Computational analysis of genetic loci required for amphid structure and functions and their possibly corresponding microRNAs in *C. elegans*

Ya-Ou HU^{1,2,3}, Yang SUN^{1,2}, Bo-Ping YE³, Da-Yong WANG^{1,2}

¹Department of Genetics and Developmental Biology, Southeast University, Nanjing 210009, China

²Key Laboratory of Developmental Genes and Human Disease, Ministry of Education, Nanjing 210009, China

³School of Life Science and Technology, China Pharmaceutical University, Nanjing 210009, China

Abstract: Objective To examine the important roles of microRNAs (miRNAs) in regulating amphid structure and function, we performed a computational analysis for the genetic loci required for the sensory perception and their possibly corresponding miRNAs in *C. elegans*. **Methods** Total 55 genetic loci required for the amphid structure and function were selected. Sequence alignment was combined with E value evaluation to investigate and identify the possible corresponding miRNAs. **Results** Total 30 genes among the 55 genetic loci selected have their possible corresponding regulatory miRNA(s), and identified genes participate in the regulation of almost all aspects of amphid structure and function. In addition, our data suggest that both the amphid structure and the amphid functions might be regulated by a series of network signaling pathways. Moreover, the distribution of miRNAs along the 3' untranslated region (UTR) of these 30 genes exhibits different patterns. **Conclusion** We present the possible miRNA-mediated signaling pathways involved in the regulation of chemosensation and thermosensation by controlling the corresponding sensory neuron and interneuron functions. Our work will be useful for better understanding of the miRNA-mediated control of the chemotaxis and thermotaxis in *C. elegans*.

Keywords: microRNA; cilia structure; sensory neuron; interneuron; computational analysis; *C. elegans*

1 Introduction

A family of small (approximately 20 – 24 nucleotide-length), non-coding RNAs are known as microRNAs (miRNAs). MiRNAs constitute a large family of genomically encoded, regulatory RNAs and these regulatory genes function to control the expression of protein-coding genes^[1]. By binding with the 3' untranslated region (UTR) of the target mRNAs in virtue of sequence complementarity, miRNA restrains the post-transcriptional progress of target genes^[1,2]. *C. elegans* has proved to be the pioneer organism for miRNA research, and miRNAs are now believed to exist in all multicellular organisms. The finally mature ~22 nt forms that down-regulate the protein expression of genes

with antisense complementarity were produced by multiple processing steps from the miRNA genes^[1].

Pioneering work in the Ambros and Ruvkun labs uncovered the first miRNA, *lin-4*, and its protein-coding target, *lin-14*, which established a novel form of gene regulation^[3]. *lin-4*, discovered in 1993, was identified to perform as the regulators of temporal gene expression^[3]. *lin-4* participates in the regulation of the developmental timing by binding to seven sites in the 3'UTR of its target gene *lin-14*^[3]. Similarly, the second miRNA gene discovered, *let-7*, also plays an important role in developmental timing by binding to the 3'UTR of *lin-41* to down-regulate LIN-41 during the larval stage^[4]. Till now, various experimental studies have already been confirmed the key roles of miRNAs in some biology activities, such as cell fate specification, life span, metabolism, and hematopoietic lineage differentiation^[1,5,6]. In addition, miRNAs are usually exhibit complementarity with multiple target genes.

Sensory cilia are present in 60 of the 302 neurons in the *C. elegans* hermaphrodite^[7]. Amphid neurons, the prin-

Corresponding author: Da-Yong WANG
Tel: 86-25-83272504-817
E-mail: dayongw@seu.edu.cn
Article ID: 1673-7067(2007)01-0009-12
CLC number: Q429
Document code: A
Received date: 2006-12-04

ciple sensory neurons, responsible for chemosensory and thermosensory behaviors, have been identified through behavioral assay and killing defined neurons using a laser microbeam. Each of two bilaterally symmetric amphids in the animal's head contains the endings of 12 types of sensory neurons^[7]. Among these 12 sensory neurons, 8 types of neurons (ADF, ADL, ASE, ASG, ASH, ASI, ASJ and ASK) detect mostly water-soluble chemicals with one or two long slender cilia that are directly exposed to the environment through the amphid pore. Three types of neurons (AWA, AWB and AWC) detect volatile odorants with flattened, branched cilia that are enclosed by an amphid sheath cell. One type of neuron (AFD) detects thermal cues with a complex, brush-like dendritic membrane structure at the sensory ending. These chemosensory and thermosensory neurons function in the regulation of chemotaxis, thermotaxis, behavioral avoidance, dauer formation, learning, memory and learning choice^[8]. Especially, because chemotaxis and thermotaxis are two central behaviors responded to the environment signals, the model organism *C. elegans* has been exploited to systematically study the molecular mechanism of learning and memory via chemotaxis and thermotaxis assays. Corresponding to the functions of these 12 sensory neurons, 5 types of interneurons (AIY, AIZ, RIA, AIA and AIB) were identified. Functions of AIY, AIZ, RIA, AIA and AIB interneurons involve the regulation of thermophilic movement, cryophilic movement and thermotaxis, respectively.

Although miRNAs are abundant in animal genomics, the researches of biological pathways in which miRNAs operate are just beginning to be performed. Recently, Chang *et al.* (2004) indicated that miRNAs can act sequentially and asymmetrically to control the chemosensory laterality in the nematode^[9], suggesting the possibly important roles of miRNAs in the regulation of amphid structure and function. In addition, for *C. elegans*, the miRBase Target, a comprehensive new database of predicted miRNA target genes has primarily been finished and available at <http://microrna.sanger.ac.uk>^[10]. Nevertheless, many of the complementing sequences of target genes still need to be further confirmed. To further investigate the possibly important functions of miRNAs in regulating amphid structure and function, we performed the computational analysis for the genetic loci required for the amphid structure and function and their possible corresponding miRNAs in *C. elegans*. Our work here will be useful for further attempt

to systematically determine the roles of miRNAs in learning and memory regulation, as well as the lifespan and dauer formation regulation, in *C. elegans*.

2 Materials and methods

2.1 Genetic loci Total 55 genetic loci were selected for assay in the current work (Tab. 1). Among these 55 genes, 16 genetic loci are required for the construction of cilia structure. They are *che-2* (abnormal CHEmotaxis)^[11], *che-3*^[11], *che-11*^[11], *che-13*^[11], *daf-10* (abnormal DAuer Formation)^[11], *osm-1* (OSMotic avoidance abnormal)^[11], *osm-5*^[11], *osm-6*^[11], *osm-3*^[11], *daf-6*^[11], *daf-19*^[11], *mec-1* (MEChano sensory abnormality)^[11], *mec-8*^[11] and *che-14*^[12], *dys-1* (abnormal DYe Filling)^[12], and *dys-13*^[12]. Thirty-one genetic loci are required for one or a few cell types of sensory neuron functions. They are *lim-6* (LIM domain family)^[9], *gcy-5* (Guanylyl CYclase)^[9], *gcy-7*^[9], *unc-37* (UNCoordinated)^[9], *die-1* (Dorsal Intercalation and Elongation defect)^[9], *ceh-36* (C.Elegans Homeobox)^[9], *tax-2* (abnormal chemoTAXis)^[13], *tax-4*^[13], *daf-11*^[14], *daf-21*^[14], *che-1*^[12], *odr-7* (ODOrant response abnormal)^[12], *odr-1*^[12], *ttx-1* (abnormal ThermoTAXis)^[12], *odr-3*^[12], *osm-9*^[12], *glr-1* [GLutamate Receptor family (AMPA)]^[12], *odr-2*^[12], *odr-4*^[12], *odr-10*^[12], *tax-6*^[15], *daf-2*^[16], *odr-9*^[17], *osm-10*^[18], *qui-1* (QUinine non-avoider)^[19], *gpc-1* (G Protein, Gamma subunit)^[20], *lim-4*^[21], *ceh-14*^[21], *osm-12*^[22], *bbs-8* [BBS (Bardet-Biedl Syndrome) protein]^[22], and *daf-7*^[23]. Eight genetic loci are required for the functions of interneuron AIY, AIZ and AIA. They are *unc-86*^[12], *lin-32* (abnormal cell LINEage)^[12], *ttx-3*^[24], *sra-11* [Serpentine Receptor, class A (alpha)]^[24], *ceh-10*^[25], *ceh-23*^[25], *lin-11*^[26], and *ins-1* (INSulin related)^[27].

2.2 Sequence alignment The sequence alignment was analyzed by DNAassist software, a C++ program for editing and analysis of nucleic acid and protein sequences^[28]. We checked out the sequences of transcript 3'UTR of the targets and aligned them directly with their corresponding miRNA sequences with this software.

2.3 E value The E value was calculated as described^[10,29]. E value means expect value, reflecting the extent of complement between the sequence of miRNAs and the 3'UTR of their corresponding targets.

3 Results

3.1 Computational analysis of genes required for cilia structure and their possible corresponding miRNAs Sensory organs, cilia, in *C. elegans* hermaphrodites appear

Tab. 1 Genetic loci required for aphid structure and function

Locus	Gene	Concise description	Structure or neuron(s) affected
<i>che-2</i>	F38G1.1	WD40 repeat-containing protein	Cilium structure
<i>che-3</i>	F18C12.1	Dyneins, heavy chain	Cilium structure
<i>che-11</i>	C27A7.4	a large, unfamiliar protein	Cilium structure
<i>che-13</i>	F59C6.7	Huntingtin interacting protein 1 (Hip1) interactor Hippi	Cilium structure
<i>daf-10</i>	F23B2.4	unknown	Cilium structure
<i>osm-1</i>	T27B1.1	a predicted intraflagellar transport (IFT) raft protein	Cilium structure
<i>osm-5</i>	Y41G9A.1	TPR repeat-containing protein	Cilium structure
<i>osm-6</i>	R31.3	Sensory cilia assembly protein	Cilium structure
<i>osm-3</i>	M02B7.3	Kinesin-like protein	Cilium structure
<i>che-14</i>	F56H1.1	Predicted patched transmembrane receptor	Cilium structure
<i>daf-6</i>	F31F6.5	unknown	Cilium structure
<i>daf-19</i>	F33H1.1	RFX family transcription factor	Cilium structure
<i>mec-1</i>	T07H8.4	Serine proteinase inhibitor (KU family)	Cilium structure
<i>mec-8</i>	F46A9.6	RNA binding protein (contains RRM repeats)	Cilium structure
<i>dyf-1</i>	F54C1.5	Uncharacterized conserved protein	Cilium structure
<i>dyf-13</i>	C27H5.7	Uncharacterized conserved protein	Cilium structure
<i>tax-2</i>	F36F2.5	orthologous to the human gene CNGB1	ASE,AWC,AWA
<i>tax-4</i>	ZC84.2	Cyclic nucleotide-gated cation channel CNGA1-3	ASE,AWC,AWA
<i>daf-11</i>	C29F5.4	guanylate cyclase	ASE,AWC
<i>daf-21</i>	C47E8.5	a member of the Hsp90 family of molecular chaperones	ASE,AWC
<i>tax-6</i>	C02F4.2	an ortholog of calcineurin A	AFD
<i>che-1</i>	C55B7.12	a C2H2-type zinc finger-containing transcription factor	ASE
<i>odr-7</i>	T18D3.2	an olfactory-specific member of the nuclear receptor superfamily	AWA
<i>odr-1</i>	R01E6.1	a putative guanylyl cyclase	AWC
<i>ttx-1</i>	Y113G7A.6	a member of the conserved OTD/OTX homeodomain protein family	AFD
<i>odr-3</i>	C34D1.3	Gi-like G- α protein	AWC,AWA,ASH
<i>osm-9</i>	B02I2.5	a capsaicin receptor homolog	ASH
<i>glr-1</i>	C06E1.4	an AMPA (non-NMDA)-type ionotropic glutamate receptor	ASH
<i>odr-2</i>	T01C4.2	three isoforms of a predicted membrane-associated protein	AWC
<i>odr-4</i>	Y102E9.1	a novel type II membrane protein	AWC,AWA
<i>odr-10</i>	ZK512.6	a member of 7-transmembrane family of odorant receptors	AWA
<i>daf-2</i>	Y55D5A.5	a predicted receptor tyrosine kinase	ADF
<i>odr-9</i>	F55A8.2	a cyclic GMP-dependant protein kinase	AWA,AWC
<i>osm-10</i>	T20H4.1	a novel protein	ASH
<i>qui-1</i>	Y45F10B.10	WD40 repeat	ASH
<i>gpc-1</i>	K02A4.2	heterotrimeric guanine nucleotide-binding protein	ASH
<i>lim-4</i>	ZC64.4	LIM homeodomain protein homolog	AWB
<i>lim-6</i>	K03E6.1	LIM class homeodomain protein	ASE
<i>ceh-14</i>	F46C8.5	LIM homeodomain protein ortholog	AFD
<i>osm-12</i>	Y75B8A.12	Uncharacterized conserved protein	ASE
<i>bbs-8</i>	T25F10.5	tetratricopeptide repeat-containing protein	ASE
<i>daf-7</i>	B04I2.2	member of the transforming growth factor beta superfamily	ASI

To be continued

Tab. 1 (Continued)

Locus	Gene	Concise description	Structure or neuron(s) affected
<i>gcy-5</i>	ZK970.6	a predicted guanylate cyclase	ASE
<i>gcy-7</i>	F52E1.4	a putative guanyl cyclase receptor	ASE
<i>unc-37</i>	W02D3.9	a transducin-like WD-repeat protein homolog	ASE
<i>die-1</i>	C18D1.1	a C2H2 zinc finger protein	ASE
<i>ceh-36</i>	C37E2.4	a paired-like homeodomain transcription factor	ASE
<i>ttx-3</i>	C40H5.5	a LIM homeodomain protein	AIY
<i>sra-11</i>	F44F4.13	an orphan, G protein-coupled seven-transmembrane receptor	AIY
<i>ceh-10</i>	W03A3.1	a member of the Paired-like class of homeodomain proteins	AIY
<i>ceh-23</i>	ZK652.5	a homeodomain protein	AIY
<i>unc-86</i>	C30A5.7	a transcription factor with a POU-type homeodomain	AIZ
<i>lin-11</i>	ZC247.3	a predicted LIM homeodomain transcription factor	AIZ
<i>lin-32</i>	T14F9.5	a basic helix-loop-helix transcription factor	AIZ
<i>ins-1</i>	F13B12.5	an insulin-like peptide orthologous	AIA

to be chemosensory and thermosensory, based on the fact that they are associated with openings in the cuticle that allow the enclosed neurons to contact the outside environmental cues^[30]. However, whether the miRNAs are involved in the regulation of cilia structure assembly and the possible regulation mechanisms are still unknown. Previous study has showed that there are more than 100 miRNAs already predicted in *C. elegans*^[10]. Here we investigated

the possible miRNA regulation on the genes required for cilia structure assembly. Sixteen genetic loci and 114 miRNAs were selected for assay. Mutations of these genes will cause various defects in sensory cilia, including the absence of cilia (*daf-19*)^[11], deletion of the middle and distal segments (*che-2*, *che-13*, *osm-1*, *osm-5* and *osm-6*)^[11], reduced or irregular cilial segments (*che-3*, *che-11* and *daf-10*)^[11], socket and sheath cell defects (*che-14* and *daf-6*)^[12],

Tab. 2 Complementary nucleotide sequences of genes required for cilia structure assemble and their possible corresponding miRNAs

Locus	Gene	Corresponding miRNA	3'UTR sequence
<i>che-13</i>	F59C6.7	<i>mir-2</i> (65.2%)	gtgtagtccgccttaaacact
		<i>mir-49</i> (68.2%)	atacgtccgagaaccacaacg
<i>osm-5</i>	Y41G9A.1	<i>mir-238</i> (69.6%)	gatttgcccgtaatttaccatttt
		<i>mir-243</i> (78.3%)	ctgtagcgcggctgaaaattgatatcatgg
<i>che-14</i>	F56H1.1	<i>mir-2</i> (59.1%)	cgggtttttggaccatttcaa
		<i>mir-71</i> (63.2%)	agagatcgttgagagaaa
<i>daf-6</i>	F31F6.5	<i>mir-236</i> (52.2%)	ttgcaaaatgctgctgtcaaaa
		<i>mir-83</i> (70%)	tgatttcatgacatcacagat
		<i>mir-49</i> (63.6%)	aaccgaagagctgaaagta
<i>daf-19</i>	F33H1.1	<i>mir-57</i> (75%)	tggtgtgcgagaaagaggtgatcat
		<i>mir-2</i> (56.5%)	ttttattataaaacgatacttt
		<i>mir-80</i> (69.6%)	aggtaaaagatggtatctagagt
<i>mec-1</i>	T07H8.4	<i>mir-67</i> (50%)	gaaacattttttcaattat
<i>mec-8</i>	F46A9.6	<i>mir-67</i> (80%)	gagaaaggagattagctcaaacact
<i>dyf-13</i>	C27H5.7	<i>mir-245</i> (63.6%)	ctatttcaaccttcgaaatca

Note: The numbers in the brackets indicate the identity between miRNA and its predicted target site.

Fig. 1 Distribution of imperfect complementarity locations for miRNAs at 3' untranslated region (UTR) of 16 genes required for cilia structure assembly.

not reconstructed (*dyf-1* and *dyf-13*)^[12], and defects in amphid cilia fasciculation (*mec-1* and *mec-8*)^[11]. All transcripts used here were from the date-bases of website (<http://www.wormbase.org>). The predicted 114 miRNAs have 54 050 corresponding targets according to the database from Griffiths-Jones *et al*^[10].

The computational screen of the corresponding miRNAs was performed according to the E values. Our screen standard is that the E value of the transcript ID should be less than E-03 (including E-03), as described previously^[10]. Usually, it is considered that if the value is below E-03, the complement between miRNA-mRNA would be more reasonable and valuable^[10]. According to our computational analysis, 8 genes from these genetic loci might have their possibly corresponding regulatory miRNA(s). They were *daf-19*, *che-13*, *osm-5*, *daf-6*, *mec-1*, *mec-8* and *dyf-13* (Tab. 2). Their functions covered most of the important aspects for cilia structure assembly as indicated above, suggesting the important roles of miRNAs in cilia structure assembly. However, genes, whose mutation would cause reduced or irregular ciliary segments, were not found to have their possible corresponding miRNAs (Tab. 2), suggesting that these genes are only under the control of RNA coding genes.

To further understand the possible regulation of miRNAs at the 3'UTR of their targets, we examined the complemen-

tary regions of corresponding miRNAs for these identified 8 genetic loci. The position of the complementary region was identified basing on the minimization of miRNA-mRNA free-energy and gaps^[10]. We found that most of the identified genetic loci might have more than one corresponding miRNA, suggesting their multiple upstream regulation pathways. In contrast to this, genetic loci of *mec-1*, *mec-8* and *dyf-13* might have the possible specificities for their miRNA regulation. Moreover, we noticed that *mir-2* might have three targets (*che-13*, *che-14* and *daf-19*), *mir-49* might have two targets of *che-13* and *daf-6*, and *mir-67* might have two targets of *mec-1* and *mec-8*. These data suggest the possible cross-talk for the signaling pathways involved in the control of cilia structure assembly. Furthermore, as shown in Fig. 1, most of these 8 genes were found to have dispersedly distributed miRNAs along the 3'UTR.

3.2 Computational analysis of genes affecting one or a few cell types of sensory neurons and their possibly corresponding miRNAs Chemosensation and thermosensation are two important sensory mechanisms, by which *C. elegans* communicate with its environment. Two bilaterally symmetric amphid neurons are responsible for these behaviors (Fig. 4A). Next, we performed the computational screen for the possibly corresponding miRNAs of genes affecting one or a few cell types of sensory neurons in amphid. Thirty-one genetic loci were selected for assay. Mutations of these genes

could cause various defects in sensory neuron functions. Mutation of *tax-2*, *tax-4*, *daf-11*, *daf-21*, *odr-3*, *odr-4*, *odr-9* would disrupt the functions of a few types of sensory neurons^[12-14,17]. However, mutation of *che-1*, *lim-6*, *osm-12*, *bbs-8*, *gcy-5*, *gcy-7*, *unc-37*, *die-1* and *ceh-36* would cause the ASE function defects^[9, 12, 22]; mutation of *osm-9*, *glr-1*, *osm-10*, *qui-1* and *gpc-1* would affect the ASH function^[12,18-20];

mutation of *daf-2* would result in the ADF function defects^[16]; *daf-7* predominantly regulate the ASI functions^[23]; *odr-7* and *odr-10* control the aspects of AWA functions^[12]; *lim-4* is involved in the regulation of AWB functions^[21]; AWC functions are under the control of *odr-1* and *odr-2*^[12]; and the expression of *tax-6*, *ttx-1* and *ceh-14* is essential for the AFD functions^[12, 15, 21].

Tab. 3 Complementary nucleotide sequences of genes affecting one or a few cell types of sensory neurons and their possibly corresponding miRNAs

Locus	Gene	Corresponding miRNA	3'UTR sequence
<i>tax-2</i>	F36F2.5	<i>mir-60</i> (60.9%)	ctcaattttctatacctatca
		<i>mir-83</i> (59.1%)	acgtcatcaatgtacaaaa
<i>tax-4</i>	ZC84.2	<i>mir-254</i> (69.6%)	atgtcaaatgctttttaaatt
		<i>mir-357</i> (52.4%)	atctggttgataccggtattt
<i>odr-1</i>	R01E6.1	<i>mir-246</i> (68.2%)	tcgagaagaagcatgtacat
<i>ttx-1</i>	Y113G7A.6	<i>mir-242</i> (66.7%)	gtccgatattccggcggaatt
		<i>mir-243</i> (45.5%)	cgatatccggcggaatttcgg
		<i>mir-254</i> (60.9%)	atctctccggttttttagtacg
		<i>mir-256</i> (61.9%)	atttcggagcatcgtactg
<i>odr-2</i>	T01C4.2	<i>mir-54</i> (52.4%)	actctagtaacactaactcctt
		<i>mir-53</i> (51.2%)	cgtttcttcattttttctcaac
		<i>mir-55</i> (62.5%)	aaaagtcttagtacttgacaat
		<i>mir-56</i> (80.1%)	attgtgggtctgtccatagttgt
		<i>mir-72</i> (65%)	ggtactgtagtagtactg
		<i>mir-52</i> (51.7%)	ttgagcatttccatcacgaagcgc
		<i>mir-51</i> (56.5%)	tgatcatatctctgaaacattt
		<i>mir-71</i> (73.7%)	agttattggttgacagaa
<i>odr-4</i>	Y102E9.1	<i>mir-86</i> (56.5%)	acttcatttaaataatcgaat
		<i>mir-240</i> (54.5%)	tacttcatttaaataatcg
<i>daf-2</i>	Y55D5A.5	<i>mir-265</i> (65%)	tttagagttaaagggggaggt
<i>osm-10</i>	T20H4.1	<i>mir-252</i> (56.5%)	aattgacgccttcaactgaat
<i>qui-1</i>	Y45F10B.10	<i>mir-243</i> (60.9%)	cgatgtgtcggccgcagggc
<i>lim-6</i>	K03E6.1	<i>mir-269</i> (58.3%)	ttaaaacaatcaaatcaagaagg
<i>ceh-14</i>	F46C8.5	<i>let-7</i> (73.9%)	ttgaaatgttcagatggaggt
		<i>mir-241</i> (71.4%)	gaaatgttcagatggaggt
		<i>mir-84</i> (73.1%)	atattgaaatgttcagatggaggt
<i>daf-7</i>	B0412.2	<i>mir-265</i> (55%)	aattgtaagaaaaagcttgt
		<i>mir-90</i> (63.6%)	ctgagtaatatggttatacagt
<i>gcy-5</i>	ZK970.6	<i>mir-360</i> (80%)	aacacgtctaagccagc
<i>die-1</i>	C18D1.1	<i>let-7</i> (72.3%)	tgaggctggtagacttttagaggt
		<i>mir-273</i> (55%)	gtgagcggggtcaagaacta
<i>unc-37</i>	W02D3.9	<i>lsy-6</i> (73.9%)	tctttactgtgcacgagctgtattaa
<i>osm-12</i>	Y75B8A.12	<i>mir-272</i> (61.1%)	acaaauuggacacaaaaaac

Note: The numbers in the brackets indicate the identity between miRNA and its predicted target site.

Fig. 2 Distribution of imperfect complementarity locations for miRNAs at 3' untranslated region (UTR) of 31 genes affecting one or a few cell types of sensory neurons.

According to our computational analysis, 16 genes from these 31 genetic loci might have their possibly corresponding regulatory miRNA(s). They were *tax-2*, *tax-4*, *odr-1*, *odr-2*, *odr-4*, *ttx-1*, *daf-2*, *osm-10*, *osm-12*, *qui-1*, *lim-6*, *ceh-14*, *daf-7*, *gcy-5*, *die-1* and *unc-37*(Tab. 3). Similarly, their functions cover most of the important aspects for sensory neuron functions as indicated above, suggesting the essential roles of miRNA that these genes are only under the control of coding RNA genes.

Further, we found that many of the identified genetic

loci might have only one corresponding miRNA, suggesting the possible specificities for their miRNA regulation. Compared to this, genetic loci of *tax-2*, *tax-4*, *ttx-1*, *odr-2*, *odr-4*, *ceh-14*, *die-1* and *daf-7* might have more than one corresponding miRNA, suggesting their multiple upstream regulation pathways. In addition, we noticed that *mir-254* might have two targets (*tax-2* and *ttx-1*), *mir-243* might bind to 3'UTR of its targets of *ttx-1* and *qui-1*, *let-7* might suppressed the expression of its targets of *die-1* and *ceh-14*, and *mir-265* might upstream regulate its targets of *daf-7* and *daf-2*,

which also suggest the possible cross-talk for the signaling pathways involved in the control of aspects of different sensory neuron functions. Moreover, the distribution

of miRNAs along the 3'UTR of these 16 genes exhibits different properties or patterns. Most of them were found to have dispersedly distributed miRNAs along their 3'UTR,

Tab. 4 Complementary nucleotide sequences of genes required for interneuron function and their possible corresponding miRNAs

Locus	Gene	Corresponding miRNA	3'UTR sequence
<i>unc-86</i>	C30A5.7	<i>mir-231</i> (58.3%)	aagccagagtatttattcccaaat
		<i>mir-47</i> (81.8%)	acctcttcgaggagccctgt
		<i>mir-79</i> (59.1%)	cgaacttttaacgaaaaga
<i>lin-11</i>	ZC247.3	<i>mir-58</i> (59.1%)	aaagaatgactaaattgatt
		<i>mir-265</i> (65%)	atggtaaagaaatgagt
		<i>mir-86</i> (56.5%)	cttaaacattttccacaccgatt
<i>ins-1</i>	F13B12.5	<i>mir-82</i> (65%)	atacaaatgataatagtg
		<i>mir-265</i> (55%)	tgccggactaacgggcctgt
		<i>mir-81</i> (70%)	atacaaatgataatagtgga
		<i>mir-60</i> (65%)	caatcttaaacatttttat
<i>ttx-3</i>	C40H5.5	<i>mir-1</i> (71.4%)	attaaaaaagaacatgtaaag
		<i>mir-265</i> (55%)	gatataaggagggaagt
		<i>mir-80</i> (69.6%)	aaaagaacatgtaagactagagt
		<i>mir-81</i> (70%)	atcgtagtgcgacttttat
		<i>mir-58</i> (55%)	atgtaagactagagtcagt
<i>sra-11</i>	F44F4.13	<i>mir-82</i> (65%)	atcgtagtgcgacttttat
		<i>mir-1</i> (85.7%)	aagtttgagatgagaaaatgtaagg
		<i>mir-240</i> (68.2%)	tgtattctaacaccgatca
<i>lin-32</i>	T14F9.5	<i>mir-124</i> (61.9%)	gaagccttacgataaaggcca

Note: The numbers in the brackets indicate the identity between miRNA and its predicted target site.

Fig. 3 Distribution of imperfect complementarity locations for miRNAs at 3' untranslated region (UTR) of 8 genes required for functions of interneuron AIY, AIZ and AIA.

Fig. 4 Circuit and anatomy sketch map of amphid neurons. **A:** The amphid sensory neuron classes consist of one pair of two bilaterally symmetric cells. This figure displays the left sensory neurons' location in amphid^[31]. **B:** Interaction of sensory neurons with downstream interneurons^[18,32]. The possibly corresponding microRNAs and corresponding interneurons, which potentially regulate the functions of amphid neurons, were list in the figure.

such as *tax-2*, *tax-4*, *daf-7* and *ttx-1* (Fig. 2). However, some genes were also found to have specific regions which allow the appearance of centralized distribution of corresponding miRNAs along these regions, such as *odr-2* and *ceh-14* (Fig. 2).

3.3 Computational analysis of genes required for functions of interneuron AIY, AIZ and AIA and their possibly

corresponding miRNAs The ASE, ASH, AFD, ASI, AWA, AWB and AWC neurons, which are important in chemotaxis and thermotaxis, all synapse extensively onto the AIY, AIZ, AIA, AIB and RIA interneurons^[12, 24]. Lastly, we analyzed the possible miRNA regulation on the genes required for interneuron functions. Eight genetic loci were selected for this assay. Mutations of these genes will cause various

defects in interneuron functions, including the deficient in AIY function (*ttx-3*, *sra-11*, *ceh-10* and *ceh-23*)^[24,25], AIZ functions (*unc-86*, *lin-11* and *lin-32*)^[12,26], and AIA functions (*ins-1*)^[27].

Based on our computational analysis, 6 genes from these 8 genetic loci might have their possible corresponding regulatory miRNA(s) (Tab. 4). They were *ttx-3*, *sra-11*, *unc-86*, *lin-11*, *lin-32* and *ins-1*, respectively (Tab. 4). Their functions cover most of the functions of interneuron AIY, AIZ and AIA, suggesting the possible involvement of miRNA regulation in the interneuron functions. Moreover, we found that most of the identified genetic loci might have more than one corresponding miRNA (Tab. 4), suggesting their multiple upstream regulation pathways. Whereas the genetic loci of *lin-32* might have only one corresponding miRNA (Tab. 4), suggesting the possible specificities for its miRNA regulation. Furthermore, we found that *mir-1* might have two targets of *sra-11* and *lin-32*, *mir-81* might have two targets of *ins-1* and *ttx-3*, and *mir-265* might have two targets of *lin-11* and *ins-1* (Tab. 4), suggesting the possible cross-talk for the signaling pathways involved in the control of aspects of different interneuron functions. In addition, these identified genes were found to have dispersedly distributed miRNAs along their 3'UTR (Fig. 3).

4 Discussion

In the current work, we performed the computational analysis for the genetic loci required for the amphid structure and function to identify their possibly corresponding regulatory miRNAs in *C. elegans*. According to the data in this report, miRNAs might participate in almost all aspects of regulation of amphid structure and function. First, miRNAs might exert their effects on the formation of cilia, construction of the middle and distal segments, assembly of socket and sheath cell defects, cilia reconstruction and amphid cilia fasciculation. Second, miRNAs control might be involved in the regulation of ASE, ASI, ASH, AFD, ADF and AWC functions, as well as the integration of multiple sensory neuron functions. Third, the functions of interneuron AIY, AIZ and AIA might be upstream regulated by specific miRNAs.

Again, our data suggest that both the structure and function of amphid might be regulated by a series of network signaling pathways. On the one hand, *mir-2*, *mir-49* and *mir-67* might have three or two corresponding targets required for cilia structure assembly, *mir-254*, *mir-243*, *let-7*

and *mir-265* might suppress the expression of two targets affecting one or a few cell types of sensory neurons, and *mir-1*, *mir-81* and *mir-265* might contain two targets required for interneuron functions. On the other hand, most of the identified genetic loci required for cilia structure and interneuron functions might have more than one corresponding miRNA. Genetic loci of *tax-2*, *tax-4*, *ttx-1*, *odr-2*, *odr-4*, *die-1*, *ceh-14* and *daf-7* might also have more than one corresponding miRNA, suggesting that they might have multiple upstream regulation pathways.

Moreover, we present the possible miRNA-mediated signaling pathways involved in the regulation of chemosensation and thermosensation by controlling the corresponding sensory neuron and interneuron functions (Fig. 4B). Although these signaling pathways still need to be further experimentally confirmed, the information presented here will be very useful for our better understanding of the miRNA-mediated control of the chemotaxis and thermotaxis. The confirmation of these signaling pathways, combined with the elucidation of single and integrated sensory neuron and/or interneuron functions, will help us deeply reveal the molecular basis of chemotaxis and thermotaxis, as well as that of learning and memory. Especially, the upregulation of *die-1* and *unc-37* expression by *mir-273* and *lsy-6* has already been confirmed by experiments^[9]. In addition, since most of the miRNAs sequences are much conserved between different species, our data could also provide some valuable cues for the research on behavior and behavioral plasticity in other species.

Acknowledgements: This work was supported by the Southeast University Foundation for Excellent Young Scholars (No. 4023001013).

References:

- [1] Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 2004, 116: 281-297.
- [2] Brennecke J, Cohen SM. Towards a complete description of the microRNA complement of animal genomes. *Genome Biol* 2003, 4: 228.
- [3] Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNA with antisense complementary to *lin-14*. *Cell* 1993, 75: 843-854.
- [4] Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, *et al.* Conservation of the sequence and temporal expression of *let-7* heterochronic regulatory RNA. *Nature* 2000, 408: 86-89.
- [5] Chen J, Li WX, Xie D, Peng JR, Ding SW. Viral virulence

- protein suppresses RNA silencing-mediated defense but upregulates the role of microRNA in host gene expression. *Plant Cell* 2004, 16: 1302-1313.
- [6] Tsuchiya S, Okuno Y, Tsujimoto G. MicroRNA: biogenetic and functional mechanisms and involvements in cell differentiation and cancer. *J Pharmacol Sci* 2006, 101: 267-270.
 - [7] White JG, Southgate E, Thomson JN, Brenner S. The structure of the nervous system of the nematode *C. elegans*. *Phil Trans R Soc* 1986, 314: 1-340.
 - [8] Ishihara T, Iino Y, Mohri A, Mori I, Gengyo-Ando K, Mitani S, *et al*. HEN-1, a secretory protein with an LDL receptor motif, regulates sensory integration and learning in *Caenorhabditis elegans*. *Cell* 2002, 109: 639-649.
 - [9] Chang S, Johnston RJ Jr, Frokjaer-Jensen C, Lockery S, Hobert O. MicroRNAs act sequentially and asymmetrically to control chemosensory laterality in the nematode. *Nature* 2004, 430: 785-789.v
 - [10] Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res* 2006, 34 (Database issue): D140-D144.
 - [11] Apfeld J, Kenyon C. Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. *Nature* 1999, 402: 804-809.
 - [12] Bargmann CI, Mori I. Chemotaxis and thermotaxis. In: Riddell DL, Blumenthal T, Meyer BJ, Priess JR (Eds.). *C. elegans II*. New York: Cold Springs Harbor Laboratory Press 1997, 717-737.
 - [13] Peckol EL, Zallen JA, Yarrow JC, Bargmann CI. Sensory activity affects sensory axon development in *C. elegans*. *Development* 1999, 126: 1891-1902.
 - [14] Vowels JJ, Thomas JH. Multiple chemosensory defects in *daf-11* and *daf-21* mutants of *Caenorhabditis elegans*. *Genetics* 1994, 138: 303-316.
 - [15] Kuhara A, Inada H, Katsura I, Mori I. Negative regulation and gain control of sensory neurons by the *C. elegans* calcineurin TAX-6. *Neuron* 2002, 33: 751-763.
 - [16] Estevez AO, Cowie RH, Gardner KL, Estevez M. Both insulin and calcium channel signaling are required for developmental regulation of serotonin synthesis in the chemosensory ADF neurons of *Caenorhabditis elegans*. *Dev Biol* 2006, 298: 32-44.
 - [17] L'Etoile ND, Coburn CM, Eastham J, Kistler A, Gallegos G, Bargmann CI. The cyclic GMP-dependent protein kinase EGL-4 regulates olfactory adaptation in *C. elegans*. *Neuron* 2002, 36: 1079-1089.
 - [18] Hart AC, Kass J, Shapiro JE, Kaplan JM. Distinct signaling pathways mediate touch and osmosensory responses in a polymodal sensory neuron. *J Neurosci* 1999, 19: 1952-1958.
 - [19] Hilliard MA, Bergamasco C, Arbucci S, Plasterk RH, Bazzicalupo P. Worms taste bitter: ASH neurons, QUI-1, GPA-3 and ODR-3 mediate quinine avoidance in *Caenorhabditis elegans*. *EMBO J*, 2004, 23: 1101-1111.
 - [20] Hilliard MA, Apicella AJ, Kerr R, Suzuki H, Bazzicalupo P, Schafer WR. *In vivo* imaging of *C. elegans* ASH neurons: cellular response and adaptation to chemical repellents. *EMBO J* 2005, 24: 63-72.
 - [21] Sarafi-Reinach TR, Melkman T, Hobert O, Sengupta P. The *lin-11* LIM homeobox gene specifies olfactory and chemosensory neuron fates in *C. elegans*. *Development* 2001, 128: 3269-3281.
 - [22] Blacque OE, Reardon MJ, Li C, McCarthy J, Mahjoub MR, Ansley SJ, *et al*. Loss of *C. elegans* BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. *Genes Dev* 2004, 18: 1630-1642.
 - [23] Koga M, Takeuchi M, Tameishi T, Ohshima Y. Control of DAF-7 TGF-(alpha) expression and neuronal process development by a receptor tyrosine kinase KIN-8 in *Caenorhabditis elegans*. *Development* 1999, 126: 5387-5398.
 - [24] Remy JJ, Hobert O. An interneuronal chemoreceptor required for olfactory imprinting in *C. elegans*. *Science* 2005, 309: 787-790.
 - [25] Altun-Gultekin Z, Andachi Y, Tsalik EL, Pilgrim D, Kohara Y, Hobert O. A regulatory cascade of three homeobox genes, *ceh-10*, *ttx-3* and *ceh-23*, controls cell fate specification of a defined interneuron class in *C. elegans*. *Development* 2001, 128: 1951-1969.
 - [26] Hobert O, D'Alberti T, Liu Y, Ruvkun G. Control of neural development and function in a thermoregulatory network by the LIM homeobox gene *lin-11*. *J Neurosci* 1998, 18: 2084-2096.
 - [27] Tomioka M, Adachi T, Suzuki H, Kunitomo H, Schafer WR, Iino Y. The insulin/PI 3-kinase pathway regulates salt chemotaxis learning in *Caenorhabditis elegans*. *Neuron* 2006, 51: 613-625.
 - [28] Patterson HG, Graves S. DNAassist, a C++ program for editing and analysis of nucleic acid and protein sequences on PC-compatible computers running Windows 95, 98, NT4.0 or 2000. *Biotechniques* 2000, 28: 1192-1197.
 - [29] Sun Y, Zhao YN, Wang DY. Computational analysis of genetic loci required for synapse structure and function and their corresponding microRNAs in *C. elegans*. *Neurosci Bull* 2006, 22: 339-349.
 - [30] Ye HY, Ye BP, Wang DY. Learning and learning choice in the nematode *Caenorhabditis elegans*. *Neurosci Bull* 2006, 22: 355-360.
 - [31] Starich TA, Herman RK, Kari CK, Yeh WH, Schackwitz WS, Schuyler MW, *et al*. Mutations affecting the chemosensory neurons of *Caenorhabditis elegans*. *Genetics* 1995, 139: 171-188.
 - [32] Tsalik EL, Niacaris T, Wenick AS, Pau K, Avery L, Hobert O. LIM homeobox gene-dependent expression of biogenic amine receptors in restricted regions of the *C. elegans* nervous system. *Dev Biol* 2003, 263: 81-102.

线虫中影响化学传感器结构与功能的遗传位点及其可能对应的 microRNA 的生物信息学分析

胡亚欧^{1,2,3}, 孙阳^{1,2}, 叶波平³, 王大勇^{1,2}

¹ 东南大学基础医学院遗传与发育系, 南京 210009

² 发育与疾病相关基因教育部重点实验室, 南京 210009

³ 中国药科大学生命科学技术学院, 南京 210009

摘要 目的 为了系统评价microRNA (miRNA)在调控化学传感器结构和功能方面的重要作用,我们进行了线虫中有关影响化学传感器结构与功能的遗传位点以及和其可能对应的miRNA的生物信息学分析。**方法** 我们选择了55个与化学传感器结构与功能相关的遗传位点,并且进一步通过评价序列比对后的E值等手段来分析和确认其可能对应的miRNA。**结果** 在55个遗传位点中,我们发现其中30个遗传位点存在与其可能对应的miRNA。这些所鉴定的基因在功能上几乎涵盖了化学传感器结构和功能调控的诸多方面。而且,我们的数据还表明化学传感器结构和功能可能受到一系列网状交联的上游信号通路所调节。另外,对应miRNA在这30个基因的3'端非编码区上的分布也呈现出各自特有的模式。**结论** 我们勾勒出了可能存在的由miRNA介导的通过控制相应感觉神经元与中间神经元功能从而调控化学趋向性与温度趋向性的信号通路。本研究将有助于我们更好的了解线虫中可能由miRNA调控的化学趋向性和温度趋向性行为。

关键词: microRNA; 化学传感器结构; 感觉神经元; 中间神经元; 生物信息学分析; 秀丽线虫