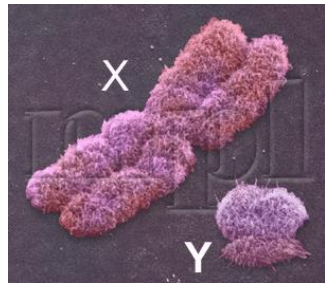
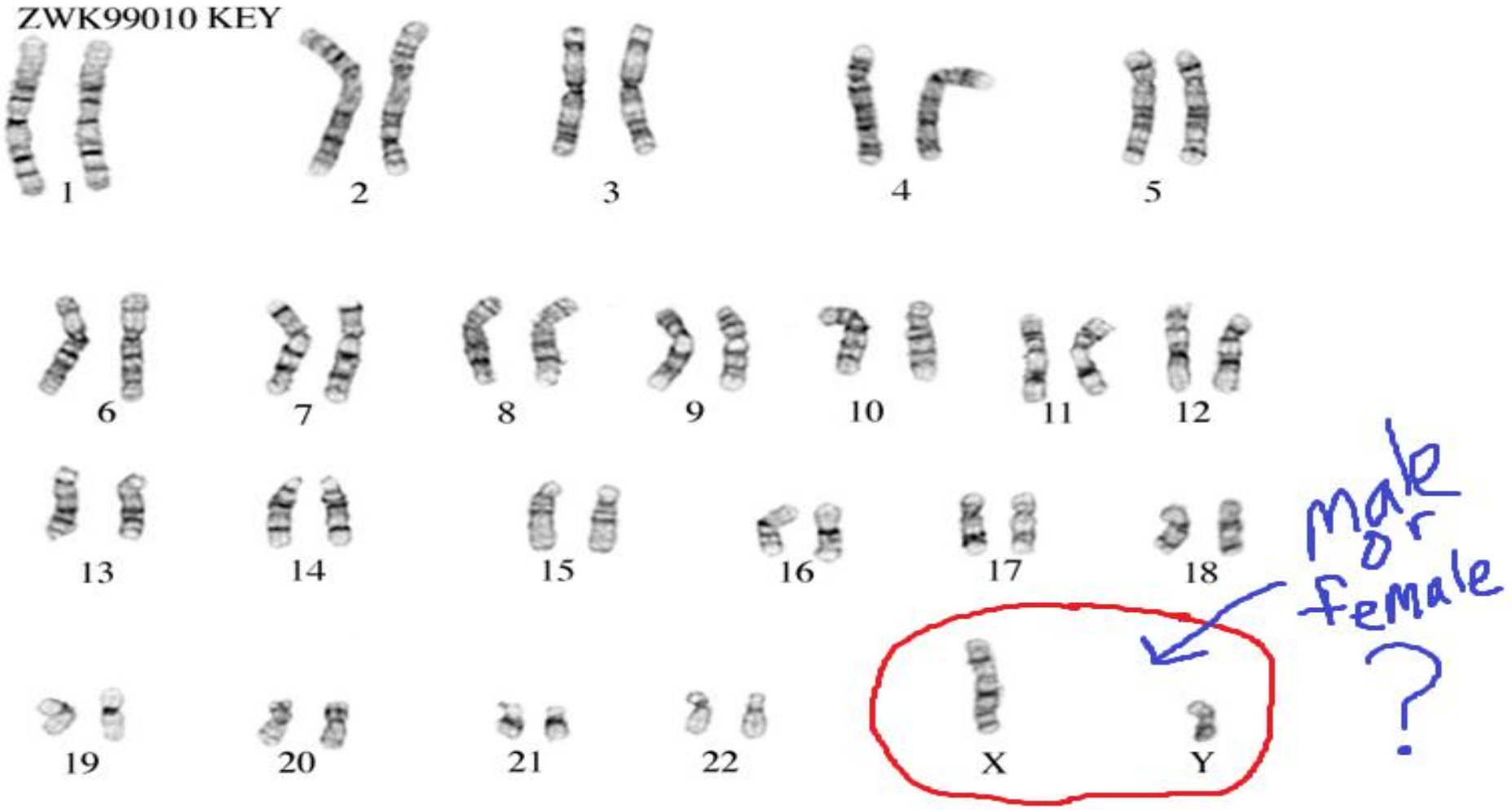
X Inactivation and Dosage Compensation

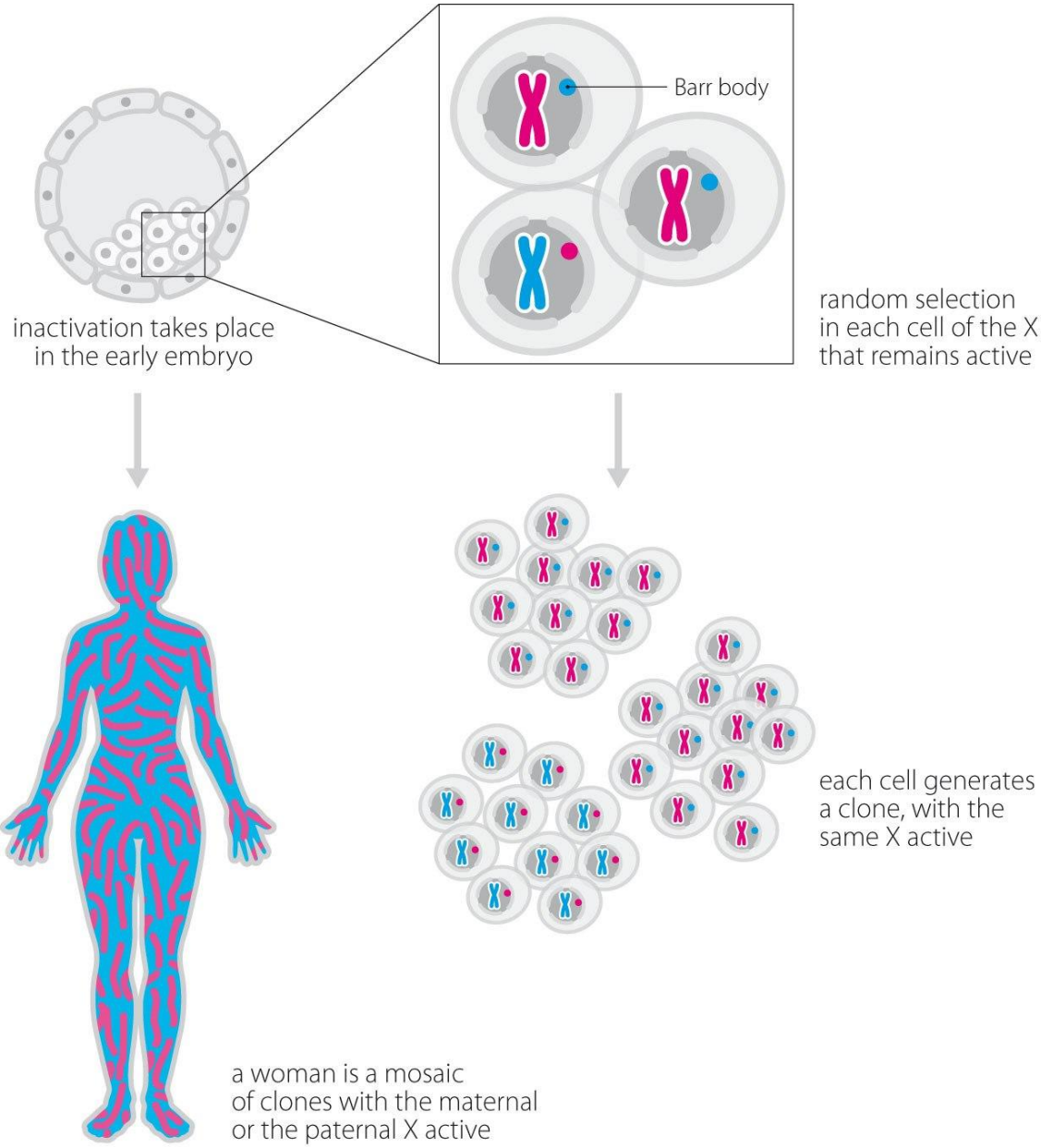
Dr.Rasime Kalkan,Ph.D.



Human Karyotype

Picture of Human Chromosomes
22 Autosomes and 2 Sex Chromosomes





X-inactivation is an epigenetic process.

Because of X-inactivation every female is a mosaic of cell lines with different active X chromosomes

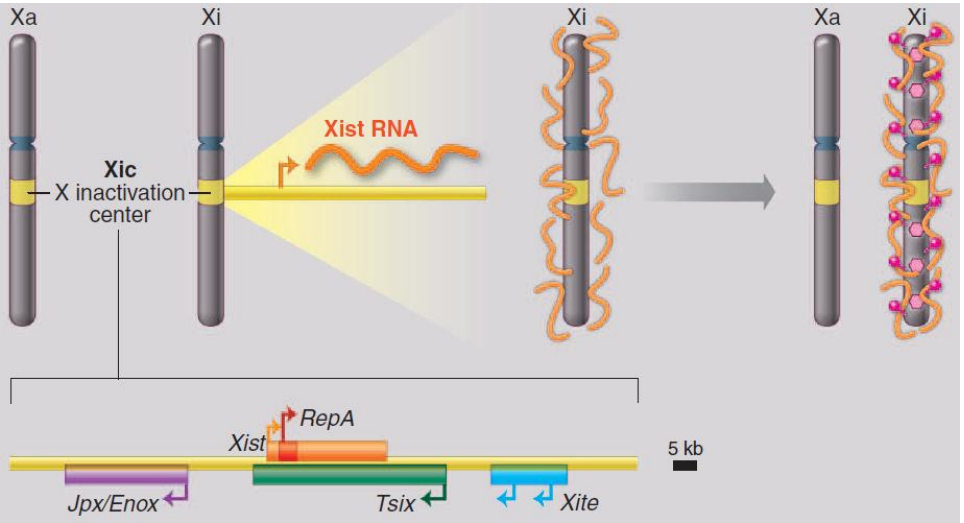
X-inactivation, Dosage compensation, and the expression of X-linked genes

Same amount of X-linked gene products between males and females

- Males
  - One X chromosome
- Females
  - Two X chromosomes
- And yet, the mean amounts of gene products of X-linked genes are the same in males as in females
- HOW?
  - Through X chromosome inactivation

X Chromosome Inactivation

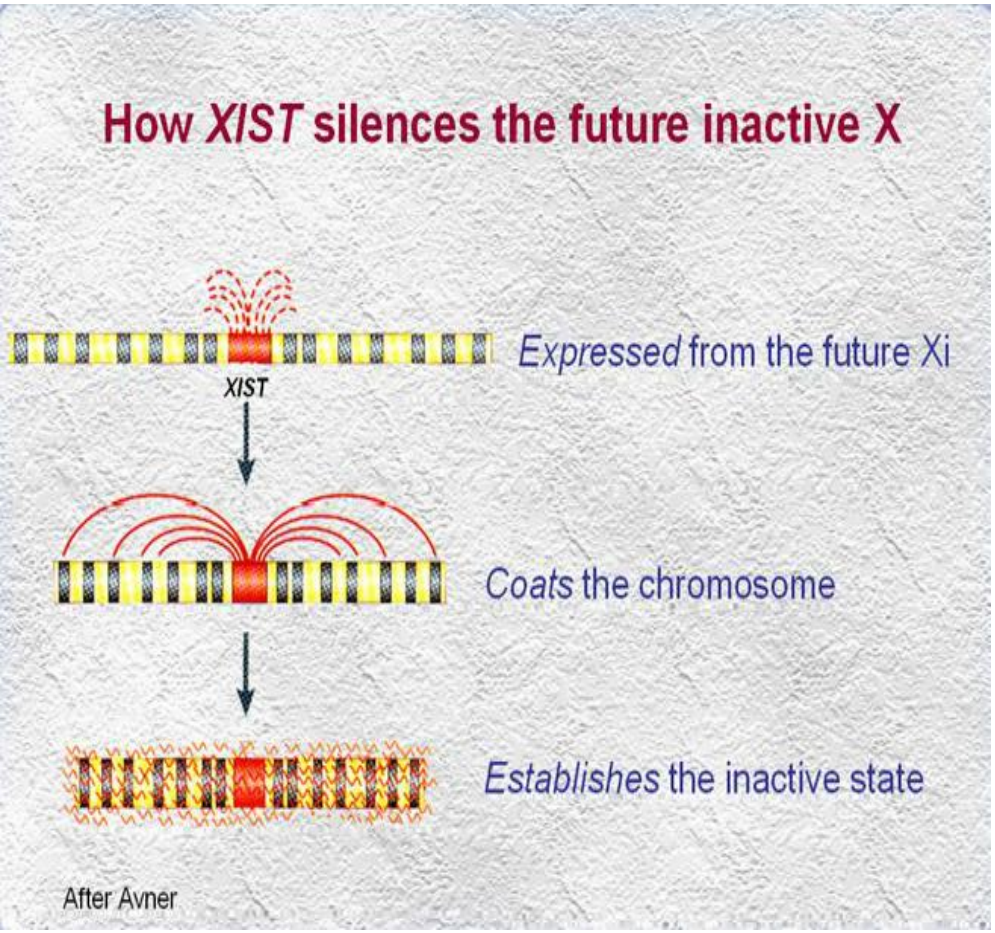
- Mechanism of X Chromosome inactivation
- XIC – X chromosome Inactivation Center
  - XIC controls expression of the *XIST* gene
  - *XIST*: X-inactive-specific transcript
  - *XIST* produces a non-coding 17 kb RNA molecule
  - "Coats" the entire *local* X-chromosome – *cis*-acting

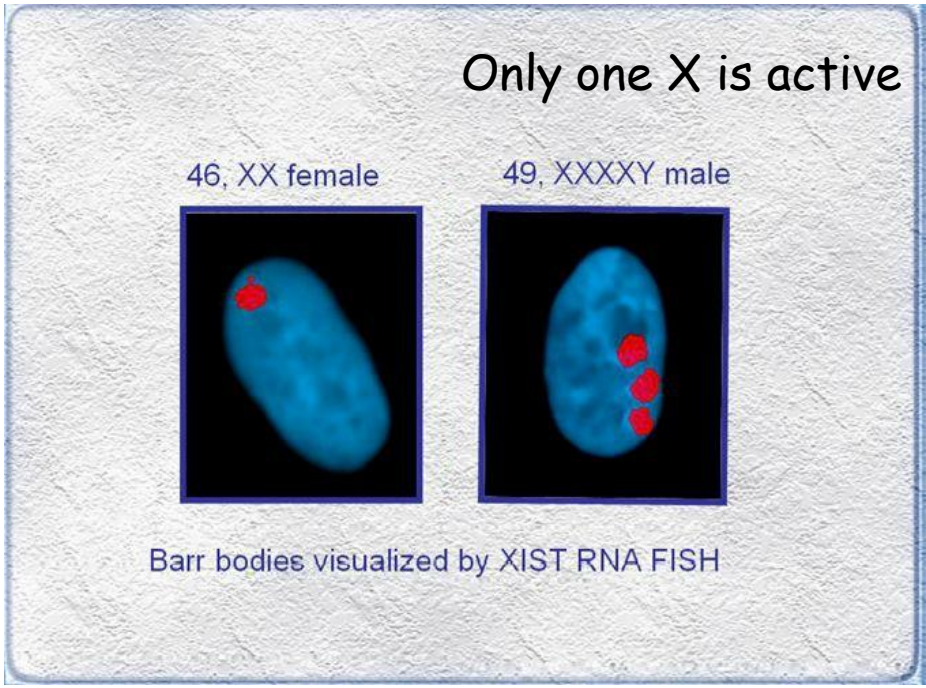


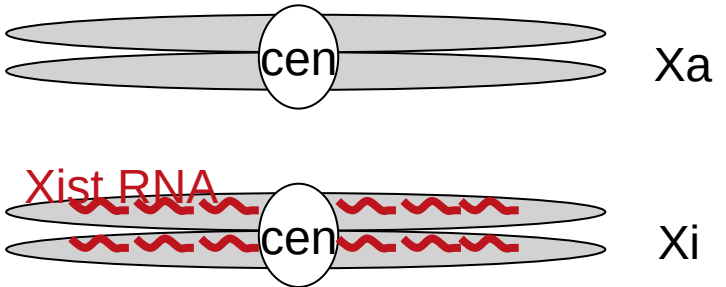
from Lee, *Science* 338, 1435 (2012)

Characteristics of XIST Gene

- Located in XIC
- Transcribed only from the inactive X

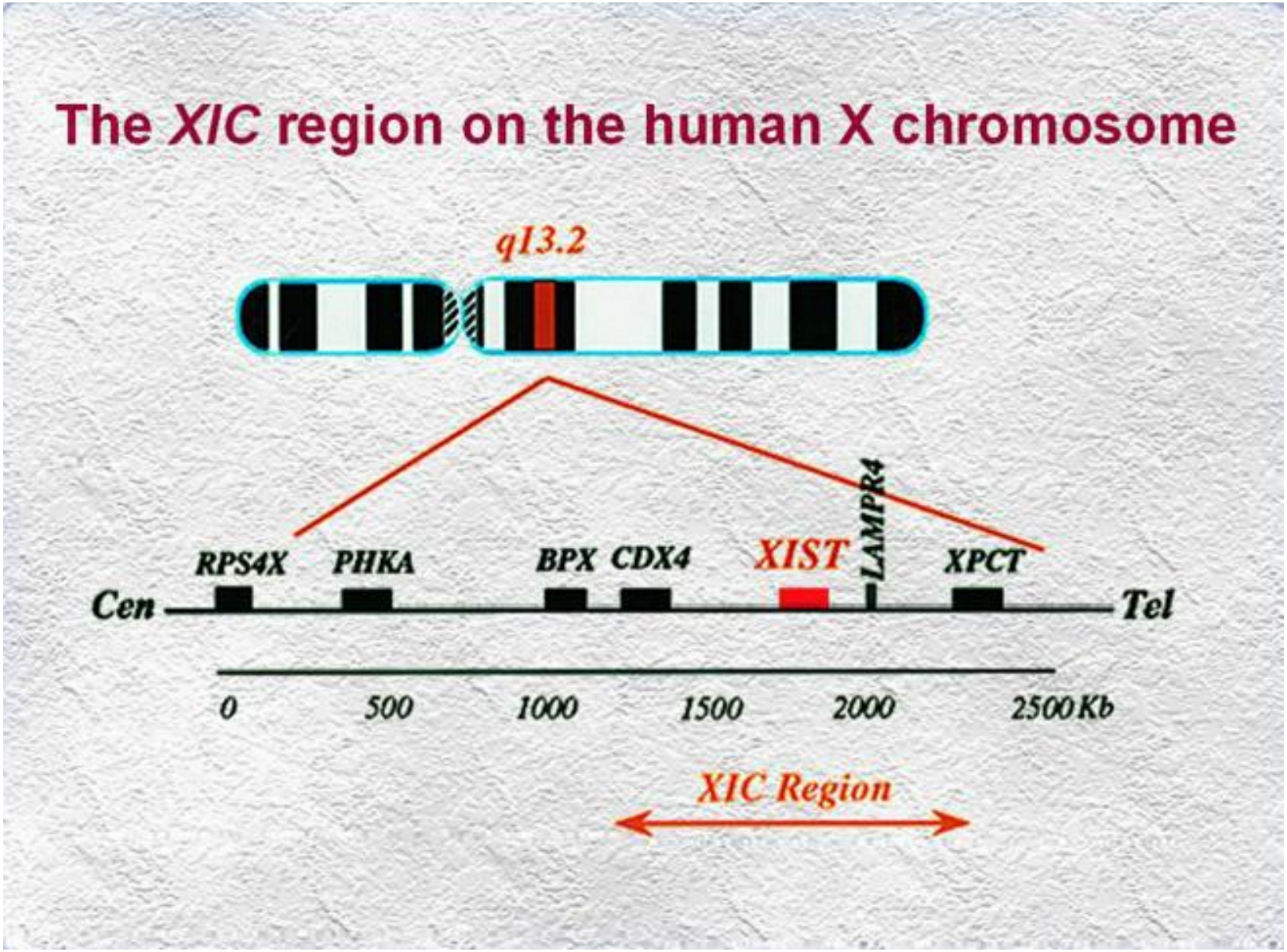






Doug Brutlag 2011 Inactive X has unacetylated histone H4 Barbara Migeon, Henry Stewart Talks

XIC Region


The XIC region on the human X chromosome
q13.2
RPS4X
PHKA
BPX
CDX4
XIST
LAMPR4
XPCT
Cen
Tel
0
500
1000
1500
2000
2500 Kb
XIC Region

The molecular mechanism behind X-inactivation

- The key player is the X-linked gene XIST
  - X(inactive)-specific transcript
  - Chromosome Xq13.2
- XIST is transcribed to produce a non-coding RNA that "coats" the X-chromosome and inactivates it
- XIST is uniquely expressed from the inactive X
- *XIST* RNA does not travel over to any other X chromosome in the nucleus (i.e., *cis* action).
- **Barr bodies** are inactive X chromosomes "painted" with *XIST* RNA.

- XIST: key master regulator for X inacitvation
- It is expressed only from the allel on the inactive X
- It is transcriptionally silent on the active X in both male and female cells.
- Have a functions in the initiation phase of X inactivation.

- Transcription of *XIST* **ceases** on the other X chromosome allowing all of its hundreds of other genes to be expressed.

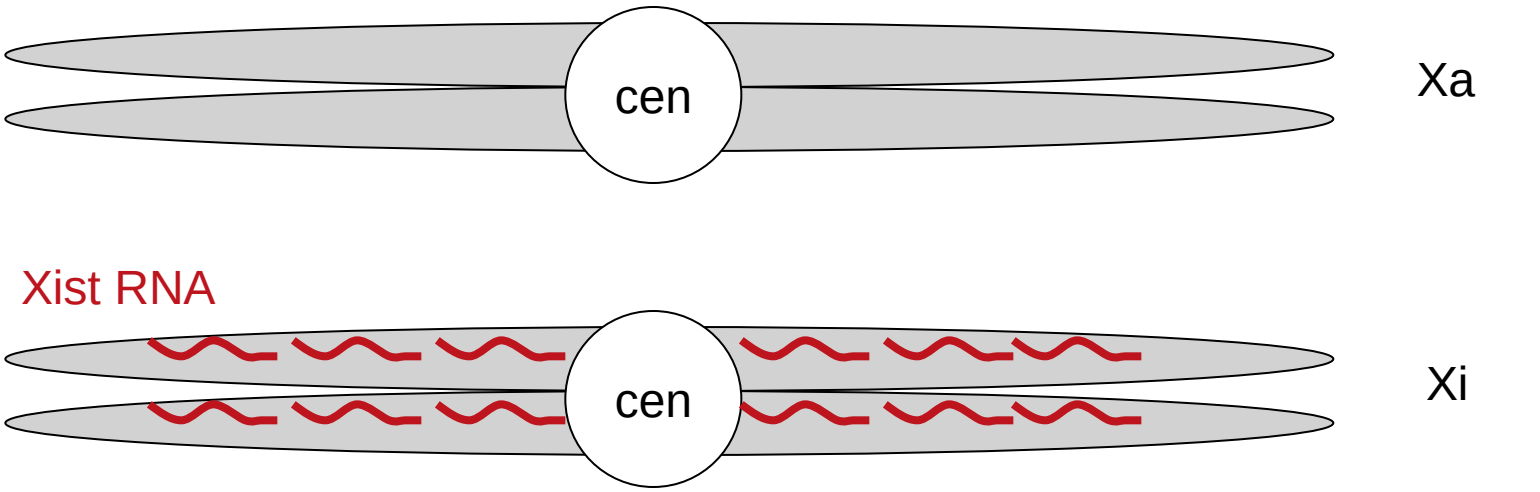
- The shut-down of the *XIST* locus on the active X chromosome is done by methylating *XIST* regulatory sequences.

- So methylation permanently blocks *XIST* expression and permits the continued expression of all the other X-linked genes.

Process

- Xist gene (pronounced "exist")
  - Encodes a large RNA molecule
    - Coats Xi from the XIC near the centromere outward along the X chromosome

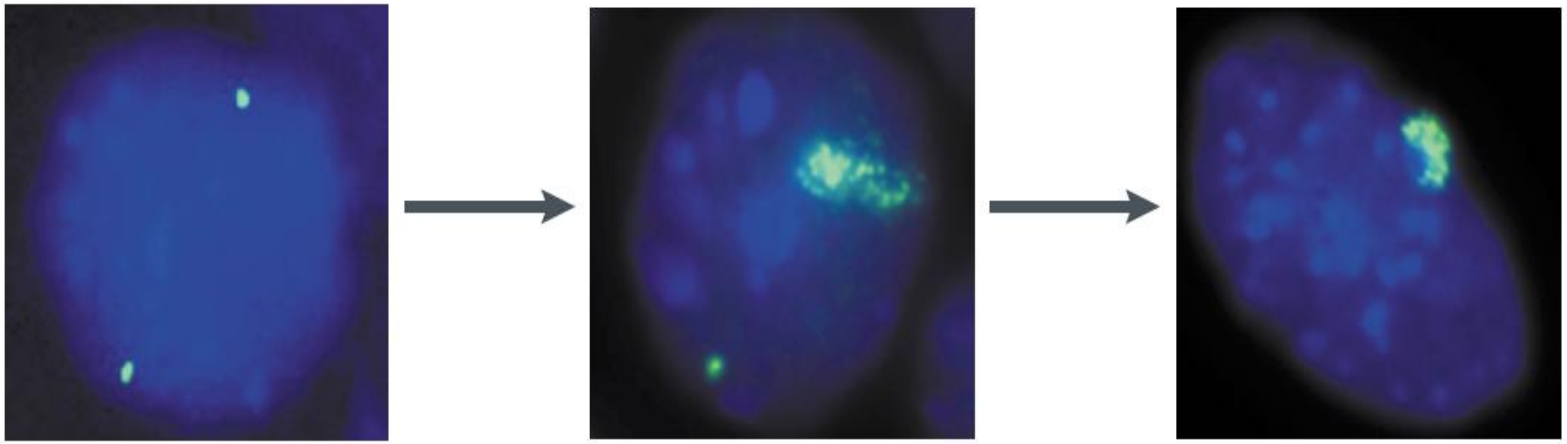
(Lyon, 2003)



Process

- Mechanism for compacting Xi (Barr body)
  - Enzymes cause the following to occur:
    - High levels of DNA methylation (CH_3) (Chadwick et al., 2003)
    - Low levels of histone substitution of the acetyl group (CH_3CO) for a H atom in a -OH group

Xist Transcription in Embryonic Stem Cells



Differentiation ⟶ *48 hrs.*

Adapted from Avnir and Heard (2001) *Nature Reviews Genetics*

Dosage compensation

- Ensures an equal expression of genes from the sex chromosomes even though females have 2 X chromosomes and males have only 1
- In each female cell, 1 X chromosome is inactivated and is highly condensed into a Barr body
- Females heterozygous for genes on the X chromosome are genetic mosaics

X-Chromosome Inactivation

X-Chromosome Inactivation

British geneticist Mary Lyon discovered that in female cells, one X chromosome is randomly switched off.

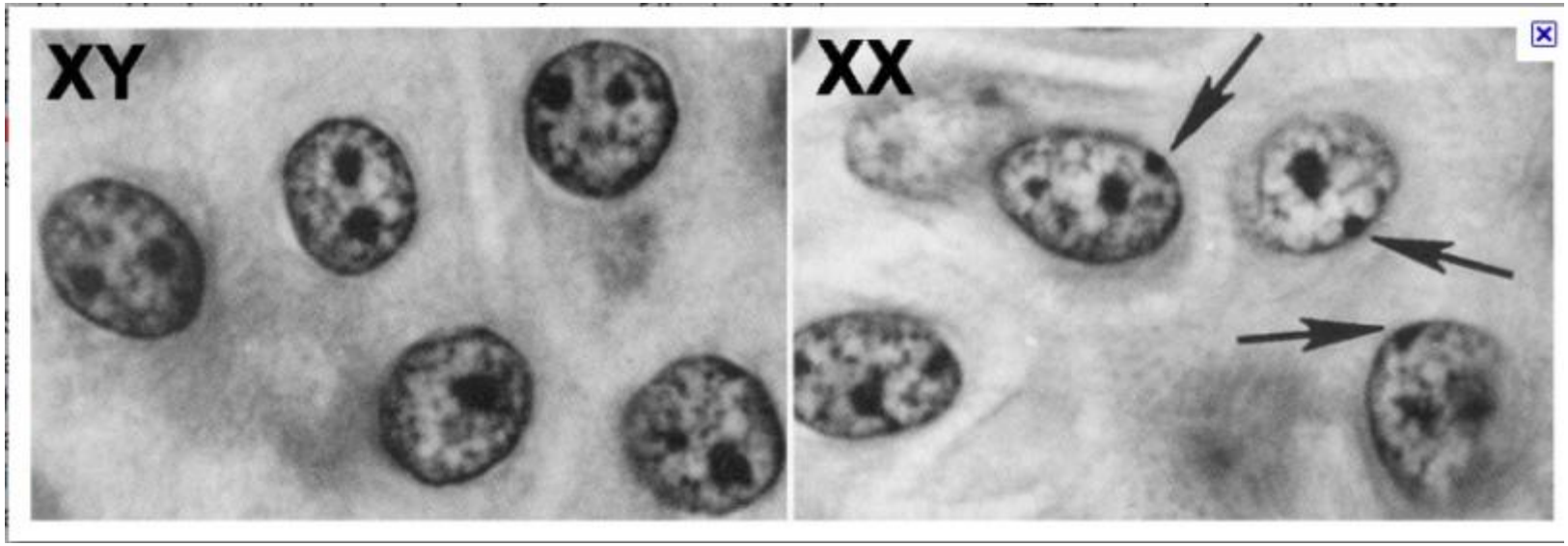
This chromosome forms a dense region in the nucleus known as a **Barr body**.

Barr bodies are generally not found in males because their single X chromosome is still active.

This explains why XXX females don't show symptoms.

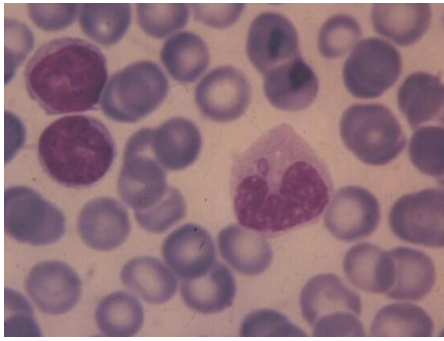
The inactive X chromosome was presence of a heterochromatic mass (Barr Body) in interphase cells

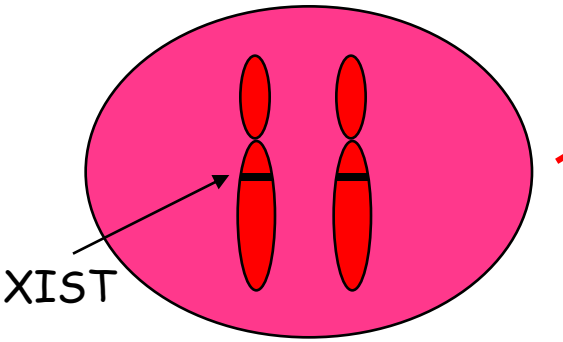
X Chromosome Inactivation: Barr Bodies



Barr, M. L., Bertram, E. G., (1949), A Morphological Distinction between Neurones of the Male and Female, and the Behaviour of the Nucleolar Satellite. *Nature.* ***163*** *(4148): 676-7.*

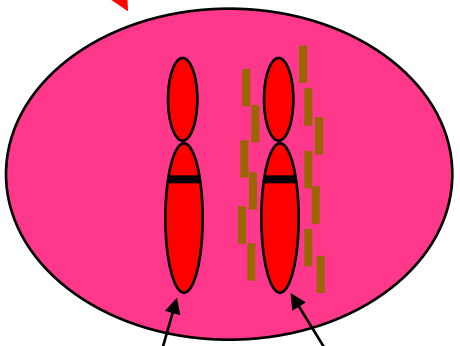
Lyon, M. F., (2003), The Lyon and the LINE hypothesis. j.semcdb 14, 313-318. (Abstract)



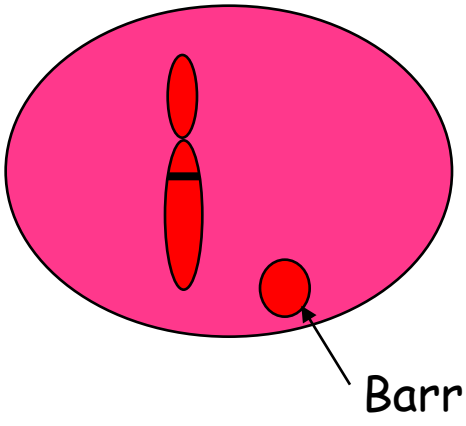


The XIST gene on one of the two X-chromosomes is randomly inactivated by DNA methylation

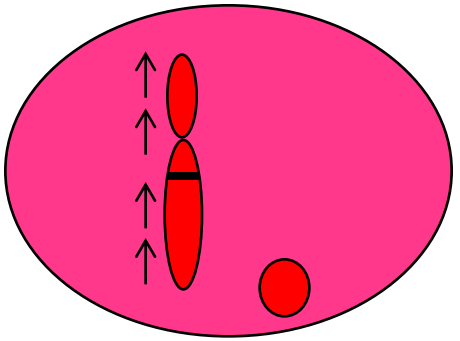
The active XIST is transcribed and its RNA product coats the X-chromosome



<u>The histones</u> on the coated X undergo methylation which causes the chromosome to condense, forming a Barr body, and renders it inactive

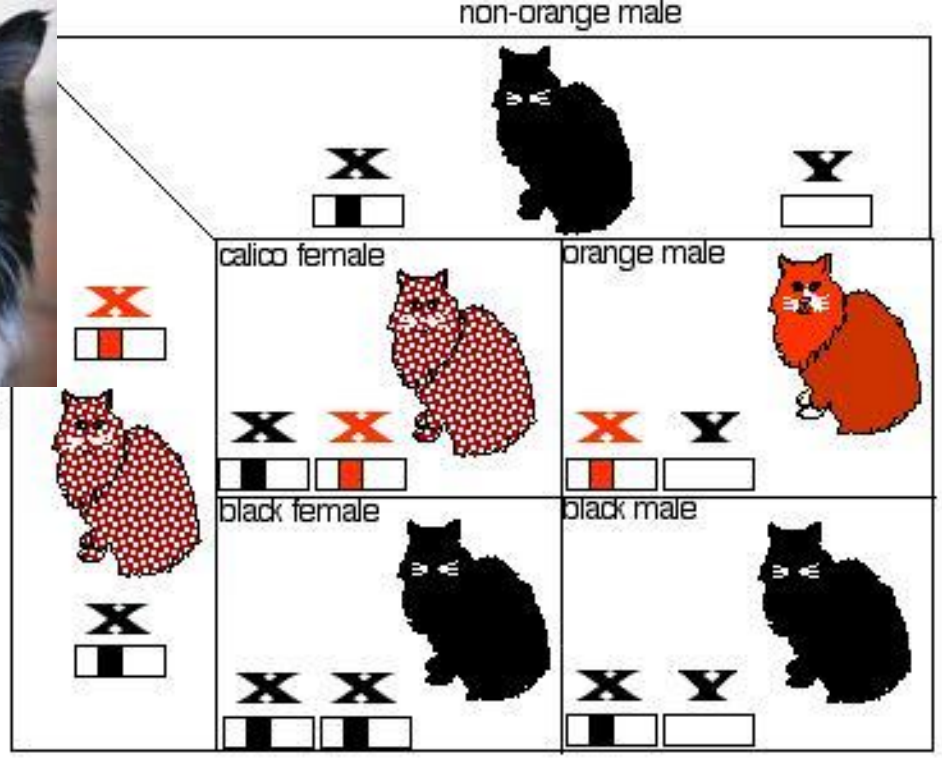


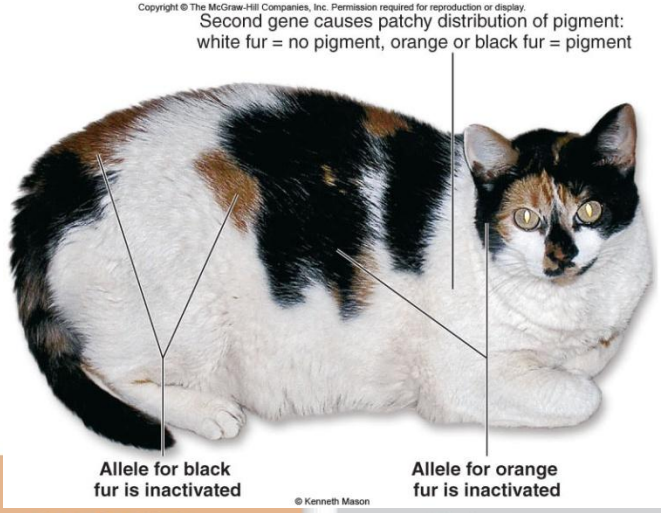
The uncoated X is left transcriptionally active



The Lyon Hypothesis of X Inactivation

- Proposed by Mary Lyon and Liane Russell (1961)
- Which X is inactivated? Inactivation of X chromosome occurs randomly in somatic cells during embryogenesis
- Progeny of cells all have same inactivated X chromosome as original, creating mosaic individual

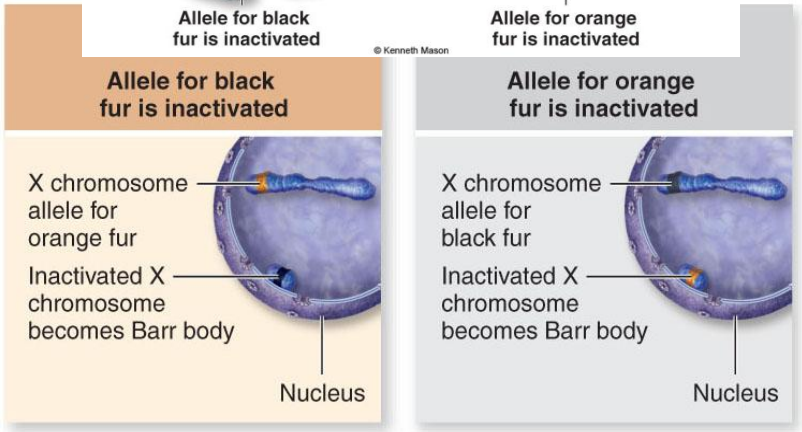




Y no color genes

X chromosome with orange gene

X chromosome with non-orange gene



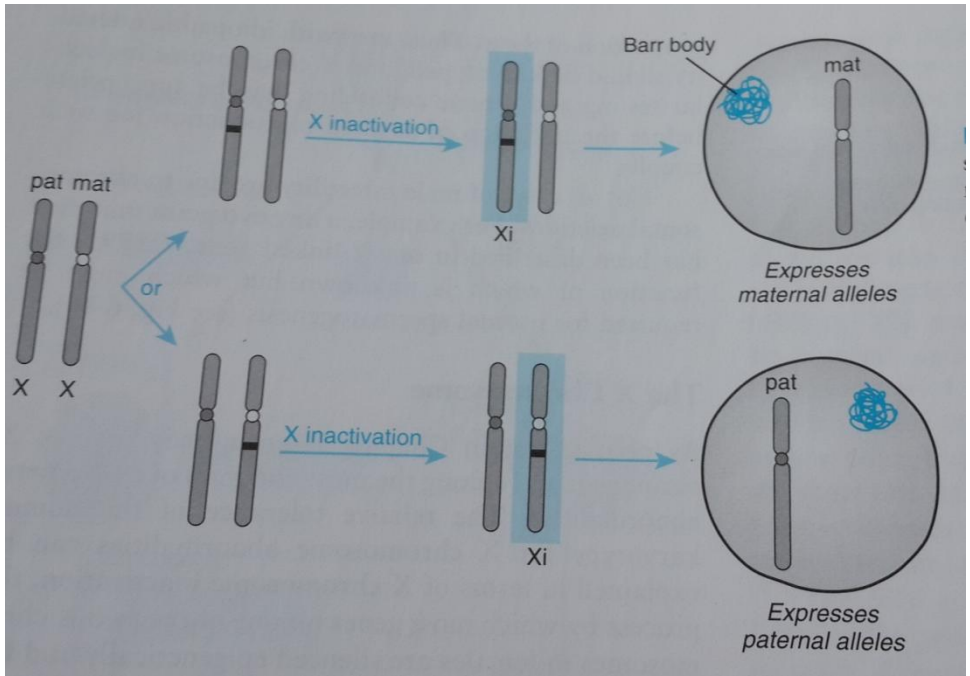
X chromosome Inactivation

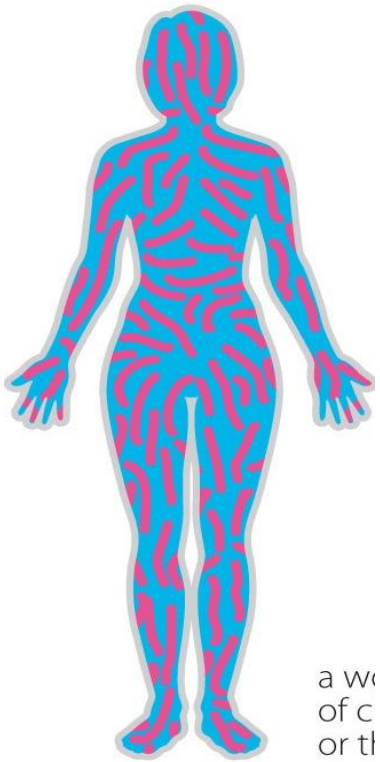
- Inactivation is not always random
  - A structurally abnormal X is preferentially inactivated, e.g., isochromosome X
  - E.g., extraembryonic membranes (that go on to form the amnion, placenta, and umbilical cord). In all the cells of the extraembryonic membranes, it is father's X chromosome that is inactivated.

- Inactivation is not complete
  - Some genes are known to escape inactivation (i.e. those with a functional homolog on the Y, e.g., genes located in the pseudoautosomal region, still others are specific to X chr.)

- Inactivation is not permanent
  - reversed in development of germ cells (not passed on to gametes)

- In normal female cells, the choice of which X is to be inactivated is random.
- Females are mosaic with respect to X linked gene expression, some cells expres allels on the paternally inherited X but not maternally inherted X, other cells do opposite.

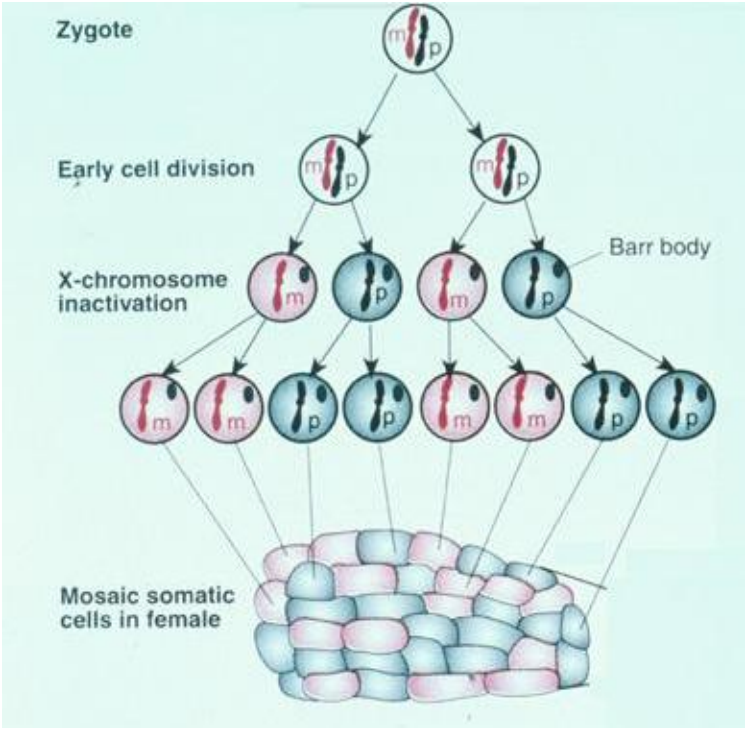




Thomson and Thomson , medical in genetics , 6th edition

Functional Mosaicism Resulting from X-inactivation

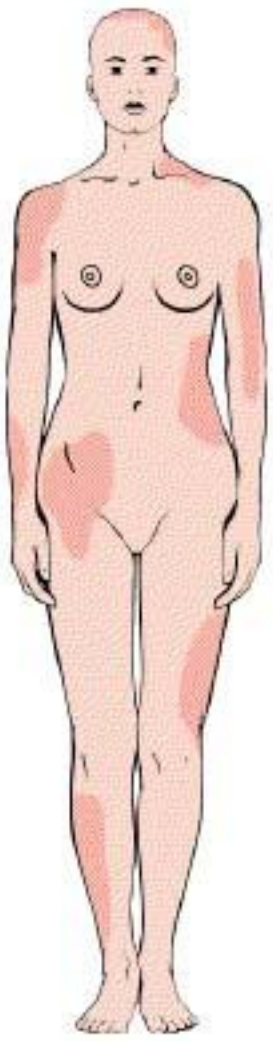
- Females are mosaics with their X-linked genes
- Mosaicism is readily detected for some disorders e.g., DMD



Thomson and Thomson , medical in genetics , 6th edition

Mosaicism Reveals the Random Inactivation of one X chromosome

Anhidrotic ectodermal dysplasia in a heterozygous woman



Regions where sweat glands are absent.

Inconsistencies between syndromes and X inactivation

If normal XX female has one X inactivated, why is a X Turner female not normal?

Similarly, if XXY male has one X inactivated, why does he have Klinefelter syndrome?

Perhaps not complete inactivation
Or inactivation does not happen immediately,
Then some overexpression of X-linked genes

Chrosomal features of X inactivation

- Inactivation of most X linked genes on the inactive X
- Random choice of one of two chromosomes in female cells
- Inactive X:

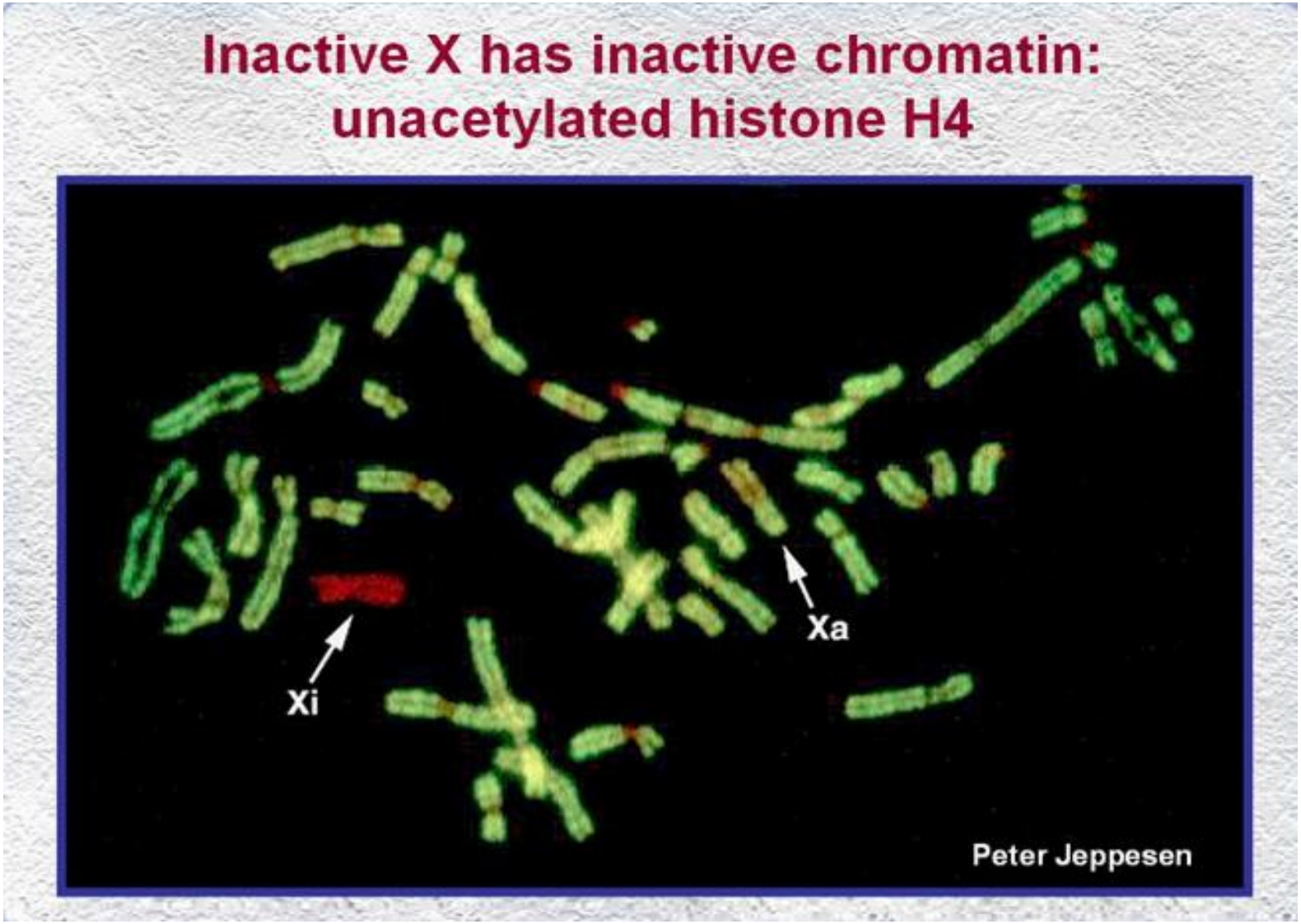
❑ Heterochromatic (Barr Bosy)

❑ Late-replicating in S phase

❑ Expresses XIST RNA

❑ Associated with macroH2A histone modifications in chromatin

Inactive X has unacetylated histone H4



Expression of X-linked Genes in Heterozyotes

- Inactivation is random, established when embryo < 100 cells → fraction of cells in carrier female with normal or mutant allele tend to be variable
- Thus, clinical variation in expression of X-linked disorders is common in heterozygotes ranging from normal to affected
- A manifesting heterozygote is a female in whom the deleterious allele is on the active X in most or all of cells (an extreme e.g., of unbalanced or skewed X-inactivation)

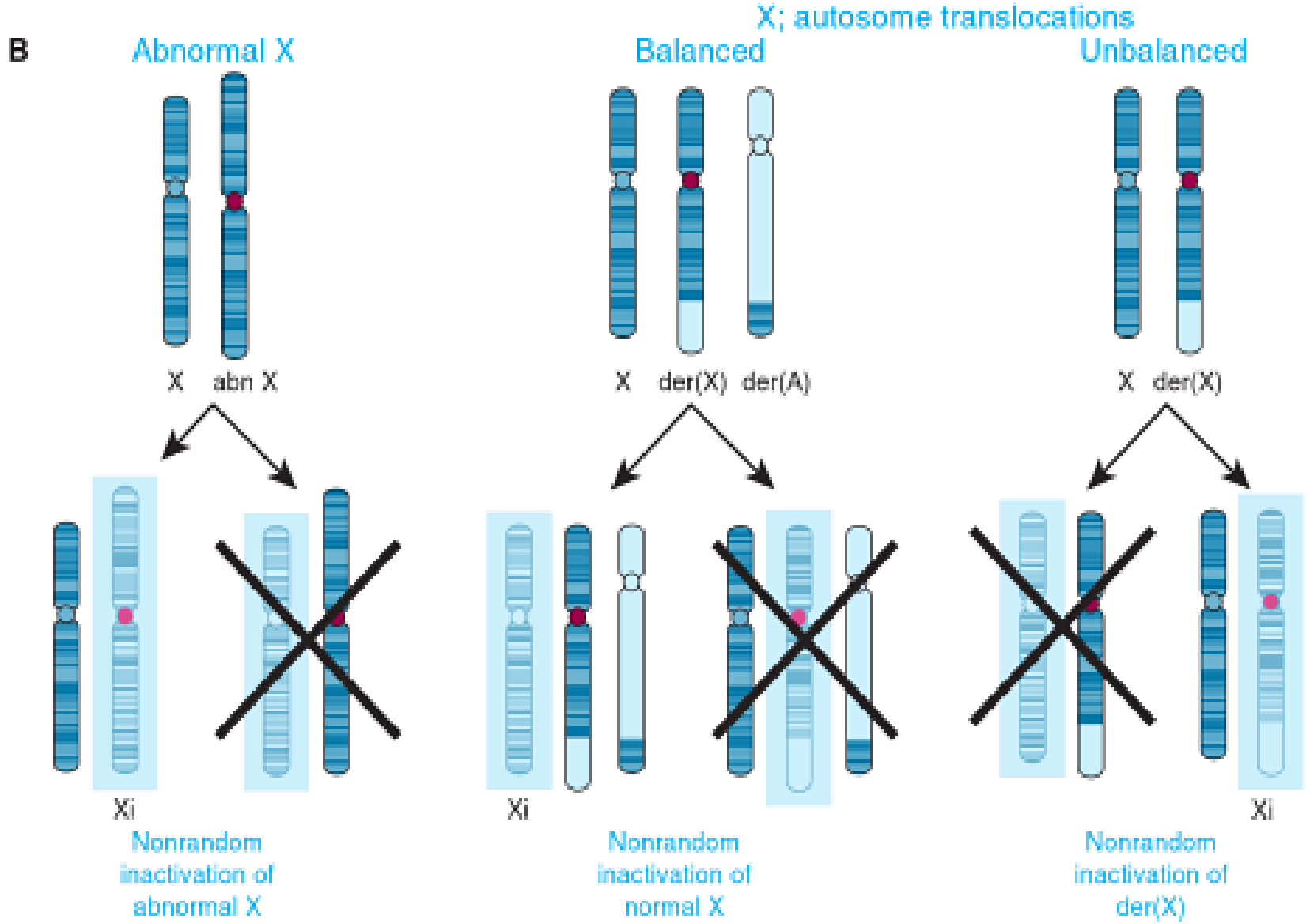
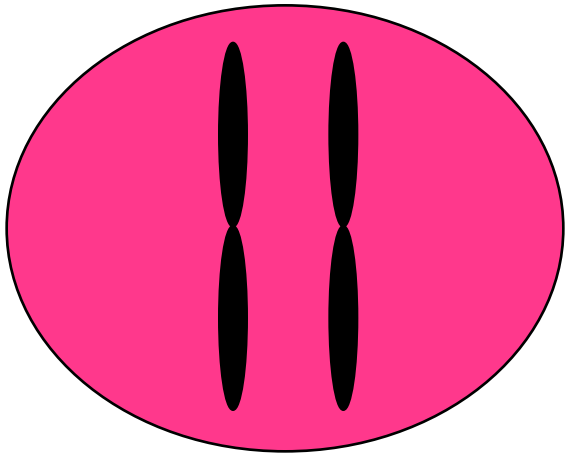
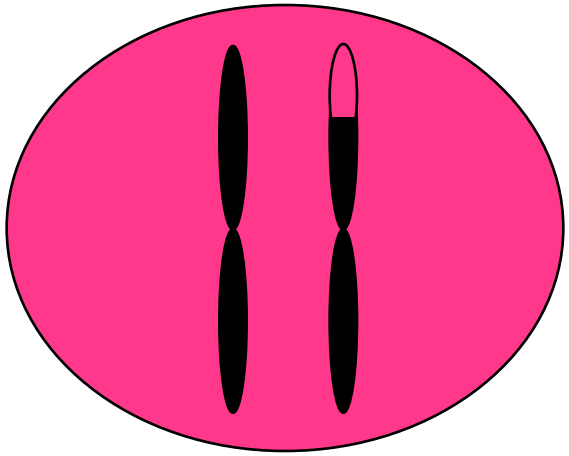


Figure 6-13 X chromosome inactivation in karyotypes with normal or abnormal X chromosomes or X;autosome translocations. A, Normal female cells (46,XX) undergo random X inactivation,

X-autosome translocation





There is normally a 50% chance that a particular X will be inactivated in a cell from a female

If an X bears a piece of autosome (translocation) then the untranslocated X is always inactivated since the cell needs both copies of the autosomal genes to be active

If the translocated X has a mutant allele, all the woman's cells will be mutant