Regulation of aging by *unc-13* and *sbt-1* in *Caenorhabditis elegans* is temperature-dependent

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Abstract: Objective To investigate the role of environmental factor—temperature in the regulation of aging process by *unc-13* and *sbt-1* in *Caenorhabditis elegans*. **Methods** The lifespan, the speed of pharynx pumping, and the intestinal autofluorescence of *unc-13* and *sbt-1* mutants were examined at different temperature conditions. In addition, to exclude the possible influences from other factors in *unc-13* and *sbt-1* mutants, the dauer formation, the thermotaxis, the brood size and the population percentage of the mutants expressing *hsp16.2-gfp* were further investigated. **Results** Mutations of *unc-13* and *sbt-1* significantly increased the mean and the maximum lifespans of nematodes cultured at 20 °C and 25 °C, while no noticeable increase was found at 15 °C in either the mean or the maximum lifespan. Investigations on the speed of pharynx pumping and the intestinal autofluorescence suggested that at 20 °C and 25 °C, mutations of *unc-13* and *sbt-1* could slow the aging process and delay the accumulation of aging-related cellular damage. Meanwhile, mutations of *unc-13* or *sbt-1* did not affect the dauer formation or the thermotaxis to different temperatures in nematodes. In contrast, at 20 °C and 25 °C conditions, mutations of *unc-13* and *sbt-1* significantly decreased the brood size and the percentage of nematodes expressing *hsp16.2-gfp*, while no such differences were detected at 15 °C. Moreover, the thermotolerance of *unc-13* and *sbt-1* mutants could be greatly strengthened after the 16-h heat shock at 35 °C. **Conclusion** The regulation of aging by *unc-13* and *sbt-1* is temperature-dependent. And the alterations in reproduction capability and stress response may be associated with the formation of this temperature-dependent property.

Keywords: aging; *unc-13*; *sbt-1*; temperature-dependent; synaptic function; *C. elegans*

1 Introduction

Caenorhabditis elegans (*C. elegans*) remains the best tool for genetic analysis of longevity so far^[1, 2]. In *C. elegans*, aging is controlled by an endocrine system, and the nervous system is the major site contributing to insulin/insulin-like

growth factor (IGF-1)-dependent lifespan extension^[3]. When *daf-2* pathway signaling is restored to neurons, muscle or intestine, insulin-like signaling in neurons alone is sufficient to specify wild-type lifespan^[3]. In addition, neuronal expression of *daf-16* is sufficient for lifespan extension, increasing lifespan by 5%-20%, compared to that of wild-type^[4]. It has also been reported that mutations of genes resulting in defects in sensory cilia, in their supporting cells, or in sensory signal transduction, can extend the lifespan, implying that the sensory neurons can link environmental cues to lifespan^[5]. Moreover, mutants lacking active TUB-1 in the ciliary neurons are chemotaxis defective, and exhibit a DAF-16-depen-

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dent lifespan extension^[6]. Besides, cholinergic synapses and muscarinic receptors may be required for the transduction of the neuronal signals^[7].

Furthermore, mutations of *unc-64* and *unc-31*, genes of which are involved in mediating Ca²⁺-regulated synaptic transmission, could extend the adult lifespan and induce the constitutive dauer formation^[8]. Previous study also suggests the important roles of the synaptic transmission in the regulations of the lifespan and the dauer formation in nematodes^[9]. Among the genes required for synaptic function, *unc-10*, *syd-2*, *h1b-1*, *dlk-1*, *mkk-4*, *scd-2*, *snb-1*, *ric-4*, *nrx-1*, *unc-13*, *sbt-1*, and *unc-64* are involved in aging control in nematodes^[9]. The present study further suggests that regulation of aging by *unc-13* and *sbt-1* is temperature-dependent in *C. elegans*.

2 Materials and methods

2.1 Strains and culture conditions Strains in the present study included wild-type N2, NM1657 *unc-10(md1117)*, CZ900 *syd-2(ju37)*, RB878 *h1b-1(ok725)*, CZ5730 *dlk-1(ju476)*, CZ4213 *mkk-4(ju91)*, RB783 *scd-2(ok565)*, NM467 *snb-1(md247)*, RM956 *ric-4(md1088)*, MC339 *unc-64(md130)*, SG1 *nrx-1(ds1)*, CB450 *unc-13(e450)* and RB987 *sbt-1(ok901)*. They were all obtained from the *Caenorhabditis* Genetics Center (funded by the NIH National Center for Research Resource, USA). KC136 (*hsp-16.2::gfp*) was a generous gift from Dr. King CHOW's lab. Besides, *unc-13(RNAi)* and *sbt-1(RNAi)* were the gifts from Dr. Chonglin YANG's lab. All the strains were cultured on nematode growth medium (NGM) seeded with *Escherichia coli* OP50 at 15 °C, 20 °C or 25 °C as described^[10]. Gravid nematodes were washed off the plates into a centrifuge tube, and were lysed with a bleaching mixture (0.45 mol/L NaOH, 2% hypochlorous acid). Young adult animals that were synchronously aged were collected as previously described^[11].

2.2 Lifespan assay The lifespan assay was performed at 15 °C, 20 °C or 25 °C, based on previous descriptions^[9,12]. In the present study, the hermaphrodites were daily transferred during the first 4 d of adulthood. The worms were checked every 2-4 d and were identified as dead if they did not move even after repeated taps with a pick. Approximately 50 ani-

mals for each strain were picked out and used for the lifespan trial. Pharynx pumping rate was scored at 15 °C, 20 °C or 25 °C at day 10 since they developed into adults, under a Nikon stereomicroscope (Nikon, Melville, NY, USA) according to previous reports^[13, 14].

2.3 Endogenous intestine autofluorescence assay The photography of autofluorescence was performed as described^[9, 15]. Images of endogenous intestine fluorescence were captured using a 525 nm bandpass filter, in order to preserve the relative fluorescence intensity of different animals. On day 12, adults cultured at 15 °C, 20 °C or 25 °C were simultaneously photographed to avoid the interference of fluorescence intensity from the light source variance. Images were taken with Magnafire software (Olympus, Irving, TX, USA). Lipofuscin level was measured by observing the expression of green fluorescence protein (GFP) using ImageJ software (NIH Image) and determining the average pixel intensity in each animal's intestine. More than 50 animals were counted for the statistical analysis.

2.4 Dauer formation assay The dauer formation was analyzed as previously described^[9]. Briefly, when the food was almost exhausted, the nematodes cultured at 15 °C, 20 °C, 25 °C or 27 °C were flooded with 1% (*m/V*) sodium dodecyl sulfate (SDS) solution to select the dauers, based on their resistance to SDS.

2.5 Brood size measurement The brood size was measured as previously described^[16]. Briefly, single nematode was cultured onto a plate at 15 °C, 20 °C or 25 °C, and then it was transferred to a new plate every 1.5 d. The total number of eggs released on the plates was recorded.

2.6 Stress response analysis The analysis of transgenic strain KC136 was performed as described^[17]. To analyze the changes of *hsp-16.2* expression pattern, the KC136 strain cultured at 15 °C, 20 °C or 25 °C was fed with *unc-13(RNAi)* and *sbt-1(RNAi)*, respectively. The treated nematodes were pipetted onto an agar pad on a glass slide, mounted and observed for the fluorescent signals with a fluorescent microscope. Observation of the GFP was recorded and color images were taken for the documentation of results with Magnafire software (Olympus, Irving, TX, USA). The GFP level was measured using ImageJ software (NIH Image) by

determining the average pixel intensity in each animal's intestine.

2.7 Thermotaxis to cultivating temperature According to previous descriptions^[18,19], approximately 50 nematodes of wild-type or each mutant strain were grown at 15 °C, 20 °C or 25 °C overnight in the presence of a fresh lawn with bacteria strain of OP50 on a 6 cm Petri dish. Young adults were transferred onto a fresh plate without bacteria and stayed for 2 min. Then the individual nematode was placed on a 9 cm Petri dish. A radial temperature gradient was created by placing a vial containing frozen acetic acid on the bottom of the plate and incubating at 25 °C for 90 min in the presence of a constant humidity of 60%. Upon removal of the nematodes from the plate, the tracks were photographed and analyzed. A trace would be considered as the isothermal if more than half of the trace left on the agar surface by a single nematode was circular or exhibited an arc of circle near the isotherm of the cultivating temperature.

2.8 Thermotolerance assay Thermotolerance of adults on day 4 was detected as previously described^[20]. Briefly, the cultivating temperature of 20 °C was raised to 35 °C, and after 16 h, the touch-provoked movement of the nematodes was scored. Approximately 80 adults were employed in this assay.

2.9 Statistical analysis Data were presented as mean±SD and analyzed with one-way analysis of variance (ANOVA), followed by a Dunnett's *t*-test. Graphs were generated using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). *P* < 0.05 was considered as significantly different.

3 Results

3.1 Regulation of aging by *unc-13* and *sbt-1* is temperature-dependent In *C. elegans*, lifespan can be affected by temperature^[21]. To determine whether functions of the genes responsible for synaptic functions in aging control are influenced by temperature, the mean lifespans of several strains were investigated, including *unc-10(md1117)*, *syd-2(ju37)*, *hlb-1(ok725)*, *dlk-1(ju476)*, *mkk-4(ju91)*, *scd-2(ok565)*, *snb-1(md247)*, *ric-4(md1088)*, *unc-64(md130)*, *nrx-1(ds1)*, *unc-13(e450)* and *sbt-1(ok901)*, at 15 °C, 20 °C and 25 °C, respectively. Among these strains, mutations of *unc-10*, *syd-2*, *hlb-1*, *dlk-1*, *mkk-4*, *scd-2*, *snb-1*, *ric-4*, and *nrx-1* signifi-

cantly decreased the mean lifespan of nematodes at 20 °C and 25 °C (*P* < 0.01); however, mutations of *unc-13*, *unc-64*, and *sbt-1* significantly extended the mean lifespan of nematodes (*P* < 0.01) at 20 °C and 25 °C (Fig. 1A). Similarly, mutations of *unc-10*, *syd-2*, *hlb-1*, *dlk-1*, *mkk-4*, *scd-2*, *snb-1*, *ric-4*, and *nrx-1* significantly decreased the mean lifespan of nematodes (*P* < 0.01) at 15 °C, while mutation of *unc-64* significantly extended the mean lifespan of nematodes (*P* < 0.01) at 15 °C. In contrast, mutations of *unc-13* and *sbt-1* did not show any significant effect on the mean lifespan of nematodes cultured at 15 °C (Fig. 1A).

Meanwhile, the maximum lifespans of *unc-13(e450)* and *sbt-1(ok901)* mutants cultured at 15 °C, 20 °C, and 25 °C were detected, respectively. Mutations of *unc-13* and *sbt-1* could significantly (*P* < 0.01) increase the maximum lifespan of nematodes cultured at 20 °C and 25 °C, while no significant effect was detected at 15 °C (Fig. 1B), indicating that the effects of *unc-13* and *sbt-1* mutations on nematode aging were temperature-dependent.

3.2 Mutations of *unc-13* and *sbt-1* could slow the aging process in nematodes cultured at 20 °C and 25 °C To examine whether mutations of *unc-13* and *sbt-1* could slow the aging process in *C. elegans*, the speed of pharynx pumping was analyzed, which could predict the aging-related physiological changes^[13]. As shown in Fig. 2A, the *unc-13(e450)* and *sbt-1(ok901)* mutants both exhibited higher pumping rates on day 10, compared to wild-type nematodes cultured at 20 °C and 25 °C, but not at 15 °C. Similar results were also found on day 8 (data not shown). In addition, from day 1 to day 4, no significant changes of pumping rate were observed in either *unc-13(e450)* or *sbt-1(ok901)* mutant cultured at 20 °C, 25 °C, or 15 °C, compared to wild-type (data not shown). These results suggested that mutations of *unc-13* and *sbt-1* could slow the aging process in nematodes cultured at 20 °C and 25 °C.

3.3 Mutations of *unc-13* and *sbt-1* at 20 °C and 25 °C could delay the accumulation of aging-related cellular damage Intestinal autofluorescence, which is caused by lysosomal deposits of lipofuscin, accumulates during aging process, and is a valuable marker of aging and cellular damage in aging cells^[9,15]. To examine whether the altered lifespans in *unc-*

Fig. 1 Effects of mutations of the genes required for synaptic function on the lifespan of nematodes cultured at 15 °C, 20 °C, and 25 °C, respectively. **A:** mutations of *unc-10*, *syd-2*, *h1b-1*, *dlk-1*, *mkk-4*, *scd-2*, *snb-1*, *ric-4*, and *nrx-1* significantly decreased the mean lifespan of nematodes at 20 °C and 25 °C, while mutations of *unc-13*, *unc-64*, and *sbt-1* significantly extended the mean lifespan of nematodes at 20 °C and 25 °C. **B:** mutations of *unc-13* and *sbt-1* significantly increased the maximum lifespan of nematodes at 20 °C and 25 °C, but not at 15 °C. ** $P < 0.01$ vs wild-type N2.

13(e450) and *sbt-1(ok901)* mutants were due to the suppression of aging-related cellular damage, the lipofuscin levels in the intestines of *unc-13(e450)* and *sbt-1(ok901)* mutants were further examined on day 12 after L4-larvae. Mutations of *unc-13* and *sbt-1* significantly ($P < 0.01$) decreased the intestinal lipofuscin level compared to that of wild-type nematodes at 20 °C and 25 °C (Fig. 2B, C). In contrast, no obvious changes of intestinal lipofuscin were detected in either *unc-13(e450)* or *sbt-1(ok901)* mutant cultured at 15 °C compared to wild-type (Fig. 2B, C). Similar observations were also found in *unc-13(e450)* and *sbt-1(ok901)* mutants at day 8 after L4-larvae (data not shown). These data suggested that mutations of *unc-13* and *sbt-1* could delay the accumulation of aging-related cellular damage at 20 °C and 25 °C.

3.4 Effects of *unc-13* and *sbt-1* mutations on dauer formation Under unfavorable conditions such as high temperature, overcrowding, food scarcity, or functional loss of a specific gene, larvae of nematodes will molt into dauers with typical diapause features (high resistance to stress, hypometabolism, and extremely long life)^[1]. To examine whether the observed prolonged lifespans in *unc-13(e450)* and *sbt-1(ok901)* mutants were due to the altered dauer formation, the dauer formations in *unc-13(e450)* and *sbt-1(ok901)* mutants at 15 °C, 20 °C, 25 °C, and 27 °C were examined, respectively. However, no significant differences in dauer formation were observed

in either *unc-13(e450)* or *sbt-1(ok901)* mutants at 15 °C, 20 °C, 25 °C or 27 °C, compared to wild-type (Fig. 3A).

3.5 Effects of *unc-13* and *sbt-1* mutations on brood size To examine whether the observed prolonged lifespans in *unc-13(e450)* and *sbt-1(ok901)* mutants were associated with altered fertility, the brood sizes of *unc-13(e450)* and *sbt-1(ok901)* mutants cultured at 15 °C, 20 °C and 25 °C were examined, respectively. Mutations of *unc-13* and *sbt-1* significantly ($P < 0.01$) reduced the brood size compared to wild-type nematodes cultured at 20 °C and 25 °C (Fig. 3B). In contrast, no obvious changes of brood size were observed in either *unc-13(e450)* or *sbt-1(ok901)* mutant at 15 °C, compared to wild-type nematodes (Fig. 3B).

3.6 Effects of *unc-13* and *sbt-1* mutations on stress response Considering the fact that stress resistance may be critical for the lifespan of *C. elegans*^[1,22], the stress responses in *unc-13(e450)* and *sbt-1(ok901)* mutants cultured at 15 °C, 20 °C and 25 °C were investigated, respectively. Expression of *hsp-16*, a classical stress gene, can be induced by an array of environmental stresses^[23]. In the present study, the stable transgenic line of KC136 (*hsp16.2-gfp*) was employed to investigate the stress response. The percentage of nematodes expressing *hsp16.2-gfp* was significantly ($P < 0.01$) reduced in *unc-13(e450)* and *sbt-1(ok901)* mutants cultured at 25 °C. Similar results were also found in *unc-13(e450)* ($P < 0.01$) and *sbt-1(ok901)* ($P < 0.05$) mutants cultured at 20 °C.

Fig. 2 Effects of mutations of *unc-13* and *sbt-1* on the speed of pharynx pumping and the mean autofluorescence from intestine lipofuscin of nematodes cultured at 15 °C, 20 °C, and 25 °C. **A:** *unc-13(e450)* and *sbt-1(ok901)* mutants both exhibited higher pumping rates compared to wild-type nematodes at 20 °C and 25 °C, but not at 15 °C. **B:** mutations of *unc-13* and *sbt-1* significantly decreased the intestinal lipofuscin level compared to that of wild-type nematodes at 20 °C and 25 °C, but not at 15 °C, on day 12 after L4-larvae. **C:** the fluorescent images of lipofuscin accumulation in *unc-13(e450)* and *sbt-1(ok901)* mutants cultured at 15 °C, 20 °C and 25 °C. ****** $P < 0.01$ vs wild-type N2.

Fig. 3 Changes of dauer formation and brood size in *unc-13(e450)* and *sbt-1(ok901)* mutants. **A:** no significant differences in dauer formation were observed in either *unc-13(e450)* or *sbt-1(ok901)* mutant at 15 °C, 20 °C, 25 °C or 27 °C, compared to wild-type. **B:** mutations of *unc-13* and *sbt-1* significantly reduced the brood size compared to wild-type nematodes cultured at 20 °C and 25 °C, but not at 15 °C. ****** $P < 0.01$ vs wild-type N2.

However, no such difference was detected at 15 °C, compared to wild-type nematodes (Fig. 4A).

3.7 Effects of *unc-13* and *sbt-1* mutations on thermotolerance

Fig. 4 Stress response, thermotolerance and thermotaxis to cultivating temperature of *unc-13(e450)* and *sbt-1(ok901)* mutants. **A**: the percentage of nematodes expressing *hsp16.2-gfp* was significantly reduced in *unc-13(e450)* and *sbt-1(ok901)* mutants cultured at 20 °C and 25 °C. **B**: mutations of *unc-13* and *sbt-1* conferred increases in thermotolerance by approximately 36.7% and 44.1% of that in wild-type, respectively, after 16-h heat shock at 35 °C. **C**: mutation of *sbt-1* exerted no influence on the percentage of nematodes performing isothermal tracking (IT) at 15 °C, 20 °C, or 25 °C, compared to wild-type nematodes. * $P < 0.05$, ** $P < 0.01$ vs wild-type N2.

Since long-lived strains of *C. elegans* are also thermotolerant^[24], it is worth studying whether mutations of *unc-13* and *sbt-1* could also protect against a lethal heat shock. As shown in Fig. 4B, mutations of *unc-13* and *sbt-1* conferred increases in thermotolerance by approximately 36.7% and 44.1% of that in wild-type, respectively, after 16-h heat shock at 35 °C.

3.8 Effects of *unc-13* and *sbt-1* mutations on percentage of nematodes performing isothermal tracking

As shown in Fig. 4C, mutation of *sbt-1* exerted no influence on the percentage of nematodes performing isothermal tracking (IT) at 15 °C, 20 °C, or 25 °C, compared to wild-type nematodes. Similarly, at 15 °C, 20 °C, and 25 °C, mutation of *sbt-1* induced no significant alteration in the locomotion behavior which was monitored by body bend of nematodes (data not shown). In addition, since *unc-13* mutant nematodes are usually severely paralyzed^[10], the IT and locomotion behavior phenotype in *unc-13* mutant were not investigated in the present study.

4 Discussion

In this study, our data suggest that regulation of aging by *unc-13* and *sbt-1* is temperature-dependent. That is, mutations of *unc-13* and *sbt-1* could significantly extend the lifespan of nematodes cultured at 20 °C and 25 °C, but not at 15 °C (Fig. 1A, B). The strain of *unc-13(e450)* employed in the present study had a point mutation of *unc-13*, and *sbt-1(ok901)* strain had a deletion mutation of *sbt-1*. The altered lifespans in *unc-13(e450)* and *sbt-1(ok901)* mutants cultured at 20 °C and 25 °C were further confirmed by detecting the speed of pharynx pumping and the intestinal autofluorescence (Fig. 2). The results suggested that mutations of *unc-13* and *sbt-1* could slow the aging process, and delay the accumulation of aging-related cellular damage in nematodes cultured at 20 °C and 25 °C.

In our previous study, mutations of *unc-13* and *sbt-1* both induced long-lived phenotypes^[9]. The presynaptic protein UNC-13 has emerged as a key synaptic priming factor^[25]. The *sbt-1* gene encodes a seven B two (mammalian 7BT prohormone convertase chaperone) homolog, and may be involved at least in the synaptic secretion^[9, 26]. Moreover, recent data suggest that UNC-13 can interact with syntaxin

to regulate the synaptic docking process^[27]. In addition, the synaptic priming process can be modulated by the balance between Tomosyn and UNC-13, which can control the availability of open-syntaxin^[28]. Therefore, the temperature may be critical in regulating the synaptic docking or priming process to influence the alteration of longevity, although the direct linkage between temperature sensitivity and synaptic secretion may not be formed in *C. elegans*.

Previous studies have suggested that several factors may influence or be associated with the aging process of nematodes^[1,29]. For example, the dauer formation results in a long-lived phenotype in *C. elegans*^[1]. In addition, the reduced fertility may be associated with the formation of long-lived phenotype^[29]. In the present study, the mutation of *unc-13* or *sbt-1* exhibited no significant influence on the dauer formation in nematodes cultured at different temperatures (Fig. 3A), while they could significantly reduce the brood size of nematodes cultured at 20 °C and 25 °C, but not at 15 °C (Fig. 3B). Moreover, it has been reported that resistance to some kinds of stress is tightly correlated with the lifespan in all known longevity mutants in *C. elegans*^[30]. In this study, the percentage of the population expressing *hsp16.2-gfp* was significantly reduced in *unc-13(e450)* and *sbt-1(ok901)* mutants cultured at 20 °C and 25 °C, but not at 15 °C (Fig. 4A). Mutations of *unc-13* and *sbt-1* further resulted in an increase of thermotolerance after the 16-h heat shock at 35 °C (Fig. 4B). Therefore, the alterations in reproduction and stress response of *unc-13(e450)* and *sbt-1(ok901)* mutants may be involved in the formation of temperature-dependent regulation of the aging process.

Previous studies have suggested that the perception regulates nematode lifespan, and thus the lifespan may be regulated by environmental cues^[5,6]. Mutation of *sbt-1* did not result in deficit in the thermotaxis to the cultivating temperature of 15 °C, 20 °C or 25 °C (Fig. 4C), suggesting that no perception defects were formed in *sbt-1* mutant during aging process compared with wild-type nematodes.

In conclusion, the regulation of aging by *unc-13* and *sbt-1* is temperature-dependent in *C. elegans*. That is, the aging process of nematodes is not invariant, and can be affected by environmental factors, such as temperature.

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***unc-13* 和 *sbt-1* 基因以温度依赖的方式调控秀丽线虫衰老**

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摘要: 目的 研究环境因素—温度对秀丽线虫 *unc-13* 和 *sbt-1* 基因调控衰老作用的影响。方法 在不同培养温度下,测定 *unc-13* 和 *sbt-1* 突变线虫的寿命、咽泵运动速率和肠道荧光。此外,为排除其他可能的影响因素,对 *unc-13* 和 *sbt-1* 突变体的永久性幼虫形成率、对培养温度的趋向性、子代数以及表达 *hsp16.2-gfp* 线虫在群体中的比例进行了测定。结果 在 20 °C 和 25 °C 培养条件下, *unc-13* 和 *sbt-1* 突变能显著提高线虫的平均寿命和最高寿命;然而在 15 °C 条件下, *unc-13* 和 *sbt-1* 突变体的平均寿命和最高寿命与野生型相似。咽泵运动速率和肠道脂褐质自发荧光分析进一步验证了在 20 °C 和 25 °C 培养条件下, *unc-13* 和 *sbt-1* 突变可以减慢线虫衰老或延缓衰老相关的细胞损伤累积。同时, *unc-13* 和 *sbt-1* 突变不影响永久性幼虫发生率以及动物对不同温度的趋向性。相反,在 20 °C 和 25 °C 培养条件下, *unc-13* 和 *sbt-1* 突变能显著降低秀丽线虫的子代数以及群体中表达 *hsp16.2-gfp* 的线虫比率,而在 15 °C 条件下未出现此种差异。此外, *unc-13* 和 *sbt-1* 突变体经 35 °C 热激 16 h 后,其耐热性得到增强。结论 *unc-13* 和 *sbt-1* 对衰老的调节具有温度依赖性。*unc-13* 和 *sbt-1* 突变体的生殖功能和应激反应的改变可能与此温度依赖性的形成有关。

关键词: 衰老; *unc-13*; *sbt-1*; 温度依赖性; 突触功能; 秀丽线虫