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Meiotic sex chromosome inactivation

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X chromosome inactivation is most commonly studied in the context of female mammalian development, where it performs an essential role in dosage compensation. However, another form of X-inactivation takes place in the male, during spermatogenesis, as germ cells enter meiosis. This second form of X-inactivation, called meiotic sex chromosome inactivation (MSCI) has emerged as a novel paradigm for studying the epigenetic regulation of gene expression. New studies have revealed that MSCI is a special example of a more general mechanism called meiotic silencing of unsynapsed chromatin (MSUC), which silences chromosomes that fail to pair with their homologous partners and, in doing so, may protect against aneuploidy in subsequent generations. Furthermore, failure in MSCI is emerging as an important etiological factor in meiotic sterility.

Introduction

Meiotic sex chromosome inactivation (MSCI) is the process of transcriptional silencing of the X and Y chromosomes that occurs during the meiotic phase of spermatogenesis. This silencing occurs at pachytene, when maternal and paternal autosomal homologues have completed pairing, or synapsis, and is mediated by large-scale chromatin remodelling of the X and Y chromosomes. MSCI is found in the male germ line of almost all organisms possessing differentiated sex chromosomes, but its importance to developmental biologists, particularly to those studying germ cell development and infertility, is only just beginning to be understood. MSCI has also received a great deal of attention in the somatic X-inactivation field. Specifically, it has been suggested that MSCI plays a role in establishing the silencing of the paternally inherited X chromosome in preimplantation mouse female embryos (Huynh and Lee, 2003).

In this primer, I outline recent advances in our understanding of MSCI in mammals. MSCI is a manifestation of a general meiotic-silencing mechanism called meiotic silencing of unsynapsed chromatin (MSUC) (see Table 1), which is beginning to throw much-needed light on our understanding of meiotic-derived sterility. MSCI is initiated by several DNA-repair proteins and is maintained by virtue of histone modifications that are associated with transcriptional silencing in a wide variety of developmental contexts. New studies are beginning to challenge the dogma that MSCI is restricted to meiosis, because the X and Y chromosomes retain a repressed state throughout round spermatid development. These findings from studies of MSCI have far-reaching implications for our understanding of germ cell epigenetics, meiotic chromosome dynamics and, more generally, infertility.

An introduction to sex chromosome activity in spermatogenesis

The transcriptional activity of the sex chromosomes varies considerably as germ cells progress through the successive stages of spermatogenesis (see Fig. 1). During the early stages of spermatogenesis, spermatogonial stem cells divide by mitosis to generate progressively more-differentiated progeny; here, genes on the X and Y chromosomes are transcriptionally active. One study (Wang et al., 2001) has revealed that a disproportionately large number of spermatogonial-specific transcripts originate from the X and Y chromosomes. This is in agreement with the predictions of Fisher (Fisher, 1931) and Rice (Rice, 1984) that the X chromosome should accumulate sexually antagonistic genes that are beneficial to one sex but harmful to the other (Khil and Camerini-Otero, 2005). When a recessive mutation that provides a reproductive advantage to males appears on the X chromosome, that advantage is immediately apparent because the X chromosome is present in only a single copy (i.e. the mutation is hemizygous). Thus, sexually antagonistic genes beneficial to males (i.e. those involved in spermatogenesis) should accumulate on the X chromosome (see Khil and Camerini-Otero, 2005).

Following spermatogonial divisions, germ cells enter meiosis. During the earliest meiotic substage, leptotene, when the DNA double-strand breaks (DSBs) that initiate meiotic recombination are formed, the X and Y chromosomes are still transcriptionally active, and remain so throughout zygotene (Fig. 1) (Turner et al., 2005), when DSBs are processed to form the single-stranded tails that drive homologous synapsis (Box 1). However, shortly after the zygotene-to-pachytene transition, when meiotic synapsis between autosomes is complete, the X and Y chromosomes are rapidly silenced and compartmentalized into a peripheral nuclear subdomain called the sex- or XY-body (Solari, 1974; McKee and Handel, 1993). MSCI then persists throughout the rest of pachytene and diplotene.

Following diplotene, germ cells undergo two successive rounds of cell division, when homologous chromosomes and their respective sister chromatids separate (the paired homologues, each comprised of two sisters, are termed bivalents). The resulting haploid daughter cells then undergo spermiogenesis, during which the DNA of these cells undergoes increasing compaction, facilitated by the replacement of histones with protamines. Protamines are small arginine- and cysteine-rich proteins that facilitate the high level of chromatin compaction required during sperm formation. Until recently, the transcriptional status of the sex chromosomes during spermiogenesis was poorly understood; few published studies focused on the activity of specific sex-linked genes, but these studies did conclude that the transcription of these genes was reactivated on the X and Y chromosomes (Hendriksen et al., 1995; Hendriksen, 1999; Wang et al., 2005). It was recently reported, however, that genes that are expressed in late spermatogenesis are under-represented on the X chromosome (Khil et al., 2004). Subsequently, three further studies (Namekawa et al., 2006; Turner et al., 2006; Greaves et al., 2006) have found cytological evidence that gene silencing is maintained on the X and Y chromosomes in spermatids, as described in more detail below. In summary, these studies indicate that the X and Y chromosomes are transcriptionally active during spermatogonial divisions and early meiotic stages, but become transcriptionally repressed from pachytene onwards, through to the end of spermatogenesis.

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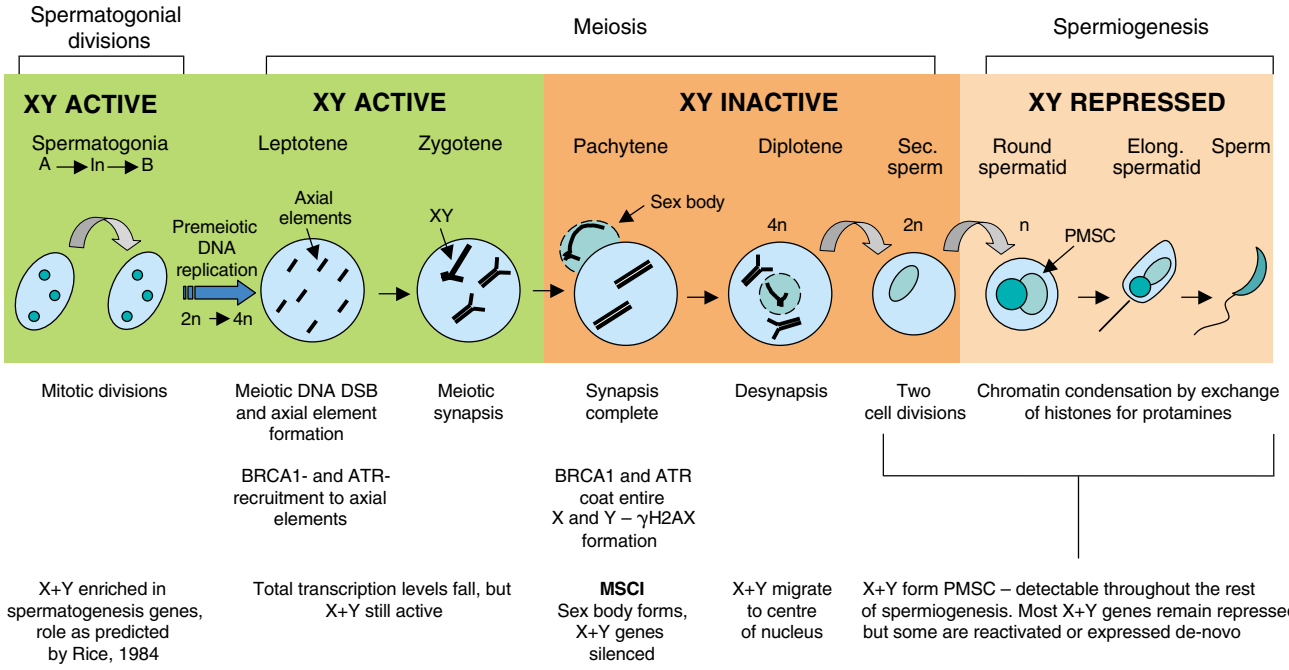


Fig. 1. Overview of XY activity during spermatogenesis. Transcriptional activity of the X and Y chromosomes is shown, with green indicating high expression and orange indicating low expression. The X and Y chromosomes are transcriptionally active during type A, intermediate (In) and type B spermatogonial divisions. They remain active during leptotene and zygotene, although total nuclear transcription is low during this time. On entry into pachytene, BRCA1 and ATR coat the entire length of the unsynapsed regions of the X and Y axial elements, followed by the translocation of ATR to the surrounding chromatin, where H2AX phosphorylation and MSCI takes place. Following meiosis, X and Y chromosome repression is maintained and the X and Y chromosomes appear as heterochromatic domains called post-meiotic sex chromatin (PMSC). This is shown as the light-turquoise structure located next to the dark-turquoise chromocentre – the site at which centromeres cluster. Elong., elongated; sec., secondary.

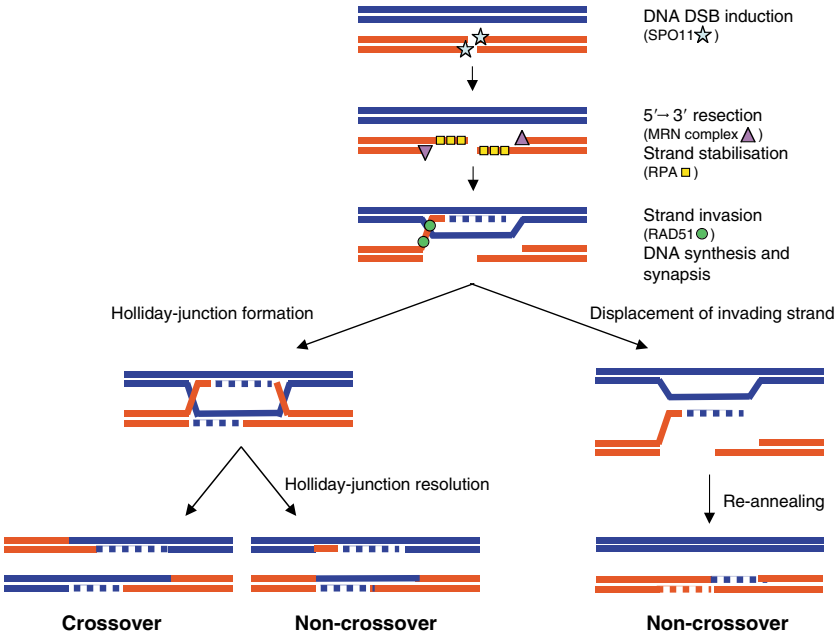
The epigenetics of MSCI

MSCI was previously thought to be mediated in much the same way as somatic X chromosome inactivation (XCI) (see Table 1). XCI is initiated by an X-encoded RNA called *Xist* (inactive X specific transcript), which coats the X chromosome from which it is transcribed (termed acting in-cis) (Penny et al., 1996; Marahrens et al., 1997). *Xist* recruits an array of chromatin-modifying enzymes to the future inactive X chromosome that induce gene silencing by catalysing methylation, ubiquitylation and deacetylation of defined histone residues (Heard and Disteche, 2006). In males, *Xist* is expressed exclusively in the testis (McCarrey and Dilworth, 1992; Salido et al., 1992; Richler et al., 1992; Ayoub et al., 1997) and has been reported to coat the sex body in a manner analogous to that in female somatic cells. The presence of *Xist* on the Y as well as on the X chromosome in the sex body spawned the 'quasi-cis' model of MSCI, in which X chromosome-derived *Xist* transcripts spread from the X chromosome to the Y chromosome via the region of X-Y synapsis (Ayoub et al., 1997). However, subsequent studies have found that, although *Xist* is essential for XCI (Penny et al., 1996; Marahrens et al., 1997), it is dispensable for MSCI (McCarrey et al., 2002; Turner et al., 2002). Attention has since focused on defining the molecular events that lead to MSCI.

A central player in MSCI is the histone H2A variant H2AX (histone family, member X) (Fig. 2). H2AX is abundant in the mammalian testis, in comparison to other tissues (Mahadevaiah et al., 2001), and is a core component of the nucleosome of meiotic cells (Fernandez-Capetillo et al., 2003). H2AX plays an essential role in the DNA-damage response (Celeste et al., 2002): following DNA DSB-induction (Box 1), H2AX is rapidly phosphorylated at serine-139 to form γH2AX (Rogakou et al., 1999) and recruits members of the DNA-repair machinery (e.g. MDC1, mediator of DNA damage checkpoint 1) to the sites of breaks (Stucki et al., 2005) (Fig. 2 and Table 1). H2AX phosphorylation occurs in response to the formation of DNA DSBs during leptotene (Mahadevaiah et al., 2001) (Fig. 2A). However, an additional wave of H2AX phosphorylation also takes place at the zygotene-pachytene transition, when MSCI commences. This second wave of phosphorylation occurs only on the chromatin of the X and Y chromosomes (Mahadevaiah et al., 2001; Turner et al., 2005) (Fig. 2D). Because post-translational histone modifications are known to control gene expression by altering higher-order chromatin structure, Mahadevaiah et al. (Mahadevaiah et al., 2001) proposed a causative role for H2AX phosphorylation in MSCI. A subsequent study revealed this to be the case – *H2AX*-null male mice display complete meiotic arrest associated with MSCI failure (Fernandez-Capetillo et al., 2003). In order to demonstrate unequivocally that H2AX phosphorylation is essential for the initiation of MSCI, a mouse with a point mutation of serine-139 needs to be generated.

Two recent studies indicate that the DNA-repair protein ATR (ataxia telangiectasia and Rad3 related) (see Table 1), a member of the PI3-like kinase family, phosphorylates H2AX and is therefore required for MSCI (Turner et al., 2004; Bellani et al., 2005). In contrast to the other PI3-like kinases [ATM (ataxia telangiectasia mutated) and DNA-PK (DNA-dependent protein kinase, also known as PRKDC – Mouse Genome Informatics)], ATR colocalises with γH2AX on the sex chromosomes once MSCI initiates until γH2AX dephosphorylation occurs at the diplotene-metaphase I transition. Mice with an *Atr* mutation die early in embryogenesis and thus cannot be used to directly address the role of ATR in MSCI (Brown and Baltimore, 2000). However, normal H2AX phosphorylation and

Box 1. A simplified model of the formation and repair of DNA DSBs during meiosis



DNA DSBs are the initiating events in meiotic recombination and are created by the SPO11 enzyme. This results in phosphorylation of H2AX by ATM. The resulting DNA DSB is 5′-to-3′ resected by the MRE11-RAD50-NBS1 (MRN) complex (MRE11 is also known as MRE11A and NBS1 as NBN), and the resulting tail is stabilised by RPA (also known as RPA1), before the loading of RAD51, which enables strand invasion and drives synapsis. The resulting intermediate can follow one of at least two fates: Holliday-junction formation, which is in turn cleaved, giving rise to either crossovers or non-crossovers; or strand displacement, giving rise to only non-crossovers. For a detailed description of players in each stage of meiotic recombination, see Krogh and Symington (Krogh and Symington, 2004).

MSCI occurs in *Atm*- or *DNA-PK*-deficient mice, indicating that *Atm* and *DNA-PK* do not normally contribute to MSCI (Turner et al., 2004; Bellani et al., 2005) (Fig. 2). The correct targeting of ATR to the X and Y chromosomes depends on the tumour suppressor protein BRCA1 (breast cancer 1, early onset) (see Table 1), which also localises to the X and Y chromosomes during MSCI (Turner et al., 2004) (Fig. 3). *Brca1* is placed upstream of *Atr*, based on studies of mice with a deletion of exon 11 of *Brca1* (Xu et al., 2003; Turner et al., 2004). As in *H2AX*-null males, MSCI is defective in *Brca1* mutant mice, because H2AX phosphorylation does not take place on the X and Y chromosomes. Instead, it occurs at ectopic sites throughout the meiotic nucleus. This ectopic H2AX phosphorylation is the result of the defective localization of ATR to the XY bivalent. Thus, recruitment of ATR to the X and Y chromosomes depends on BRCA1, either directly or indirectly.

At or shortly after the initiation of MSCI, the X and Y chromosomes undergo further post-translational modifications (reviewed in Hoyer-Fender, 2003; Handel, 2004) (Fig. 2E and see below), including H2A ubiquitylation (forming uH2A) (see Table 1) (Baarends et al., 1999), deacetylation of histones H3 and H4 (Khalil et al., 2004), dimethylation of H3 (forming H3K9me2) (Khalil et al., 2004) and the sumoylation of an, as yet, undefined target(s) (Rogers et al., 2004; Vigodner and Morris, 2005). H2A ubiquitylation may depend on the E2-conjugating enzymes UBE2A and UBE2B (see Table 1), which colocalise with uH2A in meiotic cells (Baarends et al., 2005). The methyltransferase that catalyses H3 lysine dimethylation is unknown. One candidate, SUV39H2 (suppressor of variegation 3-9 homolog 2) (see Table 1), localizes to the sex body, but the appearance of H3K9me2 is unperturbed in SUV39H2-deficient mice (Peters et al., 2001). Some of these post-translational modification products (i.e. uH2A, deacetylated H3 and H4, and H3K9me2) may serve in the maintenance of MSCI, because they remain associated with the X and Y throughout the meiotic divisions and into spermiogenesis, long after H2AX dephosphorylation has taken place.

MSCI may also depend on the incorporation of specific histone variants, such as H2AFY (H2A histone family, member Y; previously MACROH2A1.2) (Hoyer-Fender et al., 2000) and H2AZ (H2A histone family, member Z; also known as H2AFZ – Mouse Genome Informatics) (Table 1) (Greaves et al., 2006). MacroH2A1.2 is an unusually large histone variant comprised of a full-length H2A domain together with a large non-histone domain, and has previously been implicated in XCI (Costanzi and Pehrson, 1998). H2AZ plays an essential role in the maintenance of heterochromatin and in chromosome segregation, and is unique in that it associates with the X and Y chromosomes only after meiosis is complete (Greaves et al., 2006). This implies that H2AZ may function in the maintenance of MSCI (see below). The chromodomain proteins CBX1 (chromobox homolog 1) (Motzkus et al., 1999; Metzler-Guillemain et al., 2003) and CBX3 (chromobox homolog 3) (Metzler-Guillemain et al., 2003) have also been implicated in MSCI (see Table 1).

MSCI: a consequence of synaptic failure

Over recent years it has become apparent that MSCI is in fact a manifestation of MSUC, a more general meiotic-silencing mechanism (Schimenti, 2005) (see Table 1). In meiotic cells, homologues synapse via a proteinaceous scaffold called the synaptonemal complex (SC). The SC consists of two axial elements, which form during leptotene between the sister chromatids, and of a central component, which forms as synapsis takes place (de Boer and Heyting, 2006). Meiotic DNA is arranged in loops that attach at their base to these axial elements (see Fig. 2C). As the X and Y chromosomes only synapse via a homologous distal segment, such that much of the X and Y axial elements are unsynapsed during pachytene, the proteins involved in MSCI might be expected to localise to the chromatin of the arms of the DNA loops, surrounding

Table 1. Summary of terms used

Term	Meaning	Explanation	Reference
MSCI	Meiotic sex chromosome inactivation	Transcriptional silencing of the X and Y chromosomes that takes place during the pachytene sub-stage of male meiosis. MSCI is MSUC affecting the X and Y.	(McKee and Handel, 1993)
MSUC	Meiotic silencing of unsynapsed chromatin	Transcriptional silencing of unsynapsed autosomal segments that takes place during the pachytene sub-stage of male and female meiosis.	(Schimenti, 2005)
MSUD	Meiotic silencing by unpaired DNA	Transcriptional silencing of genes by virtue of their homology to other genes that are themselves unpaired during meiosis.	(Shiu et al., 2001)
PMSC	Post-meiotic sex chromatin	The heterochromatic, transcriptionally repressed conformation of the X and Y chromosomes during spermiogenesis.	(Namekawa et al., 2006)
PSCR	Post-meiotic sex chromosome repression	Transcriptional repression of the X and Y chromosomes during spermiogenesis.	(Turner et al., 2006)
XCI	X chromosome inactivation	Silencing of one of the two X chromosomes that takes place early in female mammalian pre-implantation development.	(Heard and Disteche, 2006)
BRCA1	Breast cancer 1, early onset	Tumour suppressor protein linked to a wide variety of processes, including DNA DSB repair, cell cycle progression and MSCI/MSUC.	(Boulton, 2006)
ATR	Ataxia telangiectasia and Rad3-related	Member of the PI3 kinase-like kinase family. Involved in DNA DSB repair, cell cycle progression and MSCI/MSUC.	(Traven and Heierhorst, 2005)
γH2AX	Phosphorylated histone H2AX	Modification carried out by the PI3 kinase-like kinases and involved in the early stages of DNA DSB repair – mainly via recruitment of MDC1. Also functions in MSCI/MSUC.	(Fillingham et al., 2006)
MDC1	Mediator of DNA damage checkpoint 1	DNA-repair protein that interacts with γH2AX at the sites of DNA DSBs. Loss of MDC1 results in impaired recruitment of DNA DSB-repair factors (e.g. ATM to DNA DSBs) and chromosome instability.	(Stucki et al., 2005)
uH2A	Ubiquitylated histone H2A	Modification carried out by the concerted efforts of E1 (activating), E2 (conjugating) and E3 (ligating) enzymes and involved in transcriptional silencing both in the context of somatic XCI and MSCI/MSUC.	(Baarends et al., 2005)
UBE2A/B	Ubiquitin-conjugating enzyme E2A/B, RAD6 homolog	Enzymes involved in the second step of histone ubiquitylation – conjugation. Both are candidates for the generation of uH2A during MSCI/MSUC.	(Baarends et al., 2005)
Suv39h2	Suppressor of variegation 3-9 homolog 2	Histone methyltransferase that acts with Suv39h1 to regulate histone H3 K9 dimethylation at pericentric heterochromatin. Suv39h1/2 double mutants are sterile, but sex body-associated H3K9Me2 and MSCI are unaffected.	(Peters et al., 2001)
H2AFY	H2A histone family, member Y	Large H2A variant implicated in somatic XCI and MSCI/MSUC. May exert function via inhibition of PARP-1 activity.	(Hoyer-Fender et al., 2000) (Ouararhni et al., 2006)
H2AZ	H2A histone family, member Z	Histone variant implicated in chromosome segregation and heterochromatin formation. May also function in PSCR.	(Greaves et al., 2006)
CBX1, CBX3	Chromobox homolog 1, 3	Chromobox-containing 'adaptor' proteins that bind specific post-translational histone modifications and recruit effectors of gene silencing.	(Motzkus et al., 1999) (Metzler-Guillemain et al., 2003)

the axial element, where most genes reside. Indeed, this is exactly where γH2AX is found (Turner et al., 2004). However, prior to MSCI initiation, both BRCA1 and ATR associate exclusively with the axial element of the X and Y chromosomes (Turner et al., 2004) (Fig. 2C). Shortly after, ATR translocates from the axial element to the chromatin loops, concomitant with the appearance of γH2AX at those sites (Fig. 2D). Based on the association of BRCA1 and ATR with the unsynapsed X and Y chromosome axial element, and on their absence from the distal regions of synapsed sex chromosomes, it was proposed that MSCI and a lack of synapsis were intimately linked (Turner et al., 2004). It became apparent that BRCA1 and ATR were recognizing the axial elements of the X and Y chromosomes simply because they were unsynapsed, rather than because of some special feature of these chromosomes.

Turner et al. (Turner et al., 2005) questioned whether unsynapsed autosomes would also attract BRCA1, and whether this would ultimately lead to autosomal silencing. Using T(X;16)16H male mice, in which an X-16 reciprocal translocation frequently results in errors in chromosome 16 synapsis, it was demonstrated that regions of unsynapsed chromosome 16 were indeed positive for BRCA1, ATR and γH2AX and were silenced (Turner et al., 2005). Baarends et al. (Baarends et al., 2005) also reported similar findings in mice that contained a translocation between chromosome 1 and 13, using uH2A as a marker of silencing. Both authors then studied the localization of MSCI proteins in the XO female mouse (Speed, 1986). These mice have only one X chromosome instead of two, and the single X chromosome has no homologous partner with which to synapse during meiosis. Both studies found that the unsynapsed chromosomes were also silenced during female meiosis. In a later study, Turner et al. (Turner et al., 2006) tested whether MSCI could be prevented by providing the normally unsynapsed X or Y chromosome with a synaptic partner. This proved to be the case. For example, in XYY mice, in which the Y chromosome is provided with an additional Y chromosome, fully synapsed YY bivalents were negative for γH2AX and evaded MSCI. These results are summarized in Fig. 3B,C,E.

Taken together, these findings demonstrate that unsynapsed chromosome regions are silenced during meiosis. Related meiotic-silencing mechanisms have previously been shown to operate in both *Caenorhabditis elegans* (Bean et al., 2004) and *Neurospora crassa* (Shiu et al., 2001), in which they may function in genome defence (Shiu et al., 2001). In *C. elegans*, the single X chromosome

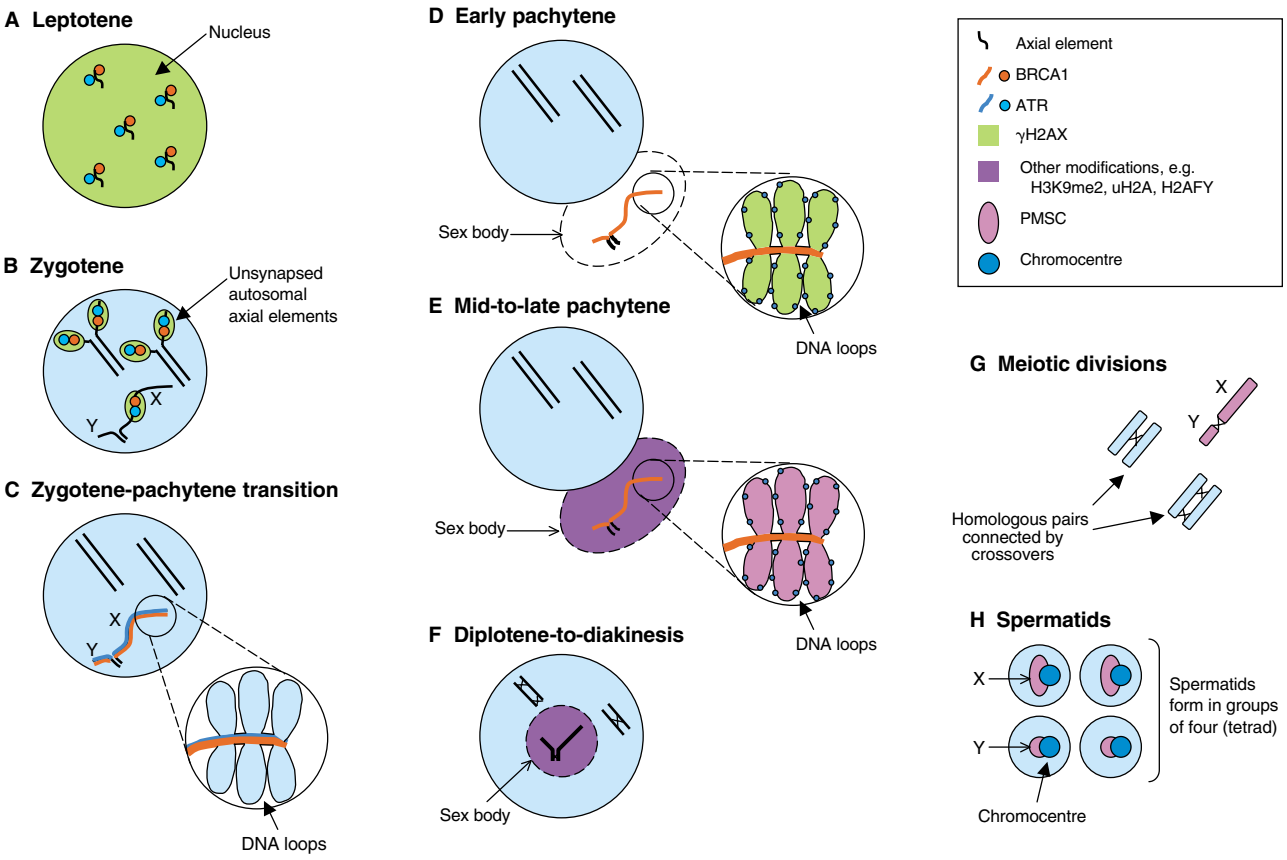


Fig. 2. Schematic representation of MSCI. (**A**) During leptotene, widespread ATM-dependent H2AX phosphorylation occurs in response to meiotic-DNA DSB formation. BRCA1 and ATR form foci on newly forming axial element (AEs). (**B**) During zygotene, synapsis coincides with the loss of BRCA1, ATR and γH2AX from autosomal AEs. BRCA1, ATR and γH2AX remain as foci on the AEs of autosomes that have not yet synapsed and on the AE of the X chromosome. (**C**) Zygotene-pachytene transition. Autosomal synapsis is complete and recombination-related γH2AX disappears. BRCA1- and ATR-staining becomes linear on the X and Y AEs. Meiotic DNA is arranged in loops attached at their bases to the AEs. (**D**) Early pachytene. ATR translocates along DNA loops, where it phosphorylates H2AX, resulting in MSCI and in the formation of the sex body. (**E**) Mid-to-late pachytene. Other histone modifications [e.g. the production of H3K9me2, uH2A and histone variants (e.g. H2AFY)] ensure the maintenance of MSCI. (**F**) Diplotene-to-diakinesis. The X and Y chromosomes migrate to the centre of the nucleus. BRCA1, ATR and γH2AX are lost from the X and Y chromosomes, but the other modifications remain. These modifications ensure the maintenance of MSCI throughout the meiotic divisions (**G**) and into spermatids (**H**), and is termed post-meiotic sex chromosome repression (PSCR).

of male meiotic cells is enriched in H3K9me2, and, when autosomes fail to synapse, they acquire the same repressive histone mark (Bean et al., 2004). In *Neurospora*, DNA that is unsynapsed during meiosis is silenced but, in contrast to the situation in mammals (Okamoto et al., 2005), this silencing affects all homologous DNA sequences, whether those sequences are synapsed or not. For this reason, meiotic silencing in *Neurospora* has been termed meiotic silencing by unpaired DNA (MSUD) (see Table 1). MSUD is thought to function post-transcriptionally, because it uses components of the RNAi pathway, including the RNA-dependent RNA polymerase (RdRP) *sad-1* (*suppressor of ascus dominance 1*) (Shiu et al., 2001) and the argonaute-like protein Sms-2 (suppressor of meiotic silencing 2) (Lee et al., 2003). RdRPs function in RNAi by converting single-stranded RNA precursors into double-stranded RNA, which are then cleaved by Dicer to form short interfering RNAs (siRNAs). These small RNA molecules induce destruction of homologous mRNA via an argonaute-containing protein complex RISC (RNA-induced silencing complex) (Dawe, 2004). The data of Turner et al. (Turner et al., 2005) and Baarends et al. (Baarends et al., 2005) indicate that MSUC operates at the transcriptional level, but this does not preclude the possibility that RNAi is involved, because the core RNAi machinery can silence genes at the transcriptional level (Grewal and Jia, 2007). Indeed, a recent study has found that maelstrom (MAEL), whose *Drosophila* orthologue is implicated in RNAi (Findley et al., 2003), localises to the sex body (Costa et al., 2006).

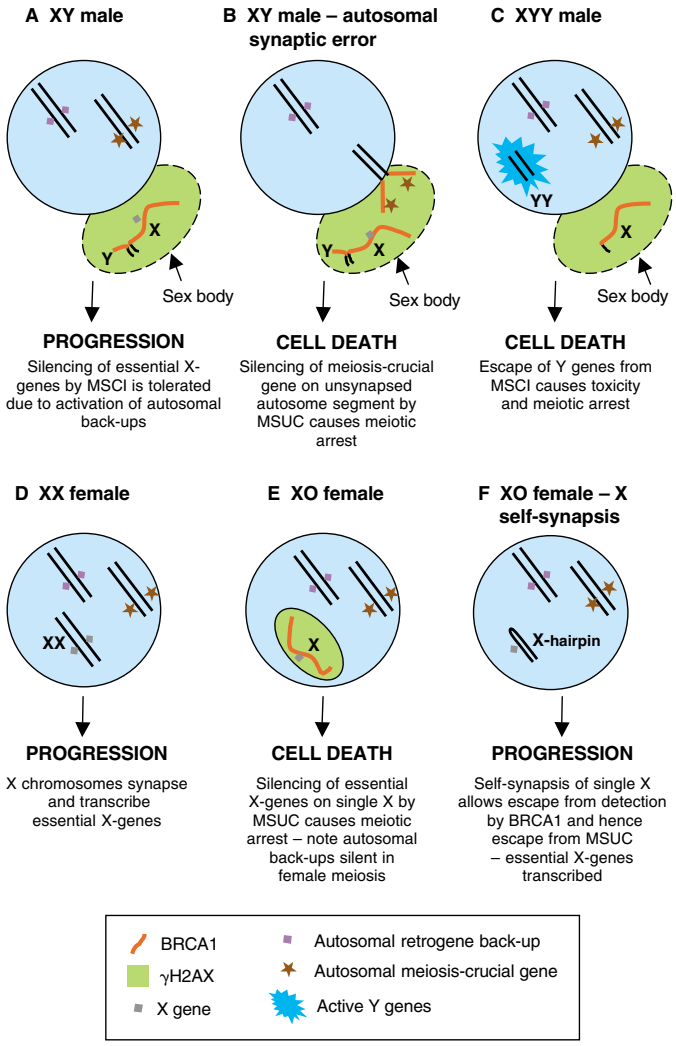


Fig. 3. Meiotic sterility caused by MSUC and by MSCI failure. (**A**) In normal (XY) males, silencing of the single X chromosome by MSCI is tolerated because essential X-encoded genes have autosomally integrated retrogene copies that are expressed during the precise time-window of MSCI-to-PSCR. (**B**) When autosomes fail to synapse, they are also silenced by MSUC. If unsynapsed autosomal segments contain a gene or genes crucial for meiosis, those genes will be silenced, causing meiotic arrest. (**C**) Allowing either the X or Y chromosome to synapse, as seen in XYY males, allows MSCI escape, with the ensuing expression of sex-linked genes causing meiotic arrest. (**D**) In XX females, all chromosomes have homologues and are thus completely synapsed. (**E**) In the XO female mouse, the single X chromosome has no synaptic partner and is therefore silenced by MSUC. Because no autosomal retrogenes are activated in the female gonad, these XO oocytes perish. (**F**) In approximately one-third of XO oocytes, the single X chromosome circumvents MSUC by synapsing non-homologously either with itself, to form a hairpin, or with other chromosomes.

Two studies have found that mice with a mutation in *Spo11*, which encodes an enzyme responsible for meiotic DSB formation, have defective MSCI, indicating a requirement for DSBs in meiotic silencing (Bellani et al., 2005; Barchi et al., 2005). However, other data suggests that meiotic DSBs actually antagonize the MSCI response, possibly by sequestering the MSCI machinery and thereby preventing its relocation to the XY bivalent (Barchi et al., 2005; de Vries et al., 2005).

MSUC and failure of MSCI as causes of infertility

In mice and humans, errors in autosomal synapsis are usually associated with meiotic arrest and impaired fertility, with the severity of the impairment increasing in proportion to the degree of asynapsis (Ashley, 2000). Because the formation of DSBs required for recombination precedes synapsis in mammals, autosomes that fail to synapse retain numerous unrepaired DNA DSBs. In yeast, a recombination checkpoint halts meiosis in response to unrepaired meiotic DSBs (Roeder and Bailis, 2000), and the existence of an equivalent checkpoint in female mammals has recently been inferred (Di Giacomo et al., 2005).

Theoretically, MSUC may also contribute to meiotic arrest when errors in synapsis take place, by silencing 'meiosis-critical' genes (Shiu et al., 2001) (Fig. 3B,E). This concept is most easily understood in the context of the XO female mouse (Fig. 3E). During normal XX female meiosis, the two X chromosomes synapse completely; this permits gene expression from both X chromosomes (Fig. 3D). However, in the XO mouse, the absence of a synaptic partner should trigger an MSUC response (Baarends et al., 2005; Turner et al., 2005), which would ultimately result in the inactivation of the whole X chromosome. Thus, all X-encoded genes essential to cell survival would be silenced, with the obvious result being that the oocyte would perish. XO mice are, nevertheless, fertile because a proportion of the oocytes successfully complete meiosis; it has been hypothesized that these survivors are those in which the single X chromosome forms a hairpin structure during pachytene, effectively engaging in non-homologous self-synapsis (Speed, 1986). Significantly, this self-synapsis would allow the X

chromosome to evade MSUC (Baarends et al., 2005; Turner et al., 2005) (Fig. 3F), thereby allowing the continued transcription of its genes.

As in XO females, the single X chromosome in normal (XY) males is silent throughout meiosis, but, clearly, without ill-effect. Here, MSCI is tolerated because male germ cells are equipped with an X-gene 'back-up' system, in which genes carrying out essential metabolic functions (e.g. *Pgk1* and *Pdha1*), integrate at autosomal sites by virtue of a retroposon-mediated duplication event (Wang, 2004). The expression of these retroposed copies is male-specific and occurs at the initiation of MSCI, thus compensating for the silencing of X-encoded products. The loss of function of at least one of these retrogenes, *Utp14b*, has been shown to cause spermatogenic arrest (Bradley et al., 2004; Rohozinski and Bishop, 2004), highlighting the importance of this back-up system for male meiosis.

Somewhat counter-intuitively, it seems that the failure of MSCI, which results in X and/or Y chromosome gene transcription, leads to spermatocyte death during pachytene. Mutations in genes required for MSCI, including *H2ax* (Fernandez-Capetillo et al., 2003) and *Brca1* (Xu et al., 2003), cause meiotic arrest midway through pachytene; however, in these instances it is difficult to determine whether the primary cause of the meiotic arrest is due to MSCI failure, because these mutants also have defective meiotic recombination and could therefore trigger a putative recombination checkpoint. A better model for addressing the requirement for MSCI in meiosis is the XYY mouse, because the Y chromosomes evade MSCI but without defective meiotic recombination (Fig. 3C). A quantitative analysis of synaptic configurations has shown that, although cells with YY bivalents are abundant during early pachytene, their numbers drop as pachytene proceeds (Turner et al., 2006). The only cells that reach late pachytene are those in which all three sex chromosomes are unsynapsed at the zygotene-pachytene transition and, thus, are completely silenced. These findings reveal how the escape of the Y chromosome from MSCI is cell-lethal at some point between early and mid-pachytene, presumably due to the toxic effects of the misexpression of one or more Y chromosome genes during meiosis. Escape of the X chromosome from MSCI, as occasionally seen in T(X;16)16H males, has an equally lethal effect (Turner et al., 2006).

Post-meiotic transcriptional repression of the sex chromosomes

Recently, two studies have found that the paternally inherited X chromosome of female-mouse pre-implantation embryos is silenced from a much earlier stage than previously thought (Huynh and Lee, 2003; Okamoto et al., 2004). One study (Huynh and Lee, 2003) found evidence for paternal X chromosome inactivation from the two-cell stage, whereas the other (Okamoto et al., 2004) found that inactivation begins at the four-to-eight-cell stage, when *Xist* expression is initiated. In light of their findings, Huynh and Lee (Huynh and Lee, 2003) have suggested that the paternal X chromosome is already inactive at the point of fertilization, and is therefore pre-inactivated during spermatogenesis, by means of MSCI (Huynh and Lee, 2003). This model fitted well with data from *C. elegans*: in XX offspring, the paternal X chromosome exhibits hallmarks of transcriptional silencing that persist from fertilisation until the 10- to 15-cell stage, but this imprint is lost at an earlier embryonic stage in hermaphrodites sired by males that lack MSCI (Bean et al., 2004). In their model, Huynh and Lee (Huynh and Lee, 2003) proposed that *Xist* functions to maintain this pre-inactivated state. If MSCI were to underlie imprinted XCI, then it would clearly have to be maintained beyond meiosis and throughout the rest of spermatogenesis. The role of MSCI in imprinted XCI in mammals has been the subject of controversy (Reik and Ferguson-Smith, 2005). A recent study (Okamoto et al., 2005) has found that transgenes that contain *Xist* and its surrounding genes are not subject to MSCI and can subsequently undergo imprinted XCI in female embryos, demonstrating that *Xist* expression alone is sufficient for imprinted XCI. The debate over the role of MSCI in imprinted XCI has encouraged a re-examination of X and Y chromosome activity in the post-meiotic period. Previous observations had suggested that transcriptional repression of the sex chromosomes was restricted to the period of meiosis (hence MSCI) (see Table 1). For example, some sex-linked genes were found to be reactivated in round spermatids (Hendriksen et al., 1995). In addition, proteins functioning in the initiation phase of MSCI (BRCA1, ATR and γH2AX) were seen to disassociate from the XY bivalent prior to the first meiotic division (Mahadevaiah et al., 2001; Turner et al., 2004), suggesting that MSCI was reversed at this stage.

The view that MSCI is restricted to meiosis was first challenged by Khalil et al. (Khalil et al., 2004), who carried out a comprehensive immunoassay of histone modifications associated with the active and inactive chromatin state on post-pachytene spermatogenic cells. They revealed that some modification products associated with heterochromatin, most notably H3K9me2, remained on the X and Y chromosomes throughout the first and second meiotic divisions and in round spermatids (Fig. 2E-H), indicating that repressive chromosome marks persist on the X and Y chromosomes post-meiotically. However, based on RNA polymerase II antibody staining, they concluded that the X and Y chromosomes were, nevertheless, transcriptionally reactivated. Shortly after this study, three other groups reported that the X and Y chromosomes were heterochromatic, and therefore under-transcribed, in round spermatids (Namekawa et al., 2006; Turner et al., 2006; Greaves et al., 2006) (see Fig. 1). In one of these studies (Namekawa et al., 2006), this 'post-meiotic sex chromatin' (PMSC; Table 1), so-called to reflect its inactive state, was detected at even later stages, during the period of spermatid elongation. An examination of X chromosome transcriptional activity on a gene-by-gene basis using microarray and quantitative reverse transcriptase-PCR analysis revealed that approximately 87% of X chromosome genes are relatively repressed in the post-meiotic period (Namekawa et al., 2006).

The continued presence on the X and Y chromosomes from meiosis into spermiogenesis of histone marks that are associated with a transcriptionally repressed state suggests that post-meiotic sex chromosome repression (PSCR) (see Table 1) is a direct consequence of MSCI. Because MSCI is essentially X and Y chromosome-specific MSUC, Turner et al. (Turner et al., 2006) next investigated whether, in general, meiotic silencing persists into spermiogenesis by studying the transcriptional fate of an unsynapsed autosomal segment in spermatids using RNA FISH (fluorescence in situ hybridization). This unsynapsed autosomal segment did indeed retain a repressed state, brought about by a condensed chromatin structure. PSCR is therefore dependent on MSUC, but it is less penetrant, because the reactivation of genes that reside within the unsynapsed autosomal segment do occur in a small population of spermatids. Thus, the emerging picture is that the X and Y chromosomes are silenced during the pachytene stage of meiosis, and that most sex-linked genes remain repressed post-meiosis. The fact that the X chromosome is transcriptionally repressed during most of spermatogenesis may, at face value, seem to contradict the prediction that spermatogenesis genes accumulate on the X

chromosome (Fisher, 1931; Rice, 1984). However, it is clear that these genes are expressed prior to MSCI taking place, during the spermatogonial divisions (Khil et al., 2004). Nevertheless, a significant minority of X- and Y-linked genes are transcribed during spermiogenesis, and some of these serve an indispensable function in sperm differentiation. For instance, the loss of the long arm of the mouse Y chromosome, which contains several spermatid-expressed genes, including *Ssty1* and *Ssty2* (spermiogenesis specific transcript on the Y 1 and 2, respectively) and *Sly* (Sycp3 like Y-linked), is associated with sperm head abnormalities and male infertility (Toure et al., 2004; Toure et al., 2005). How these genes evade the repressive effects of PSCR remains an intriguing question.

Conclusion

In summary, research into MSCI has proved insightful on many levels. It has revealed unexpected links between the DNA DSB-repair proteins and chromatin silencing, provided functional insight into the link between chromosome-pairing failure and infertility, and may eventually have far-reaching implications in our understanding of imprinting mechanisms. An important avenue for future study will be how, if at all, DNA DSBs are required for the initiation of MSCI and MSUC. If MSUC really contributes to meiotic arrest in the face of chromosome asynapsis, it might also be possible to genetically manipulate MSUC in order to rescue asynapsis-associated germ cell loss, as seen in the XO female mouse. Although the role of MSCI in imprinted XCI in eutherians remains controversial, the possibility that it underlies imprinted XCI in marsupials, which do not have a *Xist* gene (Duret et al., 2006), is an attractive possibility, and could be addressed by examining whether MSCI and PSCR operate in these organisms. Finally, an intriguing question is how are some X and Y chromosome genes expressed during spermatid differentiation, despite the repressive effects imposed by PSCR? These and other questions are likely to keep those of us interested in the fascinating field of MSCI busy for a long time to come.

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