New twists in X-chromosome inactivation
Jennifer A Erwin¹,² and Jeannie T Lee¹,²

Dosage compensation, the mechanism by which organisms equalize the relative gene expression of dimorphic sex chromosomes, requires action of a diverse range of epigenetic mechanisms. The mammalian form, ‘named X-chromosome inactivation’ (XCI), involves silencing of one X chromosome in the female cell and regulation by genes that make noncoding RNAs (ncRNA). With large-scale genomic and transcriptome studies pointing to a crucial role for noncoding elements in organizing the epigenome, XCI emerges as a major paradigm and a focus of active research worldwide. With more surprising twists, recent advances point to the significance of RNA-directed chromatin change, chromosomal trans-interactions, nuclear organization, and evolutionary change. These findings have impacted our understanding of general gene regulation and are discussed herein.

Address
¹ Howard Hughes Medical Institute, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA, USA
² Howard Hughes Medical Institute, Department of Genetics, Harvard Medical School, Boston, MA, USA

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Introduction
In mammals, X-chromosome inactivation (XCI) achieves transcriptional silencing of one X chromosome in the XX female cell, thereby equalizing gene dosage to the XY male [1]. This epigenetic phenomenon involves non-coding RNAs (ncRNAs), antisense transcription, histone modifications, and DNA methylation to distinguish two genetically identical X chromosomes as active and silent entities within the same nucleus. Because of the numerous interesting epigenetic mechanisms involved, XCI remains an active field of research spanning over half a century. Two forms of XCI have been described: in extra-embryonic mouse tissues and in all tissues of marsupial species [10,11]. On the future Xi, loss of Tsix expression prevents upregulation of Xist and silencing of the cis chromosome. In parallel on the future active X (Xa), persistence of Tsix expression permits upregulation of Xist and thereby prevents silencing on that chromosome. Tsix is in turn regulated by Xite [12], a gene located ~10 kb upstream of Tsix and bears a Tsix-specific enhancer [13].

Here, we highlight advances in the field of XCI over the past two years. Greatest advances have been made in the areas of antisense regulation, chromosome-wide changes to chromatin, nuclear organization, and evolutionary progression. Because of space limitations, we regret that other important works cannot be discussed. For a summary of earlier works, we refer the reader to a number of existing reviews [4,14–18].

Tsix-mediated repression of Xist
Large-scale genomic analyses in multiple mammalian species have confirmed the disparity between their large genome sizes and their relatively small number of coding genes [19]. Surprising findings by the Fantom 3 project established that 62% of the mouse genome is transcribed [20]. Of 181,000 unique transcripts, half are ncRNAs. In addition, 70% of transcripts have a component that is antisense to another transcript. Such studies suggest that noncoding elements – including antisense transcripts – may play a major role in regulating the epigenome and speak to the importance of learning more.
Perhaps one of the best-studied cases of mammalian antisense regulation is Tsix, a ~40-kb gene complementary to Xist [10]. Because of the sense–antisense nature of Xist and Tsix, models describing potential mechanisms of Tsix-mediated repression of Xist invoke either transcriptional or post-transcriptional mechanisms [21]. Genetic evidence points to a mechanism involving either antisense transcription through Xist or overlapping sense–antisense RNA [22,23]. Earlier evidence suggested that Xist RNA stabilization leads to initiation of XCI on the future Xi [24,25]. However, several recent papers argue against a role for Xist stabilization and instead support a model of transcriptional regulation. Specifically, it was shown that Tsix transcription through Xist modulates Xist chromatin structure and determines whether Xist will be activated or repressed [26**,27**,28**].

Two studies found that, in post-XCI cells and differentiating female ES cells, truncations of Tsix lead to an open chromatin structure within Xist, allowing for upregulation of Xist expression [26**,27**]. These changes include increased H3-K4 dimethylation, increased RNA Pol II binding, decreased histone H3-K27 methylation, and decreased DNA methylation at the Xist promoter, suggesting that Xist is upregulated when Tsix is downregulated with consequent conversion of the Xist/Tsix locus to a more euchromatic configuration. However, a third study directly investigated Xist RNA levels and transcription in differentiating female ES cells and came to slightly different conclusions [28**]. This study found that Xist RNA is indeed regulated by promoter upregulation and not by RNA stabilization, but it also proposed that a transient heterochromatic state in Xist pre-empts Xist upregulation. Histone H3-K27 trimethylation, a known heterochromatic mark, was found specifically at the Xist promoter of the future Xi, as revealed by allele-specific analysis of a Tsix+/− female mutant. On the future Xa, persistent Tsix transcription through the Xist promoter creates a euchromatic state that correlates with Xist repression. Upon silencing of Tsix on the future Xi, the Xist promoter adopts a more heterochromatic signature, which paradoxically correlates with Xist activation. Regardless of the differences among the three studies (which are likely owing to differences in experimental systems), all three clearly point to the importance of Tsix-directed chromatin change as a key regulator of Xist transcription. All agree that chromatin change occurs co-transcriptionally with Tsix expression.

Additional evidence supports the fundamental role of Tsix transcription through Xist. By eliminating all splicing products of mature Tsix, a recent work demonstrated that processed Tsix transcripts are dispensable for proper Xist repression [29**]. Indeed, processed Tsix transcripts account for only a fraction of total Tsix RNA [30]. Thus, Tsix seems more likely to act as a full-length unspliced RNA. Tsix RNA has also been implicated in the recruit-ment of Dnmt3a, a de novo DNA methyltransferase, to the Xist promoter for stable silencing of the allele on the future Xa [28**]. Consistent with this idea, Dnmt3a-deficient embryos have dysregulated Xist [31]. Tsix-dependent recruitment of Dnmt3a is likely involved in maintaining Xist silencing, but does not appear to be involved in the initiation of its silencing. Initiation seems instead to be controlled by changes in histone modifications [26**,27**,28**].

**X-chromosome crosstalk**

Because the two X chromosomes in the female must adopt mutually exclusive fates of Xa and Xi, a mechanism of crosstalk must be utilized (in principle) to ensure that no cell inappropriately inactivates both or neither X [32]. Recent reports have shed light onto this mechanism and suggest involvement of physical interactions in trans [33**,34**]. Strikingly, the Xi region of the two X chromosomes appears to touch or ‘pair’ just before the onset of XCI. The pairing model proposes that the two X chromosomes are epigenetically equivalent before the onset of XCI. Pairing then enables the asymmetric localization of factors upon separation of the Xs. Transgene analysis implicates the noncoding genes, Tsix and Xite, as mediators of pairing. Deletions of Tsix and Xite results in either loss or severe delay of pairing with consequences for counting and the pattern of XCI in female cells [35].

More recent work has provided a glimpse of mechanism. Apparently, relatively small and diverse 1–2 kb DNA elements which lie within Tsix and Xite are sufficient to establish ectopic pairing when they are placed onto autosomes [36**]. Even sequences of very low complexity, such as the 34mer repeat of DXPas34, contain sufficient information to instruct pairing. Interestingly, common to these sequences are binding sites for Ctcf, the zinc finger transcription factor and chromatin insulator. By transient Ctcf RNAi knockdown assays, CTCF was found to be essential for X chromosome pairing. Furthermore, pairing requires new transcription, as inhibition of pol-II transcription by actinomycin D and α-amanitin blocks the formation of new pairs without apparently affecting the stability of already established pairs. The requirement for new transcription suggests that RNA might also be involved, especially given that the essential genes, Tsix and Xite, make ncRNA.

**Nuclear organization and compartments**

In 1949, Barr and Bertram [37] described a unique nuclear structure present only in XX female cat neurons. Although they did not associate this structure with an Xi (that realization would only come later with the identification of XCI by Mary Lyon [1]), they noted that the structure frequently resided near the nucleolus. Nearly 60 years later, the significance of this association became clearer with the discovery that the Xi visits a perinucleolar compartment during S phase when it under-
goes DNA replication [38**]. This compartment is enriched for Snf2h, a component of the ISWI ATPase chromatin remodeling complex. Perinucleolar localization was furthermore shown to depend on Xist. Deleting Xist on the Xi results in loss of nucleolar association and reactivation of some previously silenced genes. Perinucleolar localization to a compartment rich in silencing factors provides a means of replicating not only the DNA but also the associated epigenetic factors for the maintenance of heterochromatin. This temporal and spatial separation of chromatin replication may explain how euchromatin and heterochromatin can be maintained in distinct states despite residing in the same nucleus.

The role of nuclear organization in regulating XCI has been further demonstrated by two other recent studies [39**,40**]. By detailed cytologic analysis, the studies showed that the core of the ‘Barr body’ consists of silenced nongenic sequences involving centromeric and other repetitive DNA elements – the so-called ‘Cot1’ fraction of the genome. Genic elements that are silenced by XCI move into the core region, while genes that escape apparently lie outside of or at the edge of the Xi core. Surprisingly, while genic silencing requires an intact Xist allele – notably the Repeat A element [41], nongenic silencing can occur even in its absence. Without Repeat A, nongenic regions are silenced, but genic regions are not. It was shown that Repeat A is required to relocate genic sequences into the repressive nuclear compartment. Thus, XCI can be separated into genic and nongenic silencing. The significance of nongenic silencing is currently not known.

**Chromatin modifying proteins for XCI**

What Xist RNA recruits to the X chromosome has been an active field of research since Xist was first discovered 17 years ago. No interacting factors have been published to date. In the search for silencing proteins, BRCA1 has emerged as a major point of debate. BRCA1 is known to function in DNA damage pathways as a ubiquitin ligase and play a role in meiotic sex chromosome inactivation [42]. BRCA1 mutations predispose carriers to hereditary breast and ovarian cancer. Interestingly, many breast cancers associated with BRCA1 deficiency no longer display an obvious Barr body, suggesting that the second X has either been lost or reactivated. Following upon this longstanding observation, a report from several years ago proposed that BRCA1 protein is responsible for localizing Xist RNA to the Xi and implied the high frequency of Barr body loss in breast cancers may be attributed to loss of BRCA1 [43**]. The study reported co-localization of Xist RNA and BRCA1 on the Xi, absence of Xist RNA on the Xi in BRCA1-deficient tumor cell lines, and reactivation of the Xi when BRCA1 is knocked down by RNAi in normal cells. In tumor cells, Xist RNA can apparently be relocalized to the Xi when BRCA1 is restored. These findings provided a first candidate transfactor involved in Xist localization and suggested a link between XCI and cancer.

However, more recent reports have failed to reproduce the original connection between Xist RNA and BRCA1 in the same cell lines described previously [44**,45**]. The authors found that Xist RNA and BRCA1 do not colocalize and that BRCA1 RNAi does not disrupt Xist coating of the Xi. Similarly, X-linked gene expression and XCI were not significantly disrupted in somatic cells and mammary tumors in BRCA1-deficient mice. In response, the original authors have in fact provided additional evidence to support the original claims [46**]. For example, conditionally deleting BRCA1 in mice was shown to decrease Xist RNA staining of the Xi. These disparate sets of data are clearly hard to reconcile with each other and will require additional work to resolve the differences.

Other groups have focused on the polycomb group proteins as regulators of XCI. Xist RNA upregulation is quickly followed by a number of chromatin changes, including the recruitment of polycomb repressive complexes 1 (PRC1) and 2 (PRC2) to the Xi (reviewed in [15,19]). At other loci, these complexes are known to catalyze histone H2A-K119 ubiquitination and histone H3-K27 methylation and to be essential for gene repression. For this reason, several labs have focused on characterizing PRC1 and PRC2 components genetically. The results suggest that polycomb function in XCI is complex and may be partially redundant. Surprisingly, when individually knocked out, Ring1b (a component of PRC1) was reported to not be required for random XCI [47**,48**], although Ring1b deletion clearly resulted in a lack of H2A-K119 ubiquitination and derepression of lineage-specific genes in ES cells.

Analysis of PRC2 knockouts revealed equally perplexing results [47**,49**,50**]. Because H3K27 tri-methylation marks the Xi, XCI was examined in embryos lacking one of the subunits of PRC2, Eed. Eed is believed to be a canonical component of PRC2, though Eed itself is not the H3-K27 methylase (Ezh2 is the catalytic subunit). Unexpectedly, when using an X-linked GFP reporter line, one report indicated that Eed is not required for establishment of random XCI [49**]. Similar results were obtained by a second report using other gene expression assays [47**]. Furthermore, it was reported using the X-linked GFP reporter that, while Eed is not required for the establishment of imprinted XCI in extraembryonic tissues of the mouse [51], the protein is needed to maintain silencing of the paternal X [50**].

These surprising findings implied that Eed protein – though not necessarily PRC2 – is not required for random XCI but is required for maintenance of imprinted XCI. They also suggest that Ring1b – and again not necessarily
PRC1 as a whole – may be dispensable for random XCI. One possibility is that PRC2 and PRC1 perform redundant functions during XCI and a phenotype may not be evident unless both complexes are knocked out [47**]. For the Eed knockouts, the somewhat contradictory results may in part be explained by use of different assays and an X-linked GFP transgene which may not represent the activity status of the rest of the Xi. Also, maternal pools of Eed may still present in the perimplantation embryo to establish imprinted and perhaps also random XCI. More surprises will undoubtedly surface in this system.

**XCI evolution**

While XCI is largely studied in mouse and human systems, the significance of XCI in the marsupial has not been overlooked. Marsupials undergo imprinted X inactivation, likely reveal an ancestral form of XCI, and should therefore help elucidate the evolutionary history of dosage compensation in mammals. Recent work comparing the genomes of vertebrate species suggests that the Xic region shows broken synteny in marsupials [52**,53**,54**] and that the Xist/Tsix genes evolved in eutherians from a protein coding gene [55**]. Xist/Tsix is unique to eutherians and may have been fundamental in the evolution of random XCI. Because of the lack of Xist in marsupials, it has been postulated that marsupial and eutherian XCI evolved independently of each other [55**,56]. However, it is also known that the eutherian form of imprinted XCI in the extraembryonic tissues depends on Xist/Tsix. Thus, it seems as plausible that random and imprinted XCI would be mechanistically related and that random XCI represents an evolutionary progression from imprinted XCI [2,3,16]. It is possible that an Xist-independent form of XCI evolved first in the early mammal (200–300 million years ago), that this form of imprinted XCI persisted in the early eutherian, and that it eventually gave way to random XCI with the appearance of the Xic.

Many have speculated about the role of meiotic sex chromosome inactivation (MSCI) in both the mechanism and evolution of imprinted XCI [2,3,16,57,58]. During the pachytene stage of meiosis I, both marsupials and eutherians silence the X and Y chromosomes [59,60**]. Very recent work showed unexpectedly that the sex chromosomes only partially reactivate after meiosis is completed [60**,61**,62**,63**]. During the postmeiotic stages, ~85% of X-linked genes remain transcriptionally suppressed. Termed ‘postmeiotic sex chromatin’ (PMSC), the X and Y heterochromatin is enriched in HP1α, HP1β, and histone H3-K9 methylation in the marsupial and mouse [59,60**,61**]. MSCI is thought to descend from MSUD (meiotic silencing by unpaired DNA) [64,65] and apparently does not require Xist [66,67]. These findings are consistent with the hypothesis that inheritance of a pre-inactivated spermatic X chromosome may provide the Xist-independent mechanism for imprinted XCI in marsupials and that aspects of this form of silencing may be retained in eutherian mammals, accounting in particular for imprinted paternal XCI in the early embryo [2,3,16,57,60**,68]. Further work is required to establish both the potential evolutionary and mechanistic links.

**Future questions in XCI**

While substantial progress has been made in our understanding of XCI in recent years, there is clearly still much that remains unanswered. Some hints of what will come derive from some of the most recent developments. For example, DNA methylation may play a role in both silencing of the Xi and maintaining activation on the Xa. Silenced chromatin regions are usually hypermethylated, while active regions are undermethylated [69]. However, allele-specific DNA methylation analysis of the human Xi revealed surprisingly that the Xa has twice the methylation as the Xi, with hypermethylation occurring on Xa gene bodies and hypomethylation occurring at CpG islands [70**]. Another study uncovered a pattern of distinct X-chromosome states even in pre-XCI cells in a manner that predicts which X will become Xi, implying that choice may occur earlier than prevailing models postulate [71**]. Furthermore, it has also been reported that a region of ~300 kb upstream of Xist is involved in pairing and that it initiates pairing even in the pre-XCI state, 1–4 days earlier than previously proposed [72**]. Finally, several reports now sport the idea that dosage compensation in the mammal may involve both X-silencing on Xi and X-upregulation on Xa, suggesting yet another layer of regulation [73**,74**]. The next few years will for sure uncover more intrigue.

**References and recommended reading**

Papers of particular interest, published within the period of the review, have been highlighted as:

** of outstanding interest

New twists in X-chromosome inactivation

Erwin and Lee


The authors demonstrate that RNA polymerase II is recruited to the Xist promoter during Xist upregulation, suggesting transcriptional control of Xist. Using male ES cells with a Tsix-truncation, the authors also report that Tsix is required for maintaining H3-K4 methylation over the entire locus, implying that Tsix regulates Xist through chromatin modification.


Upon examining a Tsix-deleted chromosome in cis to a disrupted Xist gene, the authors find altered chromatin within Xist (e.g. H3-K27 methylation) and DNA hypomethylation of the Xist promoter in both the embryo proper and extraembryonic tissues. These data support a model by which Tsix controls Xist by directing chromatin change at the Xist promoter.


This study directly demonstrates that RNA stabilization is not the mechanism by which Xist control in differentiating ES cells. Rather, Xist is controlled transcriptionally by Tsix in two ways: By directing chromatin change across Xist/Tsix and by recruiting Dnmt3a to the Xist promoter. Paradoxically, a euchromatic configuration at the Xist promoter is associated with Xist repression, while a more heterochromatic configuration correlates with Xist activation.


The authors block splicing of Tsix and demonstrate that fully processed mature transcripts are not required to regulate Xist, suggesting that either full-length Tsix transcripts or the act of transcription may be more significant. Together with other data in the literature, the authors support the role of Tsix transcription as a key aspect of Tsix-mediated Xist repression.


This study demonstrates through the use of DNA FISH that the Xic of the two X chromosomes transiently colocalize at the onset of XCI. The interaction requires elements within a 65 kb region downstream of Xist. Deleting this region causes loss of pairing, while autosomal insertion in males causes ectopic X-autosome interactions. It is proposed that Xic colocalization is required for proper counting.


By DNDNAFISH and 3C technology, this study shows that two female Xs pair and physically touch just before Xist upregulation. Pairing occurs exclusively at the Xic (not elsewhere on the X) and requires Xite and Tsix. X-X pairing can be blocked by deleting Tsix/Xite and by ectopic X-autosome interactions (through insertion of Xite/Tsix sequences onto autosomes) that compete away X-X interactions. These interactions block the initiation of XCI. It is proposed that X-X pairing provides the necessary crossovers for counting and mutually exclusive choice.


This study shows that very little sequence complexity and length are required to induce X-X pairing. Small Tsix/Xite transgenes are sufficient to cause X-autosomes interactions when the transgenes are inserted into autosomes. Pairing is shown to require Ctcf protein and de novo transcription. The half-life of X-X pairs is estimated to be less than 0.5-1.0 hours.

Chromosomal silencing

41. Wutz A, Rasmussen TP, Jaenisch R: Gene silencing states. Using RNA and DNA FISH, the authors demonstrate that the core of the Xist RNA territory, while genes that escape XCI are localized within the core of the silent compartment (near the inner DNA elements of the X chromosome. Genes which are subject to XCI are demonstrated to be linked to chromosomal subcompartments.

40. Turner JM, Aprelikova O, Xu X, Wang R, Kim S, Chandramouli GV, Hu Y, Li W, Cao L, Buetow K, Ried T: The X chromosome is organized into a gene-rich outer rim and an internal core containing silenced nongenic sequences. Proc Natl Acad Sci U S A 2006, 103:6788-6793. Using RNA and DNA FISH, the authors demonstrate that the core of the Barr body in female human cells is composed of intergenic and repetitive DNA elements of the X chromosome. Genes which are subject to XCI are localized within the core of the silent compartment (near the inner periphery of the Xist RNA territory), while genes that escape XCI are localized at the outer edge of the compartment. This study demonstrates the presence of chromosomal subcompartments linked to gene expression states.


Ganesan S, Silver DP, Greenberg RA, Breen M, Duke SE, Samollow PB, McCarrey JR, Leeb M, Wutz A: Co-localization of silencing and inactive X chromosome core. Nature Struct Mol Biol 2006, 13:977-987. The authors demonstrate a repressive nuclear compartment dependent on Xist silencing. Interestingly, this compartment is composed of silenced intergenic DNA and formation is independent of genic silencing but requires the Repeat A element of Xist. Relocation of X-linked genes into a more interior portion of the compartment correlates with silencing. Active genes remain at the periphery of the compartment. This study demonstrates the presence of chromosomal subcompartments linked to gene expression states.


This study makes the provocative observation that BRCA1 co-localizes with XIST RNA and may be required to target XIST RNA to the Xi. BRCA1-deficient tumor cells lack an Xi (Barr body). By adding BRCA1 back to deficient cells, the authors demonstrate a re-association of the XIST RNA foci. Similarly, depletion of BRCA1 by RNAi leads to a loss of proper Xist localization to the Xi. The authors propose that BRCA1 is required for breast cancer.

44. pageau GJ, Hall LL, Lawrence JB: BRCA1 does not paint the X chromosome to localize XIST RNA but may contribute to broad changes in cancer that impact XIST and Xi heterochromatin. J Cell Biochem 2007, 100:835-850.


The Pageau et al. and Xiao et al. studies [44**, 45**] together argue against the hypothesis that BRCA1 is required for Xist localization (Ref. [43**]). They find that BRCA1 does not co-localize with the X and that depleting cells of BRCA1 by RNAi knockdown does not affect Xist localization. They further suggest that Xist and breast cancer are not causally linked in a simple way, if linked at all.

46. Silver DP, Dimitrov SD, Feunteun J, Gelman R, Drapkin R, Lu SD, Shestakova E, Velurungan S, Denunzio N, Dragomir S et al.: Further evidence for BRCA1 communication with the inactive X chromosome. Cell 2007, 128:991-1002. In response to Refs. [44**] and [45**, the authors provide further supporting evidence that BRCA1 is involved in XIST localization to the Xi. By depleting BRCA1 either by RNAi or conditional depletion in mice, they demonstrate loss or decreased XIST localization onto Xi.


Using inducible Xist ES cell line, the authors delete Eed (a subunit of the PRC2 complex) and examine recruitment of PRC1 and the initiation of XCI. The authors find that PRC1 recruitment is not disrupted, implying that PRC2 is not required to recruit PRC1 (contrary to conventional wisdom). Furthermore, Eed- cells can still undergo some aspects of XCI, but they conclude that Eed is not absolutely essential for silencing (although PRC2 may still be required). This supports the idea that PRC2 and PRC1 may be somewhat redundant.

48. Leeb M, Wutz A: Ring1B is crucial for the regulation of developmental control genes and PRC1 proteins but not X inactivation in embryonic cells. J Cell Biol 2007, 178:219-229. The authors delete Ring1B (a component of PRC1) in ES cells and demonstrate that Ring1B is still capable of silencing nearby genes independent of PRC1. Also, PRC2 is still recruited upon Xist induction. This further supports the idea that PRC2 and PRC1 may be somewhat redundant.

49. kalantry S, Magnuson T: The polycomb group protein EED is dispensable for the initiation of random X-chromosome inactivation. FASEB J 2006, 20:3056-3064. See Ref. [50**].

50. kalantry S, Mills KG, Yee D, Otte AP, Panning B, Magnuson T: The Polycomb group protein EED protects the inactive X-chromosome from differentiation-induced reactivation. Nat Cell Biol 2006, 8:195-202. These studies [49*, 50**] examine the results of an Eed deletion on XCI in mouse embryos. Together with previous work from the laboratory (Ref. [51]), these studies show that Eed is required for imprinted XCI but not obviously for random XCI in the mouse. Surprisingly, for imprinted XCI, Eed seems to be necessary for maintaining silencing but not to establish it. Eed KO mice still suppress a paternal X-linked GFP transgene in peri-implantation embryos but cannot prevent its reactivation at later stages.


54. Davidow LS, Breen M, Duke SE, Samollow PB, McCarrey JR, Lee JT: The search for a marsupial XIC reveals a break with elaborate synten. Chromosome Res 2007, 15:137-146. The studies in Refs. [52**, 53**, 54**] search for XIC sequences in the opossum (marsupial) and find that, while protein coding regions flanking the XIC are well conserved throughout vertebrates, the ncRNAs genes of the XIC are missing. In fact, the region around XIC shows broken synteny with that of eutherian mammals. Thus, marsupial mammals are apparently lacking XIST/TSIX and may undergo imprinted XCI using a different mechanism.


60. Namekawa SH, VandeBerg JL, McCarrey JR, Lee JT: Sex chromosome silencing in the marsupial male germ line. Proc Natl Acad Sci U S A 2007, 104:9730-9735; Homecker JL, Samollow PB, Robinson ES, VandeBerg JL, McCarrey JR: Meiotic sex chromosome inactivation in the marsupial Monodelphis domestica. Genesis 2007, 45:696-708. These two papers show that MSCI also occurs in the opossum (a marsupial) and that the X and Y continue to remain transcriptionally suppressed in the post-meiotic spermatids. Both X and Y exhibit properties of post-meiotic sex chromatin (PMS), as previously described for the mouse (a eutherian mammal) (Refs. [61**,62**,63**]). The existence of MSCI and PMS in marsupials is consistent with the hypothesis that imprinted paternal X silencing may be directly derived from the silent germ line X.


63. Turner JM, Mahadevaiah SK, Ellis PJ, Mitchell MJ, Burgoyne PS: Pachytene asynapsis drives meiotic sex chromosome inactivation and leads to substantial postmeiotic repression in spermatids. Dev Cell 2006, 10:521-529. The studies in Refs. [61**,62**,63**] make the surprising finding that sex chromosome silencing does not end with meiosis. By a combination of immunostaining with chromatin markers, RNA FISH, RT-PCR, and microarray analysis, the authors show that the X and Y continue to behave differently in early and late-stage spermatids. By expression profiling, it is estimated that ~85% of X-linked genes remain transcriptionally suppressed. The sex chromosomes are marked by H3-K9me2, HP1, and H2A.Z. These studies are consistent with the idea that imprinted silencing of the paternal X may in part be derived from a previously inactivated X in the paternal germ line (Ref. [68]).


70. Hellman A, Chess A: Gene body-specific methylation on the active X chromosome. Science 2007, 315:1141-1143. Using allele specific methylation analysis on over 1000 loci of the human X chromosome, the authors and make the unexpectedly discovery that DNA methylation is concentrated on the Xa, contrary to the previous notion that the Xi is hypermethylated relative to Xa. On the Xa, CpG methylation occurs at gene bodies, while promoters are relatively unmethylated. These results suggest a role of DNA methylation on the Xa.

71. Mlynarczyk-Evans S, Royce-Tolland M, Alexander MK, Andersen AA, Kalantry S, Gribnau J, Panning B: X chromosomes alternate between two states prior to random X-inactivation. PLoS Biol 2006, 4:e159. The authors describe a cytological mark that differentiates the two Xa in undifferentiated female ES cells. In a high percentage of cells, the two Xa look different, with one showing two hybridization signals at the Xic and the other showing only one. The underlying cause of this is unknown; however, the marks appear to predict the future active and inactive states of the chromosome.

72. Augui S, Filion GJ, Huart S, Nora E, Guggiari M, Maresca M, Stewart AF, Heard E: Sensing X chromosome pairs before X inactivation via a novel X-pairing region of the Xic. Science 2007, 318:1632-1636. The authors identify a region several hundred kilobases upstream of Xist that pairs even before the onset of XCI. The authors claim this region allows for sensing of the two X chromosomes independently of Tsix/Xite and affects whether Xist is expressed. Evidence is presented that pairing can occur even in the absence of the previously identified 65 kb critical region. The putative element lies within the Xpct coding gene.


74. Lin H, Gupta V, Vermilyea MD, Falciani F, Lee JT, ONeill LP, Turner BM: Dosage compensation in the mouse balances upregulation and silencing of X-linked genes. PLoS Biol 2007, 5:e326. The studies in Refs. [73**] and [74**] present evidence that dosage compensation involves two mechanisms, one on the Xi that silences X-genes and the other on Xa to ensure that X-linked gene expression is on par with the autosomal average. Thus, dosage compensation in mammals may involve an X-upregulating mechanism like the one described in the fruitfly. Molecular players in the mammalian mechanism are not currently known.