Alkaline Ceramidase Mediates the Oxidative Stress Response in *Drosophila melanogaster* Through Sphingosine

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Abstract

Alkaline ceramidase (*Dacer*) in *Drosophila melanogaster* was demonstrated to be resistant to paraquat-induced oxidative stress. However, the underlying mechanism for this resistance remained unclear. Here, we showed that sphingosine feeding triggered the accumulation of hydrogen peroxide (*H*<sub>2</sub>*O*<sub>2</sub>). *Dacer*-deficient *D. melanogaster* (*Dacer* mutant) has higher catalase (CAT) activity and CAT transcription level, leading to higher resistance to oxidative stress induced by paraquat. By performing a quantitative proteomic analysis, we identified 79 differentially expressed proteins in comparing *Dacer* mutant to wild type. Three oxidoreductases, including two cytochrome P450 (*CG3060, CG9438*) and an oxoglutarate/iron-dependent dioxygenase (*CG17807*), were most significantly upregulated in *Dacer* mutant. We presumed that altered antioxidative activity in *Dacer* mutant might be responsible for increased oxidative stress resistance. Our work provides a novel insight into the oxidative antistress response in *D. melanogaster*.

Key words: ceramidase, *Dacer*, antioxidative, catalase, sphingosine

Generation of reactive oxygen species (ROS), including superoxide anion (*O*<sub>2</sub><sup>-</sup>), hydrogen peroxide (*H*<sub>2</sub>*O*<sub>2</sub>), and hydroxyl radical (*HO*<sup>-</sup>), might be a result of cellular mitochondrial oxidative metabolism or exogenous xenobiotic application (Ray et al. 2012). ROS overproduction leads to oxidative stresses, which is harmful to homeostasis by directly damaging DNA, protein, and lipids or dysregulating signaling pathways. For example, ROS overproduction in human beings caused a variety of pathological consequences, such as atherosclerosis (Singh and Jialal 2006), diabetes (Piconi et al. 2003), pulmonary fibrosis (Cheresh et al. 2013), neurodegenerative disorders (Pitocco et al. 2013), and arthritis (Wruck et al. 2011). Neutral ceramidase is involved in the stress responses in brown planthopper (*Nilaparvata lugens* (Stål)) (Shi et al. 2018) and alkaline ceramidase in *Drosophila melanogaster* associated to antioxidative stress capacity (Yang et al. 2010). To protect themselves against harmful ROS, organisms increase ROS cleavage through upregulating the expression of antioxidants, which in turn neutralize the ROS and maintain ROS homeostasis (Pham-Huy et al. 2008). To date, a variety of ROS-scavenging molecules have been identified. The superoxide dismutase (SOD) scavenges the *O*<sub>2</sub><sup>-</sup> and produces *H*<sub>2</sub>*O*<sub>2</sub> (Buettner 2011); catalase (CAT) and glutathione peroxidase (GPX) detoxify *H*<sub>2</sub>*O*<sub>2</sub> and form *H*<sub>2</sub>*O* (Winterbourn 2013). Additionally, non-enzymatic molecules such as glutathione, peroxiredoxin, and flavonoids (Vanderauwera et al. 2011) are built up to overcome the injury of the oxidative stress (Nikolova-Karakashian and Reid 2011).

Sphingolipids are important components of eukaryotic cell membranes. Dysregulation of sphingolipid metabolism can trigger the generation of ROS, thereby leading to oxidative stress in mammalian cells and animal models (Andreiu-Abadie et al. 2001, Apel and Hirt 2004). For examples, ceramides have been shown to regulate cellular redox homeostasis through regulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Zhang et al. 2003). Sphingosine (SPH) or its natural analogue dihydroSPH has been shown to increase ROS production in various species (Abrahant et al. 2010). On the other hand, the oxidative stress in turn increase the production of ceramides (Roy et al. 2013, Zigdon et al. 2013, Facho et al. 2017). For example, treatment of GSH or *H*<sub>2</sub>*O*<sub>2</sub> in human cells regulated SPH-recycling pathway and promote SPH generation (Sultan et al. 2006). Some sphingolipid metabolites, such as ceramides and SPH, also act as signaling molecules to mediate various biological processes (Jeffries and Krupenko 2018). These observations suggested that sphingolipid metabolism and the oxidative antistress response are intimately interconnected.

Although SPH has been implicated to involve in the oxidative antistress response, several questions remained to be resolved. These
included the regulation mechanism of SPH in response to oxidative stress, the ROS-producing pathway associated with endogenous SPH, and the roles of Dacer in ROS regulation. In many species, ceramides are hydrolyzed to form SPH in the presence of ceramidases (Mao et al. 2003). Five ceramidase genes have been identified in mice or humans, including one acid, one neutral, and three alkaline ceramidases. In *D. melanogaster*, two ceramidases were identified, including one neutral ceramidase (nCDase) (Yoshimura et al. 2002) and one alkaline ceramidase (Dacer) (Yang et al. 2010). In previous work, we demonstrated that knockout of Dacer rendered *D. melanogaster* resistant to oxidative stress that induced by paraquat. However, the underlying mechanism remained unclear.

In this study, we demonstrated that SPH feeding triggered the H2O2 formation. Dacer mutant has higher CAT activity and CAT transcription level, leading to higher resistance to oxidative stress induced by paraquat. Furthermore, we unveiled the potential regulated target proteins and metabolic pathways by proteome analysis.

**Methods**

**Stock Maintenance**

*D. melanogaster* wild-type (WT) *w1118* and Dacer mutant *bwae02081* (BL-18012, Dacer) lines were obtained from the Bloomington *Drosophila* Stock Center (Indiana University, Bloomington, IN). In the *bwae02081* mutant, the Dacer gene was inactivated by a P-element insertion on the left of the second chromosome. The fly stocks were maintained on standard cornmeal medium at 25°C on a 12:12 h light: dark cycle at constant humidity. Standard corn meal medium contained 8.6% sucrose, 1% yeast, 11.3% corn flour, 1% agar, and 1% propionic acid.

**CAT Activity**

Day 3 adult flies were transferred to cornmeal medium containing 10 mmol/L paraquat for CAT activity detection. After feeding for 24 h, approximately 20 mg flies were used for each replication and homogenized in 100 μl lysis buffer from the kit with 1% Triton. Supernatant of the homogenization was transferred to a new test tube that contains RNA was transferred to a new tube, then 0.5 ml of chloroform were added to 50 mg flies. The mixture was centrifuged at 7,500 × g for 5 min at 4°C. The RNA sample was dried and dissolved in an appropriate amount of diethyl pyrocarbonate (DEPC) water. RNA concentration was measured by NanoDrop. The RNA samples were reverse-transcribed to cDNAs using the PrimeScript RT reagent kit. cDNAs were then analyzed by quantitative PCR (qPCR) with SYBR Premix Ex TaqTM GC. The PCR was subjected to amplification in a PCR apparatus under the following conditions: 98°C for 10 s, 55°C for 30 s, 72°C for 1 min, and 30 cycles. All qPCR reagents were purchased from Takara (Takara, Kusatsu, Shiga Prefecture, Japan). All tests were done in duplicate. Primers were used: CAT, qRT-F: ATGGCTTGGACCGCATGCCG, qRT-R: GCTCCATTGCGGTGGTA; β-actin, qRT-F: TGGAAGTGGACAGGGAG.

**H2O2 Measurement**

Day 3 WT adult fruit flies were maintained on 1% agar containing 0.01 M C14-SPH. Ethanol was used as a control for 12 h before H2O2 levels were measured. Every 12 h, H2O2 was recorded till the 60th hour. H2O2 Assay Kit (Beyotime Biotechnology, Shanghai) was used to assay the H2O2 concentration. The H2O2 concentration determination was achieved by the oxidation of divalent iron ions. Their oxidation produced ferric ions and formed a purple product with xylene orange in a particular solution. Tissue samples were homogenized at a ratio of 100 μl in lysis buffer from the kit per 5 mg of tissue. The lysis buffer could be substituted by phosphate-buffered saline (PBS, containing 135 mM NaCl, 4.7 mM KCl, 10 mM Na2HPO4, 2 mM NaH2PO4, pH 7.4) buffer. The samples were centrifuged at about 12,000 g for 5 min at 4°C and the supernatant was used for H2O2 measurement. All of the above operations need to be performed at 4°C or on ice. All tests were done in triplicate. H2O2 detection reagent was thawed on ice or on ice water bath. The test was performed using 50 μl of samples and standard curve was set up by 50 μl of 1, 3, 10, 30, and 100 μM H2O2 standard solution. The absorbance at 560 nm was recorded on a uQuantMicroplate spectrophotometer (Biotek Instruments), and the concentration of H2O2 in the sample was calculated based on the standard curve.

**Proteomics Sample Preparation**

Adult flies were collected 3 d after emergence. To extract the protein, 50 μg of the samples were lysed on ice with 500 μl of lysis buffer and sonicated for 5 min. The samples were spun at 10,000 g for 10 min at 4°C to remove debris and transferred to a new tube. The BCA assay (Thermo Scientific) was used to quantify the protein levels for tandem mass tag (TMT) labeling (Thermo Scientific). TMT was performed according to the manufacturer’s instructions. A 50 μg sample of each condition was transferred to a new tube and the volume was adjusted to 100 μl with 100 mM triethylammonium bicarbonate (TEAB) buffer came with the kit. Reducing reagent (tris[2-carboxyethyl]phosphine [TCEP]), 5 μl of 200 mM, came with the kit was added to each sample, and the sample was incubated at 55°C for 1 h. Then, 5 μl of 375 mM iodoacetamide was added to each sample, and the sample was incubated at room temperature for 30 min in the dark, to obtain an alkylated cysteine. Pre-cooled (−20°C) six volumes (about 600 μl) of acetonitrile were added for precipitation and frozen at −20°C for at least 4 h. The sample was centrifuged at 8,000 g for 10 min at 4°C, the acetonitrile was removed, and the remaining sample was air dried for 2–3 min. The sample was reconstituted in 100 μl of 100 mM TEAB. Proteolytic digestion was carried out by adding 1.25 μl trypsin to the protein. The samples were incubated overnight (≥12 h) at 37°C to complete digestion. The TMT labeling reagent was reconstituted in 41 μl of anhydrous acetonitrile for 5 min, 41 μl of each label was added to the corresponding peptide sample. The
reaction was allowed to proceed at room temperature for 1 h. Eight microliters of 5% hydroxyamine was added to each sample and incubated for 15 min to quench the labeling reaction. Samples were combined in equal amounts in new microcentrifuge tubes and stored at −80°C until mass spectrometry identification.

Proteomics Mass Spectrometry
The peptide fraction was dissolved in the sample solution (0.1% formic acid, 2% acetonitrile), vortexed thoroughly, and centrifuged at 13,200 rpm for 10 min at 4°C. The supernatant was transferred to a sample tube for mass spectrometry identification; TMT-labeled tryptic peptides were fractionated using Pierce High pH Reversed-Phase Peptide Fractionation Kit (Thermo Scientific); 300 µm i.d. × 5 mm, packed with Acclaim PepMap RPLC C18, 5 µm, 100 Å, nanoViper, and Acclaim PepMap 75 µm × 150 mm, C18, 3 µm, 100 Å were used in the assay. Mobile phase A: 0.1% formic acid, 2% ACN; mobile phase B: 0.1% formic acid, 80% ACN, for 78 min at flow rate: 300 nl/min. Data for TMT-labeled samples were generated using Thermo Scientific Q Exactive (Thermo Scientific) at MS1 resolution: 70,000, AGC target: 3e6, maximum IT: 40 ms, scan range: 350–1,800 m/z, MS2 resolution: 75,000, AGC target: 1e5, maximum IT: 60 ms, TopN:20, NCE/stepped NCE: 27. All tests were done in triplicate.

Proteomics Data Analysis
To assure biological reproducibility, protein samples in four independent experiments were prepared. TMT labels (Dacer: TMT0-126, TMT0-127N, TMT0-128N, TMT0-128C and WT: TMT0-129N, TMT0-129C, TMT0-130N, TMT0-131) were separately used to label the samples from mutant group and the WT samples. In the differentially expressed protein screening process, fold change (FC) ≥1.5 or ≤1/1.5 was used as a screening standard. The protein with FC ≥1.5 was considered to be upregulated, and the protein with FC ≤1/1.5 was considered to be downregulated. Gene Ontology (GO) database (http://www.geneontology.org/) was used in differentially expressed protein GO enrichment analysis. In the partial least squares discriminant analysis (PLS-DA) analysis, the component 1 was set as X variable and component 2 as Y variable. X variable was used for spatial projection screening with the supervision method. It is the covariance between the independent variables X and the corresponding dependent variable Y of highly multidimensional data by finding a linear subspace of the explanatory variables.

Statistical Analyses
Statistical analyses were performed using Student’s t-test; SPH (Fig. 1) using multiple t-test; both qPCR and CAT activity (Fig. 2) using unpaired t-test; and GO enrichment (Fig. 4) using Fisher’s exact test. A P value of <0.05 was considered statistically significant.

Results

Treatment With C14-SPH Induced the Generation of H2O2
Dacer is the enzyme that catalyzes the decomposition of ceramide to SPH in sphingolipid pathway. To test the hypothesis that H2O2 accumulation might be triggered by SPH, flies were fed with SPH for 12, 24, 36, 48, and 60 h, respectively, and then the H2O2 levels were tested separately. The results showed that H2O2 levels in SPH-treated flies were significantly higher than that of the control at 12, 24, and 36 h—16.61% (t = 8.944, df = 4, P < 0.05), 12.14% (t = 6.250, df = 4, P < 0.05), and 11.16% (t = 7.087, df = 4, P < 0.05), respectively (Fig. 1)—while no significant difference was observed at 48 h (t1.687, df = 4, P = 0.167) and 60 h (t = 1.268, df = 4, P = 0.274), suggesting that exogenous SPH feeding could induce H2O2 accumulation in flies.

Dacer Enhanced CAT Activity in Paraquat-Induced Oxidative Stress
To investigate if Dacer has a role in CAT regulation, we measured the CAT activity in Dacer mutants and WT flies reared in medium with and without paraquat. Paraquat treatment significantly (t = 5.658, df = 3, P < 0.05) increased the CAT activity in both Dacer mutants (comparing 9.046 ± 0.6570 with paraquat to 2.247 ± 0.2305 without paraquat) (Fig. 2B) and WT flies (comparing 5.901 ± 0.6034 with paraquat to 2.496 ± 0.3167 without paraquat), respectively (Fig. 2A).

CAT Transcript Level Is Upregulated by Oxidative Stress
We investigated the transcript level of CAT in response to paraquat stress. qPCR results demonstrated that Dacer mutants has significantly (t = 7.009, df = 2, P < 0.02) higher CAT transcript level (1.824 ± 0.05415) (Fig. 2D) than WT (1.090 ± 0.08972) (Fig. 2C). These results suggested that Dacer mutants are resistant to paraquat-induced oxidative stress, possibly because of the higher CAT mRNA levels.

Dacer Regulates Expression of Oxidative Stress Protein
To investigate the protein expression changes in Dacer mutant flies, we performed quantitative proteomic analysis based on a TMT labeling method, and a total of 3,004 proteins were identified (Supplementary Table S1). PLS-DA demonstrated that the four replicates of each fly strain were well clustered, indicating that Dacer has profound influences on D. melanogaster (Fig. 3). According to the criteria (fold change ratio 1±1.5 or ≤0.67, df = 4, P < 0.05) for defining differentially expressed proteins, 33 upregulated and 46 downregulated proteins were identified in comparing Dacer mutant to WT.
GO analysis assigned these differentially expressed proteins into three main categories (biological process, cellular component, and molecular function), with the terms ‘cellular process’, ‘cell’, and ‘catalytic activity’ played the dominant role (Fig. 4). The top five most upregulated genes included: mitochondrial amidoxime reducing component (CG1665), acyl-CoA binding protein 3 (CG8628), uncharacterized protein (CG17807), seele (CG12918), and odorant-binding protein 56d (CG11218) (Supplementary Table S2); while, the five most downregulated genes included: uncharacterized protein (CG10550), aldo-keto reductase (CG3397), WNK kinase (CG7177), and uncharacterized protein (CG11035) (Supplementary Table S2). Eight proteins associated with oxidoreductase activity were significantly altered. Among them, three proteins, uncharacterized protein (CG17807), CYP6d5 (CG3050), and CYP6a2 (CG9438), were significantly upregulated; five proteins, aldo-keto reductase (CG3397), CYP28d1 (CG10833), aldo-keto reductase (CG18547), ferric-chelate reductase (CG8399), and cytochrome c oxidase (CG44296) were significantly downregulated (Table 1). Significantly changed oxidative reductases were classified into four groups, including three cytochrome P450, two aldo-keto reductases, one oxoglutarate/iron-dependent dioxygenase, and one cytochrome c oxidase. There were 10 proteins participated in hydrolysis. It is worth mentioning that, the majority of them (9 proteins) were significantly downregulated, including serine proteases, glycoside hydrolase, and carboxylesterase (Table 2).
Discussion

We previously demonstrated that D. melanogaster deficient in Dacer was more resistant to oxidative stress-induced aging than that of WT flies, possibly because that Dacer mutant has higher H₂O₂ cleavage ability (Yang et al. 2010). However, the mechanism of Dacer-regulated H₂O₂ clearance remained unclear. In this study, we demonstrated that paraquat had less impact on oxidative stress in Dacer mutant, probably because of high CAT activity and antioxidative stress capacity.

Sphingolipids are a class of bioactive lipids that regulate diverse cell functions and may act as second messengers to increase oxidant production (Ren and Hannun 2019). Many studies have been conducted to test this theory. However, the cellular mechanism remains elusive. SPH is a long-chain base (LCB) that acts as a building block of sphingolipids. They are important lipid components of eukaryotic cell membranes (van Meer et al. 2008). In addition to the role of SPH in sphingolipid metabolism, an increasing number of studies suggested that SPH accumulated in tissues such as liver, brain, and lung under conditions of cellular stress (Nikolova-Karakashian and Reid 2011). Yang and Yuan’s previously studies have shown that Dacer mutant had lower production of H₂O₂ (Yuan et al. 2011) and lower level of SPH than that of WT (Yang et al. 2010). SPH might be responsible for higher H₂O₂ production in WT flies. Based on this assumption, we supplied SPH into flies’ diet, and found that SPH feeding significantly increased the H₂O₂ level in fruit flies (Fig. 1). SPH and ROS formed a positive feedback loop. SPH could increase the generation of ROS in different cell types in various species (Sultan et al. 2006), such as

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**Table 1.** The differentially expressed proteins in related with oxidoreductase activity

| Gene names  | Annotation                        | Fold change | P value | Molecular function                  |
|-------------|-----------------------------------|-------------|---------|-------------------------------------|
| CG17807     | Uncharacterized protein           | 2.77        | 0.003   | Methyltransferase, oxidoreductase   |
| CG3050      | Cytochrome P450, CYP6d5           | 1.74        | 0       | Monooxygenase, oxidoreductase       |
| CG9438      | Cytochrome P450, CYP6a2           | 1.59        | 0       | Monooxygenase, oxidoreductase       |
| CG44296     | Cytochrome c oxidase              | 0.63        | 0.002   | Oxidoreductase                      |
| CG8399      | Ferric-chelate reductase          | 0.62        | 0       | Oxidoreductase                      |
| CG18547     | Aldo-keto reductase               | 0.61        | 0.001   | Oxidoreductase                      |
| CG10833     | Cytochrome P450, CYP28d1          | 0.59        | 0.001   | Monooxygenase, oxidoreductase       |
| CG3397      | Aldo-keto reductase               | 0.33        | 0       | Oxidoreductase                      |
Table 2. The differentially expressed proteins in related with hydrolysis activity

| Gene names | Annotation           | Fold change | P value | Molecular function                  |
|------------|----------------------|-------------|---------|-------------------------------------|
| CG6984     | Enoyl-CoA hydratase  | 1.68        | 0       | Enoyl-CoA hydratase activity        |
| CG40160    | Serine protease      | 0.66        | 0       | Hydrolase                           |
| CG9311     | Protein-tyrosine phosphatase | 0.64     | 0       | Hydrolase activity                  |
| CG1014     | Adenosine triphosphatase | 0.61      | 0.003   | Hydrolase; motor protein            |
| CG11909    | Glycoside hydrolase  | 0.61        | 0.018   | Glycosidase; hydrolase              |
| CG8329     | Serine protease      | 0.59        | 0.02    | Hydrolase; protease                 |
| CG15739    | HAD-superfamily hydrolase | 0.56      | 0       | Hydrolase                           |
| CG18258    | Carboxylesterase     | 0.56        | 0.012   | Hydrolase                           |
| CG18444    | Serine protease      | 0.54        | 0       | Hydrolase; protease                 |
| CG32209    | Chitin deacetylase   | 0.53        | 0.001   | Glycosidase; hydrolase              |

increase in rat retina neurons in response to paraquat (Abrahán et al. 2010), inducing ROS in intestinal tract in nematode (Gao et al. 2016) and in fruit flies (Kawamura et al. 2009, Kitchen and Weis 2017). However, the mechanism for the formation of this feedback loop is unclear. Our result provides evidence that exogenous sphingolipid could trigger the generation of H$_2$O$_2$.

Sphingolipids regulate cellular redox homeostasis through regulation of NADPH oxidase, mitochondrial integrity, nitric oxide synthase, and antioxidant enzymes (Marinho et al. 2014). SPH has long been known to induce ROS production in various cell types in different species (Coursol et al. 2015). We found that CAT was activated in both WT and Dacer mutants after feeding paraquat, and Dacer mutants had significantly higher CAT than WT in the presence of paraquat (Fig. 2A and B). H$_2$O$_2$ was the major redox metabolite operative in redox sensing, signaling, and redox regulation (Marinho et al. 2014, Sies 2017). CAT is an enzyme that breaks down H$_2$O$_2$ into water and oxygen, which protects the cell from oxidative damage by ROS. These results indicated that the decreased ROS level in Dacer mutants might be the result of higher CAT activity.

We previously demonstrated that Dacer mutant reduces stress-induced ROS production (Yang et al. 2010), and now we know CAT activity increased after paraquat treatment (Fig. 2A and B). To address these issues, we investigated the transcript level of CAT in response to paraquat stress (Fig. 2C and D). However, we found that the induction of mRNA level in Dacer mutants (Fig. 2D) before and after paraquat-treated samples was not correlated with CAT activity (Fig. 2B). This might be because of the low correlation of protein expression with mRNA expression in this specific case. CAT protein could accumulate during the treatment of paraquat, but we could not capture the window that mRNA was induced. Also, CAT protein maturation and activation might be promoted during paraquat treatment. Further study was needed to clarify this protein and mRNA correlation during paraquat treatment.

SPH cannot be de novo synthesized. It is only derived from hydrolysis of ceramide via ceramidas (Mao and Obeid 2008). In D. melanogaster, two ceramidase genes have been identified: one neutral ceramidase gene (Yoshimura et al. 2002) and one Dacer (Yang et al. 2010). Dacer belongs to the alkaline ceramidase family whose members are found in various species, including mammals (Mao et al. 2001, 2003). Previous study demonstrated that there was a twofold decrease in SPH in Dacer mutant flies compared to WT and the upregulation of Dacer induced the production of SPH, and Dacer mutant has lower level of SPH (Yuan et al. 2011). Abrahán and Xu’s studies demonstrated that treatment with (1S,2R)-D-erythro-2- (N-myristoylamo)-1-phenyl-1-propanol (D-e-MAPP), a specific alkaline ceramidase inhibitor, blocked the generation of SPH in rat retina neurons in response to oxidative stress (Abrahán et al. 2010, Xu et al. 2010). This suggests that alkaline ceramidase involved in the production of SPH in response to oxidative stress in higher eukaryotes. These studies together suggest that the Dacer SPH pathway is a conserved signaling pathway that mediates the oxidative antistress response.

To investigate the mechanisms that Dacer influenced CAT expression, we performed TMT proteomic analysis to identify differentially expressed proteins between Dacer mutant and WT. The results showed that uncharacterized protein (CG17807), CYP6d5 (CG3050), and CYP6a2 (CG9438) were positively regulated in Dacer mutant (Table 1). Uncharacterized protein CG17807 is an oxoglutarate/iron-dependent dioxygenase. High iron levels promote the accumulation of sphingolipid LCBS and long-chain base phosphate (LCBP) (Chen et al. 2016, Rockfield et al. 2018). The toxicity of a high level of iron was shown to be correlated with the accumulation of LCBS and LCBP s and activation of the Pkh1-Ypk1-Smp1 protein kinase pathway that is essential for lethality (Lee et al. 2012, Dixon and Stockwell 2014).

CYP6d5 (CG3050) and CYP6a2 (CG9438) were, respectively, annotated as CYP6d5 (Le Goff et al. 2006) and CYP6a2 (Wan et al. 2014). Enzymes with the cytochrome P450 domain are a superfamily of heme-containing monoxygenases found in all living organisms and exhibits extraordinary diversity in their reaction chemistry (Giraudo et al. 2010). Previous work demonstrated that CYP6d5 and CYP6a2 could be induced by xenobiotics such as phenobarbital (Le Goff et al. 2006, Coelho et al. 2015), atrazine (Miotaa et al. 2000), and caffeine (Le Goff et al. 2006, Coelho et al. 2015). Sphingolipids are required for the induction of Cyp1a1 by 3-methylcholanthrene (Merril et al. 1999). Cyp2c11 is downregulated in response to interleukin-1b (IL-1b) and the response involves the hydrolysis of ceramide to SPH. Also ceramidase activation appears to account for the loss of expression of Cyp2c11 (Nikolova-Karakashian et al. 1997). Upregulation of these proteins in Dacer mutant might help flies confer paraquat-induced stresses, thus decreased ROS response. Those findings showed sphingolipid metabolism could influence the regulation of cytochrome P450, which suggested an unexpected relationship between sphingolipid metabolism and oxidation or reduction mechanisms.

Taken together, we found that Dacer mutant has lower level of SPH and higher CAT activity, leading to higher resistance to oxidative stress. One oxoglutarate/iron-dependent dioxygenase and two P450 are upregulated in Dacer mutant, which might be responsible for decreased H$_2$O$_2$ production. Our study provides a novel insight into the oxidative antistress response in D. melanogaster and possibly in other species. Further study should be conducted to explore the underlying mechanism of sphingolipid and oxidative stress feedback loop.
Supplementary Data
Supplementary data are available at Journal of Insect Science online.

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