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REVIEW

Transcellular chaperone signaling: an organismal strategy for integrated cell stress responses

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ABSTRACT

The ability of each cell within a metazoan to adapt to and survive environmental and physiological stress requires cellular stress-response mechanisms, such as the heat shock response (HSR). Recent advances reveal that cellular proteostasis and stress responses in metazoans are regulated by multiple layers of intercellular communication. This ensures that an imbalance of proteostasis that occurs within any single tissue 'at risk' is protected by a compensatory activation of a stress response in adjacent tissues that confers a community protective response. While each cell expresses the machinery for heat shock (HS) gene expression, the HSR is regulated cell non-autonomously in multicellular organisms, by neuronal signaling to the somatic tissues, and by transcellular chaperone signaling between somatic tissues and from somatic tissues to neurons. These cell non-autonomous processes ensure that the organismal HSR is orchestrated across multiple tissues and that transmission of stress signals between tissues can also override the neuronal control to reset cell- and tissue-specific proteostasis. Here, we discuss emerging concepts and insights into the complex cell non-autonomous mechanisms that control stress responses in metazoans and highlight the importance of intercellular communication for proteostasis maintenance in multicellular organisms.

KEY WORDS: *Caenorhabditis elegans*, Chaperones, Proteostasis, Stress response, Cell non-autonomous, Metazoans

Introduction

The maintenance of a highly functioning proteome is an ever-present challenge throughout the life of every cell and therefore crucial for its optimal function and cellular viability. Although the information required for the folding of a polypeptide into a functional 3-dimensional stable conformation is encoded in the primary sequence of the protein (Anfinsen, 1973), folding efficiency and stability are challenged by the appearance of multiple on- and off-pathway intermediates, and a crowded cellular environment (Ellis and Minton, 2006). Moreover, protein folding and stability are strongly influenced by mutations within the coding sequence, error-prone synthesis, and the compromising effects of acute and chronic cell stress conditions, aging, pathology and disease. Consequently, to prevent the mismanagement of the proteome, leading to misfolding and aggregation, every cell expresses a complex network of quality control mechanisms, the proteostasis network, for efficient protein synthesis, folding, trafficking, secretion and degradation (Balch et al., 2008).

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When challenged, the proteostasis network faces a daunting task to maintain protein quality control and a balanced proteome. Studies with unicellular organisms and isolated tissue cells in culture have shown that each cell can respond cell autonomously to stress conditions by activation of the heat shock response (HSR). For metazoans composed of tissues with thousands to trillions of cells, the question is whether cellular stress responses are coordinated across tissues through additional levels of regulation to maintain balance during development, adulthood and aging, and in response to metabolic and environmental stress. For example, if a single tissue or organ experiences a proteotoxic challenge, does this tissue respond alone and independent of neighboring tissues, or is the stress sensed by adjacent cells and tissues, leading to organism-wide consequences?

Recent advances on the regulation of cell stress responses at the organismal level now show that proteostasis maintenance of individual cells can be regulated by direct communication between different cell types, such as signaling from neurons to somatic tissues, between somatic tissues, and between somatic tissues to neurons (Garcia et al., 2007; Prahlad et al., 2008; Durieux et al., 2011; Sun et al., 2011; Qi et al., 2012; Taylor and Dillin, 2013; van Oosten-Hawle et al., 2013; Zhang et al., 2013). In this review, we will discuss recent findings and emerging concepts that focus on organismal stress responses between and across tissues.

Maintaining proteome integrity by cellular stress responses

The HSR

Chronic proteotoxic stress inevitably leads to the accumulation of misfolded or aggregated proteins that result in the induction of the HSR and elevated expression of heat shock (HS) proteins, many of which function as molecular chaperones. Increased levels of chaperones have been shown to be highly protective against a wide range of proteotoxic conditions by restoring the folding and function of regulatory proteins that are essential for the re-establishment of proteostasis and cellular function. This is primarily controlled by the master regulator of the HSR, which in eukaryotes is heat shock transcription factor HSF-1. HSF-1 functions as a rheostat for acute stress, to prevent cell death, and monitors chronic proteotoxic stress that affects fecundity and lifespan (Abravaya et al., 1991; Morley and Morimoto, 2004; Åkerfelt et al., 2010; Lindquist and Kelly, 2011; McMullen et al., 2012). HSF-1 is a highly conserved member of the HSF gene family that is constitutively expressed and negatively regulated in most cell types in the absence of stress (Åkerfelt et al., 2010). The activation of HSF-1 depends on many regulatory mechanisms including interaction with a multi-chaperone complex (Abravaya et al., 1992; Shi et al., 1998; Zou et al., 1998), post-translational modifications such as phosphorylation (Kline and Morimoto, 1997; Holmberg et al., 2001; Guettouche et al., 2005), sumolyation (Hietakangas et al., 2003; Anckar and Sistonen, 2007) and acetylation (Westerheide et al., 2009). These modifications influence the formation of HSF-1 trimers (Baler et al., 1993; Sarge

et al., 1993), nuclear localization, and high-affinity binding of HSF-1 to consensus HS elements in the promoter region of target genes. The HSF-1 activation and attenuation cycle is strongly dependent on the concentration of specific cytoplasmic chaperones. For example, high constitutive levels of the chaperone Hsp90 prevent HSF-1 trimerization from its inactive monomeric state (Ali et al., 1998; Zou et al., 1998), whereas HS results in dissociation of this complex and conversion of inert HSF-1 monomers to the high-affinity DNA-binding trimers. The duration and intensity of HSF-1 activation is therefore proportional to the expression of chaperones. In this negative feedback mechanism, elevated levels of chaperones attenuate HSF-1 activity (Shi et al., 1998), which together with acetylation in the HSF-1 DNA-binding domain (Westerheide et al., 2009) provides multiple regulatory steps for both transcriptional attenuation and release of HSF-1 from the promoter element of the HS genes.

Compartmental stress responses: the UPR^{ER} and the UPR^{mt}

The functional complement to the HSR in the organelles is the unfolded protein response (UPR) that serves in a similar role to prevent misfolding in the endoplasmic reticulum (UPR^{ER}) and in mitochondria (UPR^{mt}). The ER is the primary site for synthesis and modification of membrane and secretory pathway proteins. Activation of the UPR^{ER} is triggered when unfolded proteins accumulate in the ER and exceed the folding capacity of the lumen chaperones including BiP/GRP78, GRP94, calnexin, calreticulin and PDI. To compensate, the three canonical branches of the UPR^{ER} are induced by three distinct ER stress-response factors: IRE1, PERK and ATF6. Each of these ER stress-response factors activates a distinct downstream signal transduction pathway that increases the ER capacity to counteract protein misfolding (Ron and Walter, 2007). PERK phosphorylates translation initiation factor 2 to attenuate protein synthesis, leading to reduced flux of nascent chains in the ER during stress. Activated ATF6 translocates to the Golgi, where it forms an active transcription factor that enters the nucleus to upregulate the transcription of ER chaperone proteins. IRE1 activates the transcription factor XBP-1 through splicing, which transcribes genes involved in ER homeostasis, export and degradation of misfolded proteins (Schröder and Kaufman, 2005; Ron and Walter, 2007). Interestingly, genes upregulated by the HSF-1-dependent HSR can overlap with some UPR targets and have been suggested to crosstalk during stress to promote cellular survival (Liu and Chang, 2008).

Analogous to the HSR and the UPR^{ER}, the UPR^{mt} senses and responds to the level of damaged proteins that accumulate in the mitochondrial matrix. Misfolded proteins are degraded by the matrix protease ClpXP, and are transported to the cytosol by the peptide transporter HAF1, which in turn activates the transcription factor ATFS-1 (ZC367.7) to translocate into the nucleus and induce the transcription of mitochondrial chaperones (Haynes and Ron, 2010; Nargund et al., 2012).

Although these cellular stress responses have been thought to be induced in a strict cell-autonomous manner, recent advances now demonstrate that the cytoplasmic HSR, the UPR^{ER} and the UPR^{mt} are all regulated by cell non-autonomous control in *Caenorhabditis elegans*, as will be discussed in the following sections.

Perceiving environmental stress: regulation of proteostasis via neuronal signaling

In metazoans, stress-responsive signaling is a combination of cell-autonomous and non-autonomous events that ensures cellular health across all tissues for overall organismal survival. Among these signaling pathways are neuroendocrine pathways, and neurosensory cues such as the insulin-like signaling (ILS) pathway in *C. elegans*, which influences longevity in response to different conditions (Cypser and Johnson, 2001; Alcedo and Kenyon, 2004; Baumeister et al., 2006). Although other neuroendocrine pathways such as the transforming growth factor-β (TGF-β) pathway and the nuclear hormone receptor (NR) pathway have the capability to modulate stress tolerance, the ILS pathway has gained most attention over the past years (Prahlad and Morimoto, 2009). High temperature, starvation and oxidative stress are conditions shown to inhibit this signaling cascade, leading to de-phosphorylation of the transcription factor DAF-16 and its subsequent translocation into the nucleus where it upregulates the transcription of heat shock- and other stress-inducible genes (Baumeister et al., 2006; Samuelson et al., 2007). Moreover, the ILS pathway not only requires DAF-16 but also depends on HSF-1 function (Morley and Morimoto, 2004). Interestingly, HSF-1 itself can be regulated by ILS via interaction with a DDL-1/DDL-2-containing protein complex (Hsu et al., 2003; Chiang et al., 2012).

Cell non-autonomous stress responses regulated by neurons in *C. elegans*

The biological detection of temperature relies on receptor proteins, represented by the transient receptor potential (TRP) channel protein family (Caterina et al., 1997; Clapham, 2003; Voets et al., 2004; Gallio et al., 2011), which directs the temperature signal to a signaling cue that initiates a cellular response. In *C. elegans*, TRP channels that respond to different environmental cues have been identified (Xiao and Shawn Xu, 2011). However, only the cold-sensitive TRPA-1 channel functions as a thermo-responsive TRP channel in *C. elegans* (Xiao et al., 2013).

Environmental changes in *C. elegans* are also perceived through specialized sensory neurons (Mori, 1999; de Bono and Maricq, 2005) and fluctuations of ambient temperature are sensed through two thermosensory AFD neurons (Mori et al., 2007). Input from the AFD neurons regulates temperature-dependent growth and behavior, and mutations in the AFD-specific receptor-type guanylyl cyclase *gcy-8* have been shown to disrupt thermotaxis behavior (Inada et al., 2006; Mori et al., 2007; Lee and Kenyon, 2009).

A link between thermosensory neuronal control and the HSR was demonstrated using mutations in *gcy-8* and other AFD-specific null mutations that were shown to be deficient in the HSF-1-dependent regulation of the HSR (Prahlad et al., 2008). When thermosensory *gcy-8* mutants were challenged by an acute HS treatment, the HSF-1-dependent HSR was blocked across multiple tissues of the animal, leading to reduced thermotolerance (Prahlad et al., 2008). Although it was shown previously that neuronal input at the synaptic junction regulates protein homeostasis in post-synaptic muscle cells of *C. elegans* (Garcia et al., 2007), the observation by Prahlad and colleagues provided the first evidence for a cell non-autonomous control of HSF-1 transcriptional activity through a neurosensory input. This ultimately led to the realization that stress responses and maintenance of proteostasis in multicellular organisms must be orchestrated cell non-autonomously through a centralized control. While the effector of AFD-dependent signaling in response to heat is HSF-1, the signaling pathway and molecules that transduce this signal remain to be fully identified (Fig. 1). Different neurosensory inputs induce different stress responses, because the HSF-1-dependent response to heavy metal stress, which is perceived through chemosensory neurons, remained stress responsive in AFD-deficient animals (Prahlad et al., 2008). Moreover, the AFD signal(s) appears to be critical for the response to acute heat stress but not for

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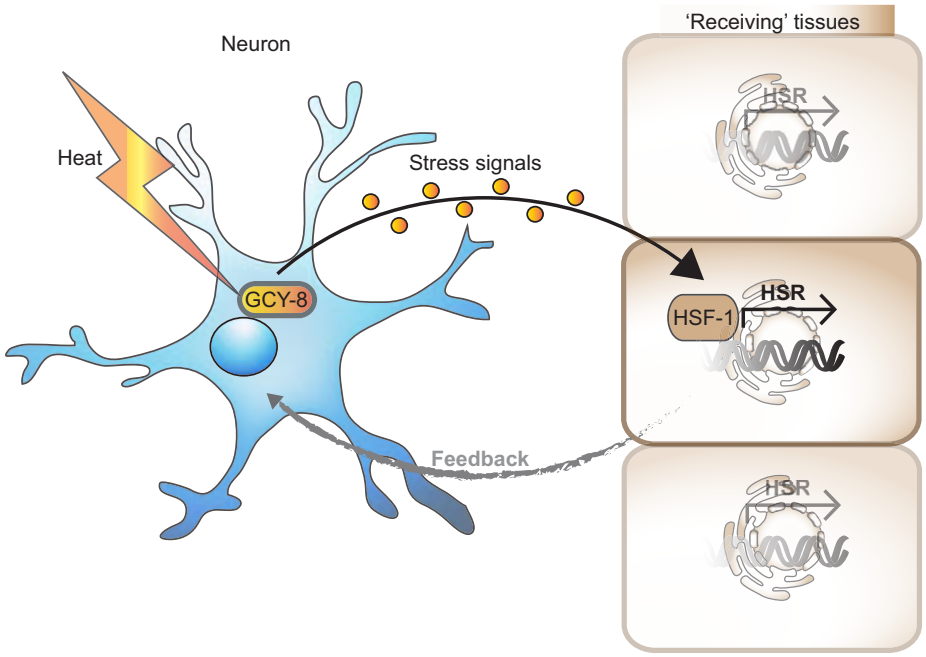

Fig. 1. Cell non-autonomous control of the heat shock response (HSR) by neurons. Organismal control of heat shock transcription factor 1 (HSF-1) transcriptional activity in peripheral tissues by the thermosensory AFD neuron via a guanylyl cyclase GCY-8-dependent signaling cascade. A steroid signalling-dependent feedback loop from peripheral cells reports HSR activity back to the AFD neurons.

chronic proteotoxic stress, as AFD-deficient animals could activate the HSR leading to suppression of protein aggregation in peripheral tissues (Prahlad and Morimoto, 2011). In this regard, it is of interest to note that AFD neurons also affect lifespan by controlling the nuclear hormone receptor DAF-12 through steroid signaling (Lee and Kenyon, 2009).

Remarkably, HSF-1 activity in peripheral tissues influences thermosensory neurons in a reverse feedback that alters thermotactic behavior in *C. elegans* (Fig. 1). This HSF-1-dependent signaling reports on temperature changes in non-neuronal cells to the nervous system, and is regulated by estrogen signaling through NHR-69 activity in AFD neurons (Sugi et al., 2011). The relationship between AFD-dependent coordination of the HSR in peripheral cells and AFD-dependent endocrine signaling involved in lifespan regulation (Lee and Kenyon, 2009) or thermotaxis (Sugi et al., 2011) remains to be established.

A comparable feedback loop exists in *Drosophila* where somatic tissues report transcriptional changes to neurons: transgenic FOXO activity in muscle cells leads to reduced insulin expression and secretion from neurosecretory cells, resulting in decreased feeding behavior. This feedback mechanism from muscle tissues to neurons in *Drosophila* involved the systemic activation of FOXO through the ILS pathway, and resulted in enhanced organismal proteostasis and lifespan (Hwangbo et al., 2004; Demontis and Perrimon, 2010). Thus, activation of the neuronal circuitry through environmental signals and intrinsic physiological signals provides a feedback on proteostasis perturbations in specific cells.

Additional support for the role of the nervous system in the response to environmental stimuli to maintain organismal homeostasis was provided in studies that identified a neural circuit controlling innate immunity in *C. elegans* via the G-protein-coupled receptor (GPCR) OCTR-1, expressed in ASH and ASI sensory neurons (Styer et al., 2008; Sun et al., 2011; Sun et al., 2012). OCTR-1 was shown to control the innate immune response to bacterial infections by negative regulation of *pqn/abu* genes in peripheral, non-neuronal tissue (Sun et al., 2011). This gene family is part of the non-canonical UPR pathway and is transcriptionally regulated by the apoptotic phagocytosis receptor CED-1. Whereas the OCTR-1-expressing sensory neurons regulate the non-canonical UPR during development, the classical IRE1/XBP1 UPR pathway is controlled by the same set of neurons in adult animals (Sun et al., 2012). Consistent with this, regulation of one of the canonical branches of the UPR^{ER} affects organismal stress signaling in *C. elegans* (Taylor and Dillin, 2013). In this study, a constitutively activated form of the UPR^{ER} stress factor XBP-1 (*xbp-1s*) in neurons was capable of restoring ER stress resistance in aged animals as well as extending lifespan. Neuronal *xbp-1s* expression also activated the UPR^{ER} in the intestine, which was dependent on the presence of functional *ire-1* and *xbp-1* in the receiving cells. These observations have led the authors to postulate that transmission of the stress signal from the neurons to the intestine may be achieved via an as-yet unidentified neurotransmitter (Taylor and Dillin, 2013).

Another example of cell non-autonomous regulation of stress responses in *C. elegans* is the UPR^{mt} (Durieux et al., 2011). Neuron-specific knockdown of *cco-1*, a component of the electron transport chain, activates the UPR^{mt} in peripheral tissues and thereby influences organismal survival. Although the mechanism by which the stress signal is transmitted in a cell non-autonomous manner is unclear, Durieux and colleagues proposed the existence of 'mitokines', signals that are generated in cells experiencing mitochondrial stress and that can be perceived by the entire animal (Durieux et al., 2011).

Proteostasis 'crosstalk' between somatic tissues

Neuronal signaling provides a rapid way to communicate vital information on cellular processes over long distances in multicellular organisms. From an evolutionary perspective, intercellular communication was also important to multicellular societies of unicellular organisms (Shapiro, 1998) in the absence of a complex centralized cellular control. Indeed, the ability of a cell to communicate with neighboring cells and sense their local microenvironment likely formed the basis for coordinated cellular activity in multicellular organisms. These include cell-to-cell communication methods in animal cells including cell junctions, adhesion contacts for soluble messengers, as well as the horizontal transfer of secreted microRNAs (miRNAs) packaged into exosomes

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or the exchange of cytoplasmic material via tunneling nanotubes (TNTs) (Singer, 1992; Denef, 2008; Kjenseth et al., 2010).

A recent observation in *C. elegans* reveals that non-neuronal regulated cell-to-cell communication, transcellular chaperone signaling, is essential for organismal proteostasis to ensure that stressed cells do not compromise the overall health of the organism (van Oosten-Hawle et al., 2013). Thus, an imbalanced proteostasis network within a single tissue not only triggers a cell-autonomous protective response but also induces increased chaperone expression in adjacent (and distant) tissues to protect the organismal proteome. By this novel form of stress signaling, expression of a metastable myosin expressed in muscle leads to upregulation of muscle-autonomous *hsp90* and a PHA-4-dependent transcriptional feedback that equalizes *hsp90* expression among different tissues. This coordination among multiple tissues to provide an organism-level protection suggests that different cell types can function as sentinels to signal local proteotoxic challenges throughout the organism. How this integrative transcellular signaling affects the entire organismal response to environmental challenges is shown by imbalanced tissue-specific chaperone expression: while increased *hsp90* expression can be beneficial for metastable proteins requiring a higher concentration of this chaperone, more severe environmental challenges could lead to a detrimental outcome due to repression of HSF-1-dependent HS proteins (van Oosten-Hawle et al., 2013) (Fig. 2).

Transcellular chaperone signaling, therefore, complements the neuronal control of the HSR, and peripheral tissues predisposed to transcellular chaperone signaling can override the neuronal influence leading to different outcomes for organismal survival. Even though the pathways that regulate transcellular chaperone signaling remain to be identified, the FoxA transcription factor PHA-4 appears to have a central role as an effector in stress signaling. PHA-4 activity is increased in the 'donor tissue', harboring an imbalanced proteostasis network, which influences PHA-4 activity in the 'receiving tissues', possibly via a signaling cascade or signaling molecule released from the donor tissue (van Oosten-Hawle et al., 2013).

Together with observations on cell non-autonomous control of proteostasis via neuronal pathways, these studies reveal the importance for an organismal level of control. Thus, the proteostasis condition in one tissue can influence the proteostasis network in other tissues, and this cell non-autonomous process requires the exchange of signals that regulate the proteostasis network in the receiving tissues. Therefore, transcellular chaperone signaling complements the different neuronal cues that regulate organismal proteostasis, by providing an independent pathway to monitor the proteostasis condition between somatic tissues (Fig. 3).

Transfer of the stress signal: modes of intercellular communication in metazoans

An important question brought forth by the discovery of the neuronal control of stress responses and transcellular chaperone signaling is: what is the identity of the intercellular stress signal(s) and how is it transduced to restore proteostasis balance? Below, we consider select examples of intercellular communication mechanisms that may be used in the regulation of cell non-autonomous proteostasis in metazoans.

Humoral control, gap junctions and nanotubes

Different specialized structures have emerged throughout evolution to provide cell-to-cell communication via direct contact, such as septal pores in fungi, plasmadesmata in plants and gap junctions in

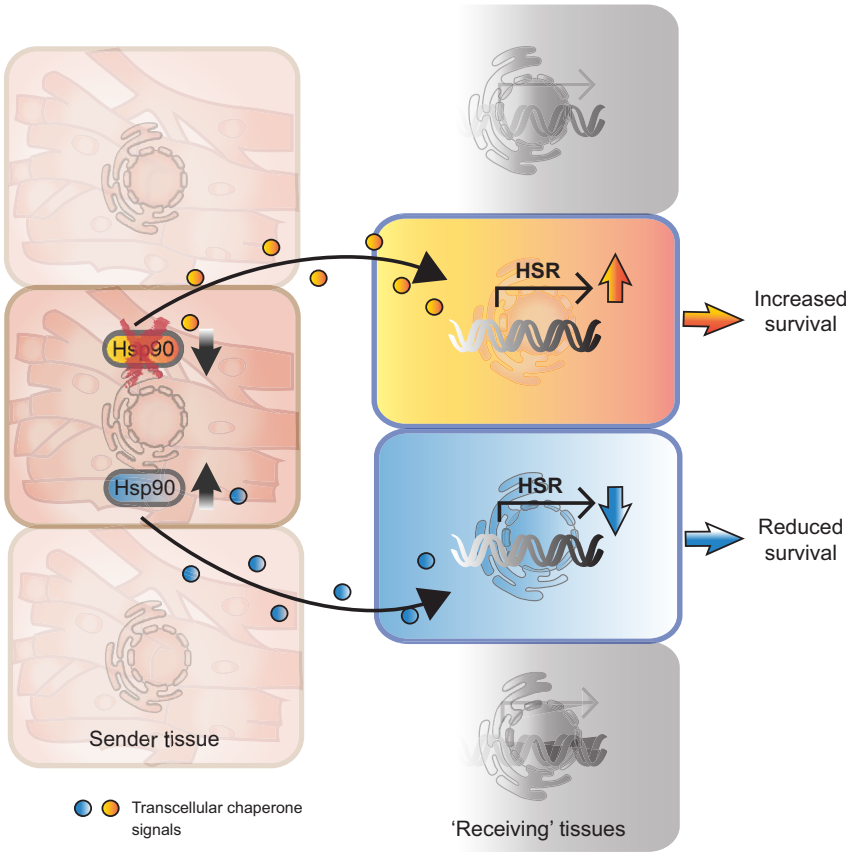

Fig. 2. Transcellular chaperone signaling regulates the organismal HSR via tissue-to-tissue crosstalk. An imbalance of proteostasis in a single tissue, through reduced (orange) or elevated (blue) expression of Hsp90, is detected and responded to in a different tissue via transcellular chaperone signaling. Because of the key role of Hsp90 as a negative regulator of the HSF-1-dependent HSR, the HSR is either induced (orange) or repressed (blue) at a cell non-autonomous level, leading to different outcomes for organismal survival during stress conditions.

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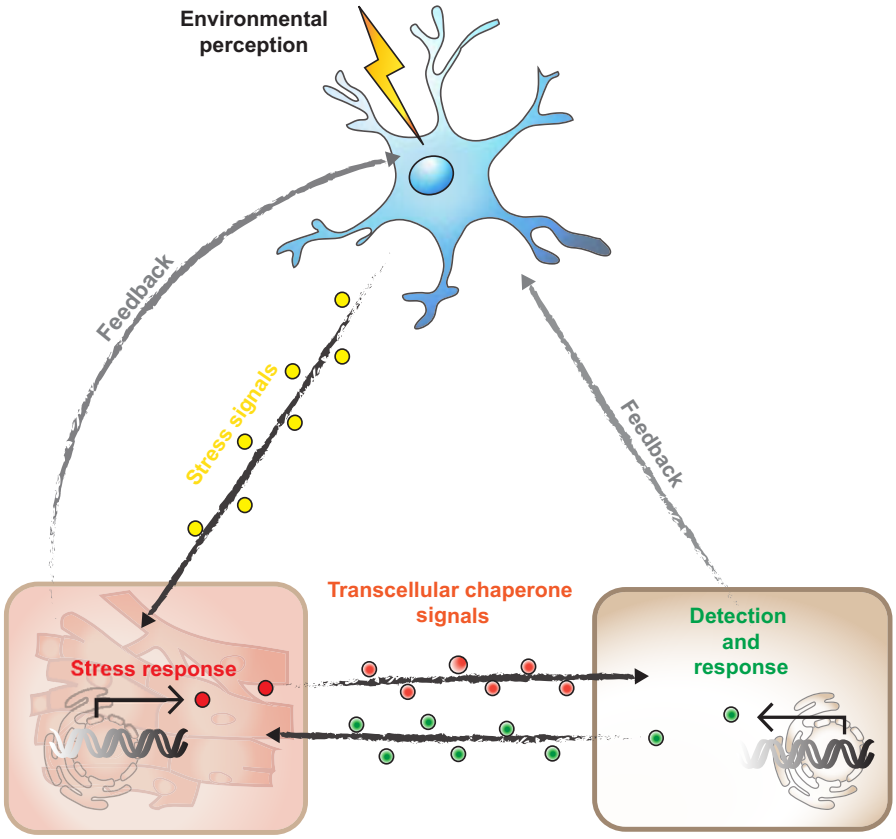

Fig. 3. Cell non-autonomous control of proteostasis via the nervous system and transcellular chaperone signaling. Sensory neurons perceive environmental stimuli and integrate the environmental challenge to fine-tune proteostasis in peripheral tissues. Non-neuronal tissues report altered proteostasis conditions back to the nervous system. At the same time, tissue-to-tissue signals via transcellular chaperone signaling can override the neuronal component to control cell-specific proteostasis. This allows proteostasis crosstalk between somatic cells independent of neural control.

animals. While gap junctions provide portals for the direct exchange of cytoplasmic contents to efficiently propagate a signal from cell to cell, intracellular signals can also be transmitted through the extracellular space, relying on communication mechanisms such as autocrine or paracrine signaling through cytokines, ligand–receptor signaling across tight cell–cell junctions or the long-range transfer of other soluble messengers such as hormones or RNA molecules to distant target cells (Singer, 1992; Denef, 2008; Kjenseth et al., 2010). The exchange of molecules over longer distances between non-adjacent cells relies on intercellular membrane bridges that include TNTs and cytonemes (Sherer and Mothes, 2008). Moreover, recent evidence in metazoans showed that prions expressed in *C. elegans* can spread between tissues via autolysosomal vesicles and be released into the pseudocoelom by vesicular trafficking (Nussbaum-Krammer et al., 2013), thus providing another means for the transport of proteinacious factors between tissues.

Gap junctions have also gained attention for a phenomenon called the radiation-induced bystander effect, in which non-irradiated cells exhibit similar effects to irradiated cells as a result of signals received from nearby irradiated cells via gap junctions (Azzam et al., 2004; Azzam and Little, 2004; Mitchell et al., 2004b; Mitchell et al., 2004a). In this process, gap junctional intercellular communication may also be involved in a range of cellular stress responses including ionizing radiation and oxidative stress (Upham et al., 1998), UV radiation (Provost et al., 2003) and HS (Hamada et al., 2003). Thus, between cells in close proximity, gap junctions may have an important role in the exchange of signaling molecules for proteostasis communication. Although in *C. elegans* some gap junction homologues (called innexins) have been well studied, for example *unc-9* and *unc-7*, which are required for electrical coupling of body wall muscle cells (Liu et al., 2006), the function of many *C. elegans* innexins remains to be established (Altun et al., 2009).

The transport of signaling molecules such as RNA across tissues and cellular boundaries has been shown in multiple organisms, including nematodes and plants, and often relies on secretion into a blood-like system for systemic transport. In *C. elegans*, the pericellular system, including the pseudocoelom, may provide a means for systemic cell-to-cell communication, triggered by both the nervous system and non-neuronal ‘sender tissues’. The pseudocoelom, analogous to the blood circulatory system in larger metazoans, is a fluid-filled body cavity bathing all tissues with nutrients, ions and oxygen, and may also function in intercellular signaling. Indeed, signaling via the pseudocoelom is often accomplished through hormones. For example, neuropeptides such as serotonin released from neurons into the pseudocoelom have been shown to act as signaling molecules to communicate with all tissues in an animal (Sieburth et al., 2007). Larger molecules can also be reliably transported through the pseudocoelom into other tissues, such as yolk lipoprotein particles that are synthesized in the intestine and secreted into the pseudocoelom. These particles are subsequently taken up into oocytes via RME-2 receptor-mediated endocytosis (Grant and Hirsh, 1999). Thus, the pseudocoelom could have a prominent role for signaling molecules secreted from non-neuronal tissues to other cells.

Moreover, the pseudocoelomic cavity in *C. elegans* is involved in distributing double-stranded (ds)RNA molecules throughout all tissues. In *C. elegans*, dsRNA-mediated gene interference (RNAi) systemically inhibits gene expression throughout the organism and, like in plants (Voinnet, 2001; Baulcombe, 2004), has a vital role in the immune response to foreign genetic material including viruses (Lu et al., 2005; Wilkins et al., 2005). Central for systemic distribution of dsRNA molecules in *C. elegans* is the transmembrane channel-forming protein SID-1, which mediates passive cellular uptake and cell-to-cell transfer (Feinberg and Hunter, 2003). Export

does not require this transmembrane channel (Jose et al., 2009), suggesting that dsRNA may be endocytosed into vesicles and released into the pseudocoelom where it can be taken up via SID-1 through most tissues (Saleh et al., 2006; Jose et al., 2009). In *C. elegans*, as in plants and mammals, non-coding 22 nucleotide miRNAs have important gene-regulatory roles by targeting mRNAs for cleavage or translational repression (Lee et al., 1993; Saumet and Lecellier, 2006). miRNAs are involved in the regulation of development, particularly in the timing of morphogenesis (Carrington and Ambros, 2003), and are capable of regulating entire gene networks during development by modulating the expression of key regulatory transcription factors in plants (Jones-Rhoades et al., 2006). Several studies have highlighted a novel role for these molecules in intercellular communication. miRNAs can be secreted into the extracellular space, often packaged into cell-derived exosomes (Valadi et al., 2007), and circulate in human body fluid (Chim et al., 2008; Lawrie et al., 2008), which allows them to act at distant sites within an organism to regulate multiple target genes or signaling events in the recipient cells (Chen et al., 2012).

Implications for aging and disease

Aging cells and tissues accumulate misfolded and aggregated proteins as a consequence of a functional decline of the proteostasis network, leading to the development of protein-misfolding diseases such as Alzheimer's or Huntington's disease. Folding challenges that arise during aging or the onset of protein-misfolding diseases likely arise from the cell-type specific regulation of protein expression that results in distinct functional proteomes within an organism. This implies that distinct cells and tissues must be prepared to detect and resolve unique folding challenges that arise during development, and in response to stress and aging (Powers et al., 2009). Likewise, many pathologies and diseases are due to limitations of specific tissues, for example dictated by the 'selective' expression of metastable or misfolded proteins in the tissue where they cause the pathology (Lage et al., 2008). In particular, neurons are vulnerable to protein damage as demonstrated by the remarkable prevalence of protein-misfolding diseases (Lee et al., 2006; Drummond and Wilke, 2008). Prominent examples of this class of diseases include the neurodegenerative diseases, such as Huntington's disease, Parkinson's disease or Alzheimer's disease, myopathies, diabetes mellitus and many forms of cancer. For example, in Huntington's disease, the brain tissue of affected individuals harbors amyloid aggregates that contain mutant Huntingtin proteins and other cellular proteins, including chaperones (Orr and Zoghbi, 2007).

Important breakthroughs in understanding the cellular disease processes in the context of post-mitotic cells within an organism have come through the development of invertebrate models of neurodegenerative protein-misfolding diseases. Among these studies is the use of *C. elegans* as a model system for the expression of polyQ repeats fused to yellow fluorescent protein (YFP) in body wall muscle cells, as seen in CAG expansion disorders such as Huntington's disease, spinocerebellar ataxias and Kennedy's disease (Morley et al., 2002). In both *C. elegans* and *Drosophila* models, the expression of polyQ repeats of different lengths in neurons, intestine and muscle recapitulates many of the molecular and cellular events associated with Huntington's disease in humans (Orr and Zoghbi, 2007), including the formation of intracellular aggregates, and has revealed a clear relationship between polyQ length, aggregation and toxicity (Satyal et al., 2000; Morley et al., 2002; Brignull et al., 2006b). The aggregation-dependent toxicity associated with polyQ proteins affects the specific cell type in which the protein is expressed in *C. elegans*: while polyQ protein expression in muscle tissue causes muscle cell dysfunction, expression in neuronal cells causes neural dysfunction (Satyal et al., 2000; Brignull et al., 2006a). While this disruption of proteostasis seems to cause strict cell-autonomous effects and the misfolding of other co-expressed metastable proteins (Gidalevitz et al., 2006), some features of this response in *C. elegans* seem to be cell non-autonomous, as disease onset is age dependent and can be modulated by the neuroendocrine ILS pathway (Morley and Morimoto, 2004). Activation of DAF-16 or HSF-1 through the ILS pathway suppresses protein aggregation and toxicity (Morley et al., 2002; Cohen et al., 2006; Ben-Zvi et al., 2009; Cohen et al., 2009), by upregulation of chaperones and other transcriptional targets of DAF-16 and HSF-1 (Balch et al., 2008). Likewise, lifespan extension does not only rely on decreased ILS signaling originating from neural tissues as endocrine signals; signals originating from mouse adipose tissue can also coordinate ageing between cells and tissues (Arantes-Oliveira et al., 2002; Blüher et al., 2003). One such potential mediator of crosstalk between tissues in mammals is associated with the hormone-like protein Klotho, as overexpression of Klotho in certain tissues is sufficient to prolong lifespan in mice (Kurosu et al., 2005). Thus, the intersection of neuroendocrine signaling pathways and cell stress-protective responses reinforces the importance of complex signaling events between cells for the organismal regulation of proteostasis.

Another line of evidence that supports the view that proteostatic disruptions in specific tissues initiate a signal to which other distant tissues can respond comes from the observation that endodermal DAF-16 can suppress certain aspects of toxicity associated with the expression of Aβ in *C. elegans* muscle cells (Zhang et al., 2013). Likewise, an imbalance of proteostasis through aggregation-prone proteins in non-neuronal tissue may influence proteostasis conditions in other tissues (Demontis and Perrimon, 2010). This also implies that different tissues may engage a communication system to sense proteotoxic stress occurring in distal cell types. Indeed, *C. elegans* seems to respond to tissue-specific imbalances of proteostasis even before the disease becomes prevalent and toxic, as demonstrated by the expression of a metastable muscle protein that induces a systemic upregulation of Hsp90 (van Oosten-Hawle et al., 2013). This response occurs before age- or temperature-dependent onset of misfolding takes place, indicating that even the slightest local imbalances are detected and responded to in a cell non-autonomous manner. Thus, transcellular chaperone signaling may function to sensitize the entire organism, and the increased expression of chaperones may serve to protect the entire organism against an oncoming challenge, such as one arising through aging or other proteotoxic stress stimuli (van Oosten-Hawle et al., 2013).

Thus, although most diseases are associated with specific tissues, the existence of a transcellular chaperone response as observed in *C. elegans* places multiple tissues or even the entire organism in a state of 'alert' to initiate protective responses when necessary. Whether organisms other than *C. elegans* employ transcellular chaperone signaling to communicate local proteotoxic stress remains to be established. Larger metazoans may communicate such stress events by a combination of direct signaling between cells and tissues and via lymphatic and circulatory systems to achieve events at a distance to coordinate cellular and organismal proteostasis.

Concluding remarks

The complexity of the regulation of proteostasis in metazoans provides multiple control points to ensure the robustness of a process that is crucial for viability of the entire organism. Clearly, cellular behavior in an organism must have evolved to benefit the

whole animal, rather than individual cells. Consistent with this notion, proteostasis maintenance in metazoans relies on multiple modes of cell non-autonomous mechanisms, including the integration of environmental signals through neuronal pathways and neuroendocrine signaling, as well as the emerging concept of transcellular chaperone signaling that integrates cell-specific and organismal stress responses independent of neural control. Transcellular chaperone signaling may rely on many different modes of intercellular communication that are protective to organismal lifespan on the one hand, and become pathological when mismanaged during disease on the other. Understanding how these concepts extend to larger metazoans together with the identification and comprehensive understanding of the underlying mechanisms will be instrumental for the development of novel approaches for the treatment of protein-misfolding diseases and other diseases associated with aging.

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The authors declare no competing financial interests.

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