

# *Caenorhabditis elegans*: An important tool for dissecting microRNA functions

Ziwen Zhu, Duo Zhang, Heedoo Lee and Yang Jin\*

Division of Pulmonary and Critical Care Medicine, Department of Medicine, Boston University, Boston, USA

## Abstract

*Caenorhabditis elegans* (*C. elegans*), a member of the phylum Nematoda, carries the evolutionarily conserved genes comparing to mammals. Due to its short lifespan and completely sequenced genome, *C. elegans* becomes a potentially powerful model for mechanistic studies in human diseases. In this mini review, we will outline the current understandings on *C. elegans* as a model organism for microRNA (miRNA)-related research in the pathogenesis of human diseases.

## Introduction

In the 1960s, Dr. Sydney Brenner first proposed using *C. elegans* as a model organism for the investigations focused on the neural development in animals [1]. Two decades later, the lineage of all 959 cells in the adult *C. elegans* were identified in Dr. John Sulston's lab [1]. Furthermore, the first *C. elegans* gene was cloned, and the physical map was constructed [2]. One important milestone in biological research is that *C. elegans* is also the first multi-cellular organism with complete genome sequenced [3].

*C. elegans* is a round worm, a member of the phylum Nematoda, and lives in the aqueous layer between the soils [1]. Its adult body only is approximate 1 mm in full length and 70 µm in diameter [4]. Bacteria (usually *E. Coli* strains in laboratory) serve as the food for *C. elegans* [5]. Interestingly, the bodies of *C. elegans* are transparent, providing convenience for investigators to observe the worm organs such as the intestine, gonads, etc under the microscope with no needs of staining. Furthermore, under a high-power phase-contrast microscope, *C. elegans* can be observed at a single cell resolution [6]. Therefore, *C. elegans* is a good model to observe cell division, differentiation and death. The life cycle of *C. elegans* is short. It only takes 3-4 days to reach the adult stage [7]. The embryonic development of *C. elegans* is much quicker comparing to other animals. Only after approximate 12 hours at 25°C, one can observe the evolution of each single cell through the entire development [8]. The culture of *C. elegans* is also convenient. Generally, agar plates using the Petri dishes serve as a good condition for them [9]. Thus, hundreds of plates for genetic mutation screening can be easily prepared in one lab. Conveniently, the long-term storage of *C. elegans* only requires -80°C freezer. The L1 or L2 stage of worms can recover in the room temperature. Moreover, *C. elegans* can be immersed in a solution containing the designated nucleic acids to acquire exogenously delivered gene modifiers, instead of requiring tedious transfection procedures. Also, the RNA interference can be performed by feeding the *C. elegans* with bacteria expressing siRNA [9].

MicroRNAs (miRNAs) are a group of highly conserved noncoding RNAs and approximately 22 nucleotides in length [10]. Most of primary miRNAs (pri-miRNAs) are transcribed in the nucleus. After transcription, pri-miRNAs are then processed by microprocessor complex. Microprocessor complex binds to the stem-loop structure

of pri-miRNAs and cleaves the primary transcripts to generate a hairpin-shaped RNA molecule known as precursor miRNAs (pre-miRNAs) [10]. These double-stranded pre-miRNAs are composed of 70-100 nucleotides each and subsequently transported from nucleus to the cytoplasm. Dicer is required for the mature of pre-miRNAs in the cytoplasm. The mature miRNA duplex is recognized by the RNA induced silencing complex (RISC) containing Dicer and AGO2 (argonaute RISC catalytic component 2), which are essential to miRNA-induced silencing [10]. Only one strand of miRNA duplex can be incorporated into the RISC to form miRISC, while the other strand, named miRNA\* is mostly degraded. The miRNA loaded RISC binds to the target mRNAs and silence these gene expression through either degradation of mRNA or inhibition of translation at post-transcriptional level [10].

In the process of discovering miRNAs, *C. elegans* played an important role as a model organism. In 1993, Lee *et al.* found that the gene *lin-4* is not encoded for the protein, but encoded a small RNA [11]. They predicted that the *lin-4* binds to the *lin-14* three prime untranslated regions and downregulates the expression of LIN-14 protein. After 7 years, Reinhart *et al.* discovered the second miRNA involved in the development-let-7 [12]. These two miRNAs open the door of miRNA research for other species. A variety of labs then proved that the miRNAs are present in *C. elegans*, *Drosophila* and mammals [13-15].

## miRNA functions in *C. elegans*

### Cell death

*C. elegans* is an easy model to study survival and death. *C. elegans* has also been used as space animals for several times. Gao *et al.* found 17 miRNAs which are involved in the space radiation- caused death [16].

**Correspondence to:** Yang Jin, MD, Ph.D., Division of Pulmonary and Critical Care, Department of Medicine, Boston University Medical Campus, 72 East Concord St., Boston, MA 02118, USA, **E-mail:** yjin1@bu.edu

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Among these miRNAs, miR-797 is related to apoptosis by targeting *ced-10* and miR-81 targets both *drp-1* and *hsp-1* [16]. Recent research further found that the let-7 family miRNAs, miR-48 and miR-84, have the strongest effect on *ced-3*, homolog of caspases 3,7 [17].

## Aging

The different miRNAs can control the same target gene. On the other hand, one miRNA can regulate a variety of target genes [18]. *C. elegans* can be used as a good model for this type of studies, given its relatively easy-signaling pathways. MiR-34 was the first identified miRNA up-regulating in *C. elegans* [19]. MiR-34 can inhibit autophagy by targeting *atg-9a* [20]. Lencastre *et al.* identified that 17 miRNAs are altered during aging processes. Moreover, they prove that miR-71, miR-238, and miR-246 have the function of increasing longevity, demonstrated using mutant worms. On the other hand, miR-239 can decrease life span [21]. MiR-71 interacts with the DNA damage response signaling pathways *via* *CDC-25.1* and *CHK-1* mediated pathways. The expression of *CDC-25.1* in aged miR-71 knock-out worms is increased by 8-folds [21]. Despite that several screenings have suggested that the aging process relates to miRNAs, the mechanisms underlying the miRNA-mediated regulation of longevity remain to be further determined [21,22].

## Metabolism

Dietary restriction (DR) is the most effective way to reduce age-related phenotypes and to extend lifespan, it can also promote longevity and protect against age-associated disease across species. *C. elegans* is a great model to examine the molecular mechanisms by which miRNAs coordinate food intake with health-promoting metabolism [23]. Vora *et al.* found that miR-80 is a major regulator for the DR state [24]. MiR-80 knock-out worms maintain cardiac and skeletal muscle-like function at older age, reduce accumulation of lipofuscin, and extend lifespan. These functions of miR-80 is very similar to the physiological features of DR [24]. With food limitation, decreased miR-80 levels upregulate CBP-1 protein levels to engage metabolic loops that promote DR [24]. Also, miR-71 and miR-228 mediate dietary-restriction-induced longevity [25]. Furthermore, PHA-4 and SKN-1 are negatively regulated by miR-228. On the other side, miR-71 represses PHA-4 [25].

## Innate immunity

The let-7 family is initially found relating to the innate immunity of *C. elegans* [26]. The let-7 family mutant worms are more resistant to pathogen infections by downregulating of heterochronic genes and the p38 MAPK pathways [26]. The developmental timing signal or ATF-7 regulate *hbl-1* to control seam cell proliferation *via* regulating miR-48, miR-84 and miR-241 [26].

## Development

*C. elegans* is an easy model to study the development, given that it only takes 2-3 days for this round worm to grow from eggs to adults. Karp *et al.* identified 14 miRNAs which are related the development of *C. elegans*. Most development miRNAs are altered during the stages from L2 to L2d or L2 molt to dauer [27]. The second found miRNA, let-7, has an important role in embryonic developing [28]. Let-7 regulates the transition process from L4 stage to adult stage. Without let-7, worms can't grow into mature stage due to uncontrolled downregulation of *hbl-1*, *lin-4* and *daf-12* [12,29]. Further studies found that let-7 family members miR-48, miR-84, and miR-241 all regulate developmental timing from the stage L2 transit into L3. The *hbl-1* is also a target similar to let-7 [29]. MiR-51 family contains

**Table 1.** miRNAs in *C. elegans* with known functions.

miRNA family	miRNA name	Function	References
let-7	let-7	DNA damage response, mitochondrial aging DNA damage response, mitochondrial respiration, germline maintenance	[21,22,36]
let-7	miR-48	Death, development	[17,21]
let-7	miR-84	Death, development	[17,21]
let-7	miR-241	Development	[19,21]
lin-4	lin-4	Aging, stress response	[37]
miR-2	miR-797	Death	[16]
miR-34	miR-34	Aging, DNA damage response, Cell death, radiosensitive	[38]
miR-51	miR-51	Development	[30,31]
miR-51	miR-52	Development	[30,31]
miR-51	miR-53	Development	[30,31]
miR-51	miR-54	Development	[30,31]
miR-51	miR-55	Development	[30,31]
miR-51	miR-56	Development	[30,31]
miR-71	miR-71	Aging, DNA damage response, germline signaling, metabolism	[39]
miR-80	miR-81	Aging, death, metabolism	[16]
miR-228	miR-228	Aging, metabolism	[21,25]
miR-238	miR-238	Aging	[21]

miR-51, miR-52, miR-53, miR-54, miR-55, and miR-56. This family has also been proved involved in the early development stage. Mutant worms of these miRNAs have the phenotypes of unattached penetrant pharynx [30,31]. Additionally, CDH-3, the mammalian fat cadherin homolog, is the target of the miR-51 family miRNAs [30].

## Stress response

The miR-360 knock-out worms were also proved to increase the function of glycyrrhizic acid [32]. And the same group prove that graphene oxide can regulate miR-360 to decrease DNA damage-apoptosis signaling by targeting CEP-1 [33,34]. Also miR-355 could regulate MWCNTs toxicity by target *daf-2*, the gene encodes for the insulin-like growth factor 1 [35] (Table 1).

## Conclusions and perspectives

*C. elegans* has been used as a model organism for more than 50 years, its biological properties facilitate the miRNA research robustly. That being said, there remain many uncertainties and undiscovered functions in the field of *C. elegans* miRNA research which require further investigations. Current research suggests that a lot of miRNAs was involved in development, cell death, aging, innate immunity, metabolism, and stress response. But further research is needed to identify the functions and detailed signaling pathways to better understand the systems-level role of miRNAs.

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