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Involvement of genes required for synaptic function in aging control in *C. elegans*

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Abstract: Objective To identify new genes required for neurosecretory control of aging in *C. elegans*. **Methods** In view of the importance of nervous system in aging regulation, we performed the screen for genes involved in the aging regulation from genetic loci encoding synaptic proteins by lifespan assay and accumulation of lipofuscin autofluorescence. We further investigated the dauer formation phenotypes of their corresponding mutants and whether they were possibly up-regulated by the insulin-like signaling pathway. **Results** The genetic loci of *unc-10*, *syd-2*, *hlb-1*, *dlk-1*, *mkk-4*, *scd-2*, *snb-1*, *ric-4, nrx-1, unc-13*, *sbt-1* and *unc-64* might be involved in the aging control. In addition, functions of *unc-10*, *syd-2*, *hlb-1*, *dlk-1*, *mkk-4*, *scd-2*, *snb-1*, *ric-4* and *nrx-1* in regulating aging may be opposite to those of *unc-13*, *sbt-1* and *unc-64*. The intestinal autofluorescence assay further indicated that the identified long-lived and short-lived mutants were actually due to the suppressed or accelerated aging. Among the identified genes, *syd-2*, *hlb-1*, *mkk-4*, *scd-2*, *snb-1*, *ric-4* and *unc-64* were also involved in the control of dauer formation. Moreover, *daf-2* mutation positively regulated the expression of *syd-2* and *hlb-1*, and negatively regulated the expression of *mkk-4*, *nrx-1*, *ric-4*, *sbt-1*, *rpm-1*, *unc-10, dlk-1* and *unc-13*. The *daf-16* mutation positively regulated the expression of *syd-2* and *hlb-1*, and negatively regulated the expression of *mkk-4*, *nrx-1*, *sbt-1*, *rpm-1*, *unc-10, dlk-1* and *unc-13*. **Conclusion** These data suggest the possibly important status of the synaptic transmission to the animal's life-span control machinery, as well as the dauer formation control.

Keywords: aging; neurotransmission; synapse; dauer formation; insulin pathway; *C. elegans*

1 Introduction

The nematode *Caenorhabditis elegans* is transparent, easy to be cultured in laboratory, prone to genetic manipulation, and living for only a few weeks. Especially, old animals become more flaccid and move more slowly with age, whereas long-lived insulin/insulinlike growth factor-1 (IGF-1) signaling mutants remain active much longer than normal[1], which made worms become a perfect animal model for the study of aging regulation.

Many processes have ever been proposed to influence the lifespan, such as a failure to replicate telomeres, to combat infectious agents effectively, or to withstand oxidative damage. In *C. elegans*, the lifespan is regulated hormonally by an insulin/IGF-like signaling pathway. Wild type animals live just a few weeks; however, reduction-of-function mutations affecting the insulin/IGF-1-like receptor DAF-2 (*da*uer *f*ormation), or components of a downstream phosphatidylinositol 3-kinase/ phosphatidylinositol (3,4, 5) P3-dependent kinase/protein kinase B (PI 3-kinase/PDK/PKB) pathway, can double the worm's lifespan[2,3]. This lifespan extension requires DAF-16, a fork-head-family transcription factor[4]. In DAF-2 pathway mutants, DAF-16 accumulates in the nuclei of many cell types, where it results in changes in the expression of a wide variety of response, and thereby extends lifespan[5]. Under normal conditions, DAF-2 activates a conserved PI 3-kinase/PDK/PKB signaling cascade that phosphorylates DAF-16, thereby inhibiting its nuclear localization[5]. When the insulin/IGF-1 signaling is prevented, DAF-16 accumulates in the nucleus

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and regulates downstream genes that extend lifespan. Thus, loss-of-function mutations in *daf-16* suppress the lifespan extension of *daf-2* and *age-1*(an *a*ltered ag*e* rate) mutants[6]. In addition, SIR-2.1 (yeast *SIR* related) protein may be able to couple longevity to nutrient availability in *C. elegans*. Increased dosage of *sir-2.1* can significantly extend the life span and the *sir-2.1* transgene functions upstream of *daf-16* in the insulin-like signaling pathway[7].

Besides the genetic or signaling pathways involved in the aging control, how signaling between tissues coordinates the physiology of an animal is another fundamental problem in endocrinology. Several tissues were found to act as signaling centers, and they include neurons and intestine[8]. Especially, DAF-16 activity in the intestine completely restores the longevity of *daf-16*(-) germline-deficient animals, and increases the lifespan of *daf-16*(-) insulin/IGF-1 pathway mutants substantially[8]. The neuronal *daf-16* activity is also sufficient to extend lifespan[8]. In addition, the *unc-64* (*unc*oordinated) and *unc-31* genes also function in the insulin receptor signaling pathway and these two genes are involved in mediating Ca^{2+}-regulated neurotransmission and secretion, suggesting an important neurosecretory signal participates in the regulation of lifespan and dauer formation[9]. Moreover, Berman and Kenyon[10] recently further found that germ-cell loss extends the worm lifespan through the regulation of DAF-16 by *kri-1* (*Kr*ev *i*nteraction trapped/cerebral cavernous malformation 1) and lipophilic-hormone signaling[10].

To understand how the nervous system affects aging, we conducted a screen for new genes required for the synaptic structure and function to lengthen or shorten lifespan. We identified several genes, whose mutations caused the significant changes of lifespan in *C. elegans*. The involvement of these genetic loci in aging control was further confirmed by the assay for accumulation of lipofuscin autofluorescence. Furthermore, we examined the roles of identified genes in dauer formation and whether they were up-regulated by the insulin signaling pathways. These data suggest and reflect the possibly important status of the synaptic transmission to the animal's lifespan control machinery.

2 Materials and methods

2.1 Genetic loci Total 16 genetic loci were selected for this work. All these genes encode synaptic proteins and are required for the synaptic functions. They are *unc-10*[11], *unc-13*[12], *syd-2* (*sy*napse *d*efective)[13], *hlb-1* (*h*omologue of *l*iprin *b*eta) (Wang *et al*., personal communication), *dlk-1* [*DAP* (Death Associated Protein kinase) *l*ike *k*inase][14], *mkk-4* [*MKK* (MAP Kinase Kinase) homolog][14], *scd-2* (*s*uppressor of *c*onstitutive *d*auer formation)[15], *rpm-1* (*r*egulator of *p*resynaptic *m*orphology)[15], *fsn-1* (*F*-box *syn*aptic protein)[15], *snb-1* (*syn*apto*b*revin)[16], *snt-1* (*syn*ap*t*otagmin)[17], *unc-64*[18], *unc-18*[19], *ric-4* (*r*esistance to *i*nhibitors of *c*holinesterase)[20], *nrx-1* (*neurex*in-like)[21] and *sbt-1* [*s*even *B t*wo (mammalian 7BT prohormone convertase chaperone) homolog][22], respectively, as shown in Tab. 1. Wild-type N2 and *unc-64* mutants were used as controls. All the mutants used in the current work were provided by the *Caenorhabditis* Genetics Center (CGC) at the University of Minnesota.

2.2 Culture conditions *C. elegans* culture and manipulation were performed using standard methods[23] and all strains were maintained at 20 ºC.

Tab. 1 Mutant strains screened for aging control

Mutants tested	Products of the gene/phenotype relevant to synaptic function
unc-10	Rab3 effector RIM1
unc-13	Neurotransmitter release regulator
syd-2	LAR-interacting protein
hlb-1	LAR transmembrane tyrosine phosphatase-interacting protein liprin
dlk-1	Serine/threonine protein kinase
mkk-4	Mitogen-activated protein kinase (MAPK) kinase MKK4
scd-2	Protein tyrosine kinase
rpm-1	Inhibitor of type V adenylyl cyclases
fsn-1	SOCS box protein SSB-1
snb-1	Synaptobrevin/VAMP-like protein
ric-4	SNAP-25 (synaptosome-associated protein) component of SNARE complex
unc-64	SNARE protein Syntaxin 1
unc-18	Vesicle trafficking protein Sec1
snt-1	Ca^{2+}-dependent phospholipid-binding protein Synaptotagmin
nrx-1	Neurexin III-alpha
sbt-1	Neuroendocrine protein 7B2

2.3 Lifespan assay The lifespan assays were performed basically as described at 20 ºC and were initiated at the fourth larval (L4) stage[7]. In this test, the medium contains

400 μg/mL 5'fluoro–2'deoxyuridine (FUDR), which can inhibit DNA synthesis and causes animals to lay eggs that cannot hatch and then eliminates the disturbance of the offspring. The worms were checked every 2–4 d and would be scored as dead when they did not move even after repeated taps with a pick.

2.4 Photography of autofluorescence The method was performed as described[24]. The images were collected for endogenous gut fluorescence using a 525-nm bandpass filter and without automatic gain control in order to preserve the relative intensity of different animal's fluorescence. Day 4, day 8 and day 12 adults were photographed on the same day to avoid effects of light source variance on fluorescence intensity.

2.5 Dauer formation assay The starvation assay method was basically performed as described[25]. About 4 pregnant worms per plate were allowed to lay eggs on nematode growth medium (NGM) plates for 4–6 h at 20 ºC. These progeny were shifted to 27 ºC, and 72 h later, the number of dauer.or adult animals was assayed. While the food was almost exhausted, the plates were flooded with 1% sodium dodecyl sulfate (SDS) solution to select for dauers (Dauers are resistant to SDS).

2.6 Semi-quantitative RT-PCR assay Total RNA was isolated using guanidinium thiocyanate/phenol method as described[26]. The frozen worm pellets were harvested from 1 L of mixed stage liquid cultures. Purified Poly $(A)^+$ RNA through two rounds of selection on oligo (dT)-cellulose was used for the RNA blotting. The PCR condition was 94 ºC for 45 s, 60 ºC for 45 s, and 72 ºC for 90 s, 30 cycles. *act-1* was used as control to determine the loading amount for each sample.

2.7 Statistical analysis All data in this article were expressed as mean±SD and analyzed by SPSS 13.0 software. Paired-sample *t* test were performed between the control and mutant animals. $P<0.05$ was considered statistically significant.

3 Results

3.1 Screen for new genetic loci involved in aging control from genes required for synaptic function In nematode *C. elegans*, *unc-64* and *unc-31* regulate the lifespan and dauer formation through the insulin receptor pathway[9], suggesting that the function of neurotransmission is required for worms to age normally. To identify new genes involved in aging control and determine the possible roles of synapse in senescence, we investigated the lifespans of 16 mutants (corresponding to 16 genes) with various defects in synaptic structure and function[27] (Tab. 1). As shown in Tab. 2, we found that the mutation of *unc-10*, *syd-2*, *hlb-1*, *dlk-1*, *mkk-4*, *scd-2*, *snb-1*, *ric-4* and *nrx-1* significantly reduced the worm lifespan. Compared with the wild-type having a mean lifespan of (14.8±0.8) d, the *unc-10 (md1117)*, *syd-2 (ju37)*, *hlb-1 (ok725)*, *dlk-1 (ju476)*, *mkk-4 (ju91)*, *scd-2 (ok565)*, *snb-1 (md247)*, *ric-4 (md1088)* and *nrx-1(ds1)* mutants had a mean lifespan of 13.3±0.5 ($P<0.01$), 10.8±0.6 ($P<0.01$), 11.9±0.9 ($P<0.05$), 10.9±0.6 ($P<0.01$), 13.3±0.7 ($P<0.05$), 10.9±0.5 ($P<0.01$), 8.8±0.5 ($P<0.01$), 11.9±0.9 ($P<0.05$), 10.6±0.6 ($P<0.01$), respectively. However, mutations of *unc-13*, *sbt-1* and *unc-64* remarkably lengthened the animals' lifespan. The *unc-13 (e323)*, *sbt-1 (ok901)* and *unc-64 (md130)* mutants had a mean lifespan of 19.6±2.0 ($P<0.01$), 20.6±1.0 ($P<0.01$) and 20.6±1.5 ($P<0.01$), respectively. Therefore, the genetic loci of *unc-10*, *syd-2*, *hlb-1*, *dlk-1*, *mkk-4*, *scd-2*, *snb-1*, *ric-4, nrx-1, unc-13*, *sbt-1* and *unc-64* might be involved in the aging control in *C. elegans*. In addition, functions of *unc-10*, *syd-2*, *hlb-1*, *dlk-1*, *mkk-4*, *scd-2*, *snb-1*, *ric-4* and *nrx-1* in regulating worm aging may be opposite to those of *unc-13*, *sbt-1* and *unc-64*.

3.2 The identified long-lived and short-lived mutants are due to changed aging Intestinal autofluorescence, caused by lysosomal deposits of lipofuscin, accumulates over time in the aging animal and is a valuable marker for aging[24]. To examine whether the long-lived and short-lived mutants identified in the current work are due to accelerated or suppressed aging, or to an unrelated, pleiotropic cause, we monitored the accumulation of intestinal autofluorescence in adult animals (Fig. 1). In agreement with the short lifespan phenotypes, mutants of *unc-10 (md1117)*, *syd-2 (ju37)*, *hlb-1 (ok725)*, *dlk-1 (ju476)*, *mkk-4 (ju91)*, *scd-2 (ok565)*, *snb-1 (md247)*, *ric-4 (md1088)* and *nrx-1 (ds1)* accumulated intestinal autofluorescence more rapidly than wild type (especially at day 12, $P<0.01$ or $P<0.05$). These results were resembled those found for short-lived strains with *lin-4 (lf)* and *daf-16 (lf)* mutations[24]. Early at day 4, the mutants of *syd-2 (ju37)*, *snb-1 (md247)* and *nrx-1 (ds1)* displayed remarkably increased lipoduscin-like intestinal autofluorescence. In contrast to this, the mutants of *unc-13 (e323)*, *sbt-1 (ok901)* and *unc-64 (md130)* displayed significantly decreased lipoduscin-like intestinal fluorescence at relatively old ages compared with wild type (especially at day 12, $P<0.01$). In *unc-18 (md299)*, *fsn-1*

Tab. 2 Mean life span of various mutants

Genotype	Mean lifespan (d)	Ratio *vs* wt[a]	Maximum lifespan (d)[b]	N	*P* value *vs* wt[c]
wild type	14.8±0.8		26, 32	24	
unc-10(md1117)	13.3±0.5	0.89	17, 15	32	<0.01
unc-13(e323)	19.6±2.0	1.32	30, 32	31	<0.01
syd-2(ju37)	10.8±0.6	0.73	16, 15	25	<0.01
hlb-1(ok725)	11.9±0.9	0.80	22, 24	19	<0.05
dlk-1(ju476)	10.9±0.6	0.74	18, 20	19	<0.01
mkk-4(ju91)	13.3±0.7	0.90	20, 24	21	<0.05
scd-2(ok565)	10.9±0.5	0.74	16, 17	25	<0.01
rpm-1(ju41)	14.1±1.1	0.95	24, 26	26	NS
fsn-1(gk429)	14.4±0.9	0.97	22, 24	30	NS
snb-1(md247)	8.8±0.5	0.59	12, 14	27	<0.01
ric-4(md1088)	11.9±0.9	0.80	24, 24	26	<0.05
unc-64(md130)	20.6±1.5	1.39	36, 38	24	<0.01
unc-18(md299)	16.4±0.6	1.11	20, 24	19	NS
snt-1(md290)	15.5±0.6	1.05	24, 26	26	NS
nrx-1(ds1)	10.6±0.6	0.72	16, 16	28	<0.01
sbt-1(ok901)	20.6±1.0	1.39	30, 34	27	<0.01

Results of one typical experiment are shown. [a]The ratio of mean life span divided by wild type. [b]Maximum life span in duplicated plates. [c]Probability of survival being different from wild type. NS, no significant differences. wt, wild type.

(gk429), *snt-1 (md290)* and *rpm-1 (ju41)* mutants, we did not find obvious differences of intestinal autofluorescence accumulation from that of wild type. These results were consistent with the life span analysis above, suggesting that the identified long-lived and short-lived mutants were actually due to the suppressed or accelerated aging.

3.3 Effects of identified mutants on dauer formation The results above indicate that the identified genes are required for aging control. Again, we tested the effects of identified mutants on dauer formation using starvation assay. The dauer larva is an alternative third larval (L3) form that is usually induced by lack of food, high temperature, and high concentration of dauer pheromone[25]. The dauer is long-lived reproductively immature, and resistant to desiccation and starvation. The lifespan extension and regulation refquire the dauer pathways[8]. In the starvation assay, dauer formation is induced by allowing plates of worms to starve naturally, which causes a very strong dauer induction[25]. Dauer formation assays are often taken at 25 ºC, a high growth temperature favoring the dauer state. In view of most of the screened mutants with shortened lifespan and wild-type N2 without dauer at 25 ºC, we performed the dauer formation assays at a higher temperature (27 ºC), which is a more severe dauer-inducing temperature[8]. As shown in Tab.3, mutants of *syd-2 (ju37)*, *snb-1 (md247)*, *snt-1 (md290)* and *scd-2 (ok565)* formed very few dauers and the dauers formed by *syd-2 (ju37)*, *snb-1 (md247)*, *snt-1 (md290)* and *scd-2 (ok565)* were 3.4±0.6, 3.3±1.1, 3.3±1.3 and 0, respectively, under this condition. Mutants of *hlb-1 (ok725)*, *mkk-4(ju91)*, *rpm-1(ju41)*, *ric-4 (md1088)* and *unc-64 (md130)* formed much more dauers than wild type. The dauers formed by *hlb-1 (ok725)*, *mkk-4 (ju91)*, *rpm-1 (ju41)*, *ric-4 (md1088)* and *unc-64 (md130)* mutants were 14.0±4.1, 14.9±5.2, 22.2±3.9, 16.3±3.3 and 19.8±3.7, respectively. In contrast, dauer formation by the *unc-10 (md1117)*, *unc-13 (e323)*, *dlk-1 (ju476)*, *fsn-1 (gk429)*, *unc-18 (md299)*, *nrx-1 (ds1)* and *sbt-1 (ok901)* mutants was indistinguishable from the wild type. Therefore, among the identified genes, *syd-2*, *hlb-1*, *mkk-4*, *scd-2*, *snb-1*, *ric-4* and *unc-64* were also involved in the control of dauer formation.

3.4 Effects of dauer pathway on expression patterns of identified genes In response to dauer pheromone, the Daf-d (*da*uer *f*ormation *d*efective) genes negatively regulate *daf-2*, which, in turn, is a negative regulator of *daf-16*[28]. Since the insulin/IGF pathway is also a central pathway in aging

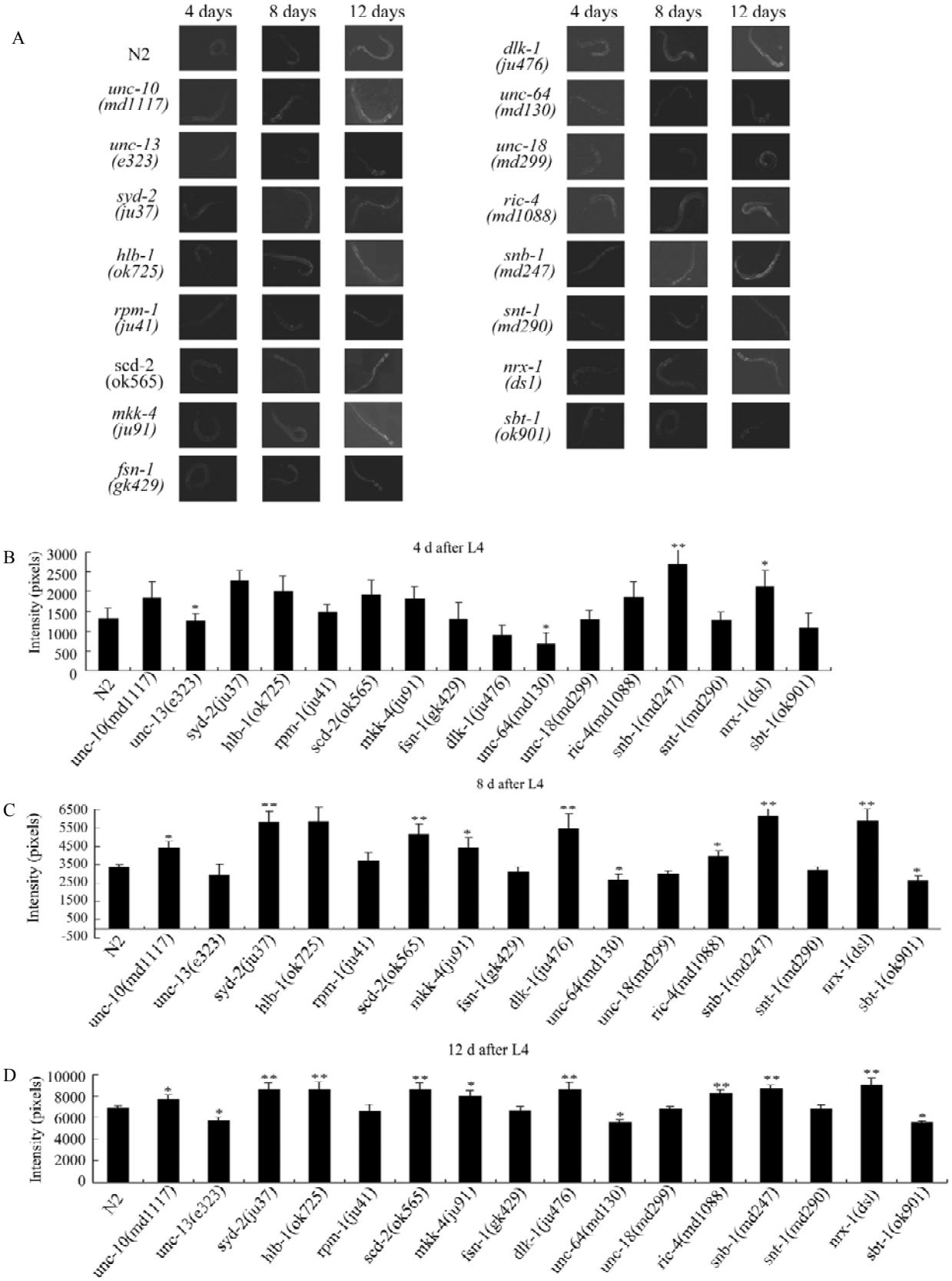

Fig. 1 Mutations of genes required for synaptic function display accelerated and delayed rates, respectively, of lipofuscin accumulation. A: The fluorescent pictures show the lipofuscin accumulation in intestines of N2 and the sixteen mutants cultured in 20 ºC at 4, 8, 12 d after L4 larva. B: Quantification of the N2 and mutants' gut autofluorescence at day 4 after the larval-to-adult transition at 20 ºC. C: Quantification of the N2 and mutants' gut autofluorescence at day 8 after the larval-to-adult transition at 20 ºC. D: Quantification of the N2 and mutants' gut autofluorescence at day 12 after the larval-to-adult transition at 20 ºC. * $P < 0.05$, ** $P < 0.01$.

Tab. 3 Percentage dauer formation by various mutants at 27 ºC

Genotype	dauer formation at 27 ºC (%)	Ratio *vs* wt[a]	*P value vs* wt[b]
wild type	8.7±4.1		
unc-10(md1117)	3.5±2.1	0.40	NS
unc-13(e323)	8.4±2.3	0.97	NS
syd-2(ju37)	3.4±0.6	0.39	<0.05
hlb-1(ok725)	14.0±4.1	1.61	<0.05
dlk-1(ju476)	6.0±4.4	0.68	NS
mkk-4(ju91)	14.9±5.2	1.71	<0.05
scd-2(ok565)	0	0	<0.01
rpm-1(ju41)	22.2±3.9	2.55	<0.01
fsn-1(gk429)	9.4±0.8	1.08	NS
snb-1(md247)	3.3±1.1	0.38	<0.05
ric-4(md1088)	16.3±3.3	1.87	<0.05
unc-64(md130)	19.8±3.7	2.28	<0.01
unc-18(md299)	8.0±3.6	0.92	NS
snt-1(md290)	3.3±1.3	0.38	<0.05
nrx-1(ds1)	6.2±2.3	0.71	NS
sbt-1(ok901)	6.1±3.5	0.70	NS

[a]The ratio of dauer formation divided by wild type. [b]Probability of dauer formation being different from wild type. NS, no significant differences. wt, wild-type.

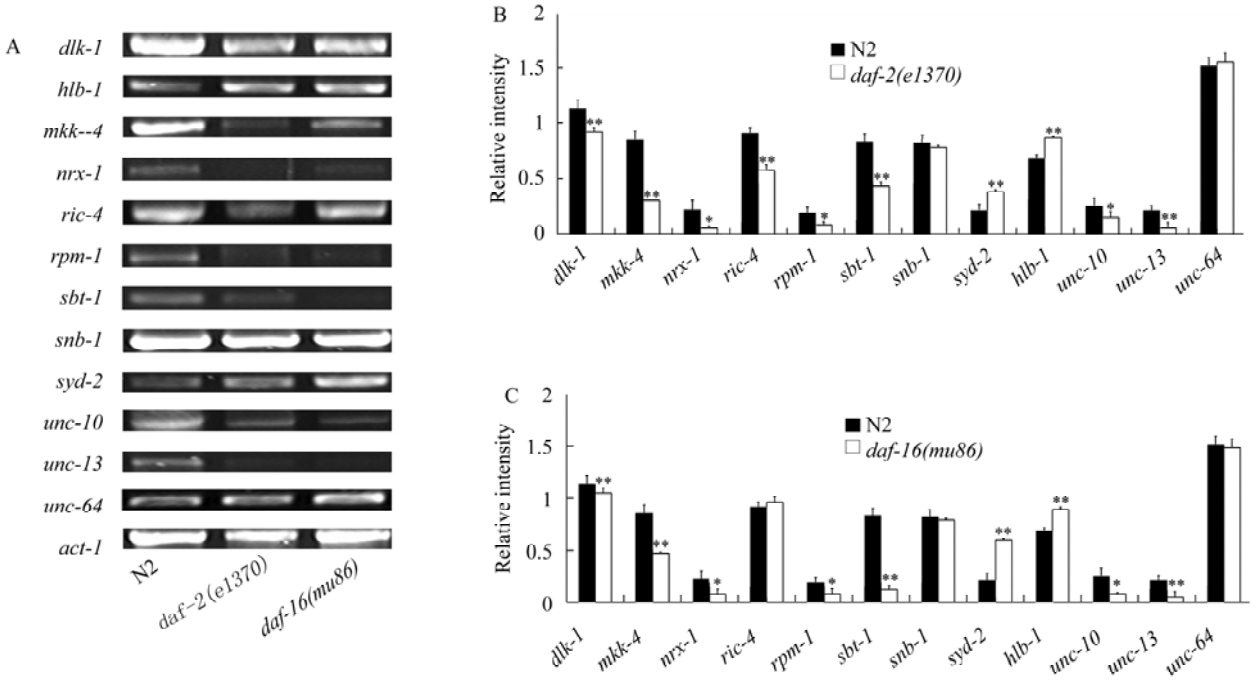

Fig. 2 Identification of possible downstream targets for *daf-2* and *daf-16* mutations. A: Semi-quantitative RT-PCR assay of expression patterns of genes required for synaptic function in wild-type and *daf-2* and *daf-16* mutants. B: Comparison of expression levels of genes required for synaptic function in wild-type and *daf-2* mutants. C: Comparison of expression levels of genes required for synaptic function in wild-type and *daf-16* mutants. * $P < 0.05$, ** $P < 0.01$.

regulation, we wanted to explore the possible effects of this pathway on the expression patterns of screened genes. As shown in Fig.2, six groups of expression patterns were observed under the genetic background of *daf-2 (e1370),*

daf-16 (mu86) mutants and wild type animals. The expression of *syd-2* and *hlb-1* belongs to the first group and their expression levels were all enhanced to an almost equal degree in *daf-2 (e1370)* and *daf-16 (mu86)* mutants, compared with those in wild type. The second group contained four genetic loci and they were *nrx-1*, *rpm-1*, *unc-10*, *dlk-1* and *unc-13*, respectively. Their expression levels were all decreased to an almost equal degree in *daf-2 (e1370)* and *daf-16 (mu86)* mutants, compared with those in wild type. Especially, we noticed that the expression of *nrx-1*, *rpm-1* and *unc-13* were almost undetectable in *daf-2 (e1370)* and *daf-16 (mu86)* mutants. In the third group, although the expression of *mkk-4* was suppressed in both *daf-2 (e1370)* and *daf-16 (mu86)* mutants, its expression was suppressed more severe in *daf-2 (e1370)* mutants than in *daf-16 (mu86)* mutants. In contrast to this, in the fourth group, the expression of gene *sbt-1* was decreased more severe in *daf-16 (mu86)* mutants than that in *daf-2 (e1370)* mutants. In the fifth group, the expression of *ric-4* was only affected under the background of *daf-2 (e1370)* mutants. The mutation of *daf-2* caused a significant decrease of *ric-4* expression. The expression of *unc-64* and *snb-1* belongs to the sixth group and their expression was not affected in *daf-2 (e1370)* and *daf-16 (mu86)* mutants, compared with those in wild type. Therefore, *daf-2* mutation positively regulated the expression of *syd-2* and *hlb-1*, and negatively regulated the expression of *mkk-4*, *nrx-1*, *ric-4*, *sbt-1*, *rpm-1*, *unc-10, dlk-1* and *unc-13*. The *daf-16* mutation positively regulated the expression of *syd-2* and *hlb-1*, and negatively regulated the expression of *mkk-4*, *nrx-1*, *sbt-1*, *rpm-1*, *unc-10, dlk-1* and *unc-13*.

4 Discussion

Many genes affect lifespan by executing their effects in a limited number of cell types, especially in neurons[8]. Nervous system functions as one of central regulators to regulate the life span at several steps[9]. Previous work from Ailion *et al*. has already suggested such a possibility that genes required for synaptic function might regulate classical neurotransmission to the cells that release insulin[9].

One objective of this study was to leaning which genes required for synaptic function may participate in the regulation of aging in *C. elegans*. Because at synaptic junctions, chemical synapses provide information flow between pre- and post-synaptic cells, and active zones function in vesicle docking and neurotransmission release[29], we screened the genes involved in the aging control from 16 genetic loci, encoding synaptic proteins, in this report. According to the analysis in the current work, mutations of *unc-10*, *syd-2*, *hlb-1*, *dlk-1*, *mkk-4*, *scd-2*, *snb-1*, *ric-4* and *nrx-1* negatively regulated lifespan, whereas mutations of *unc-13*, *unc-64* and *sbt-1* positively regulated the worm aging. Interestingly, although *syd-2*, *unc-10* and *unc-13* all encode proteins localized at the active zones, functions of *syd-2* and *unc-10* in regulating lifespan were different from that of *unc-13*. In *syd-2* mutants, presynaptic densities are altered and appear less electron-dense than normal, indicating that SYD-2 is one important organizer of presynaptic density[13]. UNC-10 localizes to the subdomain of the presynaptic terminal and regulates the vesicle docking in neurotransmission[11]. The presynaptic protein UNC-13 has emerged as a key priming factor[12]. Priming refers to the molecular events following vesicle docking that lead to vesicle fusion competence, suggesting that different aspects and/or different stages of neurotransmission regulation may exert different effects on the aging control in *C. elegans*. Roles of *snb-1*, *unc-64* and *ric-4* further support this notion. It is originally proposed that the soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptors (SNARE) proteins synaptobrevin, syntaxin and SANP-25 constitute the vesicle docking complex[30]. In *C. elegans*, the vesicle associated SNARE synaptobrevin is encoded by *snb-1*, and the plasma membrane associated SNAREs syntaxin and SNAP-25 are encoded by *unc-64* and *ric-4*, respectively[16,18,20]. The regulation of *snb-1*, *unc-64* and *ric-4* on lifespan suggest that different components of the vesicle docking complex could still have opposite effects in aging control, which might at least partially due to their different roles in vesicle docking regulation. *sbt-1* is also a neuroendocrine protein, which is involved in synaptic secretion as a proprotein convertase (PC) 2 chaperone[22]. The analysis above suggests that the vesicle docking event may mainly function as a positive regulator of lifespan, whereas the vesicle priming event may mainly serve as a negative regulator for aging control. However, specific components participating in the vesicle docking or priming may also be able to exhibit different roles due to their specific functions or regulation pathway(s).

hlb-1 is another member of liprin family in worms and might function as an important link between the active zone and the synaptic cleft (Wang *et al*., unpublished data). *dlk-1* and *mkk-4* are identified from a genetic suppressor screen

of *rpm-1*, a large conserved protein with homologs known as Highwire[14]. *scd-2* functions as a target of *fsn-1*, an RPM-1 binding partner[15]. The subsynaptic localization of *dlk-1*, *mkk-4* and *scd-2* imply that they may generate/maintain a "boundary" between cellular transporting domain and synaptic domain. Functions of *nrx-1* is largely similar to that of *hlb-1* and is involved in the assembly and function of pre- and post-synaptic via binding to neuroligin at post-synaptic[21]. All these information suggest that the synaptic assembly event may serve as an importantly positive regulator for aging control in *C. elegans*.

Another goal of this study was to learn which genes among the identified genetic loci affecting the aging may participate in the regulation of dauer formation aging in *C. elegans*. In our study, mutations of *syd-2*, *snb-1*, *snt-1* and *scd-2* caused decreased dauer formation, and mutations of *hlb-1*, *mkk-4*, *ric-4* and *unc-64* resulted in accelerated dauer formation, indicating the possibly important roles of the vesicle docking event (the vesicle docking complex) and synaptic assembly in aging control. However, we also noticed that mutations of the *hlb-1*, *mkk-4* and *ric-4* did not suppress the dauer formation. Thus, the lifespan regulation and the dauer formation control may require largely two different mechanisms. And some signaling pathways involved in lifespan regulation and dauer formation control may not be overlapped.

The third goal of this study was to learn which genes among identified genetic loci may act as the downstream targets of *daf-2* or *daf-16* mutations. We found that *dlk-1*, *nrx-1*, *unc-10, unc-13*, *mkk-4* and *sbt-1* may serve as downstream targets for both *daf-2* and *daf-16* mutations. Moreover, *mkk-4* may more likely act as the downstream target for *daf-2* mutation, and *sbt-1* may more likely act as the downstream target for *daf-16* mutation. In addition, *ric-4* may specially function as the downstream target of *daf-2* mutation.

Worms can adapt their lifespan by sensing the environmental signals through the ciliated sensory neurons. Mutations that cause defects in sensory cilia or their support cells, or in sensory signal transduction could extend the lifespan[31]. The data in the current work will be helpful for our further understanding the mechanism of sensory perception in lifespan regulation, as well as the interaction of lifespan regulation and dauer formation control.

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突触功能必需基因参与线虫衰老的调控

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摘要：目的 鉴定参与线虫衰老的神经内分泌调控的新基因。**方法** 鉴于神经系统在衰老调控中的重要作用，通过寿命分析和脂褐质自发荧光的检测，从编码突触蛋白的遗传位点中筛选参与衰老调控的基因。我们还进一步检查了这些遗传位点相应的突变体的永久性幼虫形成情况，探讨它们是否可能受胰岛素样信号通路的调控。**结果** 遗传位点 *unc-10*，*syd-2*，*hlb-1*，*dlk-1*，*mkk-4*，*scd-2*，*snb-1*，*ric-4*，*nrx-1*，*unc-13*，*sbt-1*，*unc-64* 可能参与线虫衰老的调控。而且在衰老的调控中，*unc-10*，*syd-2*，*hlb-1*，*dlk-1*，*mkk-4*，*scd-2*，*snb-1*，*ric-4*，*nrx-1* 的功能可能与 *unc-13*，*sbt-1*，*unc-64* 相反。肠道脂褐质自发荧光的检测进一步证明了筛选出的各基因对应突变体的长寿或短寿表型，是由减慢或缩短的组织衰老所致。在筛选出的基因中，*syd-2*，*hlb-1*，*mkk-4*，*scd-2*，*snb-1*，*ric-4*，*unc-64* 也参与了永久性幼虫形成的调控。另外, *daf-2* 突变增强了 *syd-2* 和 *hlb-1* 的表达，降低了 *mkk-4*，*nrx-1*，*ric-4*，*sbt-1*，*rpm-1*，*unc-10*，*dlk-1*，*unc-13* 的表达。*daf-16* 突变提高了 *syd-2* 和 *hlb-1* 的表达，降低了 *mkk-4*，*nrx-1*，*sbt-1*，*rpm-1*，*unc-10*，*dlk-1*，*unc-13* 的表达. **结论** 突触功能可能在个体寿命和永久性幼虫形成的调控机制中具有重要的作用。

关键词：衰老；神经递质释放；突触；永久性幼虫形成；胰岛素信号通路；秀丽线虫