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Genetic Link between Heme Oxygenase and the Signaling Pathway of DNA Damage in *Drosophila Melanogaster*

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Heme oxygenase (HO) is a rate-limiting step of heme degradation, which catalyzes the conversion of heme into biliverdin, iron, and CO. HO has been characterized in microorganisms, insects, plants, and mammals. The mammalian enzyme participates in adaptive and protective responses to oxidative stress and various inflammatory stimuli. The present study reports that eye imaginal disc-specific knockdown of the *Drosophila* HO homologue (dHO) conferred serious abnormal eye morphology in adults, resulting in the generation of reactive oxygen species and apoptosis in third-instar larvae. Oxidative stress frequently induces DNA lesions that are recognized by damage sensors, including ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia and rad3-related (ATR) proteins. The knockdown of dHO took place in G0/G1-arrested cells posterior to the morphogenetic furrow and thus prevented these cells from entering S-phase, with an increase in the level of histone H2A.V, a DNA damage marker. Moreover, the knockdown of dHO resulted in the enhancement of the rough eye phenotype in ATM-deficient flies or was lethal in ATR-deficient flies. These results indicate that dHO functions in control of the signal pathway of DNA damage. On the other hand, genetic crosses with a collection of *Drosophila* deficiency stocks allowed us to identify eight genomic regions, each deletion of which caused suppression of the rough eye phenotype induced by dHO knockdown. This information should facilitate the identification of HO regulators in *Drosophila* and clarification of the roles of HO in eye development.

Keywords: ataxia-telangiectasia mutated; DNA damage; *Drosophila melanogaster*; genetic interaction; heme oxygenase
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Introduction

Heme oxygenase (HO) catalyzes the rate-limiting step in the degradation of heme, producing biliverdin IXα, CO, and free iron in the presence of molecular oxygen and a suitable electron donor (Shibahara et al. 2002). HO is present in various organisms including microorganisms, plants, and mammals (Shibahara et al. 2002; Alam et al. 2004). Two isoforms of the mammalian HO, HO-1 and HO-2, have been identified and are encoded by different genes (Alam et al. 2004; Shibahara et al. 2007). HO-1/-2 share a highly conserved domain involved in the catalytic reaction, but the expression of HO-1 is induced by various stimuli such as stress and inflammation, and is high in the spleen and liver, while that of HO-2 is constant and abundant in the brain and testis (Alam et al. 2004; Andoh et al. 2004; Shibahara et al. 2007). The physiological importance of HO-1 was demonstrated using HO-1-deficient mice; homozygous HO-1-deficient mice showed partial prenatal lethality (Poss and Tonegawa 1997). The adult *ho-1 (−/−)* mice developed anemia associated with low serum iron levels and iron deposits in the liver and in kidney. The adult *ho-1 (−/−)* mice were more vulnerable to mortality and hepatic necrosis when exposed to endotoxin, indicating the involvement of the induction of HO-1 in inflammation against oxidative stress. The HO-2-deficient mice were fertile and survived normally, but were susceptible to hyperoxic lung damage and ejaculatory abnormality (Zakhary et al. 1997; Adachi et al. 2004). The deficiency of HO-2 was not restored by compensation with the expression of HO-1 (Shibahara et al. 2007). Thus, both HO-1 and HO-2 play important roles in physiological iron homeostasis, anti-

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inflammation, anti-oxidant, and possibly cGMP signaling pathways.

DNA damage repair and checkpoint mechanisms are critical components of normal cellular function to maintain the integrity of genomic DNA. Oxidative stress frequently induces DNA lesions, which is recognized initially by damage sensors that trigger the activation of transducing kinases including ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia and rad3-related (ATR) proteins (Hurley and Bunz 2007; Reinhardt and Yaffe 2009; Smith et al. 2010). Both ATM and ATR can phosphorylate multiple targeting proteins consisting of p53 and other DNA repair components. Furthermore, in response to DNA damage or structural modifications of chromatin, histone H2A is phosphorylated by ATM, ATR, or DNA-dependent protein kinase (Szilard et al. 2010; Firsanov et al. 2011). These phosphorylations are essential for the DNA repair process, and in turn amplify damage signals to committed cells for cell-cycle progression, DNA repair, and apoptosis.

Drosophila HO (dHO) is an orthologue of mammalian HOs (Zhang et al. 2004). *Drosophila* provides a powerful genetic tool for functional analysis of the *HO* gene *in vivo*. The *Drosophila* system was used to characterize the actions of dHO for tissue-specific reduction of the expression level of HO in eye development. We previously found that the knockdown of dHO by RNAi resulted in severe abnormal eye morphology with rough eye in adult flies and the over-expression of dHO also led to a rough eye phenotype, indicating that relevant expression of dHO is indispensable for the normal development of tissues (Cui et al. 2008). Apoptosis of eye imaginal discs in dHO-deficient flies occurred, suggesting that the lack of dHO has a genotoxic effect for the DNA structure.

In the present study, we found that dHO knockdown led to cessation of DNA synthesis, accompanied by an increase in the level of histone H2A.V, a marker of chromatin damage. Finally, we demonstrated that knockdown of dHO in ATM mutant induces enhancement of the rough eye phenotype, indicating that HO is required for the repair of DNA damage. These results indicate that HO is required for the repair of DNA breaks.

Materials and Methods

Fly stocks

Fly strains were maintained at 25°C on standard food. The Canton S fly was used as a wild-type strain. The transgenic fly line (line number 16) carrying GMR-GAL4 on the X chromosome was described previously (Cui et al. 2008). The stocks of ATR and ATM mutant flies were obtained from the Kyoto Drosophila Genetic Resource Center or the Bloomington Drosophila Stock Center.

Establishment of transgenic flies

GMR-GAL4>*dHO*IR lines were established as described previously (Cui et al. 2008). We used the line (line 21-1) carrying GMR-GAL4 on the X chromosome and UAS-dHOIR on the 3rd chromosome in the following studies.

5-Bromo-2′-deoxyuridine (BrdU) labeling

Detection of cells in S-phase was performed by a BrdU-labeling method as described previously with minor modifications (Wilder and Perrimon 1995). Third-instar larvae cultured at 25°C were dissected in Grace's insect medium, and then incubated in the presence of 75 μg/ml BrdU (Roche, Palo Alto, CA) at 25°C for 30 min. The samples were fixed in Carnoy's fixative (ethanol/acetic acid/chloroform, 6:1:3) for 20 min at 25°C, and further fixed in 80% ethanol/50 mM glycine buffer, pH 2.0, at −20°C for 16 h. Incorporated BrdU was visualized using the 5-bromo-2′-deoxyuridine Labeling and Detection Kit I (Roche).

Immunostaining

For immunohistochemistry, larval eye imaginal discs and pupal retinae were dissected, and fixed in 4% paraformaldehyde / phosphate-buffered saline (PBS) at 4°C for 10 min and 30 min at 25°C, respectively. After washing with PBS containing 0.3% Triton X-100, the samples were blocked with PBS containing 0.15% Triton X-100 and 10% normal goat serum for 30 min at 25°C, and incubated with diluted primary antibodies in PBS containing 0.15% Triton X-100 and 10% normal goat serum for 16 h at 4°C. The following antibodies were used: mouse anti-Elav (diluted at 1:200, Developmental Studies Hybridoma Bank [DSHB] 9F8A9), anti-histone H2A.V (diluted at 1:400, Cell Signaling, Tokyo, Japan), and activated caspase 3 (1:200, Futsch). After extensive washing with PBS containing 0.3% Triton X-100, samples were incubated with secondary antibodies labeled with either Alexa 546 or Alexa 488 (1:400; Invitrogen, San Jose, CA) for 3 h at 25°C. After extensive washing with PBS containing 0.3% Triton X-100 and PBS, samples were mounted in Fluoroguard Antifade Reagent (Bio-Rad, Herculus, CA) and analyzed by confocal laser scanning microscopy under a Zeiss LSM510 or Olympus Fluoview FV10i.

Flip-out experiments

RNAi clones in eye discs were generated with a flip-out system (Sun and Tower 1999). Female flies with *hs-flp*; *Act5C>FRT y FRT>* GAL4, UAS-*GFP* were crossed with male flies with UAS-*dHO*IR and clones were identified by the presence of green fluorescent protein (GFP) expressed under control of the *Act5C* promoter. Flip-out was induced by heat shock (60 min at 37ºC) at 24-48 h after egg laying.

Scanning electron microscopy

Adult flies were anesthetized, mounted on stages and observed under a scanning electron microscope VE-7800 (Keyence Inc., Kobe, Japan) in the low vacuum mode.

Cell culture and immunoblotting

Human embryonic kidney HEK293T cells were grown in Dulbecco's modified Eagle's medium supplemented with fetal calf serum (FCS) and antibiotics. The cells (1×10^5) in a 1.5-cm-diameter dish were transfected using Lipofectamine RNAiMAX (Invitrogen Co., San Jose, CA) with siRNAs for HO-1 (Santa Cruz Biotechnology, Santa Cruz, CA, No. sc-37007) and HO-2, then incubated in the presence of 10% FCS at 37°C for 48 h (Ohgari et al. 2011). HO-2 siRNA used was synthesized by SIGMA-ALDRICH (Tokyo, Japan): sense r(CCACCACGGCACUUUACUUCA) and antisense r(AAG UAAAGUGCCGUGGUGGCC). All other chemicals used were of analytical grade. After collecting and washing cells, the cellular pro-

teins were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting was carried out with antibodies for HO-1 (Ohgari et al. 2011), HO-2 (Ohgari et al. 2011), phospho-ATM (Ser1981) (Cell Signaling), ATM (Cell Signaling), phosho-p53 (Ser15) (Cell Signaling), phospho-Chk1 (Ser296) (Cell Signaling), Chk1 (Cell Signaling), p53 (Santa Cruz), histone γ-H2A.X (phospho-Ser139) (Abcam, Cambridge, UK), and actin (Santa Cruz), as the primary antibodies (Sawamoto et al. 2013).

Results

Knockdown of dHO in eye imaginal discs induces morphologically aberrant rough eyes

Fig. 1 shows functional analysis of *dHO* by knockdown with GMR-GAL4 driver lines in the visual system. Specific knockdown of dHO with UAS-*HO*IR flies produced abnormal eye morphology, rough in appearance (Fig. 1B), which is consistent with the previous findings (Cui et al. 2008). Flies carrying GMR-GAL4 alone exhibited apparently normal eye morphology (Fig. 1A).

To investigate whether excessive apoptosis was induced by oxidative stress, we first treated the larval eye imaginal discs with 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-DCFHDA), which can detect the generation of reactive oxygen species (ROS). The observed results revealed that ROS were extensively generated in eye imaginal discs by dHO-knockdown (Fig. 2A and B). Finally we monitored apoptotic cells in third-instar larval eye imaginal discs by immunostaining with an anti-cleaved caspase-3 antibody that specifically detects activated caspase-3. In eye imaginal discs of flies expressing GAL4 alone, there were very few apoptotic cells (Fig. 2C), while in eye imaginal discs of GMR-GAL4> UAS-*dHO*IR flies, a significant increase in cell death signals posterior to the morphogenetic furrow was observed (Fig. 2D). These results indicate that knockdown of dHO can induce excessive apoptosis in some cells in eye imaginal discs.

To determine whether the reduced expression of HO disrupted or delayed the onset of neuronal differentiation, eye discs were incubated with an anti-Elav antibody that stains all cells that have been recruited into preclusters and been committed to a neuronal fate (Moses and Rubin 1991). The Elav staining pattern of flies carrying GMR-GAL4> UAS-*dHO*IR was indistinguishable from that of control flies carrying GMR-GAL4 alone (Fig. 3A and B), indicating that dHO-knockdown did not disturb the normal differentiation of photoreceptor cells.

Knockdown of dHO inhibited cells in eye imaginal discs from entering S-phase, leading to enhancement of DNA damage

To determine the effect of dHO knockdown on the synchronized S-phase posterior to the furrow, we employed a flip-out experiment with flies carrying the hs-*flp*; *Act5C* > FRT y FRT> GAL4, UAS-*GFP* and the UAS-*dHO*IR. The third-instar larval eye imaginal discs were labeled with BrdU and stained with an anti-BrdU antibody. Cells marked with GFP express dHO dsRNA. Although BrdU signals corresponding to the S-phase zone were clearly

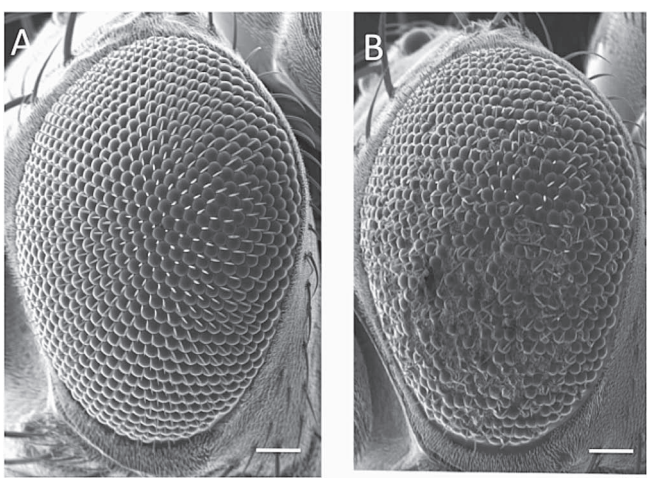

Fig. 1. Knockdown of dHO in the eye imaginal discs induces a rough eye phenotype.
Scanning electron micrographs of adult compound eyes. (A) GMR-GAL4/Y; +; +, (B) GMR-GAL4/+;+; UAS-*dHO*-IR/+ (line 21-1). The flies were developed at 25°C. The bar indicates 50 μm.

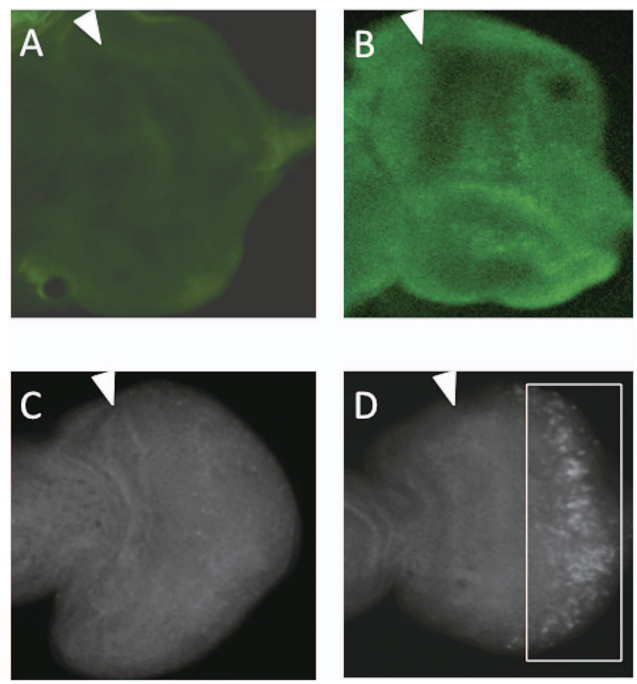

Fig. 2. Knockdown of dHO in eye imaginal discs increases the generation of ROS and induces apoptosis.
Upper two panels: Eye imaginal discs were treated with 10 μM CM-DCFHDA. Eye imaginal discs of third-instar larvae carrying GMR-GAL4/Y; +; + (A) and GMR-GAL4;+;UAS-*dHO*IR/TM3 (B). Lower two panels: Detection of apoptotic cells in eye imaginal discs with anti-cleaved caspase-3 antibody. (C) GMR-GAL4/Y; +; +, (D) GMR-GAL4/+;+; UAS-*dHO*-IR/+ (line 21-1). The induced caspase 3-positive region is enclosed. White arrowhead indicates morphogenetic furrow.

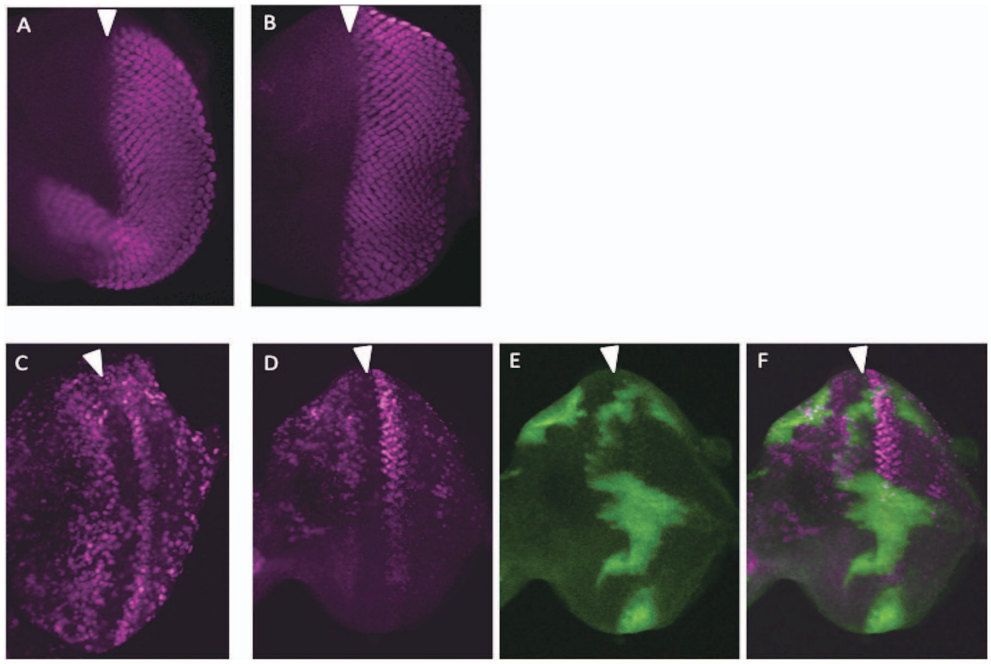

Fig. 3. Decrease of the progression of S-phase in larval eye imaginal discs by dHO knockdown.
(A, B) Immunostaining of eye discs with anti-Elav antibody revealed that the expression of dHO dsRNA exerted no effect on the differentiation of eight photoreceptor cells. (C) Patterns of BrdU incorporation in eye discs from the wild-type fly. The position of the S-phase zone is behind the morphogenetic furrow. (D-F) Flip-out experiments. In the dHO RNAi clone area (labeled with GFP) (E), levels of BrdU incorporation are reduced (D). (F) Merged image of panels D and E. Arrowhead shows morphogenetic furrow.

detected as a stripe in the control fly discs (Fig. 3C), the level of BrdU signal was markedly reduced in the RNAi clone area (Fig. 3D and F). Thus, knockdown of HO very likely prevents the G1-arrested cells at the furrow from entering S-phase.

Then, to observe whether dHO-knockdown triggers DNA damage, the level of histone H2A.V in third instar larval eye imaginal discs was examined. As shown in Fig. 4A and B, the number of foci of H2A.V increased by about 2.5-fold, indicating that HO deficiency leads to increased DNA damage. When we crossed GMR-GAL4> UAS-*dHO*IR fly with ATR null mutant, all ATR mutant flies into which dHO-knockdown was introduced died (data not shown). Knockdown of dHO in the ATM null mutant flies resulted in enhancement of the rough eye phenotype (Fig. 5), indicating that dHO functions in control of the signal pathway of DNA damage. In the separate experiment, we performed knockdown of HO-1/-2 by siRNA in human kidney HEK293T cells. By the knockdown of HO-1/-2, the activation of p53-dependent signal molecules including ATM and Chk1 was observed (Fig. 6). The level of histone γ-H2A.X, a marker of mammalian DNA damage, was increased in the HO-1/-2 deficient HEK cells.

Identification of genomic regions in which deletions modify the rough eye phenotype induced by knockdown of dHO

GMR-GAL4> UAS-*dHO*IR flies exhibit a rough eye phenotype with induced DNA damage. Therefore, they can be used as a genetic tool to identify mutations that enhance or suppress the rough eye phenotype. A collection of *Drosophila* deficiency stocks were crossed with GMR-GAL4> UAS-*dHO*IR flies for this purpose. Eye morphology of their F1 progeny was compared with that of F1 progeny between the transgenic flies and the wild-type Canton S. Deficiencies involving eye phenotype alone, such as *roe*, were not included in this screening.

Typical results for the modification of the rough eye phenotype induced by UAS-*dHO*IR when transgenic flies were made heterozygous for deletions are shown in Fig. 7A, and genomic regions whose deletion suppressed the rough eye phenotype are summarized in Fig. 7B. A total of 65 third-chromosome deficiency lines were examined. Eight lines exhibited a reduced rough eye phenotype by a reduction of gene dose of specific genomic regions. The other deficiency lines showed no detectable effects in term of the rough eye phenotype induced by knockdown of dHO.

We identified eight genomic regions, (3L) M21, h-i22, Tp/10, E299, ry615, e-N19, slo8, and D605 (Fig. 7), which would possibly contain candidate genes encoding factors that interacted with dHO. As described above, since we suggested that dHO is involved in defense against oxidative stress and DNA damage, we focused on one of the DNA repair-related genes. We tried to cross GMR-GAL4> UAS-*dHO*IR fly with flies carrying deletion in narrower genomic regions of *Df(3R)E229*, and found that the deletion of Excel8155 was effective (Fig. 7C). Then, we tried several

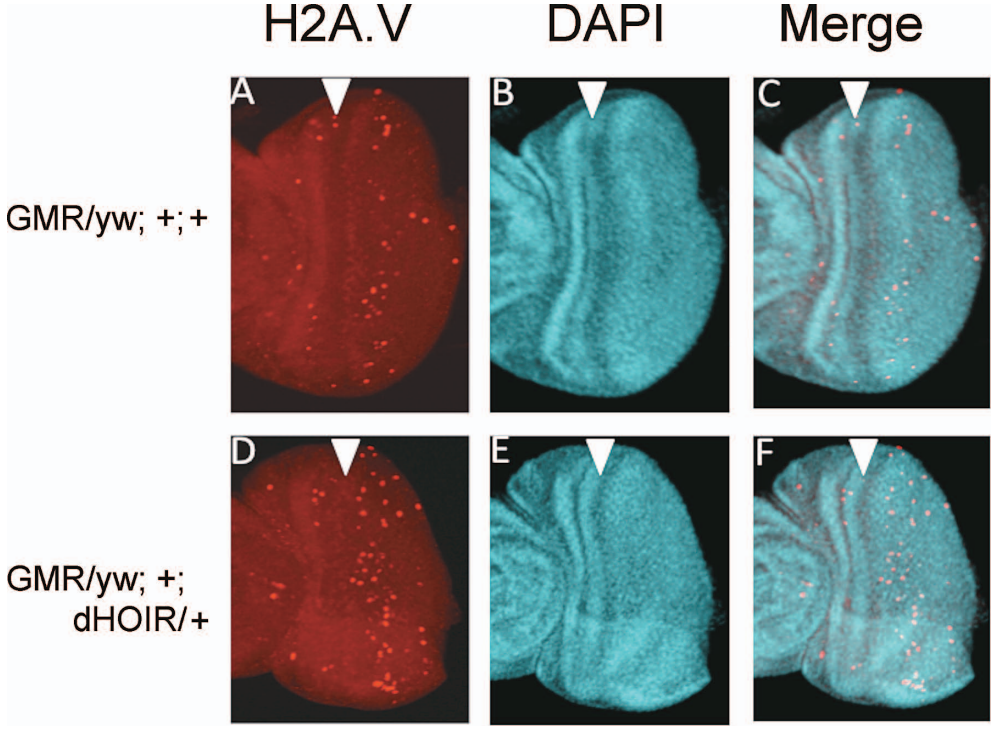

Fig. 4. The increased expression of histone H2A.V in the imaginal discs by dHO knockdown.
The eye imaginal discs were incubated with anti-γ-H2A.V antibody (A, D), followed by staining of nuclei with DAPI (B, E). (A, B, C) GMR-GAL4/Y; +; +, (D, E, F) GMR-GAL4/+;+; UAS-*dHO*-IR/+ (line 21-1). The merged images are shown in C and F. Arrowhead shows morphogenetic furrow.

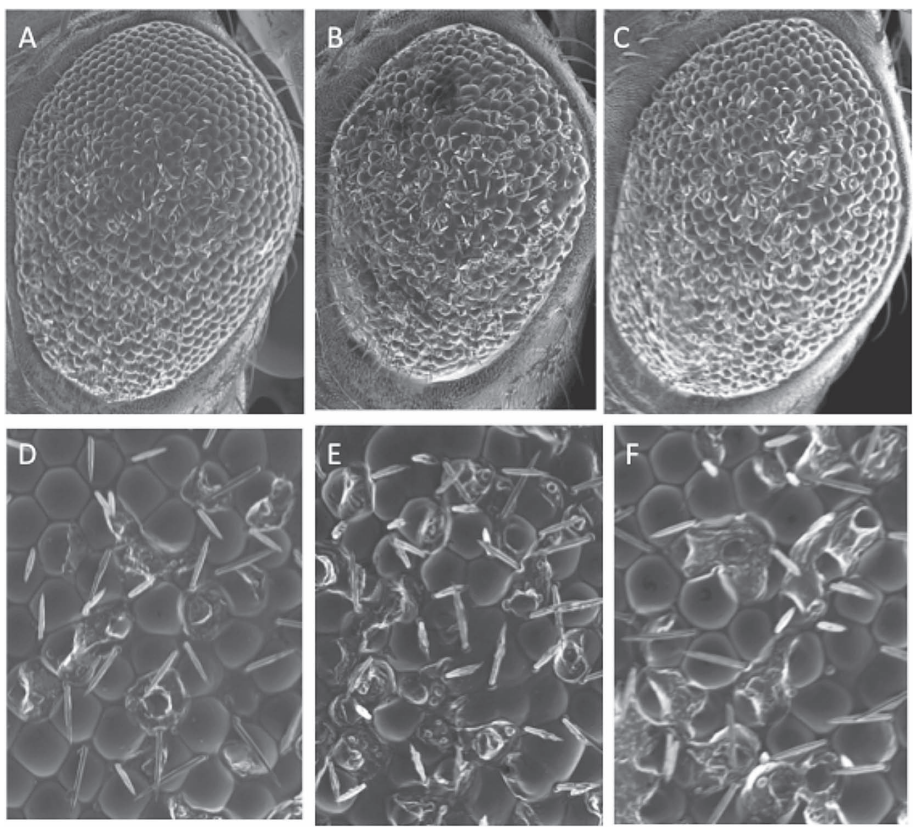

Fig. 5. Half-dose reduction of the *atm* gene dose enhances the rough eye phenotype induced by knockdown of dHO.
Scanning electron micrographs of adult compound eyes. (A) *GMR-GAL4/yw;+ ; UAS-dHOIR/+* (A, D) *GMR-GAL4/yw; + ; UAS-dHOIR/atm*^{6} (B, E) *GMR-GAL4/yw; + ; UAS-dHOIR/atm*^{3} (C, F). Magnification, (A-C) 200x; (D-F) 800x.

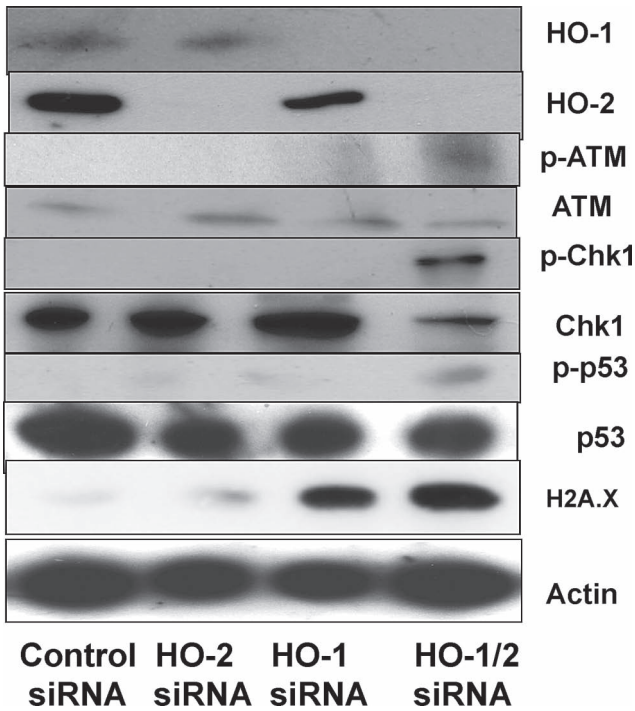

Fig. 6. Activation of ATM and p53 in human HEK cells by the knockdown of HO-1 and HO-2.
The cells (5×10^4) transfected with HO-1 and HO-2 siRNAs were cultured for 48 h, followed by incubation for 48 h. The cellular protein from the cells was analyzed by SDS-PAGE. Immunoblots were performed with antibodies for HO-1, HO-2, p-ATM, ATM, p-p53, p53, p-Chk1, Chk1, γ-H2A.X, and actin.

genes including KLH18; however, there was no detectable effect on the rough eye phenotype, suggesting that some other gene(s) in this genomic region may be responsible for the suppression.

Discussion

The present study demonstrated that the knockdown of dHO in *Drosophila* caused oxidative stress, leading to the genotoxic effect on the chromosomes. We found that the cessation of degradation of heme by the depletion of dHO led to an increase in the levels of heme and iron (Cui et al. 2008). The increase in the level of heme by the knockdown was due to blockage of the metabolic pipeline, which was seen in the case of yeast lacking the HO homologue HMX1 (Protchenko and Philpott 2003). Considering that HMX plays a role in the reutilization of iron from heme, the supply of iron from heme by dHO is important for the homeostasis of iron in *Drosophila*. Cui et al. (2008) also demonstrated that the accumulation of iron, but not heme, was found in dHO-deficient tissues, which was similar to that seen in liver and spleen of HO-1-deficient humans and mice (Poss and Tonegawa 1997; Yachie et al. 1999; Yet et al. 2003). In iron-heme metabolically active tissues, the generation of heme was reduced substantially by a decrease in the expression of 5-aminolevulinic acid synthase-1 (Furuyama et al. 2007), which contributes to a lack of utilization of iron, and then the accumulation of iron.

Cui et al. (2008) demonstrated that knockdown of dHO in transgenic fly lines induced either lethality or abnormal morphology in adult eyes, indicating that the recycling of heme-iron by dHO is indispensable for the normal development of cells. There is mounting evidence that in vertebrates, phase and period in the circadian cycle are regulated by levels of CO and NO, which are derived from light and dark environmental signals (Feng and Lazer 2012; Girvan and Munro 2013). Furthermore, the circadian clock reciprocally regulates heme biosynthesis, with heme levels rising and falling in a cyclical manner (Kaasik and Lee 2004). Given the related regulatory roles for heme, NO, and CO, it is important to elucidate the relationship between heme chemistry and the transcriptional activities of these nuclear receptors (Marvin et al. 2009).

The biosynthesis of heme in fruit flies can be tightly regulated according to developmental stage and the intracellular level of heme (Ruiz de Mena et al. 1999). Heme is a key regulator of cellular homeostasis. Heme sensor proteins are ubiquitous in regulatory responses to the bioactive gases O_2, CO, and NO (Yet et al. 2003). Regulations of blood pressure, cell division, cell death, inflammation, metabolism, hypoxia, diurnal cycles, behavior, and memory have all been attributed to the action of NO and CO (Dulak and Józkowicz 2003; Lahiri et al. 2006). Furthermore, it is suggested that heme, as well as CO and NO, may play an important role as a regulator of circadian rhythm, since heme-bound transcriptional factor E75 is one of the components involved in the circadian rhythm in *Drosophila* (Reinking et al. 2005; Aicart-Ramos et al. 2012). Therefore, knockdown of dHO may disrupt the phase and period of the circadian cycle, especially leading to dysfunction of E75. Since E75 plays redundant roles in molting and developmental progression of this direct-developing insect (Mané-Padrós et al. 2008), disruption of this function by the knockdown of HO impaired normal development in insects.

dHO knockdown flies exhibit a rough eye phenotype, associated with G1/S arrest of the cell cycle, increased generation of ROS, and cell death in the eye imaginal disc. At this stage, foci of a DNA damage marker, histone γH2A.V (Madigan et al. 2002), were markedly increased by dHO knockdown, which was consistent with observations that the level of histone γ-H2A.X, a homologue of H2A.V, increased in various tissues from HO-1-deficient mice (Otterbein et al. 2011). These observations suggest that the loss of function of HO causes a genotoxic effect such as DNA break and may activate signals of DNA repair. As expected, the rough eye phenotype of dHO knockdown flies is enhanced in ATM mutated flies, confirming that extensive apoptosis at least partially contributes to the rough eye phenotype (Fig. 5). The dHO knockdown in ATR mutant became lethal. Considering that ATM and ATR are sensors of ROS and oxidative stress to cellular macromolecules

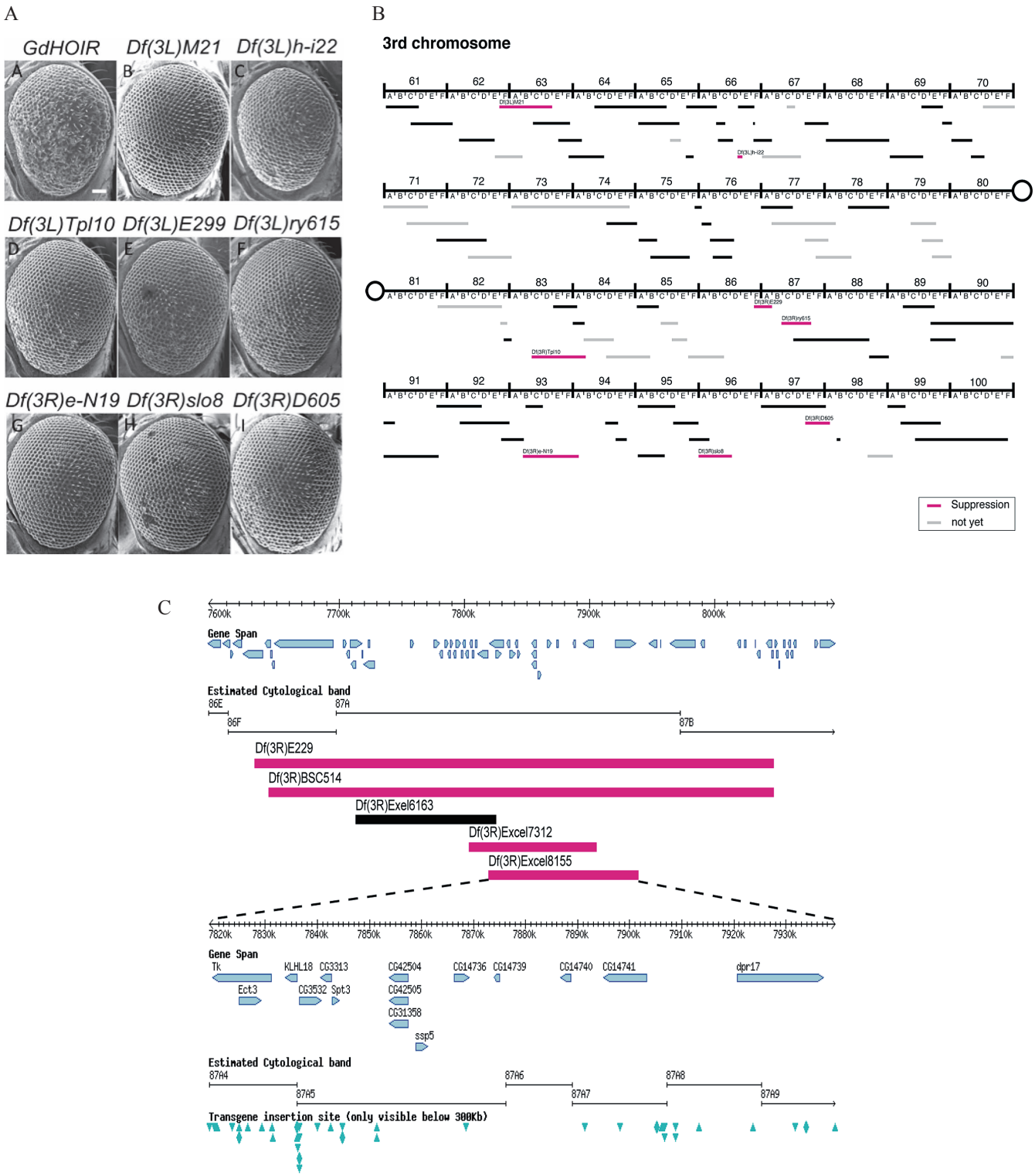

Fig. 7. Link of the chromosomal deletion to the suppression of the rough eye phenotype induced by knockdown of dHO. (A) Eight deficiency lines exhibit suppression of the rough eye phenotype induced by knockdown of dHO. Scanning electron micrographs of adult compound eyes. (B) Genetic screen to identify mutations that suppresses the rough eye phenotype. Summary of deficiencies screened. The numbered divisions and lettered subdivisions of salivary gland polytene chromosomes are marked. The boxes indicate the suppression of each deficiency on the knockdown of dHO for the rough eye phenotype: magenta lines, suppression; black lines, no effect; grey lines, not yet. (C) Identification of genomic region whose deletion suppresses the rough eye phenotype induced by knockdown of dHO in the case of Df(3R)E229.

(Hurley and Bunz 2007; Smith et al. 2010), it is possible that the observed apoptotic cells may reflect cell death as a result of a checkpoint of the cell cycle upon facing oxidative damage. When knockdown of HO-1/-2 by siRNA was carried out in human kidney HEK293T cells, the activation of p53-dependent signal molecules including ATM and Chk1 was observed (Fig. 6). Furthermore, the level of γ-H2A.X in HEK cells increased by the knockdown of HO-1/-2, supporting the observation that the deficiency of dHO caused DNA damage (Fig. 4). Conversely, ATM-deficient cells were extremely sensitive to ionizing radiation as well as oxidative stress by potent pro-oxidants. Because HO plays a cytoprotective role, HO-1/-2 was markedly induced in ATM-deficient cells upon exposure to oxidative stresses, thus indicating that the interaction of HO, a protector against stress and ATM, as a sensor for oxidative damage is indispensable for the DNA repair process.

How does dHO regulate the DNA repair? One possibility is that it produces CO. Alternatively, dHO may function in the removal of harmful uncommitted heme and exert an indirect influence. The former idea is supported by studies of mouse HO-1, showing that knockout of HO-1 impaired DNA repair through activation of ATM, including an increase in γ-H2A.X (Shen et al. 2003; Otterbein et al. 2011). Then, the phosphorylation of ATM in response to anti-cancer drugs was markedly decreased in HO-1-deficient cells, resulting in a marked increase in DNA damage. The treatment of HO-1/-2-deficient cells with CO restored the activation of ATM in vitro and that with the mice blocked DNA damage (Otterbein et al. 2011). The latter model is supported by previous studies (Wagener et al. 2013), which indicate that the generation of bioreactive heme caused lipid peroxidation, and protein and DNA degradation. Numerous reports have demonstrated the potent cytoprotective effects of CO in various models of liver failure (Zuckerbraun et al. 2003), organ transplantation (Neto et al. 2006), and ischemia/reperfusion injury (Nakao et al. 2005). It is thought that the increased production of CO by HO has a survival benefit on various insults including DNA damage.

Here, we identified eight genomic regions that suppressed the rough eye phenotype induced by knockdown of dHO (Fig. 7). These genomic regions may contain genes encoding factors that interact with dHO. Although it is still unclear at present which genes in these genomic regions are responsible for modification of the rough eye phenotype induced by knockdown of dHO, the results provide useful clues for future studies.

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Conflict of Interest

The authors declare no conflict of interest.

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