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Regulation of Organismal Proteostasis by Transcellular 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 show that expression of endogenous metastable proteins in muscle cells, which rely on chaperones for proper folding, 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 effectively by elevated expression of *HSP90* in intestine or neuronal cells. This cell-nonautonomous control of *HSP90* expression relies upon transcriptional feedback between somatic tissues that is regulated by the FoxA transcription factor PHA-4. This transcellular chaperone signaling response maintains organismal proteostasis when challenged by a local tissue imbalance in folding and provides the basis for 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 postmitotic 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), which upregulates an intrinsic network of molecular chaperones through the activity of HSF-1, a master stress 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 thermosensory 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-nonautonomous 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-nonautonomous 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 body wall muscle of *C. elegans* (Epstein and Thomson, 1974; Miller et al., 1986). Expression of *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,

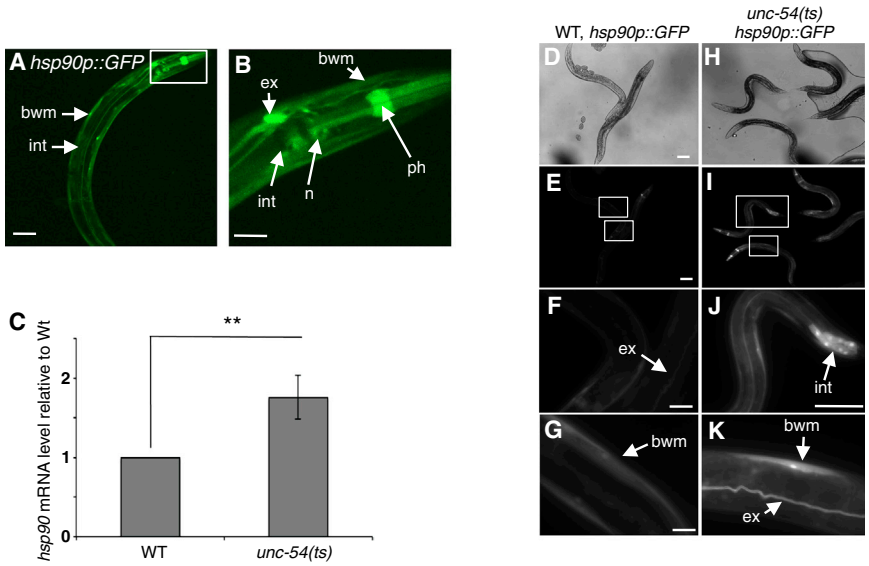

Figure 1. Tissue-Specific Perturbation of Proteostasis Is Recognized across Multiple Tissues in a Cell-Nonautonomous Manner

(A and 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 multiple tissues, including pharyngeal muscle (ph), intestine (int), pharyngeal nerve ring (n), body wall muscle (bwm), and the excretory cell (ex). Scale bar, 100 μm. (B) 63× magnification of the head region. Expression is detected in the body wall muscle (bwm), the excretory cell (ex), the pharyngeal muscle (ph), pharyngeal nerve ring (n), and the intestine (int). Scale bar, 10 μm.

(C) Total mRNA levels of *hsp90* in *unc-54(e1301)* animals relative to wild-type at 15°C. Bar graphs represent combined mean values of three independent experiments (means ± SEM). Bar graphs represent combined mean values of three independent experiments. Error bars represent ±SEM. $^{**}p < 0.01$.

(D–K) *hsp90p::GFP* reporter expression in *unc-54(e1301)* animals compared to wild-type. Scale bars, 100 μm (D–K). (F and G) 100× image of wild-type expressing the *hsp90* reporter in the excretory canal (ex) and body wall muscle (bwm). (J and K) *hsp90* expression in intestinal cells, body wall muscle, and excretory canal in *unc-54(e1301)* animals. (J) 40× image. (K) 100× image. All fluorescent images were taken at equal exposure times.

See also Figure S1.

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 available online).

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), body wall muscle (bwm), and excretory cell (ex), as observed with an *hsp90p::GFP* transcriptional reporter (Figures 1A and 1B). In *unc-54(ts)* animals, however, *hsp90* messenger RNA (mRNA) levels are induced almost 2-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 *(unc-54, e1301 and unc-54, e1157)* as well as paramyosin (*unc-15* and *e1402*), another component of muscle thick filaments (Miller et al., 1986) (Figures 1I–1K, S1D–S1F, and S1H–S1L, respectively) relative to control animals (Figures 1E–1G, S1C, S1G, and S1J). These results are consistent with increased requirements for *HSP90* in body wall muscle cells (Figure 1K, 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 (Figures 1J, 1K, S1E, S1F, 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-nonautonomous 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 body wall muscle. We therefore established *C. elegans* lines expressing *HSP90* (*HSP90::GFP*) in body wall 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 (Figures 2E and 2F).

Because the *hsp90* reporter was also induced in nonmuscle 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 ($HSP90^{neuro}$) (Figures 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 nonmuscle tissues of myosin *(ts)* or paramyosin *(ts)* mutants

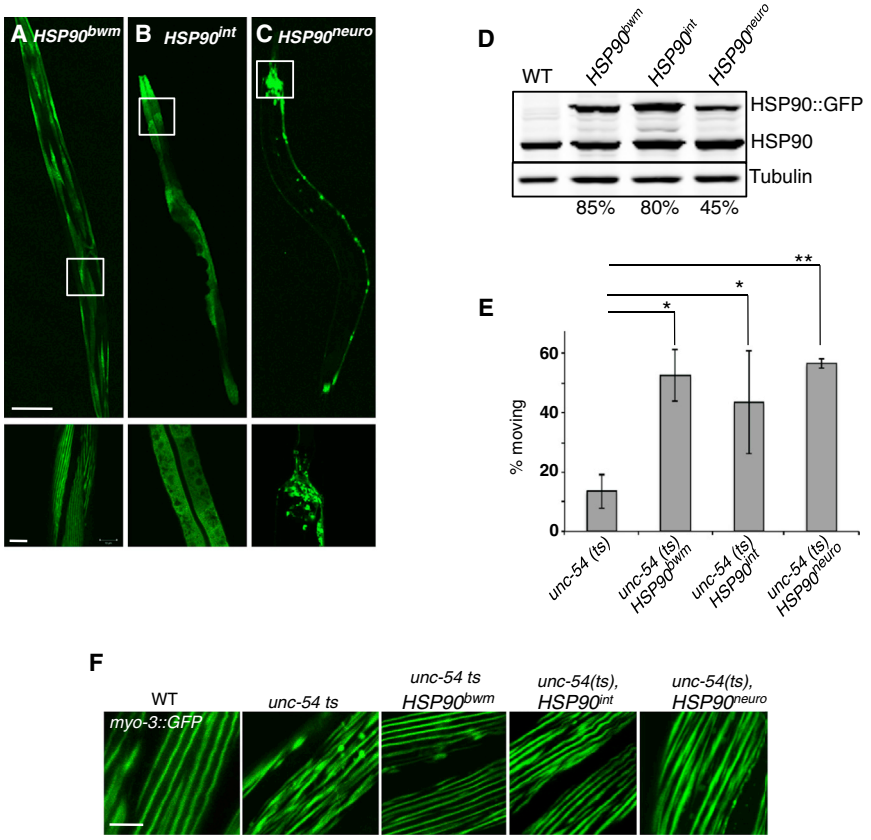

Figure 2. Tissue-Specific Increased Levels of *HSP90* Improves the Organismal Folding Environment of *myosin ts* Mutants

(A–C) Confocal images of young adult *C. elegans* animals overexpressing *HSP90::GFP* in the (A) body wall muscle (*HSP90^{bwm}*), (B) the intestine (*HSP90^{int}*), or (C) the neurons (*HSP90^{neuro}*) (Scale bar, 100 μm), with 63× 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 body wall 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 hr. n = 20 adult animals per strain per experiment. Bar graphs represent the combined results of three independent experiments. Error bars represent ±SEM. *p < 0.05; **p < 0.01.

(F) Confocal images of the body wall 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 (25°C) for 12 hr, using *myo-3::GFP* for visualization. Scale bar, 20 μm. See also Figure S2.

(Figures 1D–1K and S1C–S1L) 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-nonautonomous 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 hr 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 hr 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 form of transcellular 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* have 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 was suppressed 20-fold in *HSP90^{bwm}* animals and 5- and 3-fold in *HSP90^{int}* and *HSP90^{neuro}* animals, 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* RNA interference (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

2-fold reduction of HSF-1 DNA binding (Figures 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* induce a cell-nonautonomous 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), body wall muscle (m), and intestine (i) (Figure 3C, *i–iv)* of wild-type animals. In contrast, animals expressing *HSP90::GFP* in body wall muscle (Figure 3C, *v–viii*; Figure S3D, *i*), intestine (Figure 3C, *ix–xii*; Figure S3D, *iv*), and neurons (Figure 3C, *xiii–xvi*; Figure S3D, *vii*) showed a reduction in heat shock 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*; 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 (Figure S3D, *ii*, *v*, and *viii*) or *HSP90::GFP* levels by *GFP* RNAi (Figure S3D, *iii*, *vi*, and *ix*) prior to HS (Figure S3D, *i–ix*). The 3- and 5-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 heat-shock-induced genes (Figures S3D, *viii* and *ix*, S3E, and 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 2-fold in isolated intestinal cells of $HSP90^{bwm}$, 20-fold in the intestine of $HSP90^{int}$, and 5-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-nonautonomous 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 nonmuscle tissues can cell-nonautonomously rescue the muscle-specific phenotype of myosin *(ts)* mutants, tissue-specific elevated levels of *HSP90* are detrimental under severe heat stress conditions through cell-nonautonomous repression of HSF-1 transcriptional activity. One explanation for repression of HSF-1 activity in nontarget tissues or improved myosin folding when *HSP90* is expressed in nonmuscle 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 body cavity exposed to all tissues of the animal, before they can be taken up by surrounding tissues (Altun and Hall, 2009). Materials secreted in the pseudocoelom are also taken up nonspecifically by coelomocytes, scavenger cells that perform a primitive surveillance function in the animal (Altun and Hall, 2009; Fares and Greenwald, 2001). Therefore, we examined whether *HSP90::mCherry* overexpressed in neurons, body wall 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-nonautonomous effect giving rise to improved myosin maintenance or repression of HSF1 in nontarget 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 (Figures S4A–S4F). Quantitation of *mCherry* fluorescence intensity shows that *HSP90* levels are decreased significantly only in the targeted tissue, albeit with slight variation among animals

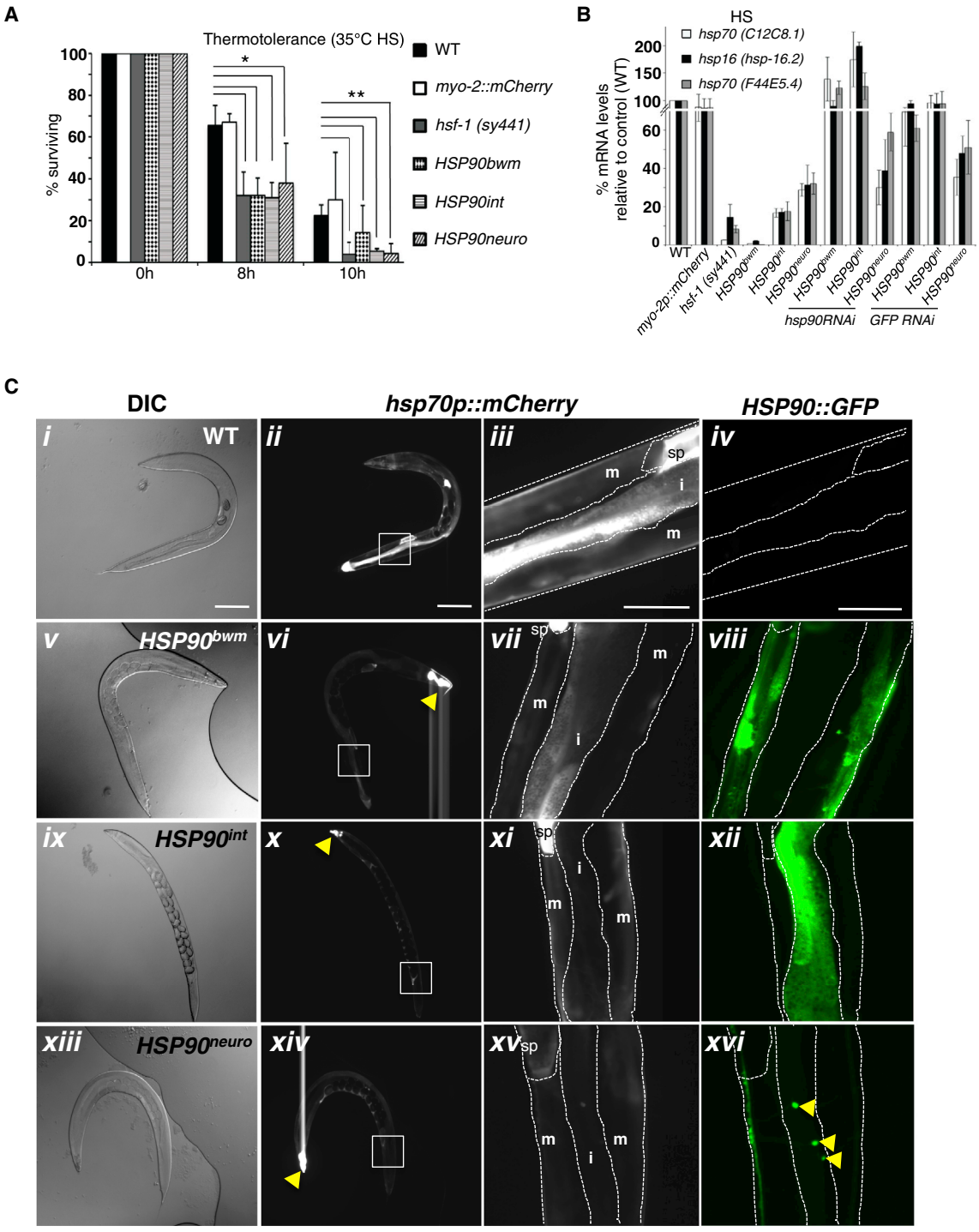

Figure 3. Elevated Tissue-Specific *HSP90* Levels Repress the HSR at an Organismal Level

(A) Thermosensitivity of young adult animals (n = 100) with indicated genotypes exposed to 35°C heat stress. *p < 0.05; **p < 0.01. Bar graphs represent combined mean values of three independent experiments. Error bars represent ±SEM.

(B) Total mRNA levels of *hsp70* (*C12C8.1* and *F44E5.4*) and *hsp16* (*hsp-16.2*) after heat shock (1 hr at 33°C) in young adult *myo-2p::mCherry*, *hsf-1 (sy441)* mutant, $HSP90^{neuro}$, $HSP90^{int}$, and $HSP90^{bwm}$ animals and upon RNAi-mediated *GFP* or *hsp90* knockdown prior to heat shock in the transgenic *HSP90* lines, relative to wild-type. Bar graphs represent combined mean values of three independent experiments. Error bars represent ±SEM.

(C) Differential interference contrast (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 hr after heat shock (33°C, 1 hr) in representative (*ii*) wild-type, (*vi*) $HSP90^{bwm}$, (*x*) $HSP90^{int}$, and (*xiv*) $HSP90^{neuro}$ animals. The yellow arrow in (*vi*), (*x*), and (*xiv*) indicates the pharyngeal *myo-2::mCherry* coinjection marker, present in all transgenic *HSP90::GFP*

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(Figures S4A–S4F). 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 was unaffected by muscle-specific hairpin RNAi (Figures 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 *HSP90^{int}* animals (Figures S4C and S4D) and to 50% in *HSP90^{neuro}* animals (Figures S4E and S4F), respectively.

Tissue-specific knockdown of *hsp90* in the neurons, intestine, or body wall 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, and 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 upregulation 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 body wall muscle significantly upregulated *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* double-stranded RNA (dsRNA) in neurons, however, exhibited an increased *hsp70* expression in only body wall 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 body wall muscle, pharynx, and vulval muscle (Figure S4I), corroborating previous observations that body wall 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-nonautonomous regulatory mechanism that modifies organismal HSF-1 activity in response to tissue-specific alteration of *HSP90* levels.

HSP90 Expression Is Regulated in a Cell-Nonautonomous Manner, Independent of Neuronal Activity

The cell-nonautonomous 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 upregulated across multiple tissues when *HSP90* levels were elevated in a single tissue. As shown in Figure 5A, increased expression of *HSP90* in body wall 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 body wall 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-nonautonomously 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-nonautonomous regulation of *hsp90* expression. We therefore tested whether inhibition of the major modes of neurosecretion, 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 nonneuronal 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-nonautonomous regulation of *hsp90* expression is independent of neuronal signaling and therefore communicated directly between somatic tissues.

PHA-4-Dependent Transcriptional Response Regulates Cell-Nonautonomous *hsp90* Expression

To examine the cell-nonautonomous 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 (Figures 3 and 6A), treatment with *hsf-1* RNAi did not affect the levels of *hsp90* in

lines. (*iii*, *vii*, *xi*, and *xv*) 20× magnifications of the posterior region of (*iii*) wild-type and *HSP90* overexpression lines (*vii*, *xi*, and *xv*), indicating *hsp70* induction in spermatheca (sp), body wall muscle (m), and the intestine (i). (*iv*, *viii*, *xii*, and *xvi*) *HSP90::GFP* expression in true color. The yellow arrows in (*xvi*) indicate neuronal cells expressing *HSP90::GFP*. (*i–xvi*) Scale bar, 100 μm.
See also Figure S3.

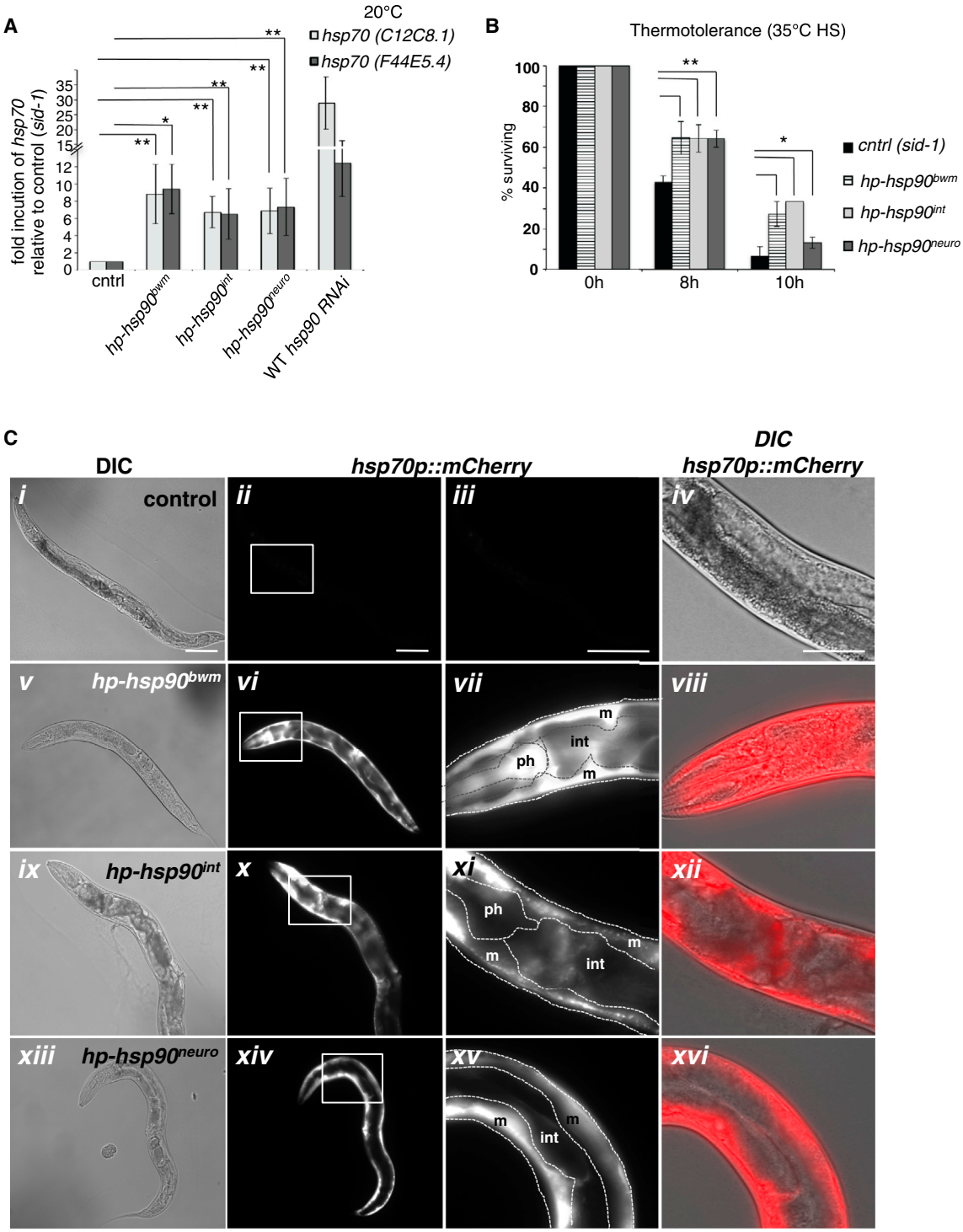

Figure 4. Tissue-Specific Knockdown of *hsp90* Cell-Nonautonomously Induces the HSR

(A) Body wall 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. Bar graphs represent combined mean values of three independent experiments. Error bars represent ±SEM. $^{**}p < 0.05$.

(B) Thermosensitivity of young adult animals (n = 100) expressing the indicated tissue-specific *hp-hsp90* construct exposed to 35°C heat stress. Bar graphs represent combined mean values of three independent experiments. Error bars represent ±SEM. $^{**}p < 0.01$; $^{*}p < 0.05$.

(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*

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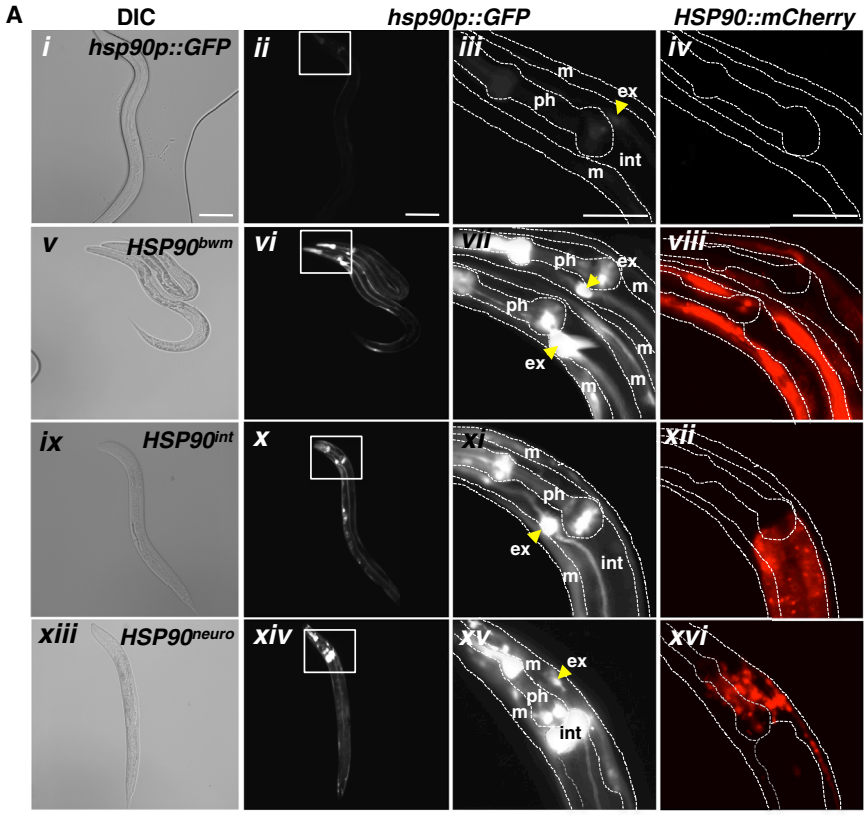

Figure 5. Coordination of Cell-Nonautonomous *hsp90* Expression Is Regulated Independent of Neural Control

(A) Tissue-selective increased levels of *HSP90* upregulates expression of the transcriptional *hsp90p::GFP* reporter at normal conditions (20°C). DIC images of (*i*) wild-type, (*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*, and *xiv*) and magnified head region (*iii*, *vii*, *xi*, and *xv*) in (*ii* and *iii*) wild-type, (*vi* and *vii*) *HSP90*^{bwm}, (*x* and *xi*) *HSP90*^{int}, and (*xiv* and *xv*) *HSP90*^{neuro}, indicating *hsp90p::GFP* in the pharynx (ph), intestine (int), body wall muscle (m), and excretory cell (ex). (*iv*, *viii*, *xii*, and *xvi*) *HSP90::mCherry* (red) expression in true color. Yellow arrows in (*iii*), (*vii*), (*xi*), *and* (*xv*) indicate *hsp90p::GFP* expression in the excretory cell. Scale bars, 100 μm.

(B) Schematic representation of the major modes of neurosecretion 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 gray bars) relative to control animals (dark gray bars). Bar graphs represent combined mean values of three independent experiments. Error bars represent ±SEM.

See also Figure S5.

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 chromatin immunoprecipitation sequencing (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-nonautonomous *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-nonautonomous 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 upregulated *hsp90* expression through this transcriptional mechanism also represses the HSR (Figures 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

control animals, (*vi*) *hp-hsp90*^{bwm}, (*x*) *hp-hsp90*^{int}, and (*xiv*) *hp-hsp90*^{neuro}. (*iii*, *vii*, *xi*, and *xv*) 20× magnification of control (*iii*) and tissue-specific *hsp90* knockdown lines (*vii*, *xi*, and *xv*) indicating expression of *hsp70p::mCherry* in the pharynx (ph), intestine (int), and body wall muscle (m). (*iv*, *viii*, *xii*, and *xvi*) Overlay of DIC Nomarski and *hsp70p::mCherry* (red). Scale bars, 100 μm.

See also Figure S4.

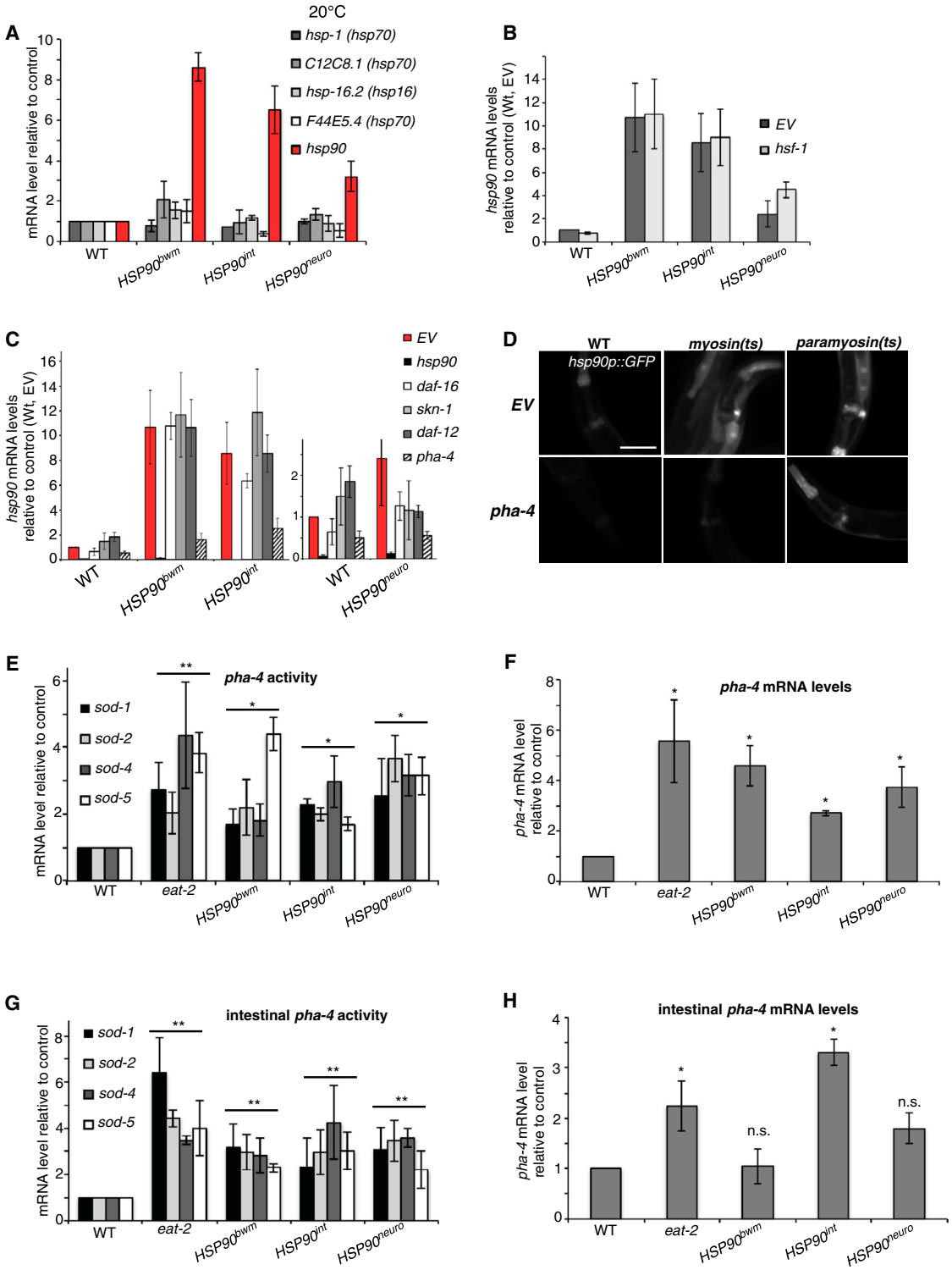

Figure 6. A PHA-4-Dependent Transcriptional Feedback Coordinates Cell-Nonautonomous *hsp90* Expression

(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 body wall 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.

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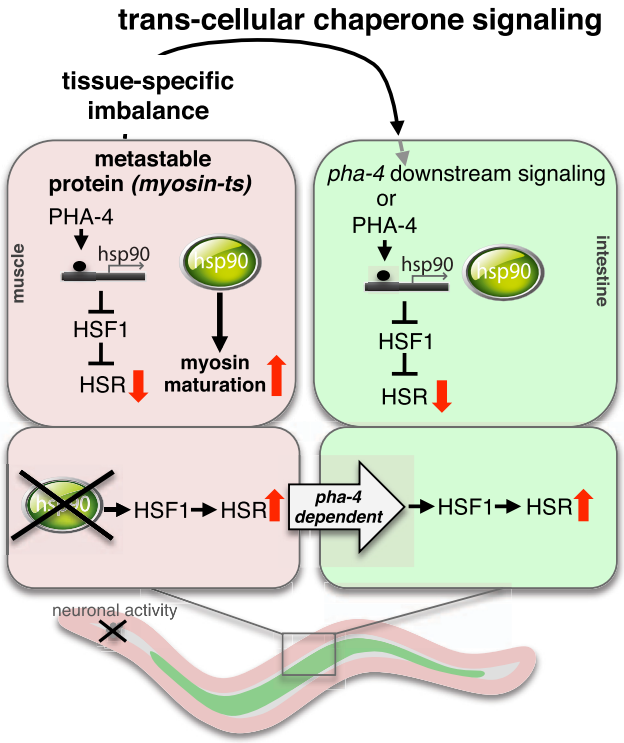

Figure 7. Model for the Cell-Nonautonomous Regulation of *HSP90* Expression in *C. elegans*

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 transcellular chaperone signaling to other tissues, independent of neural activity. Increased *pha-4* activity is required in the signaling and receiving tissue, but *pha-4* may act from a distance to regulate gene expression in the receiving tissue via a downstream 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-nonautonomous repression of HSF-1 transcriptional activity. Likewise, a tissue-specific perturbation through reduced *hsp90* levels (bottom) 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.

PHA-4 for this intertissue 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 intertissue communication, we examined *pha-4* activity and expression levels in the *HSP90* overexpression lines. *Pha-4* activity was measured by examining the levels of the *pha-4*-regulated *sod* genes (*sod-1*, *sod-2*, *sod-4*, and *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 are consistent with the observation that functional PHA-4 is required for the systemic effects through transcellular chaperone signaling.

To understand how this intertissue signaling is regulated in the receiving tissue, we examined *pha-4* activity and expression levels in isolated intestinal cells of the *HSP90* overexpression lines (Figures 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 transcellular 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 transcellular 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 nonautonomous 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 as sentinels that disseminate local proteotoxic challenges to tissues within the organism to mount a protective response.

(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*). Scale bar, 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. *$p < 0.05$; **$p < 0.02$.
(F) *pha-4* mRNA expression levels in *eat-2* and *HSP90* overexpression lines are upreguated relative to the wild-type control. *$p < 0.05$.
(G) *pha-4* activity in intestinal cells of *eat-2(ad1113)*, $HSP90^{bwm}$, $HSP90^{int}$, and $HSP90^{neuro}$ animals relative to wild-type. **$p < 0.02$.
(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). *$p < 0.05$. n.s., not significant.
All bar graphs represent combined mean values of three independent experiments (three biological replicates). Error bars represent ±SEM. See also Figure S6.

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 predispositions to 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 transcellular chaperone signaling. Indeed, increased expression of *HSP90* at the organismal level is beneficial for the folding of myosin *(ts)* mutants under mild temperature stress (Figures S6B and 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 an HSR in multiple tissues upon exposure to severe heat shock, thus affecting organismal survival (Figures S6C and 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-nonautonomous 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 transcellular 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 intertissue response functions at low basal levels in wild-type animals and becomes activated in response to tissue-specific perturbations to restore organismal proteostasis.

Transcellular 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 thermosensory AFD neuron to coordinate the regulation of HSF-1 activity in nonneuronal tissue (Prahlad et al., 2008). Yet, the two forms of cell-nonautonomous regulation complement to provide a protective mechanism for the unique proteomes expressed in different cells and tissues. Such modulation of proteostasis between nonneuronal tissues in *C. elegans* could similarly be achieved via exchange of small signaling molecules such as metabolites, reactive oxygen species, 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 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-nonautonomous 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, 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 mechanism of how tissues within an organism respond to disturbances of proteostasis to regulate a cell-nonautonomous 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 transcellular 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 previously (Prahlad et al., 2008). Animals were heat shocked by sealing plates with parafilm and Ziploc bags and immersing into a water bath equilibrated at 33°C for 1 hr or at 34°C for 30 min and allowed to recover for 1 hr at 20°C before they were harvested for quantitative RT-PCR (qRT-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). Five samples, each consisting of 20 adult animals, were used for one time point, and the experiment was repeated at least three times (three 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 hr and 10 hr) and animals were allowed to recover for 2 hr 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 nematode growth medium (NGM) plates at 25°C and scored 12 hr later for touch-induced movement. For the survival assays of *unc-54(ts)* mutants, young adult animals were allowed to lay eggs at 25°C for 3 hr. After removal of the adults, plates were incubated at the restrictive temperature for 24–48 hr and then scored for surviving and moving progeny (n = 50). All

experiments were repeated three times (three 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, UK) as described 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.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and six figures and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2013.05.015.

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