Review

Worms under stress: *C. elegans* stress response and its relevance to complex human disease and aging

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Many organisms have stress response pathways, components of which share homology with players in complex human disease pathways. Research on stress response in the nematode worm *Caenorhabditis elegans* has provided detailed insights into the genetic and molecular mechanisms underlying complex human diseases. In this review we focus on four different types of environmental stress responses – heat shock, oxidative stress, hypoxia, and osmotic stress – and on how these can be used to study the genetics of complex human diseases. All four types of responses involve the genetic machineries that underlie a number of complex human diseases such as cancer and neurodegenerative diseases, including Alzheimer's and Parkinson's. We highlight the types of stress response experiments required to detect the genes and pathways underlying human disease and suggest that studying stress biology in worms can be translated to understanding human disease and provide potential targets for drug discovery.

C. elegans as a model for complex human disease and aging

Since its introduction in the early 1970s [1], the nematode *C. elegans* (Nematoda: Rhabditidae) has been instrumental as a platform for biological research and the implementation of a vast array of technologies [2,3]. The tiny and transparent worm has been used extensively in many areas of genetics, developmental and evolutionary biology, and complex disease research [4–6]. *C. elegans* serves as an important model for human diseases because it has many biological features in common with humans, such as the development of muscles, nerves, and digestive tract and the production of sperm and eggs [7]. Although relatively short-lived (approximately 3 weeks), worms do age, and studying this process has been informative for understanding human aging [8]. Many signaling pathways underlying lifespan elongation, apoptosis (see Glossary), and complex behaviors are conserved between worms and mammals [9–11]. Most notable is the insulin/insulin-like growth factor (IGF) signaling pathway, which involves the forkhead transcription factor DAF-16, a key regulator of lifespan changes in response to environmental and gonadal stimuli [8].

C. elegans has received much attention as a model for complex human diseases, including cancer, neurodegenerative, and mitochondrial disease [12–15]. It is also an effective model species for studying complex human neurological diseases [11]. Moreover, *C. elegans* has orthologs of amyloid precursor protein, suggesting worms may be a good model for studying Alzheimer's disease [16]. A specific example of a finding from worms that has been successfully translated into an improvement in human health comes from studies on the kindlin protein family. Mutations in one member of this family, Kindlin-1, lead to Kindler syndrome in humans, which is characterized by skin blistering. The defect in Kindler patients suggested a role of Kindlin-1 in integrin adhesion, but it was in *C. elegans* that the interaction between UNC-112, the ortholog of mammalian kindlins, and integrin was demonstrated [17]. This discovery paved the way to the development of more efficient therapy for this rare disease, and it demonstrates the relevance of using *C. elegans* to understand human disease.

C. elegans stress pathways as a model for complex disease pathways in humans

Many common stress-induced effects on physiology, gene expression, and signaling pathways among animals, including *C. elegans* and mammals, have been found [18]. For instance, heat-shock experiments showed that the genes *hsf-1* and *daf-16* are part of the heat-shock response and affect lifespan in *C. elegans* [19]. Homologs of these genes

Glossary

Amyloid precursor protein: precursor of amyloid, that accumulates as plaques in brain neurons in Alzheimer's disease.
Apoptosis: genetically determined process of programmed cellular death.
Molecular chaperones: a diverse group of proteins prevent and correct intracellular folding and assembly of polypeptides.
Orthologous: two genes are to be orthologous if they diverged after a speciation event
P53: the gene encoding p53 is a tumor suppressor gene; its activity stops the formation of tumors.
PolyQ: polyglutamine (PolyQ) repeats are implicated in several neurodegenerative diseases, such as Huntington's disease.
Proteostasis: protein homeostasis in cells.
von Hippel–Lindau tumor suppressor protein: a protein which regulates gene expression and tumor growth.

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play a key role in the development of age-related diseases in humans [20]. Lack of oxygen induces the transcription factor HIF-1 in *C. elegans*, which protects the germline from apoptosis by antagonizing the function of CEP-1, the homolog of the human tumor suppressor p53 [21]. Studying these effects in *C. elegans* with mutations in genes that have human homologs [e.g., *daf-18* is the homolog of the human tumor suppressor PTEN and *daf-16* is the ortholog of human FOXO (Forkhead transcription factor)] provides a tractable genetic system to explore the stress response and its relation to disease in humans.

Here we review how *C. elegans* pathways and genes underlying the stress response to heat shock, oxidative stress, hypoxia, and osmotic stress can inform research on complex human disease pathways. The type of experiments and methods used in *C. elegans* to study the genetics of stress responses are described in Box 1. Table 1 shows which type of stress experiments are used to identify and characterize genes in *C. elegans*, their human homologs, and the types of disease with which these genes are associated.

Hitherto, stress response studies in *C. elegans* have mainly been conducted from the view of understanding the genetics of longevity, neurobiology, and developmental biology. However, stress in worms affects genes and pathways that share a high homology with humans and play an important role in various complex diseases. Because our knowledge of both the genetics of human disease and stress response in worms has increased, it is now possible to draw comparisons between worms and humans that may suggest new avenues for research or illuminate previously unknown connections in complex human diseases. Therefore, we recommend continuing to study stress biology in the worm with an eye towards understanding complex diseases in humans.

Heat shock

Following a heat shock all cells exhibit a heat-shock response, which is a program of stress-inducible gene expression, to prevent cellular degeneration and increase thermal tolerance. The heat-shock response has been well studied in *C. elegans*, revealing the involvement of three neuroendocrine signaling pathways [22]: the nuclear hormone receptor (NR) pathway, the transforming growth factor-β (TGF-β) pathway, and the IGF/insulin-like signaling (ILS) pathway, which is the most thoroughly studied. The ILS pathway is involved in elongating lifespan by regulating the entry of the transcription factors DAF-16 and heat-shock factor-1 (HSF-1) into the nucleus. Starting with the receptor DAF-2, the ILS pathway consists of chained phosphorylation that, in normal conditions, keeps DAF-16 and HSF-1 cytoplasmic and inactive. Conversely, in stress conditions this pathway promotes the dephosphorylation of these factors, allowing their entry into the nucleus and, in consequence, their transcription factor activity [23]. HSF-1 regulates the heat-shock response by controlling the expression of small heat-shock proteins (HSP), which are molecular chaperones that function to maintain cellular proteostasis in eukaryotes and prevent protein and cellular damage following stress [24]. For instance HSF-1 and HSP protect *C. elegans* from heat-stroke-associated neurodegeneration [25].

Recent studies have demonstrated that both DAF-16 and HSF-1 are required for lifespan extension mediated by ILS, but DAF-16 acts during nematode adulthood, whereas HSF-1 is more active during early development in larva

Box 1. Methods used for stress response experiments in *C. elegans*

Heat shock

For heat-shock assays, NGM plates with OP50 are preheated to 35 °C prior to placing young adult hermaphrodites (stage L4) onto the plates. Worms are exposed to 35 °C for 2 h after which the plates are returned to 20 °C. Animals are scored as dead when they fail to respond to prodding with a platinum wire [52,70].

Oxidative stress

Oxidative stress assays are performed using strongly oxidizing agents such as the herbicide paraquat. Age-synchronized young adult (24 h post-larval stage L4) worms are grown on nematode growth medium (NGM) agar seeded with bacteria *E. coli* OP50 (20 °C). The animals are transferred into 300 μl of M9 + 200 mM paraquat (in 24-well plates, six animals per well) and scored for survival at 20 °C every 30 min [52,55].

Paraquat resistance can also be measured [71]. Worms are exposed as 3-day old adults, in groups of 30, to varying concentrations of paraquat (0–85 mM) in liquid survival medium at 20 °C. Medium with paraquat was replaced daily, and the number of live worms was counted after 3 days of exposure.

Oxidative stress can also be measured using the hydrogen peroxide resistance assay [71]. Worms aged 5 days are sampled in replicate groups of 50 adult worms and washed in magnesium-free M9 medium, after which they are incubated for 4 h at 20 °C with 4–12 mM H_2O_2. Viability was scored as described above.

OxICAT is a quantitative redox proteomics technique for *in vivo* monitoring of global changes in redox environment by quantifying oxidative thiol modifications of proteins. Synchronized populations of *C. elegans* are lyzed with 10% trichloracetic acid (TCA). After lysis, proteins are precipitated, washed, resuspended, and *in vivo* reduced and oxidized thiols are labeled by thiol-reactive isotope-coded affinity tag (ICAT). HPLC then is used to separate the ICAT-labeled peptides, followed by mass spectrometry and tandem mass spectrometry to identify the thiol-peptides and quantify their oxidation status. In parallel, stable transgenic lines expressing the peroxide sensor HyPer were generated to monitor endogenous peroxide levels over the lifespan of *C. elegans*. About 30 worms of different stages of synchronized populations were analyzed for HyPer ratio. To avoid pH changes that might modify HyPer ratio in transitional stage from larva to adult stage, release of peroxide was monitored using the peroxide-specific Amplex UltraRed reagent [72].

Osmotic stress

Age-synchronized young adult worms (L4) are transferred from isotonic (50 mM NaCl NGM) to non-lethal hypertonic medium (200 mM NaCl NGM). To assess both acute and chronic responses to hypertonicity, exposure times are: 15 min, 1 h, 6 h, and one full generation of growth (96 h) [53].

Hypoxia

Worms are grown at 20 °C on standard NGM plates seeded with OP50 *E. coli*. For hypoxia, animals are kept in a hypoxia chamber (C-174 chamber, Biospherix) for 24 h at 20 °C and recovered in ambient oxygen for 12 h at 20 °C. The oxygen level is automatically maintained with an oxygen controller (ProOx P110, Biospherix) supplied with compressed nitrogen gas [73].

Table 1. Types of stress experiments to identify and characterize genes in *C. elegans*, their human homologs, and the types of disease with which these genes are associated

Stress	*C. elegans* gene	Human homolog/ortholog	Disease	Refs
Heat	*daf-18*	*PTEN*	Cancer	[74,75]
Heat-shock	*daf-16*	*FOXO3A*	Cancer	[28]
Heat-shock	*hsf-1*	*HSF1*	Cancer	[24,76]
Oxidative	*pink-1*	*PINK1*	Parkinson's	[38]
Oxidative	*lrk-1*	*LRRK2*	Parkinson's	[38]
Oxidative	*sod-1, -2, -3*	*SOD1, 2, 3*	Amyotrophic lateral sclerosis (ALS)	[77–79]
Hyperoxia	*gcy-35*	*NPR-3*	Skeletal overgrowth	[80,81]
Hypoxia	*hif-1*	*HIF*	Ischemia, cancer	[45,48,82,83]
Hypoxia	*vhl-1*	*VHL*	Cancer	[84]
Hypoxia	*egl-9*	*EGLN*	Cancer	[45]
Hypoxia/heat-shock	*cep-1*	*P53*	Cancer	[21]
Osmotic	*gpdh-1*	*GPD1*	Transient infantile hypertriglyceridemia	[52,85]
Osmotic	*elt-2, elt-3*	*GATA4–6*	Pancreatic agenesis and cardiac defects	[86,87]
Osmotic	*osm-12*	*BBS7*	Bardet–Biedl syndrome	[53]
Osmotic/oxidative/heat-shock	*skn-1*	*NFE2* (and others)		[52]

stages [26]. It appeared that the activity of HSF-1 is regulated at an early step by ILS via two HSF-1 regulators, DDL-1 and DDL-2 (*daf-16-dependent* longevity genes) [27]. Inhibition of DDL-1/2 increases longevity and thermotolerance. DDL-1/2 negatively regulate HSF-1 activity by forming a protein complex with HSF-1 which is affected by the phosphorylation status of DDL-1 (homologous to human coiled-coil domain-containing protein 53). The formation of the protein complex and the phosphorylation of DDL-1 are controlled by ILS [27].

DAF-16 and HSF-1 play an important role in aging and age-related disease in humans. DAF-16 is orthologous to human FOXO3A, which has been shown to be strongly associated with human longevity [28]. A number of other aging phenotypes, such as the prevalence of cancer and cardiovascular disease and loss of various physical and cognitive functions, are also associated with the FOXO3A genotype [28]. This is supported by many other studies showing that polymorphisms in FOXO3A are associated with the ability to reach a very old age in humans [29]. In addition to its effects on aging, there is much evidence that the insulin/IGF-1 signaling (IIS) pathway (the human equivalent of ILS in worms) is a major regulatory axis underlying cancer in humans [30]. The IIS pathway can induce cellular proliferation in both healthy conditions and cancer [31].

As in worms, HSF1 also plays an essential role in stress responses by maintaining proteostasis in humans through regulation of insulin signaling and other age-related pathways [20]. The ability of HSF to bind to DNA is inhibited by acetylation at Lys^{80} [32], which is regulated by the de-acetylase SIRT1. SIRT1 regulates cell survival (apoptosis mechanism), inflammation, and metabolism through stress activation by de-acetylation of different factors such as p53, NF-κB, and different FOXO family members. Severe stress-mediated activation of SIRT1 likely leads to negative regulation of p53 [33] and subsequent cancer formation. A recent study demonstrates that HSF1 also regulates specific transcription programs of particular types of human cancer [34].

In addition to aging and cancer, the heat-shock response, in combination with the ILS pathway, is also involved in protein aggregation in both worms and humans. When raised at 25 °C, nematodes expressing polyglutamine (polyQ) in muscle accumulate protein aggregates and become paralyzed [35]. Aberrant protein aggregation is a key characteristic of neurodegenerative diseases, such as Alzheimer's disease, which is associated with the misassembly and aggregation of the $A\beta_{1–42}$ peptide. In *C. elegans* aggregation of $A\beta_{1–42}$ was reduced when aging was slowed by decreased ILS activity. The downstream transcription factors HSF-1 and DAF-16 regulate opposing disaggregation and aggregation activities [36]. This suggests that therapeutics which prevent the age-related decline in proteostasis and promote the upregulation of chaperones would slow down disease manifestation.

Taken together, we conclude that conducting heat-shock experiments in *C. elegans* provides fundamental insights into the role of HSPs, HSF-1, and the ILS pathway, and may be useful for understanding cancer, aging, and age-related neurodegenerative diseases in humans.

Oxidative stress

High doses of reactive oxygen species (ROS) cause oxidative stress. In most cells the primary source of ROS is the mitochondrion due to inefficiencies in oxidative phosphorylation. Oxidative damage is especially known to disrupt proteostasis, but it can also affect lipids, membranes, and DNA. In *C. elegans*, environmental perturbations that induce ROS can lead to reduced levels of dopamine, an important neurotransmitter which is released by nerve cells. One study looked at the stress response to paraquat, a herbicide that can lead to the formation of ROS [37], and its effect on neurite outgrowth in *C. elegans* [38]. They studied the effect of mutations in the genes *pink-1* and *lrk-1* to paraquat sensitivity. They demonstrated that *lrk-1* mutation suppressed all phenotypic aspects of the *pink-1* mutation, suggesting that PINK-1 may antagonize LRK-1 in humans. Both genes encode putative kinases highly similar to human PINK1 [phosphatase and tensin (PTEN) homolog-induced putative kinase 1] and LRRK2 (leucine-rich repeat kinase 2), mutations in which have been associated with Parkinson's disease [39]. Many other chemical perturbations causing ROS have been used in *C. elegans* to

model Parkinson's disease [13], illustrating the utility of studying oxidative stress in worms to understand human neurodegenerative diseases.

Mutations in *mev-1*, encoding the *C. elegans* cytochrome *b* subunit of the mitochondrial respiratory chain complex II (ubiquinol–cytochrome *c* reductase), result in increased sensitivity to oxidative stress. *mev-1* is orthologous to the human Isoform 1 of succinate dehydrogenase cytochrome *b* (SDHC). Recent studies showed that the *mev-1(kn-1)* mutation, which results in an amino acid substitution at position 71 from glycine to glutamate (G71E), dramatically reduced mitochondrial complex II activity. The accumulation of ROS is twofold greater in *mev-1(kn-1)* worms relative to wild type, and consequently the mutant animals have shorter lifespans. *Mev-1(kn-1)* worms show reduced glutathione concentrations, and this metabolic imbalance might be caused by the role played by succinate dehydrogenase (SDH) in the citric acid cycle.

In humans, mutations in mitochondrial enzymes from SDH family genes cause a genetic predisposition to develop certain types of tumors [40]. SDH deficiencies might trigger hypoxic conditions, resulting in increased activity of hypoxia inducible factor, a transcription factor involved in regulating cellular oxygen balance that can cause changes in cells and metabolism, and in some cases plays an essential role in angiogenesis, metastasis, and cell proliferation [41]. This factor and other features of hypoxia stress are discussed below.

Hypoxia and CO_2 fluctuation

Low levels of O_2 (hypoxia) can lead to decreased metabolic rate, increased glycolysis, and pausing or slowing of the cell cycle. Oxygen levels are severely affected by disease states such as cancer and various heart and lung diseases, where cells and tissues suffer from very low oxygen conditions (pathological hypoxia) [42].

Work in *C. elegans* has greatly increased our knowledge about the underlying mechanisms of hypoxic effects. In contrast to mammals, nematodes do not have specific respiratory organs but instead depend on diffusion for exchange of O_2 and CO_2. The body cavity is filled with fluid which allows rapid exchange of gases and chemicals across the cells. Hypoxia activates hypoxia inducible factor 1 (*hif-1)* in *C. elegans* [43,44]. In normal O_2 conditions, the protein HIF-1 is targeted for degradation by a prolyl-hydroxylase encoded by *egl-9* in *C. elegans*, the mammalian ortholog of this gene being EGLN/PHD [45].

C. elegans shows a wide CO_2 tolerance compared to other animals. However different studies have shown that *C. elegans* avoids high levels of CO_2 [46]. Furthermore, it was found that the intensity of CO_2 avoidance was suppressed under starvation conditions. Food-sensing pathways are closely related to O_2 and CO_2 sensitivity in *C. elegans*. One of the most important pathways related to food sensing is the ILS pathway. It is known that high activity of the ILS pathway is directly correlated with a well-fed state. In *daf-2*, *pdk-1*, or *akt-1* mutants, all of which mimic starvation conditions, CO_2 avoidance was suppressed. However in *daf-2; daf-16* double mutants, where the starvation signal was suppressed, the worms did show high avoidance of CO_2. This could suggest that starvation suppresses CO_2 avoidance by downregulating the ILS pathway, activating translocation of DAF-16 into the nucleus to act as a transcription factor [46]. This response is common to stress conditions, which suggests that stress conditions such as starvation and heat shock may be linked to a disruption in O_2/CO_2 levels. Furthermore, hypoxia is also associated with oxidative stress, highlighting another link between these stress response pathways (Figure 1) [47].

As in worms, hypoxic environments activate HIF in humans (HIF is the ortholog of HIF-1 in *C. elegans*). This activation plays a central role in tissue repair, ischemia, and cancer [48]. The hydroxylation of the HIF proline residue, an evolutionarily conserved mechanism, leads to degradation of HIF by the von Hippel–Lindau tumor suppressor protein (VHL). In hypoxic conditions, the proline hydroxylation and degradation of HIF are decreased. HIF then activates target genes to increase oxygen transport. Within tumors, hypoxia can lead to HIF-1α (consisting of subunits HIF-1a, HIF-2a, and HIF-3a), overexpression of which has been shown to increase patient mortality in different types of cancer [49].

Osmotic stress

Hypertonic or osmotic stress induces protein damage by aggregation. Under desiccated conditions resulting from hypertonic stress, the loss of water leads to an intracellular ionic imbalance, causing protein aggregation. Studies in *C. elegans* suggested that nematodes may have independent pathways to control proteotoxic effects and survive osmotic shock [50,51]. In hyperosmotic stress conditions, *gpdh-1*, which encodes a glycerol-3-phosphate dehydrogenase (GPDH-1), is strongly upregulated. GPDH-1 induces *de novo* biosynthesis of glycerol, leading to rapid accumulation of organic osmotic glycerol in cells. This is a typical effect of hyperosmotic stress in cells [52]. Expression of *gpdh-1* is regulated by two GATA transcription factors, *elt-2* and *elt-3* [53]. Both factors are also required for other developmental as well as non-developmental processes [53,54]. The enzymatic activity of GPDH-1 is regulated by osmotic regulatory genes, including *osm-7*, *osm-11*, and *osm-8*, which have been described as critical regulators in osmotic disorders [55]. In *C. elegans*, disruptions in osmotic regulatory genes lead to physiological responses similar to the response to hyperosmotic stress conditions [52,53]. Recent studies showed that osmotic regulatory genes regulate osmotic stress resistance independent from other stress response mechanisms [56,57]. It has been reported that the response mediated by *gpdh-1* is very specific, and its activation occurs rapidly after the osmotic shock (<15 min) and at relatively low levels of salt (200 mM NaCl) [58]. This is in contrast to the osmotically induced accumulation of damaged proteins, which occurs only at high salt concentrations (>500 mM NaCl) and takes approximately 1 h. Both mechanisms (glycerol production and accumulation of damaged proteins) could be employed by cells against different levels of osmotic stress; in other words, they are not cooperative, but instead work independently in different situations depending on stress conditions. Because the accumulation of damaged proteins is an important feature of diseases such as Alzheimer's and

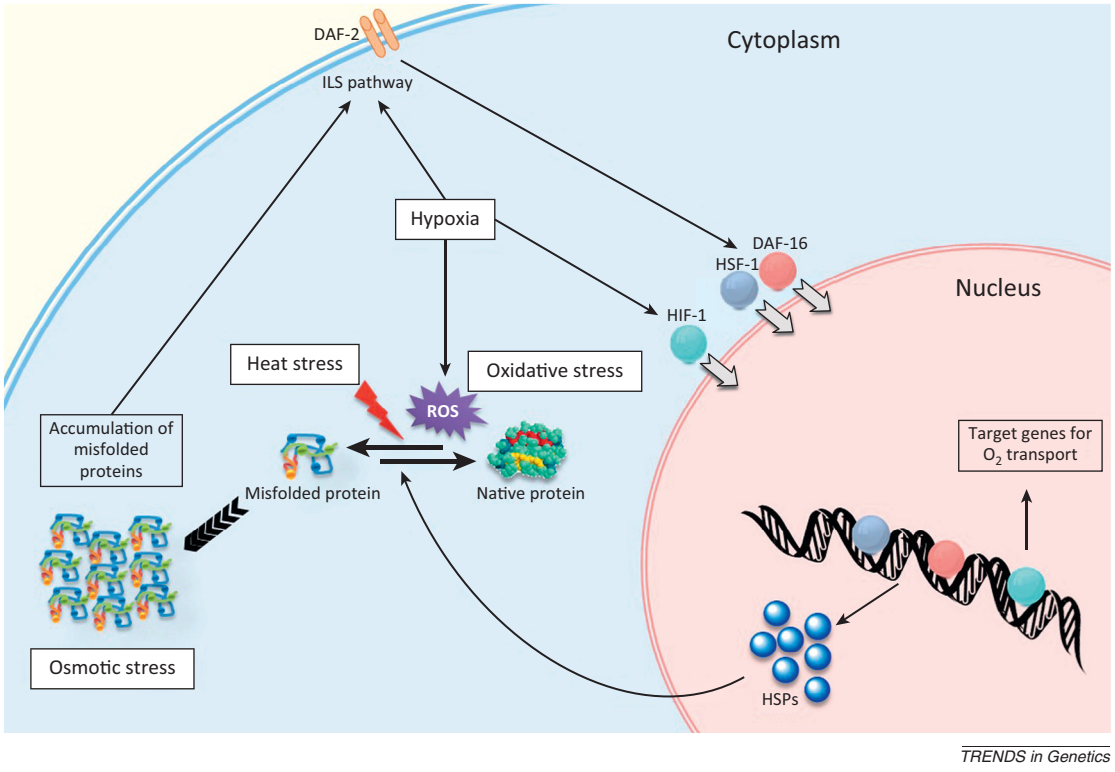

Figure 1. Schematic representation of stress response in a *Caenorhabditis elegans* cell. Hypoxia, heat stress, and oxidative stress provoke an abnormal conformation of native proteins. The accumulation of misfolded proteins leads to a proteostasis imbalance in the cytoplasm resulting in cellular osmotic stress. In these conditions the stress response mechanism is activated mainly by enhancing the insulin-like signaling (ILS) pathway. The transmembrane receptor DAF-2 initiates the intracellular signaling that directs the transcription factors DAF-16 and HSF-1 into the nucleus where they activate gene expression of heat-shock proteins (HSPs). HSPs are molecular chaperones that help re-establish proteostasis by rescuing misfolded proteins. Hypoxia activates the stress response through ILS and expression of hypoxia inducible factor (HIF-1) and its entry into nucleus to act as a transcription factor to activate the expression of target genes for O_2 transportation.

Parkinson's [59], insight into the molecular mechanisms of osmotically induced protein damage in *C. elegans* may help to unravel these complex phenotypes genetically. We suggest that the mechanisms of glycerol production and accumulation of damaged proteins in worms might be useful targets for developing potential Alzheimer's and Parkinson's therapeutics.

Implications for drug discovery

The mechanisms of stress response we presented demonstrate that different stressors share a common and well-conserved molecular response to changes in proteostasis in cells. There are several oncological, neurodegenerative, and metabolic disorders that are triggered by an accumulation of misfolded proteins as a result of cellular stress. Therefore, controlling misfolded protein imbalance may be a therapeutic tool to control damage that leads to complex human diseases. Many of the diseases related with proteostasis are associated with aging [60]. During aging, the accumulation of protein aggregates leads to an amplification of protein damage that contributes to cellular toxicity.

Stress response analysis in *C. elegans* offers the opportunity to discover new receptors and targets for drugs to treat various diseases. Heat-shock experiments can be used to investigate the genetics and molecular biology of inhibitors of HSF-1. This type of experiment further opens up new ways for assessing if small HSPs or particular protein aggregates or aggregation mechanisms are druggable. For instance, specific activators of HSF1 such as geldanamycin were effective in both polyglutamine (polyQ) disease models as well as other neurodegenerative disease models [61]. Along these same lines, many strategies are emerging to restore proteostasis to alter the clinical course in complex age-associated diseases in humans [62], which can be investigated using heat-shock experiments in *C. elegans*.

New trends in therapeutics are pointing at small-molecule pharmacological chaperones as proteostasis regulators. These regulators have been shown to increase the capacity of cells to correctly re-fold damaged proteins [62]. Many proteostasis regulators have been described recently that control HSF1 and in turn upregulate levels of cytoplasmic HSPs. More recently, the identification of around 300 chemical inducers of the heat-shock response was reported by screening for HSF-1 dependent activators of expression of chaperones in human cells [63]. This suggests that pharmacological manipulation of the heat-shock response could be of benefit in a variety of multiple conformational diseases like Alzheimers' disease and Parkinsons' disease.

Currently, clinical trials are testing chaperone inhibitors such as an inhibitor of HSP in combination with proteasome inhibitors on cancer cell fate to control endoplasmic reticulum (ER)–Golgi homeostasis (see www.clinicaltrials.org) based on the fact that a high proteostasis capability may lead to proliferation of cancer cells. It was demonstrated that the adaptive biology to proteostasis by

developing an effective stress response mechanism, such as overexpression of chaperones like HSP-70, confers protection from proteotoxicity in cases of polyQ aggregates in Huntington's disease [64] or α-synuclein aggregates in Parkinson's disease pathogenesis [65].

Furthermore, human HSF1 plays an important role in cancer cells, and its aberrant regulation can lead to disrupted signaling and malignant changes in DNA, protein, and energy metabolism, thus promoting tumorigenesis [66]. Very interestingly, recent studies in humans have shown how HSF1 not only regulates disease though the classic heat-shock response and therefore HSPs, but also regulates transcriptional programs specific to malignant cells [34]. Hypoxia experiments in *C. elegans* focus on HIF-1, which is directly relevant to human disease. HIF-1α activity has been used to induce angiogenesis (the growth of new capillary blood vessels) for use in ischemic disease (disease of reduced blood supply). In models of hind-limb ischemia, active HIF-1α was shown to be beneficial alone or in combination with bone marrow-derived angiogenic cells [67,68]. More information on drug development on the basis of HIF and the response to hypoxic stress can be found in [69].

Concluding remarks

Although there are clear limitations to using *C. elegans* stress response as a model for human disease, we suggest that nematodes are an ideal first proxy for studies that can be followed-up in closer models such as rodents. One of the *C. elegans* limitations is that in the ILS pathway *C. elegans* only has one receptor for both insulin and IGF-1, whereas mammals have separate insulin and IGF1 receptors. The central role of this signaling pathway in many stress response and complex diseases poses a challenge to translating knowledge from worms to humans. Worms also lack specific respiratory organs, which is important to consider when hypoxia experiments are used to gain insight into human disease. Further, current *C. elegans* experiments are carried out in a single genotype, Bristol N2, which might lead to biased conclusions (Box 2).

Despite these drawbacks, *C. elegans* provides researchers with a rapid and versatile system for exploring features of complex human diseases. The conservation of the components of the pathways responding to heat shock, hypoxia, osmotic, and oxidative stress makes these experiments valuable endeavors into understanding the genetics underlying various human diseases. As such, stress response experiments in *C. elegans* might provide potential new insights towards the development of new therapies that are translatable to the clinic.

Box 2. Importance of background genotype in studying stress responses

Most research on stress responses in *C. elegans* to date has made use of forward and reverse genetic screens. A common feature of all these genetic perturbation screens is that they are carried out in a single genotype (i.e., the wild type strain Bristol N2): in other words, mutants and RNAi screens are studied within a single genotype. Although these approaches have been very valuable for dissecting genetic pathways, if we want to gain an understanding of how variation within these pathways gives rise to differences among individuals, studying the pathways in different genotypes under different stress regimes becomes essential [88]. Steps have already been taken into this area through studies focusing on heat shock [89]. Using genomic mosaics derived from a cross between wild types N2 and CB4856 natural variation in heat-shock response was detected. The ability of worms to recover from heat shock was linked to a small region on chromosome II. Although more research is required to identify the causal polymorphic genes, these approaches indicate how novel genes and alleles can be detected which affect the heat-shock response, and hence potential new candidates of human disease genes. In particular, it allows understanding complex gene–environment interactions focusing on the interaction between many alleles of small effect interacting with the environment. This is of interest for translational research of human disease because many complex diseases are regulated by the interaction of small-effect genes and the environment. These developments emanate from a vast number of studies that have shown the power of natural genetic variation for identifying genes underlying many different complex traits and combination of stressors in *C. elegans* [4,90–97].

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