‘X’- inactivation in human

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Dosage compensation - X-chromosome inactivation

Males are heterogametic (with only one X-chromosome) while females are homogametic (two X-chromosomes).
Despite this, female cells do not have a **double-dose** of gene products from their X-chromosomes.
In females, one X-chromosome is **partially inactivated** early in embryonic development.

----------**This is dosage compensation**

The regions inactivated are those that are **lacking on Y-chromosome** (Deacetylation of histones, methylation of bases).

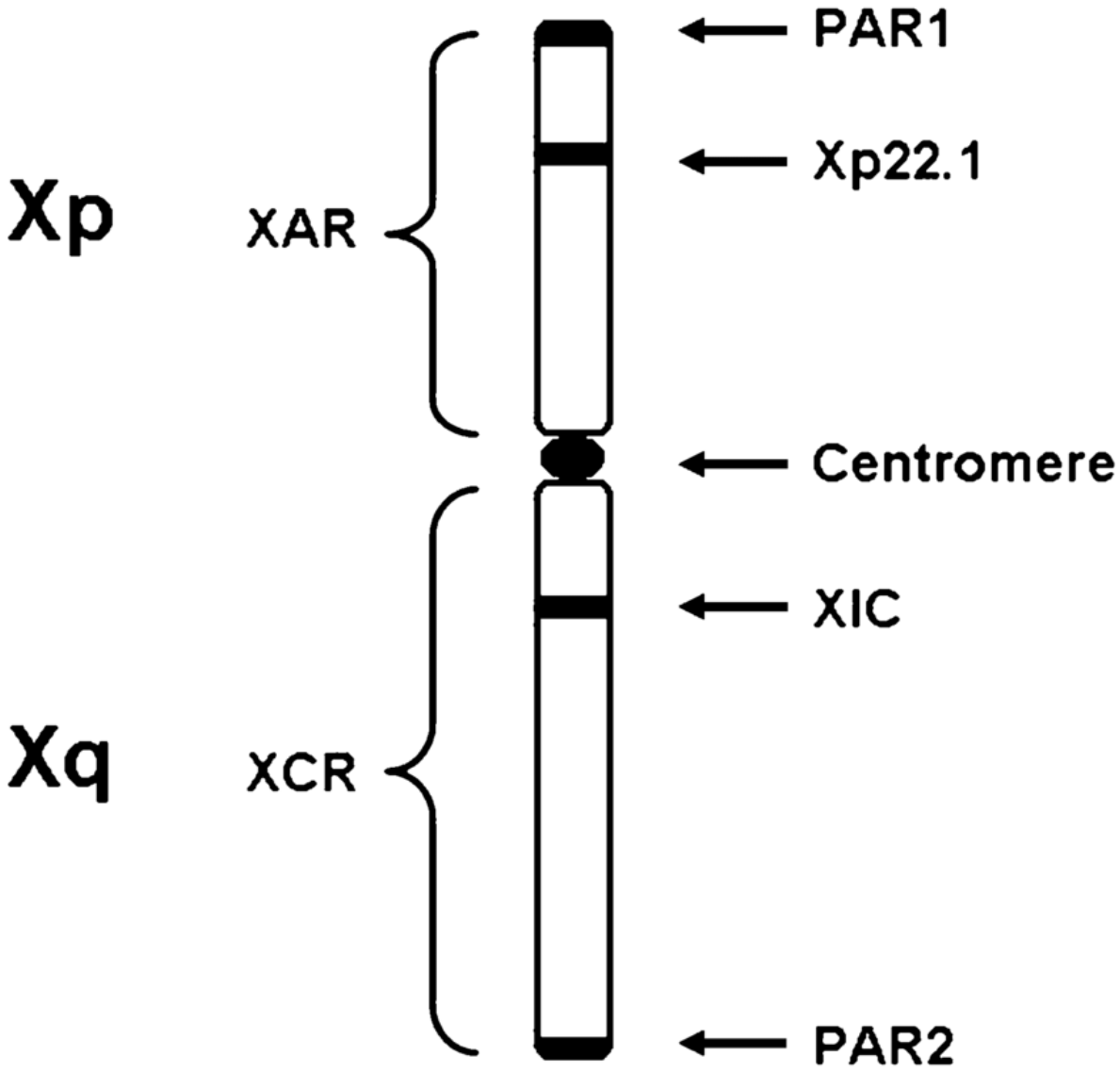
The inactivated X-chromosome shows up as a highly condensed region on the inside of the nuclear membrane – a **Barr body**.

From the beginning…….

- X chromosome inactivation process was first proposed by Mary Lyon in 1961.
- In 2011 it becomes a scientific fact.
- The inactivation process is termed as Lyonization.
- Inactive X chromosome is referred as **Barr body.**

X chromosome

- The human X chromosome is about 155 mega bases (MB) in length, consisting of approximately 51 MB of genes—somewhat less gene-dense than other chromosomes of its size.
- The rest of the chromosome consists of interspersed DNA sequences that are not unique to the X because they occur as repeats all over our genome. There are 826 protein coding genes on the X chromosome.
- There are more than 8,000 X-linked CpG islands that occur all along the chromosome, with a frequency of 5.2 islands per MB of DNA.

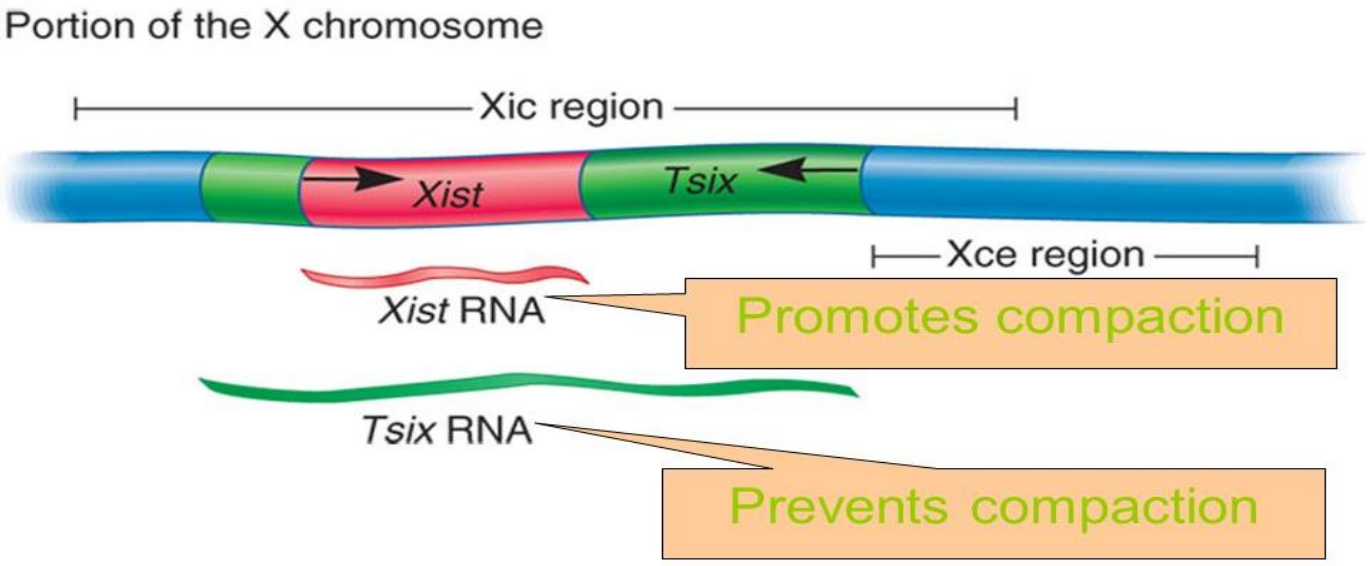


XAR - X-added region
XCR – X-conserved region

- X-chromosome contains X Inactivation Center (XIC) which located in long arm.
- XIC contains an unusual gene called inactive X (*Xi*)-specific *t*ranscripts (*XIST*).
- *XIST* expresses a noncoding functional 17 (kb) RNA molecule.
- *XIST* expressed only when more than one X-chromosome found in same cell.

XIST and TSIX RNAs

- Both the genes are located within XIC part of the X chromosome and transcribe RNAs.
- These RNAs are transcribing across the entire XIST gene in opposite direction, thus may down regulate their production.
- Both of them are *cis*- acting and remained within nucleus.
- TSIX expression is regulated by another upstream enhancer- XITE also present within XIC.



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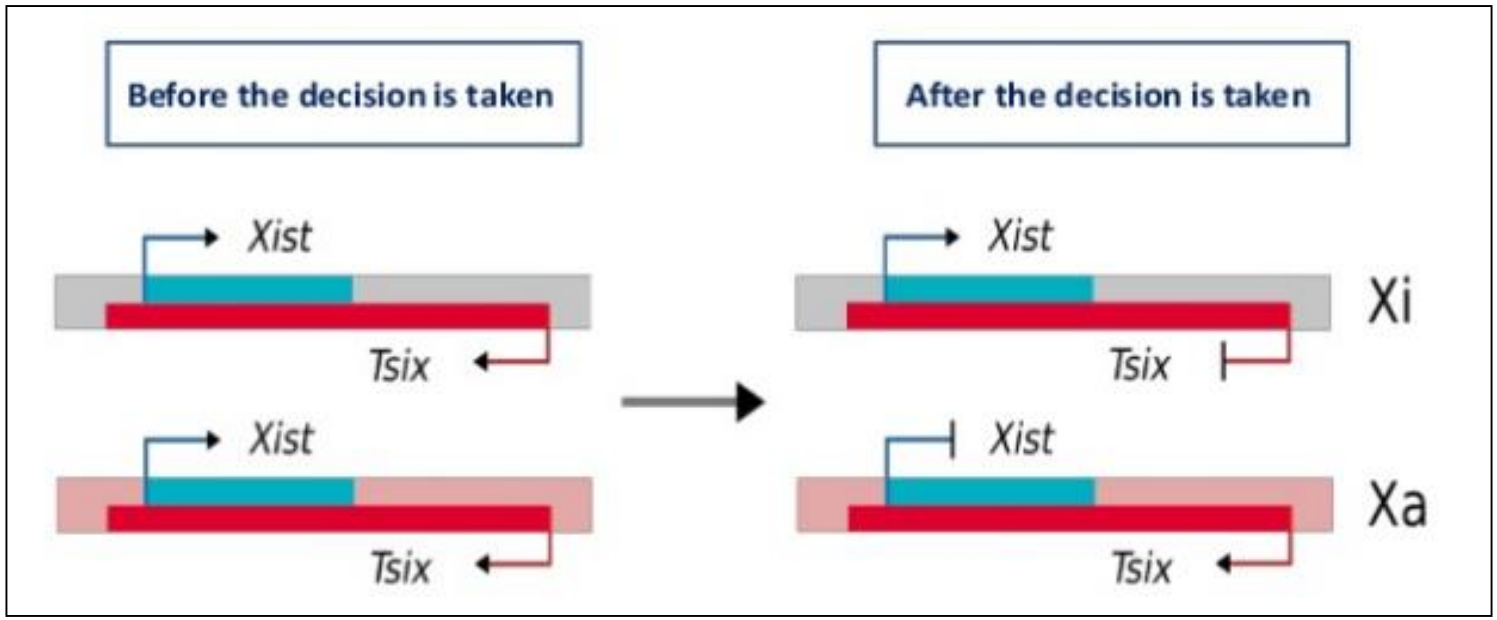
- Tsix is an element transcribed from the antisense strand relative to Xist.
- Tsix is expressed in early embryos, and has been proposed to control Xist expression at the onset of X inactivation.



X-Chromosome Inactivation: Making Two Equal One

It involves following steps:

- Chromosome counting (determining the number of X s in the cell).
- Selection of an X for inactivation.
- Inactivation itself.

Counting chromosomes

Counting refers to the process by which a cell determines its X/A ratio in order to maintain a single active X chromosome per deploid chromosome set.

Involves the X-inactivation center (XIC in humans, and Xic in mice). Experiments in transgenic mice show that:-

- Inactivation requires the presence of at least two Xic sequences, one on each X chromosome.
- Autosomes with an Xic inserted are randomly inactivated, showing that Xic is sufficient for chromosome counting and initiation of Lyonization.

Selection of X

Choice can be influenced by a locus known as the X-controlling element (*Xce*).

- Selection of an X for inactivation is made by the X-controlling element (Xce) in the Xic region.

- There are different alleles(at least 3 – a, b and c) of Xce, and each allele has a different probability that the X chromosome carrying it will be inactivated.

Inactivation

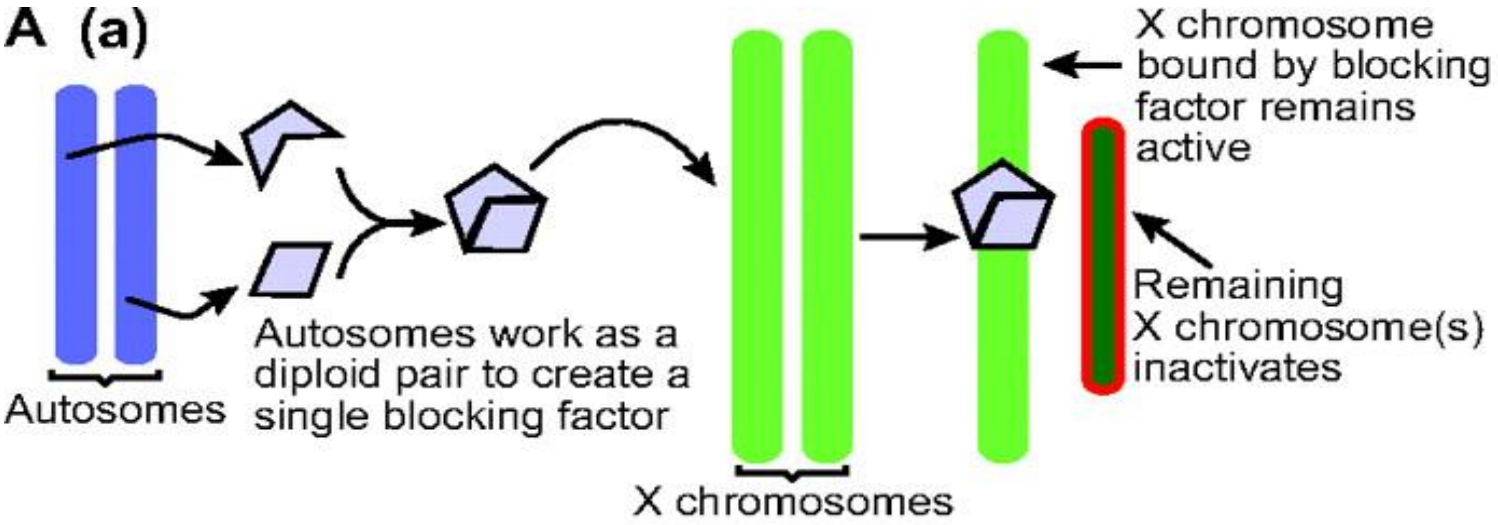
Xist is the main player for inactivation. It is expressed from the **inactive X**.

- The Xist gene transcript is 17-kb. Although it has no ORFs, it receives splicing and a poly(A) tail.
- During X inactivation, this RNA coats the chromosome to be inactivated and silences most of its genes.

How inactivation of X chromosome ensured?

1. **The blocking factor model proposed by Rastan 1983**

The model states that a diploid cell produces autosomally encoded blocking factor just enough to block one Xic, during initiation of inactivation. The blocked Xic is prevented from transcribing *Xist* RNA and so is marked as Xa. Blocking factor binds to either the maternal (Xm) or paternal (Xp) X chromosome with equal probability and in a cell-autonomous manner.



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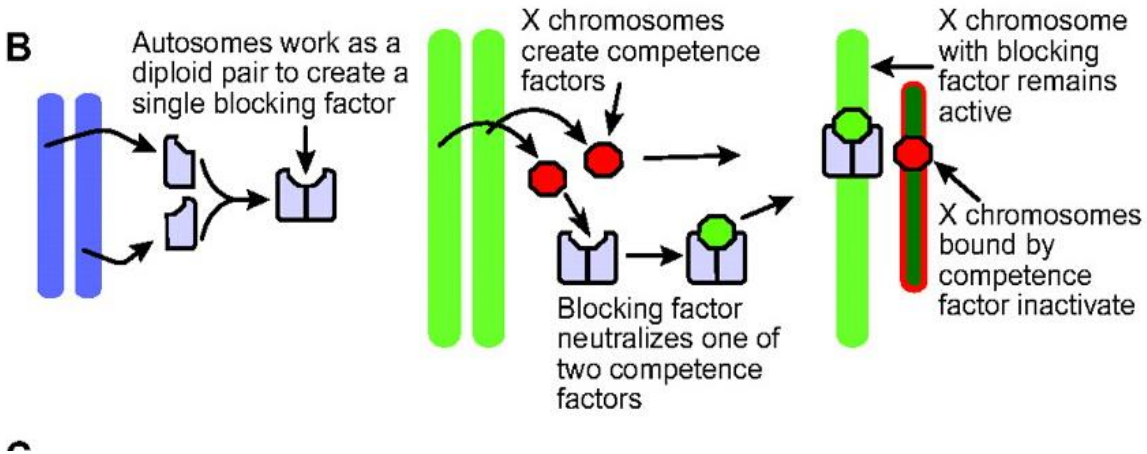
2. Two factor model

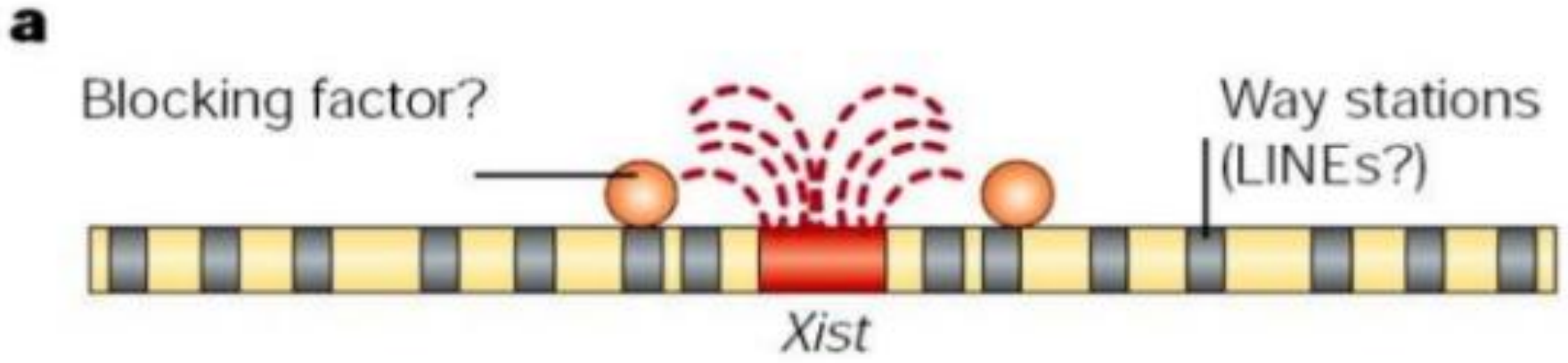
- Autosomes produces blocking factors (BF) while X chromosome produces transacting competence factors (CF).
- Blocking factors bond to competence factors and form complexes(2BF and 1 CF).
- This complex binds to X chromosome and makes it active, while single competence factor can bind to X chromosome and makes it inactive.



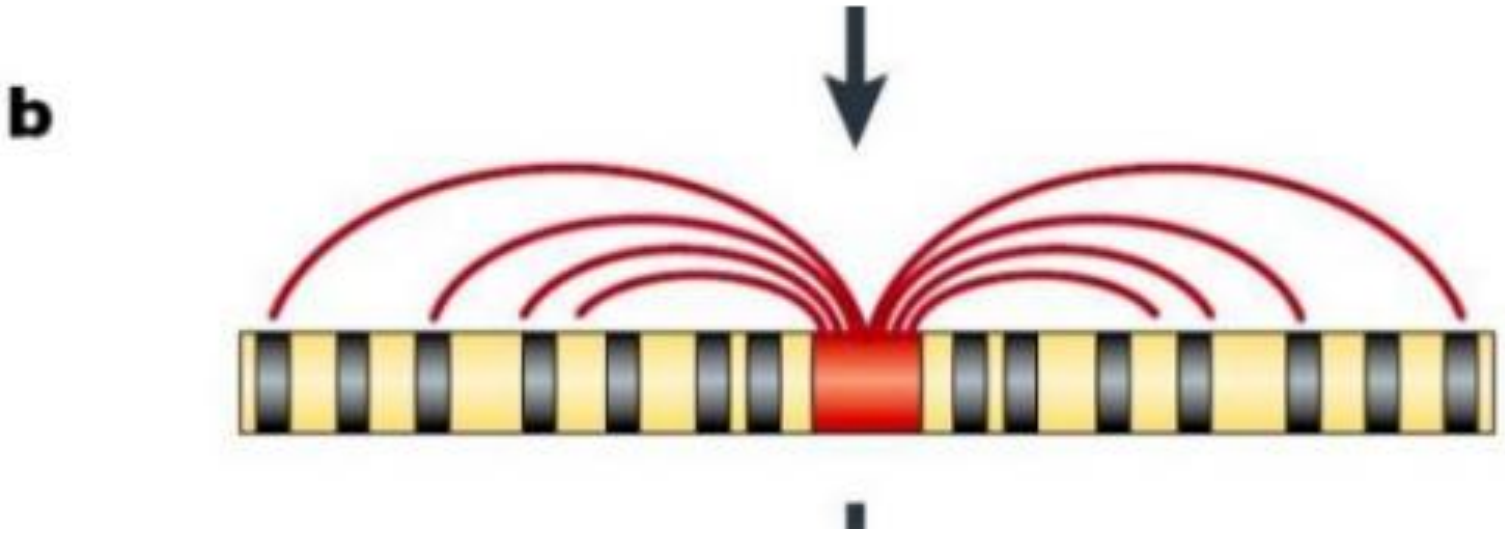
XIST the main player of x inactivation after choice

These are the following steps -

- *XIST* transcripts remains in the nucleus.
- While *Xist* is being transcribed, blocking factors prevent the association with X chromosome.



- Blocking factores are released, Xist RNA is stablized and upregulated.



- Before inactivation the X chromosome coated with stabilized Xist RNA.

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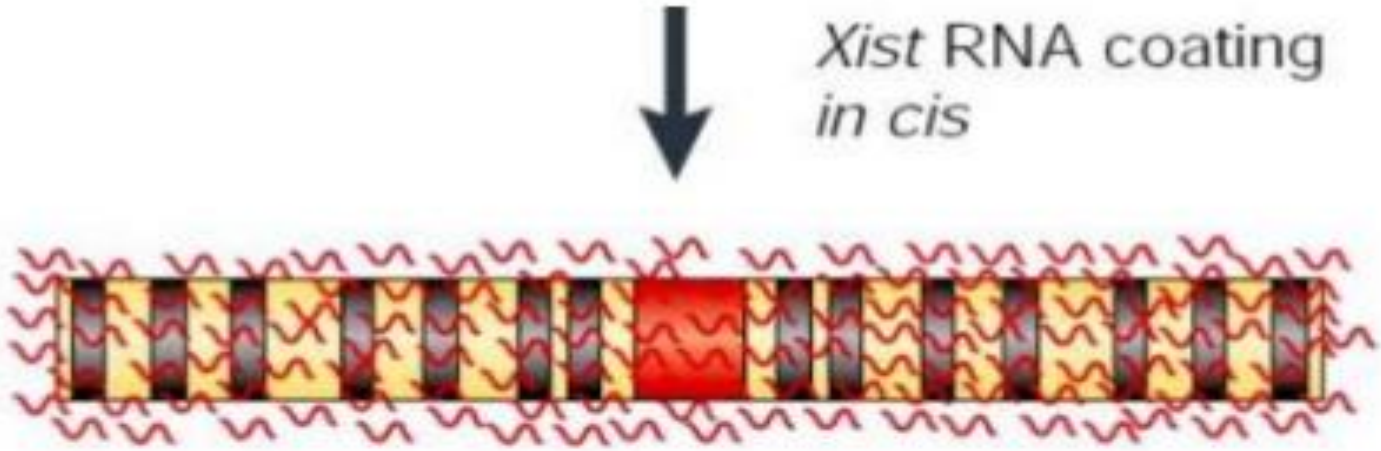
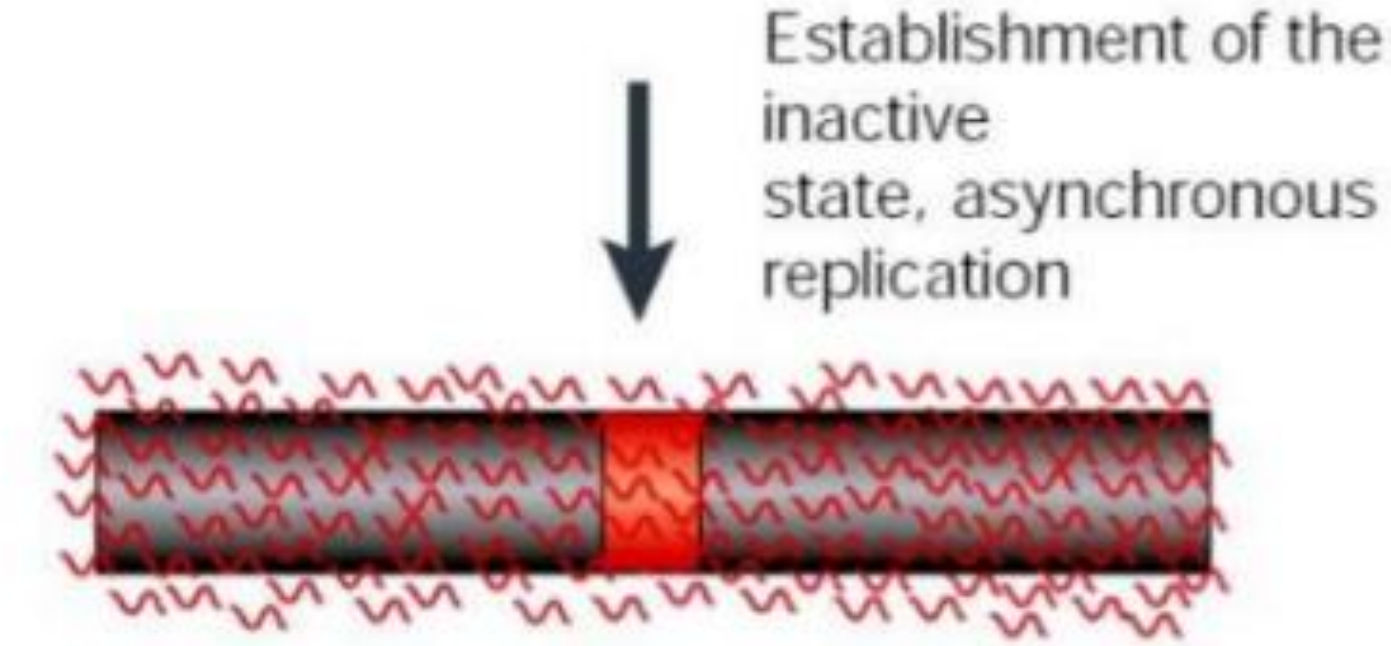
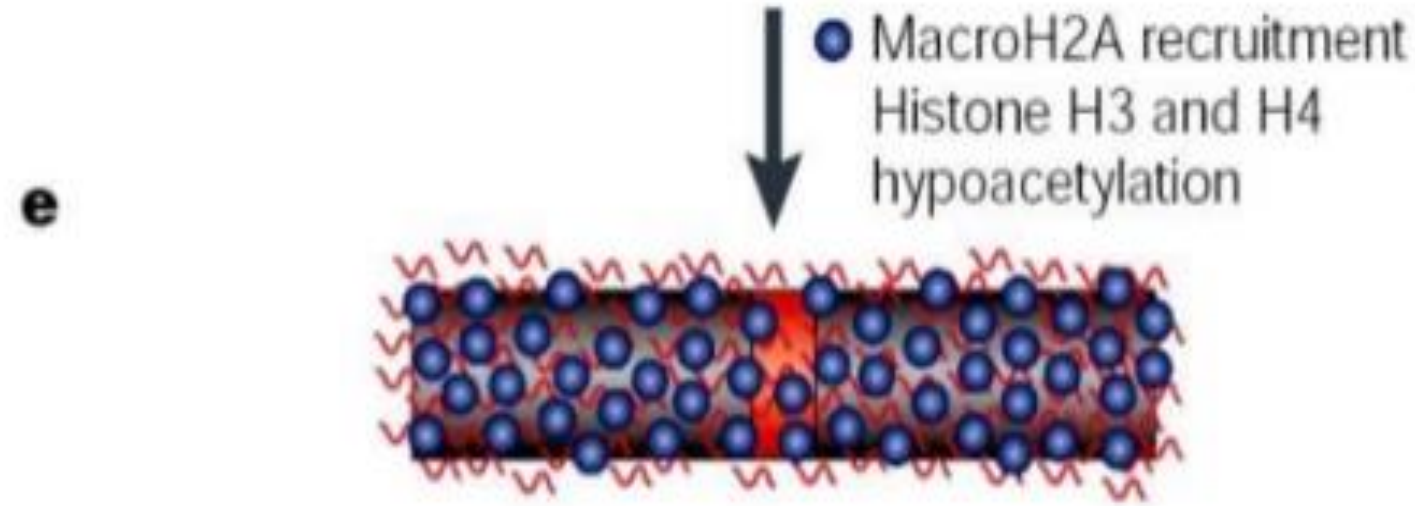



Establishment of the
inactive
state, asynchronous
replication

- Chromatin modifications, including histone deacetylation and methylation of promoters of X-linked.



Silencing and maintenance : a brief story

- X inactivation is an example of an **epigenetic** change
- One that is passed from one cell generation to the next but that does not alter the DNA base sequence.
- We can observe X inactivation at the cellular level because the turned-off X chromosome absorbs a stain much faster than the active X.
- This differential staining occurs because inactivated DNA has chemical methyl (CH 3) groups that prevent it from being transcribed into RNA and also enable it to absorb stain.

With its starting…….

- XIST RNA coats the X chromosome and promotes compaction beginning at XIC and progressing toward both ends until the entire chromosome is inactivated.

- At the late morula blastula stages the XIST signal gradually accumulated to a full cloud with heterochromatin marks on one X chromosome.

- The speed of travel of *XIST* RNA along the X chromosome may be boosted by some **''way stations''** that act as promoters of spreading and **LINE**s on X chromosome can be the candidates.

- L1 elements (LINE 1) serve as binding sites for the *XIST* RNA, perhaps acting through an RNA–protein complex.

Stabilization of inactive state

- Stabilization of the inactive state is independent of *XIST* and involves a complex series of chromatin changes, including histone modifications and methylation of CpG islands

- L1s might affect the binding of histone-modifying enzymes or might affect CpG island methylation.

- After initiation of X chromosome inactivation, XIST and its associated proteins, including YY1 transcription factor and the Polycomb Repressive Complex 2 (PRC2) spread along the X.

- Although It is not clear how these heterochromatic markers are targeted to the X chromosome, it is thought that PRC2 is recruited to the X chromosome may influence histone modification (For H3K27me3)

Xi undergoes……

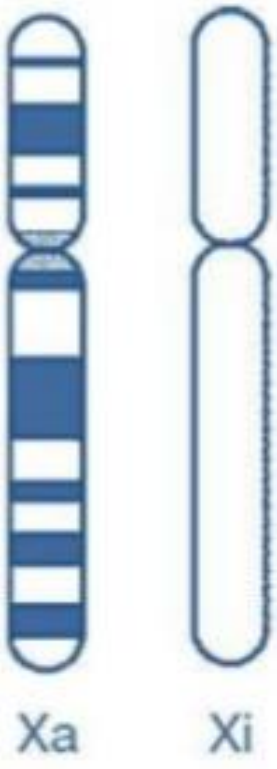
- H2A, H2B, H3 and H4 acetylation is low –like hypoacetylation of lysine 9 on histone H3 (H3K9ac)
- Methylation of lysine increases – accumulation of tri-methylation of lysine 27 on H3 (H3K27Me3)
- Macro-H2A replaces many of H2A on Xi – like enrichment of the histone variant Macro H2A 1
- DNA methylation on CpG islands increases

- These are the so-called hallmarks of XCI.

- Inactivation leads to changes in chromatin structure.
- Xi becomes late replicating.
- Its transcription pattern also changes.
- Finally, the inactivated X chromosome transferred into a cytogenetically observable Barr body.

FINAL APPEARANCE

Xi *vs.* Xa



Comparing active and inactive X

Appearance at Metaphase	*Same*
Appearance at Interphase	*Different*
DNA Replication	*Different*
DNA Content	*Same*
DNA Methylation	*Different*
Chromatin Composition	*Different*
Transcriptional Competence	*Different*

X chromosome Inactivation ...

- **Is not always random**
  - A structurally abnormal X is preferentially inactivated.
  - In extraembryonic membranes, only the maternal X is activated.
- **Is not complete**
  - Some genes are known to escape inactivation (genes located in the pseudoautosomal region-PAR).
- **Is not permanent**
  - Reversed in development of germ cells (not passed on to gametes).

Types of X inactivation

1. Random X inactivation

- Occurs in embryos around gastrulation.
- Inactive X can be maternal or paternal.
- Once established it will be maintained in the cell descendants.

2. Imprinted X inactivation

- Paternal X chromosome is selectively silenced.
- Occurs in pre-implantation embryos and extra embryonic tissues.