Review

The role of nutrition and oxidative stress as aging factors in *Caenorhabditis elegans*

Kayo Yasuda,[1,*] Masaki Miyazawa,[1] Takamasa Ishii,[2] and Naoaki Ishii[3]

[1]Department of Health Management, Undergraduate School of Health Studies and [3]Office of Professor Emeritus, Tokai University, 4-1-1 Kitakaname, Hiratsuka, Kanagawa 259-1292, Japan
[2]Department of Molecular Life Science, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan

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The molecular mechanism of aging, which has been a "black box" for many years, has been elucidated in recent years, and the nematode *C. elegans*, which is a model animal for aging research, has played a major role in its elucidation. From the analysis of *C. elegans* longevity-related mutant genes, many signal transduction systems, with the insulin/insulin-like growth factor signal transduction system at the core, have emerged. It has become clear that this signal transduction system is greatly affected by external nutrients and is involved in the downstream regulation of oxidative stress, which is considered to be one of the main causes of aging.

Key Words*: nutrition, oxidative stress, aging, *Caenorhabditis elegans

Research in the field of on aging began with comparisons of different animals or cells; the general findings are that larger animals and animals with lower specific metabolic rates tend to live longer.[1,2] This suggests that energy metabolism is deeply involved in aging. However, it has not been possible to clarify the mechanism of aging from comparative studies. A breakthrough occurred with the isolation of the mutant *age-1* from *Caenorhabditis elegans* (*C. elegans*).[3] The *age-1* mutant showed a tendency for longevity that was not affected by the period until maturity; the age-related mortality rate (Gompertz function) was lower than the wild-type, and the speed of aging was slowed. Thus, *age-1* was recognized as the first longevity gene. Since then, a large number of lifespan-related mutants have been isolated from *C. elegans*, and analysis of their genetics has elucidated the molecular mechanism of aging. The longevity and senescence genes isolated so far can be searched from The Aging Gene Database (http://genomics.senescence.info/genes/).

The gene for *age-1* is PI3 kinase; the gene for another long-lived mutant, *daf-2*, has a conserved sequence similar to that of the human insulin-like growth factor (IGF) receptor.[4,5] Subsequently, these two proteins were shown to be key components of the insulin/IGF-1 signaling pathway involved in energy metabolism. Furthermore, it was also revealed that abnormal Dauer formation (DAF)-16, which is homologous to the human forkhead box protein (FOXO) family of genes, is a transcription factor that regulates gene expression downstream of this pathway.[6] DAF-16 is involved in many signaling pathways, such as aging, development, stress response, immunity, and metabolism.[7,8] It is now believed that the mechanism of longevity control via the insulin/IGF-1 signaling system is common not only in *C. elegans*, but also in many other organisms, including *Drosophila*, mice, and humans.[9]

Target of Rapamycin (TOR) regulates insulin resistance and autophagy-mediated energy supply through nutritional status-responsive transcription factor activation, translation, and proteolysis. The inhibition of TORC1 has been reported to extend lifespan in yeast, *C. elegans*, *Drosophila*, and mouse, and to suppress the development of various geriatric diseases.[10] In addition to being stimulated by various growth factors, TORC1 is also regulated by a different mechanism through amino acid metabolism, which is thought to reflect the nutritional status of cells. TOR was found to be a target protein of rapamycin, which is known as an immunosuppressant, and the administration of rapamycin has been reported to extend the lifespan.[11]

Signal transduction pathways do not work independently; instead, they form relationships with mutual control. AKT, which is the center of the insulin/IGF-1 signaling pathway, regulates AMP-activated protein kinase (AMPK) as well as the FOXO family of genes. AMPK is evolutionarily conserved and is known to be activated when intracellular energy is low and to act as an energy sensor involved in energy production and inhibition of adenosine triphosphate (ATP) utilization.[12] Furthermore, AMPK regulates the phosphorylation of Raptor, one of the TORC1 complexes, and S6 kinase (S6K), which is downstream of mTOR, regulates AMPK phosphorylation. It has become clear that the relationship controls downstream genes.[13]

In contrast, skinhead-1 (SKN-1) is thought to have antioxidant function in *C. elegans* because it shares homology with nuclear respiratory factor (NRF), which is involved in the oxidative stress response. TORC1 and TORC2 negatively regulate SKN-1.[14,15] It has become clear that SKN-1 is regulated by AMPK through mTOR and AKT serine/threonine kinase (AKT) via the insulin/IGF signaling system.[13]

The insulin/IGF signal transduction system and SKN-1 function cooperatively to regulate various genes, especially antioxidant enzyme genes, to remove active oxygen that is generated by growth factors, nutrition, energy, and stress and shortens lifespan.

C. elegans as a Model Animal for Aging Research

C. elegans is a type of roundworm (adult length, approximately 1 mm) that can be easily grown in Petri plates on a simple diet of *Escherichia coli*. They reproduce rapidly with a life cycle of approximately 3.5 days at 20°C. *C. elegans* consists of a hypodermis, neurons, musculature, and somatic gonadal structures. Adults contain 959 somatic cells. *C. elegans* has received much attention as a genetic model, partly because its hermaphroditic mode of reproduction permits the ready isolation of mutants and allows rapid inbreeding. These genetic approaches have been useful for identifying and mapping the genes that regulate aging.[16] Mutations can be readily analyzed at the molecular level, thus providing specific insights to the various biochemical

*To whom correspondence should be addressed.
E-mail: Yasuda@tokai.ac.jp

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and physiological elements of lifespan determination.

The elucidation of the entire nucleotide sequence of *C. elegans*, which has approximately 100 Mb bases, was the first achieved for a multicellular organism.[17,18]

C. elegans offers several clear advantages for aging research, including a short maximum lifespan of approximately 30 days. In addition to these advantages, an adult soma consisting of fewer than 1,000 cells, all of which are postmitotic, offers the ability to detect cumulative age-related cellular alterations.

Oxidative Stress and Aging

Various theories have been proposed for the cause of aging, but the free radical theory, which is currently the most popular, was first proposed by Harman in 1956.[19] Reactive oxygen species (ROS) are mainly generated as a by-product in the process of energy production from the mitochondrial electron transport system. This ROS attacks molecules in the body, causing the deterioration of cell function, aging due to apoptosis, and transformation due to gene mutation. We found that the *C. elegans* with a mutation in the mitochondrial electron transport complex II subunit succinate dehydrogenase complex subunit C (SDHC) generated excessive ROS, prematurely accumulated oxidized proteins, which are indicators of aging, had increased frequencies of apoptosis and mutation, and has been shown to have a short lifespan.[20]

However, organisms have evolved enzymes to scavenge ROS. It is known that the long-lived mutant *age-1* has an age-dependent increase in the gene expression of the antioxidant enzymes superoxide dismutase (SOD) and catalase, which makes it resistant to oxidative stress and suppresses lifespan shortening under oxidative stress.[21] DAF-16, a transcription factor downstream of this pathway, regulates Mn-SOD gene expression, suggesting that insulin/IGF-1 signaling regulates the expression of antioxidant genes.[22] Thus, long-lived mutants tend to exhibit resistance to many stresses, including oxidative stress. The balance between the amount of active oxygen in the body and the antioxidant capacity has a notable influence on lifespan determination.

In contrast, in many cases, the expected longevity effect cannot be obtained in animals transfected with antioxidant enzyme genes.[23] This is probably because the types and sites of generation of ROS differ among cells and individuals, and the expression of the introduced antioxidant enzyme gene is not specifically adapted to this.

Nutrition on Aging

Food intake. The lifespan of *C. elegans* varies greatly depending on rearing conditions, including the concentration and temperature of the *E. coli* food.[24] In the laboratory, *C. elegans* is generally cultured on *E. coli* bacteria that are seeded on agar plates. Generally, the slow-growing OP50 strain, which is auxotrophic for uracil, is used because the thin bacterial lawn makes microscopic studies easier.

Several methods have been used to study dietary restriction (DR) in *C. elegans*, although all methods are associated with some problems. One such problem is that the normal food source in the laboratory, *E. coli*, is toxic to *C. elegans*. In old worms, it was observed that *E. coli* cells frequently accumulate in the pharynx and the intestine of the worm.[25] Feeding *C. elegans* with *E. coli* killed by ultraviolet irradiation or by antibiotics resulted in a 16–40% extension of lifespan.[25,26] It is therefore likely that reducing the *E. coli* intake not only lengthens the worm's lifespan through DR, but also through reduced *E. coli* toxicity.

Klass decreased the bacterial concentration in suspension culture to impose DR on *C. elegans*. He found a mean lifespan extension of 60% when the bacterial density was decreased from 109 to 108 bacterial cells per milliliter (higher concentrations led to decreased lifespan and decreased reproductive capacity, likely caused by hypoxic stress).[27] When grown in axenic media, the lifespan of worms is approximately twice as long as that for populations maintained on *E. coli*.[28] Axenically cultured worms have much slower development and a severely affected brood size compared with those grown in monoxenic culture conditions, consistent with nutrient deprivation observed in other DR regimens.

In addition, the use of bacteria other than *E. coli* also greatly alters the lifespan. *C. elegans* does not have an enzyme [e.g., nitric oxide synthase (NOS)] that synthesizes nitric oxide (NO), but using NO-producing bacteria (Bacilli) as food prolongs its lifespan.[29] DAF-16 and HSF-1, the master activator of heat shock, are involved.

With the goal of healthy longevity, research is also exploring the possibility of ingesting probiotics using nematodes.[30] Providing probiotics to nematodes not only reduces heat and oxidative stress but also extends their lifespan.[31] Feeding *Streptococcus thermophilus*, a type of lactic acid bacterium, appeared to extend lifespan, reduce the accumulation of lipofuscin, an indicator of aging, and maintain activity.[32] This effect is thought to be due to DAF-16-mediated antioxidant activity.

As *C. elegans* feeds on bacteria, there is the disadvantage that it is difficult to distinguish whether a substance acts directly on the cells or through the metabolites taken up by the bacteria.[33] Therefore, treatment methods for minimizing the metabolism of *E. coli* are also under investigation.[34]

Dietary restriction. DR is the simplest means of altering metabolism and has been shown to be involved in extending the lifespan in a variety of animals. However, it has been found that the genes corresponding to DR differ depending on the method used, such as changes in calorie intake, changes in nutritional components, and changes in meal times.

Insulin/IGF-1 is thought to be involved in *C. elegans* DR.[35] However, there are many reports that insulin/IGF-1 and its downstream DAF-16 are not involved,[3] and defective PHArynx development (PHA-4), SKN-1. There is a report that one acts specifically on calorie restriction (CR).[36] FOXA, which is homologous to PHA-4, is involved in the regulation of glucose homeostasis, and CR increases the transcriptional activity of PHA-4 and induces SOD gene expression. It has also been suggested that SKN-1 induces a longevity effect by reducing the oxidative stress of CR. SKN-1 is thought to act on oxidative stress suppression through a different pathway from that of FOXO. There is one report that SKN-1 is involved in lifespan extension in *C. elegans*, but it does not act as an antioxidant;[37,38] however, this conclusion is questionable because a very strong oxidant was used.

Sugars, amino acids, and lipids. When considering diet, it is more important to note that nutrient-detecting factors and fat and carbohydrate metabolism vary with each nutrient and are involved in longevity. Glucose is the most basic energy source; although it is known that glucose loading also promotes fat accumulation in *C. elegans* similar to in humans. Glucose loading in *C. elegans* is known to increase glucose uptake via insulin/IGF-1 signaling (IIS) and shorten lifespan.[39,40] Lee *et al.*[39] speculate that this is due to the suppression of DAF-16 in the insulin signaling system via the aquaporin gene, *aqp-1*, involved in glucose metabolism. However, glycated proteins are produced by the aminocarbonyl (Maillard) reaction, and advanced glycation endproducts further denature proteins, increase inflammation and oxidative stress, and accelerate aging.

When 2-deoxy-D-glucose (2DG) is used to suppress glycolysis and glucose metabolism, AMP-activated kinase (AAK)-2, one of the subunits of AMPK, is involved and lifespan is extended.[41] This may be due to the effect of DR on suppressing the insulin/

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IGF-1 signaling pathway. The inhibition of glucose utilization by 2DG promotes β-oxidation of fatty acids and oxygen respiration in mitochondria, resulting in increased production of ROS and increased oxidative stress. This has a paradoxical effect of increasing oxidative stress and thereby extending the worm's lifespan. As a potential explanation, it has been shown that increased oxidative stress activates transcription factors such as NRF2, FOXO3a, and phosphatidylglycerol phospholipase C (PGC) 1α, and enhances the expression and activity of antioxidant enzymes and detoxification enzymes. Consequently, resistance to various stresses is enhanced, and it is thought that the effects of delaying aging and extending lifespan are obtained.[41]

In *C. elegans*, the essential dietary amino acids are arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, isoleucine, and valine.[42] Many amino acids extend the lifespan of *C. elegans*, but the mechanism varies greatly depending on the type and concentration of the amino acid.[43] Moreover, methionine has been shown to be an important nutrient in regulating lifespan through caloric restriction in many organisms.[42,44] Recently, it has been reported that feeding *C. elegans* with *S*-adenosyl-L-homocysteine (SAH), a metabolite of methionine, prolongs their lifespan.[45]

Obesity occurs when the rate of fat accumulation exceeds the rate of breakdown; fat accumulation is known to cause insulin resistance and heart disease. However, lipid metabolism plays an important role in cells not only as an energy source but also as a raw material for cell membranes, cholesterol, and digestive enzymes. *C. elegans* stores lipids and glycogen in the gut and subcutaneous tissue.[8] The *C. elegans* transcription factor max-like (MXL-3), known to be involved in the regulation of lipid metabolism, which is closely related to aging and geriatric diseases, suppresses the gene expression of lipolytic enzymes normally localized in lysosomes. Starvation inhibits MXL-3 transcription, and HLH-30, a transcription factor homologous to TFEB, induces lysosomal-localized lipolytic enzymes and autophagy genes, thereby activating lipophagy. Consequently, fat breakdown is accelerated.[46] It is reported that HLH-30 cooperates with DAF-16 to control stress resistance and lifespan.[47] Mutants lacking the MXL-3 gene are starved and live longer. It has been reported that MXL-3 binds to the transcription factor SKN-1 involved in oxidative stress response.[48] We also found that MXL-3 is also involved in oxidative stress responses (unpublished data). It has also been suggested that SKN-1 may regulate lipid metabolism in response to lipid signals.[48]

The β-oxidation of fatty acids is enhanced by DR and AMPK activation, and the overexpression of genes involved in β-oxidation extends lifespan in *Drosophila*. It has been reported that the enhancement of β-oxidation of fatty acids itself has a life-extending effect through a mechanism similar to caloric restriction.[49] It was found that the overexpression of fatty acid-binding protein and dodecenoyl-CoA delta-isomerase gene, which are involved in fatty acid β-oxidation, extended lifespan through a similar mechanism to caloric restriction. The activation of the transcription factor FOXO has been suggested as a possible mechanism.

Ketone bodies are made from acetyl-CoA, which is produced in excess when the β-oxidation of fatty acids actively occurs. In mitochondria, β-hydroxybutyrate (βHB) is ultimately catabolized to acetyl-CoA and metabolized as part of the TCA cycle. Class I histone deacetylases are inhibited when the ketone body βHB levels increase owing to starvation or caloric restriction. βHB induces the hyperacetylation of histones in the gene promoter regions, resulting in the increased expression of genes that constitute the FOXO3a transcription factor network. Furthermore, the activation of FOXO3a induces the expression of Mn superoxide dismutase and catalase, and suppresses oxidative stress.[50] The histone deacetylase (HDAC) inhibitors sodium butyrate and valproic acid have extended lifespan in *C. elegans*,[51,52] and RNAi-mediated knockdown of HDACs class I (*hda-2* or *hda-3*) increased lifespan. The addition of βHB to *C. elegans* increased life expectancy by approximately 20%.[53] The addition of βHB also upregulated βHB dehydrogenase activity and increased oxygen consumption in *C. elegans*. The RNAi-mediated knockdown of short-chain dehydrogenase and SKN-1 target genes inhibited βHB-induced lifespan extension and βHB dehydrogenase activity. Moreover, βHB supplementation enhanced thermotolerance in *C. elegans* and partially prevented glucose toxicity. βHB-mediated lifespan extension required the DAF-16/FOXO and SKN-1/Nrf longevity pathways, the sirtuin SIR-2.1, and the AMP kinase subunit AAK-2. This result suggests that D-βHB extends lifespan by inhibiting HDAC and activating conserved stress response pathways.

Resveratrol. The polyphenol resveratrol, abundant in grapes and other plants, has a lifespan-prolonging effect in *C. elegans* and *Drosophila*, and increases the health and lifespan of mice and monkeys fed a high-fat diet.[54] This effect is due to activation of one of the nicotinamide adenine dinucleotide (NAD)-dependent sirtuin genes, Sir2p.[55] Silent information regulation 2 (SIR2) is a deacetylase that removes the acetyl group of histones, which are proteins that make up chromosomes. For this enzyme to deacetylate histones, it requires NAD^+, which is responsible for carrying energy in the cell. NAD^+ is involved in the process through which cells produce energy from nutrients they have taken up, and SIR2 is thought to be involved in its regulation. The overexpression of SIR2 has been reported to induce longevity in *C. elegans*.[56] There is also a report that resveratrol activates AMPK.[55] *SirT1*, one of the sirtuin genes, is also involved in FOXO acetylation.[57] The regulation by MXL-3 and HLH-30 is thought to function independently of SIR-2.1. DR activates the sirtuin gene. This is thought to be due to the increase in NAD^+ caused by the activation of mitochondrial metabolism resulting from the decrease in glycolysis by DR.

Conclusion

The analysis of gene mutations conferring longevity in *C. elegans* has revealed that the insulin/IGF signaling pathway is closely involved in aging. An antioxidant enzyme gene that scavenges ROS is regulated downstream in this pathway. It has also been clarified that SKN-1 (Nrf) regulates genes coding for antioxidant enzymes. SKN-1 is regulated by AMPK involved in the insulin signaling pathway, and it is thought that the FOXO and SKN-1 pathways cooperate to regulate antioxidant enzyme genes. Raptor, one of the mTORC1 complexes, is regulated by AMPK and regulates SKN-1. The insulin signaling pathway is affected by sugars, such as glucose and amino acids, but Rictor, one of the mTORC2 complexes, regulates lipid metabolism, is involved in lifespan,[58] and regulates AMPK. Therefore, it is thought to play a role in the overall metabolism of sugars, amino acids, and lipids, as well as stress response, and is involved in aging (Fig. 1).

Basic research on aging is a major part of anti-aging research, which is currently receiving much attention from researchers.

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Abbreviations

AAK	AMP-activated kinase
AKT	AKT serine/threonine kinase
AMPK	AMP-activated protein kinase
ATP	adenosine triphosphate

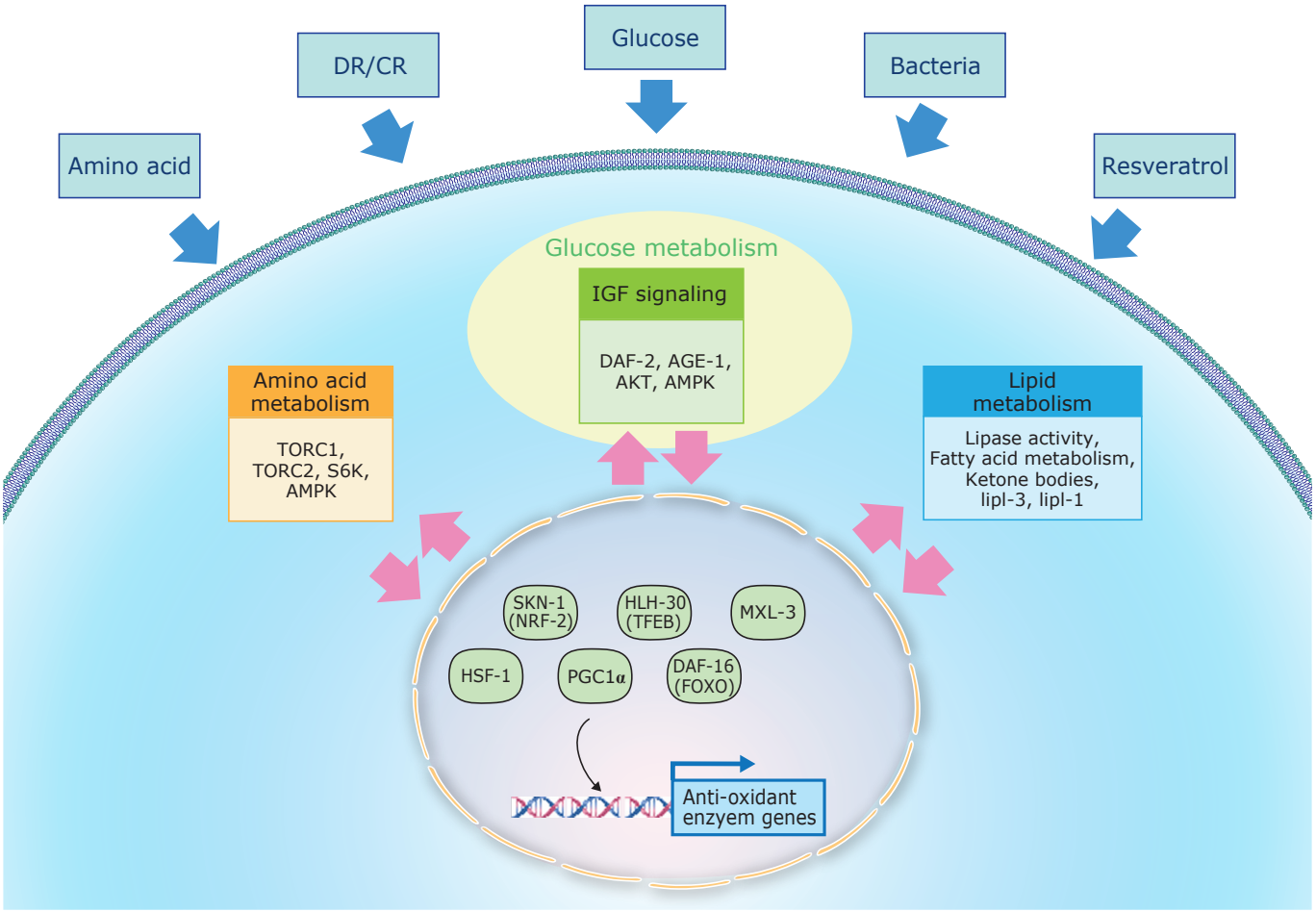

Fig. 1. Summary of recent progress in the roles of nutrition and oxidative stress in *Caenorhabditis elegans* longevity.

βHB	β-hydroxybutyrate
C. elegans	*Caenorhabditis elegans*
CR	calorie restriction
DAF	abnormal Dauer formation
2DG	2-deoxy-D-glucose
DR	dietary restriction
FOXO	forkhead box protein
HDAC	histone deacetylase
IGF	insulin-like growth factor
MXL-3	max-like
NAD	nicotinamide adenine dinucleotide
NO	nitric oxide
NOS	nitric oxide synthase
NRF	nuclear respiratory factor
PGC	phosphatidylglycerol phospholipase C
PHA-4	defective PHArynx development
ROS	reactive oxygen species
S6K	S6 kinase
SDHC	succinate dehydrogenase complex subunit C
SIR2	silent information regulation 2
SKN-1	skinhead-1
SOD	superoxide dismutase
TOR	target of rapamycin

Conflict of Interest

No potential conflicts of interest were disclosed.

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