Life with Two X Chromosomes

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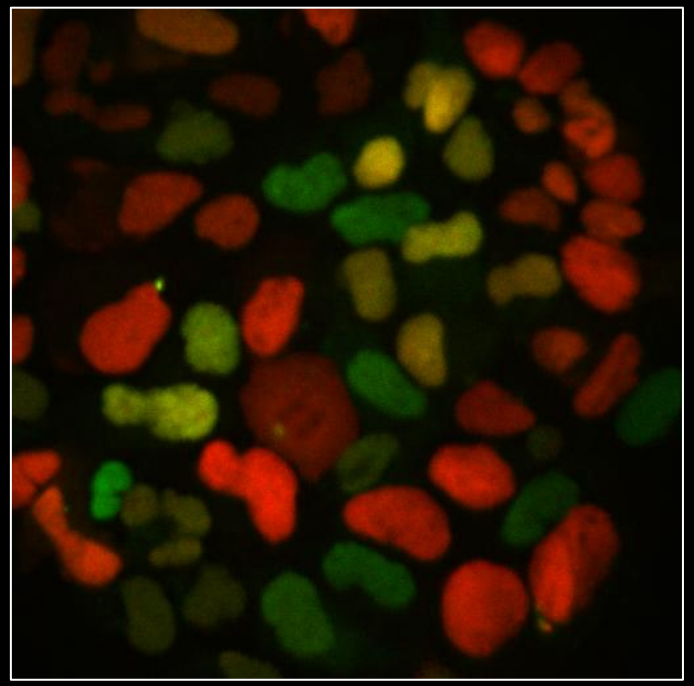

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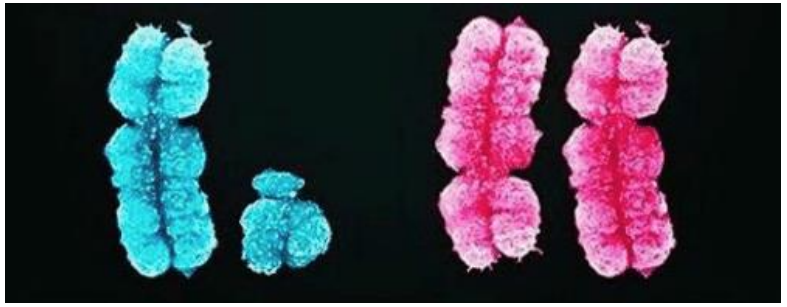
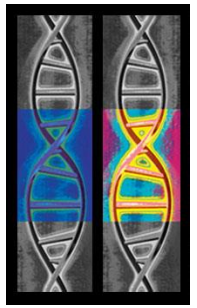
Life with sex chromosomes

Differences between the sexes
Sexual dimorphism

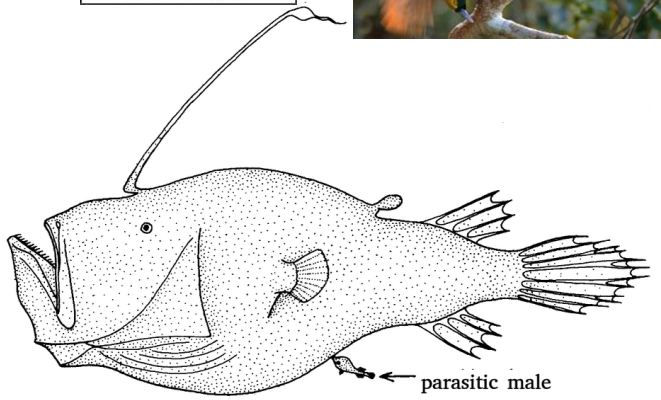
Genetics

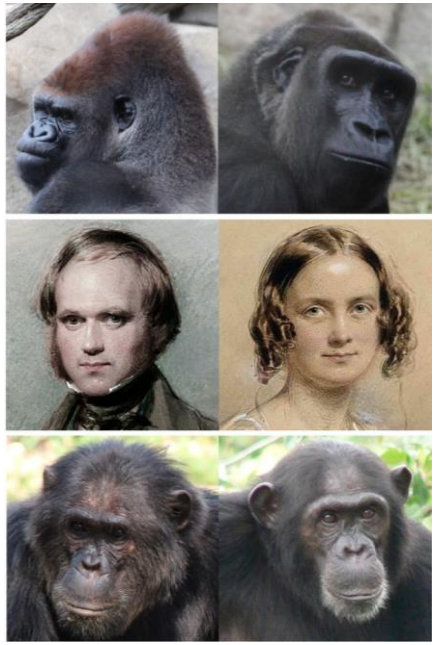




Epigenetics

Sexual dimorphism



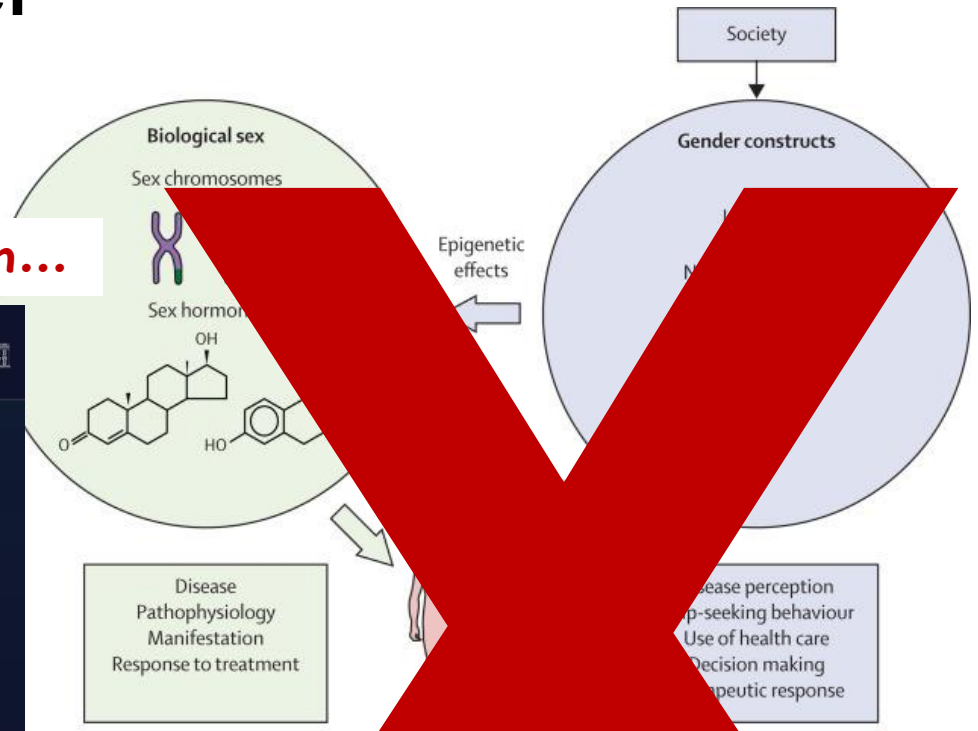


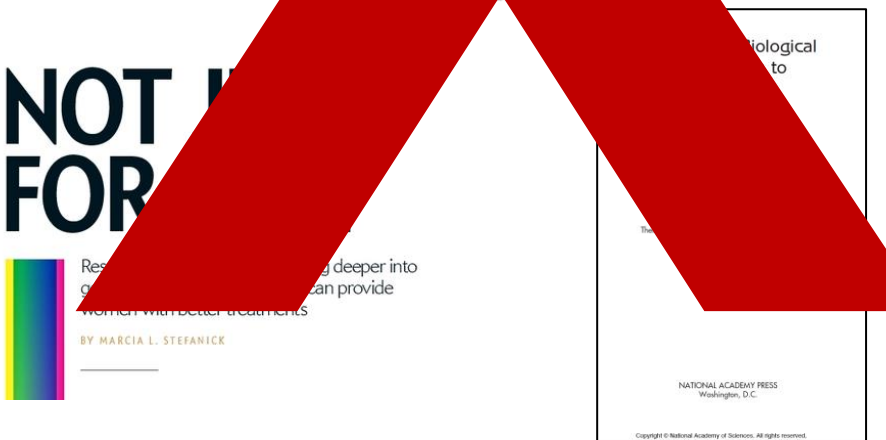
XY ♂ XX ♀

Biomedical science has shown a historic bias and blindness to sex and gender

Female biology and **health** are still understudied, after centuries of misconceptions and mis-information.

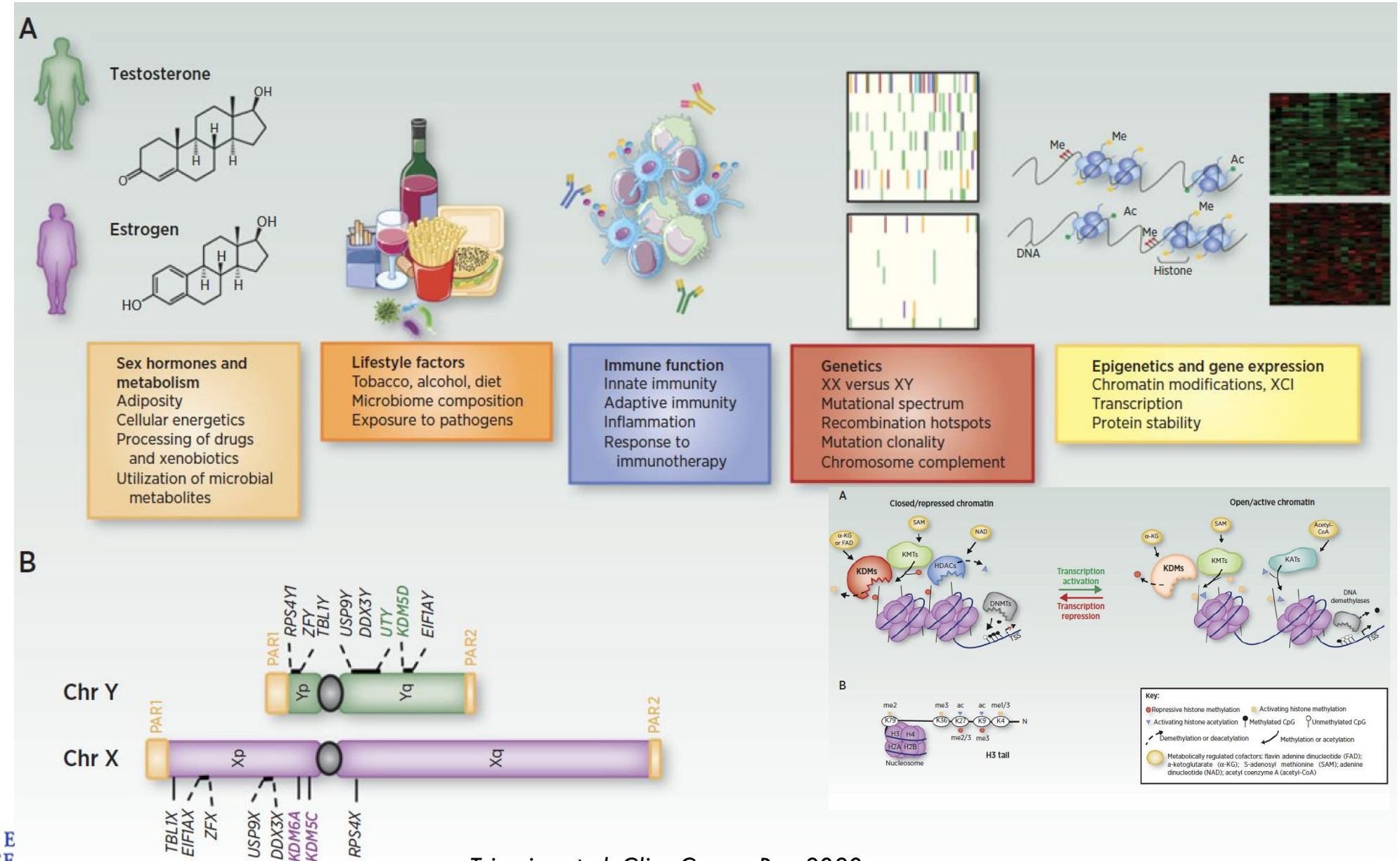
and, since January 2025, we may revert to this situation…





Environmental factors and life style differences are also crucial.

Sexual Dimorphism and Human Health



Tricarico et al, Clin.. Cancer Res. 2020

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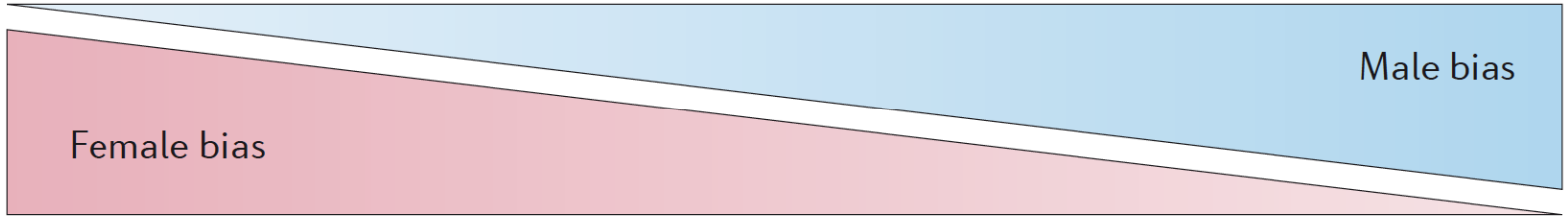
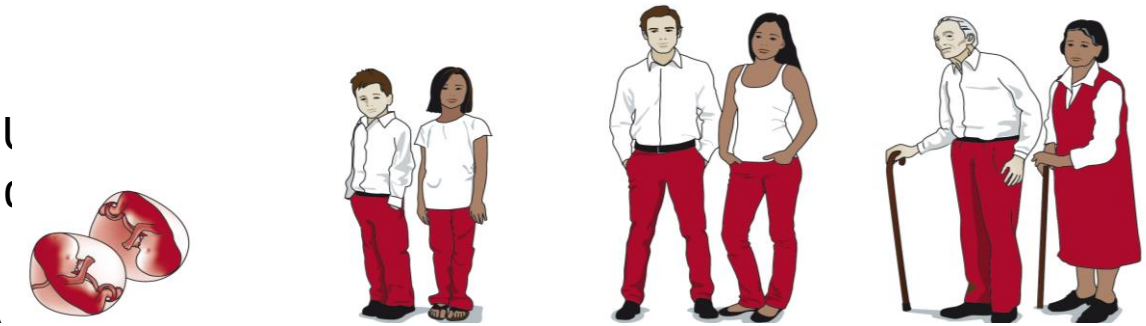
Sex Bias in Disease



Autoimmune diseases	Infectious diseases		Non-reproductive cancers
• Graves disease	• HIV	• Ebola	• Bladder
• Hashimoto thyroiditis	• Influenza	• MERS	• Bowel
• Multiple sclerosis	• Toxoplasmosis	• Hepatitis B	• Kidney
• Rheumatoid arthritis	• Legionella	• Tuberculosis	• Leukaemia
• Systemic lupus erythematosis	• Malaria	• Leptospirosis	• Liver
• Type 1 diabetes	• Zika	• Campylobacter	• Lung
		• Schistosomiasis	• Malignant melanoma
		• Amebiasis	• Oesophagus
		• Aspergillosis	• Stomach

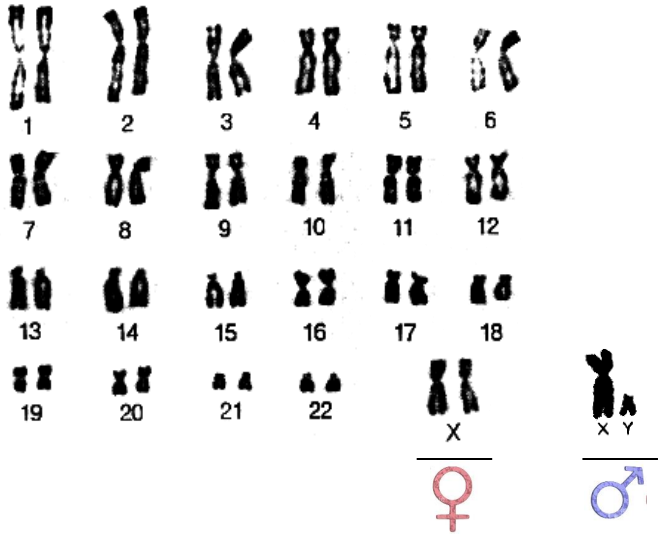
Klein and Flanagan, Nat Rev Immunol, 2016

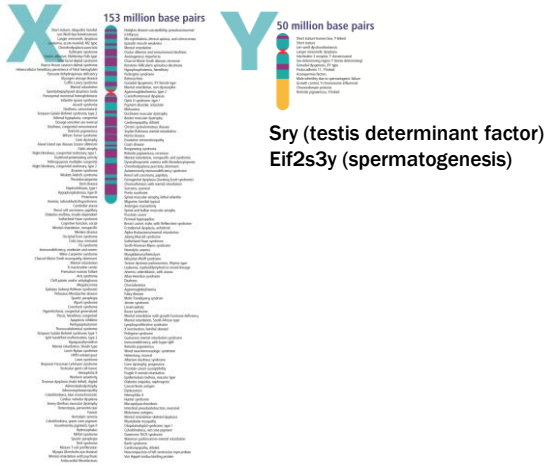


To achieve effective treatment for all individuals in the era of precision medicine:
Men and women will have to be <u>treated differently,</u> in order to be protected <u>equally</u>.

Life with two X chromosomes

In order to achieve dosage compensation one of the 2 X chromosomes is silenced in mammals
The process of X-chromosome inactivation is a paradigm of epigenetics

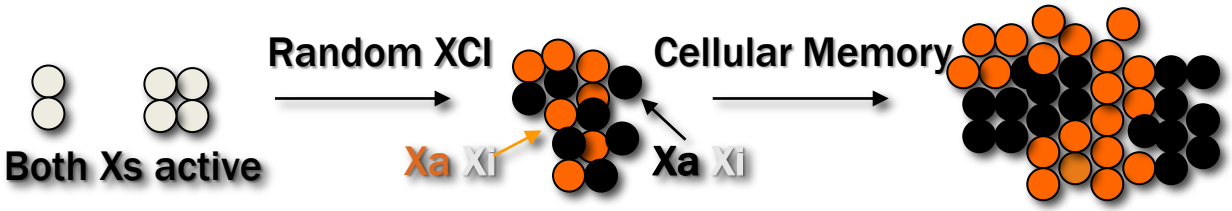




Sry (testis determinant factor)
Eif2s3y (spermatogenesis)

X: 1300 genes
Y: ~100 genes

Mary Lyon
(1929-2014)



Phenotypic variation within the same individual...

Females are mosaics for X-linked traits

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

EMBL

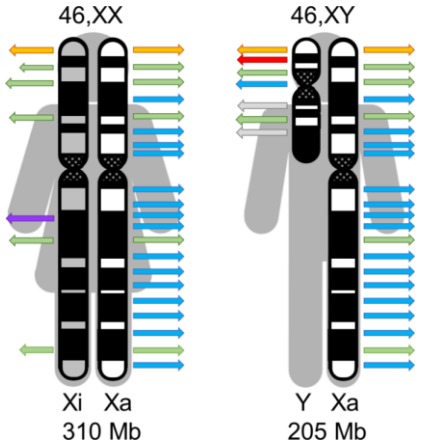
Some genes can *escape* from XCI in females

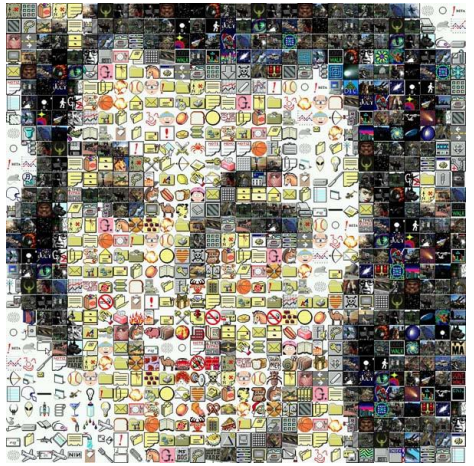
An added layer of cellular mosaicism for X-linked gene expression in females

In humans, up to 25% of X-linked genes can escape XCI
10% of these escape constitutively
15% of these genes show variability between individuals

Variable escape: genetically and environmentally controlled?

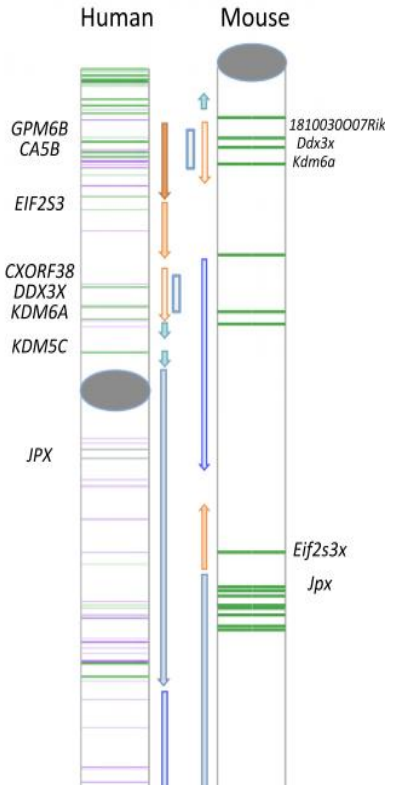
"Heritability of skewed X-inactivation in female twins is tissue-specific and associated with age " (Zito et al, 2019)





X-inactivation profile reveals extensive variability in X-link gene expression in females

Carrel and Willard (2005) *Nature* 434, 400-4



- A few escapees have Y-linked homologs, many do not
- Different tissues/lineages in the mouse show different degrees of escape
- Escape may be *accidental* (epigenetic instability) or *purposeful* (requirement of a double dosage in XX)
- It may be neutral, deleterious or advantageous
- Escape from XCI may underlie several sex-biased metabolic, immune, neurological and other diseases: eg TLR7 in lupus

Some genes can *escape* from XCI in females

An added layer of cellular mosaicism for X-linked gene expression in females

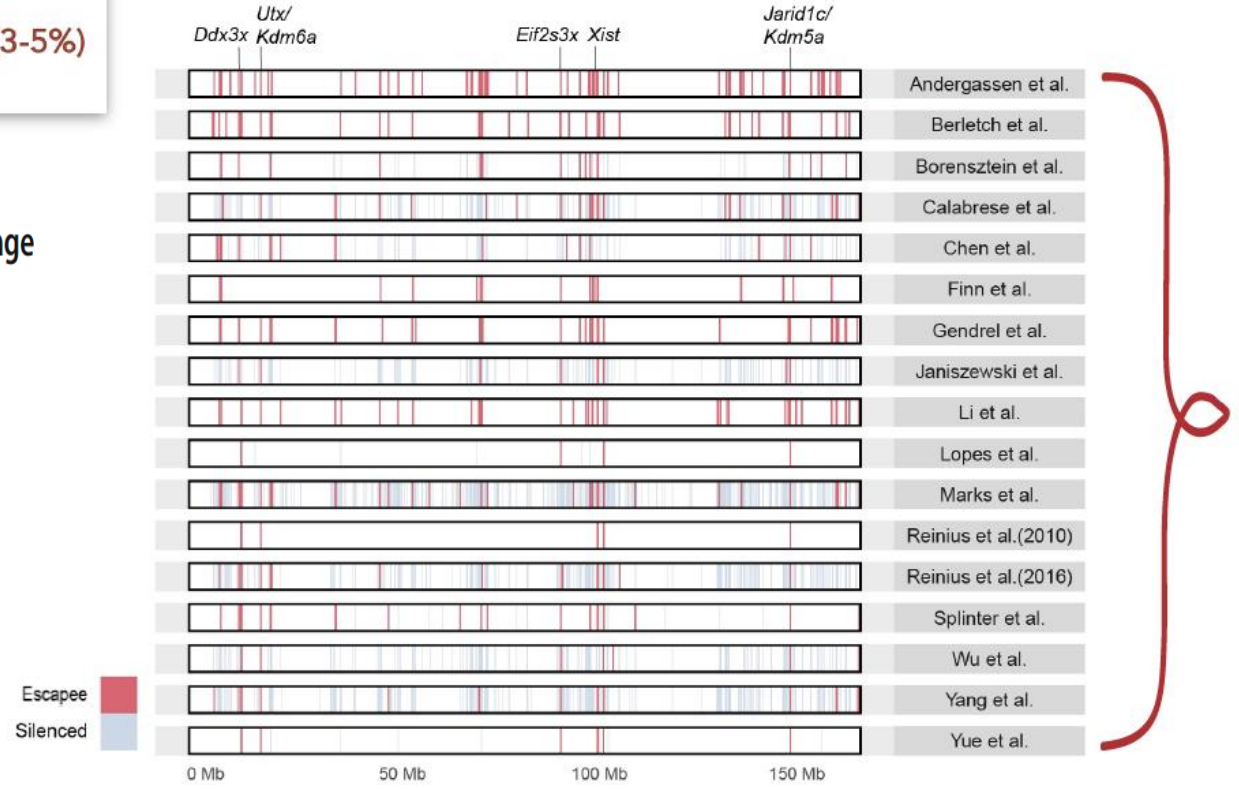
constitutive (3-5%)

Female predisposition to TLR7-driven autoimmunity: gene dosage and the escape from X chromosome inactivation

Mélanie Souyris[1] · José E. Mejía[1] · Julie Chaumeil[2] · Jean-Charles Guéry[1,3]

Altered X-chromosome inactivation predisposes to autoimmunity

Christophe Huret[1], Léa Ferrayé[2], Antoine David[3], Myriame Mohamed[1], Nicolas Valentin[4], Frédéric Charlotte[5], Magali Savignac[2], Michele Goodhardt[3], Jean-Charles Guéry[2], Claire Rougeulle[1]*, Céline Morey[1]*



Facultative (>20%)

- A few escapees have Y-linked homologs, many do not
- Different tissues/lineages in the mouse show different degrees of escape
- Escape may be *accidental* (epigenetic instability) or *purposeful* (requirement of a double dosage in XX)
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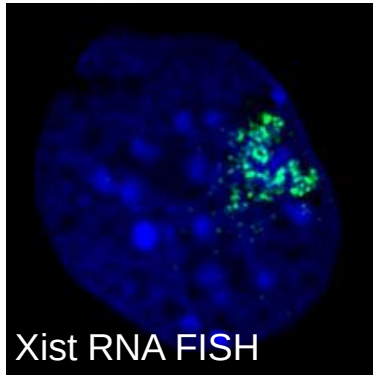
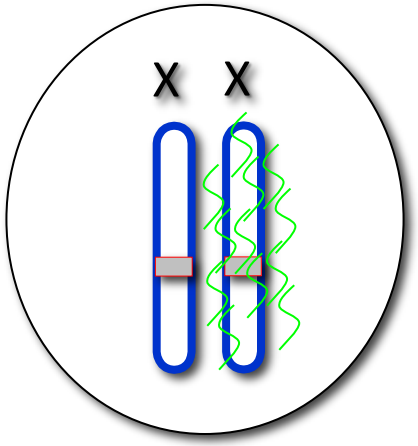
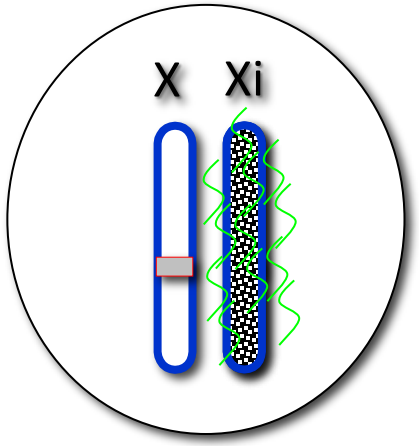
The inactive X chromosome

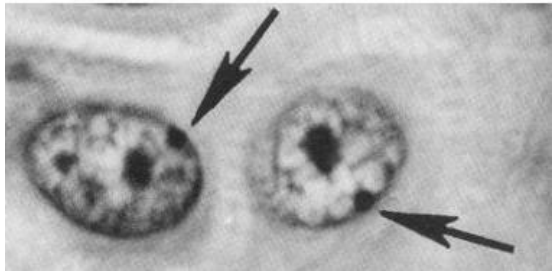
Classic example of facultative heterochromatin

Differential treatment of the two X chromosomes in same nucleoplasm





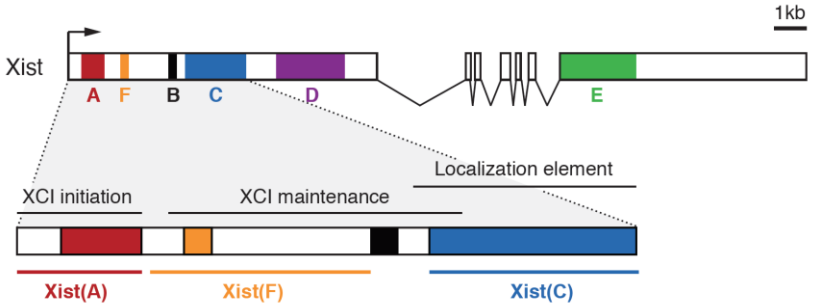




Bertram et Barr, 1949

Initiation: the Xic

Xist - a multitasking lncRNA



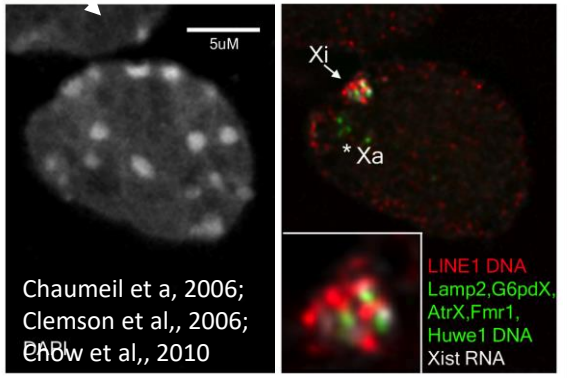
1990s: Avner, Brockdorff, Brown, Rastan, Willard, Jeanisch labs and others

Maintenance

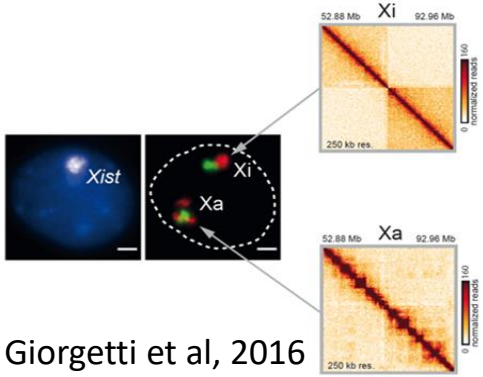
Chromatin, Nuclear organisation,

Asynchronous replication,

Chromosome structure

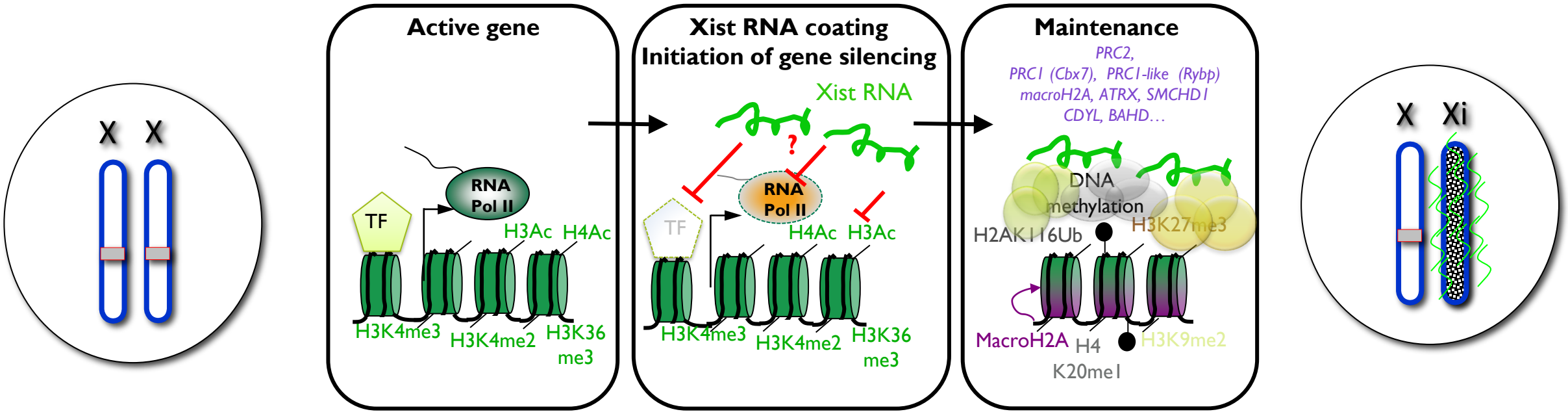


Chaumeil et a, 2006;
Clemson et al,, 2006;
Chow et al,, 2010



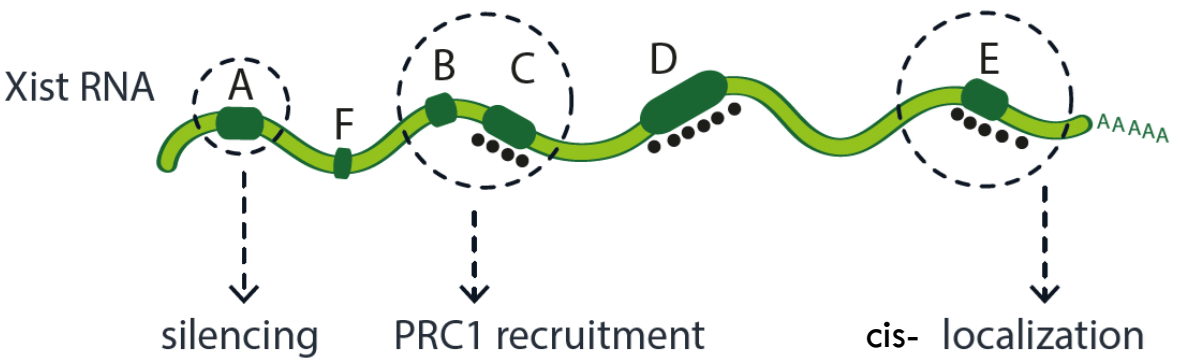
Giorgetti et al, 2016

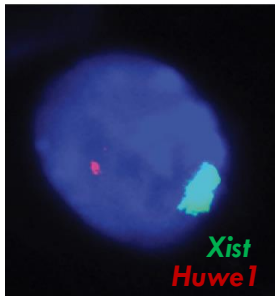
How are genes regulated during XCI?



The long non-coding Xist RNA is the key trigger for X inactivation

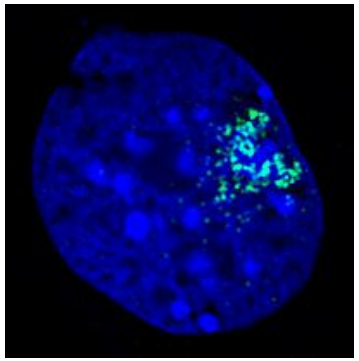
(Penny et al, 1996; Mahrarens et al, 1997; Wutz and Jaenisch, 2000)

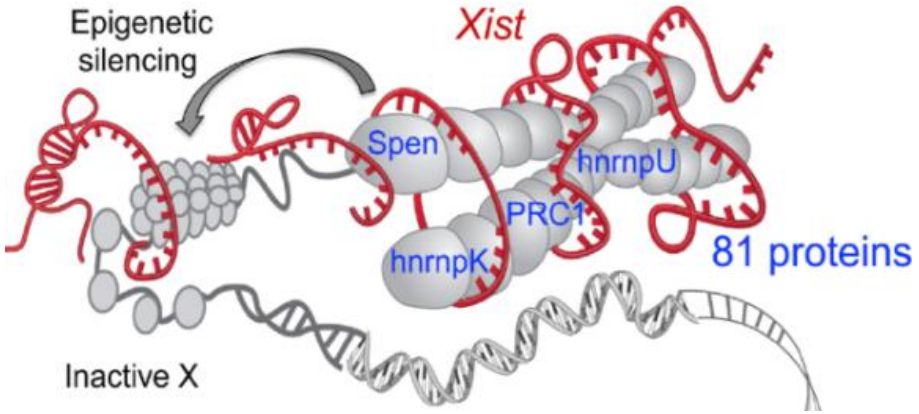




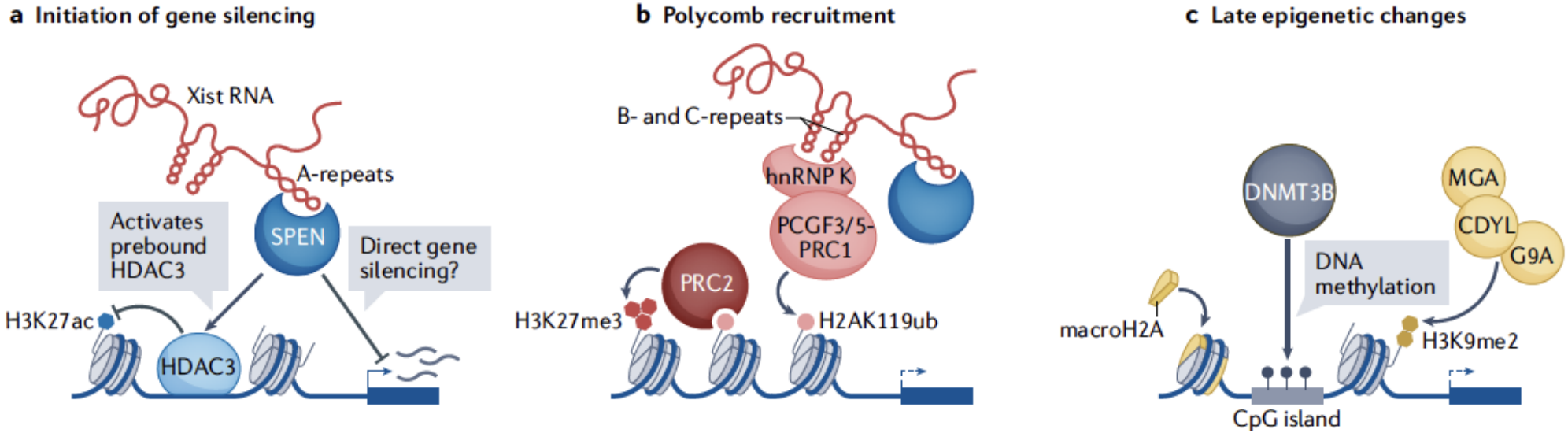
erc XPRESS

Xist RNA is a multi-tasking molecule that triggers gene silencing and chromatin changes





Chu et al, 2015
McHugh et al, 2015
Chen et al, 2016
Minajigi et al, 2015
Moindrot et al, 2015
Monfort et al, 2015

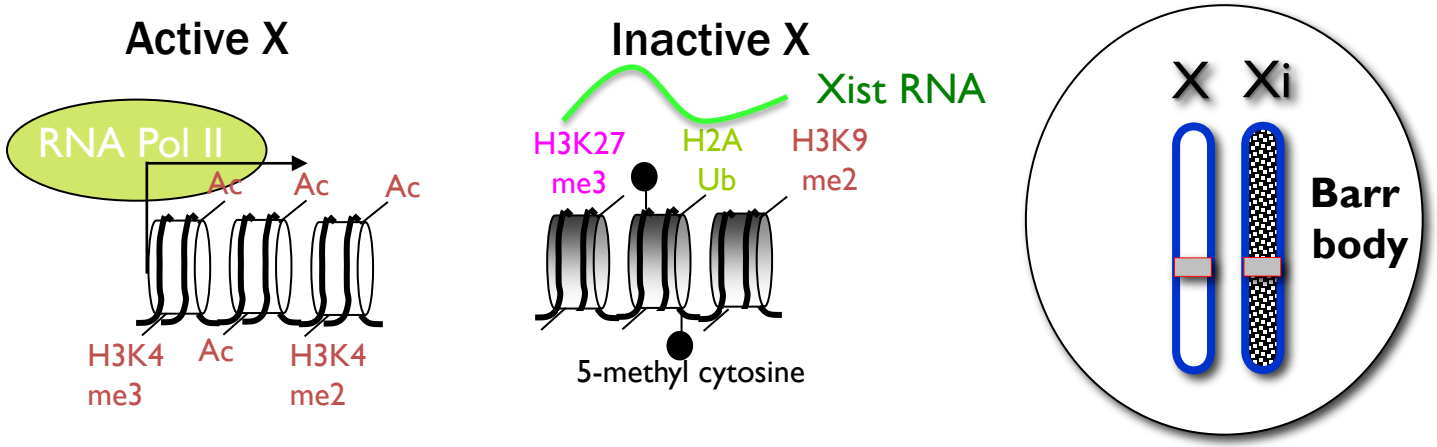


Zylicz et al, 2019
Bousard et al, 2020
Dossin et al, 2020

Dogma: Xist RNA only matters at the very beginning of XCI…

From review by Loda et al, 2021

Why, Where and How do some genes escape XCI?

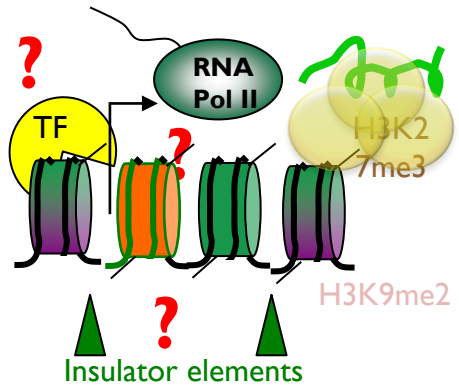


Remarkable stability of the inactive state

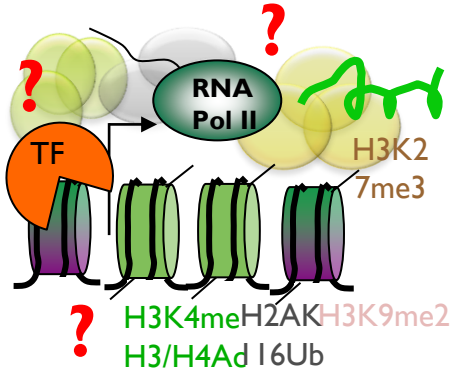
Rate of reactivation of most X-linked genes $<10^{-9}$ in somatic cells

X-linked genes with a homolog on the Y chromosome

Constitutive escape from XCI
(eg *Jarid1c, Utx*)

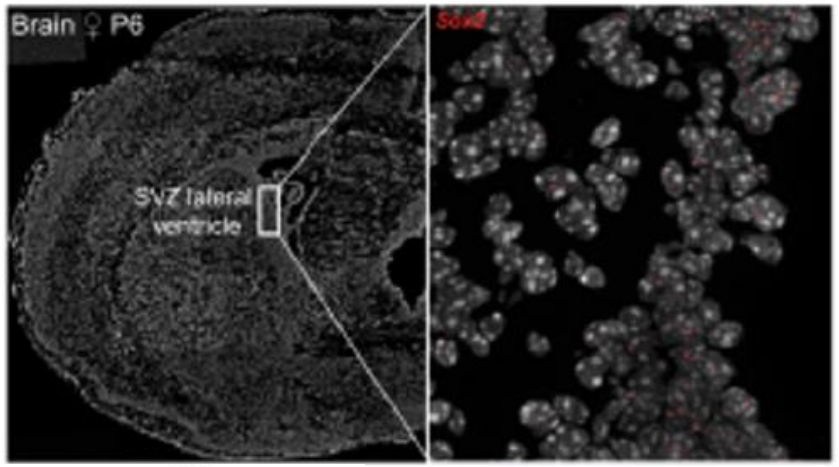


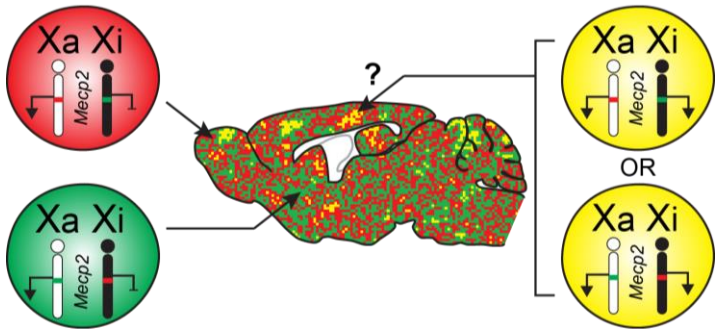
Facultative escape:
Silencing then reactivation from the Xi
(eg *Atrx* in trophoblast; *Mecp2* in brain …)



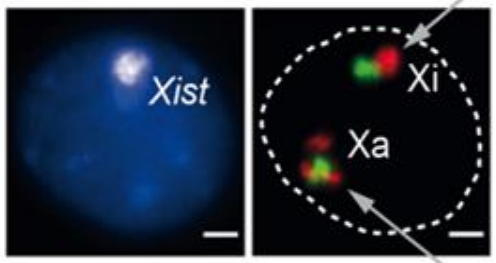
No homolog on the Y
Facultative escapees end to occur in clusters…

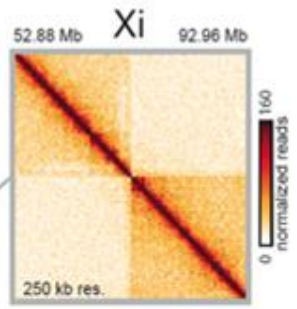
An example of facultative escape: the *Mecp2* gene in the brain

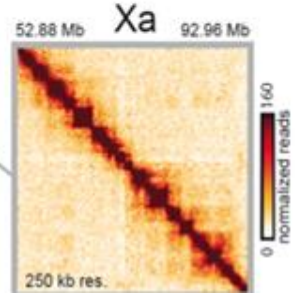


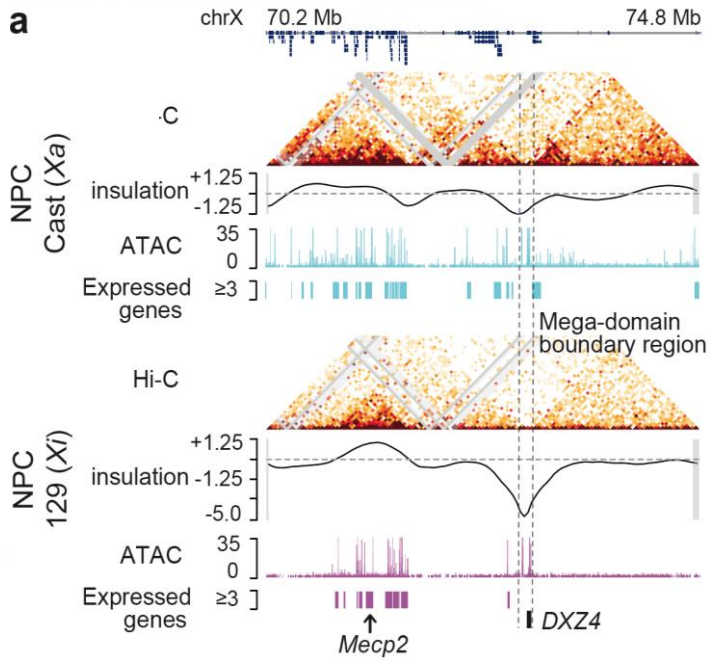


Lucile Marion-Poll









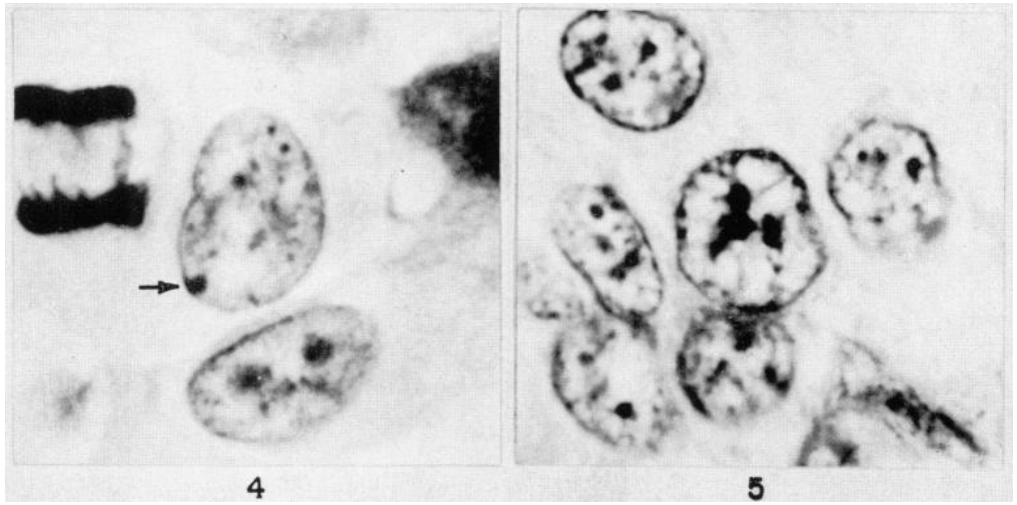
Giorgetti et al, Nature 2016

- *Mecp2* is silenced during early development
- *Mecp2* escapes in some cells in female brain
- Epigenetic plasticity in specific cell types?
- When *Mecp2* escapes, multiple genes around it also escape

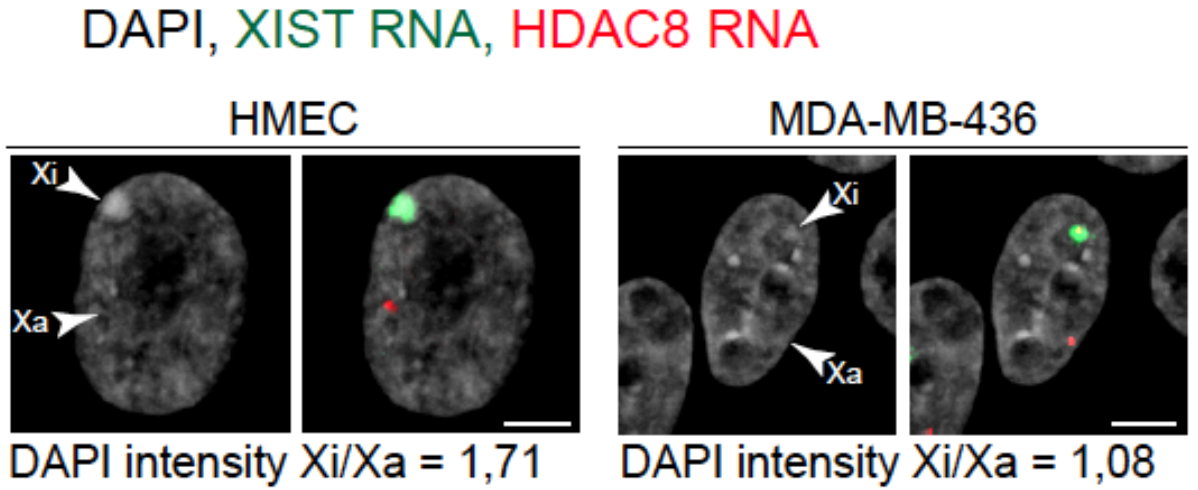
Is escape required, does it lead to phenotypes?

Facultative escapees tend to occur in clusters

How much escape from XCI can be found in cancer? Does it matter?



The Sex Chromatin in Human Malignant Tissues
K. L. Moore and M. L. Barr, 1957



Chaligné *et al.* Genome Res., 2015

Epigenetic instability and increased or aberrant escape from XCI may promote or facilitate cancer in XX individuals

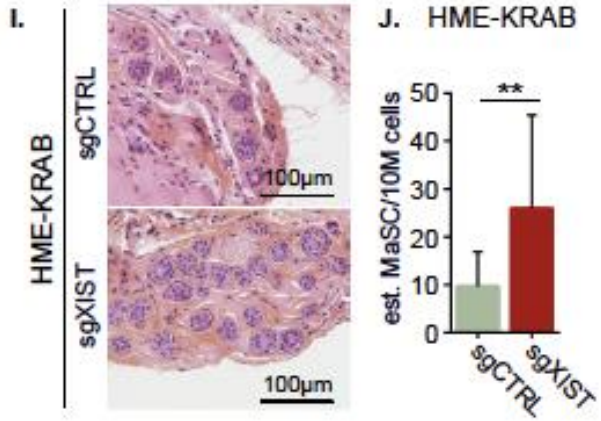
X-linked escapees may protect XX individuals from cancer: EXITS Hypothesis

Loss of XIST leads to up-regulation of some escapee genes (eg *Med14*) on the Xi with impact on mammary stem cell differentiation

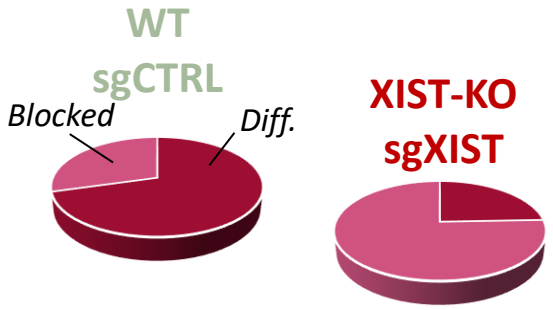
Laia Richart Ginés

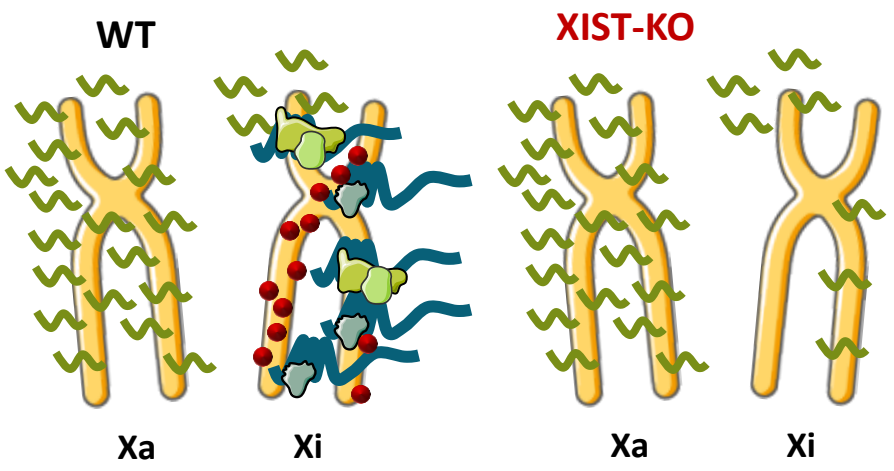
XIST LOSS DISRUPTS MAMMARY STEM CELL DIFFERENTIATION POTENTIAL

XIST KO/inhibition blocks cells in the stem cell compartment

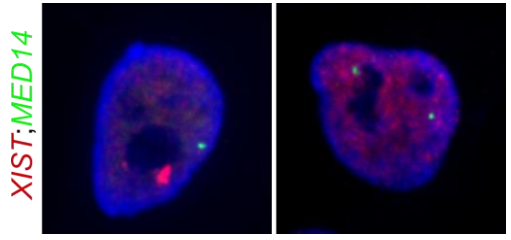


MaSC frequency is significantly higher in the sgXIST cells injected into humanised mammary fat pads of NSG mice





REACTIVATION OF A SUBSET OF X-LINKED GENES



Re-activation of MED14 is accompanied by increased protein levels of other Mediator subunits and increased stem cell enhancer activity

Raphael Margueron (Institute Curie, Paris)
Christophe Ginestier (CRCM, Marseille)

Laia Richart Ginés et al, Cell 2022

XIST loss impairs mammary stem cell differentiation and increases tumorigenicity through Mediator hyperactivation


Laia Richart Ginés

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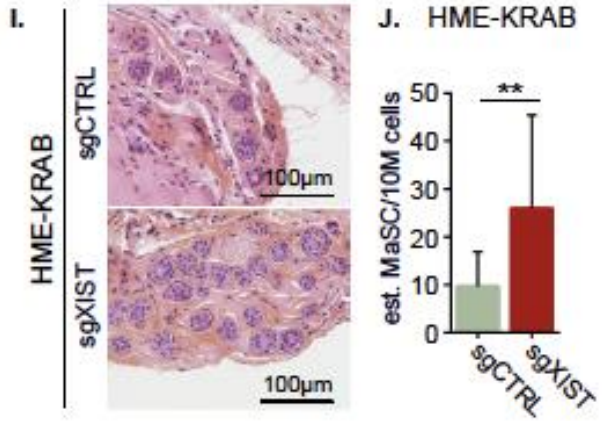

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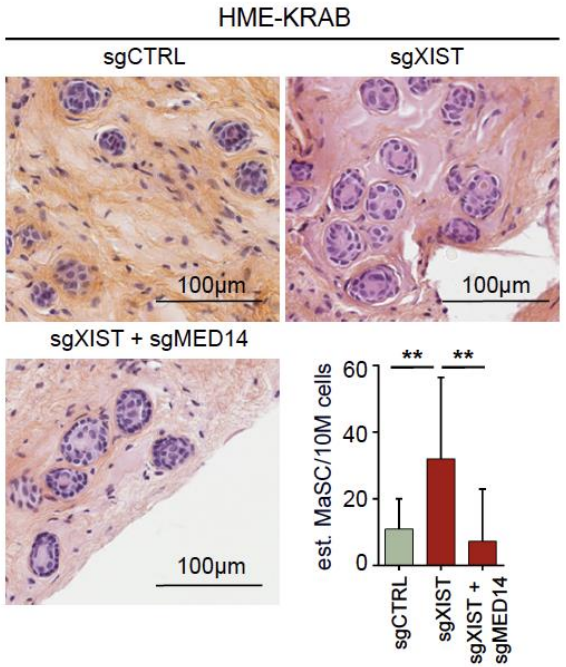


Rescue of the MaSC phenotype in XIST KO when MED14 is reduced

- Precise dosage of MED14 regulates luminal differentiation
- Mammary stem cell enhancers display chromatin features of hyperactivity in the absence of *XIST*

Raphael Margueron (Institute Curie, Paris)
Christophe Ginestier (CRCM, Marseille)

Laia Richart Ginés et al, Cell 2022

Xist RNA may be required to maintain XCI and/or control escape in differentiated cells

- XIST KD in mammary epithelial results in higher dosage of MED14 - which leads to increased breast tumorigenesis (Richart-Gines et al. 2021)
- Xist KO, KD or delocalization in somatic cells leads to sex-specific phenotypes (eg hematologic cancers – Yildirim et. al 2013; Ridings-Figueroa et. al 2017; Yang et. al 2020)
- In human B cells XIST prevents escape of X-linked genes with DNA hypomethylated promoters (Yu et al. 2021)

Do Xist RNA levels directly influence escape?

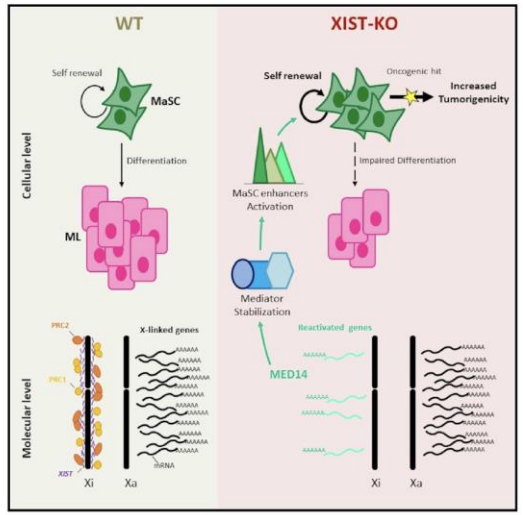
If so, how?

Cell

Article

***XIST* loss impairs mammary stem cell differentiation and increases tumorigenicity through Mediator hyperactivation**

Graphical abstract



Authors

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In brief

Outside the context of initiating X chromosome inactivation, XIST contributes to human mammary stem cell homeostasis, and loss of XIST and Xi transcriptional instabilities enhances tumorigenesis and is a common feature among human breast tumors with poor prognosis.

Highlights

- *XIST*-null cells display reactivation of a few X-linked genes, including *MED14*
- *MED14* overdosage impacts stem cell homeostasis through Mediator stabilization
- Loss of *XIST* enhances the tumorigenic potential of cells upon transformation
- Xi transcriptional reactivation is common among aggressive breast tumors

Mechanisms of Escape from XCI?

Role for Xist RNA? Role for specific factors?
Role for chromosome organization and 3D topology?

Agnese Loda

Christel Picard

Antonia Hauth

Isabell Rall

Yuvia Perez Rico

Jasper Panten
(Oli Stegle)

Emma Kneuss

2) Xist RNA levels?

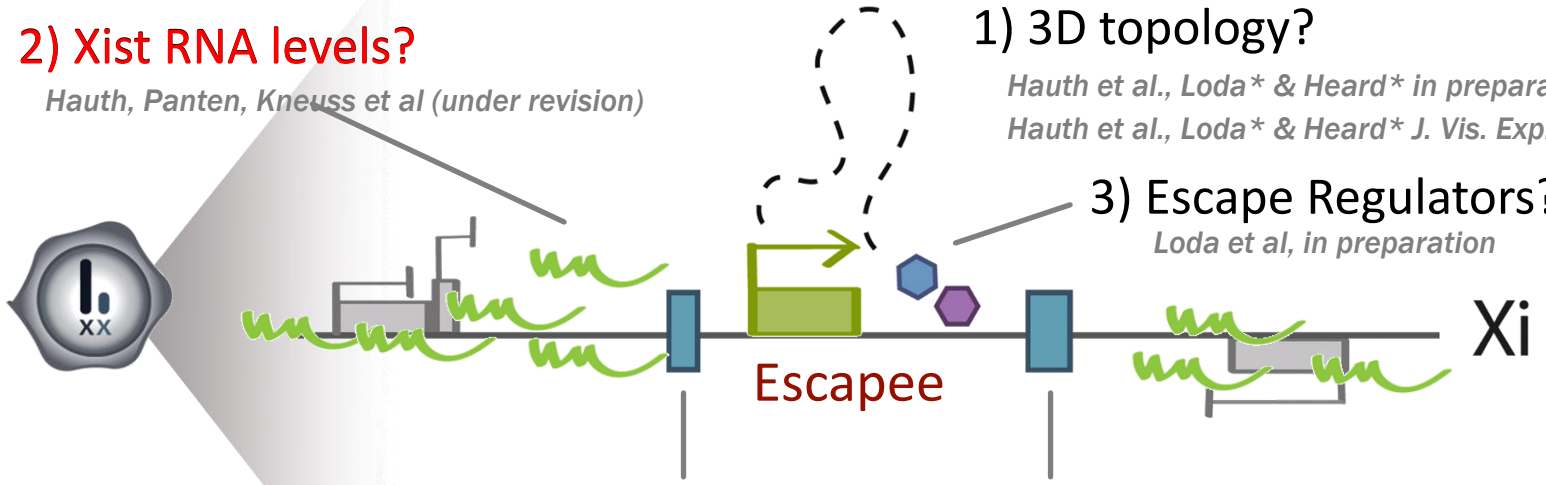
Hauth, Panten, Kneuss et al (under revision)

1) 3D topology?

Hauth et al., Loda & Heard* in preparation*
Hauth et al., Loda & Heard* J. Vis. Exp. (2022)*

3) Escape Regulators?

Loda et al, in preparation



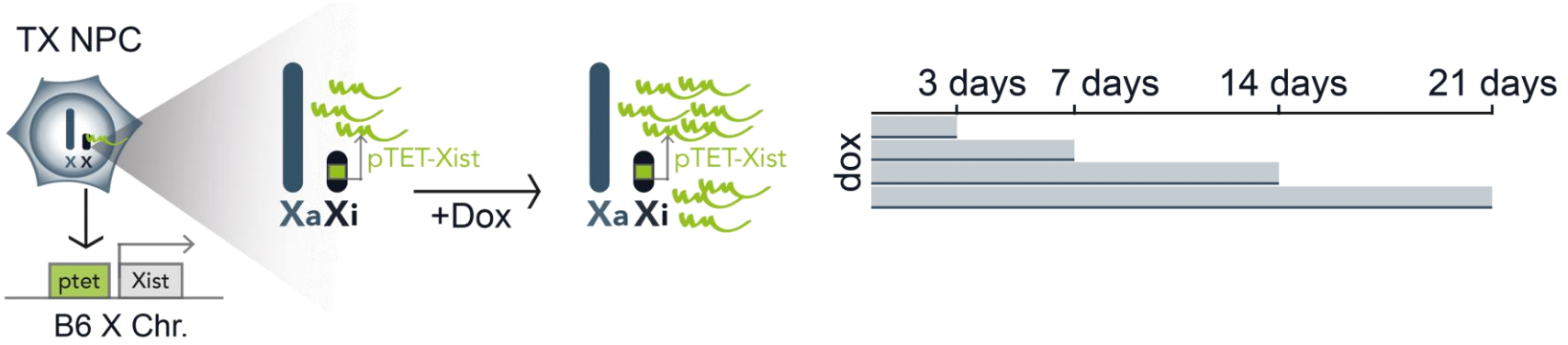
4) Boundary elements?

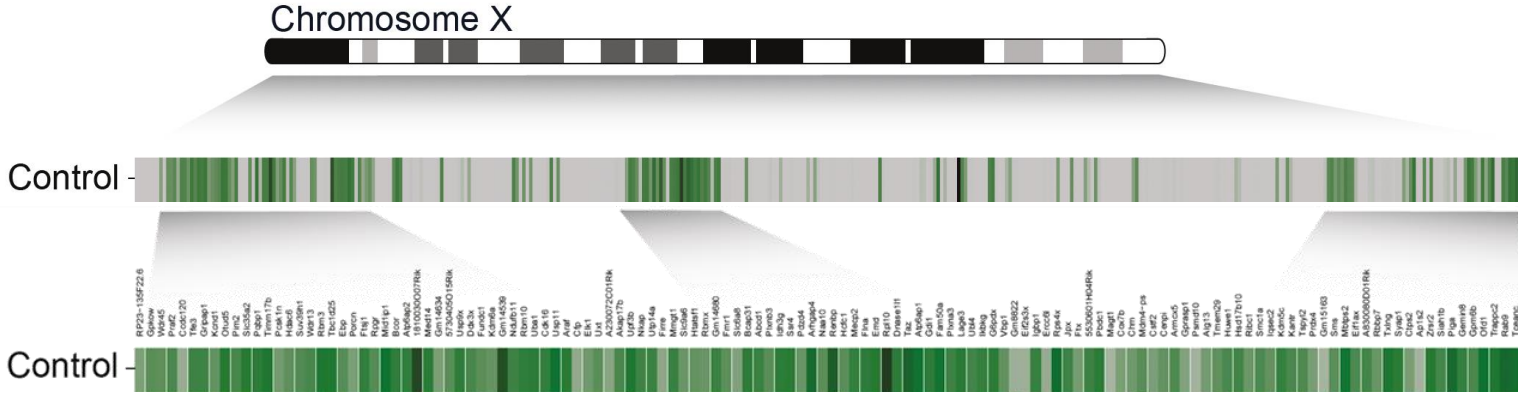
In collaboration with:

Duncan Odom (DKFZ)
Oli Stegle (DKFZ/EMBL)
Nicolas Servant (Institut Curie)

Increased Xist RNA leads to progressive silencing of escapees in NPCs

Clonal F1 Hybrid (B6/Cast) Neuronal Progenitor Cells (NPCs) with Dox-inducible *Xist* on one X (B6)



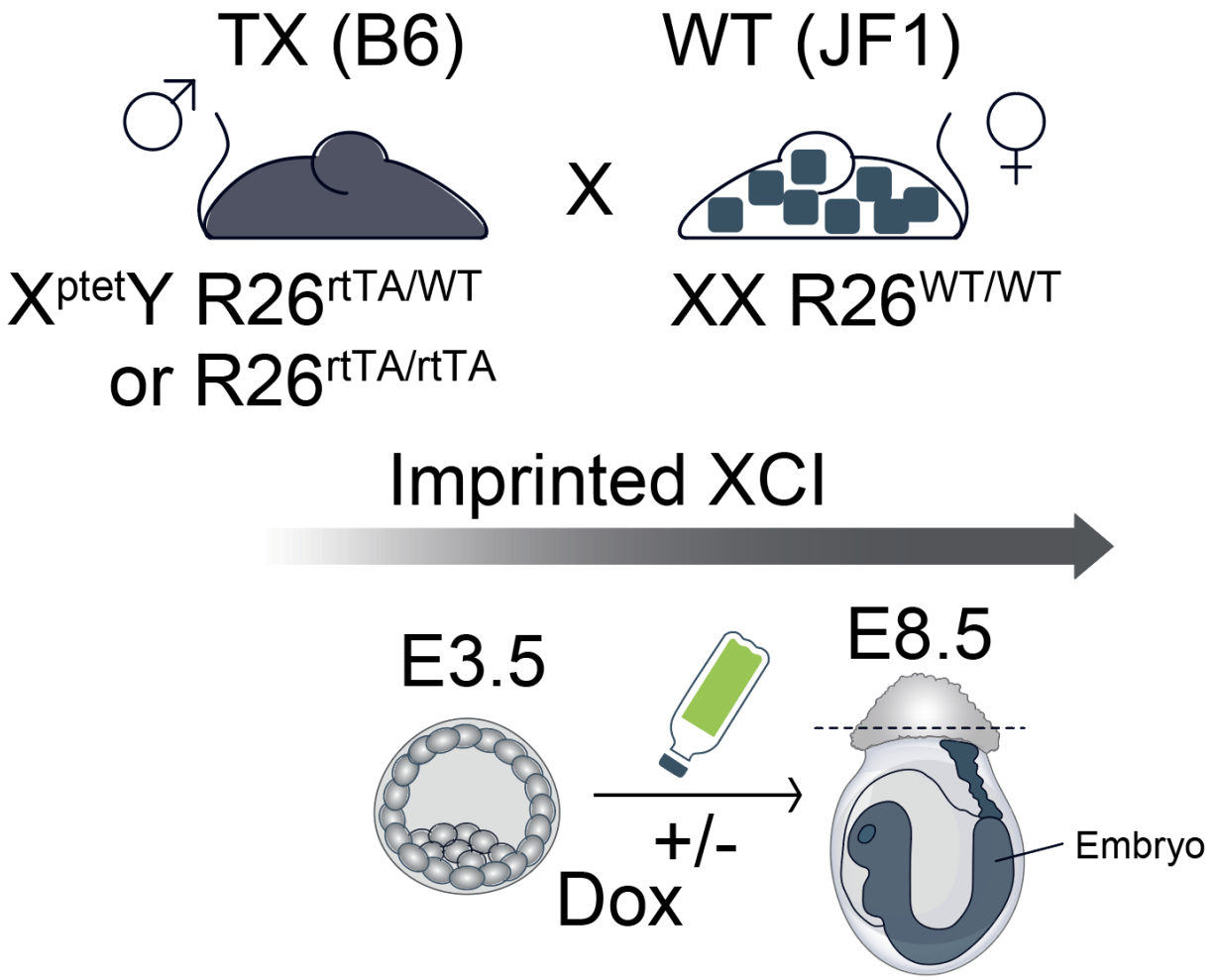


Higher Xist-induced escapee silencing:

- is SPEN dependent
- leads to loss of TAD-like structures
- becomes irreversible at facultative but not constitive escapees
- can be induced in dividing (NPCs) and non-dividing cells (astrocytes)

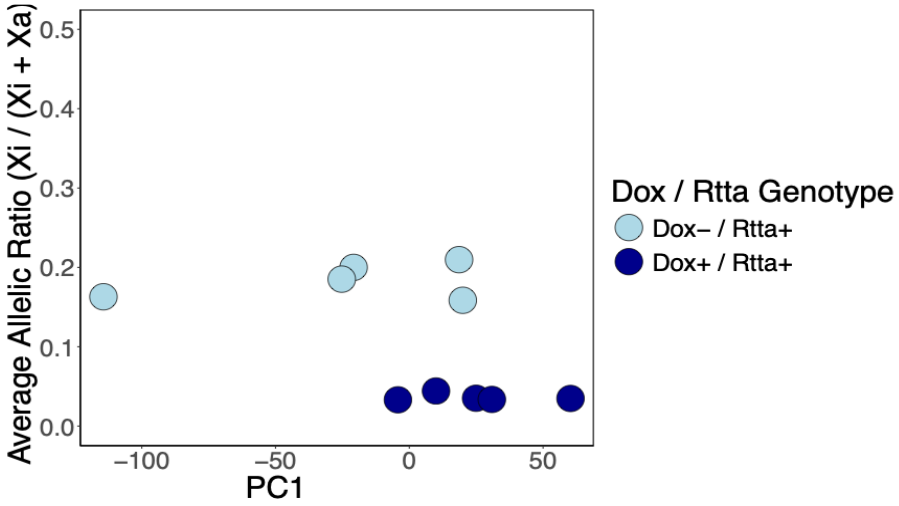
Hauth, Panten, Kneuss et al, "Escape from X inactivation is directly modulated by Xist
Nature Cell Biol. 2025

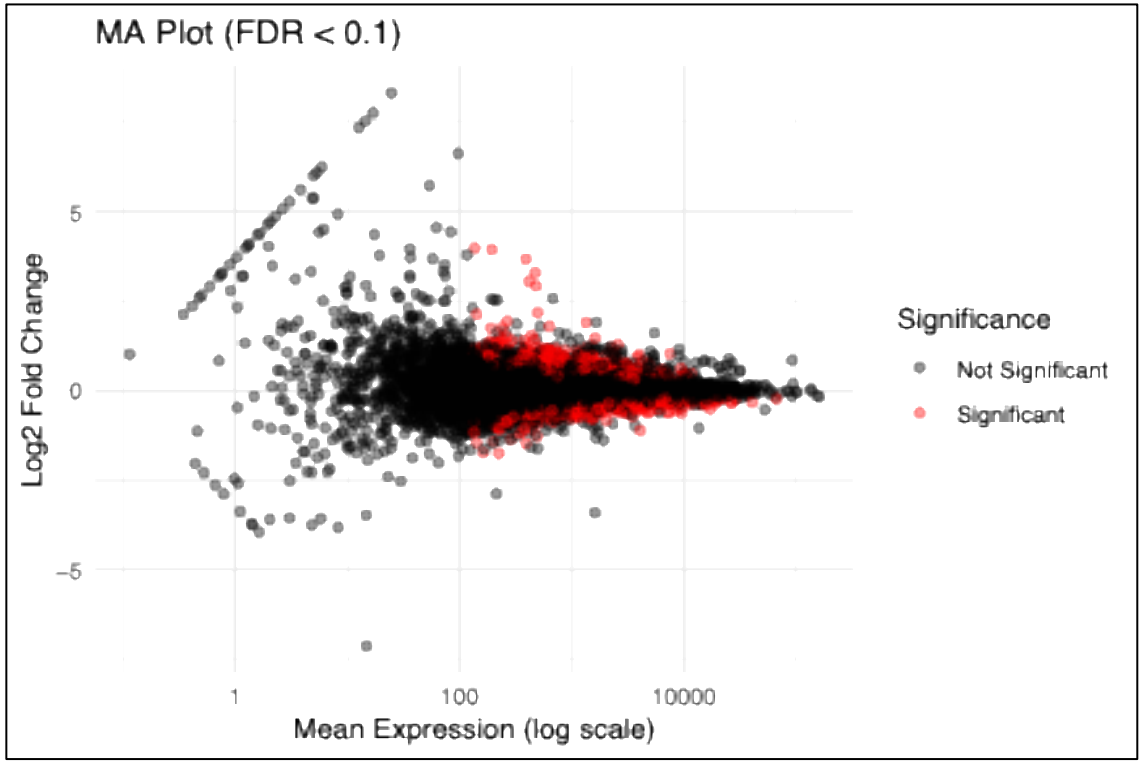
Inducing higher Xist expression in E3.5 - E8.5 embryos

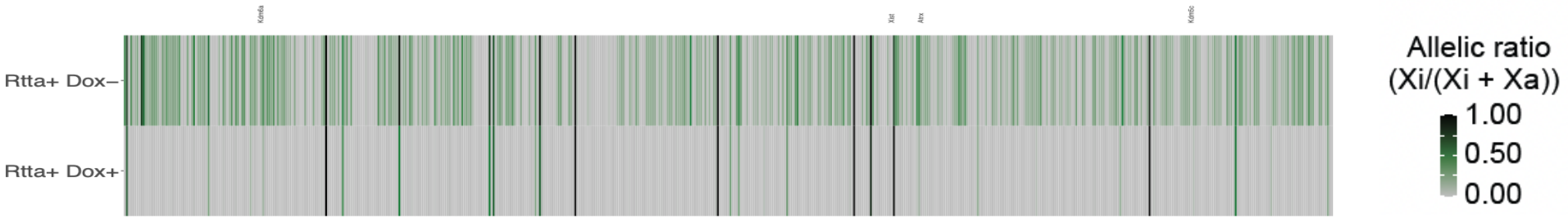


EMBL

Higher Xist-induced embryos show silencing of escapee expression as well as genome-wide changes in gene expression

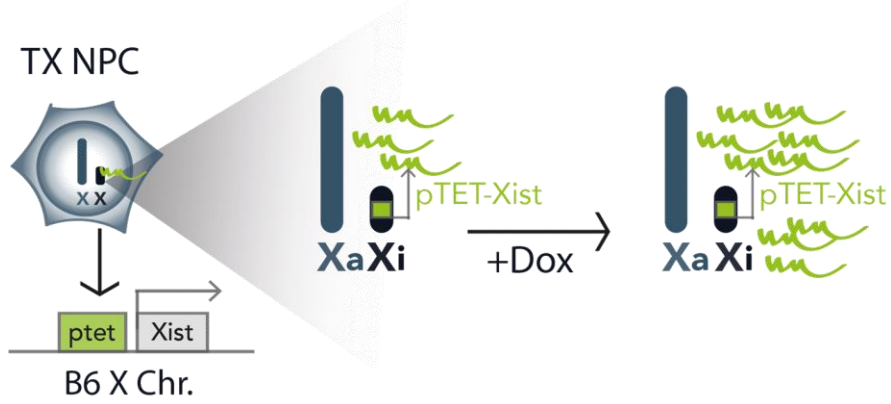


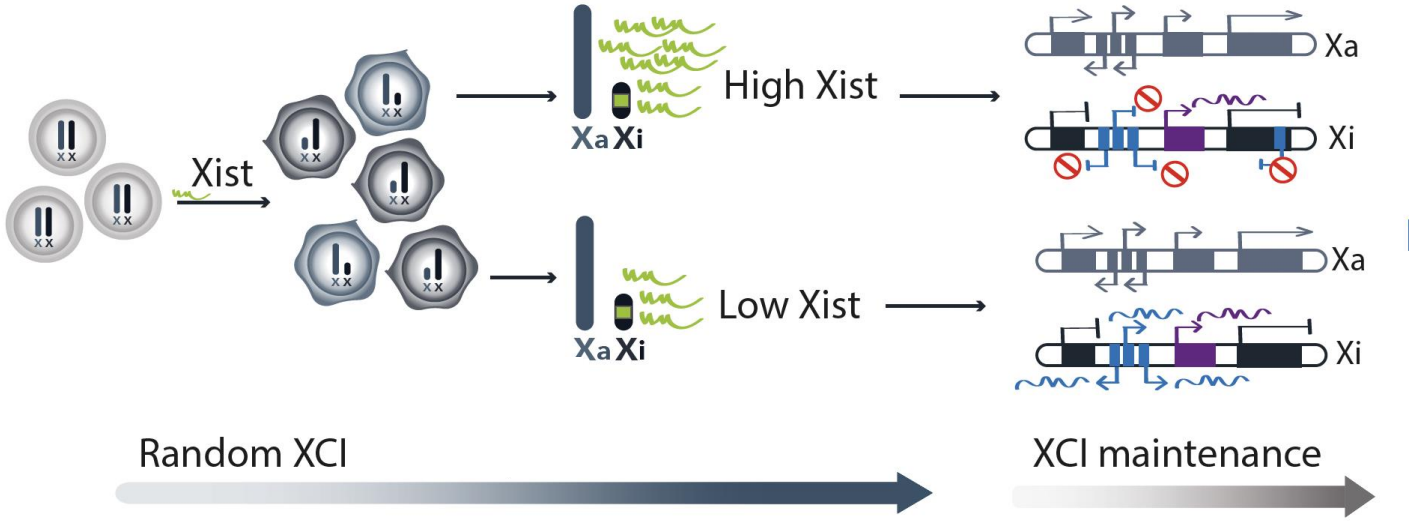




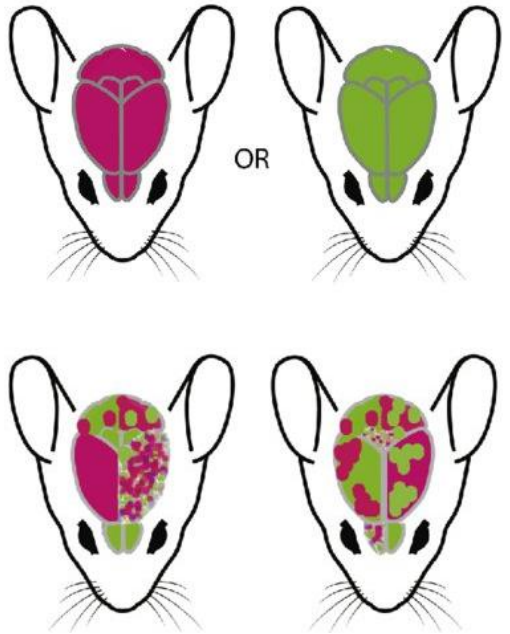
EMBL

Impact of variation of Xist RNA levels on escapee gene expression and resulting phenotypes *in vivo*





No escape: *phenotypes*?



Variable escape: *phenotypes?*

Conclusions

- Sex differences in disease are widespread and can be due to life-style, hormones and sex chromosomes (notably the inactive X and the Y chromosome)
- Escape from X-chromosome inactivation is widespread (up to 1/3 of X-genes) and varies between individuals and within tissues, in both humans and mice
- Expression from the inactive X can lead to increased protein dosage with important implications for female-specific development and disease
- We show that Xist RNA plays a central role even in somatic cells: increased *Xist* expression leads to silencing and loss of 3D domains at escapee loci
- Natural or stress-induced Xist RNA variation may lead to important X-linked dosage variation in development and disease.

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Flow Cytometry Core Facility (FCCF)
Advanced Light Microscopy Facility (ALMF)

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CHROMDESIGN Training the next generation of researches

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