Mammalian X-chromosome inactivation
An example of LncRNA action (Lnc=long noncoding)
Chapter 17
pg 323 in Allis et al.

Also

Mammalian X-inactivation
This is an extreme example of an epigenetic change. It is useful to discuss it so that we can see what is possible.

Reading Assignment
Chapter 17 Dosage compensation in mammals in Epigenetics Allis, Jenuwein and Reinberg CSHL Press 2007

Definitions
Facultative heterochromatin - heterochromatin in a chromosomal region that might be euchromatin in a different cell from the same species.

Constitutive heterochromatin - heterochromatin in a chromosomal region that is heterochromatin in all cells from the species.

Purpose of X-inactivation to avoid an imbalance in the gene dosage
Dosage Compensation
why?

In dosage compensation the goal is to have equal output.
Halve the output of two X’s in the homogametic sex, double the output of X in the heterogametic sex or halve output of the X’s in the homogametic sex. Examples of all three exist.

Mammals
Mary Lyon – 1961 – X chromosome inactivation to explain patterns produced by X-linked coat color genes in mice. Lyonization is the production of Barr bodies in mammals.

n-1 rule
n = number of X chromosomes, n-1 X’s will be inactivated.
In humans, XXX and XXXX human females and XXY human males have fewer symptoms than expected.
Initiation of silencing

Two promoters that point at each other.
**Initiation of silencing**

Xic stands for X-inactivation center. One product from it is Xist (X inactive specific transcript).

*Increased expression of XIST RNA (non-coding) from the X to be inactivated*

*Tsix regulates Xist expression*

*Tsix overlaps Xist and transcribes in the antisense direction and keeps Xist off. This may or may not involve a RNAi type mechanism.*
How is the X to be inactivated chosen?

n-1 rule - no matter how many X's one will be active.

Led to the idea that X-inactivation is the **DEFAULT** state and that the cell must prevent one X from being inactivated.

A single Xic locus is blocked in all cells.

It is thought that the cell producing enough of a blocking factor to bind one Xic. Once bound it cannot express Xist. **The idea was that this is responsible for the n-1 rule.**

XIST RNA expressed from ONE X coats this X. This silences the X.

XIST stays on even though inactivation is stable w/o it. Xist RNA expression is sufficient and necessary for the induction of heterochromatin formation BUT it is not required to maintain it!

Expression of Xist later after development cannot trigger inactivation.

More changes follow

*Rastan’s Blocking Factor model*

might block the Xist promoter or help activate the Tsix promoter or both
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**Xist RNA is not found on expressed regions eg pseudoautosomal region or on constitutive heterochromatin (centromeres, telomeres).**

**Xist acts in CIS**
RNA, known as "Repeat A. The Repeat A motif directly interacts with EZH2, the catalytic subunit of PRC2, both in vivo and in vitro. PRC2 in turn decorates the X-chromosome and silences it as it trimethylates histone H3 at lysine 27 (H3K27me3) [29–32]. Along the X, PRC2 first binds ~150 "strong sites", which have canonical features of known PRC2 binding sites, including a CpG-rich content and the presence of bivalent domains [33]. From the strong sites, PRC2 migrates laterally and locally, giving rise to thousands of non-canonical domains that may represent sites of dynamic spreading along the X chromatin [33].

H3 K27me3 also spreads out from the strong sites, and H3K27me3 occupancy is anti-correlated with LINE density [33,34], an intriguing finding given a long-standing hypothesis that LINE elements serve as "booster elements" that help X-inactivation spread across the whole chromosome [35]. When expressed ectopically from autosomal transgenes, Xist RNA also recruits PRC2 and silences genes located in cis [3,29,36], demonstrating that Xist RNA is both necessary and sufficient to recruit PRC2 and inactivates genes on a multi-megabase scale.

While it is clear that Xist RNA spreads PRC2 to targets on the X-chromosome, mechanisms that localize Xist RNA itself are just beginning to emerge. Localization begins with loading of Xist RNA at a "nucleation center" located within exon 1 of the Xist locus [3]. The transcription factor, YY1, is required for Xist RNA loading onto the nucleation site. Knocking down YY1 or mutating its three binding sites within the Xist locus results in reduced X-inactivation.

### Table 1. Summary of lncRNAs and proposed interacting protein partners for X-inactivation

<table>
<thead>
<tr>
<th>RNA Function</th>
<th>cis- or trans-acting</th>
<th>Known protein interactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xist</td>
<td>Required for initiation of X-inactivation</td>
<td>cis, can in some cases act in trans at autosomal Xist transgenes [3]</td>
</tr>
<tr>
<td>Tsix</td>
<td>Represses Xist expression by silencing the Xist promoter [7–10], also required for X-chromosome pairing, counting the number of XICs, and mutually exclusive allelic choice [11–13]</td>
<td></td>
</tr>
<tr>
<td>Dnmt3a</td>
<td>[7,8]</td>
<td></td>
</tr>
<tr>
<td>RepA</td>
<td>Independent transcript from Xist 5′ end, helps activate Xist [4]</td>
<td></td>
</tr>
<tr>
<td>Jpx</td>
<td>Activator of Xist transcription; counting of X-chromosomes [15,16]</td>
<td></td>
</tr>
<tr>
<td>Ftx</td>
<td>Potential activator of Xist expression [17]</td>
<td></td>
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### Fig. 1. Model for Xist RNA-mediated recruitment of PRC2.

**Weak PRC2 binding site**

PRC = polycomb repressive complex

of the female cell transiently come into close contact with each other in the nucleus ([Fig. 2](#)[59–62]). This transient “pairing” of the two Xic’s may allow a redistribution of transcriptional activators between the two alleles ([61,63–65]), resulting in asymmetric binding of activators to one X-chromosome and thereby enabling expression of Tsix RNA only on one chromosome. Tsix and its enhancer, Xite, are necessary for pairing ([61]) and are also each sufficient to induce pairing when integrated onto an autosome ([62]). Interestingly, inhibition of transcription with actinomycin D prevents the formation of new pairing complexes but has little effect on the half-life of paired complexes already formed, suggesting that RNA may be required to attract two X-chromosome to each other but not to keep them paired ([61],[62]). Whether transcripts emanating from Tsix and Xite are required is currently not known, though the two loci are clearly necessary and sufficient to induce pairing.

It seems likely that RNAs may generally participate in long-range chromosome interactions. There are several pieces of evidence implicating RNA as a structural component that determine higher-order structures. RNA has long been known to co-fractionate with chromatin in eukaryotic nuclear extracts ([66–69]). The nuclear matrix consists of a network of ribonucleoprotein particles ([70]), and it has been suggested that Xist RNA is a component of the nuclear matrix, interacting with factors such as hnRNP-U/SAF-A and ASF ([5,6,71,72]). Recent experiments more directly implicate a relationship between non-coding RNA and chromatin architecture. A newly discovered class of RNAs that appear to activate nearby genes in *cis* called “activating RNAs” (a-RNAs) ([73]) are required for three-dimensional contacts between enhancers and promoters of nearby genes regulated by a-RNAs ([74]). These RNAs are hypothesized to be predominately *cis*-acting non-coding transcripts that

**Fig. 2.** Model for XIC pairing before XCI onset. The two X-chromosomes are epigenetically identical and euchromatic in the pre-XCI stage. The two Xs are brought together by *Tsix* and *Xite* (pairing) during cell differentiation to enable cross-talk and mutually exclusive choice of Xa and Xi. Because it is thermodynamically favorable to do so, hypothetical transcription factors, potentially OCT4 and CTCF (blue circles), that were previously randomly distributed between the two *Tsix/Xite* alleles stochastically shift to one X, which would then become future Xa. This shift results in monoallelic *Tsix* expression and differential chromatin modifications within the Xist region, which lead to repression of Xist on Xa and upregulation of Xist on Xi.
## Silencing at multiple levels

**Gross comparison of Xi modifications**

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- **H2A, H2B, H3 and H4 acetylation is low**
- **Methylation of lysine increases**
- **Macro-H2A replaces many of H2A on Xi**
- **DNA methylation on CpG islands increases**
# Silencing at multiple levels

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Derived from a Figure 8 Ch 17 in Epigenetics by Allis Jenuwein and Reinberg CSHL Press and Figure 4 from Heard, E (2004) Recent advances in X-chromosome inactivation. Curr Opin Cell Biol, 16:247–255.
Inactivation is a multi-step process

Pulripotent cells → Differentiated cells

- Xist RNA
- H3K4 demethylation, H3K9 deacetylation
- H3K27 methylation
- Histone deacetylation
- H3K9 methylation
- macro H2A
- DNA methylation

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zygotic gene activation is thus likely to lead to the rapid silencing of the Xp by default. It is striking to note that the exclusively paternal expression of Xist in the early embryo parallels the situation at other imprinted loci, where non-coding RNAs are systematically found to be paternally expressed and maternally repressed [50].

The recent discovery that Xp inactivation takes place much earlier than was previously thought, in cleavage-stage embryos [44/C15/C15,49/C15/C15] (Figure 4), means that the long-standing idea that this process is normally associated with cellular differentiation no longer holds. The very early timing of imprinted Xp inactivation raises the question of how random X inactivation can occur subsequently in cells of the embryo proper. This has been addressed in two studies, which have shown that all cells indeed have an inactive Xp by the early blastocyst stage but that this inactivity is reversed during ICM growth as cells rapidly lose Xist RNA coating, Eed/Enx1 enrichment and histone modifications on the Xp [49/C15/C15,51/C15/C15]. In trophectoderm


Extraembryonic tissues (Xp inactivation persists)

Early replication timing

ICM

Epiblast

Xp or Xm inactivation

Epiblast will become the embryo.

Germline

Soma

Current Opinion in Cell Biology
X inactivation is a multi-step process

Xp is imprinted
Early in embryogenesis (a few cells) the paternal X is inactivated in all cells

At blastocyst stage - just prior to implantation 50 -100 cells
Xp is reactivated about the time of implantation. Both X’s are active. In extraembryonic tissues - placenta and yolk sac the Xp stays in the inactivated state.

Back to the embryo which has more than 100 cells and two active X’s.

A random selection is made and either Xp or Xm is inactivated
The product is the Barr body.

Very stable inactivation - has not yet been reversed experimentally even though it is reversed as a normal part of development.

Mice show the early Xp inactivation that is then reversed. Marsupials do this as a matter of course and I don’t think that they reverse it.
**X inactivation is a multi-step process**

*Xp is imprinted*

*Early in embryogenesis (a few cells) the paternal X is inactivated in all cells*

*At blastocyst stage - just prior to implantation 50-100 cells Xp is reactivated about the time of implantation. Both X’s are active. In extraembryonic tissues - placenta and yolk sac the Xp stays in the inactive state.*

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**Kinetics of X inactivation during pre-implantation mouse development.** The dynamic X-inactivation events that occur in pre-implantation embryos are summarised, based on new studies [44**,49**,51**]. At the two-cell stage, Xist expression has just begun and is localised to its site of transcription (shown as a small spot). At the four-cell stage, Xist RNA is accumulated over the Xp chromosome (shown as a domain) [44**,49**]. Although the initial silencing event triggered by Xist RNA coating occurs prior to Eed/Enx1 recruitment and H3K27 or H3K9 methylation of the X chromosome, it does appear to be linked with H3 hypoacetylation and loss of H3K4 methylation. The former could be explained by recruitment of an HDAC (see Figure 3a), but the latter requires loss of the histone (or its tail). Given the absence of a histone demethylase to date, this would appear unlikely. Another possibility is that a histone exchange activity could be recruited by Xist RNA, which would replace the histones present in active chromatin with unmodified histones. In the blastocyst, essentially all cells contain an inactive Xp associated with the PcG proteins Eed and Enx1. In the trophoderm and primitive endoderm (extraembryonic lineages), this inactive state is maintained and perhaps further locked in by a shift in replication timing (early replication in this case). In the ICM, however, the Xp becomes reactivated, losing its Xist RNA coating and the Polycomb group proteins, and the histone methylation marks are gradually reversed. In this way, cells that will subsequently contribute to the epiblast (embryo-proper) contain two active X chromosomes, prior to the random inactivation of either the Xp or Xm. In the female germ line, which is set aside subsequently, the inactive X becomes reactivated just prior to meiosis. PE, primitive endoderm; TE, trophectoderm.
Role of LncRNA in manipulating the higher order structure of chromosomes is not unique to Xist.

- **Lnc RNA =** long non-coding RNA
- **Xist is just extreme - entire chromosome**
- **It happens in a more local way in other places.**


**Aa  Xist, Kcnq1ot1 and Airn**

Some people also have data saying that PRC2 can be recruited without Xist.