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Drosophila Damaged DNA Binding Protein 1 Contributes to Genome Stability in Somatic Cells

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The damaged DNA-binding protein (DDB) complex consists of a heterodimer of p127 (DDB1) and p48 (DDB2) subunits and is believed to have a role in nucleotide excision repair (NER). We used the GAL4-UAS targeted expression system to knock down DDB1 in wing imaginal discs of *Drosophila*. The knock-down was achieved in transgenic flies using over-expression of inverted repeat RNA of the D-DDB1 gene [*UAS-D-DDB1(650)-dsRNA*]. As a consequence of RNA interference (RNAi), the fly had a shrunken wing phenotype. The wing spot test showed induced genome instability in transgenic flies with RNAi knock-down of D-DDB1 in wing imaginal discs. When *Drosophila* larvae with RNAi knock-down of D-DDB1 in wing imaginal discs were treated with the chemical mutagen methyl methanesulfonate (MMS), the frequency of flies with a severely shrunken wing phenotype increased compared to non-treated transgenic flies. These results suggested that DDB1 plays a role in the response to DNA damaged with MMS and in genome stability in *Drosophila* somatic cells.

Key words: damaged DNA binding protein 1 (DDB1), DNA repair, RNAi, XPE.

The damaged DNA-binding protein (DDB) complex is a heterodimeric protein composed of 127-kDa and 48-kDa subunits, termed DDB1 and DDB2. The complex has been shown to recognize many types of DNA lesions (*1–7*). *DDB2* is mutated in *Xeroderma pigmentosum complementation group E* (*XPE*) and cells with mutations in this gene are mildly defective in NER (*8–10*) and selectively defective in global genomic repair (GGR) (*11*). However, despite the fact that damaged DNA-binding activity of the DDB complex is absent in cells of a subset of XPE patients (*4*, *12–14*), DDB was found not to be required in NER reconstitution studies *in vitro* (*15–17*).

DDB1 is evolutionarily conserved in mammals, worms, insects and plants. No mutations have been found in mammalian DDB1. However, DDB2 is less conserved and DDB2 sequence homologues have been identified in mammals and plants (*18*).

Recent studies have shown that DDB1 is a component of E3 ubiquitin ligase complexes (*19–22*). In mammalian cells, DDB1 or DDB1-like proteins have been found in the other complexes that have a connection with repair of DNA damage and with chromatin organization. (*7*, *23–25*). Nevertheless, the functions of DDB1 remain elusive.

The present study was initiated to investigate the role of DDB1 in a multicellular organism, in this instance *Drosophila melanogaster*. The wing spot test (also known as the somatic mutation and recombination test) was used to study the effect of knock-down of *D-DDB1* by RNAi or by P-element insertion. The *Drosophila* wing spot test has been shown to be very useful for the study of induced genetic damage in somatic cells. The test detects loss of heterozygosity (LOH) resulting from gene mutation, chromosomal rearrangement, chromosome breakage or chromosome loss. When *multiple wing hairs* (*mwh*) is used as a marker, genetic alterations induced during mitosis of cells in wing discs can give rise to a clone(s) of *mwh* cells visible on the wing of the adult fly (*26*). Herein, we report that D-DDB1 is required for genomic stability in response to the endogenous DNA lesions and for the response induced by MMS treatment in somatic cells.

MATERIALS AND METHODS

Plasmid Construction—The plasmid p5′-*D-DDB1*(650)-dsRNA contains the *D-DDB1* ORF (1 to 750 bp) and the *D-DDB1* ORF (101 to 750 bp) in head-to-head orientation (3′; 750 to 1 bp of *D-DDB1* ORF and 5′; 101 to 750 bp of *D-DDB1* ORF), in a P element vector (*27*).

Establishment of Transgenic Flies—P element–mediated germ line transformation was carried out as described earlier (*28*). F1 transformants were selected on the basis of *white* eye color rescue (*29*). Established transgenic strains carrying pUAS-*D-DDB1*(650)-dsRNA and their chromosomal linkages are listed in Table 1 (*27*).

Fly Stocks—Fly stocks were cultured at 25°C on standard food. The OregonR fly was used as the wild-type strain. Mutant alleles of the DDB1^{EY01408}, mei-9^{L1}, mei-41^{RT1}, mus201^{D1}, mus205^{A1}, mus308^{D2}, CSN5^{L4032}, Rpn6^{k00103}, lin19^{k01207}, RnrL^{k06709}, cana^{k07716}, CSN4^{k08018}, cul-2^{02074}, mus209^{02448}, cmet^{04431}, E2f^{07172}, Mi-2^{i3D4}, DNAprim^{i10B2},

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Table 1. **Transformants carrying the 650 bp *D-DDB1* double strand RNA.**

P-element plasmid	Strain	Chromosome linkage
pUAS-*D-DDB1*(650)-dsRNA	10	II
	23	II
	31	III
	41	III

gft^{06430}, $lin19^{BG02329}$, $cul\text{-}4^{KG02900}$, $Nap1^{KG03959}$, $Iswi^{KG03354}$, $Acf1^{KG06618b}$, $HDAC4^{KG09091}$, dup^{PA77} and dup^{k03308} genes were obtained from the Bloomington Indiana Stock Center and the *Drosophila* Genetic Resource Center, Kyoto Institute of Technology.

Ectopic Expression of UAS-D-DDB1(650)-dsRNA—Ectopic expression of *D-DDB1*(650)-dsRNA was induced essentially as described by Brand and Perrimon (*30*). A line carrying heterozygous MS1096-GAL4 on the second chromosome was crossed with lines carrying the homozygous P[UAS-*D-DDB1*(650)-dsRNA] on the second chromosome. The flies were maintained at 25 or 28°C.

Wing Spot Test—The wing spot test is based on loss of heterozygosity in somatic cells of larvae (*31*). Larvae heterozygous for mwh^1 were obtained by crossing either *UAS-D-DDB1(650)-dsRNA/UAS-D-DDB1(650)-dsRNA;* mwh^1/mwh^1 males with *MS1096-GAL4/MS1096-GAL4* virgin females or mwh^1/mwh^1 males with $DDB1^{EY01408}$/TM3 virgin females. All surviving flies were collected and stored in isopropanol. Wings were dissected and mounted in 1:1 methyl salicylate/Canada balsam (Sigma). Only cells with three or more hairs were scored as the $mwh^{-/-}$ phenotype.

DNA Damaging Agent Treatments—Parent flies were removed after an egglaying period of about 1 day. After 2 days, chemicals were administered as 0.6 ml of aqueous solution per bottle onto the surface of the medium.

Modifier Screen—We observed that the *D-DDB1* knockdown transgenic flies that developed at 28°C exhibited a more severe rough eye phenotype than did the transgenic flies that developed at 25°C. This occurs because transcriptional stimulation activity of GAL4 is temperature-dependent. Therefore, we performed a genetic screen for modifiers of D-DDB1 as follows: male flies expressing 650 bp dsRNA of *D-DDB1* [+/+;*eyGAL4*, *UAS-D-DDB1(650)-dsRNA/CyO;*+/+] were crossed with individual females carrying the mutant alleles of the $mei\text{-}9^{L1}$, $mei\text{-}41^{RT1}$, $mus201^{D1}$, $mus205^{A1}$, $mus308^{D2}$, $CSN5^{L4032}$, $Rpn6^{k00103}$, $lin19^{k01207}$, $RnrL^{k06709}$, $cana^{k07716}$, $CSN4^{k08018}$, $cul\text{-}2^{02074}$, $mus209^{02448}$, $cmet^{04431}$, $E2f^{07172}$, $Mi\text{-}2^{i3D4}$, $DNAprim^{i10B2}$, gft^{06430}, $lin19^{BG02329}$, $cul\text{-}4^{KG02900}$, $Nap1^{KG03959}$, $Iswi^{KG03354}$, $Acf1^{KG06618b}$, $HDAC4^{KG09091}$, dup^{PA77} and dup^{k03308} genes. The F1 progeny were allowed to develop at either 25 or 28°C.

RESULTS

D-DDB1 Is Necessary for Normal Drosophila *Wing Development*—To investigate DDB1 function in DNA repair, we prepared transgenic flies with knock down of *D-DDB1* in the wing discs. Ectopic expression of a 650-bp dsRNA fragment of *D-DDB1* (the *D-DDB1* ORF is 3420 bp long) in living flies was performed using the GAL4-mediated expression system described in Materials and Methods and our previous paper (*27*). To verify the effectiveness of the *D-DDB1* gene silencing in the wing disc cells, transgenic flies carrying UAS-*D-DDB1*(650)-dsRNA were crossed with flies carrying *GAL4* cDNA under the control of the MS1096 enhancer-promoter (MS1096-GAL4). The flies were maintained at 25 or 28°C. We previously showed that D-DDB1 can be detected in most of the cells of the wing imaginal disc (*32*). In larvae, GAL4 is expressed preferentially in the dorsal compartment of the wing imaginal disc (*30*). At 25°C, knock down of *D-DDB1* in the wing imaginal discs caused a shrunken wing phenotype (Fig. 1A), although the severity of the

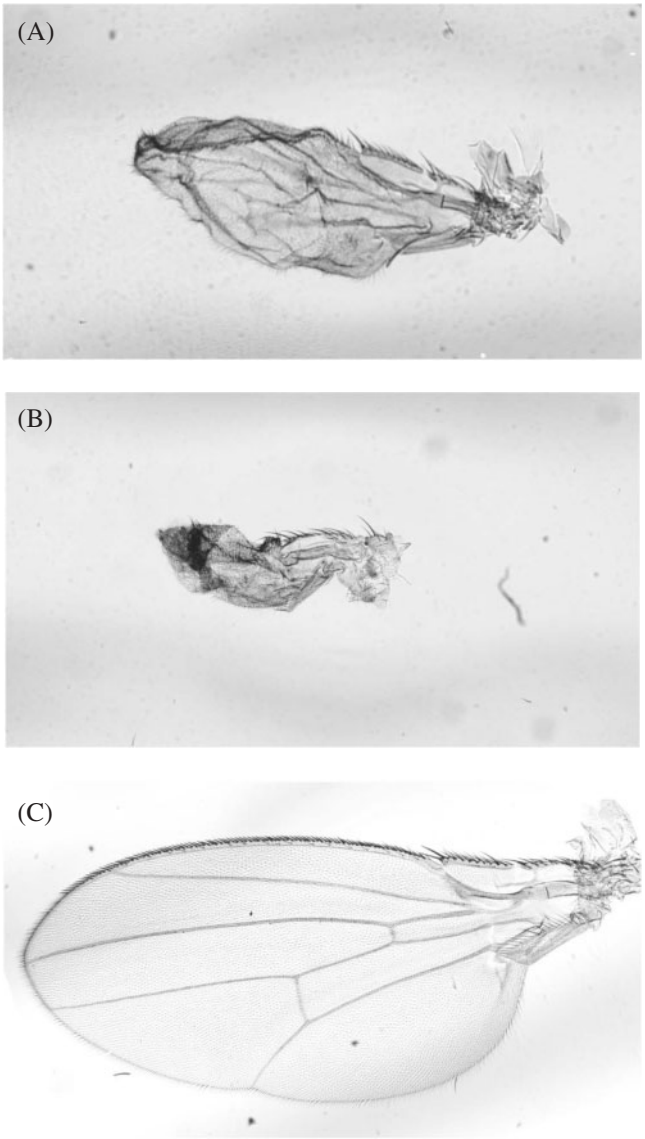

Fig. 1. **Knock down of D-DDB1 in the wing imaginal disc depresses tissue growth.** UAS-*D-DDB1*(650)-dsRNA insertion lines were crossed with MS1096-GAL4 wing drivers. MS1096 expresses GAL4 preferentially in the dorsal compartment of the wing imaginal disc. The wings of [*MS1096-GAL4/+;UAS-D-DDB1(650)-dsRNA/+*] flies that developed at (A) 25°C and (B) 28°C. (C) Negative control *MS1096-GAL4/+* flies that developed at 28°C. All are at the same magnification.

phenotype varied between individual flies. The most severe phenotype at 25°C is illustrated in Fig. 1A. Transcriptional stimulation activity of GAL4 is temperature-dependent (*33*). We observed that in the flies developing at 28°C, RNAi knock down of *D-DDB1* caused a more severe phenotype (Fig. 1B) than in the transgenic flies that developed at 25°C (Fig. 1A). At 28°C all the flies showed a severe shrunken wing phenotype. These results suggest that D-DDB1 is critical for normal *Drosophila* wing development.

RNAi Knockdown of D-DDB1 Induced Genomic Instability—The wing spot test was used to confirm that DDB1 contributes to genomic stability in somatic cells. The wing hairs of flies homozygous for *mwh* show multiple short hairs per cell (Fig. 2, A and B). Flies heterozygous for mwh^1 and MS1096-GAL4 (*MS1096-GAL4/+;*mwh^1*/+*) have normal wing hair (Fig. 2, C and D). Heterozygous mwh^1 flies with knock-down of *D-DDB1* [*MS1096-GAL4/+;UAS-D-DDB1(650)-dsRNA/+;*mwh^1*/+*] have multiple short hairs per cell (Fig. 2, E and F). Genome instability was observed in *D-DDB1* knock-down flies but not in control flies. *D-DDB1* knock-down promoted the genetic changes and induced loss of *mwh* heterozygosity in somatic cells of the wing imaginal discs.

Treatment with MMS Caused a More Severe Shrunken Wing Phenotype—We next tested whether the treatment with DNA-damaging agents enhanced the frequency of short hairs in flies with RNAi knock-down of *D-DDB1*. Larvae heterozygous for mwh^1 and with knock-down of

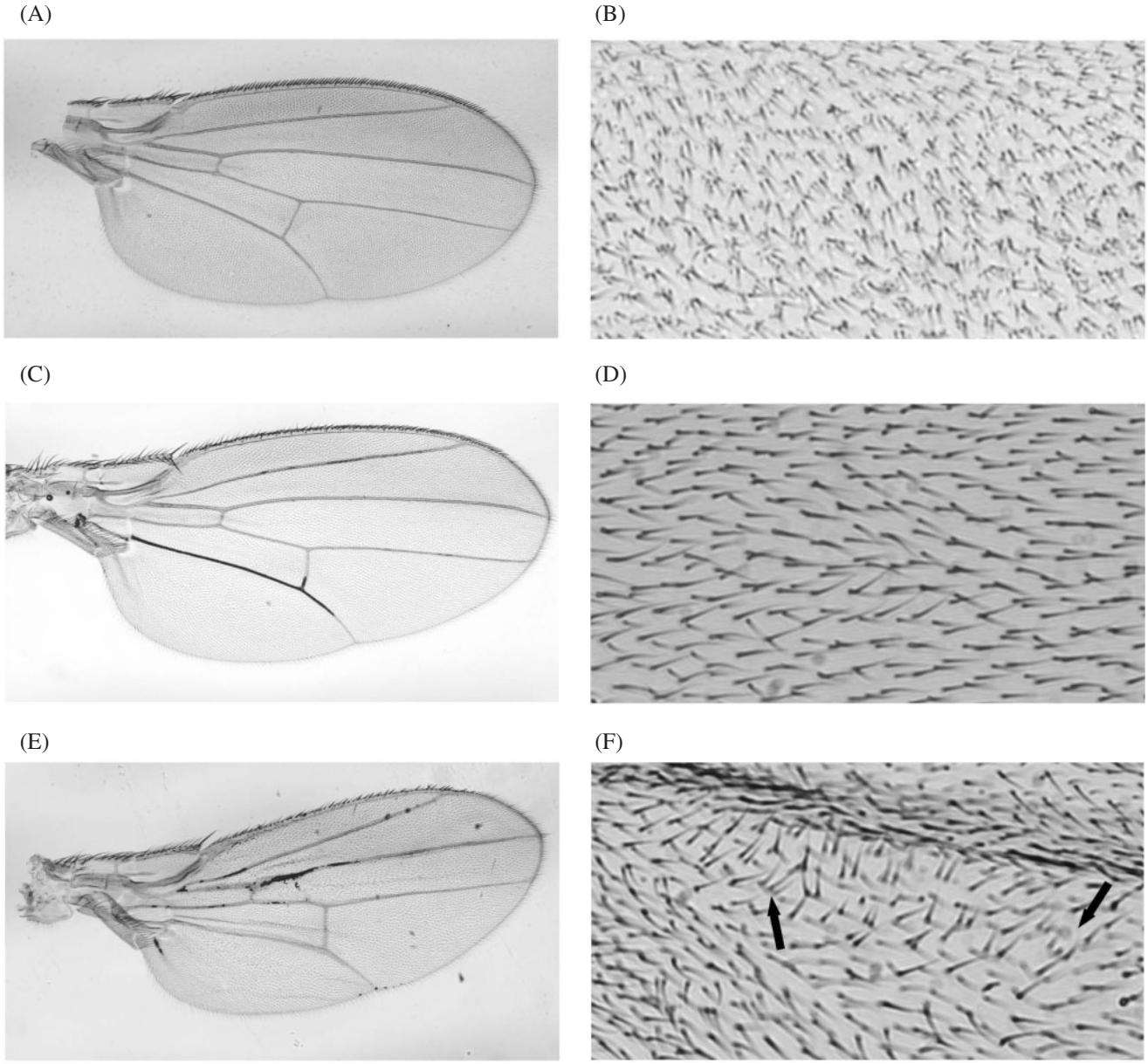

Fig. 2. **Overexpression of D-DDB1(650)-dsRNA induces genomic instability.** Multiple wing hair spot test in the wings: (A) and (B), mwh^1/mwh^1 *flies*; (C) and (D), *MS1096-GAL4/+;*mwh^1*/+* flies; (E) and (F), *MS1096-GAL4/+; UAS-D-DDB1(650)-dsRNA/+;*mwh^1*/+* flies. A spot with multiple wing hairs is indicated by the arrow in (F). Right panels show a higher magnification of the left panels.

D-DDB1 [*MS1096-GAL4/+;UAS-D-DDB1(650)-dsRNA/+;mwh*^{1}*/+*] were treated with MMS (0.0125, 0.025, and 0.05%), mitomycin C (0.5, 2.0, and 8.0 μg/ml), bleomycin (0.2 and 1.0 μg/ml), or H_2O_2 (5 and 10 mM) and allowed to develop at 25°C. Only MMS treatment caused a more severe shrunken wing phenotype than in the controls. We were unable to count the number of wing spots because the wings of flies with RNAi knock-down of *D-DDB1* were too abnormal. We determined the effects of the chemicals using the frequency of abnormal wing phenotype. As shown in Fig. 3, we classified the flies according to the severity of the shrunken wing shrinking phenotype. The most severely affected phenotype (Fig. 3A) was designated as type A, a moderately affected phenotype (Fig. 3B) was designated as type B, and a mild phenotype (Fig. 3C) was designated as type C. Although type C looks superficially normal, the wing shows defects in the pattern of the bristles on the anterior wing margin (circle, Fig. 3E) and an increased vein width (arrow, Fig. 3E) phenotype compared to the normal wing phenotype in controls (Fig. 3F). The images in Fig. 3, C and D, are shown at higher magnification in Fig. 3, E and F, respectively. We further categorized the flies depending on whether only one or both wings were affected. In flies assigned to Class I, the both wings were type C; in Class II flies, either both wings of fly were type B or one of wings was type B and the other was type A or C; in Class III flies, both wings were type A. The frequencies of different phenotypes after treatment with DNA-damaging agents are given in Table 2. The frequencies were calculated based on the report of Szakmary *et al*. (*34*). MMS treatment increased the frequency of the severe shrunken wing phenotype and did so in dose-dependent manner.

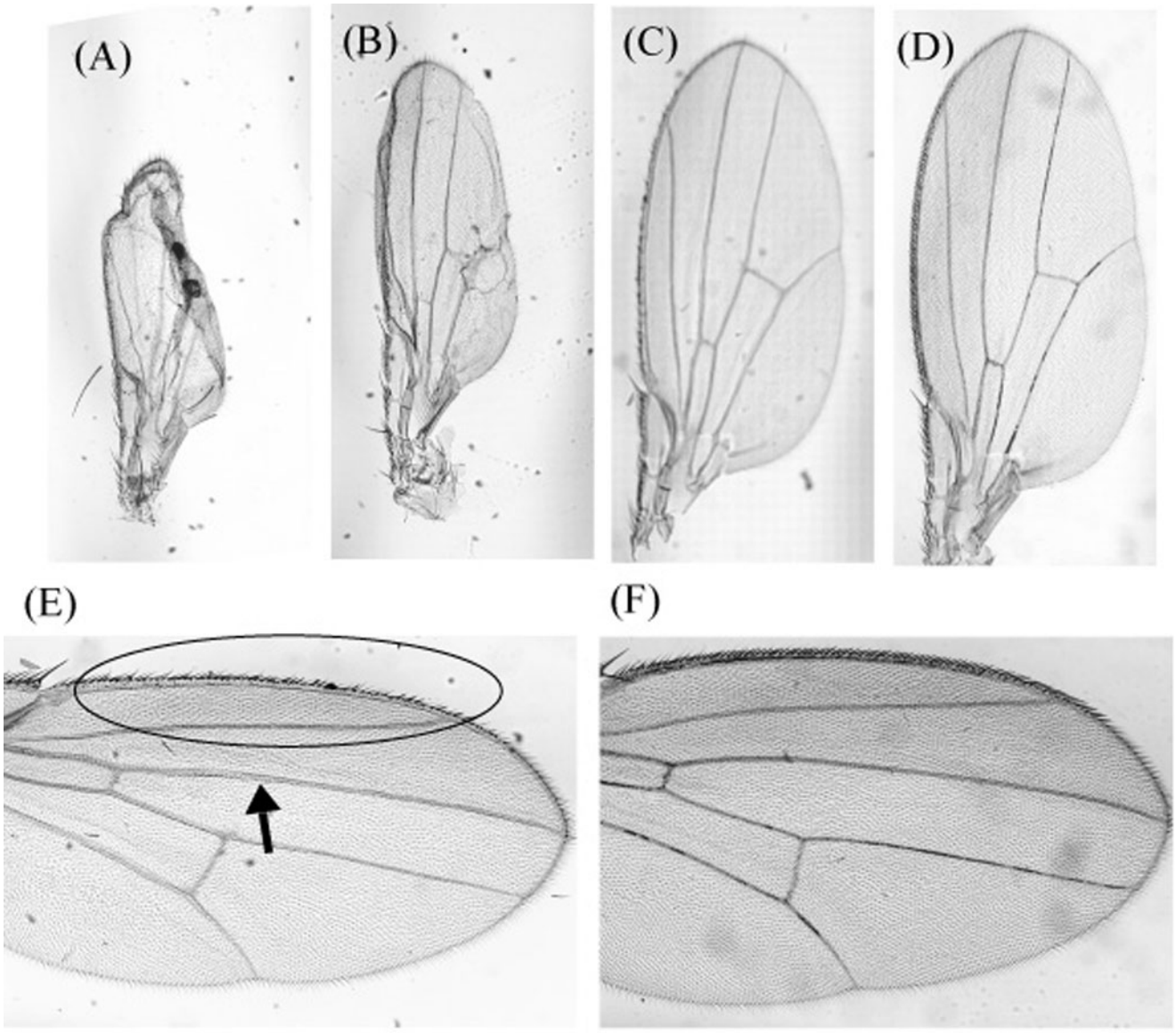

Fig. 3. **Classification of the flies [*MS1096-GAL4/+;UAS-D-DDB1(650)-dsRNA/+;mwh1/+*] by wing phenotype (A) The most severely affected phenotype, type A** (B) The intermediate, less severely affected phenotype, type B. (C) The least affected phenotype, type C. (D) The control (*MS1096-GAL4/+;mwh*^{1}*/+*) phenotype. (E) and (F), higher magnification images of (C) and (D), respectively.

Table 2. **Frequencies of flies with different wing phenotypes following treatment with DNA damaging agents.**

Treatment	MS1096-GAL4/+; UAS-*D-DDB1*(650)-dsRNA/+; mwh^1/+					MS1096-GAL4/+; +/+; mwh^1/+				
	No. of Flies Scored	no detectable phenotype (Fr.)	abnormality of wing morphlogy (Fr.)			No. of Flies Scored	no detectable phenotype (Fr.)	abnormality of wing morphlogy (Fr.)		
			I	II	III			I	II	III
H_2O (control)	189	0	0.68 ± 0.05*	0.19 ± 0.02	0.14 ± 0.04	130	1.00 ± 0	0	0	0
MMS (%)										
0.0125	52	0	0.06 ± 0.06	0.25 ± 0.10	0.69 ± 0.16	334	0.99 ± 0.02	0.01 ± 0.02	0	0
0.025	36	0	0.03 ± 0.03	0	0.97 ± 0.03	82	0.98 ± 0.03	0.02 ± 0.03	0	0
0.05	194	0	0	0	1.00 ± 0	233	0.92 ± 0.08	0.08 ± 0.08	0	0
MMC (μg/ml)										
0.5	17	0	0.59 ± 0.15	0.18 ± 0.02	0.24 ± 0.13	24	1.00 ± 0	0	0	0
2.0	24	0	0.42 ± 0.06	0.46 ± 0.10	0.13 ± 0.06	32	1.00 ± 0	0	0	0
8.0	34	0	0.26 ± 0.05	0.24 ± 0.03	0.50 ± 0.08	23	1.00 ± 0	0	0	0
Bleomycin (μg/ml)										
0.2	50	0	0.34 ± 0.20	0.26 ± 0.25	0.40 ± 0.23	47	1.00 ± 0	0	0	0
1.0	254	0	0.28 ± 0.24	0.26 ± 0.03	0.46 ± 0.26	239	1.00 ± 0	0	0	0
H_2O_2 (mM)										
5	68	0	0.72 ± 0.09	0.18 ± 0.06	0.10 ± 0.03	83	1.00 ± 0	0	0	0
10	108	0	0.47 ± 0.16	0.31 ± 0.08	0.21 ± 0.09	136	1.00 ± 0	0	0	0

No.: number of flies. Fr.: proportion of flies with the same genotype that show a particular phenotype. The proportions were calculated by using data from two or more experiments. *SD from the average value in each experiment. Average values were calculated but not given in the table.

None of the other agents affected the rate of abnormal wing phenotypes. Our observation that *D-DDB1* knock-down enhanced the sensitivity to MMS suggests that D-DDB1 is involved in the response induced by MMS.

Knock-Down of D-DDB1 Exerts an Effect on the Genome Stability in Response to Treatment with DNA Damaging Agents—We performed the wing spot test using a line carrying a P-element insert in the 5′ exon of *D-DDB1* (DDB1^{EY01408}) and maintained at 25°C. The heterozygous DDB1^{EY01408} has a normal wing phenotype (Fig. 4B and data not shown). The wing hairs of flies homozygous for mwh show multiple short hairs per cell (Fig. 4A). After MMS treatment, the frequency of spots was higher than in the controls (Fig. 4, C and D). None of the other chemicals used, mitomycin C (0.5, 2.0, and 8.0 μg/ml), bleomycin (0.2 and 1.0 μg/ml), H_2O_2 (5 and 10 mM) (data not shown), affected the rate of wing spots. These data indicate that MMS induces genome instability even in the heterozygous DDB1^{EY01408} wing.

D-DDB1 Interacts Genetically with mus205, Nap1, dup—To better understand the role of D-DDB1 *in vivo*, we identified genes whose mutations modify the rough eye phenotype induced by expression of *D-DDB1* dsRNA. For this propose it was convenient to use a transgenic fly line whose phenotype is easily detected. We therefore used a previously established fly line with constant RNAi knock-down of *D-DDB1* in the eye (*27*). A collection of *Drosophila* mutant alleles was used in crosses with the transgenic flies expressing *D-DDB1* dsRNA, and the eye morphologies of their F1 progeny were compared with those of the F1 progeny between transgenic flies and OregonR. We identified mus205^{A1}, and Nap1^{KG03959} and dup^{PA77} as dominant enhancers of *D-DDB1* RNAi-induced rough eyes (Fig. 5). These enhancers did not promote small eye morphology but occasionally promoted *D-DDB1* RNAi-induced aberrant differentiation (Fig. 5). In flies with the ey-GAL4, UAS-*D-DDB1*(650)-dsRNA/mus205^{A1} genotype, we observed the phenotype that the compound eye was divided into

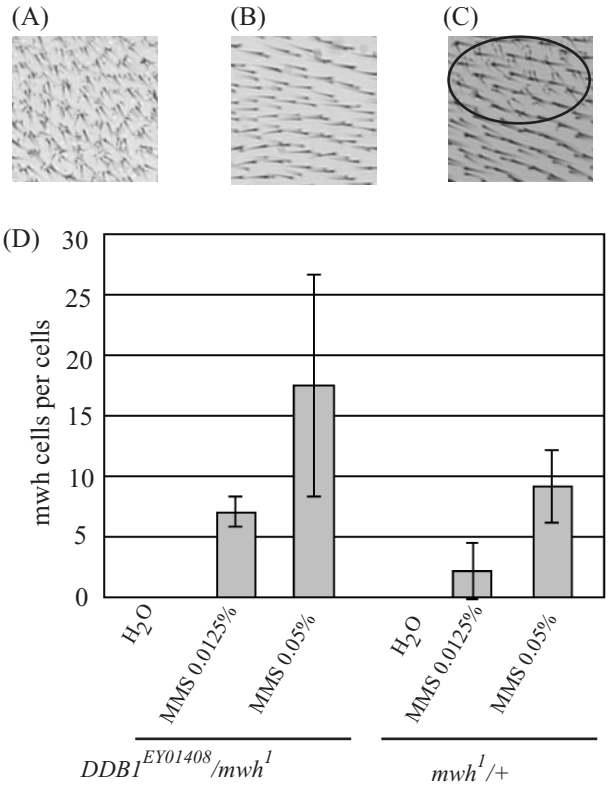

Fig. 4. **Wing spot test using DDB1^{EY01408}.** (A) *mwh*^{1}/*mwh*^{1}, (B) DDB1^{EY01408}/TM3, (C) DDB1^{EY01408}/*mwh*^{1}. Cells within the circle have lost the wild-type copy of *mwh*^{1}, uncovering the recessive *mwh* phenotype. (D) The frequencies of mwh clones per wing in *DDB1*^{EY01408}/*mwh*^{1} and *mwh*^{1}/+. Error bars, SD.

two (filled arrowhead) and that the number and location of the ocelli was aberrant (open arrowhead) in 42.9% of the flies (Fig. 5, E and F). In flies with ey-GAL4, UAS-D-*DDB1*(650)-dsRNA/Nap1^{KG03959} genotype, the location

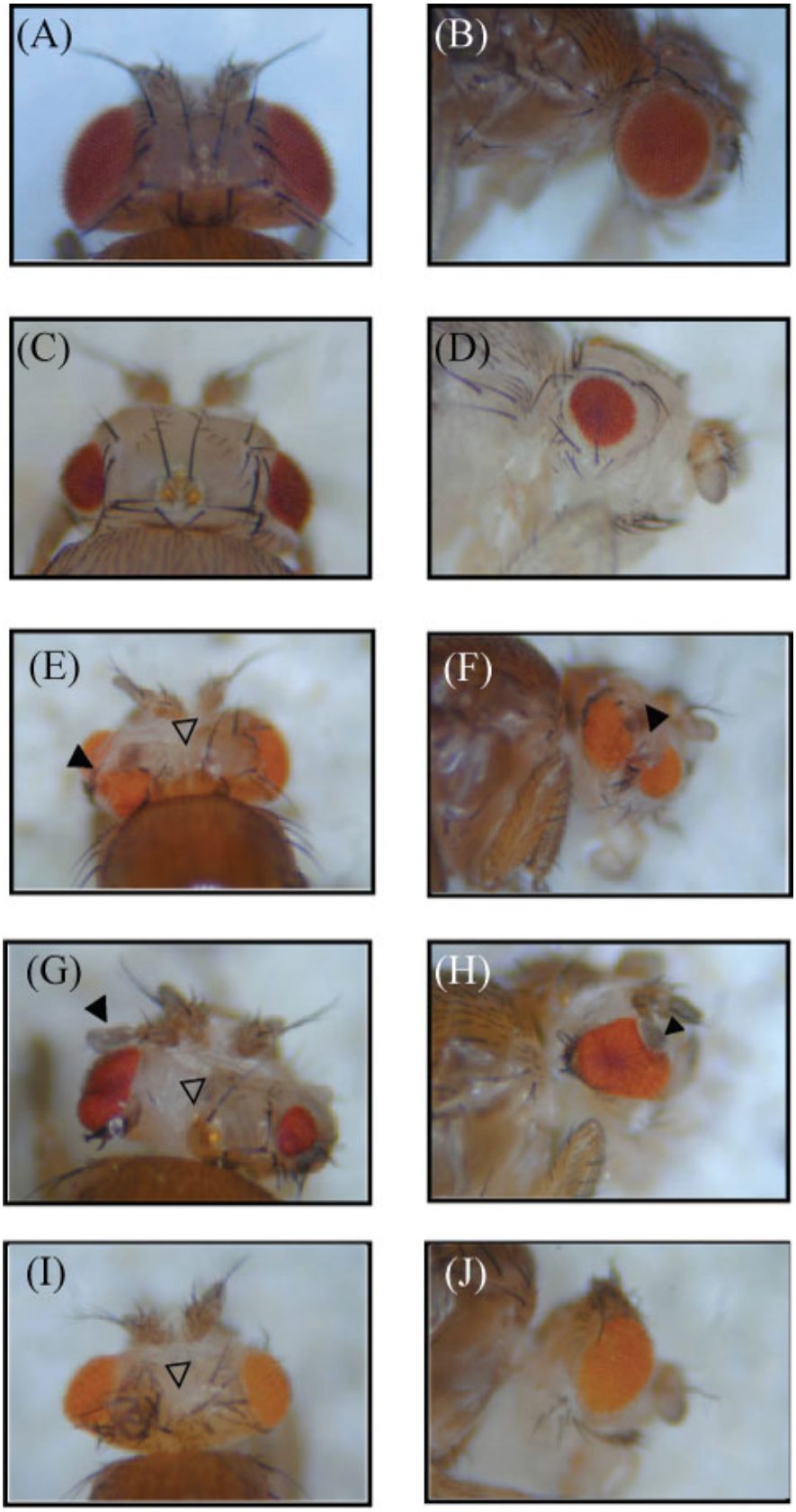

Fig. 5. **The sensitivity of ey-GAL4/UAS-D-DDB1(650)-dsRNA transgenic flies is enhanced by mutant alleles of mus205, Nap1 and dup.** (A, B) wild type fly (+/+). (C, D) *ey-GAL4, UAS-D-DDB1(650)-dsRNA*/+. (E, F) *ey-GAL4,UAS-D-DDB1(650)-dsRNA*/*mus205*^{A1}. (G, H) *ey-GAL4,UAS-D-DDB1(650)-dsRNA*/*Nap1*^{KG03959}. (I, J) *ey-GAL4,UAS-D-DDB1(650)-dsRNA*/*dup*^{PA77}.

or number of the ocelli was aberrant (open arrowhead) and/or the antennal segment was aberrant (filled arrowhead) in 53.8% of the flies (Fig. 5 G and H). With the ey-GAL4, UAS-*D-DDB1*(650)-dsRNA/ dup^{PA77} genotype, the location and/or number of the ocelli was aberrant (open arrowhead) in 90.5% of the flies (Fig. 5, I and J). Other mutant alleles including mei-9^{L1}, mei-41^{RT1}, mus201^{D1}, mus308^{D2}, CSN5^{L4032}, Rpn6^{k00103}, lin19^{k01207}, cana^{k07716}, CSN4^{k08018}, cul-2^{02074}, mus209^{02448}, cmet^{04431}, E2f^{07172}, Mi-2^{i3D4}, DNAprim^{i10B2}, gft^{06430}, lin19^{BG02329}, cul-4^{KG02900}, Iswi^{KG03354}, Acf1^{KG06618b} and HDAC4^{KG09091}, RnrL^{k06709} and dup^{k03308} did not enhance the *D-DDB1* RNAi phenotype in our test system.

DISCUSSION

DDB1 was originally identified as a component of damaged DNA-binding heterodimeric complex DDB (*1*, *4*, *12*). It is involved in NER through the interaction with DDB2 or CSA (*20*). In addition to DNA repair functions in NER, DDB1 has been implicated in other pathways. DDB1 and DDB2 were demonstrated to act as a co-factor of the E2F1 transcription factors (*19*). DDB1 also interacts with the X protein of hepatitis B virus to induce cell death (*35*). DDB1 is a component of centromere complex (*36*). In this study, RNAi knock-down of *D-DDB1* in the wing discs induced a shrunken wing phenotype. We previously reported D-DDB1 appears to be an essential development-associated factor (*27*). Therefore DDB1 might be an important multifunctional protein.

Wing spot tests showed that RNAi knock down of *D-DDB1* induced genomic instability. This is evidence that DDB1 contributes to genomic stability in somatic cells. MMS treatment of larvae with RNAi knock-down of *D-DDB1* background induced a more severe shrunken wing phenotype. This indicates that D-DDB1 has an important role in response to MMS-induced DNA damage. Although the basis for sensitivity to MMS remains unclear, the wing spot test showed that frequencies of spots were higher than controls even in heterozygous DDB1, indicating that D-DDB1 prevents MMS-induced genomic instability. The number of *mwh* cells in *D-DDB1* knock-down flies is, in fact, slightly different to that of control flies, because apoptosis of *mwh* cells may prevent an increase in their number. It has been reported that RNAi knock down of *D-DDB1* induce the failure of differentiation and apoptosis in eye imaginal disc cells (*27*).

As described in the Introduction, D-DDB1 is a member of various protein complexes. We showed that mus205^{A1}, Nap1^{KG03959} and dup^{PA77} interacted genetically with D-DDB1. mus205^{A1} has a mutation in the catalytic subunit of DNA polymerase ζ. Flies carrying the mutation are hypersensitive to MMS (*37*). RNAi knock-down of *D-DDB1* also increases MMS sensitivity, suggesting that polymerase ζ and DDB1 may cooperate in the repair of DNA damage induced by MMS. Nap1 has been shown to be required for chromatin assembly *in vitro* and is associated with the core histones H2A and H2B as a chaperone in embryos (*38*). DDB1 may participate in chromatin assembly for normal DNA synthesis or after DNA repair. Dup (Double-parked) is the Drosophila ortholog of Cdt1, a replication licensing factor. DDB1 targets Cdt1 for ubiquitination by a CUL4-dependent ubiquitin ligase, CDL4A, in response to UV irradiation (*21*). Our results suggest that DDB1 interacts with the replication licensing factor and prevents the replication of damaged DNA in *Drosophila*.

In summary, genome instability was induced in the RNAi knock-down of *D-DDB1* background, and the wings of adult flies showed a shrunken phenotype. Although further analysis is necessary to understanding the precise function of DDB1, the present study suggests that DDB1 plays an important role in the response to DNA damage induced by MMS and in the maintenance of genomic integrity.

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REFERENCES

1. Feldberg, R.S. (1980) On the substrate specificity of a damage-specific DNA binding protein from human cells. *Nucleic Acids Res.* **8**, 1133–1143
2. Carew, J.A. and Feldberg, R.S. (1985) Recognition of a cytosine base lesion by a human damage-specific DNA binding protein. *Nucleic Acids Res.* **13**, 303–315
3. Hirschfeld, S., Levine, A.S., Ozato, K., and Protic, M. (1990) A constitutive damage-specific DNA-binding protein is synthesized at higher levels in UV-irradiated primate cells. *Mol. Cell Biol.* **10**, 2041–2048
4. Keeney, S., Chang, G.J., and Linn, S. (1993) Characterization of a human DNA damage binding protein implicated in -xeroderma pigmentosum E. *J. Biol. Chem.* **268**, 21293–32100
5. Reardon, J.T., Nichols, A.F., Keeney, S., Smith, C.A., Taylor, J.S., Linn, S., and Sancar, A. (1993) Comparative analysis of binding of human damaged DNA-binding protein (XPE) and *Escherichia coli* damage recognition protein (UvrA) to the major ultraviolet photoproducts: T[c,s]T, T[t,s]T, T[6–4]T, and T[Dewar]T. *J. Biol. Chem.* **268**, 21301–21308
6. Payne, A. and Chu, G. (1994) Xeroderma pigmentosum group E binding factor recognizes a broad spectrum of DNA damage. *Mutat. Res.* **310**, 89–102
7. Wittschieben, B.B. and Wood, R.D. (2003) DDB complexities. *DNA Repair (Amst)* **2**, 1065–1069
8. Rapic Otrin, V., Kuraoka, I., Nardo, T., McLenigan, M., Eker, A.P., Stefanini, M., Levine, A.S., and Wood, R.D. (1998) Relationship of the xeroderma pigmentosum group E DNA repair defect to the chromatin and DNA binding proteins UV-DDB and replication protein A. *Mol. Cell Biol.* **18**, 3182–3190
9. Shiyanov, P., Hayes, S.A., Donepudi, M., Nichols, A.F., Linn, S., Slagle, B.L., and Raychaudhuri, P. (1999) The naturally occurring mutants of DDB are impaired in stimulating nuclear import of the p125 subunit and E2F1-activated transcription. *Mol. Cell Biol.* **19**, 4935–4943
10. Liu, W., Nichols, A.F., Graham, J.A., Dualan, R., Abbas, A., and Linn, S. (2000) Nuclear transport of human DDB protein induced by ultraviolet light. *J. Biol. Chem.* **275**, 21429–21434
11. Hwang, B.J., Ford, J.M., Hanawalt, P.C., and Chu, G. (1999) Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. *Proc. Natl. Acad. Sci. USA* **96**, 424–428

12. Chu, G. and Chang, E. (1988) Xeroderma pigmentosum group E cells lack a nuclear factor that binds to damaged DNA. *Science* **242**, 564–567
13. Kataoka, H. and Fujiwara, Y. (1991) UV damage-specific DNA-binding protein in xeroderma pigmentosum complementation group E. *Biochem. Biophys. Res. Commun.* **175**, 1139–1143
14. Keeney, S., Wein, H., and Linn, S. (1992) Biochemical heterogeneity in xeroderma pigmentosum complementation group E. *Mutat. Res.* **273**, 49–56
15. Aboussekhra, A. and Wood, R.D. (1995) Detection of nucleotide excision repair incisions in human fibroblasts by immunostaining for PCNA. *Exp. Cell Res.* **221**, 326–332
16. Mu, D., Park, C.H., Matsunaga, T., Hsu, D.S., Reardon, J.T., and Sancar, A. (1995) Reconstitution of human DNA repair excision nuclease in a highly defined system. *J. Biol. Chem.* **270**, 2415–2418
17. Kazantsev, A., Mu, D., Nichols, A.F., Zhao, X., Linn, S., and Sancar, A. (1996) Functional complementation of xeroderma pigmentosum complementation group E by replication protein A in an in vitro system. *Proc. Natl. Acad. Sci. USA* **93**, 5014–5018
18. Zolezzi, F. and Linn, S. (2000) Studies of the murine DDB1 and DDB2 genes. *Gene* **245**, 151–159
19. Hayes, S., Shiyanov, P., Chen, X., and Raychaudhuri, P. (1998) DDB, a putative DNA repair protein, can function as a transcriptional partner of E2F1. *Mol. Cell Biol.* **18**, 240–249
20. Groisman, R., Polanowska, J., Kuraoka, I., Sawada, J., Saijo, M., Drapkin, R., Kisselev, A.F., Tanaka, K., and Nakatani, Y. (2003) The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. *Cell* **113**, 357–367
21. Hu, J., McCall, C.M., Ohta, T., and Xiong, Y. (2004) Targeted ubiquitination of CDT1 by the DDB1-CUL4A-ROC1 ligase in response to DNA damage. *Nat. Cell Biol.* **6**, 1003–1009
22. Yanagawa, Y., Sullivan, J.A., Komatsu, S., Gusmaroli, G., Suzuki, G., Yin, J., Ishibashi, T., Saijo, Y., Rubio, V., Kimura, S., Wang, J., and Deng, X.W. (2004) Arabidopsis COP10 forms a complex with DDB1 and DET1 in vivo and enhances the activity of ubiquitin conjugating enzymes. *Genes Dev.* **18**, 2172–2181
23. Neuwald, A.F. and Poleksic, A. (2000) PSI-BLAST searches using hidden markov models of structural repeats: prediction of an unusual sliding DNA clamp and of beta-propellers in UV-damaged DNA-binding protein. *Nucleic Acids Res.* **28**, 3570–3580
24. Martinez, E., Palhan, V.B., Tjernberg, A., Lymar, E.S., Gamper, A.M., Kundu, T.K., Chait, B.T., and Roeder, R.G. (2001) Human STAGA complex is a chromatin-acetylating transcription coactivator that interacts with pre-mRNA splicing and DNA damage-binding factors in vivo. *Mol. Cell Biol.* **21**, 6782–6795
25. Brand, M., Moggs, J.G., Oulad-Abdelghani, M., Lejeune, F., Dilworth, F.J., Stevenin, J., Almouzni, G., and Tora, L. (2001) UV-damaged DNA-binding protein in the TFTC complex links DNA damage recognition to nucleosome acetylation. *EMBO J.* **20**, 3187–3196
26. de Andrade, H.H., Reguly, M.L., and Lehmann, M. (2004) Wing somatic mutation and recombination test. *Methods Mol. Biol.* **247**, 389–412
27. Takata, K., Yoshida, H., Yamaguchi, M., and Sakaguchi, K. (2004) Drosophila damaged DNA-binding protein 1 is an essential factor for development. *Genetics* **168**, 855–865
28. Spradling, A.C. and Rubin, G.M. (1982) Transposition of cloned P elements into *Drosophila* germ line chromosomes. *Science* **218**, 341–347
29. Robertson, H.M., Preston, C.R., Phillis, R.W., Johnson-Schlitz, D.M., Benz, W.K., and Engels, W.R. (1988) A stable genomic source of P element transposase in *Drosophila melanogaster*. *Genetics* **118**, 461–470
30. Brand, A.H. and Perrimon, N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401–415
31. Graf, U., Wurgler, F.E., Katz, A.J., Frei, H., Juon, H., Hall, C.B., and Kale, P.G. (1984) Somatic mutation and recombination test in *Drosophila melanogaster*. *Environ. Mutagen.* **6**, 153–188
32. Takata, K., Ishikawa, G., Hirose, F., and Sakaguchi, K. (2002) Drosophila damage-specific DNA-binding protein 1 (D-DDB1) is controlled by the DRE/DREF system. *Nucleic Acids Res.* **30**, 3795–808
33. Hirose, F., Ohshima, N., Shiraki, M., Inoue, Y.H., Taguchi, O., Nishi, Y., Matsukage, A., and Yamaguchi, M. (2001) Ectopic expression of DREF induces DNA synthesis, apoptosis, and unusual morphogenesis in the *Drosophila* eye imaginal disc: possible interaction with Polycomb and trithorax group proteins. *Mol. Cell Biol.* **21**, 7231–7242
34. Szakmary, A., Huang, S.M., Chang, D.T., Beachy, P.A., and Sander, M. (1996) Overexpression of a Rrp1 transgene reduces the somatic mutation and recombination frequency induced by oxidative DNA damage in *Drosophila melanogaster*. *Proc. Natl. Acad. Sc.i USA* **93**, 1607–1612
35. Bontron, S., Lin-Marq, N., and Strubin, M. (2002) Hepatitis B virus X protein associated with UV-DDB1 induces cell death in the nucleus and is functionally antagonized by UV-DDB2. *J. Biol. Chem.* **277**, 38847–38854
36. Obuse, C., Yang, H., Nozaki, N., Goto, S., Okazaki, T., and Yoda, K. (2004) Proteomics analysis of the centromere complex from HeLa interphase cells: UV-damaged DNA binding protein 1 (DDB-1) is a component of the CEN-complex, while BMI-1 is transiently co-localized with the centromeric region in interphase. *Genes Cells* **9**, 105–120
37. Eeken, J.C., Romeijn, R.J., de Jong, A.W., Pastink, A., and Lohman, P.H. (2001) Isolation and genetic characterisation of the *Drosophila* homologue of (SCE)REV3, encoding the catalytic subunit of DNA polymerase zeta. *Mutat. Res.* **485**, 237–253
38. Ito, T., Bulger, M., Kobayashi, R., and Kadonaga, J.T. (1996) Drosophila NAP-1 is a core histone chaperone that functions in ATP-facilitated assembly of regularly spaced nucleosomal arrays. *Mol. Cell Biol.* **16**, 3112–3124