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Sex chromosome recombination failure, apoptosis, and fertility in male mice

Imrul Faisal^{1,2} · Liisa Kauppi^{1,2}

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Abstract Lack of crossing-over in meiosis can trigger an apoptotic response at metaphase I by the spindle assembly checkpoint (SAC). In contrast to females, segregation of sex chromosomes in males poses a particular challenge as recombination and chiasma formation is restricted to the pseudoautosomal region, the small region of homology between X and Y chromosomes. Existing data indicate that low levels of crossover failure in male meiosis can be tolerated without compromising fertility, while high levels of X-Y dissociation (in ≥ 70 % of cells) result in widespread apoptosis and subsequent infertility, demonstrated earlier, e.g., in *Spo11* β -only mice. Here, we explore the threshold of X-Y recombination failure frequency that is compatible with fertility. We show that in *Spo11* β -only^{mb} mice with a mixed genetic background, in contrast to *Spo11* β -only mice with a C57BL/6 background, X-Y pairing fails in ~50 % of cells but this still allows for sperm production without any overt impact on fertility. We also review data on apoptosis and

fertility from other achiasmate mouse models and propose that the incidence of homolog dissociation that can be tolerated in vivo without compromising male fertility lies between 50 and 70 %.

Keywords Meiosis · Spindle assembly checkpoint · Sex chromosome · Recombination · Apoptosis · Fertility

Introduction

During meiosis, cells undergo a carefully orchestrated sequence of pre-meiotic DNA replication and two rounds of cell division to generate haploid gametes. Of these, the first meiotic division (meiosis I) is unique in its property to separate one chromosome per each pair of homologs into two individual daughter cells. In mid-prophase I, homologs are physically connected to each other by crossovers that by metaphase I mature into cytologically visible structures known as chiasmata. In meiosis I, at least one crossover event per homolog pair is presumably needed to satisfy the spindle assembly checkpoint (SAC). Persistent SAC activation by achiasmate chromosome(s) has been inferred in multiple studies (Barchi et al. 2008; Burgoyne et al. 1992; Eaker et al. 2001; Kauppi et al. 2011). In the most severe example, mice lacking the recombination protein MLH1, virtually all homolog pairs were achiasmate and misaligned at metaphase (Baker et al. 1996; Eaker et al. 2002). Other mouse models present less frequent chromosome misalignment, often involving the X-Y chromosome pair (Burgoyne et al. 1992; Kauppi et al. 2011; Mahadevaiah et al. 1988; Matsuda et al. 1991, 1992).

Unlike autosomes, male sex chromosomes are a particularly sensitive indicator of recombination defects, due to the fact that X-Y pairing and recombination is restricted to a short region of homology, the pseudoautosomal region (PAR)

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(Perry et al. 2001). In mammalian male meiosis I, the presence of achiasmate sex chromosomes can trigger apoptosis and result in infertility (Barchi et al. 2008; Eaker et al. 2002; Kauppi et al. 2011). In this robust apoptotic response, the Y chromosome-encoded transcription factor ZFY2 plays a pivotal role (Vernet et al. 2011, 2014).

SPO11 is the protein responsible for catalyzing meiotic double-strand breaks (DSBs), the initiating lesions for recombination (Keeney et al. 1997). In mouse and human, *Spo11* has two main mRNA-splicing isoforms, *Spo11 α* and *Spo11 β* (Bellani et al. 2010; Keeney et al. 1999; Romanienko and Camerini-Otero 1999). Expression timing of these two isoforms is different, such that *Spo11 β* appears earlier; its protein product, SPO11 β , is sufficient for DSB formation and recombination of autosomes and for X-X chromosome pairing (Kauppi et al. 2011, 2012). The only detectable defect in transgenic mice that lack the later-appearing isoform SPO11 α (*Spo11 β* -only mice) was inefficient PAR DSB formation and subsequently, defective X-Y pairing that affected ~70 % of meiotic cells (Kauppi et al. 2011, 2012). As demonstrated by the metaphase I arrest and infertility in *Spo11 β* -only males, efficient X-Y recombination is essential for normal spermatogenic progression.

Fortuitously, upon crossing *Spo11 β* -only mice with mice of a mixed genetic background (see **Materials and methods** section), we observed restoration of spermatogenic progression. Here, we report on meiotic progression, apoptosis, and fertility in these *Spo11 β* -only^{mb} mice (mb for “mixed background”). We show that these mice have an intermediate level of X-Y pairing (~50 %) at pachynema and elevated levels of apoptosis compared to control mice. Interestingly, meiotic defects in approximately half of spermatocytes are tolerated in vivo and still compatible with sperm production and fertility.

Materials and methods

Mice *Spo11 β* -only mice were described earlier (Kauppi et al. 2011); these mice were in the C57BL/6 strain background. The *Spo11 β* -only mice analyzed in this paper were generated in crosses with *Mad2*^{+/-} mice of mixed genetic background, with contributions from C57BL/6 and 129/Sv strains (Dobles et al. 2000). To distinguish *Spo11 β* -only mice analyzed here from the originally described *Spo11 β* -only animals, we refer to them as *Spo11 β* -only^{mb} mice (mb for “mixed background”).

All *Spo11 β* -only^{mb} mice were of the genotype *Mad2*^{+/+} *Spo11*^{-/-} *Tg(Xmr-Spo11 β_B)*^{+/+}, where *Tg(Xmr-Spo11 β_B)* stands for the transgenic *Spo11 β_B* construct expressed from a meiosis-specific promoter (Kauppi et al. 2011). Control mice used in these experiments varied in their genotypes, due to availability of males within the same litter or from closely related matings. Genotypes of control mice were *Mad2*^{+/+}

Spo11^{+/-} *Tg(Xmr-Spo11 β_B)*^{+/+} and *Mad2*^{+/+} *Spo11*^{+/-} *Tg(Xmr-Spo11 β_B)*^{+/-}.

All experiments were performed with at least two animals per group, with the exception of the test for breeding performance (see below), where only one control male was used. All cytological and histological experiments were performed on tissues derived from males between 7 and 9 weeks of age, unless otherwise specified. No significant inter-individual differences were found within the groups.

Genotyping Genotyping was performed by lysing either tail or ear pieces in DirectPCR tail lysis buffer (Viagen) supplemented with proteinase K, according to the manufacturer’s protocol. Of the lysate, 0.5 μ l was added to Platinum Blue PCR SuperMix (Invitrogen). PCR primers and cycling conditions for *Tg(Xmr-Spo11 β_B)* transgene, *Mad2* and endogenous *Spo11*, were previously described (Baudat et al. 2000; Dobles et al. 2000; Kauppi et al. 2011), with the exception of the reverse primer for *Mad2* (5’GGGGTTCGCTTCTCTACTTGC 3’).

Histology of testes and cauda epididymis Four percent paraformaldehyde (PFA)-fixed paraffin-embedded tissues were cut into 3- μ m-thick sections which were deparaffinized according to standard protocol (xylene, absolute, 95, 90, and 70 % ethanol and finally into sterile water) and oxidized in 0.5 % periodic acid solution for 5 min, followed by three washes in distilled water. Next, sections were incubated in Schiff’s reagent (Sigma) for 15 min and subsequently counterstained with Mayer’s hematoxylin solution (Sigma). After that, sections were dehydrated in an ethanol series (70, 90, 95 %, and absolute ethanol) followed by rinsing in two changes of xylene. Finally, coverslips were mounted on slides using a xylene-based mounting medium.

Spermatocyte chromosome preparation

and immunoincubation Spermatocyte chromosome preparations and immunoincubations were performed as described previously (Kauppi et al. 2011; Moens et al. 2000), with the following modifications. Ilford Ilfotol wetting agent (Harman Technology Limited, UK, catalog no. 1905162) was used instead of Kodak Photo-Flo 200. Slides were either used for immunoincubation immediately or stored at -80 °C. For immunoincubation, slides were blocked for 30–45 min with 1 $\times$ phosphate-buffered saline (PBS) containing 0.2 % bovine serum albumin (BSA), 0.2 % gelatin, and 0.05 % Tween 20. Slides were then incubated with primary antibodies against synaptonemal complex protein 3 (SYCP3) (1:400, Santa Cruz, catalog no. sc-74569) and SYCP1 (1:600, Novus Biologicals, catalog no. NB300-229) overnight at 4 °C in a humidified chamber. Next, slides were washed four to five times in 1 $\times$ PBS containing 0.1 % Triton X-100. Appropriate secondary antibodies (Alexa Fluor 488 Goat anti-Mouse IgG and Alexa Fluor 568 Goat anti-Rabbit IgG,

Invitrogen) were applied for 1 h at 37 °C in a humidified chamber. Both primary and secondary antibodies were diluted in 1× PBS containing 0.2 % BSA, 0.2 % gelatin, and 0.05 % Tween 20. After secondary antibody incubation, four to five washes were performed in 1× PBS containing 0.1 % Triton X-100 and the slides were mounted with VECTASHIELD mounting medium containing 4',6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, catalog no. H-1200). Nuclei were imaged on a Zeiss Axio Imager microscope with ZEN2 software at the Biomedicum Imaging Unit facility at the University of Helsinki and further processed with CorelDRAW image processing software (version X7).

Pairing of X and Y chromosomes at pachynema was scored based on their physical contact at the PAR. Nuclei in which X and Y were clearly separated from each other (based on SYCP3 staining) were scored as “X-Y apart.”

Combined terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and immunofluorescent staining of testis sections Testis sections were deparaffinized according to standard protocol (xylene, absolute, 95, 90, and 70 % ethanol and finally into sterile water), then permeabilized with either proteinase K (20 µg/ml) in 10 mM Tris-HCl (pH 7.5) for 15 min at room temperature or by boiling in citrate buffer (pH 6.0) for 10–15 min in a microwave oven. After two washes with 1× PBS, sections were blocked with 2 % BSA and 3 % normal serum in 10 mM Tris-HCl (pH 7.5) for 30–45 min. Fifty microliters of TUNEL reagent mix (In Situ Cell Death Detection Kit, Fluorescein, Roche, catalog no. 11684795910) was applied to each slide and incubated for 45–60 min according to the manufacturer's protocol. Sections were then washed with 0.1 % phosphate-buffered Triton X-100 (PBT) (0.1 % Triton X-100 in 1× PBS) four times, and an antibody against phosphorylated histone H3Ser10 (1:500, Millipore, catalog no. 06-570) was applied overnight at 4 °C. Four washes with 0.1 % PBT were performed prior to secondary antibody incubation at 37 °C for 1 h. Finally, sections were mounted with VECTASHIELD mounting medium containing DAPI (Vector Laboratories). Sections were imaged on a Zeiss Axio Imager microscope with ZEN2 software at the Biomedicum Imaging Unit at the University of Helsinki and further processed with CorelDRAW image processing software (version X7).

Seminiferous tubules containing four or more TUNEL-positive cells were considered TUNEL-positive tubules. Similarly, seminiferous tubules with four or more phospho-H3Ser10 positive cells were considered H3Ser10-positive tubules. One representative testis section from each animal was chosen, and all H3Ser10- and TUNEL-positive seminiferous tubules in that particular section were counted.

Matings for fertility test Each male was mated with five wild-type 2–3-month-old Naval Medical Research Institute (NMRI) virgin females. NMRI females were used in these

experiments because they produce large litters, and hence, limitation from the female contribution to fertility was minimized. Females were checked for the presence of vaginal plugs daily until a plug was observed (or for a maximum of 10 days), and the plug dates were recorded. Per each male examined in the fertility test, two females that displayed a vaginal plug were dissected at 11–16 days *post coitum*, and the number of embryos was recorded. For the remaining females with a vaginal plug, pregnancy was followed to term and the number of neonatal pups was recorded.

Statistical tests Unpaired two-tailed *t* test was performed to compare groups using GraphPad Prism software (version 6.0).

Results

X-Y chromosome pairing defect in *Spo11*β-only^{mb} mice

Meiotic pairing, recombination, and synapsis between male sex chromosomes are restricted to the PAR. We scored X-Y pairing status at the PAR in pachytene stage nuclei in *Spo11*β-only^{mb} males, and in agreement with Kauppi et al.'s findings in *Spo11*β-only males (Kauppi et al. 2011), observed two distinct classes of pachytene stage nuclei: those with successful X-Y pairing (Fig. 1a) and those with X and Y chromosomes apart (Fig. 1b).

X and Y chromosomes failed to pair in 48 % of pachytene stage nuclei (Fig. 1c). This is in contrast to control mice, where X and Y PARs touched each other in almost all pachytene nuclei. Compared to published data from *Spo11*β-only mice (C57BL/6 background) where X and Y chromosomes were paired in 30 % of cells (Kauppi et al. 2011), the percentage of nuclei with X-Y pairing in *Spo11*β-only^{mb} mice was increased.

Apoptotic response in *Spo11*β-only^{mb} mice

Pairing and recombination failure of the X and Y chromosomes in pachynema is expected to lead to an achiasmate sex chromosome pair at metaphase I. Chromosome pairs that are not physically connected to each other will lack inter-homolog tension and thus should be unable to successfully congress on the metaphase plate. In such a case, the SAC should be active and is expected to arrest spermatogenic progression at metaphase I and may trigger an apoptotic response. We assessed apoptosis in seminiferous tubules at epithelial stage XII, i.e., in tubules containing metaphase I cells. Epithelial stage XII of the seminiferous tubules was identified with an antibody against phospho-H3Ser10, and the apoptotic response in these tubules was observed by TUNEL. It should be noted that depending on the presence of achiasmate chromosomes, cells within a seminiferous tubule might either be arrested at metaphase I and subsequently face an apoptotic

Fig. 1 X-Y chromosome pairing at pachynema in *Spo11* β -only^{mb} mice. Nuclei were stained with anti-SYCP3 (red), anti-SYCP1 (green), and DAPI (blue). Shown are two spermatocyte nuclei from the same *Spo11* β -only^{mb} mouse, one with X and Y PARs touching (a) and another with X and Y chromosomes apart (b). Insets in a and b show schematic of X and Y chromosomes. Scale bars 5 μ m. c Percentages of pachytene nuclei with X and Y PARs touching in mice of the indicated genotypes. Data collected from three *Spo11* β -only^{mb} and two control mice (see Online Resource 1). Data for *Spo11* β -only mice from Kauppi et al. (2011). No significant difference was observed between control mice used in Kauppi et al. (2011) and this study. Error bars indicate SEM. *P* value from unpaired *t* test

response (marked by TUNEL positivity) or progress normally. Thus, it is difficult to accurately quantify the proportion of apoptotic cells with these markers, as also reported by earlier studies (Mahadevaiah et al. 2000; Vernet et al. 2011).

In *Spo11* β -only^{mb} testes, two types of H3Ser10-positive tubules were observed (Fig. 2b, c): those with no or very few TUNEL-positive cells (classified as TUNEL-negative tubules) and those with many TUNEL-positive cells (classified as TUNEL-positive tubules). In control mice, TUNEL-negative tubules (Fig. 2a) were predominant and TUNEL-positive tubules were rarely observed (Fig. 2d). This is in contrast to *Spo11* β -only^{mb} mice, where 64 % of H3Ser10-positive seminiferous tubules were TUNEL-positive (Fig. 2d). Although most TUNEL-positive tubules in *Spo11* β -only^{mb} mice were also H3Ser10-positive (i.e., stage XII), some were H3Ser10-negative (see Online Resource 2). These likely correspond to epithelial stage IV seminiferous tubules. It is also possible that in some apoptotic stage XII tubules, phosphorylation of H3Ser10 is already lost due to degradation.

Testis and epididymis morphology of *Spo11* β -only^{mb} mice

Mice of different backgrounds might display variable body weight or size between litters and/or individuals, and their

testis weight might vary accordingly. In order to account for such inter-individual differences, we compared relative testis weights (i.e., testis weight/body weight ratio) between the groups. Body weight was similar across all genotypes, but testes in *Spo11* β -only^{mb} mice were smaller than in controls (Table 1). Compared to *Spo11* β -only mice (data from the study by Kauppi et al. 2011), however, relative testis weights of *Spo11* β -only^{mb} mice were substantially increased. We examined the morphology of testes and cauda epididymis of control and *Spo11* β -only^{mb} mice. Seminiferous tubules appeared normal in testis sections of control mice (Fig. 3a). In testis sections of *Spo11* β -only^{mb} mice, both normal and atrophic seminiferous tubules were observed (Fig. 3b). Despite the increased atrophy in *Spo11* β -only^{mb} testes, sperm were nevertheless observed in the cauda epididymis (Fig. 3d). The amount of epididymal sperm appeared somewhat lower in *Spo11* β -only^{mb} mice compared to controls (Fig. 3c, d; not quantified).

Fertility of *Spo11* β -only^{mb} mice

Presence of sperm in the cauda epididymis of *Spo11* β -only^{mb} mice prompted us to evaluate the fertility of these animals. To do so, three *Spo11* β -only^{mb} males were each mated with five wild-type NMRI females, and the number of embryos and pups

Fig. 2 Apoptosis in *Spo11* β -only^{mb} testes. **a–c** Testis sections were stained with an antibody against phosphorylated H3Ser10 (red), with TUNEL (green) and DAPI (blue). **d** Quantification of TUNEL-positive and TUNEL-negative tubules among all H3Ser10 positive tubules. Data collected from four *Spo11* β -only^{mb} and three control mice (see Online Resource 2). Scale bars 50 μ m

produced from each mating was recorded. All *Spo11* β -only^{mb} males tested were fertile (Table 2). Pups sired by *Spo11* β -only^{mb} males appeared healthy and developed normally.

Discussion

Like *Spo11* β -only mice, *Spo11* β -only^{mb} mice lack the SPO11 α isoform and hence are expected to have defective

PAR DSB formation resulting in X-Y pairing failure. Although we did not quantify PAR DSB formation directly in the context of this study, we assessed X-Y pairing frequency in *Spo11* β -only^{mb} mice. In *Spo11* β -only mice of the C57BL/6 background, X-Y pairing was successful in just 30 % of cells and most *Spo11* β -only males were infertile (Kauppi et al. 2011). In *Spo11* β -only^{mb} mice, X-Y pairing was improved, such that in approximately half of pachytene cells, the PARs contacted each other (Fig. 1c). This percentage of nuclei with

Table 1 Body weights and testis weights of *Spo11* β -only^{mb} and control mice

	Body weight in grams, mean $\pm$ standard deviation (SD)	Weight of one testis in grams, mean $\pm$ SD	Relative testis weight in grams, mean $\pm$ SD
Control	29.81 $\pm$ 3.260	0.107 $\pm$ 0.009	3.578 $\pm$ 0.083
<i>Spo11</i> β -only ^{mb}	26.25 $\pm$ 0.464	0.065 $\pm$ 0.001	2.464 $\pm$ 0.338
<i>Spo11</i> β -only	25.83 $\pm$ 1.272	0.042 $\pm$ 0.004	1.637 $\pm$ 0.171
<i>p</i> value, control versus <i>Spo11</i> β -only ^{mb}			0.0072 (**)

N=2 for control mice, *n*=5 for *Spo11* β -only^{mb} mice, and *n*=9 for *Spo11* β -only mice. Data for *Spo11* β -only mice from Kauppi et al. (2011). The weight of both testes in control and each *Spo11* β -only^{mb} male was similar. Unpaired *t* test was performed to compare groups

***p*<0.01

Fig. 3 Testis morphology and sperm formation in *Spo11* β -only^{mb} mice. Testis (a, b) and cauda epididymis (c, d) sections from control and *Spo11* β -only^{mb} mice stained with periodic acid Schiff and counterstained with Mayer's hematoxyline solution. *Spo11* β -only^{mb} mice testes contain both atrophic (asterisks in b) and normal-looking seminiferous tubules. Scale bars 100 μ m

successful PAR pairing was substantially higher (Fig. 1c) than previously reported for *Spo11* β -only mice (Kauppi et al. 2011). The difference is likely due to their genetic background (mixed for *Spo11* β -only^{mb} mice versus pure C57BL/6 for *Spo11* β -only mice).

The mouse PAR is a remarkably dynamic structure, as evidenced by large structural differences observed, e.g., between randomly chosen individuals of an inbred hybrid strain (Kipling et al. 1996). Structural differences that are present on different haplotypes and, for instance, affect just a small portion of the PAR may have an impact on X-Y recombination frequency. Tease and Cattanch (Tease and Cattanch 1989) reported X-Y pairing behavior influenced by the sex-reversal factor (*Sxr*) in different genetic backgrounds: with *Sxr* present on the X PAR, X-Y separation at pachynema was less frequent than when *Sxr* was present on the Y PAR. Although at this time we have no information on PAR structure in *Spo11* β -only^{mb} versus *Spo11* β -only mice, we propose that the partial rescue of X-Y pairing might

be explained by differences in PAR structure or, e.g., simply PAR length between the C57BL/6 and C57BL/6 $\times$ 129/Sv mixed backgrounds. Such differences could make the *Spo11* β -only^{mb} PAR more conducive to DSB formation and subsequently to X-Y pairing and recombination. Unfortunately, the PAR DNA sequence in any mouse strain at present remains largely unknown, as sequencing of this area is hampered by high repeat density.

Improved X-Y pairing was accompanied by partial rescue of testis cellularity (Fig. 3) and weight (Table 1). Although many apoptotic seminiferous tubules were observed in *Spo11* β -only^{mb} testes, 36 % of stage XII seminiferous tubules were TUNEL-negative. It is possible that some of the tubules containing cells positive for H3Ser10 but negative for TUNEL might subsequently become apoptotic. However, the majority of these tubules are expected to maintain normal spermatogenesis, as indicated by the X-Y pairing data at pachynema (Fig. 1) and the presence of sperm in the epididymis (Fig. 3). Pachytene cells where X

Table 2 Breeding performance of *Spo11* β -only^{mb} mice

	Breeding i Number of embryos (dissected between 11 and 16 dpc)	Breeding ii Number of pups (0.5 dpp)	Breeding iii Number of pups (0.5 dpp)	Breeding iv Number of pups (0.5 dpp)	Breeding v Number of pups (0.5 dpp)	Successful pregnancies/females with vaginal plugs	Average number of pups per successful pregnancy
Control male	4	12	14	0	0	3/5	10
<i>Spo11</i> β -only ^{mb} male A	5	2	12	0	NA	3/4	6.3
<i>Spo11</i> β -only ^{mb} male B	9	8	0	0	NA	2/4	8.5
<i>Spo11</i> β -only ^{mb} male C	6	0	1	12	NA	3/4	6.3

dpc day post coitum, dpp day post partum, NA not applicable (no plug observed after 10 days)

Table 3 Homolog association in meiosis I and fertility outcomes—a review from different mouse models

Mouse model	X-Y unpaired at pachytene stage	Other chromosomes unpaired	Metaphase I apoptosis	Fertility	Reference
<i>Mlh1</i> ^{+/−}	100 %	100 %	Elevated	No	Eaker et al. (2002)
(C57BL/6× <i>M. spretus</i>) F1	83 % (92 % ^a)	23 %	ND	No	Matsuda et al. (1991) and Matsuda et al. (1992)
<i>Spo11</i> ^{+/−} <i>Atm</i> ^{−/−}	73 % (80–85 % ^a)	ND	Elevated	No	Barchi et al. (2008)
<i>Spo11</i> β-only	70 %	ND	Elevated	Mostly infertile (males only)	Kauppi et al. (2011)
XY, <i>Sxr</i>	52 % (one mouse, 8 weeks old)	ND	Not analyzed directly but reduced testes weight	Not stated; apparently variable (Lyon et al. 1981)	Mahadevaiah et al. (1988)
<i>Spo11</i> β-only ^{mb}	48 %	ND	Elevated	Yes	This study
X <i>Sxr</i> ^a /Y* ^x	37 % (50 % ^a)	ND	No histological abnormalities	No metaphase I block but infertile due to spermiogenesis defects	Burgoyne et al. (1992)
Robertsonian translocation heterozygous (Rb/+)	ND	23 %	Elevated	Yes	Davisson and Akeson (1993) and Eaker et al. (2001)

ND no data

^a At metaphase I

and Y chromosomes successfully recombine are expected to proceed normally through metaphase I, without persistent SAC activation. We propose that this cell population is sufficient to maintain sperm production and hence fertility of *Spo11*β-only^{mb} mice.

Despite the substantial rescue, approximately half of pachytene stage nuclei in *Spo11*β-only^{mb} mice were defective for X-Y pairing. When such cells progress to metaphase, they are expected to lack chiasmata connecting the X and Y chromosomes, i.e., to lack inter-homolog tension, and thus to have the potential to activate the SAC. Consistent with this idea, in *Spo11*β-only^{mb} mice, an apoptotic response was observed in many stage-XII seminiferous tubules, that is, in tubules containing metaphase I cells (Fig. 2d). Metaphase I apoptosis is likely not cell-autonomous, due to the fact that within seminiferous tubules, developing germ cells are connected through intercellular bridges. Thus, apoptotic signals may spread beyond the original cell experiencing, e.g., chromosome segregation problems. Further studies are needed to understand the role of the SAC in triggering apoptosis. A number of studies have documented a stringent apoptotic response in male meiosis upon presence of achiasmate chromosomes (Barchi et al. 2008; Davisson and Akeson 1993; Eaker et al. 2001, 2002; Kauppi et al. 2011; Sutcliffe et al. 1991; Vernet et al. 2014), whereas female meiosis was observed to be more permissive (Hunt et al. 1995). Our findings on the apoptotic response in *Spo11*β-only^{mb} testes add to the growing number of reports that suggest SAC activation in response to homologous recombination defects, especially involving the X and Y chromosomes (Table 3). If a high enough proportion of cells contain an achiasmate chromosome pair, spermatogenesis typically arrests at metaphase I and programmed cell death follows. Different mouse models characterized by sex chromosome pairing defects have shown variable meiotic phenotypes and fertility. For instance, *Spo11*^{+/−} *Atm*^{−/−} (Barchi et al. 2008), *Spo11*β-only (Kauppi et al. 2011), etc. mouse models displayed metaphase I arrest, elevated levels of apoptosis, and infertility (Table 3). It appears that X-Y recombination failure in ≥70 % of cells results in a testis-wide metaphase I halt to spermatogenesis. On the other hand, low to intermediate levels of X-Y dissociation are apparently tolerated without overt effects on spermatogenesis (this study and Table 3). As demonstrated by our observations in *Spo11*β-only^{mb} mice, X-Y chromosome dissociation in ~50 % of cells is still compatible with sperm production and fertility. These findings indicate that male meiosis has a surprisingly high-tolerance buffer for achiasmate chromosome incidence. Even when a substantial cell population is eliminated by an apoptotic response (likely SAC-mediated), fertility can be maintained, presumably thanks to the large excess of sperm normally produced.

Conclusion

Crossover defects can arrest meiotic progression at metaphase I. X-Y chromosome dissociation is the most common perturbation in male meiosis. Low levels of such defects can be tolerated without detectable impact on fertility. We show here in *Spo11* β -only^{mb} mice that an intermediate level of unpaired sex chromosomes (affecting ~50 % of spermatocytes) is still compatible with sperm production. On the other hand, a somewhat higher level (≥ 70 %) of X-Y dissociation was previously shown to impair fertility (Table 3). Combining our findings with previous studies, we propose that the incidence of homolog dissociation that can be tolerated in vivo without compromising male fertility lies between 50 and 70 %.

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Compliance with ethical standards

Conflict of interest Imrul Faisal declares that he has no conflict of interest. Liisa Kauppi declares that she has no conflict of interest.

Ethical approval All applicable national and institutional guidelines for the care and use of animals were followed.

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