CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2022-2023

"Biais liés au sexe dans la susceptibilité aux maladies: causes génétiques et épigénétiques"

27 mars, 2023

Cours IV

L'importance de la régulation du dosage des gènes sur le chromosome X dans la susceptibilité à certaines maladies

COLLÈGE DE FRANCE 1530

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Année 2022-2023

"Biais liés au sexe dans la susceptibilité aux maladies: causes génétiques et épigénétiques"

Cours I - Introduction : les maladies ont-elles un sexe ? *6 mars*

Cours II - Biais liés au sexe : comment distinguer les effets dus aux chromosomes sexuels, hormones ou mode de vie ? *13 mars*

Cours III - L'impact de l'expression des gènes liés aux chromosomes X inactif et Y sur les différences entre les sexes. *20 mars*

Cours IV - L'importance de la régulation du dosage des gènes sur le chromosome X dans la susceptibilité à certaines maladies. *27 mars*

Colloque – en lien avec le sujet du cours, le **21 avril, 2023**

CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Image : *La chute de l'homme* (détail), Cornelis Cornelisz van Haarlem, 1592. © Rijksmuseum

COLLOQUE

The Genetic and Epigenetic Basis of Sex Bias in Disease

21 avril 2023

COLLÈGE DE FRANCE 1530

Thomas Römer
Administrateur du Collège de France
11, place Marcelin-Berthelot, 75005 Paris
www.college-de-france.fr

Année académique 2022/2023

21 avril 2023 de 9h à 18h
Amphitheatre Maurice Halbwachs

The Genetic and Epigenetic Basis of Sex Bias in Disease

Edith Heard, Chaire Épigénétique & mémoire cellulaire
Scientific co-organisers: **James Cleland** and **Agnese Loda**

Daniel Andergassen
Technical University of Munich, Germany

Richard Festenstein
Imperial College, London, UK

Cornelius Gross
EMBL-Rome, Italy

Jean-Charles Guéry
INSERM, University of Toulouse, France

Jamie Hackett
EMBL-Rome, Italy

Irene Miguel-Aliaga
Imperial College, London, UK

Jessica Tollkuhn
Cold Spring Harbor Lab, New York, USA

Taru Tukiainen
FIMM, Helsinki, Finland

Judith Zaugg
EMBL Heidelberg, Germany

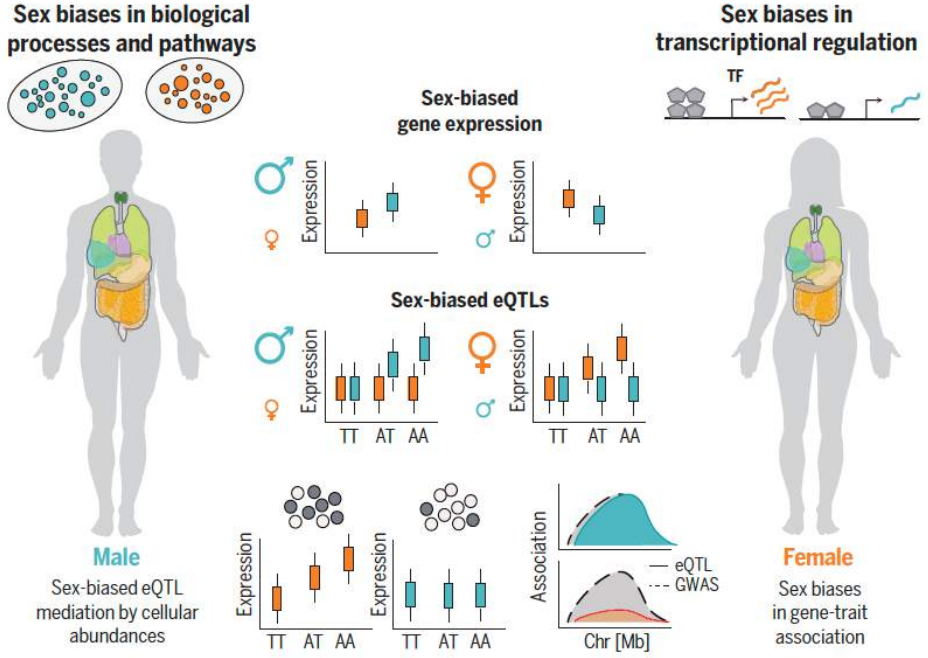
Colloquium in English, free entry, no registration required

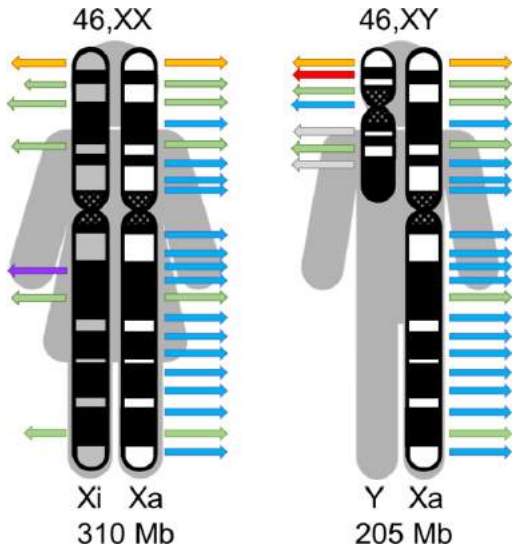
COLLÈGE DE FRANCE 1530

E. Heard, March 27th 2023

SUMMARY of LAST WEEK

- Sex- biased gene expression is a fundamental characteristic that is common across species.
- In humans, genes demonstrate sex- biased expression within and between tissues, during development, in the context of disease and under different environmental conditions
- This sex bias is both at the individual gene level and on a genome- wide scale
- Sex- biased gene expression is a common characteristic of genes encoded both on the sex chromosomes and on the autosomes, with the X being enriched for sex differentially expressed genes, largely due to escapees genes on the Xi





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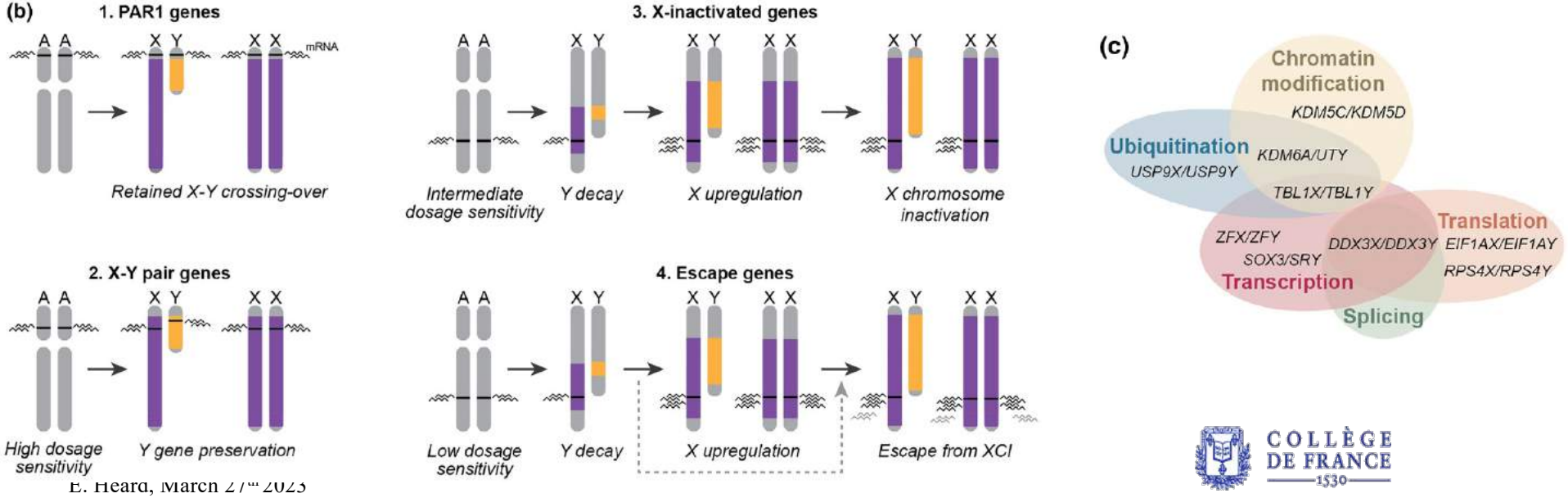
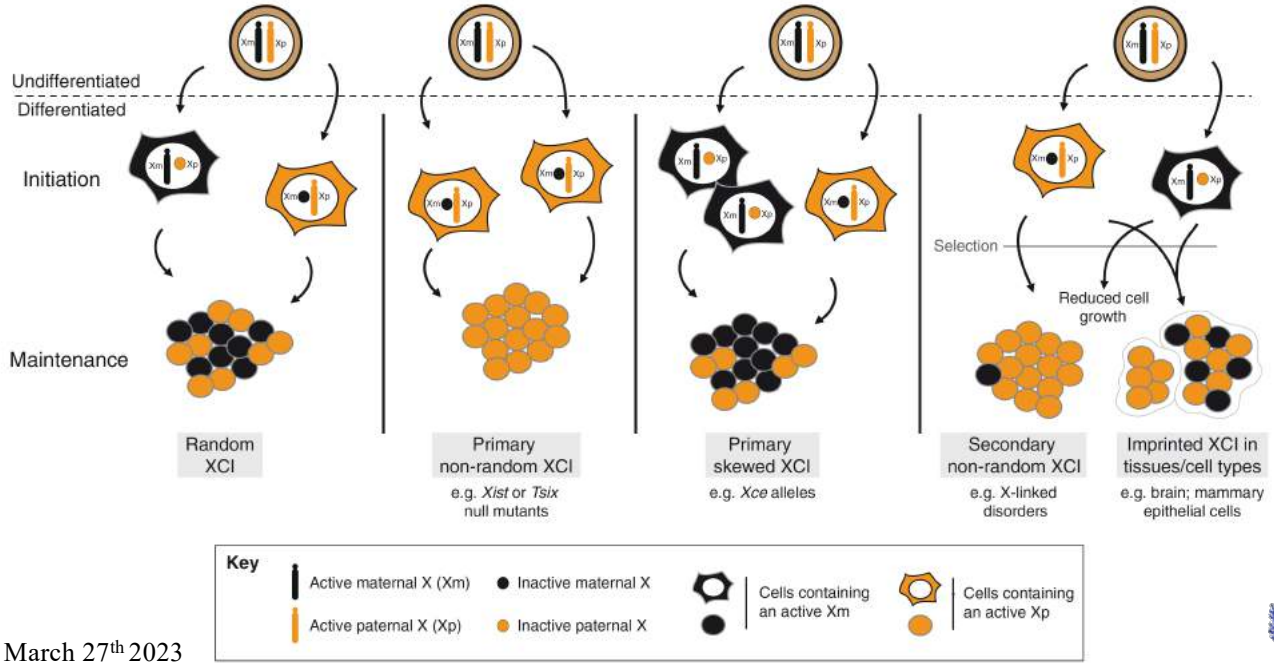
- The sex chromosomes were largely excluded from GWAS analyses until recently
- The evolution of the sex chromosomes resulted in X-inactivated genes, PAR1 genes, X-Y pairs, X-only escapees



COLLÈGE DE FRANCE 1530

SUMMARY of LAST WEEK

- Random X-chromosome inactivation early in female development leads to cellular mosaicism for proteins encoded by paternal or maternal X-chromosome genes
- Most X-linked genes are characterized by monoallelic expression but many genes can escape XCI and this can be constitutive or facultative and variable
- X-linked mutations lead to more severe phenotypes in males than females owing to the presence in XX individuals of a second X and mosaicism due to X inactivation, as well as the presence of escapees
- X inactivation contributes to a variety of differences between females in prevalence and severity of X-linked disorders



Deng et al,

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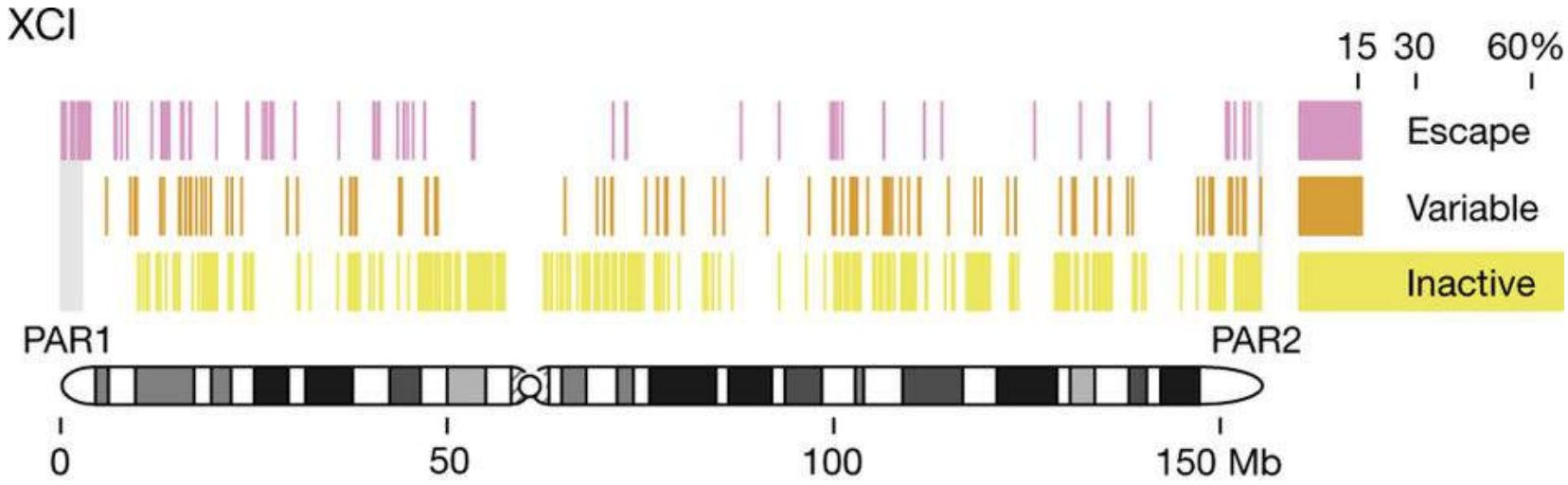

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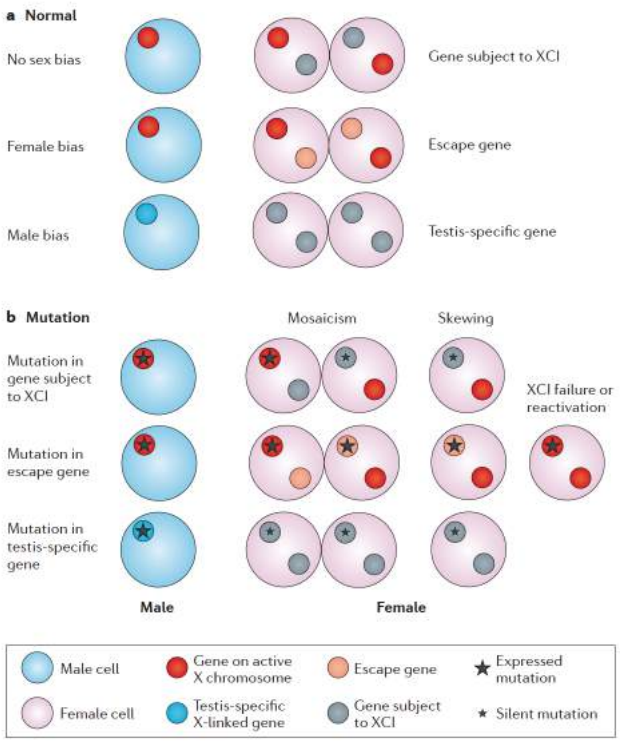


Deng et al, 2014

Xi Escapees in Twin Studies: Genetic and Stochastic/Environmental

Escape from X-inactivation in twins exhibits intra- and inter-individual variability across tissues and is heritable

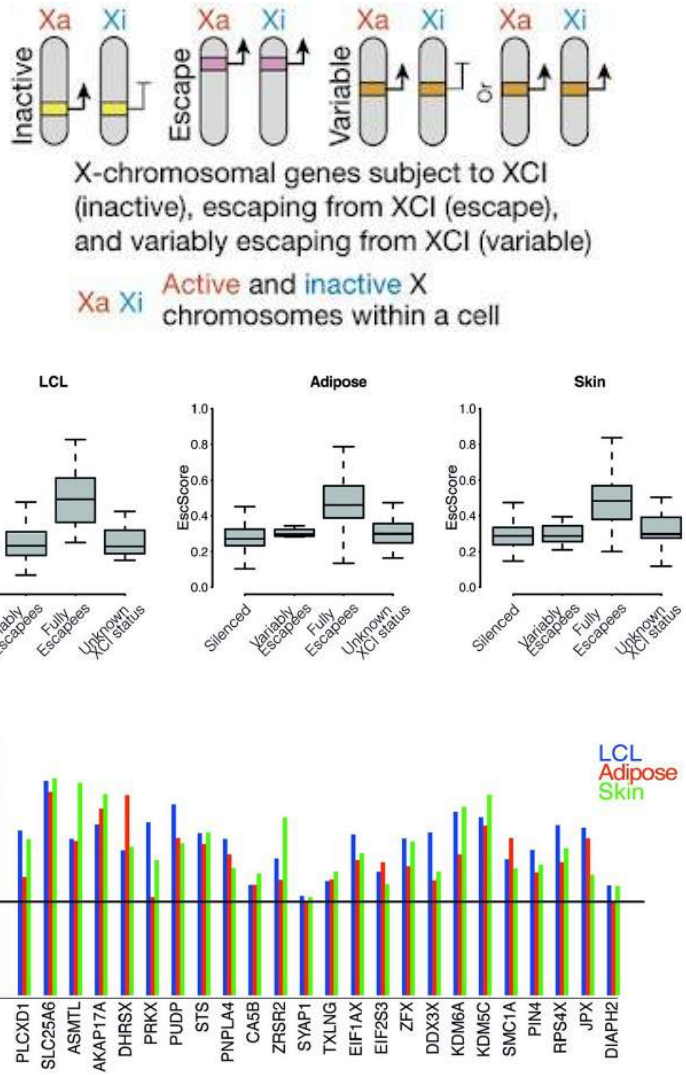
Antonino Zito[1¤a¤b]*, Amy L. Roberts[1☯], Alessia Visconti[1☯], Niccolo' Rossi[1], Rosa Andres-Ejarque[2], Stefano Nardone[3], Julia S. El-Sayed Moustafa[1], Mario Falchi[1], Kerrin S. Small[1]*

XCI escape analysed using paired bulk RNAseq and DNAseq data in a multi-tissue dataset sampled from 248 skewed female twins of the TwinsUK bioresource.

Adipose and skin tissue, lymphoblastoid cell lines and purified immune cells (monocytes, B-cells, T-CD4+, T-CD8+ and NK cells), across individuals.

Identify novel genes exhibiting tissue- and immune cell type-specific escape, and genes escaping XCI with high variability across tissues and individuals.

Escape varies across tissues and immune cells within an individual and across individuals.



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Escape from X-inactivation exhibits intra- and inter-individual variability

And may be influenced by both heritable and environmental factors

Twin studies can assess contribution of genetic factors to complex traits

Using 27 complete twin pairs (17 monozygotic (MZ or identical); 10 dizygotic (DZ or fraternal)), we quantified the concordance in the escape in LCLs between co-twins and compared such concordance between MZ and DZ twins.

MZ twins share significantly more similar escape genes than DZ twins

⇒There is a significant genetic component of escape

Nevertheless, MZ twin discordance suggests non-genetic influences.

=> both genetic and environmental factors – or stochastic variation - interplay to influence XCI escape.

Variability between immune cell types may also suggest an immune cell type-specific response to environmental influences.

Escape may influence disease risk and phenotype differences between the sexes, and within females.

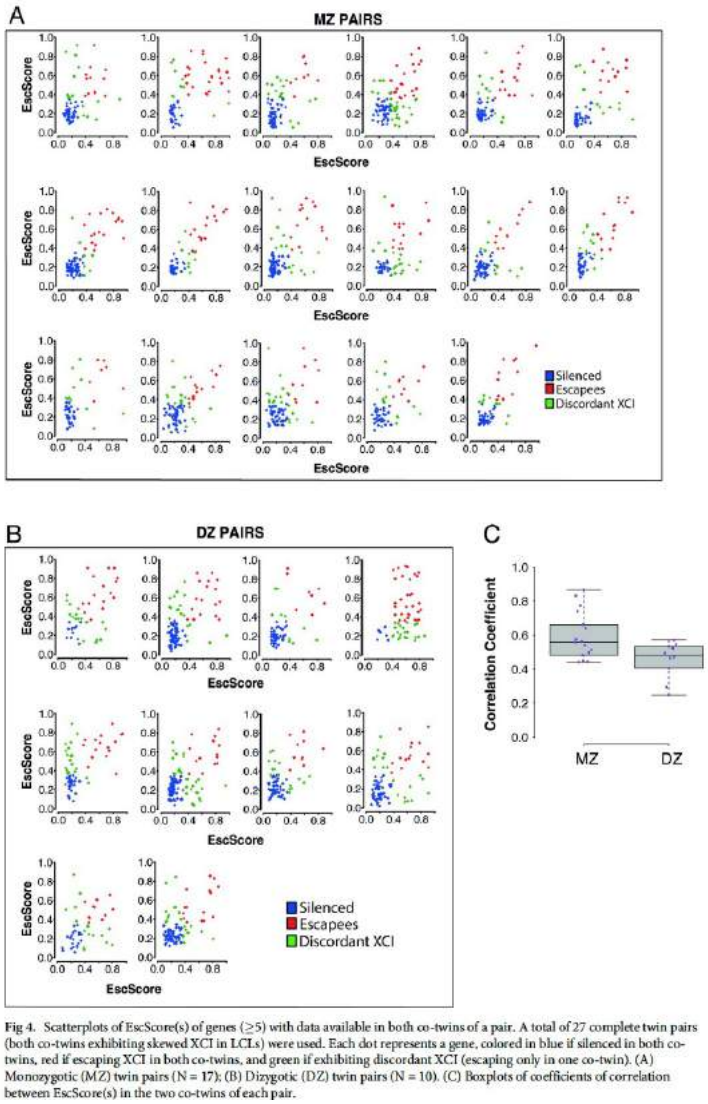
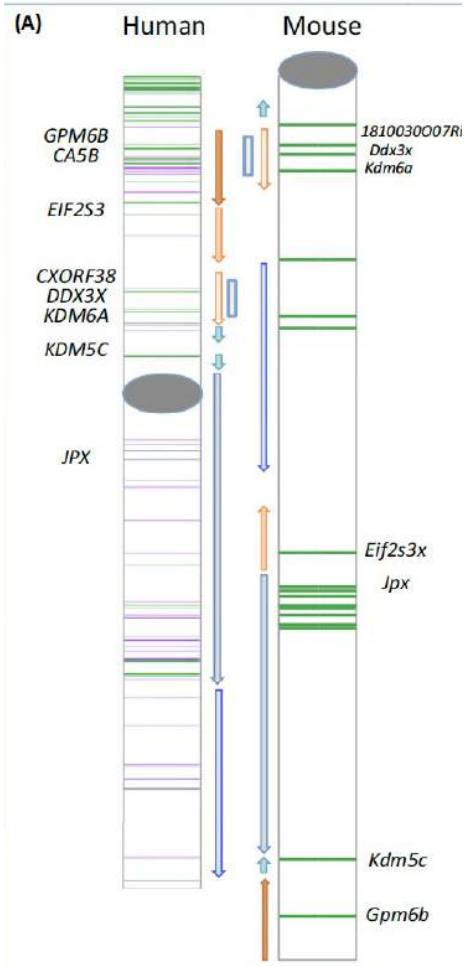
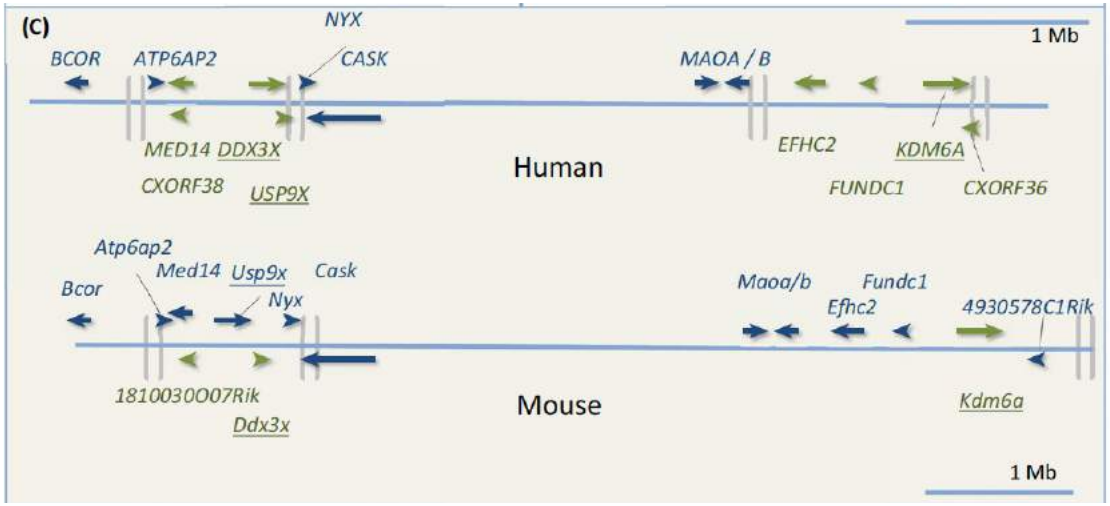


Fig 4. Scatterplots of EscScore(s) of genes (≥5) with data available in both co-twins of a pair. A total of 27 complete twin pairs (both co-twins exhibiting skewed XCI in LCLs) were used. Each dot represents a gene, colored in blue if silenced in both co-twins, red if escaping XCI in both co-twins, and green if exhibiting discordant XCI (escaping only in one co-twin). (A) Monozygotic (MZ) twin pairs (N = 17); (B) Dizygotic (DZ) twin pairs (N = 10). (C) Boxplots of coefficients of correlation between EscScore(s) in the two co-twins of each pair.

Xi escape in Humans and Mice



The human and mouse X chromosomes, showing the locations of 62 human escapee genes (green lines, intensity reflects escape gene density), 16 variable escapee genes (purple), and 17 mouse escape genes (genes shown were observed in two or more studies/tissues)



Genes subject (blue) or escaping (green) XCI

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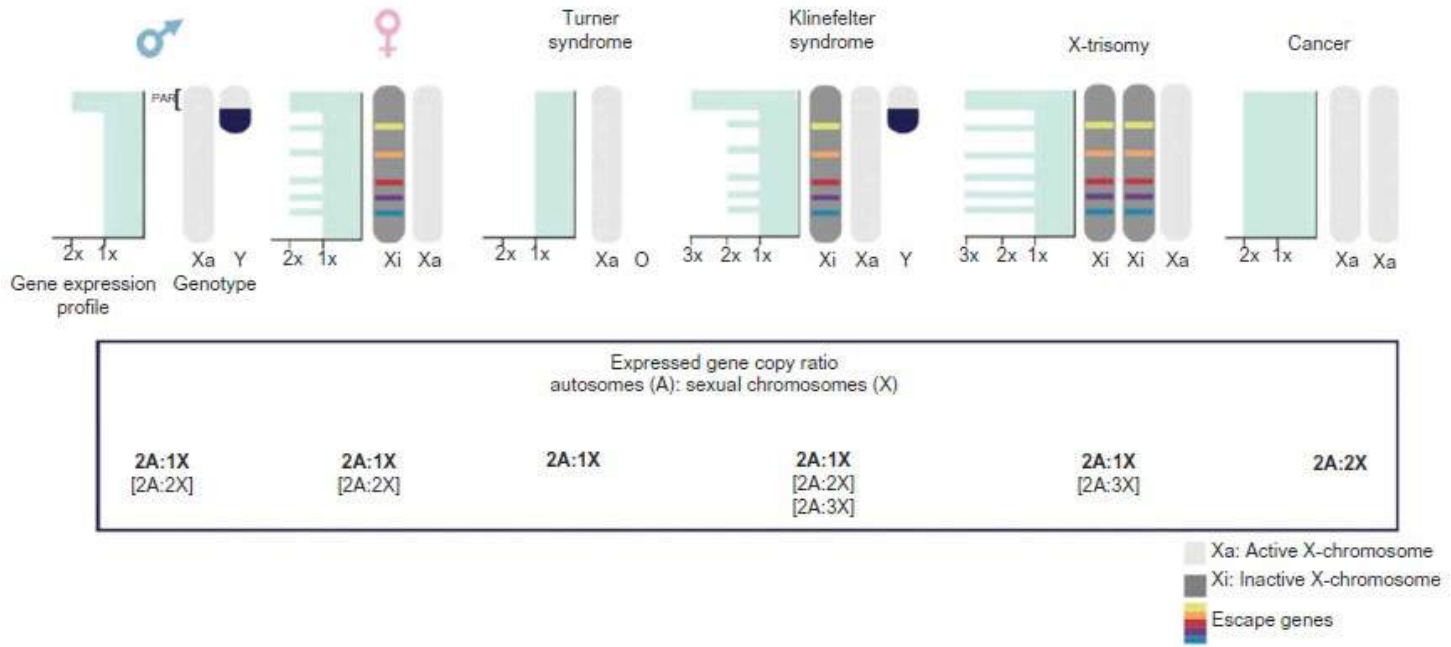
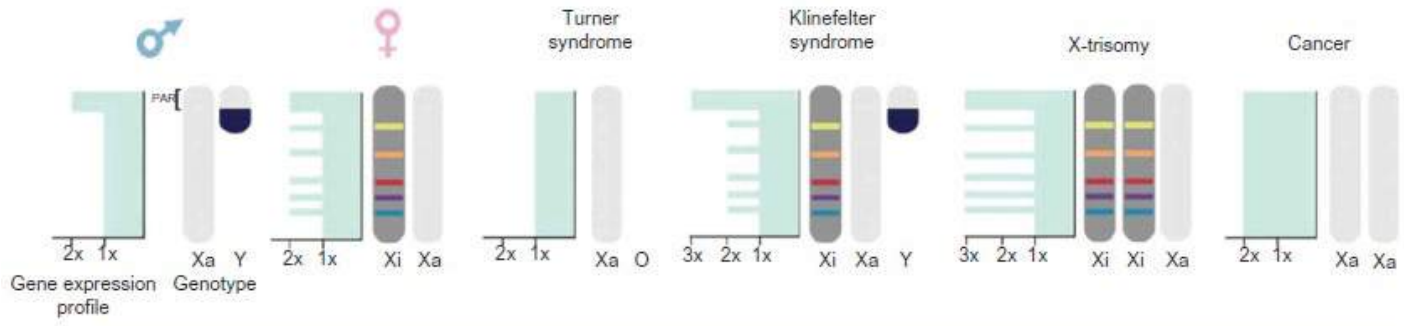
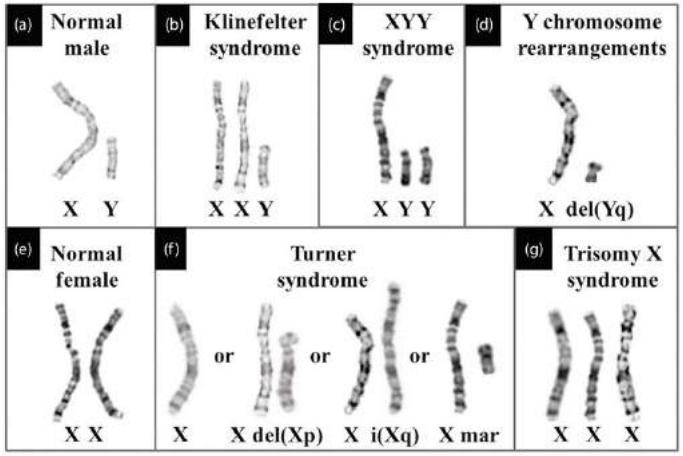
Aberrant X-linked gene dosage can have deleterious consequences



- Humans are sensitive to chromosomal dosage and most aneuploidies are lethal
- Only trisomies 13, 18, and 21 (respectively the Patau, Edwards, and Down syndromes).
- Aneuploidies of the X are common viable chromosomal abnormalities, and affected individuals w moderate phenotypes.
- The best known are Turner syndrome (XO females) and Klinefelter syndrome (XXY males).
- The reason that X chromosome aneuploidies are better tolerated than autosomal ones is due to X inactivation (XCI), in which all X chromosomes are transcriptionally silenced except for one.
- XO, XX, XXX, or XXXX, or XXY will each have only one active X chromosome with all supernumerary X's being inactivated.

Aberrant X-linked gene dosage can have deleterious consequences





E. Heard, March 27th 2023

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Aberrant X-linked gene dosage can influence multiple functions including intellectual capacity and somatic functions

TABLE I. IQ Score Mean Values, Across Studies, by Sex Chromosome Aneuploidy

	Full-scale IQ: general intelligence	Verbal IQ: verbal skills	Performance IQ: nonverbal skills
XO	90–94 (Hong et al., 2011; Rovet, 1990, 1993)	93–99 (Hong et al., 2011; Rovet, 1993)	88–91 (Rovet, 1993; Hong et al., 2011)
XYY	91–97 (Bardsley et al., 2013; Tartaglia et al., 2012)	88–92 (Bardsley et al., 2013; Tartaglia et al., 2012)	95–102 (Bardsley et al., 2013; Tartaglia et al., 2012)
XXY	92–98 (Rovet et al., 1995; Tartaglia et al., 2012)	84–93 (Rovet et al., 1995; Tartaglia et al., 2012)	98–99 (Rovet et al., 1995; Tartaglia et al., 2012)
XXX	83–93 (Tartaglia et al., 2012; Tartaglia et al., 2010b)	82–87 (Tartaglia et al., 2012; Rovet et al., 1995)	87–100 (Tartaglia et al., 2012; Rovet et al., 1995)
XXYY	78–79 (Tartaglia et al., 2008b; Tartaglia et al., 2012)	74–77 (Tartaglia et al., 2008b; Tartaglia et al., 2012)	84–87 (Tartaglia et al., 2008b; Tartaglia et al., 2012)

% CASES OF AUTISM SPECTRUM DISORDER AND ATTENTION-DEFICIT/ HYPERACTIVITY DISORDER

	XO	XXY	XYY	XXX	XXYY
ASD	3%–4% (Creswell and Skuse, 1999; Saad et al., 2013)	11%-27% (Bruining et al., 2009; Bishop et al., 2011)	19%–36% (Bishop et al., 2011; Tartaglia et al., 2012)	No increased risk (Bishop et al., 2011)	28%–34% (Tartaglia et al., 2008a; Tartaglia et al., 2012)
ADHD	20%–50% (Green et al., 2015; Saad et al., 2013)	36%–63% (Bruining et al., 2009; Tartaglia et al., 2010a)	46%–76% (Ross et al., 2012; Tartaglia et al., 2012)	25%–52% (Bender et al., 1993; Tartaglia et al., 2012)	72.2% (Tartaglia et al., 2012)

Multiple X chromosomes lead to intellectual deficiencies and somatic abnormalities

THE CANADIAN MEDICAL ASSOCIATION
LE JOURNAL DE
L'ASSOCIATION MÉDICALE CANADIENNE

JANUARY 21, 1961 • VOL. 84, NO. 3

AN XXXX SEX CHROMOSOME COMPLEX IN TWO MENTALLY DEFECTIVE FEMALES*

DAVID H. CARR, M.B., Ch.B.,†
MURRAY L. BARR, M.D.† and
EARL R. PLUNKETT, M.D., Ph.D.,‡
London, Ont.

The additional X chromosomes probably arose through non-disjunction of X chromosomes during oögenesis or an early division of the fertilized ovum. X chromosomes in excess of the normal female complement are apparently compatible with normal maturation of ovaries and other components of the reproductive system, but the tetra-X complex is perhaps an etiological factor in the mental deficiency.

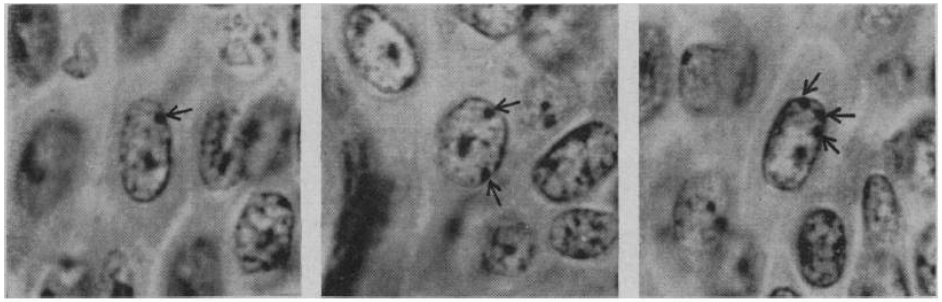

Fig. 3.—Nuclei with one, two and three masses of sex chromatin in a skin biopsy specimen. Hematoxylin and eosin stain. ×2000.

Arch. Dis. Childh., 1966, **41**, 82.

The XXXXY Sex Chromosome Abnormality

J. GALINDO and H. S. BAAR*
From the Pathology Department, Pineland Hospital, Pownal, Maine, U.S.A.

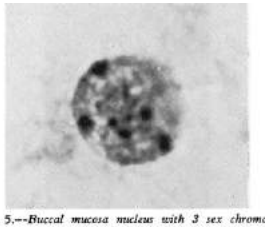

FIG. 5.—*Buccal mucosa nucleus with 3 sex chromati bodies.*

Lyon (1962) postulated the inactivation of one of the two X chromosomes of normal females, occurring early in development, and that the sex chromatin body in interphase nuclei is precisely this inactivated X. She further suggested that, in cases of anomalies concerning the number of sex chromosomes, all the X chromosomes in excess of one would be inactivated. However, the fact that multiple X karyotypes, particularly the quadruple X, are always associated with several somatic anomalies and testicular atrophy, suggests that inactivation is only partial, as has been suggested by Grumbach (1963), and there is some influence at work of chromosomal environment on the individual's somatic constitution.

The X chromosome of eutherian mammals exists in two distinct epigenetic states that are referred to as ‘‘active’’ (Xa) and ‘‘inactive’’ (Xi).

The ‘‘n-1’’ rule (where n is the number of X chromosomes per cell) states that **all diploid human somatic cells possess one X chromosome in the active state (Xa),** while all other (i.e., n-1) copies of chromosome (Chr) X4 are transcriptionally repressed through X-chromosome inactivation (XCI).

E. Heard, March 27th 2023

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Multiple X chromosomes lead to intellectual deficiencies and somatic abnormalities

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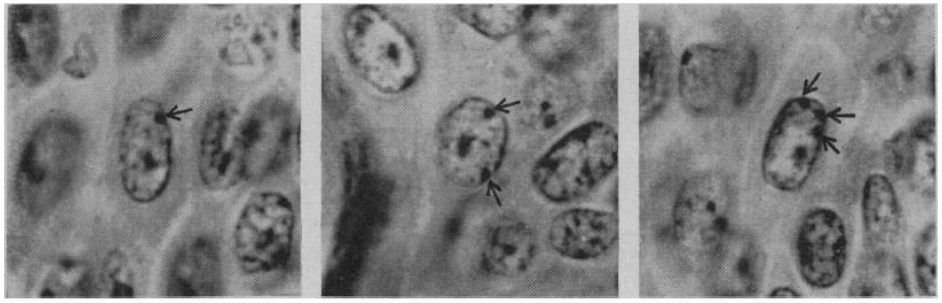

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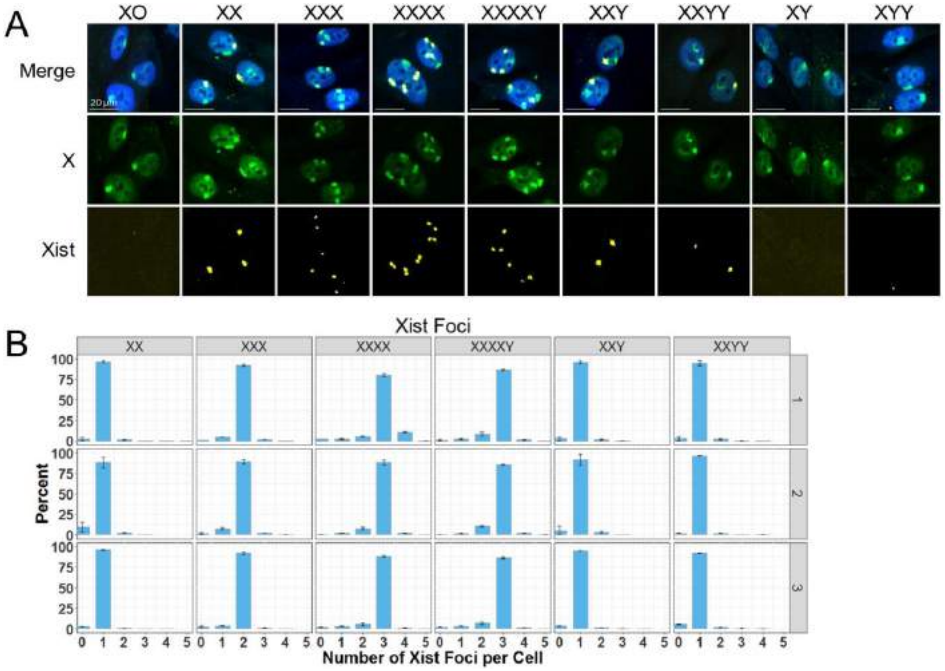
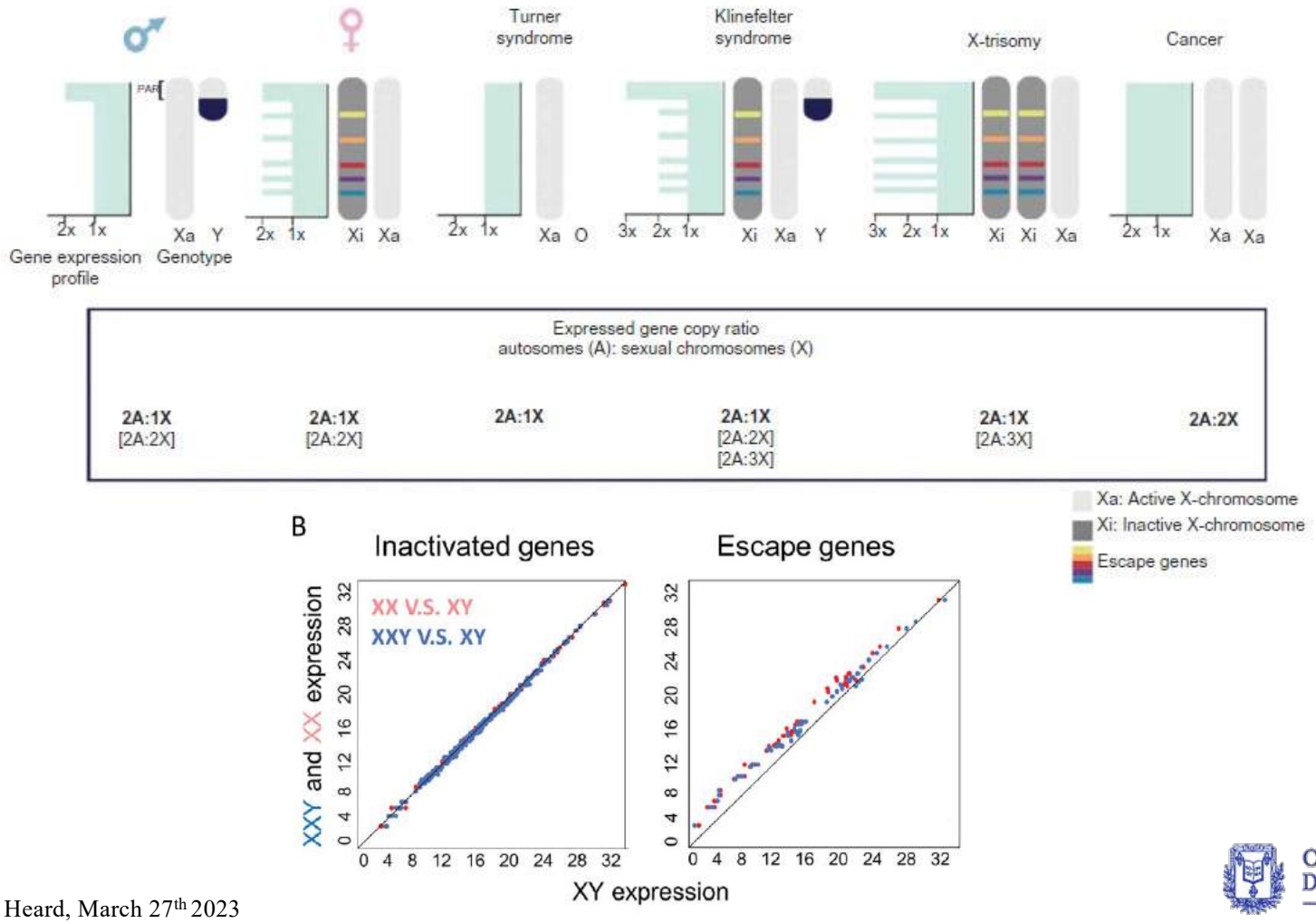


- Multiple Barr bodies and XIST RNA domains in individuals with more than two X chromosomes
- All X chromosomes but one are inactive
- Mental deficiency and somatic abnormalities in individual with supernumerary Xs implies that not all genes are silenced

COLLÈGE DE FRANCE 1530

Aberrant X-linked gene dosage can have deleterious consequences



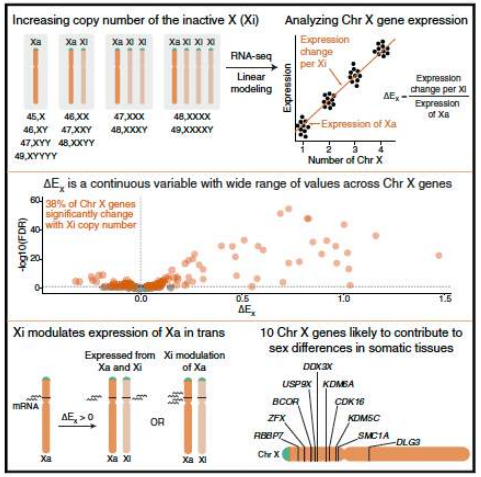
Using Aneuploidies to show that the Human Inactive X modulates expression of the Active X

Cell Genomics

Article

The human inactive X chromosome modulates expression of the active X chromosome

Graphical abstract



Authors

Adrianna K. San Roman, Alexander K. Godfrey, Helen Skaletsky, ..., Carole Samango-Sprouse, Maximilian Muenke, David C. Page

Correspondence

dcpage@wi.mit.edu

In brief

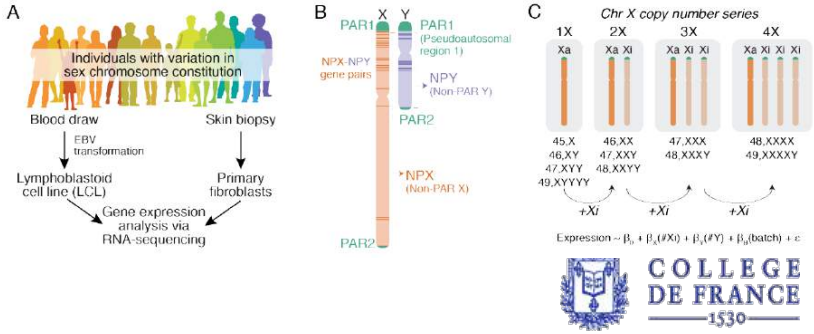
Through RNA sequencing of individuals with sex chromosome aneuploidy, San Roman et al. identify modular "active" (Xa) and "inactive" (Xi) X chromosome transcriptomes. Looking beyond classical X inactivation, which acts in *cis*, they find that Xi modulates Xa transcript levels in *trans*. They identify 10 X chromosome genes most likely to contribute to male-female differences in common disease.

Highlights

- Analyzed gene expression in sex chromosome aneuploidy samples using linear models
- Xi and Xa transcriptomes are modular
- 38% of X chromosome genes are affected by Xi copy number—in *cis* and in *trans*
- 10 X chromosome genes likely contribute to male-female differences in somatic tissues

Table 1. Samples included in sex chromosome aneuploidy analysis

Karyotype	# LCLs	# Fibroblast cultures
45,X	17	23
46,XX	22	20
46,XY	17	14
47,XXX	7	4
47,XXY	11	30
47,XYY	10	5
48,XXXX	1	0
48,XXXY	4	1
48,XXYY	3	0
49,XXXXY	12	1
49,XYYYY	2	1
Total:	*106*	*99*



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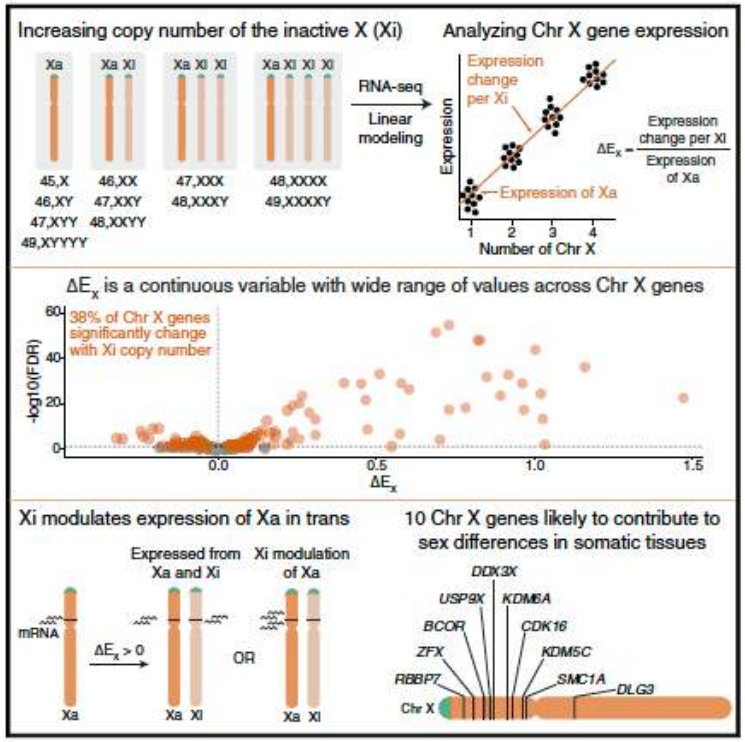
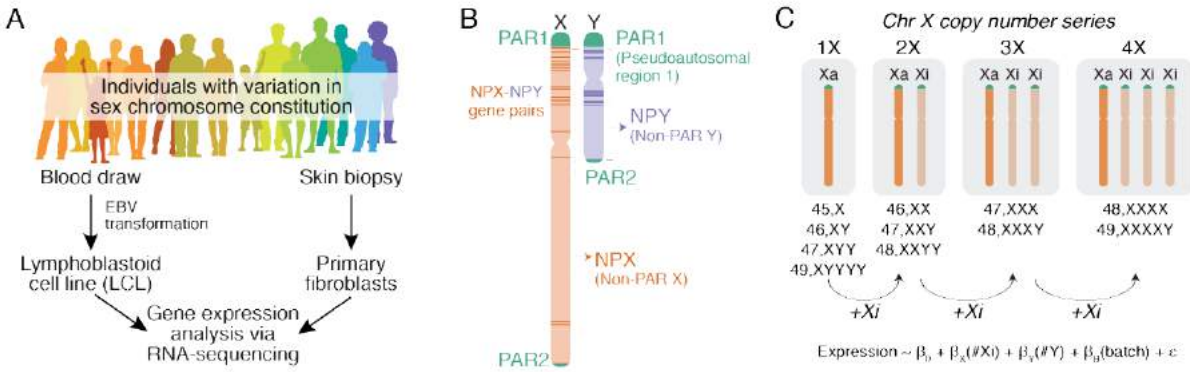
The Human Inactive X modulates expression of the Active X





The human inactive X chromosome modulates expression of the active X chromosome

Quantified Xi and Xa gene expression in individuals with one active X and zero to three inactive X chroms.

Linear modeling revealed modular Xi and Xa transcriptomes and significant Xi-driven expression changes for 38% (162/423) of expressed X-chromosome genes.

This model confirmed the "n-1" rule (ie only one Xa per diploid cell. All other Xs are inactivated)

By integrating allele-specific analyses, the Xi modulates Xa transcript levels (³ 121 genes).

10 X-chromosome genes identified that may drive sex differences in common disease and sex chromosome aneuploidy syndromes.

By comparing samples that vary in Xi copy number with and without a Y chromosome, expression from Xa was found to be quantitatively indistinguishable in phenotypic males and females—as is expression from Xi.

=> Human X chromosomes are regulated both in cis, through Xi-wide transcriptional attenuation, and in trans, through positive or negative modulation of individual Xa genes by Xi. The sum of cis and trans effects differs widely among genes.

E. Heard, March 27th 2023

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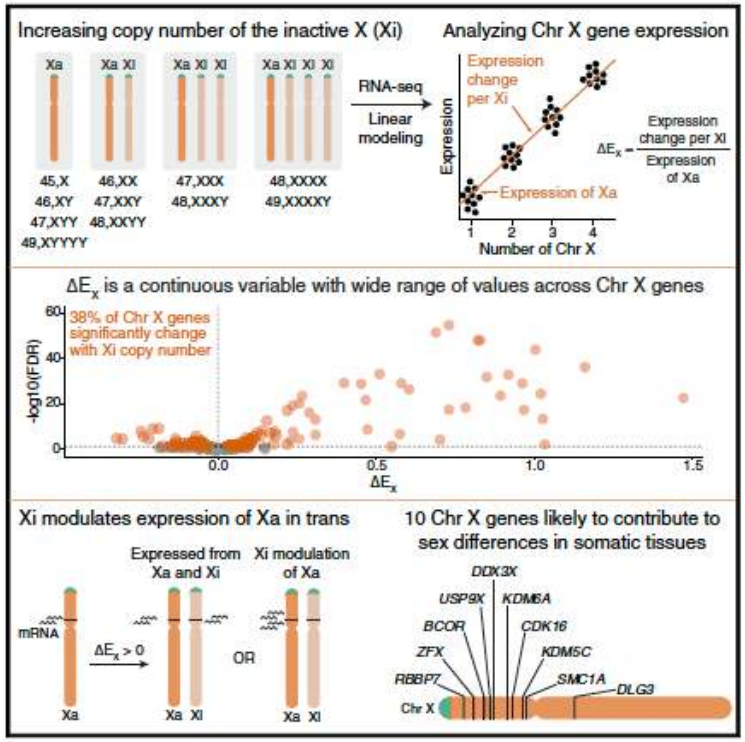
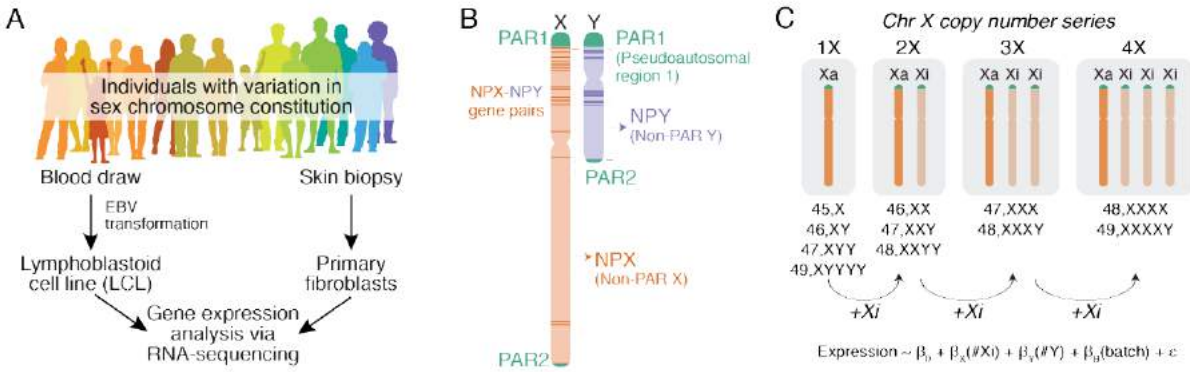




- Quantification de l'expression des gènes Xi et Xa chez des individus possédant un X actif - et de 0 à 3 chromosomes X inactifs.
- La modélisation linéaire a révélé des transcriptomes Xi et Xa modulaires et des changements d'expression significatifs induits par le Xi pour 38 % (162/423) des gènes exprimés du chromosome X. Ce modèle a confirmé le caractère "n-1" de l'expression des gènes du chromosome X actif.
- Ce modèle a confirmé la règle "n-1" (c'est-à-dire un seul Xa par cellule diploïde, tous les autres X étant inactivés).
- En intégrant les analyses allele-spécifiques, le Xi module les niveaux de transcription du Xa (³ 121 gènes).
- 10 gènes sur le chromosome X susceptibles d'être à l'origine de différences entre les sexes dans les maladies courantes et les syndromes d'aneuploïdie des chromosomes sexuels.
- En comparant des échantillons dont le nombre de copies de Xi varie avec et sans chromosome Y, il a été constaté que l'expression du Xa était quantitativement indiscernable chez les hommes et les femmes, tout comme l'est l'expression de Xi.

- => **Les chromosomes X humains sont régulés à la fois en cis, par l'atténuation transcriptionnelle à l'échelle du Xi, et en trans, par la modulation positive ou négative des gènes Xa individuels par le Xi.**
- **La somme des effets cis et trans diffère largement d'un gène à l'autre.**

E. Heard, March 27th 2023

DE FRANCE 1530

Escapees on the Human Inactive X modulate expression of the Active X

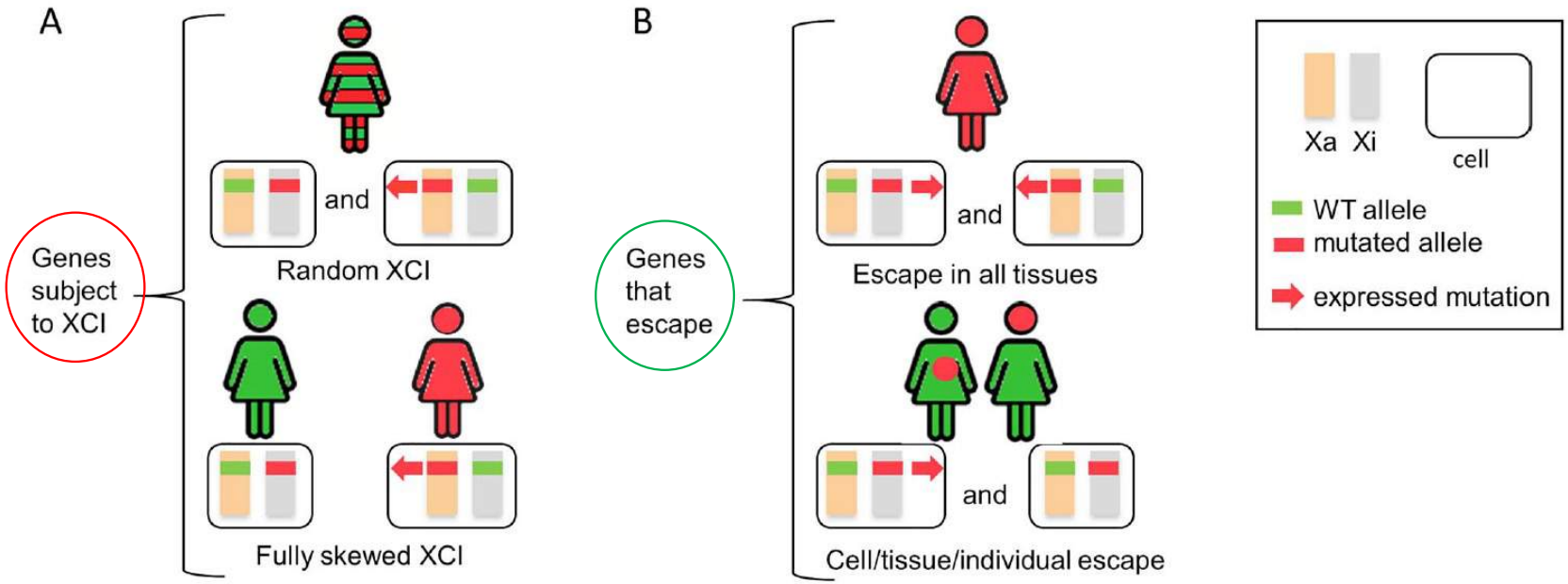
Table 2. X chromosome genes that may drive the phenotypic impacts of variation in Xi copy number

Region	Gene symbol	Gene name	NPY gene symbol	ΔE_X LCL	ΔE_X Fib.	Gene constraint (average % ranking)[a]	Disease associations: Phenotype	Disease associations: Inheritance[b]	Disease associations: MIM #
NPX	*KDM6A*	lysine demethylase 6A	*UTY*	0.83	0.45	93.3	Kabuki syndrome	XLD	300867
	KDM5C	lysine demethylase 5C	*KDM5D*	0.73	0.58	90.3	Claes-Jensen syndrome	XLR	300534
	SMC1A	structural maintenance of chromosomes 1A	–	0.58	0.43	87.6	Cornelia de Lange syndrome; developmental and epileptic encephalopathy	XLD	300590, 301044
	ZFX	zinc finger protein X-linked	*ZFY*	0.45	0.47	83.0	–	–	–
	RBBP7	RB-binding protein 7, chromatin remodeling factor	–	0.01	0.29	82.5	–	–	–
	DDX3X	DEAD-box helicase 3 X-linked	*DDX3Y*	0.26	0.16	89.2	syndromic IDD,[c] Snijders Blok type	XLD, XLR	300958
	CDK16	Cyclin dependent kinase 16	–	0.09	0.24	83.8	–	–	–
	DLG3	discs large MAGUK scaffold protein 3	–	0.18	0.07	82.8	IDD	XLR	300580
	USP9X	ubiquitin-specific protease 9 X-linked	*USP9Y*	0.14	0.17	94.4	IDD	XLR, XLD	300919, 300968
	BCOR	BCL6 corepressor	–	0.12	0.01	91.1	oculofaciocardiodental syndrome	XLD	300166
PAR1	*SLC25A6*	solute carrier family 25 member 6	N/A	1.0	0.74	67.4	–	–	–
	SHOX	short stature homeobox	N/A	N/A[d]	N/A	58.4	Leri-Weill dyschondrosteosis; Langer mesomelic dysplasia; short stature idiopathic familial	PD, PR	127300, 249700, 300582

Expression from the inactive X can offer protection against de novo and inherited X-linked mutations, and has also been proposed to contribute to the over-representation of females for some complex traits, such as the autoimmune disorders

Impact of the inactive X chromosome on human disease

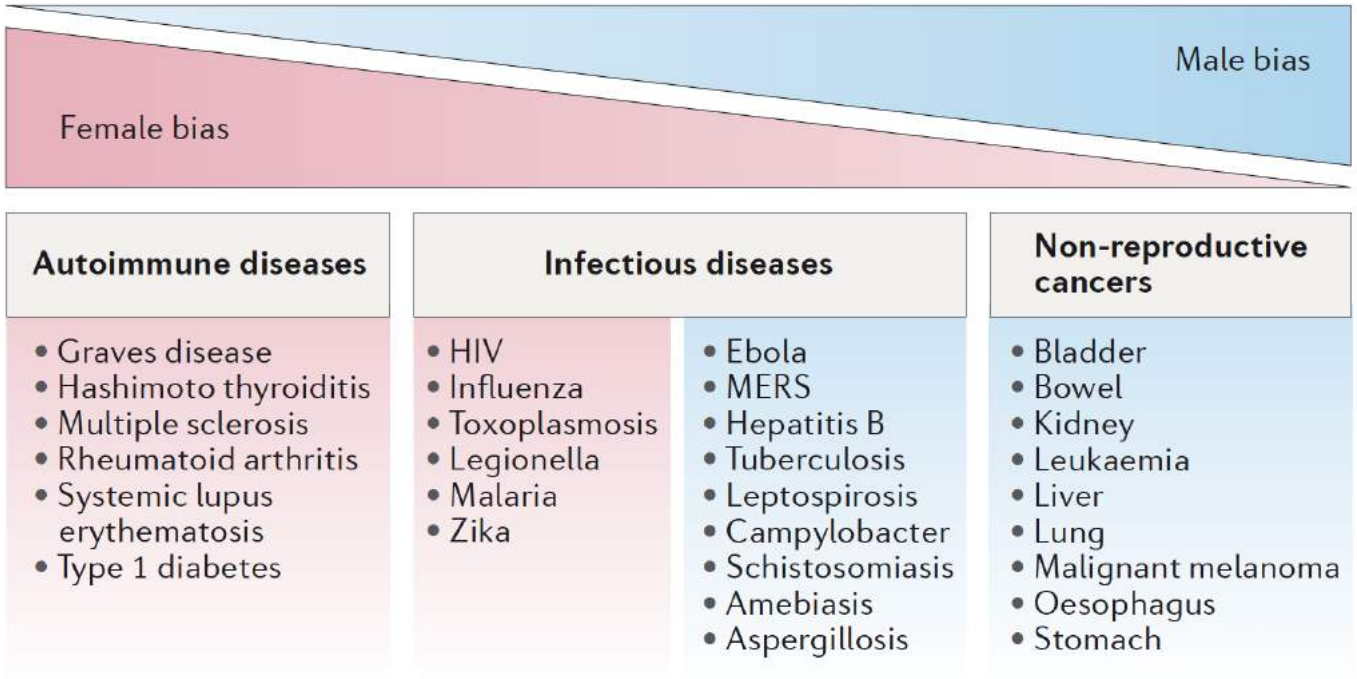
X-Chromosome Inactivation and Escape



- 5–25% of X-linked genes are known to escape X-inactivation (escapees).
- The expression levels of these genes are attributed to sex-dependent phenotypic variability.
- Mutations in escape genes are an especially common cause of XLID (and are often lethal in males eg *MECP2*)
- Autoimmune diseases, common in women, are likely caused by abnormal expression of escape genes.
- Abnormal escape gene dosage due to X aneuploidy contributes to a milieu of deleterious phenotypes including infertility, intellectual disability, immune diseases and cancer.

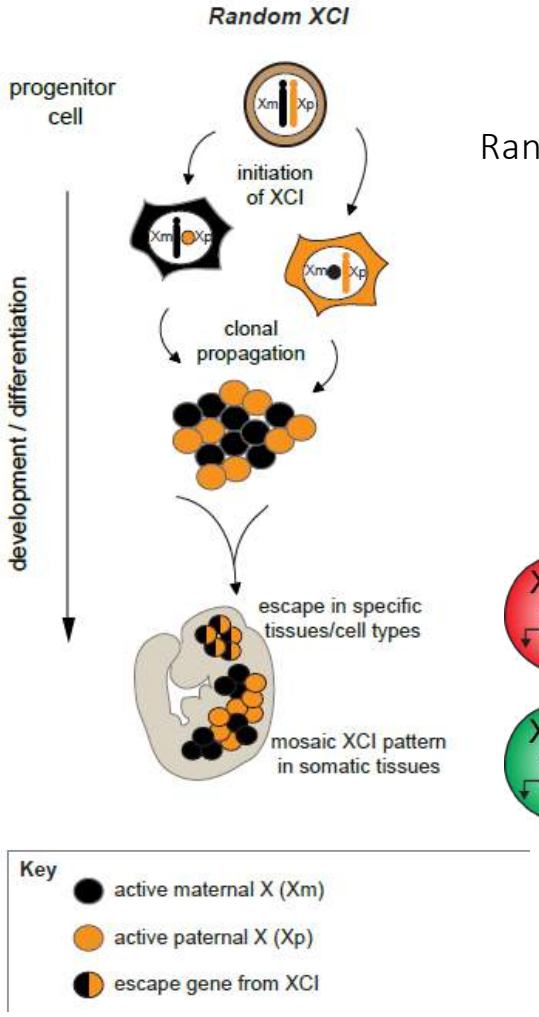
How, when, and where does escape from X inactivation occur?

How do escapees contribute to disease?

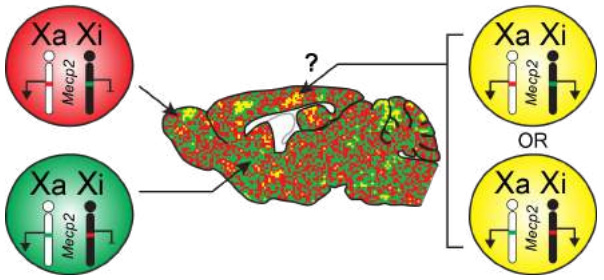


Gene silencing and escape during X inactivation

Females are cell mosaics
for X-linked gene expression



Random X-inactivation
(XCI)



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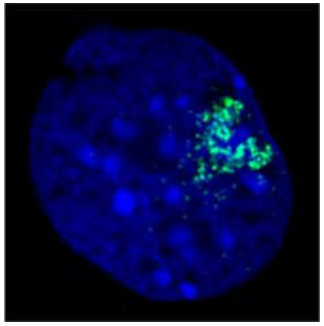
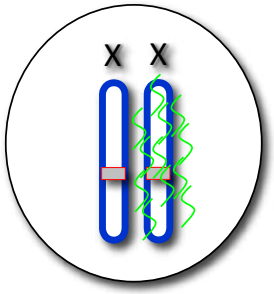
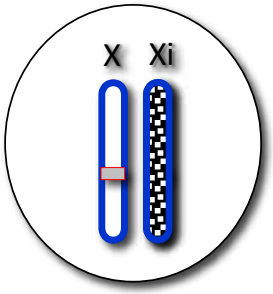
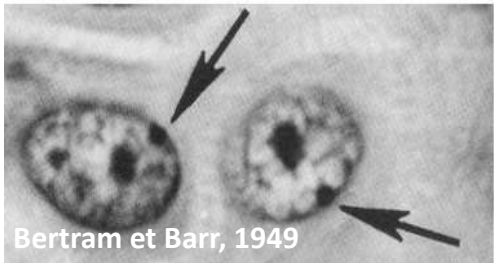
Gene silencing and escape during X inactivation

Xist RNA triggers the differential treatment of the two X chromosomes in same nucleoplasm









Initiation: the Xic

Xist - a multitasking lncRNA

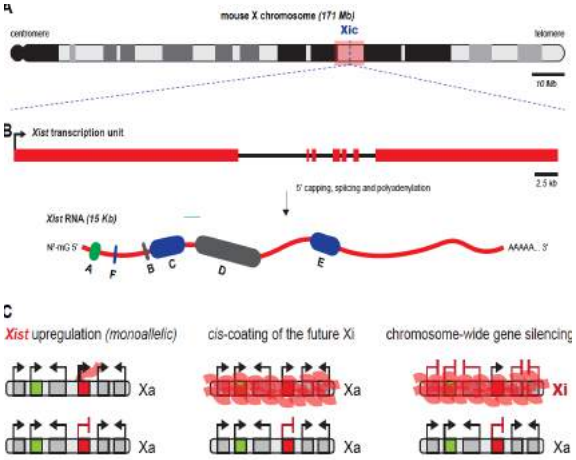
Xist lies in the Xic

Maintenance

Chromatin (Polycomb, DNA methylation)

Nuclear organisation, Asynchronous replication

Chromosome structure



E. Heard, March 27th 2023

COLLÈGE DE FRANCE 1530

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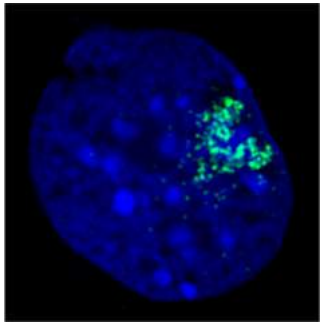

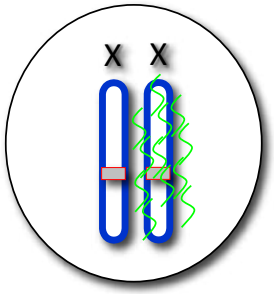

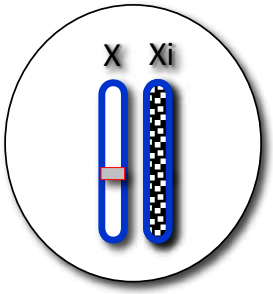

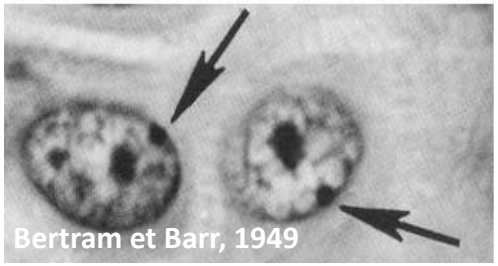

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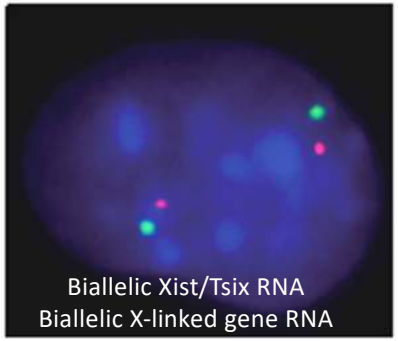

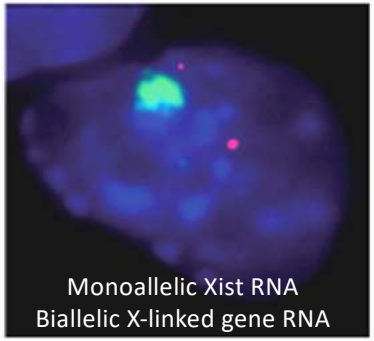

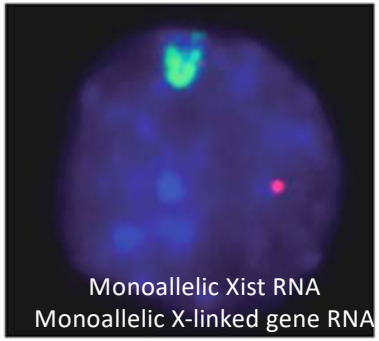

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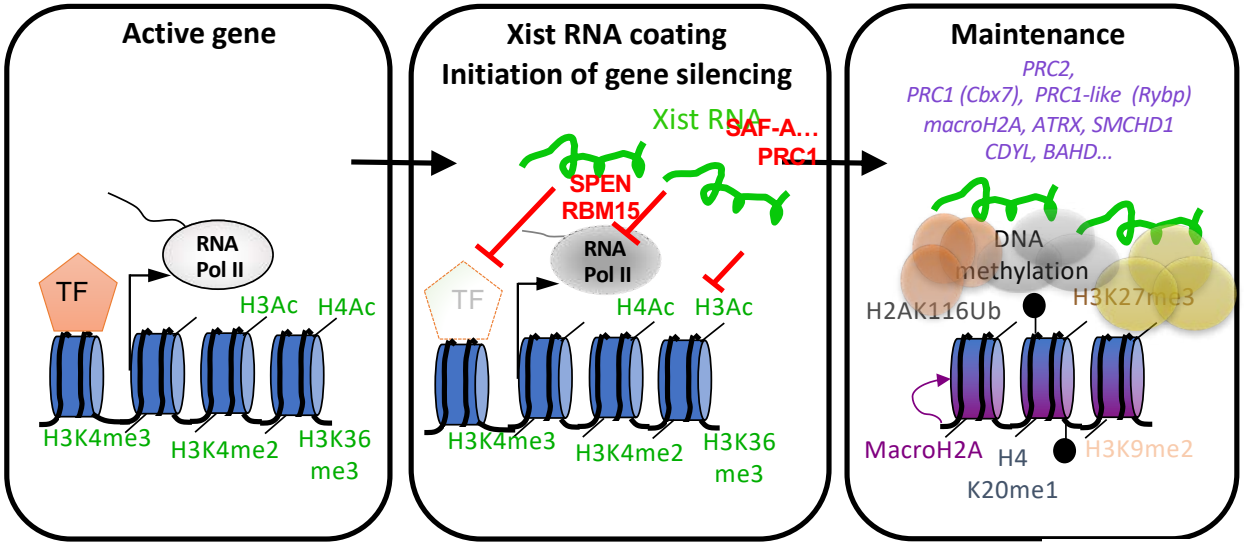
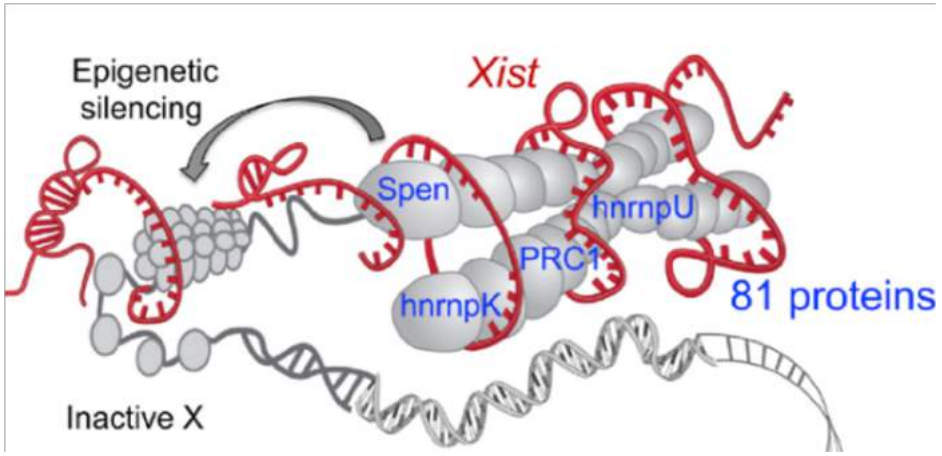
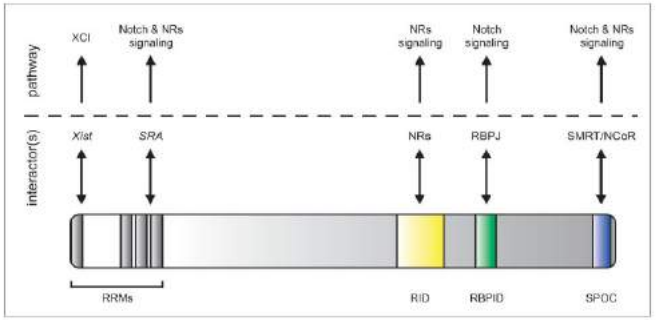
Xist RNA and it protein partners initiate XCI



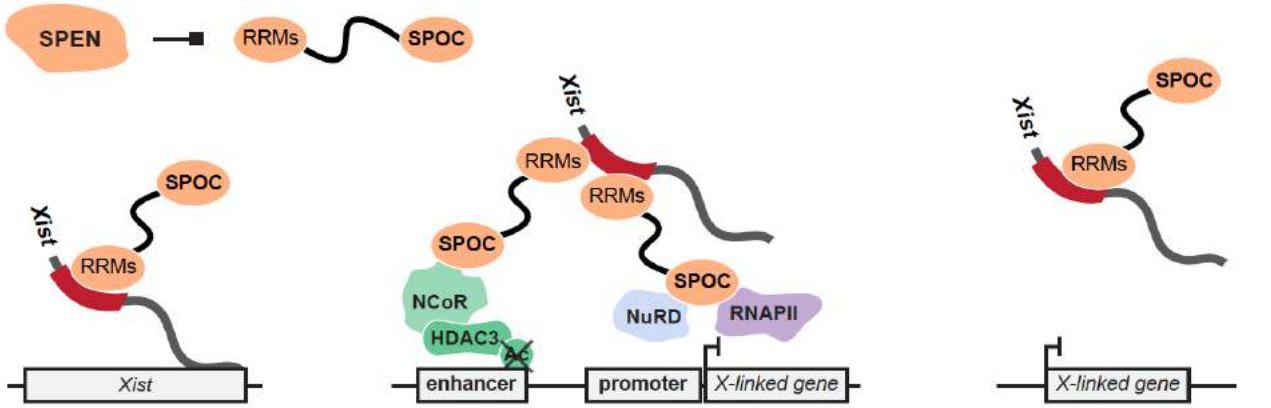




Chu et al, Cell 2015
McHugh et al, Nature 2015
Chen et al Science 2016
Minajigi et al, Science 2015
Moindrot et al, Cell Rep. 2015
Monfort et al, Cell Rep. 2015

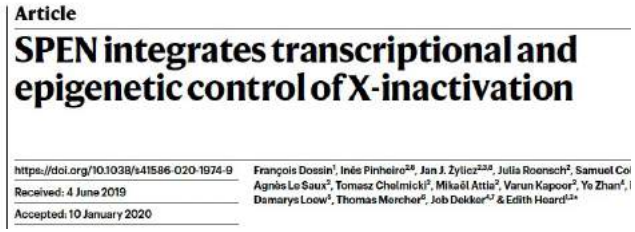
SPEN / SHARP : 3,664 a.a. protein
Implicated in RNA-directed transcriptional regulation in the context of hormone responsive nuclear receptor pathways

SPEN is a key regulator of gene silencing during initiation of X inactivation



upregulation of *Xist* | SPEN-dependent gene silencing | SPEN disengages silenced chromatin

SPEN is recruited by Xist RNA. Tethered to chromatin by its SPOC domain via RNA PolII CTD?

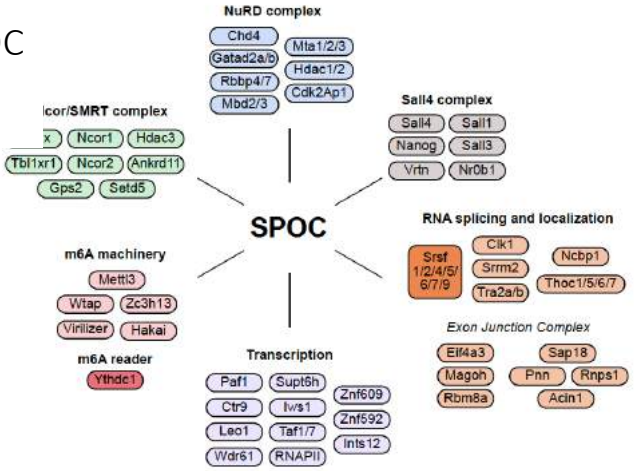


Article

SPEN integrates transcriptional and epigenetic control of X-inactivation

https://doi.org/10.1038/s41586-020-1974-9
Received: 4 June 2019
Accepted: 10 January 2020

François Dossin[1], Inés Pinheiro[2,8], Jan J. Zylicz[2,3,8], Julia Roensch[2], Samuel Co
Agnès Le Saux[2], Tomasz Cholmicki[2], Mikaël Attia[2], Varun Kapoor[2], Ya Zhan[4],
Damarys Loew[5], Thomas Mercher[6], Job Dekker[4,7] & Edith Heard[1,2*]



Dossin et al, Nature (2020)

SPEN is a key regulator of gene silencing in X inactivation: interacting with transcriptionally active promoters and enhancers and disengaging from chromatin as soon as gene silencing occurs

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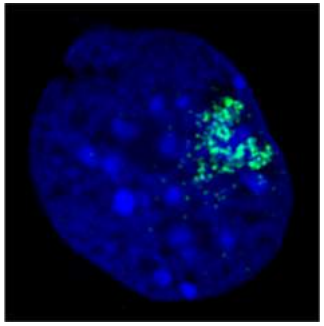
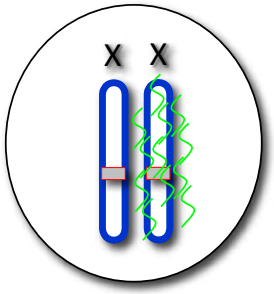
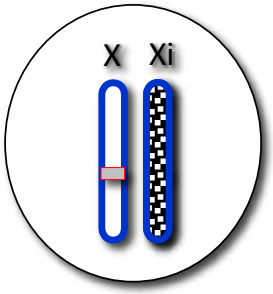
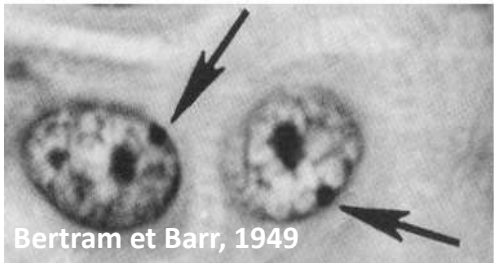
Gene silencing and escape during X inactivation

Differential treatment of the two X chromosomes is stably mitotically inherited









Initiation: the Xic

Xist - a multitasking lncRNA

Xist lies in the Xic

Maintenance

Chromatin (Polycomb, DNA methylation)

Nuclear organisation, Asynchronous replication

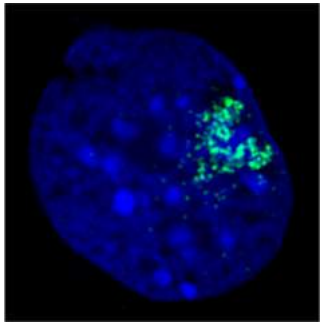
Chromosome structure

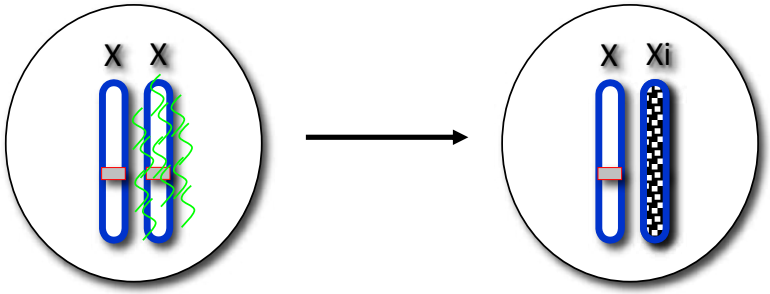
Stability of the inactive state

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

Gene silencing and escape during X inactivation

Differential treatment of the two X chromosomes is stably mitotically inherited

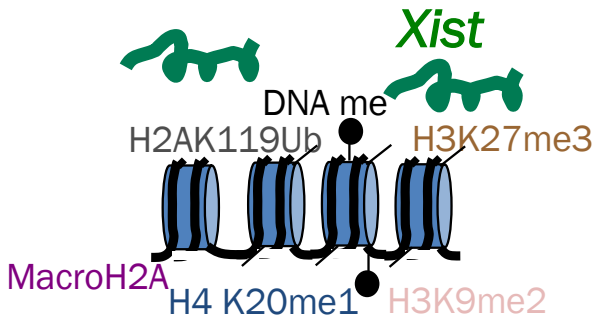


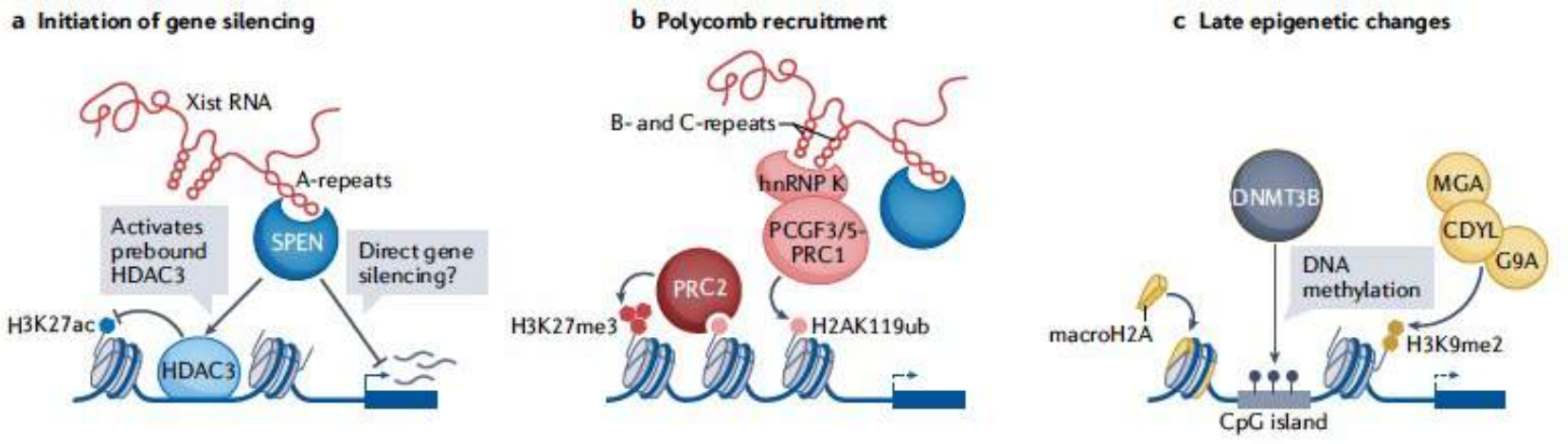


Initiation: the Xic

Maintenance

Maintenance of silencing





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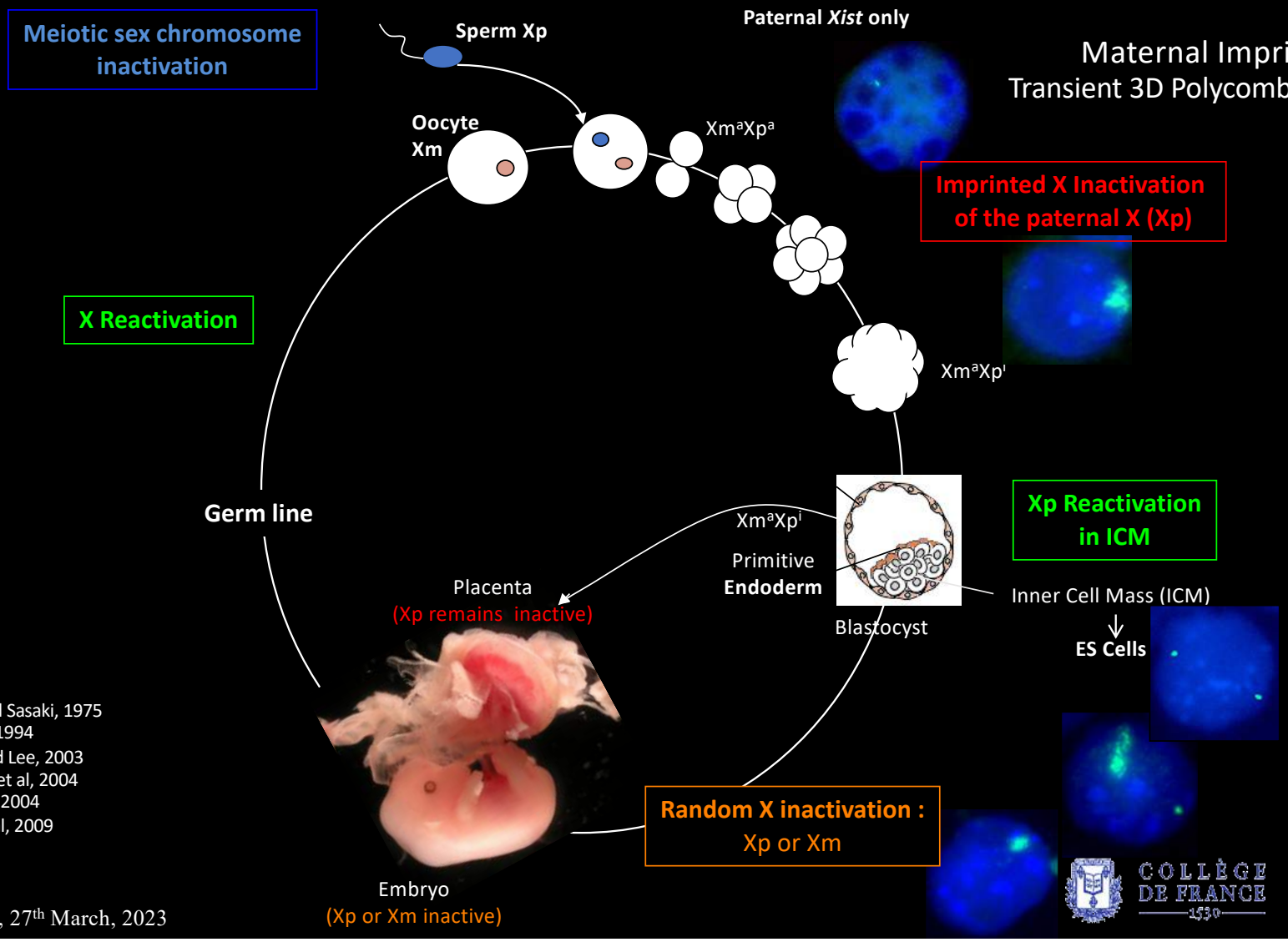
In vivo Dynamics of murine X inactivation



Takagi and Sasaki, 1975
Kay et al, 1994
Huyhn and Lee, 2003
Okamoto et al, 2004
Mak et al, 2004
Patrat et al, 2009

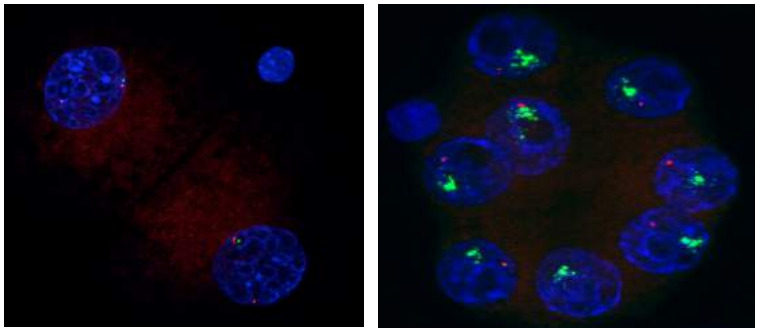
E. Heard, 27th March, 2023

COLLÈGE DE FRANCE 1530

Single cell allelic profiling of X-chromosome inactivation and reactivation in mouse embryos

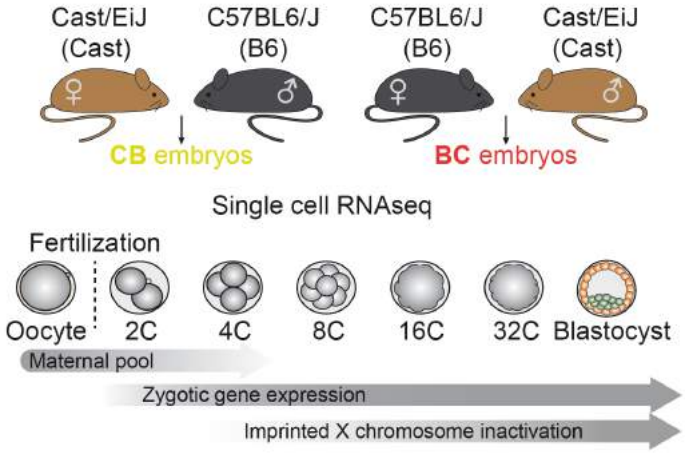
Ikuhiro Okamoto

RNA FISH:
Gene by gene analysis



Okamoto et al, Science 2004
Okamoto et al, Nature 2005
Patrat et al, PNAS 2009

Single cell RNA-seq analysis



M. Borensztein

Inter-species crosses
=> F1 embryos
19 Millions SNPs; 1 SNP/100bp
1 SNP/650bp for the X
(Frazer *et al*, Nature, 2007)

Borensztein *et al*. Xist-dependent imprinted X inactivation and the early developmental consequences of its failure. *Nature Structural & Molecular Biology* **24:**226-233 (2017)

Borensztein, Okamoto et al . Contribution of epigenetic landscapes and transcription factors to X-chromosome reactivation in the inner cell mass. *Nature Communications* 8:1297 (2017)

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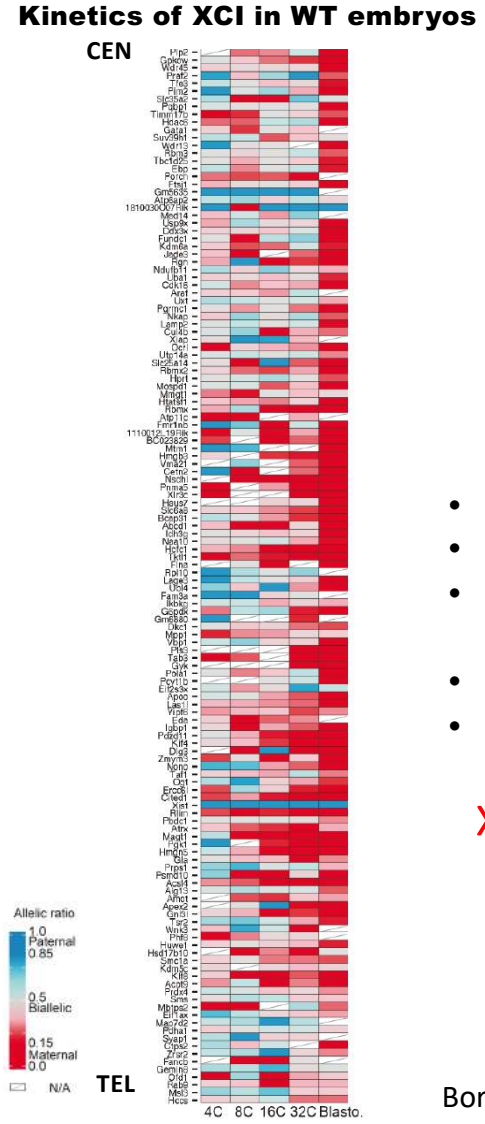
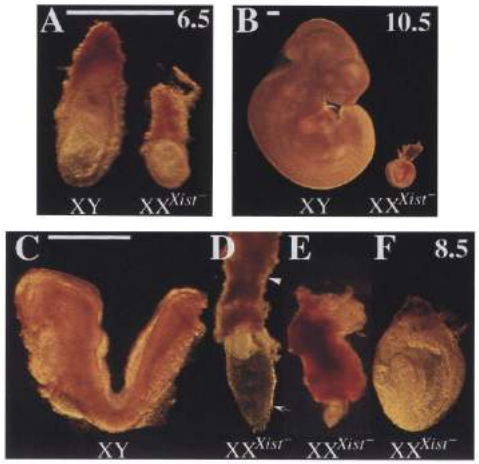
E. Heard, March 27th 2023

How does a lack of paternal *Xist* impact XCI and early female development?

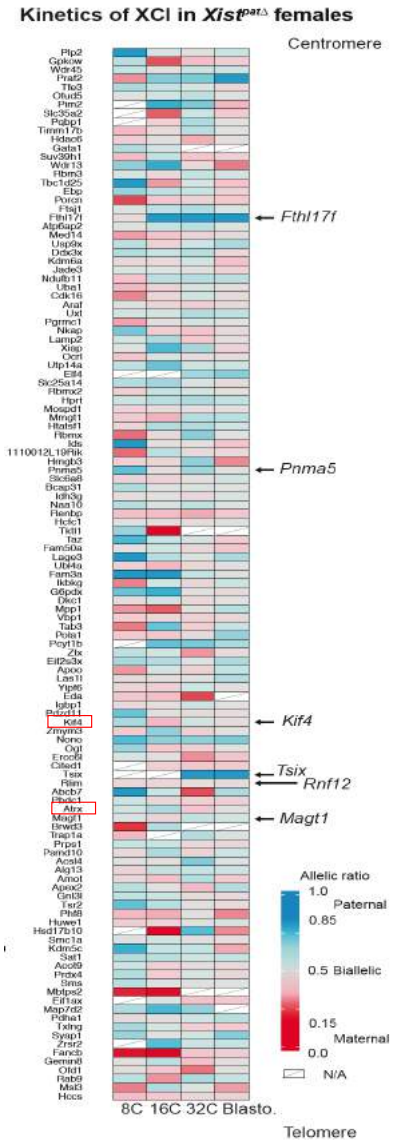




- No initiation of paternal X gene silencing
- No maternal *Xist* up-regulation
- Significant mis-regulation of X-linked and autosomal genes by 32-cell stage
- No dosage compensation
- Early post implantation lethality

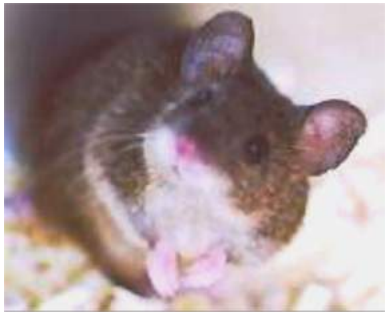
X inactivation is essential for early female development to proceed

Borensztein et al, *Nature Struct. Mol. Biol,* 2017



What about Human XCI?



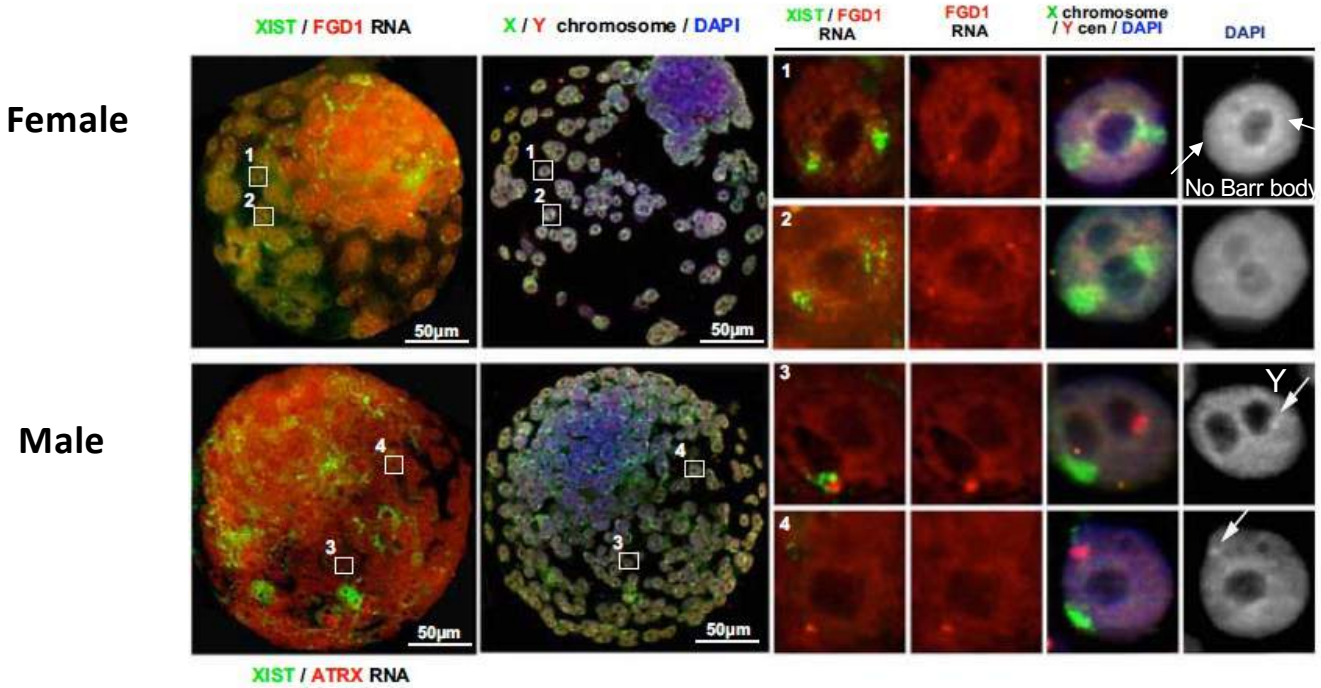


Very different modes of *XIST* Regulation and XCI kinetics in mice, rabbits and humans!

Okamoto et al, 2011, *Nature* 472 : 370-374

Pre-implantation is morphologically similar in eutherians
Yet developmental timing, gene expression and signalling requirements for pluripotency and lineage segregation are very different…

What about Human XCI?



- No imprinted *XIST* regulation in human or rabbit embryos
- *XIST* up-regulation from both Xs then resolution to one X
- In humans, XIST RNA accumulates in male and female embryos in TE and ICM cells but genes are biallelically expressed (based on nascent RNA FISH)
- No signs of H3K27me3 or Barr body **up to day 7** (Teklenburg et al, 2012)
- No inactivation and reactivation of the Xi in the ICM…relationship with pluripotency factor network must be quite different in humans and rabbits
- Primate-specific XACT lncRNA prevents efficient XIST coating? (Vallot et al, 2016)
- X-chromosome « dampening »: partial gene repression of both Xs early on?(Petropoulos et al, 2016)

Okamoto et al, 2011, *Nature* 472 : 370-374

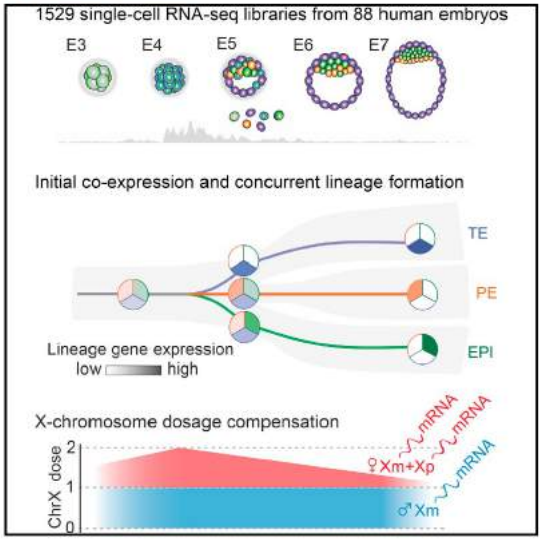
Dampening of both Xs prior to monoallelix XCI in Humans?

Resource

Cell

Single-Cell RNA-Seq Reveals Lineage and X Chromosome Dynamics in Human Preimplantation Embryos

Graphical Abstract



Authors

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In Brief

A comprehensive transcriptional map of human preimplantation development reveals a concurrent establishment of trophectoderm, epiblast, and primitive endoderm lineages and unique features of X chromosome dosage compensation in human.

Highlights

- Transcriptomes of 1,529 individual cells from 88 human preimplantation embryos
- Lineage segregation of trophectoderm, primitive endoderm, and pluripotent epiblast
- X chromosome dosage compensation in the human blastocyst

Complete XCI dynamics during *in vivo* embryogenesis in Macaque

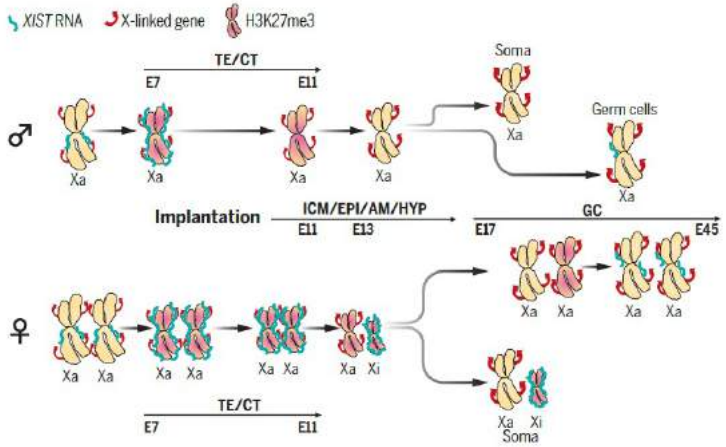
Both Xs have XIST coating, H3K27me3 and H2AK119u1 enrichment, and a compacted structure for up to 17 days

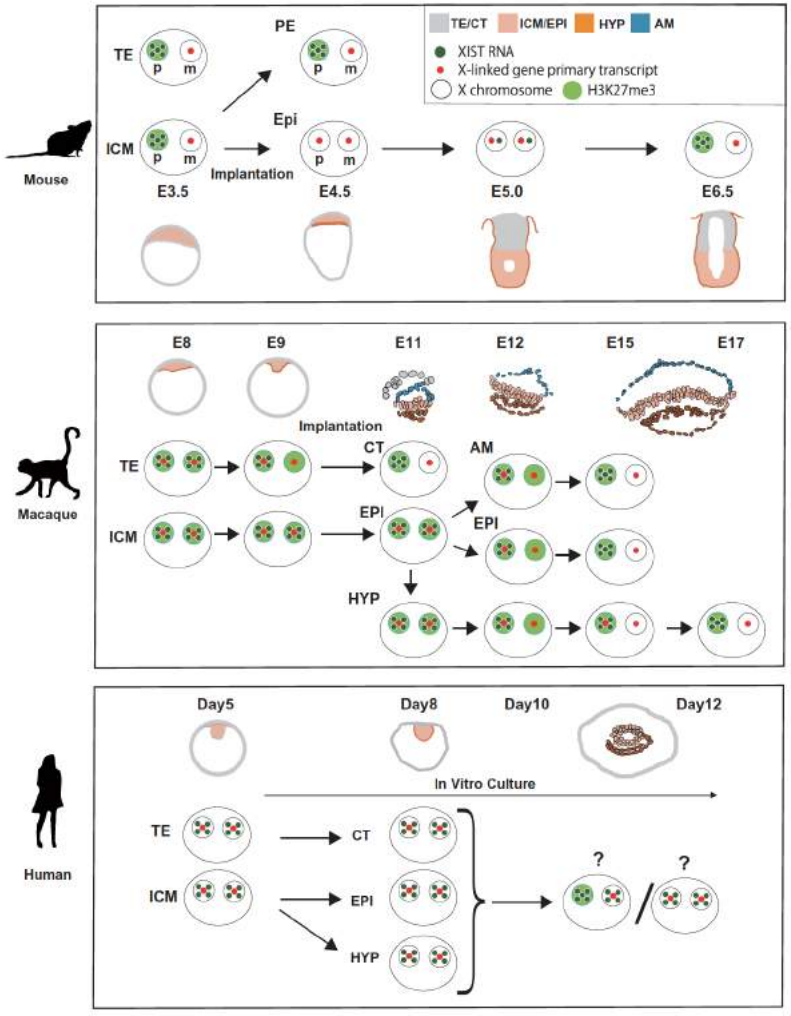
An active intermediate persists after implantation in the cynomolgus epiblast

Very divergent from mice in timing, and order of events. Also not identical to humans!

Different mechanism(s) for triggering chromosome-wide XCI in primates?

X chromosome ""dampening" of both Xs may not be a major mechanism for dosage compensation





Okamoto et al (2021) The X chromosome dosage compensation program during the development of cynomolgus monkeys. Science 374(6570)

Very diverse dosage compensation strategies even between mammals

Monotremes

Stochastic – partial
Monoallelic

Marsupials

Imprinted - leaky
Rsx RNA?

Eutherians

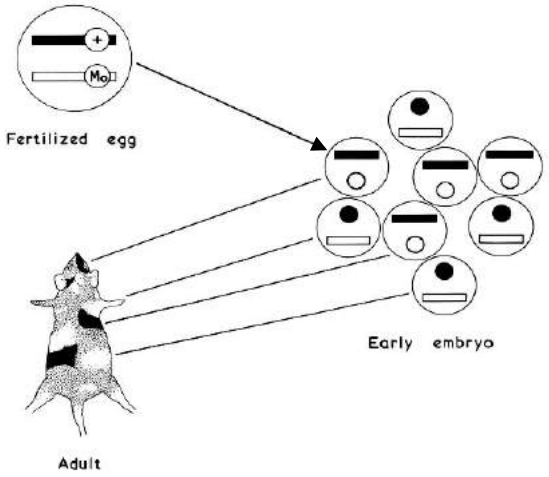
Imprinted & Random
Xist RNA

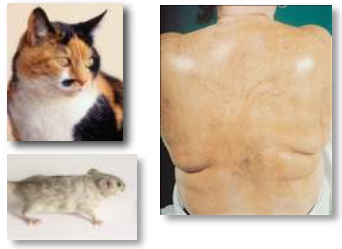
Random
Xist RNA

Random
XIST RNA

Mary Lyon

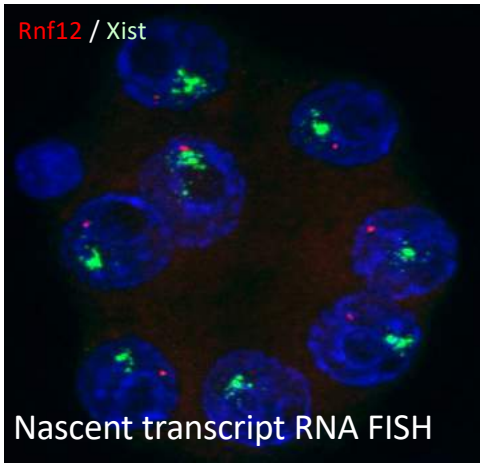
Gene Action in the X-chromosome of the Mouse (1961)

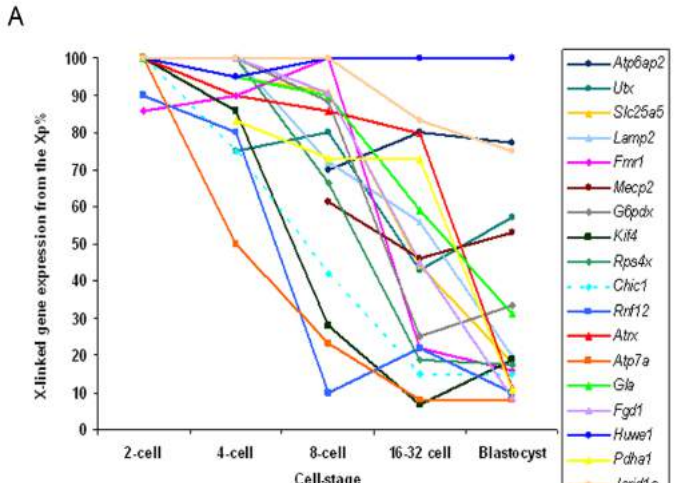


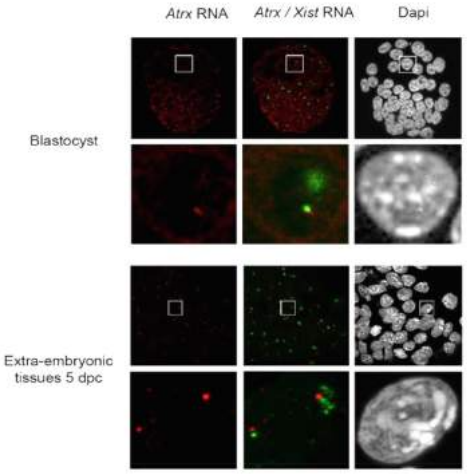


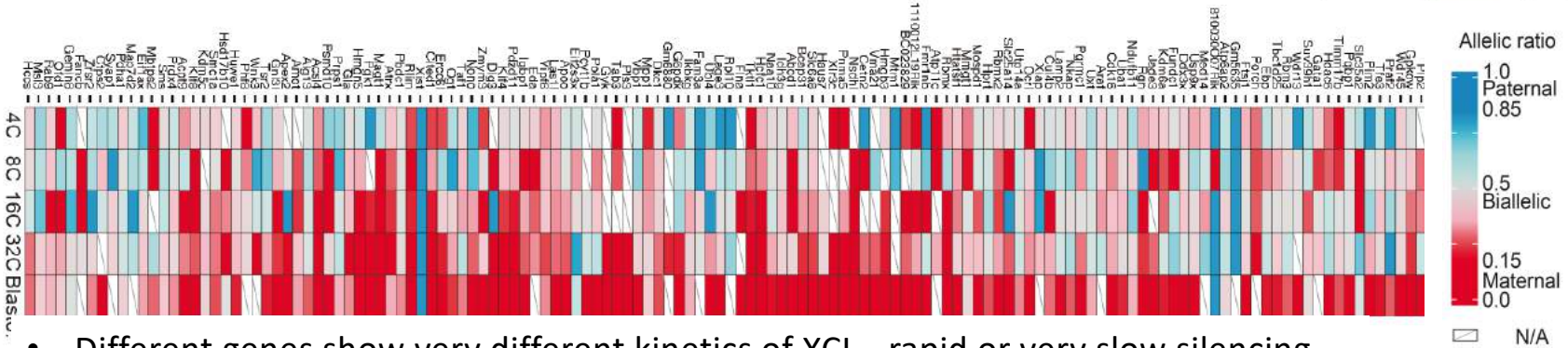
Lyon, M. F. (1961), Gene Action in the X-chromosome of the Mouse (Mus musculus L.) *Nature* 190: 372-3.

Gene Silencing and escape from XCI during mouse development









- Different genes show very different kinetics of XCI – rapid or very slow silencing
- Some genes show escape from the outset (eg *Utx, Jarid1c*)
- Others are inactivated and then reactivated in specific lineages (eg *Atrx*)
- Global Xi reactivation happens in the inner cell mass of the murine blastocyst

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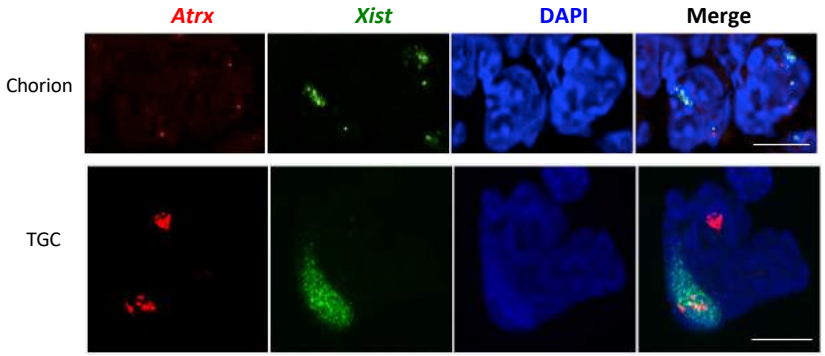
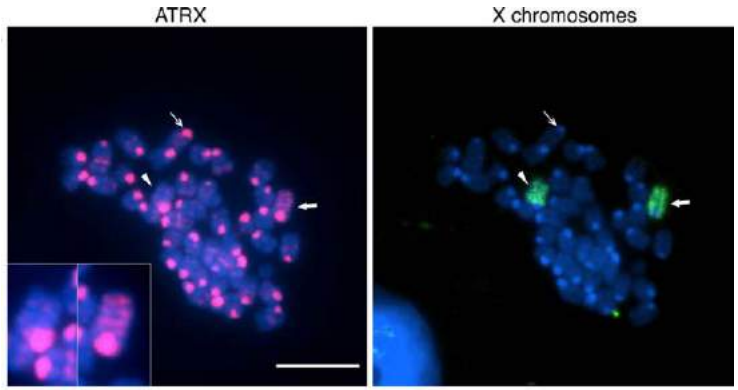
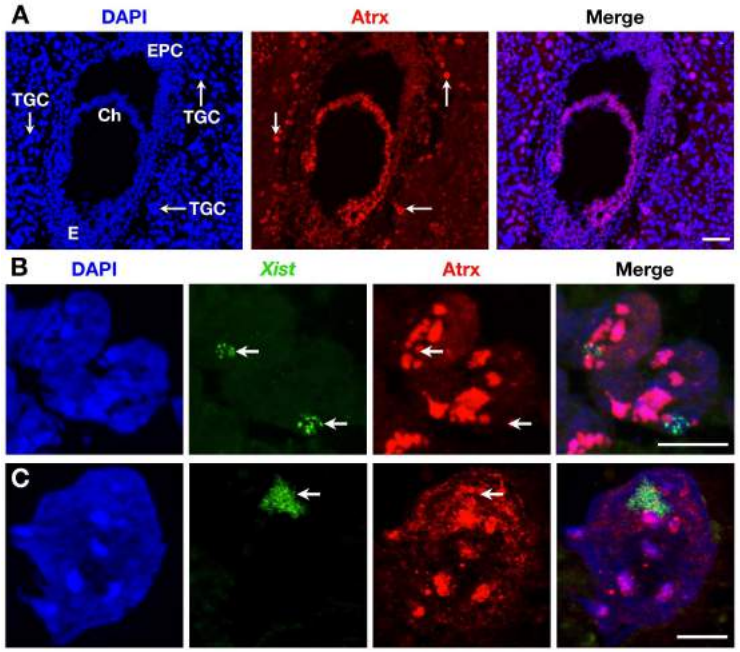
Atrx escape from XCI may be required for imprinted XCI



Atrx protein is highly expressed in Trophoblast Giant Cells, where the gene escapes from XCI.

Corbel et al, Development 2013





Atrx marks the inactive X in Trophoblast Stem cells.

Baumann and De La Fuente, 2009

Loss of Atrx causes Trophoblast failure and is associated with escape from imprinted XCI.

Garrick et al, 2010

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In vivo Dynamics of murine X inactivation

Meiotic sex chromosome inactivation

Sperm Xp

Oocyte Xm

Xm^aXp^a

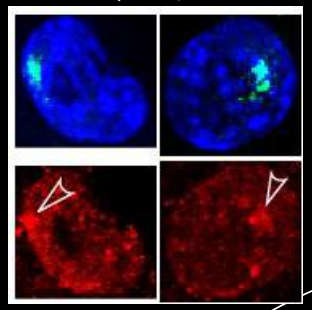
Paternal *Xist* only

Maternal Imprint
Transient 3D Polycomb domain

Imprinted X Inactivation of the paternal X (Xp)

Xm^aXp^i

PRC2 and PRC1 are required for maintenance (Masui, Corbel et al 2023)



nature cell biology

Article https://doi.org/10.1038/s41556-022-01047-y

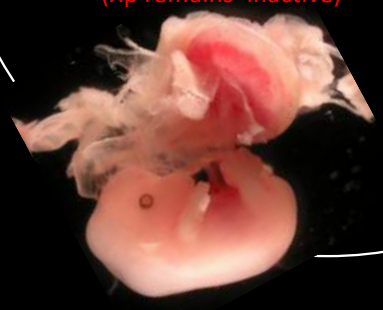
Polycomb repressive complexes 1 and 2 are each essential for maintenance of X inactivation in extra-embryonic lineages

Received: 28 April 2021
Accepted: 8 November 2022
Published online: 12 January 2023

Osamu Masui[1,2,9], Catherine Corbel[3,9], Koji Nagao[4], Takaho A. Endo[5], Fuyuko Kezuka[1], Patricia Diabangouaya[3], Manabu Nakayama[6], Mami Kumon[1], Yoko Koseki[1], Chikashi Obuse[4], Haruhiko Koseki[1,2] & Edith Heard[3,7,8]

(no DNA methylation)

Placenta
(Xp remains inactive)



Embryo
(Xp or Xm inactive)

Xm^aXp^i

Primitive **Endoderm**

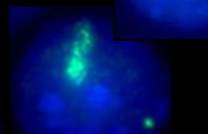
Blastocyst

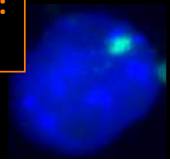
Xp Reactivation in ICM

Inner Cell Mass (ICM)

↓

ES Cells





Random X inactivation :
Xp or Xm

Takagi and Sasaki, 1975
Kay et al, 1994
Huyhn and Lee, 2003
Okamoto et al, 2004
Mak et al, 2004
Patrat et al, 2009
Masui, Corbel et al, 2023

E. Heard, 27th March, 2023

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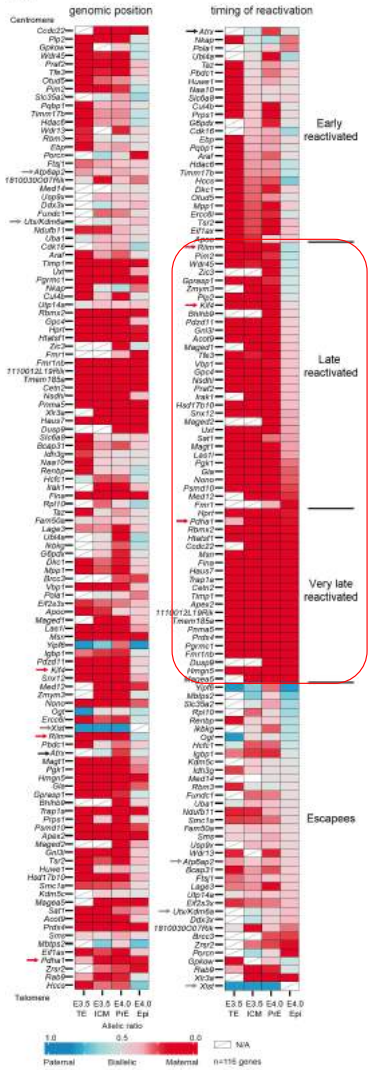
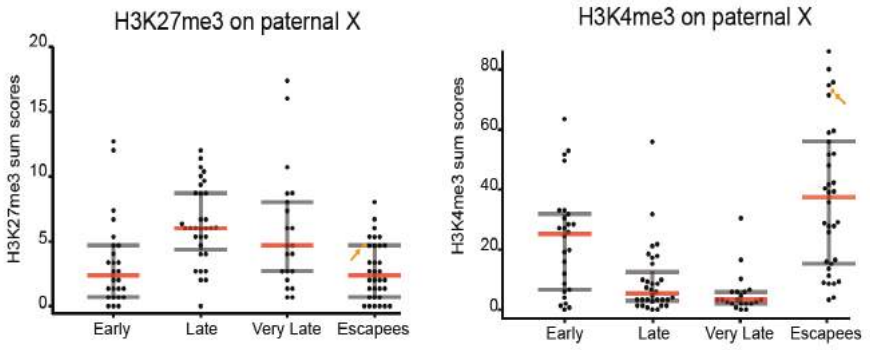
Different genes show very different kinetics of Xi reprogramming

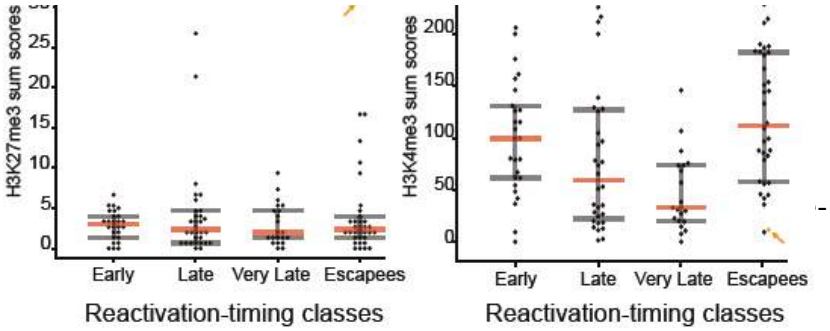


E. Heard, March 27th 2023

Epigenetic "memory" at some regions of the Xp?



Late-reactivated genes are significantly enriched in H3K27me3 on the paternal X



Zheng, H. *et al.* Resetting Epigenetic Memory by Reprogramming of Histone Modifications in Mamma[illegible] *Mol. Cell* **63,** 1066–1079 (2016).

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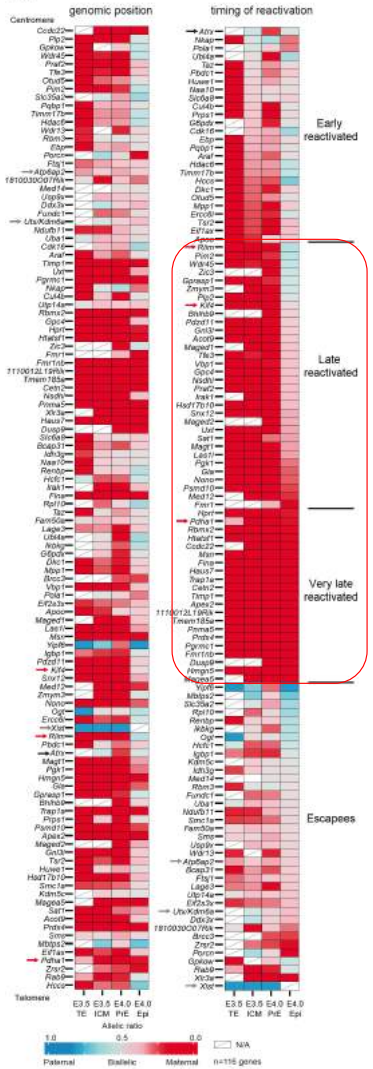
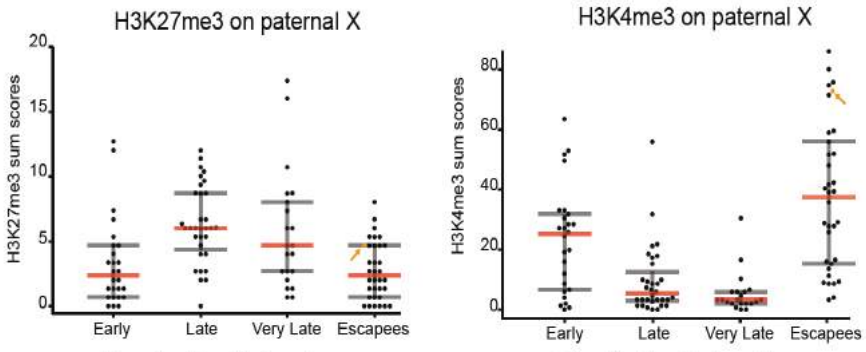


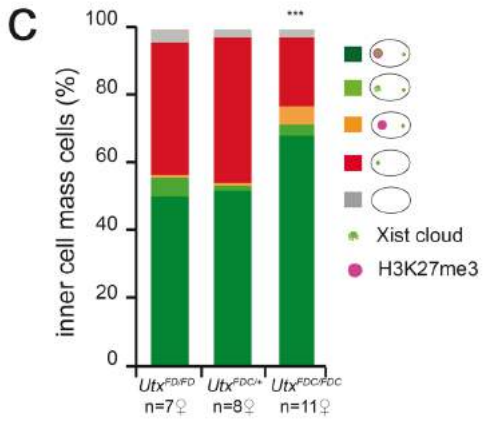
Differences in epigenetic “memory” at different regions of the X?

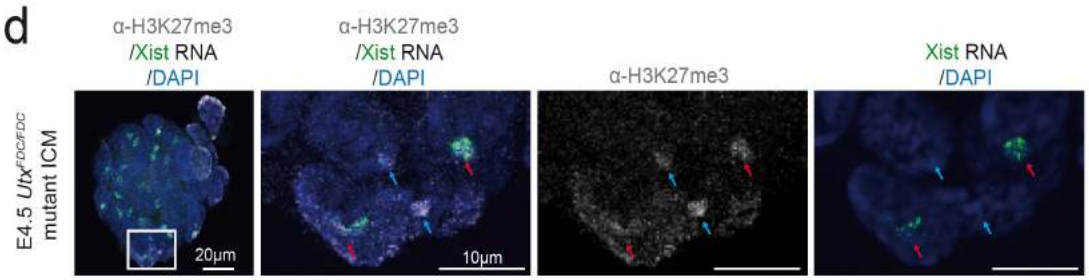


How is this repressive epigenetic memory removed from late-reactivated genes?

Is it lost passively (cell division) or is it actively erased (eg histone demethylase)?

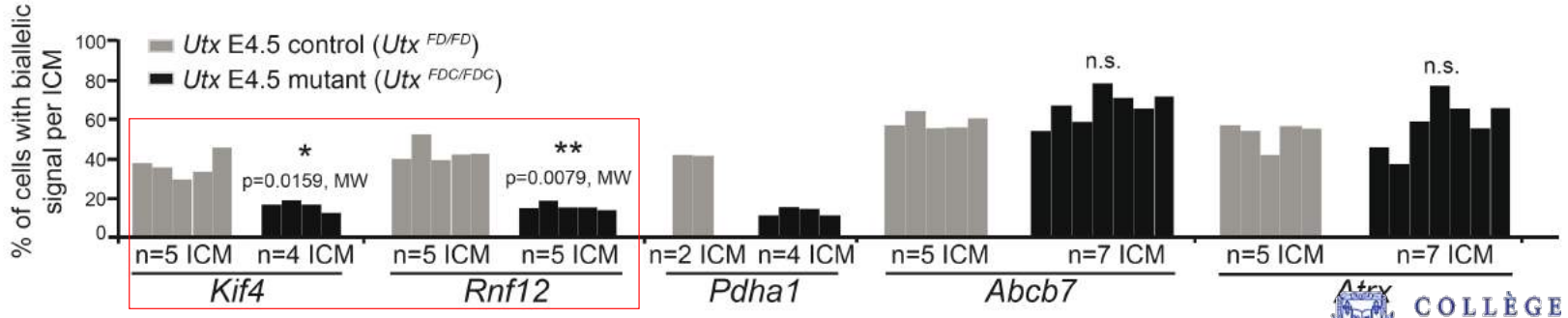
The escapee Utx/Kdm6a facilitates loss of epigenetic silencing at some loci during Xp reactivation in the ICM





In Utx mutant E4.5 female blastocysts:

- H3K27me3 remains aberrantly enriched on the Xp
- Only late-reactivated genes are affected by absence of *Utx*
- Observe <u>even slower reactivation</u> of X-linked genes that have an epigenetic memory associated with H3K27me3



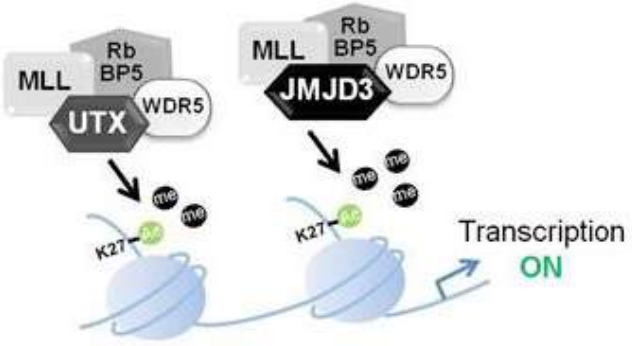
Borensztein, Okamoto et al (2017) Nature Communications 8:1297

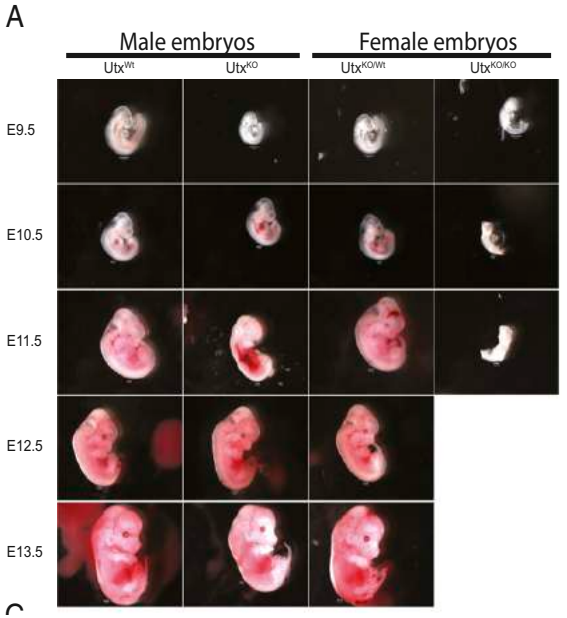
The escapee Utx/Kdm6a H3K27me3 demethylase: A developmental role in Xi reactivation and multiple disease roles

Kdm6a /Utx:

- H3K27 demethylase
- Ubiquitously expressed in embryos & somatic tissues
- Escapes X-chromosome inactivation
- Gender-specific tumor suppressor in T-cell acute lymphoblastic leukemia
- Sex-specific earlier lethality observed in *UTX* deleted mice (Jaenisch, Magnuson and Hanna labs)

Yoo *et al*, 2012





Welstead *et al*, PNAS, 2012

In Utx mutant E4.5 female blastocysts:

- H3K27me3 remains aberrantly enriched on the Xp
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- Observe <u>even slower reactivation</u> of X-linked genes that have an epigenetic memory associated with H3K27me3

Borensztein, Okamoto et al (2017) "Contribution of epigenetic landscapes and transcription factors to X-chromosome reactivation in the inner cell mass". Nature Communications 8:1297

Kdm6a/UTX controls NK cell-intrinsic sex differences

nature immunology

Article

https://doi.org/10.1038/s41590-023-01463-8

The X-linked epigenetic regulator UTX controls NK cell-intrinsic sex differences

Received: 27 April 2022

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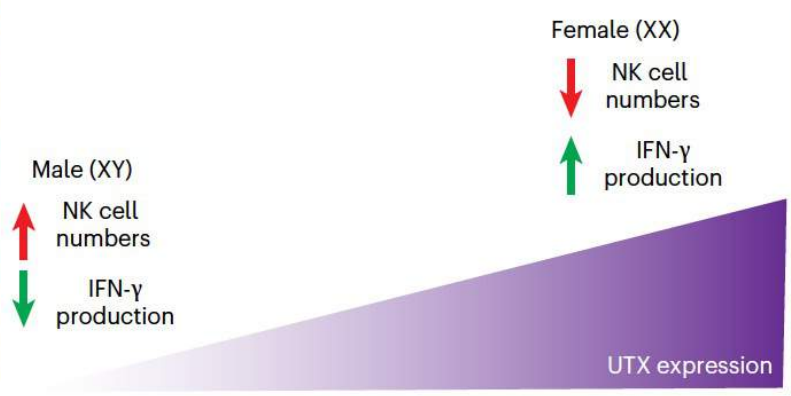
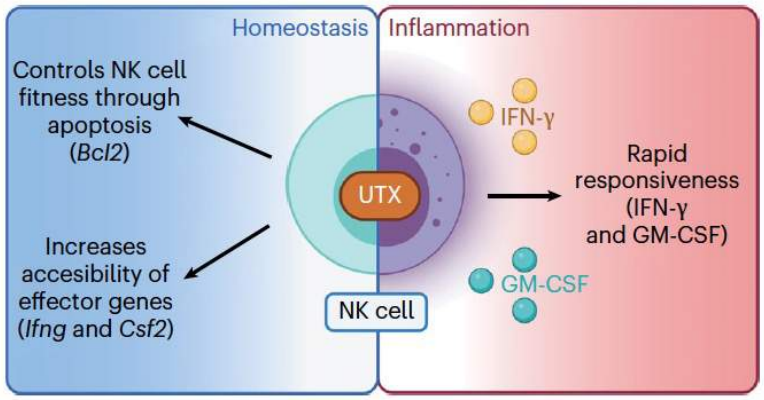
Published online: 16 March 2023

Check for updates

Mandy I. Cheng[1,2], Joey H. Li[1,2], Luke Riggan[1,2,3], Bryan Chen[1], Rana Yakhshi Tafti[1,2], Scott Chin[1], Feiyang Ma[3,4], Matteo Pellegrini[3,4], Haley Hrncir[5], Arthur P. Arnold[5], Timothy E. O'Sullivan[1,2] & Maureen A. Su[1,2,6]

Mice with NK cell-intrinsic UTX deficiency showed increased lethality to mouse cytomegalovirus. Integrative multi-omics analysis revealed a role for UTX in regulating chromatin accessibility and gene expression critical for NK cell homeostasis and effector function





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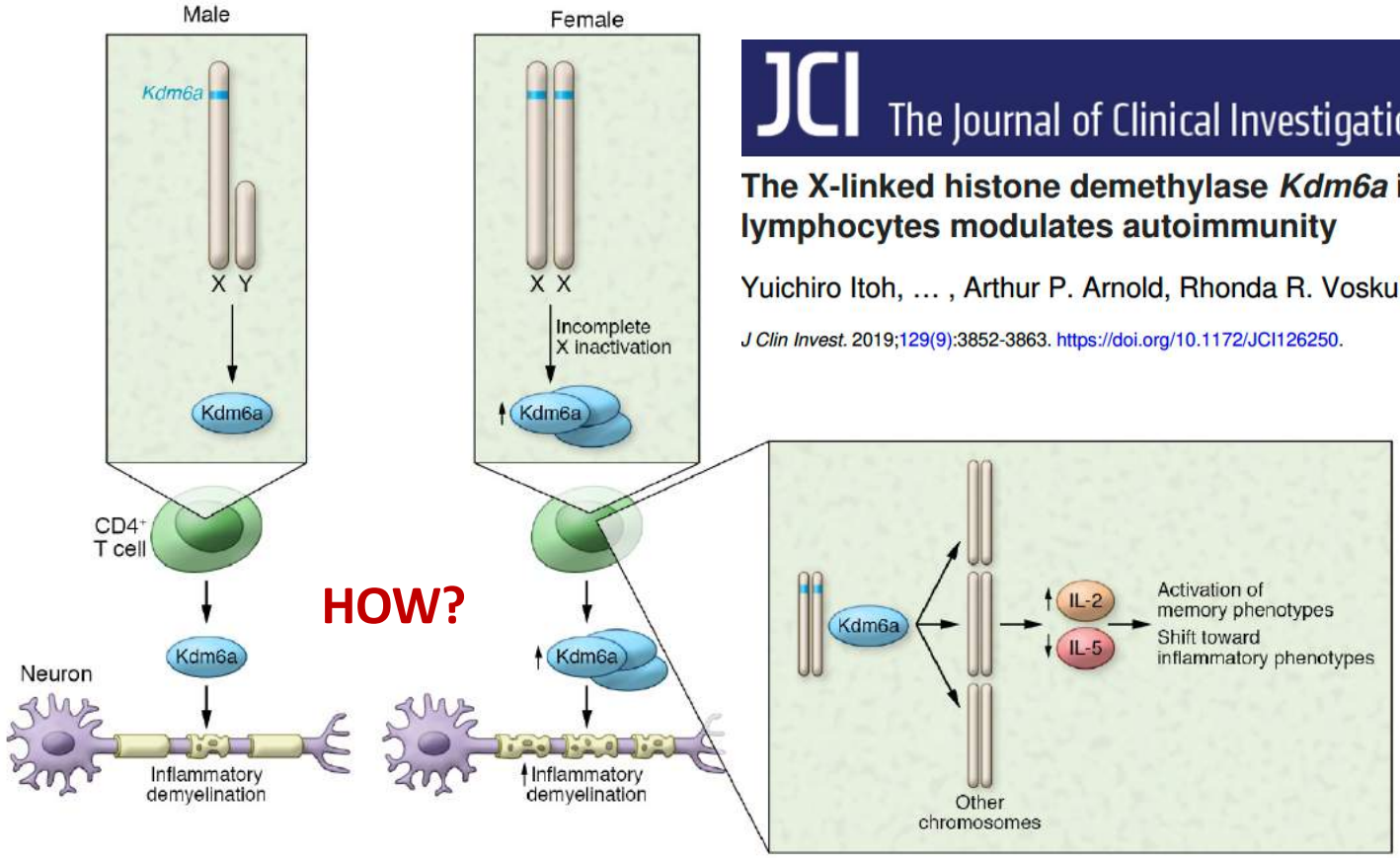
Role of Kdm6a/UTX an X-linked escapee and histone demethylase modulates autoimmunity in CD4+ T lymphocytes

Role of Kdm6a dosage in female-biased Multiple Sclerosis?



JCI The Journal of Clinical Investigation

The X-linked histone demethylase *Kdm6a* in CD4+ T lymphocytes modulates autoimmunity

Yuichiro Itoh, … , Arthur P. Arnold, Rhonda R. Voskuhl

J Clin Invest. 2019;129(9):3852-3863. https://doi.org/10.1172/JCI126250.

Wu, JCI 2019 "X-tra X: An escape to autoimmunity"

Role of Kdm6a/UTX an X-linked escapee and histone demethylase is a tumor suppressor that protects females from B-cell lymphomas

ARTICLE
DOI: 10.1038/s41467-018-05084-w OPEN

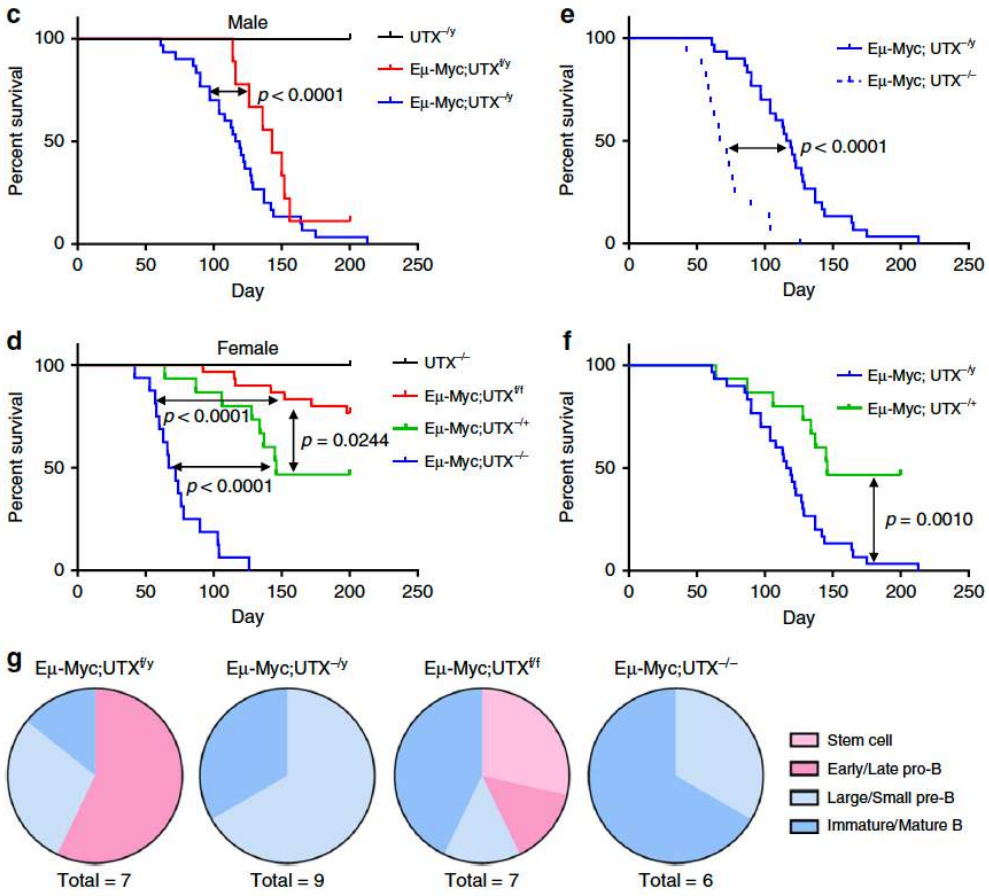
UTX is an escape from X-inactivation tumor-suppressor in B cell lymphoma

Xiaoxi Li[1,2], Yanli Zhang[1], Liting Zheng[3], Mingxian Liu[1], Charlie Degui Chen[3] & Hai Jiang[1]

The "EXITS hypothesis" of cancer sex bias

Escape from X inactivation Tumour Suppressor

- Up to 25% of X-linked genes escape XCI "constitutively" or "facultatively".
- Several constitutive escapees (e.g. *KDM6A/UTX*, *KDM5C/JARID1C*, *DDX3X*) are known tumour suppressors.
- TSG XCI escape endows female cells with both higher expression and "buffering" against loss-of-function mutation, compared with male ones.



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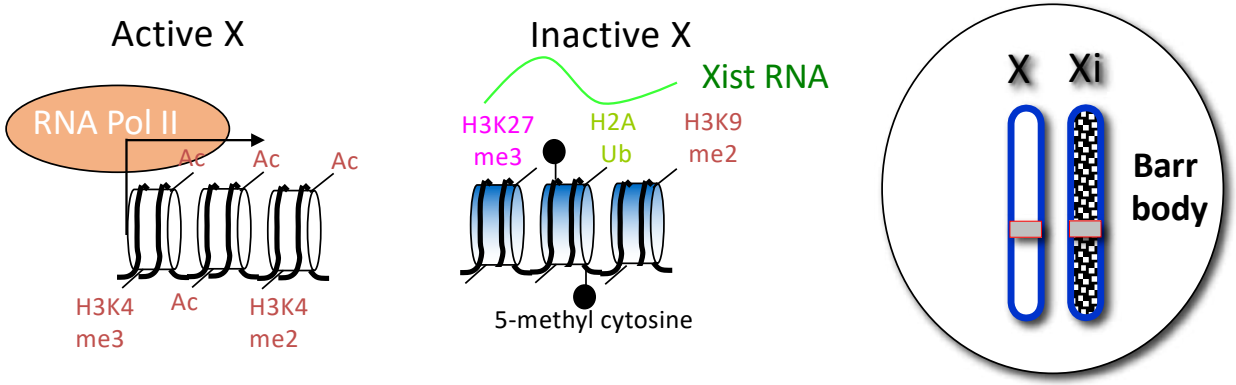
How do genes escape XCI?

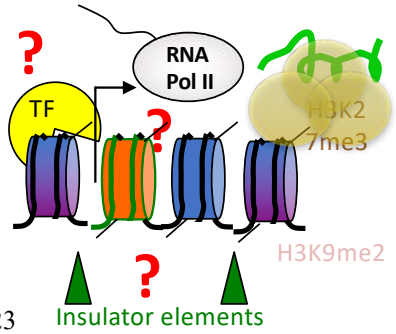


Remarkable stability of the inactive state

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

Constitutive escape from XCI

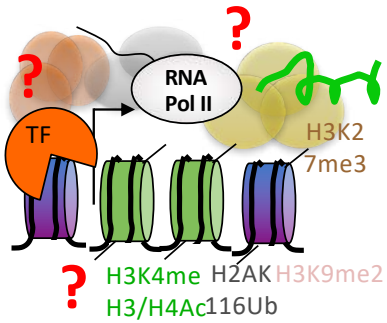
(eg *Jarid1c, Utx*)

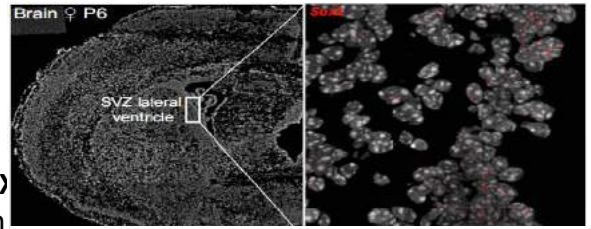


Facultative escape:

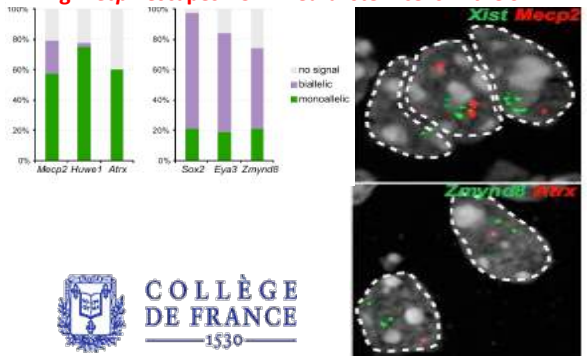
Silencing then reactivation from the X

(eg *Atrx* in trophoblast; *Mecp2* in brain





Eg *Mecp2* escapes XCI in neural stem cells in the SVZ



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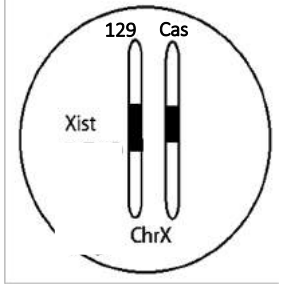
Constitutive escape is present throughout XCI
Facultative escape arises stochastically and is then stably propagated

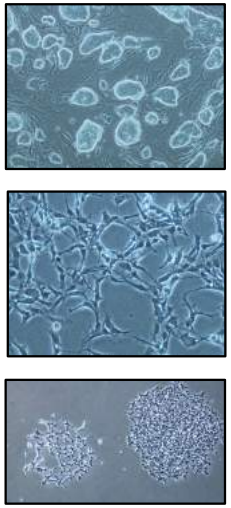


$Xa^{129}Xa^{Cas}$

$Xa^{129}Xi^{Cas}$

or

$Xi^{129}Xa^{Cas}$



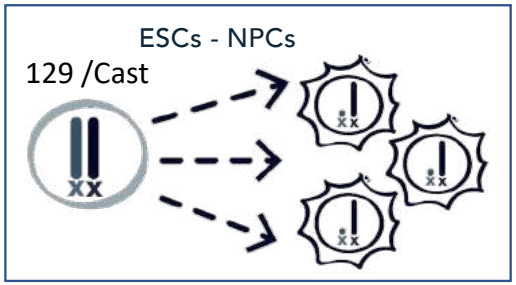
Highly polymorphic F1 Hybrid ES cell line
(129xCast or B6xCast => 1 SNP /~100bp)

Differentiation (Conti et al, 2005)
X-chromosome inactivation

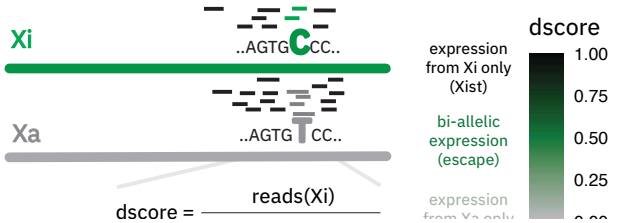
Neural Progenitor cells

Plate low density
Generation of clonal cell lines

NPC clone isiolation
RNA Seq – allele-specific analysis of gene expression



Allele-specific
RNA-Seq

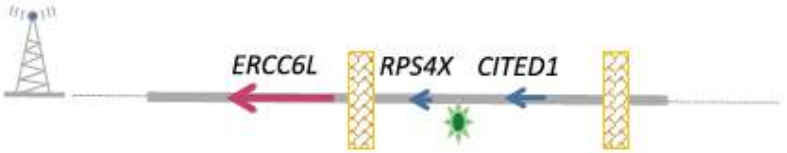


Antonia Hauth
Agnese Loda
Yuvia Alheli Perez Rico
Nicolas Servant

Clonal variation in escape from XCI

(Gendrel et al, 2014, Hauth et al in prep)

How do genes escape XCI?



Waystations:

Evidence:

- Less silencing on autosomes
- Able to spread over 200kb

Proposed Factors:

- LINE retroelements
- Polycomb-enriched regions

Escape Element:

Evidence:

- BAC integration of *Kdm5c*
- BAC integration of *RPS4X*
- Conservation of escape genes

Proposed Factors:

- YY1
- CTCF

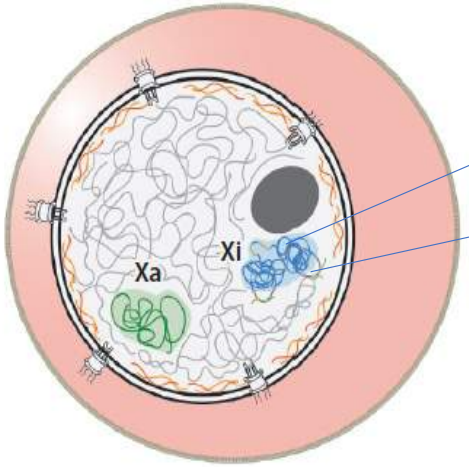
Boundary Element:

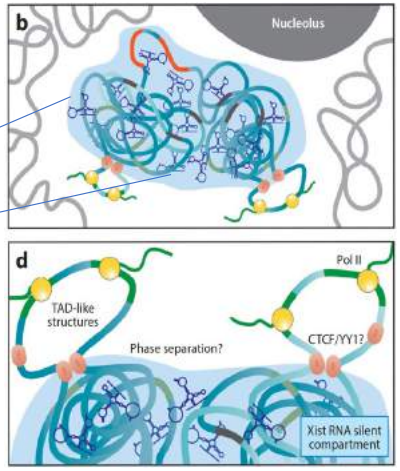
Evidence:

- Truncation of BAC integration of *Kdm5c* 'spread' escape
- CTCF element missing in human large domains
- Correlation with TADs

Proposed Factors:

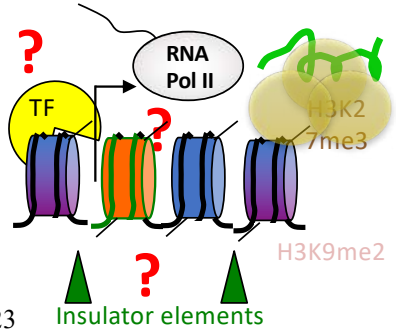
- CTCF
- TAD boundaries



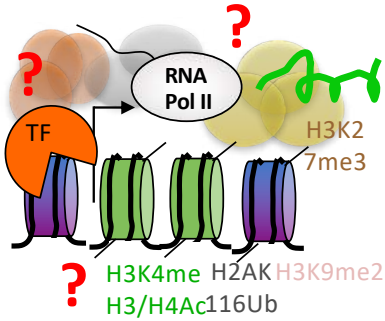


From Dossin and Heard, 2021

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



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



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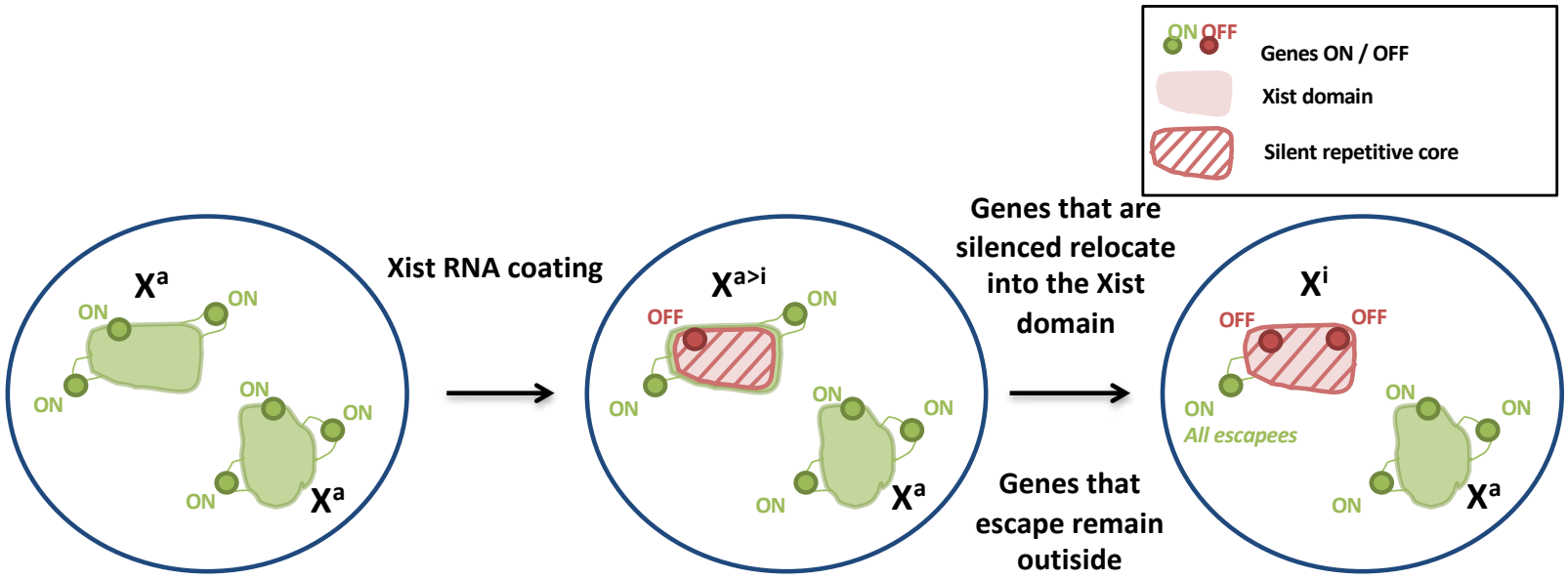
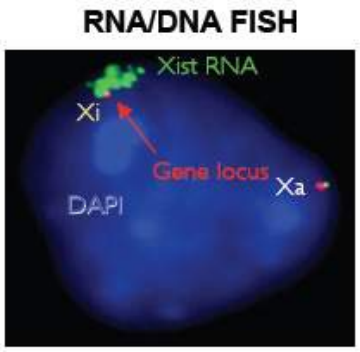
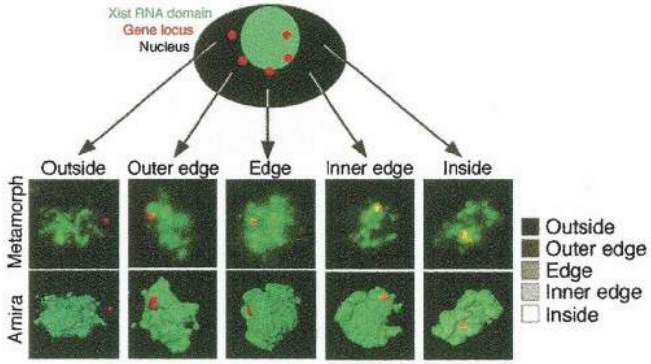
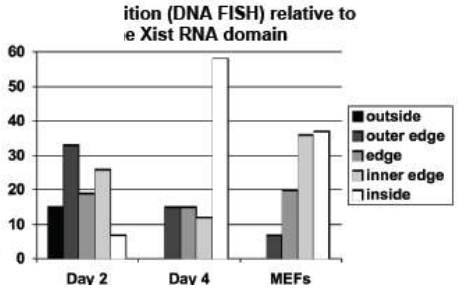
Escapees remain external to the Xist RNA coated X chromosome





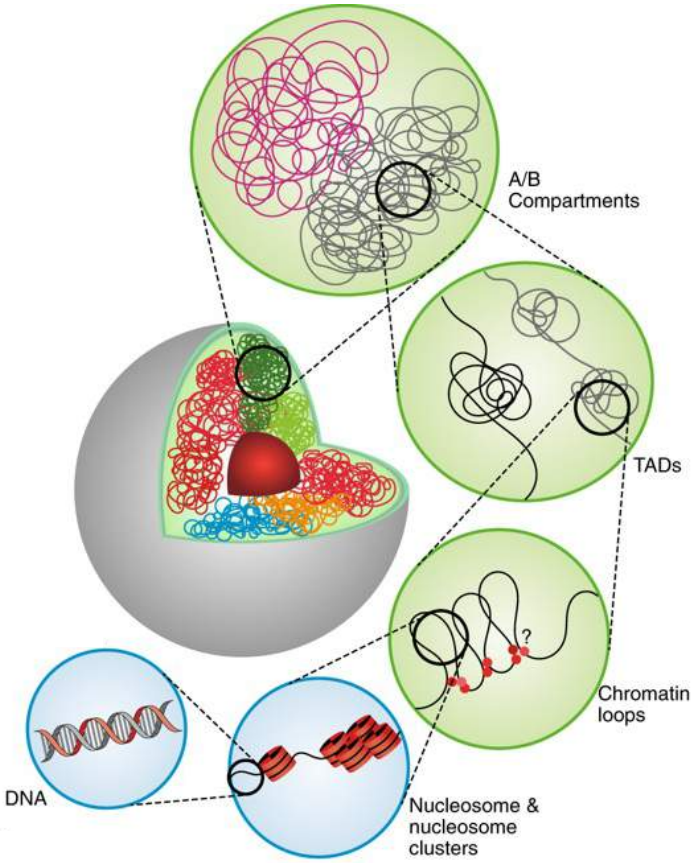




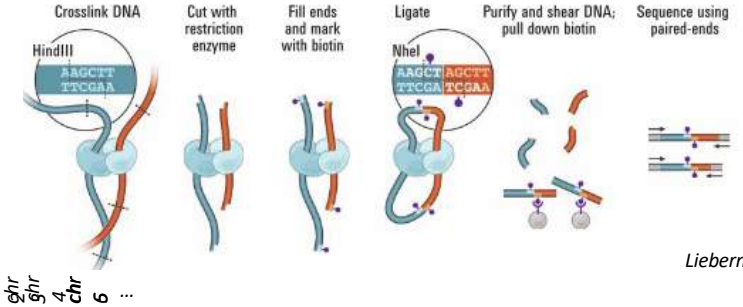
Chaumeil et al, Genes Dev. 2006

Chaumeil et al, Genes Dev. 2006;

Molecular insight chromosome organsation using chromosome conformation capture technologies

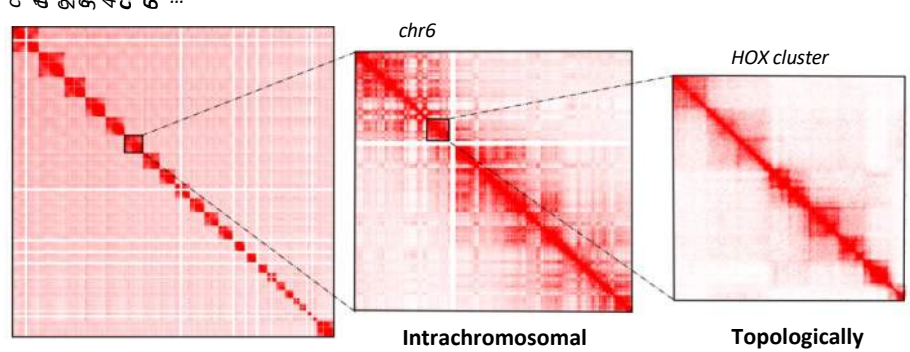


Dogan and Liu, Nat. Plant 2018



Job Dekker

Lieberman-Aiden et al. 2009



Whole genome map

Intrachromosomal contact maps

Compartments with distinctive patterns of epigenomic features
Variable between tissues,
Cell-type specific

Topologically associating domains

TADs (100kb-1Mb scale)
Invariant (almost) between tissues
Nora et al, 2012
Conserved (man/mouse)
Dixon et al, 2012

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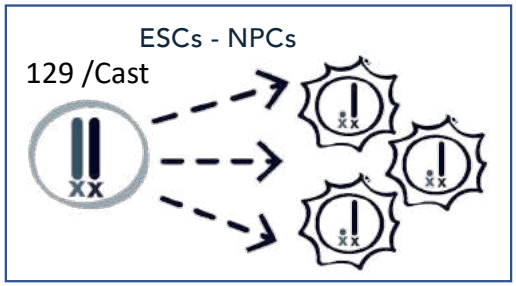
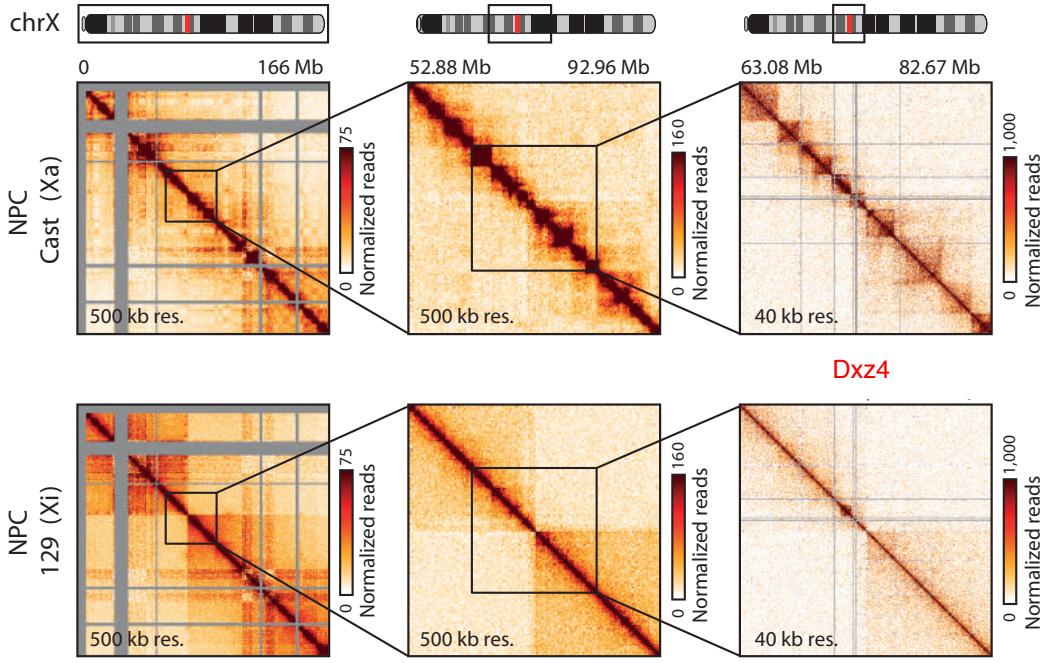
The inactive X Chromosome is partitioned into two megadomains and is globally devoid of TADs except at clusters of expressed escapees

- Allele-specific HiC in clonal neural progenitor cells (NPCs)
- The Xi is organised into two **mega-domains** with **global absence of TADs**
- Facultative escape occurs in clusters and correlates with local 3D-organisation (TAD-like domains)
- Facultative escape varies between clonal populations of NPCs
- (and *in vivo* between individual females)



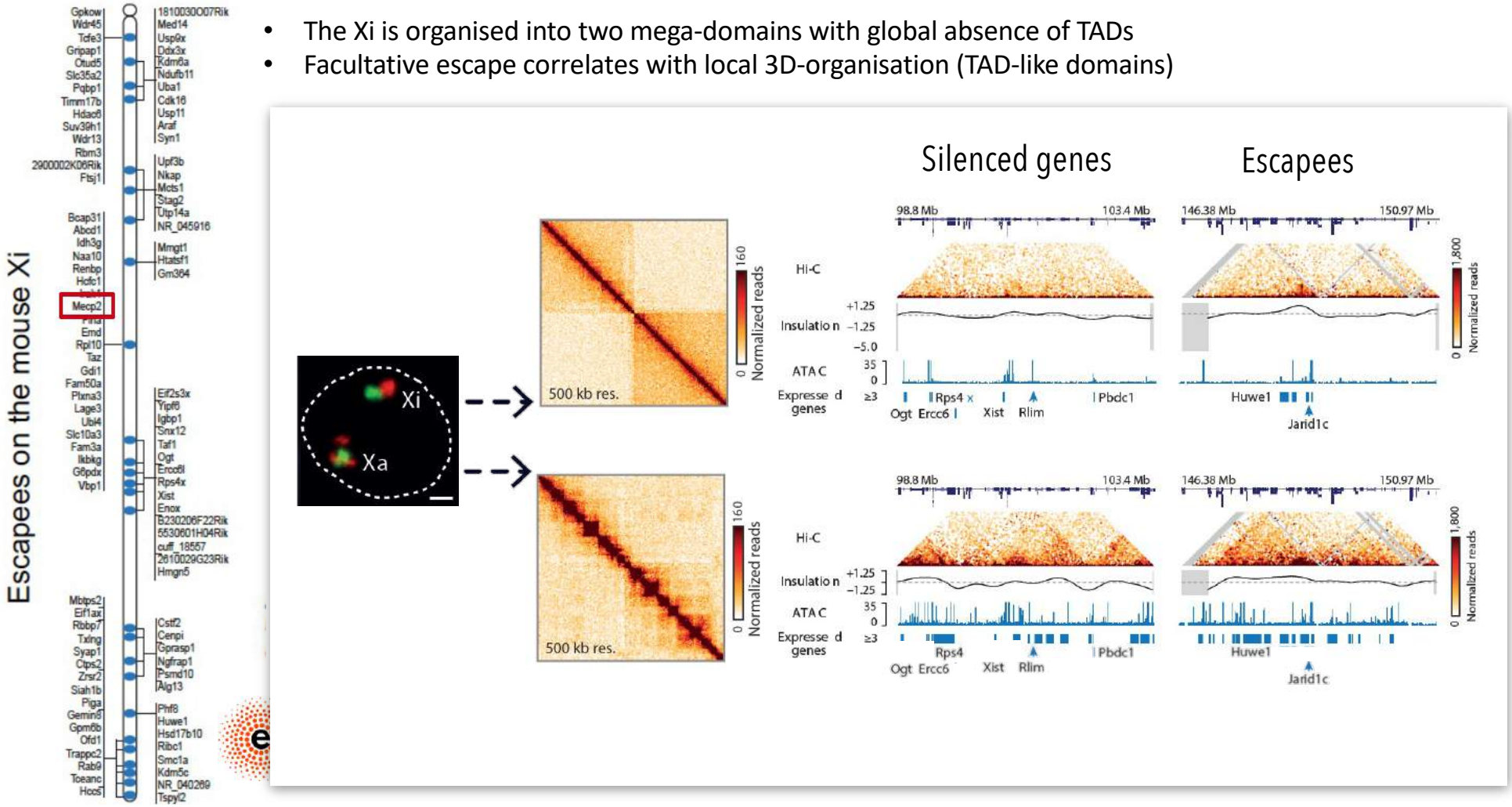


Giorgetti L et al. "Structural organization of the i

Giorgetti et al, Nature 2016

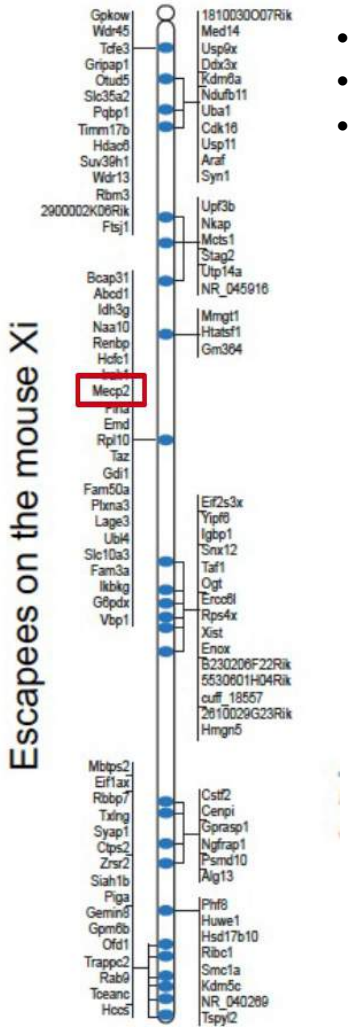
Clusters of genes that escape XCI are organised as TADs on the Xi

- The Xi is organised into two mega-domains with global absence of TADs
- Facultative escape correlates with local 3D-organisation (TAD-like domains)

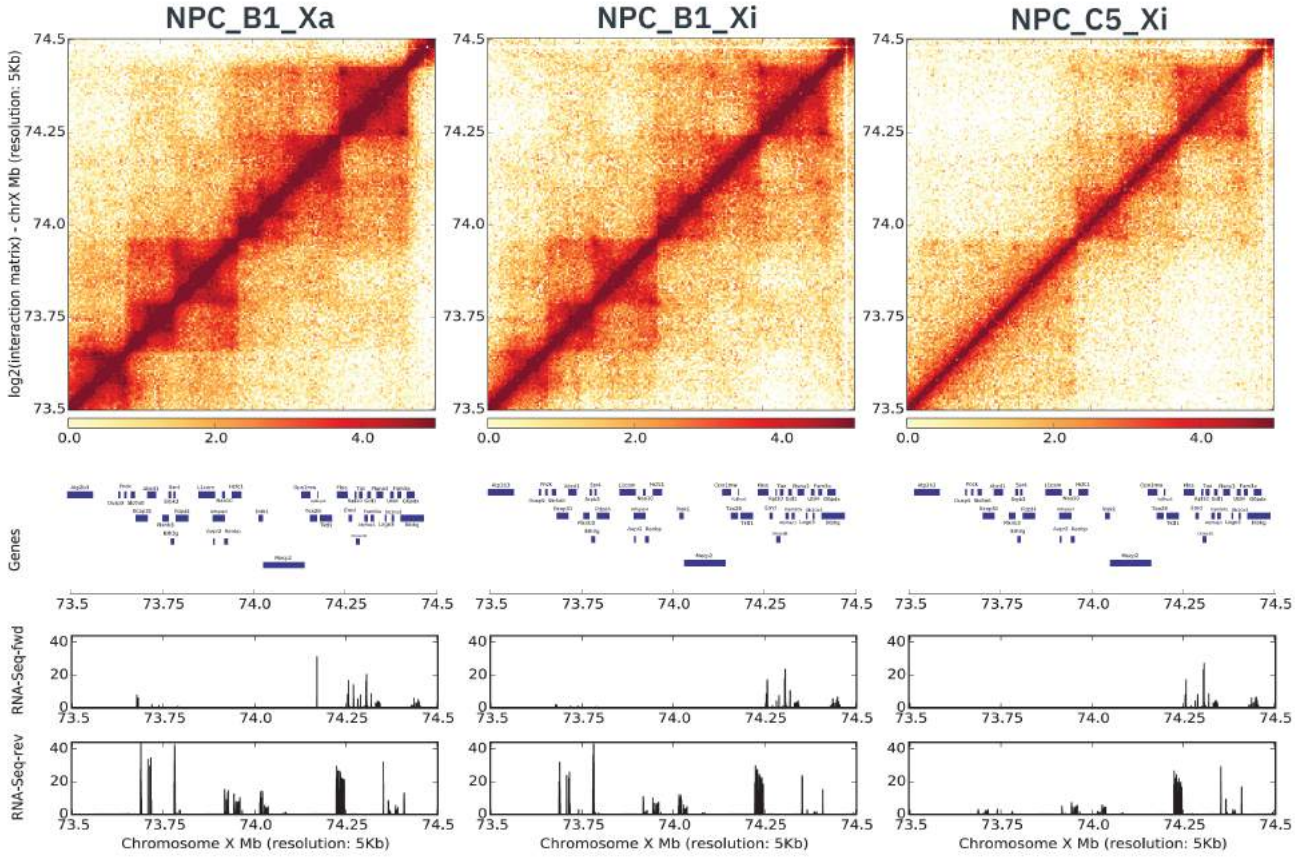


E. Heard, March 27th 2023

Clusters of genes that escape XCI are organised as TADs on the Xi

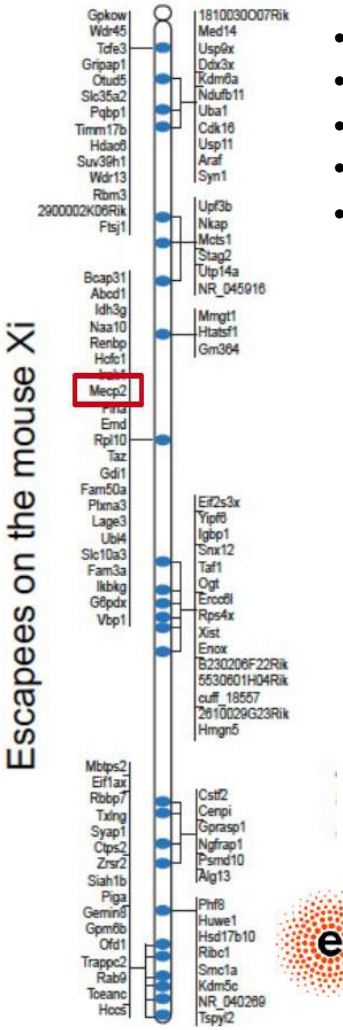


- The Xi is organised into two mega-domains with global absence of TADs
- Facultative escape correlates with local 3D-organisation (TAD-like domains)
- Different clonal populations show different escapees and 3D structure

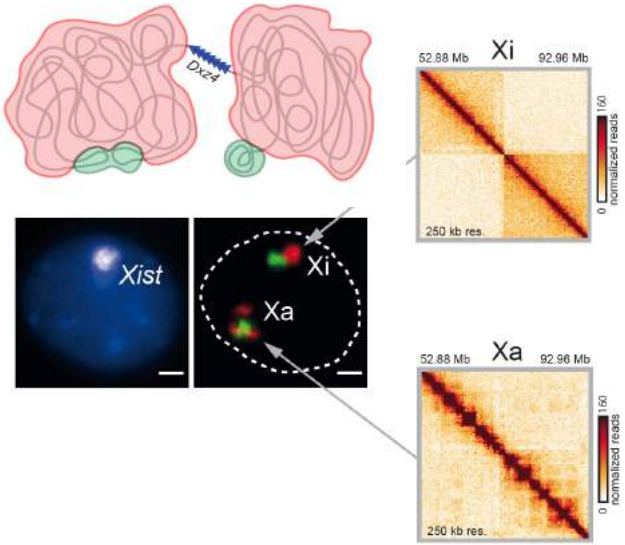


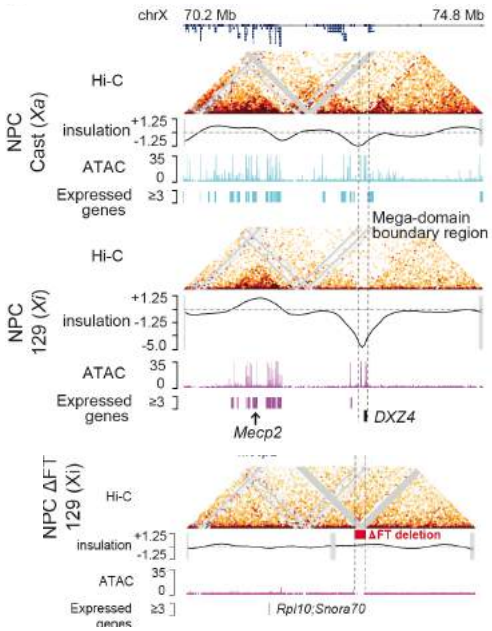
Antonia Hauth
Agnese Loda
Nicolas Servant

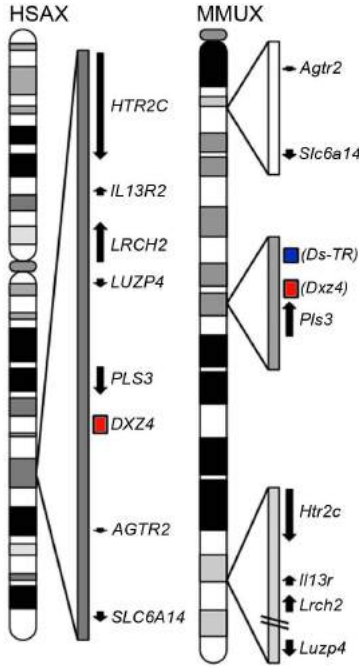
Facultative escape may be influenced by the DXZ4 locus?



- The Xi is organised into two mega-domains with global absence of TADs
- Facultative escape correlates with local 3D-organisation (TAD-like domains)
- DXZ4 macrosatellite: a long range, CTCF-rich "super-enhancer" that can influence facultative escape?
- DXZ4 KO mice show decreased facultative escape in trophectoderm and brain (Attia et al, in preparation)
- Physiological impact of facultative escape?







Giorgetti L et al. "Structural organization of the inactive X chromosome in the mouse". *Nature* 535:575-9 (2016)

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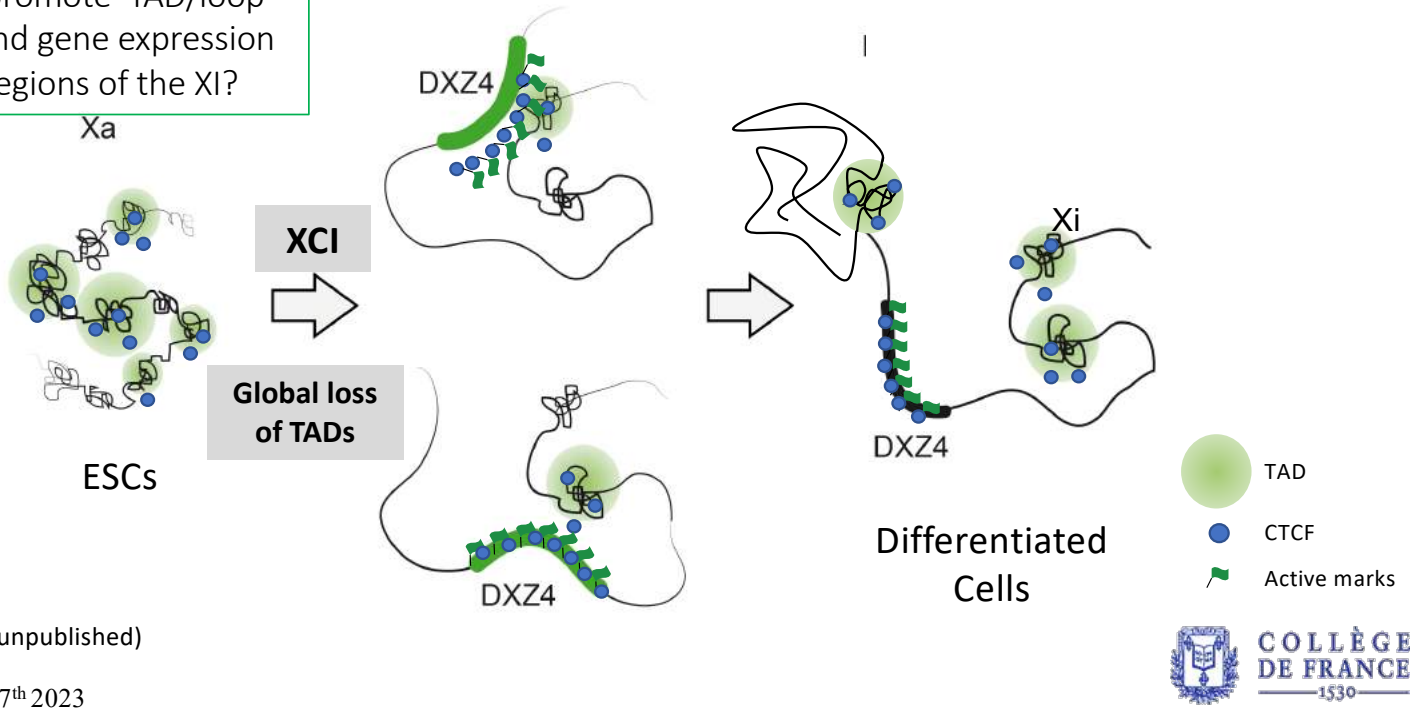
Facultative escape may be influenced by the DXZ4 locus?

- Allele-specific ChIP-seq in ESCs and NPCs:
- DXZ4 is enriched in CTCF and is euchromatic with enhancer-like marks only on the inactive X
- DXZ4 is required for *establishment* but not the maintenance of facultative escapees

Model: Transient long-range interactions of DXZ4 with some regions of the Xi during development contribute to creating local euchromatin, thus facilitating facultative escape?

DXZ4 may promote TAD/loop formation and gene expression at some regions of the XI?



Attia, Corbel et al (unpublished)

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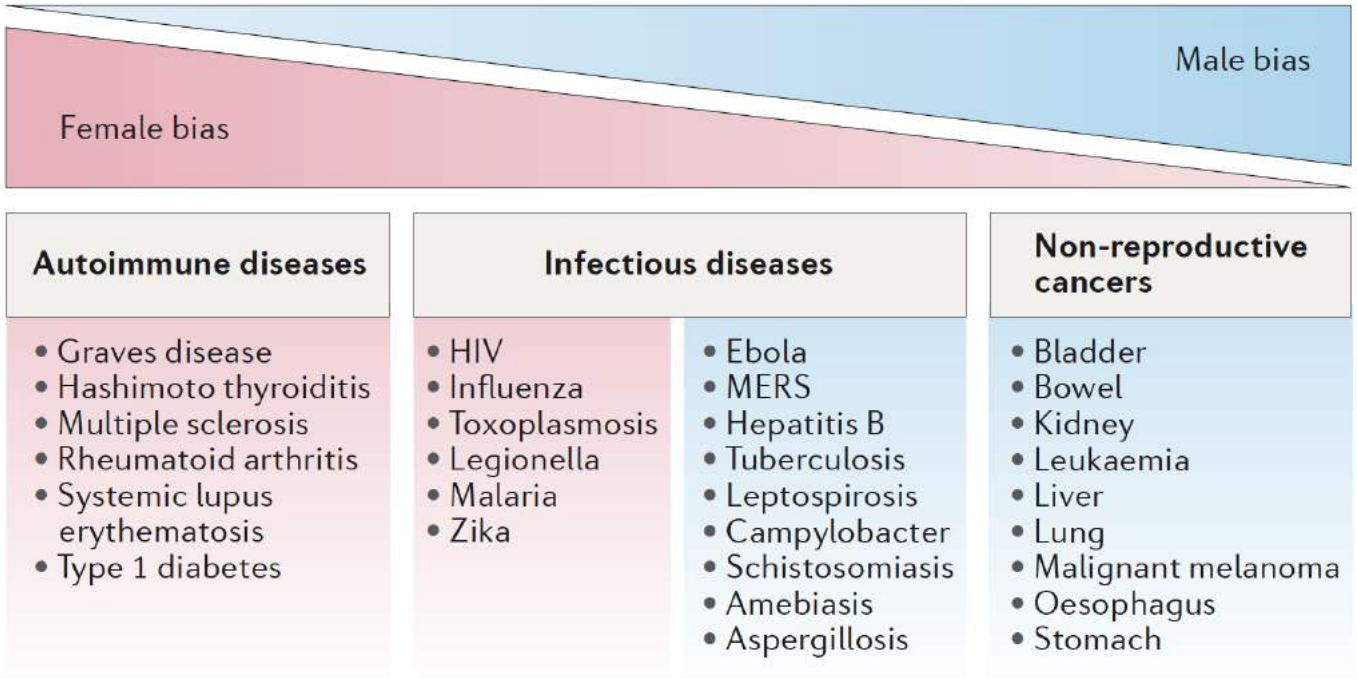
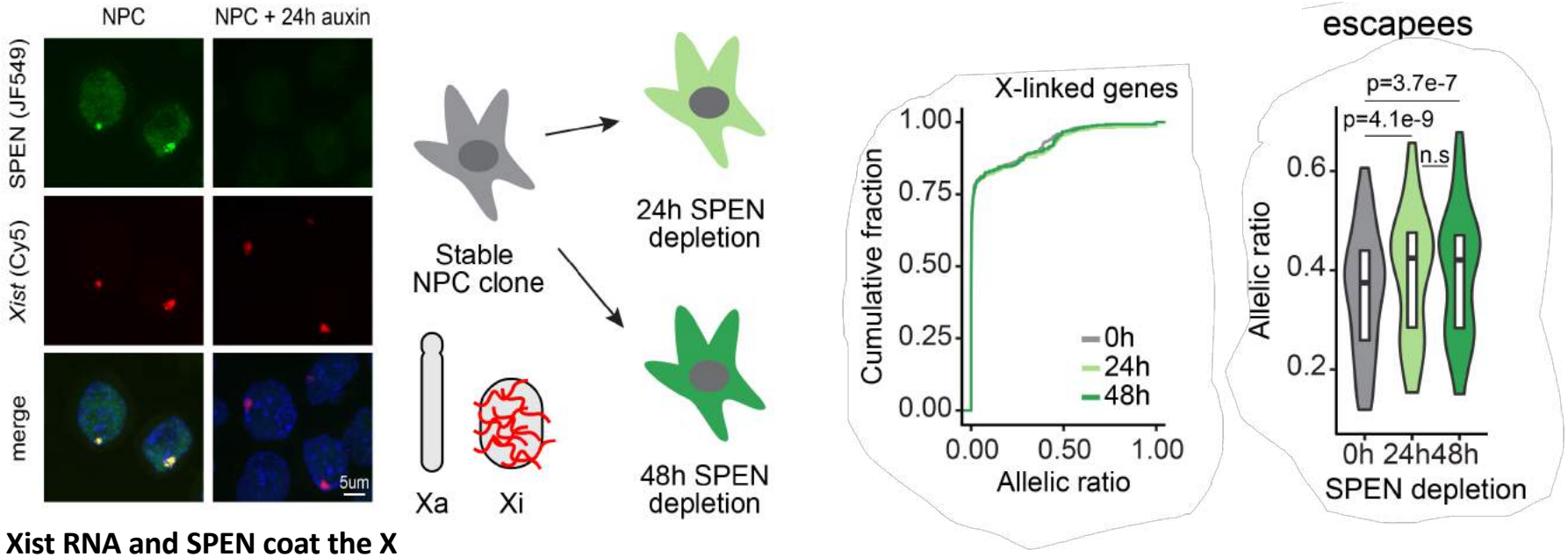
How, when, and where does escape from X inactivation occur?

How do escapees contribute to disease?



Does SPEN play a role in the maintenance of X inactivation?

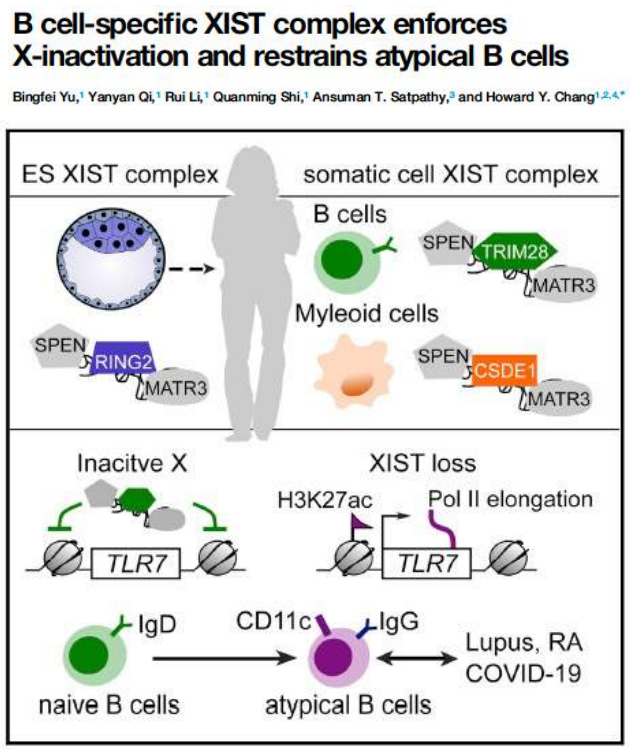


Xist RNA and SPEN coat the X chromosome in NPCs

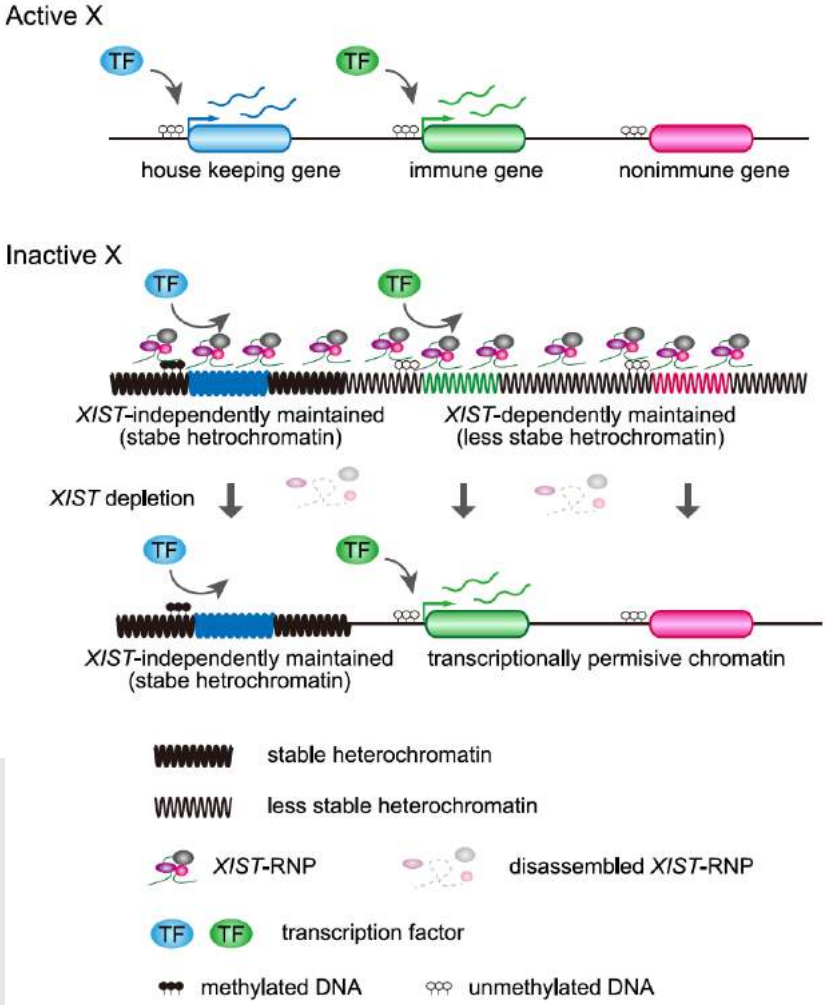
Xist + SPEN can dampen transcription of escapees in somatic cells

Implications for dosage regulation in XX cells during development or in disease?

Xist RNA (and its partners) may be required in somatic tissues to maintain silencing on the inactive X chromosome



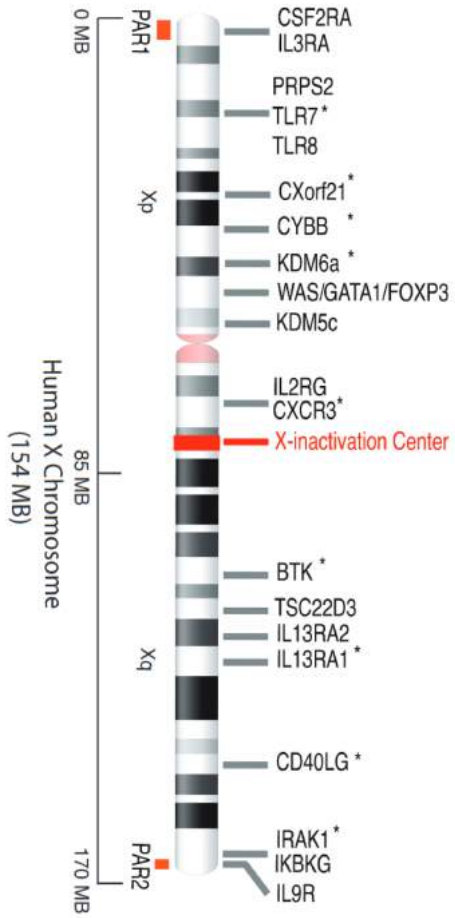
Yu et al. (2021) demonstrate that a subset of X-linked immune genes is repressed on the inactive X chromosome (Xi) in a manner dependent on XIST RNA in B cells, and derepression of these genes upon XIST depletion could bias differentiation of naive B cells and be involved in etiology of female-biased autoimmune diseases.



X-linked genes implicated in Autoimmune Diseases

Many genes on the X chromosome are involved in **immune response**
Several have been found to variably escape XCI

- X-linked genes involved in immune functions, may be responsible for overproduction of autoantibodies.
- Alternatively, overexpression of X-linked genes could disrupt the equilibrium in the mechanism of fine-tuning protein expression and generate protein aggregates that would trigger responses against self antigens.



A Tlr7 translocation accelerates systemic autoimmunity in murine lupus.

BXSB-Yaa lupus model

Tlr7 translocation on Y-chr.

Subramanian et al *PNAS* 2006

- BXSB-Yaa mouse model for lupus.
- *Male* mice develop lupus like symptoms,
- due to translocation of the X-linked TLR7 gene region on the Y chr.

⇒ **double the dosage of TLR7 in males**
⇒ **develop lupus like symptoms.**

Altered XCI maintenance in the immune system

Loss of Xist RNA from the inactive X during B cell development is restored in a dynamic YY1-dependent two-step process in activated B cells

Camille M. Syrett[1], Vishal Sindhava[1,2], Suchita Hodawadekar[1], Arpita Myles[2], Guanxiang Liang[2], Yue Zhang[1], Satabdi Nandi[1], Michael Cancro[2], Michael Atchison[1], Montserrat C. Anguera[1]*

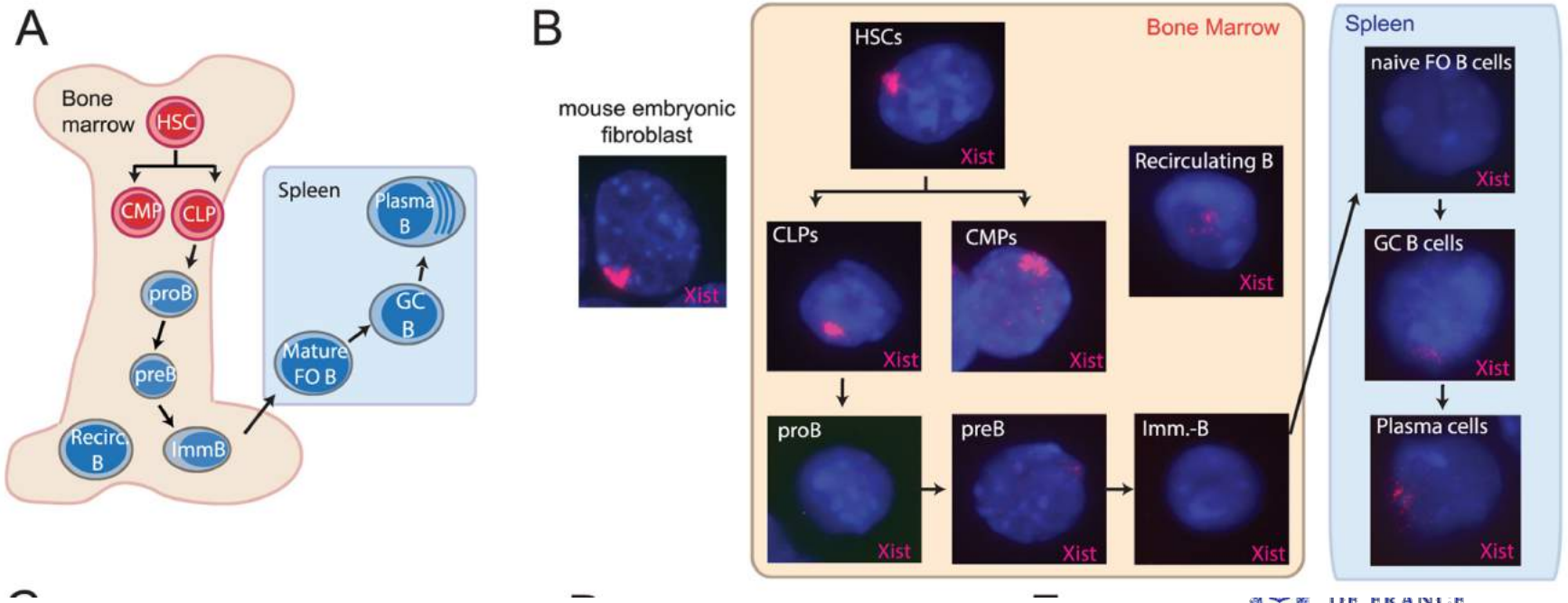
Unusual maintenance of X chromosome inactivation predisposes female lymphocytes for increased expression from the inactive X

Jianle Wang[a], Camille M. Syrett[a], Marianne C. Kramer[b], Arindam Basu[a,1], Michi and Montserrat C. Anguera[a,2]

Altered X-chromosome inactivation in T cells may promote sex-biased autoimmune diseases

Camille M. Syrett, … , Michael Atchison, Montserrat C. Anguera

JCI Insight. 2019;4(7):e126751. https://doi.org/10.1172/jci.insight.126751.



Increased expression of the X-linked *TLR7* gene leads to SLE

Autoreactive B Cell Responses to RNA-Related Antigens Due to *TLR7* Gene Duplication

Prapaporn Pisitkun,[1] Jonathan A. Deane,[1] Michael J. Difilippantonio,[2] Tatyana Tarasenko,[1] Anne B. Satterthwaite,[3] Silvia Bolland[1]*

A *Tlr7* translocation accelerates systemic autoimmunity in murine lupus

Srividya Subramanian*, Katalin Tus*, Quan-Zhen Li*, Andrew Wang*, Xiang-Hong Tian*, Jinchun Zhou*, Chaoying Liang*, Guy Bartov†, Lisa D. McDaniel†, Xin J. Zhou†, Roger A. Schultz†, and Edward K. Wakelar

Control of Toll-like Receptor 7 Expression Is Essential to Restrict Autoimmunity and Dendritic Cell Proliferation

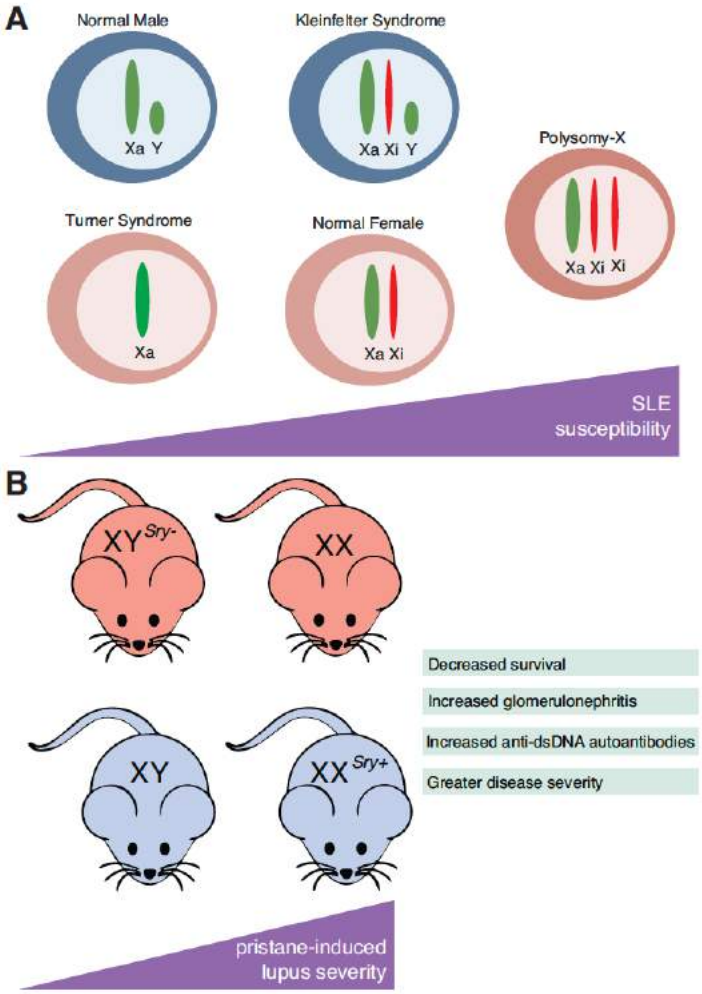
Jonathan A. Deane,[1] Prapaporn Pisitkun,[1] Rebecca S. Barrett,[1] Lionel Feigenbaum,[3] Terrence Town,[4] Jerrold M. Ward,[2] Richard A. Flavell,[4] and Silvia Bolland[1,*]

TLR7 escapes X chromosome inactivation in immune cells

Mélanie Souyris,[1] Claire Cenac,[1] Pascal Azar,[1] Danièle Daviaud,[1] Astrid Canivet,[1] Solange Grunenwald,[2] Catherine Pienkowski,[3] Julie Chaumeil,[4] José E. Mejía,[1] Jean-Charles Guéry[1]*

- TLR7 increased in women and XXY men, owing to consistent escape (expressed from Xi) in high % (>30%) primary B lymphocytes, monocytes, and plasmacytoid dendritic cells (pDCs)
- Biallelic B lymphocytes from women show greater TLR7 transcriptional expression than the monoallelic cells, correlated with higher TLR7 protein in female vs male leukocytes
- Variability in escape between individuals – correlation with disease?

X-linked genes implicated in Autoimmune Diseases



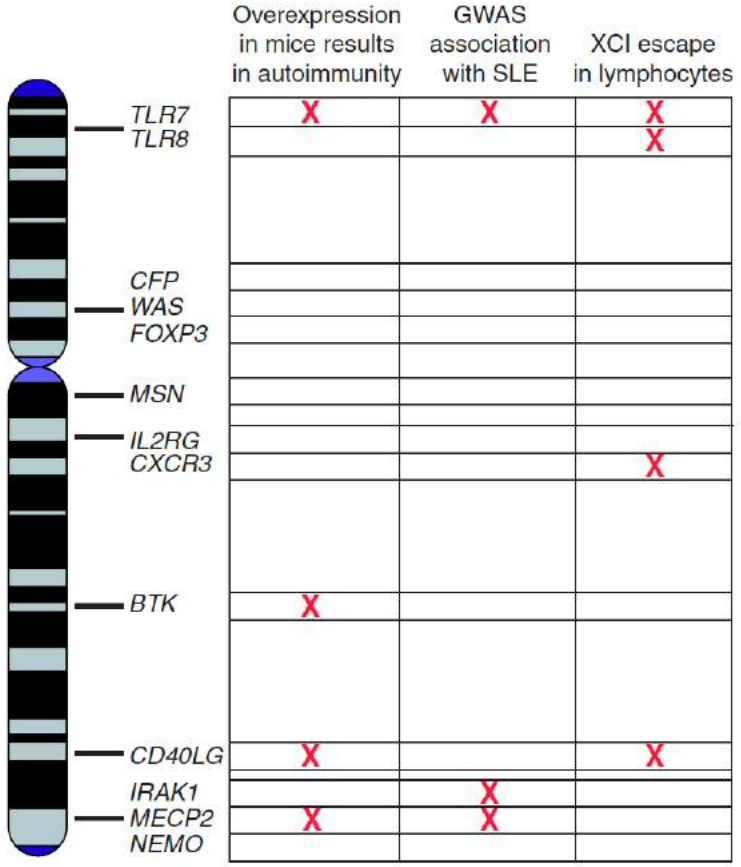


FIGURE 2 The role of the X chromosome in humans and mice

X-linked genes implicated in Autoimmune Diseases

TABLE 1 Mouse models of X-linked gene overexpression

Mouse name	Gene	Autoimmune phenotypes	References
CD19-hBtk	*Btk*	Increase in spontaneous GC and plasma B cells, enhanced B cell activation, anti-dsDNA and antinucleosome autoantibodies, glomerulonephritis and proteinuria, peripheral perivascular inflammation	Kil et al., 2012, Corneth et al., 2016
Lckgp39	*Cd40lg*	Disrupted thymocyte development, lymphoid tissue hypertrophy, mononuclear cell infiltration in peripheral tissues, myeloid hyperplasia, splenomegaly, glomerulonephritis, chronic inflammatory bowel disease and lethal wasting	Clegg et al., 1997
V_H/IgH/Ig_K:CD40L	*Cd40lg*	antinuclear antibody (ANA), anti-DNA, antihistone IgG autoantibodies, proteinuria, glomerulonephritis, in some animals	Pérez-Melgosa et al., 1999
CD40Ltg+	*Cd40lg*	Higher titers of high-affinity IgG and IgG1 Ab in response to T cell-dependent Ags	Higuchi et al., 2001
MECP2-Tg	*Mecp2*	Elevated ANAs in sera	Koelsch et al., 2013
BXSB	*Tr7*	Splenomegaly, lymph node enlargement, hemolytic anemia, glomerulonephritis, ANA autoantibodies, increased mortality	Andrews et al., 1978, Murphy and Roths 1979, Pisitkun et al., 2006, Subramanian et al., 2006,
TLR7.Tg	*Tlr7*	RNA-specific antibodies, ANA autoantibodies, glomerulonephritis, splenomegaly, dendritic cell (DC) expansion, spontaneous lymphocyte activation, increased mortality	Hwang et al., 2012, Deane et al., 2007

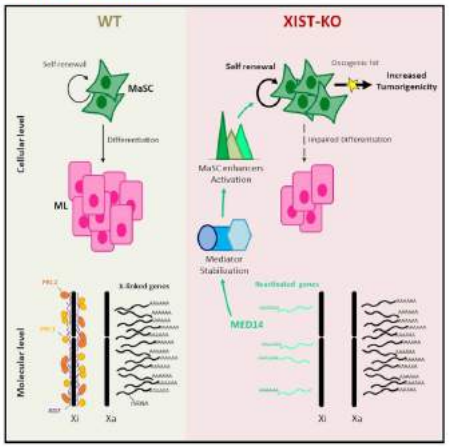
Xist RNA (and its partners) may be required in mammary stem cells to maintain silencing on the inactive X chromosome

Cell

Article

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

Graphical abstract



Authors

Laia Richart, Mary-Loup Picod-Chedotel, Michel Wassef, ..., Edith Heard, Raphaël Margueron, Christophe Ginestier

Correspondence

raphael.margueron@curie.fr (R.M.), christophe.ginestier@inserm.fr (C.G.)

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.

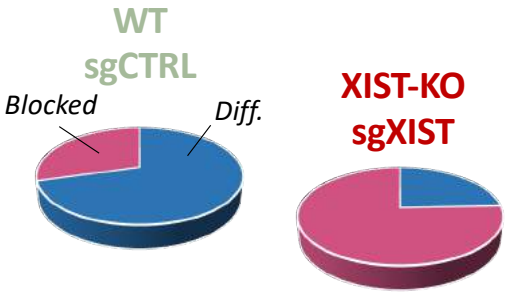
Highlights

- *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

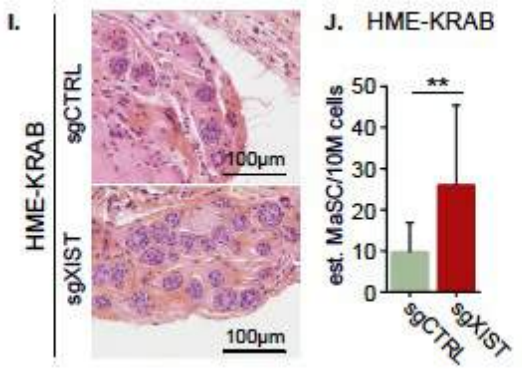
XIST disruption in mammary epithelial cells: impact on gene silencing and mammary cell differentiation?

1 - DISRUPTION OF MAMMARY STEM CELLS DIFFERENTIATION POTENTIAL

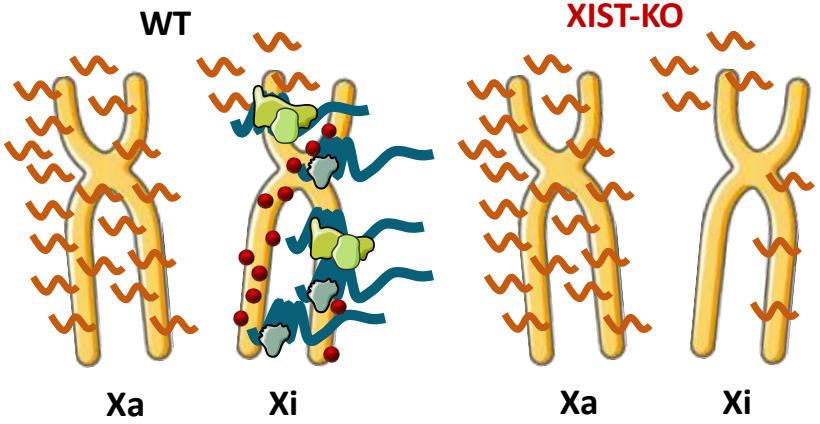
XIST KO or silencing blocks cells in the stem compartment



Laia Richart Ginés



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



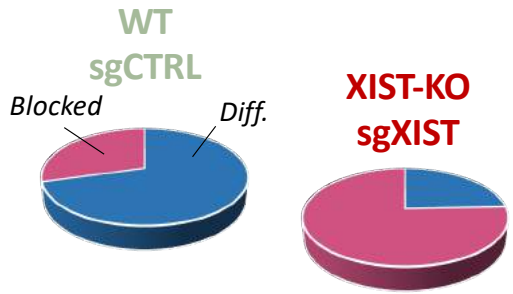
Collaboration with
Raphael Margueron (Institute Curie, Paris)
Christophe Ginestier (CRCM, Marseille)

Laia Richart Ginés et al, Cell 2022

XIST disruption in mammary epithelial cells: impact on gene silencing and mammary cell differentiation?

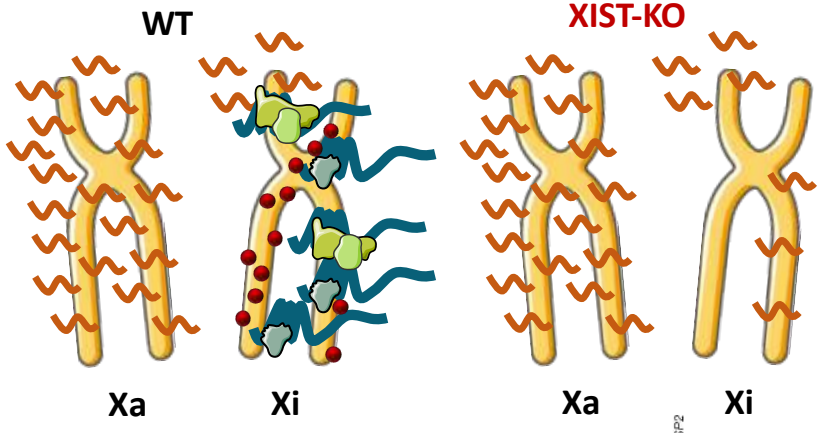
1 - DISRUPTION OF MAMMARY STEM CELLS DIFFERENTIATION POTENTIAL

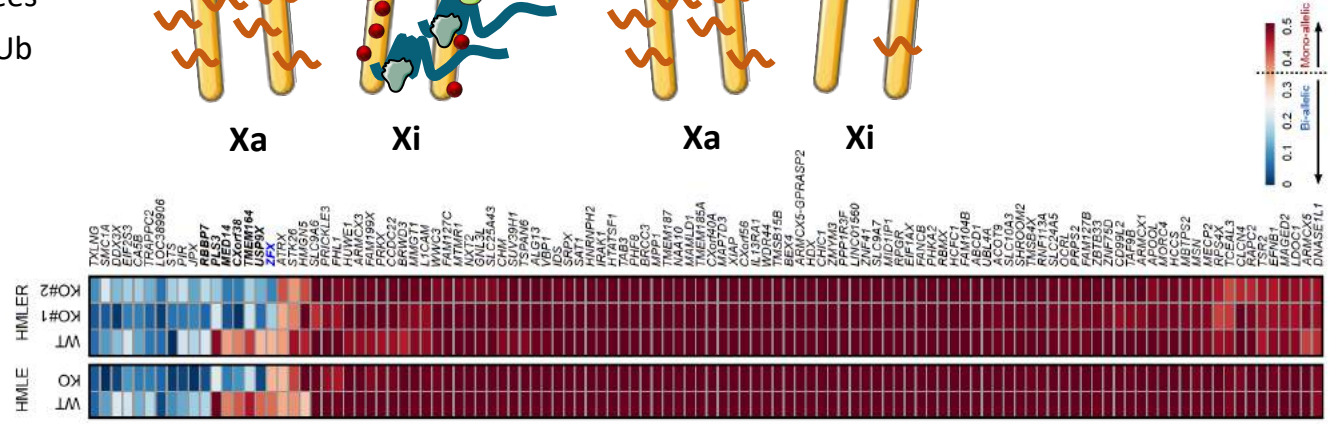
- XIST KO or silencing blocks cell in the stem compartment



2 - REACTIVATION OF A SUBSET OF GENES ON THE Xi

- Facultative escapee genes
- Clustering & proximity to escapees
- Overlap with H3K27me3 & H2AUb
- XIST and PRC1 mediate their repression





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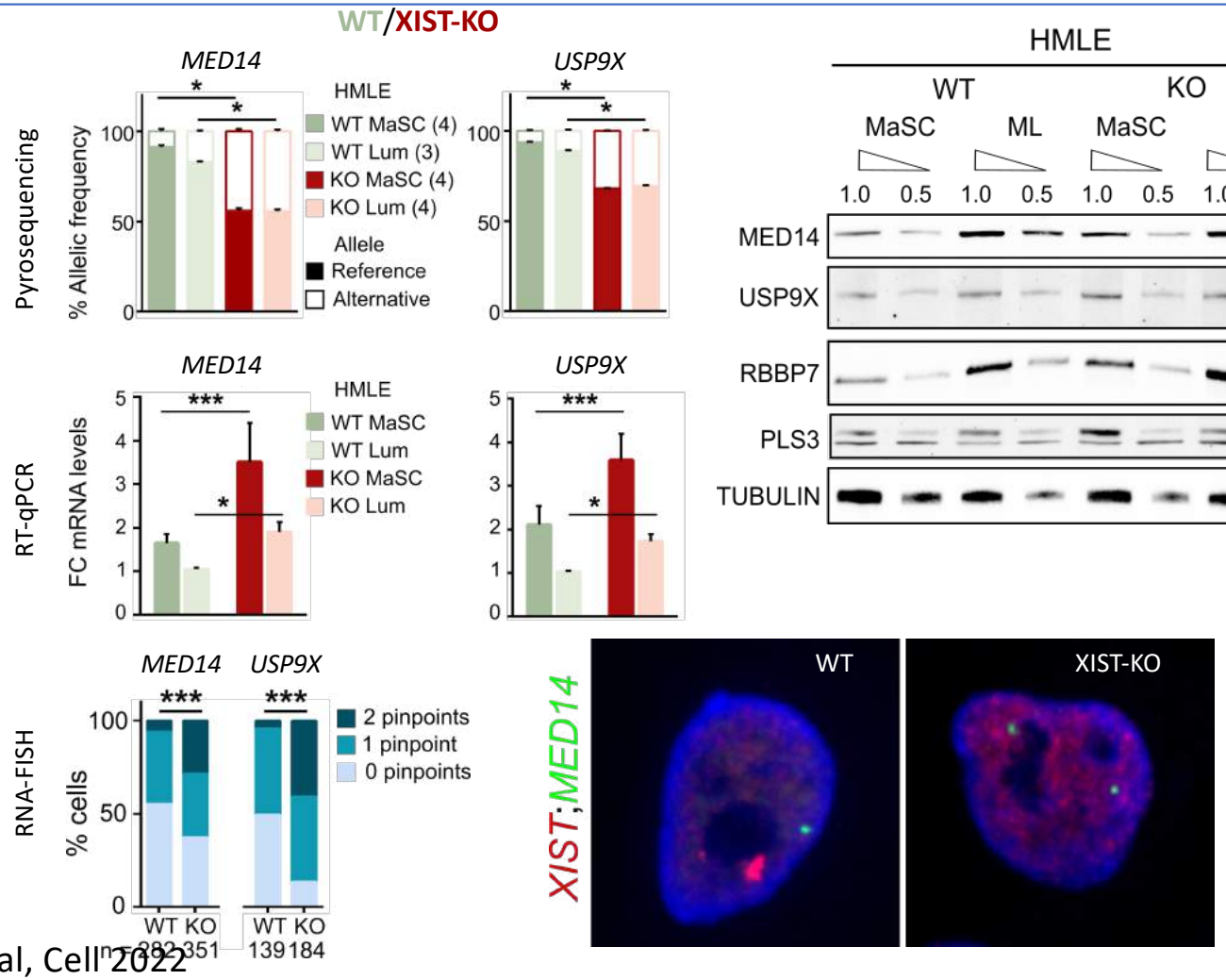
Loss of *XIST* triggers epigenetic erosion and partial transcriptional reactivation on the Xi

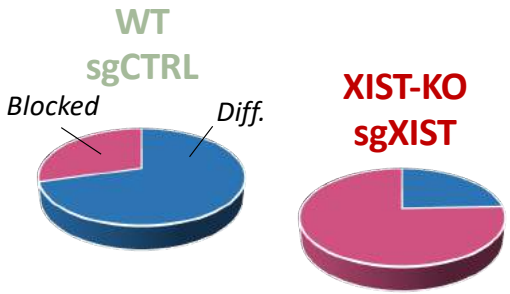


Laia Richart Ginés et al, Cell 2022

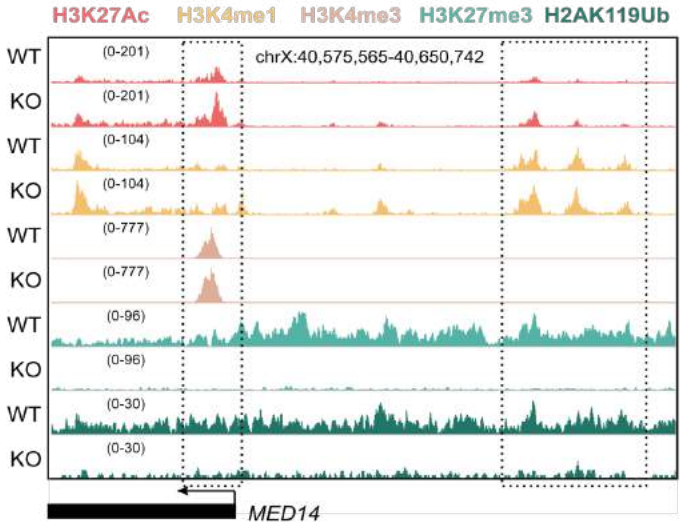
Loss of XIST leads to up-regulation of some genes on the Xi with impact on mammary stem cell differentiation

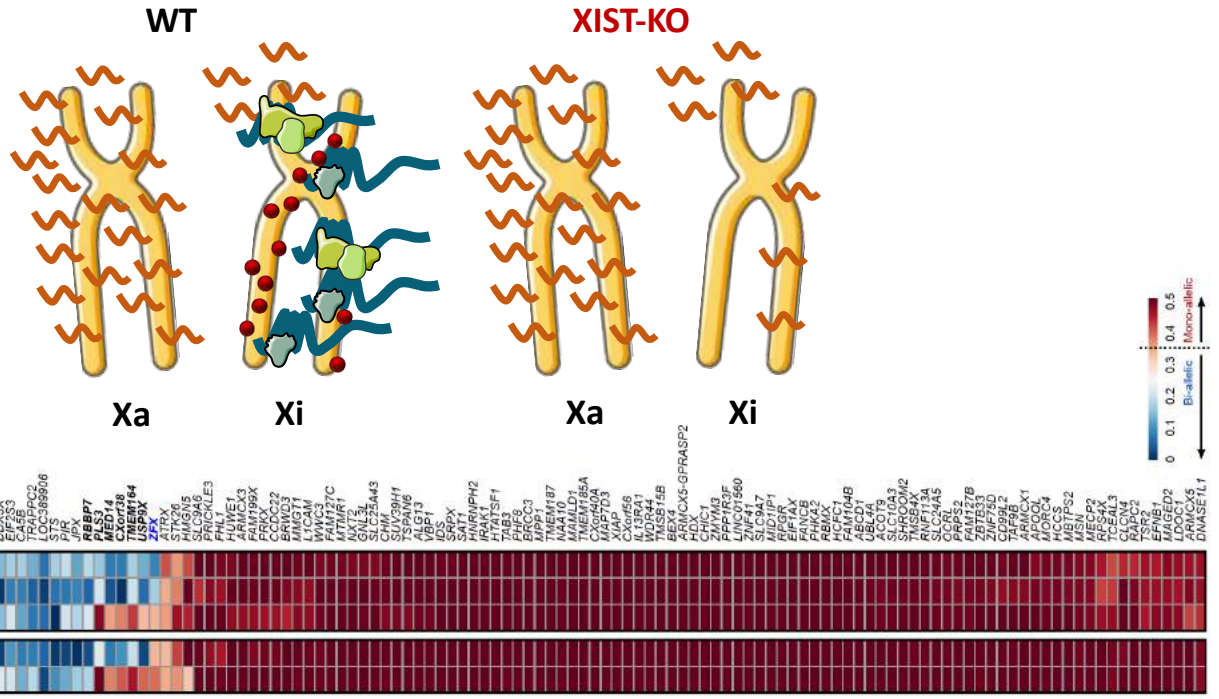
1 - DISRUPTION OF MAMMARY STEM CELLS DIFFERENTIATION POTENTIAL

- XIST KO or silencing blocks cell in the stem compartment



Loss of Polycomb (PRC1/2) at MED14 gene

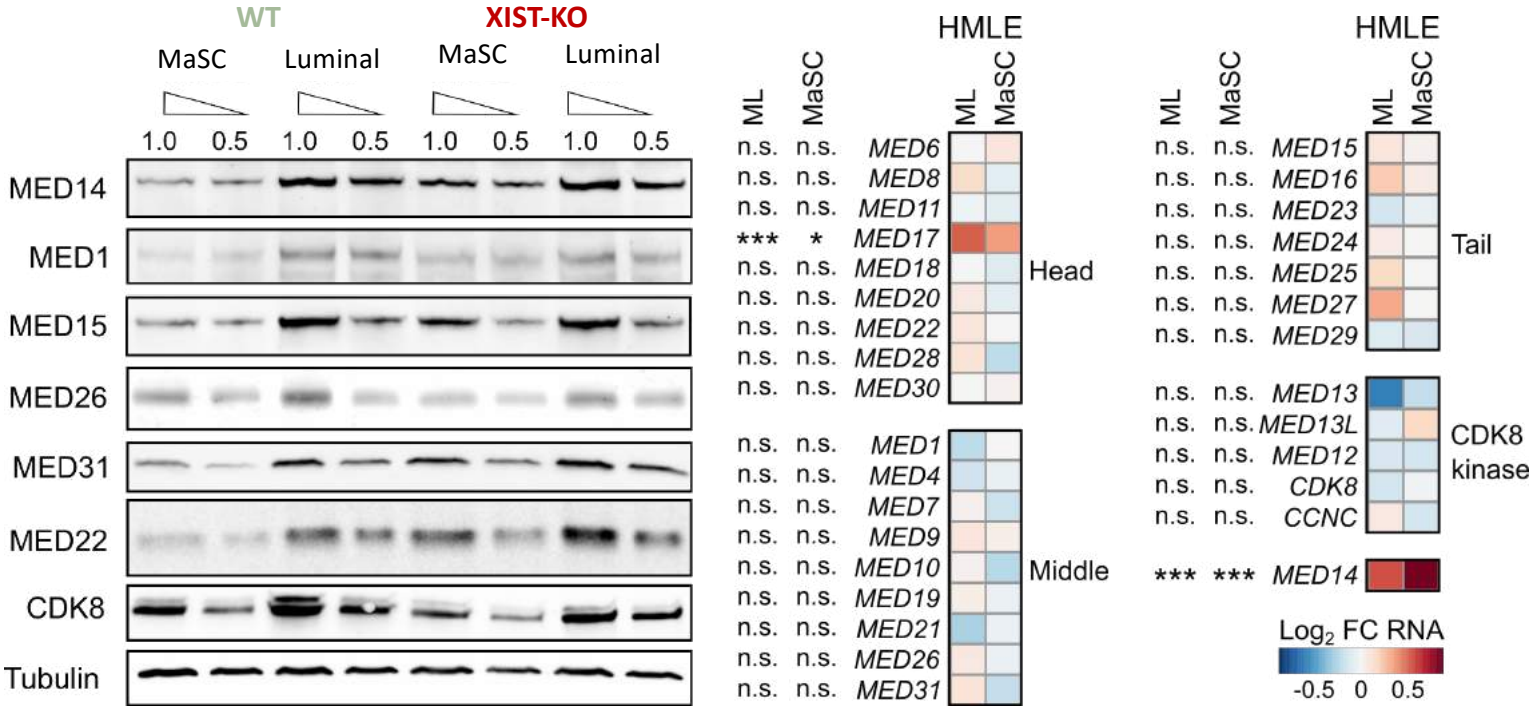




Laia Richart Ginés et al, Cell 2022

Increased levels of MED14 result in increased Mediator complex levels in *XIST*-KO cells

Protein stabilization > transcriptional regulation of Mediator subunits



* Up-regulated > Down-regulated genes in MaSC and ML cells (RNA-Seq)

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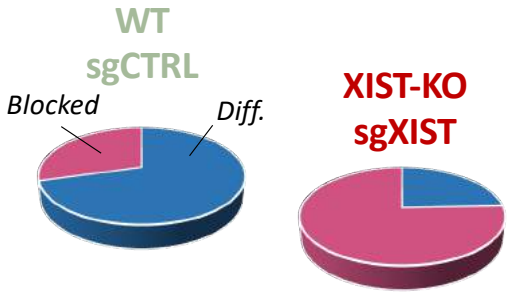

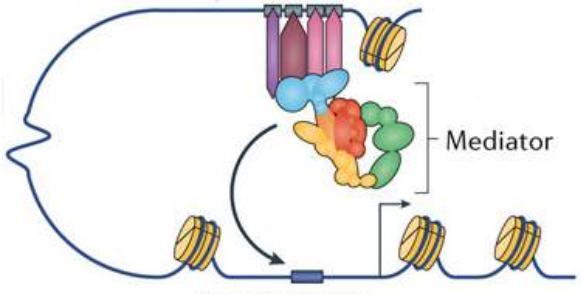

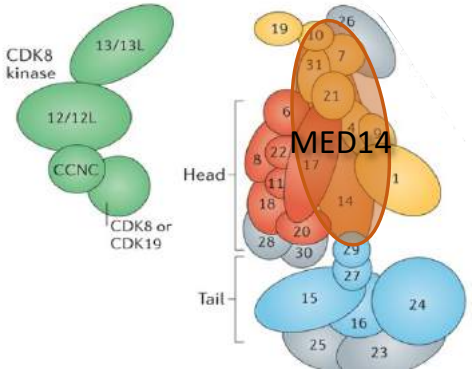

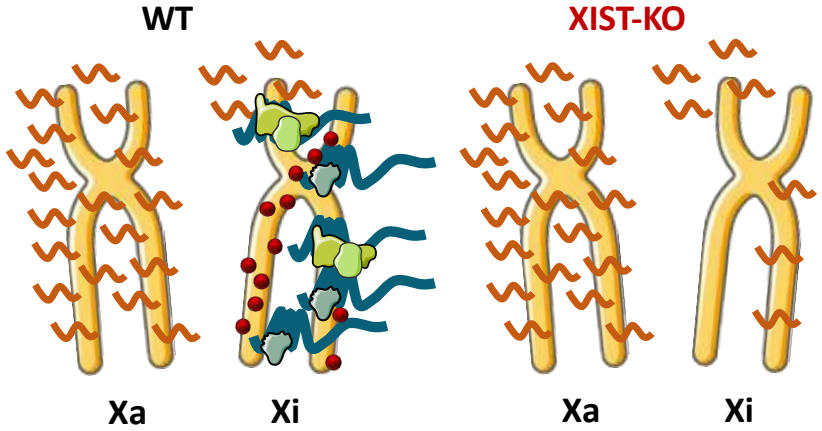

3 - MECHANISTIC LINK WITH MED14

- Critical structural and functional subunit of the Mediator complex.
- Reactivation of MED14 leads to higher protein levels Mediator subunits
- Global increase in enhancer activity (H3k27Ac and H3K4me1) at MaSC enhancers
- This may modulate tumor fate through enhancer landscape remodelling

Laia Richart Ginés

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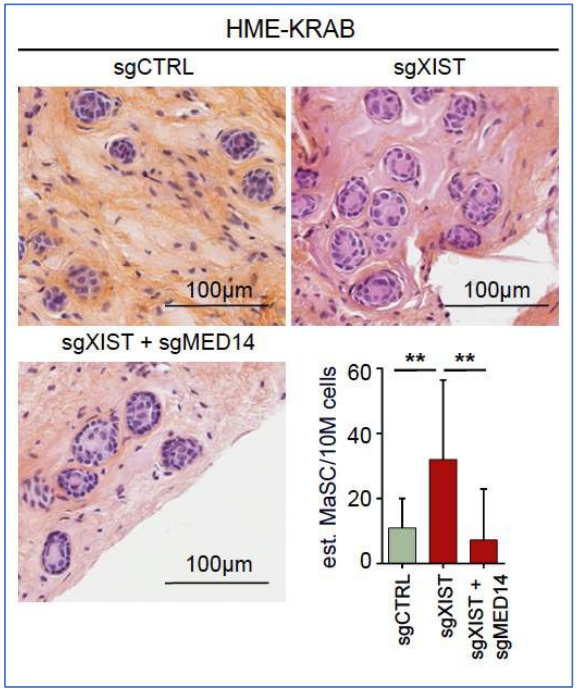
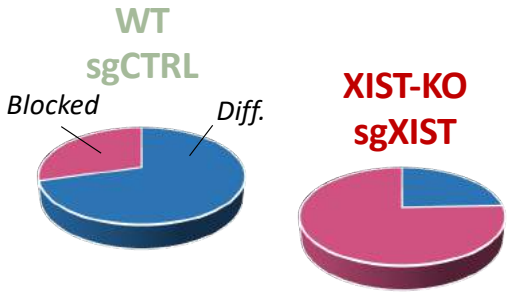
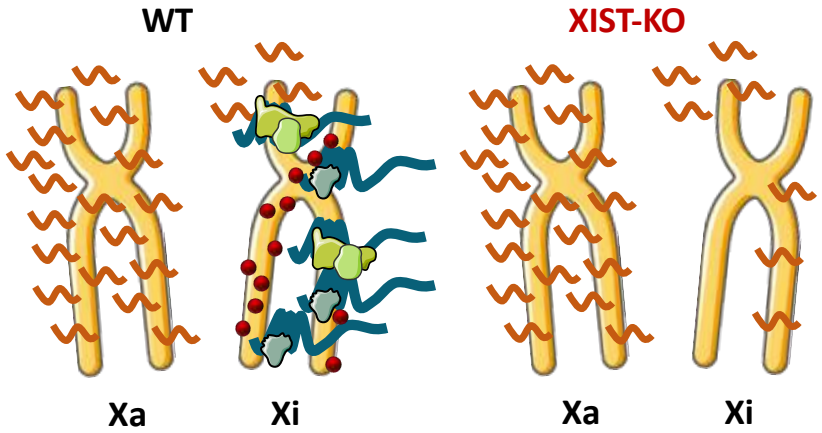






3 - MECHANISTIC LINK WITH MED14

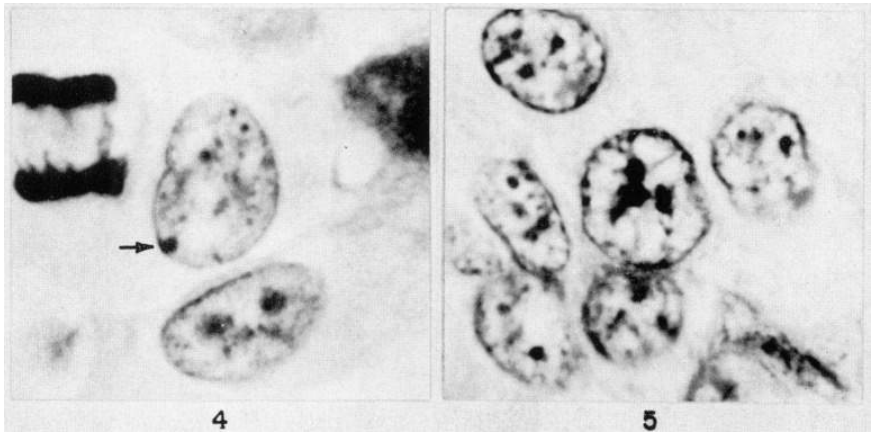
- MED14 precise dosage regulates luminal differentiation
- Mammary stem cell enhancers display chromatin features of hyperactivity in the absence of *XIST*

Laia Richart Ginés et al (under revision)

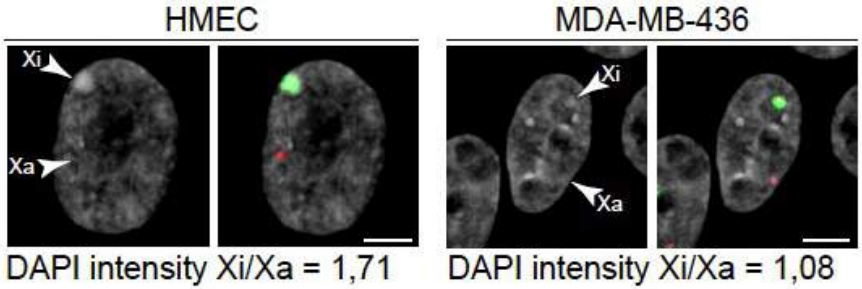
X-chromosome inactivation Escapees and Cancer

X-linked escapees may PROTECT XX individuals from Cancer?
EXITS Hypothesis

Epigenetic instability and increased or aberrant escape from XCI may PROMOTE or FACILITATE Cancer in XX individuals (eg Breast cancer)?



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

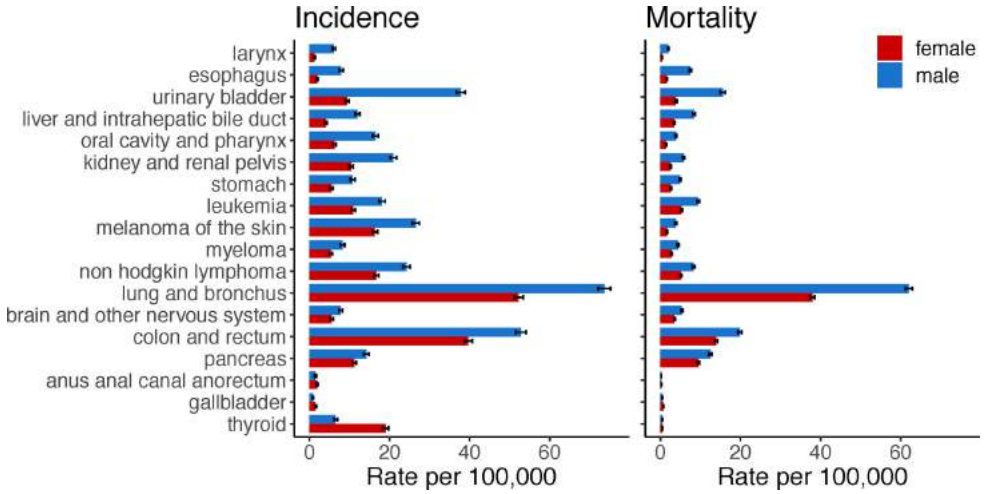


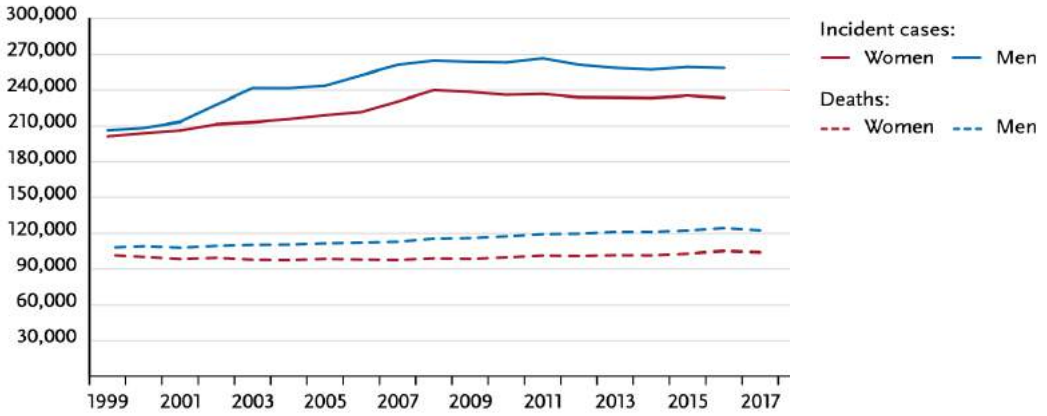
Article

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

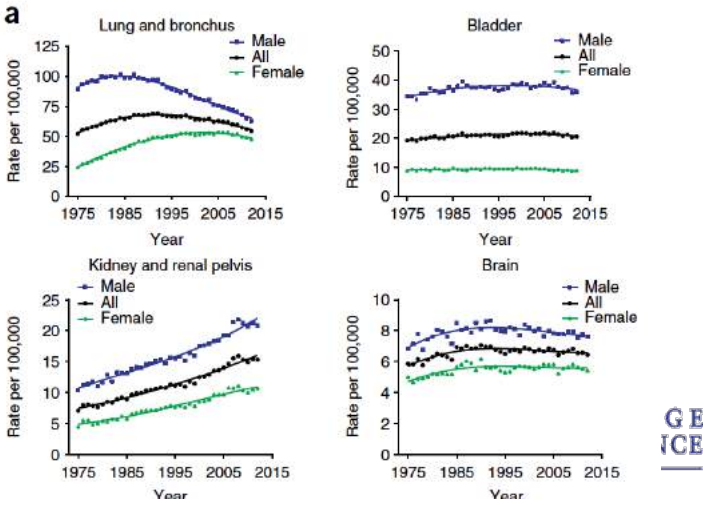
Laia Richart,[1,8] Mary-Loup Picod-Chedotel,[2,8] Michel Wassef,[1] Manon Macario,[2] Setareh Aflaki,[1] Marion A. Salvador,[2] Tiphaine Héry,[1] Aurélien Dauphin,[1] Julien Wicinski,[2] Véronique Chevrier,[2] Sonia Pastor,[3] Geoffrey Guittard,[3] Samuel Le Cam,[1] Hanya Kamhawi,[2] Rémy Castellano,[4] Géraldine Guasch,[2] Emmanuelle Charafe-Jauffret,[2,5] Edith Heard,[6,7] Raphaël Margueron,[1,9,*] and Christophe Ginestier[2,9,10,*]

Women are less susceptible than men to many non-reproductive cancers





- Disparities between men and women occur across the world, even after adjusting for differences in gross domestic product, geographical region, and environmental risk factors, including tobacco exposure.
- In fact, changes in tobacco use among males and females over the past two decades have resulted in a marked reduction in the male:female (M:F) ratio of lung and bronchus cancer.
- But over the same time period, the M:F ratios for several cancers have remained >2:1, including for those associated with tobacco use such as kidney and renal pelvis, urinary bladder, and oral cavity and pharynx cancer



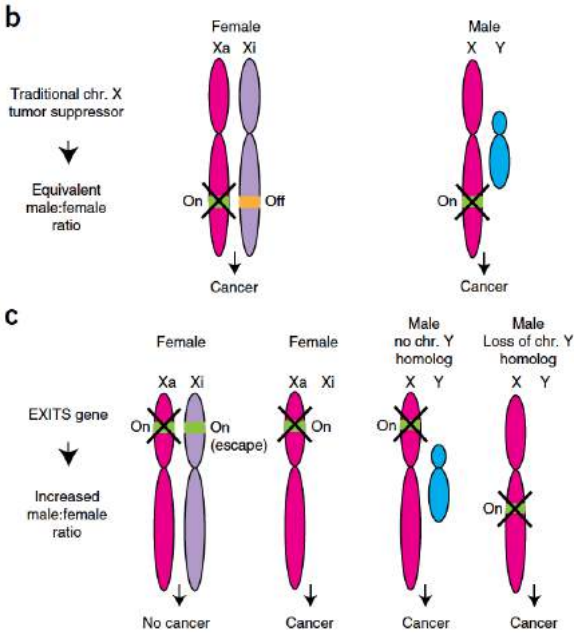
E. Heard, March 27th 2023

The EXITS Hypothesis

Escape from X inactivation Tumour Suppressor

Tumor-suppressor genes that escape from X-inactivation contribute to cancer sex bias

Andrew Dunford[1,6], David M Weinstock[1,2,6], Virginia Savova[3,4], Steven E Schumacher[1,3], John P Cleary[2], Akinori Yoda[2], Timothy J Sullivan[1], Julian M Hess[1], Alexander A Gimelbrant[1,3,4], Rameen Beroukhim[1–3], Michael S Lawrence[5,7], Gad Getz[1,5,7] & Andrew A Lane[1,2,7]



- Male predominance across many cancer types: genetic, horminal or environmental?
- Hypothesis: X-linked genes that can escape X-inactivation might protect females from complete functional loss by a single mutation.
- Identify putative 'escape from X-inactivation tumor-suppressor' (EXITS) genes,
- Look at somatic alterations in >4,100 cancers across 21 tumour types for sex bias.
- 6 out of 783 non-pseudoautosomal region (PAR) X-chromosome genes (*ATRX*, *CNKSR2*, *DDX3X*, *KDM5C*, *KDM6A*, and *MAGEC3*) had loss-of-function mutations more frequently in males (false discovery rate < 0.1), compared to zero of 18,055 autosomal and PAR genes (Fisher's exact $P < 0.0001$)
- Male-biased mutations in X-linked escapees were observed in combined analysis across many cancers and in several individual tumor types, suggesting a generalized phenomenon.
- Thus biallelic expression of EXITS genes in females explains a portion of the reduced cancer incidence in females as compared to males across a variety of tumor types.

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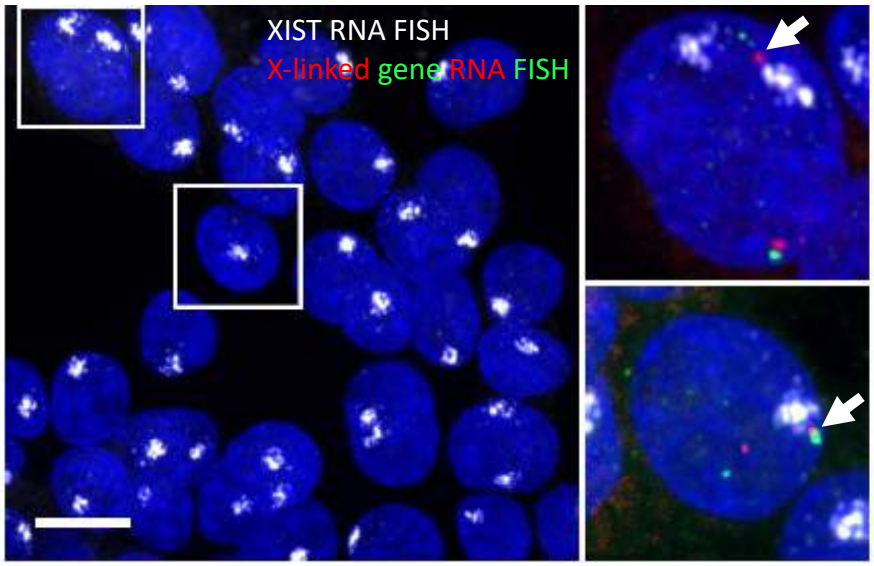
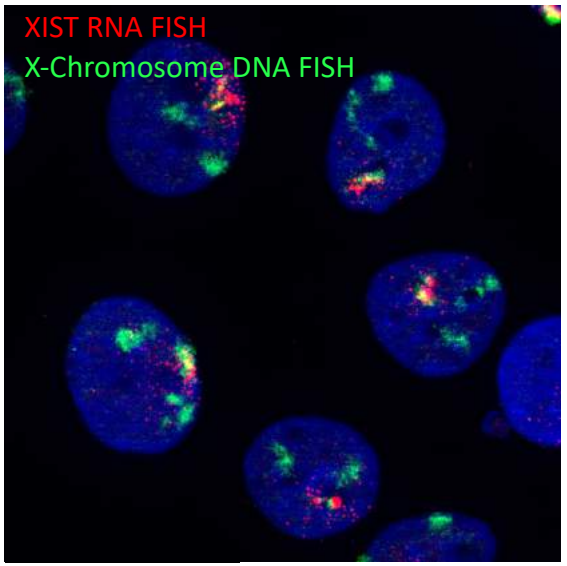
Table 1 Genes with significantly (FDR < 0.1) increased M:F mutation ratios identified by permutation analysis

Gene	Analysis set	LOF mutations	Total cancers	*P* value	*Q* (FDR) value
ATRX	All	70 M: 47 F	2,440 M: 1,686 F	0.000001	0.00066
ATRX	LGG	45 M: 19 F	98 M: 72 F	0.000001	0.000071
CNKSR2	All	30 M: 10 F	2,440 M: 1,686 F	0.00037	0.049
DDX3X	All	34 M: 9 F	2,440 M: 1,686 F	0.000026	0.0075
KDM5C	All	31 M: 10 F	2,440 M: 1,686 F	0.000092	0.015
KDM5C	KIRC	14 M: 1 F	216 M: 118 F	0.0003	0.044
MAGEC3	All	15 M: 1 F	2,440 M: 1,686 F	0.000034	0.0075
Gene	Analysis set	LOF mutations or CN deletions	Total cancers	*P* value	*Q* (FDR) value
KDM5C	All	24 M: 5 F	1,225 M: 769 F	0.00022	0.079
KDM5C	KIRC	14 M: 1 F	216 M: 118 F	0.00047	0.08
KDM6A	All	50 M: 18 F	1,225 M: 769 F	0.00025	0.079

Significance values are based on deviation of the observed mutation incidence in a specific gene relative to that expected in a given set. This approach normalizes to the number of male and female cancers (and to the number of X chromosomes) as well as to the background mutation incidence in male and female cancers in a given set. LGG, lower-grade glioma; KIRC, clear cell kidney cancer; all, pooled data from all included cancer types; LOF, loss of function (Online Methods); CN, copy number; FDR, false discovery rate.

Spatial disorganisation and Epigenetic disruption of the inactive X in Breast cancer cell lines and Primary Tumors





- Loss of the inactive X (Barr body) in cancer: genetic or epigenetic instability?
- Aberrant XIST RNA coating (Pageau et al, 2007), global chromatin disorganisation and epigenetic instability of the Xi are accompanied by significant gene reactivation (Chaligné et al, 2015)
- Chromosome-wide epigenetic changes - as well as more local promoter/regulatory sequence perturbations accompany gene reactivation
- Aberrantly re-expressed Xi genes can result in increased/abnormal protein level
  ⇒ impact on tumor growth and development?

Chaligné *et al.* Genome Res., 2015

X-linked cancer-specific escapees in primary breast tumors

Data from Shah et al. Nature, 2012

104 primary breast tumors (BLC)

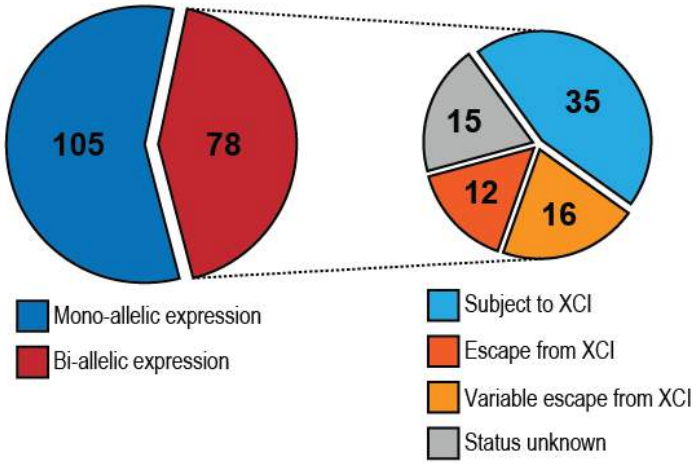
SNP6 array dataset:
No LOH at XIC / XIST locus
High purity and high quality tumors

25 primary breast tumors

RNA-seq dataset:
> 20 reads per SNP
0,3 < RNA Allelic imbalance < 0,7

Allelic expression status of X-linked genes

Allelic expression of
X-linked genes in primary tumors

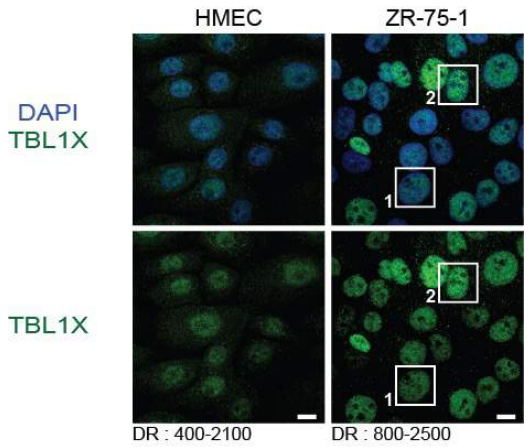


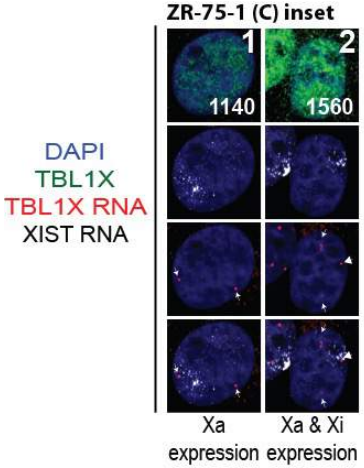
Gene	Tumor number: Bi-allelic expression	Tumor number: Mono-allelic expression
GNL3L	14	2
TMEM164	4	1
XIAP	4	9
CYBB	3	1
DOCK11	3	7
NXT2	3	3
TBL1X	3	5
CLCN4	2	6
PJA1	2	8
SASH3	2	8
SH3BGRL	2	3
SLC25A43	2	5
TCEAL4	2	4
ZNF275	2	9

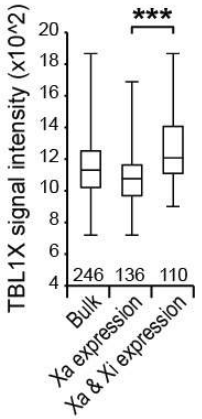
Approx. 20% of informative X-linked genes display aberrant bi-allelic expression in breast tumors of all subtypes

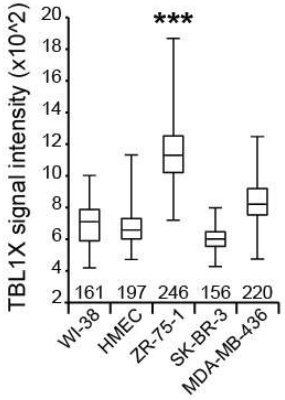
Consequence of X-linked gene reactivation on protein dosage ?

The perturbed epigenetic status of the Xi, is accompanied by significant reactivation of X-linked genes in tumor cells (>10% of genes are aberrantly reactivated)









- Xi reactivation lead to increase protein dosage which might give a selective advantage in tumorigenesis
- Several cancer specific escapees are potentially involved in cancer (HDAC8, TBL1X…)

CONCLUSIONS

X-linked dosage is critical for development and disease

X-linked escapees are directly involved in autoimmune and other diseases with sex bias

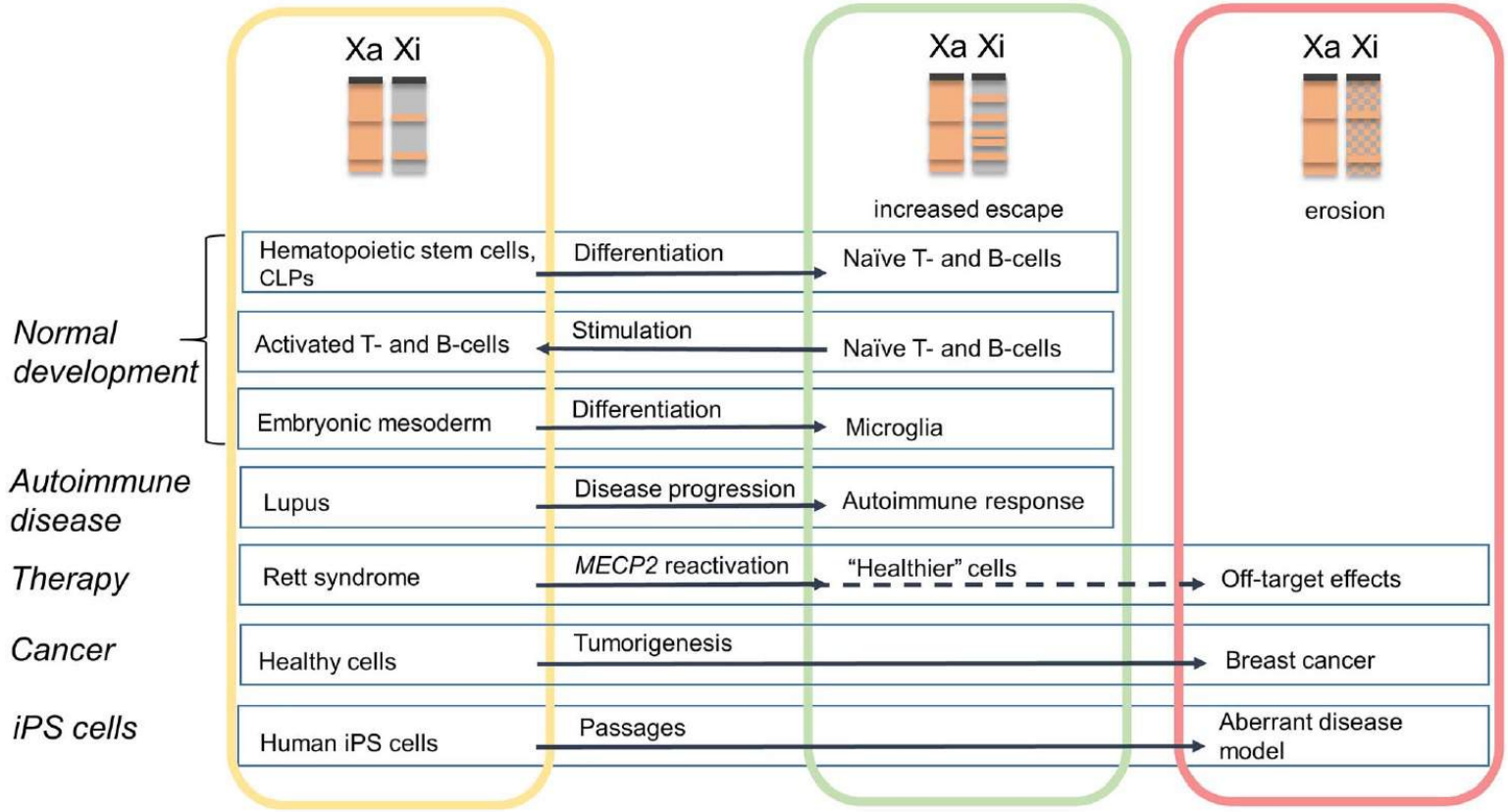
X-linked escapees can play critically roles in cancer : for XX protection or for promotion of cancer progression

Loss of XIST and Xi transcriptional instability is common among human breast tumors of poor prognosis.

XIST is not just the key trigger for XCI during development but a gatekeeper of tissue homeostasis

The involvement of genes that are variable in their escape from XCI, highlight the need to consider the inactive X chromosome as a potential contributor to disease, rather than a silent evolutionary oddity of sex determination.

Expression from the inactive X is important for normal development, disease, therapeutic treatmentd and cell engineering



Adapted from Fang et al, 2021

CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Image : *La chute de l'homme* (détail), Cornelis Cornelisz van Haarlem, 1592. © Rijksmuseum

COLLOQUE

The Genetic and Epigenetic Basis of Sex Bias in Disease

21 avril 2023

COLLÈGE DE FRANCE 1530

Thomas Römer
Administrateur du Collège de France
11, place Marcelin-Berthelot, 75005 Paris
www.college-de-france.fr

Année académique 2022/2023

21 avril 2023 de 9h à 18h
Amphitheatre Maurice Halbwachs

The Genetic and Epigenetic Basis of Sex Bias in Disease

Edith Heard, Chaire Épigénétique & mémoire cellulaire
Scientific co-organisers: **James Cleland** and **Agnese Loda**

Daniel Andergassen
Technical University of Munich, Germany

Richard Festenstein
Imperial College, London, UK

Cornelius Gross
EMBL-Rome, Italy

Jean-Charles Guéry
INSERM, University of Toulouse, France

Jamie Hackett
EMBL-Rome, Italy

Irene Miguel-Aliaga
Imperial College, London, UK

Jessica Tollkuhn
Cold Spring Harbor Lab, New York, USA

Taru Tukiainen
FIMM, Helsinki, Finland

Judith Zaugg
EMBL Heidelberg, Germany

Colloquium in English, free entry, no registration required

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E. Heard, March 27th 2023