

# Progression of meiotic recombination requires structural maturation of the central element of the synaptonemal complex

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## Summary

The synaptonemal complex is an elaborate meiosis-specific supramolecular protein assembly that promotes chromosome synapsis and meiotic recombination. We inactivated the meiosis-specific gene *Tex12* and found that TEX12 is essential for progression of meiosis in both male and female germ cells. Structural analysis of the synaptonemal complex in *Tex12*<sup>-/-</sup> meiotic cells revealed a disrupted central element structure, a dense structure residing between the synapsed homologous chromosomes. Chromosome synapsis is initiated at multiple positions along the paired homologous chromosomes in *Tex12*<sup>-/-</sup> meiotic cells, but fails to propagate along the chromosomes. Furthermore, although meiotic recombination is initiated in *Tex12*<sup>-/-</sup> meiotic cells, these early recombination events do not

develop into meiotic crossovers. Hence, the mere initiation of synapsis is not sufficient to support meiotic crossing-over. Our results show that TEX12 is a component of the central element structure of the synaptonemal complex required for propagation of synapsis along the paired homologous chromosomes and maturation of early recombination events into crossovers.

Supplementary material available online at  
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Key words: Chromosome synapsis, Meiosis, Meiotic recombination, Synaptonemal complex, TEX12

## Introduction

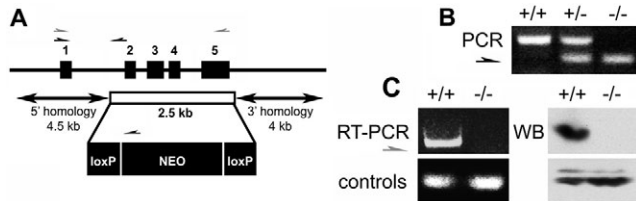
During mammalian meiosis, one round of DNA replication is followed by two successive rounds of chromosome segregation: meiosis I (movement of the homologous chromosomes, each consisting of one pair of sister chromatids, to opposite poles) and meiosis II (separation of the sister chromatids into haploid cells). The prophase of meiosis I is highly regulated and can be subdivided in four cytological stages: leptotema (chromatin condensation), zygotema (synapsis of homologous chromosomes), pachytene (full synapsis) and diplotema (visible chiasmata) (Zickler and Kleckner, 1999). During these stages the sister chromatids are held together by cohesin complex proteins, whereas synapsis of the homologous chromosomes is achieved by formation of a large zipper-like protein complex called the synaptonemal complex (Page and Hawley, 2004; Petronczki et al., 2003).

Incorrect assembly of the synaptonemal complex leads to impaired recombination and cell death, which, in humans, causes infertility in males (Judis et al., 2004; Miyamoto et al., 2003) and a high aneuploidy rate in females (Hassold and Hunt, 2001; Hunt and Hassold, 2002).

Formation of the mammalian synaptonemal complex starts during leptotema when synaptonemal complex protein-2 and -3 (SYCP2 and SYCP3) initiate formation of axial elements, fibrous cores alongside the homologous chromosomes. In male mice, knockout of the *Sycp3* gene, which also abolishes recruitment of

SYCP2, leads to sterility; the *Sycp3*<sup>-/-</sup> spermatocytes fail to form visible axial elements and fail to develop beyond the zygotene stage (Liebe et al., 2004; Pelttari et al., 2001; Yuan et al., 2000). By contrast, the *Sycp3*<sup>-/-</sup> females are fertile. However, absence of SYCP3 in oocytes partially disrupts synapsis, impairs DNA damage repair and reduces meiotic recombination, which together eventually lead to a high aneuploidy rate and embryo death (Lightfoot et al., 2006; Wang and Hoog, 2006; Yuan et al., 2002). Similar phenotypes were also observed in *Sycp2* mutant mice, probably due to the absence of SYCP3 on the residual axial elements in the *Sycp2* mutant cells (Yang et al., 2006). Even though it is not clear how this works biochemically, both SYCP2 and SYCP3 are co-dependent and are required to initiate formation of the axial elements.

During the zygotene stage, synapsis is initiated and the axial elements of the homologous chromosomes (now referred to as lateral elements) are joined by transverse filaments shaped by SYCP1. SYCP1 molecules are long coiled-coil proteins with two globular heads that form parallel homodimers, C-terminal globular heads embedded in the lateral elements and N-terminal heads that interact in a dense region in the middle of the synaptonemal complex called the central element (Liu et al., 1996; Ollinger et al., 2005; Schmekel et al., 1996). Knockout of the mouse transverse filament gene *Sycp1* leads to apoptosis of most meiotic cells during the pachytene stage and subsequently to both male and female infertility (de Vries et al., 2005). In *Sycp1*<sup>-/-</sup> spermatocytes,



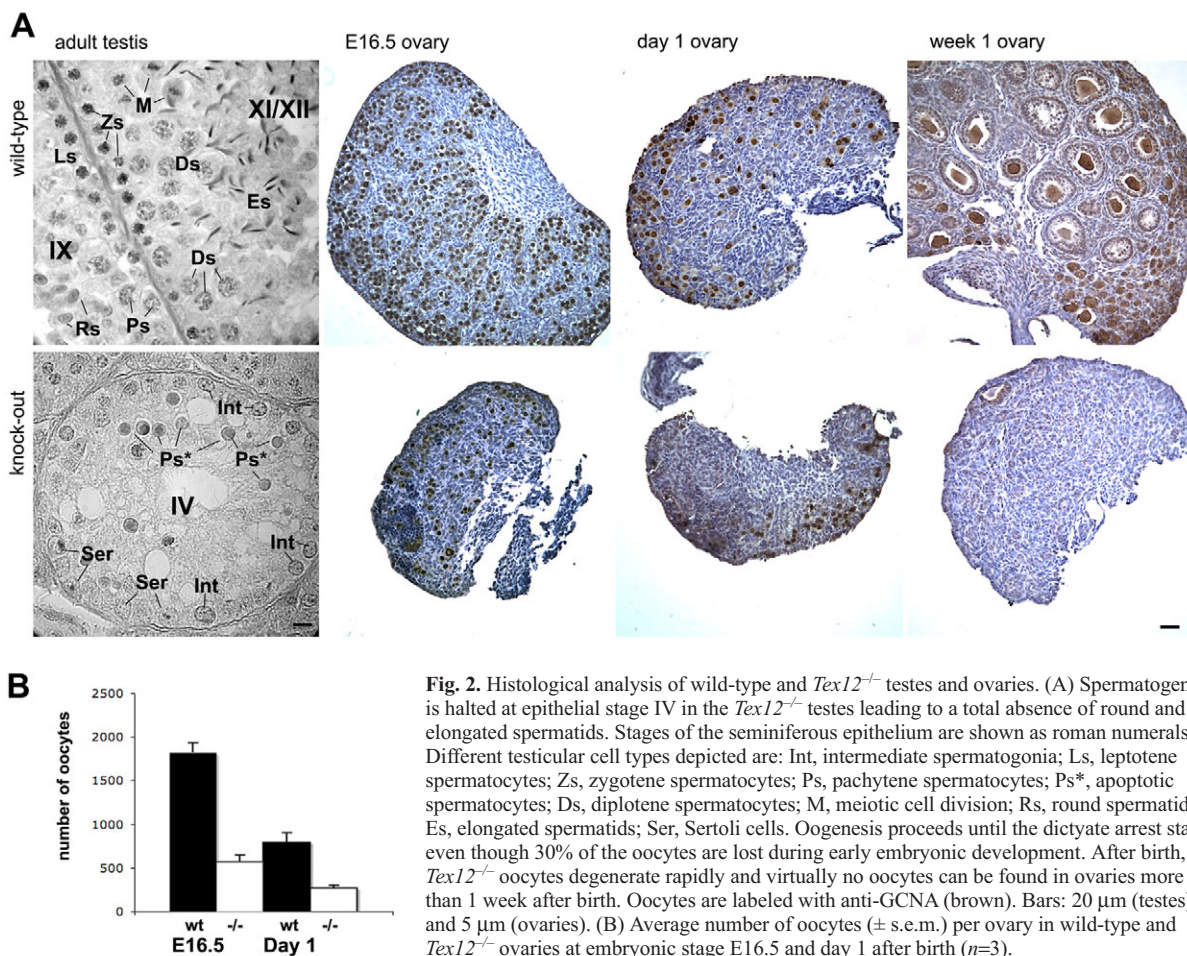
**Fig. 1.** Generation of *Tex12*<sup>-/-</sup> mice. (A) Exon 2 to exon 5 of the *Tex12* gene were replaced by a neomycin cassette to generate *Tex12*<sup>-/-</sup> mice. (B,C) The mice were genotyped using PCR (black primers in A, two in the gene, one in the neomycin cassette) and did not contain the *Tex12* RNA (RT-PCR, grey primers in A, positive control using *Trip13*) or protein (western blot, WB, positive control  $\alpha$ -tubulin).

although axial element formation and alignment of the homologous chromosomes proceed normally, chromosome synapsis and the development of meiotic crossovers do not occur (de Vries et al., 2005).

Following the association of SYCP1 with the lateral elements, further progression of synapsis gives rise to the central element structure in the middle of the synaptonemal complex. The morphology of the central element has been studied in detail by electron microscopy (Schmekel and Daneholt, 1995; Solari and Moses, 1973) and consists of three to four layers of transverse

filament components that are longitudinally connected by pillar-shaped protein structures (Schmekel and Daneholt, 1995). In mammals, two central element proteins have been identified: synaptonemal complex central element protein-1 and -2 (SYCE1 and SYCE2) (Costa et al., 2005), of which SYCE2 recently has been shown to be required for completion of synapsis (Bolcun-Filas et al., 2007). We previously identified a novel meiosis specific protein, testis-expressed protein 12 (TEX12), and showed that this protein localizes to the central element of the synaptonemal complex (Hamer et al., 2006), suggesting that TEX12 is a component of the central element. However, even though *Tex12* is conserved in vertebrates, its sequence does not tell us anything about its possible function.

To gain more insight in the function of TEX12 and its putative role in central element organization, we have now analyzed *Tex12*<sup>-/-</sup> mice. We found that TEX12 is essential for recruitment of SYCE2 to the central element region of the meiotic chromosomes and for the formation of the central element structure. In *Tex12*<sup>-/-</sup> cells, synapsis is initiated and visible as small stretches of SYCP1. However, SYCP1 fails to polymerize along the axial elements in the absence of TEX12. Recruitment of the early recombination proteins DMC1 and RPA to the meiotic chromosomes occurs independently of TEX12, but meiotic crossovers do not occur in *Tex12*<sup>-/-</sup> meiocytes. Hence, initiation of synapsis is insufficient to support meiotic crossovers in the mouse.



**Fig. 2.** Histological analysis of wild-type and *Tex12*<sup>-/-</sup> testes and ovaries. (A) Spermatogenesis is halted at epithelial stage IV in the *Tex12*<sup>-/-</sup> testes leading to a total absence of round and elongated spermatids. Stages of the seminiferous epithelium are shown as roman numerals. Different testicular cell types depicted are: Int, intermediate spermatogonia; Ls, leptotene spermatocytes; Zs, zygotene spermatocytes; Ps, pachytene spermatocytes; Ps\*, apoptotic spermatocytes; Ds, diplotene spermatocytes; M, meiotic cell division; Rs, round spermatids; Es, elongated spermatids; Ser, Sertoli cells. Oogenesis proceeds until the dictyate arrest stage, even though 30% of the oocytes are lost during early embryonic development. After birth, the *Tex12*<sup>-/-</sup> oocytes degenerate rapidly and virtually no oocytes can be found in ovaries more than 1 week after birth. Oocytes are labeled with anti-GCNA (brown). Bars: 20  $\mu$ m (testes) and 5  $\mu$ m (ovaries). (B) Average number of oocytes ( $\pm$  s.e.m.) per ovary in wild-type and *Tex12*<sup>-/-</sup> ovaries at embryonic stage E16.5 and day 1 after birth ( $n=3$ ).

## Results

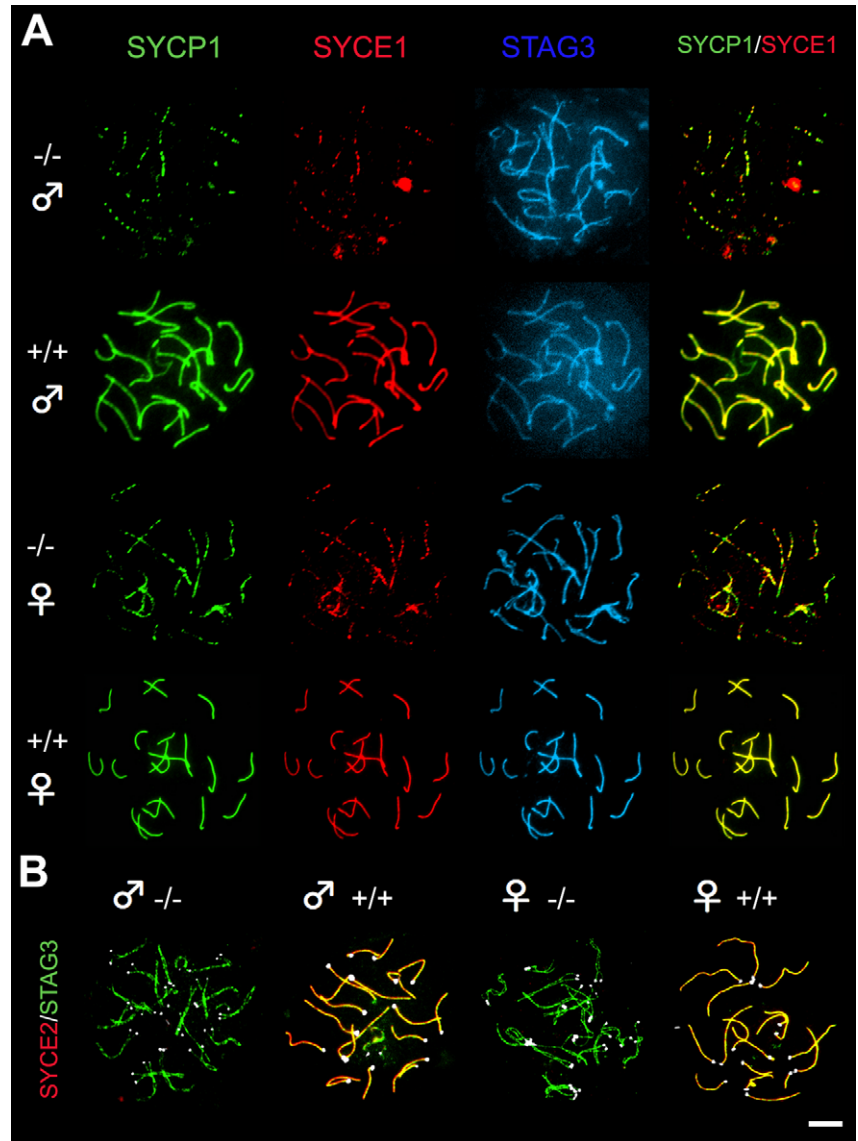
### Loss of TEX12 results in infertility

TEX12 has been shown to localize to the central element of the synaptonemal complex (Hamer et al., 2006). To gain more insight in the function of this protein during meiosis, we generated *Tex12* knockout mice. To inactivate the *Tex12* gene, a targeting vector, in which a NEO cassette replaced exon 2 to exon 5 of the gene, was electroporated into embryonic stem (ES) cells. Successfully targeted ES cells were used for generation of chimeric mice that transmitted the *Tex12*<sup>-/-</sup> allele to the germline. The *Tex12*<sup>-/-</sup> allele transmitted in a mendelian fashion in *Tex12*<sup>+/-</sup> intercrosses and the wild-type *Tex12* gene, gene transcript or encoded protein could not be detected in the *Tex12*<sup>-/-</sup> mice (Fig. 1).

Interruption of *Tex12* leads to complete elimination of spermatocytes at epithelial stage IV of spermatogenesis and subsequent infertility (Fig. 2). Analysis of *Tex12*<sup>-/-</sup> females showed that, in comparison to the wild type, only ~30% of the oocytes are present at embryonic stage E16.5 in *Tex12*<sup>-/-</sup> ovaries (Fig. 2). Notably, as the *Tex12*<sup>-/-</sup> ovary size is proportionally smaller than comparable wild-type ovaries, the density of oocytes in *Tex12*<sup>-/-</sup> ovaries is similar to the wild-type situation. The surviving *Tex12*<sup>-/-</sup> oocytes reach the dictyate arrest stage and 1 day after birth ~30% of the oocytes are still present (Fig. 2). However, *Tex12*<sup>-/-</sup> oocytes are not able to form healthy primordial follicles and eventually degenerate. As a consequence, hardly any follicles could be observed in *Tex12*<sup>-/-</sup> ovaries 1 week after birth (Fig. 2).

### TEX12 is essential for elongation of synapsis between the homologous chromosomes

To study the role of TEX12 in synaptonemal complex formation and synapsis, we analyzed meiotic cells from *Tex12*<sup>-/-</sup> and wild-type testes and ovaries using cell spread preparations and immunofluorescence microscopy. Because staining with antibodies against SYCP2 or SYCP3 interfered more with co-staining of other proteins, we used an antibody against the protein STAG3 to visualize the axial elements. STAG3 is part of the meiotic cohesin core, which keeps the sister chromatids together during meiosis, and colocalizes perfectly with the axial elements in both *Tex12*<sup>-/-</sup> and wild-type cells. By studying staining of SYCP3 (data not shown) and STAG3, we observed that the axial elements form normally and that the meiotic chromosomes align in cells without TEX12 (Fig. 3A). However, progression of synapsis was affected in *Tex12*<sup>-/-</sup> cells, resulting in only partially synapsed meiotic chromosomes (Fig. 3A). In wild-type cells, synapsis of the homologous chromosomes is marked by staining of the transversal filament protein SYCP1 and the central element protein SYCE1 along the axial elements. *Tex12*<sup>-/-</sup> meiotic cells also showed staining for SYCP1 and SYCE1, which overlapped with STAG3 on the axial elements (Fig. 3A). However, instead of covering the complete meiotic axes as observed in the wild type, SYCP1 and SYCE1 only

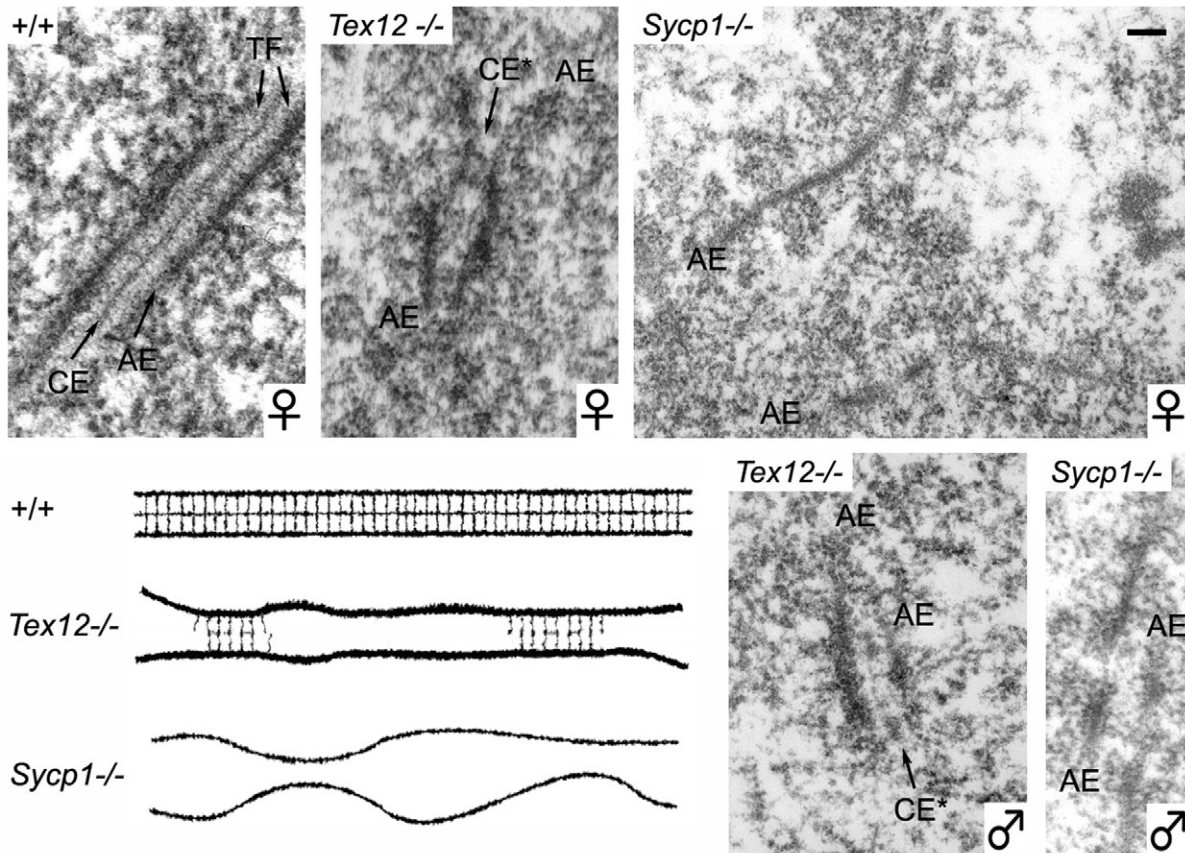


**Fig. 3.** Initiation of synapsis without an intact central element. Meiotic chromosomes in wild-type and *Tex12*<sup>-/-</sup> spermatocytes and oocytes immunolabeled for SYCP1 (transverse filaments, green), STAG3 (axial elements, blue) and SYCE1 (central elements, red) (A) or STAG3 (axial elements, green) and SYCE2 (central elements, red) (B). Centromeres are labeled with CREST (white) (B). Bar: 5 μm.

formed foci and small stretches in *Tex12*<sup>-/-</sup> meiotic cells (Fig. 3A). Importantly, the central element protein SYCE2 was completely absent from the *Tex12*<sup>-/-</sup> chromosome cores (Fig. 3B).

We also studied the structure of the meiotic chromosomes using electron microscopy (EM) and compared wild-type (full synapsis), *Tex12*<sup>-/-</sup> (partial synapsis) and *Sycp1*<sup>-/-</sup> (no synapsis) testes and ovaries (Fig. 4). In line with the immunofluorescence experiments, short synapsed axial element structures in *Tex12*<sup>-/-</sup> cells could be confirmed at the EM level in both spermatocytes and oocytes. Although partially synapsed, the *Tex12*<sup>-/-</sup> synaptonemal complex showed a weakly stained and disrupted central-element-like structure. We also observed very small areas of chromosome convergence in *Sycp1*<sup>-/-</sup> spermatocytes. However, these areas of convergence completely lacked any obvious central-element-like structure (Fig. 4).





**Fig. 4.** Initiation of synapsis without an intact central element. Electron microscopy of the synaptonemal complex in wild-type, *Tex12*<sup>-/-</sup> and *Sycp1*<sup>-/-</sup> oocytes and spermatocytes. AE, axial elements (lateral elements after synapsis); TF, transverse filaments; CE, central element; CE\*, central-element-like structure. Bar: 100 nm. A schematic representation of these results is shown in the cartoon at the bottom left.

#### DNA double-strand breaks fail to develop into meiotic crossovers

The absence of TEX12 results in a disrupted central element and only partial synapsis of the meiotic chromosomes, which could have consequences for the progression of meiotic recombination. Because the *Tex12*<sup>-/-</sup> spermatocytes are eliminated from the testis as early as epithelial stage IV, we monitored the effects of these structural deficiencies on the progression of meiotic recombination in *Tex12*<sup>-/-</sup> and wild-type oocytes, which survive until the dictyate arrest stage. As described for the *Syce2*<sup>-/-</sup> spermatocytes (Bolcun-Filas et al., 2007), the *Tex12*<sup>-/-</sup> spermatocytes show normal loading of early recombination markers but do not form an XY body marked by BRCA1 or  $\gamma$ -H2AX as observed in wild-type spermatocytes (supplementary material Fig. S1). We stained *Tex12*<sup>-/-</sup> and wild-type oocytes for proteins that mark defined stages of meiotic DNA double-strand break (DSB) processing: presence of DSBs and asynapsis ( $\gamma$ -H2AX, BRCA1), homologous recombination (DMC1, RPA) and sites of crossing-over (MLH1) (Mahadevaiah et al., 2001; Moens et al., 2002; Turner et al., 2004).

At the leptotene stage, meiotic DSBs are formed by SPO11 and marked by phosphorylation of H2AX (then referred to as  $\gamma$ -H2AX) (Mahadevaiah et al., 2001). These meiotic DSBs will be resolved by homologous recombination, which can be visualized as early recombination nodules (DMC1 foci), transformed recombination nodules (RPA foci) and sites of crossovers (MLH1 foci) (Moens et al., 2002).

In early oocytes from stage E16.5 embryos, we observed wild-type levels of DMC1 foci in both wild-type and *Tex12*<sup>-/-</sup> oocytes (Fig. 5). However, at a later stage (E18.5), high levels of DMC1 foci were still present in the *Tex12*<sup>-/-</sup> oocytes, whereas these foci were lost in wild-type oocytes (Fig. 5). Later during meiosis, the DMC1 foci are gradually replaced by RPA (Moens et al., 2002) and, in accordance, we observed high levels of RPA in both wild-type and *Tex12*<sup>-/-</sup> oocytes at stage E17.5 (Fig. 5). However, these RPA foci failed to be removed in the *Tex12*<sup>-/-</sup> oocytes and high RPA levels were still present at stage E18.5 in the *Tex12*<sup>-/-</sup> oocytes (Fig. 5). Hence, the *Tex12*<sup>-/-</sup> oocytes never complete the transition from transformed recombination nodules to meiotic crossovers. In accordance with the impaired processing of DMC1 and RPA foci, we could not observe any MLH1 foci in *Tex12*<sup>-/-</sup> oocytes (Fig. 5).

When the DSBs become properly resolved at the pachytene stage of meiosis,  $\gamma$ -H2AX is only retained on unsynapsed chromosomal regions, a process dependent on BRCA1 (Baart et al., 2000; Mahadevaiah et al., 2001; Turner et al., 2004; Turner et al., 2005). In accordance, we found  $\gamma$ -H2AX and BRCA1 to persist on the unsynapsed chromosomal regions in *Tex12*<sup>-/-</sup> oocytes (Fig. 6).

#### Discussion

TEX12 has been shown to localize to the central element of the synaptonemal complex (Hamer et al., 2006). To study the role of this protein in synaptonemal complex formation and function, we

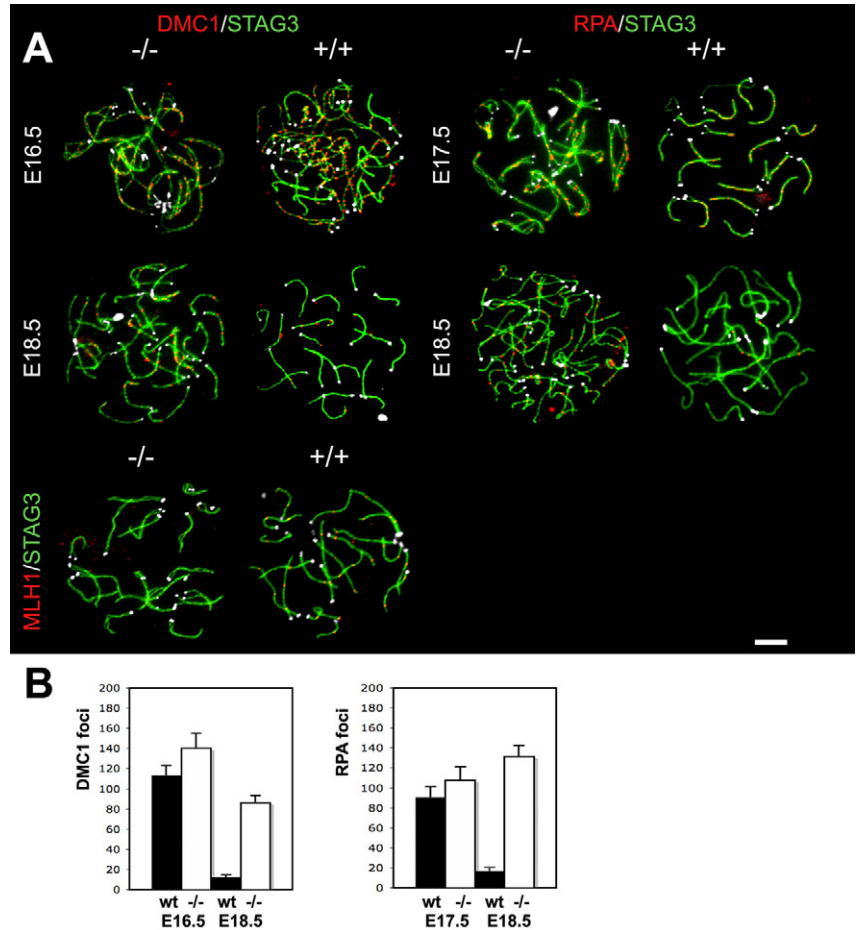
analyzed meiotic progression in *Tex12*<sup>-/-</sup> spermatocytes and oocytes. We found that without TEX12, the central element protein SYCE2 is not recruited to the meiotic chromosomes but small areas and foci containing SYCP1 and SYCE1 remain localized to the *Tex12*<sup>-/-</sup> meiotic chromosomes. Chromosome synapsis is therefore initiated without TEX12 but the *Tex12*<sup>-/-</sup> synaptonemal complex lacks a clear central element structure and synapsis is not propagated along the chromosome cores. Also, meiotic recombination is initiated in the *Tex12*<sup>-/-</sup> meiotic cells, but the recombination proteins DMC1 and RPA are not properly removed from the recombination sites and crossing-over does not occur.

Progression of synapsis requires an intact central element structure

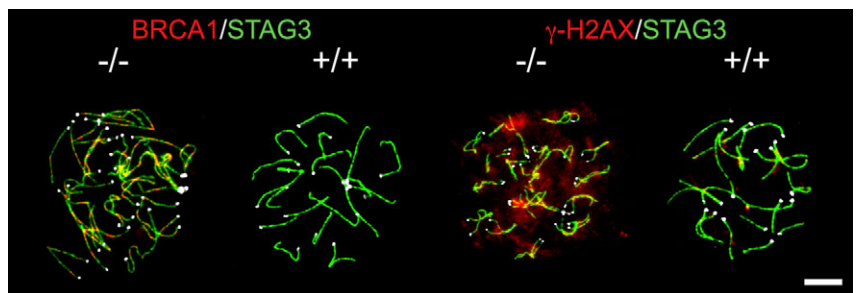
The transverse filament protein SYCP1 is required for recruitment of the central element proteins to the homologous chromosomes (Hamer et al., 2006). In cultured mammalian cells, initiation of SYCP1 fiber formation completely depends on the presence of the C-terminus (axial region) of SYCP1, whereas absence of the N-terminus (central region) only decreases the efficiency of fiber formation and elongation (Ollinger et al., 2005). Together, these results indicate that initiation of synapsis starts with the interaction of the C-terminus of SYCP1 with the axial elements. It has been proposed that the central element protein SYCE1 stabilizes the interaction between the two opposing N-termini of SYCP1 in the center of the synaptonemal complex, whereas SYCE2 would longitudinally connect these SYCP1 dimers to shape a complete central element (Bolcun-Filas et al., 2007). The central element consists of multiple layers of columns and pillars that shape an almost crystalline 3D structure in the center of the synaptonemal complex (Schmekel and Daneholt, 1995). We found that TEX12 is essential for the inclusion of SYCE2 in the central element and confirm the notion that these proteins form an independent complex (Hamer et al., 2006), possibly corresponding to the columns and pillars observed in the structural studies of the central element (Schmekel and Daneholt, 1995). Without TEX12, this structure is not properly assembled. As a consequence, the synaptonemal process does not proceed in *Tex12*<sup>-/-</sup> meiotic cells and the synapsed stretches containing SYCP1 are not elongated along the axial elements.

Progression of meiotic recombination requires structural maturation of the central element of the synaptonemal complex In *Saccharomyces cerevisiae*, initiation and elongation of the synaptonemal complex depend on a protein complex called the synapsis initiation complex, consisting of the ZMM proteins

(Zip1, Zip2, Zip3, Zip4, Mer3 and Msh4) (Fung et al., 2004; Lynn et al., 2007). Of these proteins Zip2, Zip3 and Zip4 appear functionally related to the mammalian central element proteins: they are essential for synaptonemal complex formation, they are located at the center of the synaptonemal complex and without



**Fig. 5.** The central element is required for the development of meiotic crossovers. (A) Meiotic chromosomes in wild-type and *Tex12*<sup>-/-</sup> oocytes immunolabeled for STAG3 (axial elements, green) and DMC1 (red, E16.5 and E18.5), RPA (red, E17.5 and E18.5) or MLH1 (red, E18.5). Centromeres are labeled with CREST (white). Bar: 5 μm. (B) Average number of DMC1 and RPA foci (± s.e.m.) at E16.5 (wt, n=7; -/-, n=4) and E18.5 (wt, n=12; -/-, n=9) and E17.5 (wt, n=6; -/-, n=10) and E18.5 (wt, n=10; -/-, n=9).



**Fig. 6.** Repair of DNA double-strand breaks is impaired without an intact central element. Meiotic chromosomes in wild-type and *Tex12*<sup>-/-</sup> E17.5 oocytes immunolabeled for STAG3 (axial elements, green) and  $\gamma$ -H2AX (red) or BRCA1 (red). Centromeres are labeled with CREST (white). Bar: 5 μm.



them 80% of all crossovers (Class I crossovers) do not occur (Agarwal and Roeder, 2000; Chua and Roeder, 1998; Lynn et al., 2007; Tsubouchi et al., 2006). Binding of Zip3 to synapsis initiation sites recruits Zip2 and Zip4, which in turn are responsible for polymerization of Zip1 (Agarwal and Roeder, 2000; Tsubouchi et al., 2006). The fact that these proteins are necessary for class I crossovers and that the number of synapsis initiation sites corresponds with the number of these crossovers has led to the proposal that initiation of synapsis determines the sites of future crossovers during meiosis (Henderson and Keeney, 2004; Henderson and Keeney, 2005; Zickler, 2006).

Also in mammals, synapsis and meiotic recombination are two highly intertwined events. In the mouse, although meiotic recombination initiates prior to and independently of synapsis (Baudat et al., 2000; Mahadevaiah et al., 2001), synapsis is required for recombination sites to develop into meiotic crossovers (de Vries et al., 2005). Furthermore, synapsis of the homologous chromosomes is completely dependent on the initiation of meiotic recombination (Baudat et al., 2000). However, in *Tex12<sup>-/-</sup>* oocytes, even though synapsis is initiated, crossing-over still does not occur. Hence, merely initiating synapsis is not sufficient to generate meiotic crossovers in the mouse.

How synapsis, including a fully functional central element, could promote the formation of meiotic crossovers remains unclear. The lack of TEX12 or incomplete synapsis might trigger an unknown checkpoint in the *Tex12<sup>-/-</sup>* oocytes. Moreover, TEX12 could even be part of the recombination or checkpoint machinery itself. Activation of a checkpoint in the *Tex12<sup>-/-</sup>* oocytes could possibly lead to a cell cycle arrest that would prevent the meiotic prophase to progress to the pachytene stage during which crossovers would normally occur. It has also been postulated that the mechanical properties (such as robustness or flexibility) of the synaptonemal complex affect how meiotic DSBs are resolved (Blat et al., 2002; Borner et al., 2004; Moens, 1978). The lack of crossovers in *Tex12<sup>-/-</sup>* mice could then be explained by a structural defect caused by the absence of central element proteins from the synaptonemal complex. Either way, without full synapsis along the meiotic cores and a functional central element, the limited synapsed areas on the *Tex12<sup>-/-</sup>* chromosomes fail to support the development of meiotic crossovers.

## Materials and Methods

### Animals

The *Tex12* mutant mouse line was established at the MCI/ICS (Mouse Clinical Institute/Institut Clinique de la Souris, Illkirch, France; <http://www-mci.u-strasbg.fr>). The targeting vector was constructed as follows. A 4.4 kb fragment encompassing *Tex12* exon 1 was amplified by PCR (129S2/SvPas) and subcloned in an MCI proprietary vector, resulting in a step 1 plasmid. This MCI vector has a floxed neomycin-resistance cassette. A 4.1 kb fragment was amplified by PCR and subcloned in the step1 plasmid to generate the final targeting construct (Fig. 1). The linearized construct was electroporated in 129S2/SvPas mouse embryonic stem cells. After selection, targeted clones were identified by PCR using external primers and further confirmed by Southern blot with neomycin and external probes. Two positive ES cell clones were injected into C57BL/6J blastocysts, and male chimaeras derived gave germline transmission. The *Tex12* mice were further analyzed by PCR, RT-PCR and western blot using guinea pig anti-TEX12 (Hamer et al., 2006) and mouse anti- $\alpha$ -tubulin (SIGMA).

Wild-type, *Tex12* and *Sycp1* (de Vries et al., 2005) mice were used and maintained according to regulations provided by the animal ethical committee of the Karolinska Institute who also approved of the experiments.

### Immunohistochemistry, immunocytochemistry and electron microscopy

Histology and immunohistochemistry were performed as described (Hamer et al., 2001; Wang and Hoog, 2006). Immunocytochemistry was performed as described

(Kouznetsova et al., 2005) using a 'drying-down' technique (Peters et al., 1997) and the following antibodies: rabbit anti-SYCP1 (1:50) (Liu et al., 1996), mouse anti-SYCP1 (1:200) (gift from C. Heyting, Wageningen, The Netherlands.), rabbit anti-SYCP3 (1:200) (Liu et al., 1996), human anti-CREST (1:1500) (Hadlaczky et al., 1986), rabbit anti-STAG3 (1:400) (Pezzi et al., 2000), guinea pig anti-STAG3 (1:200) (Kouznetsova et al., 2005), guinea pig anti-SYCE1 (1:1500) (Hamer et al., 2006), guinea pig anti-SYCE2 (1:400) (Hamer et al., 2006), rabbit anti- $\gamma$ -H2AX (Upstate Biotechnology) (1:100), rabbit anti-BRCA1 (gift from J. M. A. Turner, National Institute for Medical Research, London, UK) (1:1000), rabbit anti-DMC1 (1:100) and rabbit anti-RPA (1:500) (gifts from P. Moens, York University, Toronto, Canada) and mouse anti-MLH1 (1:50) (BD Biosciences). Secondary antibodies were applied as described (Kouznetsova et al., 2005; Wang and Hoog, 2006). Electron microscopy was performed on ultra thin sections of testis or ovary tissue fixed in 2.5% glutaraldehyde and 1% OsO<sub>4</sub> as described previously and according to standard protocols (Liebe et al., 2004).

### Microscopy and imaging

Slides were viewed at room temperature using Leica DMRA2 and DMRXA microscopes and 100 $\times$  objectives with epifluorescence. Images were captured with a Hamamatsu digital charge-coupled device camera C4742-95 and Openlab<sup>TM</sup> software version 3.1.4. Images were processed using Adobe Photoshop version 9.0.

We thank Christa Heyting and Albert Pastink for the *Sycp1*-deficient mice. The *Tex12* mouse mutant line was established at the Mouse Clinical Institute (Institut Clinique de la Souris, MCI/ICS) in the Targeted Mutagenesis and Transgenesis Department. This work has been supported by the Swedish Cancer Society, the Swedish Research Council, the Axel Wenner-Gren Foundation, the Deutsche Forschungsgemeinschaft (Grant Be 1168/6-3) and the Karolinska Institute.

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