COURS 2025

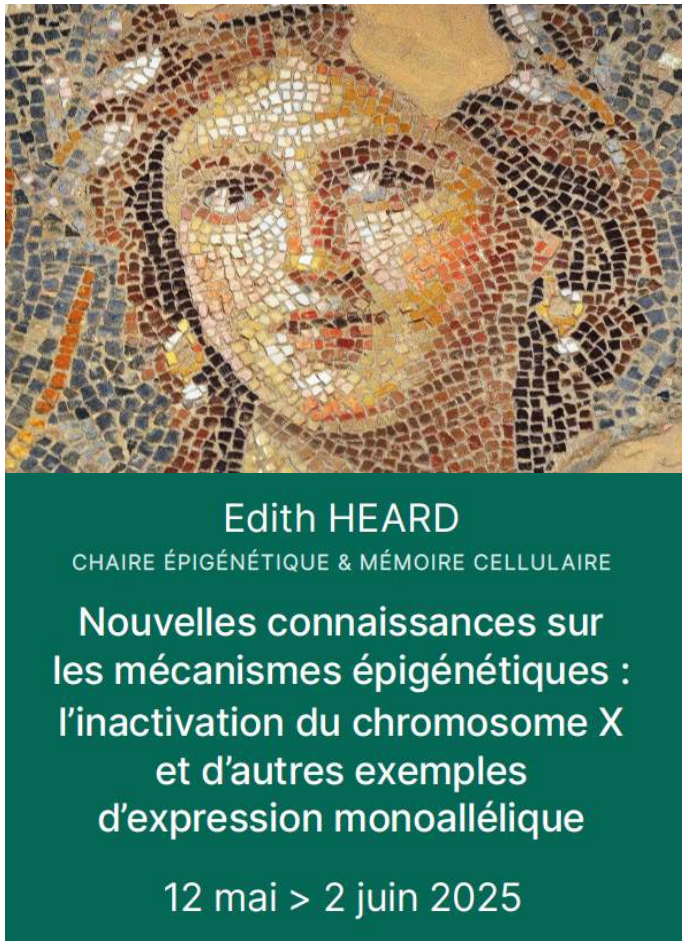
12 mai 2025
Découverte de l’inactivation du chromosome X (lyonisation)

19 mai 2025
La génétique et l’épigénétique de l’inactivation du chromosome X et d’autres exemples d’expression monoallélique

26 mai 2025
Évolution de l’inactivation du chromosome X et dynamique développementale

2 juin 2025
Implications de l’inactivation du chromosome X pour la biologie féminine

10-11 juin 2025 Colloque



CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2024-2025 :

Nouvelles conaissances sur les mécanismes épigénétiques : l'inactivation du chromosome X et d'autres exemples d'expression monoallélique

Cours I, 12 mai 2025

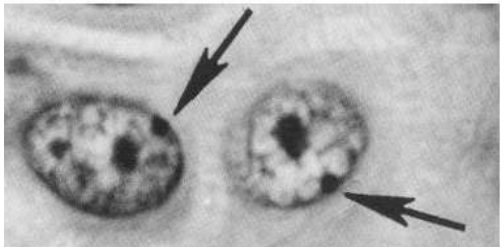
Découverte de l'inactivation du chromosome X (lyonisation)

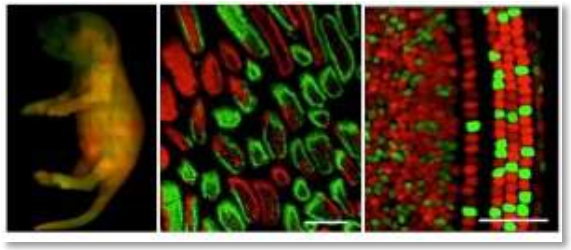
Mary Lyon (1925-2014)
née le 15 mai 1925

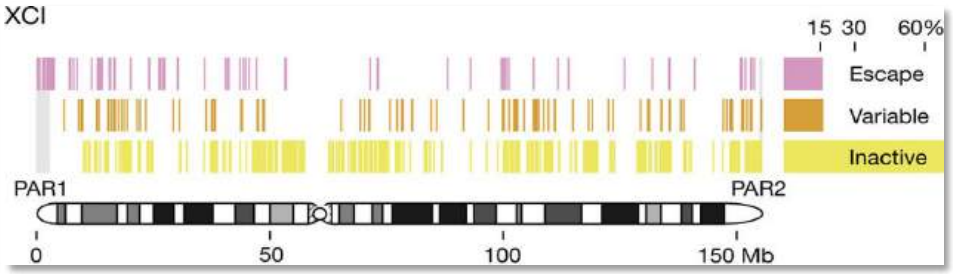
COLLÈGE DE FRANCE 1530

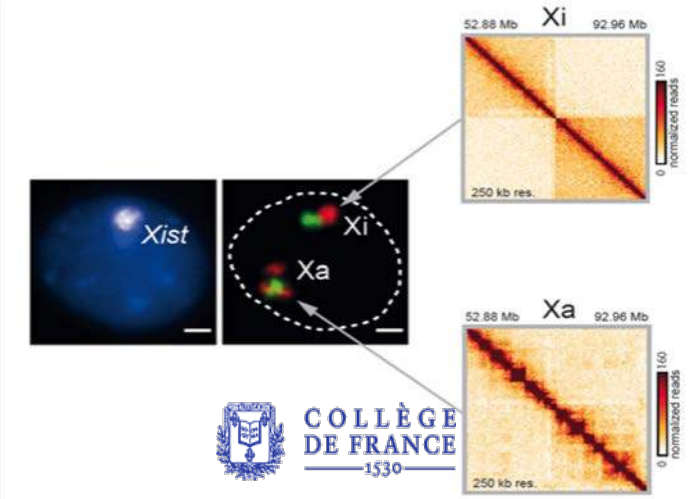
The Silencing of one of the two X Chromosomes in Mammals

The discovery of X Inactivation started with cytology (early 20th century), to genetics and cytogenetics in the 1960's-80's, to molecular genetics and cellular biology from the 1990's, and now to precision genetics, single cell biology and advanced -omics and imaging....
A time of acceleration in our understanding of XCI: its mechanisms and its implications
(How? How much? and Why?)



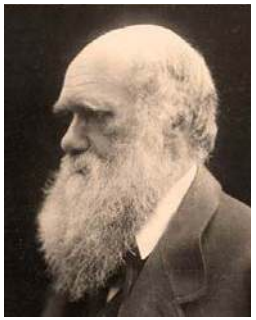




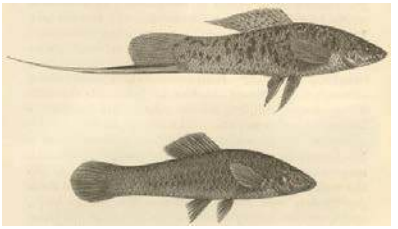


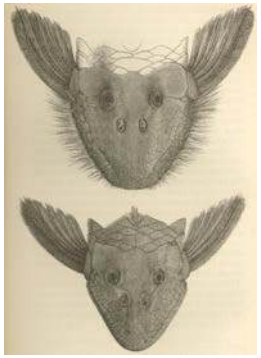
Why evolve X inactivation, why evolve sex chromosomes, why evolve sexual reproduction…?

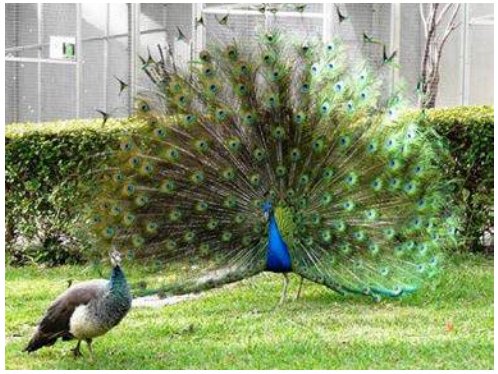
How a single species develops two strikingly different forms had fascinated early naturalists, like Charles Darwin, but it was not until the early 1900s that the genetic basis of sex started to be unravelled.



COURS 2018



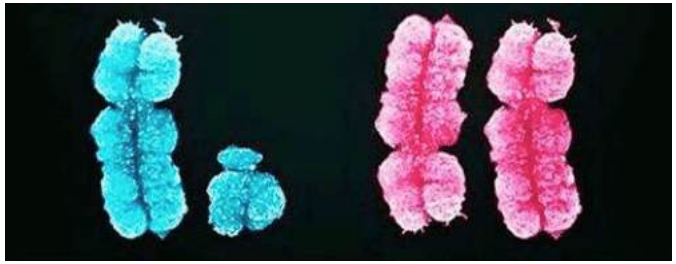




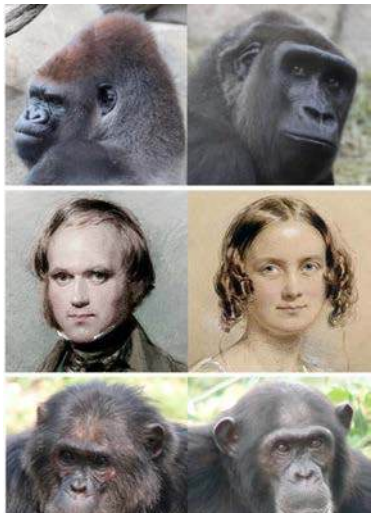
Sexual Dimorphism and Sex Chromosomes

Genetics

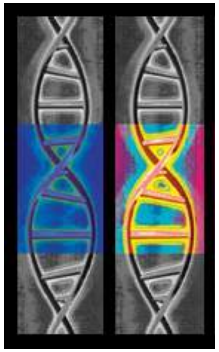
Environment



Four models for genetic determination of sex:
XY male dominant gene on Y like human
XY dosage sensitive gene on X (eg drosophila)
ZW female specific gene on W (eg Xenopus)
ZW dosage sensitive gene on Z (eg birds)



XY XX



Epigenetics

Epigenetics

Sexual dimorphism can be striking or subtle

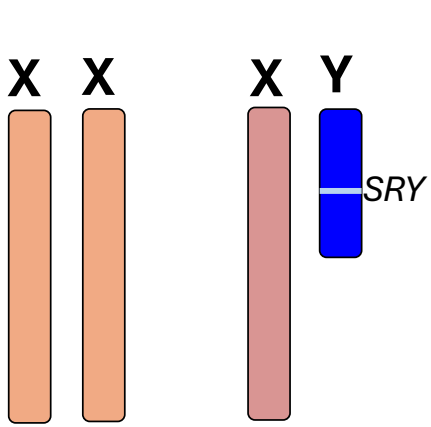
It can be manifested at the molecular, cellular and organism levels

Sex determination is “genetic” but development of different sexes can be triggered by the environment in some organisms and/or by sex chromosome differences

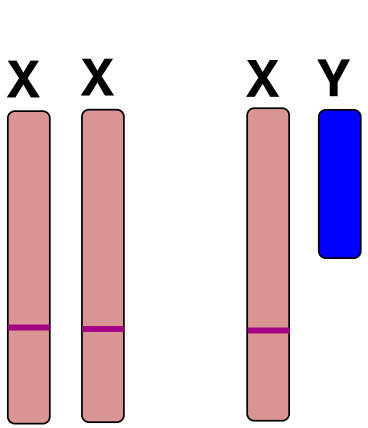
E. Heard, May 12th, 2025

Sex chromosome systems

Male heterogamety

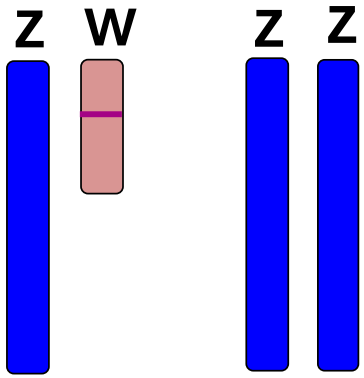


Male dominant gene on Y
e.g. SRY, human

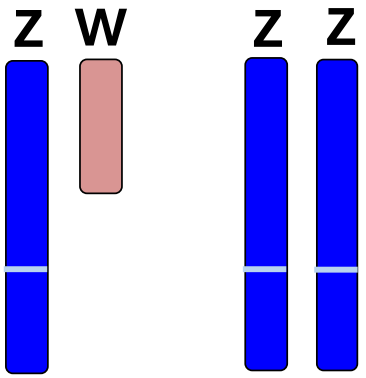


Dosage-sensitive female-determining gene on X, *e.g. SLX, Drosophila*

Female heterogamety



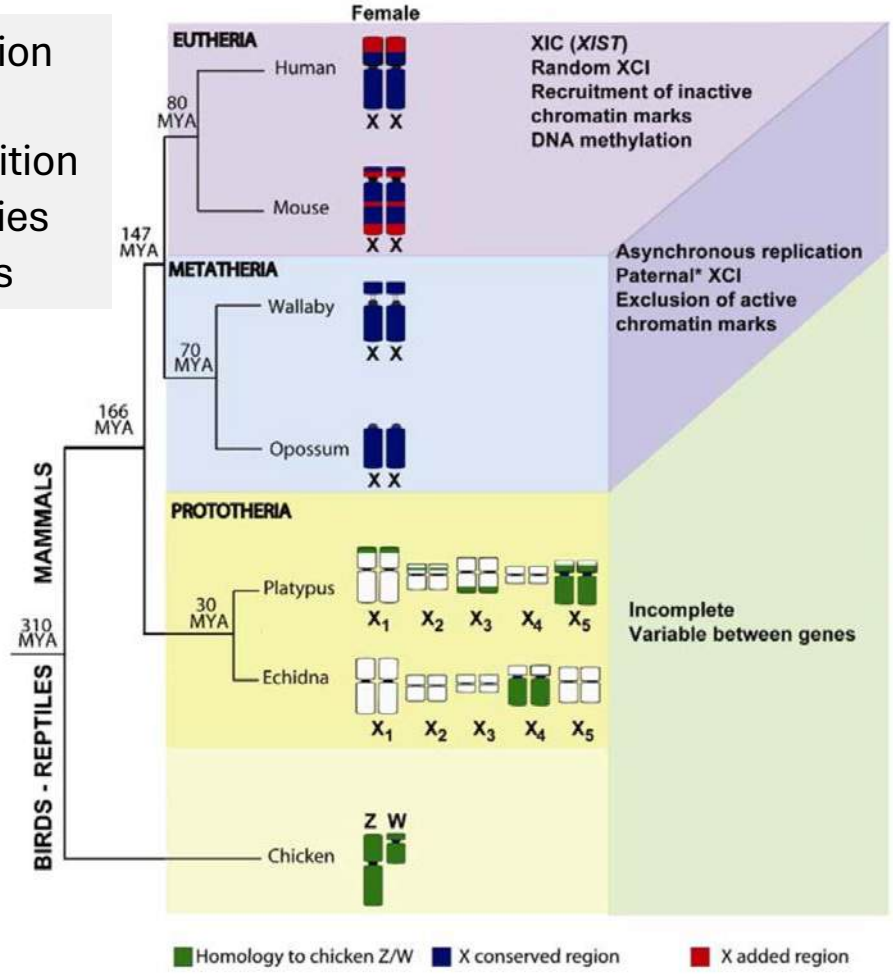
Female dominant gene on W
e.g. Xenopus laevis



Dosage-sensitive male determining gene on Z, *e.g. birds*

Mammalian Sex Chromosome Evolution

Ancestral dosage compensation
may be “gene by gene”
with the more recent superposition
of chromosome-wide strategies
eg by long non-coding RNAs



COURS 2018

E. Heard, January 29th, 2018

(Deakin, Chaumeil, Hore, Graves, Chrom Res 2009)

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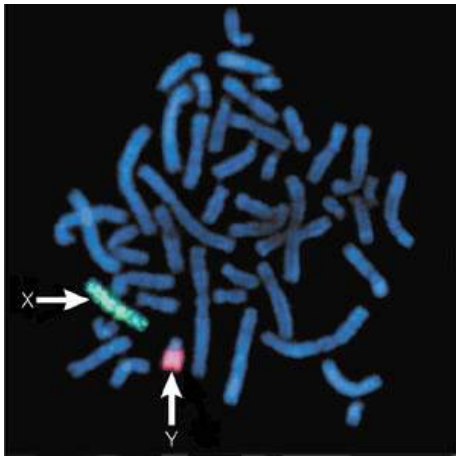
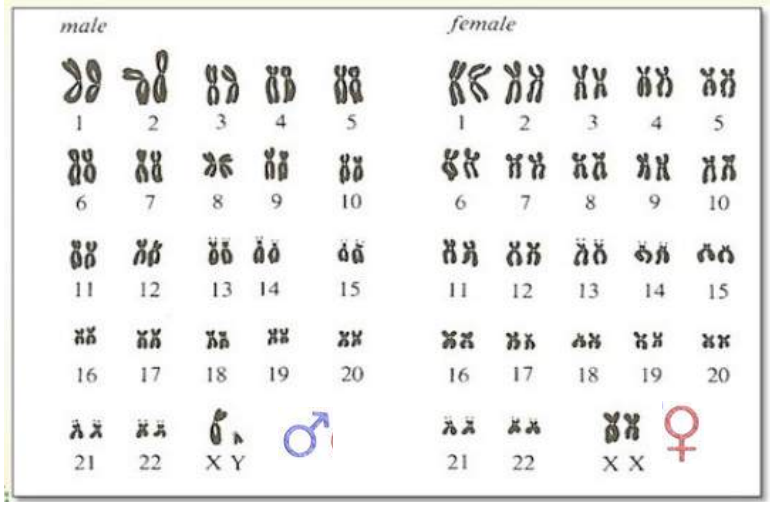
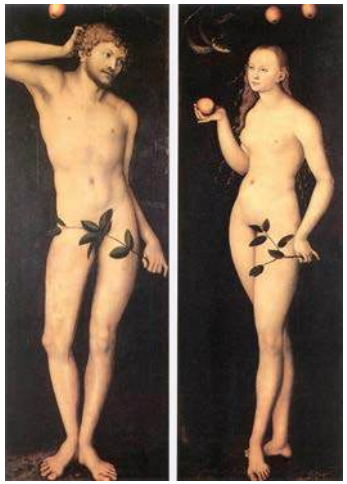
Mammalian Sex Chromosomes







- Humans normally have 46 chromosomes: 23 pairs, one set from each parent
- 22 Autosomes and one pair of Sex Chromosomes, X and Y
- Normally (46, XX) is female ; (46, XY) is male
- Rare individuals(45, XO) is female; (47 XXY) is male; (47 XXX) is female

- The evolution of mammalian sex chromosomes has led to dramatic differences in gene content and expression
- Need for dosage compensation strategies – X relative to A and XX relative to XY
- X-chromosome inactivation was the chromosome-wide strategy between XX and XY discovered in eutherian mammals (mice, humans…) for dosage compensation of X genes

Discovery of Sex Chromosomes

Hermann Paul August Otto Henking
(German cytologist 1858-1942)

- Discovered the “X” element in ~1891.
- Light microscopy: testicles of the firebug (Pyrrhocoris) Henking noted that one chromosome did not take part in meiosis.
- Named the **X element** because its strange behavior made him unsure whether it was genuinely a chromosome.
- He speculated it might play a role in sex determination

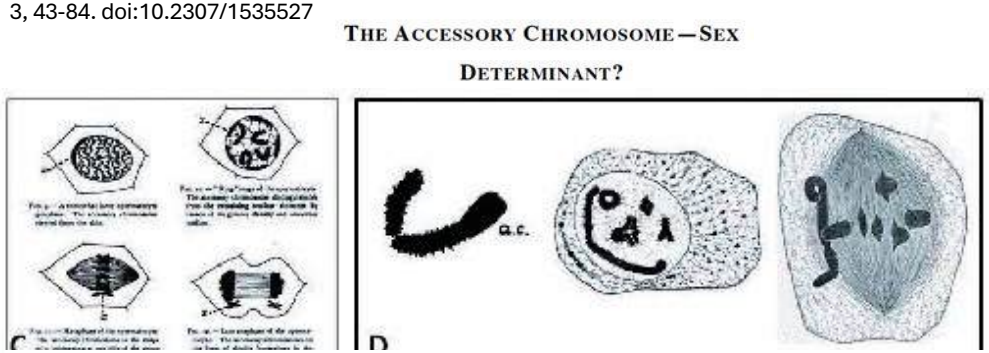
Henking H. 1891 Uber spermatogenese und deren beziehung zur entwicklung bei Pyrrhocoris apterus L. Z. Wiss. Zool. 51, 685–736.

- Henking realized that the X element (later called the X chromosome) of Pyrrhocoris is the largest chromosome in the cell and is easy to follow in meiotic divisions.
- Henking described his observations of the X element - and his work was later critical to explaining sex determination

Clarence E McClung
(US geneticist 1870- 1946)

• Renamed the X element the "accessory chromosome," because it appeared to have a separate purpose compared to the other chromosomes.
• He also noted two types of sperm cells (50/50) with or without the Accessory chromosome
• In 1901/1902- he proposed that this could influence sex determination of the zygote

McClung, C. E. (1901). Notes on the accessory chromosome. Anat. Anz. 20, 220-226.
McClung, C. E. (1902). The accessory chromosome—Sex determinant? Biol. Bull. 3, 43-84. doi:10.2307/1535527



NB precisely **why** the X element (or X chromosome) lags during meiosis in some species is still unclear…(see Paliulis et al, JCS, 2023)

Discovery of Sex Chromosomes

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- Light microscopy: testicles of the firebug (Pyrrhocoris) Henking noted that one chromosome did not take part in meiosis.
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Walter Sutton (1877-1916) and
Theodor Boveri (1862-1915)
In 1902-1904, the **chromosome theory of inheritance** was proposed independently by Sutton and Boveri who showed that the behaviour of chromosomes during meiosis could be the basis for Mendelian inheritance ; ie chromosomes bear hereditary factors, in accordance with Mendelian laws.

E. Heard, May 12th, 2025

COURS 2013, 2018

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Prior to Henking’s, McClung’s, and Sutton's reports, sex determination was attributed to factors other than gametes, such as the environment in which egg cells existed.

The pivotal study that provided this evidence was Studies in spermatogenesis (1905) by Dr Nettie M. Stevens. She showed, through careful cytological examination, that the inheritance of a small “accessory” chromosome correlates with male development in dozens of insect species. E.B Wilson made similar observations in 1905.

Stevens NM. 1905 Studies in spermatogenesis. Washington, DC:

Nettie Stevens : The Discovery of Sex Chromosomes

Nettie M. Stevens 1861-1912
American cytogeneticist

Nettie Stevens and E.B. Wilson: Discovery of Sex Chromosomes

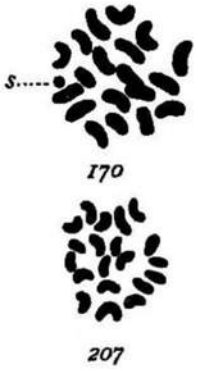
Stevens NM 1905 “Studies in spermatogenesis”. Washington, DC: Carnegie Institution of Washington.

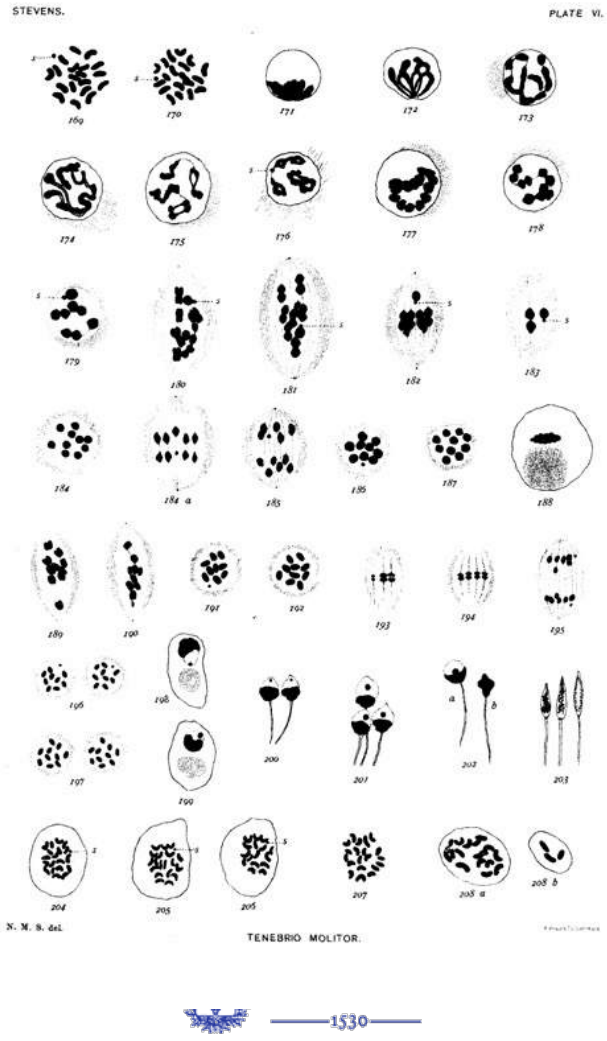
- Studied mealworm chromosomes
- Found females had 20 large chromosomes
- Males had 19 large and one small chromosome (Y)
- The small (Y) chromosome pairs with large (X) at meiosis 1
- Concluded the small chromosome determines male sex
- First strong evidence that sex is genetically determined, not environmental

«Le chromosome impair, d'après les études réalisées jusqu'à présent, se comporte exactement comme le membre le plus grand d'une paire inégale sans son compagnon plus petit. Au stade de croissance, il reste condensé et soit sphérique, soit parfois aplati contre la membrane nucléaire. Lors de la première mitose de maturation, il est attaché à un pôle du fuseau, ne se divise pas, mais se dirige vers l'un des deux seconds spermatocytes. Dans le second spermatocyte, il se divise avec les autres chromosomes, donnant deux classes égales de spermatides qui diffèrent par la présence ou l'absence de ce chromosome impair. »

« Dans les cellules somatiques et germinales des deux sexes, il existe une différence non pas dans le nombre d'éléments de chromatine, mais dans la taille de l'un d'entre eux, qui est très petite chez le mâle (170-s) et de même taille que les 19 autres chez la femelle (207). »

Nettie M. Stevens 1861-1912
American cytogeneticist





Nettie Stevens and E.B. Wilson: Discovery of Sex Chromosomes

Stevens NM 1905 "Studies in spermatogenesis". Washington, DC: Carnegie Institution of Washington.

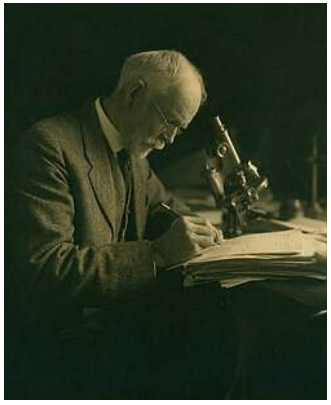
- Studied mealworm chromosomes
- Found females had two large chromosomes
- Males had one large and one small chromosome
- Concluded the small chromosome determines male sex
- First strong evidence that sex is genetically determined, not environmental

Wilson EB 1905 "The Chromosomes in Relation to the Determination of Sex in Insects"

- E.B. Wilson independently studied sex determination and made similar observations.
- He noted that in a different insect (Protenor), males have one fewer chromosome than females (five and six).
- Also sperm with a "sex chromosome" will form females, while those without it form males.
- He interpreted this as a mechanism for sex determination.
- However, he did not initially recognize the presence of a distinct "small chromosome" (now known as the Y chromosome) in males.

Wilson's paper was published just before Stevens' – though he referred to her results in his paper. Her work was more definitive as she clearly identified the small chromosome in males and also showed that it paired with a larger chromosome in females, providing solid cytological evidence that the presence or absence of this small (Y) chromosome determines sex.

Nettie M. Stevens 1861-1912
American cytogeneticist



Edmund B. Wilson (1856 –1939)
American Zoologist and Geneticist

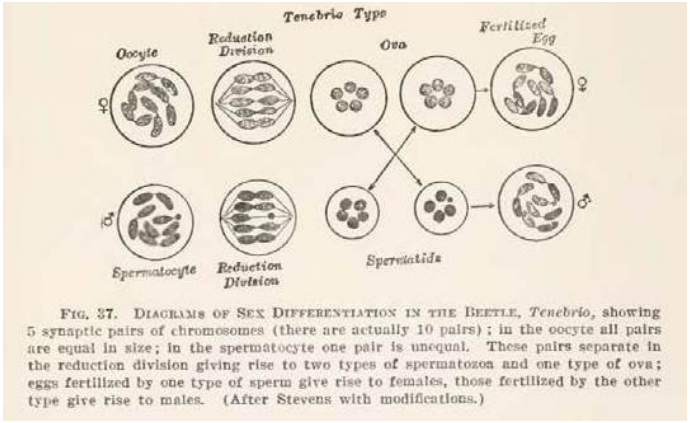


FIG. 37. DIAGRAMS OF SEX DIFFERENTIATION IN THE BEETLE, *Tenebrio*, showing 5 synaptic pairs of chromosomes (there are actually 10 pairs); in the oocyte all pairs are equal in size; in the spermatocyte one pair is unequal. These pairs separate in the reduction division giving rise to two types of spermatozoa and one type of ova; eggs fertilized by one type of sperm give rise to females, those fertilized by the other type give rise to males. (After Stevens with modifications.)

Chiefly because these two kinds of spermatozoa occur in equal numbers McClung in 1902 concluded that this accessory chromosome was a sex determinant. In 1905 Wilson discovered in a number of bugs that while there were two types of spermatozoa, one of which contained, and

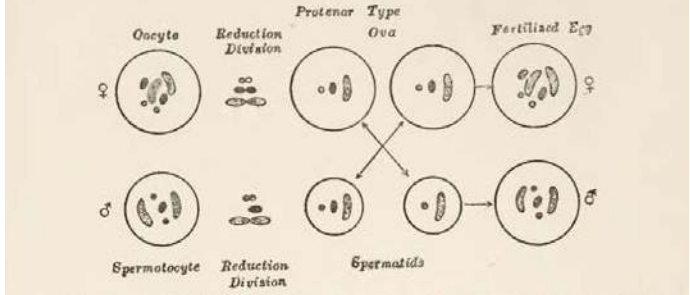


FIG. 36. DIAGRAMS OF SEX DIFFERENTIATION IN THE BUG, *Protenor*. The oocyte contains 6 chromosomes and the spermatocyte 5 chromosomes which are not yet united into synaptic pairs; the "sex" chromosomes are shown in black and white, two are present in the oocyte, but one is present in the spermatocyte. In the reduction division the synaptic pairs separate, giving rise to two types of spermatozoa, one of which has the sex chromosome and the other lacks it; all ova are alike in this regard. If an egg is fertilized by a sperm without the sex chromosome a male results; if fertilized by a sperm containing the sex chromosome a female results. (After Wilson with modifications.)

Nettie Stevens and E.B. Wilson: Discovery of Sex Chromosomes

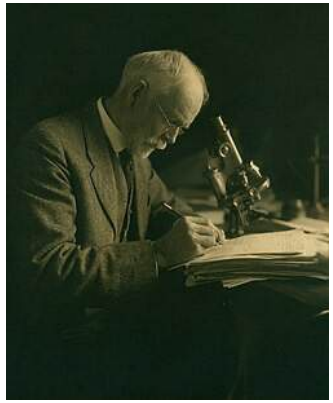
Thus Stevens and Wilson demonstrated that in some insects the cells of females have 2 X chromosomes and the cells of makes have a single X and a smaller Y chromosomes. Stevens provided evidence that the presence or absence of this small (Y) chromosome determines sex.

In 1906, Wilson first used the term 'sex chromosome'
Wilson EB. 1909. Recent researches on the determination and heredity of sex. Science 29, 53-70.

1909 the X and Y (elements) were used to delineate the heteromorphic pair
Wilson EB. 1909. Recent researches on the determination and heredity of sex. Science 29, 53-70

The findings of the many independent and complementary studies during these years laid the foundations of the modern diversity of sex chromosome systems, including XX/XO (dosage) systems where chromosome number changes between males and females, XX/XY systems where the heterogametic sex chromosome pair is found during spermatogenesis in males, and ZZ/ZW systems where the heterogametic pair is found in females during oogenesis.

Nettie M. Stevens 1861-1912
American cytogeneticist



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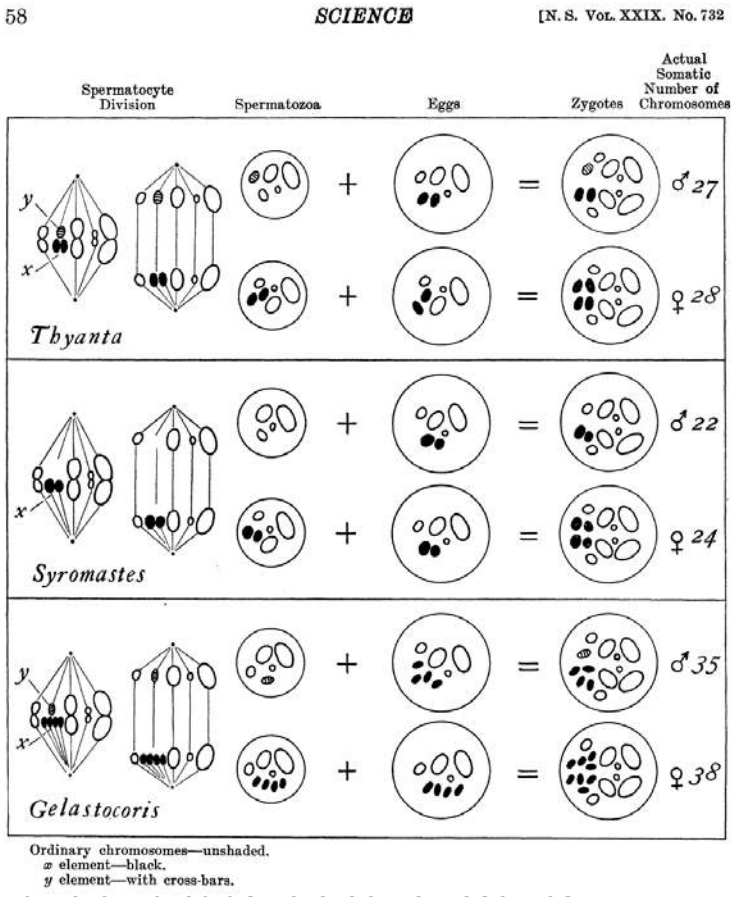


Wilson EB. 1909. Recent researches on the determination and heredity of sex. Science 29, 53-70

Timeline of the Discovery of Human Sex Chromosomes

Stevens and Wilson, working independently, discovered that sex determination in insects was linked to specific chromosomes. Stevens showed that males had XY chromosomes and females had XX. This established the chromosomal basis of sex.

1910s–1920s - Researchers attempted to extend these discoveries to mammalian cells, but due to the limits of microscope technology, chromosome counts and identification were still inaccurate.

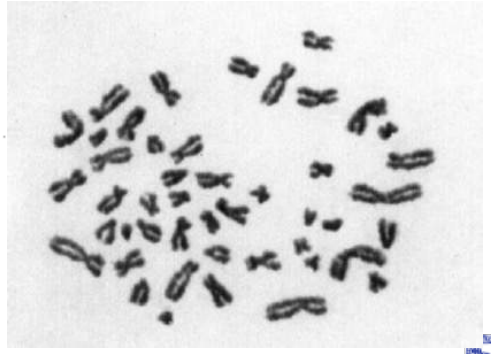
1921 – Theophilus S. Painter (1889-1969 – American zoologist)
Painter studied human spermatocytes and identified the Y chromosome as distinct from the X.
He incorrectly estimated that humans had 48 chromosomes, but his work was crucial in recognizing the X and Y chromosomes as the basis for human sex determination.

1956 – Joe Hin Tjio (1919-2001 American cytogeneticist) and Albert Levan (1905-1998 Swedish cytogeneticist)
Using improved techniques, Tjio and Levan correctly determined that humans have 46 chromosomes (23 pairs).
This was a major breakthrough in human cytogenetics and confirmed the presence of the XX (female) and XY (male) chromosome pairs.

THE CHROMOSOME NUMBER OF MAN

By *JOE HIN TJIO* and *ALBERT LEVAN*

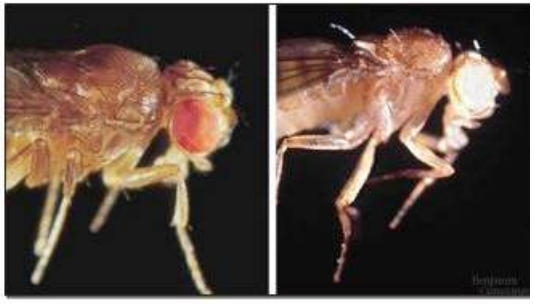
ESTACION EXPERIMENTAL DE AULA DEI, ZARAGOZA, SPAIN, AND CANCER CHROMOSOME LABORATORY, INSTITUTE OF GENETICS, LUND, SWEDEN

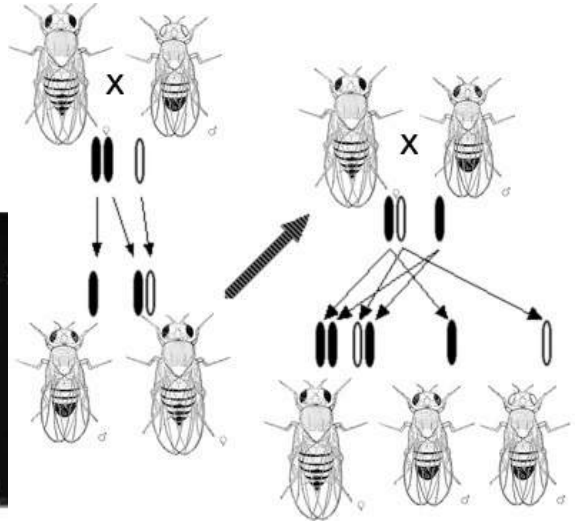


TH Morgan: Sex Chromosomes & the Gene Theory for Hereditary Material

The testing of the chromosomal theory of sex determination led to the basis for Morgan's proof that the genetic factors were physically located on the individual chromosomes.

Using Drosophila melanogaster – he identified a white eyed male and demonstrated that this trait could in fact be passed on in the same manner predicted by the inheritance of sex chromosomes.





Thomas Hunt Morgan
(1866-1945)
American evolutionary biologist, geneticist

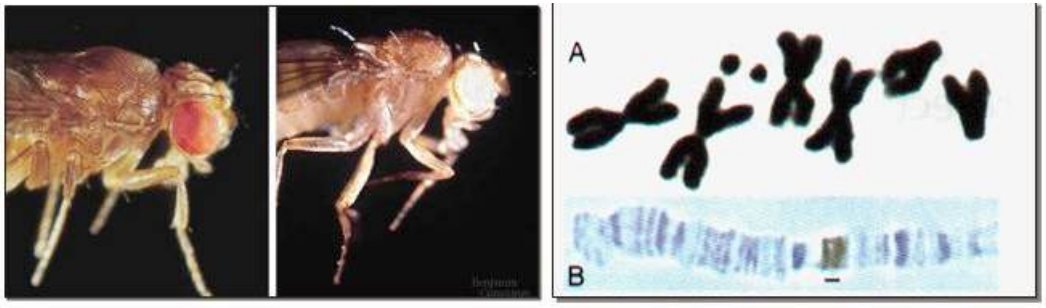
Nobel Prize in 1933
for "discoveries elucidating the role that the chromosome plays in heredity"

TH Morgan: Sex Chromosomes & the Gene Theory for Hereditary Material

Morgan concluded that the inheritance of white eye color parallels the normal meiotic segregation of the sex chromosomes in this fly. **He thus definitively linked "trait" inheritance to a specific chromosome**

This represented the first concrete evidence that chromosomes have a role in inheritance as had been suggested nearly a decade earlier by Sutton and Boveri.

His student – Muller - later discovered dosage compensation based on the the same trait (eye color).



Thomas Hunt Morgan
(1866-1945)
American evolutionary biologist, geneticist

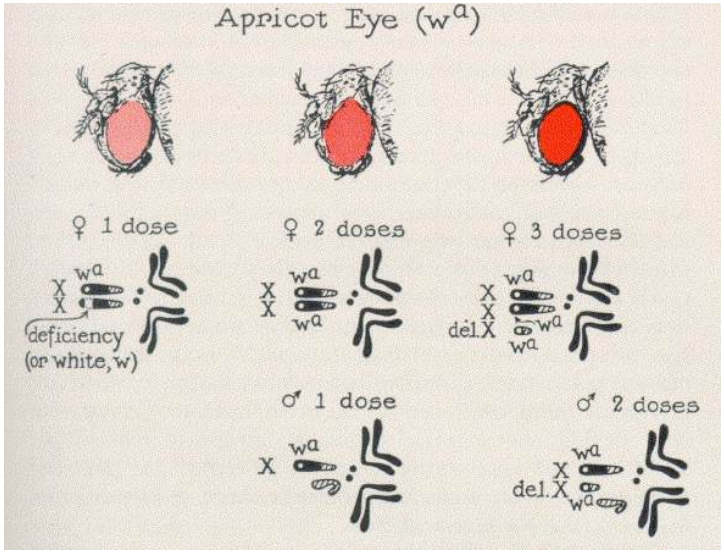
Nobel Prize in 1933
for "discoveries elucidating the role that the chromosome plays in heredity"

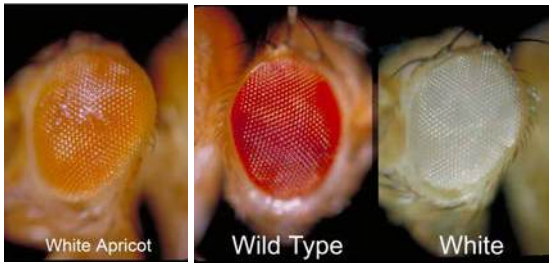
Sex Chromosome Dosage Compensation

Hermann Joseph Muller
(1890-1967)
American Geneticist

1946 Nobel Prize in Physiology or Medicine, *"For the discovery that mutations can be induced by X-rays*".

"Effects of dosage changes of sex-linked genes, and the compensatory effects of the gene differences between male and female" (Muller et al. 1932)
"Dosage Compensation" (Muller, 1947)





The w^a mutant is a partial loss-of-function allele that allows the deposition of some eye pigments in the eye. The greater the number of w^a alleles in the genotype of either females (top row) or males (bottom row) the greater the amount of pigmentation. Surprisingly, **females with two doses and males with a single dose of the allele have the same eye color**.
From Muller 1948; fThe Harvey Society.

COLLÈGE DE FRANCE 1530

Sex Chromosome Dosage Compensation

Hermann Joseph Muller
(1890-1967)
American Geneticist

1946 Nobel Prize in Physiology or Medicine, *"For the discovery that mutations can be induced by X-rays*".

"Effects of dosage changes of sex-linked genes, and the compensatory effects of the gene differences between male and female" (Muller et al. 1932)
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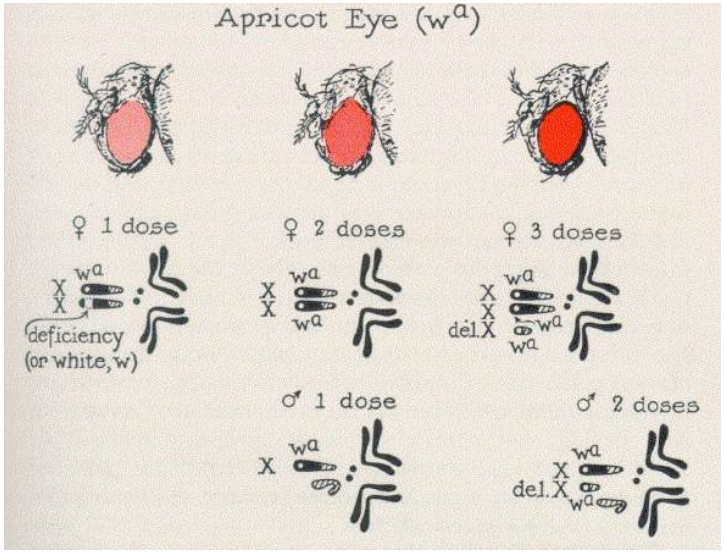

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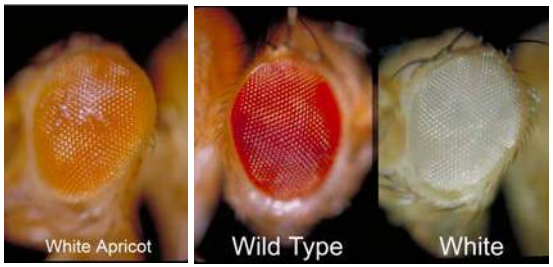


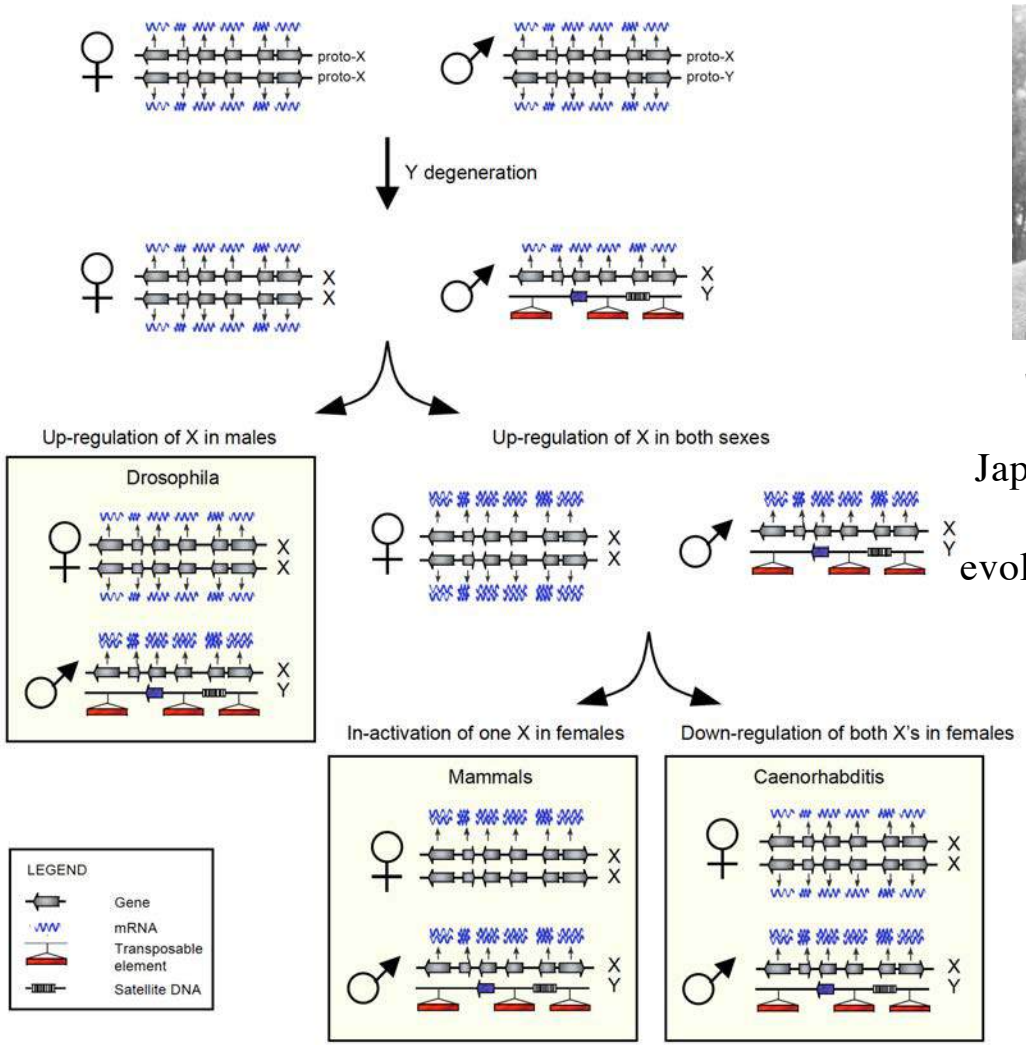
- Observing *apricot* mutation of the *white* gene: two X chromosomes in a female produce the same eye color as one X chromosome in a male.
- A male achieves with one gene dose what requires two gene doses in the female.

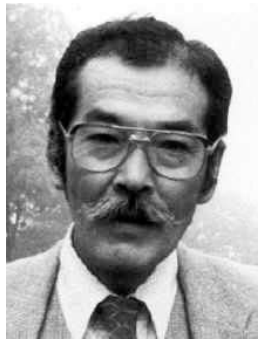
- **This constitutes the basic observation of "dosage compensation": mechanisms must exist to ensure that genes on the X chromosome produce similar effects in males (one dose) and females (two doses).**

- ***Is this due to single X hyperexression in males or to XX depression in females...***

Evolution of dosage compensation in flies, mammals & worms

COURS 2018





Susumu Ohno
(1928-2000)
Japanese-American
Geneticist,
evolutionary biologist

COLLÈGE DE FRANCE 1530

Discovery of the "Nucleolar Satellite" in Female Cat Neurons

Staining of cat neurons with cresyl violet stain revealed a "nucleolar" satellite in females but not in males: Barr and Bertram, 1949

Murray Llewellyn Barr 1908-1995
Canadian physician and researcher

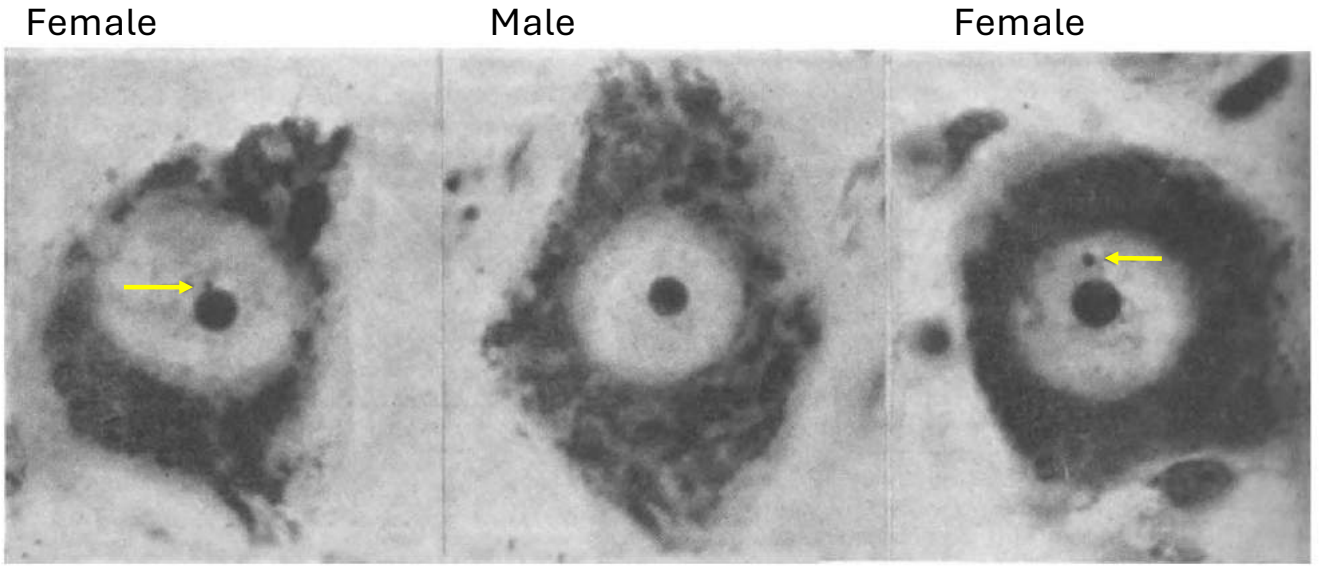


Fig. 1 Fig. 2 Fig. 3

Fig. 1. Normal motor neurone from the hypoglossal nucleus of a mature female cat showing the usual morphology of the nucleolar satellite (indicated by arrow) in the female. Cresyl violet stain, × 1,400

Fig. 2. Motor neurone from the hypoglossal nucleus of a mature male cat. The nucleolar satellite is absent, the typical condition in the mature male. Cresyl violet stain, × 1,400

Fig. 3. Motor neurone from the hypoglossal nucleus of a mature female cat 108 hours following electrical stimulation of the corresponding hypoglossal nerve for a period of 8 hours. Associated with intense synthesis of cytoplasmic ribose nucleoproteins, the nucleolar satellite (indicated by arrow) tends to move away from the nucleolus. Cresyl violet stain, × 1,400

Barr and Bertram, Nature 1949;163:676. doi:10.1038/163676a0

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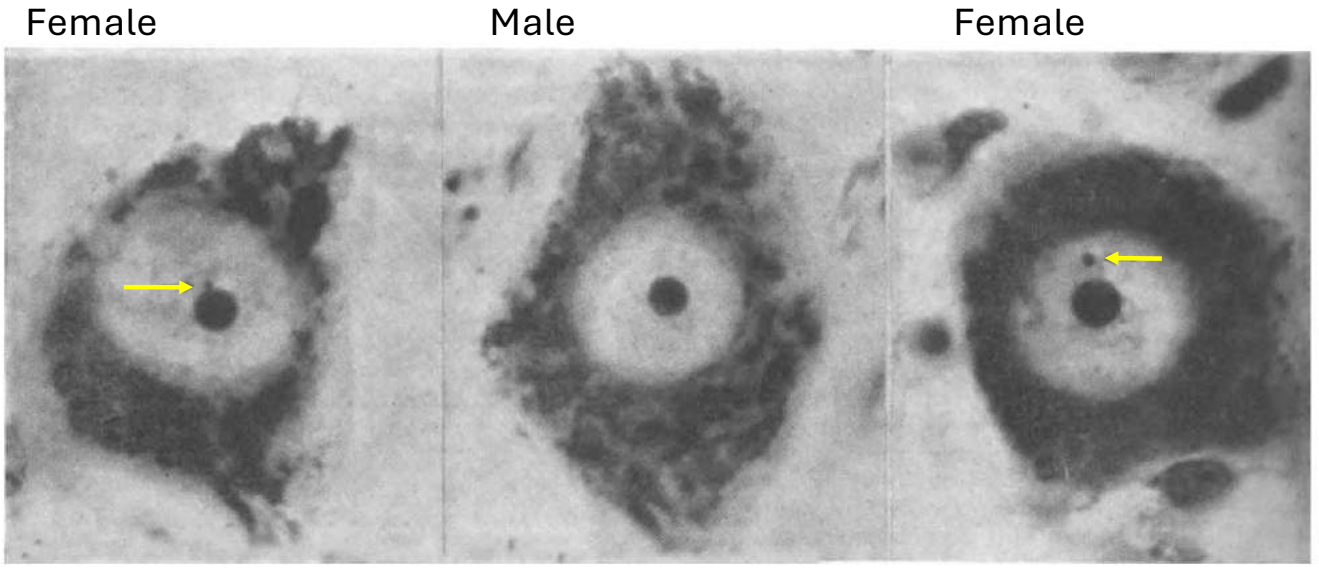

Fig. 1 Fig. 2 Fig. 3

"The sex of a somatic cell as highly differentiated as a neurone may be detected with no more elaborate equipment than a compound microscope following staining of the tissue by the routine Nissl method... The difference in nuclear structure between neurones of adult male and female cats rests on the degree of development of a second body, which is much smaller than the nucleolus."

Barr and Bertram, Nature 1949;163:676. doi:10.1038/163676a0

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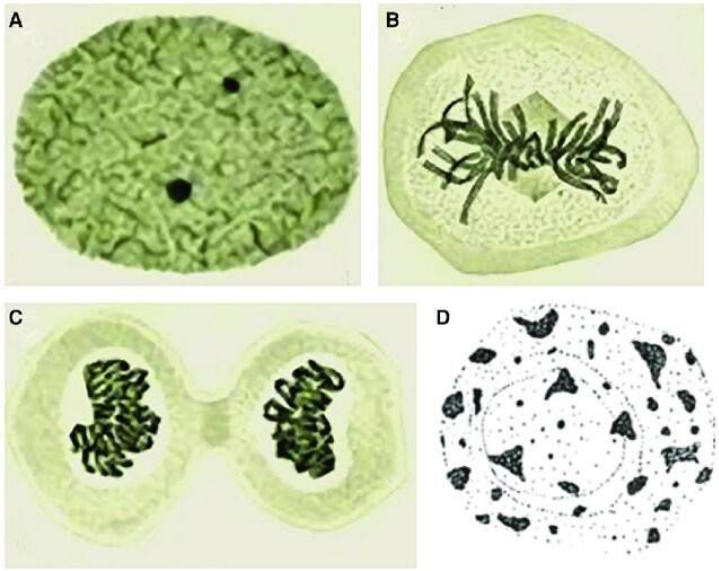
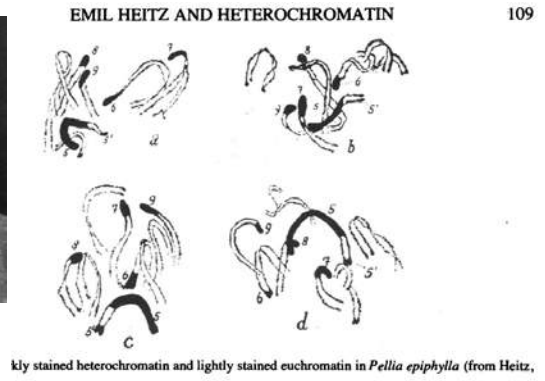
Identifying Chromatin and Chromosomes - defining Heterochromatin and Euchromatin



Walther Flemming (1843-1905)

Emil Heitz (1892-1965)



Early Depictions of Chromatin (A-C) Walther Flemming's drawing of an interphase cell (A), a cell in metaphase (B), and a cell in telophase (C). Images are from Flemming's 1882 book Cell Substance, Nucleus, and Cell Division. (D) Emil Heitz's drawing of condensed heterochromatin domains (black). Image is from his 1929 book Heterochromatin, Chromocentern, Chromomeren.

Walther Flemming (1843-1905) staining cells with aniline dyes he described chromatin (coloured material) as thread-like structures in the nucleus that takes up basophilic dyes

HW Waldeyer coined the term chromosome (coloured bodies) in 1888 to describe Flemming's nuclear threads

Emil Heitz (1892-1965) developed new techniques to obtain direct chromosome preparations from moss and many other species and in 1928 he defined heterochromatin and euchromatin:

"...heterochromatin refers to a part of a chromosome that remains heteropyknotic after telophase and thus behaves opposite to euchromatin"

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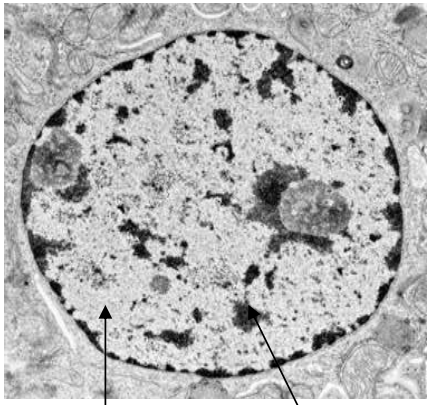
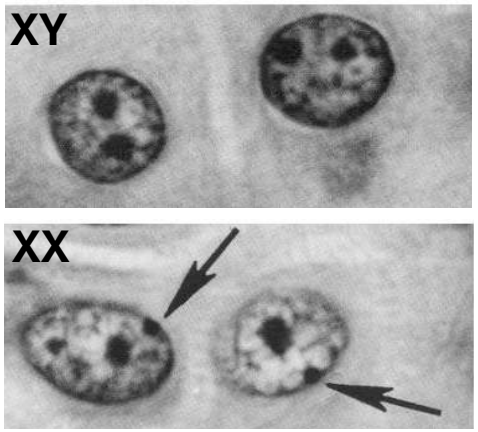
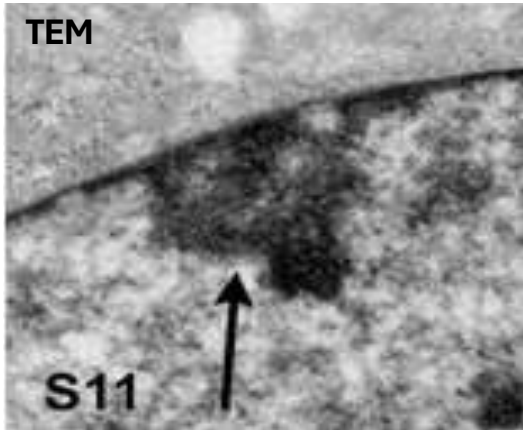
Heterochromatin and Euchromatin





Bertram et Barr, 1949



Rego et al, 2008

http://medcell.med.yale.edu/histology/

Emil Heitz (1892-1965) developed new techniques to obtain direct chromosome preparations from moss and many other species and defined heterochromatin and euchromatin: ***"...heterochromatin refers to a part of a chromosome that remains heteropyknotic after telophase and thus behaves opposite to euchromatin"***

Constitutive heterochromatin tends to be non-coding and repetitive (Pontecorvo, 1944)
Facultative heterochromatin (protein-coding chromatin) can be either active or inactive (Barr, 1949)

In 1929, Heitz proposed that heterochromatin should be enriched in those parts of chromosomes that carry no genes or are associated with genetically "passive" regions; this is still the basis of today's definition of heterochromatin.

E. H

The Sex Chromatin in Human Cells

The same body – which they now referred to as “sex chromatin” - could be seen in human female cells : Moore and Barr, 1953

Sex Chromatin and Phenotype in Man

Disagreement between nuclear sex and phenotype raises questions about the cause of sex anomalies.

Murray L. Barr

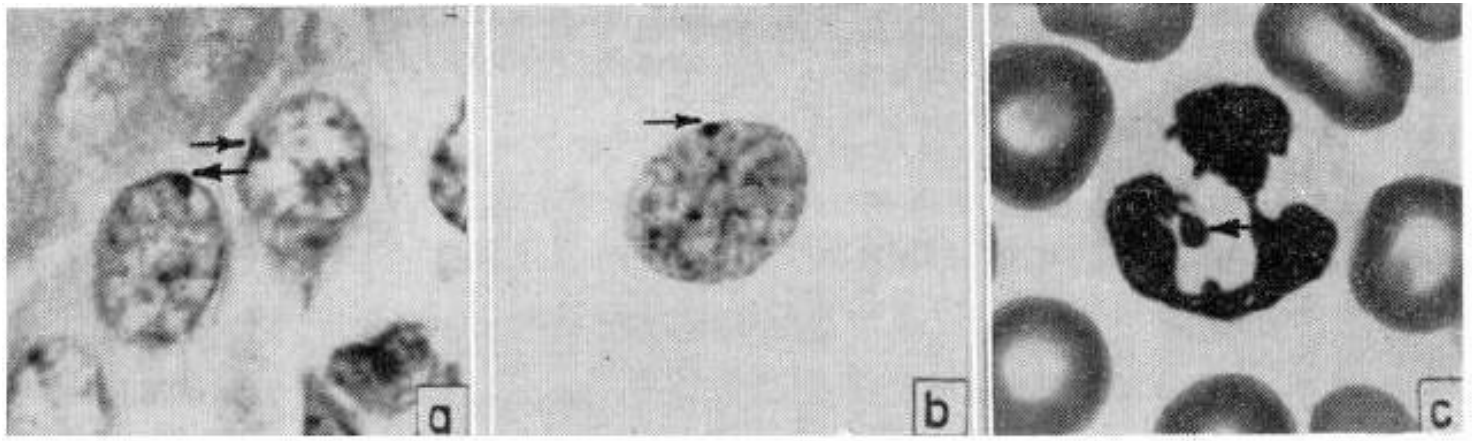
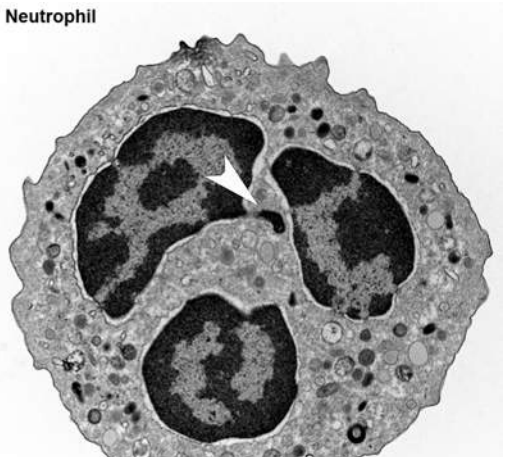


Figure 2. Nuclei of human females. A: Nuclei in epidermis of a skin b
B: nucleus in an oral mucosal smear (cresyl echt violet); C: neutroph
1,680). (From: Barr ML, Brit J Urol 1957;29:251, with permission from J



Moore KL, Barr ML. Morphology of the nerve cell nucleus in mammals, with special refere
1953;98:213–31. doi:10.1002/cne.900980203
Barr ML. Sex chromatin and phenotype in man. Science 1959;130:679–85. doi:10.1126/s

Brinkmann V & Zychlinsky A (2012) J. Cell Biol.

E. Heard, May 12th, 2025

The Sex Chromatin (Barr body) as a method of detection of chromosomeal sex in humans

From K. L. Moore et al. (1953). Surg. Gynecol. Obstet., 96, 641–648. Copyright (1953), by kind permission of the author and Surgery, Gynecology and Obstetrics

THE DETECTION OF CHROMOSOMAL SEX IN HERMAPHRODITES FROM A SKIN BIOPSY

KEITH L. MOORE, M.Sc., MARGARET A. GRAHAM, M.Sc., and MURRAY L. BARR, M.D., London, Ontario, Canada

A CASE of hermaphroditism presents to the physician an exceedingly difficult problem. The tragic status of the patient demands that measures be taken to correct the developmental error, in so far as this is possible. There is little agreement, however, concerning the etiology of hermaphroditism, the criteria on which a decision as to the dominant sex are to be based, or the management of individual cases. In brief, the problem of hermaphroditism appears to have reached an impasse and a new approach is desirable.

Painter, and Evans and Swezy have shown that in humans, females have an XX chromosome combination while males have XY chromosomes as the sex chromosomes. The Y chromosome is small relative to the size of the X chromosome. We have found that the nature of the sex chromosomes (XX or XY) in an individual may be detected by examining the epidermal nuclei in a small biopsy of skin. This technique offers a new approach to the vexatious problem of hermaphroditism.

NORMAL SEX DIFFERENTIATION

Sex is determined at fertilization depending on whether the ovum, with its single X chromosome, is fertilized by a sperm bearing an X or a Y chromosome. Sex differentiation does not begin until the seventh or eighth week of intrauterine life when the previously indifferent gonads show signs of developing into ovaries or testes. Both sex chromosomes and certain autosomes (chromosomes other than the sex chromosomes) bear genes which are concerned with gonadal differentiation. The balance between genes, with a sex-determining function, on the XX chromosomes and similar genes on the autosomes causes the indifferent gonads to develop into ovaries, while the balance between sex-determining genes on the XY chromosomes and autosomes directs indifferent gonads toward development of testes.

A wolffian or male genital duct system and a müllerian or female genital duct system both appear during early embryonic development. The external genitalia develop from sexually indifferent structures. There is disagreement concerning the factors which direct the development of wolffian or müllerian derivatives and the appropriate maturation of the anlage of the external genitalia in later stages. Since the classical work of Lillie on the freemartin in cattle, the majority of biologists have adopted the view that sex hormones of the fetal gonads are responsible for the differentiation of the genital system, once definitive ovaries or testes have been formed. This view is summarized well by Greene (7). Moore (12), on the other hand, was unable to reproduce the freemartin condition by experimental treatment of embryos with sex hormones. He expresses the minority opinion that the gonads do not produce sex hormones during fetal life and that sex differentiation during the intrauterine period is a genetically controlled process without the intervention of sex hormones. Resolution of this problem will be helpful in the eventual understanding of the etiology of developmental sex abnormalities.

TYPES OF HERMAPHRODITISM AND ABNORMAL SEX DIFFERENTIATION

In reviewing the literature on hermaphroditism one is impressed by the kaleidoscopic nature of the condition, produced by many variables appearing in diverse combinations. The Klebs classification, whatever its shortcomings may be, is in common use and has served to systematize this complex developmental abnormality.

From the Department of Anatomy, Faculty of Medicine, University of Western Ontario, London, Canada.
This work was made possible by grants-in-aid to one of the authors (M.L.B.) from the National Cancer Institute and the National Research Council of Canada.

T. V. N. Persaud (ed.), *Problems of Birth Defects*

Barr Body Analysis in Genetic Disorder Diagnosis

In 1955, Barr, in collaboration with KL Moore, developed the buccal smear test, a non-invasive method for collecting epithelial cells from the inner lining of the mouth. This technique allowed the detection of Barr bodies in somatic cells and provided a simple tool for identifying chromosomal abnormalities, such as those seen in Turner syndrome and Klinefelter syndrome.

The test became widely used in the mid-20th century and was among the earliest tools for determining chromosomal sex in clinical and research contexts

The Sex Chromatin (Barr body) as a method of detection of chromosomeal sex in humans

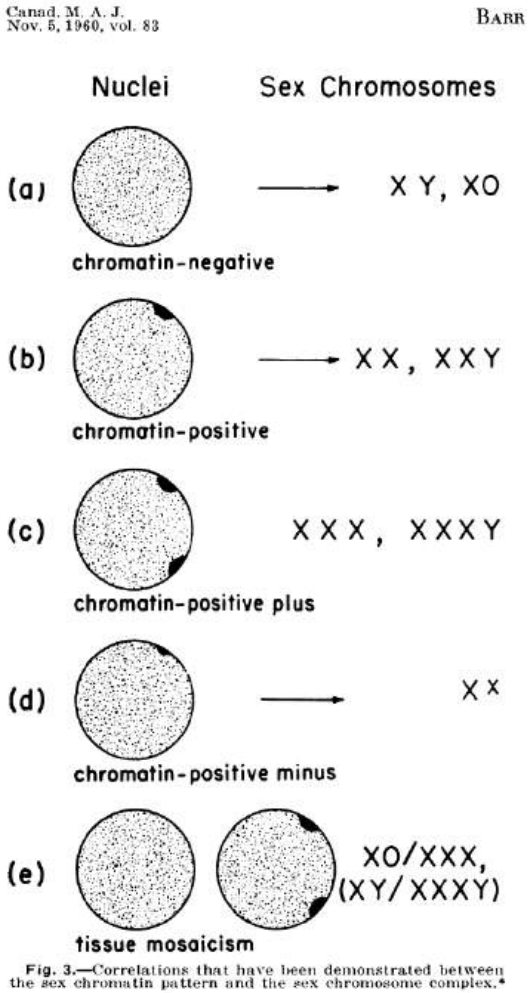


Fig. 3.—Correlations that have been demonstrated between the sex chromatin pattern and the sex chromosome complex.[4]

Barr Body Analysis in Genetic Disorder Diagnosis

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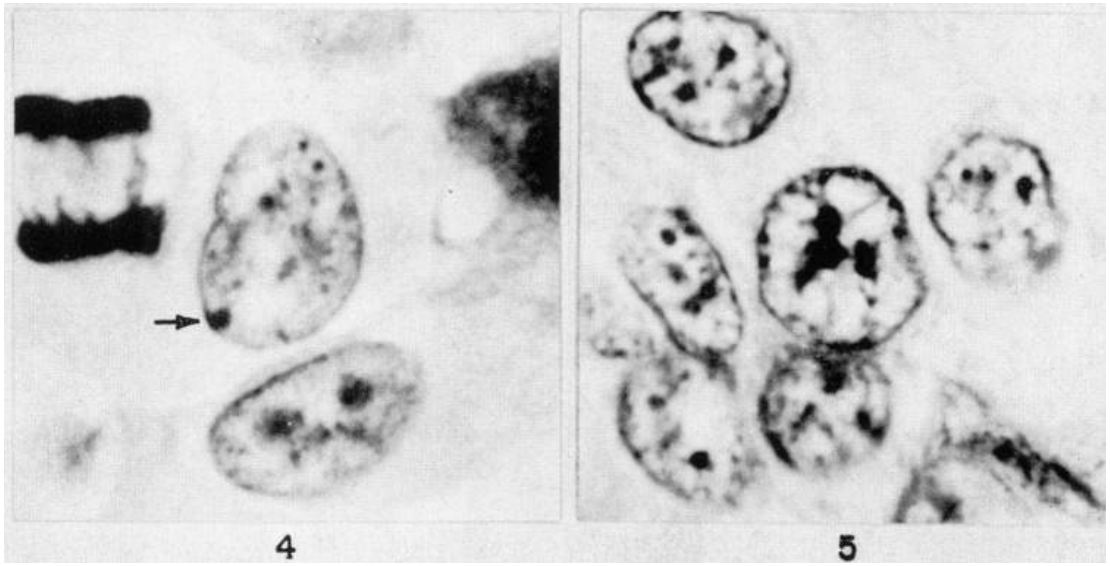
The test became widely used in the mid-20th century and was among the earliest tools for determining chromosomal sex in clinical and research contexts (complemented with karyotype analysis), for example:

Turner Syndrome (45,X) – no Barr body
Klinefelter Syndrome (47,XXY) – one Barr body
Triple X Syndrome (47,XXX) – two Barr bodies

The Barr body as a biomarker in the context of Cancer

Carcinoma of cervix



4 5

THE SEX CHROMATIN IN HUMAN MALIGNANT TISSUES

K. L. MOORE* AND M. L. BARR

From the Department of Microscopic Anatomy, University of Western Ontario, London, Ontario

Received for publication June 28, 1957

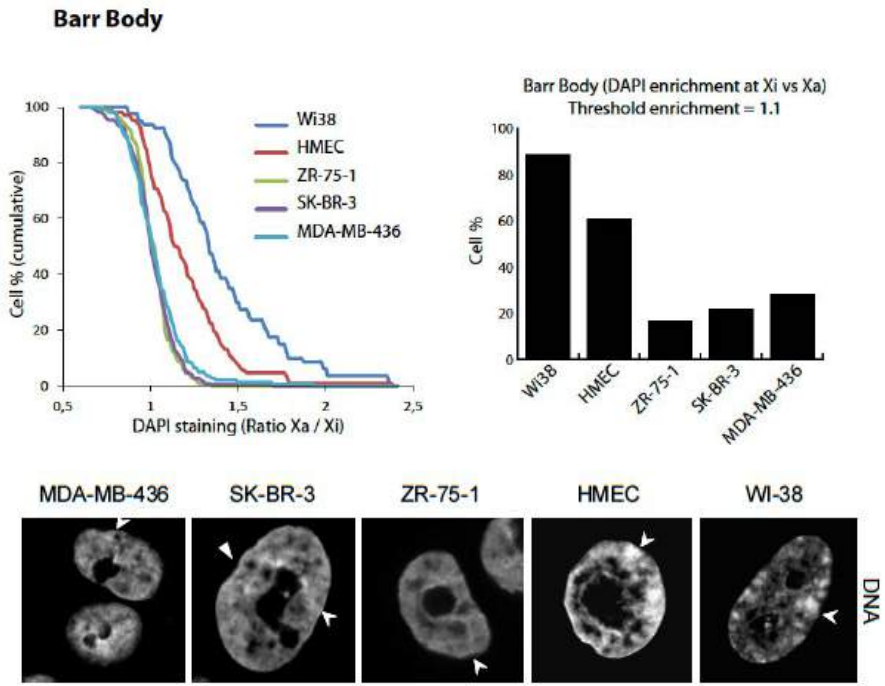
A SEXUAL dimorphism in resting nuclei has been described for man and monkey among the primates, and for several species of the orders Carnivora and Artiodactyla. It is based on the presence of a special chromocentre, known as the sex chromatin, in the nuclei of females. Graham and Barr (1952) suggested that the sex chromatin may represent heterochromatic regions of the two X-chromosomes that adhere to each other. This hypothesis is strengthened by the meticulous study of chromocentres in epidermal cell nuclei by Sachs and Danon (1956). The literature pertaining to the sex chromatin and its clinical application in anomalies of sex development has been ably reviewed by Lennox (1956), Davidson and Smith (1956) and Nelson (1956).

Several reports have appeared that deal with the sex chromatin of tumour cells and these will be referred to later in the paper. The observations recorded in the present report are a sequel to the study of sex characteristics in nuclei of benign tumours, where the nuclei were found to be like those of normal tissues (Moore and Barr, 1955).

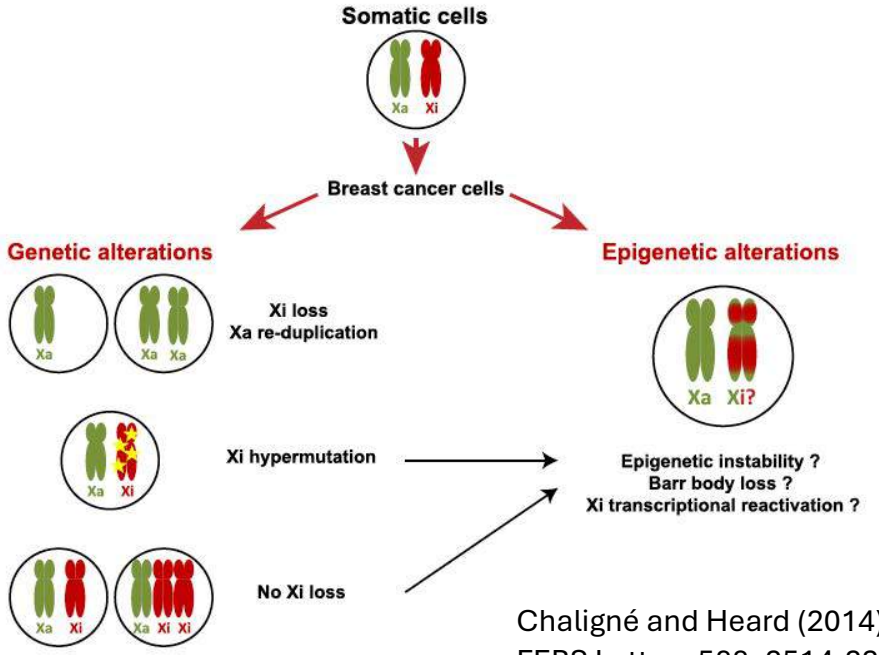
SUMMARY

The sex characteristics of cells of malignant tumours were studied in 127 specimens, 76 from females and 51 from males. In about one-third of the tumours from female hosts the incidence of sex chromatin in the nuclei was low relative to non-malignant tissues. Two or three masses of sex chromatin were present occasionally in the same nucleus. These departures from the nuclear structure of normal tissues were ascribed to various chromosomal anomalies in malignant

The Barr body as a biomarker in the context of Cancer



DAPI staining



Chaligné and Heard (2014)
FEBS Letters 588, 2514-22

Loss of the Barr body in Breast cancer

"The inactive X chromosome is epigenetically unstable and transcriptionally labile in breast cancer"

(Chaligné et al, Genome Res. 2015)

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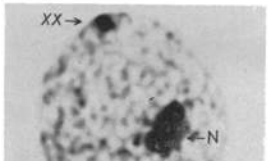
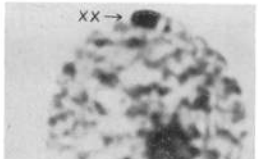
Barr body corresponds to one X chromosome, not two paired X chromosomes

Murray Llewellyn Barr 1908-1995
Canadian physician and researcher

- Barr and others (eg J. Reitalu 1957) initially believed that the sex chromatin represented the heterochromatic portion of the two X chromosomes that were somatically paired, and that the XY was too small to create such an effect. This explanation was not found to be true.
- In a letter to the journal *Science* Barr realized that other data did not support his concept of somatically paired X chromosomes representing the sex chromatin.





Observations on the So-Called Sex Chromatin in Man

by

Juhan Reitalu

Cancer Chromosome Laboratory, Institute of Genetics, Lund, Sweden

The difference in nuclear structure between male and female tissues in man has been examined in liver tissue from three embryos of each sex. The so-called sex chromatin consists of a large heterochromatic segment of the X chromosome, thus existing in duplicate in female diploid cells. The two segments have a tendency of juxtaposition resulting in a larger heterochromatic body in female than in male cells. Beside the large heterochromatic segment the X chromosome has, in the tissues studied, a euchromatic segment attached through a small terminal heterochromatic knob to a nucleolus. In male cells the euchromatic segment of the X chromosome is often joined terminally to a small heterochromatic segment believed to belong to the Y chromosome.

Barr body corresponds to one X chromosome, not two paired X chromosomes

Murray Llewellyn Barr 1908-1995
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- Barr and others (eg J. Reitalu 1957) initially believed that the sex chromatin represented the heterochromatic portion of the two X chromosomes that were somatically paired, and that the XY was too small to create such an effect. This explanation was not found to be true:
- In a letter to the journal *Science* Barr realized that other data did not support his concept of somatically paired X chromosomes representing the sex chromatin.

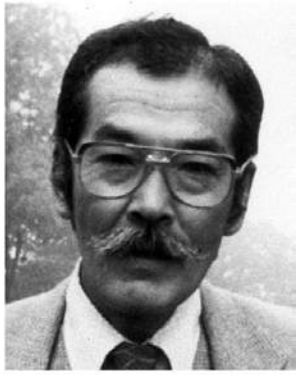
On sex chromatin:

*"…**it is formed from heterochromatic regions of a pair of homologous chromosomes** (Reitalu, 1957; Klinger and Schwarzacher, 1958; Serr et al 1958). Although an alternative interpretations has been suggested (Segal and Nelson, 1957), the weight of evidence favors the view that **the bipartite sex chromatin of females is formed by heterochromatic regios of the 2 X chromosomes and that a definite chromocenter is not formed by the non-homologous sex chromosomes of the heterogametic sex** (Graham and Barr 1952). This interpretation implies somatic pairing of the X chromosomes at any rate(Barr and Moore, 1957)."*

Barr's letter to Science, 1959

In a recent article (*1*), I favored the view that the sex chromatin represents heterochromatic regions of the two X chromosomes of female cells. The assumption of somatic pairing of the X chromosomes is an unsatisfactory aspect of this hypothesis. Somatic pairing of chromosomes is well known in many species of insects and has been described in the newt and frog (*2*). But evidence for such a relationship between the X chromosomes or other homologous chromosomes in somatic cells of mammals is admittedly scanty and inconclusive. For example, Ohno

Identification of the Sex Chromatin as one heterochromatic X chromosome



Susumu Ohno
大野 乾, おおの すすむ
1928 – 2000
Japanese American Geneticist

FORMATION OF THE SEX CHROMATIN
BY A SINGLE X-CHROMOSOME IN LIVER CELLS OF
RATTUS NORVEGICUS[1]

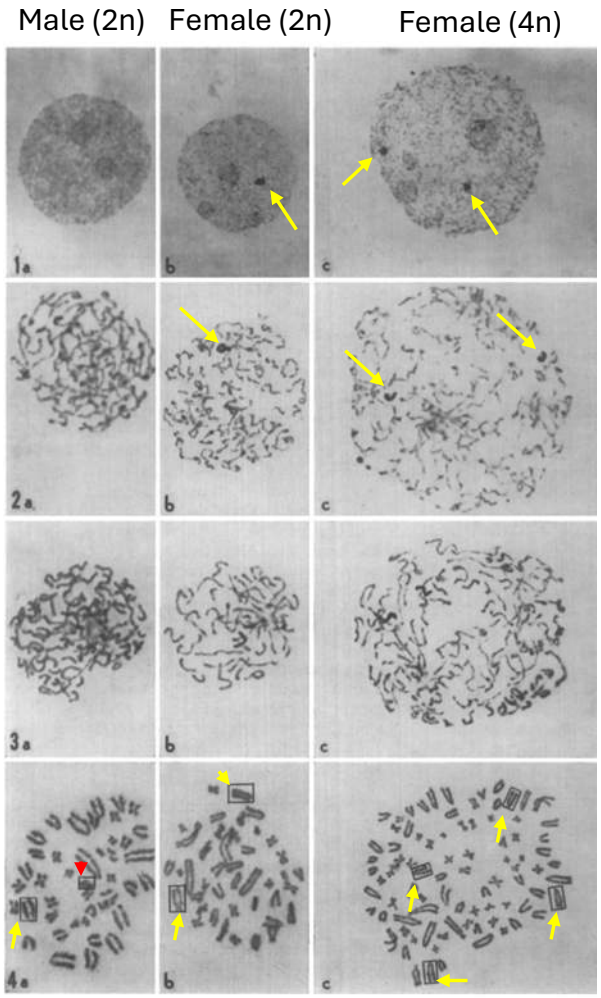
S. OHNO, W. D. KAPLAN, and R. KINOSITA

Departments of Experimental Pathology and Genetics, City of Hope Medical Center, Duarte, Calif., U.S.A.

Received August 18, 1959

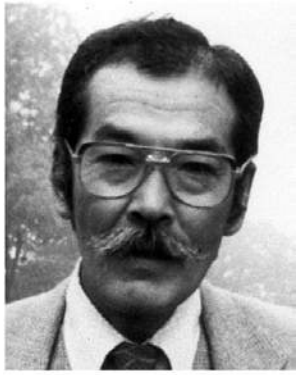
In 1958-1959, Japanese geneticist Susumu Ohno demonstrated that the previously identified "nucleolar satellite" or sex chromatin, was in fact a **single** heteropycnotic X chromosome in female XX somatic cells.

By comparison of female and male liver cells at various points in the cell cycle, female cells were seen to contain a highly condensed chromosome, absent in chromosome spreads of male cells.
They also noted that triploid cells had additional copies of this condensed structure.
From the data, Ohno deduced that the sex chromatin, was likely a condensed X chromosome.



E. Heard, May 12th, 2025

Identification of the Sex Chromatin as one heterochromatic X chromosome



Susumu Ohno
大野 乾, おおの すすむ
1928 – 2000
Japanese American Geneticist

78 JANUARY 14, 1961 Ohno S. The Lancet 1961: 1 (7168)

THE SINGLE-X NATURE OF SEX CHROMATIN IN MAN

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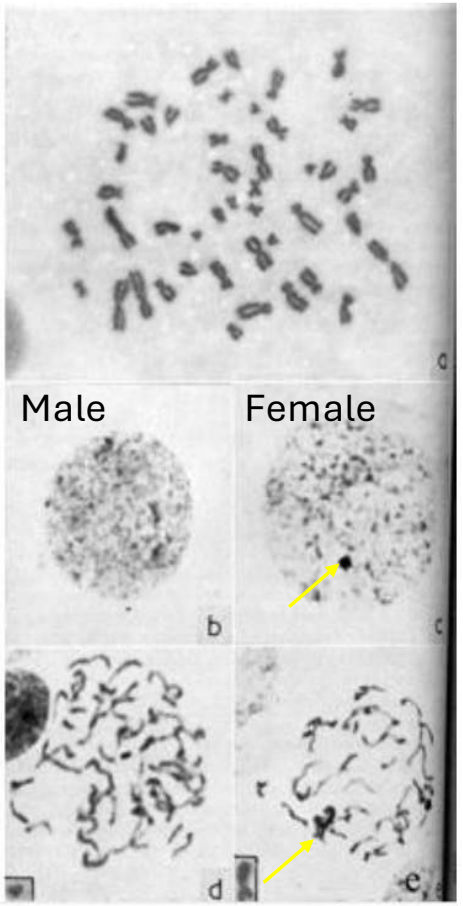
RESEARCH ASSOCIATE, DEPARTMENT OF EXPERIMENTAL PATHOLOGY,
CITY OF HOPE MEDICAL CENTER, DUARTE, CALIFORNIA
SAJIRO MAKINO
D.Sc. Hokkaido
PROFESSOR OF ZOOLOGY, ZOOLOGICAL INSTITUTE,
HOKKAIDO UNIVERSITY, SAPPORO, JAPAN

In 1958-1959, Japanese geneticist Susumu Ohno demonstrated that the previously identified "nucleolar satellite" or sex chromatin, was in fact a single heteropycnotic X chromosome in female XX somatic cells.

Looking in both human fetuses, as well as animals (rodents), he confirmed the identity of a silent X and termed it the "Barr body" in recognition of Barr's earlier discovery.

Ohno's work clarified that the Barr body was not merely a structural feature but represented the functional silencing of one whole X chromosome. Initially he suggested this was always the paternal X (ie the one unique to females).



E. Heard, May 12th, 2025

Ohno's deduction that there is one inactive X chromosome in females (1959-61)

1959

Allocycly of the X-Chromosome in Tumors and Normal Tissues*

S. Ohno and T. S. Hauschka

(City of Hope Medical Center, Duarte, California; and Roswell Park Memorial Institute, Buffalo, New York)

SUMMARY

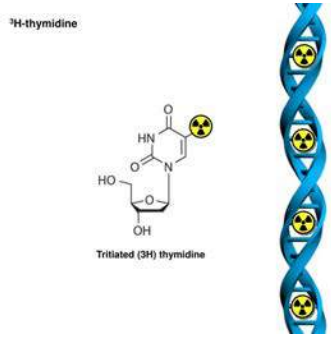
A single, deeply staining heteropyknotic chromosome, most conspicuous during prophase in neoplastic and normal diploid female cells of mouse and rat, is interpreted as one of the two X-chromosomes. Tetraploid female nuclei often contain two such elements, tetraploid male nuclei only one. Tjio and Östergren's (1958) explanation of this phenomenon as a symptom of chromosomal infection with the Bittner milk agent appears untenable. The observed allocycly of the X-chromosome has a bearing on the constitution of the "sex chromatin" in interphase nuclei which is usually composed of the heterochromatin of a single positively heteropyknotic X, rather than two paired X's.

78 JANUARY 14, 1961 ORIGINAL

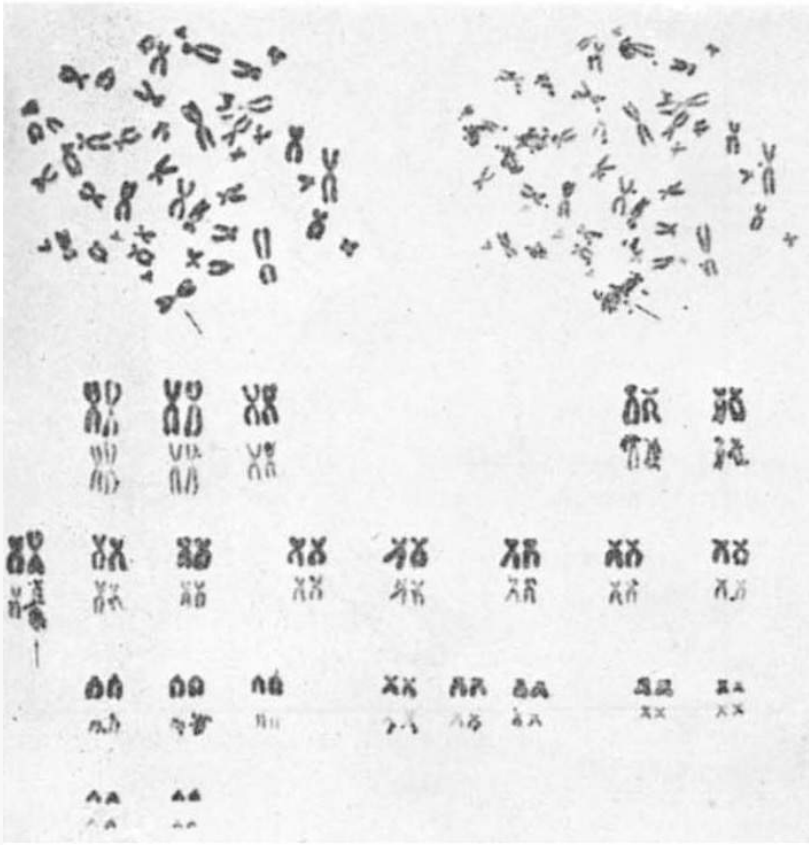
THE SINGLE-X NATURE OF SEX CHROMATIN IN MAN

Susumu Ohno
D.Med.Sc. Hokkaido
RESEARCH ASSOCIATE, DEPARTMENT OF EXPERIMENTAL PATHOLOGY, CITY OF HOPE MEDICAL CENTER, DUARTE, CALIFORNIA

Sajiro Makino
D.Sc. Hokkaido
PROFESSOR OF ZOOLOGY, ZOOLOGICAL INSTITUTE, HOKKAIDO UNIVERSITY, SAPPORO, JAPAN



Labelling with ^{3}H-thymidine by incubating cells. Incorporation into DNA in the last hours before metaphase prep
Only the latest replicating chromosome will incorporate the radioactive nucleotide
(autoradiograph from Miller et al, 1963L



Allocyclic replication of the inactive X detected by autoradiography

Ohno's deduction that there is one inactive X chromosome in females (1959-61)

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By 1959, Ohno had concluded that:

(i) One X chromosome in normal mammalian female interphase cell is heterochromatic and the other X chromosome is similar to autosomes.

(ii) The heterochromatic X chromosome accounts for the nuclear structure referred to as nuclear sex chromatin body, originally described by Barr.

(iii) The X chromosome is allocyclic (asynchronous in its replication relative to other chromosomes

Ohno and others speculated that the genes on the heterochromatic X could be largely silent, resulting in gene dosage regulation in female cells, given that genes in heterochromatic regions of chromosomes of other systems do not transcribe (cf Heitz).

Therefore, only genes on the active X chromosome would encode proteins, thus providing both male and female cells with similar dosage effects of most X chromosome-linked genes.

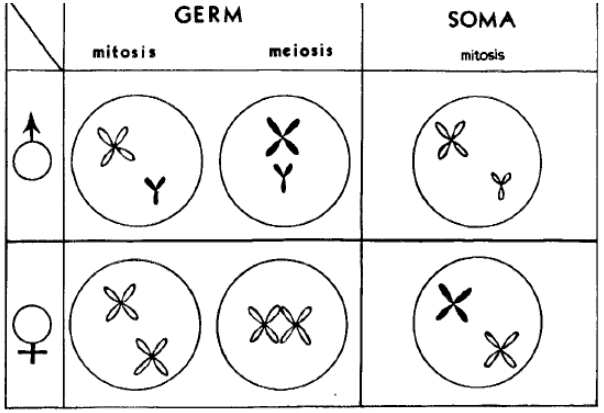

Fig. 1—Status of individual sex chromosomes in *Rattus norvegicus* is shown in germ and somatic cells of both sexes.
Heteropyknotic chromosomes are drawn solid black; chromosomes isopyknotic to the autosomes are outlined.

It was in part his work on the sex chromatin that led to Ohno's reflections on sex chromosome evolution and the requirements for dosage compensation strategies. These were elaborated a few years later, following the discovery of X-inactivation by Mary Lyon.

Mary Lyon (1925-2014)

Mary Lyon: Pioneer of X-Inactivation and Mouse Genetics

- Born 1925 in rural Norfolk; keen on biology and math; educated during WWII
- Attended Girton College, Cambridge (1943); received official degree in 1964
- Began PhD under R.A. Fisher, studying mouse mutants
- Moved to Edinburgh (1948) Institute of Animal Genetics headed by CH Waddington (who influenced her)
- Supervised by D.S. Falconer; PhD awarded in 1950
- Studied mutagenesis and radiation-induced mouse mutants post-WWII
- Moved to MRC Harwell in 1955 due to mouse breeding space constraints

Fisher and Rastan, 2024 “Mary Frances Lyon. 15 May 1925—25 December 2014”, *Biogr. Mems Fell. R. Soc. 77, 241–259 (2024).* DOI: (10.1098/rsbm.2024.0005)

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- Moved to MRC Harwell in 1955 due to mouse breeding space constraints
- Took on the Genetics Section at Harwell from 1962–1990
- She continued research after retiring, into her 80s
- Discovered X-inactivation ("Lyonization") in 1961
- Worked closely with many other mouse geneticists like Bruce Cattanach
- Developed insights into non-Mendelian inheritance via the t-complex
- Work established the mouse as a central model for human genetics and disease

E. Heard, May 12th, 2025

Fisher and Rastan, 2024 "Mary Frances Lyon. 15 May 1925—25 December 2014", *Biogr. Mems Fell. R. Soc. 77, 241–259 (2024).* DOI: (10.1098/rsbm.2024.0005)

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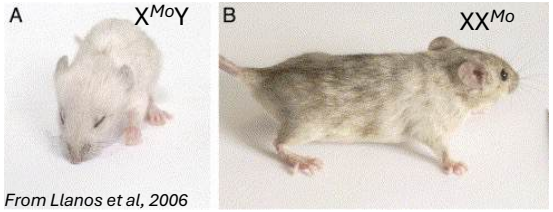
Mary Lyon (1925-2014)

Mary Lyon and the Discovery of X Inactivation

- Studied X-linked coat mutations (e.g., Tabby, mottled) at Harwell from the Edinburgh mouse lines (these were not radiation-induced mutants)
- Observed coat variegation in heterozygous females; males often died or had uniform coat phenotypes
- Discovered a rare mottled *male* and traced inheritance to mosaicism – of an early embryonic mutation
- Deduced females inherit either mutant or normal X from mosaic male
- Also realized that female mice can survive with just one X (XO)
- Connected all this with the known Barr body (sex chromatin) and Ohno's finding of a condensed X chromosome
- Proposed random X inactivation in females - each cell inactivates one X at random – both X's are present, but one is always silent
- This explained survival of XO mice and female coat mosaicism
- Published theory in Nature (1961)
- Extended hypothesis to humans and other animals - showing it applies broadly to mammals – and could be the mechanism of mammalian dosage compensation

Mary Lyon
Harwell, 1963



From Llanos et al, 2006

Female mice carrying X-linked mutations such as *Mottled* or *Tabby* show coat colour variegation

Male mutant mice show severe phenotypes and no coat colour variegation

Fisher and Rastan, 2024 "Mary Frances Lyon. 15 May 1925—25 December 2014", *Biogr. Mems Fell. R. Soc. 77, 241–259 (2024).* DOI: (10.1098/rsbm.2024.0005)

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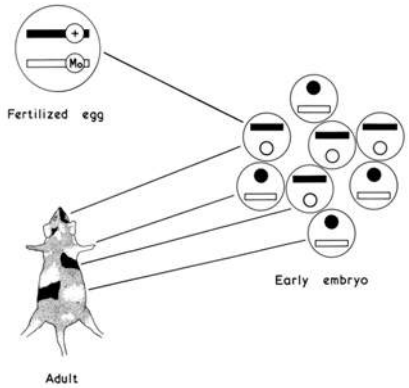
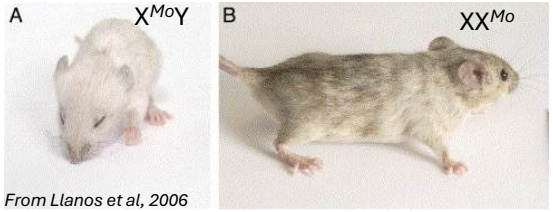




From Llanos et al, 2006

THE Y-CHROMOSOME AS THE BEARER OF MALE DETERMINING FACTORS IN THE MOUSE

By W. J. Welshons and Liane Brauch Russell

BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY,* OAK RIDGE, TENNESSEE

Communicated by Alexander Hollaender, February 19, 1959

Introduction.—Russell, Russell, and Gower, in the accompanying paper,[1] report the occasional occurrences, over the course of several years, of an unexpected class of female progeny in matings of normal males with females heterozygous for the sex-linked mutation *scurfy*. These rare, unexpected, females phenotypically resemble the hemizygous males. Since the affected females die before reproducing, genetic analysis had to be attempted by means of ovarian transplantation. This was successful in several cases and the results, described in the companion paper,[1] ruled out a number of possible explanations for the exceptional *scurfy* females. Without further work, however, no decision was possible between the remaining hypotheses. The experiments to be described here have led to an unequivocal explanation of unexpected *X*-linked inheritance.

Fisher and Rastan, 2024 "Mary Frances Lyon. 15 May 1925—25 De
Biogr. Mems Fell. R. Soc. 77, 241–259 (2024). DOI: (10.1098/rsbm.

The Discovery of X-Chromosome Inactivation in Mice

The hypothesis formulated by Mary Lyon in her Nature paper in April, 1961 was that:

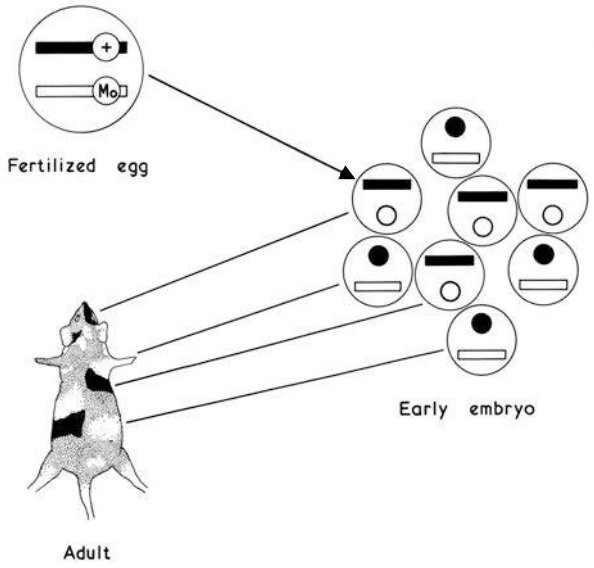
(1) the heteropycnotic X chromosome was *genetically* inactivated (wt gene could be silenced)
(2) that it could be either paternal or maternal in origin in different cells of the same animal,
(3) that the inactivation occurred early in embryonic development, and once established was stably maintained

Genetic facts underlying this hypothesis were:

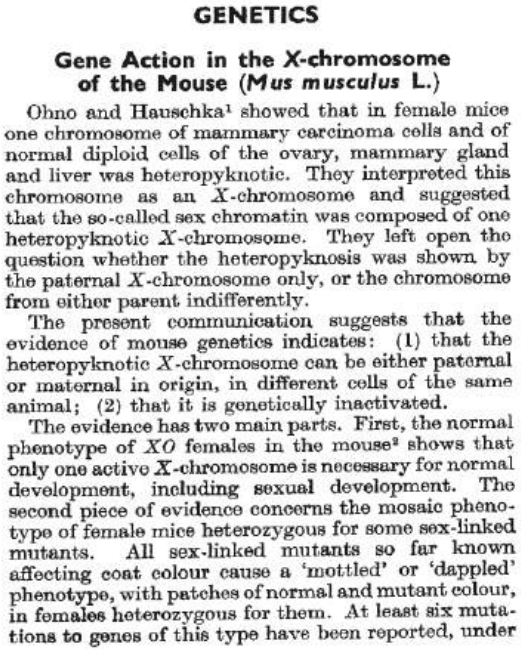
(i) that XO mice are normal fertile females => female mice needs only one X chromosome to develop normally (Welshons and Russell, 1959);

(ii) the mosaic phenotype of female mice heterozygous for some sex-linked mutants.

Adapted from Mary Lyon, Henry Stewart Talks



Lyon, M. F. (1961), Gene Action in the X-chromosome of the Mouse (Mus musculus L.) Nature. 190 (4773): 372-3.



GENETICS

Gene Action in the *X*-chromosome of the Mouse (*Mus musculus* L.)

Ohno and Hauschka[1] showed that in female mice one chromosome of mammary carcinoma cells and of normal diploid cells of the ovary, mammary gland and liver was heteropyknotic. They interpreted this chromosome as an *X*-chromosome and suggested that the so-called sex chromatin was composed of one heteropyknotic *X*-chromosome. They left open the question whether the heteropyknosis was shown by the paternal *X*-chromosome only, or the chromosome from either parent indifferently.

The present communication suggests that the evidence of mouse genetics indicates: (1) that the heteropyknotic *X*-chromosome can be either paternal or maternal in origin, in different cells of the same animal; (2) that it is genetically inactivated.

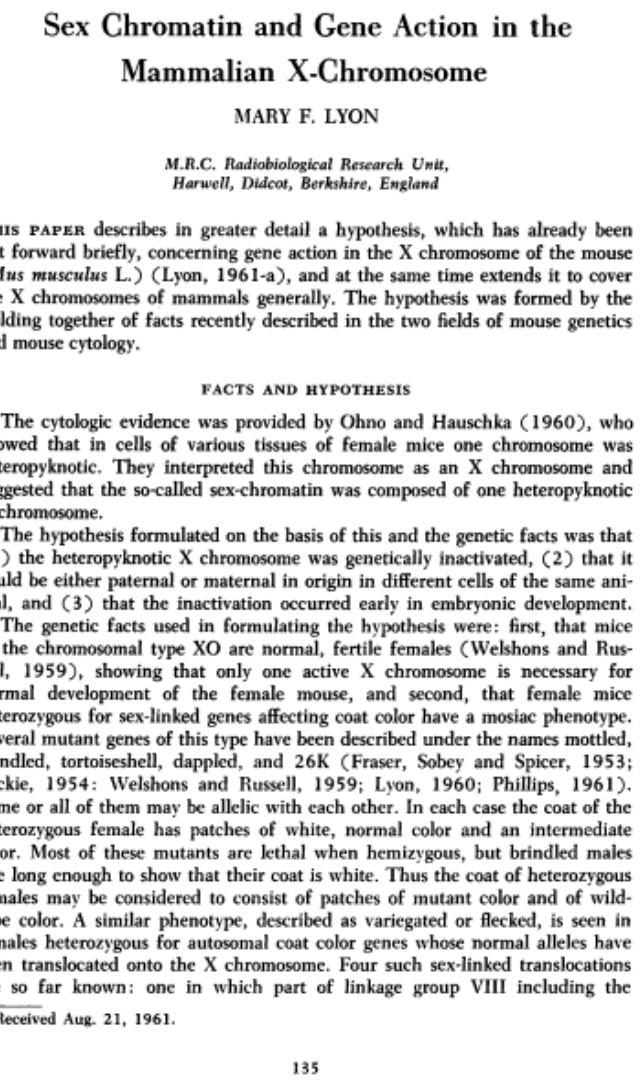
The evidence has two main parts. First, the normal phenotype of *XO* females in the mouse[2] shows that only one active *X*-chromosome is necessary for normal development, including sexual development. The second piece of evidence concerns the mosaic phenotype of female mice heterozygous for some sex-linked mutants. All sex-linked mutants so far known affecting coat colour cause a 'mottled' or 'dappled' phenotype, with patches of normal and mutant colour, in females heterozygous for them. At least six mutations to genes of this type have been reported, under

Nature, April 22, 1961

Expanding the X-Inactivation Hypothesis to Humans & other Mammals

Expanding and Defending the X Inactivation Hypothesis

- Within 4 months of her 1961 paper, Mary Lyon submitted a follow-up with 82 references across mammalian species, including humans
- With evidence that X inactivation equalizes X-linked gene expression in XX females and XY males—dosage compensation
- Cited human traits supporting her theory; key geneticists like Victor McKusick quickly recognized its significance
- Observations in XO, XXY, XXX individuals led Lyon to refine her model: one X remains active, not one becomes inactive
- Nevertheless, Mary Lyon faced resistance from Hans Grüneberg, an established geneticist and Royal Society Fellow, who felt that:
- She was too junior to propose such a major theory
- Patch patterns were too variable to support her model
- Mary countered with examples like Tabby, showing tissue mosaics explain intermediate phenotypes (e.g., in teeth)



Sex Chromatin and Gene Action in the Mammalian X-Chromosome

MARY F. LYON

M.R.C. Radiobiological Research Unit, Harwell, Didcot, Berkshire, England

THIS PAPER describes in greater detail a hypothesis, which has already been put forward briefly, concerning gene action in the X chromosome of the mouse (*Mus musculus* L.) (Lyon, 1961-a), and at the same time extends it to cover the X chromosomes of mammals generally. The hypothesis was formed by the welding together of facts recently described in the two fields of mouse genetics and mouse cytology.

FACTS AND HYPOTHESIS

The cytologic evidence was provided by Ohno and Hauschka (1960), who showed that in cells of various tissues of female mice one chromosome was heteropyknotic. They interpreted this chromosome as an X chromosome and suggested that the so-called sex-chromatin was composed of one heteropyknotic X chromosome.

The hypothesis formulated on the basis of this and the genetic facts was that (1) the heteropyknotic X chromosome was genetically inactivated, (2) that it could be either paternal or maternal in origin in different cells of the same animal, and (3) that the inactivation occurred early in embryonic development.

The genetic facts used in formulating the hypothesis were: first, that mice of the chromosomal type XO are normal, fertile females (Welshons and Russell, 1959), showing that only one active X chromosome is necessary for normal development of the female mouse, and second, that female mice heterozygous for sex-linked genes affecting coat color have a mosiac phenotype. Several mutant genes of this type have been described under the names mottled, brindled, tortoiseshell, dappled, and 26K (Fraser, Sobey and Spicer, 1953; Dickie, 1954: Welshons and Russell, 1959; Lyon, 1960; Phillips, 1961). Some or all of them may be allelic with each other. In each case the coat of the heterozygous female has patches of white, normal color and an intermediate color. Most of these mutants are lethal when hemizygous, but brindled males live long enough to show that their coat is white. Thus the coat of heterozygous females may be considered to consist of patches of mutant color and of wild-type color. A similar phenotype, described as variegated or flecked, is seen in females heterozygous for autosomal coat color genes whose normal alleles have been translocated onto the X chromosome. Four such sex-linked translocations are so far known: one in which part of linkage group VIII including the

Received Aug. 21, 1961.

135

Lyon (1962) Am. J. Hum. Genet 14:135-148.

Liane Russell: Evidence for X Inactivation based on Variagation in female X-Autosome translocation mice

Lee Russell's 1961 Contribution to Discovering X Inactivation

- Published in June 1961, shortly after Lyon's April paper
- Summarized advances in mammalian sex chromosome cytogenetics
- Described V-type (variegated) position effects in mice, adapted from Drosophila
- Studied female mice with X–chromosome 8 translocations showing patchy coat color
- Noted the X chromosome's strong heterochromatic nature, unlike autosomes
- Credited Ohno for cytological evidence supporting this genetic conclusion
- She conclude that the presence of two X chromosomes is necessary for the expression of the V-type position effect.
- Importantly, she identified XO females in her mouse stocks—carriers of the translocation that were *not* non-variegated (ie X;A no variable silencing)
- Her work on X-Autosome translocations contributed to defining and mapping the X-inactivation centre and to the concept of variable spread of autosomal cis-silencing

Liane Brauch Russell (1923 -2019)
Austrian-born American Geneticist

Liane Russell et al, Science, 9 Jun 1961
DOI: 10.1126/science.133.3467.1795

CURRENT PROBLEMS IN RESEARCH

Genetics of Mammalian Sex Chromosomes

Mouse studies throw light on the functions and on the occasionally aberrant behavior of sex chromosomes.

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Liane Brauch Russell

From Lyon, 1962

simultaneously with the original publication of the present hypothesis, Russell (1961) put forward a very similar but more limited one concerning the variegation due to sex-linked translocations in the mouse. She considered that this variegation was "presumably a heterochromatic effect," and from the fact that two X chromosomes were essential for its expression, together with cytologic evidence, postulated that "in mammals, genic balance requires the action of *one* X in a manner which precludes realization of its heterochromatic potentialities, so that only *additional* X's present assume the properties characteristic of heterochromatin."

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E. Heard, May 12th, 2025

Evidence for X inactivation based on Humane Females with G6PD deficiency

Human Females are Mosaics for X-Chromosome Activity based on G6PD deficiency as a Marker

- Test for X-chromosome dosage compensation in humans
- They hypothesise that human females are genetic mosaics with random inactivation of one X chromosome (maternal or paternal) in each cell during early embryonic development.
- By studying G6PD heterozygote females, they find two distinct red cell populations (normal and deficient), supporting a process of X inactivation.
- Variability in enzyme activity among heterozygous females is explained by early X-chromosome inactivation in a limited number of embryonic precursor cells.
- The study point to a process of stable (clonal) silencing of one X in females ie X inactivation, aligning with Lyon's independently proposed hypothesis in 1961.

*THE NORMAL HUMAN FEMALE AS A MOSAIC OF X-CHROMOSOME ACTIVITY: STUDIES USING THE GENE FOR G-6-PD-DEFICIENCY AS A MARKER**

By Ernest Beutler, Mary Yeh, and Virgil F. Fairbanks

City of Hope Medical Center, Duarte, California

Communicated by A. H. Sturtevant, November 30, 1961

The question why human females do not synthesize twice as much of those enzymes controlled by a locus on the X-chromosome as do males has never been answered satisfactorily. In human autosomal mutations in which the product of gene action can be quantitated, the heterozygote appears to produce approximately one-half of the normal quantity of the protein synthesized. Examples include acatalasemia,[1] congenital methemoglobinemia due to diaphorase deficiency,[2] PTA deficiency,[3] non-spherocytic congenital hemolytic anemia due to a deficiency of pyruvic kinase,[4] and the hemoglobinopathies.[5] This so-called "dosage effect" would lead one to believe that each gene governs the synthesis of a discrete amount of enzyme. Yet, when the gene is on the X-chromosome, such as the gene governing the synthesis of antihemophilic globulin or of glucose-6-phosphate dehydrogenase (g-6-pd), the quantity of protein produced is not twice as great in females as it is in males, in spite of the fact that females have two X-chromosomes, while males only have one.

On the basis of Drosophila genetics, attempts have been made to explain this in terms of hypothetic "dosage compensator" genes.[6, 7] However, the work of Ohno and his collaborators,[8] at this institution, has suggested to us an alternative explanation which at the same time explains the markedly variable penetrance observed in the g-6-pd–deficient heterozygote.

Ohno has shown that in somatic cells of human females, the two X-chromosomes are not alike. One behaves in exactly the same manner as the autosomes, remaining in an extended state during interphase and prophase, while the other assumes a heavily condensed state, forming the Barr sex chromatin body. In male somatic

Beutler, E., Yeh, M., Fairbanks, V. F. 1962. "The normal human female as a mosaic of X-chromosome activity:studies using the gene for G-6-PD-deficiency as a marker". Proc. Nat. Acad. Sci. USA 48:9-16

Clonal X inactivation states in Humane Females using G6PD as a marker

*DEMONSTRATION OF TWO POPULATIONS OF CELLS IN THE HUMAN FEMALE HETEROZYGOUS FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE VARIANTS**

Davidson et al, 1963, PNAS.

If the "Lyon Hypothesis" applies, the female who is heterozygous for the two electrophoretic variants should also be a mosaic: some of her cells producing A type G-6-PD, some the B type, but none producing both.

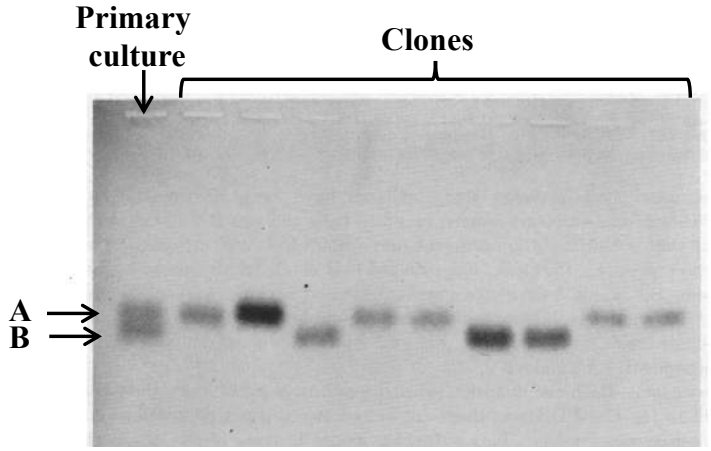
The appearance of two distinct populations of cells in the female heterozygous (for) G-6-PD variants is direct evidence in favor of the "Lyon Hypothesis."

As far as the locus for G-6-PD is concerned, in each single cell only one X chromosome is functional.

However, these data do not imply that one entire X chromosome is inactivated.

Males can have a fast migrating G-6-PD band (A) or a slow one (B).
Females can have A, B, or both A and B.

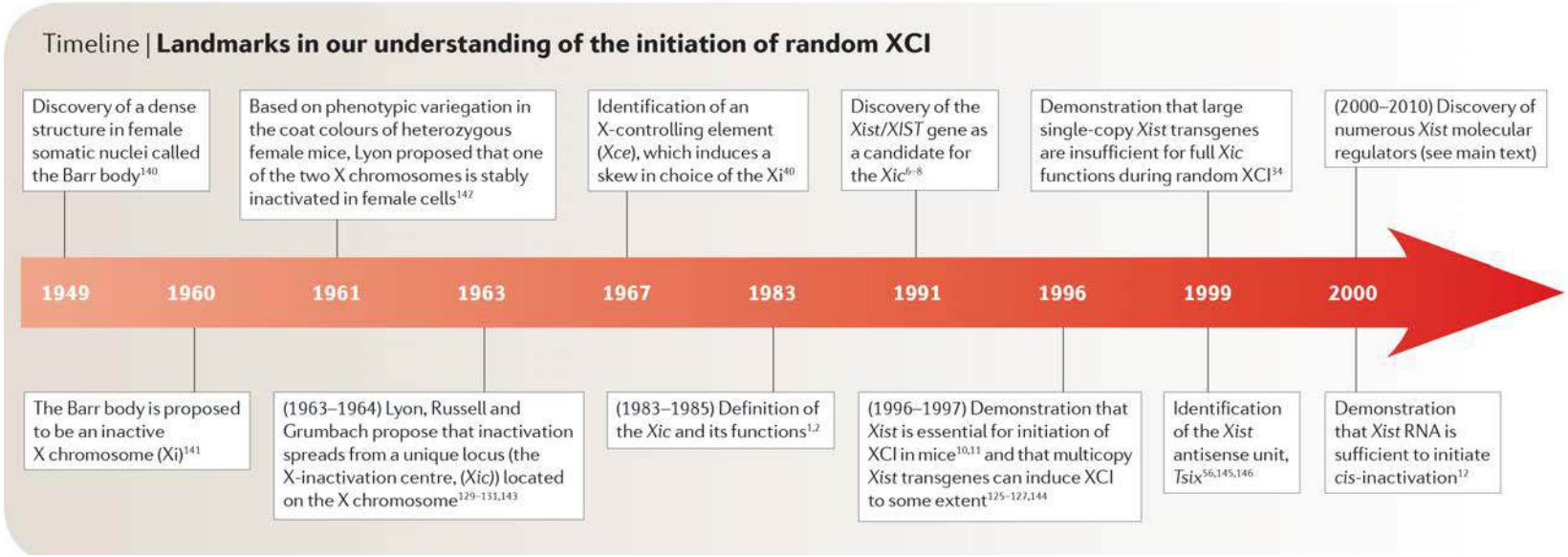
Here, a primary skin culture and nine clones (derived from single cells) were examined.
The primary skin culture contains both A and B bands - and is a mixture of cells
Each clone presents either A or B
Thus showing that only one allele is expressed (active X) in each clone
The other is silent (inactive X)



Electropheoresis patterns of G-6-PD enzyme from sonicates of cultured cells
Primary skin culture of Mrs. De. and 9 clones derived from this culture

FIG. 2.—Electrophoretic pattern of G-6-PD from sonicates of cultured cells. Samples were run singly, starting from the origin at the top of the figure. From left to right are the AB phenotype of the cell culture from Mrs. De. prior to cloning, and the single bands of nine clones derived from the original cell lines. Variation in intensity of staining is due to inequality of enzyme concentration applied to the starch gel.

The Era of Exploration: Control and Mechanism of X inactivation



In the first decades after its discovery, there were very few markers for X inactivation:

- Condensation of the Xi and its replication timing (staining techniques)
- G6PD and other X-linked enzyme isoforms (human cells)
- Genetic coat colout markers such as Tabby and Mottled in mice
- Human and mouse genetics to look at deletions and translocations to map the XIC
- Somatic cell hybrids to study isolated human X (or parts of it) on a mouse background

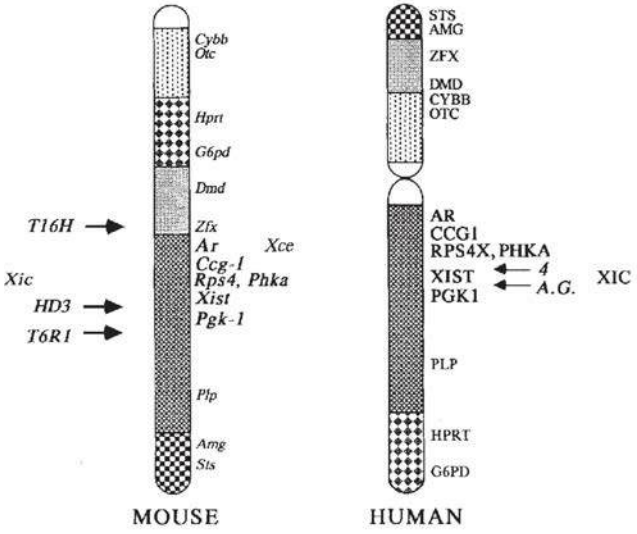
From Augui et al, Nature Rev. Genetics, 2011

Ohno's Law, 1967

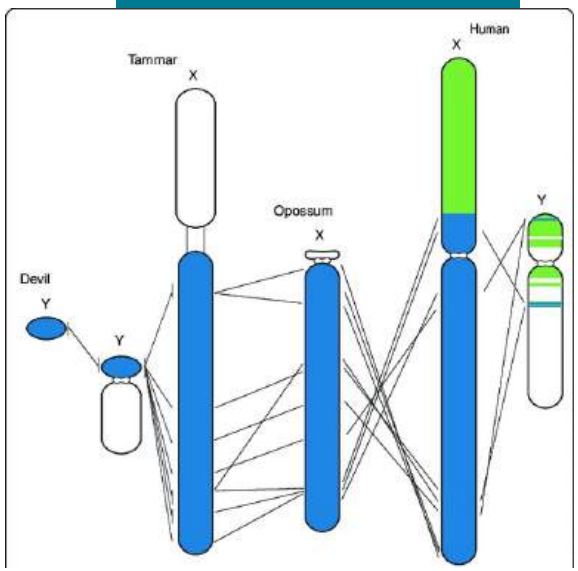
Ohno S (1967) Sex Chromosomes and Sex-Linked Genes.

A milestone came with the formulation of what is now known as Ohno's Law.

- Ohno put forward the idea that, because of the different dosage relationships of autosomal and X-linked genes, translocations between the X and autosomes that occurred during evolution would be detrimental and would be eliminated.
- **Hence, genes X-linked in one mammalian species would be X-linked in all.**
- This hypothesis opened up a way to find X-linked genes for study of X-inactivation in any species and hence enabled advances in the field. Ohno's Law is well established with no exceptions so far known among eutherian mammals.



Ohno's Law, 1967



A milestone came with the formulation of what is now known as Ohno's Law.

- Ohno put forward the idea that, because of the different dosage relationships of autosomal and X-linked genes, translocations between the X and autosomes that occurred during evolution would be detrimental and would be eliminated.
- **Hence, genes X-linked in one mammalian species would be X-linked in all.**
- This hypothesis opened up a way to find X-linked genes for study of X-inactivation in any species and hence enabled advances in the field. Ohno's Law is well established with no exceptions so far known among eutherian mammals.
- However, in marsupials and monotremes, genes on the long arm (Xq) of the present-day human X-chromosome are again X-linked in these groups, but genes on the human short arm (Xp) are autosomal both in marsupials and in the monotreme, the platypus, suggesting that these genes have been recruited from autosomes to the X-chromosome during the evolution of eutherian mammals.
- Genes on the human and mouse X-chromosomes have been rearranged relative to each other in evolution and genes from Xp are found in at least three separate segments of the mouse X-chromosome. Presumably, the arrangement on the human X is nearer to that on the primitive eutherian X-chromosome.

Identification and Mapping of the X-Inactivation Center

- After Lyon's theory, many studies confirmed X inactivation, but its control mechanisms remained unclear
- Cytogenetic analysis in mice and humans with X chromosome deletions revealed a key region: the X-inactivation center (XIC/Xic)
- Rastan & Robertson (1985) narrowed Xic in the mouse
- Use of rodent-human hybrid cell lines (from patients with X rearrangements) enabled fine mapping
- Brown et al. (1991) studied partial X chromosomes in hybrid cells to test inactivation
- Inactivated Xs identified by late replication (heterochromatic trait)
- Despite diverse rearrangements, a common region on Xq13 was always present in inactivated Xs
- In situ hybridization further defined this shared segment as the XIC
- Mouse models confirmed that this region is essential for X inactivation

An early concept concerning the mechanism of X-inactivation was that of an X-inactivation center on the X-chromosome from which inactivation was postulated to spread, take account of the effects seen in female mice with X-autosome translocations

Mammalian X-Chromosome Action:
Inactivation Limited in Spread and in Region of Origin

Abstract. *In its simplest form the hypothesis of the single-active-X chromosome does not explain variegated-type position effects in the mouse. Inactivity appears not to involve one entire X chromosome; furthermore, even those parts of the chromosome that can change to an inactive state spread inactivation not to the entire attached piece of autosome, but along a gradient to limited distances.*

Variegated-type (V-type) position effects from X-autosome translocations in the mouse have played a major role in development of the hypothesis of the single-active-X chromosome. Recent results from seven such translocations will be presented to show that this hypothesis is not valid in its simplest form (*1*).

V-type position effects were first reported by us in 1959 (*2*). Now, eight stocks of independent origin exist—seven of these (some radiation-induced, some spontaneous) at our laboratory (*1*–*5*) and one (induced with tri-ethylene melamine) in Edinburgh (*6*). All eight carry X-autosome translocations, one involving linkage-group (L.G.) VIII and the remainder L.G. I. We have explained the variegated phenotype by

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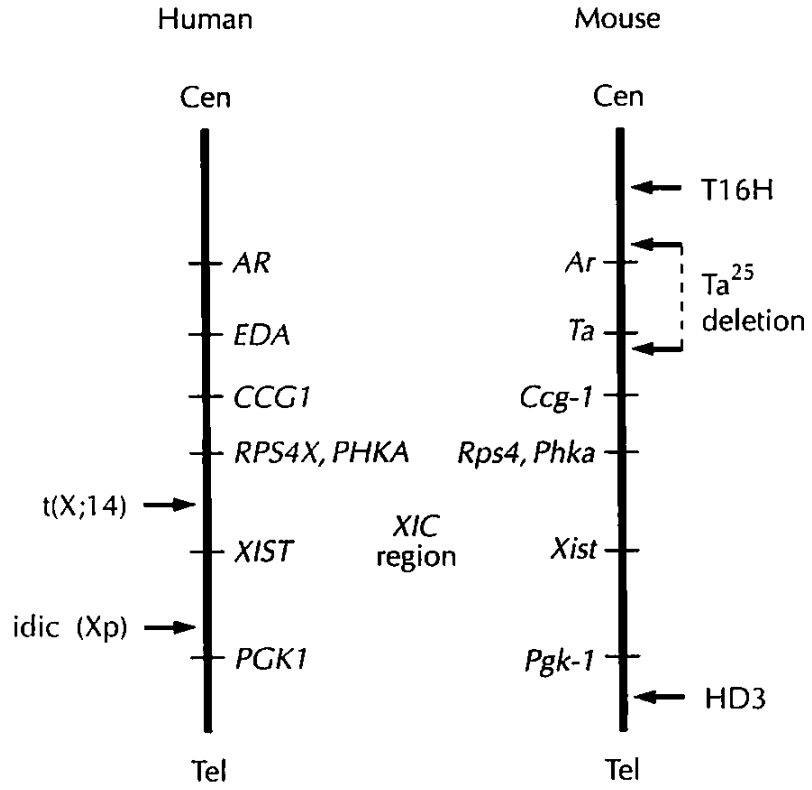


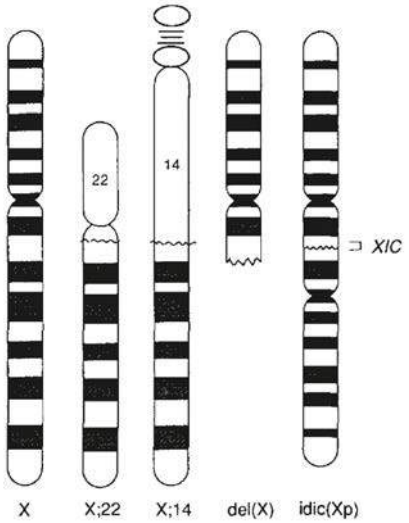




E. Heard, May 12th, 2025

Identification and Mapping of the Human and Mouse X-Inactivation Centers

CELL LINE	KARYOTYPE	SOMATIC CELL HYBRID	PORTION OF X INACTIVATED	EVIDENCE FOR INACTIVATION	LOCATION OF *XIC*
68 (GM4628)	46,X,t(X;22) (q13;p11)	A68-2A	q13->qter	Late-replication[13]	distal to break
4 (GM0074)	47,Y,t(X;14) (q13;q32) +der(14)mat	W4-1A	q13->qter	Late-replication[16]	distal to break
81	46,X,del(X) (pter>q13:)	t81-az1b	pter->q13	Late-replication[17]	proximal to break
A.G.	45,X/46,X,idic(Xp) (pter->q13:: q13->pter)	tAG-1Baz1b	pter->q13	Late-replication[18] Gene inactivation*	proximal to break

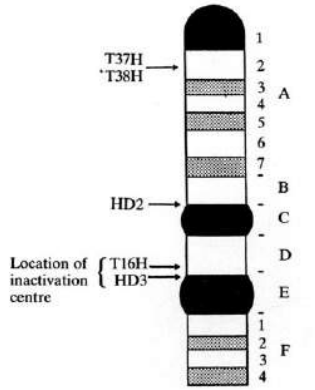


Brown et al, Localization of the X inactivation centre on the human X chromosome in Xq13. Nature 349, 82–84 (1991)

Table 1. *Proportion of metaphases in undifferentiated and differentiated states*

Cell line		Total metaphases scored	Metaphases with dark X (%)	Metaphases with no dark X (%)
HD1	U	42	0	42 (100 %)
	D	10	0	10 (100 %)
HD2	U	27	0	27 (100 %)
	D	69	0	69 (100 %)
HD3	U	18	1 (6 %)	17 (94 %)
	D	175	99 (57 %)	76 (43 %)
HD6	U	79	0	79 (100 %)
	D	23	0	23 (100 %)
CP4-2	U	44	2 (5 %)	42 (95 %)
	D	174	64 (37 %)	110 (63 %)
A13D	U	45	10 (22 %)	35 (78 %)
	D	198	79 (40 %)	119 (60 %)

U = Undifferentiated; D = Differentiated.



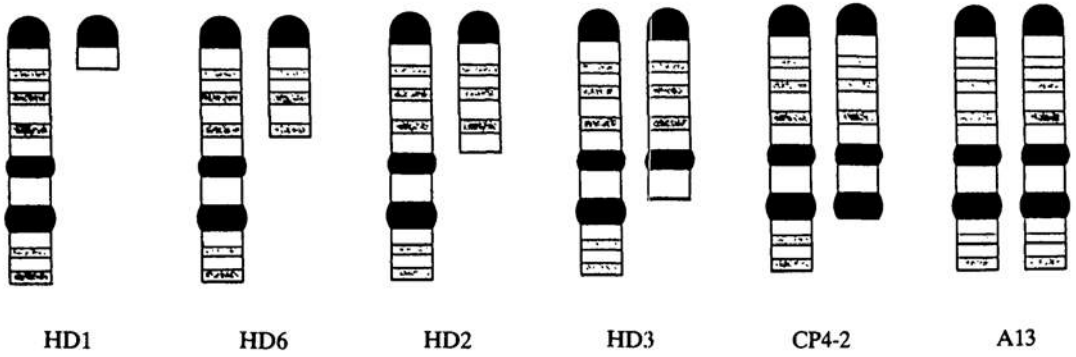


Fig. 1. Diagram of the X-chromosome constitution of the embryo-derived (EK) stem cell lines used, based on G-banded karyotypes.

Rastan and Robertson (1985) X-chromosome deletions in embryo-derived (EK) cell lines associated with lack of X-chromosome inactivation. J.Embryol. Exp. Morphol, 90: 379-388.

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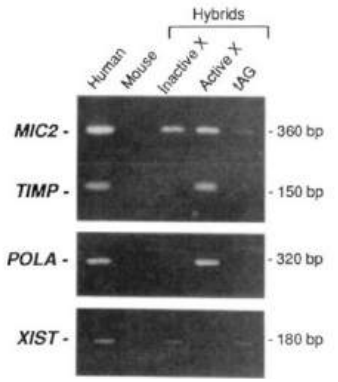
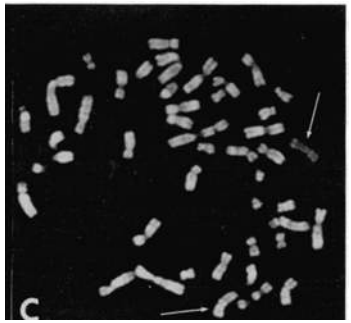


RT-PCR for X-linked gene activity



Replication timing

Kanda Staining of the inactive X

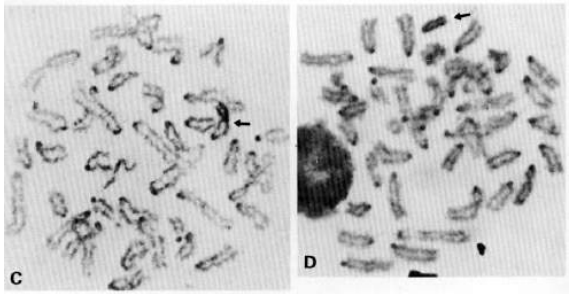
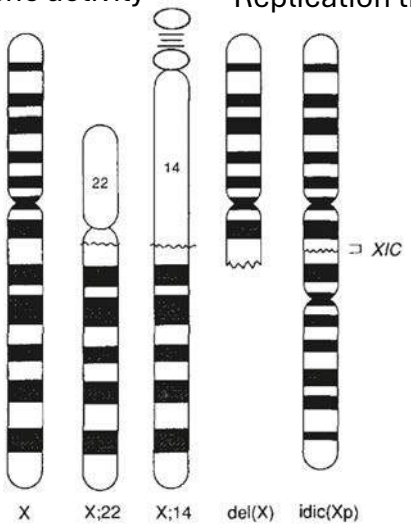
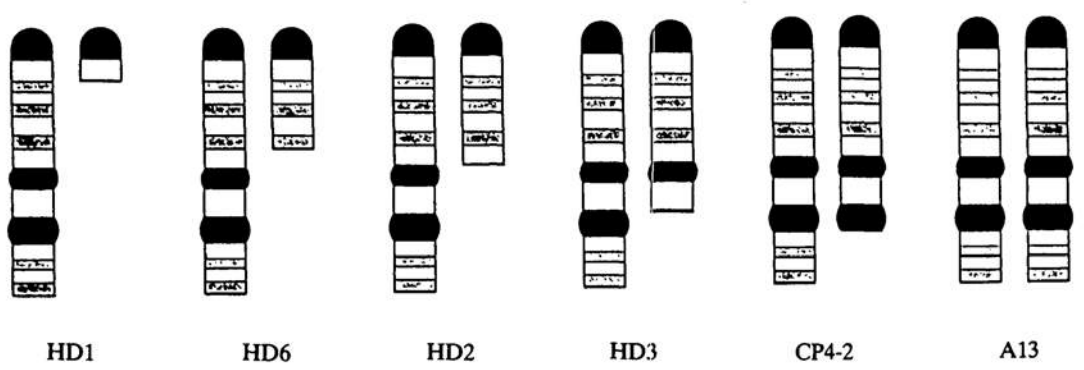


Fig. 2. Metaphase spreads from embryo-derived (EK) stem cell lines treated by the Kanda method after induction of differentiation to show dark-staining inactive X-chromosomes. (A) metaphase from line A13; (B) metaphase from line CP4-2; (C) metaphase from line HD3 with intact X inactive; (D) metaphase from line HD3 with deleted X inactive (inactive X-chromosomes arrowed).





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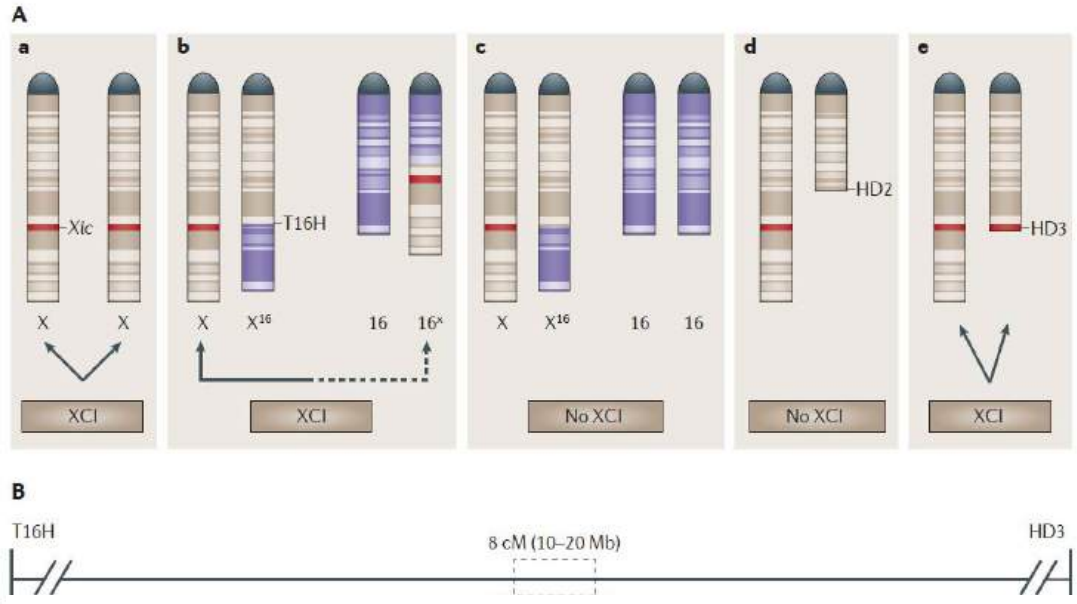

The X-inactivation centre : The Hunt Begins…

Early studies of XCI patterns in mouse embryos or embryonic cells that carried translocated or truncated X chromosomes revealed the existence of a single X-linked locus, the *Xic*, that needs to be physically linked to a chromosome to trigger its inactivation. Random XCI is only triggered in cells with at least two *Xic*-bearing chromosomes2, suggesting that the two copies of the *Xic* are able to potentiate each other in *trans,* a phenomenon that has been referred to as competence, or sensing. In XX cells, either one of the two X chromosomes will be inactivated, a process known as choice. The autosomal ploidy of a cell (the number of sets of autosomes that is contains) also seems to affect the number of X chromosomes that will be inactivated, a phenomenon known as counting. The precise mechanisms underlying these processes are only now being unravelled and recent data suggest that they are highly interconnected, both genetically and molecularly.

SUMMARY Timeline: Discovery of Sex Chromosomes

1891 – Hermann Henking
Hermann Henking, studying sperm cells in a bug species (Pyrrhocoris), observed the “X element.”
He did not fully understand its role, but it was the first observation of what would later be known as a sex chromosome.

1905 – Nettie Stevens and Edmund B. Wilson
Stevens and Wilson, working independently, discovered that sex determination in insects was linked to specific chromosomes. Stevens showed that males had XY chromosomes and females had XX. This established the chromosomal basis of sex.

1910s–1920s - Researchers attempted to extend these discoveries to mammalian cells, but due to the limits of microscope technology, chromosome counts and identification were still inaccurate.

1921 – Theophilus S. Painter
Painter studied human spermatocytes and identified the Y chromosome as distinct from the X. He incorrectly estimated that humans had 48 chromosomes, but his work was crucial in recognizing the X and Y chromosomes as the basis for human sex determination.

1956 – Joe Hin Tjio and Albert Levan
Using improved techniques, Tjio and Levan correctly determined that humans have 46 chromosomes (23 pairs). This was a major breakthrough in human cytogenetics and confirmed the presence of the XX (female) and XY (male) chromosome pairs.

1959 – Patricia Jacobs and John Strong
They discovered the first chromosomal abnormality involving sex chromosomes: Klinefelter syndrome (XXY). This, along with later identification of Turner syndrome (XO), further confirmed the role of sex chromosomes in human development.

SUMMARY Timeline: Discovery of X Chromosome Inactivation (XCI)

1949 – Murray Barr and Ewart Bertram
•Discovered a dense, dark-staining structure in the nuclei of female cat neurons — later named the Barr body.
•This structure was absent in males, suggesting a sex-linked difference.

1959 – Susumu Ohno
•Proposed that the Barr body represented a condensed, inactive X chromosome.
•Suggested that X inactivation was a way to equalize gene dosage between XX females and XY males

1961 – Mary Lyon - X chromosome inactivation
•Formulated the Lyon Hypothesis: in female mammals, one of the two X chromosomes is randomly inactivated in each cell during early embryonic development – explaining mosaicism in female mammals

1970s –Confirmation of XCI
•Cytogenetic and biochemical evidence began confirming Lyon's hypothesis.
•Studies showed that only one X chromosome is transcriptionally active in female cells.

1991 – Discovery of the XIST Gene (X-inactive specific transcript)
•Identified on the X chromosome by Brown, Willard, Ballabio, Avner, Brockdorff, Rastan
•XIST was shown to produce a non-coding RNA that coats the X chromosome (Larwrence) and initiates its inactivation, marking the first molecular mechanism of XCI.

2000s–Present – Epigenetic Regulation, Developmental and Evolutionary Dynamics, Molecular Mechanisms
•XCI in relation to chromatin marks such as DNA methylation, histone modifications, and chromosome organisation
•Escape from XCI
•Variations such as skewed XCI, imprinted XCI, reactivation in stem cells have been studied extensively.
• Discovery of random monoallelic expression at some autosomal loci

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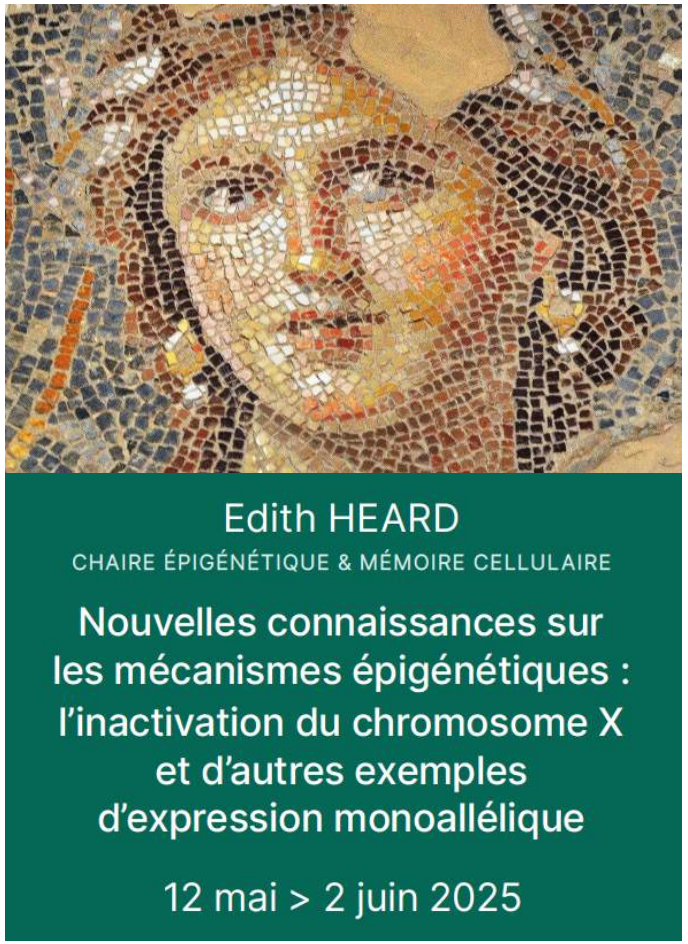

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