Drosophila Ogg1 is required to suppress 8-oxo-guanine accumulation following oxidative stress

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Reactive oxygen species (ROS) generated during energy production processes are a major cause of oxidative DNA damage. A DNA glycosylase encoded by the Ogg1 gene removes oxidized guanine bases and is widely conserved. However, the biological role of the gene in individual organisms has not yet been characterized in Drosophila, which is a suitable model to study the influence of oxidative damage on senescence. Here, we performed a genetic analysis to confirm that Ogg1 plays an essential role in the removal of 8-oxo-guanines from nuclei. We first confirmed by quantitative real-time PCR that Ogg1 mRNA expression was reduced by 30–55% in Ogg1 mutants and in flies expressing inducible Ogg1 dsRNA compared to control flies. We then showed that additional accumulation of 8-oxo-guanines occurred in the nuclei of epithelial midgut cells after paraquat feeding in flies with downregulated Ogg1 expression. We confirmed that a transposon possessing the UAS sequence was integrated in the 5′-UTR of the Ogg1 alleles and that it is oriented in the same transcriptional direction as the gene. Using the Gal4/UAS system, which enables us to induce ectopic expression in Drosophila, we induced overexpression of Ogg1 by 40-fold. We observed a lower amount of 8-oxo-guanine in the midgut epithelial cells of adults overexpressing Ogg1. These genetic data strongly suggest that the Drosophila Ogg1 ortholog CG1795 plays an essential role in the suppression of 8-oxo-guanines, consistent with its role in other organisms. Although adult flies with reduced Ogg1 expression failed to show elevated sensitivity to paraquat, those with Ogg1 overexpression showed resistance to oxidative stress by paraquat feeding and had a significantly longer lifespan in normal feeding conditions. These observations are consistent with the hypothesis that oxidative DNA damage by ROS accumulation is a major contributor to senescence.

Key words: 8-oxo-guanine, Drosophila, lifespan extension, Ogg1, oxidative stress

INTRODUCTION

Reactive oxygen species (ROS) induce damage in genomic as well as mitochondrial DNA (Lindahl, 1993; Wang et al., 1998; Cooke et al., 2003). The oxidized guanine base 8-oxo-guanine is one of the most common oxidatively damaged DNA lesions in a cell. 8-oxo-guanine forms mismatch pairs with adenine and produces G:C to T:A transversions during DNA replication (Moriya, 1993; Thomas et al., 1997). Therefore, the production of oxidized bases can lead to DNA mutagenesis, and the accumulation of such DNA damage eventually results in the development of many diseases and senescence (Barja, 2004; Maynard et al., 2009; Kryston et al., 2011). To prevent these effects, oxidized DNA bases are initially removed by the base-excision repair mechanism (Wallace, 1998; Hazra and Mitra, 2006; Nemec et al., 2010). First discovered in Escherichia coli, the DNA glycosylase MutM excises 8-oxo-guanines generated by ROS (Michaels et al., 1992; Grollman and Moriya, 1993; Boiteux and Radicella, 1999). In Saccharomyces cerevisiae, a DNA glycosylase encoded by the Ogg1 gene catalyzes the removal of 8-oxo-guanines and formamidopyrimidines from oxidatively damaged DNA (Nash et al., 1996; van der Kemp et al., 1996; Girard et al., 1997). Mammalian cells also express an ortholog of Ogg1 that plays an important role in the prevention of mutagenesis and carcinogenesis (Aburatani et al., 1997; Arai et al., 1997; Lu et al., 1997; Radicella et al., 1997; Rosenquist et al., 1997; Chevillard et al., 1998;
Sakumi et al., 2003). It was shown that the Ogg1 ortholog in human cells is a major enzyme to repair 8-oxoguanine DNA lesions (Monden et al., 1999; Boiteux and Radicella, 2000).

To understand the biological function of Ogg1 in individual organisms rather than in cultured cells, knockout mouse lines carrying the null allele generated by gene disruption have been established (Klungland et al., 1999; Minowa et al., 2000). Ogg1-deficient mice are viable, but during aging they accumulate up to 7-fold more 8-oxoguanines than wild-type mice. In addition to biochemical evidence indicating that the human OGG1 gene plays an important role in removing 8-oxo-guanines, OGG1 is a strong candidate gene for susceptibility to human cancers and metabolite-related diseases (Audebert et al., 2000; Sakumi et al., 2003). It was shown that the Ogg1 ortholog encoded by CG1795 plays an important role in the DNA repair of oxidative damage after the expression in Drosophila melanogaster (methyl viologen dichloride hydrate (Sigma-Aldrich, St. Louis, MO, USA)).

In this study, we first confirmed that Ogg1 gene expression was down-regulated by 30–55% in transposon-induced mutants as well as in Drosophila adults expressing Ogg1 dsRNA. Accumulation of 8-oxo-guanine was observed in the nuclei of epithelial midgut cells of flies fed with paraquat, which produces free radicals within a cell and thus causes oxidative stress (Cochemé and Murphy, 2008). Conversely, when we induced -40-fold over-expression of Ogg1 in adults using the Gal4/UAS system, we observed significantly less accumulation of 8-oxoguanine. A positive correlation between Ogg1 expression and the extent of 8-oxo-guanine accumulation strongly suggested that the Ogg1 ortholog encoded by CG1795 plays an important role in oxidized base accumulation in Drosophila. Interestingly, flies overexpressing Ogg1 had significantly extended adult lifespans and showed resistance to oxidative stress. Our genetic data suggest that these Drosophila mutants and flies with modified Ogg1 gene expression offer promising models to clarify the relationship between DNA oxidation and senescence or the onset of several diseases.

**MATERIALS AND METHODS**

**Drosophila stocks** All D. melanogaster stocks were maintained on standard food at 25 °C. To express Ogg1 dsRNA, P(UAS-Ogg1RNAiHMC03426) (UAS-Ogg1RNAiHMC03426) and P(UAS-Ogg1RNAi12101291) (UAS-Ogg1RNAi12101291) stocks were used. The former RNAi stock was obtained from the Bloomington Drosophila Stock Center. The latter was provided by the Vienna Drosophila RNAi Center. P(Actin5C-Gal4)25F01 (Actin5C-Gal4) was obtained from the Bloomington Drosophila Stock Center and used as a Gal4 driver. PB(WGIogg1)01284 (Ogg101284) and PB(WHIogg100603) (Ogg100603) were obtained from the Harvard Medical School Exelixis Collection. Before analyzing individual flies with modified Ogg1 expression in an adult lifespan assay, each stock was outcrossed into the w1118 background for at least three generations. Control flies (Actin5C-Gal4+), UAS-Ogg1RNAiHMS03426/+ and UAS-Ogg1RNAi12101291/+ were generated from each outcross to w1118. Flies depleted of Ogg1 mRNA were obtained from a cross between Actin5C-Gal4 and UAS-Ogg1RNAiHMS03426 or UAS-Ogg1RNAi12101291. Males over-expressing Ogg1 were generated from an outcross between Ogg1 mutant females and Actin5C-Gal4 males.

**Lifespan assay** For lifespan analysis, adult male flies were collected within 24 h after eclosion; 20 flies were reared in a single plastic vial on Drosophila instant medium (Formula 4-24, Blue, Biological Supply Company, Burlington, NC, USA) at 25 °C or 28 °C for induced gene expression dependent on the Gal4/UAS system. Dead adults were scored every day and food vials were changed every three days.

For the pararquet feeding assay, Ogg1 mutant flies and flies depleted of or overexpressing Ogg1 mRNA were reared on instant medium containing 10 mM pararquat (methyl viologen dichloride hydrate (Sigma-Aldrich, St. Louis, MO, USA)). Survival curves were analyzed by the Kaplan-Meier procedure. Significance was determined by the log-rank test.

**Quantitative real-time PCR analysis** qRT-PCR analysis was performed to determine the expression level of Ogg1 genes in adult flies with induced expression of each dsRNA or of Ogg1 with a Gal4 driver. Total RNA was extracted from adult flies with each genotype using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNA synthesis from the total RNA was carried out using a PrimeScript II High Fidelity RT-PCR kit (Takara, Shiga, Japan) with random primers. qRT-PCR was performed using FastStart Essential DNA Green Master (Roche,
Mannheim, Germany) and a LightCycler Nano (Roche, Basel, Switzerland). The following primers were used for amplification: FW, 5′-TGGCTATACATTTCACCAGA-3′; RV, 5′-GCTATGAACTTGCCCGATA-3′. RP49 was used as a normalization reference (Oka et al., 2015). Relative mRNA levels were quantified using LightCycler Nano software version 1.0 (Roche, Basel, Switzerland).

8-oxo-guanine immunostaining procedures and evaluation Anti-8-oxo-guanine immunostaining of adult midguts was largely performed as described by Park et al. (2012). We observed the surface layers of epithelial lumen cells in the posterior region using a confocal microscope. Three-day-old adult males were kept for 16 h on instant medium containing 10 mM paraquat after a 2-h starvation period. Midguts dissected from the adults were treated with 20 μg/ml DNase-free RNase (Nippongene, Tokyo, Japan) for 30 min at 37 °C as described in Yamaguchi et al. (2006). Subsequently, they were fixed with Carnoy’s solution and then incubated in 2 N HCl for 2 min. After successive washing, the fixed samples were incubated with anti-8-oxo-guanine antibody (Japan Institute for the Control of Aging, Fukuroi, Japan) as a primary antibody and then with anti-mouse IgG conjugated with Alexa594 (Invitrogen, Carlsbad, CA, USA), and mounted with Vectashield (Vector Laboratories, Burlingame, CA, USA). Samples were observed with an Olympus laser scanning confocal microscope (Fv10i, Olympus, Tokyo, Japan). Brightness and contrast of entire images were adjusted using FV10i software. For the quantitative analysis of 8-oxo-guanine-positive cells, images were processed by Photoshop. The numbers of epithelial cells were counted in a 210 × 210 μm² region of the posterior midgut and cells positive for anti-8-oxo-guanine immunostaining in the images were judged using ImageJ software (http://rsweb.nih.gov/ij/download.html). For each genotype, midguts from more than 10 individual flies were examined and at least 1,000 nuclei were observed in total.

Statistical analysis Statistical analyses were performed with Excel (Microsoft, Redmond, WA, USA) and p-values were calculated with Student’s t-tests or the log-rank test.

RESULTS AND DISCUSSION

Reduced expression of Drosophila Ogg1 in transposon-induced mutants and in flies depleted of Ogg1 mRNA by induced dsRNA expression To examine whether the D. melanogaster gene CG1795, encoding an Ogg1 ortholog, is required for the removal of oxidized guanine bases in the DNA repair process, we examined the accumulation of 8-oxo-guanines in transposon-induced mutants of the Ogg1 gene. For the two alleles, Ogg1Δ13 and Ogg1Δ226, the PiggyBac transposon PBac[w+MC] = WH (PBac(WH)) was inserted into the 5′-UTR sequences (Thibault et al., 2004). The Ogg1Δ13 insertion was integrated 277 bp upstream of the ATG codon (see Fig. 1A; http://flybase.org). The insertion in Ogg1Δ226 was integrated 13 bp upstream of Ogg1Δ226. We determined the orientation of these insertions by PCR using the DNA sequences of the Ogg1 gene and PBac[w] as primers. Both insertions were integrated within the gene in the same transcriptional orientation as the UAS sequences (Fig. 1A). Our quantitative real-time PCR (qRT-PCR) analysis revealed that Ogg1 mRNA expression was less than 60% of the level observed in the controls for both insertional mutants (Fig. 1B). Next, we further examined whether the Ogg1 mRNA was efficiently depleted by ubiquitous expression of dsRNA comprising Ogg1 mRNA and its complement. Induced expression of two different dsRNA sequences using UAS-Ogg1RNAiHMC03426 and UAS-Ogg1RNAiHMC02129 (Thibault et al., 2004). The Ogg1 mRNA expression level was reduced to 45.3% and 70.3%, respectively, relative to the normal control level (∼Actin5C-Gal4/+, Fig. 1C). Ogg1 mRNA was successfully depleted by induced expression of these dsRNAs.

Adult flies with reduced Ogg1 mRNA exhibit hyper-accumulation of 8-oxo-guanines in midgut cells after paraquat feeding Having confirmed a decrease in Ogg1 mRNA in the Ogg1 mutants as well as in flies depleted of Ogg1 mRNA, we next examined whether the number of epithelial cells containing oxidized DNA bases increased in adult midguts after paraquat feeding. We examined the presence of 8-oxo-guanines in posterior midgut cells from adult males after 16 h of feeding on diet containing 10 mM paraquat, because the midgut epithelial cells are exposed earlier to chemicals contained in the diet and continue direct contact with them for a long time. We selected adult males that ingested paraquat based on the presence of a blue dye contained in the diet as a marker.

After anti-8-oxo-guanine immunostaining of the adult midguts, we observed a layer of epithelial lumen cells at the posterior region of the midgut using a confocal microscope and examined at least 1,200 midgut epithelial cells from more than 10 individual flies (Fig. 2). In control flies (w1118), 38.6% of 2,884 midgut epithelial cells (n = 14 flies) in total were judged as 8-oxo-guanine-positive (Fig. 2, A and E). On the other hand, we scored positive cells more frequently (60.4% of 1,782 cells) in epithelial cells from Ogg1Δ13 mutant males (n = 11 flies) (Fig. 2, B and E). The frequency in the mutants was significantly higher than the control flies (p < 0.01). A statistically significant increase in the frequency of positive cells (58.3% of 2,226 cells, n = 13 flies) was also found in the Ogg1Δ226 mutant males (p < 0.01). In Ogg1 mRNA-depleted male flies (Actin5C-Gal4/UAS-Ogg1RNAiHMC03426) (Fig. 2, D and F) and Actin5C-Gal4/UAS-Ogg1RNAiHMC02129 (Fig. 2F),
the 8-oxo-guanine-positive cells were also scored more frequently (65.5% of 3,346 cells (n = 18 flies) and 70.7% of 3,762 cells (n = 16 flies), respectively), compared to the controls (Actin5C-Gal4/+/Actin5C-Gal4/UAS-Ogg1RNAiHMC03426 (31.2% of 1,934 cells, n = 11 flies, Fig. 2F). Ogg1f01384/UAS-Ogg1RNAiHMC03426 (38.5% of 1,549 cells, n = 10 flies, Fig. 2F)). The p-value indicating statistical significance was less than 0.01 in every case. These genetic data indicate that reduced expression of the Ogg1 gene resulted in additional accumulation of 8-oxo-guanines in midgut epithelial cells under oxidative stress.

Adult flies with reduced Ogg1 expression fail to show increased sensitivity to paraquat. Next, we investigated whether adult flies with reduced Ogg1 expression and additional accumulation of 8-oxo-guanines exhibit hypersensitivity to paraquat. We selected the flies (Actin5C-Gal4/UAS-Ogg1RNAiHMC03426) in which Ogg1 mRNA was depleted most efficiently and performed a paraquat sensitivity assay to examine the survival rate of male flies fed on a diet supplemented with 10 mM paraquat. As shown in Fig. 3, the viability curve of Ogg1-depleted male flies was indistinguishable from that of the controls (Actin5C-Gal4/+/Actin5C-Gal4/UAS-Ogg1RNAiHMC03426 and Actin5C-Gal4/UAS-Ogg1RNAiHMC03426) under oxidative stress. Both Ogg1 mutants, Ogg1f01384 and Ogg1f08013, showed a longer lifespan than the Ogg1 mRNA-depleted flies or the controls (data not shown). Neither the Ogg1 mutants nor the Ogg1 mRNA-depleted flies showed hypersensitivity to paraquat. The 30–55% reduction of Ogg1 expression may have been insufficient for a shift to paraquat hypersensitivity. Alternatively, it has been reported that ribosomal protein S3 (dRps3) also has 8-oxo-guanine repair activity (Yacoub et al., 1996; Cappelli et al., 2003). The dRps3 activity may be sufficient to repair the 8-oxo-guanine in midguts with reduced Ogg1 expression. It will be of interest to examine genetic interaction between Ogg1 and dRps3 in the repair of oxidative DNA damage in a future study.
Fig. 2. Immunodetection of 8-oxo-guanines accumulated in posterior midgut cells of Ogg1 mutants and adults with depleted Ogg1 mRNA after paraquat feeding. (A)–(D) Typical confocal images of epithelial cells from the posterior midgut region immunostained with anti-8-oxo-guanine antibody in Ogg1 mutant adult males and adult males with depleted Ogg1 mRNA after 16 h of feeding on food containing 10 mM paraquat. (A, A') Control epithelial midgut cells from a w1118 male, stained with DAPI (A) or anti-8-oxo-guanine antibody (A'). (B, B') Midgut cells from an Ogg1f01384/Y fly, stained with DAPI (B) or antibody (B'). (C, C') Control midgut cells from a UAS-Ogg1RNAiHMC03426/+ adult, stained with DAPI (C) or antibody (C'). (D, D') Ogg1 mRNA-depleted midgut cells from an Actin5C-Gal4/UAS-Ogg1RNAiHMC03426 adult, stained with DAPI (D) or antibody (D'). (E, F) Frequencies of epithelial midgut cells stained with the antibody after paraquat feeding. Midgut cells having 8-oxo-guanine accumulation in their nuclei significantly increased in the Ogg1 mutants (E) and in the Ogg1 mRNA-depleted adults (F). Error bars represent the standard deviation. *, p < 0.01 (Student's t-test). Scale bar, 10 μm.

Fig. 3. Paraquat sensitivity assays of Ogg1 mutant adults and adults with depleted Ogg1 mRNA. Lifespan curves of adult males depleted of Ogg1 mRNA (Actin5C-Gal4/UAS-Ogg1RNAiHMC03426), and two control males (Actin5C-Gal4/+ and UAS-Ogg1RNAiHMC03426/+). These adults were collected within 24 h after eclosion and reared on a diet containing 10 mM paraquat immediately after they were collected.
Overexpression of *Drosophila* Ogg1 using PBac{WH} insertions possessing UAS sequences and a Gal4 driver  

As described above, we showed by PCR analysis that PBac{WH} insertions in Ogg1<sup>f01384</sup> and Ogg1<sup>f08013</sup> mutants were integrated into the 5'-UTR of the gene in the same transcriptional orientation as the UAS. Because PBac{WH} possesses 14 repeats of the UAS sequence, these two alleles may allow us to induce overexpression dependent on the Gal4 transcription factor. In fact, we confirmed a 36-fold increase of Ogg1 mRNA in Ogg1<sup>f01384 /Y;Actin5C-Gal4/+</sup> adult flies by qRT-PCR, compared with the control. We also observed a 39-fold increase in Ogg1 mRNA in adult Ogg1<sup>f08013 /Y;Actin5C-Gal4/+</sup> flies (Fig. 4). We have failed to detect *Drosophila* Ogg1 ortholog polypeptide in western blot experiments using three independent Ogg1 antibodies against the human OGG1 ortholog (orb5706 (Biorbyt, Cambridge, UK), ab135940 (Abcam, Cambridge, UK), SC-376935 (Santa Cruz Biotechnology, Dallas, TX, USA)). It will be important to confirm the modified gene expression of Ogg1 described here at the protein level in our future study, and production of a specific antibody against the *Drosophila* Ogg1 ortholog is currently underway.

**Ogg1 overexpression decreases accumulation of 8-oxo-guanines in Drosophila midgut cell nuclei after paraquat feeding**  

Next, we further examined whether nuclear accumulation of 8-oxo-guanines is suppressed in adults overexpressing Ogg1. As described above, we carried out anti-8-oxo-guanine immunostaining of adult midguts for controls and flies with Ogg1 overexpression (Ogg1<sup>f01384/Y;Actin5C-Gal4/+ and Ogg1<sup>f08013/Y;Actin5C-Gal4/+</sup>) after 10 mM paraquat feeding (Fig. 5). In control flies (Actin5C-Gal4/+, Fig. 5, A and D), 8-oxo-guanine-positive cells were observed in 40.3% of total epithelial cells examined (1,269 cells, n = 10 flies). However, in Ogg1<sup>f01384/Y;Actin5C-Gal4/+</sup> flies, positive cells comprised only 13.3% of the total epithelial midgut cells (1,729 cells, n = 10 flies) (Fig. 5, B and D). The same result was also obtained in Ogg1<sup>f08013/Y;Actin5C-Gal4/+</sup> flies (Fig. 5, C and D), in which 8-oxo-guanine-positive cells appeared less frequently (13.2%) in the total epithelial cells examined (1,633 cells, n = 10 flies). In both cases, 8-oxo-guanine accumulation in midgut epithelial cells was significantly lower than with Actin5C-Gal4/+ (p < 0.01) as well as with Ogg1<sup>f01384/Y</sup> and Ogg1<sup>f08013/Y</sup> (p < 0.01). These genetic data indicated that Ogg1 overexpression suppresses the accumulation of 8-oxo-guanines in *Drosophila* adult midgut cells after paraquat feeding. Taken together with data from adults with reduced Ogg1 expression, these results lead us to conclude that CG1795 encoding *Drosophila* Ogg1 has an essential role in the suppression of oxidized guanines generated by oxidative stress, as reported in *E. coli*, budding yeast and mice (Michaels et al., 1992; Grollman and Moriya, 1993; Nash et al., 1996; van der Kemp et al., 1996; Girard et al., 1997; Klungland et al., 1999; Minowa et al., 2000).

**Adult flies with Ogg1 overexpression show significantly extended lifespan and resistance to paraquat**  

We further examined whether overexpression of the *Drosophila* Ogg1 ortholog would lead to adult lifespan extension in normal feeding conditions, i.e., without paraquat. Interestingly, Ogg1<sup>f01384/Y;Actin5C-Gal4/+</sup> adult males with 36-fold higher Ogg1 expression showed a significantly extended lifespan, compared with Ogg1<sup>f01384/Y</sup> males (p < 0.01, log-rank test) and with Actin5C-Gal4/+ males (p < 0.01, log-rank test) (Fig. 6A). We obtained essentially the same results in experiments using another PBac{WH} insertion allele, Ogg1<sup>f08013/Y</sup> (Fig. 6B). The Ogg1<sup>f08013/Y;Actin5C-Gal4/+</sup> adult males with 39-fold overexpression of Ogg1 showed a significantly extended lifespan, compared with Ogg1<sup>f08013/Y</sup> males (p < 0.01, log-rank test) and with Actin5C-Gal4/+ males (p < 0.01, log-rank test). Furthermore, we investigated whether the overexpression of Ogg1 could confer resistance against paraquat on adult flies. Ogg1<sup>f01384/Y;Actin5C-Gal4/+</sup> adult males showed a significantly longer lifespan even for diets containing 10 mM paraquat, compared with Ogg1<sup>f01384/Y</sup> males (p < 0.01, log-rank test) and with Actin5C-Gal4/+ males (p < 0.01, log-rank test) (Fig. 6C). We also obtained a similar result in experiments using another PBac{WH} allele, Ogg1<sup>f08013</sup>, although the observed lifespan extension under oxidative stress was less...
Role of Ogg1 against oxidative stress in *Drosophila*

remarkable (Fig. 6D). However, the lifespan extension in *Ogg1*/*Y;Actin5C-Gal4/+ adult males was statistically significant, compared with *Actin5C-Gal4/+* males (p < 0.05, log-rank test) (Fig. 6D).

As described above, it is reasonable to conclude that the *Drosophila* Ogg1 ortholog is required for the suppression
of 8-oxo-guanines generated by oxidative stress. Furthermore, we observed that adult flies with Ogg1 overexpression showed the acquisition of resistance to paraquat and lifespan extension in Drosophila adults. These phenotypes are probably a consequence of the efficient removal of 8-oxo-guanines in Drosophila adults, although we could not absolutely exclude the possibility that the lifespan extension and the paraquat resistance could result from a different genetic background. Several examples of gene overexpression resulting in lifespan extension in Drosophila adults have been reported. For example, overexpression of antioxidant enzymes such as SOD1, SOD2 and catalase caused lifespan extension (Orr and Sohal, 1994; Sun and Tower, 1999; Parkes et al., 1998; Sun et al., 2002). Overexpression of a Drosophila homolog of Sirtuin 2 (Sir2) (NAD+-dependent protein deacetylase) in fat bodies also led to an extended lifespan (Banerjee et al., 2012; Hoffmann et al., 2013). Although Sir2 orthologs are considered to be involved in the determination of lifespan in other organisms (Kaeberlein et al., 1999; Tissenbaum and Guarente, 2002), the ubiquitous overexpression of Drosophila Sir2 failed to extend the Drosophila adult lifespan (Burnett et al., 2011). However, its role in Drosophila lifespan determination should be reassessed. Overexpression of fatty-acid β oxidation-related genes can extend the lifespan of flies in a dietary restriction-related manner, mediated by an increased stress tolerance to oxidation and starvation (Lee et al., 2012). Moreover, overexpression of a protein repair methyltransferase that plays a role in restoring protein damage has an effect on lifespan (Chavous et al., 2001). However, it is still unknown whether overexpression of DNA repair enzymes would have a similar effect on adult lifespan in Drosophila. In this study, we first suggested that overexpression of a DNA glycosylase, Ogg1, resulted in hyper-resistance to oxidative stress and adult lifespan extension in Drosophila adults. ROS generated in the process of ATP production play a major role in oxidative DNA damage (Cadenas and Davies, 2000; Balaban et al., 2005). There is a well-known hypothesis that ROS contribute to the onset and development of many diseases such as obesity and diabetes, as well as aging (Harman, 1956, 1972; Pryor, 1987; Ames, 1989). It is possible to infer that the increased resistance to oxidative stress and the extended lifespan would be a consequence of enhanced activity to remove 8-oxo-guanines from mitochondrial and/or genomic DNA. Our results are consistent with the hypothesis that the accumulation of oxidative DNA damage is a primary cause of the senescence of organisms.

CONCLUSIONS

We showed in this study that Ogg1 mRNA levels decreased by 30–55% in Ogg1 mutants and in mRNA depletion experiments. In addition, we induced approximately 40-fold overexpression of the gene using the Gal4/ UAS system in Drosophila. Based on the positive correlation between the amounts of 8-oxo-guanines that accumulated and the level of Ogg1 expression, we could conclude that the Drosophila Ogg1 ortholog CG1795 plays an essential role in the suppression of oxidized guanine bases, consistent with previous results in other organisms. Adult flies with Ogg1 overexpression showed a resistance against oxidative stress by paraquat feeding and a significantly longer lifespan. These results are consistent with the hypothesis that oxidative DNA damage by ROS accumulation is a major contributor to senescence.

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REFERENCES


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