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Regulation of organismal proteostasis by trans-cellular chaperone signaling

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Summary

A major challenge for metazoans is to ensure that different tissues each expressing distinctive proteomes are, nevertheless, well protected at an organismal level from proteotoxic stress. We have examined this and show that expression of endogenous metastable protein sensors in muscle cells induces a systemic stress response throughout multiple tissues of *C. elegans*. Suppression of misfolding in muscle cells can be achieved not only by enhanced expression of *HSP90* in muscle cells, but as effective by elevated expression of *HSP90* in intestine or neuronal cells. This cell-non-autonomous control of *HSP90* expression relies upon transcriptional feedback between somatic tissues that is regulated by the FoxA transcription factor PHA-4. This trans-cellular chaperone signaling response maintains organismal proteostasis when challenged by a local tissue imbalance in folding and provides the basis for a novel form of organismal stress sensing surveillance.

Introduction

The expression of unique combinations of proteins that determine tissue function in metazoans must be maintained by a corresponding tissue-specific network of chaperones and quality control processes to achieve optimal proteostasis in that tissue. For example, the proteostasis network expressed in cells of the immune system, or pancreatic cells that secrete large quantities of proteins is distinct from that expressed in brain or muscle tissues (Powers et al., 2009). This would predict that differences in the proteins expressed in post-mitotic neurons, muscle, or intestinal cells in terms of proteome composition, levels of expression, protein stability, and dynamics, must also have a unique cell-type specific response to extrinsic environmental or physiological stress signals. To counteract such fluctuating conditions, cells employ highly conserved stress responses that monitor the cellular environment and prevent protein mismanagement by restoring proteostasis (Gidalevitz et al., 2011).

Within each cell, this is achieved by the Heat Shock Response (HSR), that upregulates an intrinsic network of molecular chaperones through the activity of HSF-1, a master stress

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Supplemental Information
Supplemental Information includes Extended Experimental Procedures and six figures.

transcriptional regulator (Akerfelt et al., 2010). Activation of the HSR is essential for adaptation and survival at the single cell level. The appearance of multicellularity, however, adds another challenge to maintain proteostasis, as different cell types and tissues need to exchange information to coordinate growth, metabolism, gene expression, and stress responses. For example, in *C. elegans* the HSR is regulated by thermo-sensory neurons that detect temperature changes to control HSF-1 activity throughout the somatic tissues of the animal (Prahlad et al., 2008). Yet, at the same time, the HSR is associated with numerous tissue-specific human diseases (Mendillo et al., 2012; Morimoto, 2008; Powers et al., 2009). What remains unclear is whether proteotoxic challenges that affect a single cell or tissue, such as the expression of a metastable aggregation-prone or damaged protein, would lead to a strict autonomous response or whether local protein damage within one tissue would be sensed by other tissues as an integrated organismal response.

These questions have led us to ask whether perturbation of proteostasis within a single tissue of *C. elegans* initiates a response in adjacent tissues. To address this, we used myosin temperature-sensitive *(ts)* mutations expressed only in muscle and observed induction of the myosin chaperone *HSP90* not only in muscle but also in neuronal and intestinal cells. Moreover, cell non-autonomous expression of *HSP90* suppressed myosin *(ts)* misfolding at the restrictive temperature. Consistent with these observations, activation of the HSR in one tissue had beneficial effects in other tissues. These results reveal a compensatory response to a tissue-specific imbalance in proteostasis that functions in a cell non-autonomous fashion in the nematode *C. elegans*.

Results

Tissue-specific perturbation of proteostasis is recognized at a systemic level

We monitored tissue-specific folding requirements in muscle cells using the *HSP90* client protein myosin heavy chain B (UNC-54), an essential component of thick filaments solely expressed in the bodywall muscle of *C. elegans* (Epstein and Thomson, 1974; Miller et al., 1986). Expression of temperature-sensitive myosin *(ts)* mutations [*unc-54(e1301)* or *unc-54(e1157)*] at the restrictive temperature (25°C) results in misfolded myosin and disruption of thick filaments, leading to severe movement defects and embryonic lethality (Ben-Zvi et al., 2009; Gengyo-Ando and Kagawa, 1991; MacLeod et al., 1977). Because metastable *ts* proteins are highly dependent on the cellular folding environment (Ben-Zvi et al., 2009; Gidalevitz et al., 2006), we reasoned that expression of *unc-54(ts)* mutations could place increased demands for chaperones such as *HSP90* that are required for folding of myosin and maintenance of muscle function (UNC-54) (Barral et al., 2002; Gaiser et al., 2011) (Figure S1B).

In wild type animals, the sole cytosolic *HSP90* (DAF-21) in *C. elegans* is ubiquitously expressed in the pharynx (ph), intestine (int), pharyngeal nerve ring (n), bodywall muscle (bwm) and the excretory cell (ex), as observed with an *hsp90p::GFP* transcriptional reporter (Figures 1A and 1B). In *unc-54(ts)* animals, however, *hsp90* mRNA levels are induced almost two-fold at the permissive temperature relative to wild type animals (Figure 1C). Likewise, the *hsp90* reporter was induced at the permissive temperature in animals expressing *ts* alleles of myosin *(ts)* as well as paramyosin (*unc-15, e1402*), another component of muscle thick filaments (Miller et al., 1986) (Figure 1I– 1K, Figure S1D–F and S1H–L, respectively) relative to control animals (Figure 1E – 1G and Figure S1C and S1G, J). These results are consistent with increased requirements for *HSP90* in bodywall muscle cells (Figure 1K and Figure S1E and S1K). Unexpectedly, the *hsp90* reporter was also induced in cells that do not express UNC-54, such as the intestine, pharynx, and excretory cells (Figure 1J–K; Figure S1E–F and S1L).

Thus, these results reveal that disruption of proteostasis by expression of metastable muscle proteins generates a muscle-specific stress that is sensed by multiple tissues in the animal and unexpectedly results in a cell-non-autonomous elevated expression of *HSP90.*

Tissue-specific increased expression of *HSP90* improves the organismal folding environment of myosin *(ts)* mutants

Since *hsp90* expression is induced in muscle cells of myosin *(ts)* mutants, we asked whether the defective folding of myosin that occurs at the restrictive temperature would be suppressed by increasing the expression of *HSP90* in the bodywall muscle. We therefore established *C. elegans* lines expressing *HSP90* (*HSP90::GFP*) in bodywall muscle cells (*HSP90*^{bwm}) (Figure 2A). This resulted in an 85% increase of *HSP90* above endogenous levels (Figure 2D) that suppressed myosin misfolding and reverted the paralysis *of unc-54(ts)* mutants at the restrictive temperature (Figure 2E and 2F).

Because the *hsp90* reporter was also induced in non-muscle tissues, we examined whether increasing the levels of *HSP90* in intestinal or neuronal cells would affect the folding of myosin in the muscle cell and confer protection to muscle-specific phenotypes at the restrictive temperature. Therefore, we generated transgenic lines expressing *HSP90* in the intestine (*HSP90*^{int}) or neurons (*HSP90*^{neuro}) (Figure 2B and 2C). Tissue-specific expression of *HSP90::GFP* was confirmed by measuring *GFP* and *hsp90* mRNA levels in isolated intestinal cells of *HSP90*^{bwm}, *HSP90*^{int} and *HSP90*^{neuro} (Figure S2F). *HSP90::GFP* protein levels in the *HSP90*^{int} and *HSP90*^{neuro} lines corresponded to an 80% and 45% increase relative to endogenous *HSP90*, respectively (Figure 2D). Unexpectedly, elevated expression of *HSP90* in the intestine or the neurons also suppressed muscle fiber degeneration at restrictive temperature (Figure 2F), improved the motility of *unc-54(ts)* mutants (Figure 2E), and alleviated embryonic lethality (Figure S2E).

Thus, the observation of induced *hsp90* expression in non-muscle tissues of myosin *(ts)* or paramyosin *(ts)* mutants (Figure 1D–K and Figure S1C–L), indeed serves as a protective physiological response that improves the folding environment of challenged muscle cells and enhances organismal viability of myosin *(ts)* animals during chronic proteotoxic stress.

Tissue-specific expression of *HSP90* blocks the HSR in distal tissues

The ability of elevated levels of *HSP90* to establish a protective folding environment for muscle cells in a cell non-autonomous manner in myosin *(ts)* mutants, led us to consider whether transgenic *HSP90* overexpression lines were also cross-protected against more severe heat stress conditions. In wild type animals, a stringent heat shock regimen at 35°C for 10 hours results in 20% survival, whereas by comparison all three transgenic *HSP90* lines were extremely hypersensitive to heat stress with less than 5% survival at the 10 hour time point (Figure 3A). This corresponds to the same level of heat stress sensitivity exhibited by *hsf-1(sy441)* hypomorph mutant animals (Figure 3A) (Hajdu-Cronin et al., 2004). Thus, elevated levels of *HSP90,* while protective under chronic ambient proteotoxic stress due to the expression of metastable proteins, was not tolerated under severe acute stress conditions. This suggests that metazoan cells employ a novel form of trans-cellular communication to maintain tissue proteostasis that is protective during mild fluctuating environmental conditions, but is deleterious when animals are challenged by a severe heat shock.

One explanation for the stress hypersensitivity in animals overexpressing *HSP90* in specific tissues could be that higher levels of *HSP90* has inhibitory effects on the induction of the HSR. To address this, we quantified the expression of three representative HS genes corresponding to two heat-inducible *hsp70s* (*C12C8.1* and *F44E5.4)* and the small heat

shock protein *hsp16* (*hsp-16.2*). Relative to wild type animals, the HSR in *HSP90*^{bwm} animals was suppressed 20-fold, and in *HSP90*^{int} and *HSP90*^{neuro} animals suppressed five- and three-fold, respectively (Figure 3B). This inhibition of the HSR, by tissue-specific expression of *HSP90*, was equivalent to that observed for the *hsf-1 (sy441)* hypomorph mutant (Figure 3B). Moreover, the HSR was fully restored in these transgenic lines by reducing the levels of *HSP90::GFP* using *GFP* RNAi or *hsp90* RNAi (Figure 3B). The inability to mount an organismal HSR was also not due to increased expression of other chaperones that could negatively regulate HSF-1 (Morimoto, 1998), as basal levels of constitutive *hsp70 (hsp-1)*, inducible *hsp70 (C12C8.1)*, and *hsp16* were comparable in wild type and *HSP90* overexpression lines (Figure 6A).

To identify the molecular step at which *HSP90* inhibits the organismal induction of the HSR, we examined the regulation of HSF-1 DNA binding activity by electrophoretic gel mobility shift assays in heat shocked wild type, *HSP90*^{bwm} and *HSP90*^{neuro} animals. The level of HSF-1 DNA binding activity in heat shocked wild type extracts was strongly induced relative to *HSP90*^{bwm} and *HSP90*^{neuro} animals that showed an at least two-fold reduction of HSF-1 DNA binding (Figure S3A and S3B; see Extended Experimental Procedures). This was not due to any detectable changes in the expression of *hsf-1* mRNA levels relative to wild type animals (Figure S3C). Because *HSP90* functions as a negative regulator of HSF-1 (Zou et al., 1998), we conclude that local changes in the levels of *HSP90* induces a cell non-autonomous regulatory process that inhibits HSF-1 activation in distant tissues. Thus, the molecular consequence of locally elevated *HSP90* expression is a systemic reduction in HSF-1 DNA binding activity, leading to a global reduction of the HSR.

Local changes in *HSP90* levels inhibit *HSP* expression in distal tissues

In order to directly monitor the effects of local *HSP90* overexpression on the organismal HSR in living animals, we employed an *hsp70p::mCherry (C12C8.1p::mCherry)* reporter strain to visualize the HSR across the different tissues. Heat shock induction of *hsp70p::mCherry* was readily detected in the spermatheca (sp), the bodywall muscle (m) and the intestine (i) (Figure 3C, *i–iv*) of wild type animals. In contrast, animals expressing *HSP90::GFP* in bodywall muscle (Figure 3C, *v–viii* and Figure S3D, *i*), intestine (Figure 3C, *ix–xii* and Figure S3D, *iv*) and neurons (Figure 3C, *xiii–xvi* and Figure S3D, *vii*) showed a reduction in HS-inducibility of the *hsp70p::mCherry* reporter in multiple tissues. For example, increased expression of *HSP90* in muscle cells (Figure 3C, *viii*, green) blocked induction of the *hsp70* reporter not only in muscle cells but also in intestinal cells (Figure 3C, *vii*) relative to wild type animals (Figure 3C, *iii*). Likewise, in *HSP90*^{int} animals, the *hsp70* reporter was not induced in the intestine and induced only slightly in muscle cells (Figure 3C, *xi*). Consistent with the other transgenic *HSP90* lines, animals overexpressing neuronal *HSP90* also showed very little induction of the *hsp70* reporter in both spermatheca and muscle, and no induction of the HSR in intestinal cells (Figure 3C, *xv* and Figure S3D, *vii*).

The compromised HSR could be restored to wild type levels in the individual tissues by reducing overall *HSP90* levels by *hsp90* RNAi (S3D, *ii, v, viii*) or *HSP90::GFP* levels by *GFP* RNAi (S3D, *iii, vi, ix*) prior to HS (Figure S3D, *i–ix*). The three- and five-fold decreased expression of the *hsp70p::mCherry* reporter in *HSP90*^{bwm} and *HSP90*^{int} animals respectively, was rescued by *hsp90* or *GFP* RNAi (Figure S3E). The observation that *hsp90* or *GFP* RNAi only minimally restored *hsp70* reporter expression in *HSP90*^{neuro}, is consistent with the measurement of mRNA levels of HS induced genes (Figure S3D, *viii, ix* and S3E, and Figure 3B, respectively), and that neurons are less susceptible to RNAi (Simmer et al., 2002).

To further characterize the HSR in tissues that were not targeted for overexpression of *HSP90*, and to rule out that the *hsp70p::mCherry* transgene interfered with endogenous HS gene expression, we isolated intestinal tissue from heat-shocked *HSP90*^{bwm}, *HSP90*^{int} and *HSP90*^{neuro} animals that lack the *hsp70p::mCherry* reporter transgene and quantified the expression of endogenous *hsp70* mRNA relative to intestinal cells from wild type animals. The inducible expression of *hsp70* was reduced two-fold in isolated intestinal cells of *HSP90*^{bwm}, 20-fold in the intestine of *HSP90*^{int} and five-fold in *HSP90*^{neuro}, relative to wild type levels (Figure S3F). These results are consistent with the reduction of *hsp70p::mCherry* fluorescence in the intestines of respective strains (Figure 3C, *iii* compared to *vii*, *xi* and *xv*, respectively) and provide supportive evidence that the localized expression of *HSP90* has global inhibitory effects on the organismal HSR.

Taken together, these results show that increased levels of *HSP90* in a single tissue has cell-non-autonomous inhibitory effects on *HSP* expression in other tissues within the organism, and that tissue-specific perturbations of the proteostasis network have consequences throughout the organism.

Our results reveal a potential conundrum: whereas elevated *HSP90* levels in non-muscle tissues can cell-non-autonomously rescue the muscle-specific phenotype of myosin *(ts)* mutants, tissue-specific elevated levels of *HSP90* are detrimental under severe heat stress conditions through cell-non-autonomous repression of HSF-1 transcriptional activity. One explanation for repression of HSF-1 activity in non-target tissues, or improved myosin folding when *HSP90* is expressed in non-muscle tissues, is that *HSP90* overexpressed in one tissue is released and taken up by surrounding cells where they could interact with client proteins such as myosin or HSF-1. In *C. elegans*, proteins secreted from a cell enter the pseudocoelomic space, a bodycavity exposed to all tissues of the animal, before they can be taken up by surrounding tissues (Altun-Gultekin, 2009). Thus, materials secreted in the pseudocoelom are taken up non-specifically by coelomocytes, scavenger cells that perform a primitive surveillance function in the animal (Altun-Gultekin, 2009; Fares and Greenwald, 2001). Therefore, we examined whether *HSP90::mCherry* overexpressed in neurons, bodywall muscle or intestine are secreted into the pseudocoelomic space and subsequently endocytosed by coelomocytes using a strain expressing *GFP::RAB-5* under the control of a coelomocyte promoter (Sato et al., 2005), to image an uptake of *HSP90::mCherry* into coelomocytes. However, *HSP90::mCherry* fluorescence was not detected in coelomocytes, suggesting that overexpressed *HSP90* is not exported into the extracellular space (Figure S3G). Thus rather than intercellular transmission of *HSP90*, the cell-non-autonomous effect giving rise to improved myosin maintenance or repression of HSF1 in non-target tissues must be achieved by a different mechanism.

Tissue-specific knockdown of *HSP90* induces a systemic organismal HSR

Having demonstrated that increased expression of *HSP90* in any single tissue leads to the repression of HSF-1 activity throughout the animal, we reasoned that tissue-specific *hsp90* RNAi should result in induction of the HSR in multiple tissues. To accomplish tissue-specific knockdown of *hsp90*, we employed the *sid-1* mutation (Winston et al., 2002), which allows cell-autonomous RNAi but is defective for systemic RNAi (Winston et al., 2002). To confirm that *HSP90* levels were reduced in specific tissues, animals expressing the hairpin construct in muscle (*hp-hsp90*^{bwm}), intestine (*hp-hsp90*^{int}) or neurons (*hp-hsp90*^{neuro}) were crossed with *HSP90::mCherry* lines (Figure S4A–F). Quantitation of *mCherry* fluorescence intensity shows that *HSP90* levels are decreased significantly only in the targeted tissue, albeit with slight variation among animals (Figure S4A–F). For example, muscle-specific knockdown of *hsp90 (hp-hsp90*^{bwm}*)* reduced *HSP90::mCherry* fluorescence in *HSP90*^{bwm} animals to 55%, relative to control animals, whereas *HSP90::mCherry* expression in

HSP90^{int} and *HSP90*^{neuro} animals were unaffected by muscle-specific hairpin RNAi (Figure S4A and S4B). Likewise, *hsp90* hairpin RNAi expressed in the intestine *(hp-hsp90*^{int}*)* or the neurons *(hp-hsp90*^{neuro}*)* reduced *HSP90* levels to 25% in only *HSP90*^{int} and to 50% in *HSP90*^{neuro} animals, respectively (Figure S4C–D and S4E–F, respectively).

Tissue-specific knockdown of *hsp90* in the neurons, intestine or bodywall muscle also resulted in significant developmental delays (Figure S4G), and the appearance of diverse aberrant phenotypes (Figure S4H) consistent with the proposed roles of *HSP90* in development, signal transduction, gene expression (Taipale et al., 2010), and as a capacitor of phenotypic variation (Queitsch et al., 2002; Rutherford and Lindquist, 1998).

Consistent with the function of *HSP90* as a repressor of the HSR, RNAi-mediated knockdown of *hsp90* in a single tissue induced the expression of *hsp70*, under normal conditions of growth (Figure 4A), corresponding to a 10-fold induction of *hsp70* mRNA (*C12C8.1* and *F44E5.4*) in *hp-hsp90*^{bwm} animals and 8-fold up-regulation in the *hp-hsp90*^{int} and *hp-hsp90*^{neuro} lines. By comparison, knocking down *hsp90* in all tissues of wild type animals by systemic RNAi resulted in a ~30 and 15-fold induction, respectively of two *hsp70* genes (*C12C8.1* and *F44E5.4*) (Figure 4A). This induction of *hsp70* in the tissue-specific *hsp90* knockdown lines was sufficient to ameliorate organismal survival compared to the control line (Figure 4B), indicating that the induction of *hsp70* in multiple tissues was protective.

We examined the induction of the HSR at the level of individual tissues by monitoring the *hsp70p::mCherry* reporter in living animals expressing the tissue-specific *hsp90* knockdown constructs, and observed that the HSR was induced not only in the primary tissue but also in distal tissues, that were not targeted by the hairpin RNAi (Figure 4C). Knockdown of *hsp90* in the bodywall muscle significantly up-regulated *hsp70* expression not only in muscle cells, but also in the intestine and pharynx (Figure 4C, *vi*, *vii* and *viii*, red). Likewise, in animals with reduced levels of *HSP90* in the intestine, we observed elevated *hsp70* expression in the intestine and muscle cells (Figure 4C, *x*, *xi* and *xii*, red). Animals expressing hairpin *hsp90* dsRNA in neurons however exhibited an increased *hsp70* expression in only bodywall muscle cells (Figure 4C, *xiv*, *xv* and *xvi*, red). These results are consistent with the observation that systemic knockdown of *hsp90* in wild type animals induces the HSR primarily in muscle tissue, such as the bodywall muscle, pharynx and the vulval muscle (Figure S4I), corroborating previous observations that bodywall muscle cells may be more sensitive to a reduction of *hsp90* than other tissues (Gaiser et al., 2011).

In conclusion, either enhancing or suppressing the levels of *HSP90* within a single tissue has complementary effects on the induction of the HSR across adjacent tissues of *C. elegans*. This indicates the involvement of a cell-non-autonomous regulatory mechanism that modifies organismal HSF-1 activity in response to tissue-specific alteration of *HSP90* levels.

HSP90 expression is regulated in a cell-non-autonomous manner, independent of neuronal activity

The cell-non-autonomous effect of *HSP90* on myosin maturation and organismal HSF-1 activity poses an interesting question of how *HSP90* is regulated in *C. elegans*. Expression of the *hsp90* reporter (*hsp90p::GFP*) was up-regulated across multiple tissues when *HSP90* levels were elevated in a single tissue. As shown in Figure 5A, increased expression of *HSP90* in bodywall muscle (Figure 5A, *v–viii*,) or intestine (Figure 5A, *ix – xii*) resulted in induction of the *hsp90* reporter in pharynx, excretory cell and intestine (Figure 5A, *vi – vii* and *x – xi*, respectively). Likewise, elevated *HSP90* levels in the neurons increased endogenous *hsp90* expression in the intestine, pharynx and bodywall muscle (Figure 5A, *xiv – xv*). Thus the increased activity of the transcriptional *hsp90* promoter*::GFP* fusion

indicates the involvement of a transcriptional regulatory mechanism that cell-non-autonomously regulates endogenous *hsp90* expression in response to a tissue-specific imbalance.

Since neurons are important for information exchange and coordination of transcriptional regulation at the organismal level (Prahlad et al., 2008), we examined whether neuronal signaling was essential for the cell-non-autonomous regulation of *hsp90* expression. We therefore tested whether inhibition of the major modes of neuro-secretion, the dense core vesicle (DCV) release of neurotransmitter and the small core vesicle (SCV) release of neuropeptides (Richmond and Broadie, 2002), suppressed the transcriptional tissue-feedback in response to elevated tissue-specific *HSP90* (Figure 5B), since DCV-dependent neurosecretion is also required to maintain optimal levels of chaperones in non-neuronal tissues (Prahlad and Morimoto, 2011). Organismal levels of *hsp90* mRNA were unchanged through inhibition of SCV via deletion of *unc-13* (Kohn et al., 2000), as well as through inhibition of DCV via deletion of *unc-31* (Hammarlund et al., 2008; Speese et al., 2007) (Figure 5C), which correlated with *hsp90p::GFP* expression throughout tissues (Figure S5). These results indicate that cell-non-autonomous regulation of *hsp90* expression is independent of neuronal signaling, and therefore communicated directly between somatic tissues.

PHA-4 dependent transcriptional response regulates cell-non-autonomous *hsp90* expression

To examine the cell-non-autonomous regulation of *hsp90*, we addressed the role of HSF-1, the major stress-inducible transcription factor (Akerfelt et al., 2010). *hsp90* expression in wild type animals was reduced upon *hsf-1* RNAi (Figure 6B). However, consistent with repression of HSF-1 transcriptional activity in the *HSP90* overexpression lines (Figure 3 and Figure 6A), treatment with *hsf-1* RNAi did not affect the levels of *hsp90* in $HSP90^{bwm}$, $HSP90^{int}$ or $HSP90^{neuro}$ lines (Figure 6B), revealing an HSF-1 independent process.

We next turned our attention to data from the *modENCODE* project that identified DAF-16, SKN-1, DAF-12 and PHA-4 binding to the *hsp90* promoter by ChIP-Seq ((Celniker et al., 2009); (http://modencode.oicr.on.ca/fgb2/gbrowse/worm/)). Many of these factors also have established roles in proteostasis (Hsu et al., 2003; Morley and Morimoto, 2004; Oliveira et al., 2009; Panowski et al., 2007; Wang et al., 2010; Zhong et al., 2010). Of these, RNAi-mediated knockdown experiments identified *pha-4* to have the strongest reduction of organismal *hsp90* expression in both wild type and all three *HSP90* overexpression lines (Figure 6C). *pha-4* RNAi also correlated with reduced expression of the *hsp90* reporter across multiple tissues in the *HSP90* overexpression lines (Figure S6A). These results suggest that PHA-4 is necessary for increased cell-non-autonomous *hsp90* expression. Moreover, *pha-4* RNAi suppressed the induction of *hsp90* in myosin (*ts*) and paramyosin (*ts*) mutants back to lower wild type levels (Figure 6D).

Thus, a tissue-specific imbalance through increased levels of *HSP90* or the expression of a metastable client leads to a PHA-4 dependent transcriptional feedback between different tissues that coordinates and balances expression of *HSP90* throughout the animal. This cell-non-autonomous transcriptional response regulated by PHA-4 is beneficial during mild chronic proteotoxic stress as in the case of myosin *(ts)* mutants that require higher levels of *HSP90*, but can become detrimental under severe HS conditions as up-regulated *hsp90* expression through this transcriptional mechanism also represses the HSR (Figure S6B and S6C). Consistent with this result, a tissue-specific imbalance through reduced *hsp90* expression, that induces the HSR in different tissues (Figure 4C) also requires functional

PHA-4 for this inter-tissue response, as demonstrated by using a *pha-4(zu225);smg-1* mutant (Gaudet and Mango, 2002) (Figures S6D and S6E).

To further investigate the role of PHA-4 in this inter-tissue communication, we examined *pha-4* activity and expression levels in the *HSP90* overexpression lines. *Pha4* activity was measured by examining the levels of the *pha-4* regulated *sod* genes (*sod-1, sod-2, sod-4, sod-5*) that contain a PHA-4 consensus binding site in the respective promoters (Panowski et al., 2007) as confirmed by modENCODE. All three *HSP90* overexpression lines exhibit increased *pha-4* activity (Figure 6E) as well as elevated levels of *pha-4* mRNA (Figure 6F), comparable to the long-lived *eat-2* mutant that harbors intrinsically higher *pha-4* activity and mRNA levels relative to wild type animals (Panowski et al., 2007). Thus, the higher activity and expression levels of *pha-4* in response to a tissue-specific imbalance is consistent with the observation that functional PHA-4 is required for the systemic effects through trans-cellular chaperone signaling.

To understand how this inter-tissue signaling is regulated in the receiving tissue, we examined *pha-4* activity and expression levels in isolated intestinal cells of the *HSP90* overexpression lines (Figure 6G and 6H). Whereas *pha-4* activity (Figure 6G) and expression levels (Figure 6H) are induced in the intestinal cells of $HSP90^{int}$ animals (i.e. signaling tissue), *pha-4* activity but not mRNA levels are increased in the receiving tissue (i.e. intestines of either $HSP90^{neuro}$ or $HSP90^{bwm}$) (Figures 6G and 6H). This reveals that *pha-4* expression and activity are required in the signaling tissue and suggests two possibilities for the requirement of PHA-4 in the receiving tissue: that PHA-4 has higher activity despite being expressed in relatively lower amounts, or that PHA-4 in the signaling tissue activates a downstream signaling cascade that acts independently of PHA-4 in the receiving tissue to regulate gene expression (see also Figure 7). Thus, PHA-4 or *pha-4* dependent downstream signaling likely adopts a more general role in trans-cellular chaperone signaling as a regulatory effector that contributes to organismal proteostasis surveillance.

Discussion

Local perturbations of the proteostasis network, whether caused by tissue-specific expression of metastable proteins, or by the elevated expression of individual chaperones such as *HSP90*, are compensated by a beneficial trans-cellular chaperone signaling response from adjacent tissues in *C. elegans*. This suggests that the unique complement of proteins expressed in each tissue is maintained by a combination of autonomous and non-autonomous quality control processes to prevent misfolding and aggregation from dominating the health of a tissue. We propose that individual tissues within an organism serve not only as sensors that respond to disruption of their own cell-specific proteostasis networks, but also to function as sentinels to disseminate local proteotoxic challenges to tissues within the organism to mount a protective response.

A model to describe how such compensatory responses in different tissues can protect the organism from environmental fluctuations to ensure survival of animals harboring genetic pre-dispositions for protein misfolding is shown in Figure 7. Disturbance of the tissue-specific proteostasis network by expression of a metastable client protein such as temperature-sensitive myosin induced the expression of *HSP90* not only in muscle tissue, but also in distal tissues. This response is regulated by PHA-4 activity and communicated to other tissues by trans-cellular chaperone signaling. Indeed, increased expression of *HSP90* at the organismal level is beneficial for the folding of myosin (*ts)* mutants under mild temperature stress (Figure S6B and Figure 7). However, *HSP90* also represses HSF-1 transcriptional activity (Bharadwaj et al., 1999; Zhao et al., 2002); therefore, elevated levels

of *HSP90* result in a failure to mount a HSR in multiple tissues upon exposure to severe heat shock, thus affecting organismal survival (Figure S6C and Figure 7). Our results also show that increased levels of *HSP90*, by inhibiting HSF-1, override the neuronal signal that regulates the HSR. When *HSP90* levels are elevated in a specific tissue, cell-non-autonomous regulation of endogenous *HSP90* expression is uncoupled from neural regulation of HSF-1 activity and is henceforth regulated by the FoxA transcription factor PHA-4. Although the molecular nature of the intercellular signal that mediates this trans-cellular signaling response between tissues is unclear, it is dependent upon PHA-4 (Figures 6 and S6), revealing a more general role for this transcription factor as a regulatory effector. Moreover, *pha-4* expression and activity are increased in the signaling tissue harboring higher *hsp90* levels, which leads to activation of gene expression in the downstream recipient tissues. We speculate that regulation of *hsp90* expression by PHA-4 under the conditions reported by modENCODE and the inter-tissue response, functions at low basal levels in wild type animals and becomes activated in response to tissue-specific perturbations to restore organismal proteostasis.

Trans-cellular chaperone signaling therefore communicates a local proteotoxic stress event to adjacent cells and tissues, thus providing a community-level response. By this, we propose that metazoans have developed a survival strategy to prevent the “weakest link” from compromising organismal health and survival. This form of regulation in which the community of adjacent cells and tissues restores proteostasis is distinct from the neuronal control of the HSR that transmits an external environmental signal through the thermo-sensory AFD neuron to coordinate the regulation of HSF-1 activity in non-neuronal tissue (Prahlad et al., 2008). Yet, the two forms of cell non-autonomous regulation complement to provide a protective mechanism for the unique proteomes expressed in different cells and tissues. Such modulation of proteostasis between non-neuronal tissues in *C. elegans* could similarly be achieved via exchange of small signaling molecules such as metabolites, ROS, peptides or small regulatory RNA molecules (Belting and Wittrup, 2008) that activate and change tissue-specific transcriptional programs in the target tissues.

Among the molecular chaperones, fluctuations in *HSP90* levels have been shown to affect developmental robustness in *Drosophila* and *Arabidopsis* (Gangaraju et al., 2011; Queitsch et al., 2002; Rutherford and Lindquist, 1998). Systemic reduction of *HSP90* results in larval arrest and in greater penetrance of mutations in *C. elegans* (Burga and Lehner, 2012; Casanueva et al., 2012), whereas gain of function mutations cause defective dauer signaling (Birnby et al., 2000), and defects in muscle cells (Gaiser et al., 2011). Our results provide additional support that tissue-specific reduction of *HSP90* leads to cell-non-autonomous developmental defects and phenotypes that have not been previously associated with *HSP90* dysfunction. For example, RNAi-mediated knockdown of *hsp90* in muscle cells exposes defects in other tissues, such as the excretory canal (*exc)* phenotype, aberrant hermaphrodite tail formation, or abnormal intestines (Figure S4H). These observations lend support for a role of *HSP90* to integrate transcriptional response across different cell types and tissues in *C. elegans*, consistent with a role in a complex systems network at the hub of diverse signaling processes from yeast to mammals (Taipale et al., 2010).

While our studies have only addressed the role of *HSP90* with regard to folding and stability of the client protein myosin, and how altered levels of *HSP90* transmits a signal across cells; likewise, other chaperones may also have similar effects on organismal responses. In particular, client-specific responses could ensure that different molecular chaperones could regulate complementary types of proteotoxic stress signaling events.

In summary, the work presented here provides the basis of a new mechanism of how tissues within an organism respond to disturbances of proteostasis to regulate a cell-non-

autonomous control of chaperone expression that restore balance between tissues. Future studies will address how tissue-specific perturbations in a limited number of sensor cells are transmitted to the recipient cells and tissues and whether trans-cellular chaperone signaling observed in *C. elegans* extends to other metazoans.

Experimental Procedures

Heat shock

Synchronized populations of *C. elegans* strains were grown at 20°C and animals were heat shocked at a population density of 10–15 young adult animals per plate as described (Prahlad et al., 2008). Animals were heat shocked by sealing plates with parafilm and zip-lock bags and immersing into a water bath equilibrated at 33°C for 1 hour or at 34°C for 30 minutes and allowed to recover for 1 hour at 20 °C before they were harvested for quantitative RT PCR. Each qRT-PCR experiment was repeated in triplicate.

Thermotolerance

For thermotolerance assays, a synchronized population of approximately 20 young adult animals on each plate was placed into a 35°C incubator (Fisher Scientific – Isotemp Incubator). 5 samples, each consisting of 20 adult animals were used for one time-point and the experiment was repeated at least 3 times (3 biological replicates) to achieve substantial N values. Statistically significant changes in survival were considered when $p<0.05$ (Student's T-test). Plates were collected at the indicated time-points (8 hours and 10 hours) and animals were allowed to recover for 2 hours at 20 °C before scoring for touch-induced movement and pharyngeal pumping.

Assays for temperature-sensitive phenotypes

For the paralysis assay of *unc-54(ts*) mutants, 20 young adult animals were placed onto fresh NGM plates at 25°C and scored 12 hours later for touchinduced movement. For the survival assays of *unc-54(ts)* mutants, young adult animals were allowed to lay eggs at 25°C for 3 hour s. After removal of the adults, plates were incubated at the restrictive temperature for 24 – 48 hours and then scored for surviving and moving progeny (n = 50). All experiments were repeated three times (3 biological replicates). Statistically significant changes in movement were considered if $p<0.05$ (Student's T-test).

RNAi experiments

For RNAi-mediated knockdown of indicated genes, synchronized populations of nematodes were placed on *E. coli* strain HT115(DE3) transformed with appropriate RNAi vectors (J. Ahringer, University of Cambridge, Cambridge, U.K.) as decribed previously (Nollen et al., 2004).

To knock-down *hsp90* prior to heat shock, 30 L4 larvae were placed on *E. coli* strain HT115(DE3) transformed with *hsp90* RNAi.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Imbalance of muscle proteostasis induces chaperone expression in different tissues
- Tissue-specific modulation of chaperones such as HSP90 affects the organismal HSR
- This intertissue response is regulated by interplay between HSF-1 and PHA-4
- Trans-cellular chaperone signaling integrates cell-specific and organismal responses

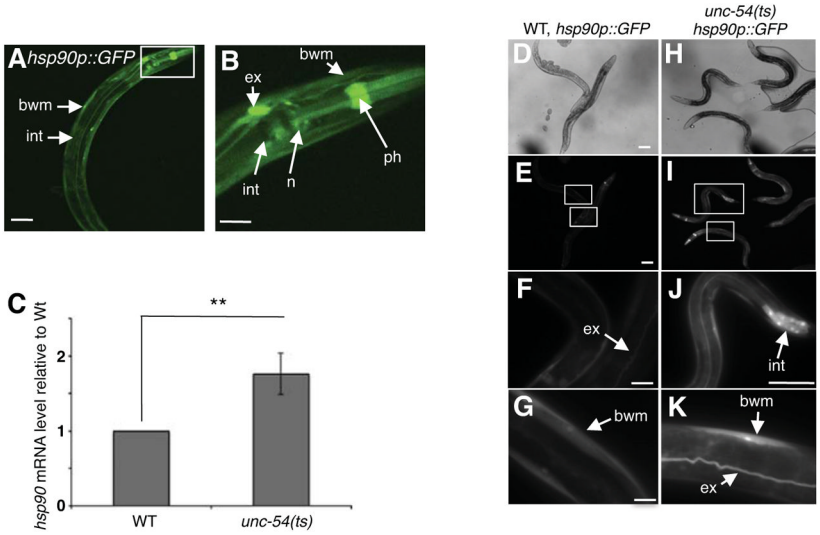

Figure 1. Tissue-specific perturbation of proteostasis is recognized across multiple tissues in a cell-non-autonomous manner

(A,B) Confocal image of a young adult animal expressing the 2.5 kb *hsp90* promoter region upstream of GFP (*hsp90p::GFP*) at 20°C. Pronounced expression was observed in mu ltiple tissues, including pharyngeal muscle (ph), intestine (int), pharyngeal nerve ring (n), bodywall muscle (bwm) and the excretory cell (ex). Scale bar is equal to 100 μm. **(B)** 63x magnification of the head region. Expression is detected in the bodywall muscle (bwm), the excretory cell (ex), the pharyngeal muscle (ph), pharyngeal nerve-ring (n) and the intestine (int). Scale bar is equal to 10 μm. **(C)** Total mRNA levels of *hsp90* in *unc-54(e1301)* animals relative to wild type at 15C°C. Bargraphs represent combined mean values of three independent experiments (means ± s.e.m.) **P-value < 0.01. **(D–K)** *hsp90p::GFP* reporter expression in *unc-54(e1301)* animals compared to wild type. Scale bar of figures **(D–K)** are equal to 100 μm. **(F,G)** 100x image of wild type expressing the *hsp90* reporter in the excretory canal (ex) and bodywall muscle (bwm). **(J,K)** *hsp90* expression in intestinal cells, bodywall muscle and excretory canal in *unc-54(e1301)* animals. **(J)** 40x image. **(K)** 100x image. All fluorescent images were taken at equal exposure times. See also Figure S1.

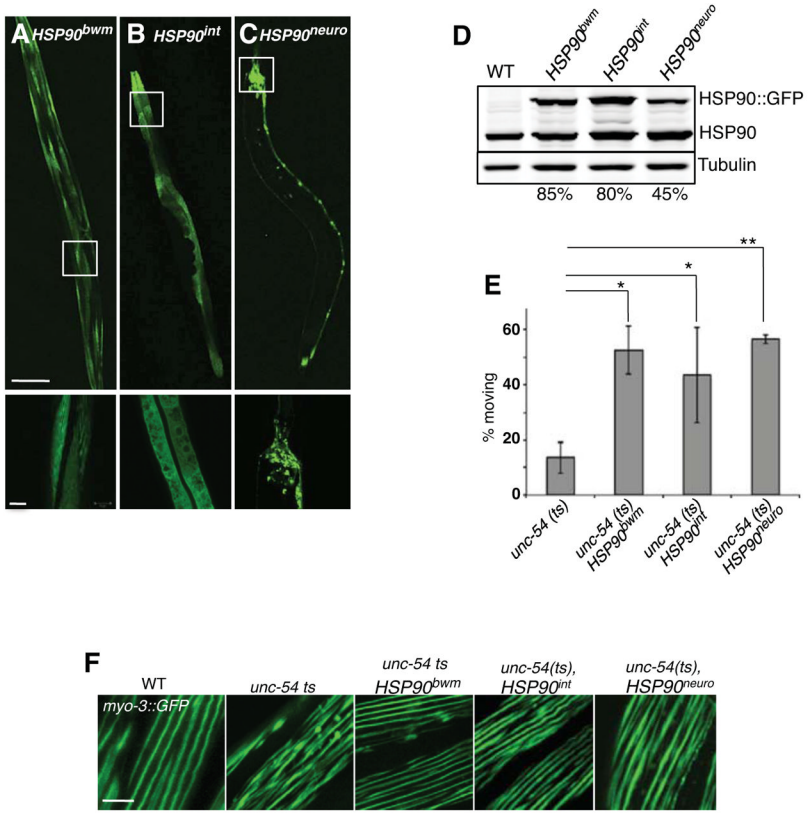

Figure 2. Tissue-specific increased levels of *HSP90* improves the organismal folding environment of *myosin(ts)* mutants

(A–C) Confocal images of young adult *C. elegans* animals overexpressing *HSP90::GFP* in the **(A)** bodywall muscle (*HSP90^{bwm}*), **(B)** the intestine (*HSP90^{int}*) or **(C)** the neurons (*HSP90^{neuro}*) (scale bar = 100 μm), with 63x magnifications of selected regions (Scale bar = 10 μm). **(D)** Western Blot analysis of young adult animals overexpressing *HSP90* using an anti-*C.elegans* HSP90 antibody. Levels of *HSP90::GFP* are normalized to the loading control (tubulin) and relative to endogenous *HSP90.* **(E)** Percentage of young adult animals expressing *ts* myosin [*unc-54 (e1301)*] alone or in the presence of bodywall muscle-specific *HSP90::GFP* overexpression (*unc-54(ts),HSP90^{bwm}*), intestinal overexpression *(unc-54(ts),HSP90^{int})*, or neuronal overexpression *(unc-54(ts),HSP90^{neuro})* showing movement after exposure to restrictive temperature (25°C) for 12 – 24 hours. n = 20 adult animals per strain per experiment. Bargraphs represent the combined results of 3 independent experiments. Error bars = ±SEM. *P-value < 0.05. **P-value < 0.01. **(F)** Confocal images of the bodywall muscle of age-synchronized wild type, *unc-54(e1301)*, *unc-54(e1301),HSP90^{bwm}*, *unc-54(e1301),HSP90^{int}*, and *unc-54(ts),HSP90^{neuro}* animals after exposure to restrictive temperature (25ºC) for 12 hours, using *myo-3::GFP* for visualization. Scalebar = 20 μm. See also Figure S2.

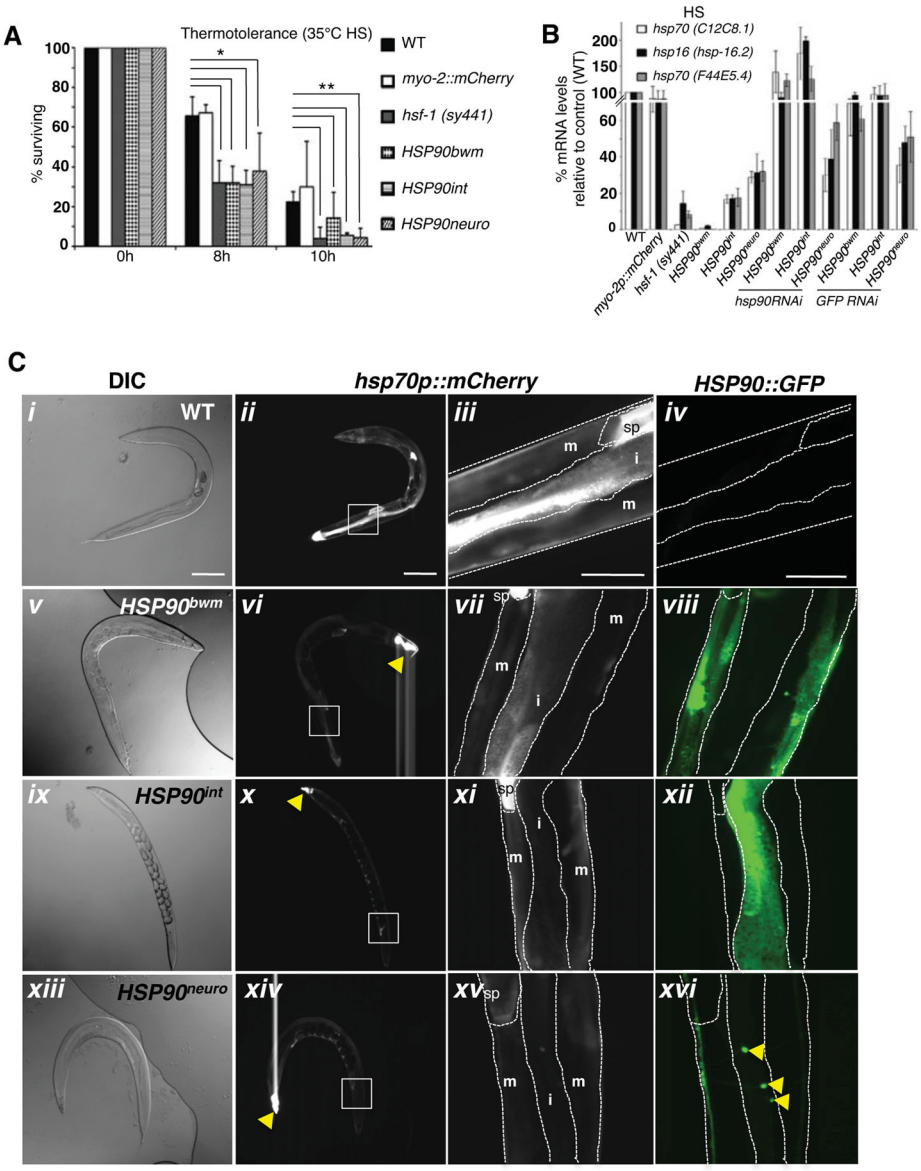

Figure 3. Elevated tissue-specific *HSP90* levels repress the HSR at an organismal level

(A) Thermosensitivity of young adult animals (n = 100) with indicated genotypes exposed to 35°C heat stress. *P-value < 0.05. **P-value < 0.01. **(B)** Total mRNA levels of *hsp70* (*C12C8.1* and *F44E5.4*) and *hsp16 (hsp-16.2*) after heat shock (1h at 33°C) in young adult *myo-2p::mCherry*, *hsf-1 (sy441)* mutant, $HSP90^{neuro}$, $HSP90^{int}$, $HSP90^{bwm}$ animals and upon RNAi-mediated *GFP* or *hsp90* knockdown prior to heat shock in the transgenic *HSP90* lines, relative to wild type. **(A and B)** Bargraphs represent combined mean values of three independent experiments (means ± s.e.m.) **(C)**

DIC Nomarski images of ***(i)*** wild type, ***(v)*** $HSP90^{bwm}$, (***ix)*** $HSP90^{int}$ and ***(xiii)*** $HSP90^{neuro}$ animals expressing the *hsp70p::mCherry* reporter. Expression of the *hsp70p::mCherry* reporter 7 hours after heat shock (33°C, 1h) in representative ***(ii)*** wild type and in ***(vi)*** $HSP90^{bwm}$, ***(x)*** $HSP90^{int}$ and ***(xiv)*** $HSP90^{neuro}$ animals. Yellow arrow in ***vi, x,*** and ***xiv*** indicates the pharyngeal *myo-2::mCherry* co-injectionmarker, present in all transgenic *HSP90::GFP* lines. ***(iii,vii,xi,xv)*** 20x magnifications of the posterior region of ***(iii)*** wild type

and *HSP90* overexpression lines ***(vii, xi, xv)***, indicating *hsp70* induction in spermatheca (sp), bodywall muscle (m) and the intestine (i). ***(iv, viii, xii, xvi)*** *HSP90::GFP* expression in true color. Yellow arrows in ***(xvi)*** indicate neuronal cells expressing *HSP90::GFP*. **(*i–xvi*)** Scale = 100 μm. See also Figure S3.

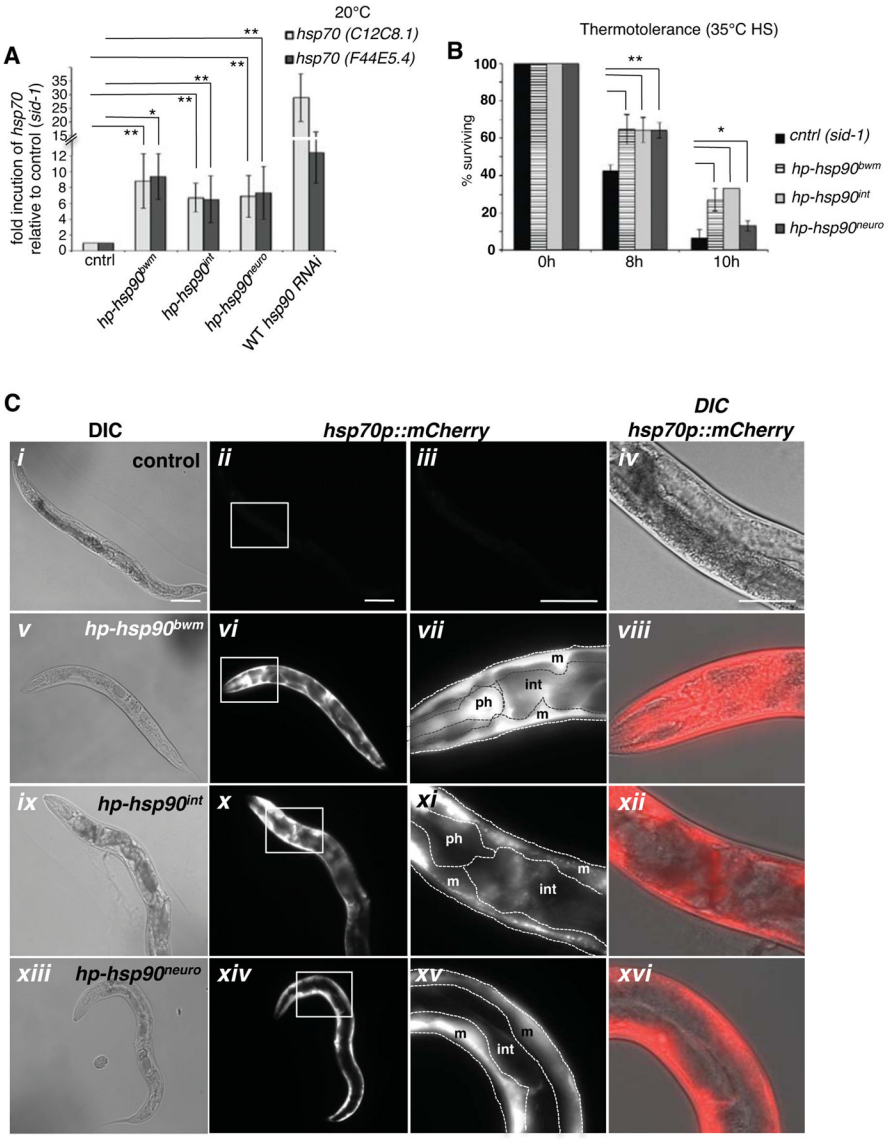

Figure 4. Tissue-specific knockdown of *hsp90* cell-non-autonomously induces the HSR

(A) Bodywall-muscle, intestine- and neuron-specific *hsp90* RNAi induces basal levels of *hsp70* (*C12C8.1* and *F44E5.4*) expression at 20°C, compared to control animals (*sid-1*). Wild type animals allow import of dsRNA from surrounding tissues, leading to higher induction of organismal *hsp70* than in the tissue-specific knockdown lines. Bargraphs represent combined mean values of three independent experiments (means ± s.e.m.) **P-value < 0.05. **(B)** Thermosensitivity of young adult animals (n = 100) expressing the indicated tissue-specific *hp-hsp90* construct exposed to 35 °C heat stress. Bargraphs represent combined mean values of three independent experiments (means ± s.e.m.) **P-value < 0.01. *P-value < 0.05. **(C)** Tissue-specific knockdown of *hsp90* induces expression of the *hsp70* reporter (*hsp70p::mCherry*) at 20°C. DIC images of synchronized young adult ***(i)*** control animals (*sid-1*), ***(v)*** *hp-hsp90*^{bwm}, ***(ix)*** *hp-hsp90*^{int}, and ***(xiii)*** *hp-hsp90*^{neuro} animals expressing the *hsp70* reporter. Expression of the *hsp70p::mCherry* in ***(ii)*** *sid-1* control animals, ***(vi)*** *hp-hsp90*^{bwm}, ***(x)*** *hp-hsp90*^{int}, and ***(xiv)*** *hp-hsp90*^{neuro}. ***(iii, vii, xi, xv)*** 20x magnification of control ***(iii)*** and tissue-specific *hsp90* knockdown lines ***(vii, xi, xv)***

indicating expression of *hsp70p::mCherry* in the pharynx (ph), intestine (int) and bodywall muscle (m). ***(iv, viii, xii, xvi)*** Overlay of DIC Nomarski and *hsp70p::mCherry* (red). Scalebars = 100 μm. See also Figure S4.

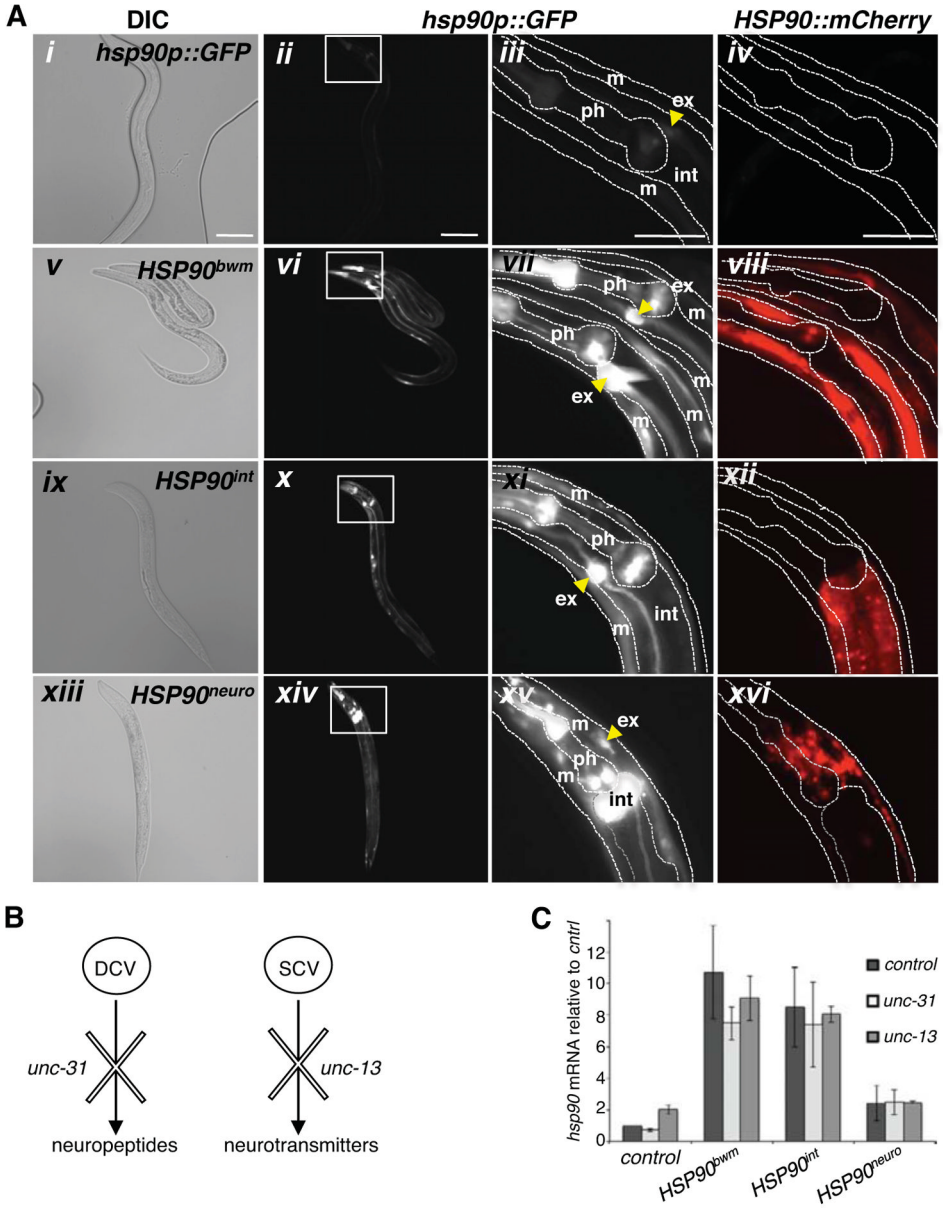

Figure 5. Coordination of cell-non-autonomous *hsp90* expression is regulated independent of neural control

(A) Tissue-selective increased levels of *HSP90* up-regulates expression of the transcriptional *hsp90p::GFP* reporter at normal conditions (20°C). DIC images o f ***(i)*** wildtype, ***(v)*** *HSP90*^{bwm}, ***(ix)*** *HSP90*^{int} and ***(xiii)*** *HSP90*^{neuro} expressing the *hsp90p::GFP* reporter. *hsp90* reporter expression in representative whole animals ***(ii, vi, x, xiv)*** and magnified head region ***(iii, vii, xi, xv)*** in (***ii, iii)*** wild type, ***(vi, vii)*** *HSP90*^{bwm}, ***(x, xi)*** *HSP90*^{int}, and ***(xiv, xv)*** *HSP90*^{neuro}, indicating *hsp90p::GFP* in the pharynx (ph), the intestine (int), bodywall muscle (m) and excretory cell (ex). ***(iv, viii, xii, xvi)*** *HSP90::mCherry* (red) expression in true color. Yellow arrows in (***iii, vii, xi and xv)*** indicate *hsp90p::GFP* expression in the excretory cell. Scalebars = 100 μm.**(B)** Schematic representation of the major modes of neuro-secretion in *C. elegans*, regulated via either dense core vesicles (DCV) through *unc-31* or via small core vesicles (SCV) through *unc-13*. **(C)** Total *hsp90* mRNA levels of

HSP90 overexpression lines in an *unc-31* deletion mutant background (white bars) or crossed to an *unc-13* deletion mutant (light grey bars) relative to control animals (dark grey bars). Bargraphs represent combined mean values of three independent experiments (means ± s.e.m.). See also Figure S5.

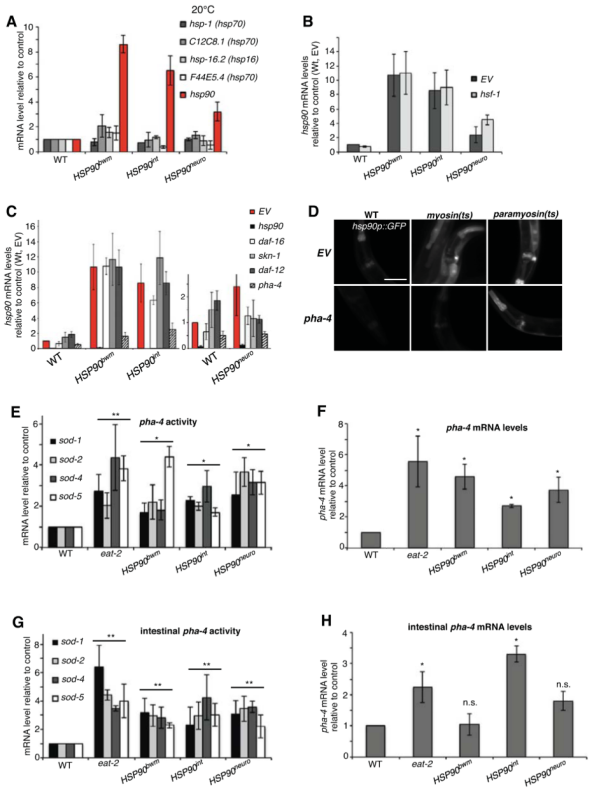

Figure 6. A PHA-4 – dependent transcriptional feedback coordinates cell-non-autonomous *hsp90* expression

(A) Total mRNA levels of *hsp-1* (constitutive hsp70), *hs*-inducible *hsp70* (*C12C8.1* and *F44E5.4*), small heat shock protein *hsp16* (*hsp-16.2)* and *hsp90* in *HSP90* overexpression lines at 20°C relative to wild type. The slightly increased *hsp* levels in the $HSP90^{bwm}$ may be indicative of the higher sensitivity of muscle cells to proteostatic perturbation, in line with the observations on tissue-specific *hsp90* knockdown, where the HSR is primarily induced in the bodywall muscle. **(B)** Organismal *hsp90* expression in *HSP90* overexpression lines is independent of *hsf-1*. *hsp90* mRNA levels in control (EV) and animals fed with *hsf-1* RNAi. Whereas *hsp90* expression in wild type is *hsf-1* dependent, RNAi-mediated knockdown of *hsf-1* leaves organismal *hsp90* levels in $HSP90^{bwm}$, $HSP90^{int}$ or $HSP90^{neuro}$ unchanged. **(C)** *pha-4* RNAi decreases elevated *hsp90* expression in the overexpression lines. **(D)** *hsp90p::GFP* reporter expression in myosin *(ts, e1157)* or paramyosin *(ts, e1402)* mutants is reduced during *pha-4* RNAi when compared to control RNAi (*EV*). Scalebar = 50 μm. **(E)** *pha-4* activity is increased in the *HSP90* overexpression lines. mRNA levels of *pha-4* regulated genes *sod-1, sod-2, sod-4* and *sod-5* are induced in *eat-2(ad1113)* mutants, $HSP90^{bwm}$, $HSP90^{int}$ and $HSP90^{neuro}$ animals relative to wild type. *P-value < 0.05. **P-value < 0.02. **(F)** *pha-4* mRNA expression levels in *eat-2* and *HSP90* overexpression lines are upregulated, relative to the wild type control. *P-value < 0.05. (**G)** *pha-4* activity in intestinal cells of *eat-2(ad1113)*, $HSP90^{bwm}$, $HSP90^{int}$ and $HSP90^{neuro}$ animals relative to wild type. **P-value < 0.02. **(H)** *pha-4* mRNA expression levels are induced in the intestines of *eat-2* mutants and $HSP90^{int}$ (=signaling tissue), but not in the intestines of $HSP90^{bwm}$ or $HSP90^{neuro}$ (=recipient tissue). *P-value < 0.05. (n.s. = not significant). **(A – H)** All bar graphs represent combined mean values of three independent experiments (3 biological replicates) (means ± s.e.m.). See also Figure S6.

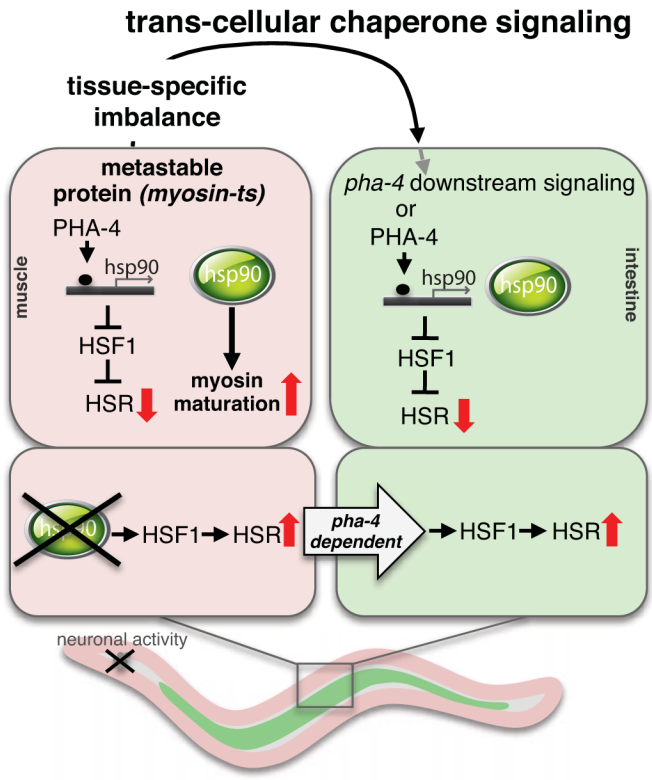

Figure 7. Model for the cell non-autonomous regulation of *HSP90* expression in *C. elegans.*

An imbalance of the proteostasis network through the presence of metastable myosin increases the expression of *HSP90* in muscle cells but also in different cell-types, such as the intestine. This is regulated by PHA-4 and communicated through trans-cellular chaperone signaling to other tissues, independent of neural activity. Increased *pha-4* activity is required in the signaling and receiving tissue, however *pha-4* may act from a distance to regulate gene expression in the receiving tissue via a down-stream signaling cascade. The resulting highly abundant *HSP90* levels in the entire animal are beneficial for myosin folding in *unc-54(ts)* mutants, but can become detrimental during severe heat shock, due to cell non-autonomous repression of HSF-1 transcriptional activity. Likewise, a tissue-specific perturbation through reduced *hsp90* levels (lower panel) leads to induction of the HSR in the same and recipient tissues. Transduction of the response to the recipient tissue is also *pha-4* dependent.