
Article:
Jones, L, Chen, Y and van Oosten-Hawle, P orcid.org/0000-0002-5713-6457 (2020) Redefining proteostasis transcription factors in organismal stress responses, development, metabolism and health. Biological Chemistry. ISSN 1431-6730

https://doi.org/10.1515/hsz-2019-0385

Redefining Proteostasis Transcription Factors in Organismal Stress Responses, Development, Metabolism and Health

Laura Jones[1], Yannic Chen[1] and Patricija van Oosten-Hawle[1,2]

[1] School of Molecular and Cell Biology and Astbury Centre for Structural Molecular Biology; University of Leeds; Leeds, United Kingdom

[2] Lead Contact

[2]Correspondence to:

Dr. Patricija van Oosten-Hawle

School of Molecular and Cell Biology

Faculty of Biological Sciences

Garstang 8.53c

University of Leeds

LS2 9JT, Leeds

United Kingdom

Tel: +44 (0) 11334 30090

e-mail: p.vanoosten-hawle@leeds.ac.uk

Abstract

Eukaryotic organisms have evolved complex and robust cellular stress response pathways to ensure maintenance of proteostasis and survival during fluctuating environmental conditions. Highly conserved stress response pathways can be triggered and coordinated at the cell autonomous and cell-non-autonomous level by proteostasis transcription factors, including HSF1, SKN-1/Nrf2, HIF1 and DAF-16/FOXO that combat proteotoxic stress caused by environmental challenges. While these transcription factors are often associated with a specific stress condition, they also direct "non-canonical" transcriptional programmes that serve to integrate a multitude of physiological responses required for development, metabolism and defence responses to pathogen infections. In this review we outline the established function of these key proteostasis transcription factors at the cell-autonomous and cell-non-autonomous level and discuss a newly emerging stress responsive transcription factor, PQM-1, within the proteostasis network. We look beyond the canonical stress response roles of proteostasis transcription factors and highlight their function in integrating different physiological stimuli to maintain cytosolic organismal proteostasis.

Keywords: Proteostasis, stress responses, transcription factors, cell-non-autonomous, *C. elegans*, metabolism

How to maintain organismal balance: The role of the proteostasis network and its transcriptional regulators

Maintaining the integrity of the proteome in the face of proteotoxic challenges is crucial for cellular and organismal survival. This is achieved by a highly fine-tuned network of components comprising the proteostasis network (PN), which includes molecular chaperones, components of the degradation machinery, including the ubiquitin proteasome system (UPS) and autophagy, as well as stress-responsive transcriptional regulators (Hipp et al., 2019; Labbadia and Morimoto, 2015a). In eukaryotic cells, stress signalling pathways have evolved to detect protein folding stresses within different subcellular compartments. These are mediated by specific stress-responsive transcription factors that promote the expression of chaperones, detoxifying enzymes or components of the degradation machinery. Canonical cellular stress responses include the cytosolic heat shock response (HSR) mediated by heat shock transcription factor 1 (HSF1) (Lindquist, 1986), the oxidative stress response mediated via SKN-1/Nrf2 (An and Blackwell, 2003), the response to hypoxia, which is regulated by HIF1 (Semenza, 1998), and organelle-specific stress responses such as the unfolded protein response (UPR) of the endoplasmic reticulum (UPR^{ER})(Gardner et al., 2013) and mitochondria (UPR^{mt}) (Pellegrino et al., 2013). For the purpose of this review, we will focus on the stress transcription factors regulating cytosolic proteostasis.

At the organismal level, maintenance of proteostasis is required for stress resilience and relies on inter-tissue stress signalling pathways to integrate physiological signals dependent on developmental stage, aging, reproduction and perturbations caused by invading pathogens. This is often mediated by endocrine signals released from neurons or signals from the reproductive system

or intestine (Labbadia and Morimoto, 2015b; Prahlad et al., 2008; Shemesh et al., 2013; van Oosten-Hawle et al., 2013), that then converge on proteostasis transcription factors to regulate protein quality control processes within the organism. Thus, proteostasis transcription factors are needed to integrate multiple demands on the organism and hence their activation is required in distinct "non-canonical" manners tailored to specific tissues and different metabolic or developmental states (see Figure 1 and Table 1 for a summary).

Canonical and "non-canonical" functions of proteostasis transcription factors in response to cellular stress and physiological stimuli

HSF1 coordinates the cell-autonomous and cell-non-autonomous HSR

Heat shock factor 1 (HSF1) is the master regulator of the heat shock response (HSR) and promotes the expression of heat shock proteins (HSPs), that ensure the folding, refolding or degradation of damaged intracellular proteins (Anckar and Sistonen, 2011; Lindquist, 1986; Wu, 1995). HSF1 is widely conserved in eukaryotes and while its function has been extensively studied in the HSR, it also plays a pivotal role in development and longevity (Hsu et al., 2003; Li et al., 2016; Morley and Morimoto, 2004). Upon heat stress HSF1 dissociates from a multi-chaperone complex containing HSP70/ HSP90, trimerises and translocates to the nucleus (Zou et al., 1998). There, it binds to cis-acting heat shock elements (HSEs) that are composed of inverted nGAAn pentamers that reside within the promoter regions of heat shock proteins and molecular chaperones (Fernandes et al., 1994). HSF1 activity is regulated by an Hsp70-dependent chaperone titration mechanism (Masser et al., 2019) and by posttranslational modifications, including

phosphorylation, acetylation and sumoylation (Akerfelt et al., 2010; Li et al., 2017; Westerheide et al., 2009; Zou et al., 1998). For example, HSF1 can be activated by phosphorylation on Ser326 via MEK, a kinase of the RAS MAPK signalling pathway (Tang et al., 2015), whereas its activity can be attenuated by acetylation via p300/CBP in the absence of the lysine deacetylase SIRT1, resulting in the release of HSF1 from heat shock promoters (Raychaudhuri et al., 2014; Westerheide et al., 2009).

In metazoans, HSF1 activity has to be coordinated among different cell types and tissues to ascertain organismal proteostasis and protection against stress conditions. For example, in rats exposed to restraint stress, adrenocorticotropin is released from the hypothalamus to activate HSF1 in the adrenal gland and upregulate HSP70 expression (Fawcett et al., 1994). This early observation was however initially not recognised to be connected with organismal proteostasis. Later on, the roundworm *C. elegans* has been instrumental for the realisation that organismal stress responses such as the HSF-1 mediated HSR can be coordinated via neural networks: AFD thermosensory neurons and their postsynaptic AIY interneurons coordinate HSF-1 activity and chaperone expression in distant tissues in a serotonergic neurotransmitter signalling dependent manner (Prahlad et al., 2008; Tatum et al., 2015). Such cell nonautonomous regulation of the HSR via the nervous system can override the cell-autonomous response in an organism, in order to achieve a highly orchestrated response to heat stress in the many heterogenous cell-types of an entire animal (Prahlad et al., 2008). The associated cell nonautonomous upregulation of chaperones protects against acute stress conditions as well as protein aggregation of e.g. human disease proteins expressed in the worm (Tatum et al, 2015). At the same this coordination of HSF-1 activity across tissues needs to be tightly regulated, because HSF1 plays important roles in

developmental programmes, as shown in both invertebrates (Li et al., 2017) and mammals (Le Masson et al., 2011). Moreover, constitutive high expression of chaperones can be detrimental for organismal proteostasis, as this disrupts processes of cell division and growth (Feder et al., 1996). Another evidence for the tight regulation of HSF-1 activity, is its diminished ability to induce stress responses after the onset of reproductive maturity which continues throughout aging. This is regulated via germ cell signalling that results in the deposition of repressive chromatin marks at the promoters of heat shock genes, thereby reducing HSF-1 accessibility at these promoters (Labbadia and Morimoto, 2015b). Cell-non-autonomous HSF1 activity is also regulated by the mTOR pathway (Chou et al., 2012), insulin/IGF-1 signalling (Barna et al., 2012; Chiang et al., 2012) and cyclic guanosine monophosphate-mediated cGMP pathways (Barna et al., 2012), linking HSF1 with the metabolic state of the organism and development. Moreover, HSF1 can be activated by food availability and sensory perception of threats such as pathogens or temperature (Prahlad et al., 2008, Ooi et al., 2017, Sugi et al., 2011), which provides a direct link between the activation of stress responses with sensory input and behavioural output, that in turn has organismal consequences for development, metabolism and aging.

Non-canonical roles of HSF1 beyond the HSR

Although the master regulator of the cell's response to heat stress, several studies have revealed numerous non-canonical transcriptional responses regulated by HSF1 (see also Figure 1). For example, a genome-wide analysis in *C. elegans* has revealed that HSF-1 controls 74 % temperature sensitive genes, which not only include heat shock proteins but also genes involved in cuticle structure, translation, regulation of growth and amine catabolic processes as well as genes lacking

a HSE in their promoter and are involved in development, metabolism, and aging (Brunquell et al., 2016; Li et al., 2016). HSF1 function is also required to repress transcription of several gene classes following heat stress including those involved in protein kinase activity, ion binding, transcription, ATP binding and reproductive development (Brunquell et al., 2016). In this way, HSF1 may ensure that stress responses are correctly balanced with growth and developmental processes. Moreover, HSF1 promotes a transcriptional programme in cancer cells that is distinct from the classical heat shock response and involves genes regulating proliferation, invasion and migration as well as inflammatory responses (Mendillo et al., 2012; Scherz-Shouval et al., 2014) corroborating an evolutionary conserved role for "non-canonical" functions.

The role of HSF1 in longevity and stress resistance

In *C. elegans* HSF-1 is required for normal lifespan (Walker, 2003;Morley, 2004; Hsu, 2003) and stress resistance (Morley, 2004). HSF-1 is required for the enhanced longevity of the *daf-2*(-) mutant, which has reduced insulin/ IGF-1 signalling. In the *daf-2(-)* mutant HSF-1 regulates the transcription of several genes together with DAF-16, including sHSP genes (Hsu, 2003), *sip-1*, which encode the stress-induced protein and *cyp-35B1* (*dod-13*), which encodes a cytochrome P450 enzyme involved in lipid storage (Iser, 2011). HSF-1 also affects the expression of certain TGFβ pathway components including DAF-7/TGFβ and DAF-9/p450, which inhibit dauer larval development (Barna, 2012). This indicates that HSF-1 functions downstream of insulin/ IGF-1 signalling to regulate the aging process.

The role of HSF1 in the oxidative stress response

HSF1 also regulates the expression of HSP genes in response to oxidative stress in *C. elegans* (Park et al., 2009) and in yeast (Yamamoto, 2007), as well as mammalian cells. HSF1 can directly sense the cellular redox state through two cysteine residues (cys35 and cys105) in its DNA binding domain, and it was shown that the strong oxidizing agent hydrogen peroxide (H_2O_2) induces trimerization of HSF1 and transcriptional activity (Ahn and Thiele, 2003). HSF1 also shares several target genes with Nf E2-related factor 2 (NRF2), a key regulator of cellular response to oxidative stress (Park et al., 2009). This suggests that HSF1 and NRF2 cooperate with each other under certain conditions (Naidu et al., 2015).

The role of HSF1 in protein degradation pathways

Defects in the UPS can lead to HSF1 over-activation and increased HSP expression in mammalian cells (Barna et al., 2018). A ChIPseq analysis on human cell lines has also revealed that both HSF1 and its related isoform HSF2 are able to bind the regulatory region of the ubiquitin genes *UBB* and *UBC*, resulting in an upregulation of these transcripts (Vihervaara et al., 2013). Autophagy is also induced following heat shock in several mammalian cell lines and model organisms (Barna et al., 2018). HSF1 overexpression induces autophagic structures and expression of autophagy (*ATG*) genes, that is required for longevity and thermotolerance (Kumsta et al., 2017). Many of these *ATG* genes contain putative HSEs in their upstream regulatory sequence and HSF1 binding has been confirmed in the regulatory region of *ATG7* (Samarasinghe et al., 2014).

The role of HSF1 in the innate immune response

The production of large amounts of secreted innate immune peptides as part of the pathogen defence response exerts considerable protein folding stress and it is therefore unsurprising that HSF1 is required for normal immune function in both invertebrate and vertebrate systems (Barna et al., 2018). For example, in *C. elegans* HSF-1, HSP-90 and small heat shock proteins are required for tolerance against several bacterial pathogens, including *Pseudomonas aeruginosa* (Singh and Aballay, 2006). An increase in HSF1 activity and HSP expression has also been observed during acute viral infection in mammalian cells (Kumar and Mitra, 2005; Rawat and Mitra, 2011). The expression of certain cytokine or cytokine receptor-encoding genes, which promote fever and inflammation, can be repressed by HSF1 (Takii, 2010 #93). Reciprocally, cytokines including IL-1 may activate HSF1 during inflammation (Sasaki et al., 2002) and HSF1 can induce key regulators of inflammatory processes such as COX-2 (cyclooxygenase) (Rossi, 2012).

Furthermore, HSF-1 regulates the hypothalamic-specific expression of the heat-inducible ion channel transient receptor potential vanilloid gene, TRPV-1 (Meng et al., 2012). Since this protein plays a crucial role in regulation of body temperature, HSF1 has a protective response during fever: By inhibiting certain cytokines to reduce fever and to protect against chronic inflammation it can lower the rate of death caused by sepsis (Hotchkiss et al., 1993).

These observations suggest that HSF1 represents a major key node linking stress signalling with the innate immune and inflammatory response, as it either controls expression of certain cytokines or can be activated by some. Such paracrine signalling through cytokines may represent an important mechanism controlling organismal proteostasis (O'Brien et al., 2018), which has indeed

been shown to be an important aspect of HSF1 activation from cell to cell in the tumor microenvironment (Scherz-Shouval et al., 2014).

FOXO/ DAF-16: a transcription factor integrating diverse signals to maintain organismal proteostasis

The Forkhead family transcription factor, FOXO/DAF-16 is a central regulator for multiple signalling pathways and is required for normal life span and stress resistance in mice, flies and worms (Tatar, 2003; Alic, 2014). In addition to its function in metazoan stress resistance and longevity, FOXO/DAF-16 is known to respond to and to regulate several biological processes including metabolism, development, cell cycle, apoptosis and reproduction (Accili and Arden, 2004; Link and Fernandez-Marcos, 2017). FOXO/DAF-16 transcription factors recognise a core consensus sequence known as the DBE (DAF-16 Binding Element), that allows regulation of numerous downstream class 1 targets genes (Murphy et al., 2003). Prominent examples are *hsp-16. 1*, *hsp-16.49* and *hsp-12.6* (Schuster et al., 2010; Zhang et al., 2013) that are required for thermotolerance in *C. elegans* (Furuhashi and Sakamoto, 2014), and antioxidant genes *sod-3* and *ctl-1,* which are required for enhanced resistance of *daf-2* mutants to oxidative stress (Honda and Honda, 1999). Class 2 target genes contain DAE (Daf-16 associated element) in their promoters and are thought to be recognised by DAF-16 in conjunction with a multitude of co-transcription factors, including PQM-1 (Tepper et al., 2013) and HLH-30 (Lin et al., 2018). In vertebrates, FOXO upregulates genes that promote DNA repair and oxidative protection, whilst downregulating genes that promote cell-cycle progression (Tatar, 2003).

One of the most extensively studied functions of FOXO transcription factors is their role in aging and longevity. FOXO transcription factor activity mediates the lifespan extending effects of diminished insulin/IGF-1 signalling, a function that is conserved in yeast, worms, flies and mice (Tatar, 2003). Other signalling pathways regulating FOXO/DAF-16 activity and correlated with a specific role in longevity and metabolism are the TOR pathway that responds to nutrients and hormone-dependent mitogenic signals (Fontana et al., 2010; Vellai et al., 2003), the AMP-activated protein kinase (AMPK) pathway that responds to high AMP/ATP ratios (Apfeld et al., 2004; Greer et al., 2007). Moreover, the reproductive system communicates with other tissues through the germline to regulate aging (Kenyon, 2010), and in *C. elegans* was found to be dependent on the intestinal ankyrin repeat protein KRI-1 and microRNAs such as *mir-71* (Boulias and Horvitz, 2012). Thus, the FOXO/DAF-16 transcription factor integrates stimuli from signalling pathways that sense nutrients, cell proliferation and developmental status and so is involved in a multi-stress response that primes the stress response machinery to increase proteostasis and hence lifespan and stress resistance.

Roles of DAF-16/ FOXO beyond aging and longevity

DAF-16 in cancer and innate immunity

In addition to its canonical function in stress resistance and longevity, DAF-16/ FOXO is known to respond to and to regulate several biological processes including metabolism and cell cycle (Figure 1) (Link and Fernandez-Marcos, 2017). Several epidemiology studies have also recently

linked impaired insulin signalling and diabetes to several forms of cancer, although the underlying molecular mechanisms remain undefined (Link and Fernandez-Marcos, 2017).

In *C. elegans*, DAF-16 is also required for the innate immune response and survival against a range of bacterial pathogens (Figure 1), including the gram-negative bacterial pathogen *P. aeruginosa* and the gram-positive pathogens *Enterococcus faecalis* and *Staphylococcus aureus* (Garsin et al., 2003), through upregulating the expression of antimicrobial peptides *lys-7* and *clec-52* (Zhang et al., 2013). It is also required for resistance to infection with *E. faecalis* through direct induction of the antioxidant genes *sod-3* and *ctl-2*, in addition to indirect induction of SKN-1-regulated genes, which protect the tissues from the ensuing oxidative stress (Chavez et al., 2007).

FOXO-to-FOXO signalling

DAF-16 is able to regulate gene expression in a cell non-autonomous fashion (Murphy et al., 2007). For example, increasing *daf-16* gene dosage in the neurons or intestine upregulates DAF-16 activity in other tissues, a process called FOXO-to-FOXO signalling (Murphy et al., 2007). A related form of this signalling can also occur without DAF-16 expression in the receiving tissue and is termed FOXO-to-FOXO(-) signalling (Alic et al., 2014; Libina et al., 2003), demonstrated in both flies {Hwangbo, 2004 #255} and worms (Kaplan et al., 2019; Murphy et al., 2007; Zhang et al., 2013). In *C. elegans,* this signalling phenomena depends on the transcriptional co-regulator MDT-15 in the sending tissue (Zhang et al., 2013), which is also required for resistance to oxidative stress (Goh et al., 2014), normal lifespan (Schleit et al., 2011) and survival against *P. aeruginosa* (Pukkila-Worley et al., 2014).

Thus like HSF-1, DAF-16/FOXO appears to function as a transcription factor hub that receives input via developmental growth signals, nutrient availability as well as neuro-endocrine signalling to maintain organismal proteostasis.

PQM-1 in cellular stress responses: a novel proteostasis transcription factor

PQM-1 (Paraquat Mediator 1) is a zinc finger transcription factor in *C. elegans* that received its name from the observation that its transcripts are upregulated under oxidative stress as induced by paraquat exposure (Tawe et al., 1998). More recently it was demonstrated that PQM-1 is required for the enhanced thermotolerance, independent of HSF1, and maintenance of proteostasis during aging (O'Brien et al., 2018; Shpigel et al., 2019). A close but antagonistic relationship has been reported between PQM-1 and DAF-16, where they do not localize to the nucleus simultaneously and appear to be oppositely regulated by insulin-like signalling (Tepper et al., 2013). From analysis of global gene expression (RNAseq) and epigenomic (ChIPseq) data it has been suggested that PQM-1 binds the DAF-16 Associated Element (DAE) to promote regulation of genes that are usually downregulated by DAF-16 (Tepper et al., 2013). However, the requirement for *pqm-1* in the enhanced longevity and thermotolerance of *daf-2* mutants makes this relationship with *daf-16* unclear (Tepper et al., 2013). The importance for PQM-1 in organismal health and lifespan was highlighted in a recent report showing how DAF-16 and PQM-1 could contribute to organismal proteostasis during aging via gonadal longevity- and dietary restriction signalling pathways (Shpigel et al., 2019). The enhanced resistance to chronic stresses, such as prolonged thermal stress or increased age-associated protein aggregation, following dietary restriction requires PQM-1 in an *eat-2* signalling pathway dependent manner. Conversely, input from the gonadal longevity

signalling pathway via DAF-16/FOXO maintains proteostasis in response to acute stress (Shpigel et al., 2019). This implies that even though proteostasis collapses at the onset of reproductive adulthood, dietary signals and signals from the gonad can enhance and sustain the cellular and organismal ability to effectively respond to acute and chronic stresses, via DAF-16 or PQM-1.

Regulation of cell-non-autonomous proteostasis by PQM-1 – Transcellular Chaperone Signalling

As a transcription factor required for multiple stress stimuli in *C. elegans*, PQM-1 is also required for an organismal stress response mechanism called transcellular chaperone signalling (TCS), that is distinct from the heat shock response and other canonical cell stress responses (van Oosten-Hawle et al., 2013). It is activated upon direct tissue-specific perturbation of proteostasis, such as tissue-specific overexpression of the major molecular chaperone HSP-90, and activates the transcription factor PQM-1 in neurons and intestinal cells to mediate the cell-non-autonomous upregulation of HSP-90 in different tissues in *C. elegans* (O'Brien et al., 2018; van Oosten-Hawle et al., 2013). While PQM-1 is involved in regulating the cell-non-autonomous expression of Hsp90, it is not clear whether Hsp90 is a direct target of PQM-1 or indirectly regulated via a PQM-1-dependent signalling pathway (O'Brien et al., 2018). The current model suggests that transcellular upregulation of Hsp90 expression in the muscle is achieved via the innate immunity associated c-type lectin, CLEC-41, and aspartic protease ASP-12, that are both directly regulated by PQM-1 in response to TCS and predicted to be secreted from the gut (O'Brien et al., 2018). The *pqm-1* dependent upregulation of Hsp90 expression in the muscle was shown to reduce amyloid beta aggregation and associated toxicity in the muscle (O'Brien et al., 2018). Thus he enhanced ability of *C. elegans* to withstand chronic proteotoxic stress when PQM-1 is activated as

is the case during TCS, is in line with the finding that PQM-1 plays a more prevalent role in cellular maintenance and responses to chronic stress (Shpigel et al., 2019).

The role of PQM-1 as a mediator of TCS was uncovered by genetically inducing mild tissue-specific stress via tissue-specific overexpression of Hsp90. Although this is an "artificially" induced condition, it unveiled an important aspect of cell nonautonomous proteostasis: namely that the orchestration of stress responses within the heterogenous cell types of an organism can be disrupted by such tissue-specific stress. This may be a mechanism utilised by cancer cells, where Hsp90 is often overexpressed in a subset of cells (Ciocca et al., 2007). The increased expression of Hsp90 in cancer cells may induce TCS, which then "overrides" the cell nonautonomously orchestrated expression of Hsp90 itself and other components to promote pro-tumorigenic transcriptional programmes within a tumor microenvironment. Although this will require further investigation, a comparable transcellular activation of HSF-1 from one cell to another has been shown in cancer associated fibroblasts in the tumor microenvironment, where HSF-1 promotes transcriptional programmes that induce growth and malignancy in neighbouring cancer cells (Scherz-Shouval et al., 2014).

PQM-1 roles beyond stress responses

Development and vitellogenin-dependent fat mobilisation

Although the downstream transcriptional program of PQM-1 has not yet been fully characterised, accumulating evidence points to an important role in intestinal fat metabolism and the production of vitellogenin in *C. elegans* (Dowen et al., 2016). Vitellogenesis is modulated by a miRNA-

driven hypodermal timing pathway, the insulin and mTORC2 pathways that converge on the transcription factor PQM-1 (Dowen et al., 2016). Moreover, it has recently been suggested that PQM-1 acts as a transcriptional repressor in association with the homeobox transcription factor CEH-60 to balance stress responses and longevity against reproduction during developmental transitions (Dowen, 2019). In this role, PQM-1 together with CEH-60 represses defence response and innate immunity associated genes, and positively regulates gene networks involved in longevity and proteasome assembly (Dowen, 2019). Thus this places PQM-1 at a key node of signalling inputs that control development, metabolism, longevity, reproduction and proteostasis (Figure 1) (Dowen, 2019).

The role of PQM-1 in the immune response

In addition to its role in longevity and proteostasis, PQM-1 has also been implicated in the innate immune response since its transcript expression is upregulated under *P. aeruginosa* infection and it is required for survival under the same gram negative pathogen (Shapira et al., 2006). Increased transcript expression in *C. elegans* has also been found under exposure to other gram negative bacterial pathogens including *S. marcescens* and the gram positive *E. faecalis* (Engelmann et al., 2011). The antagonistic relationship between DAF-16 and PQM-1 is highlighted by the observation that genes negatively regulated by DAF-16 are downregulated during yeast infection (Pukkila-Worley et al., 2011) potentially pointing towards PQM-1 being functionally active as a repressor during this time. Furthermore, PQM-1 plays a role in the signalling of the immune response across tissues, as it is required for transcellular signalling of the heat shock chaperone protein HSP-90, which has been associated with innate immunity (O'Brien et al., 2018). Although

PQM-1 seems to act as a repressor for *clec-41* and other immune-related genes during development (Dowen, 2019), it is required for the upregulation of *clec-41* and *asp-12* during TCS as discussed above (O'Brien et al., 2018). It is likely that activation of PQM-1 in the intestine promotes PQM-1 dependent expression of HSP-90 and other immune effectors in distal tissues to orchestrate a tightly regulated response that balances proteostatic responses with growth, developmental and reproductive processes.

NRF2 in the oxidative stress response

The nuclear factor-erythroid 2 p45 (NRF2) is an evolutionarily conserved transcription factor, which confers protection against a wide spectrum of stressors, including oxidative and xenobiotic stress. Its major role is to protect against oxidative stress and promote longevity has been demonstrated in mice, flies and worms (An and Blackwell, 2003; Itoh et al., 1997; Sykiotis and Bohmann, 2008). Upon activation, NRF2 translocates into the nucleus and directly binds to antioxidant responsive elements (ARE) (Friling et al., 1990) upstream of genes encoding glutathione transferase enzymes (Rushmore and Pickett, 1990), which constitute the primary cellular defence against oxidative stress.

The *C. elegans* NRF2 orthologue, SKN-1 was originally identified in a screen for genes required for mesendodermal tissue differentiation and pharyngeal formation (Bowerman et al., 1992). It has since been found to regulate the expression of several antioxidants including glutathione transferases, superoxide dismutase and glutathione peroxidase (Park et al., 2009), required to

sequester ROS and to detoxify reactive intermediates that are generated when xenobiotics are metabolized by the cytochrome p450 (Phase I) enzymes (An and Blackwell, 2003). Since the generation of reactive oxygen species (ROS) is the mode of action of many toxic chemical stressors, NRF2 plays an important role in the defence against various chemical-derived stresses, and electrophilic compounds and regulates a range of responses involved in the cellular redox statues, including redox perturbation, inflammation and nutrient/energy fluxes (Fuse and Kobayashi, 2017; Hayes and Dinkova-Kostova, 2014). Furthermore, NRF2/ SKN-1 is able to regulate gene expression in a cell non-autonomous manner (Kim and Sieburth, 2018; Staab et al., 2014; Staab et al., 2013; VanDuyn et al., 2010). For example, the oxidative stress response can be induced in the intestine via neuropeptide signalling from the neurons (Kim and Sieburth, 2018), providing evidence that SKN-1, like HSF-1, is controlled by the nervous system for activation in distal cells. Further research will need to show whether the neuronally controlled cell nonautonomous oxidative stress response is also capable to override the autonomous SKN-1-mediated response and what the consequences are for development and aging of the organism.

Roles of SKN-1/NRF2 beyond regulating the "canonical" oxidative stress response

The role of SKN-1/NRF2 in longevity and cancer

SKN-1 is regulated by the ILS pathway, a major mechanism that promotes longevity and increased stress resistance in *C. elegans* (Blackwell et al., 2015) (Figure 1). Reduced insulin-like signalling promotes nuclear localisation of SKN-1/NRF2 and leads to the increased expression of stress response genes (Table 1) (Tullet et al., 2008). More recently the canonical role of SKN-1/NRF2

in oxidative stress has been dissociated from its role in longevity in *C. elegans* (Tullet et al., 2017): SKN-1 can be transcriptionally regulated via DAF-16 and while SKN-1 is required for the enhanced oxidative stress resistance resulting from DAF-16 over-expression, it is not required for enhanced longevity (Tullet et al., 2017).

In cancer cells, NRF2 is constitutively activated (Fuse and Kobayashi, 2017). The associated enhanced cellular proteostasis in cancer cells may be partly due to the NRF2-dependent induction of proteasome subunits (Kwak et al., 2003a; Kwak et al., 2003b), which destroy unfolded proteins accumulated in the cell. Another NRF2-activating signal is the disruption of autophagy (Komatsu et al., 2010), suggesting that the NRF2 system can be activated by disorders of protein turnover to protect against age-associated toxic protein misfolding. Because NRF2 increases the resistance of cancer cells to chemotherapy, possibly through their enhanced proteostasis capacity, it has recently attracted attention as a potential therapeutic target (Fuse and Kobayashi, 2017).

SKN-1/NRF2 in innate immunity

Production of ROS is also an important defence mechanism against invading pathogens in *C. elegans*, including *P. aeruginosa* and *E. faecalis* (King et al., 2018; Liu et al., 2019) and SKN-1 is highly active in the intestine upon exposure to the these pathogens (Naji et al., 2018).

In addition to regulating genes encoding phase II detoxifying enzymes, later studies have revealed that the NRF2 system also regulates phase III xenobiotic transporters (Hayashi et al., 2003; Vollrath et al., 2006) as well as phase I-related genes (Miao et al., 2005; Shin et al., 2007). This

shows that NRF2 is involved in the entire process of xenobiotic metabolism and may play an important role in metabolizing bacterial toxins.

Thus similar to the stress transcription factors mentioned above, SKN-1/NRF2 sits at an important hub that not only regulates canonical responses to oxidative stress, but also pathogen infection and conditions associated with increased oxidative challenge, to maintain cellular and organismal proteostasis.

HIF1 in the regulation of hypoxia

The ability to respond to changes in oxygen availability is essential. During low oxygen levels, hypoxia inducible factor, HIF1α binds to hypoxia response elements (HRE) in hypoxia regulated genes (Semenza and Wang, 1992) that are required for cell survival during culture in oxygen depleted media (Semenza, 1998). The mechanism of HIF1 activation is regulated directly via oxygen levels, whereby the presence of oxygen leads to the addition of hydroxyl groups to HIF1α via prolyl hydroxylases (Bruick and McKnight, 2001). This then leads to degradation of the transcription factor by the proteasome (Huang et al., 1998). Under low oxygen conditions, HIF1α is protected from degradation and accumulates in the nucleus to induce genes involved in metabolism, cell signalling, transcription and translation (Shen et al., 2005).

Physiologically, oxygen levels often vary spatially and temporally within the different tissues and organs of multicellular organisms and HIF1 is required to respond to these alterations in oxygen levels to maintain proteostasis. Since damaged or diseased cells and tissue are often associated with hypoxic conditions, HIF1 is emerging as an important transcription factor in immunity and

human pathologies including cancer (Gonzalez et al., 2018; Nizet and Johnson, 2009). The fact that HIF needs to regulate spatially separated and heterogenous cell types within multicellular organisms raises the questions of how and whether it could regulate hypoxia in a cell nonautonomous manner, similar to HSF1 or FOXO, via e.g. neuroendocrine signalling. Whether this is the case may need further investigation. However a recent report using *C. elegans* showed that HIF-1 activity can be induced by dietary restriction, and depends on the serotonin biosynthetic enzyme TPH-1 in the neurons and the serotonin receptor SER-7 in the intestine (Leiser et al., 2015).

HIF1 role in innate immunity and cancer

It is not clear whether HIF1 has "non-canonical" functions that deviate from its role during hypoxic conditions. At least in *C. elegans* HIF-1 is also required for longevity, thermotolerance and resistance to oxidative stress, in addition to its role in response to hypoxic conditions (Figure 1) (Zhang et al., 2009). The transcriptional program for HIF-1 during heat and oxidative stress has not yet been characterised, but alterations in cellular metabolism, transcription and translation benefit recovery from cellular stress (Jones and Candido, 1999).

Since inflamed, infected or damaged tissue is typically hypoxic, specialized immune cells such as neutrophils and macrophages need to be able to operate in this demanding environment (Nizet and Johnson, 2009). Increased levels of HIF are observed in macrophages or neutrophils that are stimulated by the gram-positive bacteria, *S. aureus* and gram-negative bacteria *S. typhimurium* and *P. aeruginosa*. Furthermore, macrophages from mice deficient in HIF1α are impaired in their

ability to kill bacteria (Nizet and Johnson, 2009). A close synergistic relationship between HIF and the transcription factor nuclear factor-κB (NF-κB), a central regulator of innate immunity in flies and vertebrates, has also been found (Nizet and Johnson, 2009). For example, HIF was shown to mediate NF-κB activation in anoxic neutrophils {Walmsley, 2005 #339} and promote cytokine expression in macrophages (Jeong, 2003.

Importantly, sustained expression of HIF1 is linked to pathophysiological aspects of many human diseases, including solid tumours from various tissues (Gonzalez et al., 2018). As a result of imbalance between oxygen demand and its supply to the tissue, tumour cells are typically hypoxic and the transcriptional program activated by hypoxia-inducible factor 1 (HIF1) is a major adaptive mechanism (Qiu et al., 2015; Zhou et al., 2006). For example, HIF1α critically regulates the switch to glycolysis by activating the transcription of genes encoding for glycolytic enzymes (Semenza, 2010). Because the ability to respond to changing oxygen levels is vital for the survival of cells, tissues and entire organisms throughout life, HIF is centrally placed at one of the most important physiological adaptation processes that are tightly linked with metabolic rate, immune response and many diseases. This uniquely places HIF in conjunction with HSF1, FOXO and NRF2 as a "stress-responsive" signalling hub to regulate development, metabolism and immunity at the cell autonomous as well as organismal level.

Conclusions and future directions

Appropriate regulation of proteostasis is fundamental for stress resistance, longevity and innate immunity of eukaryotic organisms. The role of the master transcriptional regulator HSF1, along

with SKN-1/NRF2, HIF1, DAF-16/FOXO and PQM-1 have been intensively characterised over the past years. As highlighted in this review, all of the proteostasis transcription factors are regulated by signalling cues distinct from the traditional stress response pathways. The challenge for future research will be to provide a comprehensive network of how input from a variety of physiological stimuli that is present in metazoans, can tailor the activity of these transcription factors to shape organismal proteostasis.

Figure legend

Figure 1. Signalling pathways controlling metabolism, development and aging are implicated in organismal proteostasis. HSF-1 can be triggered by heat stress, oxidative stress (from e.g. Reactive Oxygen Species (ROS)) and pathogen stress stress via the accumulation of misfolded proteins, and is also regulated by ILS, TOR and cGMP signalling pathways. DAF-16/FOXO integrates responses to a variety of physiological stimuli, including oxidative and heat stress, pathogens and JNK, ILS, TOR and AMPK signalling pathways. Oxidative stress can be triggered directly by ROS released from the mitochondria and during pathogen stress and is responded to by HIF-1, SKN-1, DAF-16, HSF-1 and PQM-1; with DAF-16 and SKN-1 directly involved in the synthesis of ROS scavenging enzymes. PQM-1 also integrates signals received as a response to heat stress, TOR and ILS pathways and developmental cues.

References

Accili, D., and Arden, K.C. (2004). FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. Cell *117*, 421-426.

Ahn, S.G., and Thiele, D.J. (2003). Redox regulation of mammalian heat shock factor 1 is essential for Hsp gene activation and protection from stress. Gene Dev *17*, 516-528.

Akerfelt, M., Morimoto, R.I., and Sistonen, L. (2010). Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Bio *11*, 545-555.

Aldridge, J.E., Horibe, T., and Hoogenraad, N.J. (2007). Discovery of Genes Activated by the Mitochondrial Unfolded Protein Response (mtUPR) and Cognate Promoter Elements. Plos One *2*.

Alic, N., Tullet, J.M., Niccoli, T., Broughton, S., Hoddinott, M.P., Slack, C., Gems, D., and Partridge, L. (2014). Cell-Nonautonomous Effects of dFOXO/DAF-16 in Aging. Cell Rep *6*, 608-616.

An, J.H., and Blackwell, T.K. (2003). SKN-1 links C. elegans mesendodermal specification to a conserved oxidative stress response. Genes Dev *17*, 1882-1893.

Anckar, J., and Sistonen, L. (2011). Regulation of HSF1 Function in the Heat Stress Response: Implications in Aging and Disease. Annual Review of Biochemistry, Vol 80 *80*, 1089-1115.

Apfeld, J., O'Connor, G., McDonagh, T., DiStefano, P.S., and Curtis, R. (2004). The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in C-elegans. Gene Dev *18*, 3004-3009.

Barna, J., Csermely, P., and Vellai, T. (2018). Roles of heat shock factor 1 beyond the heat shock response. Cell Mol Life Sci *75*, 2897-2916.

Barna, J., Princz, A., Kosztelnik, M., Hargitai, B., Takacs-Vellai, K., and Vellai, T. (2012). Heat shock factor-1 intertwines insulin/IGF-1, TGF-beta and cGMP signaling to control development and aging. Bmc Dev Biol *12*.

Berendzen, K.M., Durieux, J., Shao, L.W., Tian, Y., Kim, H.E., Wolff, S., Liu, Y., and Dillin, A. (2016). Neuroendocrine Coordination of Mitochondrial Stress Signaling and Proteostasis. Cell *166*, 1553-+.

Boulias, K., and Horvitz, H.R. (2012). The C. elegans MicroRNA mir-71 Acts in Neurons to Promote Germline-Mediated Longevity through Regulation of DAF-16/FOXO. Cell Metab *15*, 439-450.

Bowerman, B., Eaton, B.A., and Priess, J.R. (1992). skn-1, a maternally expressed gene required to specify the fate of ventral blastomeres in the early C. elegans embryo. Cell *68*, 1061-1075.

Brunquell, J., Morris, S., Lu, Y., Cheng, F., and Westerheide, S.D. (2016). The genome-wide role of HSF-1 in the regulation of gene expression in Caenorhabditis elegans. Bmc Genomics *17*.

Bruick, R.K., and McKnight, S.L. (2001). A conserved family of prolyl-4-hydroxylases that modify HIF. Science *294*, 1337-1340.

Chavez, V., Mohri-Shiomi, A., Maadani, A., Vega, L.A., and Garsin, D.A. (2007). Oxidative stress enzymes are required for DAF-16-mediated immunity due to generation of reactive oxygen species by Caenorhabditis elegans. Genetics *176*, 1567-1577.

Chiang, W.C., Ching, T.T., Lee, H.C., Mousigian, C., and Hsu, A.L. (2012). HSF-1 Regulators DDL-1/2 Link Insulin-like Signaling to Heat-Shock Responses and Modulation of Longevity. Cell *148*, 322-334.

Chou, S.D., Prince, T., Gong, J.L., and Calderwood, S.K. (2012). mTOR Is Essential for the Proteotoxic Stress Response, HSF1 Activation and Heat Shock Protein Synthesis. Plos One *7*.

Cullinan, S.B., and Diehl, J.A. (2004). PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. J Biol Chem *279*, 20108-20117.
Cullinan, S.B., Zhang, D., Hannink, M., Arvisais, E., Kaufman, R.J., and Diehl, J.A. (2003). Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. Mol Cell Biol *23*, 7198-7209.
Dowen, R.H. (2019). CEH-60/PBX and UNC-62/MEIS Coordinate a Metabolic Switch that Supports Reproduction in C. elegans. Developmental Cell *49*, 235-+.
Dowen, R.H., Breen, P.C., Tullius, T., Conery, A.L., and Ruvkun, G. (2016). A microRNA program in the C-elegans hypodermis couples to intestinal mTORC2/PQM-1 signaling to modulate fat transport. Gene Dev *30*, 1515-1528.
Engelmann, I., Griffon, A., Tichit, L., Montanana-Sanchis, F., Wang, G.L., Reinke, V., Waterston, R.H., Hillier, L.W., and Ewbank, J.J. (2011). A Comprehensive Analysis of Gene Expression Changes Provoked by Bacterial and Fungal Infection in C. elegans. Plos One *6*.
Evans, E.A., Chen, W.C., and Tan, M.W. (2008). The DAF-2 insulin-like signaling pathway independently regulates aging and immunity in C-elegans. Aging Cell *7*, 879-893.
Fawcett, T.W., Sylvester, S.L., Sarge, K.D., Morimoto, R.I., and Holbrook, N.J. (1994). Effects of Neurohormonal Stress and Aging on the Activation of Mammalian Heat-Shock Factor-1. J Biol Chem *269*, 32272-32278.
Feder ME, Cartaño NV, Milos L, Krebs RA, Lindquist SL.(1996) Effect of engineering Hsp70 copy number on Hsp70 expression and tolerance of relevant heat shock in larvae and pupae of Drosophila melanogaster. J Exp Biol. 1996 Aug;199(Pt 8):1837-44.
Fernandes, M., O'Brien, T., and Lis, J.T. (1994). Structure and regulation of heat shock gene promoters. In The biology of heat shock proteins and molecular chaperones, R.I. Morimoto, A. Tissieres, and C. Georgopolis, eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), pp. 375-393.
Fontana, L., Partridge, L., and Longo, V.D. (2010). Extending Healthy Life Span-From Yeast to Humans. Science *328*, 321-326.
Friling, R.S., Bensimon, A., Tichauer, Y., and Daniel, V. (1990). Xenobiotic-Inducible Expression of Murine Glutathione-S-Transferase Ya-Subunit Gene Is Controlled by an Electrophile-Responsive Element. P Natl Acad Sci USA *87*, 6258-6262.
Furuhashi, T., and Sakamoto, K. (2014). FoxO/Daf-16 restored thrashing movement reduced by heat stress in Caenorhabditis elegans. Comp Biochem Phys B *170*, 26-32.
Fuse, Y., and Kobayashi, M. (2017). Conservation of the Keap1-Nrf2 System: An Evolutionary Journey through Stressful Space and Time. Molecules *22*.
Gardner, B.M., Pincus, D., Gotthardt, K., Gallagher, C.M., and Walter, P. (2013). Endoplasmic Reticulum Stress Sensing in the Unfolded Protein Response. Csh Perspect Biol *5*.
Garsin, D.A., Villanueva, J.M., Begun, J., Kim, D.H., Sifri, C.D., Calderwood, S.B., Ruvkun, G., and Ausubel, F.M. (2003). Long-lived C-elegans daf-2 mutants are resistant to bacterial pathogens. Science *300*, 1921-1921.
Goh, G.Y.S., Martelli, K.L., Parhar, K.S., Kwong, A.W.L., Wong, M.A., Mah, A., Hou, N.S., and Taubert, S. (2014). The conserved Mediator subunit MDT-15 is required for oxidative stress responses in Caenorhabditis elegans. Aging Cell *13*, 70-79.
Gonzalez, F.J., Xie, C., and Jiang, C.T. (2018). The role of hypoxia-inducible factors in metabolic diseases. Nat Rev Endocrinol *15*, 21-32.

Greer, E.L., Dowlatshahi, D., Banko, M.R., Villen, J., Hoang, K., Blanchard, D., Gygi, S.P., and Brunet, A. (2007). An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in C-elegans. Curr Biol *17*, 1646-1656.
Gupta, S., Deepti, A., Deegan, S., Lisbona, F., Hetz, C., and Samali, A. (2010). HSP72 Protects Cells from ER Stress-induced Apoptosis via Enhancement of IRE1 alpha-XBP1 Signaling through a Physical Interaction. Plos Biol *8*.
Hayashi, A., Suzuki, H., Itoh, K., Yamamoto, M., and Sugiyama, Y. (2003). Transcription factor Nrf2 is required for the constitutive and inducible expression of multidrug resistance-associated protein 1 in mouse embryo fibroblasts. Biochem Bioph Res Co *310*, 824-829.
Hayes, J.D., and Dinkova-Kostova, A.T. (2014). The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci *39*, 199-218.
Haynes, C.M., Petrova, K., Benedetti, C., Yang, Y., and Ron, D. (2007). ClpP mediates activation of a mitochondrial unfolded protein response in C-elegans. Developmental Cell *13*, 467-480.
Haynes, C.M., Yang, Y., Blais, S.P., Neubert, T.A., and Ron, D. (2010). The Matrix Peptide Exporter HAF-1 Signals a Mitochondrial UPR by Activating the Transcription Factor ZC376.7 in C. elegans. Mol Cell *37*, 529-540.
Heldens, L., Hensen, S.M.M., Onnekink, C., van Genesen, S.T., Dirks, R.P., and Lubsen, N.H. (2011). An Atypical Unfolded Protein Response in Heat Shocked Cells. Plos One *6*.
Hipp, M.S., Kasturi, P., and Hartl, F.U. (2019). The proteostasis network and its decline in ageing. Nat Rev Mol Cell Bio *20*, 421-435.
Honda, Y., and Honda, S. (1999). The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in Caenorhabditis elegans. Faseb J *13*, 1385-1393.
Hotchkiss, R., Nunnally, I., Lindquist, S., Taulien, J., Perdrizet, G., and Karl, I. (1993). Hyperthermia Protects Mice against the Lethal Effects of Endotoxin. Am J Physiol *265*, R1447-R1457.
Hou, J., Tang, H.T., Liu, Z.H., Osterlund, T., Nielsen, J., and Petranovic, D. (2014). Management of the endoplasmic reticulum stress by activation of the heat shock response in yeast. Fems Yeast Res *14*, 481-494.
Huang, L.E., Gu, J., Schau, M., and Bunn, H.F. (1998). Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc Natl Acad Sci U S A *95*, 7987-7992.
Hsu, A.L., Murphy, C.T., and Kenyon, C. (2003). Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science *300*, 1142-1145.
Hwangbo, D.S., Gersham, B., Tu, M.P., Palmer, M., and Tatar, M. (2004). Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. Nature *429*, 562-566.
Imanikia, S., Ozbey, N.P., Krueger, C., Casanueva, M.O., and Taylor, R.C. (2019). Neuronal XBP-1 Activates Intestinal Lysosomes to Improve Proteostasis in C. elegans. Curr Biol *29*, 2322-+.
Iser, W.B., Wilson, M.A., Wood, W.H., Becker, K., and Wolkow, C.A. (2011). Co-Regulation of the DAF-16 Target Gene, cyp-35B1/dod-13, by HSF-1 in C. elegans Dauer Larvae and daf-2 Insulin Pathway Mutants. Plos One *6*.
Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K., Hatayama, I.*, et al.* (1997). An Nrf2 small Maf heterodimer mediates the induction of

phase II detoxifying enzyme genes through antioxidant response elements. Biochem Bioph Res Co *236*, 313-322.
Jones, D., and Candido, E.P.M. (1999). Feeding is inhibited by sublethal concentrations of toxicants and by heat stress in the nematode Caenorhabditis elegans: Relationship to the cellular stress response. J Exp Zool *284*, 147-157.
Kaplan, R.E.W., Maxwell, C.S., Codd, N.K., and Baugh, L.R. (2019). Pervasive Positive and Negative Feedback Regulation of Insulin-Like Signaling in Caenorhabditis elegans. Genetics *211*, 349-361.
Kenyon, C.J. (2010). The genetics of ageing. Nature *464*, 504-512.
Kim, S., and Sieburth, D. (2018). Sphingosine Kinase Regulates Neuropeptide Secretion During the Oxidative Stress-Response Through Intertissue Signaling. J Neurosci *38*, 8160-8176.
King, C.D., Singh, D., Holden, K., Govan, A.B., Keith, S.A., Ghazi, A., and Robinson, R.A.S. (2018). Proteomic identification of virulence-related factors in young and aging C. elegans infected with Pseudomonas aeruginosa. J Proteomics *181*, 92-103.
Komatsu, M., Kurokawa, H., Waguri, S., Taguchi, K., Kobayashi, A., Ichimura, Y., Sou, Y.S., Ueno, I., Sakamoto, A., Tong, K.I.*, et al.* (2010). The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. Nat Cell Biol *12*, 213-U217.
Kumar, M., and Mitra, D. (2005). Heat shock protein 40 is necessary for human immunodeficiency virus-1 Nef-mediated enhancement of viral gene expression and replication. J Biol Chem *280*, 40041-40050.
Kumsta, C., Chang, J.T., Schmalz, J., and Hansen, M. (2017). Hormetic heat stress and HSF-1 induce autophagy to improve survival and proteostasis in C. elegans. Nat Commun *8*.
Kwak, M.K., Wakabayashi, N., Greenlaw, J.L., Yamamoto, M., and Kensler, T.W. (2003a). Antioxidants enhance mammalian proteasome expression through the Keap1-Nrf2 signaling pathway. Mol Cell Biol *23*, 8786-8794.
Kwak, M.K., Wakabayashi, N., Itoh, K., Motohashi, H., Yamamoto, M., and Kensler, T.W. (2003b). Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway - Identification of novel gene clusters for cell survival. J Biol Chem *278*, 8135-8145.
Labbadia, J., and Morimoto, R.I. (2015a). The Biology of Proteostasis in Aging and Disease. Annu Rev Biochem *84*, 435-464.
Labbadia, J., and Morimoto, R.I. (2015b). Repression of the Heat Shock Response Is a Programmed Event at the Onset of Reproduction. Mol Cell *59*, 639-650.
Le Masson F, Razak Z, Kaigo M, Audouard C, Charry C, Cooke H, Westwood JT, Christians ES, (2011). Identification of heat shock factor 1 molecular and cellular targets during embryonic and adult female meiosis. 2011 Aug;31(16):3410-23.
Leiser, S.F., Miller, H., Rossner, R., Fletcher, M., Leonard, A., Primitivo, M., Rintala, N., Fresnida, J.R., Ramos, F.J., Miller, D.L.*, et al.* (2015). Cell Non-Autonomous Activation of Flavin-containing Monooxygenase Promotes Longevity and Healthspan. Science *350*, 1375-1378.
Li, J., Chauve, L., Phelps, G., Brielmann, R.M., and Morimoto, R.I. (2016). E2F coregulates an essential HSF developmental program that is distinct from the heat-shock response. Gene Dev *30*, 2062-2075.
Li, J., Labbadia, J., and Morimoto, R.I. (2017). Rethinking HSF1 in Stress, Development, and Organismal Health. Trends Cell Biol *27*, 895-905.

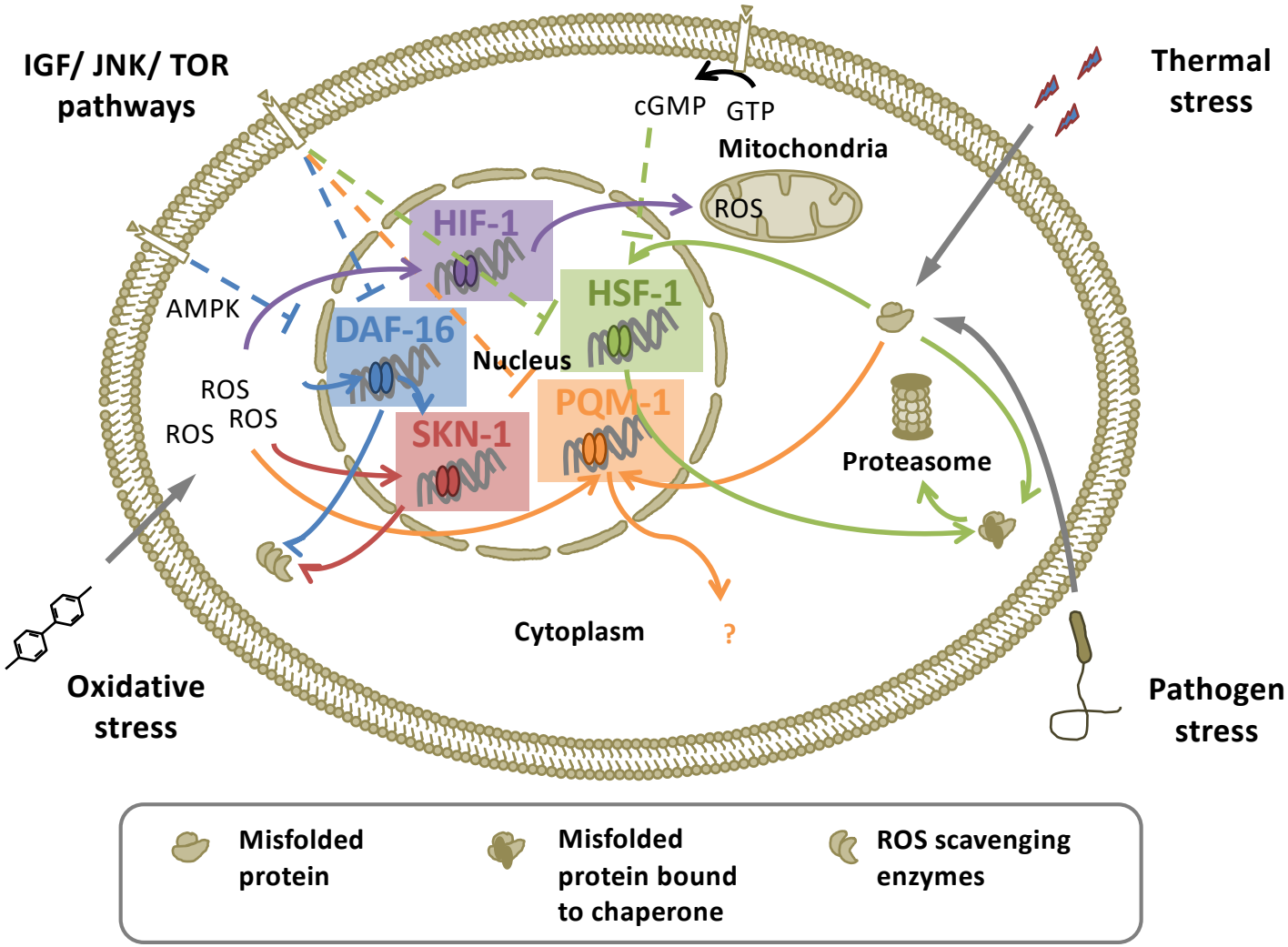
Libina, N., Berman, J.R., and Kenyon, C. (2003). Tissue-specific activities of C-elegans DAF-16 in the regulation of lifespan. Cell *115*, 489-502.
Lin, X.X., Sen, I., Janssens, G.E., Zhou, X., Fonslow, B.R., Edgar, D., Stroustrup, N., Swoboda, P., Yates, I.I.I., Ruvkun, G.*, et al.* (2018). DAF-16/FOXO and HLH-30/TFEB function as combinatorial transcription factors to promote stress resistance and longevity. Nat Commun *9*.
Lindquist, S. (1986). The Heat-Shock Response. Annual Review of Biochemistry *55*, 1151-1191.
Link, W., and Fernandez-Marcos, P.J. (2017). FOXO transcription factors at the interface of metabolism and cancer. Int J Cancer *141*, 2379-2391.
Liu, Y., and Chang, A. (2008). Heat shock response relieves ER stress. Embo J *27*, 1049-1059.
Liu, Y., Kaval, K.G., van Hoof, A., and Garsin, D.A. (2019). Heme peroxidase HPX-2 protects Caenorhabditis elegans from pathogens. PLoS Genet *15*, e1007944.
Masser, A.E., Kang, W., Roy, J., Kaimal, J.M., Quintana-Cordero, J., Friedländer, M.R., and Andréasson, C. (2019). Cytoplasmic protein misfolding titrates Hsp70 to activate nuclear Hsf1. Elife *8*.
Mendillo, M.L., Santagata, S., Koeva, M., Bell, G.W., Hu, R., Tamimi, R.M., Fraenkel, E., Ince, T.A., Whitesell, L., and Lindquist, S. (2012). HSF1 Drives a Transcriptional Program Distinct from Heat Shock to Support Highly Malignant Human Cancers. Cell *150*, 549-562.
Meng, F.X., Sun, L.M., Shi, B., Qin, X., Yang, Y., and Cao, Y. (2012). Heat shock factor 1 regulates the expression of the TRPV1 gene in the rat preoptic-anterior hypothalamus area during lipopolysaccharide-induced fever. Exp Physiol *97*, 730-740.
Miao, W.M., Hu, L.G., Scrivens, P.J., and Batist, G. (2005). Transcriptional regulation of NF-E2 p45-related factor (NRF2) expression by the aryl hydrocarbon receptor-xenobiotic response element signaling pathway - Direct cross-talk between phase I and II drug-metabolizing enzymes. J Biol Chem *280*, 20340-20348.
Mitsuishi, Y., Taguchi, K., Kawatani, Y., Shibata, T., Nukiwa, T., Aburatani, H., Yamamoto, M., and Motohashi, H. (2012). Nrf2 Redirects Glucose and Glutamine into Anabolic Pathways in Metabolic Reprogramming. Cancer Cell *22*, 66-79.
Morley, J.F., and Morimoto, R.I. (2004). Regulation of longevity in Caenorhabditis elegans by heat shock factor and molecular chaperones. Mol Biol Cell *15*, 657-664.
Murphy, C.T., Lee, S.J., and Kenyon, C. (2007). Tissue entrainment by feedback regulation of insulin gene expression in the endoderm of Caenorhabditis elegans. P Natl Acad Sci USA *104*, 19046-19050.
Murphy, C.T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Li, H., and Kenyon, C. (2003). Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature *424*, 277-284.
Naidu, S.D., Kostov, R.V., and Dinkova-Kostova, A.T. (2015). Transcription factors Hsf1 and Nrf2 engage in crosstalk for cytoprotection. Trends Pharmacol Sci *36*, 6-14.
Naji, A., Houston Iv, J., Skalley Rog, C., Al Hatem, A., Rizvi, S., and van der Hoeven, R. (2018). The activation of the oxidative stress response transcription factor SKN-1 in Caenorhabditis elegans by mitis group streptococci. PLoS One *13*, e0202233.
Nizet, V., and Johnson, R.S. (2009). Interdependence of hypoxic and innate immune responses. Nat Rev Immunol *9*, 609-617.
O'Brien, D., Jones, L.M., Good, S., Miles, J., Vijayabaskar, M.S., Aston, R., Smith, C.E., Westhead, D.R., and van Oosten-Hawle, P. (2018). A PQM-1-Mediated Response Triggers Transcellular Chaperone Signaling and Regulates Organismal Proteostasis. Cell Rep *23*, 3905-3919.

Oh, S.W., Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R.J., and Tissenbaum, H.A. (2005). JNK regulates lifespan in Caenorhabditis elegans by modulating nuclear translocation of forkhead transcription factor/DAF-16. P Natl Acad Sci USA *102*, 4494-4499.
Park, S.K., Tedesco, P.M., and Johnson, T.E. (2009). Oxidative stress and longevity in Caenorhabditis elegans as mediated by SKN-1. Aging Cell *8*, 258-269.
Pellegrino, M.W., Nargund, A.M., and Haynes, C.M. (2013). Signaling the mitochondrial unfolded protein response. Bba-Mol Cell Res *1833*, 410-416.
Prahlad, V., Cornelius, T., and Morimoto, R.I. (2008). Regulation of the cellular heat shock response in Caenorhabditis elegans by thermosensory neurons. Science *320*, 811-814.
Pukkila-Worley, R., Ausubel, F.M., and Mylonakis, E. (2011). Candida albicans Infection of Caenorhabditis elegans Induces Antifungal Immune Defenses. Plos Pathog *7*.
Pukkila-Worley, R., Feinbaum, R.L., McEwan, D.L., Conery, A.L., and Ausubel, F.M. (2014). The Evolutionarily Conserved Mediator Subunit MDT-15/MED15 Links Protective Innate Immune Responses and Xenobiotic Detoxification. Plos Pathog *10*.
Qiu, Y., Li, P., and Ji, C.Y. (2015). Cell Death Conversion under Hypoxic Condition in Tumor Development and Therapy. Int J Mol Sci *16*, 25536-25551.
Rawat, P., and Mitra, D. (2011). Cellular heat shock factor 1 positively regulates human immunodeficiency virus-1 gene expression and replication by two distinct pathways. Nucleic Acids Res *39*, 5879-5892.
Raychaudhuri, S., Loew, C., Korner, R., Pinkert, S., Theis, M., Hayer-Hartl, M., Buchholz, F., and Hartl, F.U. (2014). Interplay of Acetyltransferase EP300 and the Proteasome System in Regulating Heat Shock Transcription Factor 1. Cell *156*, 975-985.
Rossi, A., Coccia, M., Trotta, E., Angelini, M., and Santoro, M.G. (2012). Regulation of Cyclooxygenase-2 Expression by Heat: A Novel Aspect of Heat Shock Factor 1 Function in Human Cells. Plos One *7*.
Rushmore, T.H., and Pickett, C.B. (1990). Transcriptional Regulation of the Rat Glutathione S-Transferase-Ya Subunit Gene - Characterization of a Xenobiotic-Responsive Element Controlling Inducible Expression by Phenolic Antioxidants. J Biol Chem *265*, 14648-14653.
Sasaki, H., Sato, T., Yamauchi, N., Okamoto, T., Kobayashi, D., Iyama, S., Kato, J., Matsunaga, T., Takimoto, R., Takayama, T., Kogawa, T., Watanabe, N., and Niitsu, Y. (2002) Induction of heat shock protein 47 synthesis by TGF-b and IL-1b via enhancement of the heat shock element binding activity of heat shock transcription factor 1. J. Immunol. 168, 5178–5183
Samarasinghe, B., Wales, C.T.K., Taylor, F.R., and Jacobs, A.T. (2014). Heat shock factor 1 confers resistance to Hsp90 inhibitors through p62/SQSTM1 expression and promotion of autophagic flux. Biochem Pharmacol *87*, 445-455.
Scherz-Shouval, R., Santagata, S., Mendillo, M.L., Sholl, L.M., Ben-Aharon, I., Beck, A.H., Dias-Santagata, D., Koeva, M., Stemmer, S.M., Whitesell, L.*, et al.* (2014). The Reprogramming of Tumor Stroma by HSF1 Is a Potent Enabler of Malignancy. Cell *158*, 564-578.
Schleit, J., Wall, V.Z., Simko, M., and Kaeberlein, M. (2011). The MDT-15 Subunit of Mediator Interacts with Dietary Restriction to Modulate Longevity and Fluoranthene Toxicity in Caenorhabditis elegans. Plos One *6*.
Schuster, E., McElwee, J.J., Tullet, J.M.A., Doonan, R., Matthijssens, F., Reece-Hoyes, J.S., Hope, I.A., Vanfleteren, J.R., Thornton, J.M., and Gems, D. (2010). DamID in C-elegans reveals longevity-associated targets of DAF-16/FoxO. Mol Syst Biol *6*.

Semenza, G.L. (1998). Hypoxia-inducible factor 1: master regulator of O-2 homeostasis. Curr Opin Genet Dev *8*, 588-594.
Semenza, G.L. (2010). HIF-1: upstream and downstream of cancer metabolism. Curr Opin Genet Dev *20*, 51-56.
Shapira, M., Hamlin, B.J., Rong, J.M., Chen, K., Ronen, M., and Tan, M.W. (2006). A conserved role for a GATA transcription factor in regulating epithelial innate immune responses. P Natl Acad Sci USA *103*, 14086-14091.
Shemesh, N., Shai, N., and Ben-Zvi, A. (2013). Germline stem cell arrest inhibits the collapse of somatic proteostasis early in Caenorhabditis elegans adulthood. Aging Cell *12*, 814-822.
Shen, C., Nettleton, D., Jiang, M., Kim, S.K., and Powell-Coffman, J.A. (2005). Roles of the HIF-1 hypoxia-inducible factor during hypoxia response in Caenorhabditis elegans. J Biol Chem *280*, 20580-20588.
Shin, S., Wakabayashi, N., Misra, V., Biswal, S., Lee, G.H., Agoston, E.S., Yamamoto, M., and Kensler, T.W. (2007). NRF2 modulates aryl hydrocarbon receptor signaling: Influence on adipogenesis. Mol Cell Biol *27*, 7188-7197.
Shpigel, N., Shemesh, N., Kishner, M., and Ben-Zvi, A. (2019). Dietary restriction and gonadal signaling differentially regulate post-development quality control functions in Caenorhabditis elegans. Aging Cell *18*.
Singh, V., and Aballay, A. (2006). Heat-shock transcription factor (HSF)-1 pathway required for Caenorhabditis elegans immunity. P Natl Acad Sci USA *103*, 13092-13097.
Staab, T.A., Egrafov, O., Knowles, J.A., and Sieburth, D. (2014). Regulation of Synaptic nlg-1/Neuroligin Abundance by the skn-1/Nrf Stress Response Pathway Protects against Oxidative Stress. Plos Genetics *10*.
Staab, T.A., Griffen, T.C., Corcoran, C., Evgrafov, O., Knowles, J.A., and Sieburth, D. (2013). The Conserved SKN-1/Nrf2 Stress Response Pathway Regulates Synaptic Function in Caenorhabditis elegans. Plos Genetics *9*.
Sugi, T., Nishida, Y., and Mori, I. (2011). Regulation of behavioral plasticity by systemic temperature signaling in Caenorhabditis elegans. Nat Neurosci *14*, 984-U969.
Sykiotis, G.P., and Bohmann, D. (2008). Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in Drosophila. Dev Cell *14*, 76-85.
Takemori, Y., Sakaguchi, A., Matsuda, S., Mizukami, Y., and Sakurai, H. (2006). Stress-induced transcription of the endoplasmic reticulum oxidoreductin gene ERO1 in the yeast Saccharomyces cerevisiae. Mol Genet Genomics *275*, 89-96.
Takii, R., Inouye, S., Fujimoto, M., Nakamura, T., Shinkawa, T., Prakasam, R., Tan, K., Hayashida, N., Ichikawa, H., Hai, T.*, et al.* (2010). Heat Shock Transcription Factor 1 Inhibits Expression of IL-6 through Activating Transcription Factor 3. J Immunol *184*, 1041-1048.
Tang, Z.J., Dai, S.Y., He, Y.S., Doty, R.A., Shultz, L.D., Sampson, S.B., and Dai, C.K. (2015). MEK Guards Proteome Stability and Inhibits Tumor-Suppressive Amyloidogenesis via HSF1. Cell *160*, 729-744.
Tatar, M., Bartke, A., and Antebi, A. (2003). The endocrine regulation of aging by insulin-like signals. Science *299*, 1346-1351.
Tatum, M.C., Ooi, F.K., Chikka, M.R., Chauve, L., Martinez-Velazquez, L.A., Steinbusch, H.W.M., Morimoto, R.I., and Prahlad, V. (2015). Neuronal Serotonin Release Triggers the Heat Shock Response in C. elegans in the Absence of Temperature Increase. Curr Biol *25*, 163-174.

Tawe, W.N., Eschbach, M.L., Walter, R.D., and Henkle-Duhrsen, K. (1998). Identification of stress-responsive genes in Caenorhabditis elegans using RT-PCR differential display. Nucleic Acids Res *26*, 1621-1627.
Taylor, R.C., and Dillin, A. (2013). XBP-1 Is a Cell-Nonautonomous Regulator of Stress Resistance and Longevity. Cell *153*, 1435-1447.
Tepper, R.G., Ashraf, J., Kaletsky, R., Kleemann, G., Murphy, C.T., and Bussemaker, H.J. (2013). PQM-1 Complements DAF-16 as a Key Transcriptional Regulator of DAF-2-Mediated Development and Longevity. Cell *154*, 676-690.
Tullet JM, Hertweck M, An JH, Baker J, Hwang JY, Liu S, Oliveira RP, Baumeister R, Blackwell TK.Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in C. elegans. Cell. 2008 Mar 21;132(6):1025-38
Tullet, J.M.A., Green, J.W., Au, C., Benedetto, A., Thompson, M.A., Clark, E., Gilliat, A.F., Young, A., Schmeisser, K., and Gems, D. (2017). The SKN-1/Nrf2 transcription factor can protect against oxidative stress and increase lifespan in C. elegans by distinct mechanisms. Aging Cell *16*, 1191-1194.
van Oosten-Hawle, P., Porter, R.S., and Morimoto, R.I. (2013). Regulation of Organismal Proteostasis by Transcellular Chaperone Signaling. Cell *153*, 1366-1378.
VanDuyn, N., Settivari, R., Wong, G., and Nass, R. (2010). SKN-1/Nrf2 Inhibits Dopamine Neuron Degeneration in a Caenorhabditis elegans Model of Methylmercury Toxicity. Toxicol Sci *118*, 613-624.
Vellai, T., Takacs-Vellai, K., Zhang, Y., Kovacs, A.L., Orosz, L., and Muller, F. (2003). Genetics - Influence of TOR kinase on lifespan in C-elegans. Nature *426*, 620-620.
Vihervaara, A., Sergelius, C., Vasara, J., Blom, M.A.H., Elsing, A.N., Roos-Mattjus, P., and Sistonen, L. (2013). Transcriptional response to stress in the dynamic chromatin environment of cycling and mitotic cells. P Natl Acad Sci USA *110*, E3388-E3397.
Vollrath, V., Wielandt, A.M., Iruretagoyena, M., and Chianale, J. (2006). Role of Nrf2 in the regulation of the Mrp2 (ABCC2) gene. Biochem J *395*, 599-609.
Watanabe, Y., Tsujimura, A., Taguchi, K., and Tanaka, M. (2017). HSF1 stress response pathway regulates autophagy receptor SQSTM1/p62-associated proteostasis. Autophagy *13*, 133-148.
Weindling, E., and Bar-Nun, S. (2015). Sir2 links the unfolded protein response and the heat shock response in a stress response network. Biochem Bioph Res Co *457*, 473-478.
Westerheide, S.D., Anckar, J., Stevens, S.M., Sistonen, L., and Morimoto, R.I. (2009). Stress-Inducible Regulation of Heat Shock Factor 1 by the Deacetylase SIRT1. Science *323*, 1063-1066.
Wu, C. (1995). Heat shock transcription factors: Structure and regulation. Annu Rev Cell Dev Bi *11*, 441-469.
Yamamoto, A., Ueda, J., Yamamoto, N., Hashikawa, N., and Sakurai, H. (2007). Role of heat shock transcription factor in Saccharomyces cerevisiae oxidative stress response. Eukaryot Cell *6*, 1373-1379.
Zhang, P.C., Judy, M., Lee, S.J., and Kenyon, C. (2013). Direct and Indirect Gene Regulation by a Life-Extending FOXO Protein in C. elegans: Roles for GATA Factors and Lipid Gene Regulators. Cell Metab *17*, 85-100.
Zhang, Q., Wu, X.Y., Chen, P., Liu, L.M., Xin, N., Tian, Y., and Dillin, A. (2018). The Mitochondrial Unfolded Protein Response Is Mediated Cell-Non-autonomously by Retromer-Dependent Wnt Signaling. Cell *174*, 870-+.

Zhang, Y., Shao, Z.Y., Zhai, Z.W., Shen, C., and Powell-Coffman, J.A. (2009). The HIF-1 Hypoxia-Inducible Factor Modulates Lifespan in C. elegans. Plos One *4*.
Zhou, J., Schmid, T., Schnitzer, S., and Brune, B. (2006). Tumor hypoxia and cancer progression. Cancer Lett *237*, 10-21.
Zou, J.Y., Guo, Y.L., Guettouche, T., Smith, D.F., and Voellmy, R. (1998). Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. Cell *94*, 471-480.


IGF/ JNK/ TOR pathways
cGMP
GTP
Mitochondria
ROS
Thermal stress
HIF-1
HSF-1
AMPK
DAF-16
Nucleus
ROS
ROS
ROS
SKN-1
PQM-1
Proteasome
Cytoplasm
?
Oxidative stress
Pathogen stress
Misfolded protein
Misfolded protein bound to chaperone
ROS scavenging enzymes

	Target Genes related to proteostasis	Reference
HSF-1/ HSF1		
Heat shock response	hsp40, hsp70, hsp90 (yeast)	Samarasinghe et al. (2014)
	hsp-70, *-16.1,-16.49, -12.6, sip-1*, *F44E5.4; F44E5.5, hsp-90* (*C. elegans*)	Hsu et al. (2003)
Oxidative Stress	ero1, cup1, btn2, sis1, hsp10, sgt2, ssa3 (yeast)	Yamamoto et al. (2007)
	p62/SQSTM1 (yeast)	Samarasinghe et al. (2014)
Cellular Protein Recycling	ubb, ubc (human cells)	Vihervaara et al. (2013)
	atg7 (human cells)	Luo et al. (2016)
Longevity	*cyp-35b1* (*C. elegans*)	Iser et al. (2011)
	daf-7, *daf-9* (*C. elegans*)	Barna et al. (2012)
Innate Immunity	hsp-90, hsp-16 (*C. elegans*)	Singh and Aballay (2006)
	cox2 (human cells)	Rossi et al. (2012)
	trpv1 (rat)	Meng et al. (2012)
	atf3 (mouse)	Takii et al. (2010)
SKN-1/ NRF2		
Oxidative Stress Response	keap1 (*D. melanogaster*)	Sykiotis and Bohmann (2008)
	gcs-1 (*C. elegans*)	An and Blackwell (2003)
	ctl-2, *sod-1*, *gpx-7*, *gst-4*, *-10*, *-12*, *-13*, *14*, *-32*,*-38*, *-39* (*C. elegans*)	Park et al. (2009)
Xenobiotic stress	Mrp3 (Mrp4) (mouse)	Hayashi et al. (2003)
	Mrp2 (mouse)	Vollrath et al. (2006)
	cyp-13a2 (*C. elegans*)	Park et al. (2009)
	Ahr, cyp1a1, cyp1b1 (mouse)	Shin et al. (2007)
Cellular Protein Recycling	psmb5 (mouse)	Kwak et al. (2003a)
Innate Immunity	*gcs-1* (*C. elegans*)	An and Blackwell (2003)
HIF-1/ HIF1		
Hypoxia	*fmo-12*, *phy-2*, *efk-1*, *nhr-57*, *dpf-6*, *npp-6*, *fmo-14*, *smg-2*, (*C. elegans*)	Shen et al. (2005)
Innate Immunity	il-6, il-8, tnf (human cells)	Jeong et al. (2003)
	tnf, il1, il12 (mouse)	Walmsley et al. (2005)
DAF-16/ FOXO		
Heat shock response	*hsp-12.1*, *-12.2*, *-12.6*, *16.1, -16.49*, *sip-1* (*C. elegans*)	Hsu et al. (2003) Zhang et al. (2013) Schuster et al. (2010)
Oxidative Stress Response	*ctl-1*, *sod-3*, *gst-4*, *skn-1* (*C. elegans*)	Honda and Honda (1999) Zhang et al. (2013) Schuster et al. (2010)
Osmotic Stress	*hgo-1*, *tps-1*, *stps-2*, *tre-4* (*C. elegans*)	Zhang et al. (2013)
Innate Immunity	*lys-7*, *clec-52* (*C. elegans*)	Zhang et al. (2013)
PQM-1		
Heat shock response	*hsp-90* (*C. elegans*); indirect regulation	O'Brien et al. (2018)
Innate Immunity	*clec-41, asp-12* (*C. elegans*)	

Table 1. Summary of transcriptional regulators involved in cellular stress response pathways Stress responses regulated by proteostasis transcription factors and transcriptional targets which have been confirmed by RT-PCR, qPCR, western blot, EMSA, ChIP or GFP reporter studies.