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예쁜꼬마선충 IL2 뉴런에서 RFX 전사인자의 서브루틴으로서의 새로운 기전 연구

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as a regulatory subroutine in IL2 neurons of *C. elegans*

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생명과학부

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이 논문을 이학박사학위논문으로 제출함

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ABSTRACT

A novel function of RFX transcription factor as a regulatory subroutine in IL2 neurons of *C. elegans*

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The function of cilia, as sensory organelles of the eukaryotic cell, is crucial to organismal survival. In humans, cilia dysregulation leads to broad defects in various organs, including the nervous system. In this study, I used the free-living nematode *Caenorhabditis elegans* as a model system to understand the relationship between cilia function and behavior. Nictation, a dauer-specific behavior, is a crucial sensory behavior for successful interaction with carrier organisms to move worms to suitable habitats. IL2-ciliated neurons and their intact cilia structure are essential to initiate this dispersal behavior. *klp-6*, the IL2-specific kinesin, is expected to transport ciliary cargo to the distal cilia tip for IL2-specific function. From forward genetic screening, I identified a transcription factor DAF-19M that regulates the expression of *klp-6*, specifically in IL2 neurons. DAF-19M, an isoform of DAF-19, which is the worm homolog of Regulatory Factor X transcription factor (RFX TF), regulated *klp-6* gene expression through the non-canonical X-box motif in its promoter. Besides, I found two other *daf-19m* target genes, *osm-9* and *cil-7*, which have functions in cilia. I also elucidated that *daf-19m* and its target genes are constituents of a regulatory subroutine in IL2 neurons. I showed that IL2 terminal selectors *unc-86* and *cfi-1* regulate *daf-19m*, which in turn regulates a part of IL2

identity genes. I discovered that *daf-19m* regulates nictation behavior, as its target genes regulating nictation shown in a previous study. Considering the molecular identities of *daf-19m* target genes, I suggest that *daf-19m* and its target genes regulate IL2-specific neuronal differentiation as a single functional module for the nictation behavior in cilia. In summary, I report the key regulator for the terminal differentiation of IL2 neurons in *C. elegans* and the molecular connection between the gene expression in cilia and the dispersal behavior for the species.

Keywords: *C. elegans,* cilia, IL2 neurons, neuronal development, DAF-19M, regulatory subroutine, nictation behavior

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Introduction

The structure and components of cilia

In eukaryotes, specialized hair-like structures on cell have various functions and are evolutionally conserved (Sengupta, 2017). Cilia can be categorized into two types in mammals; motile cilia, and non-motile cilia (also known as primary cilia), which includes sensory cilia. Both motile cilia and non-motile cilia have axoneme, a microtubule-based structure that protrudes from the basal body. While primary cilia are comprised of only nine microtubule doublets (9+0 structure), motile cilia have extra two microtubule singlets in the center (9+2 structure). Despite different structures, both cilia structures have intracellular transport (IFT) as their core component. IFTs transport axonemal subunits, such as motor protein, tubulin, cilia-localized channel and G protein for cilia growth, maintenance and protein export (Kozminski et al., 1993; Lechtreck, 2015). IFTs show bidirectional movement along microtubules from the basal body to cilia tip and vice versa (Kozminski et al., 1993), mediated by two different motor proteins; kinesin-2 (anterograde) and dynein-1b (retrograde) (Cole et al., 1998; Porter et al., 1999).

Cilia as a sensory organelle in eukaryotes

Organisms need to sense external stimuli for environmental adaptation, which is directly connected to their survival. Primary cilia have the central role of sensation and signal transmission. For example, the kidney contains primary cilia to sense a fluid flow or mechanical stimulation (Pazour and Witman, 2003). In the liver, the cilia of cholangiocytes, located in the epithelial cells, sense changes in the flow of bile and transmit to the cell interior affecting the function of cholangiocyte (Larusso and Masyuk, 2011; Masyuk et al., 2006). The organs for sensory perception such as hearing, olfaction and light detection in mammals, have the primary cilia, and these primary cilia are also known as sensory cilia (Choksi et al., 2014; Falk et al., 2015). In all these organs, proper ciliogenesis, structure and gene expression are required for the proper function of primary cilia.

Cilia and ciliopathies

Ciliopathy is a genetic disorder that affects cilia formation or cilia maintenance. In the case of human, cilia have different roles in various organs and ciliopathies affect functions of those organs including the brain, the most studied organ about ciliopathies.

For example, some ciliopathies cause severe neural defects for sensory cilia that belong in the nervous system. In the case of Bardet-Biedl Syndrome and Joubert Syndrome, which are categorized as ciliopathies, patients show symptoms of brain abnormalities and cognitive impairment (Brinckman et al., 2013; Juric-Sekhar et al., 2012). Additionally, ciliopathies are reported to cause mental disorders, such as dyslexia, schizophrenia and bipolar disorder in recent years (Guemez-Gamboa et al., 2014).

RFX transcription factor and X-box motif

Regulatory Factor X transcription factors (RFX TFs) are the most important regulators of ciliogenesis and are well-conserved in many eukaryotes (Chu et al., 2010). Human RFX orthologs are usually broad regulators for ciliogenesis of the nervous system, mainly expressed in the cerebellum and spinal cord (Sugiaman-Trapman et al., 2018). RFX TFs commonly have DNA binding domain, and have dimerization domain except for RFX5 and RFX7 (Chakraborty et al., 2010; Morotomi-Yano et al., 2002; Nie et al., 2015; Reith et al., 1994; Smith et al., 2010). RFX TFs recognize and bind X-box motif in the promoter region of target genes. The first reported consensus X-box motif sequence is

an imperfect palindromic structure (GTNRCC/N-N_{0-3}/RGYAAC) of two 6nt half motifs with from 0 to 3 spacer nucleotides in the middle (Emery et al., 1996). In recent years, a variety of X-box motif sequences with small variants are reported in various organisms including *C. elegans* (Blacque et al., 2005; Chen et al., 2006; Swoboda et al., 2000).

C. elegans **as a model system for sensory cilia**

C. elegans has advantages to study sensory cilia in terms of the ciliogenesis, which is an evolutionally conserved process. Many researchers studied neuronal development and regulation of sensory neurons, including the cilia structure (Hobert, 2010; Inglis et al., 2007). *C. elegans* has only 302 neurons, and each of their neuronal connections have been studied extensively. Thus, cilia function, as a sensory organelle, can be analyzed easily in organismal behaviors, derived from defects of sensory neurons. Additionally, genomic analysis of other *Caenorhabditis* species facilitates motif study for regulation of neuronal development through bioinformatic approach (Kiontke and Fitch, 2005). Together, *C. elegans* is a powerful model system to study sensory cilia and its connection with neuronal development and behaviors.

RFX transcription factor in *C. elegans*

daf-19, a sole ortholog of mammalian RFX TFs in *C. elegans*, acts as a master regulator of ciliogenesis through X-box motif to express target genes (Swoboda et al., 2000). Instead of paralogs, *daf-19* has multiple isoforms to perform various functions. *daf-19c* regulates pan-sensory ciliogenesis, while *daf-19a* and *daf-19b* regulate synaptic homeostasis function (Senti and Swoboda, 2008). *daf-19m*, the isoform for mating behavior, is expressed in a subset of sensory neurons: male specific neurons (MSN; CEM, RnB, HOB) and IL2 in both males and hermaphrodites (Wang et al., 2010). However, the studies have not elucidated whether *daf-19m* shares target genes with other *daf-19* isoforms in IL2 neurons, considering the relationship between *daf-19a* and *daf-19c* (De Stasio et al., 2018).

Terminal selector and regulatory subroutine in neuronal development

Using *C. elegans* as a model system, many studies have elucidated regulators that affect a development of specific neurons and behavior elicited by those neurons.

Notably, the terminal selector, which defines and maintains neuron identity, is a crucial factor for neuronal development on the specific neurons. The combination of small numbers of terminal selector regulates a variety of neuronal differentiations (Hobert, 2008, 2011, 2016). These terminal selectors express many genes that are directly connected to neuronal identity. Among the target genes of terminal selectors and regulatory subroutines, the modules that regulate specific parts of neuronal function. Loss of terminal selector causes defects in neuronal identity and function, but the loss of subroutine does not influence neuronal identity (Altun-Gultekin et al., 2001; Etchberger et al., 2009; Etchberger et al., 2007; Gordon and Hobert, 2015; Hobert, 2016; Zhang et al., 2014). Although regulatory subroutine has the role, regulating some of neuronal identity gene expressions, the mechanism of target gene regulation and the significance of subroutine need to be studied, especially to the behaviors.

Nictation behavior and IL2 neurons

Previous studies have established genetic approach and quantitative analysis for nictation assay (Lee et al., 2017; Lee et al., 2012) after the behavior was first reported

(Cassada and Russell, 1975). The cilia structure and acetylcholine transmission of IL2 neurons, the neurons in which *daf-19m* is expressed, is essential to nictation, a dauer-specific dispersal behavior (Lee et al., 2012). Nictation behavior enables worms to ride carrier organisms in nature to move into new environments suitable for reproduction and survival (Lee et al., 2017; Lee et al., 2012). While majority of worms in nature are in their dauer stage, worms can develop into reproducing adult stage and quickly increase their population. In this boom and bust cycle, nictation plays a crucial role of finding the new environment for conservation of the species. IL2 neurons, known to regulate nictation behavior, and IL1 neurons, located in inner labial sensilla with IL2, are differentiated from common progenitor. Unlike IL1 neurons, cilia end of IL2 protrudes to the outside, implying interaction with outer environment. Therefore, IL2 specific factor for the development and activation, highly related to the terminal selector, possibly, should be investigated with the relationship with nictation behavior.

Purpose of this study

In this study using *C. elegans*, which is a suitable model system for research on

the relationship between neuronal development and organismal behavior, I tried to find out the relationship between the cilia structure and nictation behavior in IL2 neurons. Collaborators and I performed a forward genetic screening to identify IL2 specific regulator for IL2 neuronal development and functional activation of IL2 neurons. I was able to elucidate the role of the *daf-19m* regulatory subroutine, which is important to IL2 neuronal development, and the mechanism of target gene expression by *daf-19m*, and the contribution of the *daf-19m* regulatory subroutine in nictation behavior.

This study demonstrates that the *daf-19m* regulatory subroutine, as a module, regulates the terminal differentiation of IL2 neurons in *C. elegans* and suggests the biological meaning of this subroutine, in terms of evolution of species.

Materials and Methods

Worm maintenance and used strains

Worms were maintained at 20℃ and handled as previously described (Brenner, 1974) except for maintaining *daf-19(of3), daf-19(of4), daf-19(m86)* and *daf-19(rh1024)* mutants for the high frequency of dauer at 20℃. They are grown at 15℃ (Swoboda et al., 2000). The Bristol strain N2 was used as the wild type. Mutant strains used in this study have the following genotypes.

Mutants: DR86 *daf-19(m86) II*, OE3059 *daf-19(rh1024) II*, LJ897 *daf-19(n4132) II*, LU628 *daf-19(tm5562) II*, DJK218 *daf-19(sm129) II*; *him-5(e1490) V*, JT6924 *daf-19(m86) II; daf-12(sa204) X*, CB4088 *him-5(e1490) V*, LJ898 *klp-6(sy511) III*, CX10 *osm-9(ky10) IV*, JY190 *osm-9(yz6) IV*, LJ899 *cil-7(tm5848) I*, UR500 *tba-6(cxP4018) I; him-5(e1490) V*, MT1859 *unc-86(n846) III*, OS122 *cfi-1(ky651) I*

The transgenic and genome insertion lines have one of following genotypes and transgenes: LJ800 *jlIs1900* [*klp-6p::gfp*; *aqp-6p::ds-red; rol-6(su1006)*], OE4502 *daf-19(of3) II*; *jlIs1900* [*klp-6p::gfp; aqp-6p::ds-red; rol-6(su1006)*], OE4503 *daf-19(of4) II*;

jlIs1900 [*klp-6p::gfp; aqp-6p::ds-red; rol-6(su1006)*], LJ801 *daf-19(of3); jlEx1902* [*daf-19 fosmid (WRM0622dH09); act-5p::gfp, klp-6p::gfp; aqp-6p::ds-red; rol-6(su1006)*], LJ802 *daf-19(of4); jlEx1902* [*daf-19 fosmid (WRM0622dH09); act-5p::gfp, klp-6p::gfp; aqp-6p::ds-red; rol-6(su1006)*], LJ803 *daf-19(rh1024); jlEx1903* [*F28A12.3p::gfp*], PT2519 *myIs13* [*klp-6p::gfp*], LJ804 *jlEx1900* [*klp-6p::gfp; rol-6(su1006)*], LJ805 *daf-19(m86) II; jlEx1900* [*klp-6p::gfp; rol-6(su1006)*], LJ806 *daf-19(rh1024) II; jlEx1900* [*klp-6p::gfp; rol-6(su1006)*], LJ807 *jlEx1904* [*klp-6p (-641, -1)::gfp; rol-6(su1006)*], LJ808 *jlEx1905* [*klp-6p (-614, -1)::gfp; rol-6(su1006)*], LJ809 *jlEx1906* [*klp-6p (-607, -1)::gfp; rol-6(su1006)*], LJ810 *jlEx1907* [*klp-6p (-581, -1)::gfp; rol-6(su1006)*], LJ811 *jlEx1908* [*klp-6p (-1048, -624)::gfp; rol-6(su1006)*], LJ812 *jlEx1909* [*klp-6p (-1048, -608)::gfp; rol-6(su1006)*], LJ813 *jlEx1910* [*klp-6p (-1048, -600)::gfp; rol-6(su1006)*], LJ814 *jlEx1911* [*klp-6p (-1048, -590)::gfp; rol-6(su1006)*], LJ815 *jlEx1912* [*klp-6p (deletion, -614, -608)::gfp; rol-6(su1006)*], LJ816 *jlEx1913* [*klp-6p (substitution to A, -614, -608)::gfp; rol-6(su1006)*], LJ817 *jlEx1914* [*klp-6p (inverted, -614, -608)::gfp; rol-6(su1006)*], LJ818 *jlEx1915* [*klp-6p (inverted, -607, -600)::gfp; klp-6p::mcherry; unc-122p::ds-red*], LJ819 *jlEx1916* [*klp-6p (inverted, -614, -600)::gfp; klp-6p::mcherry; unc-*

122p::ds-red], LJ820 *jlEx1917* [*klp-6p (Tandem 3x, -614, -608)::gfp; rol-6(su1006)*], LJ821 *jlEx1918* [*klp-6p (Tandem 3x, -614, -602)::gfp; klp-6p::mcherry; unc-122p::ds-red*], LJ822 *jlEx1919* [*klp-6p (Tandem 3x, -628, -590)::gfp; klp-6p::mcherry; unc-122p::ds-red*], LJ823 *daf-19 (m86) II; daf-12(sa204) X; jlEx1919* [*klp-6p (Tandem 3x, -628, -590)::gfp; klp-6p::mcherry; unc-122p::ds-red*], LJ824 *daf-19 (m86) II; daf-12(sa204) X; ggEx14* [*daf-19c*; *klp-6p (Tandem 3x, -628, -590)::gfp; klp-6p::mcherry; unc-122p::ds-red*], LJ825 *daf-19 (m86) II; daf-12(sa204) X; jlEx1920* [*daf-19c(deletion, HOB, RnB element of daf-19m promoter)*; *klp-6p (Tandem 3x, -628, -590)::gfp; klp-6p::mcherry; unc-122p::ds-red*], LJ826 *daf-19 (m86) II; daf-12(sa204) X; jlEx1921* [*daf-19m*; *klp-6p (Tandem 3x, -628, -590)::gfp; klp-6p::mcherry; unc-122p::ds-red*] LJ827 *daf-19 (m86) II; daf-12(sa204) X; jlEx1928* [*daf-19c*; *daf-19m*; *klp-6p (Tandem 3x, -628, -590)::gfp; klp-6p::mcherry; unc-122p::ds-red*], LJ828 *daf-19 (tm5562) II*; *jlEx1903* [*klp-6p::gfp; myo-2p::mcherry*], LJ829 *daf-19 (m86) II; daf-12(sa204) X; jlEx1922* [*osm-9p::gfp*; *klp-6p::mcherry; unc-122p::ds-red*], LJ830 *daf-19 (m86) II; daf-12(sa204) X; jlEx1922* [*daf-19m; osm-9p::gfp*; *klp-6p::mcherry; unc-122p::ds-red*], LJ831 *daf-19 (m86) II; daf-12(sa204) X; jlEx1923* [*cil-7p::gfp*; *klp-6p::mcherry; unc-122p::ds-red*],

LJ832 *daf-19 (m86) II; daf-12(sa204) X; jlEx1923* [*daf-19m; cil-7p::gfp*; *klp-6p::mcherry; unc-122p::ds-red*], LJ833 *daf-19 (m86) II; daf-12(sa204) X; jlEx1924* [*tba-6p::gfp*; *klp-6p::mcherry; unc-122p::ds-red*], LJ834 *daf-19 (m86) II; daf-12(sa204) X; jlEx1924* [*daf-19m; tba-6p::gfp*; *klp-6p::mcherry; unc-122p::ds-red*], LJ835 *jlEx1925* [*daf-19mp::gfp; klp-6p::mcherry; rol-6(su1006)*], LJ836 *unc-86 (n846) III; jlEx1925* [*daf-19mp::gfp; klp-6p::mcherry; rol-6(su1006)*], LJ837 *cfi-1(ky651) I; jlEx1925* [*daf-19mp::gfp; klp-6p::mcherry; rol-6(su1006)*], LJ838 *jlEx1926* [*daf-19mp (deletion, -132, -116)::gfp; klp-6p::mcherry; rol-6(su1006)*], JK2868 *qIs56* [*lag-2p::gfp; unc-119*], LJ839 *daf-19(n4132) II*; *qIs56* [*lag-2p::gfp; unc-119*], LX929 *vsIs48* [*unc-17::gfp*], LJ840 *daf-19 (n4132) II*; *vsIs48* [*unc-17::gfp*], LJ841 *cfjEx90* [*myo-2p::mcherry*], LJ842 *daf-19(n4132) II; cfjEx90* [*myo-2p::mcherry*], LJ843 *daf-19(n4132) II; jlEx1921* [*daf-19m; myo-2p::mcherry*], LJ844 *daf-19(n4132) II; jlEx1927* [*F28A12.3p::klp-6::gfp; F28A12.3 p::osm-9::gfp; myo-2p::mcherry*]

See **Table 1** for details on transgenic alleles used in this study.

Generation of reporter and rescue constructs

For expression reporter and rescue constructs, standard molecular cloning, Gibson assembly and Q5 site-directed mutagenesis (New England Biolabs, MA) methods were used. See **Table 2** for information on constructs used in this study.

Promoter analysis for motif search

To find a *cis*-regulatory element of *klp-6* and *daf-19m,* fusion PCR using overlapped sequence (Hobert, 2002) and plasmid mutagenesis using Q5 site-directed mutagenesis kit methods are used. See **Table 1** for information on constructs in this study.

Transgenic worms by Microinjection

Microinjection of DNA into the gonad of 0 Day young adult hermaphrodites was carried out to express constructs (Mello et al., 1991). To isolate transgenic animal, *rol-6(su1006)* (roller phenotype), *unc-122p::ds-red* (fluorescent coelomocyte marker), *myo-2p::mcherry* (fluorescent pharynx marker), *act-5p::gfp* (fluorescent intestine marker) were used. Plasmid DNAs used for injection were extracted with the QIAGEN plasmid midi kit (Cat.

No. 12145) or Axygen midi prep kit (Cat. No. AP-MD-P-25) or Macherey-Nagel NucleoBond Xtra Midi kit (Cat. No. 740410.100). See **Table 1** for detail microinjection information, examined in this study.

Genome insertion by Gamma-ray irradiation

For efficiency of forward genetic screening, L4 synchronized transgenic worm *jlEx1900* [*klp-6p::gfp*; *aqp-6p::ds-red; rol-6(su1006)*] are irradiated to 4000 rad of gamma-ray. Single irradiated worms were moved to plates to check that transgenes were integrated into the genome completely. If the transgenic marker were shown at 100% in the plate, the expression of transgenes was examined by microscope.

EMS mutagenesis and isolating mutation

To find regulator of *klp-6* expression in IL2 neuron, *jlIs1900* [*klp-6p::gfp*; *aqp-6p::ds-red; rol-6(su1006)*] animals were treated with 50mM EMS and above 15,000 worms of F1 generation were plated. Mutations were identified by the reduced number of IL2 neuronal expression. 2 independent lines were isolated using COPAS BIOSORT large

particle flow cytometer (Union Biometrica, MA) for high throughput sorting, measuring fluorescence intensity, time-of-flight (for animal length) and extinction (optical density). Using SNP mapping and whole genome sequencing of DAF-19 coding regions, we found these two mutations were located in *daf-19*. Dauer formation assays and DiO fluorescent dye staining assays for the ciliated neuron to confirm *daf-19* mutation were performed as described (Hedgecock et al., 1985; Perkins et al., 1986).

Yeast one hybrid screening and manual reaction with candidate gene

klp-6 regulatory element of the promoter (-628, -590; gtccgtttcc tttcgtcgct tggagaccta catggcaac) was cloned into the pADE2i vector, constructed by Panbionet (Pohang, Korea) for one hybrid assay to identify DNA binding proteins. The vector, designed for Matchmaker Gold Yeast One hybrid library (Clontech, CA), contains yeast iso-1cytochrome C minimal promoter and *ADE2* gene. *C. elegans* cDNAs were inserted to the pPC86 vector, contains the GAL4 activation domain. A *cis*-acting element (a target sequence or bait) can be cloned in the MCS and used to screen GAL4 AD fusion libraries for proteins that interact with the target sequence. Positive interactions showed the *ADE2*

expression, and PCR and manual sequencing examined cDNA sequences were examined by PCR and manual sequencing.

Dauer induction

10 L4 larvae or 0-day young adults were moved to synthetic pheromone plates, including thin layered *E. coli* OP50 at 25℃ for dauer induction (Lee et al., 2015; Lee et al., 2017; Lee et al., 2012). Synthetic pheromone plates contain agar (10g/L), agarose (7g/L), NaCl (2g/L), KH_2PO_4 (3g/L), K_2HPO_4 (0.5g/L), cholesterol (8mg/L) and ascaroside 1, 2, 3 (2mg/L each) (Butcher et al., 2007; Jeong et al., 2005; Lee et al., 2017). Synthetic pheromones were provided by Young-Ki Paik's lab of Yonsei University. After 4 to 5 days, dauer can be recognized for their radially constricted bodies and dark intestines.

Nictation assays

More than 30 dauers were collected by glass capillary tube using M9 isotonic buffer and mounted on the micro-dirt chip. A micro-dirt chip was made by pouring a 3.5% agar solution onto PDMS mold (Lee et al., 2015). The solidified chip was detached PDMS

mold and dried for 90 min at 37℃. After 10-30 min, when dauers started to move, nictation was quantified as a fraction of nictating worms among moving dauers. Quiescent dauers were excluded from measuring. Nictation assays were measured at 25℃ and humidity of 30%. Assays were repeated at least seven times for quantification.

Bioinformatic analysis

The information about the binding site for *daf-19c*, *unc-86*, and *cfi-1* were from JASPAR transcription factor binding profile database (http://jaspar.genereg.net; (Sandelin et al., 2004)) and Oliver Hobert group's paper (Kerk et al., 2017). *Cis*-element conservation amongst other *Caenorhabditis* species was analyzed using pairwise sequence alignment (EMBOSS Water; https://www.ebi.ac.uk/Tools/psa/emboss_water/).

Microscopy

The transgenic animals were mounted on 3% agar pads, and the fluorescence was observed using a confocal microscope (ZEISS LSM700, Carl Zeiss, Inc) or Axioplan 2 fluorescence microscope (Carl Zeiss, Inc).

Results

Isolation of regulator for IL2 development and functional activation

klp-6 encodes kinesin motor protein, carrying crucial factors for cilia structure with another kinesin molecule, *osm-3*, in the cilia of IL2 and MSNs (Peden and Barr, 2005). Considering the importance of cilia structure in IL2 to nictation behavior (Lee et al., 2012), *klp-6* could be an IL2 specific marker to find the regulator of IL2 development and its function.

To screen IL2 regulator, I made a genome inserted transgenic line, *jlIs1900*, expressing *klp-6p::gfp* and *aqp-6::ds-red*, aquaporin in IL1 exclusive (Fig.1; Park, 2015). IL1 neurons are also ciliated neurons, which are the sibling of IL2 neurons on neuronal differentiation (Fig.2; Sulston et al., 1983). I treated ethyl methane sulfonate (EMS) to *jlIs1900*, and isolated F2 generation mutant using COPAS Biosort. To avoid the defect of IL2 progenitor, I tried to find decreased IL2 expression worms without the change of IL1 expression (Fig.1; Park, 2015).

After 15,000 F1 screenings, I isolated three independent lines, but only two of three lines were maintainable. Through Single Nucleotide Polymorphism (SNP) mapping and whole genome sequencing, I found that two mutations (*of3* and *of4*), located in *daf-*

19 (Fig.3; Park, 2015). *of3* mutation was located in the dimerization domain (DIM), and *of4* mutation was located in the DNA binding domain (DBD) of *daf-19*.

New mutations of *daf-19* phenocopy classical *daf-19* mutant phenotypes

I checked canonical *daf-19* phenotypes, reported in previous studies (Perkins et al., 1986; Swoboda et al., 2000), in our *daf-19* mutants. As described, *klp-6* expression decreased in *of3* and *of4* mutant (Fig.4; Park, 2015). I also checked *klp-6p::gfp* expression in other *daf-19* mutants (Fig.5). *daf-19* mutants showed fluorescent dye staining defect in cilia for the absence of cilia. *of3* (Fig.6) and *of4* (data not shown) alleles show dye filling defect. Another significant phenotype of *daf-19* mutants was the dauer constitutive (Daf-c) phenotype. Both *of3* and *of4* alleles show strong Daf-c phenotypes (data not shown). When I rescued *daf-19* fosmid (*WRM0622dH09*) to *of3* and *of4* alleles, all *daf-19* mutant phenotypes were restored (Figs.4 and 6; Park, 2015). I checked whether IL2 neurons are ablated in the *daf-19* mutant. In *daf-19* (*rh1024*), IL2 specific single transmembrane protein F28A12.3 (*F28A12.3p::gfp*) shows intact IL2 expression, which means IL2 cells were developed and alive (Fig.7; Park, 2015). Thus, *daf-19* regulates *klp-*

6 expression in IL2, cilia development, and dauer formation without affecting the cell fate of IL2 neurons.

Identification of *cis*-regulatory element of *klp-6*

In the previous study, it was reported that the *klp-6* promoter did not contain the X-box motif, the canonical motif for *daf-19* to regulate target genes (Fig.8; Peden and Barr, 2005). I investigated the *klp-6* promoter region to find the *cis*-regulatory element for *daf-19*. Using the Fusion PCR method, I made some of the truncated *klp-6* promoters and fused to GFP (Fig.9; Park, 2015). In these transgenic worms, LJ808, carrying *klp-6p (-614, -1)::gfp* and LJ813, carrying *klp-6p (-1048, -600)::gfp* showed intact expression (Fig.9; Park, 2015). In other words, from -614 to -600 of *klp-6* promoter region contributed *klp-6* expression. Interestingly, this region contained parts of the canonical X-box motif, especially 5' and 3' half regions with single spacer T, which is different from double spacer AT in canonical X-box motif.

To check whether this region was necessary to express *klp-6*, I made mutated *klp-6* promoters, especially from -614 to -600 region of *klp-6 cis*-element using plasmid

mutagenesis method (Fig.10). When 5' half region of the element is mutated, such as deletion, substitution to AAAAAAA, and inversion to a complementary strand, the expression of *klp-6* decreased dramatically (Fig.10). Still, one of three transgenic lines of deletion (-614, -608) showed very dim expression of various number IL2 cells, but the others showed almost off-signals. Unlike alteration of 5' half region, 3' half region inversion of the element did not affect the expression of *klp-6* that much (Fig.10). Even some transgenic worms showed expression with reduced IL2 number cells, GFP signals were still strong, and almost transgenic worms showed intact expression (Fig.10). Besides, the whole region (from -614 to -600) inversion of element showed intact IL2 expression except a few animals, meaning this regulatory element did not have any direction (Fig.10).

I also tried to check the sufficiency of this element. I cloned the plasmid, including three copies of tandem repeats to empty pPD95.77 vector using the plasmid mutagenesis method (Fig.11). Transgenic worms with tandem repeats of only 5' half site did not show any expression at all. However, tandem repeats of the whole region (from -614 to -602) of *klp-6 cis*-element and extended region (from -628 to -590) of element showed IL2 expression, though the signal was weak (Fig.11). Especially, transgenic

worms carrying tandem repeats of extended element showed intact six cell expression. Together, the *cis*-element of *klp-6* was necessary and sufficient to express *klp-6* expression. I also checked this non-canonical X-box motif could express *klp-6* in male specific neurons (MSNs) because there was a tendency that IL2 and MSN expression together (Peden and Barr, 2005; Wang et al., 2015; Wang et al., 2010). In males of LJ822, expressing tandem repeats of the extended element, CEM, RnB, and HOB; male specific neurons showed GFP signals (Fig.12).

I investigated the conservation of this element in other *Caenorhabditis* species, and I found similar sequences that were located in *C. briggsae* and *C. brenneri* (Fig.13). I concluded that this *cis*-element was a non-canonical X-box motif.

***daf-19m*, isoform for mating, regulates non-canonical X-box motif and IL2 cilia genes**

klp-6 expression was restricted in only IL2 and MSNs (Peden and Barr, 2005). I hypothesized that *daf-19* and its cofactor regulated specific expression. To figure out this, I performed yeast one hybrid (Y1H) screening in collaboration with Panbionet (Pohang,

Korea). I screened the *C. elegans* cDNA library, which could bind to a specific sequence, using tandem repeats of *klp-6 cis*-element (from -628 to -590) as a bait sequence. Surprisingly, 114 of 119 colonies were *daf-19*, and all seven sequenced colonies were *daf-19a*, the most abundant isoform in the organism (Table 3). Because *klp-6* expression was restricted in specific neurons (Peden and Barr, 2005), I tested *daf-19m*, which is exclusively expressed in IL2 and MSNs (Wang et al., 2010). I confirmed that the colony carrying *daf-19m* cDNA could bind bait sequences and could express the *ADE2* gene. Together, I concluded that all isoforms of *daf-19* could bind to the non-canonical X-box motif of *klp-6* in yeast cells.

Next, I tried to check that *daf-19* isoforms could regulate the non-canonical X-box motif. In IL2 neurons, cilia-related isoforms could be *daf-19c* and *daf-19m*, regarding that the non-canonical X-box motif came from the *klp-6* promoter (Senti and Swoboda, 2008; Wang et al., 2010). As a result, I expressed *daf-19c*, *daf-19m*, and both isoforms to *daf-19* null mutant, JT6924 *daf-19(m86); daf-12(sa204)* (Fig.14). In *daf-19c* (*pGG14* and *pJL1920*, deletion for eliminating partial *daf-19m* rescue effect in male specific neurons) rescue, non-canonical X-box motif could not be expressed in IL2 neurons (Fig.15), even

klp-6p::mcherry showed partial IL2 expression. However, in *daf-19m* rescue (with IL2/CEM enhancer insertion on promoter region), tandem repeats showed IL2 expression, though not intact expression (Fig.15; Wang et al., 2010). Additionally, in both *daf-19c* and *daf-19m* rescue, the non-canonical X-box motif showed more increased IL2 expression, almost six cells, than a *daf-19m* single rescue (Fig.15). I also checked the contribution of *daf-19a* and *daf-19b* effect to *klp-6* expression. In *daf-19(tm5562)* mutant, *klp-6* expression was an intact expression in IL2 neurons (Fig.16), suggesting both *daf-19a* and *daf-19b* did not regulate *klp-6* expression.

Because only *daf-19m* could rescue IL2 expression in *daf-19* null mutants, I also tried to find *daf-19m* downstream genes. Considering the function of *daf-19m*, target genes of *daf-19m* may not regulate cilia development in IL2 neurons. *daf-19(n4132)*, the *daf-19m*-specific mutant, disrupted cilia localization of some ciliary proteins, but not cilia development in IL2 neurons (Wang et al., 2010). *klp-6* also regulates the cilia localization of PKD-2, TRP channel protein, in male specific neurons (Peden and Barr, 2005). I checked *osm-9* (TRPV channel in many ciliated neurons including IL2s), and *cil-7* (protein with myristoyl group) and *tba-6* (IL2 and MSNs specific alpha-tubulin). Both *cil-*

7 and *tba-6* are members of polycystic kidney disease (PKD) gene battery, moving polycystin gene homolog PKD-2 and LOV-1, causing defects of cilia function in human kidney (Barr et al., 2001; Barr and Sternberg, 1999; Hurd et al., 2010; Maguire et al., 2015; O'Hagan et al., 2014; Wang et al., 2010). In *osm-9* transcriptional fusion (with 3' region after stop codon for IL2 expression (Colbert et al., 1997)) and *cil-7*, only *daf-19m* rescue can restore IL2 expression without *daf-19c* (Fig.17). However, *tba-6* showed intact IL2 expression, regardless of *daf-19m* rescue (Fig.17). Together, *daf-19m* could act as a sufficient target gene regulator in IL2 neurons.

***daf-19m* and its target genes compose regulatory subroutine of IL2 neurons**

During development, terminal selectors give neuronal identities and initiate and maintain the terminally differentiated state (Hobert, 2008, 2016). In IL2 neurons, terminal selector *unc-86* and *cfi-1* give IL2-specific identities, as a cholinergic neuron and ciliated neuron (Zhang et al., 2014). For *daf-19m* regulates IL2 functional activation, there is a possibility that *daf-19m* can interact with IL2 terminal selector genes. I checked *daf-19m* expression in *unc-86(n846)* and *cfi-1(ky651)* mutants. In terminal selector mutants, *klp-6*

expression showed impaired expression (Zhang et al., 2014). Like *klp-6*, *daf-19m* (transcriptional fusion with IL2/CEM enhancer (Wang et al., 2010)) showed off-expression totally in *unc-86(n846)* and reduced IL2 cell number expression in *cfi-1(ky651)* to 3 or 4 cells (Fig.18).

To check the possibility that *daf-19* is terminal selector under *unc-86* and *cfi-1*, I checked the expression of other IL2 marker genes, showing IL2 identities, *lag-2* and *unc-17*/*cha-1* expression. *lag-2* is a notch receptor ligand, expressed in IL2 neurons of the dauer stage (Henderson et al., 1994; Ouellet et al., 2008; Schroeder et al., 2013). *unc-17* and *cha-1* share their promoters to express, and both genes are highly connected to cholinergic neuronal function (Rand, 1989). In *daf-19(n4132)*, *daf-19m* specific mutant, both *lag-2* and *unc-17/cha-1* expression did not change at all, meaning not all identities of IL2 neurons were affected by *daf-19m* (Fig.19). In other words, *daf-19m* regulates a small set of target genes, under terminal selectors.

This regulation, governing part of the function to neurons, with regulator and target genes, is called a regulatory subroutine (Hobert, 2008, 2011, 2016). As a regulatory subroutine, to check the direct regulation of terminal selectors, I checked the *daf-19m*

promoter region, with the consensus motif of *unc-86* and *cfi-1* (Fig.20). When I changed the *cfi-1* binding site (-132, -116) of the *daf-19m* promoter, and both *unc-86* and *cfi-1* binding sites (-601, -566) to A oligomers (AAAAA-), mutated promoter phenocopied *cfi-1(ky651)*, four cell expression of the transgene in IL2 (Fig.21). Thus, *daf-19m*, the constituent of IL2 regulatory subroutine, is directly regulated by terminal selector, *cfi-1*.

Terminal selectors regulate neuronal identities in the development process and maintain its regulation in post-developmental stages. I checked *daf-19m* expression in early post developmental stages, like L4 or young adult stages as well. *daf-19m* was expressed in L1, the timepoint finished almost neuronal development, and Dauer, diapause stage in unfavorable conditions (Fig.22).

In summary, *daf-19m* was regulated by IL2 terminal selectors *unc-86* and *cfi-1*, and composed regulatory subroutine with target genes.

The *daf-19m* regulatory subroutine regulates nictation behavior

For clear genetic data, showing the existence of *daf-19m*-regulatory subroutine in IL2, I hypothesized this subroutine might regulate specific behavior. Nictation behavior

is mediated by IL2 neurons, especially through cilia structure and synaptic transmission. In the previous study, *klp-6(sy511)*, *osm-9(yz6)* mutants, defects for intact cilia of IL2, showed nictation defects (Lee et al., 2012). Because *daf-19m* could regulate *klp-6* and *osm-9* (Wang et al., 2010; Fig.17), I expected that *daf-19m* could control nictation behavior. I checked the nictation behavior of *daf-19* isoform-specific mutants (Fig.23). Though *daf-19(tm5562)*, affecting *daf-19a/b* isoforms, did not show any difference compared to N2, *daf-19(n4132)*, *daf-19m* specific isoform mutant, showed nictation defects (Figs.3 and 23). In the case of *daf-19(m86)* null mutant, including *daf-19c* deficiency, worms did not move at all, meaning that the nictation ratio was uncheckable. I checked another *daf-19m* specific mutant *daf-19(sm129)*, carrying the mutation of splicing acceptor of *daf-19m*, and this mutant also showed nictation defects (Fig.24). In other words, the biological meaning of the *daf-19m* regulatory subroutine is the regulation of IL2 cilia genes, which are crucial to nictation behavior.

Discussion

Non-canonical X-box motif regulating *klp-6* expression in IL2s and MSNs

In this study, I found a novel X-box motif on the *klp-6* promoter region through promoter analysis (Figs.9, 10, and 11). Previous studies could not show a *cis*-regulatory element of *klp-6*, though the *klp-6* expression was dependent on *daf-19m* (Peden and Barr, 2005; Wang et al., 2010). One of unique identities of the non-canonical X-box motif is single spacer T (Figs. 8 and 10). In mammal, the length of the spacer sequence of X-box motif is diverse (from 0 to 3), but the reported X-box motifs of *C. elegans* were only two spacer sequences, AT or TT (Blacque et al., 2005; Chen et al., 2006; Efimenko et al., 2005; Emery et al., 1996; Phirke et al., 2011; Swoboda et al., 2000). In previous studies, some IL2 expressing genes in hermaphrodites showed additional expression of MSNs (Wang et al., 2015; Wang et al., 2010). Interestingly, the non-canonical X-box motif of *klp-6* made MSNs expression (Fig.12). As a result, I found that IL2 and MSNs expression were correlated with the activity of the non-canonical X-box motif.

Our finding is the first example of the X-box motif with a spacer length variant in *C. elegans* and suggests that this motif plays a central role in *klp-6* expression, giving specific function among sensory cilia.

***daf-19m*, a specialized isoform of *daf-19*, gives specificity to ciliated neurons, through the non-canonical X-box motif**

In previous studies, *daf-19m* regulates some IL2 and MSNs expressing genes (Wang et al., 2010). In this study, I re-confirmed that *daf-19m* regulated its target genes using isoform-specific rescue (Fig.15). In yeast one hybrid experiments, *daf-19m* could bind to the non-canonical X-box motif, and this would be a possible mechanism for the specificity of IL2 and MSNs.

There is a remaining question about this new motif regulation, especially the role of *daf-19c*. In a supplement of *daf-19c*, there are some results about IL2 expression of *daf-19m* target genes or non-canonical X-box motif tandem repeats (Fig.15). The first explanation is that *daf-19c* may use the non-canonical X-box motif as the form of a heterodimer with *daf-19m*. In the result of yeast one hybrid experiment, this non-canonical X-box motif can bind to all of the *daf-19* isoforms without any specificity (Table 3). Interestingly, tandem repeats of the extended *klp-6* element did not show any expression with the *daf-19c* rescue, but the tandem repeats signal showed efficient

expression with *daf-19c* and *daf-19m* rescue in IL2 neurons (Fig.15). This means that *daf-19c* can bind to the non-canonical X-box motif, but *daf-19c* can regulate the target gene only with *daf-19m*, suggesting that *daf-19c* may regulate IL2 and MSNs expression through heterodimer with *daf-19m*. The second explanation is that the non-canonical X-box motif shows non-specific *daf-19* binding (Table 3). Both *daf-19a* and *daf-19m*, which are the most abundant and most scarce *daf-19* isoforms in *C. elegans*, respectively, can bind to non-canonical X-box motif in yeast one hybrid experiment. This result suggests that excessive *daf-19* isoforms can turn on the target genes of *daf-19m* in the *daf-19* isoform-specific rescue.

To discover why only *daf-19m* regulates its target gene through the non-canonical X-box motif, not showing any specificity in a heterologous expression system, N-terminal sequence of *daf-19* isoforms may be the answer. *daf-19* isoforms contain N-terminal exons with different combinations, although all isoforms share DNA binding domain and dimerization domain in the C-terminal region (Fig.25). The difference of the N-terminal amino acid sequence can give the binding specificity. To prove this, electrophoretic mobility shift assay (EMSA) may show the binding specificity of *daf-19*

isoforms to the non-canonical X-box motif.

The possibility that other *daf-19m* target genes contain the non-canonical X-box motif

IL2 expression showed co-relation with male specific neuronal expression in the previous study (Wang et al., 2015). Like *klp-6*, *osm-9* and *cil-7* showed male specific neuronal expression as well as IL2, and that expression was dependent to *daf-19m* (Fig.17). This result gives the possibility that those genes contain a non-canonical X-box motif. In the *cil-7* promoter region, I found one non-canonical X-box motif (with single T spacer) and one canonical X-box motif (with AT spacer), assuming redundant effect to *cil-7* expression, possibly. In the *osm-9* 3' region, the sequences did not contain a non-canonical X-box motif. To find a *cis*-regulatory element of *cil-7* and *osm-9*, the promoter deletion assay may be a possible option.

However, even though promoter region contains putative X-box motifs, IL2 expression of *tba-6* is independent to *daf-19m*. This suggests that *tba-6* may be regulated by a different regulatory mechanism for releasing extracellular vesicle to mediate mating

behavior in IL2 and male specific neurons (Silva et al., 2017).

daf-19m, as a regulatory subroutine, regulated by IL2 terminal selector

In the previous study, terminal selectors *unc-86* and *pag-3* can regulate a regulatory subroutine, including *ceh-14* in BDU neuron (Gordon and Hobert, 2015). I found that *daf-19m* is a constituent of IL2 regulatory subroutine, for regulating some – not all – IL2 identity genes (Figs.9, 10, 17, and 19). I made some transgenic animals, carrying mutated *daf-19m* promoter to investigate terminal selector binding sites (Fig.20) and found that *cfi-1* regulated *daf-19m* directly (Fig.21). For similarity of sequences on the *daf-19m* promoter (Fig.20), *unc-86* binding sites may act redundantly.

In this study, I suggested *daf-19m* composed IL2 regulatory subroutine for specialized function (Fig.26).

The relationship between the *daf-19m* regulatory subroutine and nictation behavior

daf-19m, IL2-specific isoforms, mediates nictation behavior (Fig.23) and its

target genes, *klp-6* and *osm-9*, constituents of subroutines as well, regulate nictation behavior (Lee et al., 2012). Considering the function of genes in the regulatory subroutine, the *daf-19m* regulatory subroutine seem to be specialized for cilia function, especially moving the channel for the mechanosensation, needed to nictation initiation (Lee et al., 2012).

klp-6 is a specialized kinesin motor protein to move the channel, PKD-2, for mating behavior in MSNs (Peden and Barr, 2005). *klp-6* is also expressed in IL2 neurons, but PKD-2 channel, a polycystin kidney disease gene, is not expressed in IL2 neurons (Barr et al., 2001; Barr and Sternberg, 1999). Meanwhile, *osm-9* is a TRPV channel mediating chemotaxis and osmotic avoidance and expressed in a subset of sensory neurons, including IL2 neurons (Colbert et al., 1997). There are reports that the role of TRP channel for the mechanosensation in cilia, including PKD-2 (Barr and Sternberg, 1999; Kang et al., 2010; Xiao and Xu, 2011). Besides, OSM-9 may regulate mechanosensations in MSNs (Zhang et al., 2018). So far, no channel for nictation behavior was reported, and *osm-9* may regulate the mechanosensation in IL2 neurons for nictation behavior. In this process, *klp-6* may regulate *osm-9* transport to cilia tip. To

prove this, the nictation assay in IL2 specific rescue of *osm-9* and subcellular localization of *osm-9* in *klp-6* mutant should be examined. *cil-7*, regulated by *daf-19m* in IL2, cannot regulate nictation behavior (data not shown), even *cil-7* regulates mating behavior. In this case, redundancy of the *cil-7* function, facilitating TRP channel transport to cilia tips (Maguire et al., 2015), regulated by *daf-19m* or not, may disturb the observation.

The *daf-19m* regulatory subroutine regulates the transport of putative channel to cilia ends for the mechanosensation. However, different IL2 neuronal function can also regulate nictation behavior. *lag-2* mutant, defect on the dauer maintenance of IL2 neurons, showed nictation defect (data not shown). *cha-1* mutant, defect on synaptic transmission of IL2, also showed a nictation defect (Lee et al., 2012).

Together, the *daf-19m* regulatory subroutine regulate nictation behavior through channel transport in cilia, not other IL2 activity. In this study, I suggested a new role of the single regulatory subroutine in a subcellular level, possibly connected to organismal behavior.

The role of subroutine-regulation in IL2 neurons

Why does *daf-19m* regulate the nictation behavior as a subroutine? There are two studies about the regulatory subroutine only in *C. elegans*. *ceh-14* regulates neuropeptide genes in BDU interneuron, and *ceh-23* regulates G-protein coupled receptor *sra-11* in AIY interneuron (Altun-Gultekin et al., 2001; Gordon and Hobert, 2015; Hobert, 2016). However, the functions of these effector genes are not reported in each of the neurons. As shown above, the subroutine regulates a variety of effector genes, but it is hard to discuss the meaning of subroutine-regulation for the limited number of studies.

Though little information about the subroutine, the property of modularity in the subroutine can give us implications. The cell can construct modules through the formation of gene interactions, and change the gene connectivity in the gene regulatory network through the evolutionary process (Melo et al., 2016). The evolutionary selection gives new interactions in the gene regulatory network of the gene pool, and this change provides a new identity to the cell, causing variations (Espinosa-Soto and Wagner, 2010). Module-level connectivity change contributes a more effect to the composition of the transcriptome than the gene-level change, meaning the modularity can vary the cellular function easily. In other words, the modularity is an efficient way to give new features to

cells.

The modularity is also shown in the process of neuronal development of *C. elegans*. Terminal selectors and subroutines can regulate neuronal identity genes in the terminal differentiation (Hobert, 2008, 2011, 2016) Both transcription factors show the modularity, regulating downstream target genes for neuronal identities. Nevertheless, terminal selectors act as the module, but subroutines control a part of identity genes in the downstream of terminal selectors, meaning subroutines act as the sub-module in the neuronal terminal differentiation. Considering the modularity of subroutine, the role of the subroutine in the regulation of nictation can be expected in IL2 neurons.

First, in the regulation of the nictation, the *daf-19m* subroutine does not affect other essential IL2 neuronal functions, except ciliary protein localization for the mechanosensation. A variety of modules regulate diverse functions in neurons. IL2 neurons have diverse roles such as dauer maintenance, chemosensation, and mechanosensation (Bargmann, 2006; Ouellet et al., 2008) and various modules regulate these functions. Nictation is an integrated behavior regulated by many IL2 functions. To regulate the nictation behavior, the control of IL2 terminal selectors, which is the

upstream regulators of *daf-19m*, can affect all of the IL2 functions. In this case, IL2 essential functions can be broken, such as dauer maintenance and synaptic transmission. *daf-19m* regulates the expression of many effector genes for the localization of ciliary proteins in IL2 as the sub-module but does not regulate *lag-2* expression for the dauer maintenance. In other words, *daf-19m* subroutine enables to sustain other essential IL2 functions in the regulation of nictation behavior.

Second, *daf-19m* can regulate the ciliary protein localization efficiently, and this can affect the decision making to execute the nictation. *daf-19m* is the upstream regulator of the subroutine, and this gives a regulation point for nictation behavior. Worms need to decide to do nictation in diverse environments. Dauer should do nictation to contact with carrier organisms in unfavorable conditions, inhibit the nictation to stay in favorable conditions. When worms decide to execute escape behavior, many changes in the neural circuit occur in less than a second, such as touch sensation and neuromodulation (Faumont et al., 2012). IL2 neurons also need the rapid and dramatic change to decide the nictation in the neural circuit. Regulating *daf-19m* is more efficient and quicker than regulating each downstream gene. The ciliary protein localization, mediated by *daf-19m*,

can affect the mechanosensation in IL2 neurons. Modulation of mechanosensation then alter the neural circuit, and worms can decide quickly to exhibit nictation in the unfavorable environment.

In summary, *daf-19m* subroutine can give neuronal identities to IL2 neurons with terminal selectors. However, the subroutine is the module for the mechanosensation, not other IL2 functions, which are also important to nictation behavior. *daf-19m* subroutine can modulate the mechanosensation in IL2 neurons, and this mediates neural circuit change for decision making of nictation behavior.

The possibility of the *daf-19m* regulatory subroutine conservation in MSNs

Considering data, described above and the previous study, I speculated the *daf-19m* regulatory subroutine might be conserved in IL2 and MSNs. Tandem repeats sequence of the *klp-6* extended element was sufficient to express MSN expression (Fig.12), and this suggested the function of the regulatory subroutine, the transport of channel to cilia tip, might be highly connected in both IL2 and MSNs. In mating behavior, PKD-2 and LOV-1, TRP channels, mediate behaviors; sensing hermaphrodite, contact,

and searching vulva (Barr et al., 2001; Barr and Sternberg, 1999). Especially, sensing and contact hermaphrodite need mechanosensations, mediated by the cilia of male tail sensory neurons. Additionally, males secrete extracellular vesicles from CEM, a male-specific sensory neuron in the head, to facilitate male specific behavior for mating (Wang et al., 2014). Both mating behavior and secreting extracellular vesicles need a transport TRP channel to the cilia tips. Considering the *daf-19m* function, TRP channel transport to cilia tip is a very critical step for both mating behavior and nictation behavior, and there is a possibility that this transport regulation may be conserved, even in different neurons. Then, in male specific neurons, do IL2 terminal selectors, *unc-86* and *cfi-1*, regulate *daf-19m*? The answer may be no. Terminal selector of male specific neurons is not reported so far, except *egl-46*, a putative terminal selector, regulating *daf-19m* in only HOB neuron.

Intriguingly, both nictation behavior and male mating behavior are related to maintaining progeny in the population level. Organisms need a suitable environment to make a progeny. Even in these distinct behaviors, cilia structure and proper regulation by the *daf-19m* regulatory subroutine may give significant contribution to species conservation (Fig. 27).

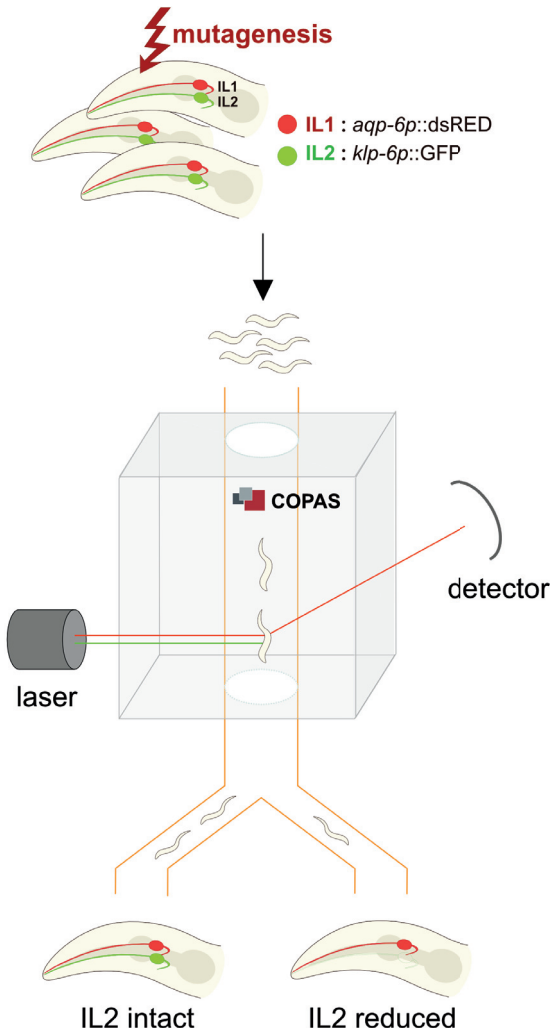

Figure 1. A schematic illustration of EMS mutagenesis screening to find IL2 regulators. We inserted transgene *ljEx1900* to the genome, to increase the efficiency of screening. The transgenic animal, LJ800, expresses IL2 marker *klp-6p::gfp* and IL1 marker *aqp-6p::gfp*. In the F2 generation from EMS treatment, we isolated worms which expressed IL1 marker only or reduced IL2 marker using COPAS biosort and confirmed

the expression of IL2 using Microscopy. F2 generation worms were screened from more than 15,000 worms of F1 generation. In collaboration with D. Park.

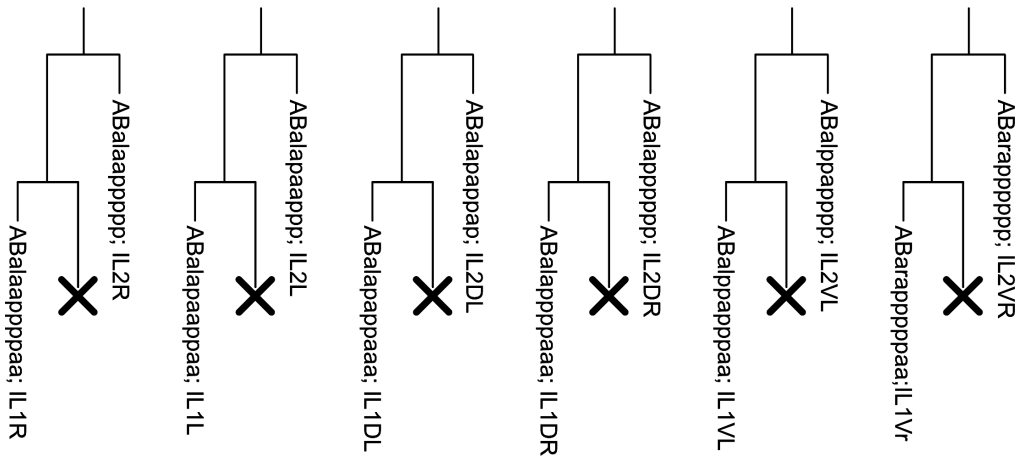

Figure 2. The illustration of inner labial sensilia neurons. Both IL2 and IL1 neurons, ciliated and located in inner labial sensilia, are differentiated from common progenitors (Sulston et al., 1983).

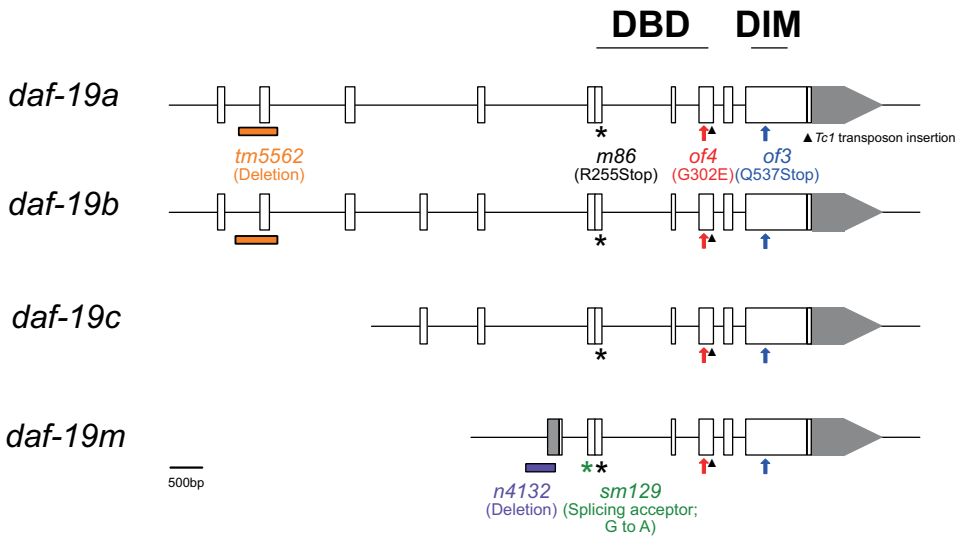

Figure 3. The structure of *daf-19* and its isoforms. *daf-19* have four isoforms, which are expressed in different location. All *daf-19* isoforms share DNA binding domain and dimerization domain. *of3* mutation is located in the dimerization domain, and *of4* mutation is located in the DNA binding domain. Both *of3* and *of4* mutations are null mutation. Scale bar is 500bp.

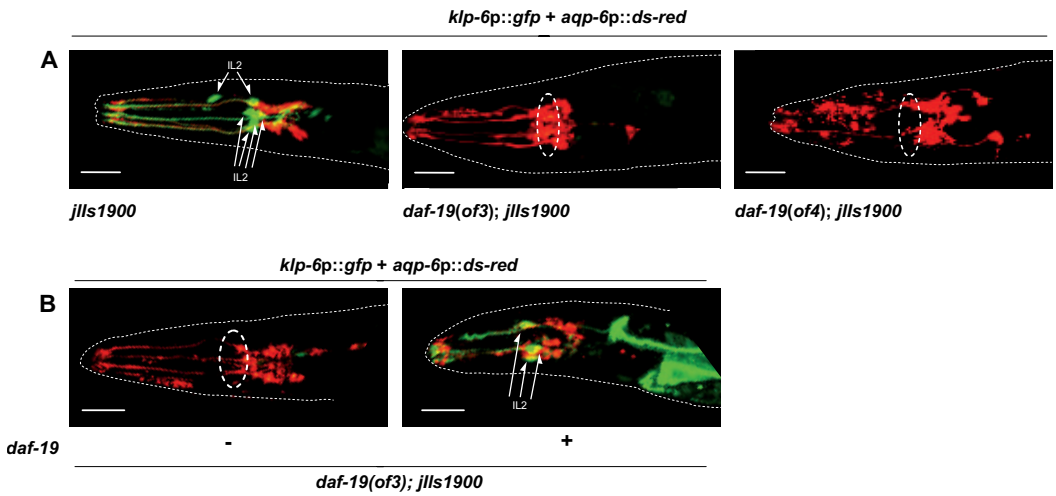

Figure 4. *daf-19* regulates *klp-6p::gfp* expression in IL2 neurons, not *aqp-6p::ds-red* expression in IL1 neurons. (A) *klp-6p::gfp* expression of IL2 neurons and *aqp-6p::ds-red* is expressed of IL1 neurons in *jlIs1900* and *daf-19* mutants (*of3*, *of4*) (At least, 20 worms per strains analyzed). (B) Rescue experiment of *daf-19* fosmid. Fosmid (*WRM0622dH09*) contains *daf-19* gene. *daf-19* fosmid was microinjected to *daf-19(of3); jlIs1900* transgenic animal (At least, 20 worms per strains analyzed). Scale bars are 20μm. In collaboration with D. Park.

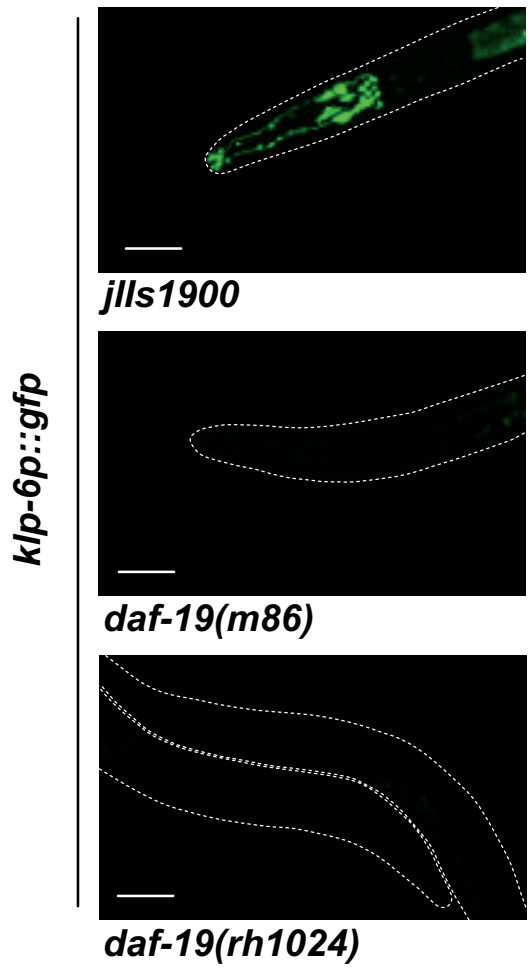

Figure 5. Other *daf-19* mutant alleles also show the defects of *klp-6p::gfp*. *klp-6p::gfp* was microinjected to *daf-19(m86)* and *daf-19(rh1024)*, containing *daf-19* null mutation (At least 15 worms per strains analyzed). Scale bars are 20μm. In collaboration with D. Park.

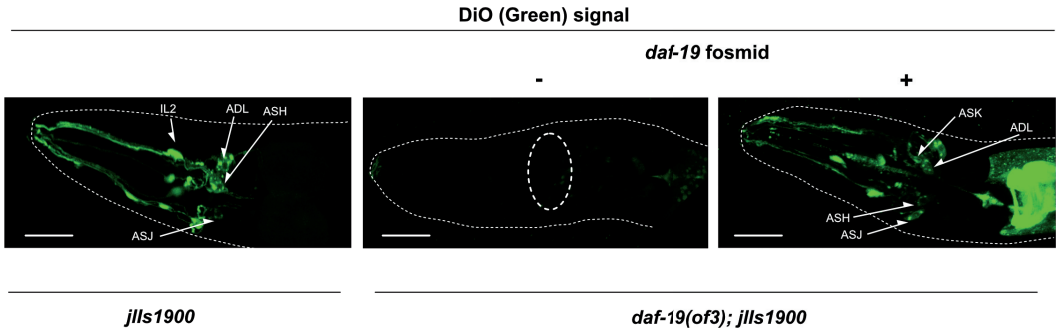

Figure 6. *daf-19(of3)* showed dye staining defect, and restored in the *daf-19* fosmid rescue worms. DiO, green fluorescent dye, signal can stain sensory cilia of *C. elegans*. The staining result of *jIIs1900*, *daf-19(of3); jIIs1900* and *daf-19(of3); jIIs1900* with *daf-19* fosmid (At least 20 worms per strain analyzed). Scale bars are 20μm. In collaboration with D. Park.

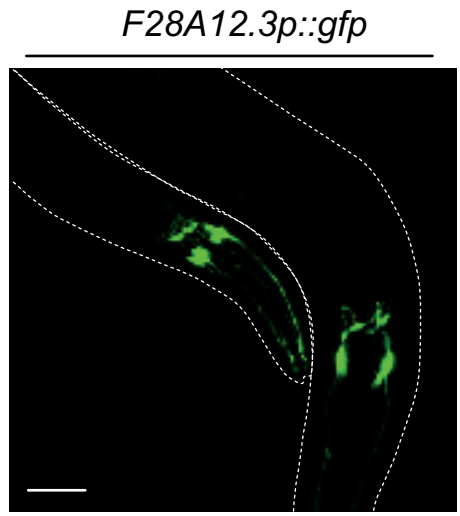

Figure 7. *daf-19(rh1024)* show F28A12.3, the single transmembrane protein, expression in IL2 neurons. The expression of *F28A12.3p::gfp* in *daf-19(rh1024)* (At least 15 worms per strain analyzed). Scale bars are 20μm. In collaboration with D. Park.

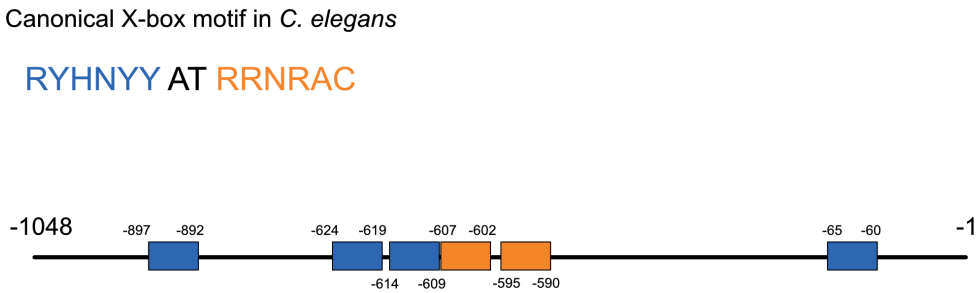

Figure 8. Absence of canonical X-box motif in *klp-6* promoter. *klp-6* promoter contains parts of canonical X-box motif, not entire motif.

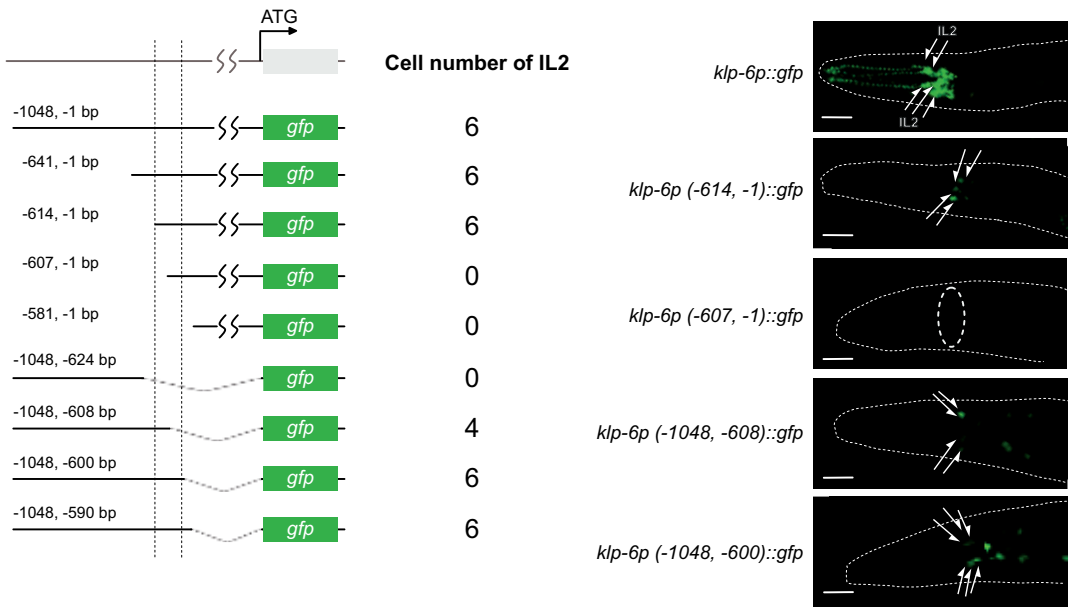

Figure 9. Promoter analysis of *klp-6* to find a *cis*-regulatory element. A schematic illustration of *klp-6* promoter deletion assay. 'Cell number of IL2' means the representative number of each transgenic animals (15 worms per strain analyzed). Scale bars are 20μm. In collaboration with D. Park.

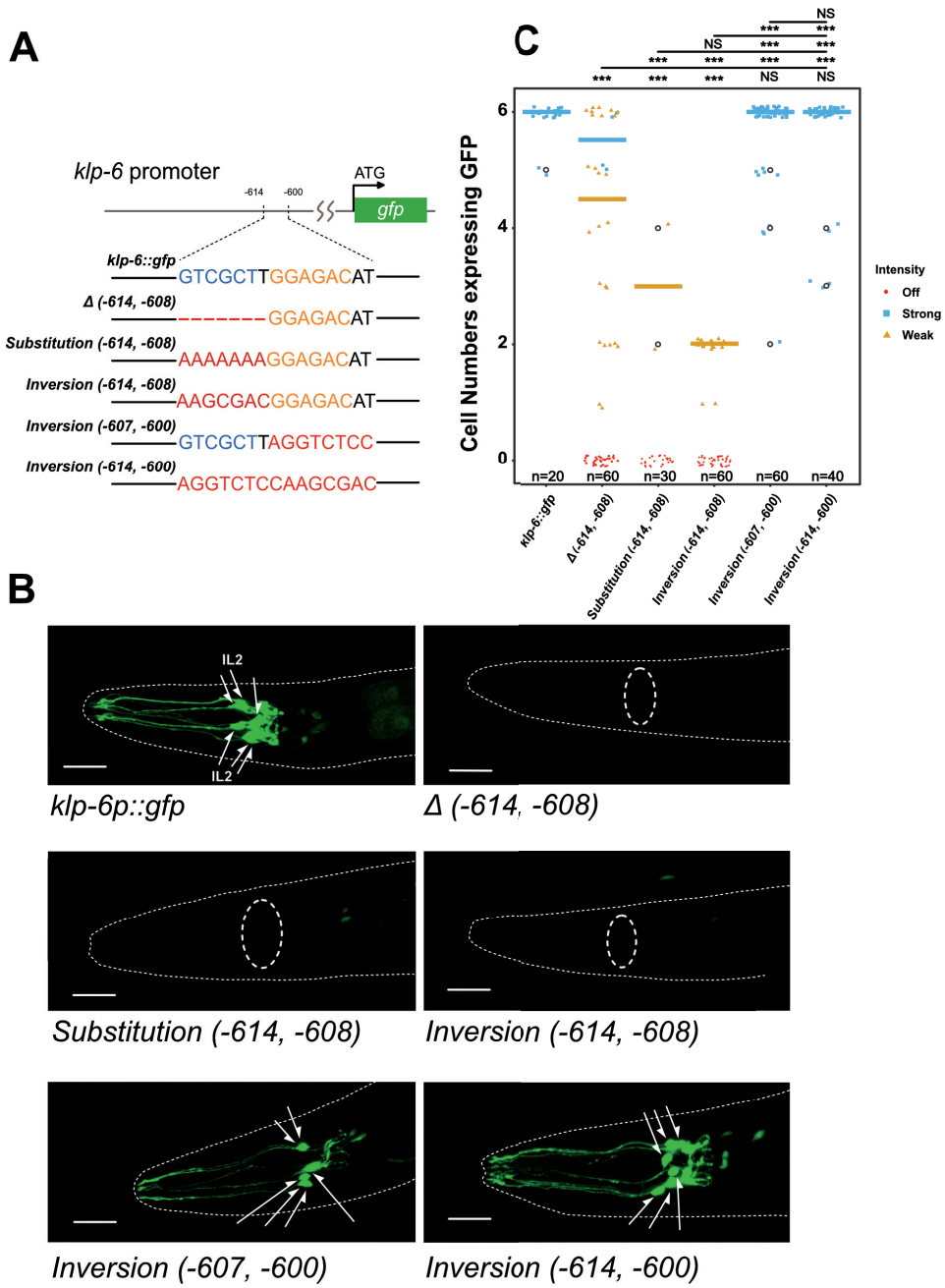

Figure 10. The *cis*-regulatory element of *klp-6* is necessary to express *klp-6*. (A) A schematic illustration of *cis*-regulatory element mutation to check necessity of *klp-6*

expression. (B) The expression of mutated *klp-6::gfp*. *Δ (-614, -608)* transgenic animals often showed off-signal or weak two cell-expression except 1 of 3 transgenic lines (At least 40 worms per strain analyzed except *klp-6p::gfp*; 20 worms and Substitution (-614, -608); 30 worms). Scale bars are 20μm. (C) The quantification of mutated *klp-6::gfp* expression. The mutation of construct was conducted by Q5 site-directed mutagenesis. Statistical significance was determined with One-way ANOVA and post-hoc Tukey HSD (*** $P \leq 0.001$, NS, not significant. Overall p value for ANOVA is less than 0.001 ($P < 0.001$).

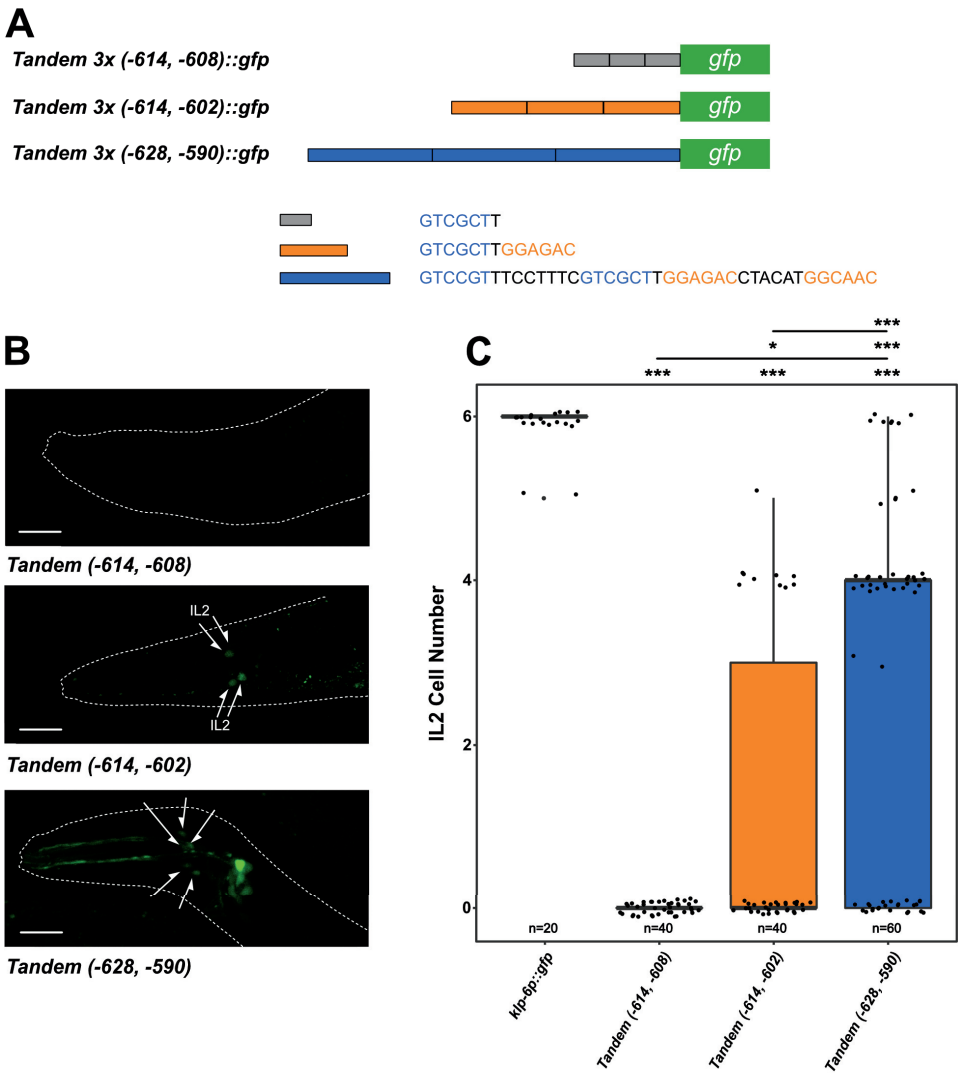

Figure 11. The *cis*-regulatory element of *klp-6* is sufficient to express *klp-6*. (A) A schematic illustration of *cis*-regulatory element tandem repeats. (B) The expression of *klp-6* element tandem repeats. The GFP signal of IL2 neurons was very weak (At least 40 worms per strains analyzed). (C) The quantification data of tandem repeats of *klp-6 cis*-

element. Scale bars are 20μm. The insertion of construct was conducted by Q5 site-directed mutagenesis. Statistical significance was determined with One-way ANOVA and post-hoc Tukey HSD (* $P \leq 0.5$, *** $P \leq 0.001$). Overall p value for ANOVA is less than 0.001 ($P < 0.001$).

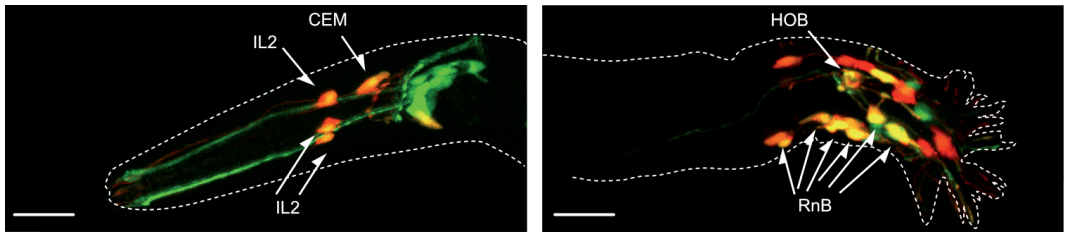

Figure 12. *klp-6 cis*-regulatory element is sufficient to express *klp-6* in male specific neurons. The expression of tandem repeats (-628, -590) in male (20 worms analyzed). *klp-6::mcherry* is used as a IL2 marker. Scale bars are 20μm.

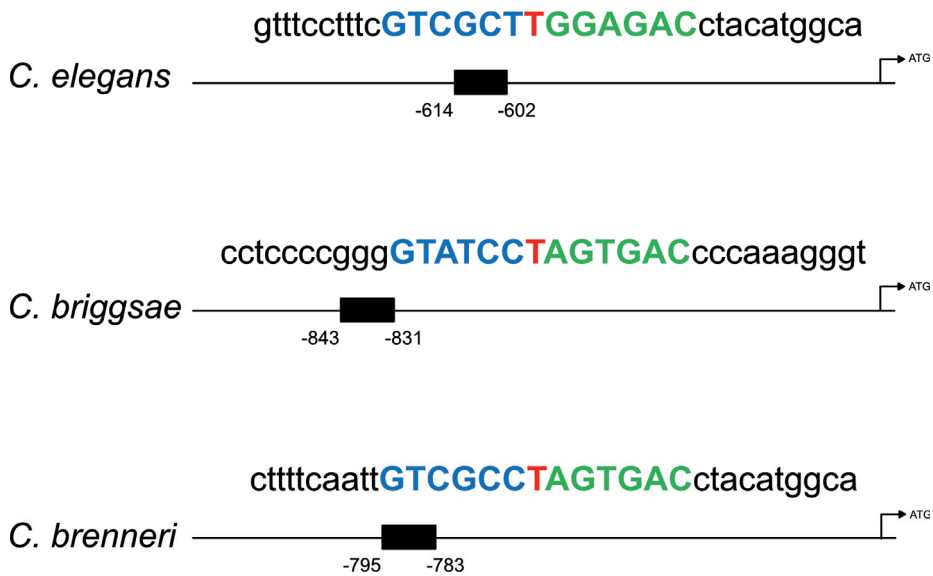

Figure 13. The non-canonical X-box motif is conserved in *Caenorhabditis* species.

The location of non-canonical X-box motif in the *klp-6* promoter of *C. briggsae* and *C. brenneri*. In collaboration with Peter Swoboda.

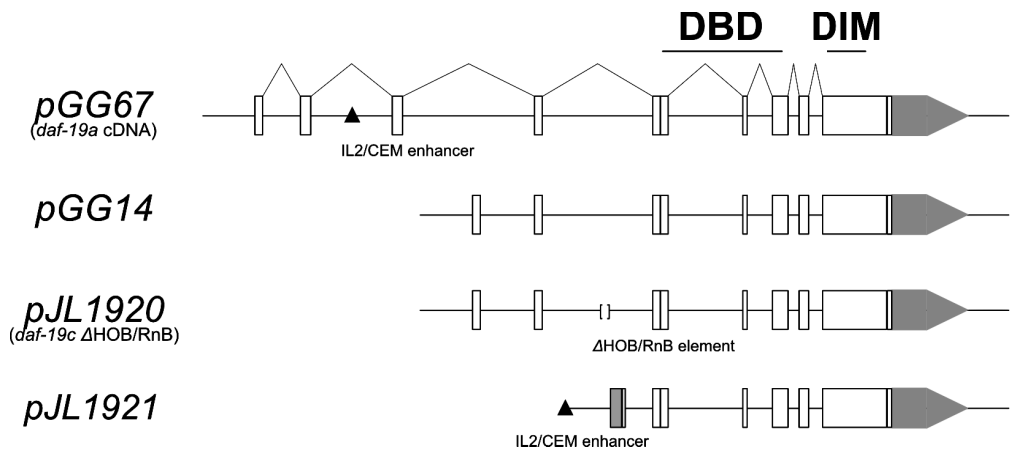

Figure 14. The rescue constructs of *daf-19* isoforms. The HOB/RnB element is removed from *pGG14*, for eliminating the possibility of *daf-19m* partial rescue in HOB/RnB neurons. *pJL1921* includes IL2/CEM enhancer in the distal promoter of *daf-19m*, located in the intron of *daf-19a* (Wang et al., 2010). The deletion of construct was conducted by Q5 site-directed mutagenesis.

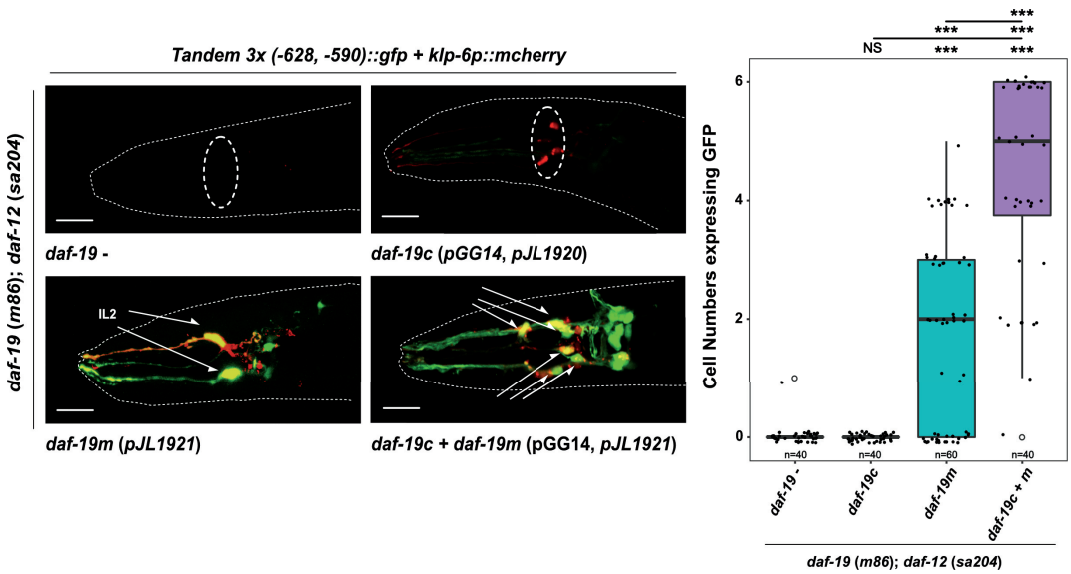

Figure 15. *daf-19m* can rescue the non-canonical X-box motif of *klp-6* expression. The rescue of *daf-19* isoforms was conducted by microinjection (At least 40 worms per strain analyzed). *klp-6::mcherry* is used as a IL2 marker. Scale bars are 20μm. Statistical significance was determined with One-way ANOVA and post-hoc Tukey HSD (*** $P \leq 0.001$, NS, not significant). Overall p value for ANOVA is less than 0.001 ($P < 0.001$).

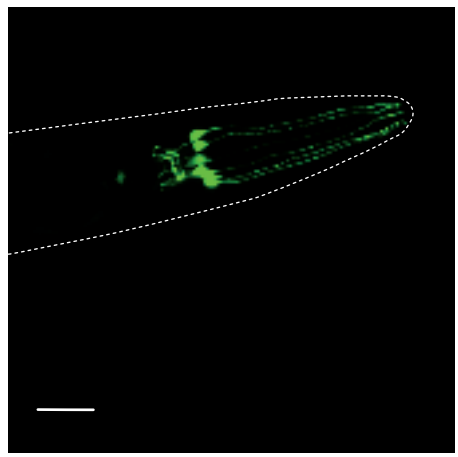

Figure 16. *daf-19a* and *daf-19b* cannot regulate *klp-6* expression. The expression of *klp-6p::gfp* in *daf-19(tm5562)* (20 worms analyzed). Scale bar is 20μm.

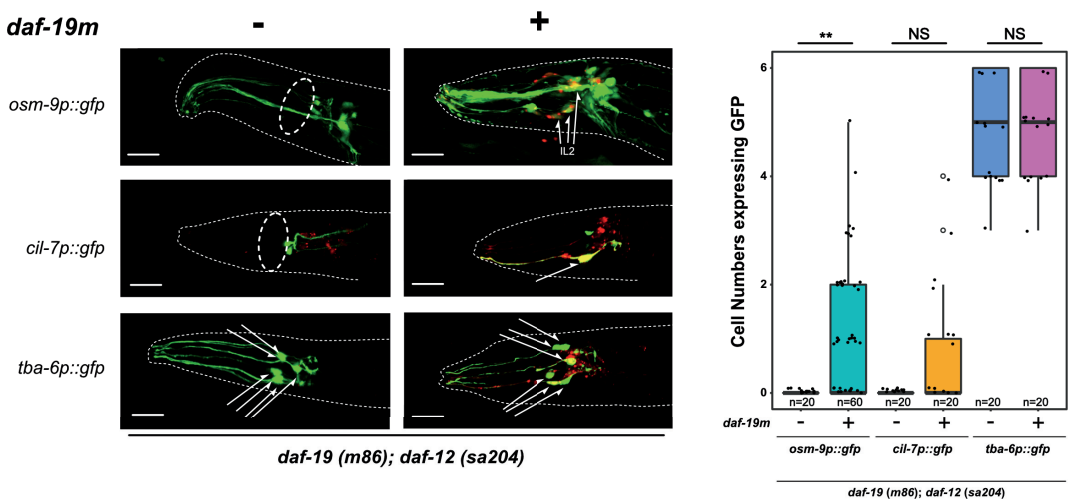

Figure 17. *daf-19m* can rescue the expression of target genes in *daf-19* null mutant.

The expression of IL2 expressing genes; *osm-9*, *cil-7*, and *tba-6*. *klp-6::mcherry* is used as a IL2 marker (20 worms analyzed per strain except for the transgenic expressing *osm-9p::gfp*; 40 worms and *osm-9p::gfp* with *daf-19m*; 60 worms). Scale bar are 20μm. Statistical significance was determined with One-way ANOVA and post-hoc Tukey HSD (** $P \leq 0.01$, NS, not significant). Overall *p* value for ANOVA is less than 0.001 ($P < 0.001$).

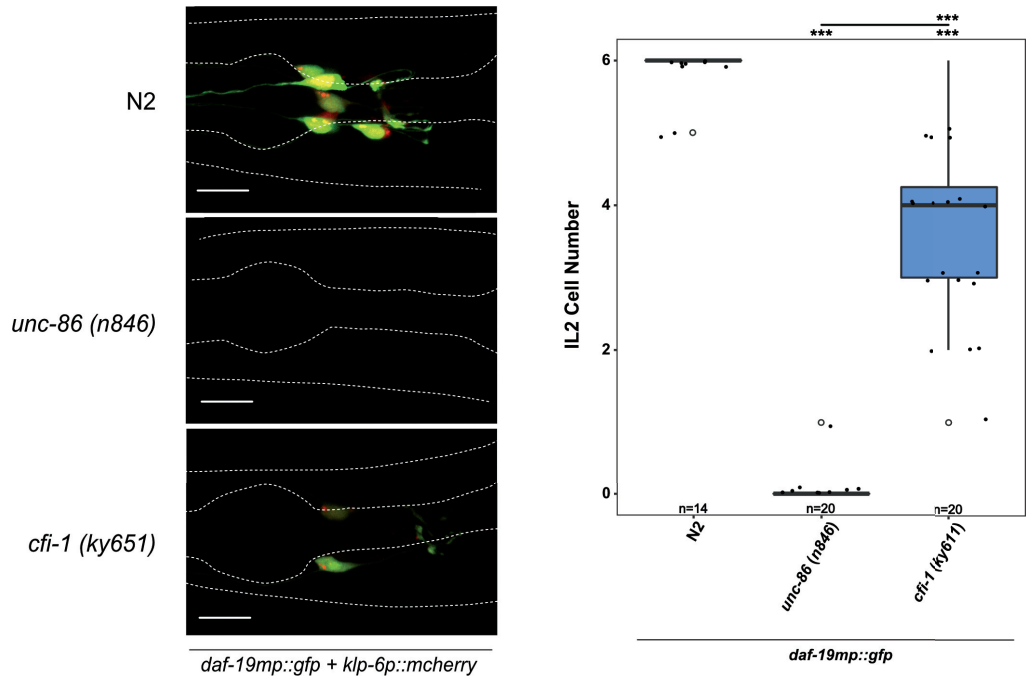

Figure 18. IL2 terminal selectors regulate *daf-19m* expression in IL2s. The expression of *daf-19mp::gfp* in *unc-86(n846)* and *cfi-1(ky651)*. *klp-6p::mcherry* is used as a IL2 marker (At least 20 worms analyzed except N2; 14). Scale bar are 10µm. Statistical significance was determined with One-way ANOVA and post-hoc Tukey HSD (*** $P \leq 0.001$). Overall *p* value for ANOVA is less than 0.001 ($P < 0.001$). In collaboration with S. Son.

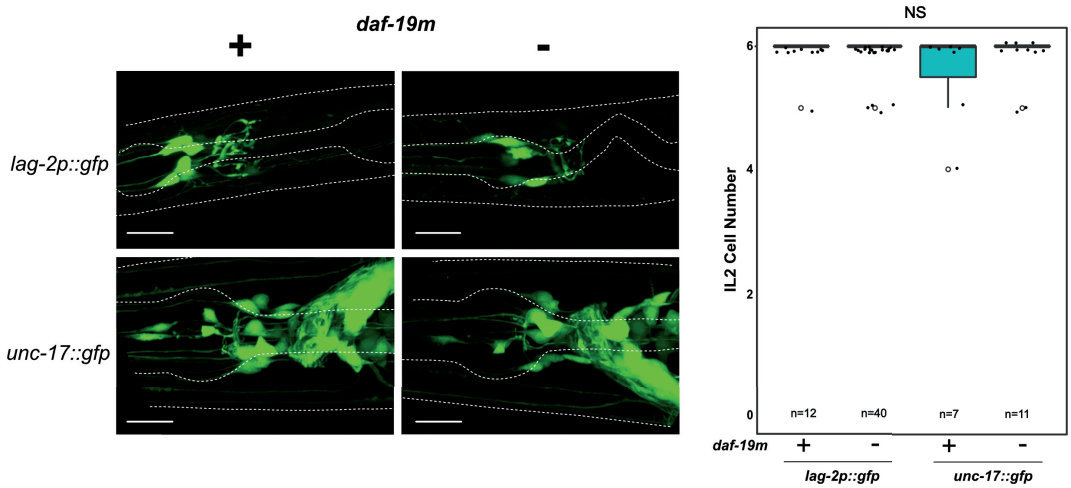

Figure 19. *daf-19m* cannot regulates some of IL2 identity genes. The expression of IL2 identity genes (*lag-2* and *unc-17*) in *daf-19(n4132)*, *daf-19m* specific mutant. (At least 10 worms analyzed except for *vsIs48*, genome insertion line of *unc-17::gfp*). Scale bar are 10μm. Statistical significance was determined with One-way ANOVA and post-hoc Tukey HSD (NS, not significant). Overall *p* value for ANOVA is 0.205 (*P*=0.205). In collaboration with S. Son.

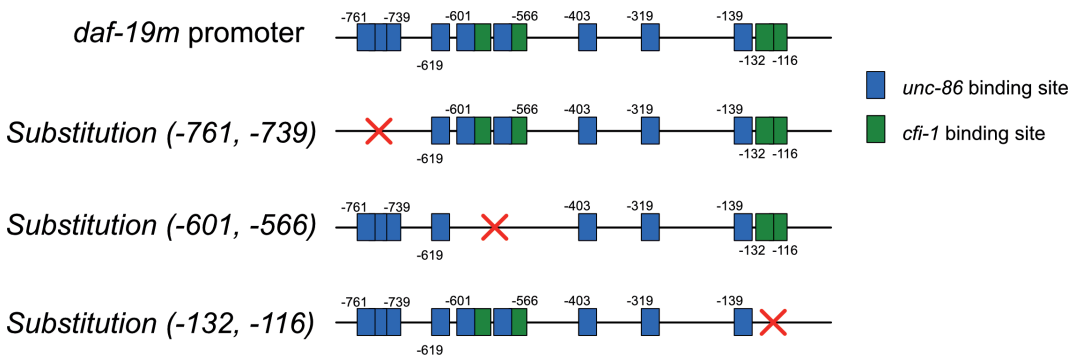

Figure 20. The putative IL2 terminal selector binding sites in *daf-19m* promoter.

There are many putative binding sites of IL2 terminal selector genes, *unc-86* and *cfi-1*. Three mutated promoter constructs of *daf-19m*, conducted by Q5 site-directed mutagenesis.

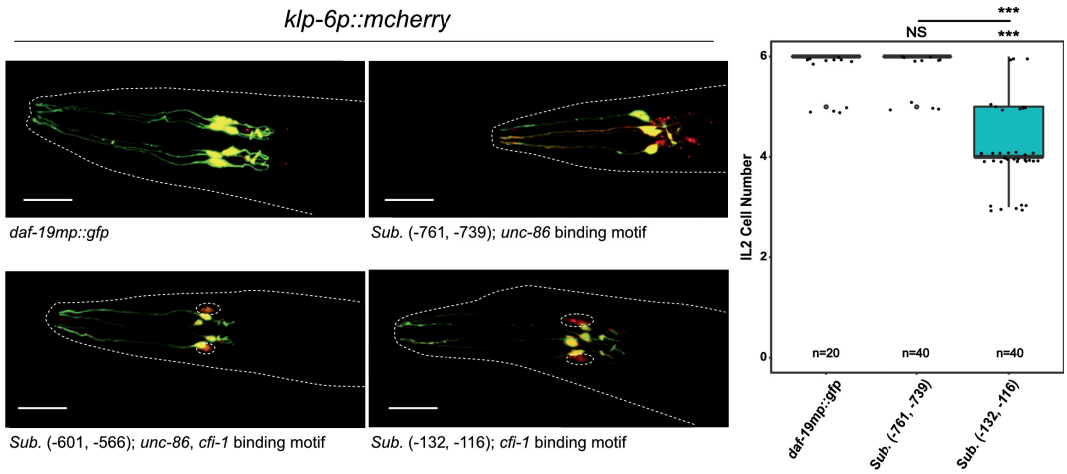

Figure 21. IL2 terminal selector regulates *daf-19m* directly. The expression of *daf-19mp::gfp* with the mutation of putative IL2 terminal selector binding sites (Fig.20). *klp-6p::mcherry* used as a IL2 marker (At least 20 worms per strain analyzed except *Δ*(-601, -566); 5 worms and *Δ*(-132, -116); 40 worms). Scale bar are 20μm. Statistical significance was determined with One-way ANOVA and post-hoc Tukey HSD (*** $P \leq 0.001$, NS, not significant). Overall p value for ANOVA is less than 0.001 ($P < 0.001$).

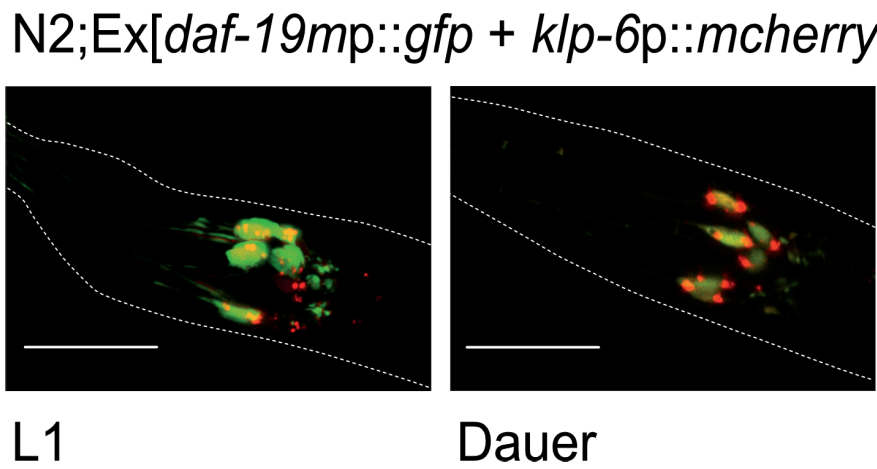

Figure 22. The maintenance of *daf-19m* expression and target gene regulation, as a regulatory subroutine. The expression of *daf-19mp::gfp* in larvae; L1 and Dauer of transgenic animals, injected to N2. 10 worms per strain analyzed. Scale bar are 20μm. In collaboration with S. Son.

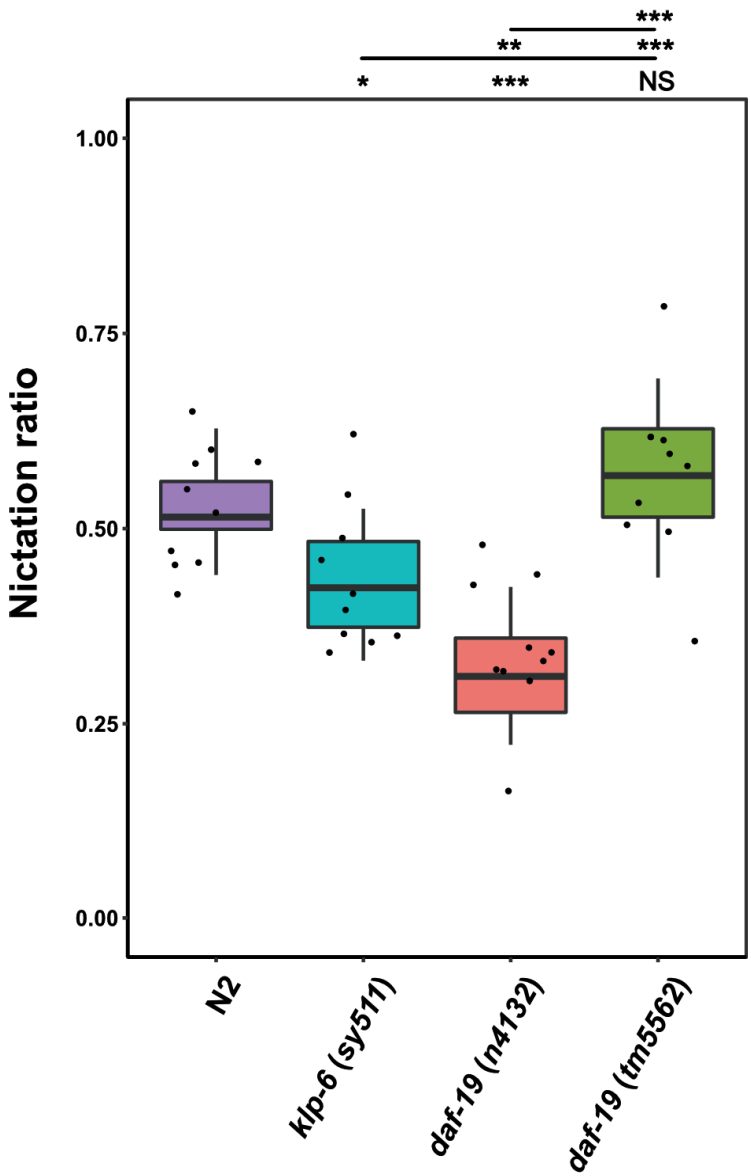

Figure 23. The nictation behavior is regulated by *daf-19m*, not *daf-19a* and *daf-19b*.

Nictation ratio of the mutant of *daf-19* isoforms and *klp-6*. At least 30 dauers analyzed per strain, and repeated 10 times. Statistical significance was determined with One-way

ANOVA and post-hoc Tukey HSD (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, NS, not significant). Overall p value for ANOVA is less than 0.001 ($P < 0.001$). In collaboration with H. Yang.

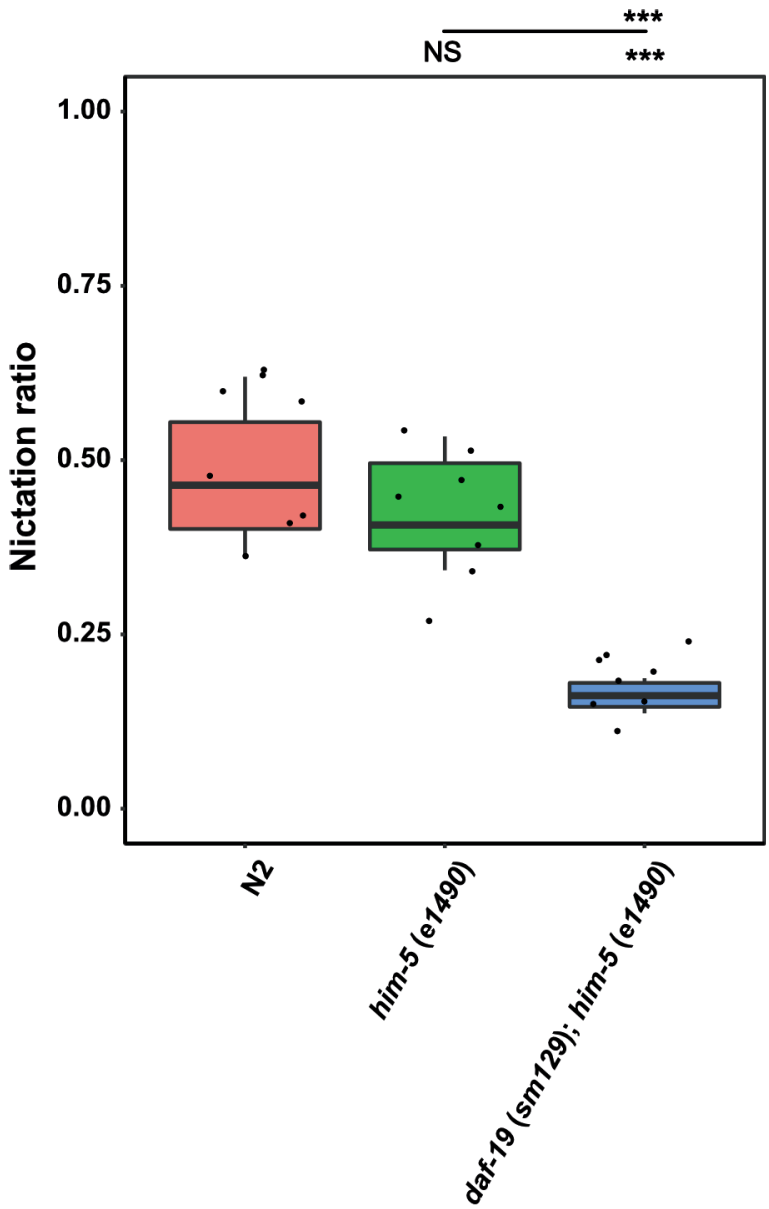

Figure 24. Another *daf-19m* specific mutant, *daf-19(sm129)* shows nictation defect. Nictation ratio of *daf-19(sm129);him-5(e1490)*. At least 30 dauers analyzed per strain, and repeated 8 times. Statistical significance was determined with One-way ANOVA and

post-hoc Tukey HSD (*** $P \leq 0.001$, NS, not significant). Overall p value for ANOVA is less than 0.001 ($P < 0.001$). In collaboration with H. Yang.

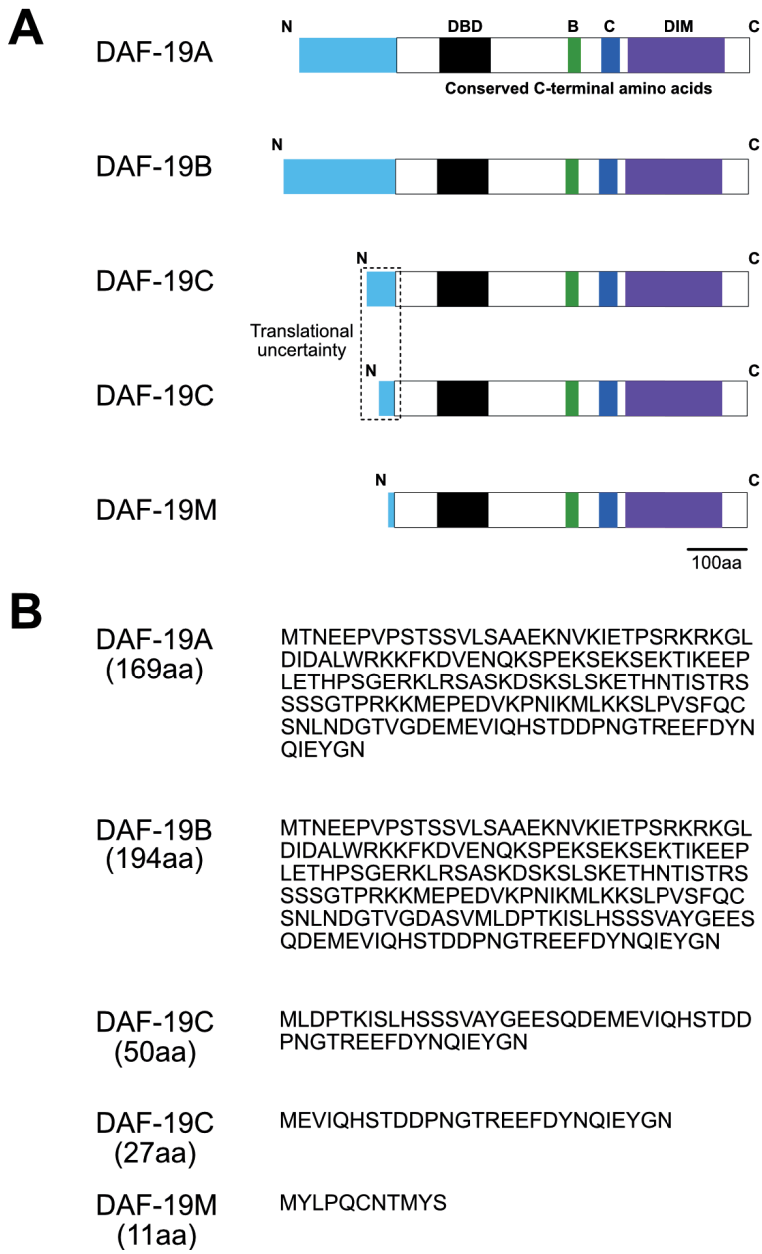

Figure 25. The amino acid sequence of *daf-19* isoforms. (A) All isoforms of *daf-19* have conserved C-terminal sequence of 611 amino acids (white area), including DNA binding domain (DBD), domain B/C (unknown functions), and dimerization domain

(DIM). Scale bars are 100 amino acids. (B) The N-terminal sequences of *daf-19* isoforms show various lengths with different combinations of exons. DAF-19B is the longest isoforms and contains all of exons of *daf-19*. The combinations of exons for isoforms are shown in Figure 3.

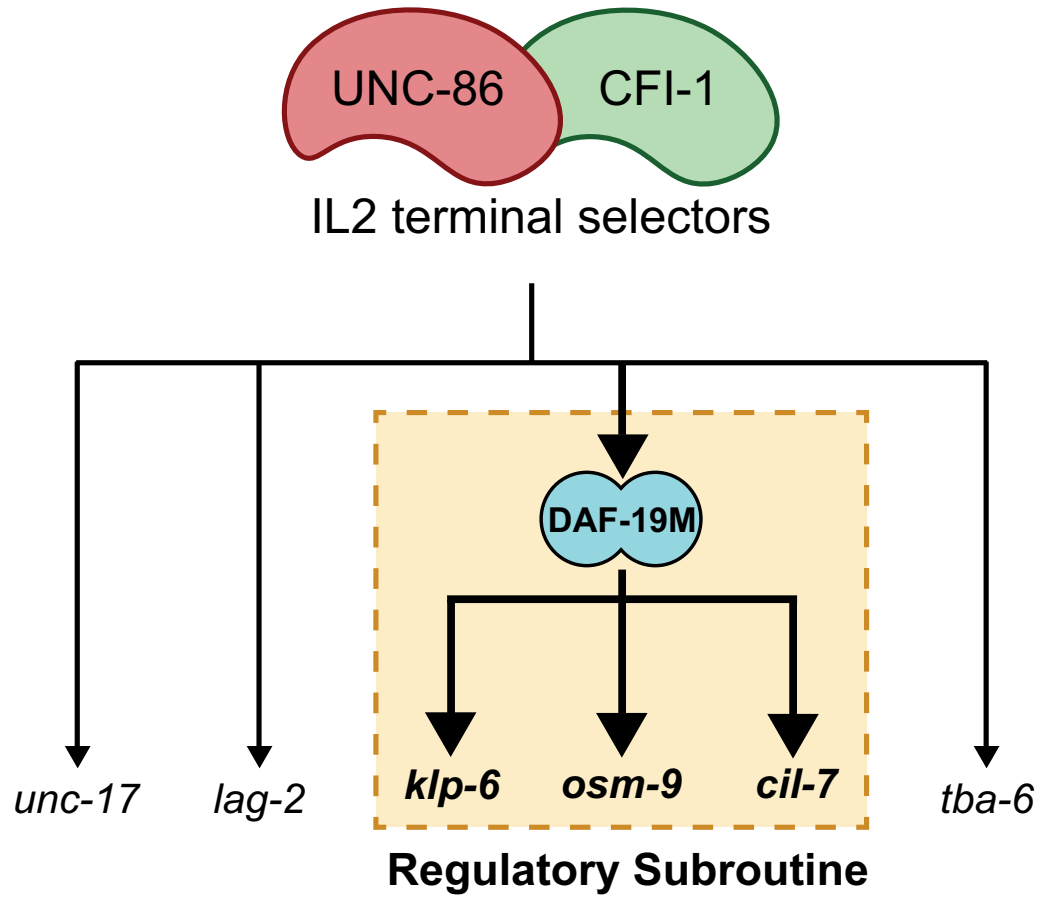

Figure 26. The hierarchy of IL2 expressing genes and *daf-19m*, as a constituent of regulatory subroutine.

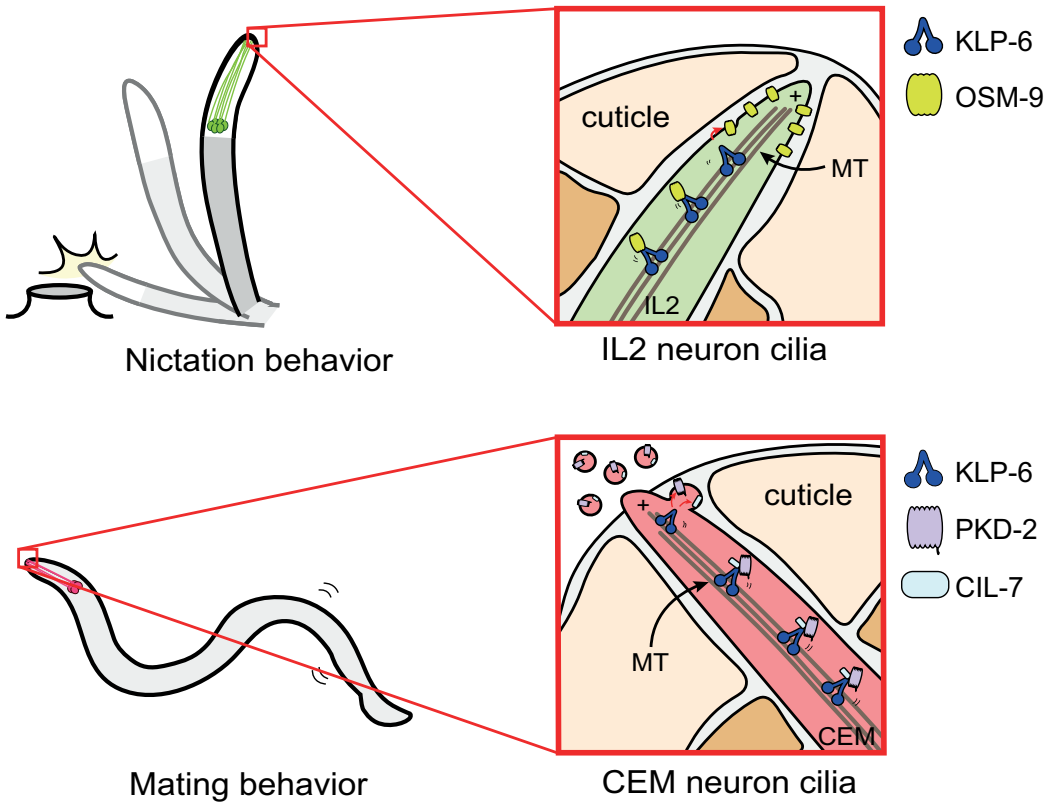

Figure 27. The possible mechanism of *daf-19m* regulatory subroutine, regulating nictation behavior and mating behavior through the transport to cilia.

Strain name	Transgene name	Genetic background	Transgene	Sequence position relative to 1st nucleotide of start codon				Remark
				Gene/Promoter		Mutation		
				5'-end	3'-end	5'-end	3'-end	
LJ800	*jIIs1900*	N2	*klp-6p::gfp*	-1048	-1			
			aqp-6p::ds-red	-3655	-1			
LJ801	*jIEx1902*	*daf-19(of3)*	*daf-19 forsmid (WRM0622dH09)*					
LJ802	*jIEx1902*	*daf-19(of4)*	*daf-19 forsmid (WRM0622dH09)*					
LJ803	*jIEx1903*	*daf-19(rh1024)*	*F28A12.3p::gfp*	-300	-1			
LJ804	*jIIs1900*	N2	*klp-6p::gfp*	-1048	-1			
LJ805	*jIIs1900*	*daf-19(m86)*	*klp-6p::gfp*	-1048	-1			
LJ806	*jIIs1900*	*daf-19(rh1024)*	*klp-6p::gfp*	-1048	-1			
LJ807	*jIEx1904*	N2	*klp-6p::gfp*	-641	-1			
LJ808	*jIEx1905*	N2	*klp-6p::gfp*	-614	-1			
LJ809	*jIEx1906*	N2	*klp-6p::gfp*	-607	-1			
LJ810	*jIEx1907*	N2	*klp-6p::gfp*	-581	-1			
LJ811	*jIEx1908*	N2	*klp-6p::gfp*	-1048	-624			
LJ812	*jIEx1909*	N2	*klp-6p::gfp*	-1048	-608			
LJ813	*jIEx1910*	N2	*klp-6p::gfp*	-1048	-600			
LJ814	*jIEx1911*	N2	*klp-6p::gfp*	-1048	-590			
LJ815	*jIEx1912*	N2	*klp-6p::gfp*	-1048	-1	-614	-608	Deletion
LJ816	*jIEx1913*	N2	*klp-6p::gfp*	-1048	-1	-614	-608	Substitution for AAAAAAA
LJ817	*jIEx1914*	N2	*klp-6p::gfp*	-1048	-1	-614	-608	Inverted to complementary strand
LJ818	*jIEx1915*	N2	*klp-6p::gfp*	-1048	-1	-607	-600	Inverted to complementary strand
LJ819	*jIEx1916*	N2	*klp-6p::gfp*	-1048	-1	-614	-600	Inverted to complementary strand
LJ820	*jIEx1917*	N2	*klp-6p (Tandem 3x)::gfp*	-614	-608			3 copies Tandem repeats
LJ821	*jIEx1918*	N2	*klp-6p (Tandem 3x)::gfp*	-614	-602			
LJ822	*jIEx1919*	N2	*klp-6p (Tandem 3x)::gfp*	-628	-590			
LJ823	*jIEx1919*	*daf-19(m86);daf-12(sa204)*	*klp-6p (Tandem 3x)::gfp*	-628	-590			Rescue effect marker (Tandem::gfp); IL2 neuron marker (klp-6p::mcherry)
LJ824	*ggEx14*	*daf-19(m86);daf-12(sa204)*	*daf-19c*	-223	11370			
LJ825	*jIEx1920*	*daf-19(m86);daf-12(sa204)*	*daf-19c (HOB, RnB deletion)*	-223	11370	2613	2625	Deletion (Senti et al., 2008; Wang et al., 2010)
LJ826	*jIEx1921*	*daf-19(m86);daf-12(sa204)*	*daf-19m*	-782	3552	-5738	-5719	Insertion (Wang et al., 2010)
LJ827	*jIEx1928*	*daf-19(m86);daf-12(sa204)*	*daf-19c*	-223	11370			pGG14
			daf-19m	-782	3552	-5738	-5719	pJL1921
LJ828	*jIEx1900*	*daf-19(tm5562)*	*klp-6p::gfp*	-1048	-1			
LJ829	*jIEx1922*	*daf-19(m86);daf-12(sa204)*	*osm-9p::gfp*	-3000	-1	6343	8697	Insertion (Colbert et al., 1997)
LJ830	*jIEx1922*	*daf-19(m86);daf-12(sa204)*	*osm-9p::gfp*	-3000	-1	6343	8697	
LJ831	*jIEx1923*	*daf-19(m86);daf-12(sa204)*	*cil-7p::gfp*	-3000	-1			
LJ832	*jIEx1923*	*daf-19(m86);daf-12(sa204)*	*cil-7p::gfp*	-3000	-1			
LJ833	*jIEx1924*	*daf-19(m86);daf-12(sa204)*	*tba-6p::gfp*	-1226	-1			
LJ834	*jIEx1924*	*daf-19(m86);daf-12(sa204)*	*tba-6p::gfp*	-1226	-1			
LJ835	*jIEx1925*	N2	*daf-19mp::gfp*	-782	-1	-5738	-5719	Insertion (Wang et al., 2010)
LJ836	*jIEx1925*	*unc-86(n846)*	*daf-19mp::gfp*	-782	-1	-5738	-5719	
LJ837	*jIEx1925*	*cfi-1(ky651)*	*daf-19mp::gfp*	-782	-1	-5738	-5719	
LJ838	*jIEx1926*	N2	*daf-19mp::gfp*	-782	-1	-132	-116	Substitution, Also contains IL2/CEM enhancer
LJ839	*qIs56*	*daf-19(n4132)*	*lag-2p::gfp*					
LJ840	*vsIs48*	*daf-19(n4132)*	*unc-17::gfp*					
LJ841	*cfjEx90*	N2	*myo-2p::mcherry*					
LJ842	*cfjEx90*	*daf-19(n4132)*	*myo-2p::mcherry*					
LJ843	*jIEx1921*	*daf-19(n4132)*	*daf-19m*	-782	3552	-5738	-5719	
LJ844	*jIEx1927*	*daf-19(n4132)*	*F28A12.3p::klp-6::gfp*	1	5394			Also contains F28A12.3 promoter 300bp
			F28A12.3p::osm-9::gfp	1	6278			pJL1928
LX929	*vsIs48*	N2	*unc-17::gfp*					
JK2868	*qIs56*	N2	*lag-2p::gfp*					
OE4502	*jIIs1900*	*daf-19(of3)*	*klp-6p::gfp*	-1048	-1			
			aqp-6p::ds-red	-3655	-1			
OE4503	*jIIs1900*	*daf-19(of4)*	*klp-6p::gfp*	-1048	-1			
			aqp-6p::ds-red	-3655	-1			
PT2519	*myIs13*	N2	*klp-6p::gfp*					

Table 1. The list of plasmid construct to make transgenic animals, used in this study.

Strain name	Transgene name	Transgene	Microinjection conditions for transgenesis		
			Transgene concentration (ng/μL)	Co-injection DNA	Co-injection DNA concentration (ng/μL)
LJ800	*jIIs1900*	*klp-6p::gfp*	60	*rol-6*	60
		aqp-6p::ds-red	60		
LJ801	*jIEx1902*	*daf-19 forsmid (WRM0622dH09)*	60	*act-5p::gfp*	60
LJ802	*jIEx1902*	*daf-19 forsmid (WRM0622dH09)*	60	*act-5p::gfp*	60
LJ803	*jIEx1903*	*F28A12.3p::gfp*	60	*rol-6*	60
LJ804	*jIIs1900*	*klp-6p::gfp*	60	*rol-6*	60
LJ805	*jIIs1900*	*klp-6p::gfp*	60	*rol-6*	60
LJ806	*jIIs1900*	*klp-6p::gfp*	60	*rol-6*	60
LJ807	*jIEx1904*	*klp-6p (-641, -1)::gfp*	60	*rol-6*	60
LJ808	*jIEx1905*	*klp-6p (-614, -1)::gfp*	60	*rol-6*	60
LJ809	*jIEx1906*	*klp-6p (-607, -1)::gfp*	60	*rol-6*	60
LJ810	*jIEx1907*	*klp-6p (-581, -1)::gfp*	60	*rol-6*	60
LJ811	*jIEx1908*	*klp-6p (-1048, -624)::gfp*	60	*rol-6*	60
LJ812	*jIEx1909*	*klp-6p (-1048, -608)::gfp*	60	*rol-6*	60
LJ813	*jIEx1910*	*klp-6p (-1048, -600)::gfp*	60	*rol-6*	60
LJ814	*jIEx1911*	*klp-6p (-1048, -590)::gfp*	60	*rol-6*	60
LJ815	*jIEx1912*	*klp-6p (Deletion, -614, -608)::gfp*	60	*rol-6*	60
LJ816	*jIEx1913*	*klp-6p (Substitution, -614, -608)::gfp*	50	*unc-122p::ds-red*	25
LJ817	*jIEx1914*	*klp-6p (Inversion, -614, -608)::gfp*	60	*rol-6*	60
LJ818	*jIEx1915*	*klp-6p (Inversion, -607, -600)::gfp*	50	*unc-122p::ds-red*	25
LJ819	*jIEx1916*	*klp-6p (Inversion, -614, -600)::gfp*	50	*unc-122p::ds-red, klp-6p::mcherry*	25, 50
LJ820	*jIEx1917*	*klp-6p (Tandem 3x, -614, -608)::gfp*	50	*rol-6*	50
LJ821	*jIEx1918*	*klp-6p (Tandem 3x, -614, -602)::gfp*	50	*unc-122p::ds-red, klp-6p::mcherry*	25, 50
LJ822	*jIEx1919*	*klp-6p (Tandem 3x, -628, -590)::gfp*	50	*unc-122p::ds-red, klp-6p::mcherry*	25, 50
LJ823	*jIEx1919*	*klp-6p (Tandem 3x, -628, -590)::gfp*	50	*unc-122p::ds-red, klp-6p::mcherry*	25, 50
LJ824	*ggEx14*	*daf-19c*	50	*unc-122p::ds-red, klp-6p::mcherry, klp-6p (Tandem)::gfp*	25, 50, 50
LJ825	*jIEx1920*	*daf-19c (HOB, RnB deletion; 2613, 2625)*	50	*unc-122p::ds-red, klp-6p::mcherry, klp-6p (Tandem)::gfp*	25, 50, 50
LJ826	*jIEx1921*	*daf-19m (IL2/CEM enhancer insertion; -5738, -5719)*	50	*unc-122p::ds-red, klp-6p::mcherry, klp-6p (Tandem)::gfp*	25, 50, 50
LJ827	*jIEx1928*	*daf-19c*	50	*unc-122p::ds-red, klp-6p::mcherry, klp-6p (Tandem)::gfp*	25, 50, 50
		daf-19m (IL2/CEM enhancer insertion; -5738, -5719)	50		
LJ828	*jIEx1900*	*klp-6p::gfp*	50	*myo-2p::mcherry*	3
LJ829	*jIEx1922*	*osm-9p::gfp (IL2 enhancer insertion; 6343, 8697)*	50	*unc-122p::ds-red, klp-6p::mcherry*	25, 50
LJ830	*jIEx1922*	*osm-9p::gfp (IL2 enhancer insertion; 6343, 8697)*	50	*unc-122p::ds-red, klp-6p::mcherry, daf-19m*	25, 50, 50
LJ831	*jIEx1923*	*cil-7p::gfp*	50	*unc-122p::ds-red, klp-6p::mcherry*	25, 50
LJ832	*jIEx1923*	*cil-7p::gfp*	50	*unc-122p::ds-red, klp-6p::mcherry, daf-19m*	25, 50, 50
LJ833	*jIEx1924*	*tba-6p::gfp*	50	*unc-122p::ds-red, klp-6p::mcherry*	25, 50
LJ834	*jIEx1924*	*tba-6p::gfp*	50	*unc-122p::ds-red, klp-6p::mcherry, daf-19m*	25, 50, 50
LJ835	*jIEx1925*	*daf-19mp::gfp (IL2/CEM enhancer insertion; -5738, -5719)*	50	*rol-6, klp-6p::mcherry*	50, 50
LJ836	*jIEx1925*	*daf-19mp::gfp (IL2/CEM enhancer insertion; -5738, -5719)*	50	*rol-6, klp-6p::mcherry*	50, 50
LJ837	*jIEx1925*	*daf-19mp::gfp (IL2/CEM enhancer insertion; -5738, -5719)*	50	*rol-6, klp-6p::mcherry*	50, 50
LJ838	*jIEx1926*	*daf-19mp::gfp (Substitution; -132, -116)*	50	*rol-6, klp-6p::mcherry*	50, 50
LJ839	*qIs56*	*lag-2p::gfp*			
LJ840	*vsIs48*	*unc-17::gfp*			
LJ841	*cfjEx90*	*myo-2p::mcherry*	3	*myo-2p::mcherry*	3
LJ842	*cfjEx90*	*myo-2p::mcherry*	3	*myo-2p::mcherry*	3
LJ843	*jIEx1921*	*daf-19m (IL2/CEM enhancer insertion; -5738, -5719)*	50	*myo-2p::mcherry*	3
LJ844	*jIEx1927*	*F28A12.3p::klp-6::gfp*	50	*myo-2p::mcherry*	3
		F28A12.3p::osm-9::gfp	50		
LX929	*vsIs48*	*unc-17::gfp*			
JK2868	*qIs56*	*lag-2p::gfp*			
OE4502	*jIIs1900*	*klp-6p::gfp*	60	*rol-6*	60
		aqp-6p::ds-red	60		
OE4503	*jIIs1900*	*klp-6p::gfp*	60	*rol-6*	60
		aqp-6p::ds-red	60		
PT2519	*myIs13*	*klp-6p::gfp*			

Table 2. The list of transgenic animals, used in this study.

Gene	Location of bait sequence	Count
daf-19	-32nt from *daf-19a* start codon	1
	-26nt from *daf-19a* start codon	1
	86th amino acid of *daf-19a*	1
	151st amino acid of *daf-19a*	1
	226th amino acid of *daf-19a*	3
	Core domain of *daf-19* isoforms (Confirmed by PCR)	107
unc-15	656th amino acid	1
	660th amino acid	1
	665th amino acid	1
cpar-1	1st amino acid	2
Total		119

Table 3. The result of yeast one hybrid experiment. The information of positive colonies, showing interaction between bait sequence and proteins, translated from the cDNA library of *C. elegans*. All colonies are examined by PCR confirmation and some colonies were sequenced to get the detail information of binding sites.

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국문 초록

예쁜꼬마선충 IL2 뉴런에서 RFX 전사인자의 서브루틴으로서의 새로운 기전 연구

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진핵세포의 감각 기관으로서 섬모의 기능은 개체의 생존을 위해 필수적이다. 사람의 경우 섬모의 이상은 신경계를 포함한 다양한 기관에 영향을 주게 된다. 신경계에서 섬모의 역할이 많이 연구되는 가운데, 그 조절 기전을 이해하는 것은 매우 중요하다. 본 연구에서는 예쁜꼬마선충을 이용해 뉴런의 분화에서 획득하는 섬모의 기능과 그 기전을 연구하였다. 닉테이션은 이동수단으로 활용할 수 있는 다른 개체와의 상호작용을 위한 중요한 다우어 특이적인 행동이며, 이를 통해 선충은 좋은 환경으로 옮겨갈 수 있다. IL2 섬모성 뉴런과 그 섬모 구조는 이 닉테이션 행동에 핵심적인 역할을 한다고 알려져 있으며, IL2 특이적인 모터 단백질 KLP-6는 섬모 구조 끝으로 단백질들을 이동시켜 IL2 특이적인 기능을 수행할 것이라 예상되고 있다. 정방향 유전학 스크리닝을 통해, 섬모의 발생과 기능을 조절하는 RFX 전사인자의 상동유전자인 *daf-19m*이 기존에 찾지 못했던 비정형적 X-box motif를 통해 *klp-6*를 조절한다는 사실을 확인했으며, 동시에 섬모에서의 기능이 있는 *daf-19m*의 다른 타겟 유전자

들을 찾았다. 또한 *daf-19m*이 IL2 뉴런에서 타겟 유전자와 함께 서브루틴을 이룬다는 사실을 확인했다. *daf-19m*은 IL2 뉴런에서 최종 분화 과정을 조절하는 터미널 셀렉터의 조절을 받으며, IL2 뉴런의 아이덴티티를 조절하는 일부의 타겟 유전자들만 조절한다. 그리고 *daf-19m*의 타겟 유전자가 그렇듯, *daf-19m*이 닉테이션 행동을 조절하는 사실을 발견했다. *daf-19m*과 하위 유전자들의 분자적 정체성을 고려해 볼 때, *daf-19m* 서브루틴은 섬모에서 IL2 뉴런의 기능 중 하나인 물리자극반응에 영향을 줘서 닉테이션을 조절할 것이라 예상된다. 본 연구에서는 정방향유전학 및 다양한 분자생물학 기법을 이용해 예쁜꼬마선충에서 IL2 뉴런의 최종 분화과정에서의 핵심적인 서브루틴을 발견하고 그 기능을 규명하였다.

주요어: 예쁜꼬마선충, 섬모, IL2 뉴런, 신경세포 발달, DAF-19M, 서브루틴, 닉테이션 행동

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