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(Pepe et al., 2007), its relationship with lifespan has, like in worms, been controversial. Kalen and co-workers detected an age-associated loss of CoQ in homogenized human tissues (Kalen et al., 1989), while a constant level of CoQ with age was reported from homogenates of rat brains and lungs (Beyer et al., 1985; Sohal and Forster, 2007).

The biosynthesis of CoQ in S. cerevisiae is a complex process. Nine genes which are designated as COQ1—COQ9 have been identified for this series of enzymatic process in S. cerevisiae (Kawamukai, 2009). For example, yeast COQ3 gene that was identified as an O-methyltransferase is required for the methylation of two intermediate products during CoQ synthesis (Jonassen and Clarke, 2000). COQ7 encodes a protein catalyzing the hydroxylation of benzoquinone (Turunen et al., 2004). Mutation of C. elegans clk-1, the homolog of yeast COQ7, leads to accumulation of demethoxyubiquinone 9 (DMQ9) instead of CoQ9 (Jonassen et al., 2001).

In this study, we characterized a Drosophila homolog of COQ2, which is named as small boy (sbo), mutation of which leads to a small larvae phenotype. The sbo null mutants are developmentally arrested at the first instar larval stage. Flies that are heterozygous for sbo show reduced CoQ9 and CoQ10 production and extended lifespan. Homozygous sbo animals exhibit reduced activities of the insulin/insulin-like growth factor signaling (IIS) pathway.

2. Materials and methods

2.1. Fly stocks and genetics

All flies were raised at 25°C unless noted. sbo<sup>152—2</sup> allele was obtained by P-element mediated jump-out, which was started from the P[Supor-P]KG09818 insertion strain (BL#15216). We identified the sbo<sup>152—2</sup> allele from about 400 independent excision events detected by PCR and further confirmed by sequencing. The primers used for PCR were: 5′-AAGGCGTGGAAAATCCGACTG-3′ and 5′-CACTGCCCTGGGT-3′. For genomic rescue experiments, a 4.8-kb DNA segment that contains a full genomic sequence of sbo but not any complete neighboring genes was cloned (with Spe I and Not I) into the pTARG to generate a pTARG-sbo construct. Transgenic flies were generated using standard technique.

2.2. Complementation assay

The cDNA of sbo was cloned into a yeast expression vector pADH1-YES2 (kindly provided by Dr. Bing Zhou, Tsinghua University, China) with EcoR I and Xho I to generate pADH1-YES2-sbo. The yeast strain ΔCOQ2, which contains a disrupted COQ2 gene (Yazaki et al., 2002) was made competent and transformed with pADH1-YES2 and pADH1-YES2-sbo, respectively. These two transformed strains together with ΔCOQ2 and the wild type strain of W303-1A were cultured in liquid media before plated on two kinds of solid SD media that used glucose or glycerol as the carbon source.

2.3. CoQ extraction and HPLC assay

CoQ was extracted and analyzed essentially following a previously described protocol by Jonassen and Clarke (2000). Briefly, about 1 g 3-day-old adults were homogenized intensively in 9 mL of methanol and 6 mL of petroleum ether. The homogenates were vortexed and centrifuged for 10 min (1811 × g at 4°C). The upper petroleum ether fraction was transferred into a new 50 mL tube and 8 mL of petroleum ether was added into the remaining methanol phase to repeat the extraction for two more times. To the total extracts of the three extractions 10 mL of ferric chloride solution (5 mg FeCl<sub>3</sub>·6H<sub>2</sub>O per mL of ethanol) was added and mixed, followed by incubation at room temperature for 30 min for the oxidation of reduced CoQ. Finally, 18 mL water was added to the mixture to extract FeCl<sub>3</sub> followed by collecting the upper petroleum ether phase, N<sub>2</sub> dry, before the sample was resuspended in 2 mL of ethanol.

The reverse-phase high pressure liquid chromatography (HPLC) with a C18 column (Dikma Technologies Inc., USA) was used to analyze the CoQ composition of the extracts. CoQs were eluted by ethanol/water (98/2, v/v) at a flow rate of 1 mL/min, and detected by UV at 275 nm. The peaks of CoQ<sub>9</sub> and CoQ<sub>10</sub> were identified according to the elution time of the CoQ<sub>9</sub> and CoQ<sub>10</sub> standards that were purchased from Sigma (USA).

2.4. ATP assay

ATP level was measured essentially according to the previously described protocol (Park et al., 2006) with some modifications. Five larvae (48 h AED, After Egg Deposition) were collected as a group. The samples were washed with distilled water for three times, homogenized in 100 μL of 6 mol/L guanidine-HCl in the extraction buffer (100 mmol/L Tris and 4 mmol/L EDTA, pH 7.8). After boiling and centrifugation, the supernatant was harvested for ATP detection assay using ENLITEN kit (Promega, USA). The ATP level was calculated as the absolute nmol of ATP in every 1 mg total proteins.

2.5. Lifespan measurements

Newly eclosed adults were collected. Fifty flies were grouped into a 33 mm × 84 mm glass vial containing standard cornmeal food. At least 10 such groups were used for each genotype. The numbers of dead flies were counted every other day and the living flies were transferred into new vials. Males and females were tested separately but in parallel. The experiments were performed at 29°C with the humidity of 60%. The statistical analysis of the lifespan curve was carried out using the GraphPad Prism program (Slack et al., 2010).

2.6. Climbing assay

The climbing assay was carried out according to a previously described protocol (Park et al., 2006). Ten age-matched
(5, 20 and 30 days old) adults were put into groups in 18 cm long testing tubes for acclimatization for 1 h before the assay. Flies were gently tapped to the bottom, before measuring the time when half of the flies climbed across the 15 cm target line. Climbing index was calculated according to the climbing performance of the flies. Five trials were conducted for each group, and the climbing index for each genotype is the average index of five independent groups. Males and females were tested separately. The illumination was stable, humidity was 60% and temperature was 25°C.

2.7. tGPH localization analysis

Larvae of the different genotypes were collected 48 h AED. Foreguts were dissected and fixed in 4% paraformaldehyde for 20 min at room temperature. After washing with PBS for 3 times, the samples were then stained with DAPI (1 µg/mL) for 20 min. Pictures were taken with a Leica TSC SP5 confocal laser scanning microscope. The pictures were adjusted and edited with Adobe Photoshop 8.0.1 program.

2.8. RT-PCR

One hundred and fifty 48 h AED larvae of correct genotypes were collected and washed with water before homogenized in Trizol (Invitrogen, USA). Total RNA was isolated following the Trizol protocol. cDNAs were produced with the Invitrogen Superscript II kit in a 20 µL reaction volume. The quantitative RT-PCR was performed employing MJ Research PTC-200 Peltier Thermal Cycler with EvaGreen (Biotium, USA). The transcription levels of the test genes were normalized to Ribosomal protein L32 (Rpl32, rp49). The following primers were used. For semiquantitative RT-PCR: Rpl32: 5'-TATGCTAAGCTGTCGCACAA-3' (Forward), 5'-ACCAGGAACCTTCTTGAAATCC-3' (Reverse); sbo: 5'-GGCCATTTTTTGAACGGTCTA-3' (Forward), 5'-CGAAGCTCTCGGAGGACCAT-3' (Reverse); for quantitative RT-PCR: 4E-BP: 5'-TCAGCTAAAGTTCGGCTTC-3' (Forward), 5'-AGATAAGTTGGTGCCCTCA-3' (Reverse); InR: 5'-GGATTACTGAACCTCTCC-3' (Forward), 5'-CTTTACAGATCATCGTA TTCCCTG-3' (Reverse).

3. Results

3.1. Generation and characterization of sbo\textsuperscript{152--2} mutant

Drosophila CG9613 is predicted to encode a prenyltransferase, known as coenzyme Q biosynthesis protein 2 (COQ2), which catalyzes the transfer of a polypropenyl group to para-hydroxybenzoate to form 3-polyprenyl-4-hydroxybenzoate which catalyzes the transfer of a polyprenyl group to para-ase, known as coenzyme Q biosynthesis protein 2 (COQ2), ATTTTTTGACTGTGCTA-3' AGTTTGGTGCCTCCA-3'.

3.2. sbo is a functional Drosophila homolog of COQ2

sbo is predicted to encode a 392 amino acid protein that shares 47% identity with human COQ2 and 33% identity with that of yeast. TargetP analysis shows that Sbo protein is a mitochondrial protein (Emanuelsson et al., 2000; Forsgren et al., 2004), which is consistent with the fact that yeast COQ2 is involved in the CoQ biosynthesis that occurs on the inner membrane of the mitochondria (Ashby et al., 1992). The TMHMM (transmembrane hidden Markov method) (Sonnhammer et al., 1998) predicts that Sbo has five potential transmembrane segments that are indicated by the solid lines in Fig. 2A. The amino acids 92 to 358 of Sbo contains a 4.8 kb genomic segment that covers the regulatory and coding sequences of sbo gene as sbo (small boy). To exclude the possibility that food-taking disability might be the cause of developmental arrest, the mutant and wild type animals were fed with a blue-colored (Blue No. 1, Sigma) yeast for food uptake behavior detection (Zinke et al., 1999). Five hours after addition of the colored food, it was detected in the midguts of both wild type and the sbo\textsuperscript{152--2} homozygous mutants (data not shown), indicating that the sbo mutant animals are not defective in food-taking during development.

To test whether loss of function of sbo specifically attributes to the lethality of the mutant, we generated a transgene, pTARG-sbo, which contains a 4.8 kb genomic segment that covers the regulatory and coding sequences of sbo gene as sbo (small boy, Fig. 1A). Introduction of pTARG-sbo into the sbo mutant rescues the "small boy" phenotype (Fig. 1C and D). The mutants that contain the rescue transgene develop to be normal adults (data not shown). However, the rescue efficiency is compromised (Fig. 1D), which is likely due to low expression level of the transgene and/or a lack of partial regulatory elements of sbo. Taken together, sbo\textsuperscript{152--2} is a null allele of sbo gene, which is essential for Drosophila development.

3.2. sbo is a functional Drosophila homolog of COQ2

sbo is predicted to encode a 392 amino acid protein that shares 47% identity with human COQ2 and 33% identity with that of yeast. TargetP analysis shows that Sbo protein is a mitochondrial protein (Emanuelsson et al., 2000; Forsgren et al., 2004), which is consistent with the fact that yeast COQ2 is involved in the CoQ biosynthesis that occurs on the inner membrane of the mitochondria (Ashby et al., 1992). The TMHMM (transmembrane hidden Markov method) (Sonnhammer et al., 1998) predicts that Sbo has five potential transmembrane segments that are indicated by the solid lines in Fig. 2A. The amino acids 92 to 358 of Sbo consists of its potential UbiA prenyltransferase domain, which was identified by Pfam analysis (Finn et al., 2010). In addition, Sbo has a potential polypropenyl diphosphate substrate binding site
To assess the possibility that Drosophila sbo gene encodes a functional homolog of yeast COQ2, we performed a complementation experiment in which the Drosophila sbo cDNA was expressed in the yeast ΔCOQ2 disruptants. Yeast ΔCOQ2 mutant cells cannot produce CoQ and thus are unable to grow on the SD medium containing non-fermentable carbon hydrates, such as glycerol, due to the lack of polyprenyltransferase activity (Ashby et al., 1992) (Fig. 2B). When Drosophila sbo was transformed into the mutant yeast cells, an ectopic expression of Drosophila sbo cDNA under the control of pADH1 promoter restores the growth of ΔCOQ2 mutant cells on glycerol medium, albeit the growth is at a slower rate than the wild type strain of W303-1A (Fig. 2B). Together, these results show that sbo serves the similar function as yeast COQ2. All these results demonstrate that Drosophila sbo is a functional homolog of the yeast COQ2 gene.

3.3. sbo is required for CoQ biosynthesis

Mutations of genes that are involved in CoQ synthesis have been shown to affect the production of CoQ in vivo. For example, mutation of clk-1, the C. elegans homolog of yeast COQ7, results in a lack of endogenous CoQ9 in worms (Jonassen et al., 2001). Given that Drosophila sbo is predicted to encode a prenyltransferase that is required for CoQ biosynthesis and that expression of Drosophila sbo in yeast rescues the lethality of ΔCOQ2 cells, we reasoned that sbo mutation should alter the CoQ level of the mutant animals. To assess this hypothesis, we determined the endogenous CoQ level of wild type and heterozygous sbo animals using a reverse-phase high pressure liquid chromatography (HPLC) system in combination with a UV detector (Liu et al., 2005). Our results indicate that, in addition to CoQ9, which appears to be the main component of CoQ in Drosophila, CoQ10 was also detected. As shown in Fig. 3A, in wild type flies, the CoQ9 and CoQ10 peaks were well separated, with the elution time of 15 min and 20 min, respectively, as determined by the CoQ9

(Forsgren et al., 2004) that is indicated by the hatched bar (Fig. 2A).

To assess the possibility that Drosophila sbo gene encodes a functional homolog of yeast COQ2, we performed a complementation experiment in which the Drosophila sbo cDNA was expressed in the yeast ΔCOQ2 disruptants. Yeast ΔCOQ2 mutant cells cannot produce CoQ and thus are unable to grow on the SD medium containing non-fermentable carbon hydrates, such as glycerol, due to the lack of polyprenyltransferase activity (Ashby et al., 1992) (Fig. 2B). When Drosophila sbo was transformed into the mutant yeast cells, an ectopic expression of Drosophila sbo cDNA under the control of pADH1 promoter restores the growth of ΔCOQ2 mutant cells on glycerol medium, albeit the growth is at a slower rate than the wild type strain of W303-1A (Fig. 2B). Together, these results show that sbo serves the similar function as yeast COQ2. All these results demonstrate that Drosophila sbo is a functional homolog of the yeast COQ2 gene.

### Table 1

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>WT</th>
<th>sbo&lt;sup&gt;152&lt;/sup&gt;-2</th>
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<td></td>
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<td>192</td>
<td>44 (41)</td>
<td>73.3</td>
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Genotypes: WT: yw, sbo<sup>152</sup>-2; yw; sbo<sup>152</sup>-2. * Denotes the time after egg deposition (AED); ** The numbers in brackets represent the number of pupa.
and CoQ_{10} standards. Interestingly, in the sbo^{152-2} heterozygous adults, the CoQ_{9} level was reduced to 81.0% of the control flies while the CoQ_{10} level showed a 34.7% reduction (Fig. 3A and B).

Since CoQ is one of the key components required for oxidative phosphorylation which produces the important energy currency, ATP, the immediate consequence of CoQ deficiency, therefore, may be the reduction of total ATP level. To determine whether the sbo^{152-2} mutation affects ATP production in Drosophila, total amount of ATP in sbo^{152-2} homozygous larvae were quantified. As shown in Fig. 3C, the ATP content of sbo^{152-2} was 6.1 nmol ATP/mg total proteins, Fig. 2. Drosophila sbo is the functional homolog of \textit{S. cerevisiae} \textit{COQ2}. A: amino acids sequence alignment of \textit{Drosophila sbo} (GenBank accession No. NP_649789) with its homologs from humans (NP_056512), \textit{C. elegans} (NP_871684), \textit{E. coli} (AA24712) and \textit{S. cerevisiae} (NP_014439). Black shading and gray shading indicate amino acid identity and similarity, respectively. The putative prenyl diphosphate binding motif is highlighted by the hatched bars above the sequence. The potential transmembrane motifs are depicted by the solid lines above the sequence. B: complementation of \textit{S. cerevisiae} \textit{ΔCOQ2} disruptant with the expression of \textit{Drosophila sbo} gene. Yeast strains with correct genotypes were plated on the SD medium which contains either glucose or glycerol as the sole carbon source. Ectopic expression of \textit{Drosophila sbo} restores the growth of \textit{ΔCOQ2} disruptant on glycerol-SD medium. WT, \textit{W303-1A}; \textit{ΔCOQ2}, \textit{W303 ΔCOQ2}; \textit{pADH1-YES2-sbo}, transformed yeast cells that express \textit{Drosophila sbo}; \textit{pADH1-YES2}, empty vector control.
about half of that of wild type control flies. Taken together, the mutation of sbo leads to a reduction of the amount of CoQ and a consequent reduction of ATP, indicating that sbo plays an important role in CoQ biosynthesis in Drosophila.

3.4. sbo152 mutation extends Drosophila lifespan

CoQ has been shown to be an antioxidant, the amount of which changes with age (Kalen et al., 1989; Battino et al., 1995). Lass and colleagues reported that the mitochondrial CoQ in mouse skeletal muscles declines with age (Lass et al., 1999). Withdrawal of CoQ from the diet of wild type C. elegans extends adult lifespan by about 60% (Larsen and Clarke, 2002). Our study, as described above, has shown that sbo is required for CoQ synthesis, thus we wondered how loss of function of sbo would affect the lifespan of Drosophila. To address this point, sbo152 mutant flies were backcrossed with w1118 for more than five generations before being used for lifespan assays. Both the median and mean lifespan of sbo152 mutant heterozygotes significantly increased for either males or females as compared with the wild type flies (Fig. 4A and B, Table 2). Interestingly, in agreement with the observation that the sbo mutation causes lifespan extension, increased dose of sbo by introducing an extra transgenic copy of sbo, pTARG-sbo, shortened the lifespan as compared with the wild type flies (Fig. 4A and B, Table 2).

Loss of negative geotaxis (climbing behavior) is one of the behaviors which accompany with fly aging (Ganetzky and Flanagan, 1978; Feany and Bender, 2000; Gargano et al., 2005). Long-lived Indy and chico mutant flies show delayed loss of negative geotaxis (Gargano et al., 2005). Climbing behavior (loss of negative geotaxis) has become one of the parameters to monitor Drosophila aging (Ganetzky and Flanagan, 1978; Feany and Bender, 2000; Gargano et al., 2005). To test whether sbo mutation affects Drosophila negative geotaxis, 5-day-old female flies of sbo152 heterozygotes and wild type were collected to perform the climbing assay. Fifteen days after the start of the test, both the mutant females and the wild type females appear to show reductions of the climbing ability, but with only slight difference (Fig. 4D). However, by the time of 30 days after eclosion, the climbing ability of the wild type females declined significantly more than that of the mutant females (Fig. 4D). Similarly, mutation of sbo enhanced the climbing ability of the males as well (Fig. 4C). Consistent with the loss of function results, extra copy of wild type sbo gene accelerated the declining of climbing ability (Fig. 4C and D). Taken together, these results that reduced dosage of sbo extends the lifespan of Drosophila and that sbo mutation delays declining of the climbing ability suggest that sbo is likely involved in the aging process.

3.5. sbo152 mutation down-regulates the function of IIS pathway

As described thus far, sbo152 mutation leads to small body size phenotype of the mutant larvae (Fig. 1C and D). Reduced function of sbo extends lifespan of the mutant flies (Fig. 4 and Table 2), which resembles phenotypes that are caused by down-regulation of the insulin/insulin-like growth factor signaling (IIS) pathway both in flies and worms (Samuelson et al., 2007; Taguchi and White, 2008; Werz et al., 2009). To test whether sbo152 mutation affects the IIS pathway, tGPH was used as a read-out to assess the insulin pathway activity (Britton et al., 2002; Bauer et al., 2007; Hyun et al., 2009). In this reporter system, green fluorescent protein (GFP) was fused with the PH domain of the Drosophila homolog of the general receptor for phosphoinositides-1 (GRP1), resulting in the GPH (GFP-PH domain) transgene that is ubiquitously expressed under the control of Drosophila β-tubulin promoter (tGPH). Because tGPH binds specifically to phosphatidylinositol-3,4,5-P3 (PIP3) that is generally anchored on the plasma membrane, the redistribution of tGPH from the cytoplasm to the cell membrane is an indication of activation of the insulin pathway (Britton et al., 2002). We examined the distribution of tGPH in the foregut cells of both wild type and homozygous sbo152 mutants. In wild type larvae, GFP is mainly located at the plasma membrane of the cells, indicating active IIS signaling (Fig. 5A). In contrast, in mutant foregut cells, the relative cell membrane-
associated GFP signals are significantly reduced, with more diffused GFP in the cytoplasm, indicating a down-regulated IIS activity (Fig. 5A, middle panel). To determine that the IIS activity down-regulation is specifically caused by sbo152/C02 mutation, genomic sbo rescue construct was introduced into the mutant, which restored the distribution of GFP preferentially to the cytoplasmic membranes (Fig. 5A, right panel). These results suggest that sbo152/C02 mutation leads to a down-regulation of the IIS pathway.

To show directly at the molecular level that the IIS activity is impaired in the sbo152/C02 mutants, we examined the expression of 4E-BP and InR, two molecular markers of the insulin signaling pathway. As the direct targets of the transcription factor dFOXO, the transcription of 4E-BP and InR is expected to be increased upon IIS pathway down-regulation (Edgar, 2006; Fuss et al., 2006). As shown in Fig. 5B, transcription level of 4E-BP and InR in sbo152/C02 mutants was about 6-fold higher than that of the control animals, indicating a distinct suppression of the insulin pathway. Taken together, these results indicate that sbo152/C02 mutation down-regulates the insulin/insulin-like growth factor signaling (IIS) pathway.

4. Discussion

4.1. A Drosophila model for studying human CoQ deficiency related diseases

CoQ is one of the critical components of the mitochondrial electron transport chain (ETC), transferring electrons from
complexes I (NADH-ubiquinone oxidoreductase) and II (succinate–ubiquinone oxidoreductase) to complex III (ubiquinol–cytochrome c reductase) (Crane et al., 1957; Turunen et al., 2004). In addition to this essential role that it plays in the process of electron transportation, CoQ has also been shown to modulate the amount of activation of mitochondrial uncoupling proteins, modulation of the amount of α2-integrins on the surface of blood monocytes (Turunen et al., 2004).

The biosynthesis of CoQ in S. cerevisiae is a complex process that involves many biochemical reactions and a lot of enzymes. Nine genes designated as COQ1–COQ9 have been identified in the series of these enzymatic reactions (Kawamura, 2009). Mutation of any of these genes disrupts CoQ synthesis, followed by the reduction of ATP generation and mitochondrial dysfunction. In humans, CoQ biosynthesis defects lead to CoQ10 deficiency diseases such as isolated myopathy, Leigh syndrome, cerebellar ataxia and infantile multisystemic disease (Sacconi et al., 2010). To date, several human genes have been linked to CoQ10 deficiency, including COQ2, ABC1/COQ8, COQ9, PDSS1 and PDSS2 (Sacconi et al., 2010). COQ2 encodes an enzyme that catalyzes the combination reaction of a polyisoprenyl side chain with a p-hydroxybenzoate ring. The first COQ2 mutation in humans was reported from two siblings, who had 18% reduction of CoQ10 and suffered encephalopathy and nephropathy (Artuch et al., 2009). Another more severe CoQ deficiency patient who had up to 24% reduction of CoQ10 was reported to suffer from a fatal infantile multiorgan disease (Artuch et al., 2009). It would be very useful to establish an experimental model system to study the human CoQ deficiency syndromes.

In this study, we identified sbo as the Drosophila homolog of COQ2 based on the following findings: 1) sbo is predicted to encode a protein that shares 47% identity of amino acids with human COQ2 and 33% identity with that of yeast. In addition, sbo encodes a protein with a potential UbIA domain that is required for prenylation of the 4-hydroxybenzoic acid, a critical step for CoQ synthesis. 2) Expression of Drosophila sbo cDNA rescues lethality phenotype of the yeast ΔCOQ2 disruptant when grown on media with non-fermentable carbon source. 3) Reducing the dose of sbo leads to a decrease of endogenous CoQ and ATP level. To experimentally verify that sbo is the functional homolog of human COQ2, we were tempted to use human COQ2 cDNA to rescue the sbo mutants. However, the rescue experiment with fly sbo using the GAL4/UAS system resulted in lethality of the animals, likely due to too high expression of the transgene in this system, which led to a cease of the rescue experiment with human COQ2 cDNA. Nevertheless, the fact that both Drosophila sbo and human COQ2 (Forsgren et al., 2004) rescue the yeast COQ2 mutants let us to propose that our sbo mutant is possibly a feasible model for studying human disease that is caused by CoQ deficiency.

4.2. sbo is a gene that controls lifespan

Aging is a complicated process that involves environmental and genetic causes. In the past decades, genes including isp-1, nua-2, cco-1, clk-1 whose mutation extends the lifespan of C. elegans, have been identified (Wong et al., 1995; Feng et al., 2001; Dillin et al., 2002). All these genes related to longevity can be grouped into three categories: 1) genes that control dietary restriction (eat-2 etc.); 2) genes whose mutation causes mitochondrial dysfunction, especially respiratory chain dysfunction (clk-1, isp-1 etc.); and 3) genes that suppress insulin/insulin-like growth factor signaling (daf-2, age-1, daf-16 etc.) (Feng et al., 2001). Based on our results described above that loss of function of sbo affects both in vivo CoQ production and insulin signaling, sbo falls likely into the second category of genes that controls lifespan, however may be linked to the IIS pathway as well (see next part of discussion).

It remains unclear thus far, because of sometimes conflicting results, how mitochondrial respiration defects, especially CoQ deficiency affect lifespan. To investigate whether a decrease in CoQ level extends Drosophila lifespan, Palmer and Sackton fed wild type flies with CoQ8-deficient yeasts. They did not observe any lifespan extension (Palmer and Sackton, 2003), which is different from the results obtained from C. elegans (Larsen and Clarke, 2002). It was interpreted that perhaps the endogenously produced CoQ was sufficient and the wild type flies did not need to acquire CoQ from their...
diet (Palmer and Sackton, 2003). Our study described here offers an excellent system to study the effect of CoQ deficiency. In our experiments, sbo<sup>152-2</sup> is a null allele of the sbo gene. Reduction of one copy of sbo leads to a decrease of the endogenously produced CoQ<sub>9</sub> and CoQ<sub>10</sub> by 19% and 34.7%, respectively. Consequently, the mean lifespan of the heterozygous sbo<sup>152-2</sup> flies were extended by 12.5% and 30.8% for males and females, respectively. Thus our study demonstrates that lower level of CoQ extends the lifespan of Drosophila.

It has been controversial about the time point when mitochondrial dysfunction starts to alter the animal lifespan. Dillin and colleagues reported that RNAi of C. elegans ETC components during adulthood does not extend the worm lifespan while it does if RNAi occurs earlier (Dillin et al., 2002). However, another study showed that knocking down of the respiratory components (CG9140, COQ7, CG4796 and CG11015) at the larval stage of Drosophila did not affect the lifespan of the adults (Rera et al., 2010). Furthermore, it has been shown by Copeland et al. (2009) that RNAi of the Drosophila ETC components (CG9762 and CG9162) specifically in adults did extend the mean lifespan of the females. Our study shows that to half the dose of sbo throughout the animal development extends adult lifespan. Our mutant flies offer a good tool to study the relations between lifespan alteration and mitochondrial dysfunction during development.

4.3. The role of sbo in the insulin/insulin-like growth factor signaling (IIS) pathway

IIS is a key pathway that controls growth, metabolism, fecundity and lifespan of multicellular organisms from worms to mammals (Werz et al., 2009). Down-regulation of the IIS pathway reduces cell size, cell number and consequently animal size. For example, homozygous chico (the insulin receptor substrate) mutant adults are about only half of the wild type flies in their body size (Bohni et al., 1999). In addition, the chico homozygous adults display an extension of the median lifespan by 48% (Clancy et al., 2001). The sbo<sup>152-2</sup> homozygotes exhibit a similar small body size phenotype to that of chico, which led us to examine whether the IIS pathway is affected in the sbo<sup>152-2</sup> mutants.

The tGPH assay and quantitative PCR detection of 4E-BP and Inr (two direct targets of the transcription factor dFOXO) both support a suppression of the IIS pathway in sbo<sup>152-2</sup> animals. To exclude the possibility that the “small boy” phenotype was caused by a lack of enough food, we examined the food intake behavior of the sbo<sup>152-2</sup> mutants. No significant difference of food intake was observed between sbo<sup>152-2</sup> homozygous larvae and wild type larvae (data not shown). Thus we conclude that the developmental arrest of the sbo<sup>152-2</sup> mutants may be resulted from a down-regulation of the IIS activity.

The mechanism by which mutation of sbo suppresses the IIS activity remains to be elucidated. There are two possibilities according to our observations. The first one is that the decreased level of energy supply would affect the IIS activity. The endogenous ATP level of sbo<sup>152-2</sup> homozygous animals is reduced to about a half of that of wild type (Fig. 3C). We propose that a decreased ATP:ADP ratio may serve as a signal for the cells to down-regulate the IIS activity; alternatively, CoQ deficiency directly triggers a signal for the cells to down-regulate the IIS pathway, which is called the retrograde response (Cristina et al., 2009).

Acknowledgements

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