Review Article

Caenorhabditis elegans as a Toolkit for Studying Mammalian Aging Pathways

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Abstract

During the last few decades the free living soil nematode *Caenorhabditis elegans* has been highlighted as an important model organism to decipher the role of several conserved signaling pathways in longevity determination. *C. elegans* is a most effective *in vivo* model for studying aging due to its cellular complexity and high homology with mammalian biochemical and genetic pathways. Despite its apparent simplicity, lately the nematode *C. elegans* has been developed into an important model for biomedical research, mostly in the functional characterization of novel drug targets identified using genomics technologies. For many decades, aging was considered to be a passive, entropic process of tissue decline that occurred in a haphazard way. We know now, however, that the aging process, like so many other biological processes, is subject to regulation by classical signaling pathways viz. insulin signaling pathway, mitochondrial pathway etc. Some of these genetic pathways were first of all reported in small, short-lived organisms such as yeast, worms and flies, and a genetic alteration in same pathways turned out to extend lifespan in mammals as well. *C. elegans* aging mechanism provides a basis to understand how age regulation of a genetic pathway might be conserved between distantly related species. Here we review some aging pathways that are evolutionary conserved and modulates lifespan from worms to mammals viz. Insulin signaling (ILS), Dietary restriction (DR), Mitochondrial respiration and Sirtuin pathway.

Introduction

The nematode *Caenorhabditis elegans* has emerged as an important model for aging research as they are readily amenable to genetic and molecular analyses [1]. The aging processes in organisms are described by a progressive loss of physiological integrity, which leads to the weakening of biological functions and increased susceptibility towards death [2]. The primary risk factor caused by this deterioration process includes major human pathologies viz. cancer, diabetes, cardiovascular diseases and neurological disorders [3]. *C. elegans* has been used extensively in aging study, owing to its several advantages viz. short lifecycle (about 3 days) and short lifespan (about 3 weeks) [4]. *C. elegans,* the free living soil nematode was only recognized as a potential genetic model organism in the early 1960s by Sydney Brenner [5]. *C. elegans* strains are easy to culture and we could easily maintain on nematode growth medium (NGM) agar plates or in liquid culture seeded with *E. coli* OP50 bacteria (Figure 1). Furthermore, the worm's short life cycle (approximately 3 days at 20ºC), small size (about 1-1.2 mm), high productivity, transparent appearance allow easy and quick manipulations of biogerontological studies using this as animal model as already reported by former researchers [5,6].

Extensive studies on complete genome sequence, its 60-80% homology with human genes [7], knockout (KO) mutant libraries, established genetic methodologies (forward and reverse) [8], mutagenesis, carcinogenesis and recently RNA interference (RNAi)

technology [9], provide a variety of options to manipulate and study *C. elegans* at the molecular level and excavate the associated genetic pathways, made us interested using *C. elegans* as a model organism to study and regulate age related genes [5,6,10,11].
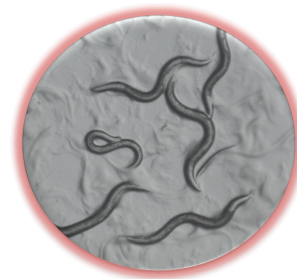

Figure 1: Microscopic view of *C. elegans*.

Partridge reported that a single gene mutation could produce a substantial increase in its lifespan [12-14]. This led to the hypothesis that same kind of mutation and further the loss of function of the equivalent genes can extend the lifespan of mammals as most of the *C. elegans* genes have human orthologues [10,15], suggesting that mechanisms found to influence aging in *C. elegans* are likely to have a conserved role in regulating longevity in humans. Many genes that affect *C. elegans* life span have already been isolated and have been ordered in pathways based on genetic analysis [16]. Michael Klass conducted the first experiment that described aging in nematode *C.*

elegans and suggested that age-related changes in this model are similar to other organisms [17]. The first gene altering aging in *C. elegans* was identified as *age-1* by Klass and Johnson in the 1980. Mutations in this *C. elegans* gene increased lifespan by 50% [12,17]. The classical work on aging by Klass and Johnson (1980) transformed the field of gerontology and proved that aging is regulated at a cellular level rather than a random process. The landmark finding of age altering gene led to many more discoveries resulting in the identification of numerous genes and pathways involved in aging. Many aging pathways are identified that are evolutionary conserved and modulates lifespan from worms to mammals viz. Insulin signaling (ILS), Dietary restriction (DR), TOR signaling, Mitochondrial respiration [18], JNK signaling, Oxidative stress pathway, Sirtuin regulated signaling etc. (Figure 2) [15,19].
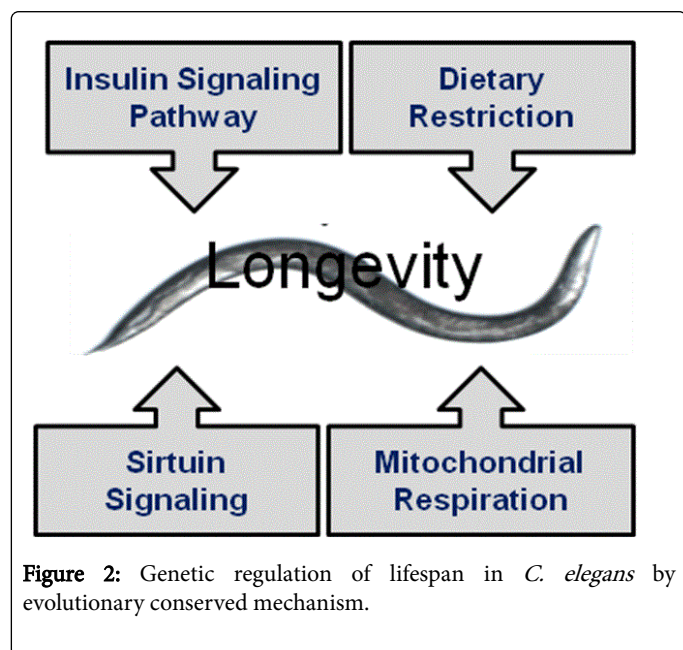
Figure 2: Genetic regulation of lifespan in *C. elegans* by evolutionary conserved mechanism.

The main pathway that regulates life span is reported to be insulin-like signaling (ILS) pathway which effects longevity in worms [15]. Mutations in various genes of ILS pathway have resulted influencing lifespan in flies, mice and also in human longevity.

Insulin Signaling Pathway (ILS)

Insulin signaling pathway is an evolutionarily conserved pathway, modulating organism's lifespan and regulating several cellular processes such as stress resistance against abiotic factors, immunity against biotic factors and aging [20,21]. The impact of this pathway was first identified using the invertebrate model *C. elegans* [13,17,22]. Insulin like growth factor (IGF)/ILS pathways have been shown to influence lifespan in vertebrates as well, including human beings (Figure 3) [20,23,24]. In *C. elegans*, the IIS signaling pathway consists of various proteins that are encoded by the genes *daf-2, age-1, akt-1, akt-2, daf-16* and *daf-18*. The gene *daf-2* encodes a homolog of the mammalian insulin/IGF-I receptor (Figure 3) [25] while *age-1* encodes the catalytic p110 subunit of phosphoinositide-3-OH kinase (PI3K) situated downstream of DAF-2 [26]. Mutations in these genes (*age-1* and *daf-2*) increase the longevity and stress resistance of nematodes in *daf-16* dependent manner. In this pathway, stimulation of *daf-2* receptor activates the phosphatidylinositol 3-kinase (PI 3-kinase). AGE 1 that consists of a p55-like regulatory subunit [27] and a p110

catalytic subunit [28] that is antagonized by DAF-18. Thereafter, PIP3 signal activates the AKT/PKB kinase homologue PDK-1, which phosphorylates AKT-1, AKT-2 and SGK-1. The AKT proteins are responsible for dauer formation as well as for lifespan extension while SGK-1 proteins regulate development, stress response and longevity in worms. These kinases then phosphorylate the transcription factor DAF-16 and inactivate it by cytoplasmic retention [6,27,29-34].

The sub-cellular DAF-16 cytoplasmic localization is regulated by the formation of a protein complex between 14-3-3 FTT-2 and DAF-16, a mechanism that appears to be conserved in mammals [35]. Mutations in *daf-2* or other upstream components of insulin signaling pathway such as *age-1, akt-1* that result in a reduction in IGF-I signaling cause dephosphorylation of DAF-16 and its subsequent translocation into the nucleus. Nucleus localized DAF-16 regulates a number of target genes that have been identified to be involved in various mechanism such as dauer formation, metabolism, development, stress response, detoxification and other signaling (Figure 3B) [36-42].
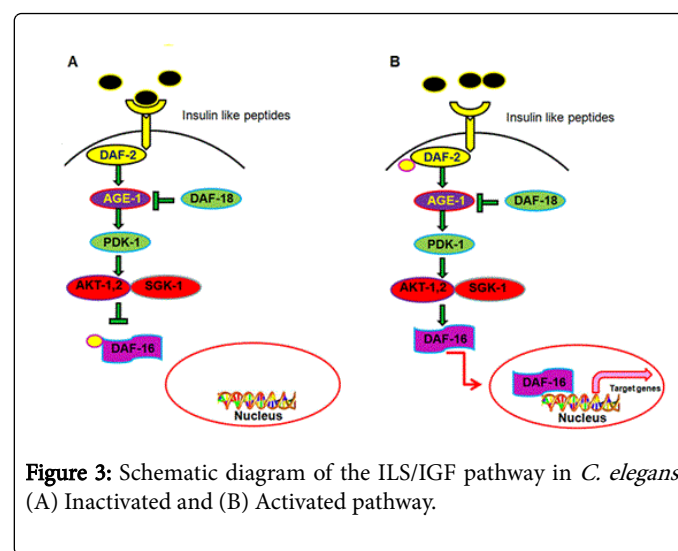
Figure 3: Schematic diagram of the ILS/IGF pathway in *C. elegans* (A) Inactivated and (B) Activated pathway.

Apart from insulin signaling pathway and its molecular players, many genes and genetic signaling pathways have been discovered in worms which are related to lifespan extension. A few important genes/ pathways discovered are: Dietary restriction (DR), SIR2 deacetylase activity (probably with importance in DR), JNK-signaling, TOR, mitochondrial mechanisms, oxidative stress signaling and others [43].

Dietary Restriction (DR) Pathway

Dietary restriction is the reduction of dietary intake without starvation that substantially increases lifespan [44]. This term is also known as caloric restriction (CR). During different time interval similar effects have been observed from lower to higher organisms such as baker's yeast (*Saccharomyces cerevisiae*), fruitfly (*Drosophila melanogaster*), mice (*Mus musculus*), dogs (*Canis lupus familiaris*) and rhesus monkeys (*Macaca mulatta*) [45,46]. Restricting food intake of rodents can extend their lifespan, and this extension is specifically due to a reduction of caloric intake. Reducing calories can also extend life span in other organisms, such as yeast and *C. elegans* [47,48]. The first study report DR mediated longevity in *C. elegans* involved dilution of food bacteria (*E. coli* OP50) in buffer [49,50]. The decreasing concentration of *E. coli* caused lifespan extension and reduction in worm's fertility. Another approach showed same result where reducing the nutrient content in the agar plate showed an

inverse relationship between lifespan and bacterial quantity [36]. The DR studies in *C. elegans* have been to correlate the biology of DR to the identified various aging genes/pathways viz. *eat-2, sir2.1, pha-4* etc. [51,52].

Sirtuin Signaling

For the study of aging and longevity SIRT1 is most extensively studied and implicated as a key mediator in Dietary restriction [53]. Sirtuins are highly conserved NAD$^+$-dependent protein deacetylases that identified early in yeast known as silent information regulator (Sir) [54]. A number of studies suggest that over expression of Sir2 increase lifespan in many models organism such as yeast, *C. elegans* and Drosophila [19]. In mammals seven SIRT proteins (SIRT1-7) are reported that are either NAD-dependent deacetylases or protein ADP-ribosyltransferases and display diversity of functions [55]. In *C. elegans,* over expression of the sirtuin gene *sir-2.1* causes longevity by activating DAF-16/FOXO [56,57]. SIR-2.1 is probably activating transcription factor DAF-16 by deacetylation. As it is reported that in response to oxidative stress mammalian SIRT1 is known to deacetylate FOXO proteins therefore it likely shifts their target towards the antioxidant genes which are play a major role in stress resistance [56]. In the *C. elegans* oxidative stress conditions may be stimulates the binding of SIR-2.1 towards DAF-16 [57] and cause lifespan extension in a *sir-2* dependent and *daf-16*-dependent manner [58]. The findings advocate that sirtuins can deacetylate FOXO proteins directly, as well as it has been reported that insulin/IGF-1 pathway mutants do not require *sir-2.1* for longevity. This phenomenon reveals that sirtuins may influence DAF-16/FOXO transcription factor and also enhance lifespan independently of insulin/IGF-1 signaling in worms. The mechanism of DR have been determined through developmental delays, reduced metabolic rate, attenuated glucocorticoid pathways [59], diminished fat levels, decreased ROS levels, increased rate of cell survival and changes in protein turnover rates.

Mitochondria and Metabolic Control of Aging Pathway

The free radical theory of aging is the most widely accepted theory that explored casual link between the rate of aging and free radical damage [60]. As the mitochondrion is known for ATP generation and one of the primary sites of ROS production in the cell, therefore these organelles are very important and are assumed to have major impact on the aging process [61,62]. Mutations in several genes that affect mitochondrial function probably have an impact on ROS generation (either increasing or decreasing ROS). The point mutations in numerous genes are directly associated with ETC function (*clk-1, isp-1,* and *nuo-6*) that can extend lifespan [18]. Mutations in any one of these genes results in the extended lifespan, cell cycle length, and a slowing down of development and behavioural activity (pharyngeal pumping, defecating, egg laying, and moving). The genetic mutation where both deletions and point mutations have been found to increase lifespan was first reported in the *clk-1* [63]. The *clk-1* gene also affects aging and several other physiological rates in *C. elegans* [64] and encodes an enzyme that is responsible for biosynthesis of ubiqunone [63,65,66] an essential cofactor in numerous redox reactions, including mitochondrial respiration, as a membrane antioxidant and an oxygen sensor [67]. The mitochondrial protein CLK-1 is conserved among eukaryotes and recognized as a hydroxylase involved in the ubiquinone biosynthesis (UQ9) in *C. elegans* [68-70]. In *clk-1* mutants reduced respiration suggests that longevity is directly linked with decrease mitochondrial function which is observed in many species.

Conclusion

In conclusion, this review found that studies in the model organism *C. elegans* have identified genes/pathways that may regulate lifespan in a conserved manner. Recent work has firmly established the role of *C. elegans* in the study of aging process. There is now strong evidence for the close evolutionary conservation of lifespan regulating mechanism in a number of model organisms, supporting a similar control in human also. Comparison of aging transcriptional profiles in worms, flies, mice and humans provides a quantitative, global view of the overall relatedness of the aging process across different species. These results provide a view of the relative proportion of the aging process that is specific to humans rather than shared across animals. The frontier of aging research now lies in the light of this information to mammals and in particular to uncover the processes most important in human aging. It is likely that the framework that has emerged from the study of model organism *C. elegans* and the pathways involved will provide a basis for an understanding of aging which can be further applied on human as well.

Acknowledgment

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