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Caenorhabditis elegans: A Convenient In Vivo Model for Assessing the Impact of Food Bioactive Compounds on Obesity, Aging, and Alzheimer's Disease

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Keywords

C. elegans, obesity, aging, Alzheimer's disease

Abstract

Caenorhabditis elegans is a small free-living nematode that lives in temperate soil environments. It has been widely employed as an animal model in research involving obesity, aging, and neurodegenerative diseases, including Alzheimer's disease, because of its various advantages, such as small size, large number of progeny, completely sequenced genome, and short life span, over traditional animal models of vertebrates. These benefits contribute to an ideal research model organism. In this review, we provide an introduction to *C. elegans* and its applications in obesity, aging, and Alzheimer's disease studies, with the aim of stimulating scientists to use *C. elegans* as an experimental model in various fields of research.

INTRODUCTION

Caenorhabditis elegans is a free-living nematode that lives in temperate soil environments. After first being adopted as a model organism for developmental biology studies in the 1960s, *C. elegans* has been widely used in research involving obesity, aging, development, locomotive activity, and neurodegenerative disorders. Compared to traditional animal models, this eukaryotic, multiorgan organism possesses many advantages, including small size (~1 mm in length for adults), short life span (~21 days for wild type), large progeny production (~300 by self-fertilization), and a completely sequenced and well-annotated genome. Furthermore, it encodes more than 65% of the characterized human disease-related genes, and research using *C. elegans* does not require approval by the Institutional Animal Care and Use Committees. All of these properties make *C. elegans* a convenient and popular animal model for research (Zheng & Greenway 2012). Specifically, *C. elegans* has been widely applied as an alternative model for genetic mechanistic studies because of the availability of broad genetic tools, including targeted deletions, mutagenesis screens, and a genome-scale RNA interference (RNAi) screen (Shen et al. 2017a). More than 3,000 mutant strains are available at low cost from the Caenorhabditis Genetics Center (University of Minnesota), which further facilitates the application of *C. elegans* (Stiernagle 2006). Therefore, with a completely sequenced genome and the well-investigated function of each gene, *C. elegans* has been extensively applied as a useful model to identify the potential effects and the underlying mechanisms of food compounds.

CAENORHABDITIS ELEGANS AS A MODEL

Gender

There are two genders of *C. elegans*, hermaphrodites and males. Males can be distinguished from hermaphrodites by their distinctive tail, which bears a copulatory apparatus (**Figure 1**) (Hall & Altun 2008). Males account for 50% of the progeny by mating but only 0.1% by self-fertilization of hermaphrodites. The isolation, maintenance, and generation of mutant strains can be achieved through mating, whereas genetically identical progeny can be obtained through self-fertilization of homozygous hermaphrodites. A hermaphrodite that self-fertilizes can produce approximately 300 progeny (consisting of 99.9% hermaphrodites) throughout its lifetime, whereas the brood size can increase to 1,200–1,400 by mating with a male (Hall & Altun 2008).

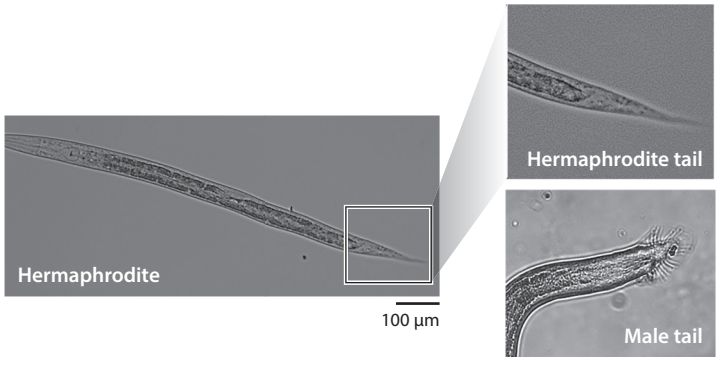

Figure 1

Microscopy images of the tails of a *Caenorhabditis elegans* hermaphrodite and male. Magnification 200×. Males can be distinguished from hermaphrodites by their distinctive tail, which bears a copulatory apparatus.

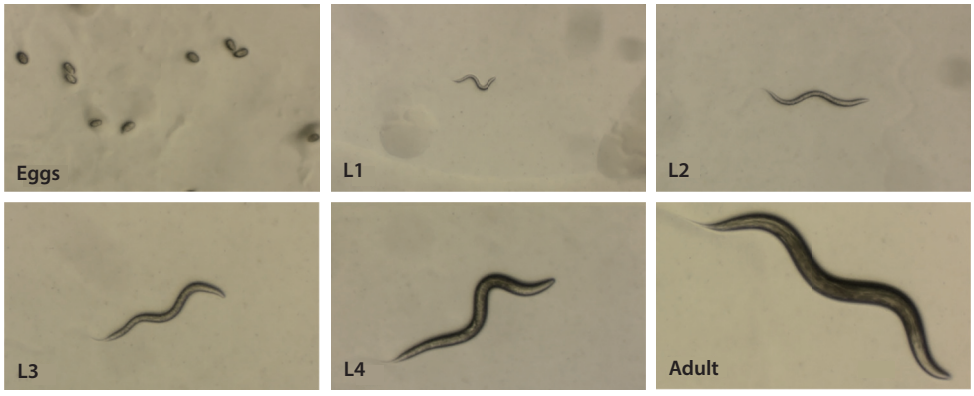

Figure 2

Images of the four larval stages of *Caenorhabditis elegans* and the adult *C. elegans* by microscopy. Magnification 50×. L1-stage nematodes hatch from eggs. Larval development proceeds through the L2, L3, and L4 stages.

Life Cycle

The life cycle of *C. elegans* consists of four larval stages (L1–L4) and adulthood (**Figure 2**) (Wood 1988). After fertilization, the zygote produces a tough shell and a vitelline membrane, which render the embryo impermeable to most solutions and able to survive outside the uterus after being laid through the vulva (Wood 1988). L1-stage nematodes hatch from eggs, and larval development then proceeds through the L2, L3, and L4 stages; the end of each larval stage is marked with a molt. If the embryos hatch in the absence of food, they arrest at the L1 stage until food becomes available. Such larvae can survive up to 6–10 days without feeding. After food becomes available, these arrested larvae go through normal molting and development (Wood 1988).

Anatomy

The basic anatomy of *C. elegans* includes a mouth, pharynx, intestine, gonad, and cuticle (**Figure 3**). The mouth of an adult nematode has six symmetrical lips, which surround the opening and form a 1–3 µm circular cavity through which food is transported to the pharynx (Colmenares et al. 2016). *C. elegans* feeds through a two-lobed pharynx, which is a nearly autonomous organ with a neuronal system, muscles, and epithelium (Altun & Hall 2009). The lumen of the pharynx is connected to that of the intestine (**Figure 3**), where the ground food can pass into the intestine via the intestinal pharyngeal valve (Altun & Hall 2009).

The *C. elegans* intestine is responsible for a variety of functions, including food digestion and nutrient absorption as well as the synthesis and storage of macromolecules (McGhee 2007); it consists of 20 cells, which are arranged to form a tube with a central lumen. The apical surface of these cells carries numerous microvilli to increase the absorption surface. The contents of the intestine are excreted to the outside via a rectal valve, which connects the gut to the rectum and anus. The four enteric muscles that work for defecation are located around the rectum and posterior intestine (Altun & Hall 2009).

The overall gonad of *C. elegans* is similar to that of other organisms. It is organized as a blind-ended tube with proliferating germ cells at the distal end, gametes at the proximal end, and intervening stages of germ-cell differentiation in between (Hubbard 2007). During the postembryonic development, *C. elegans* is enclosed within an exoskeleton called the cuticle, which is

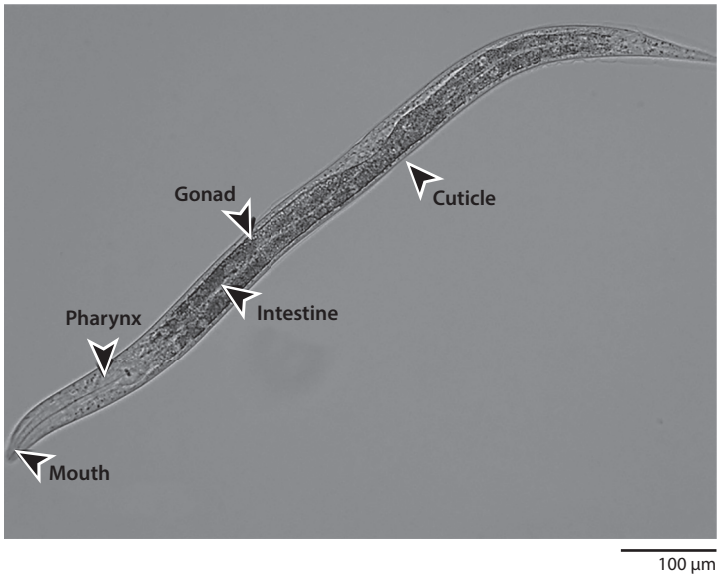

Figure 3

An image of *Caenorhabditis elegans* hermaphrodite anatomy by microscopy. Magnification 50×. The basic anatomy of *C. elegans* includes a mouth, pharynx, intestine, gonad, and cuticle.

a collagenous extracellular matrix (Page & Johnstone 2007). It is synthesized by an underlying ectodermal cell layer (the hypodermis) that surrounds the worm body. In addition to a highly impervious barrier between the animal and its environment, the cuticle is also a multifunctional exoskeleton that is essential for the maintenance of body morphology and integrity and plays a critical role in locomotion via attachments to body-wall muscles (Page & Johnstone 2007).

Uptake of Compounds into *Caenorhabditis elegans*

Supplementation with target compounds in *C. elegans* is frequently used in research, with delivery methods varying between studies. The compounds can be applied to bacteria that are ingested (bacteria are a major food for *C. elegans*), directly spread onto the surface of NGM (nematode growth medium) plates, or added to the liquid medium (Zheng et al. 2013).

Compounds enter *C. elegans* via three distinct routes: ingestion, uptake through the cuticle, and uptake via exposed sensory neuronal cilia (Kaletta & Hengartner 2006). Uptake by ingestion is a relatively slow process. Appropriate food sources could be recognized and selected by chemosensory neurons and taken up by aspiration through the pharynx. This feeding activity depends on satiety and food availability and is controlled by various neurotransmitters. Once the compounds enter the intestinal lumen, they reside in the intestine for fewer than 2 min and are then absorbed by intestinal cells and distributed throughout the body (Kaletta & Hengartner 2006).

Compounds can also be absorbed through the cuticle (Stawicki et al. 2011). The *C. elegans* cuticle allows for diffusion of some materials into and out of the body through a permeability barrier established by the hypodermis (Stawicki et al. 2011), which facilitates the access of compounds to the target tissues. However, final concentrations of dimethyl sulfoxide (DMSO) solution higher than 0.6% shorten the life span of *C. elegans* (Solis & Petrascheck 2011). In addition, some compounds may be taken up via the worm's exposed sensory neuronal cilia, as observed by fluorescein

accumulation through their exposed cilia when living animals were placed in a solution of this dye (Perkins et al. 1986).

APPLICATION OF *CAENORHABDITIS ELEGANS* IN OBESITY STUDIES

Obesity can be commonly characterized as a disorder in energy homeostasis that develops when energy intake exceeds energy expenditure (Watts 2009). It has become one of the leading contributors to a variety of chronic diseases worldwide, including diabetes, cardiovascular disease, and hypertension. Thus, research on the identification of compounds that can regulate energy homeostasis could greatly facilitate the prevention and treatment of obesity. *C. elegans* is a great model for exploring the genetic regulation of fat storage because many aspects of fat synthesis and breakdown pathways characterized in humans are conserved in this simple model organism (Watts 2009).

Factors Affecting Fat Accumulation in *Caenorhabditis elegans*

Fatty acids are known to play important roles in selective permeability, membrane fluidity, and cell signaling and can be stored in lipid droplets after esterification to glycerol to form triacylglycerides (TAGs) (Watts 2009). In *C. elegans*, TAGs make up approximately 40–55% of total lipids depending on diet and growth stage (Ashrafi 2006). The lipid droplet is a ubiquitous organelle with a monolayer phospholipid membrane coated with various proteins and serves as the main fat storage site in *C. elegans* (Mak 2012). The capacity of fat accumulation depends on the homeostasis of lipogenesis and lipolysis.

Lipogenesis. *C. elegans* obtains fatty acids from its bacterial diet or via de novo synthesis from acetyl-CoA, which is the key substrate for synthesis of fatty acids (**Figure 4*a***) (Ashrafi 2007).

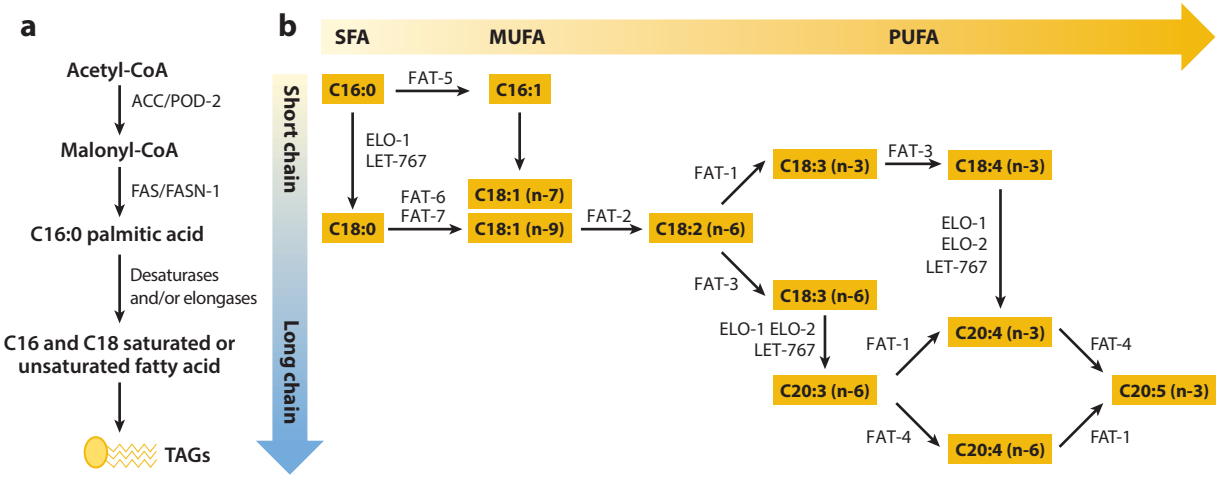

Figure 4

Synthesis pathways of (*a*) triacylglycerides (TAGs) and (*b*) polyunsaturated fatty acids (PUFAs) in *Caenorhabditis elegans*. Abbreviations: ACC/POD-2, acetyl-CoA carboxylase; FAS/FASN-1, fatty acid synthase; FAT-1, omega-3 desaturase; FAT-2, Δ12 desaturase; FAT-3, Δ6 desaturase; FAT-4, Δ5 desaturase; FAT-5, FAT-6, FAT-7, Δ9 desaturases; ELO, fatty acid elongase; LET-767, 3-ketoacyl-CoA reductase; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid.

Acetyl-CoA is carboxylated by acetyl-CoA carboxylase (ACC/POD-2) to form malonyl-CoA, which is then elongated stepwise by fatty acid synthase (FAS/FASN-1) to generate fatty acids with different lengths, mainly palmitic acid (C16:0) (Ashrafi 2007). Palmitic acid can be integrated into TAGs or phospholipids or can be modified by fatty acid elongases and/or desaturases to form a variety of long-chain polyunsaturated fatty acids (PUFAs) (Watts & Browse 2002). Storage of fatty acids involves the stepwise conversion of fatty acyl-CoAs derived from exogenous or endogenous sources to phosphatidic acid, diacylglycerol, and, ultimately, TAGs (Ashrafi 2007).

A key step of lipid synthesis and breakdown is the production of monounsaturated fatty acids (MUFAs) (**Figure 4*b***) (Watts 2009). The Δ9 desaturases catalyze the insertion of the first double bond into a saturated fatty acid at the C9 position. *C. elegans* encodes three Δ9 desaturases: FAT-5, FAT-6, and FAT-7. The FAT-5 desaturase is specific for palmitic acid (C16:0), whereas FAT-6 and FAT-7 mainly desaturate stearic acid (C18:0) (Brock et al. 2006, Watts & Browse 2000). Inhibition of the *fat-5*, *fat-6*, or *fat-7* gene is associated with reduced total TAG levels (Ashrafi 2007). In addition, another four fatty acid desaturases (FAT-1 through FAT-4), one 3-ketoacyl-CoA reductase (LET-767), and two fatty acid elongases (ELO-1 and ELO-2) have been shown to be involved in PUFA biosynthesis in *C. elegans* (**Figure 4*b***) (Watts 2009).

Lipolysis. Lipolysis plays important roles in providing energy and involves complex signaling cascades and sequential enzymatic activations (Lee et al. 2014). Mobilization of stored TAG is initiated by lipolytic enzymes, such as hormone-sensitive lipase. Liberated fatty acids are then activated into their respective acyl-CoA derivatives by acyl-CoA synthases/ligases. Breakdown of fatty acyl-CoA to acetyl-CoA occurs in peroxisomes or mitochondria via β-oxidation enzymes (Ashrafi 2007).

Food. In nature, *C. elegans* feeds on microorganisms, and in the laboratory a nonpathogenic strain of *Escherichia coli* OP50 is typically used as a standard food source, mainly owing to the fact that it forms a thin lawn on the agar in the plate that allows for optimal visualization of *C. elegans* (Brenner 1974, Stiernagle 2006). In addition, DA837 (a strep-resistant strain derived from OP50), HB101 (a B × K12 hybrid that forms a visibly thicker lawn than OP50 or DA837), and HT115 (DE3) (a K12-derived RNase III minus strain used for RNAi feeding experiments) have also been used as food sources in *C. elegans* (Boyer & Roulland-Dussoix 1969, Shtonda & Avery 2006, Timmons et al. 2001). Research showed that differences in the macronutrients of bacterial strains may be responsible for the different fat storage observed in *C. elegans* feeding on various strains. Therefore, the same strain of *E. coli* should be used for *C. elegans* in a series of experiments in which body fat is an end point (Brooks et al. 2009).

Caenorhabditis elegans as a Model for Obesity Study

Energy homeostasis, which is highly regulated by cellular and organism-wide networks, involves complex processes, including food sensation, nutrient intake, transport, storage, and energy expenditure (Watts 2009). *C. elegans* has been widely used as an in vivo model in obesity studies, typically by determining triglyceride accumulation as an end point along with the measurement of food intake and energy expenditure (Sun et al. 2016).

Food intake. The feeding of *C. elegans* depends on the action of the pharynx, a neuromuscular pump that joins the mouth to the intestine; thus, pharyngeal pumping rates determine and correlate with the amount of food intake (Avery & You 2012). Pumping is a cycle of contraction and relaxation, and pumping rate is measured by directly counting the contractions per

minute using a stereomicroscope. Alternatively, the food intake can be determined using *E. coli* XL1-blue transformed with green fluorescent protein (GFP), allowing visualization and quantification of fluorescent intensity in nematodes' bodies under a fluorescent microscope (Ding et al. 2015).

The microtiter plate-based bacterial clearing assay has been used to measure food intake by quantifying the change of the optical density of bacteria at 600 nm over time (Gomez-Amaro et al. 2015). Food intake per worm can be calculated as the bacterial clearance divided by the number of worms per well. The bacterial clearing assay provides a simple and direct quantitative measurement of food intake over time. However, the sensitivity and robustness of the assay essentially depend on several technical aspects of the protocol, including potential influence of test compounds on the growth of bacteria (Gomez-Amaro et al. 2015).

A complete understanding of feeding requires measuring not only the amount of food eaten but also the amount incorporated into the animal. To measure food absorption, a pulse-feeding assay was developed; for example, a ^{15}N pulse is delivered to *C. elegans* by feeding nitrogen-isotope-labeled (^{14}N and ^{15}N) bacteria (Gomez-Amaro et al. 2015). Nutrient (i.e., food) absorption is subsequently determined by measuring isotope incorporation into the worm proteome, which is proportional to the amount of labeled bacteria ingested. The greatest strength of the pulse-feeding method is that it directly establishes nutrient utilization independent of the culture medium and can provide meaningful information regardless of food intake status. However, the application of this assay may be limited by its complexity (Gomez-Amaro et al. 2015).

Energy expenditure. In *C. elegans*, energy expenditure can be inferred from oxygen consumption, which can be measured in a 96-well plate containing an oxygen-sensitive fluorescent film (Srinivasan et al. 2008). However, oxygen consumption alone is unlikely to be a sufficient marker of energy expenditure in many situations because of the inherent variability in the link between oxidation and phosphorylation (Salin et al. 2015).

Energy expenditure can be also estimated by locomotive activity using an automatic tracking system (Sun et al. 2016, Taki et al. 2013). This method is easy to handle and can be used for large-scale screening. However, it has been reported that movement accounts for only approximately 37% of the total energy expenditure of L4 larvae, and this fraction decreases further to 25% in adult animals (Vanfleteren & De Vreese 1996). Thus, locomotive activity may not completely reflect total energy expenditure.

Determination of total fat accumulation in *Caenorhabditis elegans*. To study the effect of specific conditions on adiposity in *C. elegans*, it is essential to quantify the amount of fat accumulation in this model. The total fat accumulation of *C. elegans* can be quantified chemically by thin layer chromatography (TLC) or biochemically by an enzymatic triglyceride assay kit (Lemieux et al. 2011, Sun et al. 2016). Furthermore, visualization of fat stores in individual worms can often be achieved by staining with fat-soluble dyes (Watts 2009). In recent years, label-free lipid analysis approaches, such as coherent anti-Stokes Raman scattering and stimulated Raman scattering, take advantage of the characteristic vibrational properties of lipid molecules to visualize fat stores in live animals (Wang et al. 2011). Lastly, transgenic strains expressing GFP fusion of lipid droplet–associated protein have been generated to label lipid droplets specifically in *C. elegans*. The size and number of GFP-marked lipid droplets correlate well with the fat storage status in vivo (Liu et al. 2014). The advantages and disadvantages of these methods are summarized in our recent review (Shen et al. 2017a).

Targets to Modulate Fat Accumulation in *Caenorhabditis elegans*

The function of genes involved in fat regulation of *C. elegans* has been extensively identified using targeted deletions, mutagenesis screens, and a genome-scale RNA interference (RNAi) screen (Ashrafi 2007). The inactivation of approximately 300 genes has been shown to cause a reduction in body fat, whereas nematodes with the inactivation of approximately 100 genes exhibited increased fat accumulation (Ashrafi 2007). Although the studies of these genes are still in their infancy, many of these identified *C. elegans* fat regulatory genes are functionally conserved in mammals.

spb-1. Sterol response element binding protein (SREBP) is a crucial regulator of cholesterol and fatty acid homeostasis in mammals (Bengoechea-Alonso & Ericsson 2007, Raghow et al. 2008). *C. elegans* possesses one SREBP ortholog, *sbp-1*, which is expressed in all metabolic tissues (McKay et al. 2003). Mutation of *sbp-1* in *C. elegans* exhibits delayed growth, reduced fat levels, and altered rates of expression of lipogenesis genes, such as *acc-1*, *fas-1*, fatty acid elongases (*elo-5* and *elo-6*), and stearoyl-CoA desaturases (*fat-6* and *fat-7*) (Ashrafi 2007). These observations suggest that SBP-1 shows the same function in *C. elegans* as SREBP-1c does in mammals.

nhr-49. Nuclear hormone receptors (NHRs) are transcription factors that function as metabolic sensors and master regulators of energy balance in mammals (Chawla et al. 2001). The *C. elegans* NHR-49 [homolog of mammalian peroxisome proliferator-activated receptors (PPARs)] plays an important role in β-oxidation of fatty acids, glycolysis, and gluconeogenesis as well as in the expression of at least 13 genes involved in energy metabolism (Watts 2009). The mutation in *nhr-49* downregulated the expression of genes that encode mitochondrial β-oxidation enzymes, including *acs-2* (encodes a mitochondrial acyl-CoA synthetase) and *ech-1* (encodes a mitochondrial β-oxidation trifunctional enzyme). Thus, deletion of *nhr-49* exhibits a high-fat phenotype (Van Gilst et al. 2005).

aak-2. AMP-activated protein kinase (AMPK) is an evolutionarily conserved heterotrimeric serine/threonine protein kinase complex that negatively regulates overall energy balance (Steinberg & Kemp 2009). It is a major cellular fuel gauge with catalytic α and regulatory β as well as γ subunits. In *C. elegans*, *aak-1* and *aak-2* encode the orthologs of mammalian AMPK catalytic subunits α1 and α2, respectively (Apfeld et al. 2004). AAK-2 is the subunit responsible for the kinase activity of AMPK, and its activation modifies several metabolic markers, such as lactate production, TAG, and oxygen consumption (Moreno-Arriola et al. 2016). Additionally, AAK-2 has been reported to regulate lipid mobilization through the adipose triglyceride lipase enzyme (ATGL-1) to maintain dauer larvae survival (Apfeld et al. 2004, Mair et al. 2011, Moreno-Arriola et al. 2016).

AAK activation was reported to increase oxidative metabolism and resulted in reduced triacylglycerol content in wild-type worms. Furthermore, AAK activation decreases the elongation steps and blocks the synthesis of PUFAs, mainly by reducing FAT-2, the rate-limiting enzyme in the conversion of MUFAs to PUFAs in *C. elegans* (Moreno-Arriola et al. 2016).

tub-1. One of the few single-gene mutations in mammals that cause obesity is *tubby* (Carroll et al. 2004). This gene is highly expressed in the hypothalamus and other tissues of the central nervous system, suggesting that this gene might be involved in controlling satiety or regulating feeding behavior (Watts 2009). *C. elegans tub-1* mutants show increased life span and increased fat accumulation compared to the wild type (Mukhopadhyay et al. 2005). It was reported that increased fat accumulation in *tub-1* mutants appears to be linked to impaired fat oxidation due to

reduced *kat-1* activity, which encodes 3-ketoacyl-CoA thiolase, a highly conserved enzyme in the mitochondrial fatty acid β-oxidation pathway (Mak et al. 2006).

cebp-2. CCAAT/enhancer binding proteins (C/EBPs) are a family of transcription factors that contain a highly conserved basic leucine-zipper domain at the C terminus, and at least six members of the C/EBP family have been isolated and characterized (C/EBPα–C/EBPζ) (Xu et al. 2015). CEBP-2, a *C. elegans* ortholog of mammalian C/EBPs, controls total body fat content by regulating fatty acid mitochondrial β-oxidation. The mechanism by which *cebp-2* influences fat storage is likely to be mainly through modulation of *ech-1.1* expression, which is the key enzyme in fatty acid β-oxidation (Xu et al. 2015). In addition, *cebp-2* affects fat storage by upregulation of the expression of *fat-5*, which participates in the formation of MUFAs in *C. elegans*. Loss of function of CEBP-2 created a low-fat phenotype in *C. elegans* (Xu et al. 2015).

Many other fat regulatory genes identified in *C. elegans* also have mammalian homologs. However, their roles in energy homeostasis have not yet been fully studied. Given that energy homeostasis is important to all living beings, *C. elegans* can be a useful organism model in the functional study of genes involved in fat metabolism as well as in the identification of food compounds that may regulate fat metabolism.

APPLICATION OF *CAENORHABDITIS ELEGANS* IN AGING STUDIES

Aging is characterized by progressive degenerative changes in tissue organization and function that increase the probability of mortality (Collins et al. 2008). Aging is associated with numerous chronic diseases, such as diabetes, cancer, and neurodegenerative disorders; thus, slowing aging and age-related degeneration would potentially be beneficial for human health (Niccoli & Partridge 2012). Although understanding human aging is an important goal, the experimental challenges of studying aging in humans or other vertebrates are substantial. By contrast, *C. elegans* is a leading model system for aging studies because of its genetics, relatively short life span, and ease of propagation of populations of synchronized individuals (Zheng & Greenway 2012).

Factors that Affect Aging in *Caenorhabditis elegans*

Typically, wild-type nematodes have a life span of ~21 days (Zheng & Greenway 2012). The longevity of *C. elegans*, as well as nematodes with target-gene deletion/inactivation, also depends on temperature and food availability.

Temperature. *C. elegans* can be best maintained at temperatures of 15°C to 25°C, and growth temperature is an important factor affecting its longevity (Stiernagle 2006). Generally, wild-type worms grown at low temperature (15°C) live more than twice as long as their high-temperature counterparts (25°C). The effect of temperature on life span in *C. elegans* is thought to be due to the differences in metabolic rate, which increases with the elevation of growth temperature (Van Voorhies & Ward 1999).

Although this temperature law is widely accepted, recently there has been a study indicating that temperature differentially regulates life span at different stages of life (Zhang et al. 2015). Exposure to low temperatures at the adult stage prolongs life span; however, it unexpectedly reduces life span at the larval stage (Zhang et al. 2015).

Food availability. When conditions are unfavorable for growth (such as limited food), a pheromone is produced to induce the larvae to enter a dormant state, namely the dauer stage

(Klass & Hirsh 1976, Van Voorhies & Ward 1999). Although dauer larvae are capable of moving, they exhibit significantly reduced activity and metabolic rate. Dauer worms can survive for months in this state without aging, and the time spent as dauer larvae is additive to their total longevity. When the animal experiences favorable conditions, the dauer worms molt to the L4 stage and enter their life cycle again (Klass & Hirsh 1976, Van Voorhies & Ward 1999).

Caenorhabditis elegans as a Model for Aging Study

In recent years, *C. elegans* has become a leading model in aging studies for the identification of mutations, compounds, and environmental factors that may significantly modulate the life span. The foundation of these studies is the measurement of the effects of potential treatments on age-related changes and life span (Collins et al. 2008).

Aging-related phenotype measurement. Because it is difficult to determine an individual's biological age, measurement of aging-related changes can provide a description of normal aging and markers of aging (Collins et al. 2008).

Pumping rate. Pumping rates, as indicative of food intake, of larval worms increase with age and reach their maximum at the L4/young adult stage, then display age-related decline (Huang et al. 2004). Therefore, pharyngeal pumping rate can be used as a marker of aging.

Body movement. The well-coordinated sinusoidal body movement characteristic of young hermaphrodites becomes progressively slower and less coordinated and ultimately ceases in old hermaphrodites (Collins et al. 2008). Body movement during aging can be measured as waves per minute observed under a dissecting microscope (Bolanowski et al. 1981) or can be measured by an automated worm tracking system (Taki et al. 2013). Additional categories of movement ability can be used as markers of aging: Class A for animals that move using rhythmic sinusoidal movement; Class B for animals that are uncoordinated and not active; and Class C for animals that are unable to progress but spontaneously move their head or tail and respond to touch (Herndon et al. 2002). Another study by Huang et al. (2004) defined two categories: fast moving and not fast moving.

Chemotaxis. *C. elegans* senses various environmental cues, such as chemical odorants (Collins et al. 2008). The assays for sensory perception typically involve exposure to a stimulus and measurement of a motor response (Collins et al. 2008). It is known that wild-type animals display progressive and age-related decline of chemotaxis in response to an attractive odorant (Glenn et al. 2004). Chemotaxis can be quantified as net displacement away from the original position, in the general direction of the odorant, as opposed to net displacement from the attractant (Glenn et al. 2004).

Accumulation of fluorescent compound. In *C. elegans*, intestinal autofluorescence (sometimes referred to as lipofuscin, or age pigment) accumulates with age and is often used as a marker of health or the rate of aging, which can be visualized by fluorescence microscopy in intact animals (Liao et al. 2011). Alternatively, a fluorescence or luminescence spectrophotometer can be used to analyze aqueous homogenates or organic extracts of pooled worms (Braeckman et al. 2002). Electron microscopy can also be used to visualize pigmented granules that appear to contain fluorescent material (Epsteina et al. 1972).

A recent study showed that this autofluorescent material is spectrally heterogeneous, and fluorescence under different excitation wavelengths has distinct biological properties (Pincus et al.

2016). Red autofluorescence (visible with a tetramethylrhodamine isothiocyanate filter set) correlates well with an individual's remaining days of life; blue autofluorescence (via a 4′,6-diamidino-2-phenylindole filter set) is an indicator of an individual's incipient or recent demise; and green autofluorescence (via a fluorescein isothiocyanate or GFP filter set) combines both properties. However, none of this autofluorescence increases under an oxidative stress condition, which indicates that this autofluorescent material in *C. elegans* is distinct from lipofuscin as reported in the mammalian literature (Pincus et al. 2016).

Life-span measurement. In addition to age-related changes, life-span study is another way to evaluate the environmental and dietary effects on aging, as the short life span of *C. elegans* makes this organism an ideal model in aging studies. Manually, the life span of the worm can be evaluated by recording the daily survival by microscopy at 25°C (traditional assay) or 37°C (high-temperature assay) (Fitzenberger et al. 2013, Shen et al. 2017b). In addition, the life span can be determined by a microtiter plate assay with SYTOX green, which is a fluorescent dye that binds to DNA only if the cell's membrane has been compromised (Gill et al. 2003). Others reported that the nematode's maximum velocity (MV) declined with age and the MV of a specific day (within one day of the first death in the cohort) showed the best correlation with life span (Hahm et al. 2015). In addition, automated systems, such as the WormFarm platform, have recently been developed to assist the life-span measurement of *C. elegans* (Xian et al. 2013). The advantages and disadvantages of these methods are summarized in our recent review (Shen et al. 2017a).

Genes and Signaling Pathway Targets to Modulate Life Span in *Caenorhabditis elegans*

In addition to environmental factors, genetic factors also play important roles in the regulation of the life span of *C. elegans* (Collins et al. 2008). Studies have shown that a large number of genes and signaling pathways that are highly conserved in mammals regulate the life span of this organism (Collins et al. 2008).

Insulin/IGF-1 signaling pathway. Insulin/insulin-like growth factor (IGF)-1 signaling (IIS) pathway is known to regulate aging and longevity in many organisms, ranging from simple invertebrates to mammals (Altintas et al. 2016). In *C. elegans*, many components of the insulin signaling pathway are evolutionarily conserved. Specifically, *daf-2* and *age-1* encode the nematode's sole insulin/IGF-1 receptor and phosphatidylinositol-3-OH kinase (PI3K), respectively, which are two key upstream components of IIS pathway (Zheng & Greenway 2012).

The activation of DAF-2 results in activation of AGE-1, which further leads to the phosphorylation and inactivation of the DAF-16 by preventing its nuclear translocation (Murphy & Hu 2013). *C. elegans daf-16* encodes an ortholog of mammalian Forkhead box O transcription factor (FOXO) and plays an essential role in mediating the downstream insulin signaling pathways, including stress response (Murphy & Hu 2013). Inhibition/deletion of either *daf-2* or *age-1* dramatically extended life span in *C. elegans* through the activation of DAF-16/FOXO via enhancing its translocation from the cytoplasm to the nucleus, which further induced the expression of genes that promote longevity (Altintas et al. 2016).

***sir-2.1*/SIRT1 signaling pathway.** The sirtuins are a family of proteins that act predominantly as nicotinamide adenine dinucleotide (NAD)-dependent deacetylases, are an evolutionarily conserved family of proteins in many organisms, and were originally characterized as regulators of life span in yeast (Kaeberlein et al. 1999, Sack & Finkel 2012). In *C. elegans*, *sir-2.1* encodes sirtuin,

which is responsive to metabolic changes in the cellular environment, including nutrient/energy availability and cellular stress (Lin & Guarente 2003). Overexpression of SIR-2.1 extends life span, suggesting *sir-2.1* is a conserved regulator of the aging process in the organism. Moreover, this life-span extension requires DAF-16/FOXO, a member of the *C. elegans* insulin/IGF signaling pathway (Tissenbaum & Guarente 2001).

***skn-1* signaling pathway.** Evidence suggests that oxidative stress plays an important role in normal aging by causing random deleterious oxidative damage to a variety of tissues (Muller et al. 2007, Park et al. 2009). The mammalian Nrf/CNC proteins (Nrf1, Nrf2, Nrf3, and p45 NF-E2) perform a wide range of cellular protective and maintenance functions (Blackwell et al. 2015). The most extensively described of these proteins, Nrf2, is best known as a regulator of antioxidant and xenobiotic defense (Blackwell et al. 2015). In *C. elegans*, *skn-1* encodes a sequence and functional ortholog of Nrf/CNC proteins. Under oxidative stress conditions, SKN-1 is activated through translocation to the nucleus, which induces the expression of genes involved in the oxidative-stress response (An et al. 2005). Mutation in *skn-1* leads to decreased resistance to oxidative stress and shortened life span, whereas overexpression of SKN-1 (constitutively localized to the nuclei of the intestine) contributes to increased longevity and resistance to oxidative stress (An & Blackwell 2003, An et al. 2005, Tullet et al. 2008).

Mitochondria-related genes. Mitochondria are essential organelles involved in energy metabolism via oxidative phosphorylation that play a vital role in diverse biological processes, including aging (Tsang & Lemire 2003). In *C. elegans*, mitochondria function has also been implicated as one of the primary determinants of the rate of aging (Anson & Hansford 2004). The *clk-1* was the first mitochondrial gene linked to life span in this nematode (Wong et al. 1995). This gene encodes a demethoxyubiquinone hydroxylase that is necessary for the biosynthesis of ubiquinone (Coenzyme Q10), which functions as a carrier of electrons and protons from mitochondrial complexes I and II to complex III (Miyadera et al. 2001). *clk-1* mutants exhibit extended life span with deceleration of a number of physiological events, such as swimming, pharyngeal pumping, and defecation (Anson & Hansford 2004).

Another mitochondrial-related gene, *mev-1*, which encodes a subunit of succinate-Coenzyme Q oxidoreductase in the mitochondrial electron transport chain, has been reported to regulate life span and aging in *C. elegans*. The mutation of *mev-1* results in hypersensitivity to oxidative stress, elevated production of mitochondrial reactive oxygen species, and shortened life span (Senoo-Matsuda et al. 2003). In addition, *isp-1* encodes an iron-sulfur protein, which is a subunit of the mitochondrial complex III in the mitochondrial membrane. Mutation of *isp-1* resulted in worms with an extended life span that exhibited greatly increased resistance to ROS (Feng et al. 2001).

Diet restriction–related genes. Dietary restriction without malnutrition has been reported to extend life span and reduce age-dependent decline and diseases in virtually all species tested (Greer & Brunet 2009). The life-extending effect of dietary restriction is also observed in *C. elegans*, where several distinct ways to impose dietary restriction have been found. Decreased bacterial concentration causes life extension in *C. elegans* (Hosono et al. 1989), and reduced bacterial concentration on agar plates (by decreasing amount of bactopeptone) extends life span without affecting reproductive capacity or body size (Hosono et al. 1989). Similarly, worms grown in a synthetic (axenic) medium in the absence of bacteria have slower development and a two-fold increase in life span (Houthoofd et al. 2003). In addition, a genetic model of dietary restriction in *C. elegans* is the strain corresponding to the mutation of *eat-2*, which encodes the pharyngeal nicotinic acetylcholine receptor subunit. The *eat-2* mutant worms exhibit a decreased pumping

rate (~40% of wild type), and *eat-2* mutants exhibit an approximately 25% extension of median life span compared to wild-type worms (Lakowski & Hekimi 1998, Schleit et al. 2011).

Because many of these genes identified in *C. elegans* are conserved in mammals, research on identification of and the mechanisms associated with the genes and signaling pathways in the organismal aging will greatly facilitate the development of preventive and/or therapeutic treatment to promote healthy aging and potentially combat age-related disease in humans.

APPLICATION OF *CAENORHABDITIS ELEGANS* IN STUDYING ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a type of neurodegenerative disease characterized by mental decline, memory loss, and cognitive deterioration (Alzheimer's Dis. Int. 2016). For people with AD, symptoms generally first appear in their mid-60s, slowly developing and worsening over time. This rising disease has been estimated to affect more than 40 million people worldwide (Alzheimer's Dis. Int. 2016) and is increasingly becoming a major concern for humankind in light of the aging population. Sadly, although a few drugs can temporarily halt or improve symptoms, no cure is currently available. Therefore, the major goal of Alzheimer's research is to find therapies that could treat the disease or prevent it from developing. *C. elegans* has emerged as a promising in vivo model for studying AD.

Factors Affecting Alzheimer's Disease

Although the cause of AD has not been fully elucidated, the pathologic hallmarks have been identified: the presence of senile plaques and neurofibrillary tangles (Kidd 1964, Krigman et al. 1965, Luse & Smith 1964). These aberrant protein aggregates were predicted to result in nerve cell death by blocking intercellular communication and disrupting the normal cell cycle, therefore contributing to the development of AD symptoms (Bloom 2014).

The major component of senile plaques is β-amyloid (Aβ), insoluble peptides consisting of 40–43 amino acids, which is cleaved from amyloid precursor protein (APP) via a proteolytic pathway (Haass et al. 1992). In mammals, there are two proteolytic pathways for APP processing: amyloidogenic and nonamyloidogenic (Alexander et al. 2014; Haass et al. 1992, 1994). The amyloidogenic pathway results in the production of Aβ peptides by β-secretase and γ-secretase, which are thought to be associated with AD development. In the nonamyloidogenic pathway, APP is cleaved by proteases α- and γ-secretase into nontoxic proteins.

The other pathologic marker of AD, neurofibrillary tangles, is formed by protein tau aggregation (Alonso et al. 1996). Tau, mainly expressed in neuron cells, is a highly soluble protein that belongs to the microtubule-associated protein family. The function of tau protein is to promote the stabilization and assembly of microtubules by regulating their phosphorylation level. In the nervous tissue of AD patients, tau protein is abnormally hyperphosphorylated, leading to the self-aggregation of soluble tau into extremely insoluble fibrillar deposits and failure to stabilize microtubules properly, therefore affecting neuron function and resulting in neuron degeneration (Alonso et al. 1996, Billingsley & Kincaid 1997).

Caenorhabditis elegans as a Model for Alzheimer's Disease

Because *C. elegans* does not naturally form abnormal Aβ and tau aggregates, various nematode strains that express human pathologic proteins were constructed for AD research. Transgenic *C. elegans* AD models can be divided into two categories: the Aβ model and the tau model, which

were specifically designed and constructed to mimic the pathologic role of Aβ and tau in AD development as described above.

Aβ model. In *C. elegans*, there is a sole APP homolog, APL-1 (Daigle & Li 1993). However, with APL-1, *C. elegans* does not form β-amyloid naturally for two reasons: First, the *C. elegans* APP homolog APL-1 does not contain an Aβ sequence (Daigle & Li 1993) and second, *C. elegans* does not have β-secretase (Link 2006). Therefore, the processing of APL-1 in *C. elegans* is rather exclusively via the nonamyloidogenic pathway (Link 2006). Thus, to mimic the pathology of Aβ peptides in AD, various transgenic nematode strains that express human Aβ peptides in either muscle cells or neurons were constructed for the investigation of potential AD treatments.

For muscle cell models, animals were generated with constitutive (Link 1995) or temperature-inducible expression (Link et al. 2003) of human $A\beta_{1-42}$ in muscle cells, which can lead to progressive or rapid paralysis with development of AD. For neuron cell models, transgenic worms were constructed by expressing human Aβ under pan-neuronal promoter *snb-1*, resulting in learning-deficit behavioral phenotypes, including deficits in odorant preference associated-learning behavior; serotonin-related, experience-dependent learning; and reproduction (Dosanjh et al. 2010). These measurable phenotype changes induced by human Aβ can be monitored as indicators to study the effects of bioactives on AD prevention or treatment.

Recently, an Aβ model expressing Aβ–green fluorescence protein (GFP) fusion protein was developed that allows visualization of the dynamics of Aβ aggregation (Ochiishi et al. 2016). Insoluble aggregation of fused protein tends to affect the proper folding of GFP, thereby leading to the loss of fluorescence. Thus, transgenic nematodes were constructed by introducing Aβ-GFP plasmids with a short linker (0–3 amino acids) and long linker (14 amino acids), and it was found that a long linker is required for GFP expression. In addition to those transgenic strains that express human proteins, a *C. elegans* model with pan-neuronal APL-1/APP expression was also generated, which exhibits deficits in olfactory and gustatory learning behavior and touch habituation (Ewald et al. 2012).

Tau model. The *ptl-1* encodes the sole tau homolog in *C. elegans* (Goedert et al. 1996). The loss of *ptl-1* function mutant showed normal development but reduced viable progeny number and touch sensitivity (Gordon et al. 2008). Moreover, PTL-1/tau was found to regulate neuron integrity and longevity in *C. elegans* (Chew et al. 2013). Because wild-type PTL-1/tau has not been reported to aggregate into fibrils, transgenic *C. elegans* strains that express human tau have been constructed for the research of tau pathology in AD.

Different transgenic *C. elegans* strains that model tau pathology were constructed by expressing human tau proteins in worm nerve cells. Many of these strains were developed to express wild-type tau or mutant tau proteins (Fatouros et al. 2012, Kraemer et al. 2003, Miyasaka et al. 2005). However, there is no mutant tau protein that has been found to be related with AD development; instead, mutant tau proteins were found to be more closely associated with frontotemporal dementia (FID) or Parkinsonism and therefore might not be appropriate models for tau pathology in AD.

A transgenic *C. elegans* strain developed by Brandt et al. (2009) expresses human tau and pseudohyperphosphorylated (PHP)-tau under the pan-neuronal *rgef-1* promoter. Animals expressing either wild-type tau or PHP-tau exhibit progressive age-dependent phenotype of uncoordinated locomotion, but only PHP-tau animals showed axonal abnormalities in inhibitory motor neurons. This might be a representative model for tau modification in AD.

Gene/Signaling Pathway Targets to Modulate Alzheimer's Disease

Based on AD transgenic *C. elegans* models listed above, numerous studies investigated AD therapies and identified several genetic pathways that were possibly associated with AD development.

Transforming growth factor beta signaling. Transforming growth factor beta (TGFβ) signaling pathway plays fundamental roles in regulating a wide range of cellular processes, such as cellular homeostasis, apoptosis, and immune function (Massagué & Gomis 2006). TGFβ could activate both canonical (Smad dependent) and noncanonical (non-Smad dependent) signaling pathways. Among them, it has been suggested that the Smad-dependent-TGFβ pathway is closely associated with AD pathology and is neuroprotective, because it was shown that deficient TGFβ-Smad signaling contributes to the accumulation of Aβ deposit and Aβ-induced neurodegeneration in various AD models (Von Bernhardi et al. 2015).

In *C. elegans*, five TGFβ-related genes have been identified (*daf-7*, *dbl-1*, *unc-129*, *tig-2*, and *tig-3*) (Gumienny & Savage-Dunn 2013). DAF-7 and DBL-1 function through canonical TGFβ signaling pathway, whereas *unc-129* is associated with noncanonical signaling (Gumienny & Savage-Dunn 2013). The functions of the other two, *tig-2* and *tig-3*, are not yet known (Gumienny & Savage-Dunn 2013). Therefore, TGFβ-mediated cell signaling maintains both canonical and noncanonical mechanisms in *C. elegans* that correlate with other animal models. Haque & Nazir (2016) demonstrated that loss of the Smad transcription factor SMAD-9 in TGFβ signaling cascade accelerated Aβ aggregation and related outcomes in the *C. elegans* AD model, suggesting the potential of TGFβ signaling as a potential drug target for AD treatment.

Proteostasis. Perturbation of proteostasis, the biological process that controls the biogenesis, folding, and degradation of proteins, leads to the accumulation of aberrantly folded and aggregated proteins, which are known to be associated with many age-dependent neurodegenerative diseases, including AD (Hipp et al. 2014, Morawe et al. 2012). Proteasomes are the protein complex that is responsible for degradation of unneeded and damaged proteins, and it has been reported that in the nervous tissues of AD patients, proteasome function is significantly impaired (Keller et al. 2000). The impaired proteasome activity affects Aβ degradation, resulting in the accumulation of Aβ aggregates, which, in turn, further aggravates proteasome deterioration (Hong et al. 2014).

In *C. elegans* AD models, many bioactives have been found to activate proteasome activity, for example, 18α-glycyrrhetinic acid, a known proteasome activator, and significantly decrease Aβ deposits, thereby lessening the progression of AD phenotype in *C. elegans* AD model (Papaevgeniou et al. 2016). Likewise, deceleration of AD pathology was also reported in quercetin (Regitz et al. 2014) and resveratrol (Regitz et al. 2016) by stimulating protein degradation in *C. elegans* AD models. In addition, stress-related pathway IIS signaling (see discussion below) is intricately connected with proteasome activity. However, how IIS modulates proteasome activity remains controversial (Matilainen et al. 2013, Stout et al. 2013).

Hassan et al. (2009) reported that AIP-1, a human arsenite-inducible, RNA-associated protein (AIRAP) homolog, is a positive regulator of proteasome function and exhibits a protective effect against Aβ toxicity. This suggests that AIP/AIRAP might also be a useful potential target for investigating the therapies of AD.

Stress-related pathways. Stress-related pathways are highly conserved in *C. elegans*. As discussed in the aging section, IIS signaling regulates several transcription factors, such as DAF-16, HSF-1, and SKN-1. In addition, the literature suggests that stress-related pathways play a role in the alleviation of AD pathology (Cohen et al. 2006, Dostal et al. 2010, Fonte et al. 2008). Cohen

et al. (2006) found that reduced IIS activity could lower the Aβ toxicity because the insulin/IGF-1 receptor gene *daf-2* RNAi significantly decreased Aβ-induced paralysis and increased life span in a *C. elegans* AD model that constitutively expresses muscle-specific $A\beta_{1–42}$. This IIS-mediated detoxification of Aβ aggregates requires both *daf-16* and *hsf-1* (Cohen et al. 2006). Moreover, it was found that overexpression of the *C. elegans* chaperone protein HSP-16.2 significantly suppresses the human $A\beta_{1–42}$ toxicity in a *C. elegans* AD model, which is the downstream target of DAF-16 and HSF-1 (Brunquell et al. 2016, Fonte et al. 2008, Murphy et al. 2003). In addition, stress-related transcriptional factor Nrf2/SKN-1 has been found to be involved in AD detoxification (Dostal et al. 2010). Dostal et al. (2010) demonstrated that the protective effect of coffee extract against Aβ toxicity was *skn-1* dependent, indicating the potential involvement of the Nrf2 detoxification pathway.

Dietary restriction also exhibits a protective effect against AD-induced proteotoxicity in *C. elegans* (Steinkraus et al. 2008). In a *C. elegans* AD model, dietary restriction suppresses progressive paralysis caused by $A\beta_{1–42}$-induced proteotoxicity, which is independent of the IIS pathway but requires *hsf-1* (Steinkraus et al. 2008).

SUT-1/SUT-2. *sut-1* encodes an RNA- and snRNP-binding protein (SUT-1/SL26p) in *C. elegans* that has been proved to be required for tau-induced neurotoxicity (Kraemer & Schellenberg 2007), although the human homolog of SUT-1 is yet to be identified.

SUT-2 is an ortholog of human MSUT-2 (zinc finger CCCH-type containing 14), and the loss-of-function *sut-2* mutant exhibits significantly increased resistance to human tau neurotoxicity (Guthrie et al. 2009). Consistently, overexpression of SUT-2 protein enhances the tau-induced neuronal dysfunction (Guthrie et al. 2011), suggesting that SUT-2 might be a potential drug target for tauopathy therapies.

CONCLUSION

C. elegans is a well-established in vivo research model for many human diseases. It not only allows studies of genes that are homologous to human genes but also favors transgenic expression of human disease-related proteins. Furthermore, *C. elegans* provides an easily traceable genetic background that permits the study of knockdown or knockout of disease-related genes. In this review, we discussed the advantages, methods, and genetic relevance of using *C. elegans* as a model for obesity, aging, and AD studies, aiming to provide instructive information to investigate food-based bioactive compounds for disease prevention and treatment using *C. elegans* as an animal model.

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