Epigenetics

The complex story of human X chromosome inactivation

MARIA FELICIA BASILICATA, ANNA EINSIEDEL
UNIVERSITY MEDICAL CENTER OF THE UNIVERSITY OF MAINZ

The X and Y sex chromosomes represent one of the key cellular differences between male and female. Here, we introduce the unique, silent nature of the inactive X in female cells (and its exceptions) and summarise recent insights to the molecular processes taking place on the X chromosome. We are curious to learn how these mechanisms drive sex differences in neurodevelopmental disorders.

DOI: 10.1007/s12268-024-2333-4

■ What are the biological mechanisms that distinguish men and women from each other? One explanation for their differences are their sex chromosomes: women have two X chromosomes, while men have one X and one Y chromosome. The X chromosome encodes approximately 2,500 genes, compared to only about 600 on the Y chromosome. Changes in gene dosage significantly impact development, as evident in conditions like trisomies 21 or 18, namely Down Syndrome or Edwards Syndrome. This imbalance raises a crucial question: how do humans balance gene dosage differences between the sexes (**Fig. 1A**)?

The answer lies in X chromosome inactivation (XCI), a process that silences one of the X chromosomes in female cells. This mechanism, first proposed by Mary Lyon in 1961 [1], balances X-linked gene expression between male and female cells. The silenced X chromosome forms a condensed structure known as the Barr body, first observed by Murray Barr and Ewart Bertram in the late 1940s (**Fig. 1B**, [2]).

The mechanism – how is the female X chromosome inactivated?

The silencing of the X chromosome occurs without altering DNA sequence, instead relying on chromatin modifications. Chromatin, composed of DNA wrapped around histone proteins, can be modified to influence gene expression. For instance, histone acetylation typically activates transcription, while certain methylation patterns, such as trimethylation of lysine 27 on histone 3 (H3K27me3), lead to gene silencing. These modifications, along with other factors, create the compact chromatin state of the inactive X (Xi). In this context, it is particularly interesting to understand how the molecular mechanism of XCI works – how is it regulated, and how is it initiated?

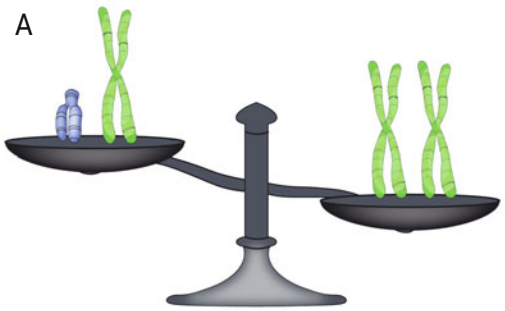
After the discovery of XCI, researchers found a region on the X chromosome that controls the initiation of the process, termed the X-inactivation centre (Xic). Specifically, the RNA "X inactive specific transcript" (*XIST*) is expressed in this region and appears to play a central role. *XIST* is a non-coding RNA, meaning it does not produce a protein and accumulates on the entirety of one of the X chromosomes, resulting in repression of gene expression. Based on the latest scientific findings, *XIST* initiates this process by recruiting the chromatin remodeler SPEN. In turn, SPEN interacts with transcriptional co-repressors and activates histone deacetylase 3 (HDAC3). In addition to this, *XIST* interacts with polycomb complexes, a family of proteins inducing transcriptional repression. Firstly, *XIST* recruits polycomb repressive complex 1 (PRC1), which catalyses the ubiquitylation of lysine residues on histones, specifically H2AK119ub1. Subsequently, PRC2 deposits the repressive H3K27me3 along the entire chromosome (**Fig. 2A, B**). Later chromatin modifications include the deposition of H3K9me2/3, which ultimately also coat the entire X. Numerous other chromatin modifications following *XIST* accumulation have been discovered and are currently still under investigation.

Besides elucidating the molecular mechanism of XCI, another important aspect is the focus on its timing. At what point during development is the X inactivated?

The timing – when is the X inactivated? And which X is chosen?

After the fusion of egg and sperm, the zygote starts dividing and transcribing its own genes. When transcription is initiated after the first cell division, most species have to balance out the gene dosage of their sex chromosomes.

Mouse studies have revealed two waves of XCI. Immediately after fertilisation, the



▲ **Fig. 1:** The imbalance in gene dosage between XY and XX individuals is compensated through X chromosome inactivation. **A**, Due to the degeneration of the Y chromosome, individuals with two X chromosomes express a higher dosage of genes. This imbalance is compensated through the inactivation of one of the X chromosomes, putting it in a heterochromatic, condense state. **B**, The inactivated X chromosome was first observed by Murray Barr in neurons of female cats and named the "Barr body" (adapted from [2]).

paternal X chromosome is always inactivated, a process known as imprinted XCI. Subsequently, in the inner cell mass, both X chromosomes briefly become active again. Then, one of the chromosomes – either paternal or maternal – is randomly inactivated. As a result, females have a mosaic pattern of X chromosome expression, with approximately half of their cells expressing the maternal X chromosome and half expressing the paternal X chromosome in a random distribution throughout the body.

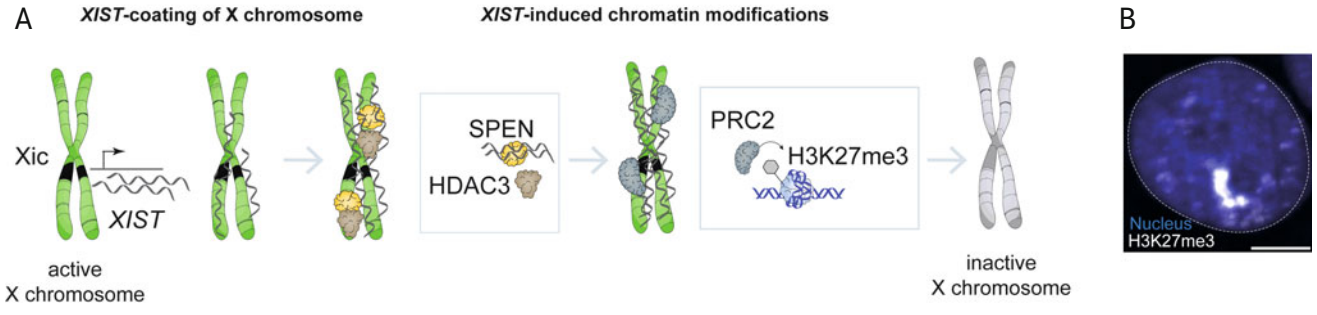
However, recent studies show that imprinted XCI does not occur in early human embryos. Instead, both X chromosomes are active, but their expression is reduced through a process called dampening. Although the theory of dampening has been controversial for a long time, recent evidence has supported its existence and importance as a dosage compensation mechanism in early human development [3,4]. Ultimately, in the first three weeks after the implantation of the embryo, random XCI is established through upregulation of *XIST*. Like in mice, the ratio of cells where the maternal or the paternal X chromosome is active is approximately 50:50 (**Fig. 3**).

How loud is the silent X?

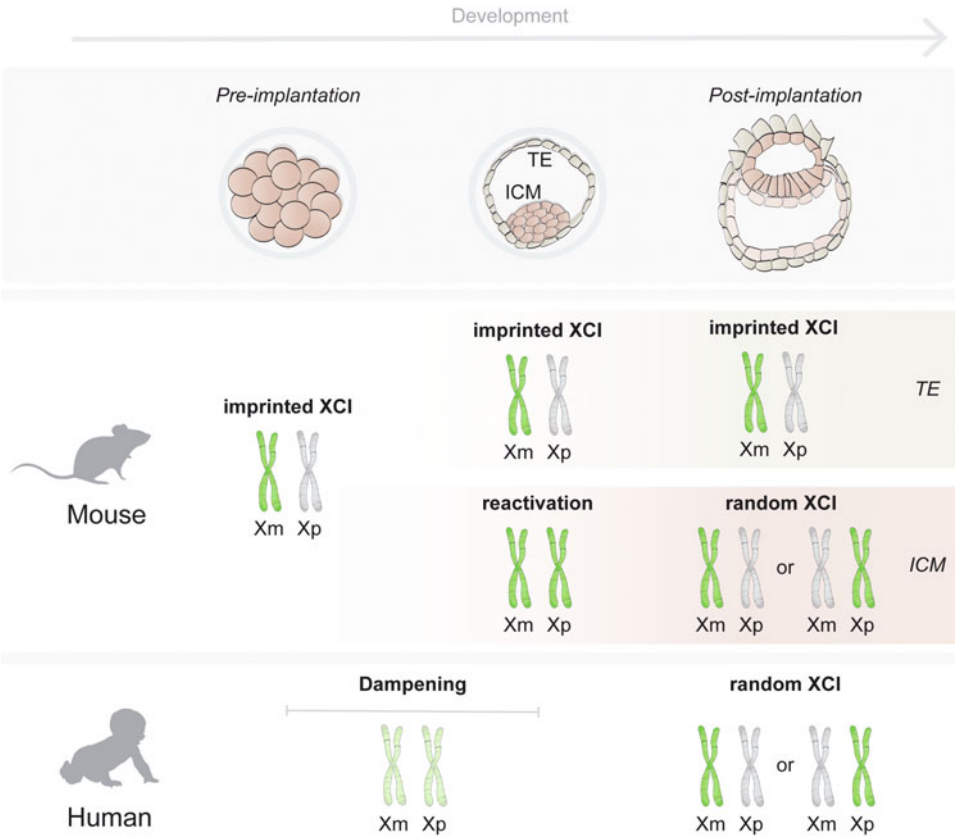
The silenced X chromosome is not completely quiet – in fact, up to a fifth of the X-coded genes can be expressed under certain circumstances, despite the inactivation of the chromosome. The expression of these "escapees" is dependent on the cell type and tissue and can even differ between individuals [5]. Since genes escaping XCI are transcribed from both X chromosomes, their expression is increased in comparison to the corresponding gene from the single X in males. Thus, it is hypothesised that escapee genes contribute to sexual dimorphism during development and diseases. For example, upregulation of escapees with immune regulatory functions has been reported during disease progression and may be implicated in increased female susceptibility to autoimmune diseases. However, activation of genes from the Xi can also protect female cells. The X chromosome is enriched in genes linked to brain function, and the escape from XCI may explain why females are often affected less severely by neurodevelopmental disorders (e. g. autism spectrum disorder). Moreover, differences in escapee expression may explain variabilities between female patients. For example, in Basilicata-Akhtar syndrome, the degree of severity to which women are affected varies greatly [6]. The gene MSL3, whose pathogenic variants cause the syndrome, is variably expressed from the Xi depending on the tissue, cell type and between individuals. This is referred to as facultative escape.

How and why to study XCI?

For years, medical research has excluded females from phase II and III clinical trials. Thus, the causes and consequences of sexual dimorphism on therapies are often poorly understood. Biological factors such as sex hormones and chromosomes, XCI, and escapees contribute to differences in medical needs between males and females. Although the mouse model is powerful in biomedical research, the recently discovered differences in XCI between rodents and humans (see above) make alternatives necessary. For example, studying human embryonic stem cells and induced pluripotent stem cells (hiPSC) has greatly improved our understanding of the early events of human development. However, XCI can be artificially switched off in these cell models, which leads to the simultaneous activity of both X chromosomes. This phenomenon is referred to as "erosion of XCI" and has been found to alter the cellular proteome globally [7]. Because of the epigenetically unstable landscape of



▲ **Fig. 2:** X chromosome inactivation is initiated through the master regulator XIST, resulting in deposition of repressive chromatin modifications such as H3K27me3. **A**, Representation of the sequence of events leading to X chromosome inactivation. The long non-coding RNA XIST is expressed from the Xic of the prospective inactivated X chromosome. XIST recruits the chromatin remodeler SPEN, which in turn activates HDAC3, resulting in the deacetylation of the histones on the X chromosome. In addition, XIST accumulation leads to PRC2-mediated enrichment of the repressive H3K27me3. Green (active), grey (inactive). **B**, Immunofluorescence staining of female hiPSC where the X chromosome is marked by H3K27me3 antibody (white) in the nucleus (blue). Scale bar 5 µm.



◀ **Fig. 3:** X chromosome inactivation dynamics in mouse and human early development. In mice, the paternal X chromosome (Xp) is inactivated during early preimplantation development in an imprinted manner. Xp inactivation is maintained in the trophectoderm (TE), while in the inner cell mass (ICM), both X chromosomes are transiently activated. In the ICM, one X chromosome is then randomly inactivated at post-implantation. In humans, during the early phases, expression of X-linked genes is reduced on both X chromosomes (dampening). At the post-implantation stage, one X chromosome is randomly inactivated, similar to mice. Free silhouette images from phylopic.org.

female hiPSC, current datasets often inaccurately reflect biological events studied with such cellular models.

Studying the X chromosome's function, its diversity across different species, and how it is regulated is a challenging but incredibly rewarding field of research. It reveals fundamental principles of genome control applicable to various areas of biology, such as the function of DNA methylation in gene regulation, 3D chromosome conformation principles, chromosome-wide chromatin regulation, and the functional contribution of noncoding RNAs. In mammalian XCI, many questions remain unanswered as to how the XIST gene is regulated during human development, how disruptions in this process affect health and ageing, and how X chromosome regulation influences sex differences in brain development. In our laboratory, we are especially interested to study how reactivation of genes on the Xi influences the phenotypes in neurodevelopmental disorders. We are only now beginning to understand the complexity of how we "balance the scales" of having two X chromosomes, and therefore, uncovering one of the central differences between the biological sexes. ■

References

[1] Lyon MF (1961) Gene action in the X-chromosome of the mouse (Mus musculus L.). Nature 190: 372–373
[2] Barr ML, Bertram EG (1949) A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. Nature 163: 676
[3] Dror I, Chitiashvili T, Tan SYX et al. (2024) XIST directly regulates X-linked and autosomal genes in naive human pluripotent cells. Cell 187: 110–129.e31
[4] Alfeghaly C, Castel G, Cazottes E et al. (2024) XIST dampens X chromosome activity in a SPEN-dependent manner during early human development. Nat Struct Mol Biol 187: 110–129
[5] Tukiainen T, Villani AC, Yen A et al. (2017) Landscape of X chromosome inactivation across human tissues. Nature 550: 244–248
[6] Basilicata MF, Bruel AL, Semplicio G et al. (2018) De novo mutations in MSL3 cause an X-linked syndrome marked by impaired histone H4 lysine 16 acetylation. Nat genet 50: 1442–1451
[7] Brenes AJ, Yoshikawa H, Bensaddek D et al. (2021) Erosion of human X chromosome inactivation causes major remodeling of the iPSC proteome. Cell Rep 35: 109032

Funding note: Open Access funding enabled and organized by Projekt DEAL.

Corresponding author:
Dr. Maria Felicia Basilicata
Institute of Human Genetics
University Medical Center of the Johannes Gutenberg University
Obere Zahlbacher Str. 63/Geb 911
D-55131 Mainz
mfbasilicata@uni-mainz.de

AUTHORS

M. Felicia Basilicata
2003 Master in Medical Biotechnology, Napoli, Italy. **2010–2014** Promotion Biology, Max Planck Institute of Immunobiology and Epigenetics, Freiburg. **2014–2020** Postdoc in Chromatin Regulation, MPI-IE Freiburg. **2020–2022** Senior Research Associate, IMB GmbH, Mainz. Since **2022** Group Leader at the University Medical Center of JGU, Institute of Human Genetics, Mainz.

Anna Einsiedel
2022 Bachelor in Biology, University of Mainz. Since **2022** Student Research Assistant, IMB GmbH. Since **2023** Master Student in the Group of Dr. M. F. Basilicata, University Medical Centre, Institute of Human Genetics, Mainz.