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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 HU and MMS 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.

Keywords

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 comprised of Hus1, Rad1, and Rad9 and the associated protein Rad17. Computer modeling suggests that Rad9, Hus1 and Rad1 (also called 9-1-1 complex) form a doughnut-like heteromeric PCNA complex that can be loaded directly onto damaged DNA (Rauen et al.,

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2000; Venclovas and Thelen, 2000; Bermudez et al., 2003). The signal transducers are comprised of four sets of conserved protein families. One family is composed of ATM and ATM-Rad3-related (ATR) proteins. Downstream of these proteins are two sets of checkpoint kinases, the Chk1 and the Chk2 kinases and their homologues. The fourth conserved family is that of the BRCT-repeat containing proteins. Finally, a diverse range of effector proteins execute the function of the DNA damage response, which can lead to cell cycle arrest, apoptosis or activation of the DNA repair machinery (reviewed in Harrison and Haber, 2006).

A number of checkpoint proteins that were initially characterized in budding and fission yeast, have counterparts in *Drosophila*, *C. elegans* and mammals, demonstrating the conservation of these surveillance mechanisms. Several checkpoint proteins have been characterized in *Drosophila*, mainly the ATM/ATR and the Chk1/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* also 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* or *Dmnk*) 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.

Material and Methods

Drosophila strains

Oregon-R was used as wild-type control. The following mutant and transgenic flies were used: *spn-B*^{BU} (Ghabrial et al., 1998), *okra*^{AA} (Ghabrial et al., 1998), *mei-41*^{D3} (Hari et al., 1995), and *chk2*^{P6} (Abdu et al., 2002), Df(3R)110 (Bloomington stock center), P{GT1}BG00590 and P{SUPor-P}KG07223 (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 *SalI* site at the 3′ end. The resulting PCR product was cut using *XhoI* and *SalI* and was cloned into the pHybLex/Zeo vector (LexA DBD, which was used as bait). The entire coding sequence of Rad1 as well as a truncated version (from amino acid 35) was introduced into the pYESTrp2 vector (B42 AD, which was used as prey) as *Sac1-EcoR1*. The entire coding sequence of Rad9 was cloned into the pYESTrp2 vector as *HindIII-EcoRI*, and also cloned into the pHybLex/Zeo vector as *SacI-XhoI*. Positive interactions were detected by selecting on SD-His plates, followed by a second screen for *β-galactosidase* expression.

RT-PCR analysis

Total RNA was obtained from 10–15 ovaries using Trizol Reagent® (Invitrogen) following the manufacturer's protocol. RT–PCR was performed using SuperScript™ One-Step RT–PCR with Platinum® *Taq* (Invitrogen). Control experiments, using Platinum® *Taq* minus RT, were performed to confirm the absence of contaminating genomic DNA. No signal was ever obtained from the RNA preparation. The primers that were used are: 1) Rad1 forward GGATGACTGATGTGGAGCCATC and reverse CAGGGGATCGCCCTTATCCCTG , 2) Hus1 forward GCCTCGGTGCTTACGTCGTCTTCAAC, reverse ACATACAAACAGCTGGCAGAATAG and 3) Rad9 forward TTGCCAATGAAATACACTTTAG, reverse CCACGGATTATATTCGGCATC.

Transgenic flies

To make the pUASp-Hus1 fusion construct the entire coding sequence of *hus1* was amplified by PCR using modified primers to create a *KpnI* restriction site at the 5′ end and a *NotI* site at the 3′ end. The resulting PCR product was cut using *KpnI* and *NotI* and was cloned into pUASp. P-element-mediated germ-line transformation of this construct was carried out according to standard protocols (Spradling and Rubin, 1982). Hus1 was expressed in the ovaries using an Act5C-Gal4 expression system.

DNA staining of ovaries

For karysome staining, ovaries were dissected in phosphate-buffered saline (PBS), fixed in 200 μl 4% paraformaldehyde in PBST (PBS + 0.2% Tween 20) plus 600 μl heptane for 20 minutes. Ovaries were incubated in 0.2 mg/ml RNase A and a 1:5000 dilution of OliGreen (Molecular Probes) or 1:10,000 Hoechst (Molecular Probes) and 1μg/ml wheat-germ Agglutinin-488 (Molecular Probes) for 1 hour. After several washes, ovaries were mounted in 50% glycerol:PBS and visualized by confocal microscopy.

Creation of *Hus1* mutants

Excision of P{SUPor-P}KG07223 was generated by crossing to a transposase-expressing line (*Sb Δ2–3/TM6B*). Seventy male progeny from this cross, of the genotype *w*; *KG07223*/ *Sb*

Δ*2–3*, were then crossed to *Pri/TM6B* females, and 145 potential excision events were identified by the loss of the w^+ marker. All of these lines were tested by genomic PCR reaction with primers that cover the first exon. Excision of P{GT1}BG00590 was done the same as above with the following modification: 167 potential excision events were identified and tested by genomic PCR reaction with primers that cover the second exon.

MMS, HU and IR sensitivity assays

Heterozygous males and females were mated in vials and eggs were collected for 24 hours at room temperature. Parents were removed and 24-48 hours later the larvae were treated with different concentrations of methyl methanesulfonate (MMS, Sigma) or hydroxyurea (HU, Sigma), or irradiated with 2500 Rads in a Faxitron X-ray cabinet. Control flies were treated with 250 μl water or not irradiated. After eclosion the percentage of mutant flies was determined, and the sensitivity was expressed as the fraction of the expected percentage of the mutant flies in the treated vial as compared to the progeny of untreated control vials. Each experiment was repeated at least three times.

Survival rates of *hus-1* larvae and pupae

First and early second instar larvae (age: 30 +/− 12 hrs after egg laying) of appropriate genotype were selected under a dissecting microscope with GFP detection filter. The larvae were put into food vials and treated with 0.08% MMS 4-6 hrs later. Control larvae were treated with 250 μl water. White non-motile pupae were counted, later, pharate adults, and finally, hatched adults were counted.

Checkpoint and apoptosis assays

Homozygous $hus1^{37}$ and $mei\text{-}41^{D3}$ larvae were tested for their ability to undergo cell cycle arrest after IR as described in Brodsky et al., 2000. Confocal stacks of 0.5 micron intervals were analyzed using Volocity 3DM software (Improvision). At least five discs from two separate experiments were used for quantification.

To determine the requirement of *hus1* for post-irradiation induction of apoptosis during larval development, climbing homozygous larvae were mock-treated or treated with 4000 Rads. Four hours after irradiation, imaginal discs were dissected, incubated in 0.5 μg/ml acridine orange for 5 minutes, washed in PBS, and visualized with a fluorescent 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. 4-5 days after MMS treatment larval brains of climbing third instar larva were dissected in PBS and incubated in 20 μg/ml colchicine in PBS for one hour. Brains were incubated in 0.5% sodium citrate for 10 minutes, fixed in 11:11:2 acetic acid/methanol/water, and squashed in 45% acetic acid. Slides were frozen in liquid nitrogen, incubated 20 minutes in cold ethanol and mounted in Vectashield mounting media with DAPI (Vector).

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 in 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. 1 C2), only a weak interaction between Hus1 and Rad9 was detected (Fig. 1 C1). 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-elements 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′ of *hus1* (Bellen et al., 2004) yielded one candidate mutant, $hus1^{98}$. 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 2kb from the 3′ of *hus1* gene, was mobilized (Bellen et al., 2004) and one candidate mutant, $hus1^{37}$, was identified. $hus1^{37}$ has deletion of 3297 bases which remove the entire ORF of *hus1* gene and delete 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. 2 A). However, no *hus1* transcript was detected in $hus1^{37}$ ovaries by RT-PCR analysis as compared to wild-type (Fig. 2 B), as expected from the molecular analysis, demonstrating that $hus1^{37}$ is a null allele. The level of *rad9* transcript was used as control (Fig. 2 B). We found that $hus1^{37}$ mutant flies are viable however, females are sterile. This line was used for further examination of *hus1* mutant phenotypes.

$hus1^{37}$ 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 $hus1^{37}$ mutants to varying concentrations of HU and MMS and to X-ray irradiation (2500 Rads) was determined. HU stalls replication through inhibition of deoxynucleotide 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, while 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 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 larva were examined for chromosomal defects. *hus1* mutant larva treated with 0.025% MMS had 15.4% aneuploid nuclei (Fig. 3B), an approximately four fold increase as compared to wild-type larva or their untreated siblings (Fig. 3A).

Treatment of $hus1^{37}$ with 2500 Rads of irradiation did not result in a decrease of homozygous flies relative to untreated controls. Similar results were also observed when we tested $hus1^{37}$/Deficiency (Table 3). In our irradiation assay we were able to detect a significant sensitivity for *spnB* mutant flies (Table 4), which have been shown to be only moderately sensitive to irradiation (Staeva-Viera et al., 2004), indicating that *hus1* mutant flies are not sensitive to irradiation.

Hus1 is not required for the G2/M checkpoint and for post-irradiation induction of apoptosis

Following irradiation, a checkpoint is activated in the imaginal discs that results in a cell cycle arrest and the induction of apoptosis (Brodsky et al., 2000). Though *hus1* is not required for survival after irradiation, Jaklevic and Su (2004) have suggested that while DNA repair is essential for surviving irradiation, proper cell cycle regulation and *p53*-dependent cell death is not essential for survival. While *grapes* (*chk1*)is required for cell cycle arrest in the imaginal discs after irradiation and *p53* is required for radiation-induced death, neither mutant exhibits a significant decrease in survivorship after irradiation (Jaklevic and Su, 2004). Therefore we tested for a requirement for *hus1* in cell cycle arrest after irradiation by examining the phospho-histone H3 levels 1 hour post-irradiation. Similar to wild-type controls, very few mitotic cells are observed in *hus1* mutant discs after irradiation (Fig. 4), indicating that cell cycle arrest is still correctly initiated. *hus1* is also not required for the post-irradiation induction of apoptosis seen in wild-type discs. Four hours after irradiation, *hus1* mutant discs exhibited wild-type levels of apoptosis (Fig. 5). For comparison, we also irradiated larvae homozygous mutant for *mei-41*. As previously reported (Jaklevic and Su, 2004), we observed that cell 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 $hus1^{37}$ mutant suppresses the pattering 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 *okr* (*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 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 in mutants affecting 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 pattering 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 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 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 $hus1^{37}$ mutant background and found that this transgene fully rescues the karyosome defects (Data not shown).

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 inactivation of *hus1* 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; Hartwell et al., 1994; Kostrub et al., 1997). In *C. elegans* although *hus1* is not absolutely required for embryonic survival, a significant fraction of *hus1* embryos die during embryogenesis, likely due to genomic instability. Also, *hus1* mutants fail to induce apoptosis and proliferation arrest following DNA damage and show increased sensitivity to DNA damage-induced lethality (Hofmann et al., 2000). We found that the *Drosophila hus1* is not an essential gene, although similarly to *C. elegans* the females are sterile; this is probably due to the defects in the organization of the DNA within the oocyte nucleus.

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 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/Dchk1* and *mei-41/ATR* 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 Chk1/Grapes 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 *okr* (*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 *spnB* 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 required for 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. While some of the functions of Hus1, such as binding to 9-1-1 complex members and an essential role in survival to 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 plants, 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.

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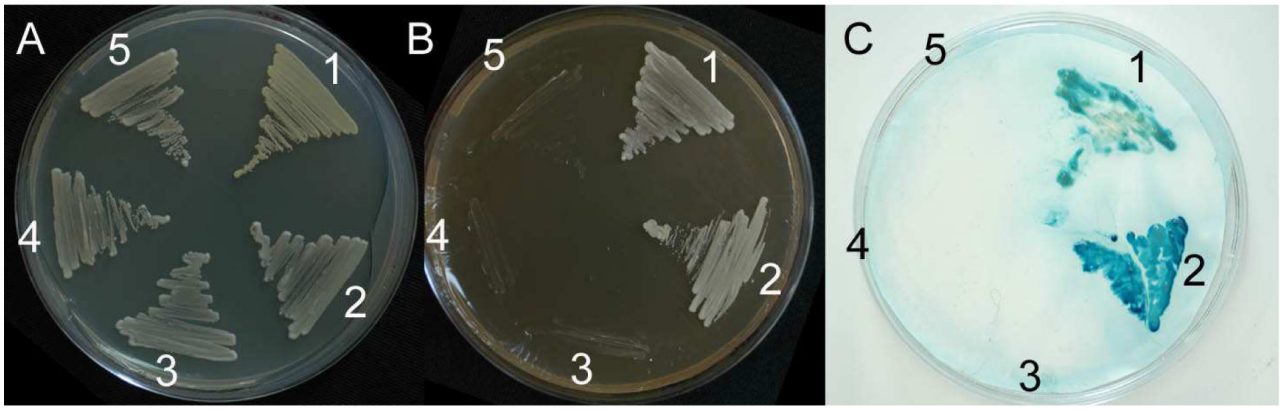

Figure 1.

Detection of the interaction between Hus1 and Rad9 or Rad1. 1, L40 bearing Hus1 in pHybLex/Zeo vector and Rad9 in pYESTrp2 vector; 2, L40 bearing Hus1 in pHybLex/Zeo vector and Rad1 in pYESTrp2 vector; 3, L40 bearing Rad9 in pYESTrp2 vector and an empty pHybLex/Zeo vector; 4, L40 bearing Hus1 in pHybLex/Zeo vector and an empty pYESTrp2 vector; 5, L40 bearing Rad1 in pYESTrp2 vector and an empty pHybLex/Zeo vector. A, Non-selective medium for detection of interaction; B, The activation of the *HIS* promoter was tested on plates without Histidine. C, Activation of the *LacZ* promoter by assay of β-*galactosidase* activity. Hus1 interacted either with Rad9 (B1, C1) or Rad1 (B2, C2).

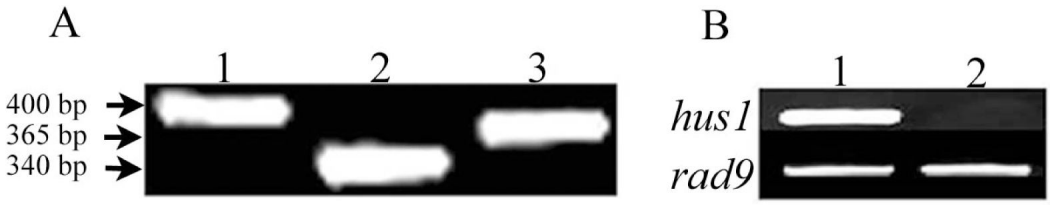

Figure 2.
RT-PCR detection of *hus1*, *rad9* and *rad1* transcripts in wild type and in *hus1*^{37} mutant ovaries. A, detection of *hus1*, *rad9* and *rad1* transcripts in wild type ovaries. 1, *hus1* transcript ; 2, *rad1* transcript; 3, *rad9* transcript. B, detection of *rad9* and *hus1*, transcripts in wild type and in *hus1*^{37} mutant ovaries. 1, wild type ovaries; 2, *hus1*^{37} mutant ovaries.

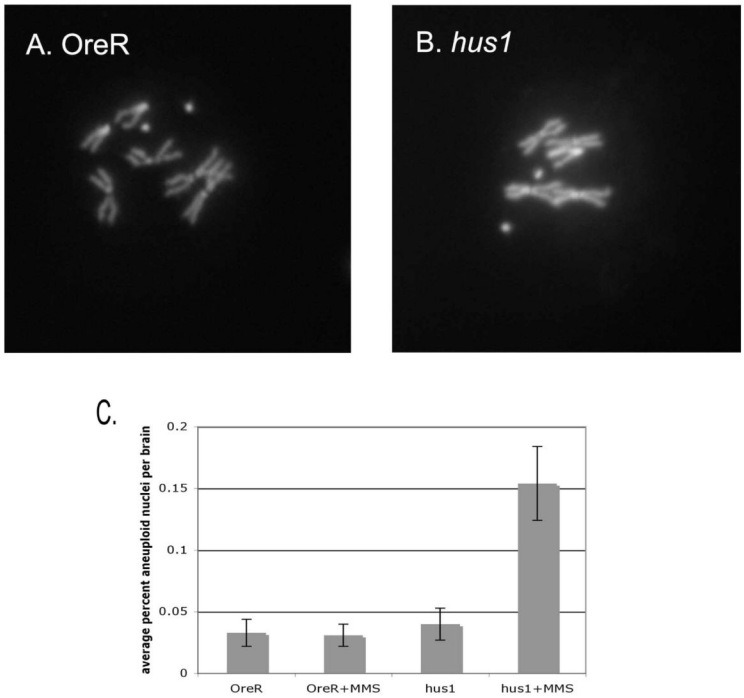

Figure 3.

hus1 mutant larva accumulates aneuploid nuclei after MMS treatment. A, wild-type neuroblast chromosome spread. B, *hus1*^{37} mutant nucleus lacking one sex chromosome. C. Frequencies of aneuploid nuclei after MMS treatment. Standard deviations represent deviation between the average percent aneuploid nuclei from four brains from two separate experiments.

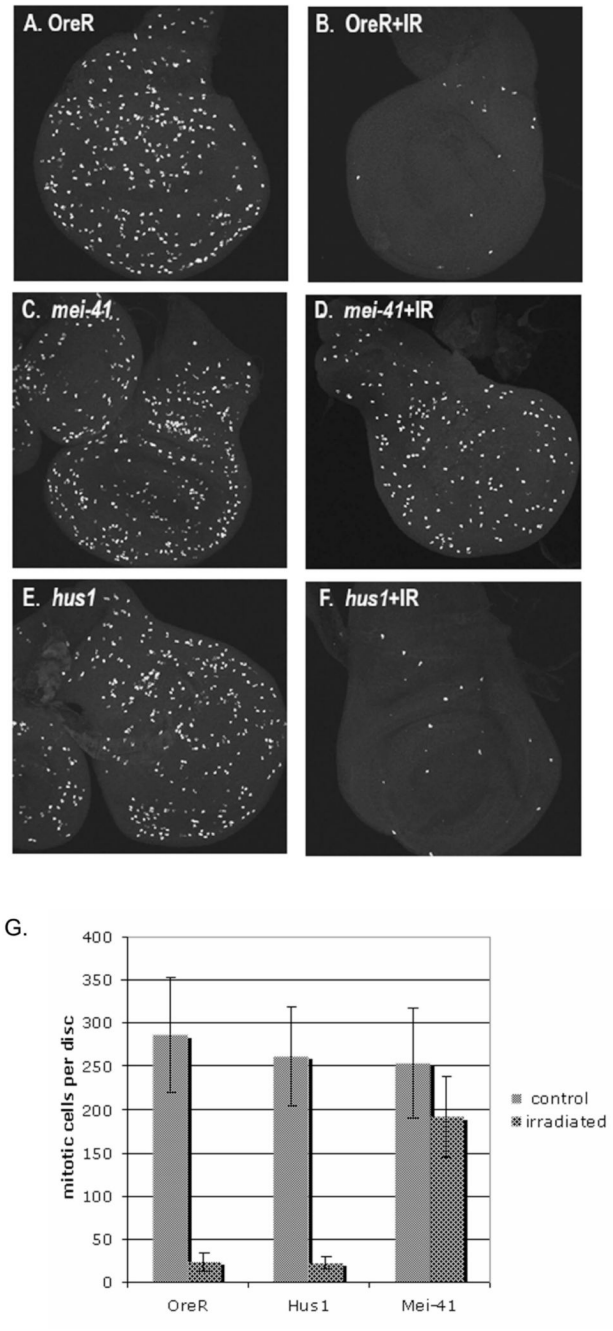

Figure 4.

hus1 is not required for the G2/M checkpoint in the developing wing disc. Larva were mock-irradiated or irradiated with 4000 rads and allowed to recover for one hour before detection prior to fixation for (I-P) phospho-histone H3 staining. G, Number of mitotic cells in imaginal wing discs. Standard deviations represent deviations in the average number of mitotic cells from at least five wing discs.

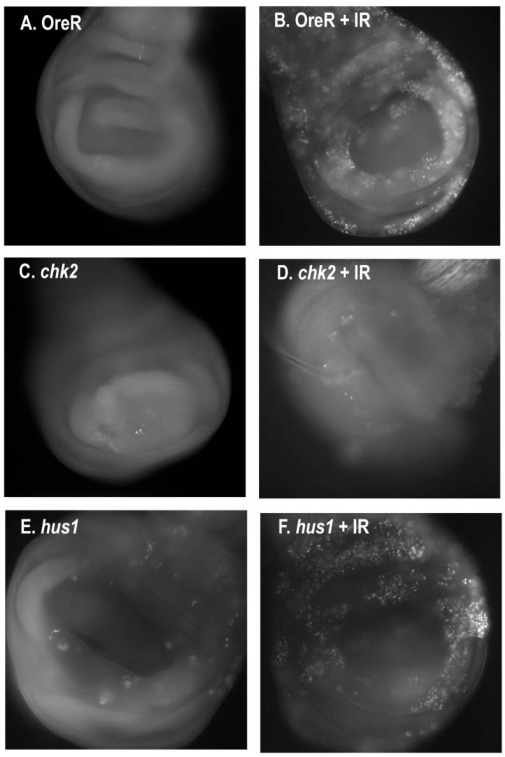

Figure 5.
hus1 is not required for post-irradiation induction of apoptosis in the developing wing disc. Larva were mock-irradiated or irradiated with 4000 Rads and allowed to recover for four hours before detection with (A-F) acridine orange. Representative discs shown. At least fifteen discs were examined for each condition.

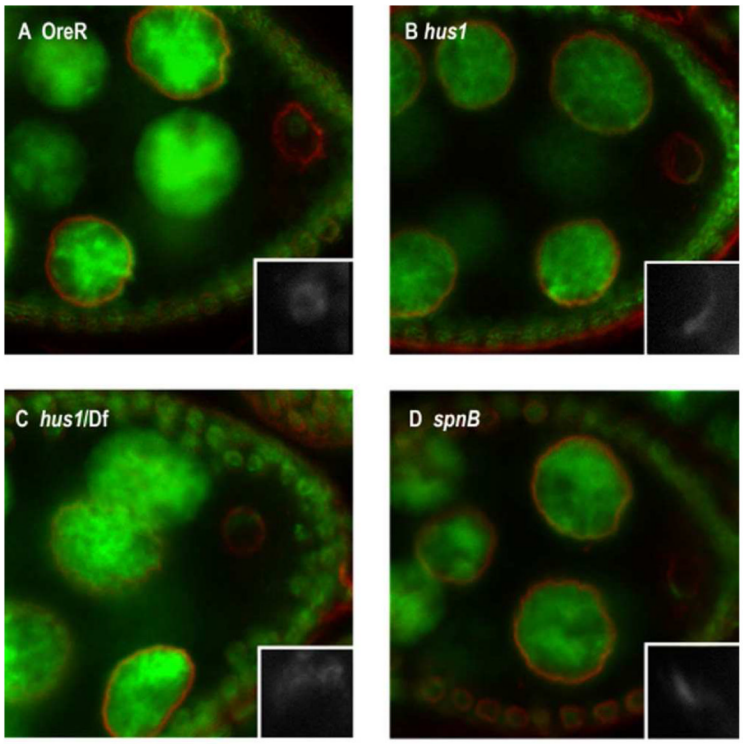

Figure 6.
Organization of the DNA in the oocyte nucleus in wild-type and *hus1* mutants. DNA in green and nucleus membrane in red. Inserts show a higher magnification of the oocyte DNA. A, wild-type egg chamber. B, hus^{37} egg chamber. C, $hus^{37}/Df(3R)110$ egg chamber. D, $spnB^{BU}$ egg chamber. The wild-type karysome is a sphere near the center of the nucleus, while the mutant karysomes are crescent-shaped DNA masses near the nuclear periphery.

Table 1

HU sensitivity of *hus1* mutant larvae

	$hus1^{37}$/TM6B in % of total	**$hus1^{37}$/ $hus1^{37}$ in % of total**	**Standard deviation 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

	$hus1^{37}$/TM3 Df(hus1)/TM6 (%)	**$hus1^{37}$/ *Df(hus1)* (%)**	**Standard deviation between experiments**	**n**
Control	33 36	31	NA	128
HU 20 mM	43 40	17	NA	120

In the first set of experiments the larvae were derived from a cross between heterozygous *$hus1^{37}$*/TM6B parents; in the second experiment (last two lines) the larvae were derived from a cross of *$hus1^{37}$*/TM6B × *Df(3R)110*/TM3, Sb.

NA - not applicable.

Table 2

MMS sensitivity of *hus1* mutant larvae

	hus1^{37}/TM6B (%)	***hus1^{37}/ hus1^{37}* (%)**	**Standard deviation 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

	hus1^{37}/TM3 Df(hus1)/TM6 (%)	***hus1^{37}/ Df(hus1)* (%)**	**Standard deviation between experiments**	**n**
Control	32 31	36	NA	358
MMS 0.08%	52 46	2	NA	246

In the first set of experiments the larvae were derived from a cross between heterozygous *hus1^{37}*/TM6B parents; in the second experiment (last two lines) the larvae were derived from a cross of *hus1^{37}*/TM6B × *Df(3R)110*/TM3, Sb.

% are given as the fraction of total surviving adults.

Table 3

Irradiation sensitivity of *hus1* mutant larvae.

	hus1^{3}/TM3 (%)	***hus1^{37}/ hus1^{37}* (%)**	**Standard deviation between days**	**n**
control	65	35	9	2119
IR 2500 rads	60	40	10	1869

	hus1^{37}/TM3 Df(hus1)/TM6 (%)	***hus1^{37}/ Df(hus1)* (%)**	**Standard deviation between days**	**n**
Control	33 33	34	6	1830
IR 2500 rads	37 25	38	5	674

In the first set of experiments the larvae were derived from a cross between heterozygous *hus1^{37}*/TM6B parents; in the second experiment (last two lines) the larvae were derived from a cross of *hus1^{37}*/TM6B × Df(3R)110/TM3, Sb. % are given as the fraction of total surviving adults. Standard deviation shown is for percent *hus1^{37}*/Df(*hus1*) surviving flies.

Table 4

Irradiation sensitivity of *spn-B* mutant larvae.

	spnB^{BU}/TM6B (%)	***spnB^{BU}/ spnB^{BU}* (%)**	**Standard deviation between days**	**n**
control	65	35	4	2355
IR 2500 rads	81	19	5	827

Larvae were derived from a cross between heterozygous *spnB^{BU}*/TM6B parents.

% are given as the fraction of total surviving adults

Table 5

Eggshell phenotypes of *spn-B* and *okra* alone and in combination with *hus1*.

Maternal genotype	Wild-type-like eggshell (%)	Abnormal eggshell (%)	n
$spn\text{-}B^{BU}$	42	58	325
$hus1^{37}$ $spn\text{-}B^{BU}$ / $spn\text{-}B^{BU}$	55	45	458
$hus1^{37}$ $spn\text{-}B^{BU}$	99	1	652
okr^{AA}	49	51	321
okr^{AA}/ okr^{AA}; $hus1^{37}$/*TM6B*	65	35	254
okr^{AA}/ okr^{AA}; $hus1^{37}$/ $hus1^{37}$	99	1	677

Wild-type-like egg shells display 2 separate dorsal appendages. Abnormal, ventralized egg shells display 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
$hus1^{37}$ $spn\text{-}B^{BU}$ / $spn\text{-}B^{BU}$	2	98	88
$hus1^{37}$ $spn\text{-}B^{BU}$	1	99	74
okr^{AA}/ okr^{AA}; $hus1^{37}$/TM6B	4	96	65
okr^{AA}/ okr^{AA}; $hus1^{37}$/ $hus1^{37}$	3	97	87
$hus1^{37}$	8	92	121