学位論文 (要約)

Regulation of body size and learning by the nervous system in *C. elegans*

(線虫 *C. elegans* の神経系が体長と学習を制御する機構の解析)

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Abstract

To adapt to their ambient environment and survive in the natural world, animals respond to environmental stimuli. The nervous system of animals senses external stimuli and modulates behavior and development. In mammals, the nervous system is complex due to the existences of many neurons and their connections. Therefore, the roles of the nervous system at the molecular, cellular and individual levels are not fully understood. In this study, I investigated the regulatory function of nervous system on appropriate responses according to nutritional conditions, using the nematode *Caenorhabditis elegans*. *C. elegans* makes a good model organism thanks to its short generation time and suitability for genetic manipulations. Many factors which act in the nervous system of *C. elegans* are homologs to those of mammals. Thus, *C. elegans* is a fitting choice for investigating the relationship between the nervous system and development. Moreover, *C. elegans* displays mechanisms of learning and memory and is suitable for analyses at single-cell levels. Investigation by using *C. elegans* allows for examination of the role of individual neurons in learning and memory.

In the chapter 1, I examined the relationship between animal body size and dopamine, which is one of the monoamine neurotransmitters that are released by sensing food. Dopamine has been known to affect the emotional and reward systems in mammals. However, little has been known about the molecular mechanism of dopamine in animal development. This study shows that dopamine negatively regulates body length through the D2-like dopamine receptors, insulin-like signaling and octopamine signaling. In addition to body length, this study also demonstrates the relationship between egg-laying and dopamine. Dopamine positively regulates egg-laying. As dopamine is thought to be released by sensing food, the egg-laying promoted by dopamine causes animals to lay eggs under well-fed condition and may act beneficially on leaving progeny. Body size regulation by dopamine works independently of the egg-laying phenotype.

Chapter 2 の Abstract の内容は、5 年以内に雑誌等で刊行予定のため非公開

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5 年以内に雑誌等に刊行予定のため非公開

Introduction

To survive in the natural world, it is important for organisms to adapt to an ever changing environment. Most animals are drawn to an environment with ample food or one with the opposite sex present in order to survive and leave behind offspring. On the other hand, animals avoid harsh environments that contains their predators, toxic substances, or lack food, thereby avoiding danger and death. The nervous system in all animals possessing one plays a key role in the sensing of external stimuli and modulating development and behavior. However, the regulatory functions of the nervous system are not fully understood at the molecular and organismal levels. The nematode *Caenorhabditis elegans* was used in this study to explore these mechanisms and systems. *C. elegans* is suitable for analyses of the nervous system at the molecular and organismal levels because of its complete neural circuit mapping and genetic tractability.

In chapter 1, I examined the function of the nervous system in body size regulation. It has previously been known that body size of *C. elegans* is regulated by the nervous system. However, the relationship between neurotransmitters and body size remained unclear. Here, I examined the molecular mechanisms by which dopamine regulates body size. Although dopamine has been known to affect regulation of the emotion and reward systems, its effect on development has remained unknown.

In chapter 2, I investigated the mechanisms of learning controlled by the nervous system. In order to avoid harsh environments, most animals memorize environmental conditions and change behavior according to past experiences. *C. elegans* memorizes salt concentrations and avoids the concentrations encountered under starvation conditions (taste avoidance learning). It has been previously reported that insulin-like signaling acts in a sensory neuron known as ASER and is required for taste avoidance learning (Tomioka et al., 2006). Moreover, genetic analyses suggest that the insulin-like signaling involved in learning is different than that of the signaling involved in regulation of longevity, development and metabolism. In this chapter, I focused on functional mechanisms of insulin-like signaling in taste avoidance learning and examined DAF-16/FOXO transcription factor, a major downstream factor in the insulin-like signaling pathway.

In this study, I attempt to clarify the functions of dopamine and DAF-16/FOXO in the nervous system with respect to development and learning, and to understand the role of the nervous system in regulatory responses to environmental changes.

Chapter 1:
Body size regulation by dopamine

1.1 Abbreviations

C. elegans	*Caenorhabditis elegans*
E. coli	*Escherichia coli*
GFP	green fluorescent protein
GH	growth hormone
IGF	insulin-like growth factor
L4	the fourth stage of larva
MAP kinase	mitogen-activated protein kinase
NGM	nematode growth media
PCR	polymerase chain reaction
PI3K	phosphatidylinositol 3-kinase
SMAD	sma- and mad-related
TGF-β	transforming growth factor β
TOR	target of rapamycin
wt	wild-type

1-2. Introduction

1-2-1. Body size regulation in animals

Body size is one of the most basic traits of organisms and is influenced by ambient environment. Food is one of the most influential factors in the body size regulation. Quality of food causes body size differences. For example, a high-fat diet during early life results in larger body size of mice (Lin et al., 2000). High-fat diet also regulates metabolism through the epigenetic modification of adiponectin and leptin genes (Masuyama and Hiramatsu, 2012). Food-rich conditions promote the increased body size via the control of cell division in *Drosophila* (de Moed et al., 1997). In addition to nutritional factors, ambient temperature affects animal body size. Tail length and surface area of the ear of mice show positive correlation with ambient temperature (Serrat et al., 2008). In *Drosophila*, lower temperatures allow females to become larger by increasing the cell size (French et al., 1998; de Moed et al., 1997; Robertson, 1959). Although lower temperatures are positively correlated with the body size of male, the increased body size is caused by not only the increased size of cells but also by an increase in their numbers. These data suggest that the mechanism of body size regulation is different between females and males. *Drosophila* also changes their body size in response to humidity. The surface area of wings become larger in the low-humidity conditions (Kennington et al., 2003). Collectively, the body size regulation is complex because it is influenced by various factors.

1-2-2. Genetic factors for body size regulation of *C. elegans*

Previously, many factors that regulate body size have been found in the nematode *Caenorhabditis elegans*. *C. elegans* is suitable for the study of body size due to its rapid development and easy handling for genetic analyses. The signaling pathways and their components that affect body size regulation are described below.

TGF-β signaling:

Transforming growth factor-β (TGF-β) signaling is involved in animal body size (Patterson and Padgett, 2000; Tuck, 2014). TGF-β superfamily ligands regulate cell function, identity, and survival, and affect many diseases (Massagué, 2012; Wu and Hill, 2009). DBL-1/CET-1, a TGF-β ligand, has been identified in *C. elegans* (Morita et al., 1999; Suzuki et al., 1999) and is homologous to *Drosophila* Decapentaplegic (Dpp) and mammalian bone morphogenetic proteins. Although DBL-1 does not affect cell division, it plays a role in the growth of hypodermal cells. *dbl-1* loss of function mutants showed smaller body size compared to wild-type animals, suggesting that DBL-1 positively regulates body size. DBL-1 is expressed in neurons and is thought to be secreted from neurons. The DBL-1 receptor is composed of DAF-4 and SMA-6 proteins. While SMA-6 is expressed in

hypodermis, pharynx and intestine, the SMA-6 expression in hypodermis regulates body size (Yoshida et al., 2001). DAF-4 and SMA-6 complex activates SMAD transcription factors, SMA-2, SMA-3, and SMA-4 (Estevez et al., 1993; Krishna et al., 1999). DBL-1 and SMAD proteins decrease the expression of the *lon-1* gene, which encodes a cysteine-rich secretory protein family homolog (Maduzia et al., 2002; Morita et al., 2002). Its expression is thought to promote the polyploidization of hypodermal cells, which in turn modulates hypodermal cell size. Therefore, the DBL-1 that is secreted from neurons binds to its receptor expressed in hypodermis, and the size of hypodermal cells are cell-autonomously modulated by SMAD transcription factors, thereby affecting body size.

Insulin-like signaling:

In mammals, insulin/IGF signaling works downstream of the growth hormone (GH) in body size regulation (Blutke et al., 2014; Lundberg et al., 2015). Insulin-like signaling also acts in invertebrates which do not possess GH. *chico* and *dInR Drosophila* mutants, which are defective in the insulin-like signaling, exhibit small body size.

Previously, forty insulin-like peptides have been identified in *C. elegans*. These insulin-like peptides are expressed in various tissues and their expression levels are changed according to developmental stages and environmental conditions (Ritter et al., 2013). The signals of these peptides are received by the sole insulin-like receptor, DAF-2. DAF-2 has been known to affect food-dependent body size regulation (So et al., 2011).

TOR signaling:

Target of rapamycin (TOR) signaling affects animal development and acts downstream of insulin-like signaling (Scott et al., 1998; Sekulić et al., 2000). Of the two TOR signaling complexes, TORC1 and TORC2, the TORC1 signaling is involved in cell growth via the initiation factor 4E-BP, which is associated with translation factors and ribosomal S6 kinase (Gingras et al., 2001). *Drosophila* TOR signaling mutants, *dTOR* and *dS6K*, show small body size.

In addition to TORC1, TORC2 signaling also affects body size regulation of *Drosophila* and *C. elegans* (Hietakangas and Cohen, 2007; Lee and Chung, 2007). RICT-1 is one of the TORC2 components, and *rict-1* mutants exhibit smaller body size compared to wild-type animals (Jones et al., 2009). Genetic analysis showed that body size regulation by RICT-1 works in parallel with that by TGF-β signaling.

MAP kinase signaling:

Mutants for *sma-5*, an ortholog of MAP kinase BMK1/ERK5, exhibit smaller body size than wild-type animals in *C. elegans* (Watanabe et al., 2005). Double mutants that have defects in both MAPK signaling and TGF-β signaling are known to be the smallest mutants of *C. elegans* (Watanabe et al., 2007). This result suggests that MAPK signaling regulates body size independently of TGF-β signaling.

Hippo signaling:

Hippo signaling was first identified in *Drosophila* and is evolutionarily conserved in most animals (Justice et al., 1995; Xu et al., 1995; Yu et al., 2015). Several genes which encode homologs of the Hippo signaling components have been found in *C. elegans*. RNAi knockdown of the *C. elegans warts* homolog, *wts-1*, causes animals to become small (Cai et al., 2009). In contrast to *C. elegans*, the *warts* gene negatively controls overgrowth in *Drosophila*. The function of Hippo signaling may be different between *C. elegans* and *Drosophila*.

Others:

The signal from the germ line has been known to affect body size in *C. elegans* (Patel et al., 2002). Ablation of germ line cells causes animals to become larger. Although the molecular mechanism of this germ line signal is currently unknown, it is at least suggested that this germ line signal is independent of TGF-β signaling.

1-2-3. Environmental factors for body size regulation of *C. elegans*

Body size is changed in response to the quality of diet in *C. elegans*. Animals fed an *E. coli* HB101 diet become larger compared to those fed an *E. coli* OP50 diet (So et al., 2011). The effect of the diet was reduced in insulin-like receptor mutants (*daf-2* mutants) indicating that food quality affects body size regulation via insulin-like signaling. Animals fed a *Comamonas* DA1877 diet show fast development and increased body size compared to those fed an *E. coli* OP50 diet (MacNeil et al., 2013). This effects of the diet are caused by vitamin B12 that is produced by *Comamonas* DA1877 (Watson et al., 2014).

In addition to the quality of diet, the quantity of diet also changes body size. *eat-2* mutants, which have defects in normal pharyngeal pumping, exhibit reduced food intake and small body size (So et al., 2011). Both *pha-2* and *pha-3* mutants, which have defects in normal pharyngeal development, have smaller body size than wild-type animals (Mörck and Pilon, 2006). The low-food conditions cause animals to become smaller (Lenaerts et al., 2008). Starvation in early life of parent animals causes production of progeny with shorter body lengths (Jobson et al., 2015), suggesting that starvation epigenetically regulates body size.

In general, ectotherms grown at lower temperatures exhibit increased body sizes, and this phenomenon is called the "temperature-size rule". Standard N2 wild-type animals grown at lower temperatures are larger than those grown at higher temperatures (Kammenga et al., 2007). In contrast, animals of another wild-isolate, CB4856, do not show the phenotype. The single nucleotide polymorphism between N2 and CB4856 also affects body size regulation. *tra-3* gene encodes a calpain-like protease. A single nucleotide polymorphism in the *tra-3* gene, which encodes a calpain-like protease, affects temperature-dependent regulation.

1-2-4. Roles of the nervous system in the regulation of body size of *C. elegans*

Previously, external stimuli have been known to affect body size regulation of *C. elegans*. Animals grown in isolation show decreased developmental rates and smaller body sizes compared to those grown in groups (Rose et al., 2005). This effect of isolation was not observed in the *mec-4* mutants, which have defects in touch-sensing. These data indicated that mechanosensory neurons play a role in body size regulation.

C. elegans can perceive environmental stimuli via ciliated sensory neurons. The *che-2* gene encodes a WD protein and is required for cilia formation and for sensing environmental stimuli (Fujiwara et al., 1999). *che-2* mutants show smaller body size, suggesting that environmental stimuli affect body size regulation through the function of ciliated sensory neurons. This reduced body size of *che-2* mutants is suppressed by *egl-4* mutation. The *egl-4* gene encodes a cGMP-dependent protein kinase and is expressed in the nervous system. *che-2* and *egl-4* are thought to regulate body size through the control of the TGF-β ligand secreted from neurons. Although the nervous system shows a certain level of control over body size, it is unclear what role each individual neurotransmitter plays.

1-2-5. Relationship between dopamine and body size

Dopamine is a one of the monoamine neurotransmitters and its signaling components are conserved among most animals. It has previously been found that dopamine defective mutants, *cat-2* mutants, are longer than wild-type animals in *C. elegans* (Nagashima et al., 2016). *cat-2* mutants are significantly longer than wild-type animals at any timepoint after eggs were laid (Fig. 1-2-1). In addition to the body length, body width of *cat-2* mutants is larger than that of wild-type animals. These data suggest that dopamine negatively regulates body size.

Although body size is influenced by nutritional factors, body size regulation by dopamine does not depend on the quantity or quality of food. *cat-2* mutants did not show increased food intake but exhibited the increased body length in the background of the *Comamonas* DA1877 or *E. coli* HB101 diets, which affects animal body size.

Octopamine, an amine neurotransmitter, is considered the biological equivalent of mammalian noradrenaline (Roeder, 1999). Genetic analyses show that octopamine signaling functions downstream of dopamine. Moreover, developmental rate has been examined. *cat-2* mutants are more developed than wild-type animals 48 hours after eggs-laying. However, when animals were synchronized at the L1 larval stage, the developmental stage of *cat-2* mutants was the same level with that of wild-type animals. These results suggested that there was not remarkable difference in developmental rate between wild-type and *cat-2* mutant animals. One possibility was that *cat-2* mutants were more developed compared to wild-type animals before the L1 larval stage.

1-2-6. Aim of this study

To find the mechanisms of body size regulation by dopamine, I examined the relationship between developmental stage and body size and searched for downstream factors of dopamine. First, to clarify the relationship between dopamine and the developmental rate before L1 larval stage, I observed the developmental stage of embryo. Next, to clarify the relationship between the developmental stage of embryo and body size of adult animals, I measured body length of animals after the developmental stage was synchronized. Finally, to find the downstream mechanisms of body size regulation of dopamine, I searched for the downstream factors of dopamine, using genetic analyses.

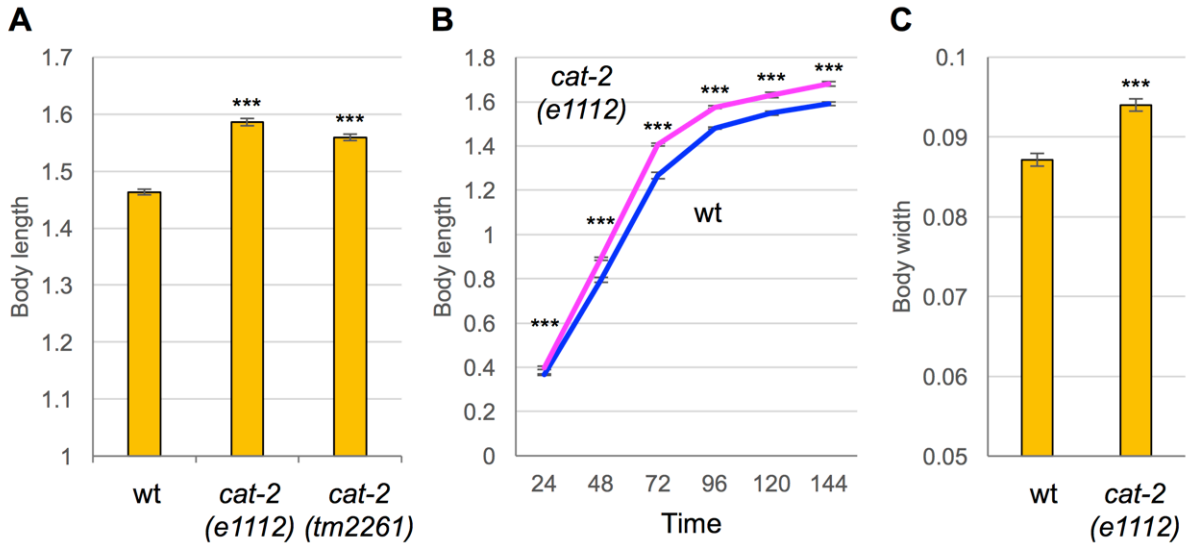

Fig. 1-2-1. *cat-2* mutants exhibit enlarged body size.
(A) Both *cat-2(e1112)* and *cat-2(tm2261)* mutants are defective in the dopamine synthesis. Body length of wild-type and *cat-2* mutant animals is shown. Animals were synchronized at the egg-laying. N > 71. Error bars: SEM. *** $p < 0.001$ (ANOVA with Tukey multiple test).
(B) Body length of wild-type and *cat-2(e1112)* mutant animals was measured after egg-laying every 24 hours for six days. N > 32. *** $p < 0.001$ (*t*-test compared to wt at any point).
(C) Body width of wild-type and *cat-2(e1112)* mutant animals is shown. N = 20. Error bars: SEM. *** $p < 0.001$ (*t*-test).

1-3. Materials and Methods

1-3-1. *C. elegans* strains

Most strains were provided by *Caenorhabditis* Genetics Center (CGC) and National BioResource Project (NBRP). These strains were stocked by Dr. Satoshi Suo. In this study, double mutants were generated by standard crossing manipulations, and their genotypes were checked by PCR.

Table 1-3-1. *C. elegans* strains

Strain	Genotype	Source	Purpose of use
N2	wild-type	CGC	wild-type control
CB1112	*cat-2(e1112)II.*	CGC	Analysis of dopamine
VN366	*cat-2(tm2261)II.*	Dr. Suo	Analysis of dopamine
	cat-2(e1112)II; Ex[cat-2(genome); glr-3^{prom}::mCherry]	This study	*cat-2* rescue
	cat-2(e1112)II; Ex[glr-3^{prom}::mCherry]	This study	*cat-2* rescue
	cat-2(tm2261)II; Ex[cat-2(genome); glr-3^{prom}::mCherry]	This study	*cat-2* rescue
	cat-2(tm2261)II; Ex[glr-3^{prom}::mCherry]	This study	*cat-2* rescue
LX645	*dop-1(vs100)X.*	CGC	Analysis of dopamine
LX702	*dop-2(vs105)V.*	CGC	Analysis of dopamine
LX703	*dop-3(vs106)X.*	CGC	Analysis of dopamine
VN384	*dop-4(ok1321)X.*	Dr. Suo	Analysis of dopamine
VN282	*lgc-53(tm2735)X.*	Dr. Suo	Analysis of dopamine
	dop-3(vs106)X; Ex[dop-3(genome); glr-3^{prom}::mCherry]	This study	*dop-3* rescue
	dop-3(vs106)X; Ex[H20^{prom}::dop-3; glr-3^{prom}::mCherry]	This study	*dop-3* rescue
	dop-3(vs106)X; Ex[tbh-1^{prom}::dop-3; glr-3^{prom}::mCherry]	This study	*dop-3* rescue
	dop-3(vs106)X; Ex[ceh-17p4^{prom}::dop-3; glr-3^{prom}::mCherry]	This study	*dop-3* rescue
	dop-3(vs106)X; Ex[myo-3^{prom}::dop-3; glr-3^{prom}::mCherry]	This study	*dop-3* rescue
	dop-3(vs106)X; Ex[rol-6^{prom}::dop-3; glr-3^{prom}::mCherry]	This study	*dop-3* rescue
	dop-3(vs106)X; Ex[rol-6^{prom}::dop-3; glr-3^{prom}::mCherry]	This study	*dop-3* rescue
	dop-3(vs106)X; Ex[glr-3^{prom}::mCherry]	This study	*dop-3* rescue

Strain	Genotype	Source	Purpose of use
MT1082	*egl-1(n487)V.*	CGC	Analysis of egg-laying
MT3188	*egl-17(n1377)X.*	CGC	Analysis of egg-laying
VN22	*tbh-1(ok1196)X.*	Dr. Suo	Analysis of octopamine
	cat-2(e1112)II; tbh-1(ok1196)V.	Mr. Oami	Analysis of octopamine
VN182	*ser-3(ad1774)I.*	Dr. Suo	Analysis of octopamine
	ser-3(ad1774)I; cat-2(e1112)II.	Mr. Oami	Analysis of octopamine
VN280	*ser-6(tm2146)IV.*	Dr. Suo	Analysis of octopamine
	cat-2(e1112)II; ser-6(tm2146)IV.	Mr. Oami	Analysis of octopamine
FK3890	*dbl-1(tm3890)V.*	NBRP	Analysis of TGF-β
	cat-2(e1112)II; dbl-1(tm3890)V.	Mr. Oami	Analysis of TGF-β
	cat-2(tm2261)II; dbl-1(tm3890)V.	This study	Analysis of TGF-β
LT121	*dbl-1(wk70)V.*	CGC	Analysis of TGF-β
	cat-2(tm2261)II; dbl-1(wk70)V.	This study	Analysis of TGF-β
NU3	*dbl-1(nk3)V.*	CGC	Analysis of TGF-β
	cat-2(tm2261)II; dbl-1(nk3)V.	This study	Analysis of TGF-β
CB444	*unc-52(e444)II.*	CGC	Analysis of muscle
	cat-2(e1112)II unc-52(e444)II.	Mr. Oami	Analysis of muscle
CB190	*unc-54(e190)I.*	CGC	Analysis of muscle
	unc-54(e190)I; cat-2(e1112)II.	Mr. Oami	Analysis of muscle
CB1370	*daf-2(e1370)III.*	CGC	Analysis of insulin/IGF
	cat-2(e1112)II;daf-2(e1370)III.	This study	Analysis of insulin/IGF

1-3-2. Cultivation

The culturing of *C. elegans* was performed as described (Brenner, 1974). Animals were cultivated on Nematode Growth Media (NGM) under 15 °C, 20 °C or 25 °C. The *E. coli* strain, OP50, was used as a bacterial food source.

NGM agar plate

0.25 %	Bacto pepton (BD)
0.3 %	NaCl
1.7 %	INA Agar (Funakoshi)
1 L	Elix water

After NGM was autoclaved, the following reagents componds were added, and then 10 ml of NGM was poured into 6cm plates.

0.5 ml	5 mg/ml cholesterol with ethanol,
1 mL	1M $MgSO_4$
1 mL	1M $CaCl_2$
25 mL	1M Potassium phosphate (pH 6.0)

1-3-3. Transgenic animals

For the *cat-2* rescue experiment, the *cat-2* gene was amplified with Ex Taq DNA polymerase (Takara, Japan). N2 genomic DNA and the primers, 5'-CGCAGTTTTCGCAAGTAGTG-3' and 5'-GAAGCCGTACCGG- GAAGTCTC-3'. The amplified DNA contained 2 kbp upstream and 0.5 kbp downstream of the *cat-2* gene. The amplified *cat-2* gene, a transformation marker plasmid (*glr-3^{prom}::mCherry*) and an empty vector (pBluescript) was purified and injected into *cat-2(e1112)* and *cat-2(tm2261)* mutants. *glr-3^{prom}::mCherry* induced mCherry expression in the RIA neuron. For the generation of marker control animals, the transformation marker plasmid and the empty vector were injected into *cat-2* mutants.

For the *dop-3* rescue experiment, the *dop-3* genomic region was amplified with Ex Taq DNA polymerase (Takara, Japan) by using N2 genomic DNA as a template. The primers used were, 5'-TGTCATTATCGCATCGGGTC-3' and 5'-AATTTGTTCCCATCGCCTCC-3' The amplified DNA contained plus 3 kbp upstream and 0.5 kbp downstream of the *dop-3* gene. The amplified DNA was purified and injected into *dop-3(vs106)* mutants with the transformation marker and the empty vector. For the generation of marker control animals, the transformation marker plasmid and the empty vector were injected into *dop-3* mutants.

To express *dop-3* in muscle and hypodermis, I generated two plasmids. The cDNA of *dop-3* gene was fused to the downstream of *myo-3^{prom}* and *rol-6^{prom}*, respectively. These plasmids were injected into *dop-3(vs106)* mutants with the transformation marker and the empty vector. Moreover, the following plasmids of *dop-3* were also injected. *tbh-1^{prom}::dop-3* and *ceh-17p4^{prom}::dop-3* plasmids have been previously constructed. *H20^{prom}::dop-3* plasmid was provided from Dr. Kotaro Kimura.

1-3-4. Measurement of body length

Adult hermaphrodites were placed on 6 cm NGM plates seeded with *Escherichia coli* OP50, and were allowed to lay eggs for two hours at 20 °C. When the body length of transgenic animals was measured, parent animals carrying transgenes and not carrying transgenes were allowed to lay eggs. And then, animals were removed from the plates. After culturing for 72 hours at 20 °C, animals were transferred to new 6 cm NGM plates and incubated for an additional 24 hours at 20 °C. Animals were collected in water, and the supernatant was removed. Animals were mounted on glass slides after they were anesthetized with M9 buffer containing 50 mM sodium azide (NaN_3). The images of individual animals were captured by using a microscope equipped with a digital camera (Olympus IX70, DP73 or Zeiss Axioplan2, Hamamatsu ORCA-ER). Body length was measured by using ImageJ software as described (Mörck and Pilon, 2006). The center line of each animal was generated by mouse clicks, and the length of the line was calculated (Fig. 1-3-2A).

Body length of male animals was measured 48 hours after L4 larval stage. L4 larva was collected according to its tale morphology.

1-3-5. Exogenous dopamine treatment

For the exogenous dopamine rescue experiment, the test plates were prepared. The test plates contained 1.7% Agar, 51 mM NaCl, 0.01 mM cholesterol, 25 mM KPO_4 (pH 6.0), 1 mM $MgSO_4$ and 1 mM $CaCl_2$. The overnight culture solution of *E. coli* OP50 was centrifuged, and the bacterial concentrate was prepared. The concentrate was spread on the test plates. Dopamine which was dissolved in water was spread on test plates (final 0 or 7.8 mM) about several hours before animals were placed.

Adult hermaphrodites were allowed to lay eggs for two hours at 20 °C. Animals were cultured for 96 hours at 20 °C. Animals were collected in water and transferred to new plates every 24 hours for 96 hours. Animals were anesthetized with M9 buffer containing 50 mM NaN_3, and the body length was measured.

1-3-6. Observation of embryo

To determine the developmental stage of laid eggs, L4 hermaphrodites were picked onto 6 cm NGM plates seeded with OP50, and incubated for 24 hours at 20 °C. Adult animals were placed on NGM plates, and were allowed to lay eggs for two hours at 20 °C. After then, the embryo in each egg was observed using a microscope (Olympus BX53), and visually categorized based on the morphology. I categorized eggs into five embryonic stages and L1 larval stage of development: gastrulation stage, bean stage, 1.5-fold stage, 2-fold stage, 3-fold stage and L1 larval stage (Fig. 1-3-2B). The embryonic morphogenesis from the first cleavage to elongation has been previously described (Chin-Sang and Chisholm, 2000).

1-3-7. Developmental stage and body length of L4 larva

The vulval morphology of L4 larva allows animals to be categorized in detail (Fig. 1-3-2C). In this study, L4 larvae were categorized into five different groups. L4 larvae that appeared to be in between "Early L4" and "Mid L4" stages were categorized as "Early-Mid L4". The developmental stage of *dbl-1(nk3)* and *cat-2;dbl-1* double mutants was examined. Gravid adult animals were treated with a hypochlorite/NaOH solution, and eggs were washed three times using M9 buffer. Eggs were collected in 1 ml M9 buffer and cultured for 24 hours at 20-25 °C. L1 animals were transferred to a seeded NGM plate and cultured for 48 hours at 20 °C. After 48 hours, animal body length was determined.

To compare the body length at the same developmental stage, the body length of L4 larva was measured. Animals were collected in water, and the supernatant was removed. Animals were mounted on glass slides after they were anesthetized with M9 buffer containing 50 mM sodium azide (NaN_3). The image of Mid L4 larva was captured by using a microscope equipped with a digital camera (Olympus IX70, DP73 or Zeiss Axioplan2, Hamamatsu ORCA-ER). And then, body length was measured by using imageJ.

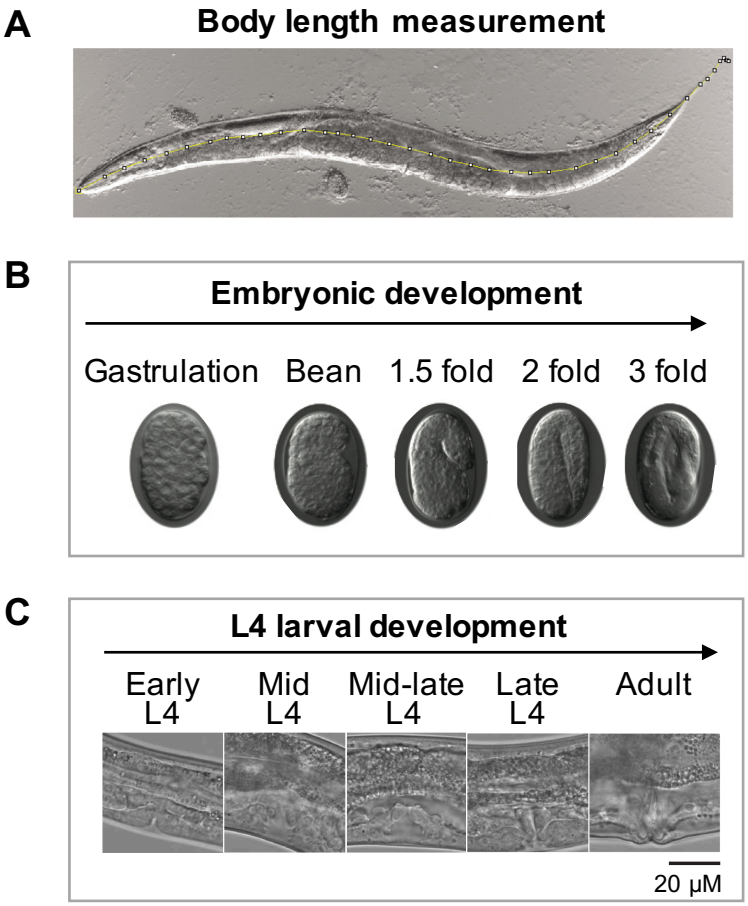

Fig. 1-3-2. Body length measurement and developmental stage.
(A) Method of body length measurement by using the ImageJ software. The image was quoted from the paper (Mörck and Pilon, 2006) and partially edited.
(B) Five stages of embryonic development were shown. The images were quoted from Wormatlas "INTRODUCTION TO *C. elegans* EMBRYO ANATOMY" (http://www.wormatlas.org/embryo/introduction/EIntroframeset.html).
(C) Five stages of L4 larval development were shown. The images were quoted from the paper (MacNeil et al., 2013) and partially edited.

1-4. Results

1-4-1. Body length of dopamine deficient mutants

To investigate the role of dopamine, an amine neurotransmitter, in body size regulation of *C. elegans*, body length of the hermaphrodites of dopamine defective mutants, *cat-2* mutants, was examined. The *cat-2* gene encodes a tyrosine hydroxylase which is a key enzyme for dopamine synthesis. First, I confirmed whether *cat-2* mutants, which are defective in dopamine synthesis, were longer than wild-type animals. In this study, two *cat-2* mutant alleles were used, *cat-2(e1112)* and *cat-2(tm2261)*, which have a point mutation and a deletion mutation, respectively. Both *cat-2* mutants showed increased body length compared to wild-type N2 animals, as previously described (Fig. 1-4-1A, B). This result suggested that dopamine negatively regulates body length in *C. elegans*. In contrast, the effects of the *cat-2* mutation on body length was not observed in males (Fig. 1-4-1C). These results suggested that body size regulation by dopamine occurred only in hermaphrodites.

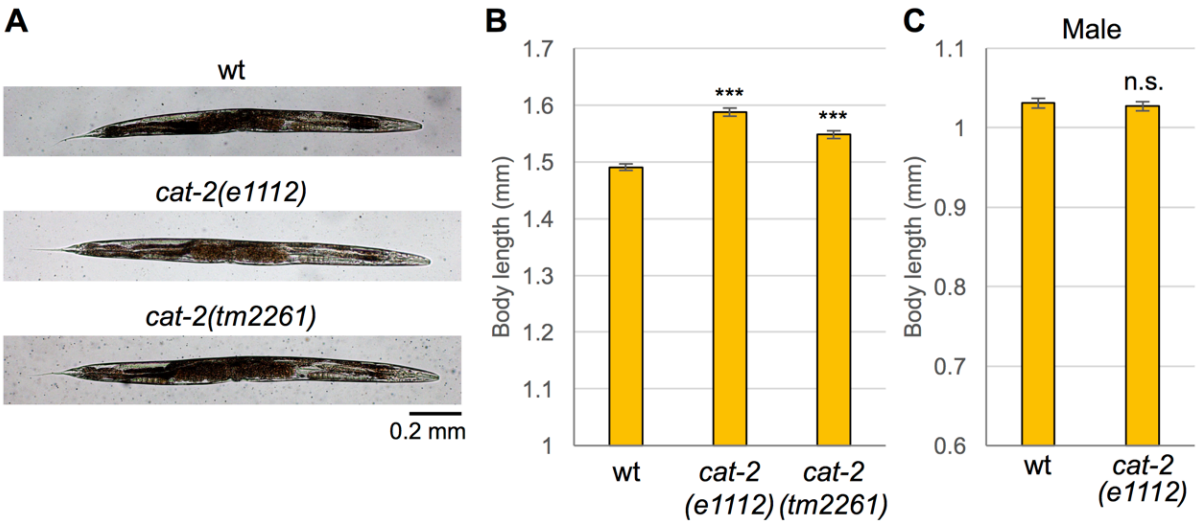

Fig. 1-4-1. Body length of *cat-2* mutants.
(A) Images of N2 wild-type, *cat-2(e1112)* and *cat-2(tm2261)* mutant animals.
(B) Body length of wild-type and *cat-2* mutant animals was measured 96 hours after egg-laying. N = 70. *** $p < 0.001$ (ANOVA with Tukey multiple test).
(C) Body length of wild-type and *cat-2(e1112)* mutant males was shown. Body length was measured 48 hours after the L4 larval stage. N > 35. Error bars: SEM. n.s. $p > 0.05$ (*t*-test).

To confirm the effects of *cat-2* gene on body size, a transgene containing wild-type *cat-2* genomic DNA was injected into *cat-2* mutants. The increased body length of *cat-2(e1112)* mutants was canceled by wild-type *cat-2* gene (Fig. 1-4-2A). The body length of *cat-2* mutants carrying a marker-only transgene was not different between transgene (+) and (-). The effect of introduction of the *cat-2* gene was seen not only *cat-2(e1112)* mutants, but also in the body length of *cat-2(tm2261)* mutants as well (Fig. 1-4-2B). These results indicated that the *cat-2* gene affected body size regulation. The *cat-2* gene encodes a tyrosine hydroxylase that synthesizes L-DOPA. Dopamine is synthesized via L-DOPA by the BAS-1 enzyme, aromatic amino acid decarboxylase. Therefore, one possibility that the increased body size of *cat-2* mutants was caused by L-DOPA. I next tested exogenous dopamine treatment. Wild-type and *cat-2* mutant animals were grown on plates containing 0 or 7.8 mM dopamine for 96 hours. The exogenous dopamine treatment reduced the increased body length of *cat-2* mutants, but the effects were not observed in the wild-type animals (Fig, 1-4-2C). This result further indicated that dopamine negatively regulated body size in *C. elegans*.

1-4-2. Effects of dopamine receptors on body length

To identify the dopamine receptor that affects body size regulation, I examined dopamine receptor mutants. Previously, five dopamine receptors have been reported, *dop-1, dop-2, dop-3, dop-4* and *lgc-53* (Chase et al., 2004; Sanyal et al., 2004; Sugiura et al., 2005; Suo et al., 2002, 2003).

The body length of *dop-1, dop-2*, and *lgc-53* mutants was as long as that of wild-type animals, whereas the *dop-4* mutants were shorter than wild-type animals (Fig. 1-4-3A). Among dopamine receptor mutants, *dop-3* mutants were the longest, suggesting that *dop-3*, which encodes a D2-like dopamine receptor, mainly regulates body size. To confirm the relationship between *dop-3* and body size, the transgene containing the wild-type *dop-3* genomic DNA was introduced into *dop-3* mutants. The increased body length of *dop-3* mutants was suppressed by the introduction of *dop-3* gene (Fig. 1-4-3B). The *dop-3* gene has been known to be expressed in the RIC neurons, SIA neurons, several neurons and body wall muscle. RIC receives dopamine signals and releases octopamine, a monoamine neurotransmitter (Suo et al., 2009). The signal is transmitted by octopamine to its receptors expressed in SIA in the regulation of food response. A major signaling of body size regulation of *C. elegans*, TGF-β signaling, functions in the hypodermis (Yoshida et al., 2001). To examined where *dop-3* function in the regulation of body length, *dop-3* was expressed in several tissues (Fig. 1-4-3C). When *dop-3* was expressed in SIA and RIC, the difference of body length was not observed between transgene (+) and (-). On the other hand, the increased body length of *dop-3* mutants was suppressed by *dop-3* expression in all neurons. This result suggested that *dop-3* acted in the nervous system, but not in RIC or SIA. The *dop-3* expression in muscle and hypodermis did not did not change body length. The dopamine signaling that regulated body length might not function in muscle and hypodermis.

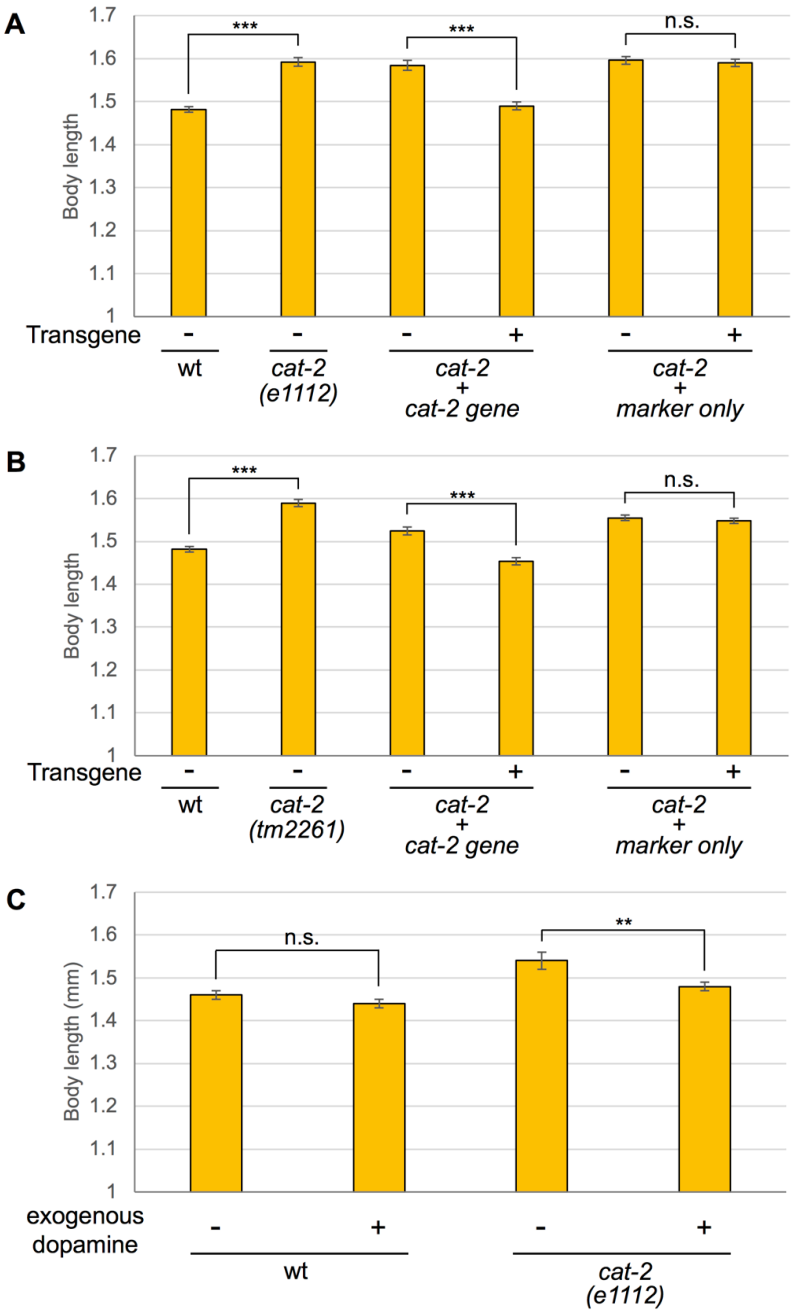

Fig. 1-4-2. Relationship between *cat-2* and body length.
(A) Body length of wild-type, *cat-2(e1112)* mutant and transgenic animals. "*cat-2* + *cat-2* gene" means *cat-2(e1112)* mutants carrying the transgene containing *cat-2* genomic DNA. "*cat-2* + marker only" means *cat-2(e1112)* mutants carrying only transformation marker (*glr-3*^{prom}*::mCherry*). N > 28.
(B) Body length of wild-type and *cat-2(tm2261)* mutant and transgenic animals were measured 96 hours after egg-laying. "*cat-2* + *cat-2* gene" and "*cat-2* + marker only" mean *cat-2(tm2261)* mutants carrying the transgene containing *cat-2* genomic DNA and only a transformation marker, respectively. N > 37. (A, B) Body length of transgenic animals was calculated from that of two independent lines. Body length of *cat-2(e1112)* marker-only animals was obtained from a line because another line was smaller than wild-type animals.
(C) Body length of wild-type and *cat-2(e1112)* mutant animals was measured after animals were treated with exogenous dopamine for 96 hours. N > 35. Error bars: SEM. (A-C) n.s. $p > 0.05$, ** $p < 0.01$ and *** $p < 0.001$ (ANOVA with Tukey multiple test).

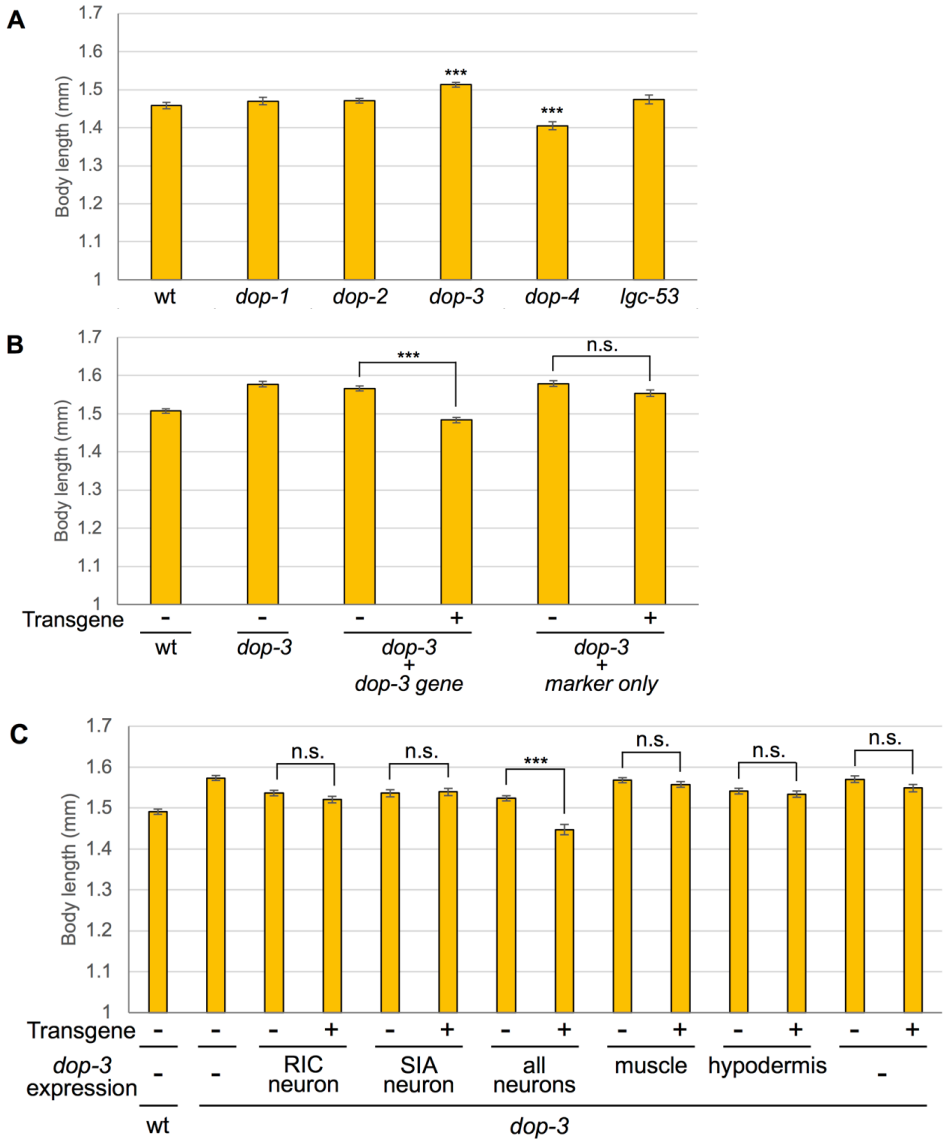

Fig. 1-4-3. Relationship between dopamine receptors and body length.
(A) Body length of dopamine receptor mutants. Body length was measured 48 hours after animals were synchronized at the L4 larval stage. N > 21. *** $p < 0.001$ compared to wt (ANOVA with Tukey multiple test).
(B) Body length of wild-type, *dop-3* mutant and transgenic animals. "*dop-3* + *dop-3* gene" and "*dop-3* + marker only" mean *dop-3(vs106)* mutants carrying the transgene containing *cat-2* genomic DNA and only a transformation marker (*glr-3*^{prom}*::mCherry*), respectively. Body length was measured 96 hours after egg-laying. N > 47.
(C) The rescue experiment by the *dop-3* tissue expression. The *dop-3* expression was driven by *tbh-1*^{prom} (RIC neuron), *ceh-17p4*^{prom} (SIA neuron), *H20*^{prom} (all neurons), *myo-3*^{prom} (muscle) and *rol-6*^{prom} (hypodermis). Body length was measured 96 hours after egg-laying. N > 29. (B, C) Body length of transgenic animals was calculated from that of two independent lines. (B, C) Error bars: SEM. n.s. $p > 0.05$ and *** $p < 0.001$ (ANOVA with Tukey multiple test).

1-4-3. Effects of dopamine on egg-laying

Previous studies suggested that dopamine negatively regulated developmental rate. *cat-2* mutants were more developed than wild-type animals at the L4 larval stage 48 hours after eggs were laid. However, the developmental stage at L4 was not different between *cat-2* mutants and wild-type animals after the developmental stage was synchronized at the L1 larval stage. There was the possibility that dopamine regulated egg-laying behavior, and *cat-2* mutants laid more developed eggs. Previously, many mutants which have reduced activity of egg-laying and laid developed eggs have been reported. There are mutants of the Egl-phenotype that means defective egg-laying. To examine whether *cat-2* mutants exhibited Egl-phenotype, the developmental stage of laid eggs was observed. The developmental stage of eggs was visually categorized based on the embryonic morphology. I found that *cat-2* mutants laid more developed eggs compared to wild-type animals (Fig. 1-4-4A). *dop-3* mutants tended to lay more developed eggs. In addition, the defects in egg-laying of *cat-2* mutants were partially suppressed by the introduction of the wild-type *cat-2* gene (Fig. 1-4-4B). These results suggested that dopamine promoted egg-laying.

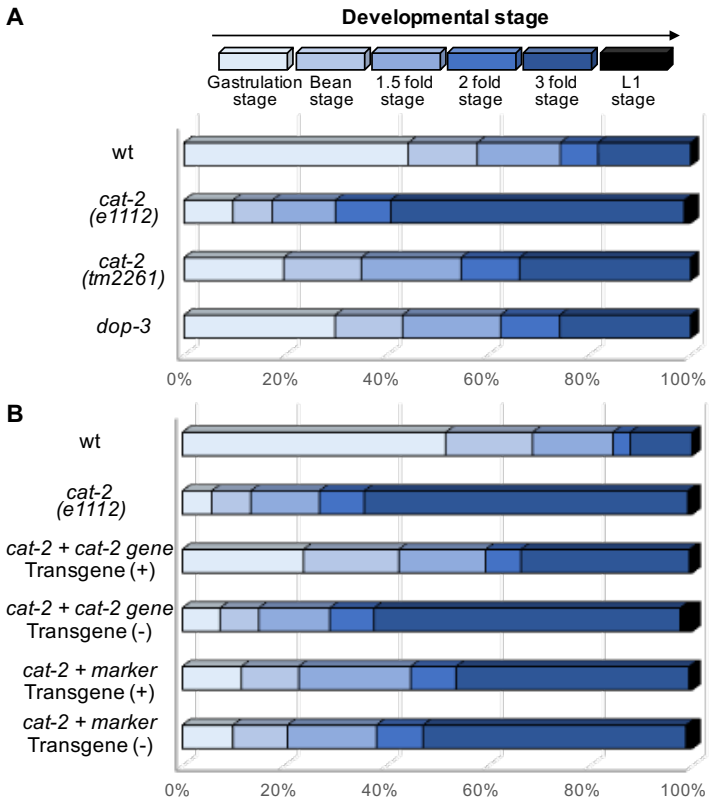

Fig. 1-4-4. Ratio of each developmental stage of laid eggs.

(A) The laid eggs of wild-type, *cat-2* mutant and *dop-3* mutant animals were observed. The developmental stage of each embryo was visually categorized based on its morphology. N > 319.
(B) The laid eggs of wild-type, *cat-2* mutant and transgenic animals were observed. "*cat-2* + *cat-2* gene" and "*cat-2* + marker only" mean *cat-2(e1112)* mutants carrying the transgene containing *cat-2* genomic DNA and only a transformation marker, respectively. N > 251.

1-4-4. Relationship between body length and egg-laying

There was the possibility that the increased body size of *cat-2* mutants was observed due to the different developmental stage between *cat-2* mutants and wild-type animals at the time of observation. When body length was measured in the experiments described above, *cat-2* mutants were more developed than wild-type animals because animals were synchronized at egg-laying (Fig. 1-4-5A). Therefore, to exclude the effect of the difference in the developmental stages, body length of the animals was measured at the same developmental stage. The L4 larval stage can be categorized in detail based on the its vulval morphology (MacNeil et al., 2013). *cat-2* mutants were longer than wild-type animals at the Mid L4 larval stage (Fig. 1-4-5B). *cat-2* mutants were also longer than wild-type animals at adult stage after animals were synchronized at L4 larval stage (Fig. 1-4-5C).

As *cat-2* mutants had defects in egg-laying, *cat-2* retain more eggs. It was possible that *cat-2* mutants were bloated with eggs, which made them longer than wild-type animals. The body length of mutants that retain more eggs was measured. *egl-1* and *egl-17* mutants have been known to exhibit strong defects in egg-laying due to defects in the serotonergic HSN neurons and egg-laying muscles, respectively (Burdine et al., 1997; Conradt and Horvitz, 1998). Although *egl-1* mutants were longer than wild-type animals, they were shorter than *cat-2* mutants (Fig. 1-4-5D). *egl-17* mutants were the same length as wild-type animals. This result suggested that retaining eggs was not sufficient for body size enlargement, and body size regulation by dopamine was not solely caused by the egg-laying phenotype.

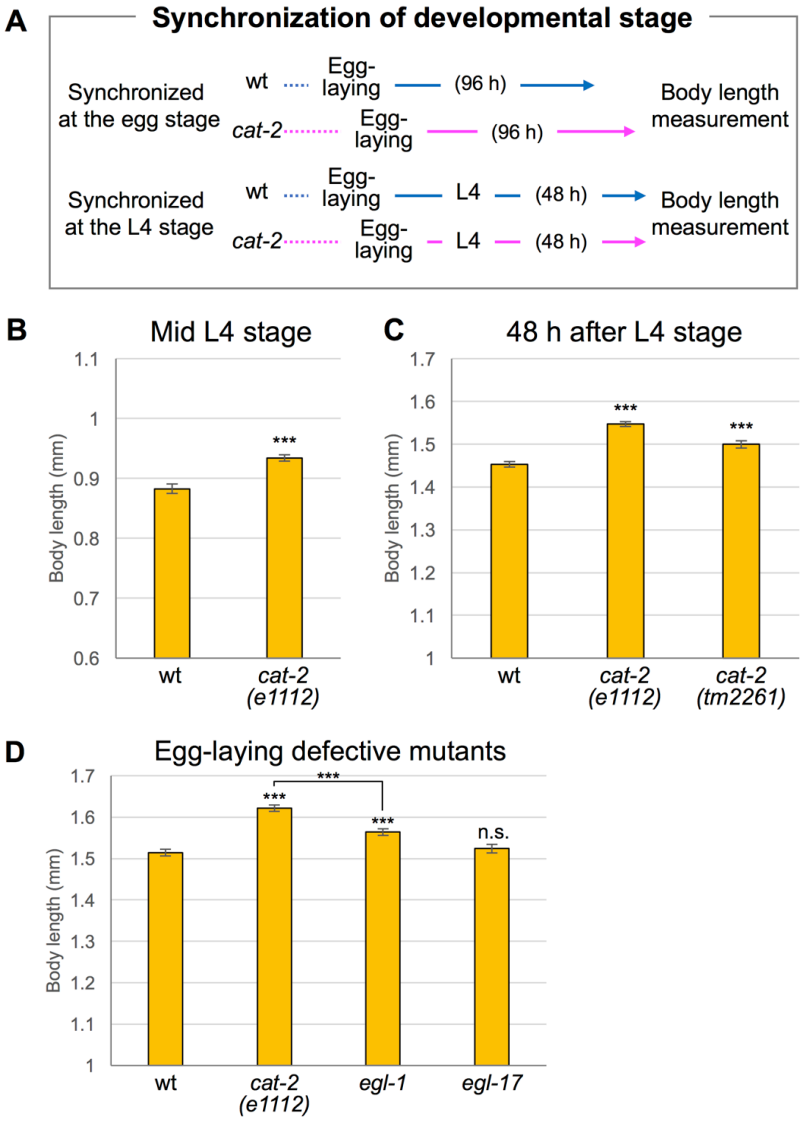

Fig. 1-4-5. Relationship between egg-laying and body length.

(A) Synchronization of developmental stage. After animals were synchronized at the egg stage, *cat-2* mutants may more developed when body length was measured. Once animals were synchronized at the L4 larval stage, there may be no difference of developmental stage between wild-type and *cat-2* mutant animals.

(B) Body length of wild-type and *cat-2* mutant animals at the Mid L4 larval stage. N > 37. Error bars: SEM. *** $p < 0.001$ (*t*-test).

(C) Body length of wild-type and *cat-2* mutant animals 48 hours after animals were synchronized at the L4 larval stage. N > 52.

(D) Body length of wild-type, *cat-2(e1112)*, *egl-1(n487)* and *egl-17(n1377)* mutant animals 48 hours after animals were synchronized at the L4 larval stage. N > 26. (C, D) Error bars: SEM. *** $p < 0.001$ (ANOVA with Tukey multiple test).

1-4-5. Downstream factors of dopamine signaling in body length regulation

The octopamine signaling has been known to function downstream of dopamine in the regulations of odor avoidance behavior and CREB-dependent transcriptions (Kimura et al., 2010; Suo et al., 2009). In this experiment, the relationship between the dopamine and octopamine in body size regulation was investigated via epistatic analyses. The *tbh-1* gene encodes a tyramine β-hydroxylase, and octopamine was lost in *tbh-1* mutants. The increased body length of *cat-2* mutants was suppressed in the background of *tbh-1* mutation at the Mid L4 stage (Fig. 1-4-6A). *ser-3* and *ser-6* encode octopamine receptors and affect the food mediated octopamine signaling of *C. elegans* (Suo et al., 2009; Yoshida et al., 2014). *cat-2;ser-3* double mutants exhibited longer body length than *ser-3* single mutants. On the other hand, *cat-2;ser-6* double mutants were as long as *ser-6* single mutants. This result suggested that octopamine signaling affected body length regulation of dopamine. When the body length of adult animals that were synchronized at the L4 larval stage was measured, the increased body length of *cat-2* mutants was also suppressed in the *tbh-1* and *ser-6* mutant background, respectively (data not shown). The octopamine signaling that regulates body length was likely to be transmitted through SER-6 receptors, rather than SER-3 receptors.

Transforming growth factor β (TGF-β) signaling play a key role in body size regulation of *C. elegans*. The TGF-β ligand, DBL-1, is a major factor of the TGF-β signaling and is thought to be secreted from neurons and bind to its receptors in hypodermal cells (Morita et al., 1999; Suzuki et al., 1999; Yoshida et al., 2001). As *dbl-1* mutants are small, *dbl-1* has been thought to positively regulate body size. In this experiment, three deferent alleles of *dbl*-1 were used. The increased body length of *cat-2* mutants was observed in the *dbl-1(tm3890)* and *dbl-1(wk70)* mutant background, respectively (Fig. 1-4-6B, C). In contrast, the increased body length was not observed in the background of *dbl-1(nk3)* mutants. I examined the developmental stage of *dbl-1(nk3)* (Fig. 1-4-6D). *dbl-1(nk3)* mutants developed more than *cat-2;dbl-1(nk3)* double mutants. The suppression by the *dbl-1(nk3)* mutation might be caused by defects in developmental rate of *cat-2;dbl-1* double mutants. These data indicated that dopamine acted in parallel with TGF-β signaling.

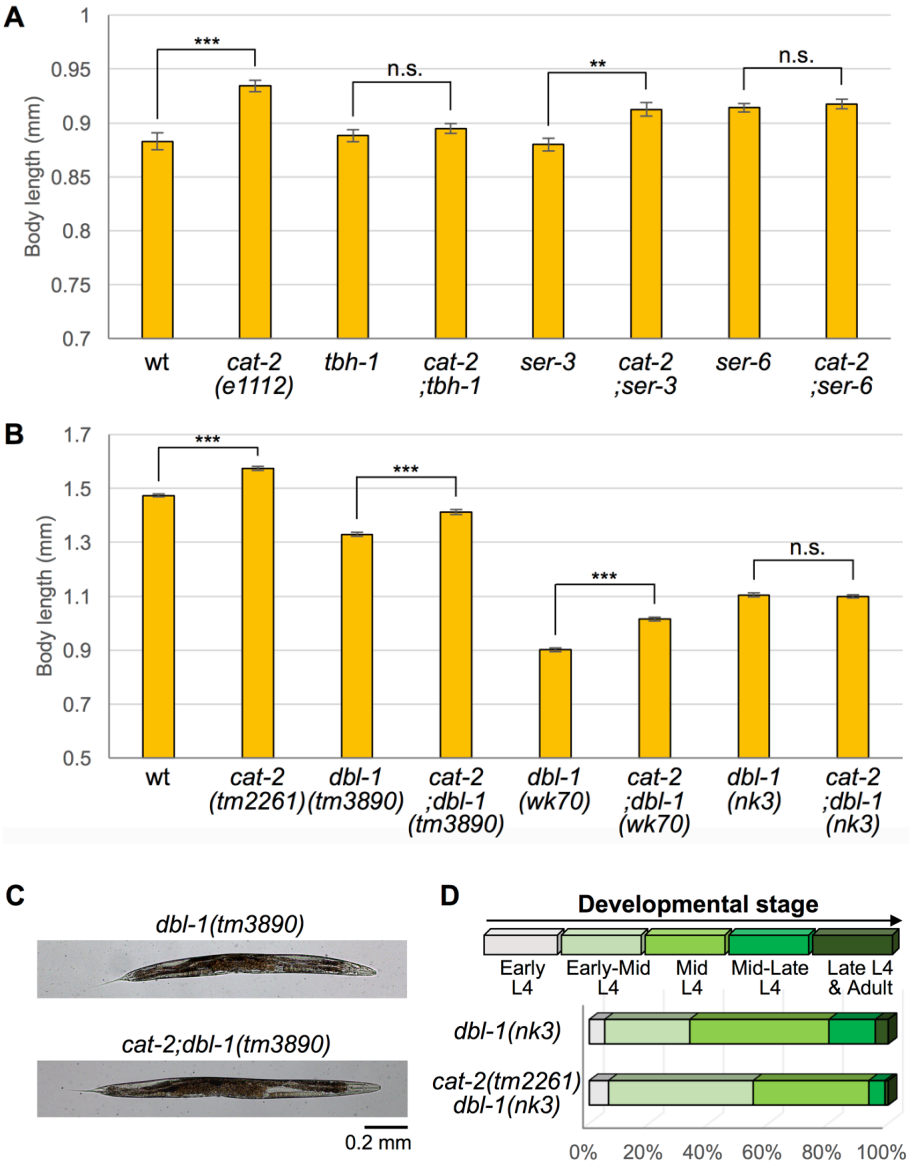

Fig. 1-4-6. The effects of octopamine signaling and TGF-β signaling on body length regulation by dopamine.

(A) Body length of octopamine-deficient mutants (*tbh-1(ok1196)*), octopamine receptor mutants (*ser-3(ad1774)* and *ser-6(tm2146)*), and the double mutants with *cat-2(e1112)* was measured at the Mid L4 larval stage. N > 23.

(B) Body length of *dbl-1* mutants, which are defective in TGF-β signaling, and the double mutants with *cat-2(tm2261)* was determined 96 hours after eggs were laid. N > 34. (A, B) Error bars: SEM. n.s $p > 0.05$, ** $p < 0.01$ and *** $p < 0.001$ (ANOVA with Tukey multiple test).

(C) Images of *dbl-1(tm3890)* and *cat-2(e1112);dbl-1(tm3890)* double mutants.

(D) The L4 larvae of *dbl-1(nk3)* and *cat-2(tm2261);dbl-1(nk3)* mutant animals were observed. The developmental stage of each larva was visually categorized based on its morphology. N > 92.

The *unc-52* gene encodes perlecan and affects myofilament organization in the body wall muscles (Rogalski et al., 1993). The *unc-54* gene encodes a muscle myosin class II heavy chain. These genes are required for normal muscle function and locomotion activity. Body length of *unc-52* and *unc-54* mutants was slightly shorter than that of wild-type animals at the Mid L4 larval stage (Fig. 1-4-7A). The body length enlargement of *cat-2* mutants was suppressed in both the *unc-52* and *unc-54* mutant backgrounds. This epistatic analysis suggested that normal muscle function was required for the body size regulation by dopamine.

Finally, I examined the relationship between dopamine and the insulin-like signaling. The insulin/insulin-like growth factor (IGF) is a well-known genetic factor that controls body size. The *daf-2* gene encodes the insulin/IGF receptor and is required for body size regulation that is modulated by kinds of food (So et al., 2011). *daf-2* mutants were longer than wild-type animals at Mid L4 (Fig. 1-4-7B). *cat-2;daf-2* double mutants were as long as *daf-2* single mutants, suggesting that the *daf-2* functioned downstream of dopamine.

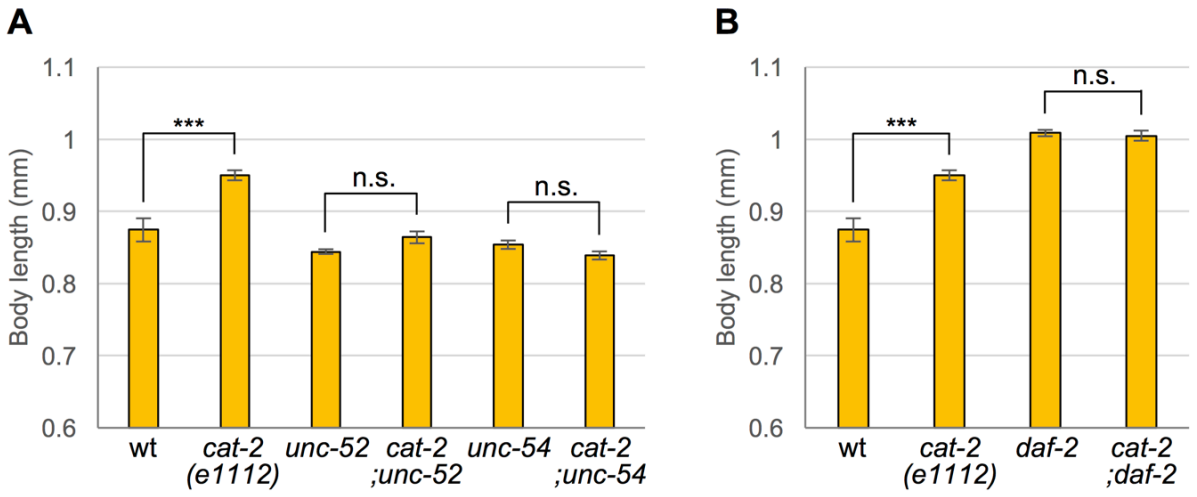

Fig. 1-4-7. Downstream factors of dopamine in body size regulation.
(A) Body length of mutants (*unc-52(e444)* and *unc-54(e190)*), which have abnormal muscle function, and the double mutants with *cat-2(e1112)* was measured at the Mid L4 larval stage. N > 9.
(B) Body length of *daf-2(e1370)* mutants, which has defects in insulin-like signaling, and the double mutants with *cat-2(e1112)* was measured at the Mid L4 larval stage. N > 11. (A, B) Error bars: SEM. n.s $p > 0.05$ and *** $p < 0.001$ (ANOVA with Tukey multiple test).

1-5. Discussion

1-5-1. Mechanisms of body size regulation by dopamine

In this study, it was shown that dopamine negatively regulated body length of *C. elegans*. Dopamine deficient mutants, *cat-2* mutants, were longer than wild-type animals. The D2-like dopamine receptor mutants, *dop-3* mutants, were longest among the dopamine receptor mutants (Fig. 1-4-3A). These results suggested that dopamine regulates body size through the D2-like dopamine receptor, DOP-3. On the other hand, the invertebrate-specific dopamine receptor mutants, *dop-4* mutants, showed shorter body length compared to wild-type animals. DOP-4 has been known to increase intracellular cAMP concentration, while DOP-3 attenuates cAMP formation (Sugiura et al., 2005). There was the possibility that DOP-4 regulated body length by reducing the effects of DOP-3. In another experiment, *dop-3;dop-4* double mutants were as long as *dop-3* mutants, but significantly longer than *dop-4* mutants. The effect of *dop-4* was suppressed by the *dop-3* mutation. This result suggests that DOP-3 plays a key role in transmitting the dopamine signal in body size regulation.

The results suggest that an amine neurotransmitter, octopamine, functioned downstream of dopamine. Octopamine deficient mutants, *tbh-1* mutants, suppressed the effects of *cat-2* mutants (Fig. 1-4-6A). The effect of *cat-2* mutants was completely suppressed in the octopamine receptor mutant, *ser-6*. In contrast to *ser-6*, another octopamine receptor mutant, *ser-3*, did not suppressed the effect of *cat-2* mutants. These results were also obtained when body length was measured 48 hours after synchronization at the L4 larval stage (data not shown). These data suggest that dopamine regulates body size through octopamine and its receptor, SER-6. Previous study showed that the octopamine signaling is negatively regulated by DOP-3 dopamine receptors expressed in the RIC neuron (Suo et al., 2009). This study supported the idea that octopamine acted downstream of dopamine. However, *dop-3* rescue experiments suggest that *dop-3* functions in neurons, except for RIC and SIA (Fig. 1-4-3C). The regulation of these amine neurotransmitters in the body size regulation may be different from that previously described. SER-6 is expressed not only in the RIC and SIA neurons but also in the ADL, ASI, AWB and the other neurons. Dopamine may negatively regulate the signal of SER-6 in these neurons.

I examined the relationship between dopamine and transforming growth factor-β (TGF-β) signaling which is thought to be a major signaling of body size regulation in *C. elegans*. DBL-1 ligands function in TGF-β signaling. Most *dbl-1* mutants did not suppress the increased body length of *cat-2* mutants, but one *dbl-1* allele, *dbl-1(nk3)*, did suppress this increase in body length (Fig. 1-4-6B). The developmental stage of *dbl-1(nk3)* mutants was different from that of *cat-2(tm2261);dbl-1(nk3)* double mutants (Fig. 1-4-6D). When body length was measured, *dbl-1* mutants were likely to be more developed than *cat-2(tm2261);dbl-1(nk3)* double mutants. The suppression by the *dbl-1(nk3)* mutation may be caused by faster development of *dbl-1(nk3)* mutants compared to *cat-2(tm2261);dbl-1(nk3)* double mutants. These results suggest that dopamine regulates body size independently of TGF-β signaling.

In mammals and flies, body size is controlled by changes of both cell size and cell number. In contrast, the body size of worms is thought to be regulated by changes of cell size because of their small number of cells and their rigid cell lineage (Morita et al., 2002). Dopamine might regulate body size via the control of cell size. Downstream factors of TGF-β signaling function in the hypodermis and are thought to change the cell size in the hypodermis. The changes of cell size of hypodermal cells may modulate body size. However, it has not yet been reported that DOP-3 dopamine receptors and SER-6 octopamine receptors are expressed in the hypodermis. Dopamine or octopamine signaling might be transmitted by other means and may change cell size of hypodermal cells.

The increased body length of *cat-2* mutants was also suppressed by the both *unc-52* and *unc-54* mutations (Fig. 1-4-7A), suggesting that body size regulation by dopamine requires normal muscle function. The *unc-52* gene encodes perlecan, which is essential for the development of muscle structure. Body wall muscle is essential for the elongation in the embryonic stage, and the strong *unc-52* mutant alleles block the elongation of epidermis (Rogalski et al., 1993). Dopamine may be involved in the developmental control of body wall muscles that extend through the epidermis and regulate body size.

1-5-2. Biological significance of body size regulation by dopamine

The body length and body width of *cat-2* mutants were larger than those of wild-type animals. Dopamine, in *C. elegans*, is known to affect food sensing and food-dependent behaviors (Hills et al., 2004; Sawin et al., 2000). It is possible that dopamine regulates body size according to ambient nutritional conditions. For example, when animals grow under low food conditions, dopamine may not be released as much, and body size may increase. The body size enlargement may allow animals to have more nutrient stores ready in case of additional starvation.

The increased body size of *cat-2* mutants was observed in hermaphrodites but not in males. The mechanisms of dopamine in body size regulation are likely to be different between hermaphrodites and males. While hermaphrodites have eight dopaminergic neurons, males have additional six neurons in the tail (Lints and Emmons, 1999; Sulston et al., 1975). *cat-2* mutant males exhibit abnormal tail morphology. The abnormal tail development may affect the body length of *cat-2* mutant males. In *Drosophila*, lower temperatures cause female animals to become larger by increasing cell size. On the other hand, males become larger by not only increased cell size, but also the increased cell number. These data suggest that the regulation of body size differs between males and females (French et al., 1998; de Moed et al., 1997; Robertson, 1959). Sexual differences of body size regulation may be observed among many species.

Is body size regulated by dopamine in mammals and humans? It was previously reported that D2 dopamine receptors affect body size through the control of growth hormone secretion in mice (Noaín et al., 2013). In humans, there is a known association between stature and a polymorphism in the promoter region of the D2 dopamine receptor gene (Arinami et al., 1999). The data demonstrated that the decreased transcriptional activity of the D2 dopamine receptor gene results in increased height. Acromegaly, a disease associated with the body size enlargement, is caused by hypersecretion of growth hormone. Acromegaly patients are treated with dopamine agonists, Cabergoline, which suppress GH secretion (Abs et al., 1998). These data imply that body size regulation by dopamine may be conserved among species.

1-5-3. Biological significance of egg-laying regulation by dopamine

cat-2 mutants laid more developed eggs (Egl phenotype), suggesting that dopamine promotes egg-laying. It has been reported that exogenous dopamine inhibits egg-laying (Schafer and Kenyon, 1995), and that this effect is modulated by the serotonin-gated chloride receptor MOD-1 (Dempsey et al., 2005). On the other hand, exogenous dopamine causes an increase in egg-laying when animals are immersed in water (Vidal-Gadea et al., 2012). It has not been known whether endogenous dopamine regulates egg-laying. I found that *cat-2* mutants exhibited an Egl phenotype (Fig. 1-4-4), indicating that endogenous dopamine promotes egg-laying. Moreover, exogenous octopamine suppresses egg-laying induced by serotonin or food. Therefore, one possibility is that endogenous dopamine promotes egg-laying by suppressing octopamine signaling.

What is the purpose of dopamine's modulation of egg-laying? Dopamine affects the sensing of food, and food-dependent behavior in *C. elegans* (Hills et al., 2004; Sawin et al., 2000). For example, when animals exist under the low food conditions, dopamine may not be released as much, and egg-laying is inhibited. Conversely, when animals exist in the well-fed conditions, dopamine may be released and promote egg-laying. Dopamine may allow progeny to hutch under well-fed conditions to help avoid starvation.

1-5-4. Insulin-like signaling, which acts downstream of dopamine

Insulin/IGF-1 signaling is involved in many metabolic and neural regulations of animals, including development and longevity (Dyer et al., 2016; Nässel et al., 2015). Insulin/IGF signaling works downstream of the growth hormone (GH) to regulate body size in mammals (Blutke et al., 2014; Lundberg et al., 2015). In invertebrates that do not possess GH, insulin/IGF signaling affects body size regulation in a cell-autonomous manner (Böhni et al., 1999; Brogiolo et al., 2001). In this study, insulin-like receptor mutants (*daf-2* mutants) were longer than wild-type animals (Fig. 1-4-7B), suggesting that *daf-2* negatively regulates body size. The increased body length of *cat-2* mutants was suppressed in the *daf*-2 mutant background, indicating that insulin-like signaling acts downstream of dopamine. DAF-2 receptors are expressed not only in the nervous system but also weakly in the hypodermis (Kimura et al., 2011). Insulin/IGF signaling may function in the hypodermis in a cell-autonomous manner and regulate body size by changing the size of cells in the hypodermis.

Insulin-like signaling has been known to affect the food-dependent body size regulation (So et al., 2011). Animals fed on an *E. coli* HB101 diet become 1.6 times as large as those fed on a standard *E. coli* OP50 diet. Part of this regulation is done by DAF-2 receptors. The effect of *daf-2* mutation is suppressed by *daf-16* mutation, indicating that DAF-16, a forkhead transcription factor, acts downstream of this DAF-2 signaling. In addition to regulation of body size, the effect of *daf-2* mutation is, in general, suppressed by *daf-16* mutation in regulation of development and longevity. However, this type of suppression by *daf-16* is not observed in salt chemotaxis learning for unknown reasons (Tomioka et al., 2006). Hence, the regulatory function of *daf-16* in learning behaviors was investigated in the next chapter.

Chapter 2:

Analysis of the DAF-16/FOXO transcription factor in taste avoidance learning

5 年以内に雑誌等で刊行予定のため非公開

Conclusion

To adapt to ambient environment and survive in the natural world, animal behavior is affected by environmental stimuli. In this study, I investigated regulatory function of the nervous system on appropriate behavioral responses according to nutritional conditions.

In the chapter 1, I examined the relationship between animal body size and dopamine, which is one of the monoamine neurotransmitters and is released when food is sensed. Dopamine has been known to affect the emotional and reward system in mammals (Baik, 2013). However, little has been known about the molecular mechanism of dopamine in animal development. This study showed that dopamine negatively regulated body size through insulin-like signaling and the octopamine signaling. In humans, a disease associated with body size called acromegaly has been treated with Cabergoline, a dopamine agonist (Abs et al., 1998). There was a correlation between stature and the polymorphism in the promoter region of the D2 dopamine receptor gene (Arinami et al., 1999). Therefore, body size regulation by dopamine may be conserved across species. In addition to body size, the results also demonstrated the relationship between egg-laying and dopamine. It was suggested that dopamine positively regulated egg-laying. As dopamine is thought to be released by sensing food, regulation by dopamine promotes egg-laying under well-fed conditions and may act beneficially on leaving progeny.

Chapter 2 については、5 年以内に雑誌等で刊行予定のため非公開

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