DNA double-strand breaks, recombination and synapsis: the timing of meiosis differs in grasshoppers and flies

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The temporal and functional relationships between DNA events of meiotic recombination and synaptonemal complex formation are a matter of discussion within the meiotic field. To analyse this subject in grasshoppers, organisms that have been considered as models for meiotic studies for many years, we have studied the localization of phosphorylated histone H2AX (γ -H2AX), which marks the sites of double-strand breaks (DSBs), in combination with localization of cohesin SMC3 and recombinase Rad51. We show that the loss of γ -H2AX staining is spatially and temporally linked to synapsis, and that in grasshoppers the initiation of recombination, produced as a consequence of DSB formation, precedes synapsis. This result supports the idea that grasshoppers display a pairing pathway that is not present in other insects such as *Drosophila melanogaster*, but is similar to those reported in yeast, mouse and *Arabidopsis*. In addition, we have observed the presence of γ -H2AX in the X chromosome from zygotene to late pachytene, indicating that the function of H2AX phosphorylation during grasshopper spermatogenesis is not restricted to the formation of γ -H2AX foci at DNA DSBs.

Keywords: γ -H2AX; Rad51; SMC3; grasshopper; meiosis; recombination; synapsis

EMBO reports (2004) 5, 385–391. doi:10.1038/sj.embor.7400112

INTRODUCTION

The nucleosomal histone family H2A has several variants with different functions. Phosphorylation of the C-terminal tails of H2A histones is one of the earliest responses to induced double-strand breaks (DSBs) and to programmed DSBs in processes such as apoptosis and meiotic recombination (Rogakou *et al*, 2000; Mahadevaiah *et al*, 2001). The amino-acid sequence of H2AX is nearly identical to that of H2A except for its C-terminal tail, which has a divergent sequence and 13 additional amino acids. H2AX is phosphorylated on serine 139 in mammals (Rogakou *et al*, 1998), and this form is referred to as γ -H2AX. DSBs also induce phosphorylation of the C-terminal tails of the core histone H2A and of the histone variant H2Av in budding yeast and *Drosophila melanogaster*, respectively (Downs *et al*, 2000; Madigan *et al*, 2002). These observations suggest that H2A phosphorylation is an evolutionarily conserved response of eukaryotic cells to DSBs (Redon *et al*, 2002).

There is general agreement that phosphorylation of H2A histones is dispensable for the initial recognition of DNA breaks and that its function is associated with the recruitment of repair factors to damage DNA in order to facilitate repair efficiency (Madigan *et al*, 2002; Celeste *et al*, 2003). However, there is also evidence suggesting additional functions of H2AX phosphorylation during spermatogenesis. γ -H2AX staining has been observed in intermediate and B spermatogonia, sex bodies and round spermatids of mouse (Mahadevaiah *et al*, 2001; Fernández-Capetillo *et al*, 2003; Hamer *et al*, 2003).

The temporal and functional relationships between DNA events of meiotic recombination and synaptonemal complex (SC) formation at prophase I are one of the subjects related to the meiotic process that have been more actively investigated in the last few years. To date, at least two different situations have been described. In budding yeast and mouse, the initiation of synapsis is dependent on the occurrence of DSBs during leptotene and zygotene (Kleckner, 1996; Mahadevaiah *et al*, 2001). However, in other species such as *D. melanogaster* and *Caenorhabditis*

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elegans, recombination events are delayed until SC formation has been completed (Dernburg *et al*, 1998; McKim & Hayasi-Hagihara, 1998). The purpose of our work was to investigate whether the *Drosophila* model could be valid for other insect species that have been considered for decades as model organisms for studying meiotic pairing and synapsis. As γ -H2AX seems to be a good marker of DSBs, we have used an antibody against this histone variant to investigate this feature in the grasshoppers *Locusta migratoria* and *Eyprepocnemis plorans* in combination with the detection of cohesin SMC3 and recombinase Rad51.

RESULTS

Immunoblotting

In western blot analyses with extracts of grasshopper and mice testes, the antibodies against γ -H2AX, SMC3 and Rad51 specifically recognized the same protein band in both species, which migrated at a similar position (see supplementary Fig 1 online), thus indicating the high degree of conservation of these proteins.

γ -H2AX labelling precedes synapsis

To determine the timing and distribution of DSBs in relation to synapsis, it was important to establish accurately the meiotic stages. For this purpose, we used an antibody against SCM3, a member of the structural maintenance of chromosomes (SMC) family of proteins, which is widely conserved in eukaryotes (Hirano, 2002). This cohesin subunit has been found along the axial elements (AEs) and lateral elements (LEs) of the SC in mammal meiosis (Peltari *et al*, 2001). We found that SMC3 labelled the axial structures of grasshopper chromosomes as early as leptotene and throughout all prophase I stages. Thus, assuming that these cohesin axes are subjacent to the chromosomal AEs, it is possible to identify accurately meiotic stages and then to establish the timing of appearance of γ -H2AX and Rad51 throughout prophase I (Fig 1 and supplementary movies 1 and 2 online). As we obtained similar results in the two grasshopper species analysed, we will only describe those observations corresponding to *L. migratoria*.

Fig 1 | Prophase I in grasshopper spermatocytes. Immunolabelling pattern for SMC3 (green) (A,C), γ -H2AX (red) (B) and Rad51 (red) (D). (A,B) Superimposition of 50 focal planes throughout several squashed prophase I nuclei double labelled for SMC3 (A) and γ -H2AX (B). Several cells at different meiotic stages are observed: leptotene (Le), zygotene (Zy), pachytene (P). (C,D) Superimposition of 50 focal planes throughout a field of squashed prophase I nuclei double labelled for SMC3 (C) and Rad51 (D).

Fig 2 | Double immunolocalization of SMC3 (green in first and third columns) with either γ -H2AX (red in the second column) or Rad51 (red in the fourth column) in prophase I squashed grasshopper spermatocytes. (A–D) Early–mid-leptotene was devoid of γ -H2AX (B) or Rad51 (D) signalling. (E–H) Leptotene–zygotene transition. A massive accumulation of γ -H2AX occurred (F), whereas only a few Rad51 foci were detected (H). (I–L) Mid-zygotene. The γ -H2AX signals observed in this stage were polarized to the *bouquet* area (J). Rad51 (L) has extraordinarily increased its foci number. (M–P) Mid-late zygotene. The γ -H2AX staining was restricted to those AEs that have not yet achieved synapsis (N), whereas the number of Rad51 foci remains similar to that found in early zygotene (P). (Q–T) Early or mid-pachytene. Only a few γ -H2AX foci were detected (R), being located in the synapsed AEs of autosomes. A similar spotted pattern of labelling and distribution was obtained for Rad51 (T).

SMC3 was first detected as short discrete stretches at leptotene. During zygotene, the labelling became more regular and appeared as thin lines that were polarized into a *bouquet* arrangement. In the *bouquet* area, these lines associated in pairs to form thick filaments (Fig 2). This sequence is similar to the process of AE and SC formation, as observed under electron microscopy (Jones & Croft, 1986). No γ -H2AX signals were detected in spermatogonia and early leptotene (Fig 2A,B). The first γ -H2AX domains appeared in the leptotene–zygotene transition as massive accumulations preferentially associated with the region where chromosome ends were clustered, and where the synapsis of autosomes begins (Fig 2E,F). These accumulations increased in number and intensity during early and mid-zygotene, whereas extensive synapsis occurred in autosomes (Fig 2I,J). However, as synapsis proceeded, the labelling of γ -H2AX started to fade along the synapsed regions but persisted encompassing those AEs that have not achieved synapsis (Figs 2M,N and 4A–C). The γ -H2AX signal concentrated on the chromatin associated with the last stretches of AEs to synapse, that is, interstitial regions in those bivalents with two synaptic initiation points, or one chromosome end in those bivalents showing a single synaptic initiation point. At late zygotene, γ -H2AX started to disappear from the autosomal chromatin. In early or mid-pachytene spermatocytes (Fig 2Q,R), only a few γ -H2AX foci were detected in the synapsed LEs of autosomes, and covering the chromatin of the unsynapsed X chromosome (Fig 3M). It must be noted that this labelling was not observed when a combined immunodetection of γ -H2AX and SMC3 was performed, perhaps as a consequence of steric interactions of both antibodies over the X chromosome (compare Figs 1A,B and 2Q,R). γ -H2AX labelling disappeared from mid-pachytene onwards and was no longer observed until the formation of spermatids. Spermatid labelling has also been reported in mouse (Hamer *et al*, 2003). Thus, SMC3 and γ -H2AX labelling confirmed that extensive γ -H2AX staining occurs before synapsis.

Appearance of γ -H2AX is followed by Rad51 location to AEs

Rad51 is homologous to the bacterial RecA protein, which is responsible for DNA strand exchange, suggesting that Rad51 may have a similar function in eukaryotic cells (Shinohara *et al*, 1992). In human and mouse spermatocytes, Rad51 localizes to chromatin regions that are undergoing, or are about to undergo, synapsis, indicating a role in meiotic recombination (Barlow *et al*, 1997; Tarsounas *et al*, 1999). The Rad51 distribution pattern found in grasshoppers is in broad agreement with these reports (Fig 2). Thus, Rad51 appeared as discrete foci on the chromosome axes at early zygotene. There were about 400 Rad51 foci per nucleus by

mid-zygotene when chromosomes were pairing and synapsing (Fig 2C,D; G,H; K,L; O,P). Rad51 labelling appeared to be closely associated with AEs/LEs localized in both unsynapsed and synapsed autosomal regions, and in many cases the foci distribution in both homologues was symmetrical (Fig 4D–F). The labelling at fully synapsed bivalents decreased very rapidly in late zygotene nuclei. During pachytene, 5–15 Rad51 foci were observed per nucleus. Interestingly, the higher number of foci matched the mean chiasmata number in *L. migratoria* male meiosis (Fig 2S,T). Rad51 foci disappeared completely by mid-late pachytene. Throughout all these meiotic stages, Rad51 labelling was not associated to the single X chromosome (Fig 3N,O). To avoid a possible steric interaction between the antibodies against SMC3 and Rad51 on the X chromosome, similar to that observed for SMC3 and γ -H2AX antibodies, we performed a single immunolabelling of Rad51. The results confirmed the absence of Rad51 foci on the X chromosome (supplementary movie 3 online).

To establish accurately the relative timing of appearance of γ -H2AX and Rad51, we performed double immunolabellings. As described above, γ -H2AX first appeared in the leptotene–zygotene transition as diffuse accumulations in the *bouquet* area. In this stage, no Rad51 labelling was detected. Furthermore, when the first Rad51 foci were detected in early zygotene, nuclei showed an extensive γ -H2AX staining (Fig 3A,B). On these grounds, we conclude that γ -H2AX appears before the Rad51 foci at prophase I. However, it is interesting to note that Rad51 foci localized over or around γ -H2AX domains. Direct evidences of such colocalization were found in mid-zygotene nuclei (Fig 4G,H). Afterwards, the γ -H2AX staining disappeared concomitantly with synapsis progression, whereas the Rad51 signals were discernible until late zygotene (Fig 3A–L). At pachytene, there is a correspondence between the number and location of γ -H2AX and Rad51 foci, but although they are in close proximity they do not strictly colocalize, as confirmed by confocal microscopy observations (Fig 3M–R).

DISCUSSION

The view that the stage of commitment to recombination might precede the full formation of the SC has been clearly established in *Saccharomyces cerevisiae*. In this species, the earliest identifiable molecular event in meiotic recombination occurs at leptotene and consists of the formation of DSBs. Mutants defective in DSB repair display defects in synapsis that are more severe in those mutants blocked at earlier steps in the repair pathway. Additionally, mutants with significant or even normal levels of meiotic recombination do not form SCs. These data are consistent with a model based on the dependence of synapsis on recombination

◀ **Fig 3** | Double immunolabelling of γ -H2AX (green) and Rad51 (red), and counterstaining with DAPI (blue). (A–C) Leptotene–zygotene transition. Massive staining of γ -H2AX was present (A), whereas there was a reduced number of Rad51 foci (B). (D–F) Early zygotene. Both γ -H2AX and Rad51 signals increased relative to the leptotene–zygotene transition. Rad51 foci were located at autosomes with and without detectable γ -H2AX (E). (G–I) Mid-zygotene. γ -H2AX remained at unsynapsed regions of the chromosomes (G), but Rad51 appeared all over the nucleus (H). (J–L) Late zygotene. γ -H2AX remained on the last stretches of autosomes to achieve synapsis and in the X chromosome (X), whereas Rad51 foci were still abundant (K). (M–O) Early pachytene. A few γ -H2AX (M) and Rad51 (N) foci were detected over the autosomes. (P–R) When early pachytene nuclei were analysed using confocal microscopy, the close proximity of γ -H2AX and Rad51 foci became evident (P), but they did not strictly colocalize (R). Note that γ -H2AX localized to the X chromosome (X) from leptotene up to late pachytene, but Rad51 was completely absent on it. Although in some figures the X chromosome seems to be labelled with Rad51 (H,K), this is an artefact produced by the superimposition of focal planes. However, confocal analyses confirmed the absence of Rad51 signals in the X chromosome.

Fig 4 | Enlarged images of unsynapsed chromosomes in zygotene. (A–C) Location of SMC3 (A) and γ -H2AX (B). The unsynapsed AEs labelled by SMC3 are surrounded by γ -H2AX (C). (D–F) Double immunolabelling of SMC3 (D) and Rad51 (E). (F) Symmetric pattern of location of Rad51 foci lying adjacent to both unsynapsed AEs. (G–I) Relative distribution of γ -H2AX and Rad51 (H). Over the broad signalling of γ -H2AX (G), a few discrete Rad51 foci appear (H). The symmetric foci in both AEs are located in close proximity to the γ -H2AX labelling (I).

(reviewed in Kleckner, 1996; Roeder, 1997). This model is also applicable in mouse and *Arabidopsis* (Grelon *et al*, 2001; Mahadevaiah *et al*, 2001), but not in *Drosophila* (McKim & Hayasi-Hagihara, 1998) and *C. elegans* (Dernburg *et al*, 1998). Recently, Jang *et al* (2003) have demonstrated in *Drosophila*, by means of an antibody that recognizes the phosphorylation of the histone variant H2Av at DSB sites, that DSB formation occurs after synapsis. All these findings have motivated many discussions regarding the interdependence of recombination and synapsis.

Our results on the time course of DSB formation in grasshopper spermatocytes, based on the distribution of γ -H2AX and Rad51 domains, indicate that extensive γ -H2AX immunostaining occurs at the leptotene–zygotene transition, just before SC formation. Thus, we conclude that in grasshoppers the initiation of recombination, as detected by formation of DNA damage signalling, occurs before the initiation of synapsis. We have also observed that Rad51 foci appear immediately after γ -H2AX labelling and, therefore, downstream of DSB formation. This situation agrees with the recombination pathway described in budding yeast. Furthermore, during zygotene, the number of γ -H2AX domains decreased and their intensity declined, being concentrated on the chromatin associated with the last autosomal stretches of AEs to synapse and the X chromosome. Therefore, the disappearance of γ -H2AX signals is temporally and spatially correlated with synapsis. Taking into account all these findings, it can be concluded that grasshoppers do not follow the sequence of meiotic events observed in *Drosophila* and *C. elegans* because initiation of recombination precedes synapsis. On these grounds, and although further studies are necessary, it is tempting to suggest that in grasshoppers certain steps in the recombination pathway might be required for normal synapsis.

Conversely, the γ -H2AX signals observed in the X chromosome at late pachytene and in spermatids indicate that the function of H2AX phosphorylation during grasshopper spermatogenesis is not restricted to the formation of γ -H2AX domains at DNA DSBs. It has been recently reported that the accumulation of γ -H2AX in the sex body of the mouse from late zygotene to early diplotene takes place in a manner independent of meiotic-recombination-associated DSBs (Fernández-Capetillo *et al*, 2003). These authors have proposed that phosphorylation of H2AX histone is required for chromatin remodelling and associated silencing in mouse meiosis. The accumulation of γ -H2AX in the X chromosome of male grasshoppers from mid-zygotene until late pachytene resembles the situation described for the sex chromosomes in mouse and, although its meaning remains to be ascertained, it could be indicative of the implication of γ -H2AX in an ancient mechanism of meiotic sex chromosome inactivation.

METHODS

Adult males of the grasshopper species *E. plorans* and *L. migratoria* (Orthoptera: Acrididae) were used for this study.

Antibodies. A polyclonal rabbit anti-SMC3 antibody (AB3914; Chemicon International) raised against a synthetic peptide from human SMC3 was used to detect SMC3. To detect γ -H2AX, we used a monoclonal mouse antibody (no. 05-636; Upstate) raised against amino acids 134–142 of human histone H2AX (Paull *et al*, 2000). This sequence has eight identical amino acids in yeast and mouse (Redon *et al*, 2002). A polyclonal rabbit anti-Rad51 antibody (Ab-1; PC130; Oncogene Research Products), generated against recombinant HsRad51 protein, was used to detect Rad51.

Immunoblotting. Western blot analyses were performed with extracts of grasshopper and mice testes to detect proteins

recognized by the antibodies against γ -H2AX, SMC3 and Rad51. Testes from adult *L. migratoria* males were processed as described in supplementary information online. Testes from adult male C57BL/6 mice were processed as described previously (Parra *et al*, 2002).

Immunofluorescence microscopy. Testes were processed using the squashing procedure described by Page *et al* (1998). For double immunolabellings with antibodies raised in the same host species, we proceeded as described by Page *et al* (2003). For further details, see supplementary information online.

Observations were performed using an Olympus BX61 microscope equipped with a motorized Z-axis and epifluorescence optics. The images were captured with a DP70 Olympus digital camera using the Olympus Analysis software, analysed and processed using Adobe Photoshop 6.0 software and the public domain software ImageJ (National Institutes of Health, USA; <http://rsb.info.nih.gov/ij/>) and VirtualDub (VirtualDub.org; <http://www.virtualdub.com>). Samples were also analysed in an Olympus IX-70 inverted microscope equipped with a confocal laser scanning system (Fluoview 300). Images were captured by sequential scanning, noise-filtered, corrected for background and processed using the Olympus Fluoview software (version 4.3). **Supplementary information** is available at *EMBO reports* online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

This work was supported by grants BMC2002-00043 and BMC2002-01171 from Ministerio de Ciencia y Tecnología (Spain). A.V. was awarded a fellowship from Fundación General de la UAM and Olympus España SA.

REFERENCES

- Barlow EL, Benson FE, West SC, Hultén MA (1997) Distribution of the Rad51 recombinase in human and mouse spermatocytes. *EMBO J* **16**: 5207–5215
- Celeste A, Fernández-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, Bonner RF, Bonner WM, Nussenzweig A (2003) Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. *Nat Cell Biol* **5**: 675–679
- Downs JA, Lowndes NF, Jackson SP (2000) A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. *Nature* **408**: 1001–1004
- Dernburg AF, McDonald K, Moulder G, Barstead G, Dresser M, Villeneuve AM (1998) Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. *Cell* **94**: 387–398
- Fernández-Capetillo O, Mahadevaiah SK, Celeste A, Romanienko PJ, Camerini-Otero RD, Bonner WM, Manova K, Burgoyne PS, Nussenzweig A (2003) H2AX is required for chromatin remodelling and inactivation of sex chromosomes in male mouse meiosis. *Dev Cell* **4**: 497–508
- Grelon M, Vezon D, Gendrot G, Pelletier G (2001) *AtSPO11-1* is necessary for meiotic recombination in plants. *EMBO J* **20**: 589–600
- Hamer G, Roepers-Gajadien LP, van Duyn-Goedhart A, Gademan IS, Kal HB, van Buul PPW, de Rooij DG (2003) DNA double-strand breaks and γ -H2AX signaling in the testis. *Biol Reprod* **68**: 628–634
- Hirano T (2002) The ABCs of SMC proteins: two-armed ATPases for chromosome condensation, cohesion, and repair. *Genes Dev* **16**: 399–414
- Kleckner N (1996) Meiosis: how could it work? *Proc Natl Acad Sci USA* **93**: 8167–8174
- Jang JK, Sherizen DE, Bhagat R, Manheim EA, McKim KS (2003) Relationship of DNA double-strand breaks to synapsis in *Drosophila*. *J Cell Sci* **116**: 3069–3077
- Jones GH, Croft JA (1986) Surface spreading of synaptonemal complexes in locusts: zygotene pairing behaviour. *Chromosoma* **93**: 489–495
- Madigan JP, Chotkowski HL, Glaser RL (2002) DNA double-strand break-induced phosphorylation of *Drosophila* histone variant H2Av helps prevent radiation-induced apoptosis. *Nucleic Acids Res* **30**: 3698–3705
- Mahadevaiah SK, Turner JM, Baudat F, Rogakou EP, de Boer P, Blanco-Rodriguez J, Jasin M, Keeney S, Bonner WM, Burgoyne PS (2001) Recombinational DNA double strand breaks in mice precede synapsis. *Nat Genet* **21**: 271–276
- McKim KS, Hayasi-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
- Page J, Suja JA, Santos JL, Rufas JS (1998) Squash procedure for protein immunolocalization in meiotic cells. *Chromosome Res* **6**: 639–642
- Page J, Berrios S, Rufas JS, Parra MT, Suja JA, Heyting C, Fernández-Donoso R (2003) The pairing of X and Y chromosomes during meiotic prophase in the marsupial species *Thylamys elegans* is maintained by a dense plate developed from their axial elements. *J Cell Sci* **116**: 551–560
- Parra MT, Page J, Yen TJ, He D, Valdeolmillos A, Rufas JS, Suja JA (2002) Expression and behaviour of CENP-E at kinetochores during mouse spermatogenesis. *Chromosoma* **111**: 53–61
- Pauli TT, Rogakou EP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM (2000) A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr Biol* **10**: 886–895
- Pelttari J *et al* (2001) A meiotic chromosome core consisting of cohesin complex proteins recruits DNA recombination proteins and promotes synapsis in the absence of an AE in mammalian meiotic cells. *Mol Cell Biol* **21**: 5667–5677
- Redon C, Pilch D, Rogakou EP, Sedelnikova O, Newrock K, Bonner WM (2002) Histone H2A variants H2X and H2Z. *Curr Opin Genet Dev* **12**: 162–169
- Roeder GS (1997) Meiotic chromosomes: it takes two to tango. *Genes Dev* **11**: 2600–2621
- Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM (1998) DNA double-stranded breaks induce histone H2AX phosphorylation in serine 139. *J Biol Chem* **273**: 5858–5868
- Rogakou EP, Nieves-Neira W, Boon C, Pommier Y, Bonner WM (2000) Initiation of DNA fragmentation during apoptosis induces phosphorylation of H2AX histone at serine 139. *J Biol Chem* **275**: 9390–9395
- Shinohara A, Ogawa H, Ogawa T (1992) Rad51 protein involved in repair and recombination in *Saccharomyces cerevisiae* is a RecA-like protein. *Cell* **69**: 457–470
- Tarsounas M, Morita T, Pearlman RE, Moens PB (1999) RAD51 and DMC1 form mixed complexes associated with mouse meiotic chromosome cores and synaptonemal complexes. *J Cell Biol* **147**: 207–219