Application of *Caenorhabditis elegans* for Research on Endoplasmic Reticulum Stress

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ABSTRACT: *Caenorhabditis elegans* is a versatile model organism that has been applied to research involving obesity, aging, and neurodegenerative diseases. *C. elegans* has many advantages over traditional animal models, including ease of handling, a short lifespan, a fully sequenced genome, ease of genetic manipulation, and a high similarity to human disease-related genes. With established *C. elegans* models of human disease, *C. elegans* provides a great platform for studying disease pathologies, including endoplasmic reticulum (ER) stress, which is characterized by the accumulation of unfolded and misfolded proteins involved in the pathologies of many diseases. ER stress can lead to activation of the unfolded and misfolded protein response, a mechanism that attenuates ER stress and recovers ER homeostasis. The current review gives an introduction to *C. elegans* and ER stress, along with the pathological role of ER stress in disease and the application of worm models in ER stress-related research.

Keywords: endoplasmic reticulum stress, unfolded protein response, *Caenorhabditis elegans*

INTRODUCTION

*Caenorhabditis elegans* is a widely used animal model that has many advantages over other *in vivo* models (1). The wildtype *C. elegans* has a short life cycle (about 2 days at 25°C), a relatively short lifespan (about 2 ~ 3 weeks), and a large brood size (more than 300 progeny) (1). In addition, experiments using *C. elegans*, which has a transparent body, only require relatively simple laboratory conditions and do not need institutional committee approval (1). For example, *C. elegans* is fed with non-pathogenic *Escherichia coli* OP50 and raised on petri dishes or in liquid media (1). Thus, research using *C. elegans* is much more time and cost-efficient than research using vertebrate models. Moreover, *C. elegans* shares the basic biology of vertebrates; 83% of its proteome is homologous to that of humans, and about two thirds of its genes are related to human diseases (2). In addition, *C. elegans* has a completely sequenced genome and more than 3,000 mutants are available, and RNA interference (RNAi), microinjection as well as genetic crosses are amenable in this worm model (2). These reasons make this animal model a powerful toolbox in many life science research areas (3).

BASIC BIOLOGY OF *C. elegans*

*C. elegans* is a free-living nematode about 1.0 ~ 1.5 mm long in its adult stage (2). It has a life cycle of about 2 days; this includes an embryonic stage, four larval stages (L1 ~ L4), and the adult stage (1). Its eggs have an impermeable shell that isolates them from the outside environment (1). Hatched eggs turn into L1-stage worms, which proceed with adequate food into the L2 ~ L4 stages in the developmental process. The cuticle of the worms is reestablished after each larval stage (1). When the nematode encounters harsh environments, including an absence of food and/or the presence of unfavorable chemicals, it arrests development and enters a dormant state named a dauer larvae stage (4). Nematodes in the dauer stage will continue to grow into L4 stage worms with sufficient food and favorable environmental conditions (4).

*C. elegans* has a high degree of cell differentiation and specialization (4). The worm has five systems; the epidermis, and muscular, digestive, nervous, and reproductive systems (4). The presence of well-defined tissues and organs makes the worm useful for research in biological processes involved in multicellular organisms (5).

The epidermis of the worm is composed of epidermal cells that secrete the cuticle, an exoskeleton that helps in the locomotion, protection and growth of the worm...
(1). The cuticle of C. elegans, which consists of collagens, proteins, glycoproteins, and lipids, is reestablished five times during its development (1). Movement is controlled by muscles that are attached to the epidermis (4). The relaxation and contraction of these muscles are necessary for the sinusoidal movement of the animal (4). For example, one of the unc (uncoordinated) genes, unc-54, encodes proteins that are needed for the typical sinusoidal movement of the worms, and alterations to unc-54 can cause a paralytic phenotype (4).

The digestive system of C. elegans is primarily composed of the pharynx, intestine, rectum, and anus (4). When the worm eats, food passes through the mouth opening first and then gets grinded at the pharynx (4). Next, the ground food reaches the intestine, where it is further digested, and where nutrients are absorbed, utilized, or stored (1). After the food has been digested in the intestine, remaining waste passes the rectum and anus and gets excreted through a rectal valve; the defecation cycle is about 50 s (1).

The nervous system of C. elegans contains 302 neurons for an adult hermaphrodite (1). C. elegans has three classes of neurons; chemosensory, mechanosensory, and thermosensory neurons (6). The worm model shares many proteins with mammalian neurons involved in the formation, trafficking, and releasing of synaptic vesicles (6). Because C. elegans uses many of the same neurotransmitters as vertebrates—including dopamine, serotonin, gamma- amino butyric acid, and acetylcholine—it has been used as a model for many neurodegenerative diseases (4).

The reproductive tissue of the adult hermaphrodite C. elegans is comprised of the somatic gonad that houses the germline and egg-laying apparatuses (1). Over 99% of the worms are self-fertilizing hermaphrodites, which helps to preserve homozygous clones (4).

ENDOPLASMIC RETICULUM (ER) STRESS

Proteins are synthesized by the ribosomes attached to the rough ER. They are modified and folded by chaperones and enzymes in the ER, and then transported out of the ER-lumen (7). As the ER is a highly dynamic organelle, many parameters in the ER microenvironment can affect the elements of protein folding, including chaperones, foldases, calcium levels, the redox milieu, and the phospholipid composition of the ER membrane, etc. (7). Once protein folding is disturbed or inadequate, unfolded and misfolded proteins can accumulate in the ER lumen and the cytoplasm, a condition named ER stress (8,9). The term ‘unfolded proteins’ in this paper refers both unfolded and misfolded proteins. The ER has a ‘quality control’ system to degrade unfolded proteins: the ER-associated degradation (ERAD) pathway delivers damaged protein to proteasome, and the autophagy pathway transports unfolded proteins to the lysosome (10). However, over accumulation of unfolded proteins leads to activation of the unfolded protein response (UPR\textsuperscript{ER}), which has both protective and apoptotic components (11). It has been reported that the UPR\textsuperscript{ER} has a pathological role in many conditions, including aging, neurodegenerative diseases, obesity, and type 2 diabetes (8). Thus, understanding the role of UPR\textsuperscript{ER} may provide important insights into the pathology and new treatment of these and other diseases.

UPR\textsuperscript{ER}

The UPR\textsuperscript{ER} is a signaling pathway that is conserved from yeasts to mammals, which allows researchers to study this pathway in a variety of models, including C. elegans (12). The UPR\textsuperscript{ER} stress response system can monitor the accumulation of unfolded proteins and correct protein folding and processing (7). The UPR\textsuperscript{ER} system tries to maintain normal cell function by reducing protein-folding workload, strengthening protein-folding capacity, as well as removing and degrading unfolded proteins (12). However, when ER stress cannot properly handle unfolded proteins, the apoptotic pathway can be triggered by the UPR\textsuperscript{ER} via UPR\textsuperscript{ER} signaling pathways (7).

THE UPR\textsuperscript{ER} SIGNALING PATHWAYS

The UPR\textsuperscript{ER} system includes three branches, each represents by one of three transmembrane proteins: the inositol-requiring kinase-1 (IRE-1), protein kinase RNA-like endoplasmic reticulum kinase (PERK), and activating transcription factor-6 (ATF-6) (12). All these branches have a luminal domain that can sense the amount of unfolded proteins and initiate responses to manage ER stress (10).

IRE-1 pathway

The IRE-1 branch of the UPR\textsuperscript{ER} is conserved from yeasts to humans (13). Although the exact mechanism of IRE-1 activation is not completely understood, one study suggests that both unfolded proteins and binding immunoglobulin globulin protein (BiP) participate in modulation of IRE-1 (14). Without unfolded proteins, BiP binds to IRE-1 to inhibit its activity (15). In the presence of unfolded proteins, BiP binds to them and activates IRE-1 (15). Activation of IRE-1 results in the dimerization and autophosphorylation of the protein (16). The activated IRE-1 acts as an endoribonuclease that cleaves a 26-base fragment from X-box binding protein-1 (XBP-1) mRNA, and the spliced mRNA is translated into spliced X-box binding
In addition, the UPRER response to protein folding and contribute to age-related diseases with age. This may allow for an accumulation of unfolding, which can further imperil the function of the UPRER of these chaperones suffer oxidative damage during aging (28). Many karyotic translational initiation factor 2 domain (15). The activated PERK can phosphorylate eukaryotic translational initiation factor 2α (eIF2α) and downregulate global protein synthesis to alleviate ER workload (22). Moreover, the activation of eIF2α can also increase the translation of protective proteins, including activating transcription factor 4 (ATF4) (23). In mammals, ATF4 controls a range of target genes, including the pro-apoptotic transcription factor, C/EBP homologous protein (CHOP) (24-26). The downstream targets of ATF4 in C. elegans is, however, still not known.

ATF-6 pathway
ATF-6 is a transmembrane transcription factor that is activated upon accumulation of unfolded proteins (24). Once activated, ATF-6 translocates to the Golgi apparatus, where it is cleaved by site-1 protease (S1P) and site-2 protease (S2P) to generate the cleaved form of ATF-6 (13). Once cleaved, ATF-6 upregulates a wide range of UPRER target genes, including chaperones, protein foldases, and components in the protein degradation ERAD pathway in mammals (13). However, in C. elegans, ATF-6 regulates less target genes than in mammals, and the specific role of ATF-6 branch in C. elegans is still to be determined (27).

CONDITIONS RELATED TO ER STRESS AND THE UPRER

Aging
The ability of UPRER to respond to ER stress declines with age. This may allow for an accumulation of unfolded proteins and contribute to age-related diseases (25). In addition, the UPRER response to protein chaperones and foldases also decreases with aging (28). Many of these chaperones suffer oxidative damage during aging, which can further imperil the function of the UPRER system (29). The decline in the protective role of the UPRER during aging is accompanied by an increase of pro-apoptotic markers in the UPRER (28). Expression of pro-apoptotic markers, including CCAAT-enhancer-binding protein (C/EBP) homologous protein CHOP and caspase-12, are upregulated in the aged, but not young rats, when treated with lactacystin, a proteasome inhibitor that disrupts protein homeostasis (30). Thus, overall, aging reduces the protective functions of the UPRER and strengthens its pro-apoptotic signaling, which further leads to age-related accumulation of unfolded protein and cell dysfunction.

Regulation of aging process in C. elegans
The homolog of insulin/insulin-like growth factor receptor (IIS), DAF-2, is well known to be associated with lifespan in C. elegans (31). The DAF-2 mutant has an extended lifespan that depends on the activation of the forkhead box transcription factor (FOXO). In addition, UPRER components ire-1 and xbp-1 are involved in lifespan extension, which occurs as xbp-1 upregulates daf-16 in the DAF-2 mutant (32).

In addition to IIS, protein skinhead-1 (skn-1), the mammalian homolog of nuclear respiratory factor 2 (Nrf2), has been associated with longevity in C. elegans (33). A recent report indicates that vitamin D can extend lifespan through activation of skn-1 in C. elegans, and the activation of skn-1 requires UPRER components ire-1 and xbp-1 (34). Consistently, others have reported that skn-1 contributes to longevity by activating the IRE-1 and PERK branches of the UPRER (23).

Improving ER protein homeostasis also contributes to longevity in C. elegans (25). As UPRER regulates many components that can improve ER protein homeostasis (31), upregulation of UPRER can improve age-related diseases (35). In fact, the activation of the hexosamine pathway, which has been linked to UPRER upregulation, can improve longevity and reduce protein aggregation by enhancing ER-associated protein degradation, proteasome function, and autophagy in C. elegans (35,36).

Neurodegenerative disease
A common pathological marker for many neurodegenerative disorders is the accumulation of unfolded proteins in neurons (37). Thus, studying stressors and potential therapies that target unfolded proteins may provide a better understanding of these disorders and play a crucial role in the development of preventive and treatment strategies for these neurodegenerative disorders (37).

Alzheimer’s disease
Alzheimer’s is a neurodegenerative disease exhibiting the presence of extracellular senile plaques and intracellular neurofibrillary tangles (38). The major component of senile plaques is β-amyloid (Aβ), while the neurofibrillary tangles are formed by aggregation of tau proteins (38). Many studies have reported that ER stress and the UPRER system are involved in the development of Alzheimer’s disease (39,40). Recent studies have shown that Aβ can
accumulate at the ER lumen and disturb calcium homeostasis in the ER (41). This causes ER stress and activation of the UPRER (41), which leads to impaired synaptic function and eventually induces apoptosis of neurons (40). In addition, a protein associated with Alzheimer’s disease, mutated presenilin-1, can both disrupt calcium homeostasis and inhibit IRE-1 activity, which worsen ER homeostasis and reduce UPRER signaling (42).

Studying Alzheimer’s disease using C. elegans
In humans, Aβ is produced when amyloid precursor protein (APP) is cleaved by β- and γ-secretase (38). However, the C. elegans homolog of APP, apl-1, does not contain the genetic information for β-amyloid (38). Thus, researchers have created C. elegans models of Alzheimer’s disease by introducing human Aβ1-42 to generate transgenic strains (43). They have applied these models to screen bioactive compounds and discover contributors to the disease (43). Using this model, resveratrol is reported to reduce the toxicity of Aβ aggregation through upregulation of autophagy and proteasomal degradation (43). Ablation of the IRE-1 branch of the UPRER may reduce the toxicity of β-amyloid, although the role of IRE-1 in Alzheimer’s disease has been controversial (44,45).

Parkinson’s disease
Parkinson’s disease is a neurodegenerative disorder marked clinically by tremors, bradykinesia, and impaired balance (2). These have been associated with two main pathological hallmarks: the formation of proteinaceous inclusions (Lewy Bodies) and the loss of dopaminergic neurons from the substantia nigra (2). Recent studies have identified a pathological role for ER stress in Parkinson’s disease (46). Aggregation of α-synuclein in the ER can impact ER homeostasis, including depletion of ER chaperones and inhibition of ER-to-Golgi trafficking (47-49). Although it is generally agreed that ER stress is involved in the neuropathology of Parkinson’s Disease, the exact mechanism for how induction of ER stress causes the loss of dopaminergic neurons in Parkinson’s disease is not yet well understood (49).

Studying Parkinson’s disease using C. elegans
In humans, α-synuclein aggregates are products of the α-syn gene (2). Because C. elegans does not possess this gene (2), C. elegans models of α-synuclein are established with human α-synuclein expression in neurons or muscle promoters (6). In addition, neurotoxins like 6-hydroxydopamine can be used to degenerate dopaminergic neurons in C. elegans, which also induces ER stress (50). Consistently, mutation of leucine-rich repeat kinase 2, a gene commonly associated with Parkinson’s disease, has been shown to inhibit UPRER responses, leading to increased ER stress in C. elegans (50). Moreover, a type of bacterial metabolite, phenazine derivatives, has been shown to exacerbate ER stress in the worm model of Parkinson’s disease (51).

Huntington’s disease
Huntington’s disease is an inherited neurodegenerative disease that results in cognitive, motor, and psychiatric changes (52). This disease results from expansion of CAG repeats in the Huntingtin gene that adds a long polyglutamine repeat to the Huntingtin protein (52). Recent studies have linked ER stress to the development of Huntington’s disease in several ways: [1] polyglutamine fragments can entrap ERAD proteins (Npl4, Ufd1, and p97) and induce ER stress (53), [2] overexpression of these ERAD proteins can reduce the toxicity caused by polyglutamine (53), and [3] polyglutamine indirectly increases accumulation of unfolded proteins by disruption of vesicular trafficking and by causing defects in lysosome-mediated protein degradation (54).

Studying Huntington’s disease using C. elegans
In C. elegans, the human huntingtin-polyglutamine protein is incorporated in muscle or neurons to generate disease models for relatively easy detection of disease development (5,55). Using these models, UPRER components and changes in the disease progression can be investigated. For example, manganese treatment can induce ER stress and increase polyglutamine aggregates as an indicator of Huntington’s disease (56). This provides further evidence that ER stress is associated with the development of Huntington’s disease (56).

OBESITY AND TYPE 2 DIABETES
Obesity is also known to be associated with increased ER stress and activated UPRER in many tissues, such as hypothalamus, liver, muscle, and adipose tissue (57). Treatment of chemical chaperones, which improve protein folding and synthesis, can improve ER function and reduce ER stress markers in the liver and adipose tissue of obese mice (58). This indicates that obesity-associated ER stress may result from a high protein synthesis load (58). In addition, increased free fatty acid load as well as changes in the composition of the ER membrane can also activate the UPRER, which suggests that the UPRER responds to lipid imbalances (59-62). The activated UPRER plays a significant role in the development of obesity-related disorders, particularly type 2 diabetes (57). Increased ER stress in obesity can lead to the induction of c-Jun N-terminal kinases, and thus reduce the activity of insulin receptor substrate-1 to down-regulate insulin signaling (58). The UPRER also interacts with hepatic gluconeogenesis by ATF-6, one of
the three branches of the UPRER, to inhibit the activity of CREB-regulated transcription coactivator 2 (CRTC2), which results in down-regulation of gluconeogenesis in the liver (63). With increased insulin demand due to insulin resistance, the pancreatic β-cells can exceed their protein folding capacity and generate ER stress (13). Eventually, prolonged ER stress in the pancreas can trigger the UPR-mediated apoptosis in β-cells, exacerbate hyperglycemia, and eventually increase insulin deficiency (11).

**C. elegans model of obesity and type 2 diabetes**

*Caenorhabditis elegans* is an established model for research in obesity and type 2 diabetes. The key pathways on energy metabolism, such as the lipid metabolism and IIS, are conserved between mammals and *C. elegans* (64). For example, researchers have found that a change of phospholipid composition in the ER membrane in *C. elegans* can activate UPRER without disturbing ER proteostasis (60). This indicates that in *C. elegans* lipid imbalance may directly activate the UPRER (60), which is consistent with observations in mammals (65). Thus, the worm model has tremendous potential for valuable research in obesity and diabetes, particularly research in ER stress- and UPR-mediated mechanisms.

**CONCLUSION**

The introduction of the *C. elegans* model of human diseases has provided a versatile and powerful platform for studying the pathology and potential treatments of these diseases. As one of the complex pathways in cells, UPRER is involved in the pathology of many diseases. Using the worm model to study the UPRER already has been proven to be fruitful, and continued discoveries using this worm model will provide important insights in research related to the UPR and to human diseases in the future.

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**AUTHOR DISCLOSURE STATEMENT**

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