



HAL
open science

Drosophila Yemanuclein associates with the cohesin and synaptonemal complexes

R. Meyer, A. Algazeery, M. Capri, H. Brazier, C. Ferry, Ounissa Ait-Ahmed

► **To cite this version:**

R. Meyer, A. Algazeery, M. Capri, H. Brazier, C. Ferry, et al.. Drosophila Yemanuclein associates with the cohesin and synaptonemal complexes. *Journal of Cell Science*, 2014, 127 (21), pp.4658-4666. 10.1242/jcs.152520 . hal-02884214

HAL Id: hal-02884214

<https://hal.science/hal-02884214v1>

Submitted on 1 Jun 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

RESEARCH ARTICLE

Drosophila Yemanuclein associates with the cohesin and synaptonemal complexes

Régis E. Meyer^{1,*§}, Ahmed Algazeery^{1,‡§}, Michèle Capri¹, Hélène Brazier¹, Christine Ferry¹ and Ounissa Ait-Ahmed^{1,2,1}

ABSTRACT

Meiosis is characterized by two chromosome segregation rounds (meiosis I and II), which follow a single round of DNA replication, resulting in haploid genome formation. Chromosome reduction occurs at meiosis I. It relies on key structures, such as chiasmata, which are formed by repair of double-strand breaks (DSBs) between the homologous chromatids. In turn, to allow for segregation of homologs, chiasmata rely on the maintenance of sister chromatid cohesion. In most species, chiasma formation requires the prior synapsis of homologous chromosome axes, which is mediated by the synaptonemal complex, a tripartite proteinaceous structure specific to prophase I of meiosis. Yemanuclein (Yem) is a maternal factor that is crucial for sexual reproduction. It is required in the zygote for chromatin assembly of the male pronucleus, where it acts as a histone H3.3 chaperone in complex with Hira. We report here that Yem associates with the synaptonemal complex and the cohesin complex. A genetic interaction between *yem*¹ (V478E) and the *Spo11* homolog *mei-W68*, modified a *yem*¹ dominant effect on crossover distribution, suggesting that Yem has an early role in meiotic recombination. This is further supported by the impact of *yem* mutations on DSB kinetics. A *Hira* mutation gave a similar effect, presumably through disruption of Hira–Yem complex.

KEY WORDS: *Drosophila*, Oocyte, Meiosis, Yemanuclein, Yem, Synaptonemal complex, Cohesin, Recombination, Hira

INTRODUCTION

Meiosis is the specialized chromosome segregation process that drives germ cells to form haploid gametes. To achieve this result two successive segregation rounds that eventually halve the chromosome number follow a single round of DNA replication. Meiosis I is the key step in the chromosome segregation process that leads to haploidy; it relies on specific structures and molecules. The side-by-side organization of the sister kinetochores, which is maintained by meiosis-I-specific cohesion in conjunction with a physical link between the homologous chromatids, ensures the accurate segregation of the

homologous centromeres at anaphase I of a recombination-proficient meiosis. Importantly, the sister centromeres are held together until anaphase II owing to the meiosis-specific cohesion (see Petronczki et al., 2003; Watanabe, 2005 and references therein). In most species, the physical link that connects the homologous chromatids, which results from the use of the homologous chromatid as a template for double-strand break (DSB) repair, can be observed as a cytological mark called chiasma. These meiotic events can be genetically measured as crossing-overs (Phadnis et al., 2011). Resolution of recombination intermediates as crossing-overs relies on specific protein complexes. The first complex whose structure and function in meiotic recombination was recognized is the synaptonemal complex, a zipper-like proteinaceous structure (Carpenter, 1975; Moses, 1969; Moses, 2006). The other complex is the cohesin complex, which is important for many aspects of chromosome structure and function (Hirano, 2000; Wood et al., 2010). Importantly, the cohesin complex is essential for the organization of meiotic chromosomes; in particular, it is intimately linked to the structure and assembly of the synaptonemal complex (Revenkova and Jessberger, 2006; Stack and Anderson, 2001). The synaptonemal complex holds the homologs together during the recombination process; therefore, its integrity is required for the chromosome segregation process, which relies on homologous recombination (Page and Hawley, 2004). In *Drosophila*, only oocyte chromosomes undergo recombination, accordingly the synaptonemal complex is specific to female meiosis; males use a different strategy for the segregation of their meiotic chromosomes (Thomas et al., 2005). The structure of the synaptonemal complex is highly conserved from yeast *S. cerevisiae* to mammals. It is a tripartite structure composed of two lateral elements, a central element and transverse filaments that cross the central element (Lake and Hawley, 2012; Page and Hawley, 2004; Zickler and Kleckner, 1999). Lateral elements originate from the axial elements, structures to which cohesin proteins localize and which organize the sister chromatids. Axial elements are considered a basis for synaptonemal complex assembly (Lake and Hawley, 2012; Page and Hawley, 2004; Zickler and Kleckner, 1999). In spite of the high conservation of the synaptonemal complex structure, its components are not necessarily conserved at the amino acid sequence level. In *Drosophila*, the first components whose requirement for synaptonemal complex structure and crossover formation was reported were C(3)G (a component of transverse filaments) and C(2)M (a component of lateral elements) (Manheim and McKim, 2003; Page and Hawley, 2001). C(2)M does appear to be distantly related to kleisins, the non-SMC cohesin subunits; however, no function in cohesion has been found for C(2)M (Heidmann et al., 2004). Later, Corona (Cona),

¹Institut de Génétique Humaine, Unité Propre de Recherche 1142, Centre National de la Recherche Scientifique (CNRS), 34396 Montpellier, France.

²Institute of Regenerative Medicine and Biotherapy (IRMB), INSERM U1040/Hôpital Saint-Eloi CHRU, 34295 Montpellier, France.

*Present address: Cell Cycle and Cancer Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104, USA. ‡Present address: Faculty of Science, Zagazig University, 44 519 El-Sharkia, Egypt.

§These authors contributed equally to this work

¹Author for correspondence (ounissa.ait-ahmed@inserm.fr)

Received 16 May 2014; Accepted 26 August 2014

another component described as a central element, has also been reported to be required for synaptonemal complex formation (Page et al., 2008), in contrast to Ord, a synaptonemal complex component that is required for sister chromatid cohesion. As evidenced by C(3)G staining, the synaptonemal complex looks normal in early *ord*-null germarial cysts, but its disassembly occurs prematurely, by stage 1 (Webber et al., 2004), whereas in wild-type synaptonemal complex disassembly does not occur before stage 6 (Page and Hawley, 2001). Interestingly, synaptonemal complex components persist at centromeric regions far beyond recombination completion, both in flies and mammals (Bisig et al., 2012; Qiao et al., 2012; Takeo and Hawley, 2012; Takeo et al., 2011). SMC1 and SMC3, the canonical cohesins, and Nipped-B, the *Scs2* homolog, have all been shown to localize to the synaptonemal complex (Gause et al., 2008; Khetani and Bickel, 2007). How these complexes form, the possible existence of subcomplexes, and how they contribute to the chromosome axes and the synaptonemal complex lateral elements, are complex issues that are not yet fully understood.

In the present work, we have investigated the role of the Yemanuclein (Yem, also known as Yem- α) (Ait-Ahmed et al., 1992) during early meiotic prophase. In our previous work we have shown that Yem has an essential role in sexual reproduction, as mutations of Yem can result in parthenogenetic offspring, suggesting that there are chromosome segregation defects in the oocyte and defective syngamy in the zygote (Meyer et al., 2010). These observations were further supported by the discovery that Yem functions in remodeling the paternal pronucleus chromatin through the complex it forms with Hira (Orsi et al., 2013). Hira is the first member of the Yem–Hira H3.3 chaperone complex to be recognized as a major player in chromatin assembly of the male pronucleus (Loppin et al., 2005). Here, we show that Yem is associated with the synaptonemal complex and that it interacts *in vivo* with components of the cohesin complex. We also show that it is required for various aspects of meiotic recombination, namely, crossover distribution and the timing of DSB formation. A *Hira*-null mutation had a similar effect on DSB kinetics, presumably through disruption of the germarial localization of Yem, unveiling for the first time a role of the Hira–Yem complex in female meiosis.

RESULTS

Yem is a new synaptonemal-complex-associated protein

The localization of Yem to the oocyte nucleus and its importance for female meiosis has already been recognized in our previous work (Ait-Ahmed et al., 1992; Meyer et al., 2010). In the present work, we aimed to investigate Yem function in early meiotic prophase. In a first experiment, this question was addressed cytologically by immunostaining whole-mount ovaries with antibodies against Yem. As shown in Fig. 1A, Yem could be detected from as early as region 2a of the germarium, which is in line with its mRNA expression and localization (Capri et al., 1997). The various stages are schematically shown up to mid-prophase in the drawing of Fig. 1B (adapted from King, 1970). Higher magnification of Yem-stained egg chambers revealed ribbon-like structures typical of the synaptonemal complex (Fig. 1Ad,e). The synaptonemal complex initially forms inside the three- and four-ring canal cells of the 16-cell cyst before being restricted to the four-ring canal cells (called pro-oocytes). Yem colocalization with well-characterized synaptonemal complex

components, such as C(3)G and C(2)M, was assessed by co-staining with a monoclonal antibody against C(3)G and an antibody against a HA-tagged version of C(2)M (Manheim and McKim, 2003; Page and Hawley, 2001). Results in Fig. 1C clearly show colocalization of Yem with C(3)G and C(2)M.

The germarial localization of Yem depends on synaptonemal complex lateral elements and transverse filaments

C(3)G, a transverse filament component, and C(2)M, a lateral element component, have been shown to be indispensable for synaptonemal complex formation (Anderson et al., 2005). As previously revealed by C(3)G staining, the threadlike structure typical of the synaptonemal complex is replaced by punctuate structures in the absence of C(2)M (Manheim and McKim, 2003). As shown in Fig. 2A, Yem staining was strongly affected in germaria of females that are homozygous for *c(2)M^{EP2115}* or *c(3)G⁶⁸* (Manheim and McKim, 2003; Page and Hawley, 2001). A higher magnification of the *c(2)M^{EP2115}* germarium shows a punctuate distribution of Yem identical to that of C(3)G (Fig. 2B). A similar observation has been previously made for the localization of the Cona protein, which is a component of the central element (Page et al., 2008). This result suggests that Yem localization to the chromosomes during early meiotic prophase is dependent on the integrity of the synaptonemal complex. However, towards the end of pachytene, Yem localization to the oocyte nucleus no longer required the presence of the synaptonemal complex (Fig. 2A). Yem and C(3)G also localize similarly in *ord* mutant background (Fig. 2B). Ord, as well as C(2)M and SMC1, is considered to be a component of lateral elements in *Drosophila* (Anderson et al., 2005; Khetani and Bickel, 2007; Manheim and McKim, 2003; Webber et al., 2004). In contrast to the *c(2)M* and *c(3)G* mutant backgrounds, the *ord* background does not totally abolish the formation of the synaptonemal complex threadlike structures. However, they are not as stable as they are in wild-type backgrounds (Khetani and Bickel, 2007; Webber et al., 2004). It is noteworthy that *yem* mutant backgrounds had no effect on the integrity of the synaptonemal complex (supplementary material Fig. S1; Fig. 5).

Yem associates *in vivo* with components of the cohesin complex

Because Yem associates with the synaptonemal complex (this work) and also has DNA-binding properties (Ait-Ahmed et al., 1992), we decided to investigate its possible interaction with components of the cohesin complex. To address this question, we performed co-immunoprecipitation experiments using ovary protein extracts. We tested the *in vivo* association of Yem with SMC3, a canonical cohesin protein, and C(2)M, which is known to form a complex with SMC3 (Heidmann et al., 2004). For this purpose, we constructed a fly stock that had both the *gSmc3-ha* and *gc(2)M-myc* transgenes described previously (Heidmann et al., 2004). Then, immunoprecipitations were performed with antibodies against the endogenous Yem protein and the Myc [C(2)M] and HA (SMC3) epitope tags. The immunoprecipitates were probed with the corresponding antibodies and also with an antibody against the endogenous kleisin Rad21 (listed as Vtd in FlyBase) (Gause et al., 2008). To assess the specificity of the immunoprecipitations and western blot experiments, we used an antibody against the FLAG epitope as a negative control. As shown in Fig. 3A, in the control experiment using the FLAG antibody, no signal was

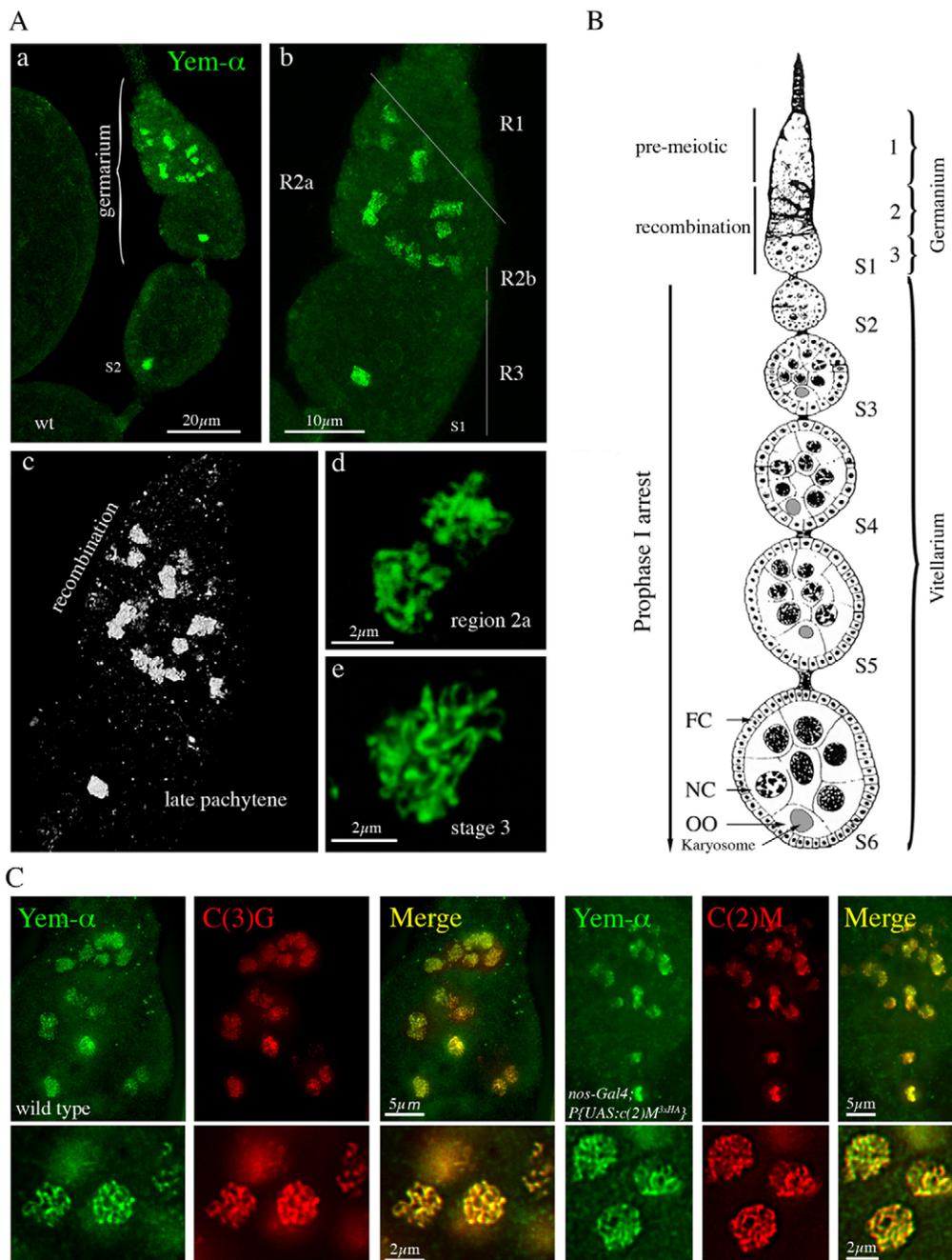


Fig. 1. Localization of Yem-alpha protein in wild-type germaria.

Whole-mount ovaries were stained as described in the Materials and Methods. (A) Yem localization at early stages in a wild-type (wt) ovariole (a–e). Images b and c display a higher magnification of the germarium shown in a. In b, a projection of confocal sections, R1 to R3 represent regions of the germarium. c and e show 3D reconstructions obtained after application of Volocity software to the stacks. The pictures d and e show the typical ribbon-like localization of Yem in the two proocytes of a germarium region 2a egg chamber and in the stage 3 oocyte. (B) Schematic representation of an ovariole up to stage 6 (S6). FC, follicle cells; OO, oocyte; NC, nurse cells (King, 1970). (C) Projection of deconvolved z-series. Colocalization of Yem staining (green) with C(3)G and C(2)M staining (red) is shown. Lower panel displays higher magnifications of the germaria.

observed in the immunoprecipitated fraction, whereas the anti-Yem polyclonal antibody precipitated a substantial amount of Yem and also SMC3–HA and C(2)M–Myc. These results were confirmed by the reciprocal experiments using, on the one hand, C(2)M–Myc as a bait for Yem and SMC3–HA, and, on the other hand, SMC3–HA as a bait for Yem and C(2)M–Myc. SMC3–HA and C(2)M–Myc have already been described to be within the same meiotic complex (Heidmann et al., 2004). Interestingly Rad21 was also found in the C(2)M–Myc immunoprecipitate, a result reported here for the first time. In addition Rad21 was consistently found in immunoprecipitates obtained with two different antibodies directed against the endogenous Yem protein (Fig. 3B). Our data suggest that Yem associates with the meiotic cohesin complex and that Rad21 is part of the complex.

Crossover distribution in *yem*^{1/+} oocytes

Several properties of Yem suggest that it has a potential role in meiotic recombination: (1) Yem is a DNA-binding protein that is specifically localized to the oocyte (Aït-Ahmed et al., 1992); (2) *yem*¹ oocytes frequently show abnormal segregation of chromosome 4 (Meyer et al., 2010), which might reflect an indirect consequence of the increase of nonexchange chromosomes in recombination-defective backgrounds (Baker and Hall, 1976; Sandler et al., 1968), and (3) Yem is associated with the synaptonemal complex and cohesin components. We first tested the role of Yem in recombination by genetically analyzing the effect of *yem*¹ mutation in the progeny of mutant mothers. Because females that are homozygous for the *yem*¹ mutation are infertile (Meyer et al., 2010), we tested meiotic recombination in

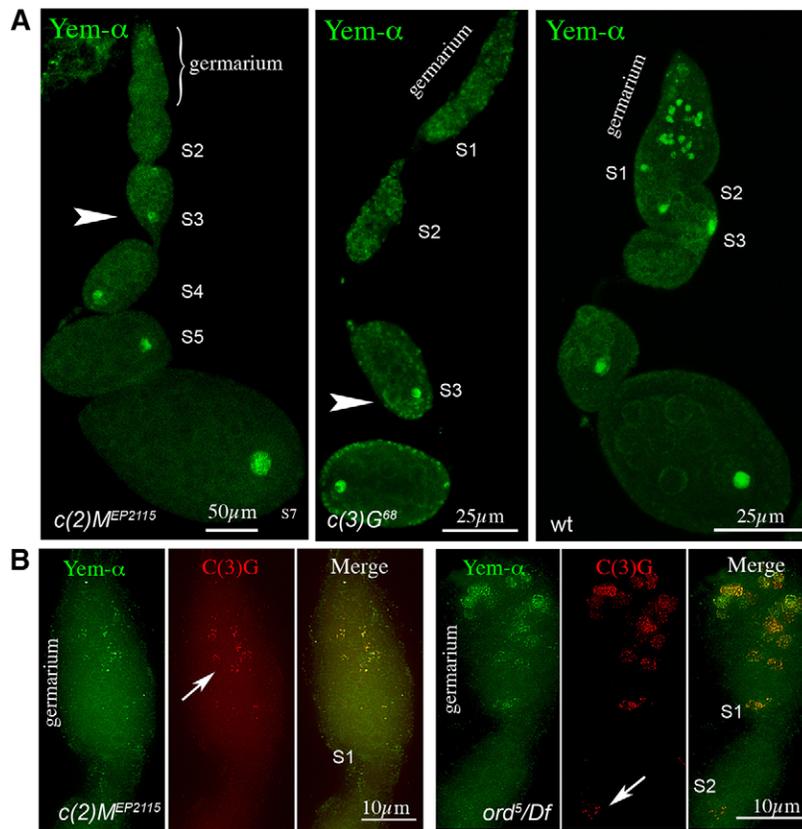


Fig. 2. Yem localization in ovaries mutant for synaptonemal complex components. Each image represents a full projection from a z-series. (A) Localization of Yem in *c(2)M* and *c(3)G* mutant and wild-type (wt) ovarioles. Yem staining is lacking in the germinal germ line nuclei of *c(2)M^{EP2115}* and *c(3)G⁶⁸* mutants but its intensity was similar to wild-type in the oocyte nucleus by stage 3 (arrowheads). (B) High magnification of germaria from *c(2)M^{EP2115}* (left panels) and *ord⁵/Df* (right panels) mutants; both C(3)G and Yem stainings appear as fragmented and spotty signals in *c(2)M^{EP2115}* germaria (arrow). In the *ord⁵/Df* mutant germaria (right panels), Yem displays a staining similar to that of C(3)G, which shows that synaptonemal complex forms at early stages, and then is severely disturbed at later germarial stages where it looks fragmented. In the stage 2 oocyte, both Yem and C(3)G stainings appear as spots (arrow). These defects are comparable to those previously described for C(2)M and C(3)G in *ord⁵/Df* mutant germaria (Webber et al., 2004). The *ord* deficiency used here is Df(2R)3-70. Some staining can be observed throughout the cyst, probably owing to background when using the Yem antiserum.

the progeny of *yem¹* heterozygous females, taking advantage of the sensitivity of meiotic recombination to gene dosage (Carpenter, 2003; Carpenter and Sandler, 1974; Hinton, 1966; Mason, 1976; McKim et al., 1998; Page and Hawley, 2001). We first determined

the effect of *yem¹* on crossover distribution along the X chromosome. Because meiotic recombination is sensitive to female age and environmental conditions, such as temperature, recombination frequency was determined in the same experimental

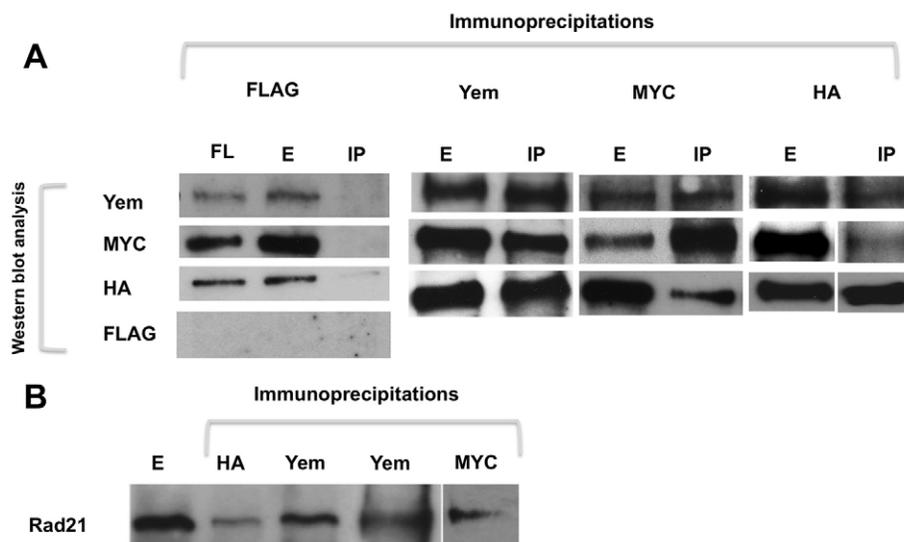
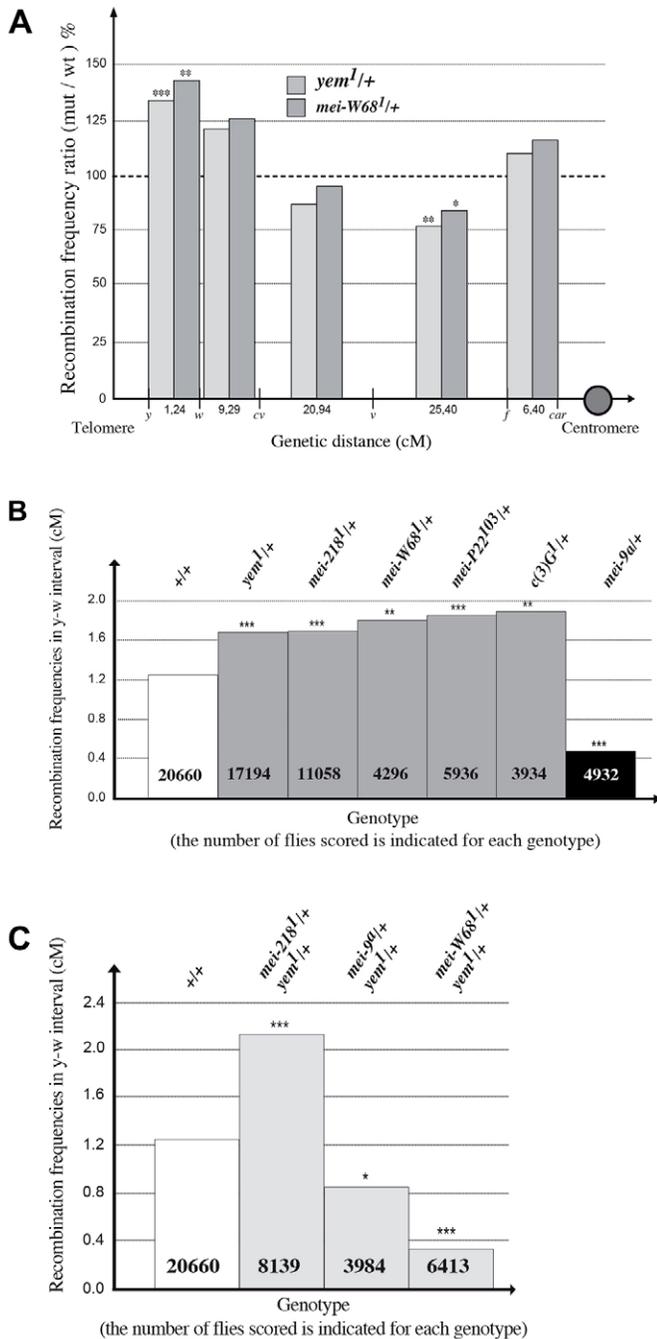


Fig. 3. Yem associates *in vivo* with C(2)M, SMC3 and Rad21. Ovary extracts were prepared from flies that express both SMC3–HA and C(2)M–Myc transgenic proteins. (A) The immunoprecipitations were performed with anti-Yem AS2 polyclonal antibody, anti-HA and anti-Myc tag antibodies. The mock immunoprecipitation was performed with an anti-FLAG monoclonal antibody. The protein extracts (E), the flow through (FL) that represents the non-retained fraction and the immunoprecipitated (IP) fractions were submitted to western blot analysis. It is noteworthy that immunoprecipitation with anti-Yem antibody gave the best results, presumably because a polyclonal antibody directed against the endogenous protein was more efficient than anti-tag antibodies. (B) Rad21 presence was assessed in the immunoprecipitated complexes described in (A) with a rabbit polyclonal antibody. Rad21 was consistently immunoprecipitated by two different anti-Yem antibodies (Yem columns).



conditions for wild-type, *mei-W68* and *yem* mutant backgrounds. The ratio of recombination frequencies tested in the progeny of heterozygous mothers to that in wild-type mothers is shown in Fig. 4A. In all the chromosome intervals tested, *yem*¹/+ and *mei-W68*¹/+ mothers had a similar effect on crossover frequency, namely an increase at telomeric and centromeric regions and a decrease in the central region. Because mutation of *yem* affected the distribution of crossovers, by definition *yem* falls into the ‘precondition’ class of meiotic genes (Carpenter and Sandler, 1974).

We then chose to score recombination frequency for the *y-w* interval in different mutant backgrounds. The *y-w* interval, which is located in the subtelomeric region of the X chromosome, was chosen because of its small size, thus reducing the probability of

Fig. 4. Recombination frequency on the X chromosome in mutant and wild-type mothers. All the recombination tests were carried out in well-controlled experimental conditions with females whose age was less than 5 days. Recombination frequency is expressed in centimorgans (cM). (A) Crossing over distribution along the X chromosome for *yem*¹/+ (light gray) and *mei-W68*¹/+ (dark gray) females. The ordinate axis represents the ratio of genetic distance observed in the mutant genotype over the genetic distance observed in the wild type (determined in our experimental conditions). The abscissa axis represents genetic intervals along the chromosome listed from distal to proximal; the wild-type genetic distance (cM) is indicated for each interval. Statistical significance is assessed with the Chi-squared test. **P*<0.05, ***P*<0.01 and ****P*<0.001. (B,C) Recombination frequency (cM) analyzed for the *y-w* interval in offspring of heterozygous meiotic mutants (B) and trans-heterozygous mutants (C). Wild-type, as determined in our experimental conditions, is indicated in white, precondition genotypes and *yem*¹/+ that display an increase in the recombination frequency are indicated in gray, and the *mei-9* exchange mutant that displays a decrease in recombination frequency is in black. Statistical significance was determined with the Chi-squared test. **P*<0.05, ***P*<0.01, and ****P*<0.001. (C) Note the high decrease of recombination rate in *mei-W68*¹/+; *yem*¹/+ mutants in comparison to the other trans-heterozygous combinations with the *yem*¹ mutation.

multiple recombination events. Moreover, the recombination frequency scored in this interval was a nearly 50% increase, which is the strongest effect of the *yem*¹ mutation when heterozygous (Fig. 4A). In our experiments, the *y-w* interval was estimated as being 1.2 cM for wild-type whereas in the same experimental conditions the same interval was estimated as being 1.7 cM for *yem*¹/+ mothers. A similar increase was observed in the *y-w* interval when analyzing progeny of mothers heterozygous for *mei-W68*¹, *mei-218*¹, *c(3)G*¹ and *mei-P22*¹⁰³. In contrast, the analysis of progeny from *mei-9*^a/+ mothers revealed a dramatic decrease (Fig. 4B). Our results are in agreement with those published earlier (supplementary material Table S1). Our finding that *mei-P22* falls in the precondition class is consistent with its role in DSB formation, as for *mei-W68* (Liu et al., 2002; McKim and Hayashi-Hagihara, 1998).

In order to gain an understanding of how these precondition and exchange mutations behave in combination with *yem*¹/+, we performed an analysis of the recombination frequency in the *y-w* interval for *mei-218*¹/+; *yem*¹/+, *mei-W68*¹/+; *yem*¹/+ and *mei-9*^a/+; *yem*¹/+ genotypes. The results are shown in Fig. 4C. In both the *mei-218*¹/+; *yem*¹/+ and the *mei-9*^a/+; *yem*¹/+ genotypes the experimentally determined recombination frequencies were close to the expected values. The experimental value in cM was 2.1 (expected 2.8) for *mei-218*¹/+; *yem*¹/+, whereas for the *mei-9*^a/+; *yem*¹/+ genotype, the observed recombination frequency was 0.8 (expected 0.8). In contrast, the result obtained for the *mei-W68*¹/+; *yem*¹/+ background was not expected from the recombination frequencies observed in the single mutants. The discrepancy between the experimentally determined recombination frequency (0.3) and the expected value if *yem* and *mei-W68* were independent (3.0) is high. In conclusion, *mei-W68*¹ and *yem*¹ display a genetic interaction, unlike the other mutations analyzed here, which might reflect a role of Yem in DSB metabolism.

DSB dynamics is modified in *yem* and *Hira* mutant backgrounds

To gain further understanding on Yem role in the meiotic recombination pathway, we analyzed DSBs in the *yem*¹ mutant background used in the genetic experiments along with *yem*-null (*yem*²) and *Hira*-null (*Hira*^{HRI}) mutant backgrounds (Orsi et al., 2013). To address this question, we monitored the DSBs with an

anti- γ -H2Av antibody. In mammals, the H2AX variant is phosphorylated in the presence of DSBs both in mitotic and meiotic cells (Mahadevaiah et al., 2001; Rogakou et al., 1999). Similarly, *Drosophila* H2Av undergoes such a modification (denoted as γ -H2Av, phosphorylation occurs on serine 137) in response to DSBs, hence it can be used as a marker for the presence of DSBs (Jang et al., 2003; Madigan et al., 2002). As schematically shown in Fig. 5A, DSBs in *Drosophila* form in late region 2a, after the synaptonemal complex is fully formed, and γ -H2Av foci are absent by late pachytene, in region 3 of the germarium, suggesting that DSBs are fully repaired at this stage (Jang et al., 2003; Mehrotra and McKim, 2006; Webber et al., 2004). Fig. 5B shows germaria of various genetic backgrounds stained for the synaptonemal complex with an anti-C(3)G monoclonal antibody and for DSBs with an anti- γ -H2Av polyclonal antibody. In wild-type germaria, the staining was essentially observed in cysts of late region 2a and region 2b. It is worth noting that γ -H2Av staining was also detected in C(3)G-negative nuclei. In absence of Mei-W68 activity, the γ -H2Av staining disappeared from all the nuclei, underscoring the role of Mei-W68 activity in DSB formation, including in C(3)G-negative nuclei. The γ -H2Av staining in *yem*¹/*Df3450* or *yem*²/*Df3450* (*yem*-null) ovaries was in striking contrast with the wild-type staining. In the *yem* mutant backgrounds, γ -H2Av staining was shifted to earlier cysts and displayed weaker signal intensity. *Hira*^{HR1} (a *Hira*-null) mutation had a similar effect (Fig. 5B). In addition, a Yem signal was not detected on the germarial meiotic

chromosomes in the *Hira*^{HR1} background (supplementary material Fig. S2), which suggests that the meiotic function of Yem on DSBs is dependent on the Hira–Yem complex. To verify whether the precocious DSBs revealed by the anti- γ -H2Av antibody in the *yem* and *Hira* mutant backgrounds were dependent on Mei-W68 activity, the *mei-w68*¹; *yem*¹/*Df3450* double mutant was assessed with the same antibody. In the absence of Mei-W68 activity the γ -H2Av foci were not observed, therefore the precocious DSBs observed in the *yem* mutant background cannot be attributed to some dysfunction such as unrepaired replication forks (Fig. 5B). It is worth noting that *Hira*-null as well as *yem*-null mutations had no effect on synaptonemal complex.

DISCUSSION

We report here on the function of Yem in early meiosis. Yem was first identified in differential screens for maternally expressed genes (Ait-Ahmed et al., 1992; Ait-Ahmed et al., 1988; Ait-Ahmed et al., 1987). The V478E mutant allele was recovered in a screen for female-sterile mutations, which fall in the genomic region that contains the *yem* locus (Ait-Ahmed et al., 1992; Meyer et al., 2010). The properties of Yem raised the question of its role in early meiotic prophase.

Yem is a new synaptonemal-complex- and cohesin-associated protein

Our results clearly show that Yem associates with the synaptonemal complex following a pattern that is very similar to

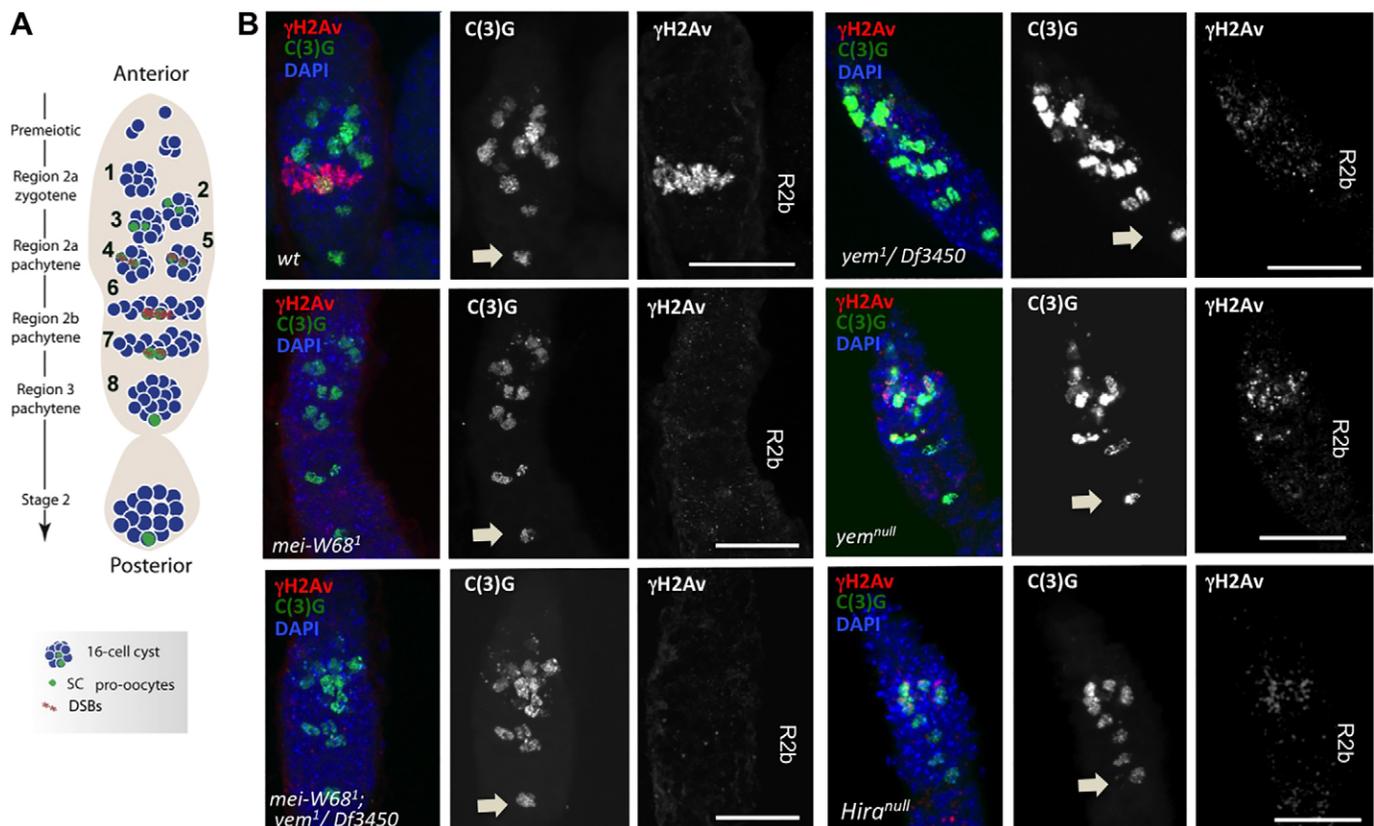


Fig. 5. Effect of *yem* and *Hira* mutations on γ -H2Av foci. (A) Schematic view of a germarium with the main events of the early meiotic prophase (adapted from Joyce and McKim, 2010; Lake and Hawley, 2012). (B) Whole-mount ovarioles were stained for DNA (blue), C(3)G (green) and γ -H2Av (red). The C(3)G and γ -H2Av stainings are shown separately for each genotype as black-and-white images. All images represent a full projection of a z-series. The following genotypes were analyzed: wild-type (wt), *yem*¹/*Df3450*, *yem*²/*Df3450* (*yem*^{null}), *Hira*^{HR1} (*Hira*^{null}), *mei-W68*¹, *mei-W68*¹; *yem*¹/*Df3450*. Note the absence of effect of *yem*^{null} and *Hira*^{null} on the synaptonemal complex. Scale bars: 20 μ m.

that described for the two well-characterized C(3)G and C(2)M proteins (Manheim and McKim, 2003; Page and Hawley, 2001). Moreover, as previously described, Yem follows a pattern that is similar to that of C(3)G in germaria that are mutant for *c(2)M* and *ord* (Manheim and McKim, 2003; Page and Hawley, 2001; Webber et al., 2004). Therefore, its localization to the chromosomes at early meiotic stages is dependent on the integrity of the synaptonemal complex. Interestingly, Yem is an *in vivo* partner of components of the cohesin complex. Cohesin is an essential component of the chromosome axes that eventually build the lateral elements of the synaptonemal complex (Lee and Orr-Weaver, 2001; Revenkova and Jessberger, 2006). From these features it is tempting to assume that Yem is part of the axial elements and as such would be essential for synaptonemal complex formation or maintenance. However, even in mutants that are null for *yem* (no staining at all with anti-Yem antibodies), the synaptonemal complex looks normal when probed with the anti-C(3)G antibody. In contrast, C(2)M, which we found in the same complex as Yem, has been reported to be an axial element component that is required for formation of the synaptonemal complex (Anderson et al., 2005; Manheim and McKim, 2003). It remains to be determined what role Yem has in the synaptonemal complex and the cohesin complex, and whether it has a function similar to Rec8, which might be obscured by functional homologs. Rec8, a Rad21/Scc1 paralog with no equivalent in *Drosophila* has been shown to be a meiosis-specific kleisin that is essential for meiotic cohesion both in *S. pombe* and *S. cerevisiae* (Klein et al., 1999; Watanabe and Nurse, 1999). Moreover, it is a component of the axial elements in *S. cerevisiae* (Klein et al., 1999). The functions of Rec8 are far less clear in mammals. First, there was a controversy as to whether or not the mammalian meiotic cohesin complex is a component of axial elements. This controversy has been partly solved with the identification of a new meiosis-specific Rad21 paralogous protein called Rad21L; axial elements only get affected substantially in a context where both Rec8 and Rad21L are simultaneously knocked out (Llano et al., 2012). However, the impact of this double deficiency on sister chromatid cohesion is still controversial (Herrán et al., 2011; Ishiguro et al., 2011; Llano et al., 2012). In fact, a variety of complexes with cohesin components might exist simultaneously and fulfil different roles during meiosis (Calvente et al., 2013). In the case of Yem, its role in cohesion and synaptonemal complex assembly or maintenance is still elusive. No effect of *yem*¹ mutation on sister chromatid cohesion could be observed in spite of a detailed analysis of the mutant phenotypes (Meyer et al., 2010), neither could we show in the present work any effect on the synaptonemal complex. Given their complexity, our results require further investigations before being fully understood. An account of our current knowledge on cohesin complexes during meiosis and the many unanswered questions has been published recently (McNicoll et al., 2013).

Crossover distribution and DSBs formation in *yem*¹ mutant oocytes

Crossovers are essential for chromosome segregation in recombination-proficient cells because they link the homologs, resulting in their segregation at meiosis I. In absence of crossovers, the homologous chromosomes segregate randomly. Therefore the mechanisms that support crossover formation must be stringently regulated, in particular, to ensure that at least one crossing over forms between each homologous pair. Beyond the obligate crossing over, the spatial distribution of crossovers is regulated. Crossing over distribution can be regulated at two levels: (1) at the DSB

formation level, and (2) in resolution of crossing over versus non-crossing over. The relative number of non-crossing over versus crossing over events depends, in particular, on crossover interference, a phenomenon that prevents a new crossover from forming too close to another crossover (Youds and Boulton, 2011). As only a few progeny can be recovered from *yem*¹ females (Meyer et al., 2010), to test the effect of *yem*¹ on meiotic recombination we took advantage of the dominant effect of recombination-defective mutations (Carpenter, 2003). We were then able to show that *yem*¹ affected crossover distribution along the X chromosome. It is noteworthy that all the meiotic mutations tested in heterozygous conditions had the same effect on the frequency of recombination in the *y-w* interval with the exception of *mei-9*, the archetypal exchange mutation (Carpenter and Sandler, 1974). All genes whose mutant alleles affected crossover distribution are classified as precondition genes (Carpenter and Sandler, 1974) regardless of their function. This group includes genes with functions as diverse as synaptonemal complex formation [*c(3)G*], DSB formation (*mei-W68*) and DSB repair (Bhagat et al., 2004), suggesting that the effect on crossover distribution is indirect. In response to a decrease in the number of crossovers in the mutant backgrounds, a back-up mechanism would allow their formation at a place where they are not usually authorized (Bhagat et al., 2004). Both in *C. elegans* and *Drosophila*, a checkpoint has been reported that delays pachytene to allow crossovers to form even in chromosome locations where they normally do not occur (Carlton et al., 2006; Joyce and McKim, 2010). Indeed, even chromosome 4 might experience recombination in a context where crossover distribution is aberrant (Sandler and Szauter, 1978). As can be observed in our data, in response to *yem*¹ mutation, more crossovers form in the telomeric and centromeric regions. However, it is not clear to what extent crossing over frequency is affected in homozygotes. Interestingly, *yem* mutations affect the timing of DSBs. A similar effect is observed in *Hira*^{HRI} mutant ovaries, a result that is in line with the absence of Yem on the meiotic chromosomes in the *Hira*^{HRI} background. Therefore, the role of Yem on DSB kinetics is dependent on its Hira partner. Taken together, these data suggest that the function of Yem during early meiotic prophase is dependent on the Hira–Yem complex. The function of the Hira–Yem H3.3 histone complex in the DSB kinetics is reported here for the first time. These data deserve further investigations in order to gain insight into the role of chromatin status in the meiotic recombination process. Moreover, this work raises new issues, such as the impact of chromatin structure on meiosis-specific protein complexes (the synaptonemal complex and cohesin complexes) and DSB metabolism.

MATERIALS AND METHODS

Fly stocks used in this work are given in supplementary material Table S2.

Whole-mount immunocytochemistry for detection of Yem, the synaptonemal complex and γ -His2Av

For Yem detection, immunostaining was performed with a polyclonal antibody developed against Yem- α ; washing and incubation with fluorescent secondary antibodies were as described previously (Ait-Ahmed et al., 1992; Capri et al., 1997; Meyer et al., 2010). Additional primary antibodies included mouse anti-C(3)G antibody, used at 1:500 (Page and Hawley, 2001), rabbit anti-C(2)M antibody, used at 1:400 (Manheim and McKim, 2003), and the monoclonal anti-HA tag antibody, used at 1:200 (Sigma). Rhodamine- or FITC-conjugated anti-rabbit-IgG and anti-mouse-IgG antibodies were from Jackson ImmunoResearch Labs Inc. The other secondary antibodies were coupled either to Alexa Fluor 488 and Alexa Fluor 555 (Molecular Probes) or Fluorophores 546 (Interchim).

For γ -His2Av detection, females were dissected and fixed as described previously (Page and Hawley, 2001). Primary antibodies included mouse anti-C(3)G antibody used at 1:500 and rabbit anti- γ -His2Av antibody used at 1:500 (Rockland Anti-Histone H2AvD pS137 Antibody). Secondary antibodies were coupled either to Alexa Fluor 555 (Molecular Probes) or Fluorophores 546 (Interchim). A Leica DM600B microscope was used for conventional epifluorescence. Confocal microscopy was performed either with a Biorad device or a Zeiss LSM 510 microscope.

Recombination frequency analysis

The flies were raised at 25°C for all the crosses designed to measure crossing over. All control experiments were carried out in exactly the same conditions as test experiments. Freshly hatched females were mated on the same day. After 5 days of egg laying, the parents were discarded. Only the 0–5-day egg-laying period was used to avoid age-dependent effects. Crossing overs were assayed for the X chromosome by crossing the different mutant stocks with stocks containing appropriate combinations of X chromosome markers to generate females that are heterozygous for *yem*¹, *mei-W68*¹, *mei-P22*¹⁰³, *mei-218*¹, *c(3)G*¹ or *mei-9*^a and heterozygous for *y w cv v f car*. To cope with the epistatic effect of *white* on the other eye markers, different set ups of marked chromosomes were used (supplementary material Table S2). Statistical significance was assessed with a Chi-squared test. For all experiments, **P*<0.05 ***P*<0.01 and ****P*<0.001.

Immunoprecipitations and western blot analysis

For co-immunoprecipitation experiments, we essentially used a protocol described previously (Jäger et al., 2001) with some modifications as indicated. 100 ovaries were dissected manually on ice in 250 μ l of ice-cold lysis buffer. Lysis buffer was essentially as described previously (Jäger et al., 2001) but was supplemented with an EDTA-free protease inhibitor cocktail (Roche tablets). PMSF was also added to a 1 mM final concentration. Before homogenization, an equal volume of ice-cold lysis buffer was added. After centrifugation, the cleared homogenate was adjusted to 1 μ l in lysis buffer. Two aliquots of 30 μ l were set aside for use as input in the western blot experiments. The diluted cleared extracts were then submitted to the immunoprecipitation procedure with 50 μ l G-Sepharose beads (Sigma) pre-incubated with the primary antibodies [AS2 rabbit polyclonal (10 μ l), M2 mouse anti-Flag (2 μ l; Sigma), monoclonal anti-c-Myc (2 μ l; Sigma), rabbit ChIP grade anti-HA (2 μ l; Abcam)]. The anti-Flag antibody was used in a mock experiment to assess the specificity of the immunoprecipitation reactions.

The protein extracts and the retained fractions were then submitted to western blot analysis. SDS-PAGE electrophoresis was carried out on 8% acrylamide gels with a Bio-Rad device. Western blotting was performed with standard procedures using either Pierce ECL Western Blotting Substrate (Thermo Scientific) or Millipore Immobilon Western Chemiluminescent substrate as recommended by the supplier. The following antibodies against the epitope tags were used: M2 anti-Flag (1:1000; Sigma), anti-c-Myc (1:400; Sigma), anti-HA (1:400; monoclonal Sigma). Yem AS2 rabbit polyclonal antibodies were used at 1:100 and rabbit anti-Rad21 polyclonal antibodies (kindly provided by Dale Dorsett, Doisy Research Center, USA) were used at 1:500 (Gause et al., 2008). Secondary antibodies were goat horseradish-peroxidase-coupled anti-mouse-IgG and anti-rabbit-IgG antibodies (1:10,000; Beckman).

Acknowledgements

We are grateful to Adelaide Carpenter (University of Cambridge, UK) for her insightful suggestions at an early stage of this work and Kim McKim (Rutgers University, USA) for his valuable comments on the manuscript. We thank Kathy Matthews and the Bloomington Drosophila stock center (Indiana, USA), Kim McKim, Trudy Schupbach (Princeton University, USA), Sharon Bickel (Dartmouth College, USA), Scott Hawley (Stowers Institute, USA), Christian Lehner (University of Zurich, Switzerland), Dale Dorsett (Doisy Research Center, USA), Margarete Heck (University of Edinburgh, UK) for providing fly stocks and/or antibodies. We thank Matthieu Schoenhals and Laure Giard for their input as masters students and Sylvaine Artero (INSERM, Montpellier) for statistical test verifications. We acknowledge Julien Cau and Nicole Lautrédu (Montpellier RIO

Imaging) for help with confocal microscopy and deconvolution analyses; Patrick Atger and Cyril Sarrauste de Menthère (CNRS, Montpellier) for help with iconography at an early phase of the manuscript.

Competing interests

The authors declare no competing interests.

Author contributions

O.A. conceived the study. R.E.M., A.A. and O.A. designed the experiments. R.E.M., A.A., M.C., H.B. and C.F. performed the experiments. R.E.M., A.A. and O.A. interpreted the data. O.A. wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Centre national de la recherche scientifique (CNRS) and Fondation ARC (grants to O.A.). R.E.M. held fellowships from the French national PhD training program (3 years) and Fondation ARC (1 year). A.A. was supported by a 4-year PhD training fellowship from the Egyptian Government.

Supplementary material

Supplementary material available online at <http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.152520/-DC1>

References

- Ait-Ahmed, O., Thomas-Cavallin, M. and Rosset, R. (1987). Isolation and characterization of a region of the Drosophila genome which contains a cluster of differentially expressed maternal genes (yema gene region). *Dev. Biol.* **122**, 153–162.
- Ait-Ahmed, O., Griffin-Shea, R., Vola, C., Capri, M., Thomas-Cavallin, M. and Pardo, D. (1988). Isolation of developmentally regulated genes in Drosophila melanogaster. *Biol. Cell* **62**, 57–64.
- Ait-Ahmed, O., Bellon, B., Capri, M., Joblet, C. and Thomas-Delaage, M. (1992). The yemanuclein-alpha: a new Drosophila DNA binding protein specific for the oocyte nucleus. *Mech. Dev.* **37**, 69–80.
- Anderson, L. K., Royer, S. M., Page, S. L., McKim, K. S., Lai, A., Lilly, M. A. and Hawley, R. S. (2005). Juxtaposition of C(2)M and the transverse filament protein C(3)G within the central region of Drosophila synaptonemal complex. *Proc. Natl. Acad. Sci. USA* **102**, 4482–4487.
- Baker, B. S. and Hall, J. C. (1976). Meiotic mutants: genetic control of meiotic recombination and chromosome segregation. In *The Genetics and Biology of Drosophila* (ed. M. Ashburner, E. Novitski, H. L. Carson and J. N. Thompson), pp. 352–434. London: Academic Press.
- Bhagat, R., Manheim, E. A., Sherizen, D. E. and McKim, K. S. (2004). Studies on crossover-specific mutants and the distribution of crossing over in Drosophila females. *Cytogenet. Genome Res.* **107**, 160–171.
- Bisig, C. G., Guiraldelli, M. F., Kouznetsova, A., Scherthan, H., Höög, C., Dawson, D. S. and Pezza, R. J. (2012). Synaptonemal complex components persist at centromeres and are required for homologous centromere pairing in mouse spermatocytes. *PLoS Genet.* **8**, e1002701.
- Calvente, A., Viera, A., Parra, M. T., de la Fuente, R., Suja, J. A., Page, J., Santos, J. L., de la Vega, C. G., Barbero, J. L. and Rufas, J. S. (2013). Dynamics of cohesin subunits in grasshopper meiotic divisions. *Chromosoma* **122**, 77–91.
- Capri, M., Santoni, M. J., Thomas-Delaage, M. and Ait-Ahmed, O. (1997). Implication of a 5' coding sequence in targeting maternal mRNA to the Drosophila oocyte. *Mech. Dev.* **68**, 91–100.
- Carlton, P. M., Farruggio, A. P. and Dernburg, A. F. (2006). A link between meiotic prophase progression and crossover control. *PLoS Genet.* **2**, e12.
- Carpenter, A. T. (1975). Electron microscopy of meiosis in Drosophila melanogaster females. I. Structure, arrangement, and temporal change of the synaptonemal complex in wild-type. *Chromosoma* **51**, 157–182.
- Carpenter, A. T. (2003). Normal synaptonemal complex and abnormal recombination nodules in two alleles of the Drosophila meiotic mutant *mei-W68*. *Genetics* **163**, 1337–1356.
- Carpenter, A. T. and Sandler, L. (1974). On recombination-defective meiotic mutants in Drosophila melanogaster. *Genetics* **76**, 453–475.
- Gause, M., Webber, H. A., Misulovin, Z., Haller, G., Rollins, R. A., Eissenberg, J. C., Bickel, S. E. and Dorsett, D. (2008). Functional links between Drosophila Nipped-B and cohesin in somatic and meiotic cells. *Chromosoma* **117**, 51–66.
- Heidmann, D., Horn, S., Heidmann, S., Schleiffer, A., Nasmyth, K. and Lehner, C. F. (2004). The Drosophila meiotic kleisin C(2)M functions before the meiotic divisions. *Chromosoma* **113**, 177–187.
- Herrán, Y., Gutiérrez-Caballero, C., Sánchez-Martín, M., Hernández, T., Viera, A., Barbero, J. L., de Álava, E., de Rooij, D. G., Suja, J. A., Llano, E. et al. (2011). The cohesin subunit RAD21L functions in meiotic synapsis and exhibits sexual dimorphism in fertility. *EMBO J.* **30**, 3091–3105.
- Hinton, C. W. (1966). Enhancement of recombination associated with the c3G mutant of Drosophila melanogaster. *Genetics* **53**, 157–164.
- Hirano, T. (2000). Chromosome cohesion, condensation, and separation. *Annu. Rev. Biochem.* **69**, 115–144.

- Ishiguro, K., Kim, J., Fujiyama-Nakamura, S., Kato, S. and Watanabe, Y. (2011). A new meiosis-specific cohesin complex implicated in the cohesin code for homologous pairing. *EMBO Rep.* **12**, 267–275.
- Jäger, H., Herzig, A., Lehner, C. F. and Heidmann, S. (2001). Drosophila separase is required for sister chromatid separation and binds to PIM and THR. *Genes Dev.* **15**, 2572–2584.
- Jang, J. K., Sherizen, D. E., Bhagat, R., Manheim, E. A. and McKim, K. S. (2003). Relationship of DNA double-strand breaks to synapsis in Drosophila. *J. Cell Sci.* **116**, 3069–3077.
- Joyce, E. F. and McKim, K. S. (2010). Chromosome axis defects induce a checkpoint-mediated delay and interchromosomal effect on crossing over during Drosophila meiosis. *PLoS Genet.* **6**, e1001059.
- Khetani, R. S. and Bickel, S. E. (2007). Regulation of meiotic cohesin and chromosome core morphogenesis during pachytene in Drosophila oocytes. *J. Cell Sci.* **120**, 3123–3137.
- King, R. C. (1970). *Ovarian Development in Drosophila Melanogaster*. San Diego, CA: Academic Press.
- Klein, F., Mahr, P., Galova, M., Buonomo, S. B., Michaelis, C., Nairz, K. and Nasmyth, K. (1999). A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. *Cell* **98**, 91–103.
- Lake, C. M. and Hawley, R. S. (2012). The molecular control of meiotic chromosomal behavior: events in early meiotic prophase in Drosophila oocytes. *Annu. Rev. Physiol.* **74**, 425–451.
- Lee, J. Y. and Orr-Weaver, T. L. (2001). The molecular basis of sister-chromatid cohesion. *Annu. Rev. Cell Dev. Biol.* **17**, 753–777.
- Liu, H., Jang, J. K., Kato, N. and McKim, K. S. (2002). mei-P22 encodes a chromosome-associated protein required for the initiation of meiotic recombination in Drosophila melanogaster. *Genetics* **162**, 245–258.
- Llano, E., Herrán, Y., García-Tuñón, I., Gutiérrez-Caballero, C., de Álava, E., Barbero, J. L., Schimenti, J., de Rooij, D. G., Sánchez-Martín, M. and Pendás, A. M. (2012). Meiotic cohesin complexes are essential for the formation of the axial element in mice. *J. Cell Biol.* **197**, 877–885.
- Loppin, B., Bonnefoy, E., Anselme, C., Laurençon, A., Karr, T. L. and Couble, P. (2005). The histone H3.3 chaperone HIRA is essential for chromatin assembly in the male pronucleus. *Nature* **437**, 1386–1390.
- Madigan, J. P., Chotkowski, H. L. and Glaser, R. L. (2002). DNA double-strand break-induced phosphorylation of Drosophila histone variant H2Av helps prevent radiation-induced apoptosis. *Nucleic Acids Res.* **30**, 3698–3705.
- Mahadevaiah, S. K., Turner, J. M., Baudat, F., Rogakou, E. P., de Boer, P., Blanco-Rodriguez, J., Jasin, M., Keeney, S., Bonner, W. M. and Burgoyne, P. S. (2001). Recombinational DNA double-strand breaks in mice precede synapsis. *Nat. Genet.* **27**, 271–276.
- Manheim, E. A. and McKim, K. S. (2003). The Synaptonemal complex component C(2)M regulates meiotic crossing over in Drosophila. *Curr. Biol.* **13**, 276–285.
- Mason, J. M. (1976). Orientation disruptor (ord): a recombination-defective and disjunction-defective meiotic mutant in Drosophila melanogaster. *Genetics* **84**, 545–572.
- McKim, K. S. and Hayashi-Hagihara, A. (1998). mei-W68 in Drosophila melanogaster encodes a Spo11 homolog: evidence that the mechanism for initiating meiotic recombination is conserved. *Genes Dev.* **12**, 2932–2942.
- McKim, K. S., Green-Marroquin, B. L., Sekelsky, J. J., Chin, G., Steinberg, C., Khodosh, R. and Hawley, R. S. (1998). Meiotic synapsis in the absence of recombination. *Science* **279**, 876–878.
- McNicoll, F., Steverson, M. and Jessberger, R. (2013). Cohesin in gametogenesis. *Curr. Top. Dev. Biol.* **102**, 1–34.
- Mehrotra, S. and McKim, K. S. (2006). Temporal analysis of meiotic DNA double-strand break formation and repair in Drosophila females. *PLoS Genet.* **2**, e200.
- Meyer, R. E., Delaage, M., Rosset, R., Capri, M. and Ait-Ahmed, O. (2010). A single mutation results in diploid gamete formation and parthenogenesis in a Drosophila yemanuclein-alpha meiosis I defective mutant. *BMC Genet.* **11**, 104.
- Moses, M. J. (1969). Structure and function of the synaptonemal complex. *Genetics* **61 Suppl.**, 41–51.
- Moses, M. J. (2006). A new chromosome structure is revealed. *Chromosoma* **115**, 152–154.
- Orsi, G. A., Algazeery, A., Meyer, R. E., Capri, M., Sapey-Triomphe, L. M., Horard, B., Gruffat, H., Couble, P., Ait-Ahmed, O. and Loppin, B. (2013). Drosophila Yemanuclein and HIRA cooperate for de novo assembly of H3.3-containing nucleosomes in the male pronucleus. *PLoS Genet.* **9**, e1003285.
- Page, S. L. and Hawley, R. S. (2001). c(3)G encodes a Drosophila synaptonemal complex protein. *Genes Dev.* **15**, 3130–3143.
- Page, S. L. and Hawley, R. S. (2004). The genetics and molecular biology of the synaptonemal complex. *Annu. Rev. Cell Dev. Biol.* **20**, 525–558.
- Page, S. L., Khetani, R. S., Lake, C. M., Nielsen, R. J., Jeffress, J. K., Warren, W. D., Bickel, S. E. and Hawley, R. S. (2008). Corona is required for higher-order assembly of transverse filaments into full-length synaptonemal complex in Drosophila oocytes. *PLoS Genet.* **4**, e1000194.
- Petronczki, M., Siomos, M. F. and Nasmyth, K. (2003). Un ménage à quatre: the molecular biology of chromosome segregation in meiosis. *Cell* **112**, 423–440.
- Phadnis, N., Hyppa, R. W. and Smith, G. R. (2011). New and old ways to control meiotic recombination. *Trends Genet.* **27**, 411–421.
- Qiao, H., Chen, J. K., Reynolds, A., Höög, C., Paddy, M. and Hunter, N. (2012). Interplay between synaptonemal complex, homologous recombination, and centromeres during mammalian meiosis. *PLoS Genet.* **8**, e1002790.
- Revenkova, E. and Jessberger, R. (2006). Shaping meiotic prophase chromosomes: cohesins and synaptonemal complex proteins. *Chromosoma* **115**, 235–240.
- Rogakou, E. P., Boon, C., Redon, C. and Bonner, W. M. (1999). Megabase chromatin domains involved in DNA double-strand breaks in vivo. *J. Cell Biol.* **146**, 905–916.
- Sandler, L. and Szauter, P. (1978). The effect of recombination-defective meiotic mutants on fourth-chromosome crossing over in Drosophila melanogaster. *Genetics* **90**, 699–712.
- Sandler, L., Lindsley, D. L., Nicoletti, B. and Trippa, G. (1968). Mutants affecting meiosis in natural populations of Drosophila melanogaster. *Genetics* **60**, 525–558.
- Stack, S. M. and Anderson, L. K. (2001). A model for chromosome structure during the mitotic and meiotic cell cycles. *Chromosome Res.* **9**, 175–198.
- Takeo, S. and Hawley, R. S. (2012). Rumors of its disassembly have been greatly exaggerated: the secret life of the synaptonemal complex at the centromeres. *PLoS Genet.* **8**, e1002807.
- Takeo, S., Lake, C. M., Morais-de-Sá, E., Sunkel, C. E. and Hawley, R. S. (2011). Synaptonemal complex-dependent centromeric clustering and the initiation of synapsis in Drosophila oocytes. *Curr. Biol.* **21**, 1845–1851.
- Thomas, S. E., Soltani-Bejnood, M., Roth, P., Dorn, R., Logsdon, J. M., Jr and McKee, B. D. (2005). Identification of two proteins required for conjunction and regular segregation of achiasmata homologs in Drosophila male meiosis. *Cell* **123**, 555–568.
- Watanabe, Y. (2005). Sister chromatid cohesion along arms and at centromeres. *Trends Genet.* **21**, 405–412.
- Watanabe, Y. and Nurse, P. (1999). Cohesin Rec8 is required for reductional chromosome segregation at meiosis. *Nature* **400**, 461–464.
- Webber, H. A., Howard, L. and Bickel, S. E. (2004). The cohesion protein ORD is required for homologue bias during meiotic recombination. *J. Cell Biol.* **164**, 819–829.
- Wood, A. J., Severson, A. F. and Meyer, B. J. (2010). Condensin and cohesin complexity: the expanding repertoire of functions. *Nat. Rev. Genet.* **11**, 391–404.
- Youds, J. L. and Boulton, S. J. (2011). The choice in meiosis - defining the factors that influence crossover or non-crossover formation. *J. Cell Sci.* **124**, 501–513.
- Zickler, D. and Kleckner, N. (1999). Meiotic chromosomes: integrating structure and function. *Annu. Rev. Genet.* **33**, 603–754.