An essential role for Drosophila hus1 in somatic and meiotic DNA damage responses

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Summary

The checkpoint proteins Rad9, Rad1 and Hus1 form a clamp-like complex which plays a central role in the DNA-damage-induced checkpoint response. Here we address the function of the 9-1-1 complex in Drosophila. We decided to focus our analysis on the meiotic and somatic requirements of hus1. For that purpose, we created a null allele of hus1 by imprecise excision of a P element found 2 kb from the 3′ of the hus1 gene. We found that hus1 mutant flies are viable, but the females are sterile. We determined that hus1 mutant flies are sensitive to hydroxyurea and methyl methanesulfonate but not to X-rays, suggesting that hus1 is required for the activation of an S-phase checkpoint. We also found that hus1 is not required for the G2-M checkpoint and for post-irradiation induction of apoptosis.

We subsequently studied the role of hus1 in activation of the meiotic checkpoint and found that the hus1 mutation suppresses the dorsal-ventral pattering defects caused by mutants in DNA repair enzymes. Interestingly, we found that the hus1 mutant exhibits similar oocyte nuclear defects as those produced by mutations in DNA repair enzymes. These results demonstrate that hus1 is essential for the activation of the meiotic checkpoint and that hus1 is also required for the organization of the oocyte DNA, a function that might be independent of the meiotic checkpoint.

Key words: Drosophila, DNA damage checkpoint, Meiotic checkpoint, Hus1

Introduction

In many cell types specific checkpoint mechanisms exist that monitor the integrity of the chromosomes. These checkpoints coordinate cell cycle progression with DNA repair to ensure the distribution of accurate copies of the genome to daughter cells. If left unrepaired, chromosomal lesions can lead to genomic instability, a major contributing factor in the development of cancer and other genetic diseases. The DNA damage checkpoint response system involves a signal transduction pathway consisting of sensors, transducers and effectors (Dasika et al., 1999; Zhou and Elledge, 2000). Damaged DNA is initially sensed by a complex consisting of Hus1, Rad1 and Rad9 and the associated protein Rad17. Computer modeling suggests that Rad9, Hus1 and Rad1 (also called the 9-1-1 complex) form a doughnut-like heteromeric clamp-like complex which plays a central role in the DNA-damage-induced checkpoint response. Here we address the function of the 9-1-1 complex in Drosophila. We decided to focus our analysis on the meiotic and somatic requirements of hus1. For that purpose, we created a null allele of hus1 by imprecise excision of a P element found 2 kb from the 3′ of the hus1 gene. We found that hus1 mutant flies are viable, but the females are sterile. We determined that hus1 mutant flies are sensitive to hydroxyurea and methyl methanesulfonate but not to X-rays, suggesting that hus1 is required for the activation of an S-phase checkpoint. We also found that hus1 is not required for the G2-M checkpoint and for post-irradiation induction of apoptosis.

A number of checkpoint proteins that were initially characterized in budding and fission yeast, have counterparts in Drosophila, Caenorhabditis elegans and mammals, demonstrating the conservation of these surveillance mechanisms. Several checkpoint proteins have been characterized in Drosophila, mainly the ATM and/or ATR and the Chk1 and/or Chk2 transducer family of proteins. An ATR homolog in Drosophila is encoded by mei-41 (Hari et al., 1995). mei-41 is essential for the DNA damage checkpoint in larval imaginal discs and neuroblasts and for the DNA replication checkpoint in the embryo (Hari et al., 1995; Brodsky et al., 2000; Garner et al., 2001). mei-41 also has an essential role during early nuclear divisions in embryos (Sibon et al., 1999). In addition, mei-41 plays important roles during meiosis, where it has been proposed to monitor double-strand-break repair during meiotic crossing over, to regulate the progression of prophase I, and to enforce the metaphase I delay observed at the end of oogenesis (Ghabrial and Schüpbach, 1999; McKim et al., 2000). Drosophila ATM and ATR orthologs are required for different functions. In Drosophila, recognition of chromosome ends by ATM prevents telomere fusion and apoptosis, by recruiting chromatin-modifying complexes to telomeres (Song et al., 2004; Bi et al., 2004; Silva et al., 2004; Oikemus et al., 2004). It has also been shown that dATM and mei-41 have temporally distinct roles in G2 arrest after irradiation (Song et al., 2004).

A Chk1 homolog in Drosophila is encoded by grapes (Fogarty et al., 1997). Similarly to mei-41, grapes is required
to delay the entry into mitosis in larval imaginal discs after irradiation and to delay the entry into mitosis after incomplete DNA replication in the embryo (Sibon et al., 1997; Brodsky et al., 2000). The Drosophila Chk2 homolog [also designated loki (lok) or Dmmk] regulates multiple DNA repair and apoptotic pathways following DNA damage (Xu et al., 2001; Peters et al., 2002; Masrouha et al., 2003; Brodsky et al., 2004). It plays an important role in a mitotic checkpoint in syncytial embryos (Xu and Du, 2003) and is important in centrosome inactivation (Takada et al., 2003). Like Mei-41, DmChk2 also plays an important role in monitoring double-strand-break repair during meiotic crossing over (Abdu et al., 2002). Although our understanding of the role of DNA damage proteins is increasing, there is still a lack of information on the function of the Drosophila PCNA-like complex, 9-1-1.

In this study, we analyzed the interaction between the Drosophila Rad9, Hus1 and Rad1 proteins using a yeast two-hybrid assay. We were able to detect interaction between Hus1 and Rad9 or Rad1, but not between Rad9 and Rad1. We decided to focus our analysis on the meiotic and somatic requirement of Hus1. A null allele of hus1 was created by imprecise excision of a P element. We observed sensitivity of hus1 mutants to hydroxyurea (HU) and to methyl methanesulfonate (MMS) but not to X-ray irradiation. This implies that hus1 is required for the DNA replication checkpoint. The ability of a mutation in hus1 to suppress the eggshell polarity defects detected in mutants affecting double strand DNA repair enzymes demonstrates that it is required for the activation of the meiotic checkpoint that leads to a strong reduction in the translation of gurken mRNA. The similarity of the defects in the organization of the DNA in the oocyte nucleus between hus1 mutants and mutations in DNA repair enzymes suggest that hus1 may act upstream of the DNA repair machinery.

Results

Functional analysis of the Drosophila Hus1 gene

Studies in yeast and humans have shown that Rad9, Hus1, and Rad1 interact in a hetrotrimeric complex, which resembles a PCNA-like sliding clamp (reviewed by Parrilla-Castellar et al., 2004). To study the interaction between the Drosophila Rad9, Hus1 and Rad1 proteins, we performed a yeast two-hybrid assay (Fig. 1) in which Hus1 was used as a bait. Our results showed that Hus1 interacts with Rad9 and Rad1 to different degrees. Whereas Hus1 and Rad1 showed strong interaction (Fig. 1C2), only a weak interaction between Hus1 and Rad9 was detected (Fig. 1C1). To analyze the interaction between Rad9 and Rad1, Rad9 was used as bait. No interaction between Rad1 and Rad9 was found in this assay (data not shown).

Generation of null mutations in Hus1

We decided to focus our study on hus1, since there were several P-element lines available in hus1 gene region (Bellen et al., 2004). To analyze the somatic and meiotic requirements of the Drosophila Hus1, genetic studies were initiated. We screened for transposase-induced imprecise excisions by loss of the w * marker and tested these lines by genomic PCR and DNA sequencing. Excision of the P transposon insertion, P{SUPor-P}KG07223, which is inserted 150 bases away from the 5’ end of hus1 (Bellen et al., 2004) yielded one candidate mutant, hus1106. This line has deletion of 230 bases, which removes the first exon. RT-PCR analysis showed that removing the first exon had no effect on the level of hus1 transcript (data not shown). To create a null allele, another P-element transposon, {GT1}BG00590, which is inserted 2 kb from the 3’ of hus1 gene, was mobilized (Bellen et al., 2004) and one candidate mutant, hus1137, was identified. hus1137 has deletion of 3297 bases, which removes the entire ORF of the hus1 gene and deletes no other predicted transcript.

Since we were interested in understanding the role of the 9-1-1 complex in meiosis, the expression pattern of hus1, rad1 and rad9 genes during Drosophila oogenesis was studied. RT-PCR analysis showed that all three genes are expressed in Drosophila ovaries (Fig. 2A). However, no hus1 transcript was detected in hus1137 ovaries by RT-PCR analysis, unlike wild-type ovaries (Fig. 2B), as expected from the molecular analysis, demonstrating that hus1137 is a null allele. The level of Rad9 transcript was used as control (Fig. 2B). We found that hus1137 mutant flies are viable, however, females are sterile. This line was used for further examination of hus1 mutant phenotypes.

hus1137 mutant flies are sensitive to HU and MMS but not to X-rays

To examine a possible requirement for hus1 in somatic checkpoints in Drosophila, the sensitivity of hus1137 mutants to varying concentrations of HU and MMS and to X-ray irradiation (2500 rad) was determined. HU stalls replication through inhibition of deoxyribonucleotide synthesis, MMS causes non-bulky adducts, which if not repaired by nucleotide excision repair or DNA base excision repair, result in DSB formation during replication, whereas X-rays cause a wide spectrum of DNA damage, including DSBs, throughout the cell cycle. Mutagen sensitivity is indicated by a decrease in the percentage of surviving homozygous flies in the irradiated cross progeny relative to unirradiated controls. We found that homozygous hus1 flies were sensitive to MMS and HU (Table 1 and 2). Exposure to 10 or 20 mM HU affected the survival of hus1 mutants, whereas treatment with 30 mM HU eliminated most of the hus1 homozygous class of progeny, indicating that hus1 mutant larvae are indeed highly sensitive
to HU, presumably reflecting a requirement for hus1 activity in a fully functional DNA replication checkpoint. Similar results were also obtained when the hus1 allele was tested over a deficiency (Table 1). Interestingly, we found that hus1 mutants were highly sensitive to MMS. Relatively low doses of MMS (0.025%) caused almost 100% death of hus1 mutant flies. Similar results were also obtained when testing the hus1 mutation over a deficiency (Table 2). Most of the hus1 homozygous individuals died as larvae. When hus1 homozygous first and early second instar larvae were separated from their heterozygous siblings before MMS treatment using a GFP balancer chromosome, we found that only 19% (29/150) survived to pupal stages, whereas 75% of their heterozygous siblings (112/150) formed pupae. For both genotypes around 20% died as pharate adults.

To determine potential causes of lethality after genotoxic stress, neuroblast squashes of MMS-treated larvae were examined for chromosomal defects. HU 30 mM 99 1 1 65
HU 20 mM 88 12 6 213
HU 10 mM 80 20 2 221
Control 65 35 4 438

Control 32 31 36 NA 358
MMS 0.08% 52 46 2 NA 246

MMS, methyl methanesulfonate. In the first set of experiments the larvae were derived from a cross between heterozygous hus137/TM3 Df(hus1) TM6B/TM3, Sb. Df(3R)110/ TM3, Sb. Df(3R)110/TM6B parents; in the second experiment the larvae were derived from a cross of hus137/TM6B x Df(3R)110/TM3, Sb.

To determine potential causes of lethality after genotoxic stress, neuroblast squashes of MMS-treated larvae were examined for chromosomal defects. HU 30 mM 99 1 1 65
HU 20 mM 88 12 6 213
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HU, hydroxyurea. In the first set of experiments the larvae were derived from a cross between heterozygous hus137/TM6B parents; in the second experiment the larvae were derived from a cross of hus137/TM6B x Df(3R)110/TM3, Sb. NA, not applicable.

**Table 1. Hydroxyurea sensitivity of hus1 mutant larvae**

| Experiment 1 | hus117/TM6B (%) | hus117/hus117 (%) | s.d. between experiments | n |
|--------------|-----------------|-------------------|--------------------------|---|
| Control      | 65              | 35                | 4                        | 438 |
| HU 10 mM     | 80              | 20                | 2                        | 221 |
| HU 20 mM     | 88              | 12                | 6                        | 213 |
| HU 30 mM     | 99              | 1                 | 1                        | 65  |

| Experiment 2 | hus117/TM3 Df(hus1)/TM6 (%) | hus117/Df(hus1) (%) | s.d. between experiments | n |
|--------------|-----------------------------|---------------------|--------------------------|---|
| Control      | 33 36                       | 31                  | NA                       | 128 |
| HU 20 mM     | 43 40                       | 17                  | NA                       | 120 |

**Table 2. Methyl methanesulfonate sensitivity of hus1 mutant larvae**

| Experiment 1 | hus117/TM6B (%) | hus117/hus117 (%) | s.d. between experiments | n |
|--------------|-----------------|-------------------|--------------------------|---|
| Control      | 47              | 53                | 4                        | 771 |
| MMS 0.025%   | 97              | 3                 | 2                        | 362 |
| MMS 0.05%    | 98              | 2                 | 6                        | 192 |
| MMS 0.08%    | 100             | 0                 | 1                        | 121 |

| Experiment 2 | hus117/TM3 Df(hus1)/TM6 (%) | hus117/Df(hus1) (%) | s.d. between experiments | n |
|--------------|-----------------------------|---------------------|--------------------------|---|
| Control      | 32 31                       | 36                  | NA                       | 358 |
| MMS 0.08%    | 52 46                       | 2                   | NA                       | 246 |

MMS, methyl methanesulfonate. In the first set of experiments the larvae were derived from a cross between heterozygous hus117/TM6B parents; in the second experiment the larvae were derived from a cross of hus117/TM6B x Df(3R)110/TM3, Sb.

Percentages are the fraction of total surviving adults.
division was not arrested in the mei-41 mutant. This result shows that the requirements for hus1 differ from those of mei-41 after IR.

The hus137 mutant suppresses the patterning defects caused by mutations in the DNA repair enzymes, but not the oocyte nuclear defects

Mutations in the spindle class of double-strand break (DSB) DNA repair enzymes, such as spn-A (RAD51), spn-B (XRCC3), spn-C (HEL308), spn-D (Rad51C) and okra (Dmr54), affect dorsal-ventral patterning during Drosophila oogenesis and cause defects in the appearance of the oocyte nucleus (Ghabrial et al., 1998; Staeva-Vieira et al., 2003; Abdu et al., 2003; Laurencon et al., 2004). Interestingly, the defects in dorsal-ventral and in the oocyte nucleus are dependent on the activation of a meiotic checkpoint (Ghabrial and Schüpbach, 1999; Abdu et al., 2002; Staeva-Vieira et al., 2003). Activation of the meiotic checkpoint prevents efficient translation of gurken (grk) mRNA, which results in a ventralization of eggs and embryos.

The patterning and the oocyte nuclear defects that occur as a result of mutations that affect double-strand DNA repair can be suppressed by blocking the formation of double-strand DNA breaks (DSBs) during meiosis using mutations in the topoisomerase mei-W68 (Ghabrial and Schüpbach, 1999) or by eliminating the checkpoint by using mutations in mei-41 and chk2 (Ghabrial and Schüpbach, 1999; Abdu et al., 2002; Staeva-Vieira et al., 2003). To study whether hus1 is required in the activation of the meiotic checkpoint due to unrepaired double-strand DNA breaks, flies double mutant for hus1 and spn-B or okra were generated. In double-mutant flies we observed suppression of the dorsal-ventral patterning defects as compared to the single mutants (Table 5). However, the oocyte nuclear defects were not suppressed by our null mutation in hus1 (Table 6). Interestingly, analyzing the organization of the oocyte nucleus DNA in the hus1 single mutant revealed similar oocyte nuclear defects (Table 6) as those produced by mutations in DNA repair enzymes. In hus1 mutants the DNA within the oocyte nucleus is found in a variety of conformations including the smooth spherical wild-type shape (Fig. 6A), oblong shape (Fig. 6B) or in several separate pieces along the nuclear periphery (data not shown) similar to the

### Table 3. Irradiation sensitivity of hus1 mutant larvae

| Treatment | hus137/TM3 (%) | hus137/hus137 (%) | s.d. between days | n  |
|-----------|----------------|------------------|------------------|----|
| Control   | 65             | 35               | 9                | 2119 |
| IR 2500 rads | 60             | 40               | 10               | 1869 |

Experiment 2

| Treatment     | hus137/TM3 Df(hus1)/TM6 (%) | hus137/Df(hus1) (%) | s.d. between days | n  |
|---------------|-----------------------------|---------------------|------------------|----|
| Control       | 33 33                       | 34                  | 6                | 1830 |
| IR 2500 rads  | 37 25                       | 38                  | 5                | 674  |

IR, irradiation. In the first set of experiments the larvae were derived from a cross between heterozygous hus137/TM6B parents; in the second experiment the larvae were derived from a cross of hus137/TM6B × Df(3R)110/TM3, Sb.

Percentages are the fraction of total surviving adults. Standard deviation shown is for percentage of hus137/Df(hus1) surviving flies.

### Table 4. Irradiation sensitivity of spn-B mutant larvae

| Treatment | spnB81/TM6B (%) | spnB81/spnB81 (%) | s.d. between days | n  |
|-----------|----------------|------------------|------------------|----|
| Control   | 65             | 35               | 4                | 2355 |
| IR 2500 rads | 81             | 19               | 5                | 827  |

IR, irradiation. Larvae were derived from a cross between heterozygous spnB81/TM6B parents. % are the fraction of total surviving adults.
karyosome defects found in the spindle class of DNA repair enzyme mutations (Fig. 6D). Similar nuclear organization defects were obtained when the *hus1* allele was tested over a deficiency (Fig. 6C). To demonstrate that the karyosome defects are due to the lack of the *hus1* gene, we expressed the entire *hus1* open reading frame using an actin-Gal4 driver line in a *hus1* mutant background and found that this transgene fully rescues the karyosome defects (data not shown).

**Fig. 4.** *hus1* is not required for the G2-M checkpoint in the developing wing disc. (A-F) Larvae were mock-irradiated or irradiated with 4000 rad and allowed to recover for 1 hour before detection prior to fixation for phospho-histone H3 staining. (G) Number of mitotic cells in imaginal wing discs. Bars indicate standard deviations in the average number of mitotic cells from at least five wing discs.

**Discussion**

In this study we analyzed the requirement of the *Drosophila* Hus1 protein in somatic and meiotic checkpoints. First, we analyzed the interaction of the 9-1-1 complex in a yeast two hybrid assay. We found that Hus1 interacted with Rad1 or Rad9, however no interaction between Rad1 and Rad9 was observed. The yeast two hybrid system may not be sensitive enough to pick up the interaction, since possibly the interaction between these two proteins is more transient than the interaction between Hus1 and the other proteins. Similar results were seen in *C. elegans* where these proteins interact in vivo (Hofmann et al., 2002).

Several studies have investigated the role of *hus1* during development. In mouse, *Hus1* is an essential gene, since its inactivation results in mid-gestational embryonic lethality due to widespread apoptosis. Also, loss of *Hus1* leads to an accumulation of genome damage (Weiss et al., 2000). Both fission and budding yeast that lack *hus1* fail to arrest the cell cycle after DNA damage or blockage of DNA synthesis (Enoch et al., 1992; Weinert et al., 1994; Kostrub et al., 1998). In *C.
Table 5. Eggshell phenotypes of spn-B and okra alone and in combination with hus1

| Maternal genotype | Wild-type-like eggshell (%) | Abnormal eggshell (%) | n   |
|-------------------|----------------------------|-----------------------|-----|
| spn-B<sup>BU</sup> | 42                         | 58                    | 325 |
| hus1<sup>17</sup> spn-B<sup>BU</sup>/spn-B<sup>BU</sup> | 55                         | 45                    | 458 |
| hus1<sup>17</sup> spn-B<sup>BU</sup> | 99                         | 1                     | 652 |
| okr<sup>AA</sup> | 49                         | 51                    | 321 |
| okr<sup>AA</sup>/okr<sup>AA</sup>; hus1<sup>17</sup>/TM6B | 65                         | 35                    | 254 |
| okr<sup>AA</sup>/okr<sup>AA</sup>; hus1<sup>17</sup>/hus1<sup>17</sup> | 99                         | 1                     | 677 |

Wild-type-like eggshells have two separate dorsal appendages. Abnormal, ventralized eggshells have partially or completely fused appendages or lack appendages altogether.

Table 6. Karyosome phenotypes of spn-B and okra alone and in combination with hus1

| Maternal genotype | Wild-type-like oocyte nucleus (%) | Abnormal oocyte nucleus (%) | n   |
|-------------------|----------------------------------|----------------------------|-----|
| hus1<sup>17</sup> spn-B<sup>BU</sup>/spn-B<sup>BU</sup> | 2                            | 98                        | 88  |
| hus1<sup>17</sup> spn-B<sup>BU</sup> | 1                            | 99                        | 74  |
| okr<sup>AA</sup>/okr<sup>AA</sup>; hus1<sup>17</sup>/TM6B | 4                            | 96                        | 65  |
| okr<sup>AA</sup>/okr<sup>AA</sup>; hus1<sup>17</sup>/hus1<sup>17</sup> | 3                            | 97                        | 87  |
| hus1<sup>17</sup> | 8                            | 92                        | 121 |

In order to test for a requirement for Drosophila hus1 in response to genotoxic stress, we examined the survival rates of flies after exposure to HU, MMS and IR during larval development and found that hus1 mutant flies were sensitive only to HU and MMS. This result suggests that hus1 is required for the activation of an S-phase checkpoint. It is possible that this requirement is due to a role of hus1 in Chk1 (Grapes) activation after genotoxic stresses that affect S phase. In yeast and mice, hus1 has been shown to be required for Chk1 activation after replicative stress (Bao et al., 2004; Weiss et al., 2003). In Drosophila, mutations affecting grapes and mei-41 fail to show a decrease in BrdU-staining after irradiation, indicating a defect in an S-phase checkpoint (Jaklevic and Su, 2004), and it would, therefore, seem likely that Hus1 signals to activate Grapes (Chk1) through Mei-41 during S phase. An increase in aneuploid nuclei in hus1 mutants after MMS treatment is consistent with a requirement for hus1 in the response to DNA damage caused during S phase as it has been suggested in budding yeast that spontaneous chromosome loss is primarily suppressed by functional S-phase checkpoints and not by G2-M checkpoints (Klein, 2001). Since the hus1 mutant still exhibits cell cycle arrest after irradiation, hus1 does not seem to be required for the G2-M checkpoint that is dependent on Mei-41. Rather, our data suggest that hus1 is only required for certain DNA damage situations, and not for the same spectrum as Mei-41.

Activation of a meiotic checkpoint, also known as the pachytene checkpoint, in response to the persistence of unrepaired DSBs appears to be a conserved regulatory feature common to yeast, worms, flies and vertebrates. However, a requirement for the 9-1-1 complex in activation of the meiotic checkpoint has only been demonstrated in budding yeast. It was found that mutations in the yeast Hus1 homologue, Mec3, and the Rad1 homologue, Ddc-1, abolish the pachytene checkpoint in budding yeast (Hong and Roeder, 2000). In Drosophila, mutations in the spindle class of double-strand break (DSB) DNA repair enzymes, such as spn-A (RAD51), spn-B (XRCC3), spn-C (HEL308), spn-D (Rad51C) and okra (Dmrad54), affect dorsal-ventral patterning in Drosophila oogenesis and cause defects in the appearance of the oocyte nucleus (Ghabrial et al., 1998; Staeva-Vieira et al., 2003; Abdu...
et al., 2003; Laurencon et al., 2004). Interestingly, the defects in dorsal-ventral patterning and in the oocyte nucleus are dependent upon activation of a meiotic checkpoint (Ghabrial and Schüpbach, 1999; Abdu et al., 2002; Staeva-Vieira et al., 2003). We found that hus1 mutants are able to suppress the dorsal-ventral defects but not the defects in the organization of the DNA within the oocyte nucleus. The suppression of the DV patterning defects of spn-B mutants demonstrates that during meiosis Hus1 is required for the meiotic checkpoint in response to persistent DSBs. This finding is interesting in light of the fact that hus1 mutants are not IR sensitive or defective in somatic checkpoints after irradiation. Either there is a fundamental difference between germline and somatic DSBs and DSB response machinery, or the non-DSB lesions created during irradiation that are not present during meiotic recombination serve as triggers for an alternative sensing mechanism that does not require hus1 and is therefore still able to activate a checkpoint mechanism. The inability of hus1 mutants to suppress the karyosome phenotype along with the hus1 mutant phenotype by itself, demonstrates that hus1 is required for the organization of the oocyte DNA, a function that might be independent of the meiotic checkpoint.

In this study we have shown that Drosophila Hus1 is required for both the meiotic and somatic DNA damage responses as well as demonstrating a novel role of Hus1 in the organization of the oocyte nuclear DNA. Whereas some of the functions of Hus1, such as binding to 9-1-1 complex members and an essential role in surviving genotoxic stress during S phase, appear to be conserved across the species studied so far, some Hus1 functions seem to be less conserved. In contrast to the findings in yeast, worms and mouse, fly Hus1 is not required for survival after irradiation. Finally, the karyosome defect of hus1 mutants demonstrates a role for Drosophila Hus1 in organizing the chromosomal DNA of the meiotic nucleus.

Materials and Methods

Drosophila strains

Oregon-R was used as the wild-type control. The following mutant and transgenic flies were used: spn-Bg6 (Ghabrial et al., 1998), okra49 (Ghabrial et al., 1998), mei-41D3 (Hari et al., 1995) and chk2P6 (Abdu et al., 2002), Df(3R)110 (Bloomington stock center), P(GT1)B90590 and P(SUP+P)KGO7223 (Bellen et al., 2004). Marker mutations and balancer chromosomes are described in the Drosophila Genome Database at http://flybase.bio.indiana.edu.

Yeast two hybrid

The two-hybrid screen was performed using the Hybrid Hunter System (Invitrogen). The entire coding sequence of Hus1 was amplified by PCR using modified primers to create an XhoI restriction site at the 5' end and a SacI site at the 3' end. The resulting PCR product was cut using XhoI and SacI and was cloned into the pHybLex/Zeo vector (LexA DBD, which was used as bait). The entire coding sequence of Rad9 was cloned into the pYESTrp2 vector (B42 AD, which was used as prey) as follows: 1.25 mg/ml colchicine in PBS for 1 hour. Brains were incubated in 0.5 mg/ml Acridine Orange for 5 minutes, washed in PBS, and visualized with a fluorescence microscope. Representative discs are shown from one of three replicate experiments. At least five discs were analyzed per experiment.

Neuroblast chromosome squashes

Larva were treated with water or 0.025% MMS as described for MMS sensitivity assays. Four to five days after MMS treatment larval brains of climbing third instar larvae were dissected in PBS and incubated for 10 min with MMS; Sigma). Four days after irradiation, imaginal discs were dissected, incubated in 0.5 mg/ml Acridine Orange for 5 minutes, washed in PBS, and visualized with a fluorescence microscope. Representative discs are shown from one of three replicate experiments. At least five discs were analyzed per experiment.
References

Abdu, U., Brodsky, M. and Schupbach, T. (2002). Activation of a mitotic checkpoint during Drosophila oogenesis regulates the translation of Gurken through Chk2/Mnk. *Curr. Biol.* 12, 1645-1651.

Abdu, U., Gonzalez-Reyes, A., Ghabrrial, A. and Schupbach, T. (2003). The Drosophila spin-D gene encodes a RAD51Cl-like protein that is required exclusively during meiosis. *Genetics* 165, 197-204.

Bao, S., Lu, T., Wang, X., Zheng, H., Wang, L. E., Wei, Q., Hittelman, W. N. and Li, L. (2004). Disruption of the Rad9/Rad1/Hus1 (9-1-1) complex leads to checkpoint signaling and replication defects. *Onco genes* 23, 5586-5593.

Bellen, H. J., Levis, R. W., Liao, G., He, Y., Carlson, J. W., Tsang, G., Evans-Holm, M., Hiesinger, P. R., Schulze, K. L., Rubin, G. M. et al. (2004). The BDGP gene disruption project: single transposon insertions associated with 40% of Drosophila genes. *Genetics* 167, 761-781.

Bermudez, V. P., Lindsey-Boltz, L. A., Cesare, A. J., Maniya, Y., Griffith, J. D., Hurwitz, J. and Sancar, A. (2003). Loading of the human 9-1-1 checkpoint complex onto DNA by the checkpoint clamp loader Rad17-replication factor C complex in vitro. *Proc. Natl. Acad. Sci. USA* 100, 1633-1638.

Bi, X., Wei, S. C. and Rong, Y. S. (2004). Telomere protection without a telomerase; the role of ATM and Meo11 in Drosophila telomere maintenance. *Curr. Biol.* 14, 1348-1353.

Brodsky, M. H., Sekelsky, J. J., Tsang, G., Hawley, R. S. and Rubin, G. M. (2000). mus304 encodes a novel DNA damage checkpoint protein required during Drosophila development. *Genes Dev.* 14, 666-678.

Brodsky, M. H., Weinert, B. T., Tsang, G., Rong, Y. S., McGinnis, N. M., Golie, K. G., Rio, D. C. and Rubin, G. M. (2004). Drosophila melanogaster Mnk/Chk2 and p53 regulate multiple DNA repair and apoptotic pathways following DNA damage. *Mol. Cell. Biol.* 24, 1219-1231.

Dasika, G. K., Lin, S. C., Zhao, S., Sung, P., Tomkinson, A. and Lee, E. Y. (1999). DNA damage-induced cell cycle checkpoints and DNA strand break repair in development and tumorigenesis. *Onco genes* 18, 7883-7889.

Enoch, T., Carr, A. M. and Nurse, P. (1992). Fission yeast genes involved in coupling mitosis to completion of DNA replication. *Genes Dev.* 6, 2035-2046.

Fogarty, P., Campbell, S. D., Abu-Shumays, R., Phalle, B. S., Yu, K. R., Uy, G. L., Goldberg, M. L. and Sullivan, W. (1997). The Drosophila grapes gene is related to checkpoint gene chklad27  and is required for late syncytial division fidelity. *Curr. Biol.* 7, 418-426.

Garner, M., van Kreeveld, S. and Su, T. T. (2001). mei-41 and bubl block mitosis at two distinct steps in response to incomplete DNA replication in Drosophila embryos. *Curr. Biol.* 11, 1595-1599.

Ghabrrial, A. and Schupbach, T. (1999). Activation of a meiotic checkpoint regulates translation of Gurken during Drosophila oogenesis. *Nat. Cell Biol.* 1, 354-357.

Ghabrrial, A., Ray, R. P. and Schupbach, T. (1998). okra and spindle-B encode components of the RAD52 DNA repair pathway and affect meiosis and patterning in Drosophila oogenesis. *Genes Dev.* 12, 2711-2723.

Hari, K. L., Santerre, A., Sekelsky, J. J., McKim, K. S., Boyd, J. B. and Hawley, R. S. (1995). The mei-41 gene of D. melanogaster is a structural and functional homolog of the human ataxia telangiectasia gene. *Cell* 82, 815-821.

Harrison, J. C. and Haber, J. E. (2006). Surviving the breakup: the DNA damage checkpoint. *Annu. Rev. Genet.* 19, 210-224.

Hofmann, E. R., Milstein, S., Boulton, C. L., Ye, M., Vladar, E. K., Langley, S. A., Bakis, E. P., Harris, D. T., Harris, N. J., Wayson, S. M. et al. (2004). A large-scale screen for mutagen-sensitive loci in Drosophila. *Genetics* 167, 217-231.

Hsu, N. Y., Wang, L., Hijal, S., Larocelhe, S. and Suter, B. (2003). The Drosophila chkl gene loki is essential for embryonic DNA double-strand-break checkpoints induced in S phase or G2. *Genetics* 163, 973-982.

Kim, K. S., Jang, J. K., Sekelsky, J. J., Laurencon, A. and Hawley, R. S. (2000). mei-41 is required for precocious anaphase in Drosophila females. *Chromosoma* 109, 44-49.

Koemis, S. R., McGinnis, N., Queiroz-Machado, J., Tukachinsky, H., Takada, S., Sunkel, C. E. and Brodsky, M. H. (2004). Drosophila atm/telomere fusion is required for telomeric localization of H3 and telomere position effect. *Genes Dev.* 18, 1850-1861.

Kostrub, C. F., Knudsen, K., Subramani, S. and Enoch, T. (1998). Hus1p, a conserved fission yeast checkpoint protein, interacts with Rad1p and is phosphorylated in response to DNA damage. *EMBO J.* 17, 2055-2066.

Laurencon, A., Orme, C. M., Peters, H. K., Boulton, C. L., Vladar, E. K., Langley, S. A., Bakis, E. P., Harris, D. T., Harris, N. J., Wayson, S. M. et al. (2004). A large-scale screen for mutagen-sensitive loci in Drosophila. *Genetics* 167, 217-231.