int}}::GFP; sid-1(pk3321);vha-6p::SID-1 animals allowing intestine-specific (Na, i and ii) control (EV) RNAi or (Na, iii and iv) hsp-90 RNAi. (Oa) HSP-90::RFP\textsubscript{int};P\textsubscript{hsp-90\textsubscript{int}}::GFP; sid-1(pk3321);myo-3p::SID-1 animals allowing muscle-specific (Oa, i and ii) control (EV) RNAi or (Oa, iii and iv) hsp-90 RNAi.

(Ab – Ob): Quantification of RFP fluorescence intensity in strains of the genotype indicated in Aa – Oa, respectively.

(Ca, Da, Ea, Ha, Ia, Ja, Ma, Na, Oa) Yellow arrow indicates a P\textsubscript{myo-2}::mCherry co-injection marker.

(A-O) ***P < 0.001; ****P < 0.0001; n.s. = not significant. Scale bar, 50 um. Bar graphs represent combined mean values of three independent experiments. Error bars represent +/- SEM.
Figure S6. Deletion of the putative PQM-1 binding motif in the hsp-90 promoter does not abolish TCS-induced and pqm-1-dependent Phsp-90::GFP expression; Related to Figure 5 and Figure 6.

(A) Promoter reporter construct for Phsp-90::GFP expression. The predicted PQM-1 binding motif is indicated.

(B) Day 1 adults expressing the low-copy Phsp-90::GFP reporter construct, either containing the predicted PQM-1 motif (Phsp-90(wt)::GFP) or a deleted motif (Phsp-90(del)::GFP). (C) Day 1 adults expressing the Phsp-90(wt)::GFP or Phsp-90(del)::GFP reporter in the HSP-90::RFPneuro strain background. (D) Quantification of total GFP fluorescence in Phsp-90(wt)::GFP compared to Phsp-90(del)::GFP and HSP-90::RFPneuro; Phsp-90(wt)::GFP compared to HSP-90::RFPneuro; Phsp-90(del)::GFP. n.s. = not significant. (E) GFP transcript levels in animals expressing the low-copy Phsp-90(wt)::GFP reporter compared to the Phsp-90(del)::GFP reporter construct and HSP-90::RFPneuro; Phsp-90(wt)::GFP compared to HSP-90::RFPneuro; Phsp-90(del)::GFP.

Bar graphs represent combined mean values of three independent experiments. Error bars represent +/- SEM. **P < 0.01
Figure S7; Related to Figure 6 and Figure 7

(A) HSP-90::RFPneuro;Phsp-90::GFP, sid-1(pk3321);unc-119p::SID-1, anterior region. (B) HSP-90::RFPneuro;Phsp-90::GFP or transcript levels in RNAi.

(B, ii and v) 20x magnification of the anterior (head) region. (D, ii and v) Related to Figure 6.

Figure S7. RNAi-mediated knockdown of genes involved in the innate immune response reduce TCS-induced hsp-90 expression; allowing for intestine-specific RNAi. (A) HSP-90::RFPneuro;Phsp-90::GFP, sid-1(pk3321);unc-119p::SID-1, anterior region. (D) HSP-90::RFPmuscle;Phsp-90::GFP;sid-1(pk3321);unc-119p::SID-1, anterior region.

Paralysis experiments of unc-31(e928); EV RNAi or fed with (A) control (EV) RNAi or B0285.9, F21F8.4 or B0365.6 RNAi. (B) HSP-90::RFPneuro;Phsp-90::GFP animals treated with (B, i and iii) EV control RNAi or (B, iv and vi) clec-41 RNAi. (B, ii and v) 20x magnification of the anterior (head) region and (C) HSP-90::RFPneuro;Phsp-90::GFP;sid-1(pk3321);unc-119p::SID-1 animals allowing for intestine-specific (C, i and iii) EV control RNAi or (C, iv and vi) clec-41 RNAi. (C, ii and v) 20x magnification of the anterior (head) region. (D) HSP-90::RFPneuro;Phsp-90::GFP animals treated with (D, i and iii) EV control RNAi or (D, iv and vi) asp-12 RNAi. (D, ii and v) 20x magnification of the anterior (head) region. (E) HSP-90::RFPneuro;Phsp-90::GFP;sid-1(pk3321);vha-6p::SID-1 animals allowing for intestine-specific (E, i and iii) EV control RNAi or (E, iv and vi) asp-12 RNAi. (E, ii and v) 20x magnification of the anterior (head) region.

(F - H) Relative GFP and hsp-90 mRNA levels in intestine-specific RNAi, relative to control (EV) or systemic RNAi. (F) HSP-90::RFPneuro;mAβ(3-42);cntrl RNAi or (H) HSP-90::RFPneuro;mAβ(3-42);pha-4 RNAi.

(K) Paralysis assays of HSP-90::RFPneuro;mAβ(3-42) treated with EV or pha-4 RNAi compared to Aβ(3-42) animals.

Paralysis data represent SEM of 3 biological replicates (100 animals per biological replicate). Statistical significance was determined by Wilcoxon matched pairs-signed rank test. *P < 0.05.