***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 oxidative 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;

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Sakumi et al., 2003). It was shown that the Ogg1 ortholog in human cells is a major enzyme to repair 8-oxo-guanine 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-oxo-guanines 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; Mao et al., 2007; Nakabeppu et al., 2007; Paz-Elizur et al., 2008; Okasaka et al., 2009; Sampath et al., 2012). However, genetic analyses that verify the relationship between *Ogg1* expression and diseases using animal models remain challenging tasks.

Drosophila melanogaster has been the subject of intensive genetic analyses investigating the role of genes implicated in various biological phenomena (Pandey and Nichols, 2011; Prüßing et al., 2013). *Drosophila* also possesses an Ogg1 ortholog, which excises 8-oxo-guanines from γ -irradiated DNA *in vitro*. It is expressed at a low level in proliferative cells (Dherin et al., 2000). The *Ogg1/CG1795* gene was also identified from a genome-wide RNAi screen as a candidate gene involved in sensitivity to methyl methanesulfonate-induced DNA damage (Ravi et al., 2009). However, because neither genetic nor developmental analyses using *Ogg1* mutants or RNAi flies have yet been performed (see Flybase, <http://flybase.org>), it is unclear whether the enzyme plays an important role in the DNA repair of oxidative damage after the incorporation of exogenous oxidants or via the ROS produced during intracellular metabolic processes.

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 overexpression of *Ogg1* in adults using the Gal4/UAS system, we observed significantly less accumulation of 8-oxo-guanine. 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-Ogg1RNAi^{HMC03426}}* (*UAS-Ogg1RNAi^{HMC03426}*) and *P{UAS-Ogg1RNAi^{kk101291}}* (*UAS-Ogg1RNAi^{kk101291}*) 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{WH}Ogg1^{f01384}* (*Ogg1^{f01384}*) and *PB{WH}Ogg1^{f08013}* (*Ogg1^{f08013}*) 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-Ogg1RNAi^{HMC03426}/+*, *UAS-Ogg1RNAi^{kk101291}/+*) were generated from each outcross to *w¹¹¹⁸*. Flies depleted of *Ogg1* mRNA were obtained from a cross between *Actin5C-Gal4* and *UAS-Ogg1RNAi^{HMC03426}* or *UAS-Ogg1RNAi^{kk101291}*. Males overexpressing *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 paraquat feeding assay, *Ogg1* mutant flies and flies depleted of or overexpressing *Ogg1* mRNA were reared on instant medium containing 10 mM paraquat (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'-TGCGTATACATTTCCACGA-3'; RV, 5'-GCTATGAACTTGGCCCGATA-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://rsbweb.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, *Ogg1*^{f01384} and *Ogg1*^{f08013}, the PiggyBac transposon *PBac*[*w*+*mC*] =

WH] (*PBac*(*WH*)) was inserted into the 5'-UTR sequences (Thibault et al., 2004). The *Ogg1*^{f08013} insertion was integrated 277 bp upstream of the ATG codon (see Fig. 1A; <http://flybase.org>). The insertion in *Ogg1*^{f01384} was integrated 13 bp upstream of *Ogg1*^{f08013}. We determined the orientation of these insertions by PCR using the DNA sequences of the *Ogg1* gene and *PBac*(*WH*) 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-Ogg1RNAi*^{HMC03426} and *UAS-Ogg1RNAi*^{kk101291} was carried out. 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 (*w*¹¹¹⁸), 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 *Ogg1*^{f01384} 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 *Ogg1*^{f08013} mutant males (*p* < 0.01). In *Ogg1* mRNA-depleted male flies (*Actin5C-Gal4/UAS-Ogg1RNAi*^{HMC03426} (Fig. 2, D and F) and *Actin5C-Gal4/UAS-Ogg1RNAi*^{kk101291} (Fig. 2F)),

Fig. 1. Insertional mutations of a gene encoding *Drosophila* Ogg1 ortholog and reduction of *Ogg1* mRNA in mutants and in flies expressing *Ogg1* dsRNAs. (A) Genomic map of two *pBac{WH}* insertional mutations in the *Ogg1* locus. Gray bars represent the 5'- or 3'-UTR of *Ogg1*. Black bars are coding exons. Open bars show the surrounding genes. Both insertions carrying UAS sequences are integrated into the 5'-UTR region of the gene in the same orientation with respect to *Ogg1* transcription. (B) Relative mRNA level of *Ogg1* mRNA from adult males of two insertional mutants (*Ogg1*^{f01384}/Y and *Ogg1*^{f08013}/Y) was calculated on the basis of real-time qRT-PCR analysis. (C) Relative mRNA level of *Ogg1* mRNA from males inducibly expressing dsRNAs for *Ogg1* (*Actin5C-Gal4/UAS-Ogg1RNAi*^{HMC03426} and *Actin5C-Gal4/UAS-Ogg1RNAi*^{kk101291}) was calculated on the basis of real-time qRT-PCR analysis. Data are represented as averages of three independent experiments and error bars represent the standard deviation.

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/+* (40.3% of 1,269 cells, $n = 10$, Fig. 2F), *UAS-Ogg1RNAi*^{HMC03426} (31.2% of 1,934 cells, $n = 11$ flies, Fig. 2, C and F), *UAS-Ogg1RNAi*^{kk101291} (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-Ogg1RNAi*^{HMC03426}) 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/+* and *UAS-Ogg1RNAi*^{HMC03426/+}) under oxidative stress. Both *Ogg1* mutants, *Ogg1*^{f01384} and *Ogg1*^{f08013}, 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 *Ogg1^{f01384}/Y* fly, stained with DAPI (B) or antibody (B'). (C, C') Control midgut cells from a *UAS-Ogg1RNAi^{HMC03426}/+* adult, stained with DAPI (C) or antibody (C'). (D, D') *Ogg1* mRNA-depleted midgut cells from an *Actin5C-Gal4/UAS-Ogg1RNAi^{HMC03426}* 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 μ m.

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-Ogg1RNAi^{HMC03426}*), and two control males (*Actin5C-Gal4/+* and *UAS-Ogg1RNAi^{HMC03426}/+*). 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*^{f01384} and *Ogg1*^{f08013} 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*^{f01384}/*Y*; *Actin5C-Gal4*/+ adult flies by qRT-PCR, compared with the control. We also observed a 39-fold increase in *Ogg1* mRNA in adult *Ogg1*^{f08013}/*Y*; *Actin5C-Gal4*/+ 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* overex-

Fig. 4. Overexpression of *Ogg1* mRNA in mutants carrying *PB{WH}* insertions that possess UAS sequences and *Actin5C-Gal4*. qRT-PCR experiments were carried out to quantify *Ogg1* mRNA expression in adult males (*Ogg1*^{f01384}/*Y*; *Actin5C-Gal4*/+ and *Ogg1*^{f08013}/*Y*; *Actin5C-Gal4*/+). Overexpression of 36- and 39-fold was observed in these adult males, compared with the controls carrying *Actin5C-Gal4* alone. Data are represented as averages of three independent experiments and error bars represent the standard deviation.

pression (*Ogg1*^{f01384}/*Y*; *Actin5C-Gal4*/+ and *Ogg1*^{f08013}/*Y*; *Actin5C-Gal4*/+) 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*^{f01384}/*Y*; *Actin5C-Gal4*/+ 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*^{f08013}/*Y*; *Actin5C-Gal4*/+ 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*^{f01384}/*Y* and *Ogg1*^{f08013}/*Y* (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*^{f01384}/*Y*; *Actin5C-Gal4*/+ adult males with 36-fold higher *Ogg1* expression showed a significantly extended lifespan, compared with *Ogg1*^{f01384}/*Y* 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 *PB{WH}* insertion allele, *Ogg1*^{f08013}/*Y* (Fig. 6B). The *Ogg1*^{f08013}/*Y*; *Actin5C-Gal4*/+ adult males with 39-fold overexpression of *Ogg1* showed a significantly extended lifespan, compared with *Ogg1*^{f08013}/*Y* 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*^{f01384}/*Y*; *Actin5C-Gal4*/+ adult males showed a significantly longer lifespan even for diets containing 10 mM paraquat, compared with *Ogg1*^{f01384}/*Y* 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*^{f08013}, although the observed lifespan extension under oxidative stress was less

Fig. 5. Immunodetection of 8-oxo-guanines in posterior midgut cells from adults with *Ogg1* overexpression after paraquat feeding. (A)–(C) Typical confocal images of epithelial cells from the posterior midgut region immunostained with anti-8-oxo-guanine antibody in control adult males and males with *Ogg1* overexpression after 16 h of feeding on food containing 10 mM paraquat. (A, A') Control epithelial midgut cells from an *Actin5C-Gal4/+* male, stained with DAPI (A) and with anti-8-oxo-guanine antibody (A'). (B, B') Midgut cells from an adult fly with *Ogg1* overexpression (*Ogg1^{f01384}/Y; Actin5C-Gal4/+*), stained with DAPI (B) and with the antibody (B'). (C, C') Midgut cells from an adult fly with *Ogg1* overexpression (*Ogg1^{f08013}/Y; Actin5C-Gal4/+*), stained with DAPI (C) and with the antibody (C'). (D) The frequencies of epithelial midgut cells stained with the antibody after paraquat feeding. Note that the frequency of midgut cells containing 8-oxo-guanines in their nuclei significantly decreased in adults with *Ogg1* overexpression. Error bars represent the standard deviation. *, $p < 0.01$ (Student's *t*-test). Scale bar, 10 μ m.

Fig. 6. Paraquat sensitivity assays and lifespan assays of adult males with *Ogg1* overexpression. (A, B) Lifespan curves of adult males with *Ogg1* overexpression in normal feeding conditions without paraquat. *Ogg1* overexpression was performed in *Ogg1^{f01384}/Y; Actin5C-Gal4/+* (A) and in *Ogg1^{f08013}/Y; Actin5C-Gal4/+* (B). (C, D) Lifespan curves of adult males fed a diet containing 10 mM paraquat. *Ogg1* overexpression was performed in *Ogg1^{f01384}/Y; Actin5C-Gal4/+* (C) and in *Ogg1^{f08013}/Y; Actin5C-Gal4/+* (D).

remarkable (Fig. 6D). However, the lifespan extension in *Ogg1^{f08013}/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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