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REVIEW

Roles of MicroRNAs in the *Caenorhabditis elegans* Nervous System

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ABSTRACT

The first microRNA was discovered in *Caenorhabditis elegans* in 1993, and since then, thousands of microRNAs have been identified from almost all eukaryotic organisms examined. MicroRNAs function in many biological events such as cell fate determination, metabolism, apoptosis, and carcinogenesis. So far, more than 250 microRNAs have been identified in *C. elegans*; however, functions for most of these microRNAs are still unknown. A small number of *C. elegans* microRNAs are associated with known physiological roles such as developmental timing, cell differentiation, stress response, and longevity. In this review, we summarize known roles of microRNAs in neuronal differentiation and function of *C. elegans*, and discuss interesting perspectives for future studies.

KEYWORDS: *C. elegans*; Nervous system; MicroRNA; Function; Differentiation

INTRODUCTION

Regulation of gene expression plays a key role in essentially all life events. The significance of transcriptional regulation has long been recognized, whereas an appreciation of the potential of posttranscriptional gene regulation mediated by various classes of noncoding RNAs is increasing (Shan, 2010; Khalil and Rinn, 2011; Hu et al., 2012; Lin et al., 2012; Liu et al., 2012). MicroRNA (miRNA) is a widespread class of ~22 nt small noncoding RNAs primarily with posttranscriptional regulatory functions found in animals and plants (Ambros, 2001).

The first miRNA *lin-4* was identified in *Caenorhabditis elegans* as a nematode specific "heterochronic" small RNA in 1993 (Lee et al., 1993; Wightman et al., 1993). It was the molecular cloning of the second miRNA *let-7* in *C. elegans* and the later discovery of its conservation across the animal kingdom in 2000 that initiated a rush to exploit biogenesis, functional mechanism, and diversity of these tiny noncoding RNAs (Pasquinelli et al., 2000; Reinhart et al., 2000; Ambros, 2001). A large number of miRNAs have now been identified owing to the contributions of many researchers and the development of new methodology in RNA sequencing, bioinformatics, and molecular biology.

Even though a large number of miRNAs have been identified, functional characterization of the majority of miRNAs is lagging. There are currently more than 250 miRNAs believed to be encoded by the *C. elegans* genome, about 30% of which are phylogenically conserved; however, functions of more than 200 *C. elegans* miRNAs remain elusive (Ambros et al., 2003; Ruvkun, 2008; Abbott, 2011).

Phenotypic characterization of mutants could provide convincing evidence for function of specific miRNA genes. Although mutants of the first two miRNAs *lin-4* and *let-7* showed obvious defects in developmental timing and cell lineage, early functional study of *C. elegans* miRNA had been discouraging (Miska et al., 2007; Alvarez-Saavedra and Horvitz, 2010). Robert Horvitz and colleagues reported their work on creating mutations for 87 miRNAs in 2007 (Miska et al., 2007). However, most miRNA mutant strains lacked grossly

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abnormal phenotypes. In the research, they argued that functional redundancy, either among target genes of miRNAs or miRNA family members, might be a reason for the mutants showing no obvious phenotype. In 2010, Robert Horvitz group generated mutant strains for 15 miRNA families, and each strain lacked multiple or all members of the individual miRNA family (Alvarez-Saavedra and Horvitz, 2010). The results were again surprising in that mutants of 12 out of these 15 families had no obvious synthetic abnormalities in development and viability (Alvarez-Saavedra and Horvitz, 2010). Mutations deleting all members of the *mir-35* or *mir-51* families were embryonic or early larvae lethal; and mutants deleting four members of the *mir-58* family had defects in locomotion, body size, egg laying, and dauer formation.

Despite challenges for assigning functions for individual miRNAs, a variety of physiological roles such as developmental timing, cell differentiation, stress response, and longevity have been associated with specific *C. elegans* miRNAs (Abbott, 2011). In the subsections below, we focus on recent findings about functions of specific miRNAs in *C. elegans* neuronal development, plasticity, function, and aging.

mir-84 REGULATES DEVELOPMENTAL TIMING OF NEURONS

mir-84 is a member of the *let-7* family in *C. elegans* (Roush and Slack, 2008), and it functions in timing the remodeling of dorsal D (DD) motor neurons (mns) during L1 to L2 stage (Thompson-Peer, et al. 2012). In the L1 stage, six GABAergic DD mns receive inputs from cholinergic dorsal A (DA) and dorsal B (DB) neurons on their dorsal processes and make synaptic outputs onto the ventral body wall muscles (White et al., 1976). At the end of the L1 stage, the presynaptic components of the DD mns shift from the ventral to the dorsal side, and at the same time the postsynaptic components shift from the dorsal to the ventral side; these phenomena occur without growth of new neurites (White et al. 1978). Several transcription factors have been identified that regulate the remodeling of the DD mns (Hallam and Jin, 1998; Zhou and Walthall, 1998; Shan et al., 2005; Shan and Walthall, 2008; Park et al., 2011; Petersen et al., 2011). HBL-1 is a hunchback like transcription factor, and is a heterochronic factor regulating several aspects of the DD plasticity (Thompson-Peer et al., 2012). DD remodeling is delayed in *hbl-1* mutants. *hbl-1* is negatively regulated by *let-7* family and contains binding sites for three *let-7* paralogs in its 3′ UTR. *mir-84* was expressed in the L1 stage, and *mir-84* mutants exhibited precocious DD remodeling. The expression of a reporter with *hbl-1* 3′ UTR was significantly increased in the *mir-84* mutant compared to *wildtype*. Correspondently, there was no significant difference in the expression between *wildtype* and *mir-84* mutants if the reporter missed the *hbl-1* 3′ UTR. This demonstrated that *mir-84* regulated *hbl-1 via* its 3′ UTR. It was interesting that the reporter with *hbl-1* 3′ UTR showed a difference in expression between *mir-84* mutant and *wildtype* only in the early stage but not in adults. This suggested that *mir-84* might regulate *hbl-1* transiently. Also, *hbl-1;mir-84* double mutants eliminated effects of *mir-84* single mutant on DD remodeling, suggesting that *hbl-1* was the primary target of *mir-84* in regulating the timing of DD remodeling (Fig. 1). *lin-14* was the first transcription factor gene identified in the timing of DD remodeling (Hallam and Jin, 1998). As *lin-4* and *let-7* are known to regulate *lin-14* directly, it is reasonable to speculate that these two miRNAs also function in timing DD remodeling (Reinhart et al., 2000).

mir-71, lsy-6, AND *mir-273* REGULATE NEURONAL CELL FATE SPECIFICATION

Anatomically the nervous system of *C. elegans* is bilaterally symmetric, yet there are striking asymmetries. The mechanisms that generate these asymmetries are largely unclear. miRNAs such as *mir-71*, *lsy-6*, and *mir-273* are involved in the generation of asymmetries among neurons (Johnston and Hobert, 2003; Chang et al., 2004; Hsieh et al., 2012).

The two AWC neurons are left/right symmetrical in morphology, but take on asymmetric fates indicated by the differential expression of chemoreceptor gene *str-2* (Troemel et al., 1999). The expression of *str-2* in AWC is defined as AWC^{ON}, and the other AWC without *str-2* expression is defined as AWC^{OFF}. These two fates of the AWC neurons are determined by a calcium-mediated UNC-43 (CaMKII)/TIR-1 (Sarm1)/NSY-1 (MAPKKK) signaling pathway (Chuang and Bargmann, 2005). This pathway suppresses the expression of *str*-2. *nsy-4* (a member of the calcium channel gamma subunit family) associates with *nsy-5* (encoding an innexin, protein that forms gap junction channels) to down regulate this pathway in AWC^{ON} (Vanhoven et al., 2006; Chuang et al., 2007). In a recent publication, researchers hypothesized that there was a miRNA functioning in suppressing the calcium-mediated signaling pathway along the establishment of AWCs asymmetry (Hsieh et al., 2012). To test this hypothesis, they took a computational approach to identify miRNAs predicted to target the 3′ UTR of genes known to be associated with fate determination of AWC neurons. They made specific criteria and selected six potential miRNAs for further investigations. Ectopic expression of *mir-71*, which was predicted to target *tir-1* 3′ UTR, resulted in double AWC^{ON} neurons. This suggested that *mir-71* plays a vital role in suppressing the UNC-43/TIR-1/NSY-1 pathway by inhibiting the adaptor gene *tir-1*. A reporter driven by the promoter of *mir-71* showed that *mir-71* was expressed at a higher level in AWC^{ON} than in AWC^{OFF}, which was consistent with the role of *mir-71* in promoting the AWC^{ON} fate. Meanwhile, expression of *mir-71* was also shown to be controlled by NSY-4/claudin-like protein and NSY-5/innexin gap junction protein, which functioned in parallel with the UNC-43/TIR-1/NSY-1 pathway to antagonize the calcium signaling pathway, and induce the AWC^{ON} fate. Mature *mir-71* levels decreased in *nsy-4* and *nsy-5* mutants. Furthermore, TIR-1 adaptor was downstream of NSY-4 and NSY-5. Thus, there is a feedback loop through which *mir-71* functions to influence AWC neuronal fate determination (Hsieh et al., 2012; Fig. 1). In this feedback loop, the expression of *mir-71* was promoted by NSY-4/NSY-5

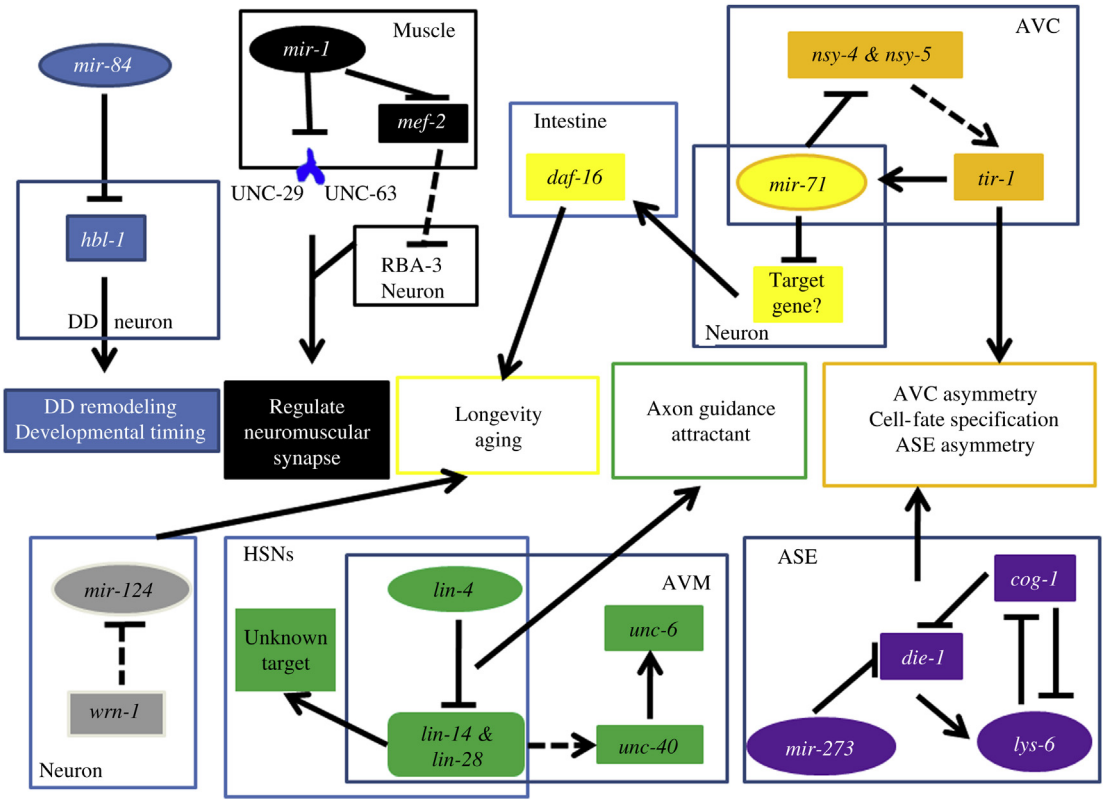

Fig. 1. miRNAs play crucial roles in *C. elegans* nervous system.
mir-84 directly suppresses *hbl-1* in the DD remodeling; *mir-71* inhibits *nsy-4* and *nsy-5* in AWCs, and *mir-71* represses some unknown targets in neurons to activate *daf-16* in intestine cells for longevity; *lsy-6* and *mir-273* act through *cog-1* and *die-1* in ASE asymmetry; *lin-4* regulates axon guidance by inhibiting *lin-14* and *lin-28* for axon guidance; *mir-124* is regulated by *wrn-1* to regulate aging; *mir-1* modifies neuromuscular synapses by suppressing *unc-29*, *unc-63* and *mef-2*. Hollow boxes represent different tissues or cells, solid blocks represent protein coding genes, and solid circles represent miRNAs.

at the transcriptional level. NSY-4/NSY-5 showed asymmetry in AWC neurons, while the asymmetric expression of *mir-71* might have no direct relationship with NSY-4/NSY-5 asymmetry. At the post-transcriptional level, *mir-71* regulated *tir-1* by targeting its 3′ UTR.

ASEL and ASER neurons are another example of left/right asymmetry in *C. elegans* nervous system (Cochella and Hobert, 2012). ASEL and ASER derive from lineage branches that diverge at the four-cell stage in early embryogenesis (Poole and Hobert, 2006). ASEL is a descendant of the ABa, while ASER derives from ABp. A Delta/Notch signal from the P2 cell represses two redundant T-box transcription factor genes, *tbx-37* and *tbx-38*, leading to the asymmetry between ASEL and ASER (Chang et al., 2004). In *tbx-37;tbx-38* double mutants, the ASEL neuron was converted into an ASER neuron (Poole and Hobert, 2006). Despite their asymmetric origins, the two lineages give rise to the ASEs due to the expression of a terminal selector, *che-1* (Etchberger et al., 2007). CHE-1 binds directly to the promoters of many genes expressed in ASE neurons, including *die-1*, which encodes a C2H2 zinc finger protein, *cog-1*, an ortholog of the vertebrate NKx6 homeodomain protein gene, and *lsy-6*, a miRNA gene. *die-1*, *cog-1*, and *lsy-6* are key factors functioning in a bistable feedback loop (Etchberger et al., 2007; Sarin et al., 2007; O'Meara et al., 2009). Null allele of *lsy-6* displayed double ASER phenotype, as the expression of ASER-specific gene *gcy-5* was detected in both ASEs, and the ASEL-specific gene *gcy-7* failed to be expressed in either of these two neurons. Ectopic expression of *lsy-6* in both ASEs resulted in double ASEL phenotype, which indicated that *lsy-6* was sufficient to promote ASEL cell fate. *die-1* was also expressed in ASEL neuron and functioned in the ASE laterality by activating the expression of *lsy-6*. *lsy-6* expression was absent in ASEs in *die-1* null mutants, while on the other hand *lsy-6* was expressed in both ASEs when *die-1* was ectopically expressed in both ASEs. *cog-1*, which was expressed in ASER, was a target of *lsy-6* (Johnston and Hobert, 2003; Chang et al., 2004). A reporter driven by *ceh-36* promoter (leading to transcription in both ASEL/ASER) containing *cog-1* 3′ UTR was expressed only in ASER in *wildtype*, but was expressed in both ASEs in *lsy-6* mutant worms. In addition, replacing the *cog-1* 3′ UTR with an *unc-54* 3′ UTR also led to the expression of the reporter in both ASEs.

A recent study has identified a temporally separated, two-step activation sequence for the expression of *lsy-6* miRNA, which controls ASEs left/right asymmetry (Cochella and Hobert, 2012). *lsy-6* might be one of the first genes expressed in ASEs. Previously a study had shown that a construct containing 932 bp of the upstream sequence of *lsy-6* and *lsy-6* hairpin could rescue the phenotype of *lsy-6* null

allele (Johnston and Hobert, 2003). The 932 bp *lsy-6* upstream sequence could be divided into two fragments, one named the upstream element and the other one named the downstream element. A reporter construct with only the upstream element was expressed in both ASEs in embryos, and the expression was restricted to ASEL in adulthood. After the addition of the downstream element to the reporter construct, it was expressed only in ASEL. It was interesting that the expression of *lsy-6* actually started in the precursor for ASEL, and was then boosted to a functional level several divisions later in the mother of ASEL (Cochella and Hobert, 2012). Further study demonstrated that CHE-1 controlled the boost of *lsy-6* expression, while TBX-37/38 controlled the priming of *lsy-6* expression. Both upstream (controlled by CHE-1) and downstream (controlled by TBX-37/38) of *cis*-regulatory elements were necessary for the precise, spatial and temporal expression of *lsy-6*. TBX-37/38 functioned in modifying the chromatin structure of the *lsy-6* gene locus to prime the expression of *lsy-6*. *tbx-37/38* mediated priming of the *lsy-6* locus and involved the decompaction and activation of chromatin. Based on these results, a 'primer and boost' model was proposed: TBX-37/38 was necessary to prime *lsy-6* for low levels of transcription; subsequently, with the action of CHE-1, *lsy-6* displayed a boosted expression.

Another miRNA, *mir-273* was identified, which is involved in ASE laterality through regulation of *die-1* (Chang et al., 2004). *mir-273* and *die-1* showed mutually exclusive expression between the two ASEs. *mir-273* directly suppressed the expression of *die-1*, and alteration of the binding site of *mir-273* in *die-1* 3′ UTR resulted in an ectopic expression of a reporter in both ASEs, confirming that *die-1* was a target of *mir-273* (Chang et al., 2004; Fig. 1).

lin-4 REGULATES AXON GUIDANCE

During development, neurons need multiple ligands and receptors to control the extension of axon. Several ligands and receptors have been identified in axon guidance, yet not much is known about the fine regulation of this event. Two cues, Netrin and Slit, participate in axon guidance in *C. elegans* (Killeen and Sybingco, 2008). UNC-6, a member of the netrin family of secreted guidance cues, which is recognized by its receptor UNC-40, could function as an attractant. SLT-1, a member of the slit family of secreted guidance cues, which is recognized by its receptors SAX-3 and EVA-1, functions as a repellent. Under the balance of UNC-6 and SLT-1 cues, axons of neurons such as HSNs and AVMs can reach the ventral midline (Killeen and Sybingco, 2008).

lin-4 could promote initiation of axon growth in HSN neurons (Olsson-Carter and Slack, 2010). HSN axons showed delayed extension in *lin-4* loss-of-function strains. HSN could not form mature morphology in *lin-14* mutants, while grew precociously in *lin-4* overexpressing animals. *lin-4* expression in HSN neurons started from L1 stage and reached the peak at the L4 stage, which was in consistent with roles of *lin-4* in axon growth of HSN during the L4 stage. *lin-4* regulated HSN axon growth by down-regulating two inhibitors, LIN-14 and LIN-28. *lin-14* and *lin-28* displayed the highest expression at the L1 stage, and the expression decreased to the lowest level at the L4 stage; this expression pattern was in reverse to that of *lin-4*. In the *lin-4* mutants, the expression of LIN-14 and LIN-28 remained at a stable level. *lin-14* was confirmed to be a direct *lin-4* target, while *lin-28* might not be a direct target of *lin-4*, as *lin-28* 3′ UTR lacked *lin-4* complementary sites (Moss et al., 1997). It seemed that *lin-4* had a cell-autonomous role in HSN axon growth, but it was surprising that its role was not dependent on the UNC-40/DCC and SAX-3/Robo receptors, guidance receptors well known to regulate axon growth (Olsson-Carter and Slack, 2010). More insight into the molecular mechanism for the role of *lin-4* in HSN axon guidance is needed.

Another study revealed a role for *lin-4* function in regulating axon guidance in the postembryonic mechanosensory neuron, AVM (Zou et al., 2012). *lin-4* is expressed at the L1 stage in AVM shortly after the cell's birth. *lin-4* regulated AVM through the UNC-6 signaling pathway. *lin-4* mutants do not show a defect in the ventral guidance of AVM axons, but the mutants exhibited a suppression of AVM axon guidance defects normally observed in *slt-1* mutants and *eva-1* mutants. Loss of *lin-4* function enhanced UNC-6-mediated attraction. However, no defect was observed on AVM cell fate in *lin-4* mutant. *lin-4* was detected in AVM neurons only after AVMs had migrated to their final position. *lin-14* might contribute to the ventral guidance of AVM axon by activating *unc-6*. It is interesting that *lin-14* was suppressed directly by *lin-4* in AVM during the L1 stage but not at other stages (Zou et al., 2012; Fig. 1).

mir-1 REGULATES SYNAPTIC FUNCTION

Synapses are formed between differentiated neurons and cells that they innervate. Synaptic transmission requires crosstalk between pre- and postsynaptic cells. A retrograde synaptic signal is needed to inhibit the release of acetylcholine (ACh) at the neuromuscular junction (Davis and Bezprozvanny, 2001). *mir-1*, a well conserved muscle-specific miRNA, is involved in adjusting synaptic transmission by affecting both pre- and postsynaptic components (Simon et al., 2008). In the postsynaptic cell, *mir-1* modified the sensitivity of muscle to ACh by regulating the mRNAs of *unc-29* and *unc-63*, two genes that encode levamisole-sensitive nicotinic acetylcholine receptor (nAChR) subunit. In *mir-1* mutants, the amount of UNC-29 and UNC-63 was increased, leading to increased sensitivity to ACh. Reporter constructs containing the *unc-29* or *unc-63* 3′ UTR was expressed at higher levels in *mir-1* mutants than in *wildtype*. *mir-1* mutants showed obvious defects in both endogenous EPSCs (excitatory postsynaptic currents) and stimulus evoked EPSCs. *mir-1* also adjusted the presynaptic release of ACh by regulating *mef-2* mRNA. A reporter construct containing an *mef-2* 3′ UTR indicated that *mef-2* expression was controlled by *mir-1* in body wall muscles. The muscle transcription factor MEF-2 controlled a retrograde signal that had effects on the presynaptic release of ACh mediated by the synaptic vesicle protein

RAB-3. Thus *mir-1* might be a central regulatory element coupling postsynaptic muscle activity to presynaptic neuronal function (Fig. 1).

mir-71 REGULATES NEURONAL ASPECT OF LONGEVITY

The insulin/insulin-like growth factor-1 (IGF-1) signaling (IIS) pathway is the first identified pathway involved in longevity based upon research with *C. elegans* (Friedman and Johnson, 1988b; Kenyon et al., 1993). Multiple genes such as *daf-2*, *age-1*, and *daf-16* function in this pathway to control aging (Friedman and Johnson, 1988a; Kenyon et al., 1993). Some miRNAs, such as *mir-71* and *lin-4,* regulate aging by directly or indirectly interacting with genes such as *daf-16* (Boehm and Slack, 2005; Boulias and Horvitz, 2012).

mir-71 is broadly expressed in intestine, body wall muscle, and the nervous system in *C. elegans* (Boulias and Horvitz, 2012). The longevity function of *mir-71* was identified from a screen of 115 miRNA mutants from Boulias and Horvitz's study. The *mir-71* mutant showed about a 40% decrease in lifespan. It was interesting that *mir-71* functioned in neurons to promote lifespan. In *mir-71* mutants, a strong rescue of lifespan was achieved with neuronal *mir-71* expression. Germline ablation resulted in a robust lifespan extension in *wildtype* animals, but it failed to extend the lifespan of *mir-71* mutants. Thus, *mir-71* is required for lifespan extension caused by germline removal. Both insulin/IGF signaling and defective mitochondrial function could extend the lifespan of *mir-71* mutants (Boulias and Horvitz, 2012). *mir-71* itself was sufficient to extend the lifespan, as worms with *mir-71* overexpression showed about 20% lifespan increase. But actually, the link between *mir-71* and *C. elegans* longevity was discovered earlier with a screen for biomarkers of aging (Pincus et al., 2011). Effect of *mir-71* overexpression on longevity could be fully suppressed by a null allele of *daf-16*, indicating that *mir-71* acted upstream of *daf-16* to promote germline mediated longevity. *mir-71* expressed in neurons extended lifespan, while *daf-16* expressed in intestine but not in neurons could rescue the *mir-71*-mediated lifespan extension. The localization and transcriptional activity of DAF-16 in intestine cells is regulated by neuronal *mir-71* expression. The accumulation of DAF-16:GFP, which represented the expression and subcellular localization of DAF-16, and the induction of *Psod-3::gfp*, which was widely used as a sensor of *daf-16* activity, were partially blocked in *mir-71* mutants (Boulias and Horvitz, 2012). It would seem reasonable that the *C. elegans* germline sends signals to neurons, leading to the targeting of *mir-71* to unidentified genes in neurons, and eventually these changes in neuronal gene expression somehow regulate *daf-16* expression in intestinal cells to control lifespan.

mir-124 MAY PLAY ROLES IN *C. ELEGANS* AGING

Just like *mir-1*, *mir-124* is highly conserved from worm to human, and it is the most abundant miRNA in the brain (Cao et al., 2007). Previous studies demonstrated that it played important roles in nervous system, such as neuronal differentiation in mice (Cao et al., 2007), dendritic branching in *Drosophila* (Xu et al., 2008), synaptic plasticity in *Aplysia* (Martin et al., 1997; Rajasethupathy et al., 2009), and brain function in rat (Chandrasekar and Dreyer, 2009). In *C. elegans*, *mir-124* is expressed in many neurons (Clark et al., 2010). *mir-124* mutants had a decrease in lifespan and ATP level, and an increase in reactive oxygen species (ROS) production and the aging marker lipofuscin. *mir-124* was downregulated in *wrn-1* mutants (Dallaire et al., 2012). Deletion of *wrn-1* resulted in a decrease in lifespan and ATP level and an increase in ROS production, and the *mir-124*; *wrn-1* double mutant showed a more severe phenotype than the single mutant. *wrn-1* is a Werner syndrome protein homolog in *C. elegans* (Lee et al., 2004). Werner syndrome is also known as adult progeria, and has a symptom of premature aging in human. Approximately 90% of individuals with Werner syndrome have mutations in *WRN* gene, which encodes a RecQ DNA helicase (Gray et al., 1997). Although the detailed molecular mechanism demands further investigation, the regulation of *mir-124* by *wrn-1* and the functional association between them are strong indications that *mir-124* may have roles in Werner syndrome, and more broadly, in the aging process.

CONCLUSIONS AND PERSPECTIVES

C. elegans was the first species in which miRNAs were identified, and the nematode continues to provide new insight into roles for miRNAs in regulating gene expression. The relative simplicity and the suitability for classical forward and reverse genetics have made this animal a great model for generating knowledge about miRNAs. Based on information summarized in this review, it is clear that miRNAs have play an important role in the *C. elegans* nervous system (Table 1 and Fig. 1).

Although miRNAs utilize a general posttranscriptional regulatory mechanism, each miRNA has a very distinct expression pattern. *lsy-6*, for example, is expressed almost exclusively in ASE neurons, while *lin-4* and *mir-71* are expressed in many cell types (Lee et al., 1993; Wightman et al., 1993; Boulias and Horvitz, 2012). In the cases of *lin-4* and *mir-71*, the miRNAs probably target different RNAs and have different functions in different cells (Fig. 1). Although miRNAs may be expressed in multiple cell types, the expression of the miRNA in one cell type is crucial for a specific physiological function. The important role for the neuronal expression of *mir-71* in longevity is a good example of this phenomenon (Boulias and Horvitz, 2012). It is true that many miRNAs execute their functions cell-autonomously, but there are also miRNAs such as *mir-1* that act in a cell nonautonomous role (Simon et al., 2008). In cases of *mir-71* and *mir-1*, whether they function cell autonomously or non-cell autonomously (or possibly both ways) remains unclear. The cell autonomous action of miRNAs is true in most cases that have been investigated; however, recent findings showing

Table 1
C. elegans microRNAs with known neuronal functions

MicroRNA	Family	Seed sequence	Conservation	Neuronal function	Target gene
lys-6	Single member	UUUGUAU	Worm specific	ASE asymmetry	*cog-1*
lin-4	Single member	CCCUGAG	Conserved from worm to human (*mir-125* in human)	Axon guidance	*lin-14* *lin-28*
mir-1	Single member	GGAAUGU	Conserved from worm to human	Synaptic transmission	*unc-29* *unc-63* *mef-2*
mir-71	Single member	GAAAGAC	Conserved in some invertebrates	AWC asymmetry aging	*nys-4* *nys-5*
mir-84	*let-7*	GAGGUAG	Conserved from worm to human	DD remodeling	*hbl-1*
mir-124	Single member	AAGGCAC	Conserved from worm to human	Aging	Unknown
mir-273	Single member	GCCCGUA	Worm specific	ASE asymmetry	*die-1*

intercellular miRNA transport in mammals may expand this view (Valadi et al., 2007; Gibbings et al., 2009).

Despite miRNAs are considered to target multiple gene products, often one or a small subset of targets are identified as primary targets responsible for the specific physiological functions of individual miRNAs. *cog-1* and *die-1* are primary target for *lsy-6* and *mir-273* respectively in regulating ASE asymmetry (Chang et al., 2004). It would be reasonable to speculate that other "secondary" targets may be present, and we have to examine the whole regulatory network for each miRNA to better understand the related physiological function.

Members of the same miRNA family can have redundant roles, as expression of any member of the *mir-35* family in mutants with all other family members deleted rescued the defective phenotype (Alvarez-Saavedra and Horvitz, 2010). On the other hand, family members also have diverse functions. As seen for *mir-84*, a *let-7* family member, which has a unique function in DD remodeling (Thompson-Peer, et al. 2012).

Although miRNAs are less conserved than protein genes, the sequences of some, such as *mir-1* and *mir-124*, have been well kept for millions of years of animal evolution (Table 1). Conservation in sequence often indicates functional conservation at least partially. For *mir-1* and *mir-124*, it is striking to see the high degree of similarity in their sequence, tissue specific expression, and functions across the gap between *C. elegans* and humans (Simon et al., 2008; Clark et al., 2010). There are also miRNAs such as *mir-71* with high conservation among invertebrates, and other miRNAs that appear only in nematodes (Table 1). A fact has to be pointed out is that a worm specific miRNA does not necessarily regulate worm specific genes. For example, *lsy-6*, a worm specific miRNA, targets *C. elegans cog-1*, a well conserved ortholog of the vertebrate Nkx6 homeodomain gene (Chang et al., 2004).

For those *C. elegans* miRNAs that have not been characterized functionally, it is possible that we did not "look careful enough", or did not "look at the right place". For example, *lsy-6* is expressed exclusively, but plays roles only in ASE neurons (Chang et al., 2004). Its function would not have been identified from a general inspection of worm morphology, locomotion, viability, or reproduction. Encouragingly, the functions of multiple *C. elegans* miRNAs were uncovered in recent years. Under relatively harsh conditions, specific physiological states, or in certain mutant backgrounds, specific functions for miRNAs have been revealed. For example, roles of *mir-71* in longevity were revealed under the condition of germline removal, while the absence of *mir-71* did not alter lifespan in intact worms (Boulias and Horvitz, 2012). The expression of miRNAs such as *mir-35* is not specific to neurons or the nervous system. In fact, two *mir-35* targets expressed in different tissues were identified, suggesting its distinct pleiotropic roles in various tissues (Liu et al., 2011). Thus, systematically examining miRNA functions and changing the condition of *C. elegans* may be reasonable ways to reveal biological roles of miRNAs.

C. elegans is in a unique position for revealing functions of miRNAs. For example, its simple nervous system has facilitated pioneering research on the roles of miRNAs in the creation of neural asymmetry. *C. elegans* sometimes is complementary to other model organisms. In the study of *mir-1*, fly mutants and mouse knockouts are lethal, whereas *C. elegans mir-1* mutants are viable and show normal muscle morphology (Simon et al., 2008; Luo et al., 2013). The link between *mir-124* and *wrn-1* in *C. elegans* model of human Werner syndrome suggests that *C. elegans* and its nervous system may be a model to further investigate the roles of miRNAs in the etiology of certain human diseases (Dallaire et al., 2012).

miRNA was first identified in *C. elegans* two decades ago (Lee et al., 1993; Wightman et al., 1993), while roles of miRNAs in *C. elegans* nervous system had only been studied for several years. Here we summarize known roles of *C. elegans* miRNAs in the nervous system. About 30% of worm cells are neurons; undoubtedly we will see more cases where miRNAs regulate biological processes in the *C. elegans* nervous system. With the development of new methodologies, new functions of miRNAs in neural and nonneural cells will continue to be uncovered.

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