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Pachytene Asynapsis Drives Meiotic Sex Chromosome Inactivation and Leads to Substantial Postmeiotic Repression in Spermatids

Short Article

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Summary

Transcriptional silencing of the sex chromosomes during male meiosis (MSCI) is conserved among organisms with limited sex chromosome synapsis, including mammals. Since the 1990s the prevailing view has been that MSCI in mammals is transient, with sex chromosome reactivation occurring as cells exit meiosis. Recently, we found that any chromosome region unsynapsed during pachytene of male and female mouse meiosis is subject to transcriptional silencing (MSUC), and we hypothesized that MSCI is an inevitable consequence of this more general meiotic silencing mechanism. Here, we provide direct evidence that asynapsis does indeed drive MSCI. We also show that a substantial degree of transcriptional repression of the sex chromosomes is retained postmeiotically, and we provide evidence that this postmeiotic repression is a downstream consequence of MSCI/MSUC. While this postmeiotic repression occurs after the loss of MSUC-related proteins at the end of prophase, other histone modifications associated with transcriptional repression have by then become established.

Introduction

During meiosis in male mammals, the behavior of the X and Y chromosomes differs markedly from that of the autosomes. Whereas autosomal homologs synapse along their entire length, the largely nonhomologous X and Y chromosomes show only limited synapsis mediated by their homologous pseudoautosomal regions (Burgoyne, 1982). Furthermore, whereas the autosomes exhibit abundant transcription during pachytene, the sex chromosomes are transcriptionally silenced, a process termed meiotic sex chromosome inactivation (MSCI), and form a peripheral, densely staining domain called the sex or XY body (Handel, 2004; Solari, 1974). MSCI initiates as cells enter pachytene, when the unsynapsed regions of the X and Y chromosomes become enriched in the tumor suppressor protein BRCA1 and the kinase ATR; this leads to the phosphorylation of the nucleosomal core histone H2AX, triggering the chromatin changes that result in silencing (Bellani et al., 2005; Fernandez-Capetillo et al., 2003; Turner et al., 2004). MSCI is widely regarded as being a transient phenomenon, because a number of X and Y genes subjected to MSCI are transcribed postmeiotically in spermatids (Hendriksen, 1999; Hendriksen et al., 1995).

In a recent study, we found that unsynapsed autosomes also accumulate BRCA1 and ATR during pachytene, with consequent H2AX phosphorylation and transcriptional silencing (Turner et al., 2005). This, together with the work of Baarends et al. (2005), demonstrated that a general silencing mechanism operates during mammalian meiosis that senses and silences unsynapsed chromosome segments; this led us to hypothesize that MSCI is a consequence of the fact that the X and Y axes remain largely unsynapsed during pachytene of normal male meiosis (Turner et al., 2005). We now refer to this general silencing mechanism as meiotic silencing of unsynapsed chromatin (MSUC) (Schimenti, 2005) in order to distinguish it from the mechanistically distinct meiotic silencing by unpaired DNA (MSUD) first described in *Neurospora* (Shiu et al., 2001). Meiotic silencing also operates in *Caenorhabditis elegans* (Bean et al., 2004). Although the role of meiotic silencing is unclear, it may have evolved as a means of genome defense or to aid in the detection and elimination of cells with synaptic errors (Bean et al., 2004; Turner et al., 2005).

Recently, it was established that in preimplantation XX mouse embryos one X chromosome, the paternal X chromosome, is transcriptionally repressed earlier than previously thought, although there has been controversy as to whether this repression occurs de novo at the four- to eight-cell stage, or whether a "preinactivated X" is provided by the sperm at fertilization (Huynh and Lee, 2003; Okamoto et al., 2004, 2005). Proponents of the preinactivated X hypothesis have suggested that the inactivation of the paternal X may be causally linked to the inactivation of the X chromosome during pachytene of male meiosis (Huynh and Lee, 2005). This implies that either a degree of X chromosomal repression or some other form of mark predisposing to paternal X inactivation is maintained from pachytene through to the mature sperm. Significantly, Khalil et al. (2004) have reported that a marker of transcriptionally repressed chromatin, histone H3 dimethylated at lysine-9 (dimH3K9), appears on the X and Y chromosomes at the end of pachytene and persists through the meiotic divisions into early spermiogenesis. However, they concluded based on RNA polymerase II antibody staining that the X and Y chromosomes were nevertheless reactivated in spermatids.

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In the present study, we set out to first test our hypothesis that MSCI is simply a manifestation of the MSUC response by studying mice in which the X or the Y chromosome is given the opportunity to synapse; the hypothesis predicted that this should circumvent MSCI, and this proved to be the case. We then asked whether, despite the known transcription of some X and Y genes in spermatids, there is any postmeiotic X and Y gene repression or other evidence that "a memory" of MSCI is carried over into the postmeiotic period.

Results

Asynapsis Drives Meiotic Y Chromosome Silencing

To test whether synapsis of the Y chromosome allows it to escape MSCI, we used XYY males, in which a proportion of cells exhibit complete YY synapsis (Rodriguez and Burgoyne, 2000). We immunostained pachytene XYY spermatocytes for the MSCI marker phosphorylated H2AX (γH2AX) and the axial element protein SCP3 (Lammers et al., 1994). Of 145 pachytene spermatocytes scored, 65 had incomplete YY synapsis, and, as is the case for the X and Y chromosomes in normal male meiosis, in the majority of these cells the three sex chromosomes were only synapsed distally via their PARs; in all 65 cells, the 3 sex chromosomes were found within the γH2AX-positive sex body domain (Figures 1A and 1B). In the remaining 80 cells, the Y chromosomes had synapsed completely (Figure 1C). In contrast to the incompletely synapsed Y chromosomes, these fully synapsed YY bivalents predominantly displayed reduced or absent γH2AX staining, with 22% being completely γH2AX negative (Figure 1C) and 45% being γH2AX positive in only a restricted region of the Y chromatin; only 33% were positive for γH2AX along the length of the bivalent. Notably, while YY bivalents showing residual γH2AX staining were associated with the X chromosome-containing sex body, those that were completely γH2AX negative were spatially separated from the sex body (Figure 1C). These results show that synapsis of the Y axis can afford protection of the Y chromosome from H2AX phosphorylation.

We next asked whether the absence of γH2AX on fully synapsed YY bivalents correlated with escape from MSCI. To do this, we combined γH2AX immunostaining with RNA FISH for the Y chromosome gene *Uty* (Figures 1D and 1E). In wild-type (XY) males, *Uty* is highly expressed in spermatogonia and early prophase cells and is then silenced during early pachytene as a result of MSCI (data not shown). As expected, we observed pachytene cells with both silent and misexpressing *Uty* genes in XYY males. Subsequent DNA FISH confirmed that when the *Uty* genes were γH2AX positive they were silent (*Uty*, n = 61; Figure 1D), but when they were γH2AX negative, and hence located on synapsed chromatin, they were active (*Uty*; n = 48; Figure 1E). We conclude that asynapsis does indeed drive Y chromosome silencing during male meiosis.

Asynapsis Drives Meiotic X Chromosome Silencing

Next, we tested whether X chromosome synapsis would also allow for escape from H2AX phosphorylation. The logical way to test this would have been to use XXY males; however, these males have a complete spermatogonial block caused by the toxic effects of double X chromosome dosage and hence are devoid of meiotic cells (Mroz et al., 1999). We instead used male carriers of the reciprocal X-autosome translocation T(X;16)16H (Ford and Evans, 1964) (Figure 2A), in which a number of different synaptic associations occur between the translocation products, the Y chromosome, and the intact chromosome 16 (for further details of configurations, see Odorisio et al., 1998; Solari, 1971). In the second commonest association, the 16^{X} translocation product synapses with the Y chromosome, and the X^{16} translocation product synapses with chromosome 16: the so-called "bivalent-bivalent" configuration (Figure 2A). Of 137 pachytene bivalent-bivalent configurations examined, 65 showed synapsis of the X^{16}/16 bivalent that was restricted to the regions of homology; the unsynapsed regions were γH2AX positive (Figure 2B). In the remaining 72 configurations, the entire X^{16} translocation product, which encompasses approximately half of the X chromosome, had achieved complete (and hence largely nonhomologous) synapsis with chromosome 16 (Figure 2C). Of these, 38 retained some γH2AX staining, and, in the majority (35/38) of these bivalents, the γH2AX-positive segment was associated with the sex body. In the remaining 34 cells, this complete synapsis was associated with the absence of γH2AX from the X^{16}/16 bivalent, and, in all of these cases, the bivalent was away from the sex body (Figure 2C). Thus, synapsis of the X axis can prevent X chromosome H2AX phosphorylation.

We then carried out RNA FISH for the X chromosome gene *Ddx3x* (which is located on the X^{16} translocation product) in T(X;16)16H males to correlate loss of γH2AX with escape from MSCI. As was the case with the Y chromosome of XYY males, we found that synapsis of the X chromosome segment can afford escape from MSCI: when *Ddx3x* was γH2AX positive, it was silent (*Ddx3x*; n = 36; Figure 2D), but when it was γH2AX negative, it was active (*Ddx3x*; n = 18; Figure 2E). These results show that asynapsis drives X chromosome silencing. In addition, they confirm our previous suggestion that nonhomologous synapsis affords escape from MSUC.

Meiotic Cells Exhibiting Escape from MSCI Are Eliminated during Pachytene

In the process of studying meiosis in XYY and T(X;16)16H males, we noted an unexpected relationship between escape from MSCI and the pachytene substage. Specifically, XYY cells containing completely synapsed YY bivalents and T(X;16)16H cells containing nonhomologously synapsed X^{16}/16 bivalents seemed to be exclusive of the early- or early-mid-pachytene subtype. In order to confirm that these cells are eliminated prior to late pachytene, we then studied sex chromosome synaptic configurations in 100 XYY and 186 T(X;16)16H late-pachytene cells. If cells exhibiting complete escape from MSCI, assayed by the absence of γH2AX, had not been subject to negative selection, we would have expected them to comprise 12% and 7% of the late-pachytene pool in XYY and T(X;16)16H males, respectively (see the legends of Figures 1 and 2 for explanation).

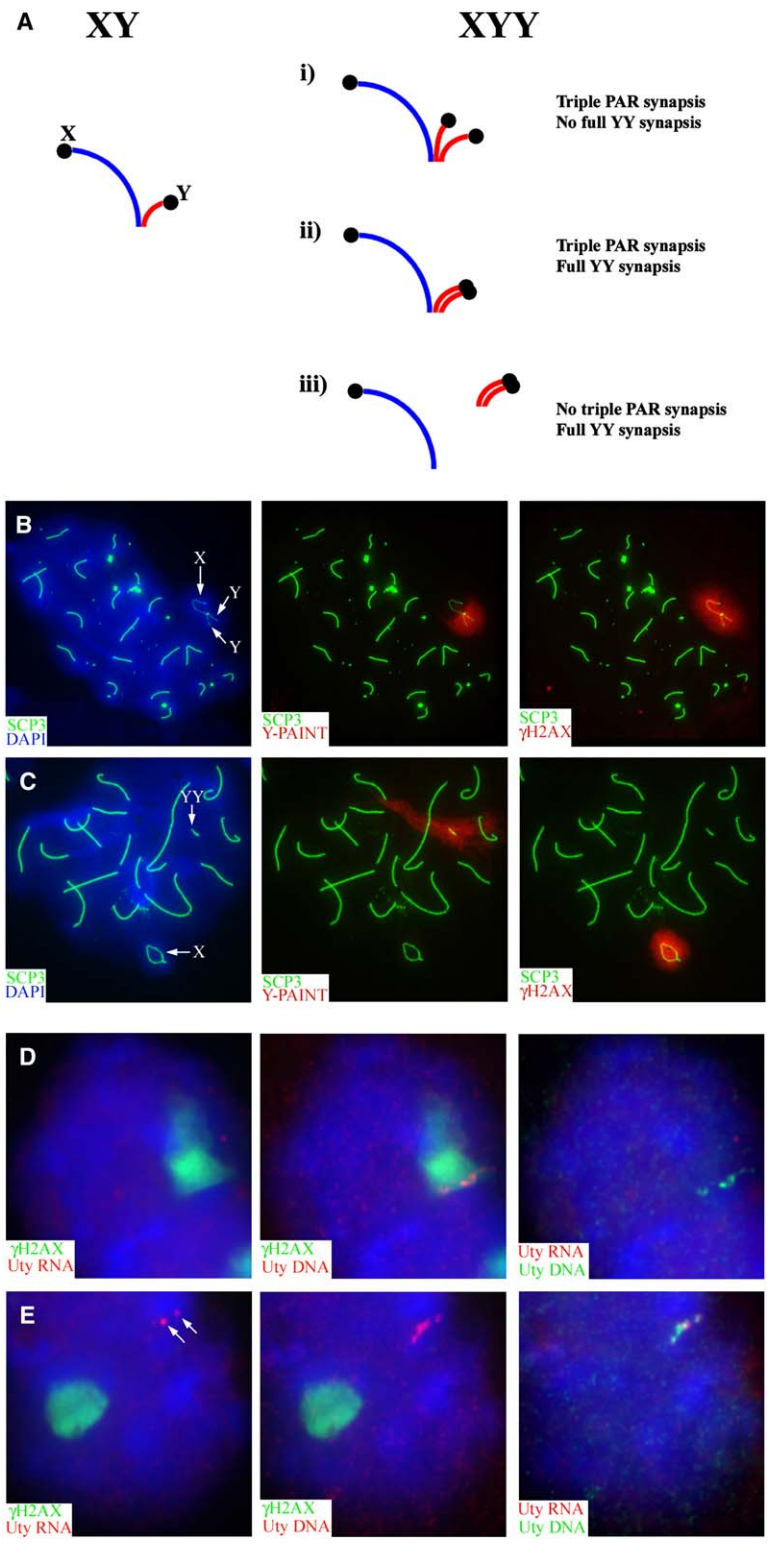

Figure 1. Synapsed Y Chromosomes Escape MSCI in XYY Males

(A) Cartoon showing synapsis in XY and XYY males. In XY males, synapsis is restricted to the pseudoautosomal region (PAR). In XYY males, synapsis is either (i) via the PARs, as in XY males (no full YY synapsis), or there is full YY synapsis. In the case of full YY synapsis, the resulting YY bivalent may (ii) or may not (iii) synapse with the X at the PAR.

(B and C) Relationship between YY synapsis and H2AX phosphorylation. (B) Example of PAR synapsis in XYY males. All three sex chromosomes are γH2AX positive and are enclosed in the sex body. (C) Example of full YY synapsis. The resulting YY bivalent in this nucleus has not synapsed with the PAR of the X chromosome, and the YY bivalent lies outside the sex body, in the autosomal compartment, and is γH2AX negative. Cells exhibiting *complete* absence of γH2AX from the YY bivalent comprise 12% (18/145) of all pachytene cells analyzed.

(D and E) Relationship between YY synapsis and *Uty* transcription. (D) Example of PAR synapsis in XYY males. The unsynapsed *Uty* genes lie within the sex body, are γH2AX positive, and are silent. (E) Example of full YY synapsis. The YY bivalent lies outside the sex body and is γH2AX negative, and the *Uty* genes are transcribing (arrows in first panel).

In fact, none of the late-pachytene XYY cells contained γH2AX-negative Y chromosomes, and only one late-pachytene T(X;16)16H cell (0.5%) contained a γH2AX-negative X^{16} chromosome. These findings suggest that there is a selective loss of XYY and T(X;16)16H cells exhibiting escape from MSCI at some point during early-mid pachytene.

Transcriptional Repression of the Sex Chromosomes during Round Spermatid Development

Next, we used Cot1 RNA FISH (Turner et al., 2005), combined with DAPI staining as a marker of chromatin density, to directly monitor the transcriptional activity of the X and Y chromatin after γH2AX disappears during

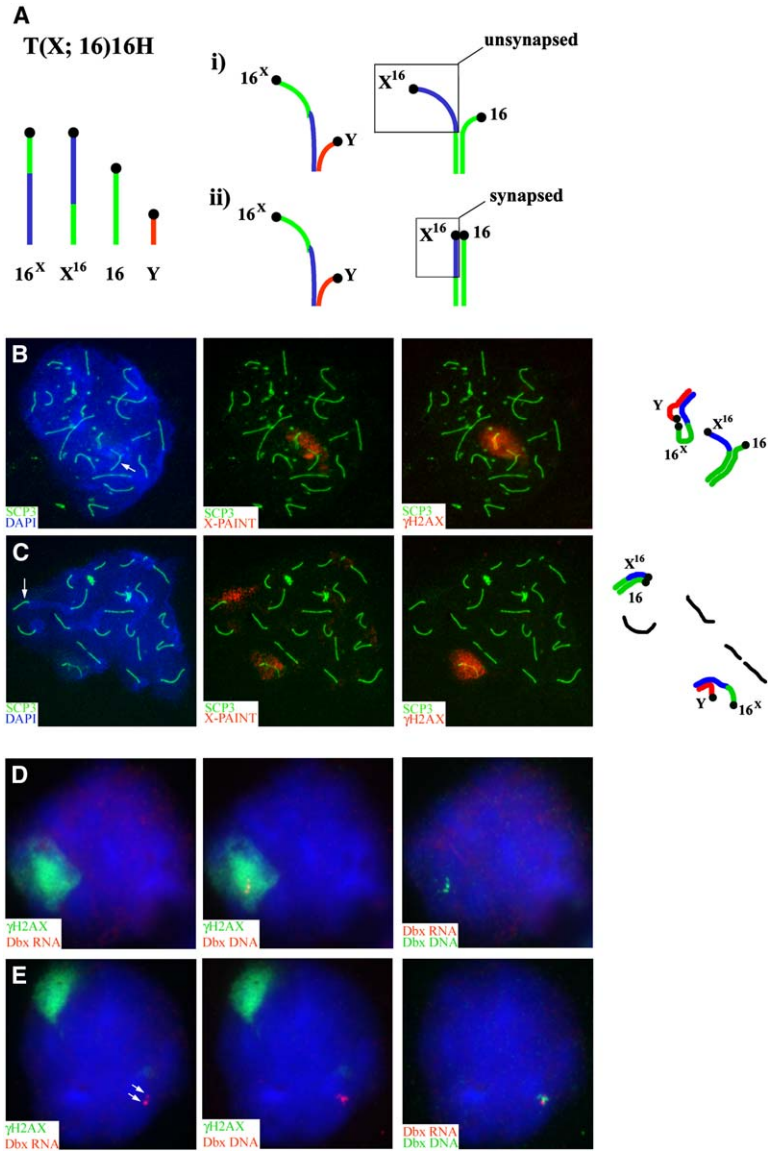

Figure 2. Synapsed X Chromosomes Escape MSCI in T(X;16)16H Males

(A) Cartoon showing the T(X;16)16H karyotype and the so-called "bivalent-bivalent" synaptic configuration, which comprises 29% of all possible synaptic configurations (Odorisio et al., 1998). In all bivalent-bivalent cases, synapsis between the 16^X and the Y chromosome is via the PARs. (i) Synapsis between the X^{16} chromosome and chromosome 16 is via the regions of homology in 47% (65/137) of cases. (ii) In the remaining 53% of cases (72/137), the entire bivalent undergoes complete nonhomologous synapsis.

(B and C) Relationship between the X^{16} chromosome and chromosome 16 synapsis and H2AX phosphorylation. The X^{16}/16 bivalent is shown by an arrow in the first panel. Synaptic configurations are shown in the last panel. (B) Example of conventional bivalent-bivalent configuration. There is no X^{16}/16 nonhomologous synapsis, and the resulting unsynapsed regions are γH2AX positive and reside within the sex body, together with the 16^X/Y bivalent. (C) Example of complete X^{16}/16 synapsis. The resulting bivalent is γH2AX negative and lies outside the sex body, in the autosomal compartment. Cells exhibiting an absence of γH2AX from the X^{16}/16 bivalent comprise 25% (34/137) of bivalent-bivalent configurations. Since bivalent-bivalent configurations comprise 29% of all possible configurations in T(X;16)16H males, the overall frequency of cells with an absence of γH2AX from the X^{16}/16 bivalent is 7%.

(D and E) Relationship between X^{16}/16 synapsis and *Ddx3x* (written as *Dbx* in the figure) transcription. (D) Example of conventional bivalent-bivalent configuration. There is no X^{16}/16 nonhomologous synapsis; the unsynapsed *Ddx3x* gene lies within the sex body, is γH2AX positive, and is silent. (E) Example of complete X^{16}/16 synapsis. The synapsed *Ddx3x* gene lies outside the sex body, is γH2AX negative, and is transcribing (arrows in first panel).

diplotene. We analyzed interphase secondary spermatocytes (n = 48), a stage that immediately follows the first meiotic division, and round spermatids (n = 300), which are the haploid products of the second meiotic division. As expected, the nuclei contained regions of centromeric heterochromatin that were brightly stained with DAPI and were Cot1 negative (transcriptionally repressed), surrounded by euchromatin, which appeared faintly DAPI stained and enriched in Cot1 signals (transcriptionally active; Figures 3A and 3B). In addition, we observed a round nuclear subdomain that was of intermediate brightness when stained with DAPI and, in round spermatids, was characteristically located next to the chromocenter (Figure 3B). We found this subdomain, as defined by DAPI staining, in all round spermatids irrespective of stage. Chromosome painting revealed that this structure was the X or Y chromosome (Figures 3A and 3B), and deconvolution revealed that it was depleted of Cot1 signals relative to the surrounding euchromatin in 93% of X-bearing and 79% of Y-bearing secondary spermatocytes or round spermatids (Figures 3A and 3B). dimH3K9 was associated with the X and Y chromosomes in the vast majority (76%, n = 100; Figure 3C) of round spermatids, and we also found that the heterochromatin protein CBX1 (formerly called M31 or HP1β) was enriched in the same domain in 44% of round spermatids (n = 100; Figure 3D). We conclude that compared to the autosomes, the sex chromosomes remain relatively repressed into the postmeiotic period.

Postmeiotic dimH3K9 Enrichment and Transcriptional Repression Is a Downstream Consequence of MSUC

We next sought to determine whether the localization of dimH3K9 to the X and Y chromosomes in spermatids is dependent on MSCI having taken place (and thus is a downstream consequence of the MSUC response to pachytene asynapsis). We were unable to use the XYY or T(X;16)16H models for this purpose, because of the elimination of those cells with Y or X MSCI failure, respectively, during pachytene. We therefore used the

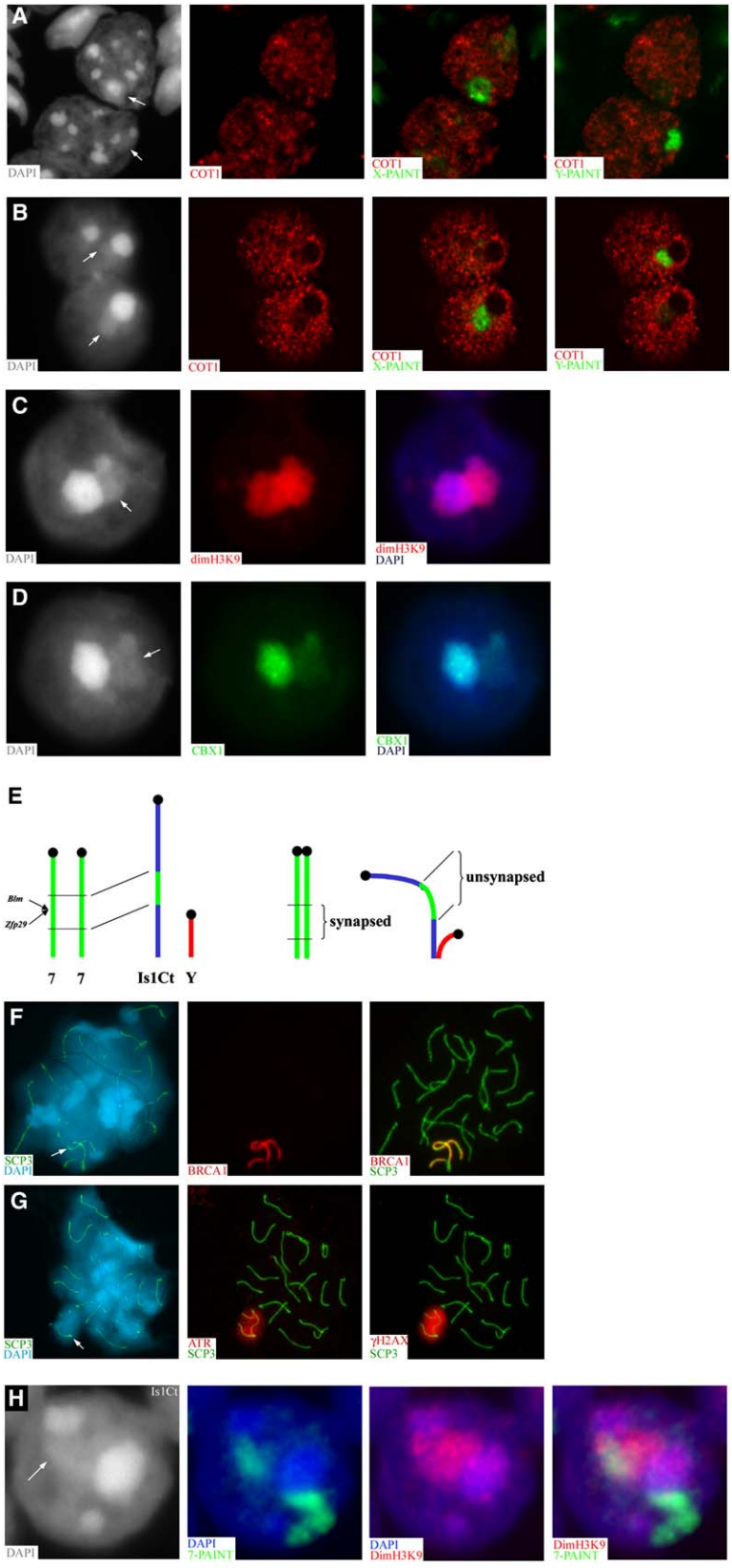

Figure 3. Transcriptional Repression of Sex Chromosomes and Unsynapsed Autosomes in Round Spermatids

(A–D) X and Y transcriptional repression in secondary spermatocytes and round spermatids. Cot1 RNA FISH in (A) secondary spermatocytes and (B) round spermatids. The sex chromosomes are indicated by arrows in the first panel. DAPI is shown in black and white to allow easier visualization of the condensed X and Y chromosomes. (C) Dimethylated H3K9 staining in round spermatids; (D) CBX1 (formerly HP1β) staining in round spermatids. Both antibodies mark the chromocenter and the X/Y DAPI dense domain.

(E–H) Transcriptional repression of unsynapsed autosomal DNA in meiotic and postmeiotic Is1Ct cells. (E) Cartoon of the Is1Ct karyotype, showing the positions of the *Blm* and *Zfp29* genes and of the synaptic configuration. The X chromosome, which contains an inserted portion of chromosome 7, is coated with (F) BRCA1 and with (G) ATR and γH2AX in all pachytene spermatocytes. (H) In Is1Ct round spermatids, the X-inserted chromosome 7 segment (arrow in first panel), like the rest of the X chromosome, is DAPI bright and dimH3K9 positive, in contrast to the normal chromosome 7.

Is1Ct male mouse model (Ohno and Cattanach, 1962), in which an extra copy of a segment of chromosome 7 is inserted into the X chromosome and has no synaptic partner (Figure 3E). SCP3 staining of spread nuclei confirmed that the X-inserted chromosome 7 segment was constitutively unsynapsed during pachytene (n = 100),

and in all cells it was enriched for the MSUC proteins BRCA1, ATR, and γH2AX; in contrast, the synapsed chromosome 7 bivalent was of course devoid of these proteins (Figures 3F and 3G). Staining for dimH3K9 revealed that in X-bearing spermatids the X-inserted chromosome 7 segment was incorporated into the spermatid "sex body" and was enriched in dimH3K9 (n = 64; Figure 3H). Thus, unsynapsed DNA in general is targeted by a dimH3K9 "imprint" that is perpetuated into spermiogenesis.

To see whether the dimH3K9-enriched X-inserted chromosome 7 segment was also transcriptionally repressed, we carried out RNA FISH for two genes mapping within the inserted chromosome 7 segment: *Blm* and *Zfp29* (Figures 3E and 4). In wild-type mice, *Blm* is expressed before and during meiosis and in early round spermatids (Seki et al., 1998) (Figures 4A–4C), while *Zfp29* is expressed in pachytene cells and over three-quarters of round spermatids (Denny and Ashworth, 1991) (Figures 4D–4F). We carried out subsequent DNA FISH, in order to ensure that chromatin accessibility problems did not influence our ability to detect the relevant transcript (van Raamsdonk and Tilghman, 2001), as well as chromosome painting, to determine unambiguously the chromosomal origin of each locus. We observed transcription of *Blm* from all three loci in early meiotic Is1Ct cells (n = 30; Figure 4A), indicating that the X-inserted chromosome 7 segment is competent to transcribe prior to the onset of MSUC. In contrast, in all late-pachytene cells examined (n = 100), expression of *Blm* was detected from the "synapsed" chromosome 7 loci, but not from the "unsynapsed" X-inserted chromosome 7 locus (Figure 4B), demonstrating that MSUC operates with high efficiency. Importantly, in 98% (n = 60) of X-bearing, *Blm*-expressing round spermatids, we also detected transcription of *Blm* from the native chromosome 7, but not from the X-inserted locus (Figure 4C). For *Zfp29*, once again transcription was detected only from the synapsed chromosome 7 loci in all late-pachytene cells examined (n = 100; Figure 4D), and the site of transcription in *Zfp29*-expressing round spermatids was again markedly skewed in favor of the native chromosome 7 locus. However, this skewing was less extreme than for *Blm*: 71% of X-bearing spermatids showed exclusive expression from the native chromosome 7 (Figure 4E), 24% showed expression from both the native and X-inserted loci (Figure 4F), and 5% showed a single transcription spot originating from the X chromosome-inserted segment only (n = 91).

These findings show that pachytene asynapsis and the MSUC response does lead to a substantial postmeiotic heterochromatinization and transcriptional repression, and, by extrapolation, they imply that the heterochromatic conformation of the X and Y chromosomes in chromosomally normal spermatids is a consequence of their unsynapsed state during pachytene.

Discussion

In this study, we have provided direct evidence that MSCI is indeed an MSUC response to the unsynapsed X and Y axes during pachytene, substantiating our earlier hypothesis (Turner et al., 2005). In mouse meiosis, the formation of meiotic DNA double-strand breaks (DSBs) precedes meiosis (Mahadevaiah et al., 2001), and chromosomes that fail to synapse, including the X chromosome, are also sites of persistent DSBs. It is therefore possible that persistent DSBs, rather than asynapsis per se, drive MSUC.

During the analysis of the XYY and T(X;16)16H males, there was a tight correlation between loss of γH2AX staining from the Y or X^{16} chromosome following synapsis and escape of Y and X genes from transcriptional inactivation. However, a substantial proportion of the fully synapsed bivalents retained at least some γH2AX staining. Why might this be? It is most probably because they have entered pachytene with incomplete synapsis, which triggers H2AX phosphorylation, and then there is subsequent completion of synapsis but dephosphorylation has not yet been completed. This phenomenon has previously been documented in the context of "synaptic adjustment" of the 1^{16} bivalent in mice heterozygous for the T70H and T1Wa translocations (Baarends et al., 2000; Mahadevaiah et al., 2001).

Notably, the retention or absence of γH2AX on synapsed bivalents is closely correlated with the proximity of that segment to the sex body. When residual γH2AX is present on either the YY or X^{16} chromosome, the bivalent associates with the sex body, while complete loss of γH2AX is associated with spatial separation of the bivalent from the sex body. Since the presence of γH2AX indicates transcriptional silencing, this suggests that transcriptionally silent DNA clusters within the meiotic nucleus. Such compartmentalization of genes according to their transcriptional state has been observed in other systems, such as in erythroid cell progenitors (Osborne et al., 2004).

In the process of studying XYY and T(X;16)16H male meiosis, we noted that pachytene cells exhibiting sex chromosome escape from MSCI are eliminated prior to late pachytene, suggesting that MSCI is essential for the completion of meiotic prophase. Thus, MSCI failure is undoubtedly a contributory cause of the pachytene losses seen in T(X;16)16H males (Reader and Solari, 1969) and provides an explanation for the marked levels of pachytene loss associated with XYY spermatogenesis in mouse and man (Palmer et al., 1990; Solari and Valzacchi, 1997). It is significant that the severe selection observed during pachytene by Palmer et al. (1990) in XYY cells exhibiting a particularly high level of full YY synapsis was not observed for the XYY cells studied by Mahadevaiah et al. (2000) (see Figure 2 therein), in which the presence of a large deletion in the long arm of one of the Y chromosomes largely prevented full YY synapsis. Although targeted mutations of *Brca1* (Turner et al., 2004) and *H2AX* (Fernandez-Capetillo et al., 2003), which result in defective MSCI, also result in pachytene spermatocyte loss, these genes also function in meiotic recombination, raising the alternative possibility of pachytene checkpoint-mediated spermatocyte elimination (Roeder and Bailis, 2000). We hypothesize that escape from MSCI in XYY and T(X;16)16H males results in pachytene loss due to the inappropriate expression of one or more sex-linked genes during meiosis.

We also show that the X and Y chromosomes remain substantially transcriptionally repressed in the postmeiotic period and present evidence that this is a downstream consequence of the MSUC response. The

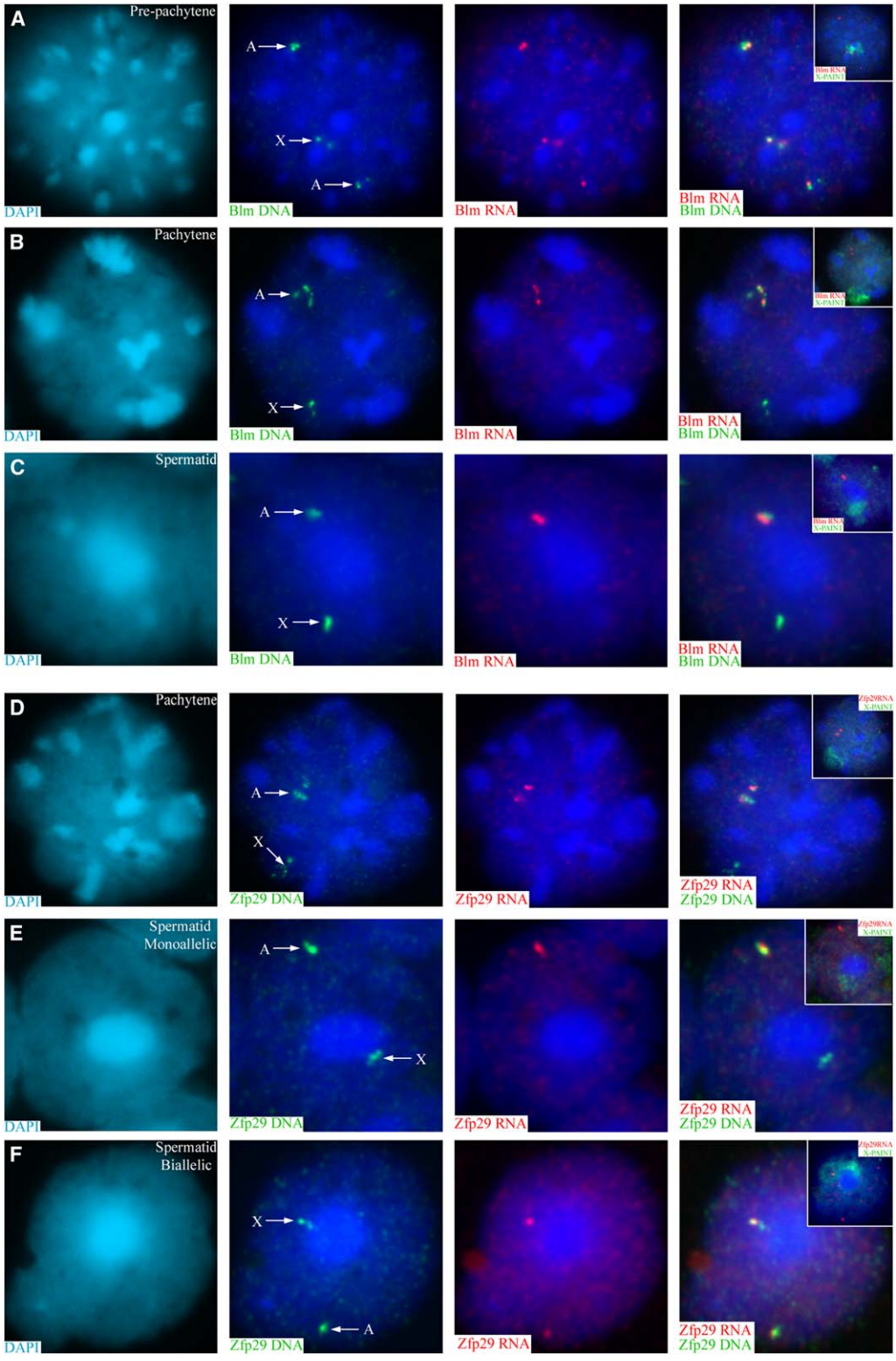

Figure 4. *Blm* and *Zfp29* Transcription in Is1Ct Spermatogenic Cells

The first panel shows DAPI pseudocolored light blue to allow for easier identification of cell types. The arrows in the second panel show autosomal (chromosome 7) versus X-inserted loci, as determined by X chromosome painting, which is shown as an inset in the last panel. (A–C) *Blm* transcription. (A) In prepachytene cells, all three *Blm* loci are transcriptionally active. (B) In pachytene cells, the synapsed chromosome 7 *Blm* loci are active, and the unsynapsed X-inserted *Blm* locus is silent. (C) In round spermatids, transcription occurs only from the native (previously synapsed) chromosome 7. (D–F) *Zfp29* transcription. (D) In pachytene cells, the synapsed chromosome 7 *Zfp29* loci are active, and the unsynapsed X-inserted *Zfp29* locus is silent. (E and F) In round spermatids, transcription occurs only from the (E) native (previously synapsed) chromosome 7 in 71% of cells, and from (F) both loci in 24% of cells.

gene-specific RNA FISH for the one X gene, one Y gene, and two autosomal genes indicated that the MSUC response to asynapsis was extremely efficient at blocking transcription. In contrast, the postmeiotic transcriptional repression was less complete. We are now systematically studying X and Y transcription in round spermatids by gene-specific RNA FISH, and this has confirmed that while some genes display complete reactivation (e.g., *Ube1x* and *Ube1y* [Hendriksen et al., 1995; Odorisio et al., 1996]) or de novo transcription (e.g., *Sly*; [Touré et al., 2005]), others show mosaic reactivation (e.g., *Ddx3x*). Consistent with these preliminary findings, another recent study has also found that the vast majority of X chromosome genes remain repressed postmeiosis, but that a small proportion reactivates (Namekawa et al., 2006). The imperfect nature of postmeiotic sex chromosome inactivation is likely to result from a balance between the repressive effects of MSUC and other factors that act to maintain expression of some X and Y genes essential for spermiogenesis, such as *Ube1x* and *Ube1y*, which function in histone ubiquitination (Baarends et al., 1999).

What might the role of postmeiotic sex chromosome repression be? We recently discovered that deletions of MSYq, encompassing multicopy spermatid-expressed genes (Touré et al., 2005), are associated with marked upregulation of a number of spermatid-expressed X genes together with some Y genes located on the short arm (Ellis et al., 2005). The amplification of X and Y gene families is considered a hallmark of a past or ongoing "genomic conflict" between sex-linked meiotic drivers and repressors (Hurst, 1992). The concept underlying this genomic conflict is that with the evolution of the mammalian X and Y chromosomes and their divergence in gene content, functional differences might have arisen between X- and Y-bearing sperm that led to a distortion of the sex ratio. Coincident with this, the progressive reduction in the extent of XY synapsis will have resulted in transcriptional repression during pachytene (MSUC). Ellis et al. (2005) suggested that one way to suppress the genomic conflict and consequent sex ratio distortion would be to repress X and Y gene transcription in spermatids, thus providing an evolutionary basis for the extension of X and Y transcriptional repression into spermatids.

Although the finding that the X chromosome remains substantially repressed into spermiogenesis might appear to support the preinactivation hypothesis (Huynh and Lee, 2003, 2005), a recent study has demonstrated that MSCI is not a prerequisite for imprinted XCI in female embryos (Okamoto et al., 2005). One way to reconcile these apparently conflicting observations has recently been suggested in which the X chromosome is inherited in an epigenetically inactive state, but then is rapidly reactivated at zygotic gene activation, prior to resilencing at the four- to eight-cell stage by an *Xist*-dependent mechanism (Reik and Ferguson-Smith, 2005).

Experimental Procedures

Mice

XYY males were generated on an MF1 background by mating XY males to XY^{d1} females. The Y^{d1} chromosome is deleted for most copies of the *Rbmy* gene cluster, but other Yp genes, including *Uty*, are left intact (Mahadevaiah et al., 1998). The T(X;16)16H and Is1Ct mice were generated on a mixed 129/SvEv-C3H/HeJ background and on an A/J background, respectively, from heterozygous female carriers.

RNA FISH, Immunofluorescence, and Chromosome Painting

RNA FISH, immunofluorescence, and chromosome painting were performed as described previously (Turner et al., 2005), with the following modifications. For dimH3K9 (Upstate; 1;100 dilution) immunofluorescence, spermatogenic cells were pelleted and then fixed in 1.85% formaldehyde/0.1 M sucrose for 10 min. They were then washed once in PBS, placed onto slides, and then permeabilized in 0.5% Triton X-100 for 10 min, before rinsing and blocking. For *Uty* RNA FISH, we used mouse BAC CITB-246A22 (Research Genetics), which has all exons of *Ddx3y* and exons 1–6 of *Uty*. Exons 1–5 of *Ddx3y* were deleted, and the *Uty* exons covering exon 7 to the 3′ UTR were derived from cDNA and added downstream of exon 6. These modifications were conducted as part of functional studies unrelated to the present work (M.J.M., unpublished data). The *Ddx3x* BAC CITB-551M19 (Research Genetics) contains the entire transcribed portion of *Ddx3x* (11,578 bp) and the 3′ end of *Usp9x* (28,285 bp). Both the *Uty* and *Ddx3x* BACs were purified by a caesium chloride gradient. For the *Blm* and *Zfp29* RNA FISH experiments, we used BAC clones (bMQ98G17 and bMQ431G03, respectively) acquired from the Wellcome Trust Sanger Institute, Cambridge, UK and prepared by a standard alkaline lysis procedure. Hybridization reactions consisted of 100 ng biotin-labeled BAC probe, 3 μg mouse Cot1 DNA, and 10 μg salmon sperm DNA and were carried out overnight at 37°C. The rabbit SCP3 antibody was obtained from Abcam (ab15092).

DNA FISH

DNA FISH was carried out after RNA FISH and immunofluorescence. Slides were prepared in exactly the same way as for chromosome painting. We used digoxigenin-labeled probes, prepared by using the Digoxigenin Nick Translation Kit (Roche), and hybridizations were carried out as for RNA FISH (see above). We used the same stringency washes as described previously (Turner et al., 2005), and DNA FISH signals were developed by using anti-DIG-FITC (Chemicon), diluted 1:10, for 1 hr at 37°C.

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References

Baarends, W.M., Hoogerbrugge, J.W., Roest, H.P., Ooms, M., Vreeburg, J., Hoeijmakers, J.H.J., and Grootegoed, J.A. (1999). Histone ubiquitination and chromatin remodeling in mouse spermatogenesis. Dev. Biol. *207*, 322–333.

Baarends, W.M., van der Laan, R., and Grootegoed, J.A. (2000). Specific aspects of the ubiquitin system in spermatogenesis. J. Endocrinol. Invest. *23*, 597–604.

Baarends, W.M., Wassenaar, E., van der Laan, R., Hoogerbrugge, J., Sleddens-Linkels, E., Hoeijmakers, J.H., de Boer, P., and Grootegoed, J.A. (2005). Silencing of unpaired chromatin and histone H2A ubiquitination in mammalian meiosis. Mol. Cell. Biol. *25*, 1041–1053.

Bean, C.J., Schaner, C.E., and Kelly, W.G. (2004). Meiotic pairing and imprinted X chromatin assembly in *Caenorhabditis elegans*. Nat. Genet. *36*, 100–105.

Bellani, M.A., Romanienko, P.J., Cairatti, D.A., and Camerini-Otero, R.D. (2005). SPO11 is required for sex-body foramtion, and Spo11 heterozygosity rescues the prophase arrest of *Atm*−/− spermatocytes. J. Cell Sci. *118*, 3233–3245.

Burgoyne, P.S. (1982). Genetic homology and crossing over in the X and Y chromosomes of mammals. Hum. Genet. *61*, 85–90.

Denny, P., and Ashworth, A. (1991). A zinc finger protein-encoding gene expressed in the post-meiotic phase of spermatogenesis. Gene *106*, 221–227.

Ellis, P.J., Clemente, E.J., Ball, P., Toure, A., Ferguson, L., Turner, J.M., Loveland, K.L., Affara, N.A., and Burgoyne, P.S. (2005). Deletions on mouse Yq lead to upregulation of multiple X- and Y-linked transcripts in spermatids. Hum. Mol. Genet. *14*, 2705–2715.

Fernandez-Capetillo, O., Mahadevaiah, S.K., Celeste, A., Romanienko, P.J., Camerini-Otero, R.D., Bonner, W.M., Manova, K., Burgoyne, P., and Nussenzweig, A. (2003). H2AX is required for chromatin remodeling and inactivation of sex chromosomes in male mouse meiosis. Dev. Cell *4*, 497–508.

Ford, C.E., and Evans, E.P. (1964). A reciprocal translocation in the mouse between the X chromosome and a short autosome. Cytogenetics *3*, 295–305.

Handel, M.A. (2004). The XY body: a specialized meiotic chromatin domain. Exp. Cell Res. *296*, 57–63.

Hendriksen, P.J. (1999). Do X and Y spermatozoa differ in proteins? Theriogenology *52*, 1295–1307.

Hendriksen, P.J.M., Hoogerbrugge, J.W., Themmen, A.P.N., Koken, M.H.M., Hoeijmakers, J.H.J., Oostra, B.A., van der Lende, T., and Grootegoed, J.A. (1995). Postmeiotic transcription of X and Y chromosomal genes during spermatogenesis in the mouse. Dev. Biol. *170*, 730–733.

Hurst, L.D. (1992). Is *Stellate* a relict meiotic driver? Genetics *130*, 229–230.

Huynh, K.D., and Lee, J.T. (2003). Inheritance of a pre-inactivated paternal X chromosome in early mouse embryos. Nature *426*, 857–862.

Huynh, K.D., and Lee, J.T. (2005). X-chromosome inactivation: a hypothesis linking ontogeny and phylogeny. Nat. Rev. Genet. *6*, 410–418.

Khalil, A.M., Boyar, F.Z., and Driscoll, D.J. (2004). Dynamic histone modifications mark sex chromosome inactivation and reactivation during mammalian spermatogenesis. Proc. Natl. Acad. Sci. USA *101*, 16583–16587.

Lammers, J.H.M., Offenberg, H.H., Van Aalderen, M., Vink, A.C.G., Dietrich, A.J.J., and Heyting, C. (1994). The gene encoding a major component of synaptonemal complexes of rat is related to X-linked lymphocyte-regulated genes. Mol. Cell. Biol. *14*, 1137–1146.

Mahadevaiah, S.K., Odorisio, T., Elliott, D.J., Rattigan, A., Szot, M., Laval, S.H., Washburn, L.L., McCarrey, J.R., Cattanach, B.M., Lovell-Badge, R., and Burgoyne, P.S. (1998). Mouse homologues of the human *AZF* candidate gene *RBM* are expressed in spermatogonia and spermatids, and map to a Y deletion interval associated with a high incidence of sperm abnormalities. Hum. Mol. Genet. *7*, 715–727.

Mahadevaiah, S.K., Evans, E.P., and Burgoyne, P.S. (2000). An analysis of meiotic impairment and of sex chromosome associations throughout meiosis in XYY mice. Cytogenet. Cell Genet. *89*, 29–37.

Mahadevaiah, S.K., Turner, J.M.A., Baudat, F., Rogakou, E.P., de Boer, P., Blanco-Rodriguez, J., Jasin, M., Keeney, S., Bonner, W.M., and Burgoyne, P.S. (2001). Recombinational DNA double-strand breaks in mice precede synapsis. Nat. Genet. *27*, 271–276.

Mroz, K., Carrel, L., and Hunt, P.A. (1999). Germ cell development in the XXY mouse: evidence that X chromosome reactivation is independent of sexual differentiation. Dev. Biol. *207*, 229–238.

Namekawa, S.H., Park, P.J., Zhang, L.F., Shima, J.E., McCarrey, J.R., Griswold, M.D., and Lee, J.T. (2006). Post-meiotic sex chromatin in the male germline of mice. Curr. Biol., in press.

Odorisio, T., Mahadevaiah, S.K., McCarrey, J.R., and Burgoyne, P.S. (1996). Transcriptional analysis of the candidate spermatogenesis gene *Ube1y* and of the closely related *Ube1x* shows that they are coexpressed in spermatogonia and spermatids but are repressed in pachytene spermatocytes. Dev. Biol. *180*, 336–343.

Odorisio, T., Rodriguez, T.A., Evans, E.P., Clarke, A.R., and Burgoyne, P.S. (1998). The meiotic checkpoint monitoring synapsis eliminates spermatocytes *via* p53-independent apoptosis. Nat. Genet. *18*, 257–261.

Ohno, S., and Cattanach, B.M. (1962). Cytological study of an X-autosome translocation in *Mus musculus*. Cytogenetics *1*, 129–140.

Okamoto, I., Otte, A.P., Allis, C.D., Reinberg, D., and Heard, E. (2004). Epigenetic dynamics of imprinted X inactivation during early mouse development. Science *303*, 644–649.

Okamoto, I., Arnaud, D., Le Baccon, P., Otte, A.P., Disteche, C.M., Avner, P., and Heard, E. (2005). Evidence for de novo imprinted X-chromosome inactivation independent of meiotic inactivation in mice. Nature *438*, 369–373.

Osborne, C.S., Chakalova, L., Brown, K.E., Carter, D., Horton, A., Debrand, E., Goyenechea, B., Mitchell, J.A., Lopes, S., Reik, W., and Fraser, P. (2004). Active genes dynamically colocalize to shared sites of ongoing transcription. Nat. Genet. *36*, 1065–1071.

Palmer, S.J., Mahadevaiah, S.K., and Burgoyne, P.S. (1990). XYY spermatogenesis in XO/XY/XYY mosaic mice. Cytogenet. Cell Genet. *54*, 29–34.

Reader, C.R., and Solari, A.J. (1969). The histology and cytology of the seminiferous epithelium of mice carrying Searle's X-autosome translocation. Acta Physiol. Lat. Am. *19*, 249–256.

Reik, W., and Ferguson-Smith, A.C. (2005). Developmental biology: the X-inactivation yo-yo. Nature *438*, 297–298.

Rodriguez, T.A., and Burgoyne, P.S. (2000). Evidence that sex chromosome asynapsis, rather than excess Y gene dosage, is responsible for the meiotic impairment of XYY mice. Cytogenet. Cell Genet. *89*, 38–43.

Roeder, S., and Bailis, J.M. (2000). The pachytene checkpoint. Trends Genet. *16*, 395–403.

Schimenti, J. (2005). Synapsis or silence. Nat. Genet. *37*, 11–13.

Seki, T., Wang, W.S., Okumura, N., Seki, M., Katada, T., and Enomoto, T. (1998). cDNA cloning of mouse BLM gene, the homologue to human Bloom's syndrome gene, which is highly expressed in the testis at the mRNA level. Biochim. Biophys. Acta *1398*, 377–381.

Shiu, P.K., Raju, N.B., Zickler, D., and Metzenberg, R.L. (2001). Meiotic silencing by unpaired DNA. Cell *107*, 905–916.

Solari, A.J. (1971). The behaviour of chromosomal axes in Searle's X-autosome translocation. Chromosoma *34*, 99–112.

Solari, A.J. (1974). The behaviour of the XY pair in mammals. Int. Rev. Cytol. *38*, 273–317.

Solari, A.J., and Valzacchi, G.R. (1997). The prevalence of YY synaptonemal complex over XY synapsis in an XYY man with exclusive XYY spermatocytes. Chromosome Res. *5*, 467–474.

Touré, A., Clemente, E.J., Ellis, P., Mahadevaiah, S.K., Ojarikre, O.A., Ball, P.A.F., Reynard, L., Loveland, K.L., Burgoyne, P.S., and Affara, N.A. (2005). Identification of novel Y chromosome encoded transcripts by testis transcriptome analysis of mice with deletions of the Y chromosome long arm. Genome Biol. *6*, R102.

Turner, J.M., Aprelikova, O., Xu, X., Wang, R., Kim, S., Chandramouli, G.V., Barrett, J.C., Burgoyne, P.S., and Deng, C.X. (2004). BRCA1, histone H2AX phosphorylation, and male meiotic sex chromosome inactivation. Curr. Biol. *14*, 2135–2142.

Turner, J.M., Mahadevaiah, S.K., Fernandez-Capetillo, O., Nussenzweig, A., Xu, X., Deng, C.X., and Burgoyne, P.S. (2005). Silencing of unsynapsed meiotic chromosomes in the mouse. Nat. Genet. *37*, 41–47.

van Raamsdonk, C.D., and Tilghman, S.M. (2001). Optimizing the detection of nascent transcripts by RNA fluorescence in situ hybridization. Nucleic Acids Res. *29*, E42.