

# Meiotic cohesin complexes are essential for the formation of the axial element in mice

Elena Llano,<sup>1,2</sup> Yurema Herrán,<sup>1</sup> Ignacio García-Tuñón,<sup>1</sup> Cristina Gutiérrez-Caballero,<sup>1</sup> Enrique de Álava,<sup>1</sup> José Luis Barbero,<sup>4</sup> John Schimenti,<sup>5</sup> Dirk G. de Rooij,<sup>6</sup> Manuel Sánchez-Martín,<sup>3</sup> and Alberto M. Pendás<sup>1</sup>

<sup>1</sup>Instituto de Biología Molecular y Celular del Cáncer, <sup>2</sup>Departamento de Fisiología, and <sup>3</sup>Departamento de Medicina, Consejo Superior de Investigaciones Científicas-Universidad de Salamanca, 37007 Salamanca, Spain

<sup>4</sup>Departamento de Proliferación Celular y Desarrollo, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, 28040 Madrid, Spain

<sup>5</sup>Center for Vertebrate Genomics, Cornell University, Ithaca, NY 14850

<sup>6</sup>Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, Netherlands

**C**ohesin is a conserved multisubunit protein complex that participates in chromosome segregation, DNA damage repair, chromatin regulation, and synaptonemal complex (SC) formation. Yeast, but not mice, depleted of the cohesin subunit Rec8 are defective in the formation of the axial elements (AEs) of the SC, suggesting that, in mammals, this function is not conserved. In this paper, we show that spermatocytes from mice lacking

the two meiosis-specific cohesin subunits RAD21L and REC8 were unable to initiate RAD51- but not DMC1-mediated double-strand break repair, were not able to assemble their AEs, and arrested as early as the leptotene stage of prophase I, demonstrating that cohesin plays an essential role in AE assembly that is conserved from yeast to mammals.

## Introduction

In mammalian spermatogenesis, a subset of spermatogonia undergoes a terminal round of DNA replication and then enters meiosis. Meiosis is a specialized process in which two successive rounds of chromosome segregation and cell divisions occur without intervening DNA replication. This reduces the number of each chromosome from four copies in the meocyte to one copy in haploid gametes. At the initiation of the prophase I, a proteinaceous structure called the axial element (AE) begins to form along replicated sister chromatids. Subsequently, the AEs of homologues become juxtaposed by transverse element proteins (e.g., SYCP1, beginning in zygonema), and the paired axes joined by transverse elements form the tripartite synaptonemal complexes (SCs) that connect all homologues at pachynema (Yang and Wang, 2009). This dynamic protein complex provides the structural framework in which homologous chromosomes undergo close juxtaposition and repair of double-strand breaks (DSBs) by recombination. Subsequently, but before the first meiotic division, homologues desynapse but retain stable connections

formed by resolution of certain recombination events as crossovers (visible as chiasmata). In most organisms, chiasmata are required to enable proper orientation of homologues on the meiosis I spindle before the first meiotic division. Sister chromatid cohesion distal to crossovers maintains chiasmata at their initial positions until anaphase I.

During meiosis, sister chromatid cohesion is lost in two consecutive steps. Loss of chromosome arm cohesion in anaphase I releases the linkage between homologues, allowing them to segregate to opposite poles (Page and Hawley, 2003). However, maintenance of centromeric cohesion by the action of Shugoshin-like-2 in mammals ensures the generation of tension by the proper attachment of sister chromatids to the meiosis II spindle, enabling their proper segregation to opposite poles (Llano et al., 2008; Gutiérrez-Caballero et al., 2012).

During the mitotic cell cycle, sister chromatid cohesion is mediated by the multisubunit cohesin complex between S phase and anaphase (Gruber et al., 2003; Unal et al., 2007; Haering et al., 2008; Zhang et al., 2008). Structurally, the mitotic cohesin complex comprises four core proteins: SMC1 $\alpha$ , SMC3, RAD21, and a HEAT repeat domain protein (STAG1

E. Llano and Y. Herrán contributed equally to this paper.

Correspondence to Alberto M. Pendás: amp@usal.es; or Elena Llano: ellano@usal.es

Abbreviations used in this paper: AE, axial element; dKO, double knockout; DSB, double-strand break; LE, lateral element; RPA, replication protein A; SC, synaptonemal complex.

© 2012 Llano et al. This article is distributed under the terms of an Attribution-Noncommercial-Share Alike-No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms>). After six months it is available under a Creative Commons License (Attribution-Noncommercial-Share Alike 3.0 Unported license, as described at <http://creativecommons.org/licenses/by-nc-sa/3.0/>).

and STAG2; Watanabe, 2005; Hirano, 2006). SMC1 $\alpha$  and SMC3 are members of the structural maintenance of a chromosome family of ATPases, which heterodimerize in an antiparallel orientation. The  $\alpha$ -kleisin subunit RAD21 closes the ring cohesin complex and is the substrate of the protease separase (Uhlmann et al., 2000). There are meiosis-specific mammalian paralogues of RAD21, SMC1 $\alpha$ , and STAG1-2, namely, REC8 and RAD21L, SMC1 $\beta$ , and STAG3, respectively (Parisi et al., 1999; Prieto et al., 2001; Gruber et al., 2003; Gutiérrez-Caballero et al., 2011), which lead to a variety of meiosis-specific cohesin complexes (Ishiguro et al., 2011; Gutiérrez-Caballero et al., 2011). Interestingly, yeast Rec8 is necessary for stepwise release of sister chromatid cohesion in meiosis, and this role is widely conserved across eukaryotes (Klein et al., 1999; Golubovskaya et al., 2006; Severson et al., 2009). Furthermore, the analysis of yeast mutants of Rec8 revealed additional functions in AE assembly, pairing of homologues, synapsis, and recombination (Klein et al., 1999; Watanabe and Nurse, 1999).

In mammals, cohesins colocalize and interact with the structural AE components SYCP3 and SYCP2 (Suja and Barbero, 2009). However, there is disagreement as to whether the mammalian cohesin complex is an integral part of the AE itself (Eijpe et al., 2003) or constitutes a different structure of the chromosomal core (Pelttari et al., 2001). Mouse spermatocytes lacking SMC1 $\beta$ , REC8, or RAD21L are able to assemble AEs but undergo meiotic arrest at the zygotene or early pachytene stages, with partially synapsed chromosomes (Bannister et al., 2004; Revenkova et al., 2004; Herrán et al., 2011). REC8 is also dispensable for AE and SC assembly in many higher eukaryotes, suggesting that cohesin may not be universally required for AE assembly (Bhatt et al., 1999; Bannister et al., 2004). However, *Caenorhabditis elegans* depleted of the three meiosis-specific kleisins (Rec8, COH-3, and COH-4) are unable to form AEs, similar to yeast bearing *rec8 $\Delta$*  or *smc3 $\Delta$*  alleles (Klein et al., 1999; Severson et al., 2009). These results suggest that this AE assembly function might be obscured in other higher eukaryotes, such as mammals, owing to the involvement of multiple kleisins (Gutiérrez-Caballero et al., 2011; Ishiguro et al., 2011; Lee and Hirano, 2011).

To better understand the role that cohesins play in AE and SC assembly in mammals, we performed a genetic depletion of the two meiosis-specific kleisins REC8 and RAD21L in mice (Bannister et al., 2004; Herrán et al., 2011). Our results reveal that either of these kleisins is each sufficient for association of the AE proteins SYCP3 and SYCP2 with chromosomes and that AE formation fails only in mice lacking both kleisins. This failure to assemble AEs leads to accumulation of cells with leptotene-like morphology, which is, to the best of our knowledge, the earliest arrest of mouse spermatogenesis ever reported. This evidence indicates that meiotic cohesin complexes are essential structural components of the AE from yeast to mammals. In addition, we show that meiotic cohesins function downstream of the SPO11-mediated DSB formation and upstream of RAD51- but not DMC1-mediated DSB repair.

## Results and discussion

### Mice lacking RAD21L and REC8 develop normally but are infertile

Mice lacking either of two meiosis-specific cohesin subunits, REC8 or RAD21L, show similar defects in meiosis. To test for possible overlap in the functions of these two kleisin proteins, we generated kleisin double-knockout (dKO) mice. *Rad21l<sup>-/-</sup>Rec8<sup>-/-</sup>*, henceforth dKO-kls animals, were obtained from crosses of double heterozygotes (*Rec8<sup>+/-</sup>* and *Rad21l<sup>+/-</sup>*) in the expected Mendelian ratios and analyzed. dKO-kls mice developed normally and displayed no overt defects besides infertility. As expected from the phenotypes of the single *Rec8* and *Rad21l* mutants, all the dKO-kls mice ( $n = 11$ ) were infertile (unpublished data; Bannister et al., 2004; Herrán et al., 2011). The testes from dKO-kls mice were reduced in size, weighing  $30 \pm 3$  mg at 6–8 wk of age compared with  $103 \pm 8$  mg in wild-type males (Fig. 1 A and Table S1). Furthermore, histological examination of testes from 6–8-wk-old dKO-kls males revealed seminiferous tubules that were always devoid of postmeiotic cell types, despite the presence of spermatogonia, and Sertoli and Leydig cells (Fig. 1 B and not depicted). Similar defects were also observed in the *Rec8*-deficient and *Rad21L*-deficient mice (Fig. 1, A and B; Bannister et al., 2004; Herrán et al., 2011).

### Meiotic kleisins are essential for the formation of AEs in spermatocytes

We staged and examined spermatocyte spreads by immunolocalization of SYCP3. In the absence of RAD21L and REC8, AE assembly and synapsis between homologues were disrupted very early (Fig. 1, C and D). dKO-kls spermatocytes arrested at a leptotene-like stage (100% of cells) were characterized by the punctate aggregates of SYCP3. In contrast, thin threads were observed in late leptotene spermatocytes from wild-type and single mutant mice (Fig. 1, C and D). SYCP2, another axial protein, colocalized with SYCP3 in these aggregates (Fig. 1 D). Antibodies against SYCP1 were used to determine whether transverse components of the SC assembled in dKO-kls mutant spermatocytes. The aggregates of AE proteins did not show reactivity with SYCP1 antibodies, indicating an absence of transverse filament assembly ( $n = 120$ ; Fig. 1 D). In agreement with previous studies, spermatocytes of *Rad21l* and *Rec8* single mutant mice arrested at a zygotene-like stage, displaying several fragmented AEs and some partially synapsed lateral elements (LEs) that never progressed to the expected 19 fully synapsed autosomal bivalent chromosomes observed in wild-type pachynema (unpublished data; Bannister et al., 2004; Herrán et al., 2011).

Tubule section in wild-type mice can be categorized in stages running from I to XII according to the spectrum of germ cell types that are present (Russell et al., 1990). Following these criteria, dKO-kls, *Rad21l*, and *Rec8* mutant mice appeared to be arrested at stage IV of the seminiferous epithelium cycle (Fig. 1 B; Herrán et al., 2011). In wild-type tubules, stage IV typically corresponds to midpachynema. At this stage, mutant spermatocytes that fail to complete recombination and/or chromosome synapsis will usually undergo apoptosis (de Rooij and de Boer, 2003). As examples, MSH5-, DMC1-, or SPO11-deficient spermatocytes

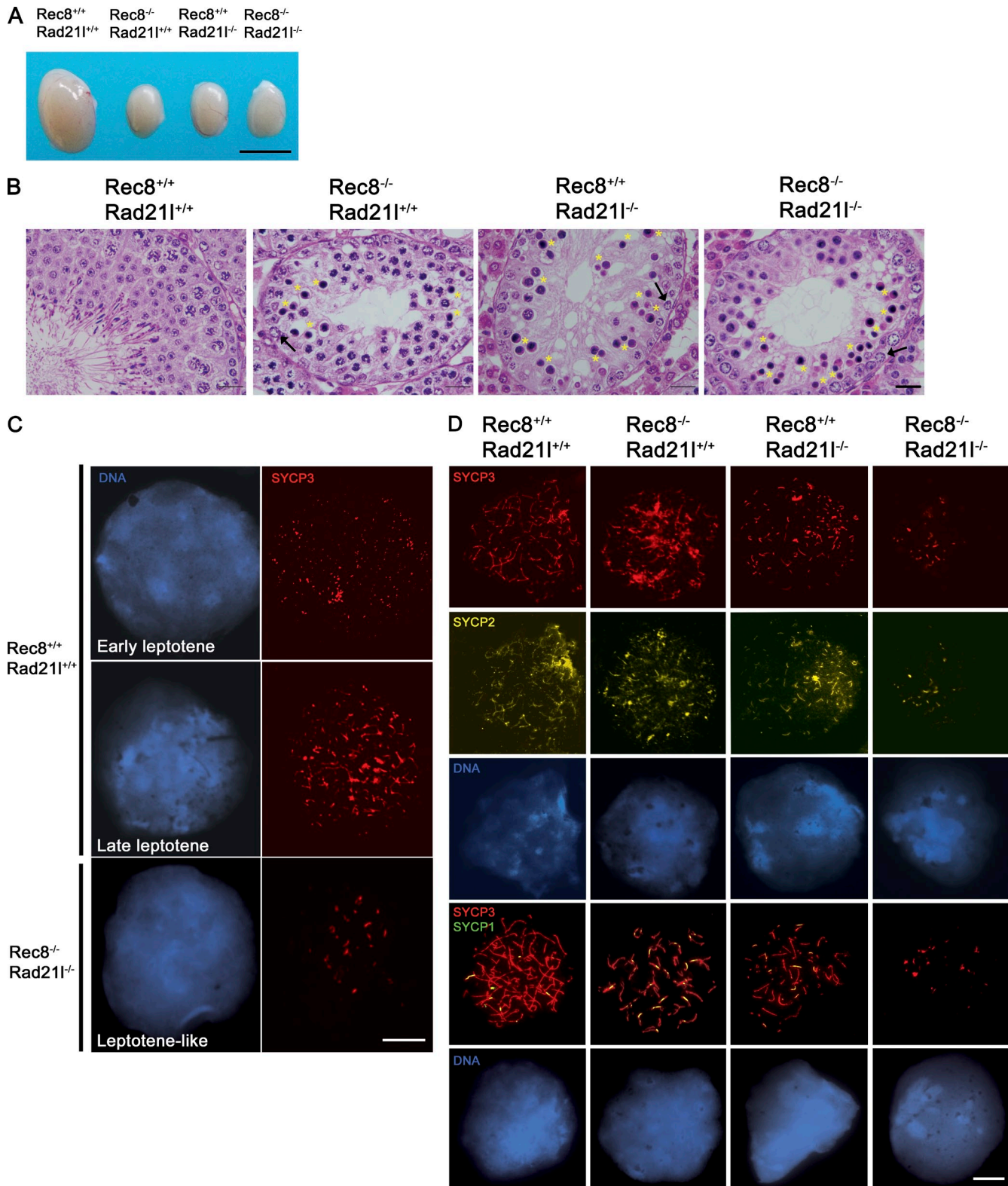


Figure 1. **Absence of RAD21L and REC8 arrest mouse spermatogenesis in early prophase I.** (A) dKO-kl mice show a 70% reduction in testes size compared with wild type. Similar reductions are observed in  $Rad21^{-/-}$  and  $Rec8^{-/-}$  males. (B) Mutation of both  $Rad21$  and  $Rec8$  elicits an arrest of spermatogenesis at stage IV characterized by intermediate spermatogonia (arrows) in a representative section of a seminiferous tubule. Massive apoptosis of spermatocytes (condensed nuclei indicated by asterisks) and absence of mature spermatozoa/spermatids are observed in the dKO-kl tubules. A similar arrest is observed in seminiferous tubules from singly mutant  $Rad21$  and  $Rec8$  mice. (C) Immunolabeling for SYCP3 in spermatocytes from a wild-type mouse at early and late leptotene and spermatocytes arrested at a leptotene-like stage from a dKO-kl mouse. (D) dKO-kl spermatocytes arrested at the leptotene-like stage show absence of chromosomal synapsis. Double immunolabeling for SYCP3 and SYCP2 or SYCP3/SYCP1 shows SYCP3/SYCP2 aggregates without synapsis as indicated by the lack of SYCP1 labeling in double mutant spermatocytes. Spermatocytes from  $Rad21^{-/-}$  (zygotene-like arrest),  $Rec8^{-/-}$  (zygotene-like arrest), and wild-type (zygotene stage) mice show AEs and synapsed LEs with stretches of SYCP1. Bars: (A) 5 mm; (B) 25  $\mu$ m; (C and D) 100  $\mu$ m.

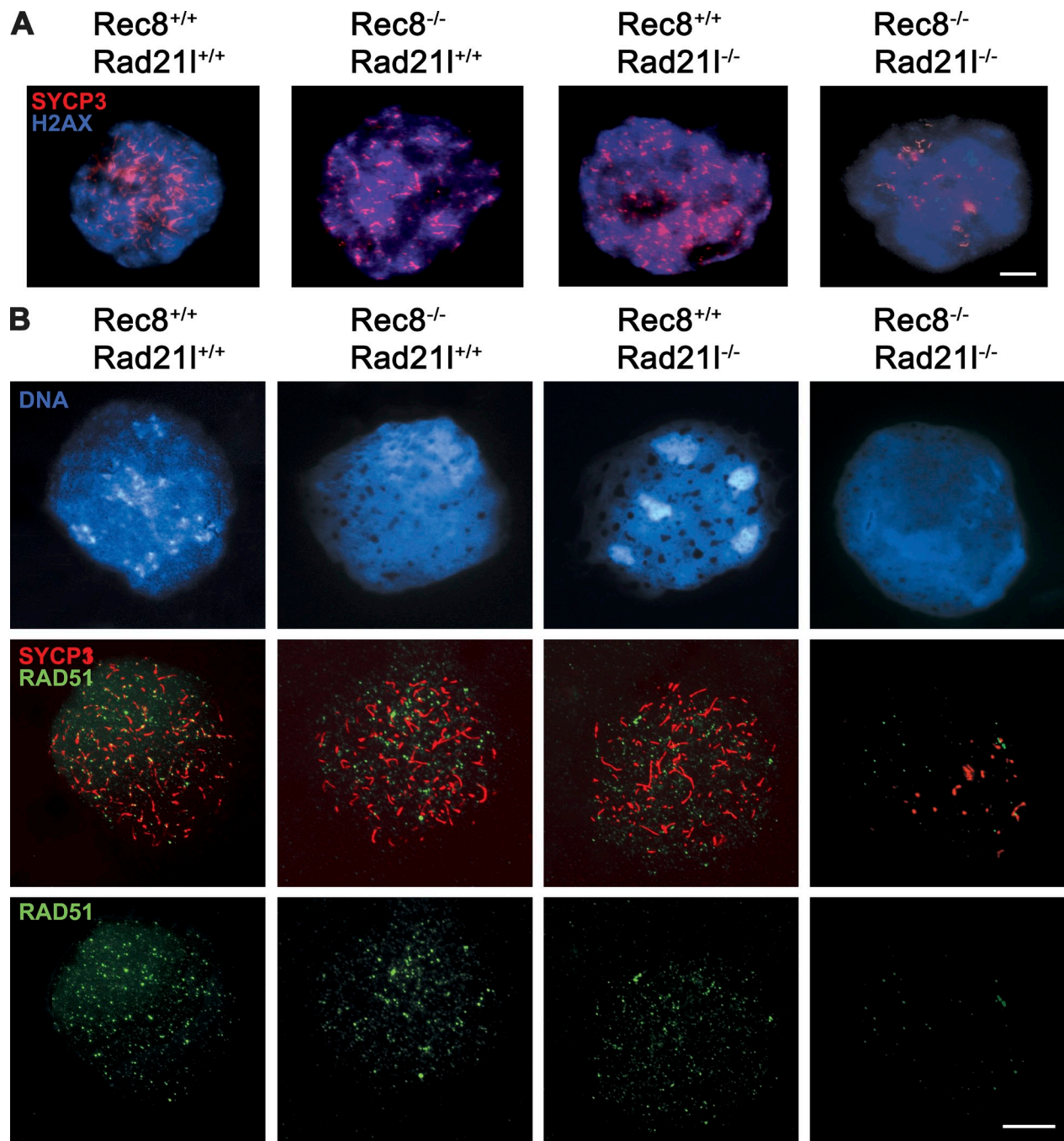


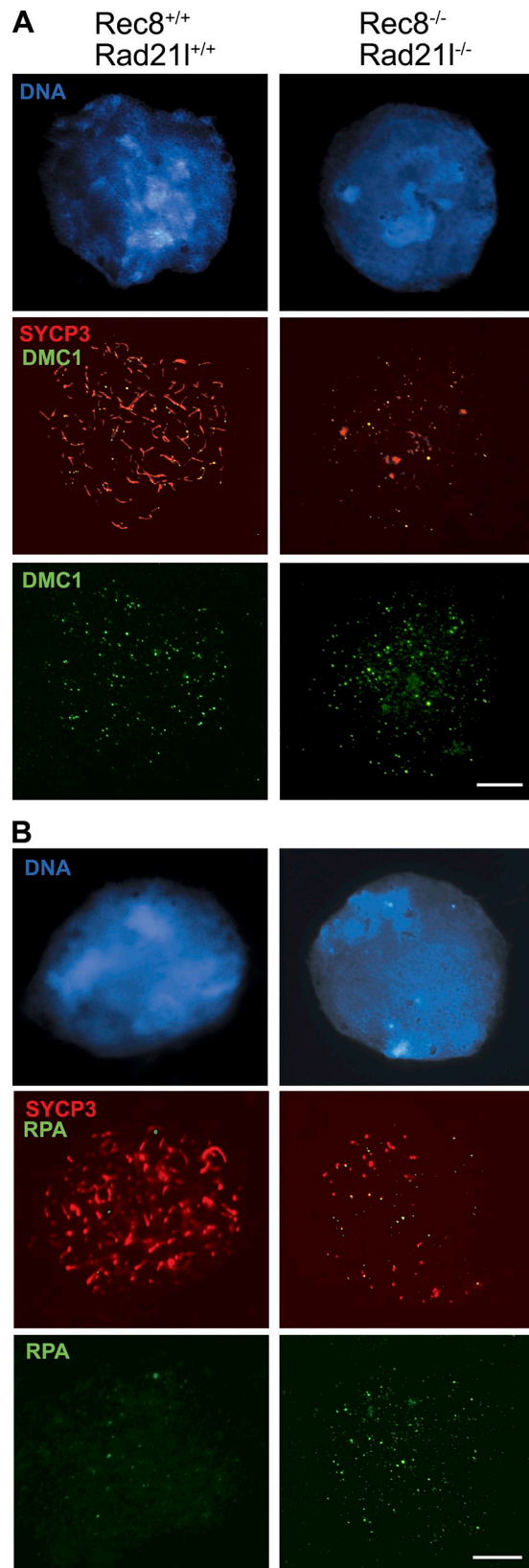
Figure 2. **Meiotic cohesins are dispensable for DSB formation but not for normal loading of RAD51.** (A) Double immunolabeling of SYCP3 and  $\gamma$ -H2AX. In wild-type spermatocytes,  $\gamma$ -H2AX labels the chromatin from leptotema to pachynema when the signal disappears from autosomal chromosomes and remains only on sex body chromatin (not depicted). In the single and dKO-kl mutants,  $\gamma$ -H2AX also labels leptotene chromatin. (B) Double immunolabeling of SYCP3 and RAD51. In wild-type spermatocytes and spermatocytes of single *Rad211* or *Rec8* mutants, RAD51 localizes to AEs/LEs at leptotema. In *Rec8<sup>-/-</sup> Rad211<sup>-/-</sup>* spermatocytes, there is an 8.5-fold reduction in the number of RAD51 foci. Bars, 100  $\mu$ m.

arrest at stage IV, but the terminal stage was described as zygonema, late zygonema, and midpachynema, respectively, based on the progression of synapsis (Yoshida et al., 1998; de Vries et al., 1999; Baudat et al., 2000). Interestingly, dKO-kl spermatocytes arrest at a stage best described as leptotema because they lack AEs yet remain viable up to stage IV. The absence of later stages indicates that these spermatocytes then undergo apoptosis, likely caused by activation of meiotic checkpoints. Thus, disruption of the meiotic cohesin complexes

leads to absence of AEs, which elicits spermatocyte death after an extended leptotene-like stage.

#### DSBs are formed but not repaired in dKO-kl male mice

We next sought to determine how recombination mechanisms are affected by the absence of RAD21L and REC8. Programmed DSBs that initiate meiotic recombination are normally generated by the nuclease SPO11 at the early leptotene stage (Keeney, 2001).



**Figure 3. Normal loading of DMC1 and RPA accumulation at leptotene-arrested dKO-kl spermatocytes.** Double immunolabeling of SYCP3 and DMC1 or RPA. (A) In wild-type and dKO-kl spermatocytes, DMC1 protein localizes to AEs/LEs at leptotene. (B) In dKO-kl spermatocytes, there is a threefold increase in the numbers of RPA foci at the leptotene-like arrest in comparison with wild-type spermatocytes. Bars, 100  $\mu$ m.

We analyzed break formation in mutant spermatocytes using antibodies against the  $\gamma$ -H2AX, a histone variant that is phosphorylated during early prophase I in response to SPO11-induced DSBs in an ATM-dependent manner (Mahadevaiah et al., 2001). As seen in Fig. 2 A, 100% of leptotene-like arrested spermatocytes from dKO-kl mice showed a positive staining that was similar to that observed in the wild-type mice ( $87 \pm 30$  vs.  $92 \pm 56$ ; Table S1). This suggests that the formation of programmed DSBs is not markedly altered in dKO-kl mice. However, the presence of  $\gamma$ -H2AX staining in all the arrested spermatocytes from dKO-kl animals indicates that breaks are not repaired efficiently.

We further analyzed the recombination process to address why DSBs are not repaired in the double mutant spermatocytes. After DSBs are induced, the broken ends are resected, and the strand invasion enzymes RAD51 and DMC1 are recruited to the resulting single-strand DNA overhangs to promote homologue pairing and DNA repair (Symington and Gautier, 2011). In wild-type leptotene spermatocytes, RAD51 and DMC1 assemble on the AEs/LEs of bivalents and disappear toward pachynema, with the exception of the unsynapsed portions of the sex chromosome AEs (Tarsounas et al., 1999). As shown in Fig. 2 B, wild-type spermatocytes showed 70–150 RAD51 foci ( $111 \pm 32$ ). In contrast, the dKO-kl contained only a few RAD51 foci ( $14 \pm 5$ ,  $P < 0.05$ ; Table S1). Next, we monitored the loading of DMC1, a meiosis-specific paralogue of RAD51. In wild-type spermatocytes, both recombinases colocalize extensively such that most recombination-associated foci contain both proteins (Tarsounas et al., 1999). Intriguingly, immunofluorescence with a DMC1-specific antibody revealed no detectable difference in numbers of DMC1 foci between wild type and dKO-kl in leptotene ( $52 \pm 20$  vs.  $54 \pm 19$ ; Table S1 and Fig. 3 A). The presence of an equivalent number of DMC1 foci in double mutant leptotene spermatocytes implies that the  $\gamma$ -H2AX labeling in the dKO-kl spermatocytes is very likely generated by the meiotic SPO11 nuclease.

Next, we determined the distribution of the replication protein A (RPA) in dKO-kl spermatocytes. RPA is a single-stranded DNA-binding protein that enhances the formation of RAD51 and DMC1 filaments in vitro. RPA is first detected in few foci at leptotene. The initial binding of RPA to single-strand DNA at the resected ends of DSBs is supposed to be too transient to be cytologically detected by immunofluorescence because RPA is rapidly displaced by RAD51/DMC1 (Yang et al., 2008). Subsequently, after RAD51/DMC1 loading, abundant RPA foci are detected in the synapsed regions of the LEs at zygonema (Krogh and Symington, 2004; Moens et al., 2007). Intriguingly, despite failure to proceed beyond leptotene, RPA foci were increased threefold in the dKO-kl spermatocytes relative to wild type ( $55 \pm 19$  vs.  $18 \pm 10$ ,  $P < 0.05$ ; Table S1 and Fig. 3 B), which is very likely caused by the sharp reduction in the loading of RAD51 (Fig. 2 B; Roig et al., 2010). TRIP13 is the mammalian orthologue of the yeast Pch2 (pachytene checkpoint 2) gene, and its deletion leads to a block of spermatogenesis and oogenesis because of defects in DSB repair (Li and Schimenti, 2007; Roig et al., 2010). Interestingly, *Tripl3* mutant spermatocytes accumulate RPA foci in leptotene and also show

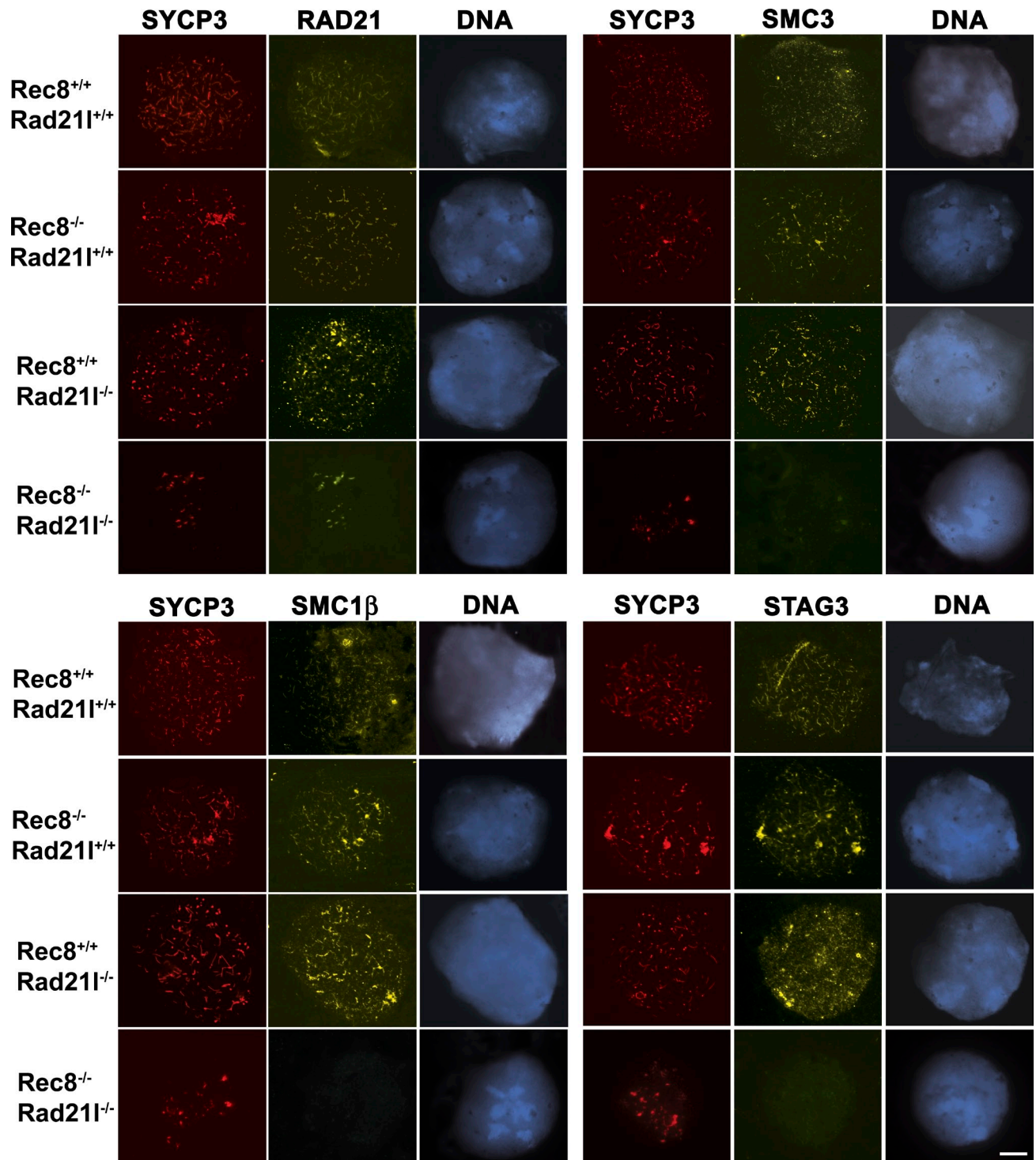


Figure 4. **Cohesin complexes in the absence of RAD21 and REC8.** Double immunofluorescence of SYCP3 and either RAD21, SMC3, SMC1 $\beta$ , or STAG3. In wild-type leptotene spermatocytes, the cohesins RAD21, SMC3, SMC1 $\beta$ , and STAG3 colocalize with SYCP3 along the AEs of the chromosomes. In *Rad21* and *Rec8* single mutant leptotene spermatocytes, RAD21, SMC3, SMC1 $\beta$ , and STAG3 colocalize also with SYCP3 along the AEs/LEs of the chromosomes. In spermatocytes from dKO-kls, arrested at leptotema, SMC1 $\beta$  and STAG3 are not detected by immunofluorescence, whereas immunolabeling for RAD21 and SMC3 renders robust and very faint fluorescence signals, respectively. Bar, 100  $\mu$ m.

a sharply reduced number of RAD51 foci and an accumulation of DMC1 foci at leptotema (Roig et al., 2010). Thus, the RAD51 and DMC1 homologous recombination pathways, although very similar biochemically (Kagawa and Kurumizaka, 2010),

can be genetically dissected. Collectively, our results indicate that meiotic cohesins are dispensable for the loading of DMC1 but are essential for normal loading of RAD51 (Xu et al., 2003; Sharan et al., 2004; Yang et al., 2008; Roig et al., 2010).

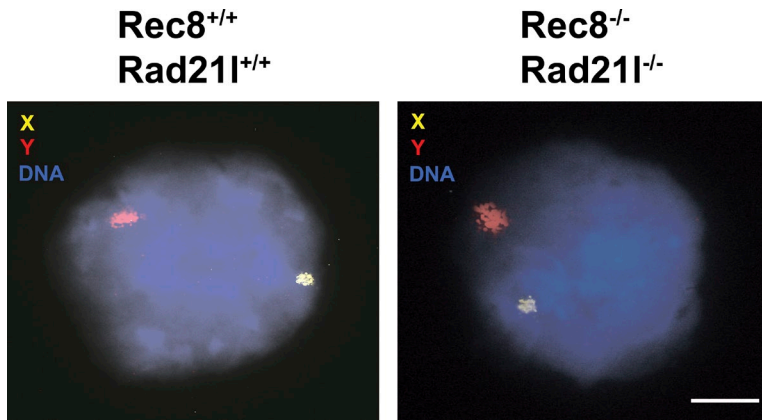


Figure 5. **dKO-kl spermatocytes are proficient for sister chromatid cohesion.** Hybridization of two DNA probes to the X and Y chromosomes renders a single dot signal for each probe in both wild-type and dKO-kl spermatocytes. Clear FISH signals for both probes can be observed in 63% of the 90 leptoneuma-like cells analyzed. Bar, 100  $\mu$ m.

### Cohesin complexes in mutant spermatocytes

In the absence of SYCP3, the cohesin complex persists at a residual AE-like chromosomal core in mouse meiocytes (Pelttari et al., 2001; Kolas et al., 2004; Kouznetsova et al., 2005). We evaluated whether deficiency of both RAD21L and REC8 compromised the loading of other cohesin subunits by immunostaining for the cohesin subunits STAG3, SMC3, SMC1 $\beta$ , and RAD21 (Fig. 4). Surprisingly, we observed robust staining only for RAD21, which colocalized with SYCP3 labeling, and much fainter staining of SMC3 (Fig. 4). We did not detect either STAG3 or SMC1 $\beta$  (Fig. 4), suggesting that these cohesin subunits are complexed in vivo with REC8 and RAD21L kleisins. Collectively, these data suggest that the two  $\alpha$ -kleisins, RAD21L and REC8, are not epistatic and sustain most, if not all, meiosis-specific cohesin complexes involved in AE formation and SC assembly. Our results are consistent with the observation that RAD21 is present on chromosome axes from leptoneuma to diploneuma in mouse spermatocytes (this paper; Herrán et al., 2011) but contrast with one study in which RAD21 was first detected in the pachytene stage (Ishiguro et al., 2011). Because we observed RAD21 immunolabeling in the *Rad21*<sup>-/-</sup>, *Rec8*<sup>-/-</sup>, and dKo-kl spermatocytes (Fig. 4), cross-reactivity of our antibody with RAD21L and/or REC8 is not likely the reason for these contrasting results, as previously claimed (Ishiguro et al., 2011). This early localization of RAD21 would suggest a direct role of this kleisin in establishing cohesion in premeiotic S phase.

### Sister chromatid cohesion in the absence of meiotic kleisins

In addition to their role in AE formation and DNA looping (Revenkova and Jessberger, 2006; Novak et al., 2008), cohesin complexes must tether the two sister chromatids from S phase until the onset of anaphase, when separase is activated, cleaves the  $\alpha$ -kleisin RAD21 subunit, and leads to opening of the cohesin ring (Hauf et al., 2001). Thus, a deficiency for two out of the three mammalian kleisins could partially affect chromosomal cohesion and/or replication of the premeiocyte/meiocyte. To test this possibility, we performed FACS analysis of whole cells from seminiferous tubules, which showed the existence of tetraploid compartment in dKO-kl testes (Fig. S1). Next, we performed FISH analysis (one probe from the X and one probe

from the Y chromosomes) in dKO-kl spermatocytes (Fig. 5). The results revealed a single signal for each chromosome probe (corresponding to two closed and tethered sister chromatids). Collectively, these results suggest that sister chromatid cohesion is properly established in the preceding S phase through the somatic  $\alpha$ -kleisin RAD21 subunit complexed with the SMC3 and SMC1 $\alpha$  or SMC1 $\beta$  subunits. This result implies that meiosis-specific cohesin complexes are dispensable for cohesion, by itself, and are specifically required for SC structure.

To our knowledge, deletion of both REC8 and RAD21L leads to the earliest arrest of mouse meiotic prophase reported to date, including the mutations for the individual cohesins REC8, SMC1 $\beta$ , and RAD21L and the structural additional components SYCP3, SYCP1, and SYCP2 (Yuan et al., 2000; Bannister et al., 2004; Revenkova et al., 2004; de Vries et al., 2005; Yang et al., 2006; Herrán et al., 2011). Interestingly, it was reported very recently that SYCP1 and SYCP3 double deficiency does not abolish the loading of the meiotic cohesin complexes nor the presynaptic pairing and early homologous recombination in mouse meiocytes (Kouznetsova et al., 2011). Thus, the meiotic cohesin complexes are required to initiate AE/LE assembly in mammals (and not vice versa), which is a prerequisite for SC formation. Our findings provide evidence for a crucial role for meiotic cohesins (REC8- and RAD21L-containing cohesin complexes) in the very early assembly of the AEs of the mammalian SC, revealing that this process is conserved from yeast to mammals.

## Materials and methods

### Immunocytology and antibodies

Testes were detunicated and processed for spreading using the “dry-down” technique (Peters et al., 1997). The slides were rinsed three times for 5 min in PBS and incubated overnight at room temperature with primary antibodies diluted in PBS. The primary antibodies used for immunofluorescence were rabbit  $\alpha$ SMC3 serum K987 (1:20), rabbit  $\alpha$ SMC1 $\beta$  serum K974 (1:20; Prieto et al., 2004), rabbit  $\alpha$ STAG3 serum K403 (1:20; Prieto et al., 2001), rabbit  $\alpha$ RAD21 IgG K854 (1:5; Prieto et al., 2002), rabbit  $\alpha$ SYCP2 serum K1035 (1:20), mouse  $\alpha$ SYCP3 IgG sc-74569 (1:100), rabbit  $\alpha$ RAD51 sc-8349 (1:30), and PC130 (1:5; EMD; provided by J. Page, Universidad Autonoma de Madrid, Madrid, Spain), rabbit  $\alpha$ DMC1 sc-22768 (1:20; Santa Cruz Biotechnology, Inc.), rabbit  $\alpha$ SYCP1 IgG ab15090 (1:200; Abcam), rabbit anti- $\gamma$ -H2AX (ser139) IgG (#07-164; 1:200; Millipore), and rabbit  $\alpha$ RPA IgG (1:300; provided by E. Marcon, Toronto University, Toronto, Canada). The secondary antibodies used were TRITC  $\alpha$ -mouse 115-095-146/ $\alpha$ -rabbit 111-025-144 and FITC  $\alpha$ -mouse 115-095-146/ $\alpha$ -rabbit 111-095-045 (Jackson ImmunoResearch Laboratories,

Inc.; all 1:100). Slides were visualized at room temperature using a microscope (Axioplan 2; Carl Zeiss) with 63× objectives with an aperture of 1.4 (Carl Zeiss). Images were taken with a digital camera (ORCA-ER; Hamamatsu Photonics) and processed with OPENLAB 4.0.3 (PerkinElmer) and Photoshop (Adobe).

### Mice

The mutation at the *Rad21*<sup>-/-</sup> locus is a null allele generated by gene targeting using an insertional targeting vector (Herrán et al., 2011). Mice were genotyped by genomic Southern blot analysis of tail genomic DNA digested with SpeI and hybridized with a 5' external probe. The probe (1 kb) was generated by PCR using the primers 5'-ACTAGTCTAAATAAAGGTCTT-3' and 5'-GATTTAAGCATGAATGAAGTAAC-3'. The mutation at the *Rec8* locus is a null allele (premature stop codon at exon 6) isolated in a forward genetic screen for mouse infertility (Bannister et al., 2004). Mice were genotyped by direct sequencing of the PCR-amplified exon 6 from genomic DNA using the primers 5'-CCTTACATCCTGTCTC-3' and 5'-ACAGGAACACCAACTAATC-3'. dKO-cls mice were obtained by genetic crossing of double heterozygote mice (*Rec8*<sup>+/-</sup> *Rad21*<sup>+/-</sup>) and compared with single mutant and wild-type littermates. All animal experiments were reviewed and approved by the Consejo Superior de Investigaciones Científicas (Spanish Research Council) and the Universidad de Salamanca National Committee on Bioethics.

### FISH analysis

The mouse Y-specific probes were obtained by PCR using the following three sets of primers: 1S, 5'-TAGGATGGTAAGCCCAATGC-3'; 1AS, 5'-TTGGTTGTTAATTGTTGGG-3'; 2S, 5'-CATGCCCTGGACTGAC-3'; 2AS, 5'-CTTTTTTTTCCCTCTGG-3'; 3S, 5'-TCCTCTGCAGAGAAGGGAC-3'; and 3AS, 5'-CCTCCGCTCAATCCTATCA-3' (Navin et al., 1996). The X-specific DNA probe is a pericentromeric DNA fragment cloned in a plasmid (Disteche et al., 1985). Both probes were labeled by Nick translation with Dig-11-deoxy-UTP and Bio-16-deoxy-UTP and hybridized to spermatocyte spreads following standard procedures. In brief, slides were pre-treated with pepsin (0.005% in 0.01 N HCl for 5 min at 37°C), dehydrated, and RNase treated (0.1 mg/ml in PBS for 1 h at 37°C). After denaturation in 70% formamide during 3 min at 75°C, slides were dehydrated in ice-cold ethanol and hybridized overnight at 37°C to a denatured DNA probe. After washing (50% formamide and 2× SSC at 42°C), biotin- and digoxigenin-labeled probes were immunodetected using streptavidin-Cy3/goat biotinylated anti-streptavidin/streptavidin-Cy3 (Jackson ImmunoResearch Laboratories, Inc.) and mouse anti-digoxigenin-FITC/goat anti-mouse/rabbit anti-mouse-FITC (Roche), respectively (Pendás et al., 1994).

### FACS analysis

Wild-type, *Rad21*<sup>-/-</sup>, and dKO-cls testicular cell preparation and their DNA content measurement were performed as previously described (Malkov et al., 1998). In brief, testes were dissected in separation medium (4 mM L-glutamine, 1.5 mM sodium pyruvate, 10% fetal calf serum, and 75 mg/ml ampicillin in DMEM containing nonessential amino acids). After decapsulation, the tubules were then treated with collagenase for 5 min at 37°C. The sedimented seminiferous cords were washed in separation medium and treated with 2.5 μg/ml trypsin and 1 U/ml DNase I for 2 min at 37°C. Using a pair of scalpels, the tubules were disintegrated, and the resulting tissue suspension was passed through a 50-μm nylon mesh, washed twice by centrifugation, and counted. Cells were brought to a concentration of 2 × 10<sup>6</sup> cells/ml in separation medium, diluted 1:1 with propidium iodide solution (10 mM Tris, pH 8, 1 mM NaCl, 0.1% Nonidet P-40, 0.7 mg/ml RNase A, and 0.05 mg/ml propidium iodide), and freshly analyzed by a cell-sorting instrument (FACSsort; BD).

### Online supplemental material

Fig. S1 shows that the absence of RAD21L and REC8 does not impair DNA replication and does not provoke loss of chromatid cohesion in dKO-cls spermatocytes. Table S1 shows the number of early recombination-associated foci in spermatocytes and the weight of testis from wild-type and dKO-cls mice. Online supplemental material is available at <http://www.jcb.org/cgi/content/full/jcb.201201100/DC1>.

We wish to express our sincere thanks to E. Marcon and J. Page for providing antibodies (RPA and RAD51, respectively), I. Ramos-Fernández and Teresa Hernandez for excellent technical assistance, and C. López-Otín for his support. We specially appreciate the comments from an anonymous reviewer.

This work was supported by grant SAF2011-25252 and Junta de Castilla y León. C. Gutiérrez-Caballero and Y. Herrán are supported by Fondo de

Investigación Sanitaria and Formación de Personal Investigador fellowships, respectively. E. Llano is the recipient of a Ramón y Cajal Research contract.

Submitted: 18 January 2012

Accepted: 21 May 2012

## References

- Bannister, L.A., L.G. Reinholdt, R.J. Munroe, and J.C. Schimenti. 2004. Positional cloning and characterization of mouse *mei8*, a disrupted allele of the meiotic cohesin *Rec8*. *Genesis*. 40:184–194. <http://dx.doi.org/10.1002/gene.20085>
- Baudat, F., K. Manova, J.P. Yuen, M. Jasin, and S. Keeney. 2000. Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking Spo11. *Mol. Cell*. 6:989–998. [http://dx.doi.org/10.1016/S1097-2765\(00\)00098-8](http://dx.doi.org/10.1016/S1097-2765(00)00098-8)
- Bhatt, A.M., C. Lister, T. Page, P. Fransz, K. Findlay, G.H. Jones, H.G. Dickinson, and C. Dean. 1999. The DIF1 gene of *Arabidopsis* is required for meiotic chromosome segregation and belongs to the REC8/RAD21 cohesin gene family. *Plant J*. 19:463–472. <http://dx.doi.org/10.1046/j.1365-313X.1999.00548.x>
- de Rooij, D.G., and P. de Boer. 2003. Specific arrests of spermatogenesis in genetically modified and mutant mice. *Cytogenet. Genome Res*. 103:267–276. <http://dx.doi.org/10.1159/000076812>
- de Vries, F.A., E. de Boer, M. van den Bosch, W.M. Baarends, M. Ooms, L. Yuan, J.G. Liu, A.A. van Zeeland, C. Heyting, and A. Pastink. 2005. Mouse Sycp1 functions in synaptonemal complex assembly, meiotic recombination, and XY body formation. *Genes Dev*. 19:1376–1389. <http://dx.doi.org/10.1101/gad.329705>
- de Vries, S.S., E.B. Baart, M. Dekker, A. Siezen, D.G. de Rooij, P. de Boer, and H. te Riele. 1999. Mouse MutS-like protein Msh5 is required for proper chromosome synapsis in male and female meiosis. *Genes Dev*. 13:523–531. <http://dx.doi.org/10.1101/gad.13.5.523>
- Disteche, C.M., U. Tantravahi, S. Gandy, M. Eisenhard, D. Adler, and L.M. Kunkel. 1985. Isolation and characterization of two repetitive DNA fragments located near the centromere of the mouse X chromosome. *Cytogenet. Cell Genet*. 39:262–268. <http://dx.doi.org/10.1159/000132155>
- Eijje, M., H. Offenber, R. Jessberger, E. Revenkova, and C. Heyting. 2003. Meiotic cohesin REC8 marks the axial elements of rat synaptonemal complexes before cohesins SMC1β and SMC3. *J. Cell Biol*. 160:657–670. <http://dx.doi.org/10.1083/jcb.200212080>
- Golubovskaya, I.N., O. Hamant, L. Timofejeva, C.J. Wang, D. Braun, R. Meeley, and W.Z. Cande. 2006. Alleles of *afd1* dissect REC8 functions during meiotic prophase I. *J. Cell Sci*. 119:3306–3315. <http://dx.doi.org/10.1242/jcs.03054>
- Gruber, S., C.H. Haering, and K. Nasmyth. 2003. Chromosomal cohesin forms a ring. *Cell*. 112:765–777. [http://dx.doi.org/10.1016/S0092-8674\(03\)00162-4](http://dx.doi.org/10.1016/S0092-8674(03)00162-4)
- Gutiérrez-Caballero, C., Y. Herrán, M. Sánchez-Martín, J.A. Suja, J.L. Barbero, E. Llano, and A.M. Pendás. 2011. Identification and molecular characterization of the mammalian α-kleisin RAD21L. *Cell Cycle*. 10:1477–1487. <http://dx.doi.org/10.4161/cc.10.9.15515>
- Gutiérrez-Caballero, C., L.R. Cebollero, and A.M. Pendás. 2012. Shugoshins: from protectors of cohesion to versatile adaptors at the centromere. *Trends Genet*. <http://dx.doi.org/10.1016/j.tig.2012.03.003>
- Haering, C.H., A.M. Farcas, P. Arumugam, J. Metson, and K. Nasmyth. 2008. The cohesin ring concatenates sister DNA molecules. *Nature*. 454:297–301. <http://dx.doi.org/10.1038/nature07098>
- Hauf, S., I.C. Waizenegger, and J.M. Peters. 2001. Cohesin cleavage by separase required for anaphase and cytokinesis in human cells. *Science*. 293:1320–1323. <http://dx.doi.org/10.1126/science.1061376>
- Herrán, Y., C. Gutiérrez-Caballero, M. Sánchez-Martín, T. Hernández, A. Viera, J.L. Barbero, E. de Álava, D.G. de Rooij, J.A. Suja, E. Llano, and A.M. Pendás. 2011. The cohesin subunit RAD21L functions in meiotic synapsis and exhibits sexual dimorphism in fertility. *EMBO J*. 30:3091–3105. <http://dx.doi.org/10.1038/embj.2011.222>
- Hirano, T. 2006. At the heart of the chromosome: SMC proteins in action. *Nat. Rev. Mol. Cell Biol*. 7:311–322. <http://dx.doi.org/10.1038/nrm1909>
- Ishiguro, K., J. Kim, S. Fujiyama-Nakamura, S. Kato, and Y. Watanabe. 2011. A new meiosis-specific cohesin complex implicated in the cohesin code for homologous pairing. *EMBO Rep*. 12:267–275. <http://dx.doi.org/10.1038/embor.2011.2>
- Kagawa, W., and H. Kurumizaka. 2010. From meiosis to postmeiotic events: uncovering the molecular roles of the meiosis-specific recombinase Dmc1. *FEBS J*. 277:590–598. <http://dx.doi.org/10.1111/j.1742-4658.2009.07503.x>



- Keeney, S. 2001. Mechanism and control of meiotic recombination initiation. *Curr. Top. Dev. Biol.* 52:1–53. [http://dx.doi.org/10.1016/S0070-2153\(01\)52008-6](http://dx.doi.org/10.1016/S0070-2153(01)52008-6)
- Klein, F., P. Mahr, M. Galova, S.B. Buonomo, C. Michaelis, K. Nairz, and K. Nasmyth. 1999. A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. *Cell.* 98:91–103. [http://dx.doi.org/10.1016/S0092-8674\(00\)80609-1](http://dx.doi.org/10.1016/S0092-8674(00)80609-1)
- Kolas, N.K., L. Yuan, C. Hoog, H.H. Heng, E. Marcon, and P.B. Moens. 2004. Male mouse meiotic chromosome cores deficient in structural proteins SYCP3 and SYCP2 align by homology but fail to synapse and have possible impaired specificity of chromatin loop attachment. *Cytogenet. Genome Res.* 105:182–188. <http://dx.doi.org/10.1159/000078188>
- Kouznetsova, A., I. Novak, R. Jessberger, and C. Höög. 2005. SYCP2 and SYCP3 are required for cohesin core integrity at diplotene but not for centromere cohesion at the first meiotic division. *J. Cell Sci.* 118:2271–2278. <http://dx.doi.org/10.1242/jcs.02362>
- Kouznetsova, A., R. Benavente, A. Pastink, and C. Höög. 2011. Meiosis in mice without a synaptonemal complex. *PLoS ONE.* 6:e28255. <http://dx.doi.org/10.1371/journal.pone.0028255>
- Krogh, B.O., and L.S. Symington. 2004. Recombination proteins in yeast. *Annu. Rev. Genet.* 38:233–271. <http://dx.doi.org/10.1146/annurev.genet.38.072902.091500>
- Lee, J., and T. Hirano. 2011. RAD21L, a novel cohesin subunit implicated in linking homologous chromosomes in mammalian meiosis. *J. Cell Biol.* 192:263–276. <http://dx.doi.org/10.1083/jcb.201008005>
- Li, X.C., and J.C. Schimenti. 2007. Mouse pachytene checkpoint 2 (trip13) is required for completing meiotic recombination but not synapsis. *PLoS Genet.* 3:e130. <http://dx.doi.org/10.1371/journal.pgen.0030130>
- Llano, E., R. Gómez, C. Gutiérrez-Caballero, Y. Herrán, M. Sánchez-Martín, L. Vázquez-Quinones, T. Hernández, E. de Alava, A. Cuadrado, J.L. Barbero, et al. 2008. Shugoshin-2 is essential for the completion of meiosis but not for mitotic cell division in mice. *Genes Dev.* 22:2400–2413. <http://dx.doi.org/10.1101/gad.475308>
- Mahadevaiah, S.K., J.M. Turner, F. Baudat, E.P. Rogakou, P. de Boer, J. Blanco-Rodríguez, M. Jasin, S. Keeney, W.M. Bonner, and P.S. Burgoyne. 2001. Recombinational DNA double-strand breaks in mice precede synapsis. *Nat. Genet.* 27:271–276. <http://dx.doi.org/10.1038/85830>
- Malkov, M., Y. Fisher, and J. Don. 1998. Developmental schedule of the postnatal rat testis determined by flow cytometry. *Biol. Reprod.* 59:84–92. <http://dx.doi.org/10.1095/biolreprod59.1.84>
- Moens, P.B., E. Marcon, J.S. Shore, N. Kochakpour, and B. Spyropoulos. 2007. Initiation and resolution of interhomolog connections: crossover and non-crossover sites along mouse synaptonemal complexes. *J. Cell Sci.* 120:1017–1027. <http://dx.doi.org/10.1242/jcs.03394>
- Navin, A., R. Prekeris, N.A. Lisitsyn, M.M. Sonti, D.A. Grieco, S. Narayanswami, E.S. Lander, and E.M. Simpson. 1996. Mouse Y-specific repeats isolated by whole chromosome representational difference analysis. *Genomics.* 36:349–353. <http://dx.doi.org/10.1006/geno.1996.0473>
- Novak, I., H. Wang, E. Revenkova, R. Jessberger, H. Scherthan, and C. Höög. 2008. Cohesin Smc1 $\beta$  determines meiotic chromatin axis loop organization. *J. Cell Biol.* 180:83–90. <http://dx.doi.org/10.1083/jcb.200706136>
- Page, S.L., and R.S. Hawley. 2003. Chromosome choreography: the meiotic ballet. *Science.* 301:785–789. <http://dx.doi.org/10.1126/science.1086605>
- Parisi, S., M.J. McKay, M. Molnar, M.A. Thompson, P.J. van der Spek, E. van Drunen-Schoenmaker, R. Kanaar, E. Lehmann, J.H.J. Hoeijmakers, and J. Kohl. 1999. Rec8p, a meiotic recombination and sister chromatid cohesion phosphoprotein of the Rad21p family conserved from fission yeast to humans. *Mol. Cell. Biol.* 19:3515–3528.
- Pelttari, J., M.R. Hoja, L. Yuan, J.G. Liu, E. Brundell, P. Moens, S. Santucci-Darmanin, R. Jessberger, J.L. Barbero, C. Heyting, and C. Höög. 2001. A meiotic chromosomal core consisting of cohesin complex proteins recruits DNA recombination proteins and promotes synapsis in the absence of an axial element in mammalian meiotic cells. *Mol. Cell. Biol.* 21:5667–5677. <http://dx.doi.org/10.1128/MCB.21.16.5667-5677.2001>
- Pendás, A.M., P. Moran, J.P. Freije, and E. Garcia-Vazquez. 1994. Chromosomal mapping and nucleotide sequence of two tandem repeats of Atlantic salmon 5S rDNA. *Cytogenet. Cell Genet.* 67:31–36. <http://dx.doi.org/10.1159/000133792>
- Peters, A.H., A.W. Plug, M.J. van Vugt, and P. de Boer. 1997. A drying-down technique for the spreading of mammalian meiocytes from the male and female germline. *Chromosome Res.* 5:66–68. <http://dx.doi.org/10.1023/A:1018445520117>
- Prieto, I., J.A. Suja, N. Pezzi, L. Kremer, C. Martínez-A, J.S. Rufas, and J.L. Barbero. 2001. Mammalian STAG3 is a cohesin specific to sister chromatid arms in meiosis I. *Nat. Cell Biol.* 3:761–766. <http://dx.doi.org/10.1038/35087082>
- Prieto, I., N. Pezzi, J.M. Buesa, L. Kremer, I. Barthelemy, C. Carreiro, F. Roncal, A. Martinez, L. Gomez, R. Fernandez, et al. 2002. STAG2 and Rad21 mammalian mitotic cohesins are implicated in meiosis. *EMBO Rep.* 3:543–550. <http://dx.doi.org/10.1093/embo-reports/kv1f108>
- Prieto, I., C. Tease, N. Pezzi, J.M. Buesa, S. Ortega, L. Kremer, A. Martínez, C. Martínez-A, M.A. Hultén, and J.L. Barbero. 2004. Cohesin component dynamics during meiotic prophase I in mammalian oocytes. *Chromosome Res.* 12:197–213. <http://dx.doi.org/10.1023/B:CHRO.0000021945.83198.0e>
- Revenkova, E., and R. Jessberger. 2006. Shaping meiotic prophase chromosomes: cohesins and synaptonemal complex proteins. *Chromosoma.* 115:235–240. <http://dx.doi.org/10.1007/s00412-006-0060-x>
- Revenkova, E., M. Eijpe, C. Heyting, C.A. Hodges, P.A. Hunt, B. Liebe, H. Scherthan, and R. Jessberger. 2004. Cohesin SMC1 beta is required for meiotic chromosome dynamics, sister chromatid cohesion and DNA recombination. *Nat. Cell Biol.* 6:555–562. <http://dx.doi.org/10.1038/ncb1135>
- Roig, I., J.A. Dowdle, A. Toth, D.G. de Rooij, M. Jasin, and S. Keeney. 2010. Mouse TRIP13/PCH2 is required for recombination and normal higher-order chromosome structure during meiosis. *PLoS Genet.* 6:e1001062. <http://dx.doi.org/10.1371/journal.pgen.1001062>
- Russell, L.D., H.P. Ren, I. Sinha Hikim, W. Schulze, and A.P. Sinha Hikim. 1990. A comparative study in twelve mammalian species of volume densities, volumes, and numerical densities of selected testis components, emphasizing those related to the Sertoli cell. *Am. J. Anat.* 188:21–30. <http://dx.doi.org/10.1002/aja.1001880104>
- Severson, A.F., L. Ling, V. van Zuylen, and B.J. Meyer. 2009. The axial element protein HTP-3 promotes cohesin loading and meiotic axis assembly in *C. elegans* to implement the meiotic program of chromosome segregation. *Genes Dev.* 23:1763–1778. <http://dx.doi.org/10.1101/gad.1808809>
- Sharan, S.K., A. Pyle, V. Coppola, J. Babus, S. Swaminathan, J. Benedict, D. Swing, B.K. Martin, L. Tessarollo, J.P. Evans, et al. 2004. BRCA2 deficiency in mice leads to meiotic impairment and infertility. *Development.* 131:131–142. <http://dx.doi.org/10.1242/dev.00888>
- Suja, J.A., and J.L. Barbero. 2009. Cohesin complexes and sister chromatid cohesion in mammalian meiosis. *Genome Dyn.* 5:94–116. <http://dx.doi.org/10.1159/000166622>
- Symington, L.S., and J. Gautier. 2011. Double-strand break end resection and repair pathway choice. *Annu. Rev. Genet.* 45:247–271. <http://dx.doi.org/10.1146/annurev-genet-110410-132435>
- Tarounas, M., T. Morita, R.E. Pearlman, and P.B. Moens. 1999. RAD51 and DMC1 form mixed complexes associated with mouse meiotic chromosome cores and synaptonemal complexes. *J. Cell Biol.* 147:207–220. <http://dx.doi.org/10.1083/jcb.147.2.207>
- Uhlmann, F., D. Wernic, M.A. Poupard, E.V. Koonin, and K. Nasmyth. 2000. Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. *Cell.* 103:375–386. [http://dx.doi.org/10.1016/S0092-8674\(00\)00130-6](http://dx.doi.org/10.1016/S0092-8674(00)00130-6)
- Unal, E., J.M. Heidinguer-Pauli, and D. Koshland. 2007. DNA double-strand breaks trigger genome-wide sister-chromatid cohesion through Eco1 (Ctf7). *Science.* 317:245–248. <http://dx.doi.org/10.1126/science.1140637>
- Watanabe, Y. 2005. Sister chromatid cohesion along arms and at centromeres. *Trends Genet.* 21:405–412. <http://dx.doi.org/10.1016/j.tig.2005.05.009>
- Watanabe, Y., and P. Nurse. 1999. Cohesin Rec8 is required for reductional chromosome segregation at meiosis. *Nature.* 400:461–464. <http://dx.doi.org/10.1038/22774>
- Xu, X., O. Aprelikova, P. Moens, C.X. Deng, and P.A. Furth. 2003. Impaired meiotic DNA-damage repair and lack of crossing-over during spermatogenesis in BRCA1 full-length isoform deficient mice. *Development.* 130:2001–2012. <http://dx.doi.org/10.1242/dev.00410>
- Yang, F., and P.J. Wang. 2009. The mammalian synaptonemal complex: a scaffold and beyond. *Genome Dyn.* 5:69–80. <http://dx.doi.org/10.1159/000166620>
- Yang, F., R. De La Fuente, N.A. Leu, C. Baumann, K.J. McLaughlin, and P.J. Wang. 2006. Mouse SYCP2 is required for synaptonemal complex assembly and chromosomal synapsis during male meiosis. *J. Cell Biol.* 173:497–507. <http://dx.doi.org/10.1083/jcb.200603063>
- Yang, F., S. Eckardt, N.A. Leu, K.J. McLaughlin, and P.J. Wang. 2008. Mouse TEX15 is essential for DNA double-strand break repair and chromosomal synapsis during male meiosis. *J. Cell Biol.* 180:673–679. <http://dx.doi.org/10.1083/jcb.200709057>
- Yoshida, K., G. Kondoh, Y. Matsuda, T. Habu, Y. Nishimune, and T. Morita. 1998. The mouse RecA-like gene Dmc1 is required for homologous chromosome synapsis during meiosis. *Mol. Cell.* 1:707–718. [http://dx.doi.org/10.1016/S1097-2765\(00\)80070-2](http://dx.doi.org/10.1016/S1097-2765(00)80070-2)
- Yuan, L., J.G. Liu, J. Zhao, E. Brundell, B. Daneholt, and C. Höög. 2000. The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. *Mol. Cell.* 5:73–83. [http://dx.doi.org/10.1016/S1097-2765\(00\)80404-9](http://dx.doi.org/10.1016/S1097-2765(00)80404-9)
- Zhang, N., S.G. Kuznetsov, S.K. Sharan, K. Li, P.H. Rao, and D. Pati. 2008. A handcuff model for the cohesin complex. *J. Cell Biol.* 183:1019–1031. <http://dx.doi.org/10.1083/jcb.200801157>