X-chromosome inactivation : a mammalian model system for epigenetics

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Genetic sex determinism in Mammals

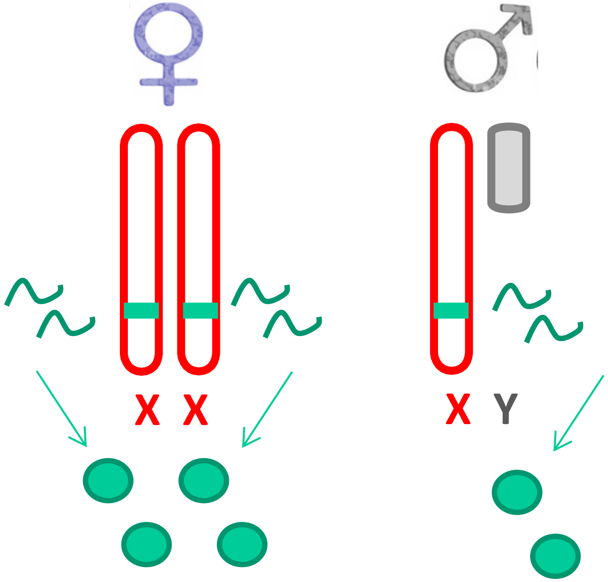
- X Chromosome : ~900 proteins
  Y Chromosome : few proteins (<100)

- Unbalance between males and females:
  Females should produce twice the amount of X-linked proteins.

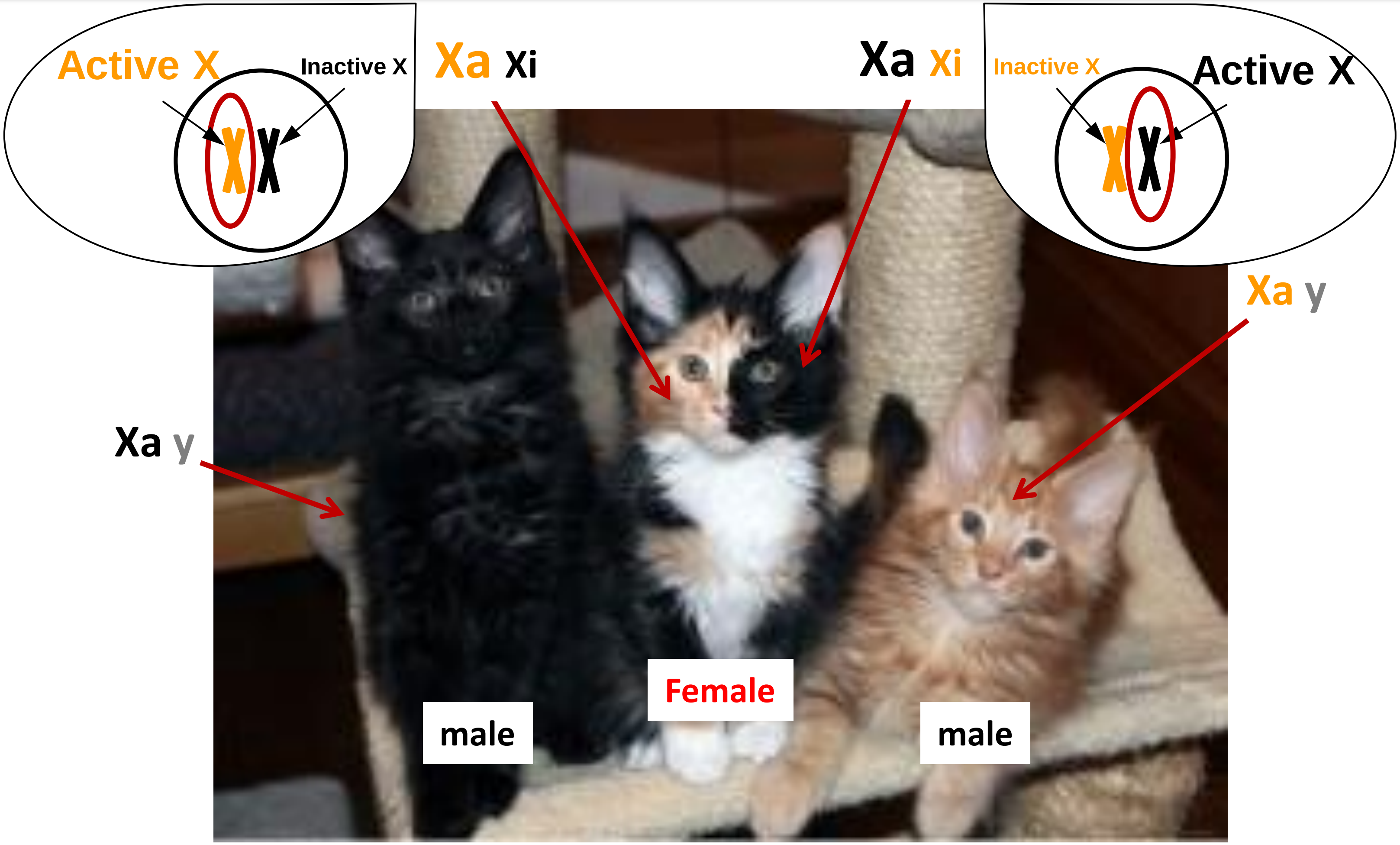
  But the majority of the X-linked genes are as important for both sexes.

Need a dosage compensation strategy!

Which one?



Dosage compensation between XX females and XY males: Random inactivation of one X chromosome in female mammals



Genotype: X / X
Phenotype: Xa or Xa

-> Depending on the inactivated X, patchs will be black or orange.
(White patchs = no melanocyte)

=> Why patches?

Dosage compensation between XX females and XY males: Random inactivation of one X chromosome in female mammals

=> Why patches?

Random silencing
during early development

Stable and clonal inheritance
(Maintenance)

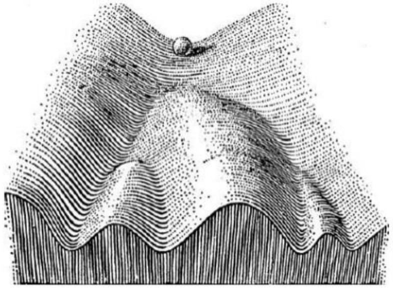
Dosage compensation strategies

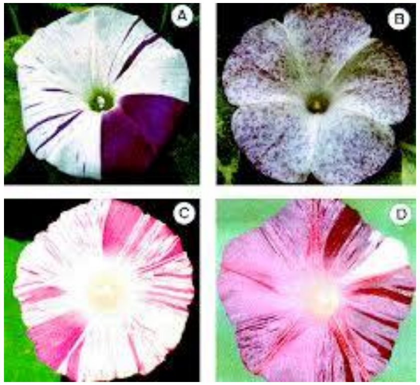
		♀	♂	
Mammals		X X_i	X Y	**Inactivation of one of the 2 X in females**
Drosophila		X X	X Y	**x2 Transcription of the male X**

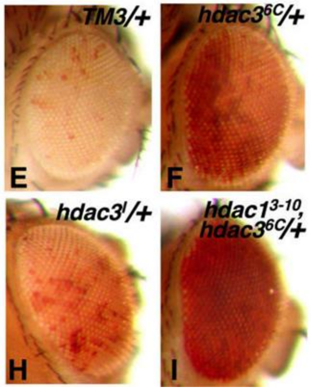
		⚥	♂	
C. elegans		x x	XO	**÷2 Transcription of the 2 X in hermaphrodites**

1. Introduction on X Chromosome Inactivation

Classical example of epigenetic mechanism





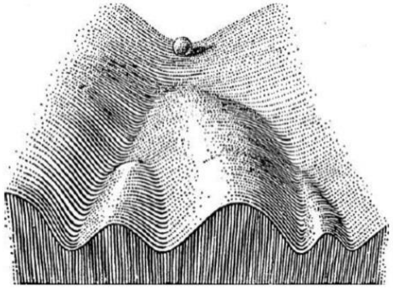


Classical example of epigenetic mechanism

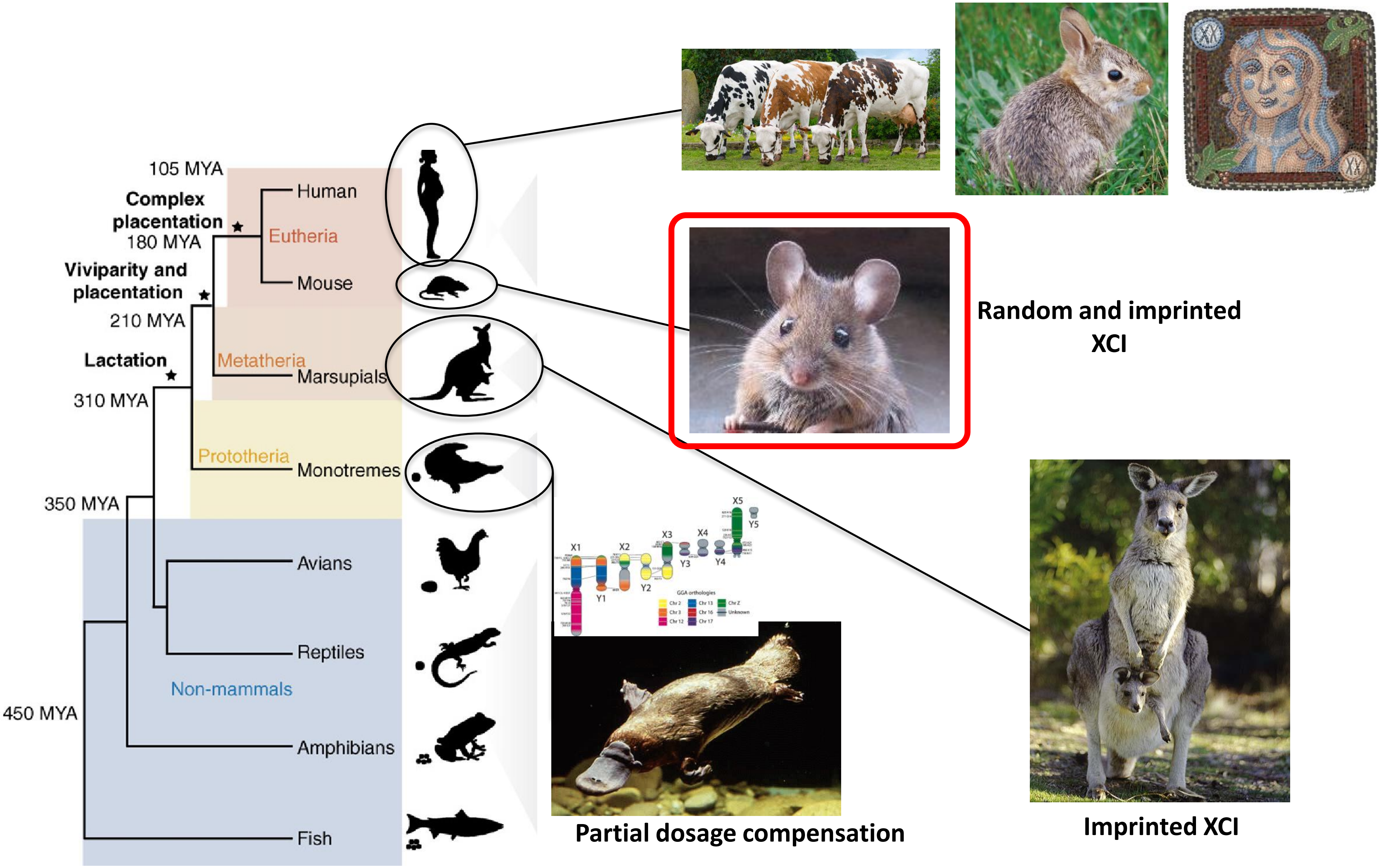
- Modification of gene expression without changes in gene sequence
- Stable through cell divisions
- Can be reversed *(in the germ line)*

Special features:

- Regulation of expression of a whole chromosome
- Differential treatment de of two homologous chromosome in the same nucleus



Different strategies to perform XCI in mammals



Random XCI

Random and imprinted XCI

Partial dosage compensation

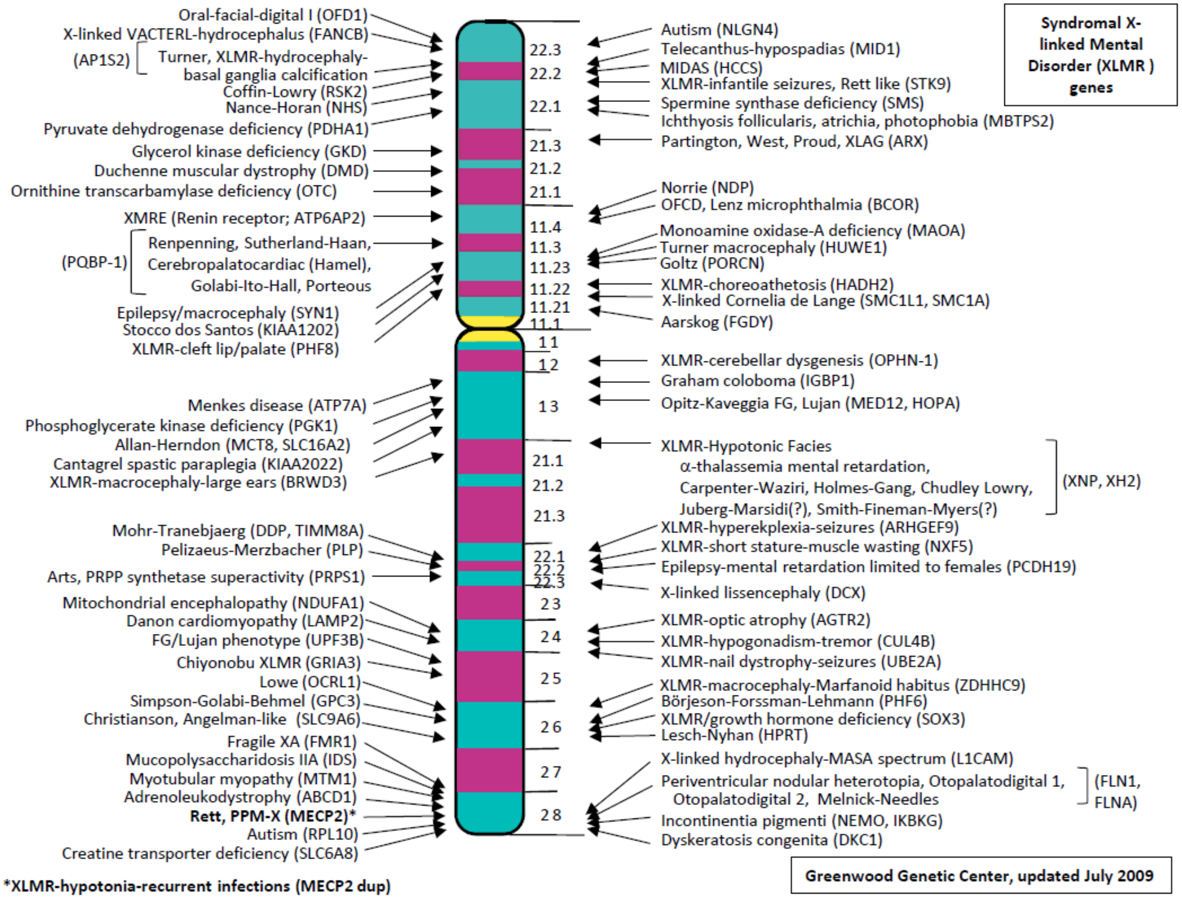
Imprinted XCI

(Hore and Graves, 2007)

Implication of X inactivation in human diseases

Severe phenotypes / lethality in men
Variable phenotypes / no phenotype in women

Eg Haemophilia, muscular dystrophy, autism, Rett syndrome …



Syndromal X-linked Mental Disorder (XLMR) genes

Oral-facial-digital I (OFD1)
X-linked VACTERL-hydrocephalus (FANCB)
(AP1S2) Turner, XLMR-hydrocephaly-basal ganglia calcification
Coffin-Lowry (RSK2)
Nance-Horan (NHS)
Pyruvate dehydrogenase deficiency (PDHA1)
Glycerol kinase deficiency (GKD)
Duchenne muscular dystrophy (DMD)
Ornithine transcarbamylase deficiency (OTC)
XMRE (Renin receptor; ATP6AP2)
(PQBP-1) Renpenning, Sutherland-Haan, Cerebropalatocardiac (Hamel), Golabi-Ito-Hall, Porteous
Epilepsy/macrocephaly (SYN1)
Stocco dos Santos (KIAA1202)
XLMR-cleft lip/palate (PHF8)
Menkes disease (ATP7A)
Phosphoglycerate kinase deficiency (PGK1)
Allan-Herndon (MCT8, SLC16A2)
Cantagrel spastic paraplegia (KIAA2022)
XLMR-macrocephaly-large ears (BRWD3)
Mohr-Tranebjaerg (DDP, TIMM8A)
Pelizaeus-Merzbacher (PLP)
Arts, PRPP synthetase superactivity (PRPS1)
Mitochondrial encephalopathy (NDUFA1)
Danon cardiomyopathy (LAMP2)
FG/Lujan phenotype (UPF3B)
Chiyonobu XLMR (GRIA3)
Lowe (OCRL1)
Simpson-Golabi-Behmel (GPC3)
Christianson, Angelman-like (SLC9A6)
Fragile XA (FMR1)
Mucopolysaccharidosis IIA (IDS)
Myotubular myopathy (MTM1)
Adrenoleukodystrophy (ABCD1)
Rett, PPM-X (MECP2)*
Autism (RPL10)
Creatine transporter deficiency (SLC6A8)

Autism (NLGN4)
Telecanthus-hypospadias (MID1)
MIDAS (HCCS)
XLMR-infantile seizures, Rett like (STK9)
Spermine synthase deficiency (SMS)
Ichthyosis follicularis, atrichia, photophobia (MBTPS2)
Partington, West, Proud, XLAG (ARX)
Norrie (NDP)
OFCD, Lenz microphthalmia (BCOR)
Monoamine oxidase-A deficiency (MAOA)
Turner macrocephaly (HUWE1)
Goltz (PORCN)
XLMR-choreoathetosis (HADH2)
X-linked Cornelia de Lange (SMC1L1, SMC1A)
Aarskog (FGDY)
XLMR-cerebellar dysgenesis (OPHN-1)
Graham coloboma (IGBP1)
Opitz-Kaveggia FG, Lujan (MED12, HOPA)
XLMR-Hypotonic Facies α-thalassemia mental retardation, Carpenter-Waziri, Holmes-Gang, Chudley Lowry, Juberg-Marsidi(?), Smith-Fineman-Myers(?) (XNP, XH2)
XLMR-hyperekplexia-seizures (ARHGEF9)
XLMR-short stature-muscle wasting (NXF5)
Epilepsy-mental retardation limited to females (PCDH19)
X-linked lissencephaly (DCX)
XLMR-optic atrophy (AGTR2)
XLMR-hypogonadism-tremor (CUL4B)
XLMR-nail dystrophy-seizures (UBE2A)
XLMR-macrocephaly-Marfanoid habitus (ZDHHC9)
Börjeson-Forssman-Lehmann (PHF6)
XLMR/growth hormone deficiency (SOX3)
Lesch-Nyhan (HPRT)
X-linked hydrocephaly-MASA spectrum (L1CAM)
Periventricular nodular heterotopia, Otopalatodigital 1, Otopalatodigital 2, Melnick-Needles (FLN1, FLNA)
Incontinentia pigmenti (NEMO, IKBKG)
Dyskeratosis congenita (DKC1)

***XLMR-hypotonia-recurrent infections (MECP2 dup)**

Greenwood Genetic Center, updated July 2009

Implication of X inactivation in human diseases

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Variable phenotypes / no phenotype in women

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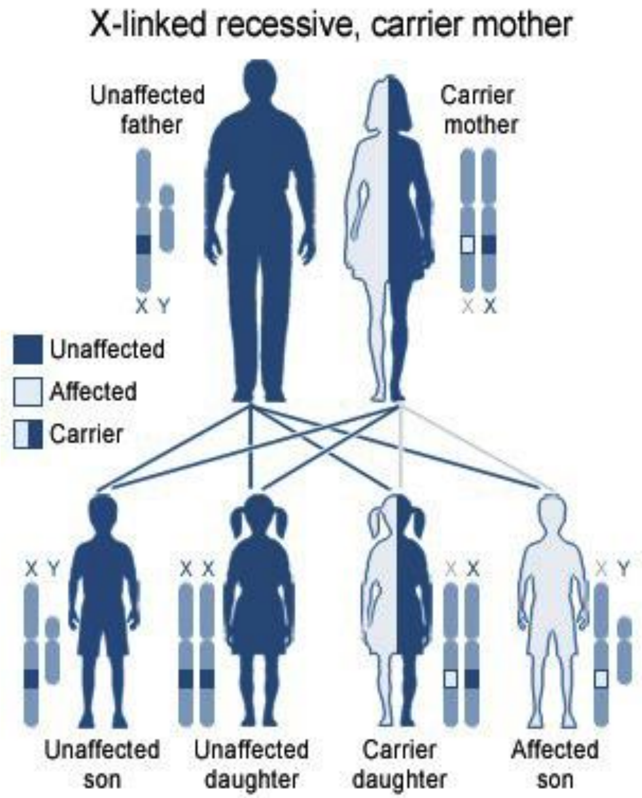
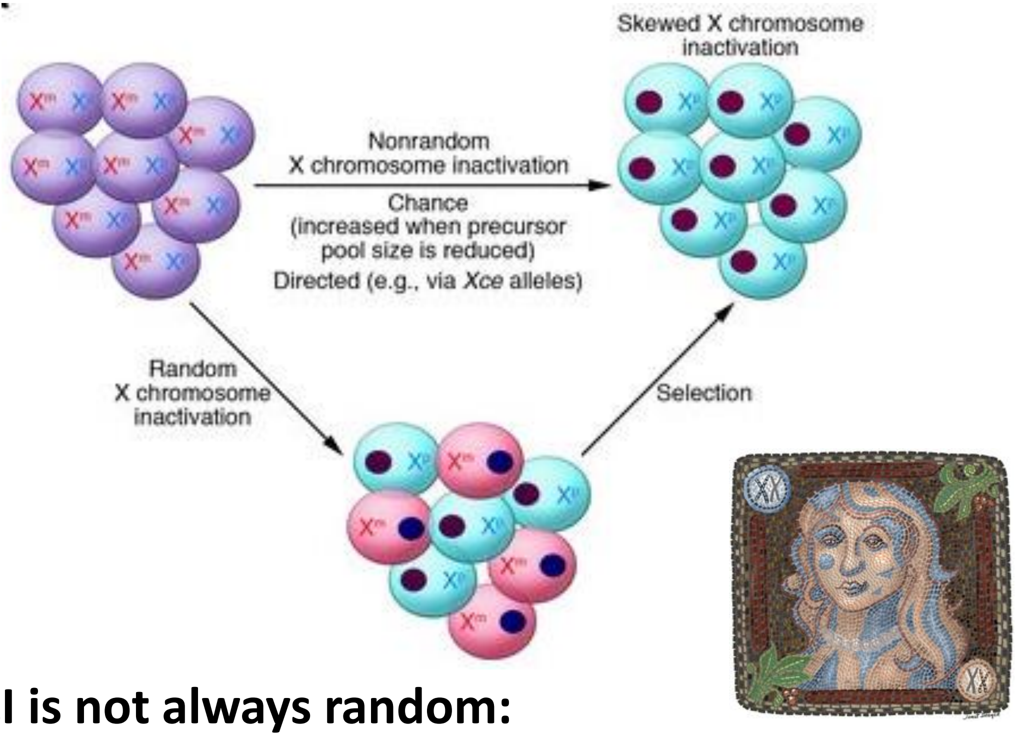


U.S. National Library of Medicine

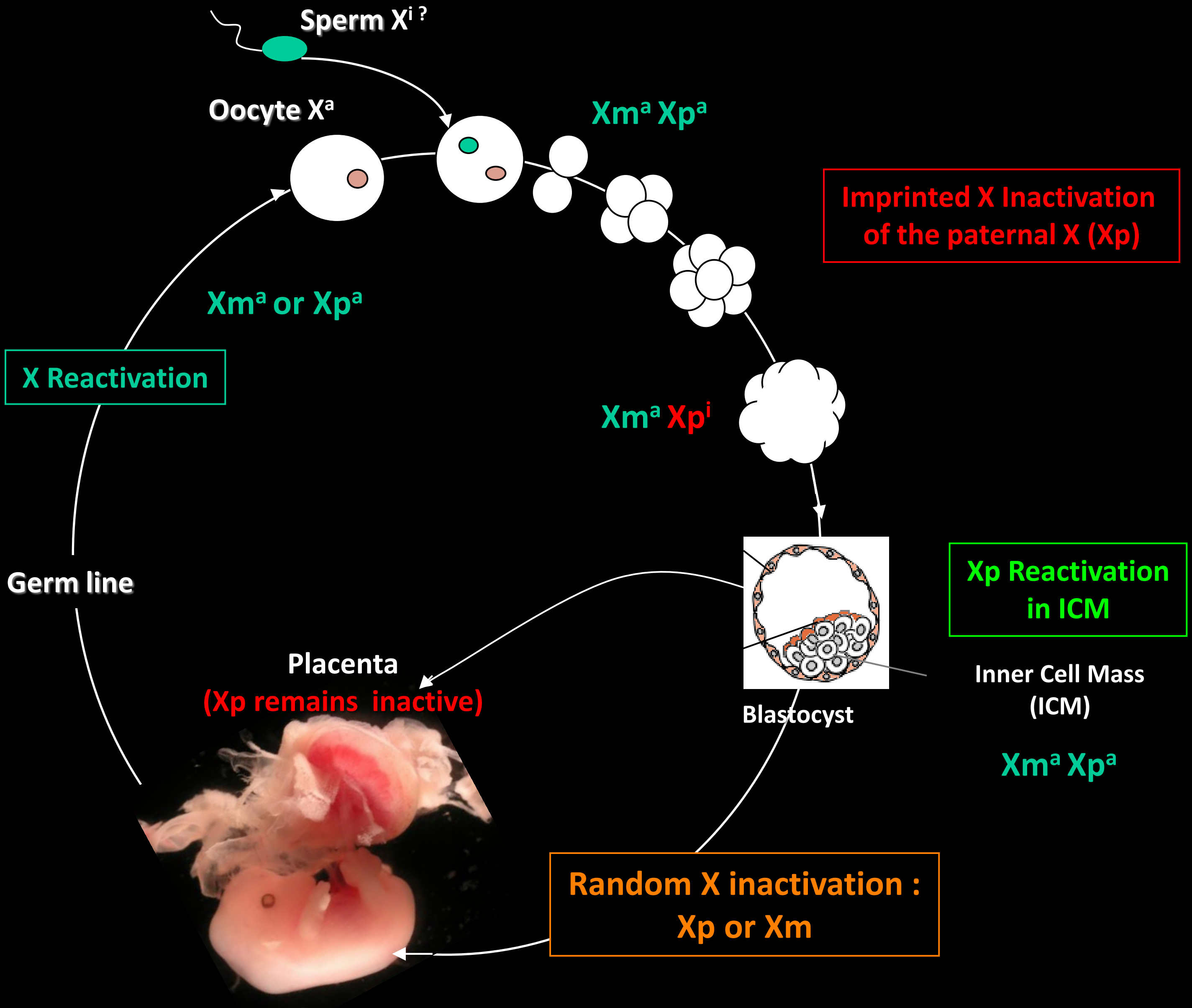


XCI is not always random:

- Skewing in the initial choice of X to inactivate – either by chance, or due to genetic differences

- Selection <u>against</u> cells expressing mutant allele

Developmental dynamics of X inactivation in mice



Sperm $X^{i\,?}$

Oocyte X^a

$Xm^a\ Xp^a$

Imprinted X Inactivation of the paternal X (Xp)

Xm^a or Xp^a

X Reactivation

$Xm^a\ Xp^i$

Germ line

Xp Reactivation in ICM

Placenta

(Xp remains inactive)

Blastocyst

Inner Cell Mass (ICM)

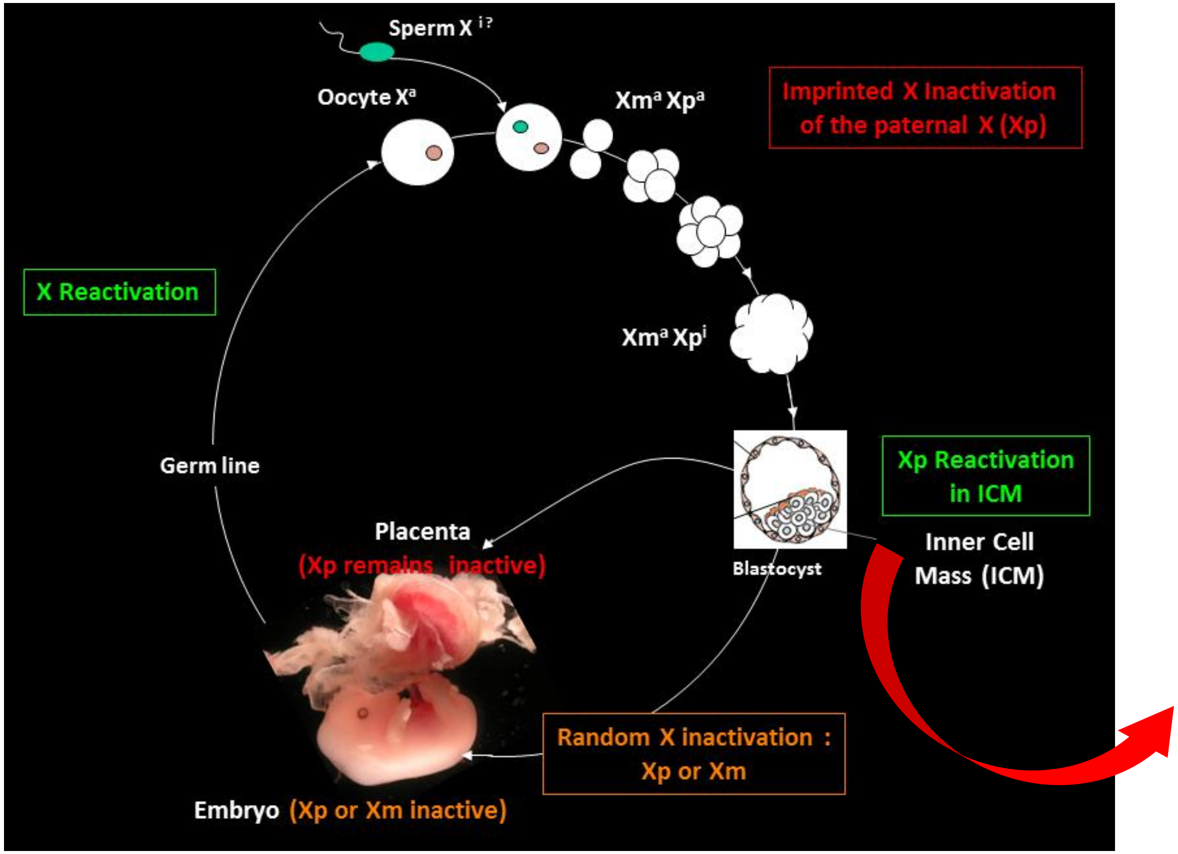
$Xm^a\ Xp^a$

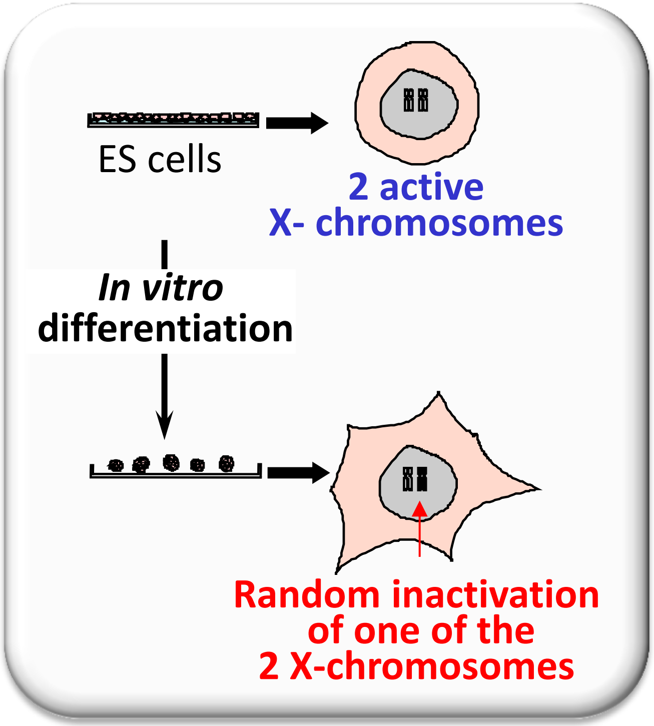
Random X inactivation : Xp or Xm

Embryo ($Xm^a\ Xp^i$ or $Xm^i\ Xp^a$)

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

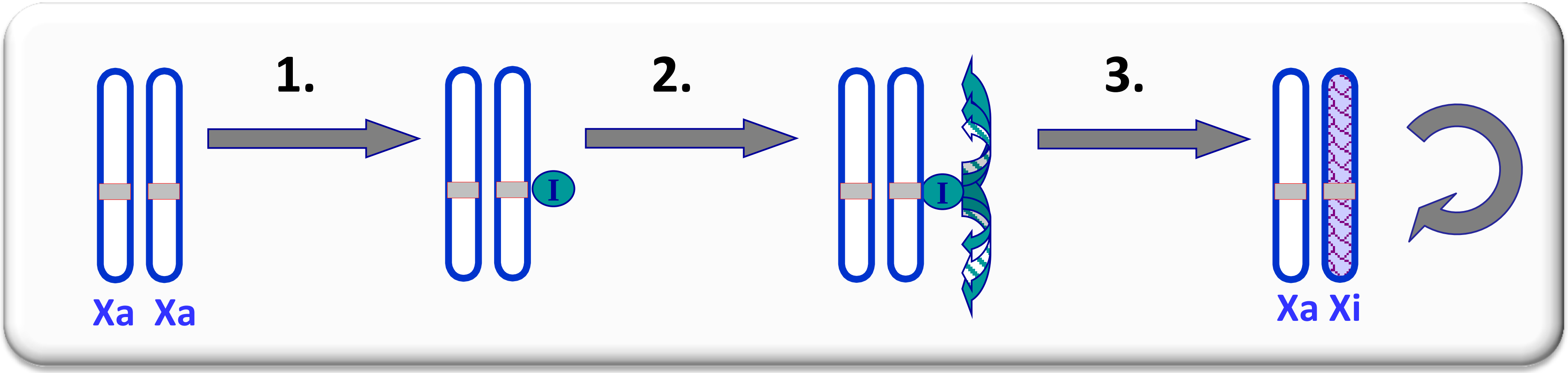
Embryonic Stem Cells: A model system for XCI





Mechanisms regulating X inactivation?

Steps of the XCI process



1. **INITIATION: It has to initiate the process (counting – choice)**

2. **PROPAGATION: It has to propagate the silencing through the entire chromosome**

3. **MAINTENANCE: It has to maintain the inactive state stable through cell divisions**

Work from Avner, Brockdorff, Brown, Gribnau, Heard, Jaenisch, Lee, Panning, Rastan, Rougeulle, Sado, Sasaki, Willard, Wutz … labs.

QUESTIONS

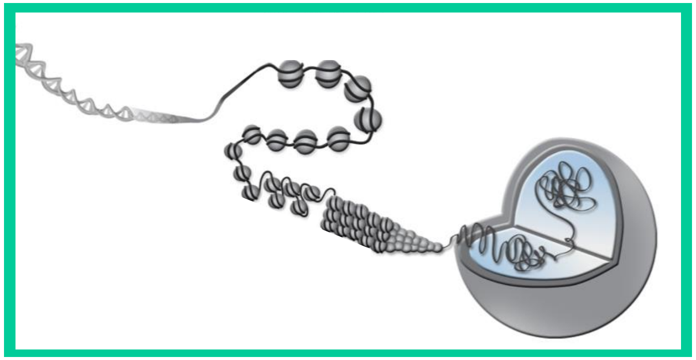
How are two genetically identical chromosomes in the same nucleoplasm differentially treated ?

How is XCI set up ? How is it maintained ? How is it reversed ?

Ideas?

➔ Epigenetic mechanisms

Genome conformation   Nuclear organization

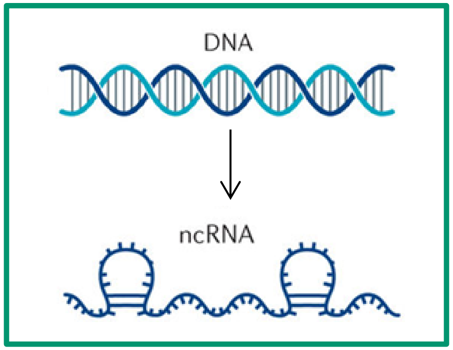


DNA compaction
Cis / trans interactions

Nuclear location
Nuclear bodies

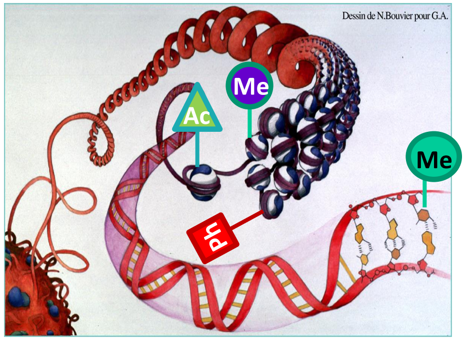
Gene regulation
Nuclear functions

Non-coding RNAs



<u>Long non-coding RNAs</u>
Small RNAs (siRNAs, miRNAs, piRNAs…)
Intergenic transcripts

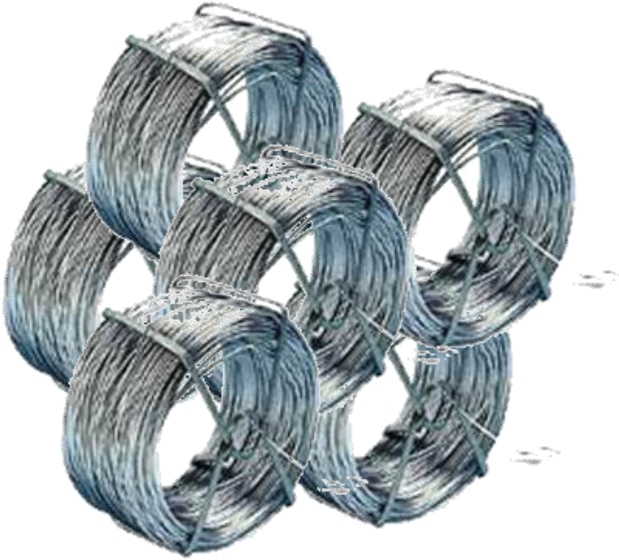
Chromatin landscape



Histone modifications and variants
Chromatin remodelling
DNA methylation
…

How to fold…

…2 meters of DNA…



20 Km of wireframe…

… In a 1000 µm^3 nucleus?

… In a tennis ball

diameter:	6µm (10^{-6}m)	6.7cm (10^{-2}m)
length:	2m	~20km (Paris-Orly airport)
thickness:	2.5nm (10^{-9}m)	~20µm (10^{-5}m)

How to fold…

…2 meters of DNA…

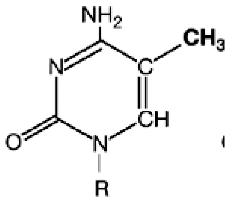
… In a 1000 μm^3 nucleus?

HOW ???

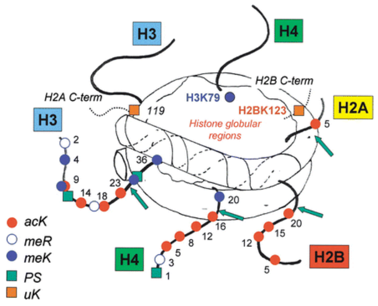
The nucleus is highly organized

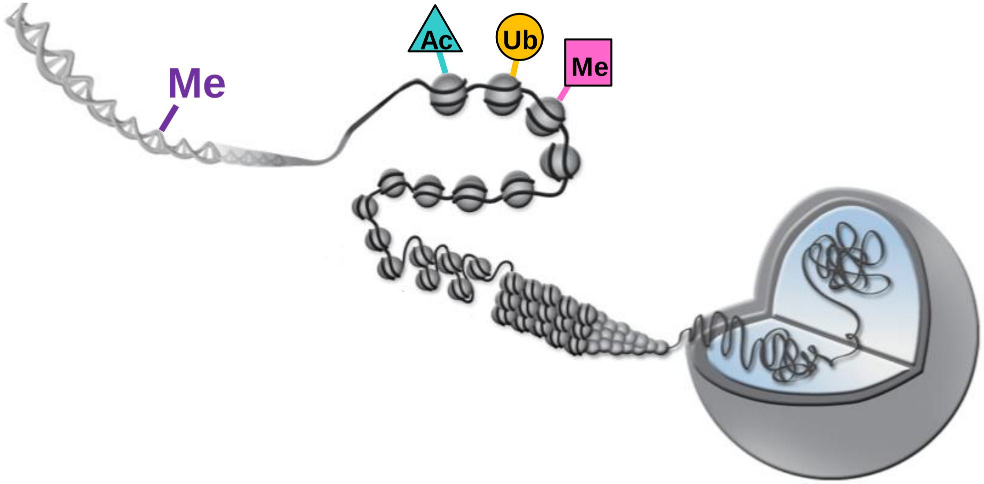
Chromatin folding - Genome conformation - Chromosome territories & positioning

DNA methylation
Histone modifications

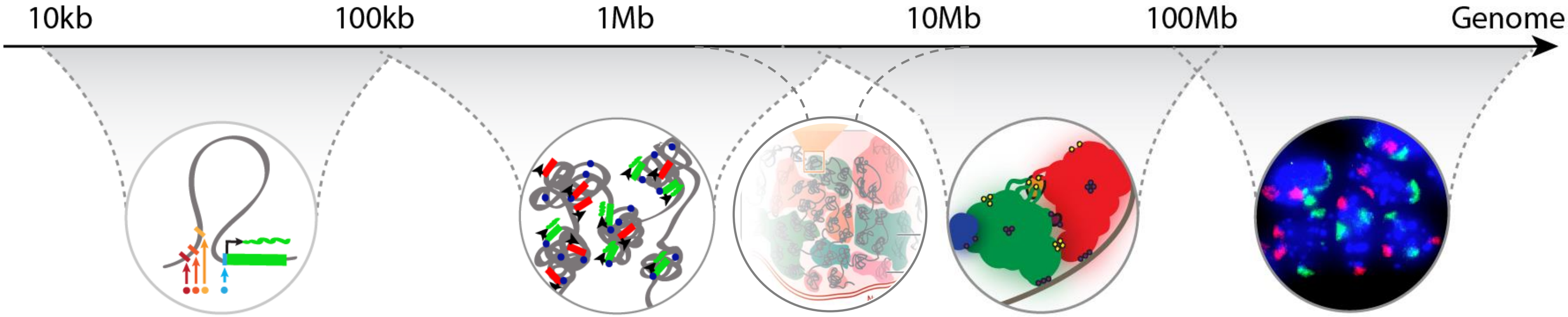


5-Methyl-cytidine





Genome organization



Local DNA loops

Topologically associating domains

A/B compartments

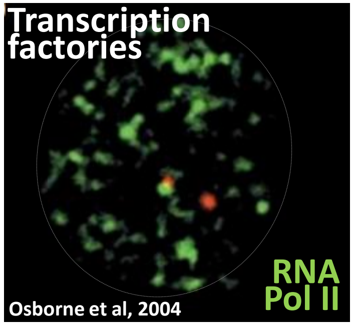
Chromosome territories

Chromosome positioning

The nucleus is highly organized

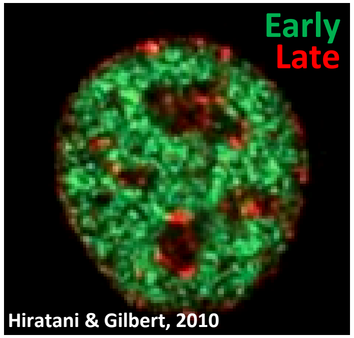
Functional compartmentalization

Transcription:

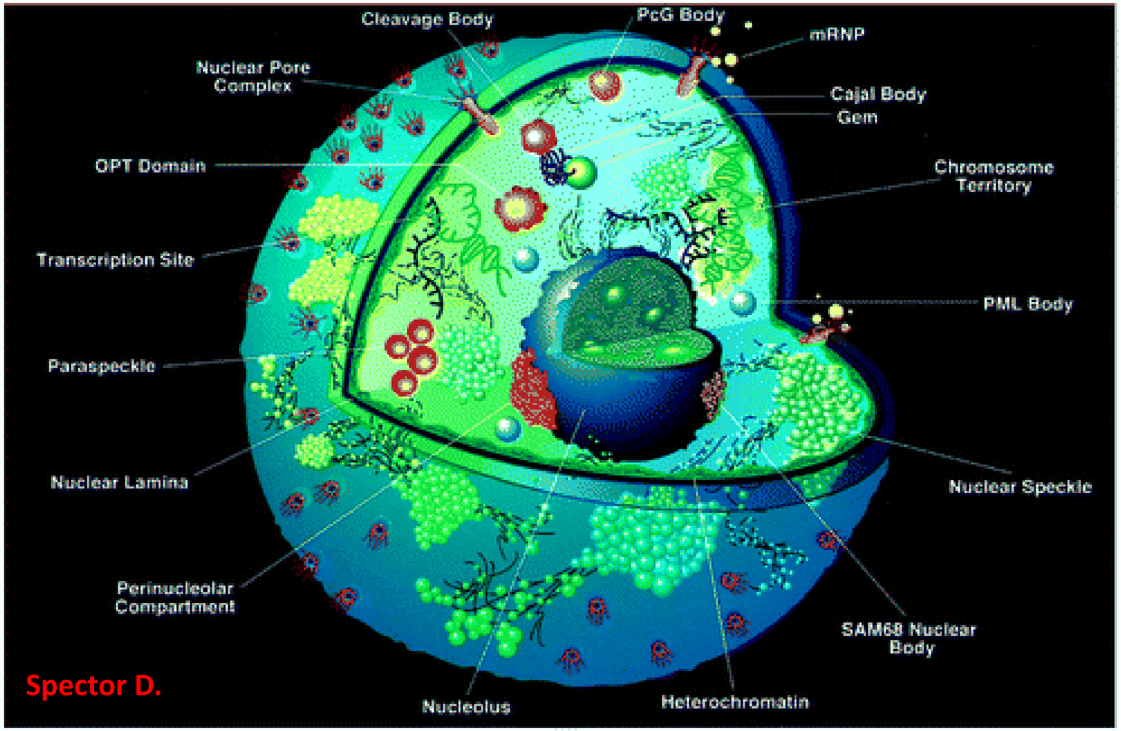


Osborne et al, 2004

Replication:

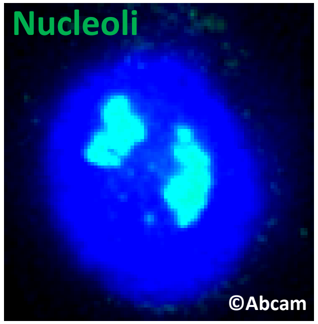


Hiratani & Gilbert, 2010



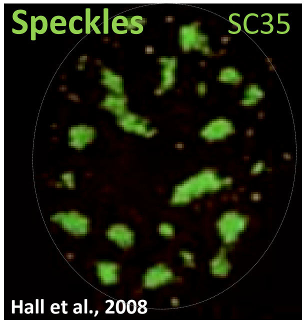
Spector D.

rRNA biogenesis:



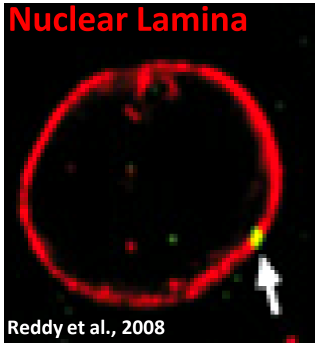
©Abcam

RNA splicing:

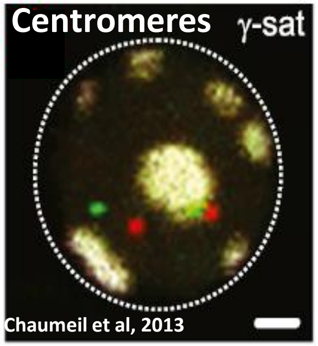


Hall et al., 2008

Transcriptional repression:



Reddy et al., 2008



Chaumeil et al, 2013

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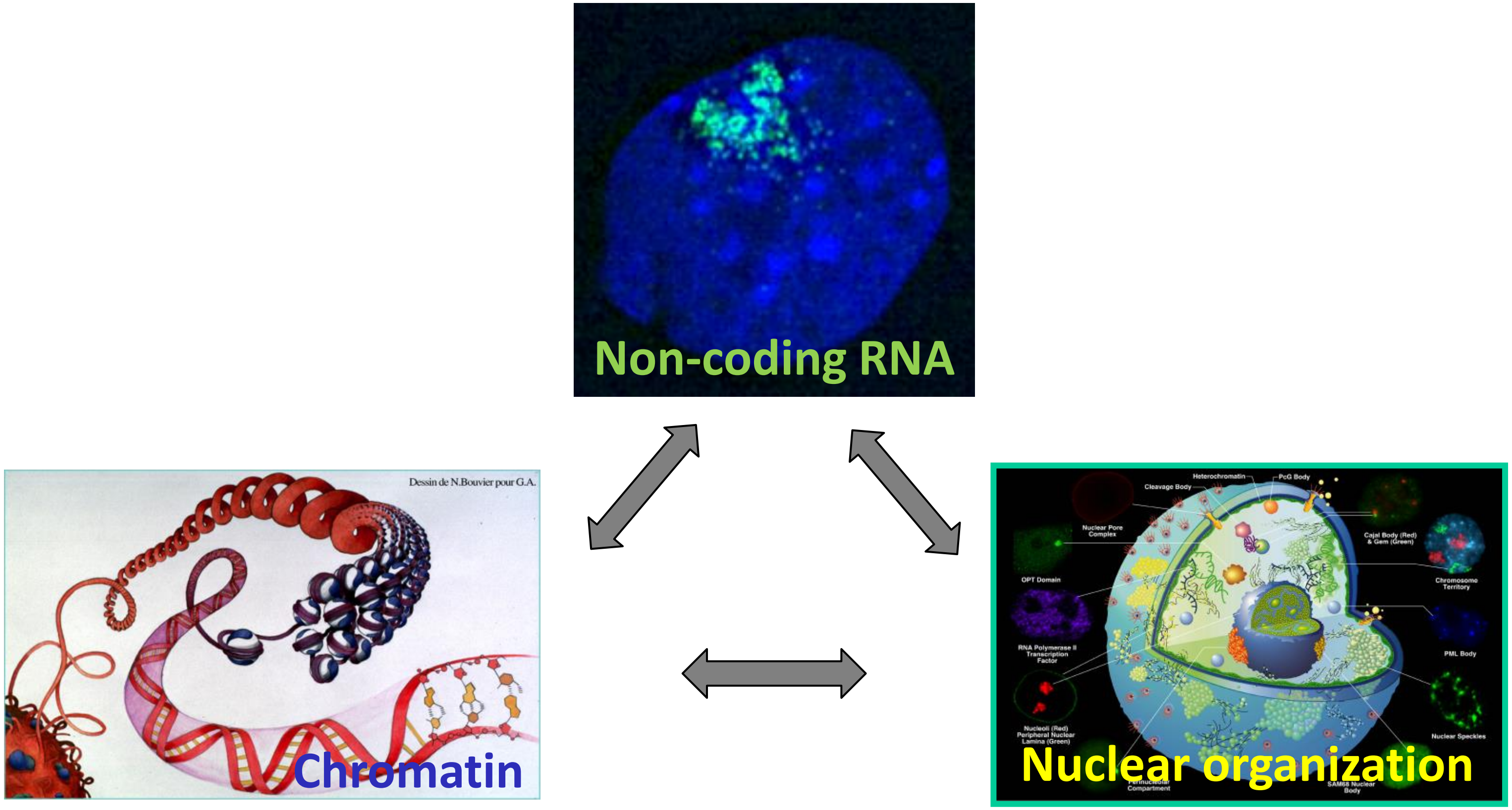
How is XCI set up ? How is it maintained ? How is it reversed ?

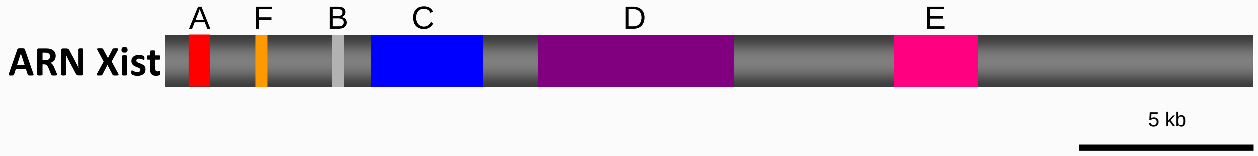


1. **Introduction on X Chromosome Inactivation**

2. **Initiation of XCI:**

What is the key player?

The Xist non-coding RNA



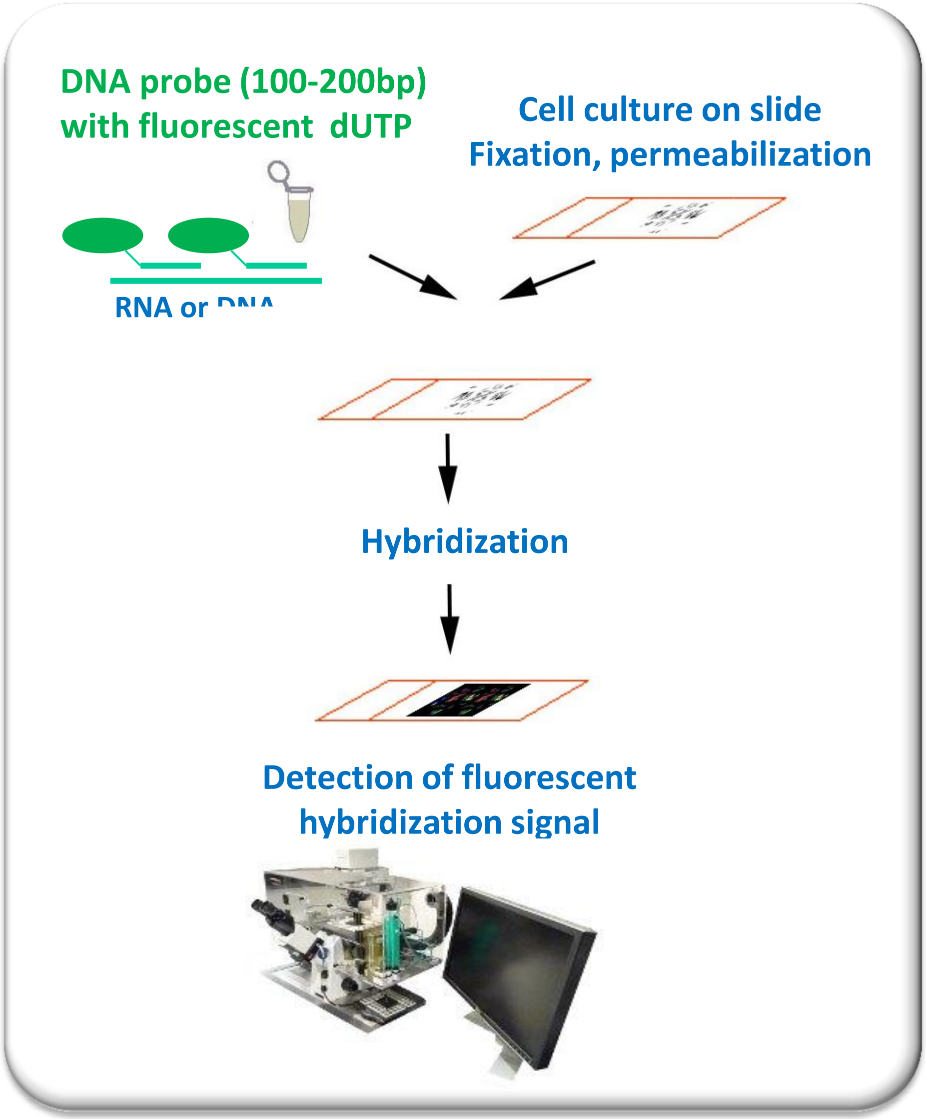
- ✓ Transcript expressed exclusively from the inactive X (gene in the Xic).
- ✓ ~17kb long.
- ✓ Poor similarity in sequence between mouse and human but similarities in the secondary structure (REPEATS).
- ✓ Splicing and polyadenylation like mRNAs.

Localization of the Xist RNA in the cell?

METHOD?

work from Avner, Brockdorff, Gribnau, Heard, Jaenisch, Lee, Panning, Rougeulle, Sado, Sasaki, Wutz…labs.

Method: In situ fluorescent hybridization – **RNA** FISH



DNA probe (100-200bp) with fluorescent dUTP

RNA or

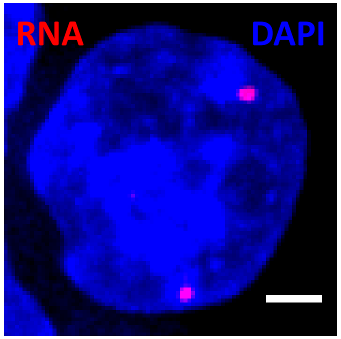
Cell culture on slide
Fixation, permeabilization

Hybridization

Detection of fluorescent hybridization signal

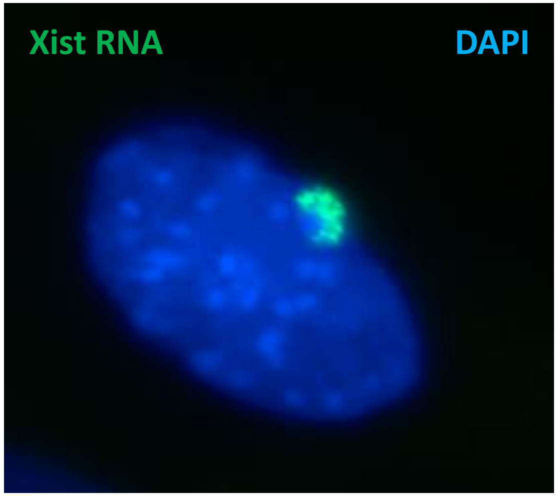
RNA FISH:

2 pinpoints = nascent RNAs
at their transcription sites



The Xist non-coding RNA

RNA FISH



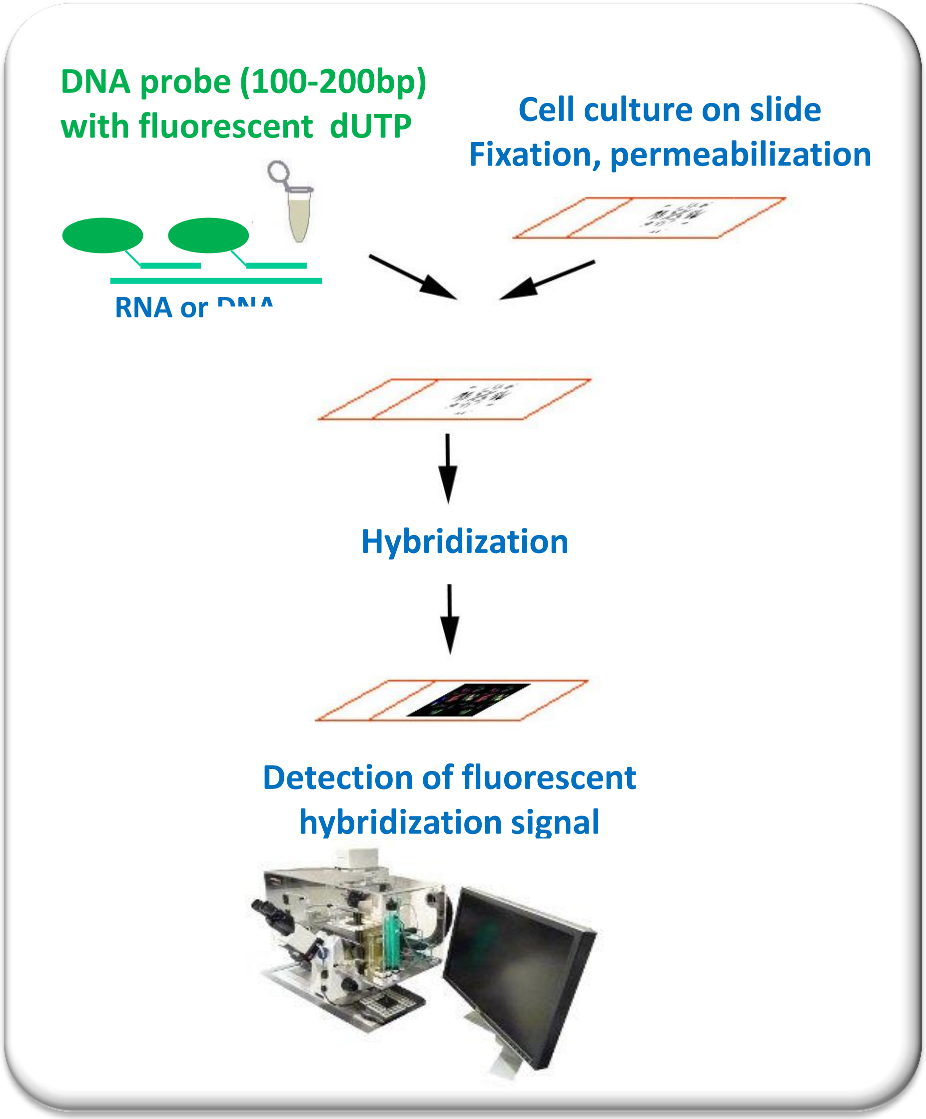
- ✓ **Xist remains in the nucleus and forms a “cloud”.**

What is this cloud?
Method?

work from Avner, Brockdorff, Brown, Gribnau, Heard, Jaenisch, Lee, Panning, Rastan, Rougeulle, Sado, Sasaki, Willard, Wutz… labs

Method: In situ fluorescent hybridization – **RNA FISH**

What is the key difference between a DNA and an RNA FISH?



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RNA or DNA

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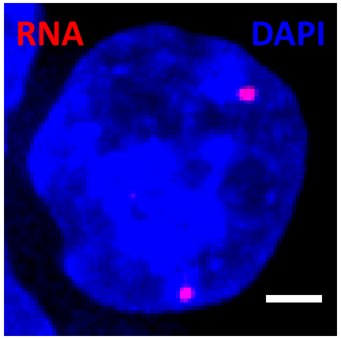
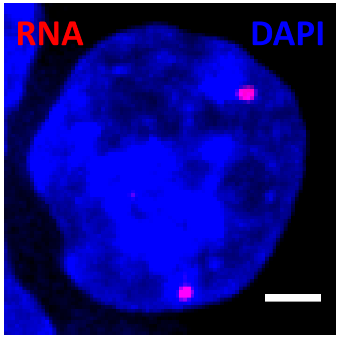


DNA FISH:

2 pinpoints = 2 alleles



Method: In situ fluorescent hybridization – **RNA FISH**

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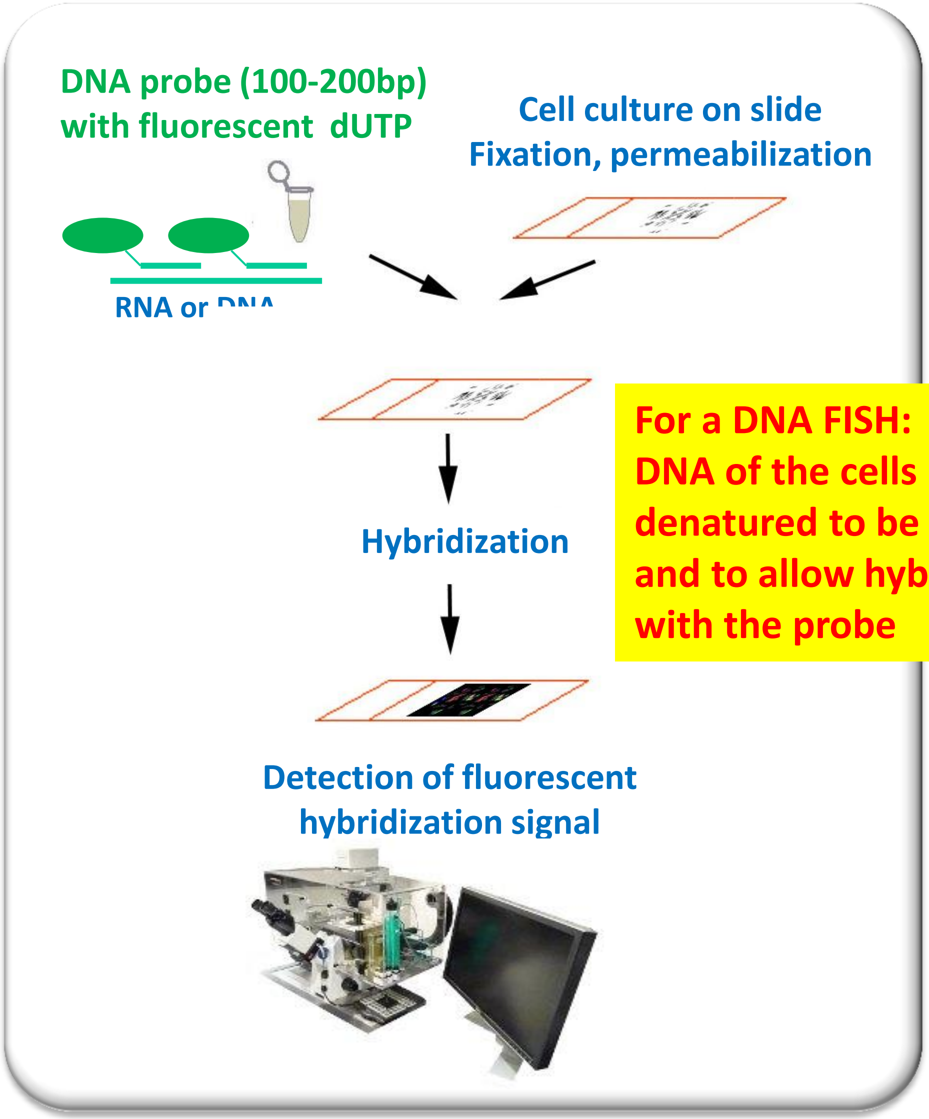


DNA probe (100-200bp) with fluorescent dUTP

RNA or DNA

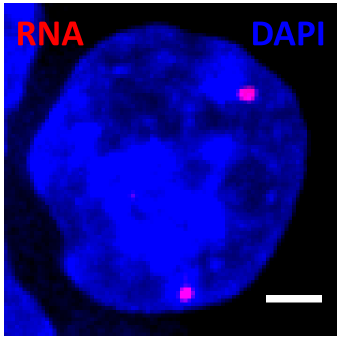
Cell culture on slide
Fixation, permeabilization

Hybridization

Detection of fluorescent hybridization signal

For a DNA FISH:
DNA of the cells must be denatured to be accessible and to allow hybridization with the probe

RNA FISH:
2 pinpoints = nascent RNAs at their transcription sites



DNA FISH:
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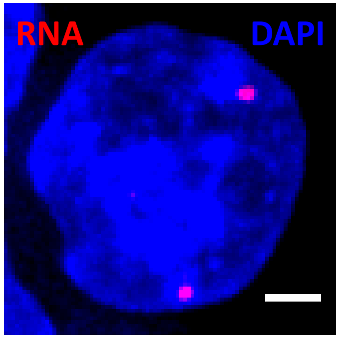
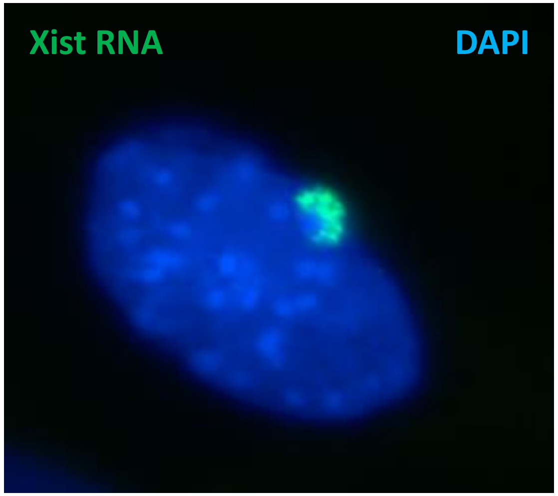
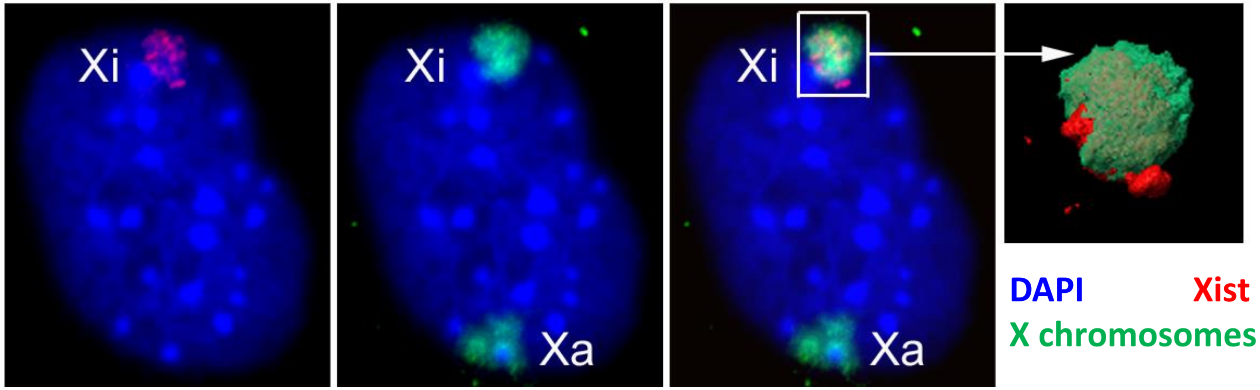


The Xist non-coding RNA

RNA FISH



RNA/DNA FISH



✓ **Xist remains in the nucleus and coats the inactive X chromosome.**

Timing of accumulation ?

work from Avner, Brockdorff, Gribnau, Heard, Jaenisch, Lee, Panning, Rougeulle, Sado, Sasaki, Wutz…labs.

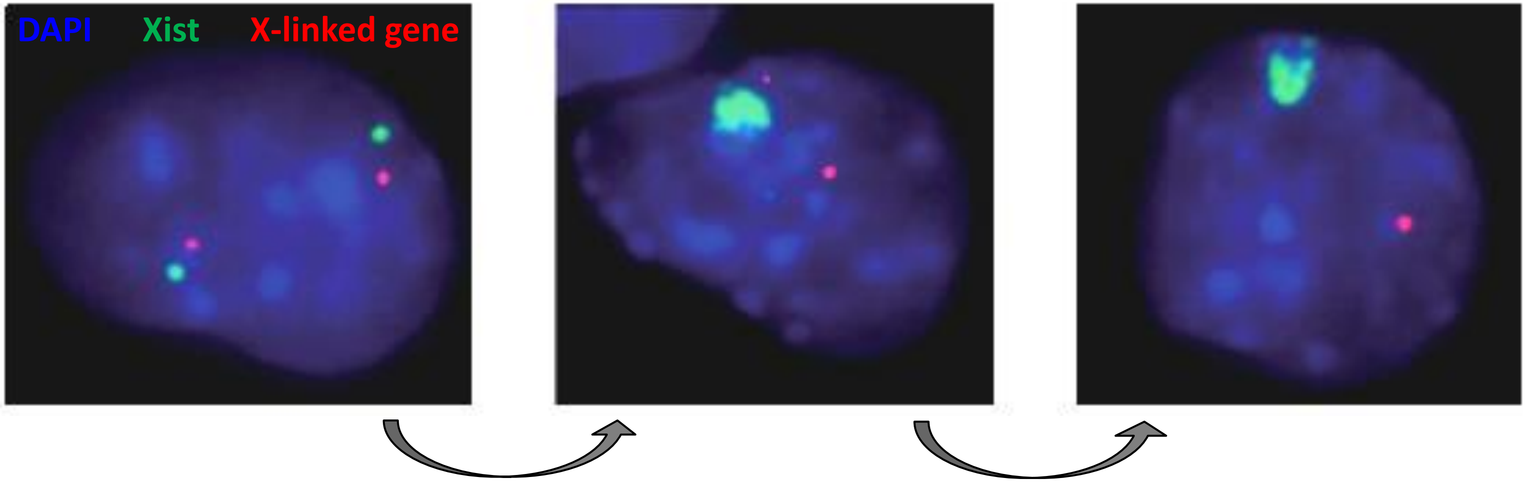
The Xist non-coding RNA

Embryonic Stem Cells

2 active X

***In vitro* differentiation**

Inactivation of one of the 2 X



FIRST: Upregulation of Xist from one X Silencing of the other allele

THEN: Gene repression of the Xist-coated X chromosome

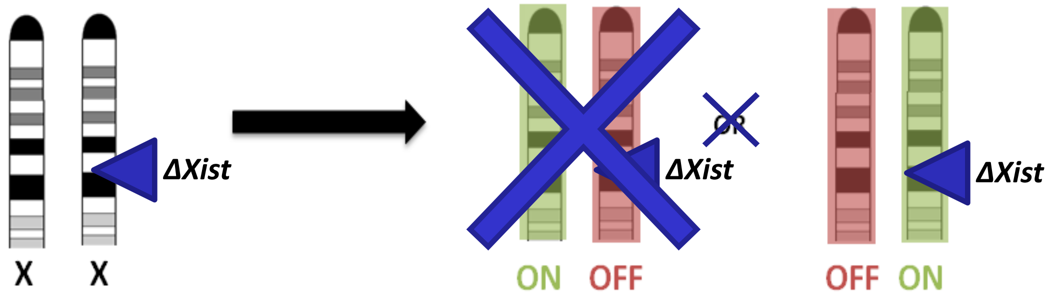
✓ **Control of *Xist* transcription: *Up-regulation during ESC differentiation before gene repression***

Role of Xist ?

work from Avner, Brockdorff, Gribnau, Heard, Jaenisch, Lee, Panning, Rougeulle, Sado, Sasaki, Wutz…labs.

Role of Xist RNA is triggering XCI ?

Deletion of *Xist* in female cells

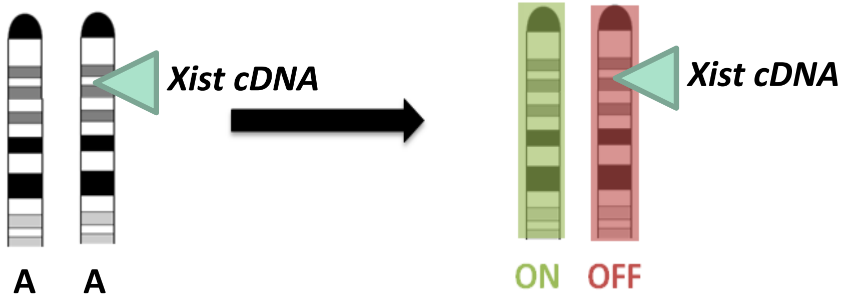


$X^{\Delta Xist/+}$: 100% Xi =WT allele

➔Xist is necessary *in cis* to trigger XCI

Inducible *Xist* cDNA in an autosome

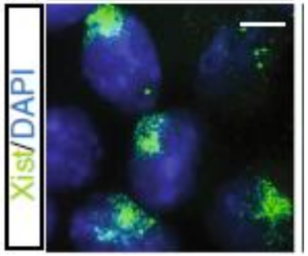
$A^{Xist\ cDNA}$ =Ai -> Xist coating and gene silencing



➔Xist is sufficient to trigger XCI

➔ Xist-mediated silencing is not X-specific

Tg on Chr 8



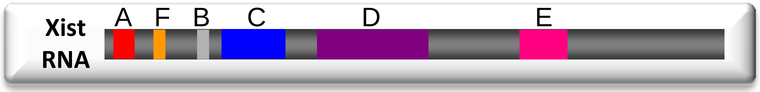
(Loda et al, 2017)

- ✓ Xist RNA is necessary and sufficient to trigger XCI.
- ✓ Xist RNA works in *cis*: it coats and silences the chromosome from where it is expressed.

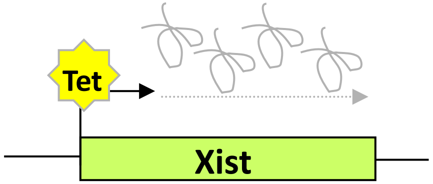
Functional domains of the Xist RNA?

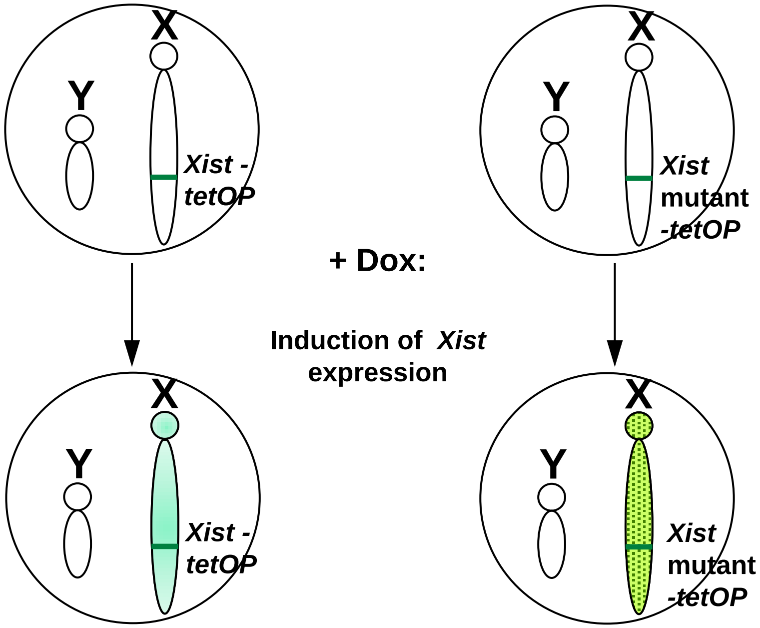
Penny et al 1996
Marahrens 1997
Wutz 2000, 2002

Functional domains of Xist ?



Inducible Xist cDNA
(Wutz & Jaenisch, 2000)



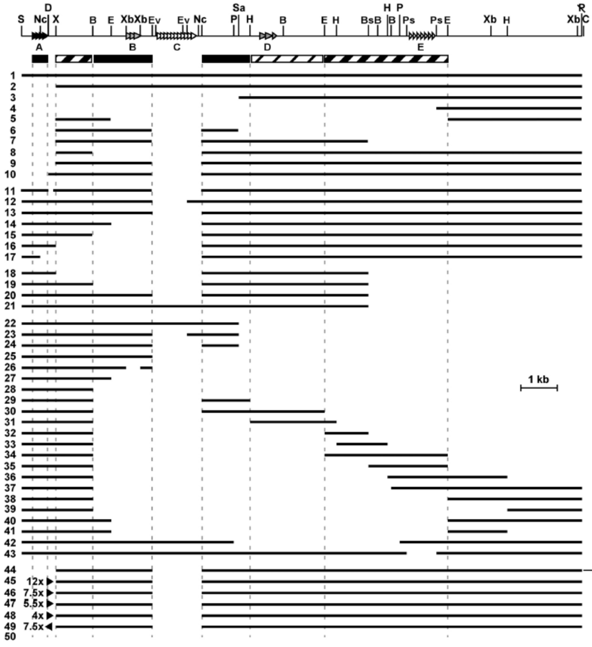


+ Dox:

Induction of *Xist* expression

INACTIVATION:
⇨ Repression of the only X chr
⇨ Cell death (XCI)

INACTIVATION ?
⇨ Xist accumulation?
⇨ Cell death (XCI) or cell survival (no XCI)?

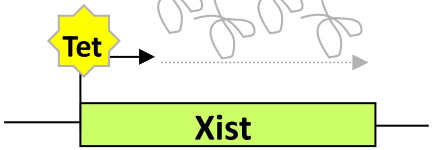


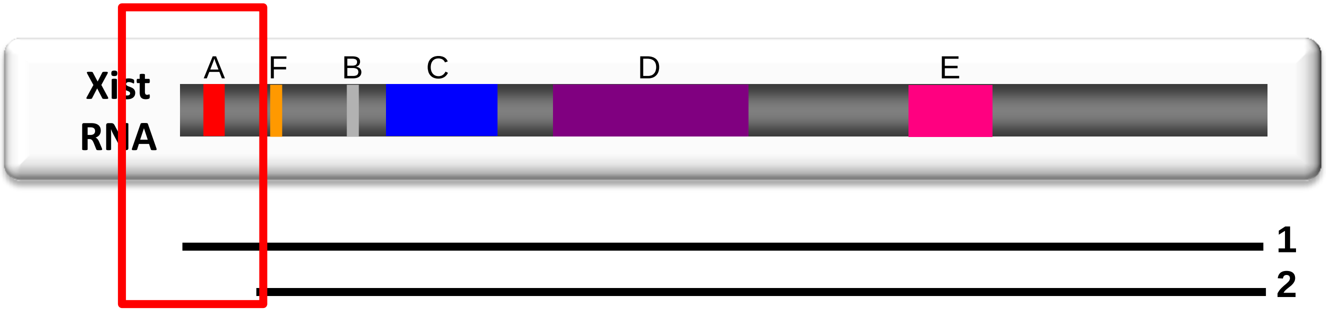
	construct	survival %	*n*	localization clusters	P	I
1	Xist	23 ± 8	12	+++++	+	
2	ΔSX	93 ± 4	3	++++	+	
3	ΔSSa	92 ± 8	2	-		
4	ΔSPs	89 ± 8	3	-		
5	ΔEΔSX	96 ± 5	3	-		
6	ΔEvNΔSCΔSX	81 ± 2	2	-		
7	ΔEvNΔBCΔSX	90 ± 1	2	-		
8	ΔBNΔSX	n.d.		+		
9	ΔEvNΔSX	88 ± 1	2	++++	+	
10	ΔEvNΔDX	103 ± 8	3	++++		
11	ΔEvNΔSD	26 ± 11	3	+++++		
12	ΔEv	25 ± 14	2	+++++		
13	ΔEvN	21 ± 7	2	+++++		
14	ΔEN	24	1	+++++		
15	ΔBN	25	1	+++++		
16	ΔXN	39 ± 5	2	+++		
17	ΔN	88 ± 6	2	-		
18	ΔXNΔBsC	66 ± 5	2	++		
19	ΔBNΔBsC	26 ± 6	4	++++		
20	ΔEvNΔBsC	21 ± 0	2	++++		
21	ΔBsC	33 ± 6	8	++++	+	
22	ΔSaC	34 ± 9	9	++++		
23	ΔEvΔSaC	44	1	++++		
24	ΔEvNΔSaC	37	1	++++		
25	ΔEvC	41 ± 11	11	+++		
26	ΔEvCΔXb	39 ± 18	2	+++		
27	ΔEC	64 ± 10	8	+		
28	ΔBC	76 ± 8	15	-		
29	ΔBNΔHC	34 ± 9	4	n.d.		
30	ΔBNΔEC	30 ± 9	4	n.d.		
31	ΔBC-H2.3	52 ± 14	4	n.d.		
32	ΔBC-EBs1.2	58 ± 1	2	n.d.		
33	ΔBC-H1.2	56 ± 0	2	n.d.		
34	ΔBC-E3.3	32 ± 14	4	n.d.		
35	ΔBC-EBs2K	77 ± 13	2	n.d.		
36	ΔBC-H3.3	63	1	n.d.		
37	ΔB	50 ± 7	9	n.d.		
38	ΔBC-ES3.7	77 ± 2	2	n.d.		
39	ΔBC-H2.1	50 ± 8	4	n.d.		
40	ΔE	65 ± 12	11	+++		
41	ΔEΔHC	60 ± 15	3	+++		
42	ΔP	27 ± 5	4	+++++	+	
43	ΔPs	22	1	+++++		
44	ΔSX-3'-SX	43 ± 3	2	n.d		
45	ΔEvN:XCR 12	17 ± 10	2	n.d		
46	ΔEvN:XCR 7.5	36 ± 13	4	+++++		
47	ΔEvN:XCR 5.5	46 ± 16	4	++++		
48	ΔEvN:XCR 4	80 ± 25	5	++++		
49	ΔEvN:XCR 7.5AS	96 ± 10	3	++++	+	
50	control T20 cells	93 ± 7	9	n.a.		

Wutz et al, 2002

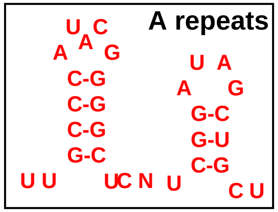
Functional domains of Xist ?

Inducible Xist cDNA
(Wutz & Jaenisch, 2000)





	Xist cloud:	Cell death:
1	+++++	77%
2	++++	7%



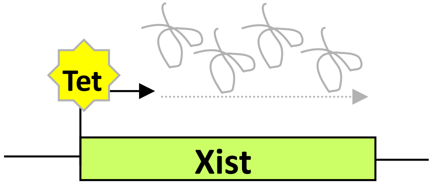
⇨ Deleting the A repeats is sufficient to inhibit gene repression.

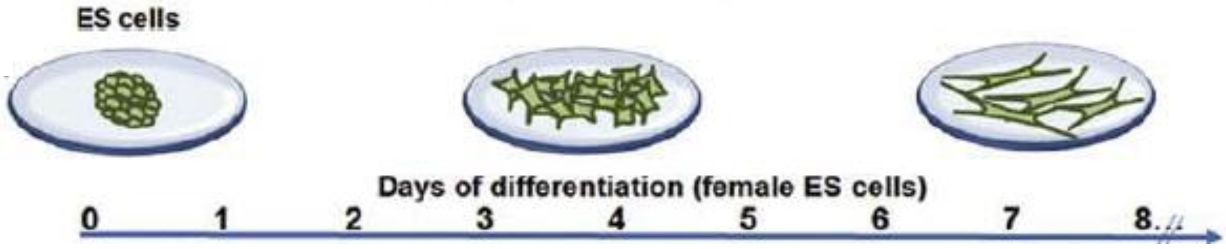
⇨ A-repeats are essential for Xist-silencing function.

Wutz et al, 2002

Xist RNA is only necessary for the onset of XCI

Inducible Xist cDNA
(Wutz & Jaenisch, 2000)





Initiation phase → **Maintenance phase**

- **Reversible inactivation**
- **Xist RNA is necessary**

- **Irreversible inactivation**
- **Xist RNA is no longer necessary**

Another characteristics of an epigenetic mechanism:
Change in gene expression stays stable even when the initial signal is lost.

How does Xist induce gene silencing: Role(s) of Xist?
How is the inactive state maintained?

1. **Introduction on X Chromosome Inactivation**

2. **Initiation of XCI: the Xist RNA**

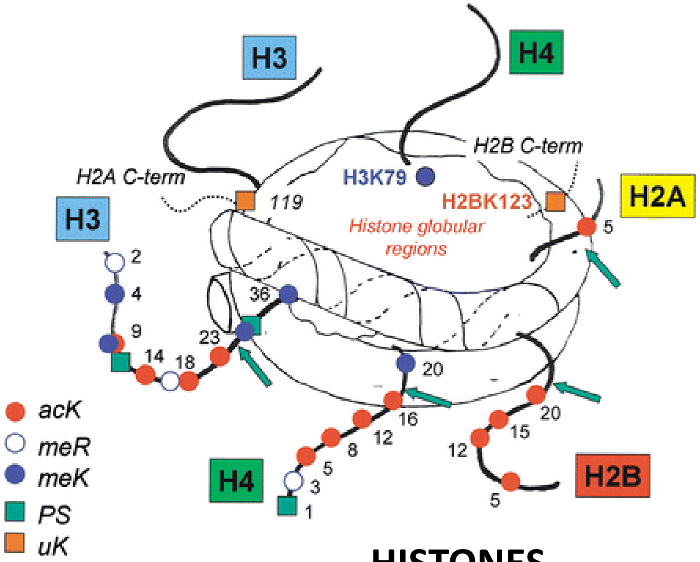
3. **Roles of Xist**

=> Ideas?

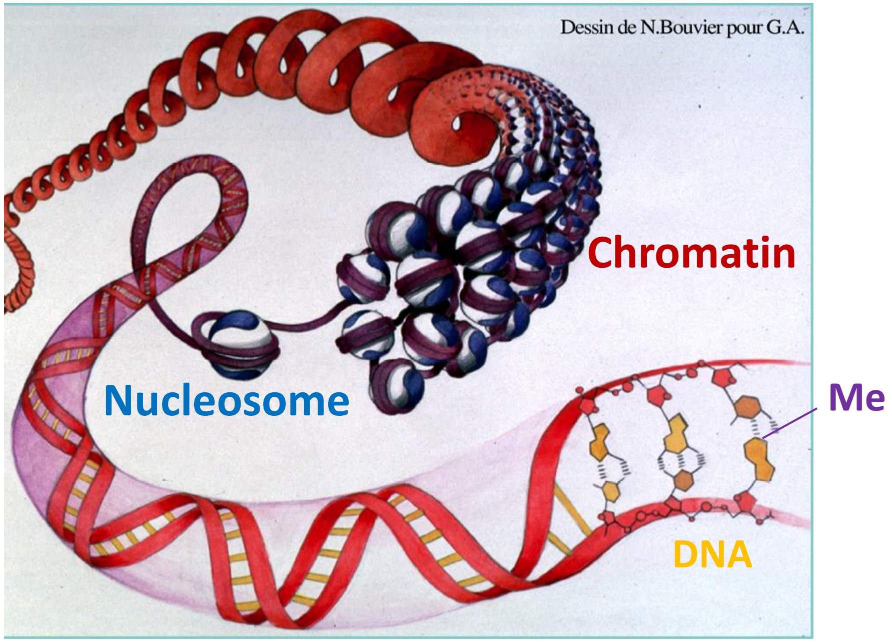
Epigenetic mechanisms?

=> What kind?

Epigenetic marks?



HISTONES



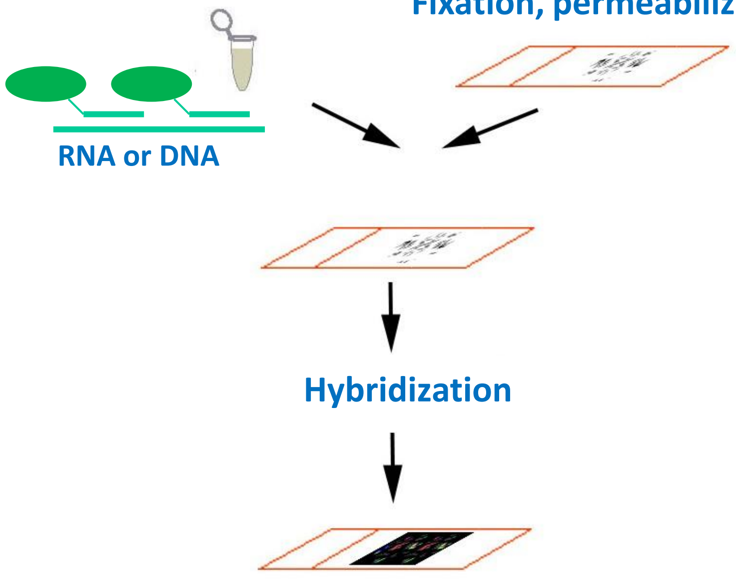
<u>Histone modifications:</u> **methylation, acetylation, etc….**

<u>DNA methylation:</u> **mark of gene silencing**

⇒ How to look at specific histone marks on the Xi?

Method: Combined Immunofluorescence and RNA or DNA FISH

Other methods: ChIP-seq, DamID, Hi-ChIP… But?



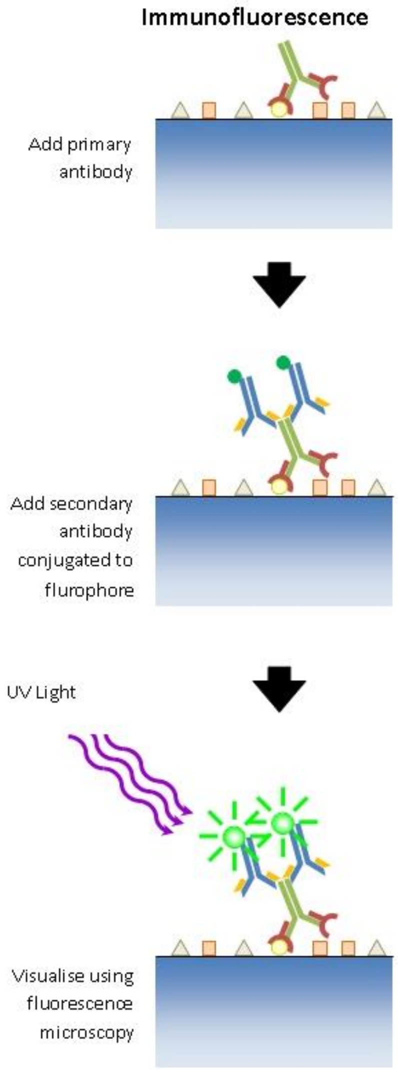
DNA probe (100-200bp) with fluorescent dUTP

RNA or DNA

Cell culture on slide
Fixation, permeabilization

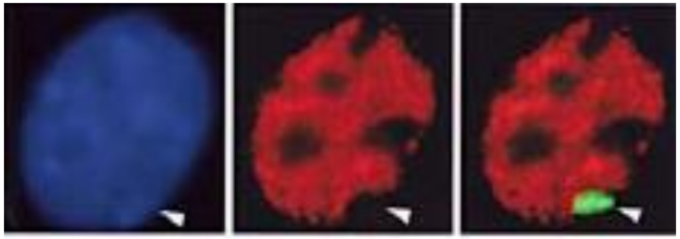
Hybridization

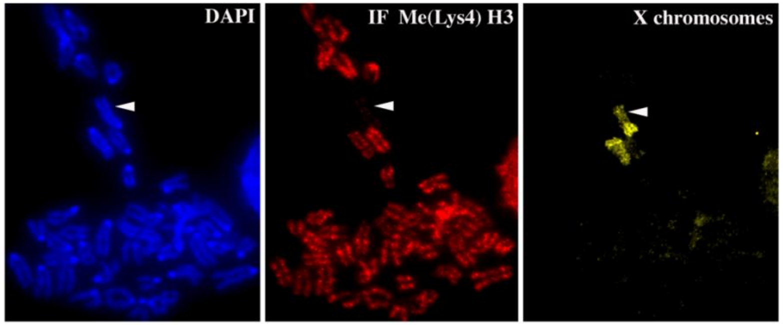
Detection of fluorescent hybridization signal



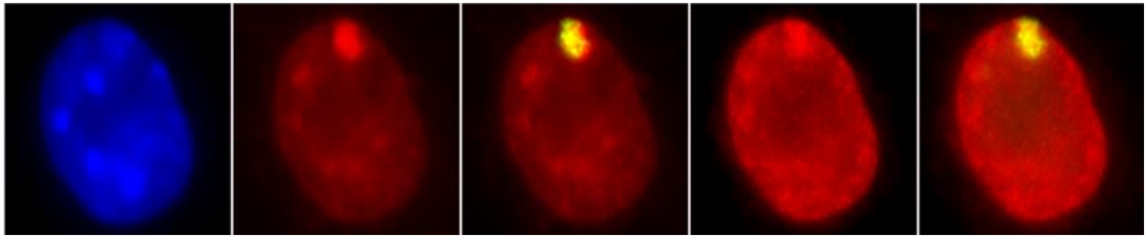
Xi-specific epigenetic marks

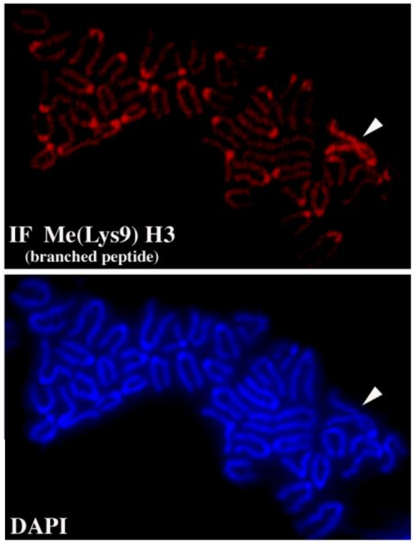
Exclusion of active histone marks


DAPI H3K4me2 / H4Kac



Recruitment of specific heterochromatic marks:
H3K27me3, H3K9me2, H4K20me1, H2AK119ub


DAPI H3K27me3 H3K9me2



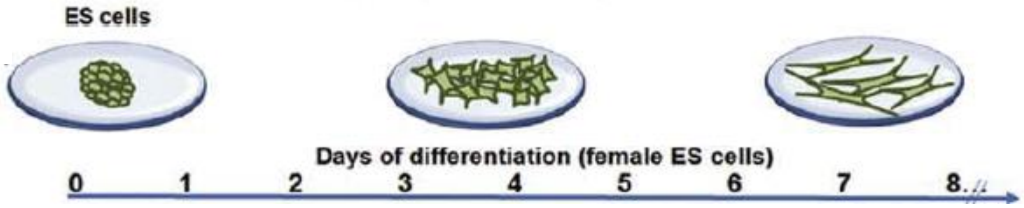
Stay on mitotic chromosomes

=> True epigenetic marks!

(Chaumeil et al., 2002; Rougeulle, Chaumeil et al., 2004)

Xi-specific epigenetic marks

Initiation — Irreversible inactivation



X-linked gene silencing

Xist coating

Loss of active marks (H3K4me, H3/H4ac…)

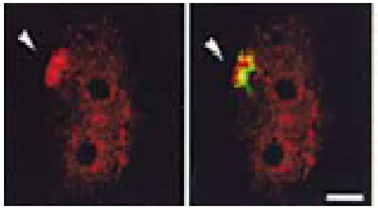
Recruitment of H3K9me2, H3K27me3, H2AK119Ub

Recruitment PRC1/2

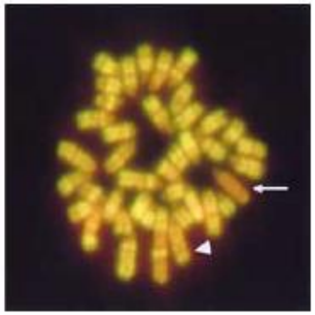
**Timing:
Could be
involved in
the silencing
process?**

Late replication timing

macroH2A association



macroH2A

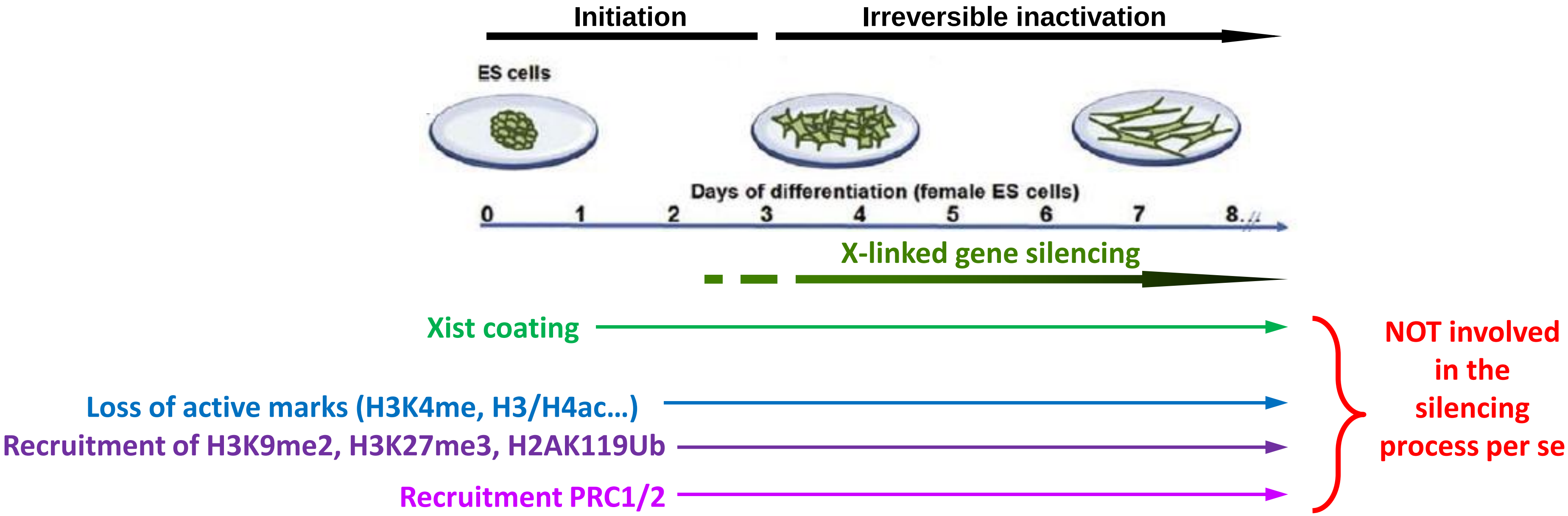


(Chaumeil et al, CGR, 2002)

DNA methylation

Chaumeil et al, CGR, 2002
Rougeulle, Chaumeil et al, MCB, 2004
Kay et al, 1994
Keohane et al, 1996
Clemson et al, 1996
Panning et al, 1997
Mermoud et al, 1999
Wutz et al, 2000,2002
Heard et al, 2001
Silva et al, 2003
Plath et al, 2003
Kohlmaier et al, 2004
De Napoles et al, 2004
Plath et al, 2004
Blewitt et al, 2008
Gendrel et al, 2012,2013
Da Rocha et al, 2014

Xi-specific epigenetic marks



X-linked gene silencing

Xist coating

Loss of active marks (H3K4me, H3/H4ac…)

Recruitment of H3K9me2, H3K27me3, H2AK119Ub

Recruitment PRC1/2

NOT involved
in the
silencing
process per se

Xist RNA is no longer required for the stable propagation of the inactive state.

Synergy of chromatin marks, DNA methylation and asynchronous replication timing could provide extremely stable, heritable silencing over hundreds of cell divisions.

-> Are these epigenetic features directly recruited by Xist?
-> What are the factors involved in X-gene silencing?

Csankovszki et al, 2001
Zhang et al, 2007
Wutz 2011 for review

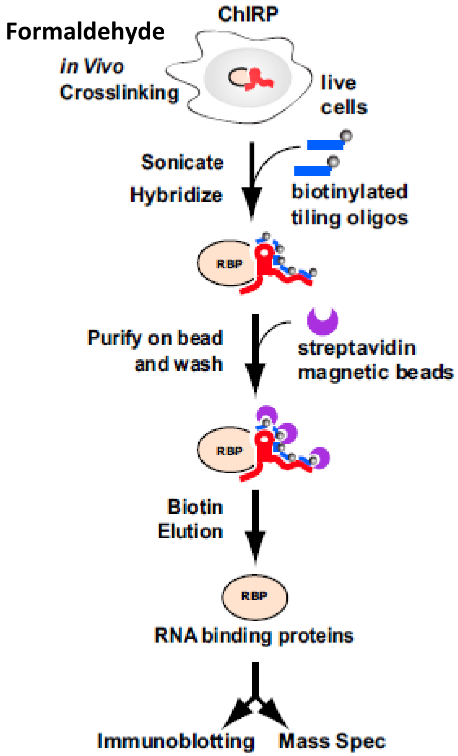
A. Xist interactors : recruitment of factors involved in XCI

Method to characterize
Xist-interactors ?

3. Roles of Xist

A. Xist interactors : recruitment of factors involved in XCI

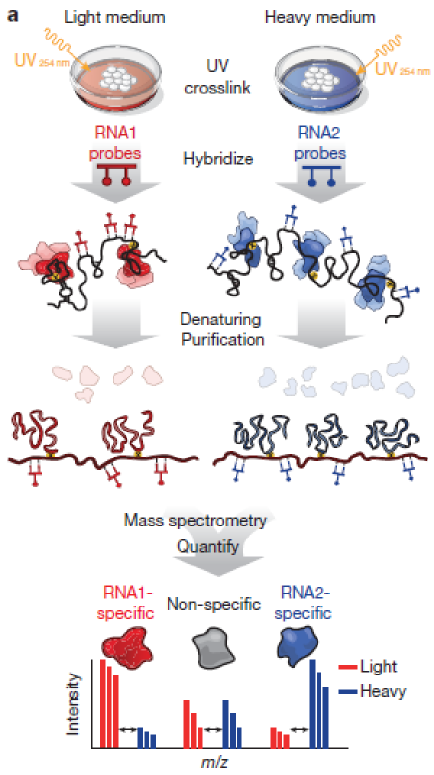
ChIRP-MS - H. Chang's lab -
(Chu et al, Cell, April 2015)



Male ES cells with a Dox inducible Xist on Chr 11
Female Epiblast Stem cells (just after inactivation)
Female Trophoblast Stem cells (imprinted XCI)

- ➢ 81 proteins (62 in all 4 conditions)

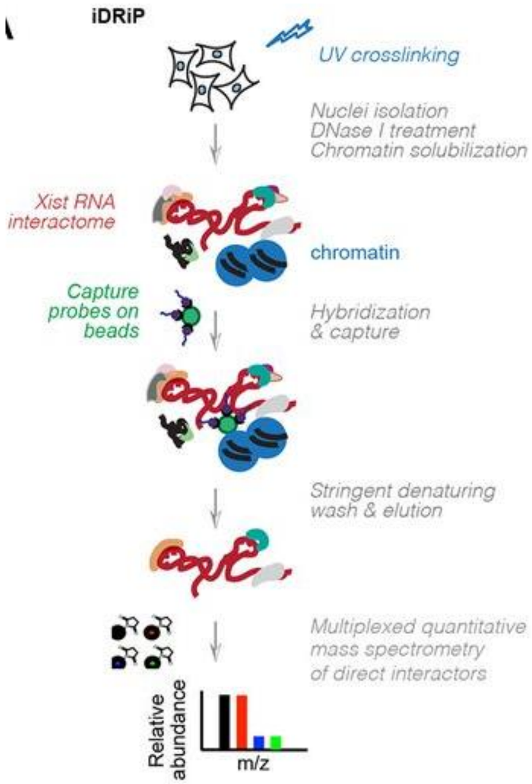
RAP-MS - M. Guttman's lab -
(McHugh et al, Nature, May 2015)



Tet inducible Xist in male ES cells

- ➢ 10 proteins

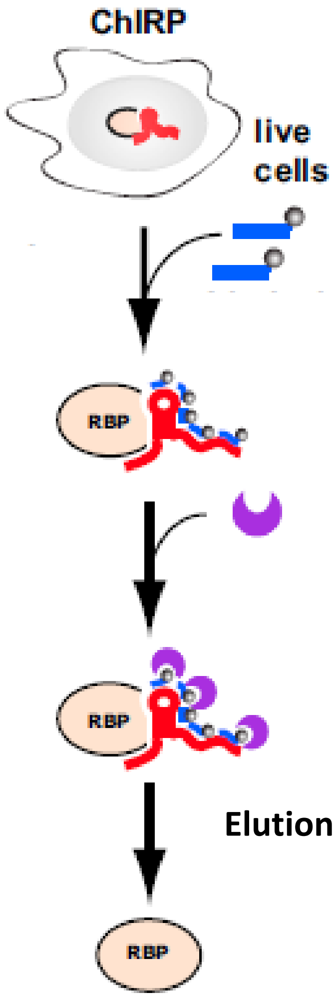
iDRIP - J. Lee's lab -
(Minajigi et al, Science, June 2015)



Female ES cells (day7)
Female fibroblasts

- ➢ 80 proteins : ≥3-fold enriched
- ➢ >200 proteins: ≥2-fold enriched

A. Xist interactors : recruitment of factors involved in XCI



Fixation : coupling RNAs and proteins

Hybridization of biotinylated probes on the Xist RNA

Purification of the Xist-protein complexes using streptavidin-coupled magnetic beads

Elution

Mass Spectrometry for Xist protein factors

ChIRP-MS - H. Chang's lab - *(Chu et al, Cell, April 2015)*

RAP-MS - M. Guttman's lab - *(McHugh et al, Nature, May 2015)*

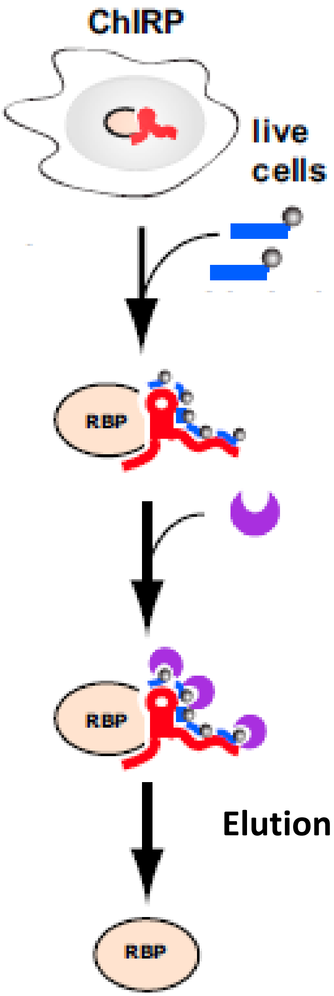
iDRIP - J. Lee's lab - *(Minajigi et al, Science, June 2015)*

- Btw 10 to 100 proteins

- Factors found in two or more studies:
  - SPEN (transcription repressor)
  - SAF-A, HnrnpK (nuclear matrix)
  - PRC1 not PRC2
  - WTAP / RBM15 (RNA methylation)

- Some validated in screen-based studies (SPEN, RBM15…) *(Monfort et al; Moindrot et al; Cell reports, August 2015)*

A. Xist interactors : recruitment of factors involved in XCI



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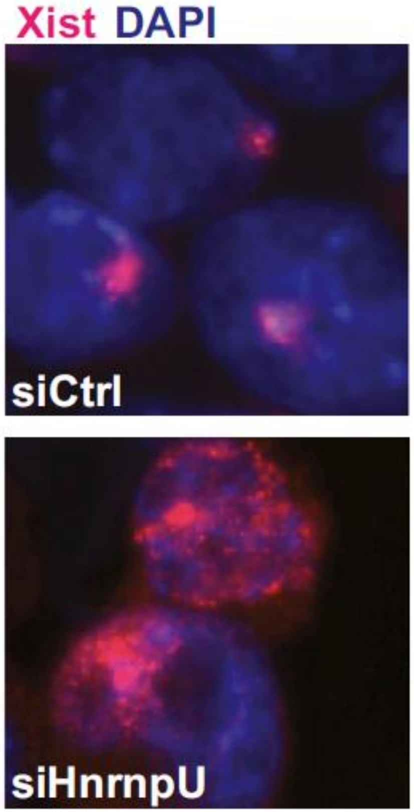
iDRIP - J. Lee's lab - *(Minajigi et al, Science, June 2015)*

Method to characterize the role of these factors?

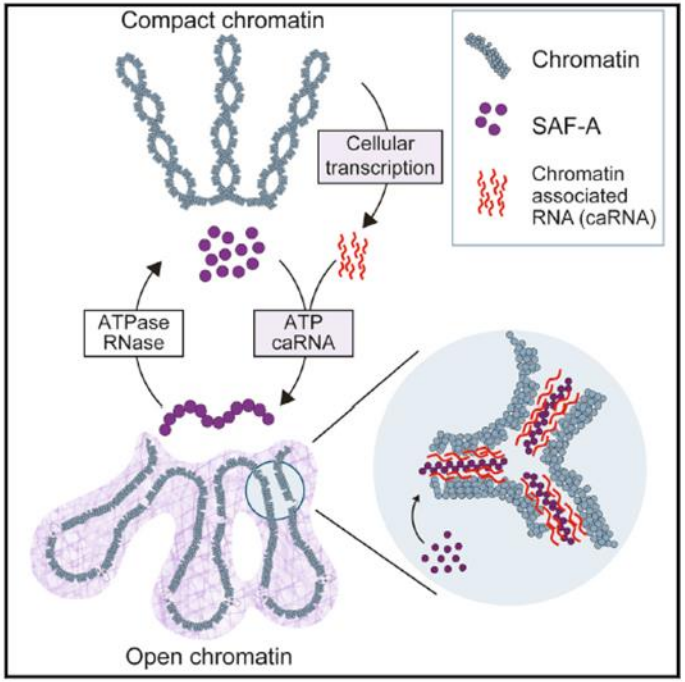
A. Xist interactors : recruitment of factors involved in XCI

SAF-A/Hnrnp-U

-> siRNA :
defect in silencing / Xist mislocalization



(Chu et al, Cell, April 2015)



-> Scaffold protein: regulates interphase chromosome structure through oligomerization with chromatin associated RNAs

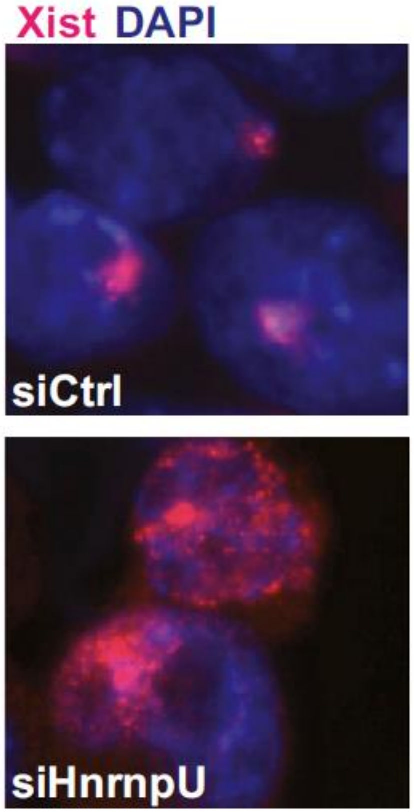
(Nozawa et al, Cell, April 2017)

Could it explain one property of the Xist RNA?

A. Xist interactors : recruitment of factors involved in XCI

SAF-A/Hnrnp-U

-> siRNA :
defect in silencing / Xist mislocalization



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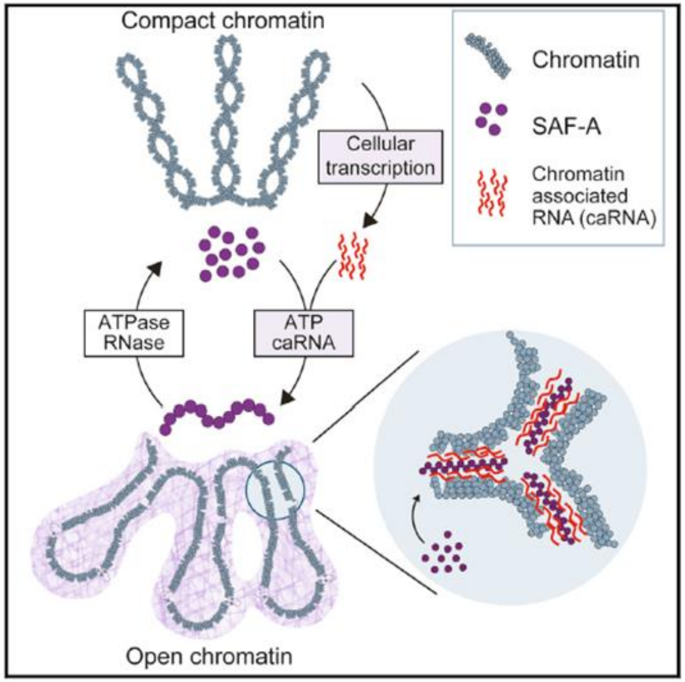


-> Scaffold protein: regulates interphase chromosome structure through oligomerization with chromatin associated RNAs

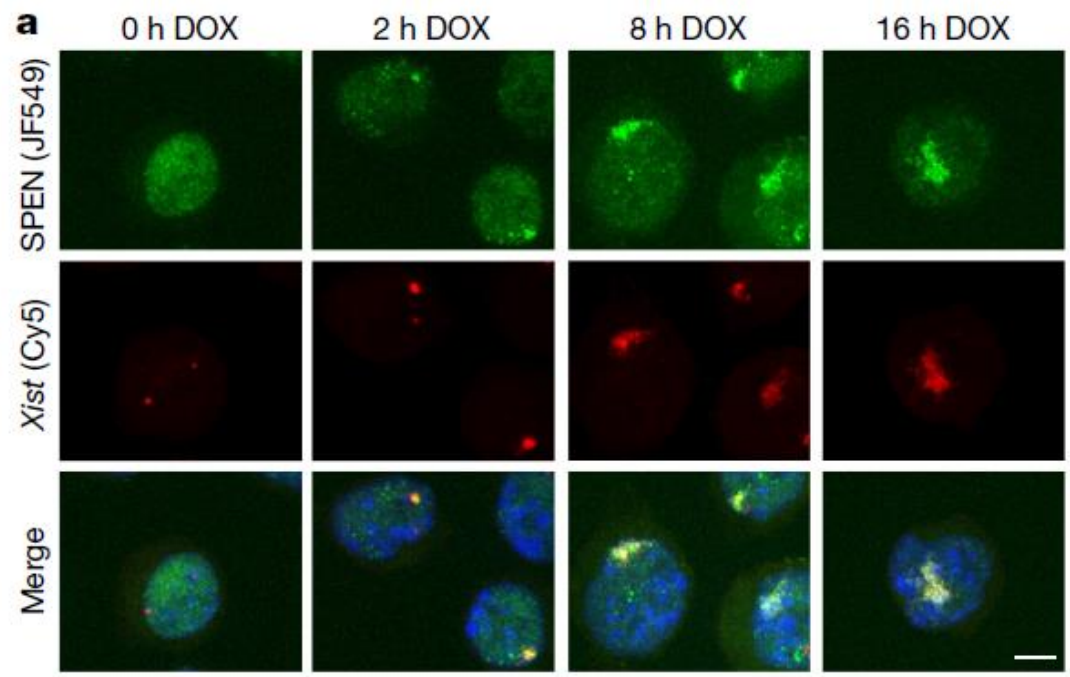
(Nozawa et al, Cell, April 2017)

The Xist RNA coats the chromosome from where it is expressed!

A. Xist interactors : recruitment of factors involved in XCI

SPEN/SHARP

ChIRP-MS on WT-Xist or ΔA-Xist:
-> 3 factors don't bind the mutant : Spen
-> Direct interaction Spen – Xist A repeats
-> SPEN localization?

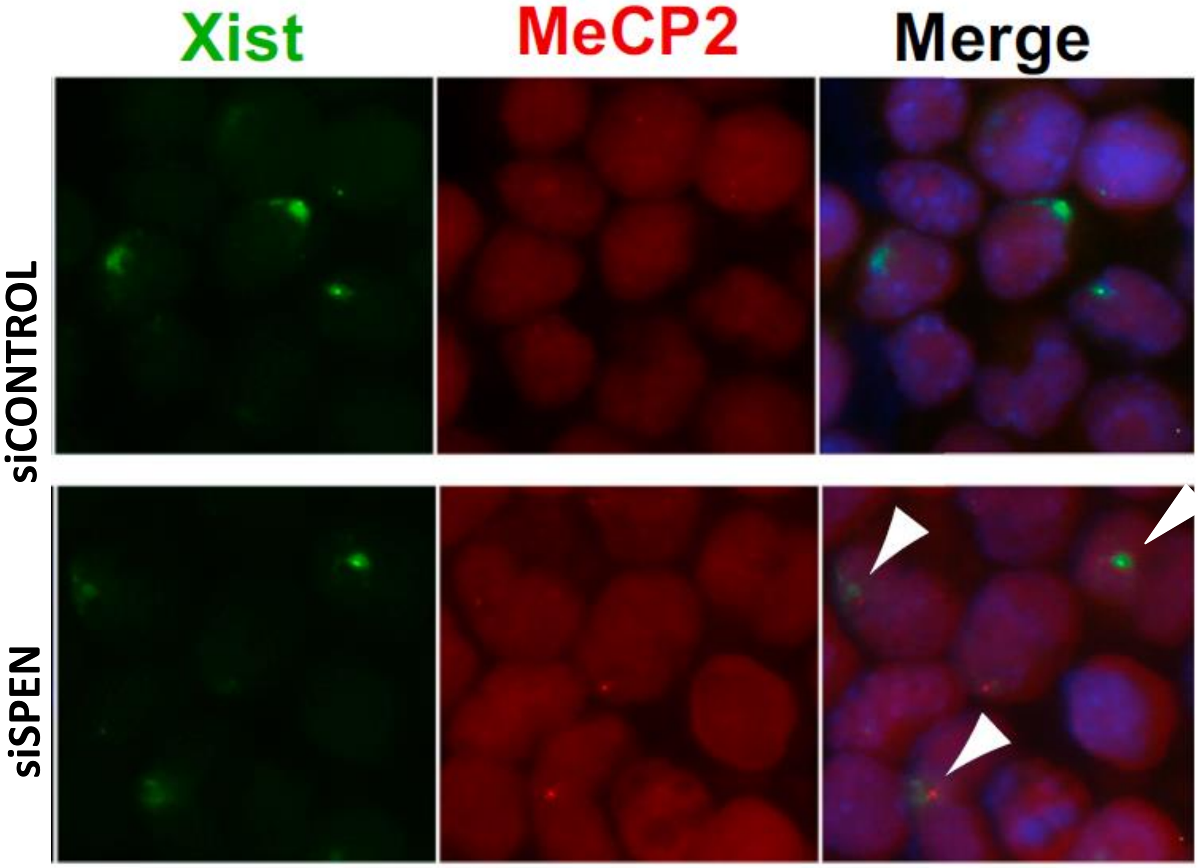


(Dossin et al 2020)

A. Xist interactors : recruitment of factors involved in XCI

SPEN/SHARP

-> Direct interaction Spen – Xist A repeats
-> SPEN localizes to the X right after Xist upregulation
-> Role of SPEN in X-linked gene silencing?



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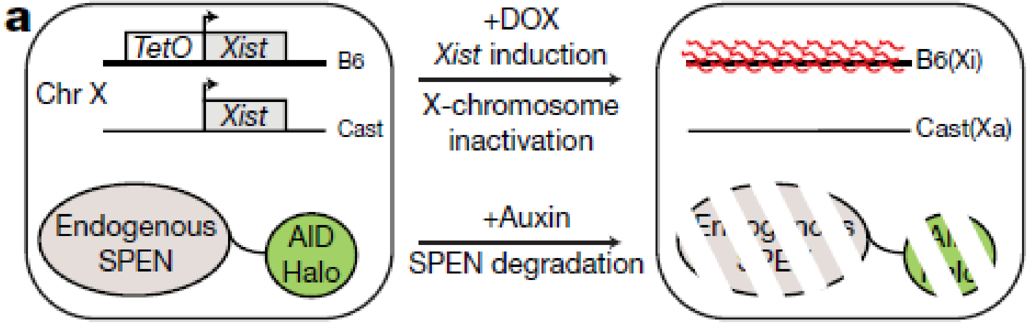
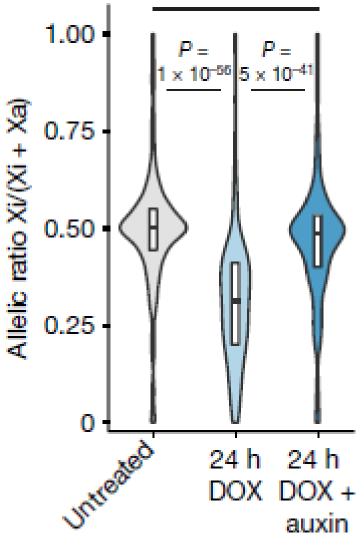
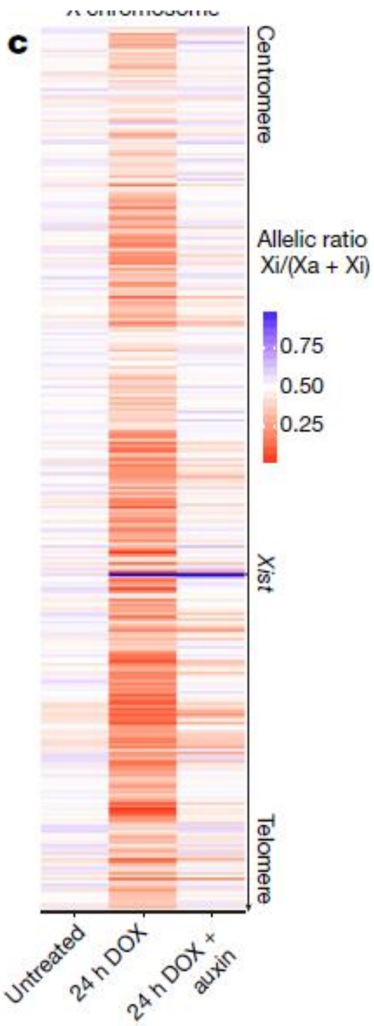
DEGRON SYSTEM







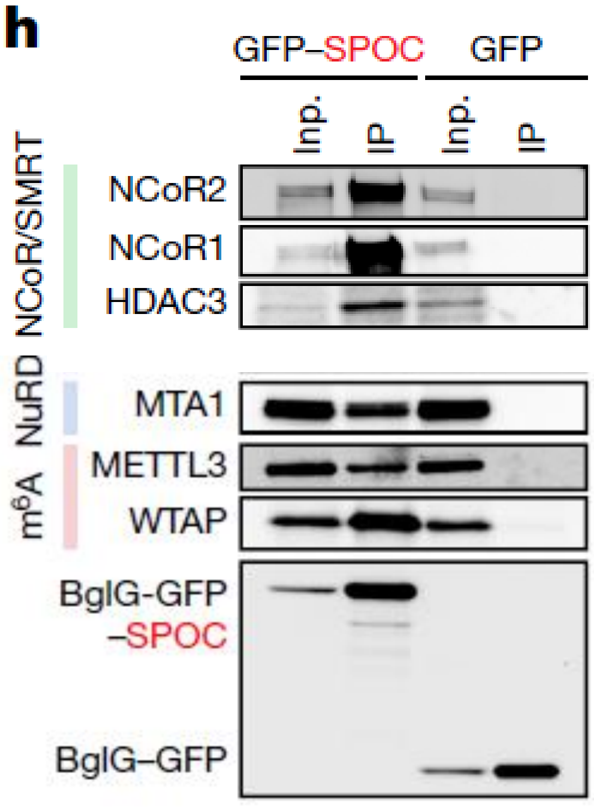
-> in absence of SPEN: no inactivation

(Dossin et al 2020)

A. Xist interactors : recruitment of factors involved in XCI

SPEN/SHARP

-> Direct interaction Spen – Xist A repeats
-> SPEN localizes to the X right after Xist upregulation
-> siRNA/DEGRON: no problem in Xist localization
but defect in silencing
-> Partners?



(Dossin et al 2020)

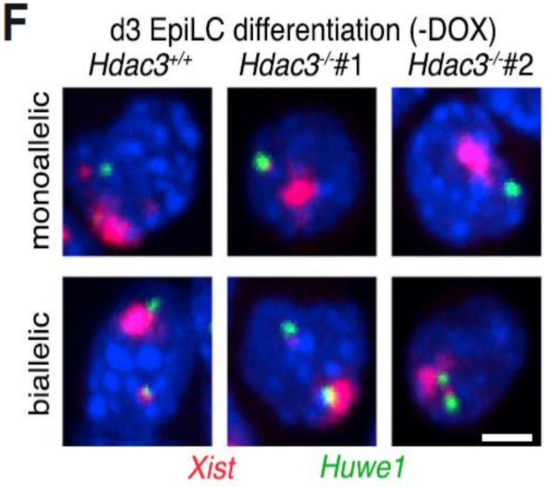
A. Xist interactors : recruitment of factors involved in XCI

SPEN/SHARP

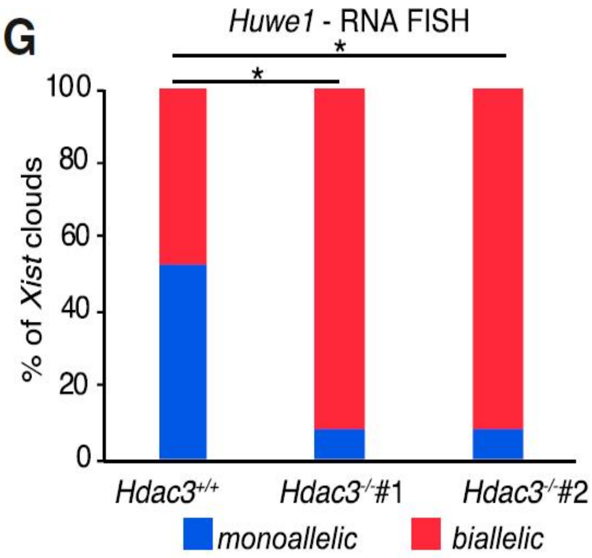
-> Direct interaction Spen – Xist A repeats
-> SPEN localizes to the X right after Xist upregulation
-> siRNA/DEGRON: no problem in Xist localization
but defect in silencing
-> The SPOC domain mediates silencing and recruits multiple factors:

- HDAC3
- Transcription machinery / NuRD complex
- m6A methyltransferase complex (RBM15/WTAP)

F



G



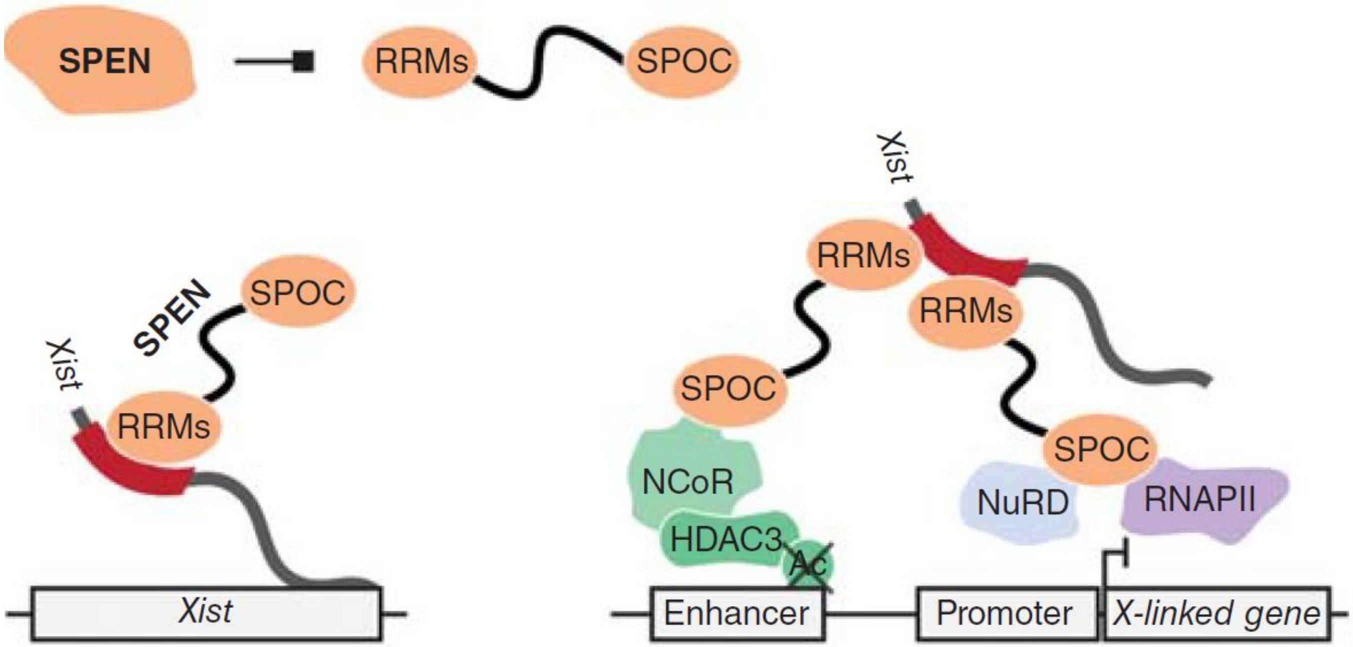
(Zylicz et al, cell, 2018)

A. Xist interactors : recruitment of factors involved in XCI

1) Xist recruits the silencing factor SPEN (A-repeats):

- Recruits HDAC3 to deacetylates the X
- Evicts the RNA PolII machinery

=> X-linked gene silencing



Up-regulation of *Xist*

SPEN-dependent gene silencing

(From Dossin and Heard 2021)

3. Roles of Xist

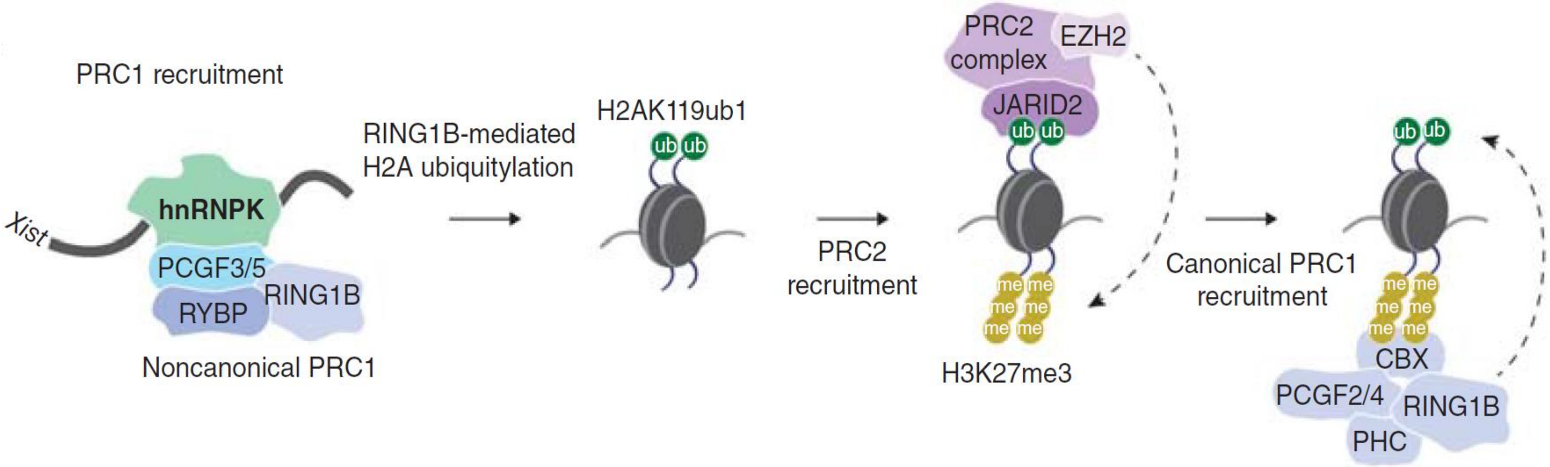
A. Xist interactors : recruitment of factors involved in XCI

1) Xist recruits the silencing factor SPEN (A-repeats):
- Recruits HDAC3 to deacetylates the X
- Evicts the RNA PolII machinery

=> X-linked gene silencing

2) Xist recruits hnRNPK (B-repeats):
- Recruits non canonical PRC1 -> H2AK119Ub
- H2AK119Ub recruits Jarid2/PRC2 -> H3K27me3

=> Maintenance of the XCI (PRC1/2 recruitment)



(From Dossin and Heard 2021)

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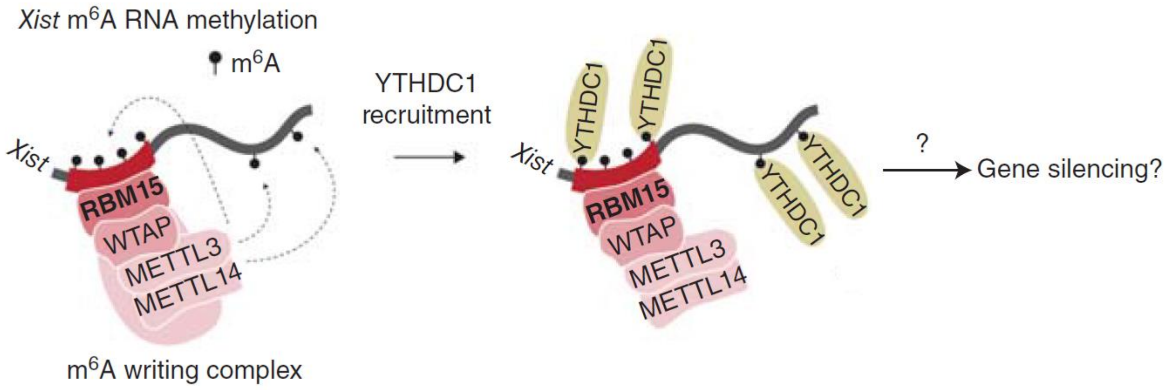
=> Maintenance of the XCI

3) Xist and SPEN recruit RBM15/WTAP:

- m^6A methylation of Xist
- Recruits YTHDC1

=> Involved in Xist stability? Gene silencing?



(From Dossin and Heard 2021)

A. Xist interactors : recruitment of factors involved in XCI

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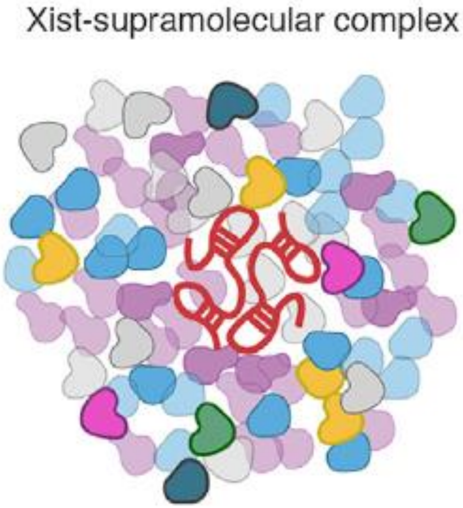
=> X-linked gene silencing

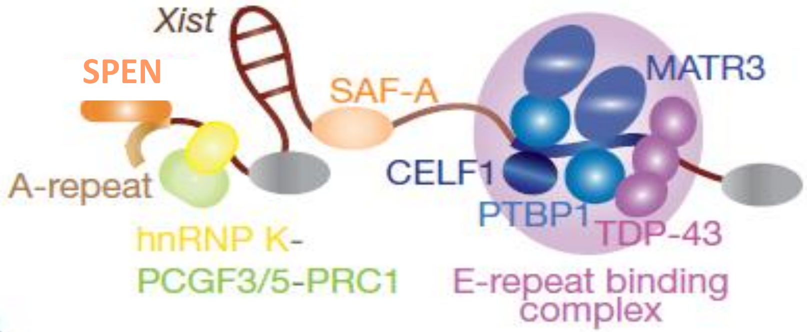
2) Xist recruits hnRNPK (B-repeats):
=> Maintenance of the XCI

3) Xist and SPEN recruit RBM15/WTAP:
=> Involved in Xist stability? Gene silencing?

4) Xist recruits 4 RNA-binding proteins (E-repeats):
- Form a condensate that can be sustained in absence of Xist
- Xist seed dynamic supramolecular complexes (SMACs: SPEN, CELF1, PCGF5, and CIZ1)
- Crowding of SPEN within SMACs is required for XCI
- Silencing on the X proceeds through chromatin compaction and clustering of SMAC

=> Gene silencing / Anchor of Xist to the Xi

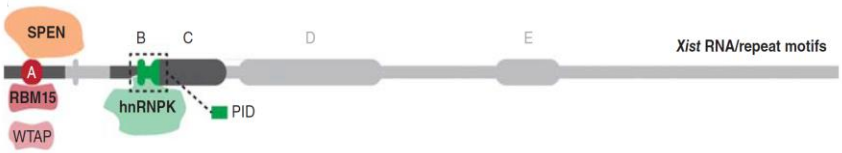




(Pandya-Jones et al, 2020; Markaki et al, 2021)
(From Dossin and Heard 2021)

3. Roles of Xist

A. Xist interactors : recruitment of factors involved in XCI



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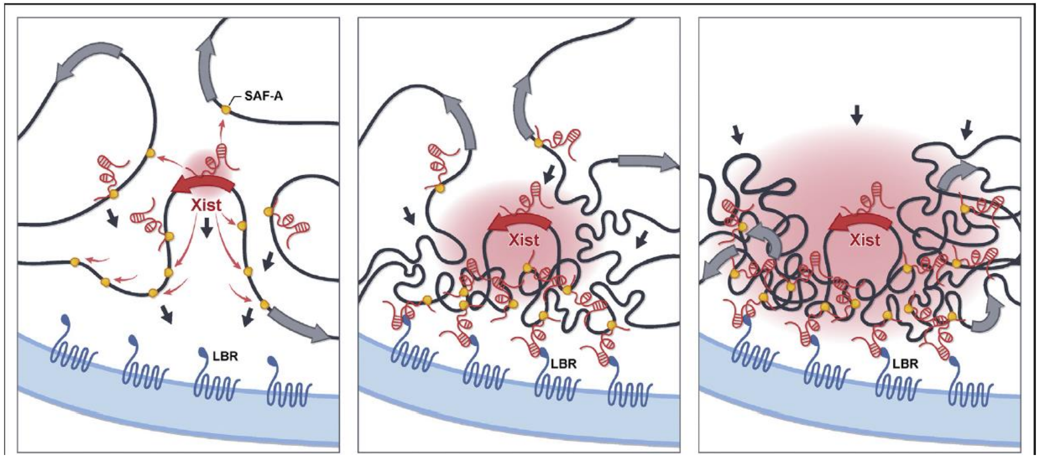
=> Involved in Xist stability? Gene silencing?

4) Xist recruits 4 RNA-binding proteins (E-repeats):
=> Gene silencing / Anchor of Xist to the Xi

5) Xist binds SAF-A and Lamin-B Receptor (LBR):
=> Sequestration for silencing?



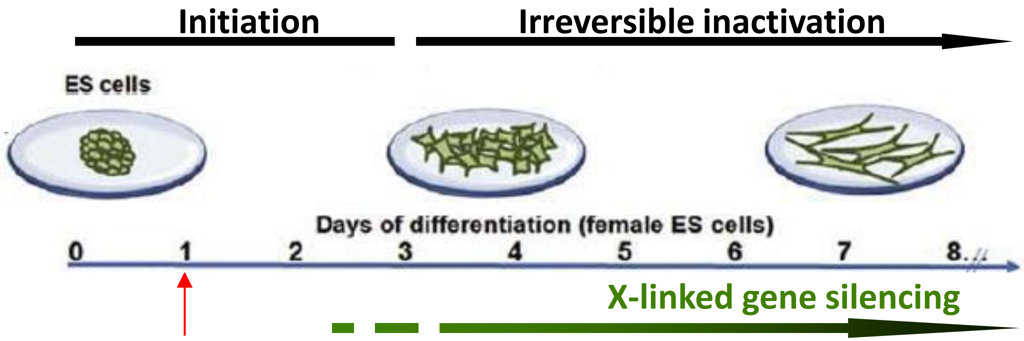
(Strehle and Guttman, 2020)
(Pandya-Jones et al, 2020; Markaki et al, 2021)
(From Dossin and Heard 2021)

1. **Introduction on X Chromosome Inactivation**

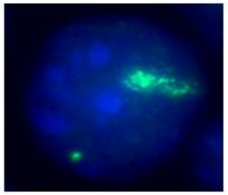
2. **Initiation of XCI: the Xist RNA**

3. **Roles of Xist:**
    - **A. Platform to recruit silencing factors**
    - **B. Platform to recruit maintenance factors**

How does Xist induce gene silencing?



Xist Accumulation

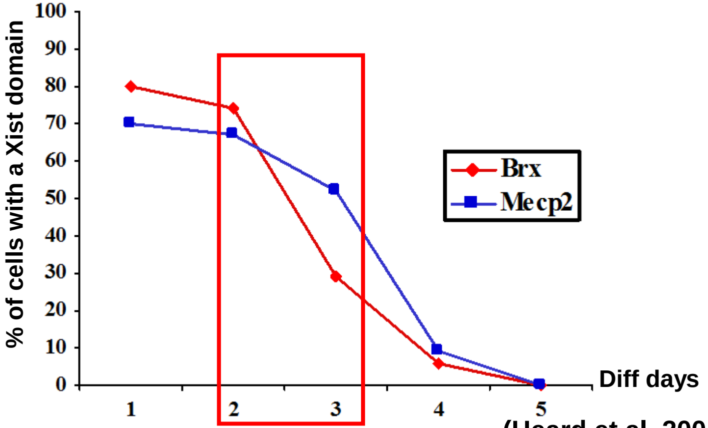


?

- ⇒ **Mechanisms involved in Xist-mediated gene silencing?**
- ⇒ **Gap between Xist coating and gene silencing?**

Kinetics of gene silencing

(RT-PCR, Northern Blot, RNA FISH)



(Heard et al.,2001)

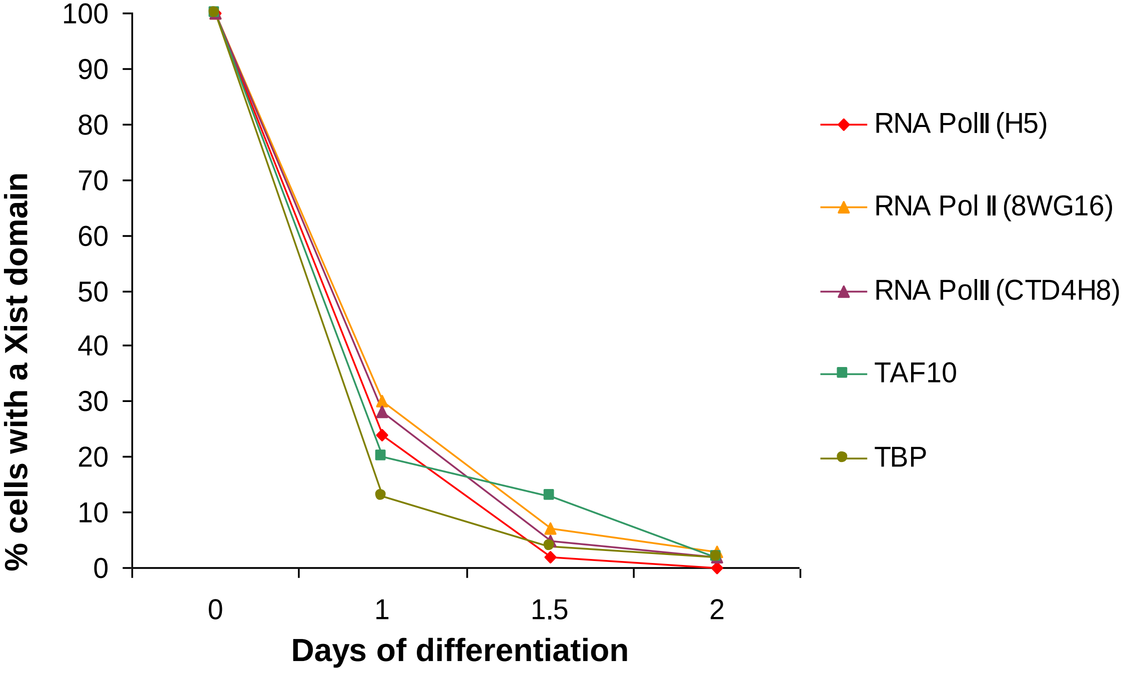
One hypothesis:

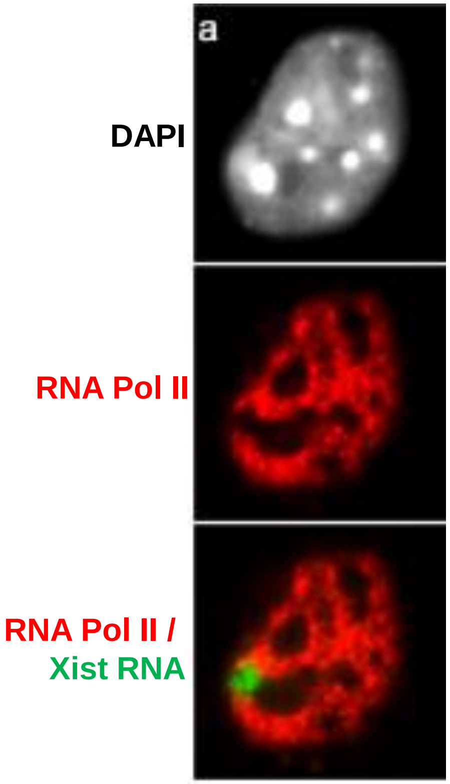
Xist coating of the X may create a nuclear repressive compartement.

(Clemson et al, 1996; Fackelmayer, 2004)

B. Xist as a nuclear and chromosome organizer

Transcription machinery?





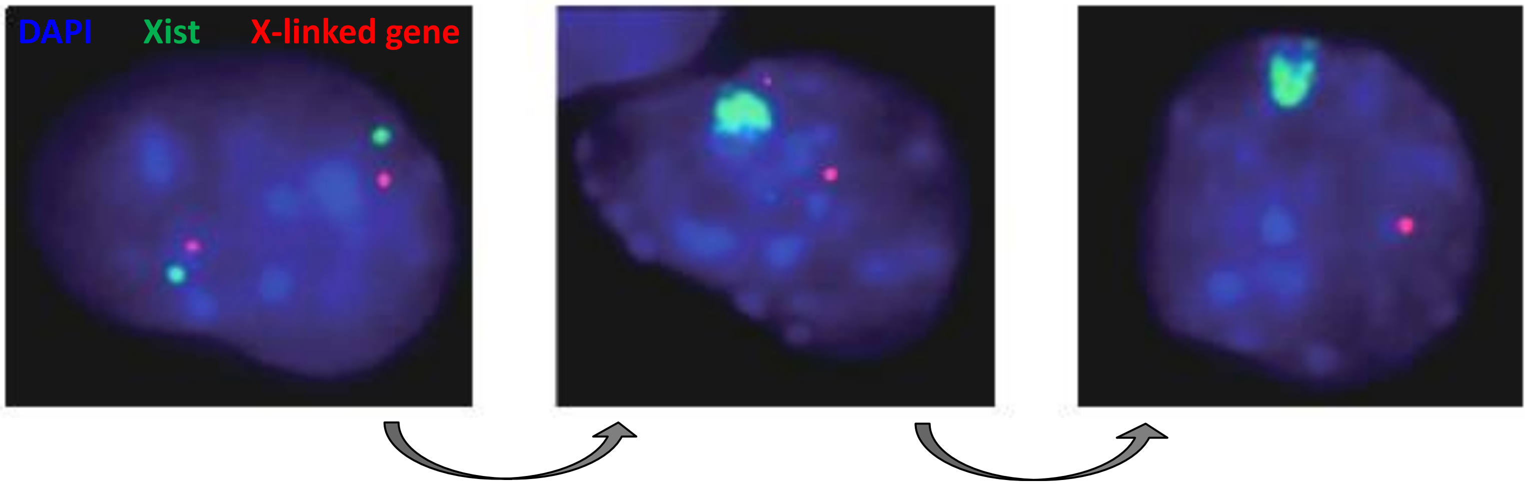
IF / RNA FISH de Xist
(Cellules ES en cours de différenciation)

Xist RNA rapidly creates a repressive compartment excluding the transcription machinery.

Why is gene silencing so delayed ?

Chaumeil et al, Genes & Dev, 2006

The Xist non-coding RNA



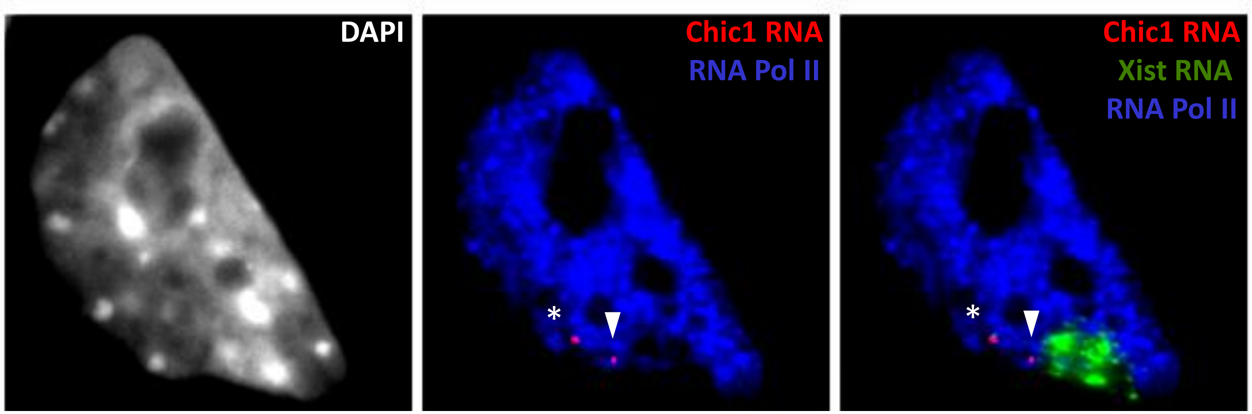
**FIRST: Upregulation of Xist from one X
Silencing of the other allele**

**THEN: Gene repression of the
Xist-coated X chromosome**

work from Avner, Brockdorff, Gribnau, Heard, Jaenisch, Lee, Panning, Rougeulle, Sado, Sasaki, Wutz…labs.

B. Xist as a nuclear and chromosome organizer

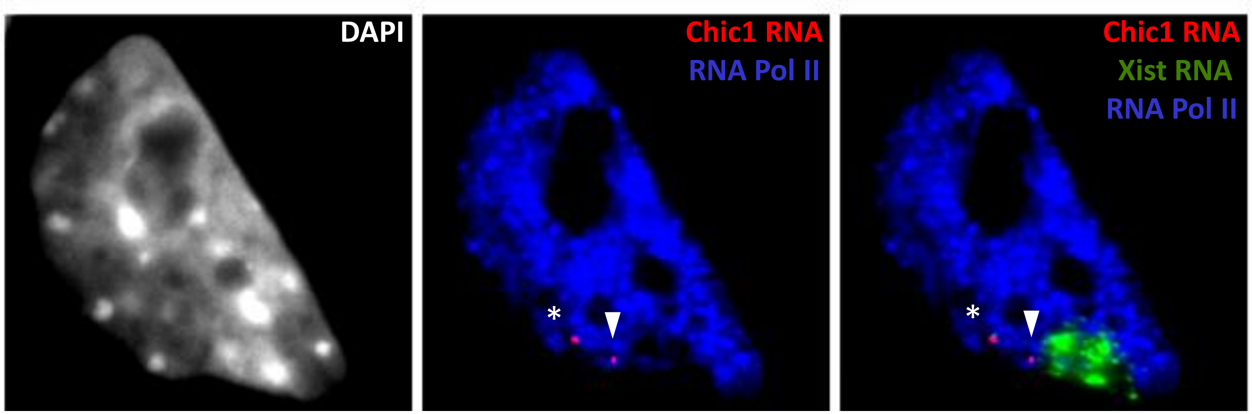
Active gene



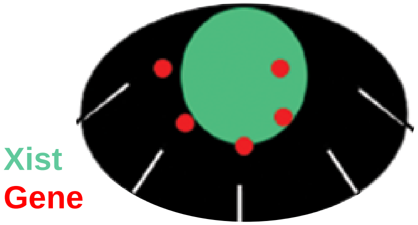
Chaumeil et al, Genes & Dev, 2006

B. Xist as a nuclear and chromosome organizer

Active gene



Before inactivation, expressed genes are located outside the Xist domain.
-> Still in contact with the transcription machinery.



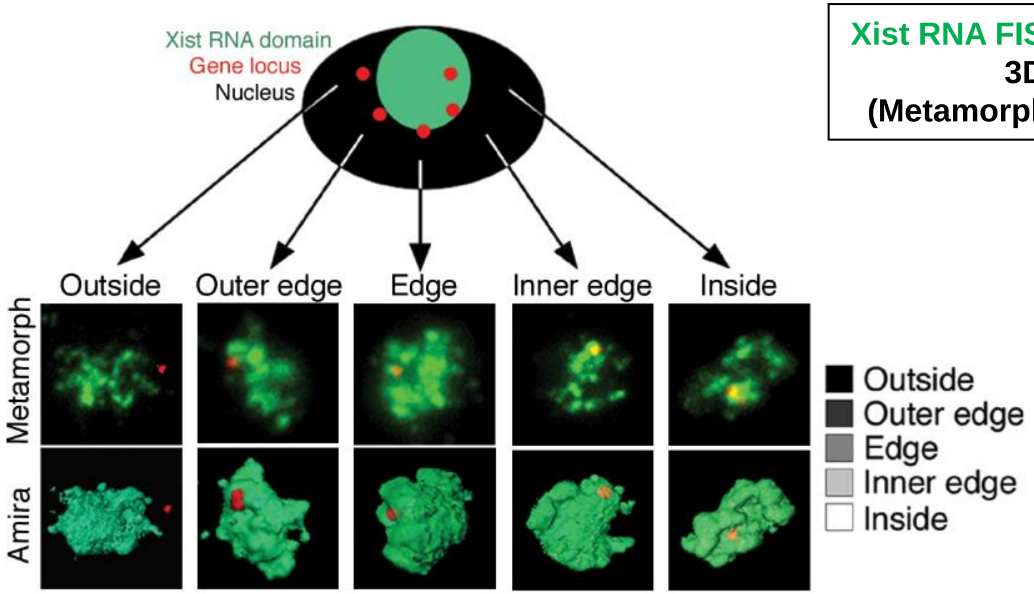
Where are the genes after their silencing?
METHOD?

Chaumeil et al, Genes & Dev, 2006

B. Xist as a nuclear and chromosome organizer

Where are the genes after their silencing?

Xist RNA FISH / Gene DNA FISH
3D analysis
(Metamorph Imaging – Amira)



Chaumeil et al, Genes & Dev, 2006

B. Xist as a nuclear and chromosome organizer

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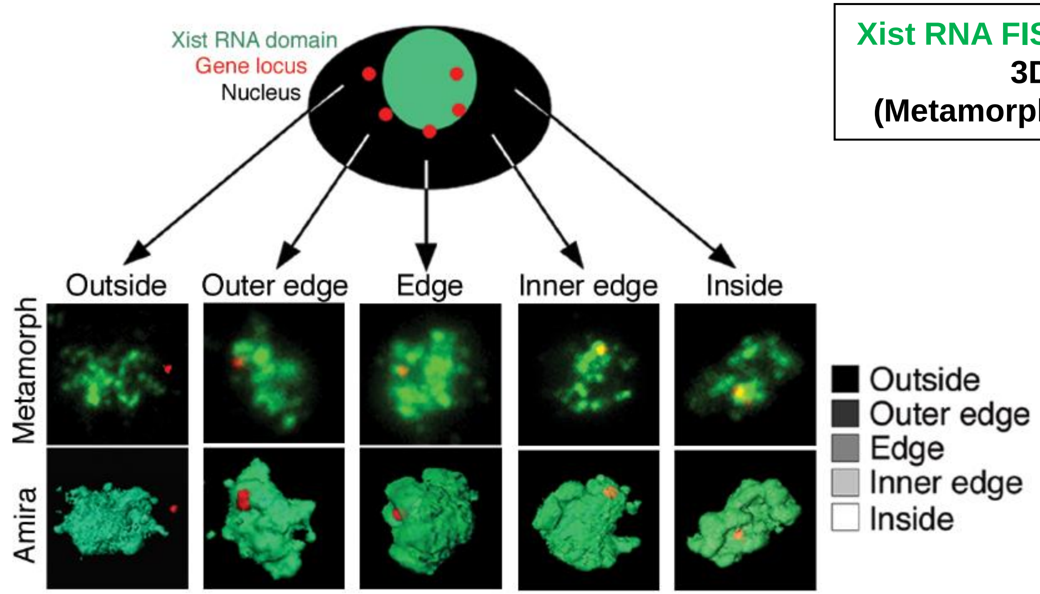
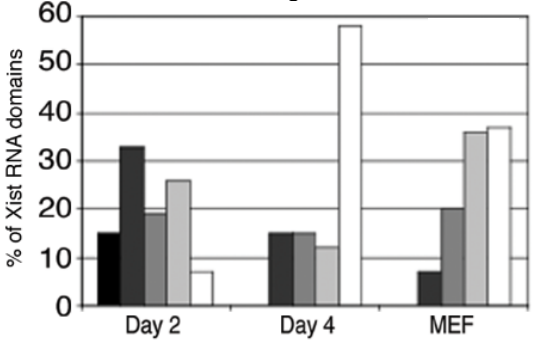


Xist RNA FISH / Gene DNA FISH
3D analysis
(Metamorph Imaging – Amira)

Pgk1



X-linked genes are relocated into
The Xi repressive compartment
as they become silenced

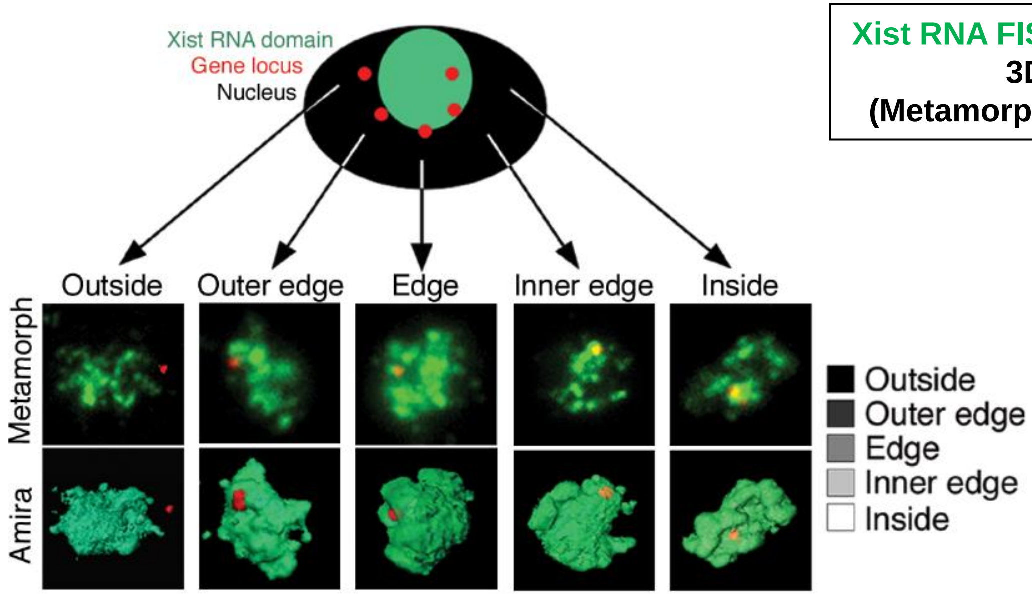
=> Is Xist involved in this reorganization?

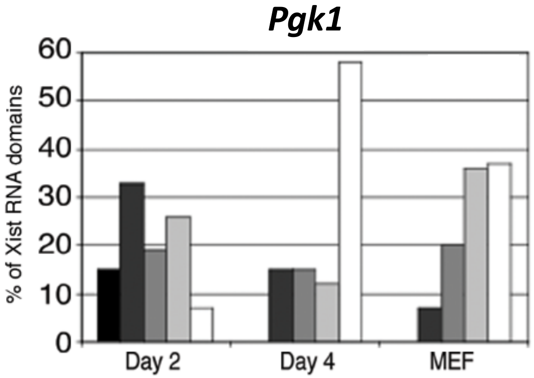
Chaumeil et al, Genes & Dev, 2006

B. Xist as a nuclear and chromosome organizer

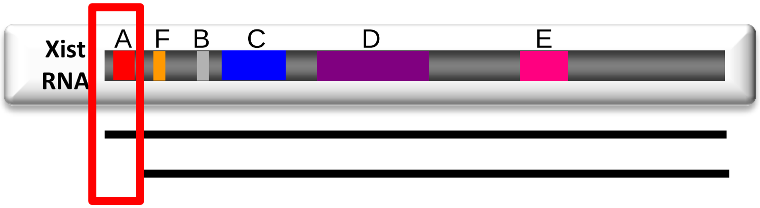
Where are the genes after their silencing?

Xist RNA FISH / Gene DNA FISH
3D analysis
(Metamorph Imaging – Amira)





Xist mutant lacking its A-repeats (inactivating domain):
- Still able to create a silent compartment
- BUT not able to induce gene silencing



Chaumeil et al, Genes & Dev, 2006

B. Xist as a nuclear and chromosome organizer

Where are the genes after their silencing?

Xist RNA FISH / Gene DNA FISH
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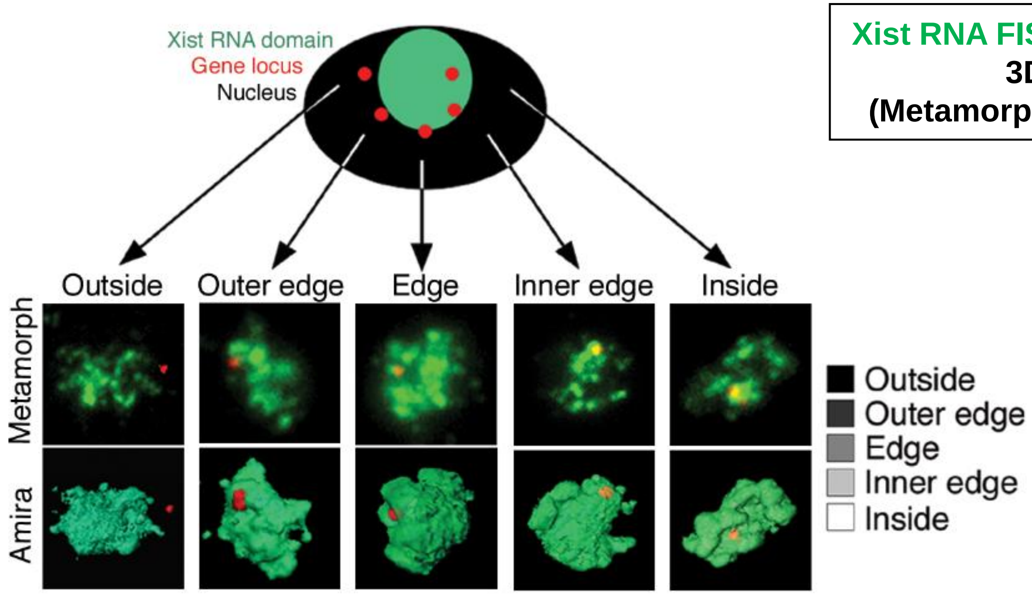
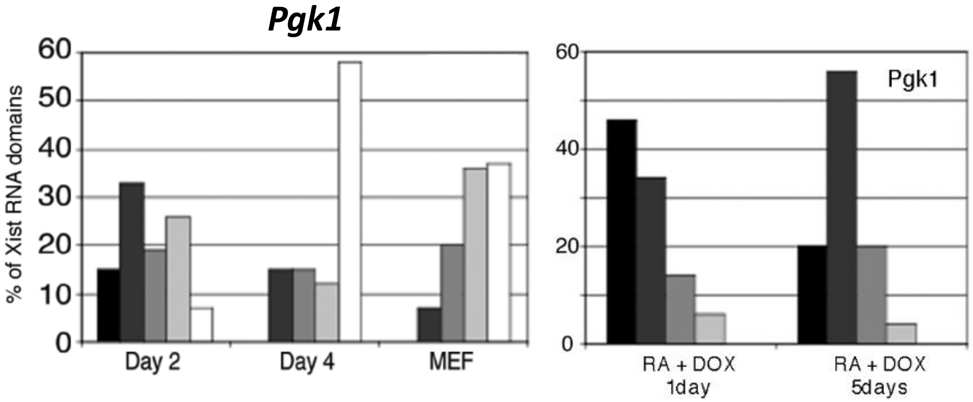


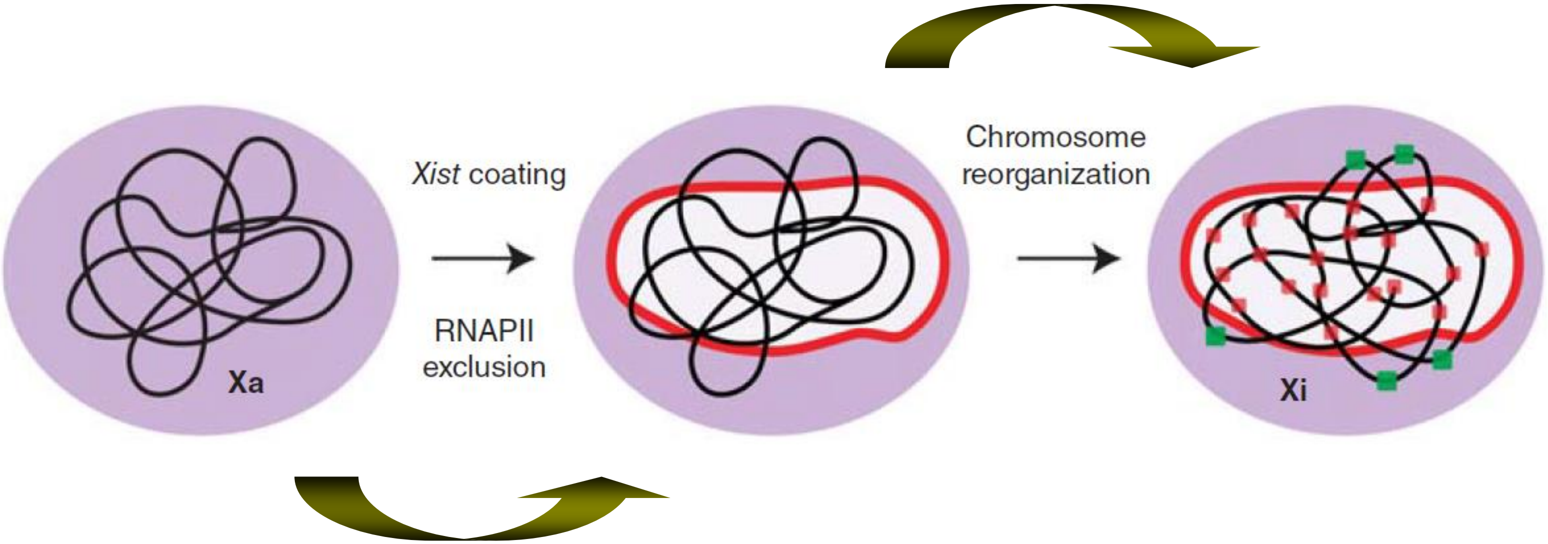


X-linked genes are relocated into
The Xi repressive compartment
<u>in a Xist-dependent manner</u>
as they become silenced

Chaumeil et al, Genes & Dev, 2006

B. Xist as a nuclear and chromosome organizer

Transcriptional shutdown of X-linked genes
Gene relocation into Xist RNA compartment
Xist RNA dependent - A repeat dependent



Xist RNA coating ⇨ Creation of a repressive compartment composed of silenced repeats: exclusion of RNA Pol II

Xist RNA dependent - A repeat independent

Mechanism?
still mostly unknow… but…

Ideas?

⬭ *Xist* compartment RNAPII concentration: 🟪 High ☐ Low 🟥 Silenced genes 🟩 Escapees

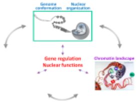
(Adapted from Dossin and Heard 2021)

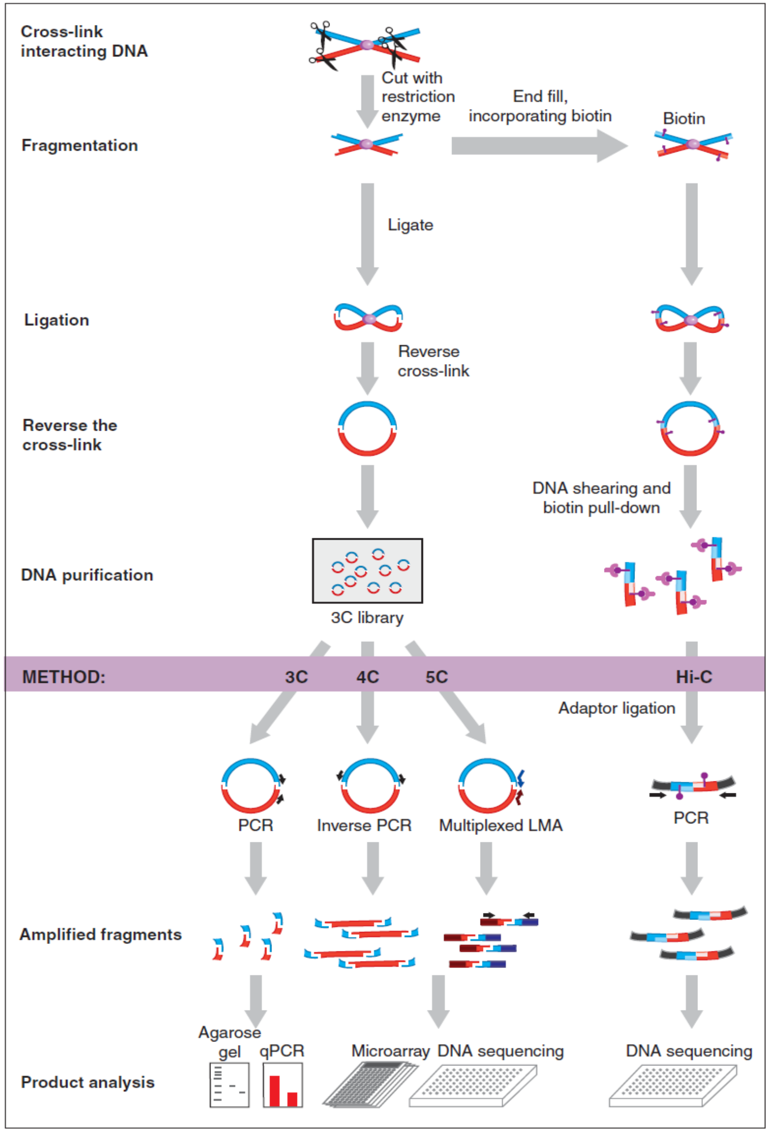
B. Xist as a nuclear and chromosome organizer

What is the global 3D architecture of the Xi and how is it related to gene silencing during X inactivation?

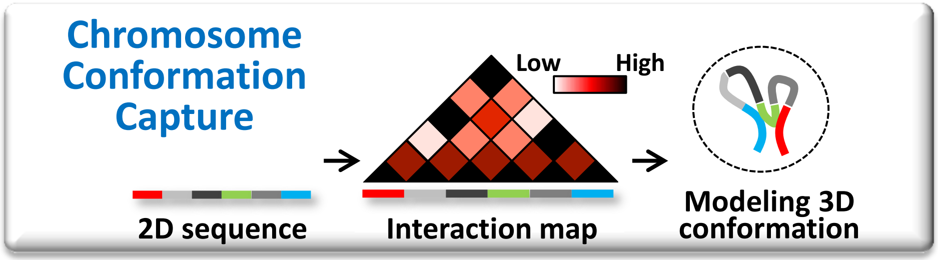
C. Disteche's lab *(Deng et al, Genome Biol, 2015)*
J. Lee's lab *(Minajigi et al, Science, June 2015)*
E. Heard's lab *(Giorgetti et al, Nature, 2016)*

METHOD?





One-to-one One-to-all Few-to-few All-to-all



Being far along the DNA sequence (in 2D) does not mean that you’re far in the nuclear space (in 3D) !

On the interaction map, high interactions away from the diagonal show 3D proximity of sequences far from each other in 2D.

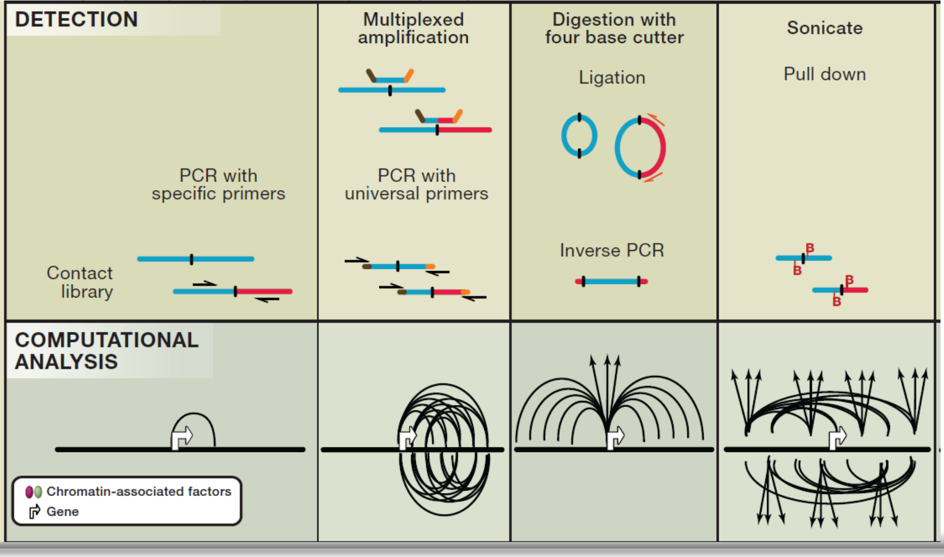
Dekker and Misteli, 2015

The nucleus is highly organized

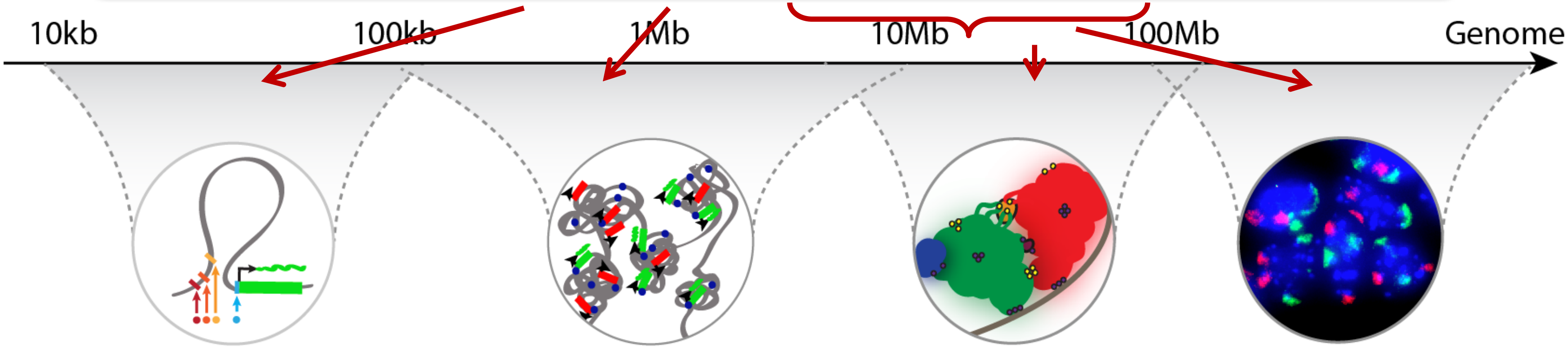
Genome conformation - Chromosome territories - Nuclear positioning

« Chromosome Conformation Capture »

3C	5C	4C	Hi-C
One-to-One	Few-to-Few	One-to-All	All-to-All



Adapted From Hakim and Misteli, Snapshot in *Cell*, 2012



Local DNA looping

Topologically associating domains

Chromosome territories

Chromosome positioning

The nucleus is highly organized

Genome conformation - Chromosome territories - Nuclear positioning

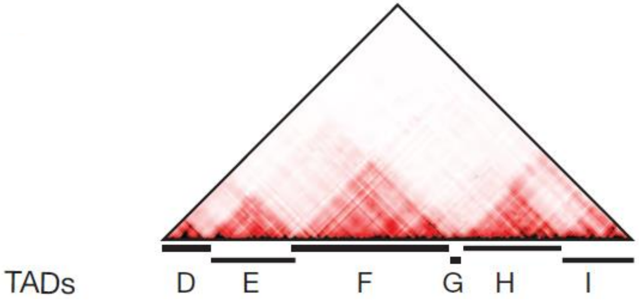
5C, HiC

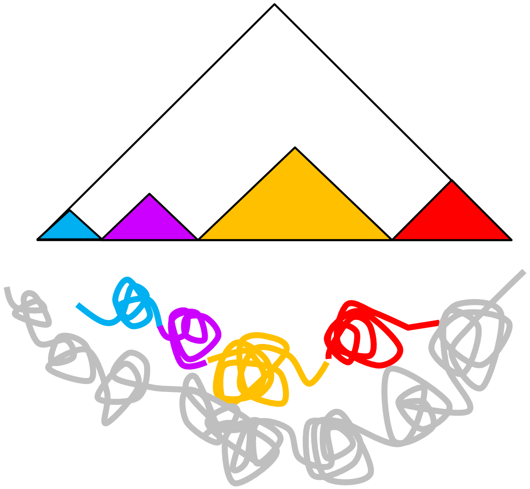
TAD = Topologically Associating Domains

Genomic regions of preferential interactions (ex: enhancer/promoter)

TADs are in general stable in different cell types, but intra-TAD interactions are dynamic

TADs cluster in bigger compartments: A (active) / B (inactive)





Other method?

Validation?

Nora et al, 2012

Dixon et al, 2012

The nucleus is highly organized

Genome conformation - Chromosome territories - Nuclear positioning

5C, HiC

TAD = Topologically Associating Domains

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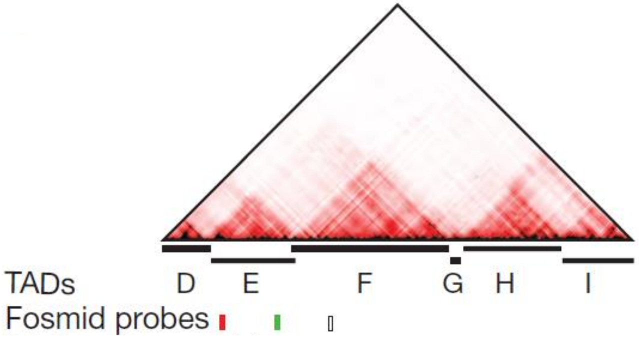
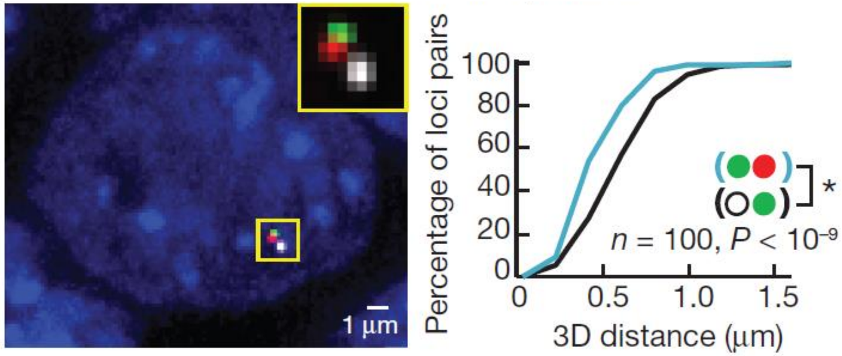
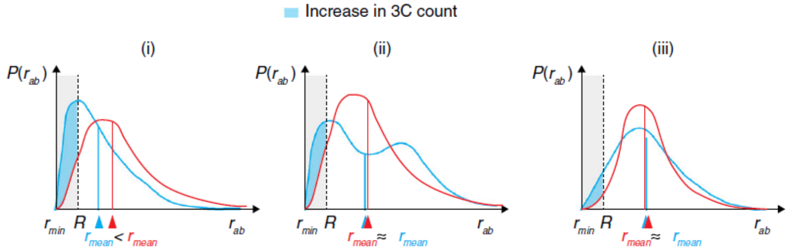




3D DNA FISH

- Still based on Formaldehyde fixation
- No PCR/sequencing artefacts
- Quantitative measurements (vs 3C=population-averaged probability of being « close enough to be crosslinked »)
- « Single-cell » resolution / « cell-to-cell » variation



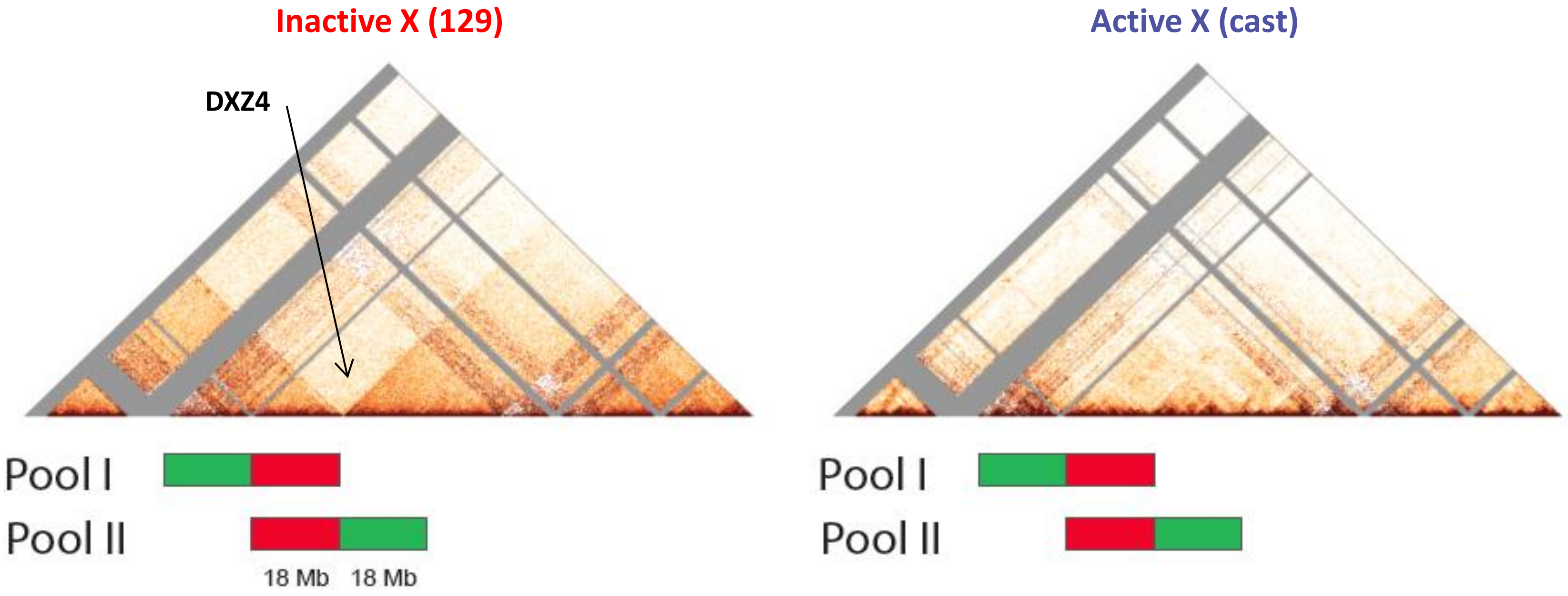
Giorgetti and Heard, 2016

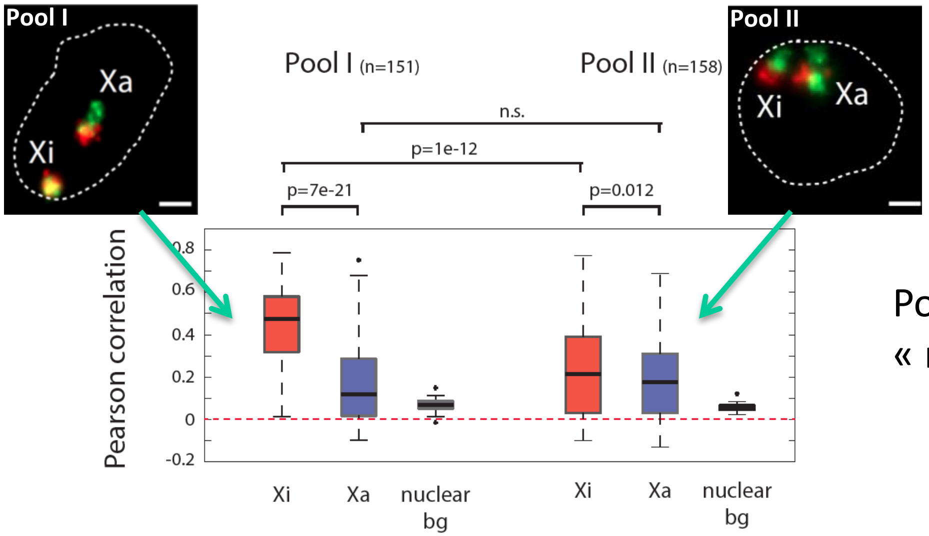
Nora et al, 2012

Dixon et al, 2012

3. Roles of Xist

B. Xist as a nuclear and chromosome organizer





Pool I: 2 probes in the same « mega-domain »

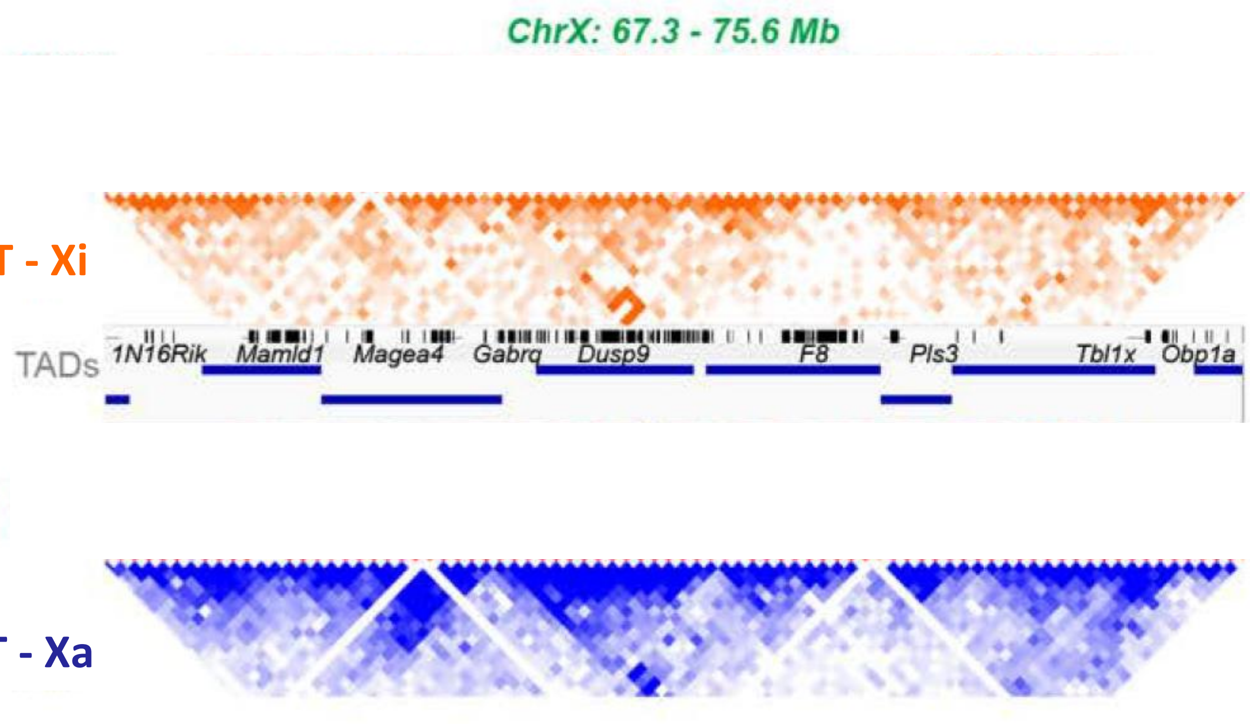
Pool II: 2 probes across the « mega-domain » boundary

- ✓ **Global loss of TAD**
- ✓ **The Xi is divided into 2 Megadomains separated by a boundary (DXZ4 macro satellite repeat)**

(Giorgetti et al, Nature, 2016)

B. Xist as a nuclear and chromosome organizer

Female fibroblasts WT or ΔXist -> TAD structure?



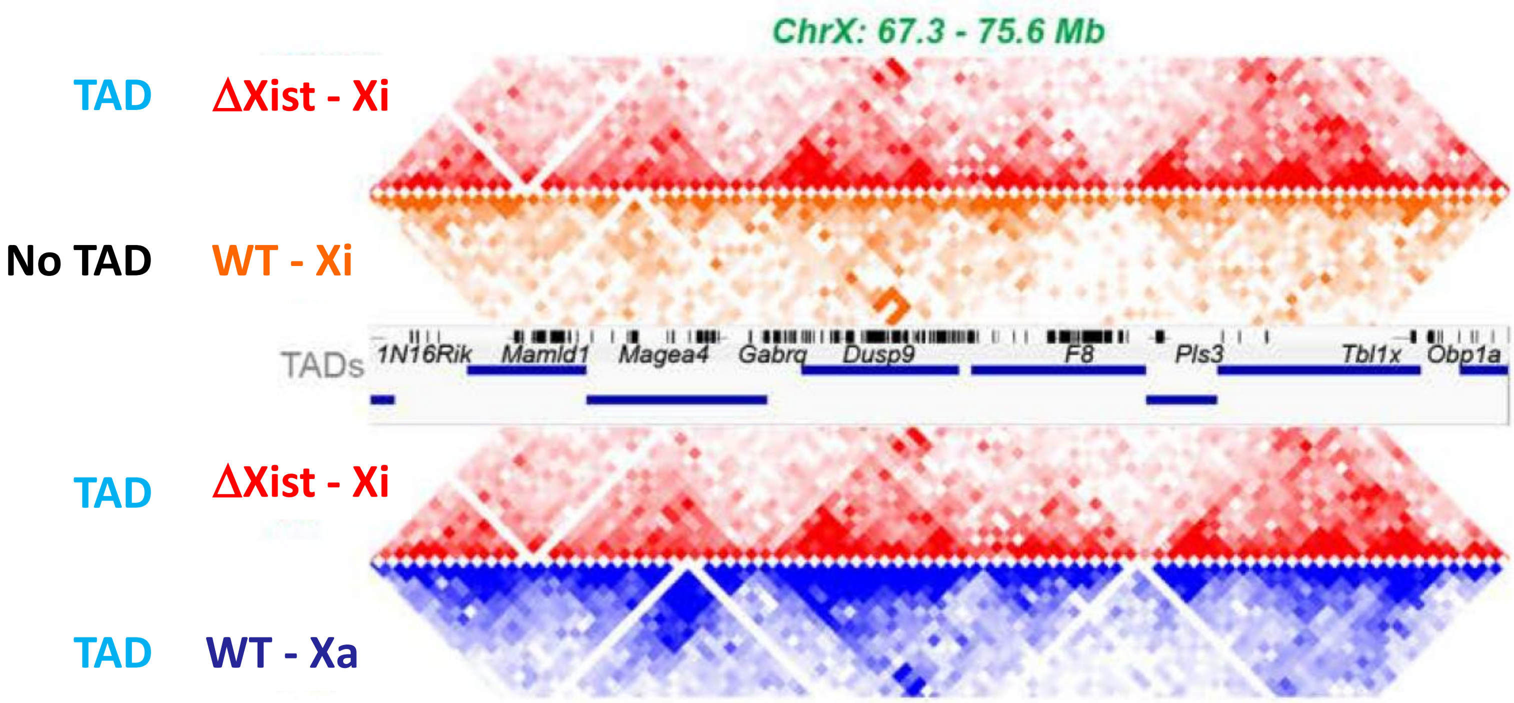
No TAD WT - Xi

TAD WT - Xa

(Minajigi et al, Science, 2015)

B. Xist as a nuclear and chromosome organizer

Female fibroblasts WT or ΔXist -> TAD structure?



- ✓ **Xi from Δxist mutant cell recovers a TAD-like structure like the Xa.**

(Minajigi et al, Science, 2015)

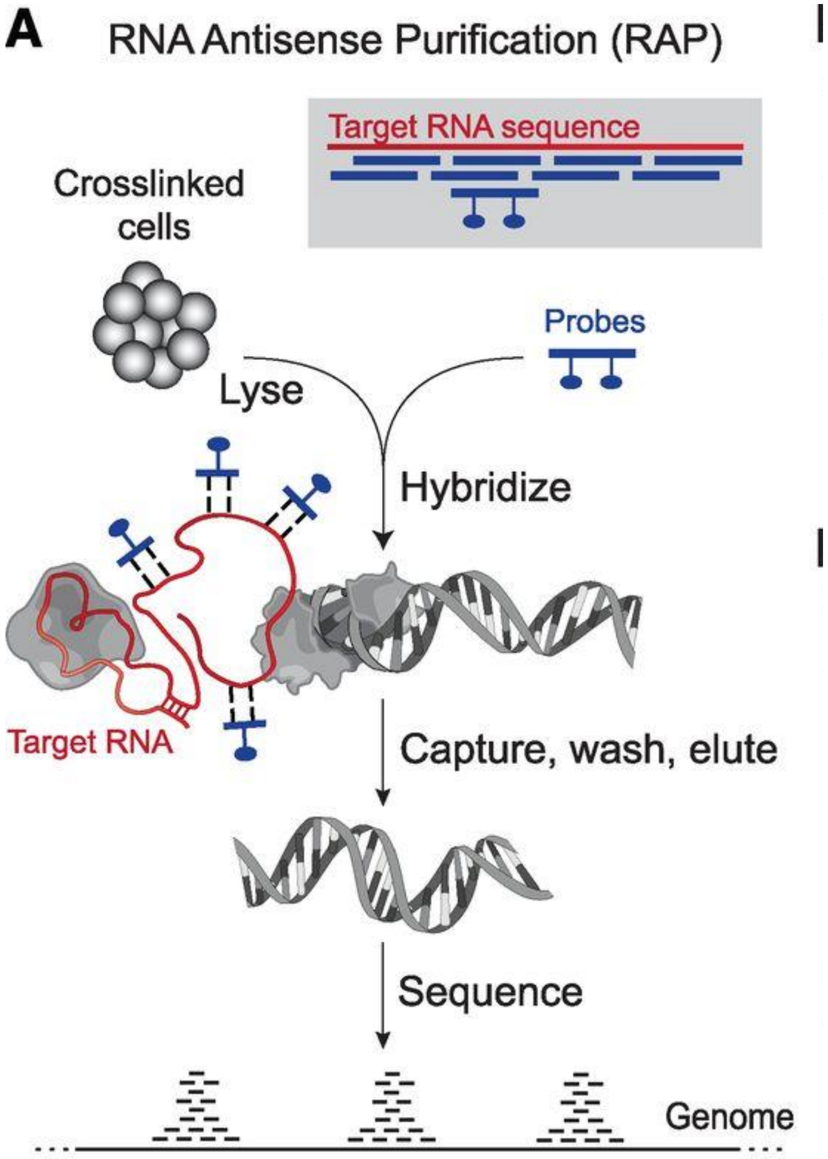
B. Xist as a nuclear and chromosome organizer

Hi-C data can also help dissecting anotehr question:
how does Xist spread along the X chromosome?

Guttman's lab *(Engreitz et al, 2013)*

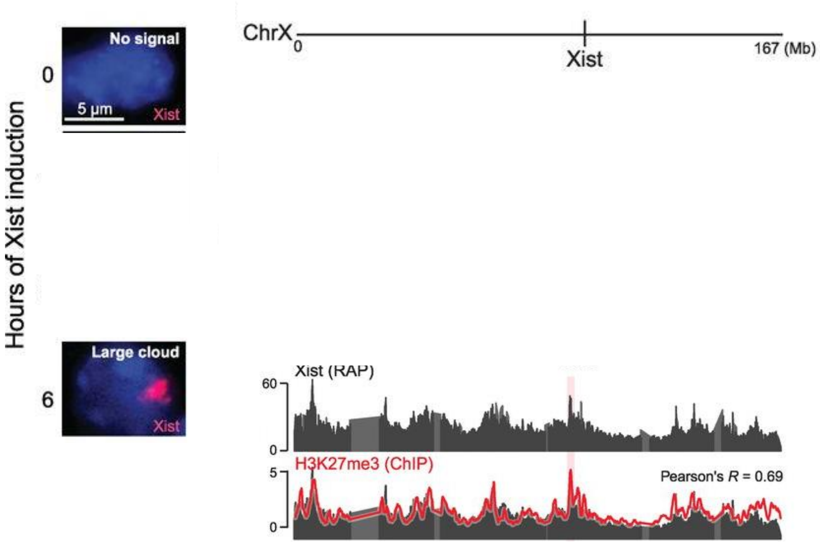
METHOD?

Method: Pull-down of RNA-DNA complexes followed by DNA-sequencing



Engreitz, et al , Science, 2013

C. Xist spreading along the X chromosome

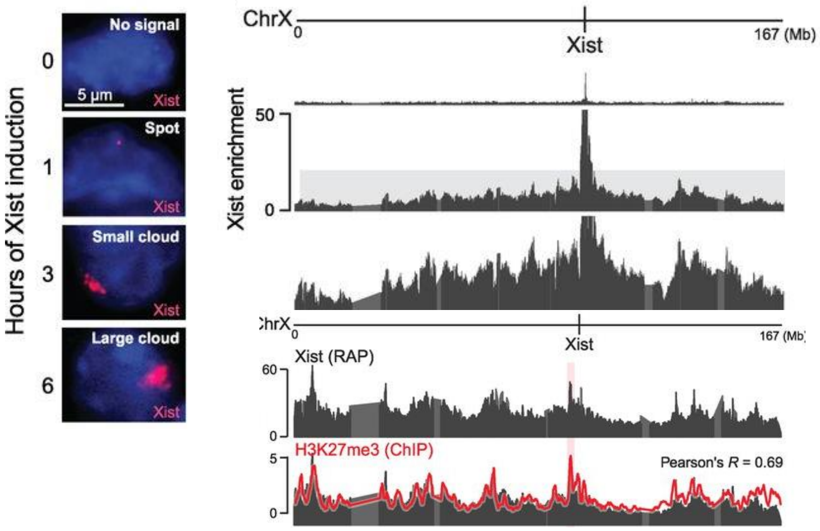


-**During the maintenance of XCI, Xist binds broadly across the X chromosome :**

- The <u>most enriched</u> regions showed higher H3K27me3 and higher gene density
- The <u>least enriched</u> regions contained genes known to escape XCI, consistent with their preferential positioning outside of the Xist domain

Engreitz, et al , Science, 2013

C. Xist spreading along the X chromosome



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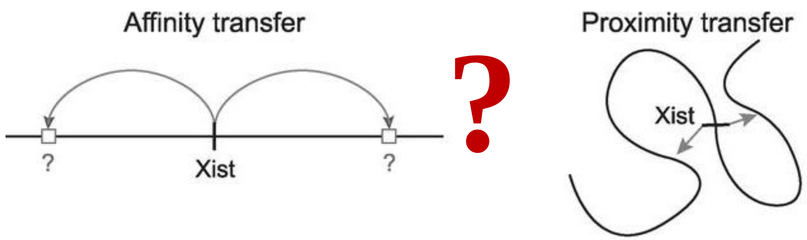
-**During initiation of XCI:**

- Xist transfers to regions proximal to its transcription site,
- then transfers to distal early localization sites across the chromosome (28 sites).

What are these early entry sites?

Engreitz, et al , Science, 2013

C. Xist spreading along the X chromosome



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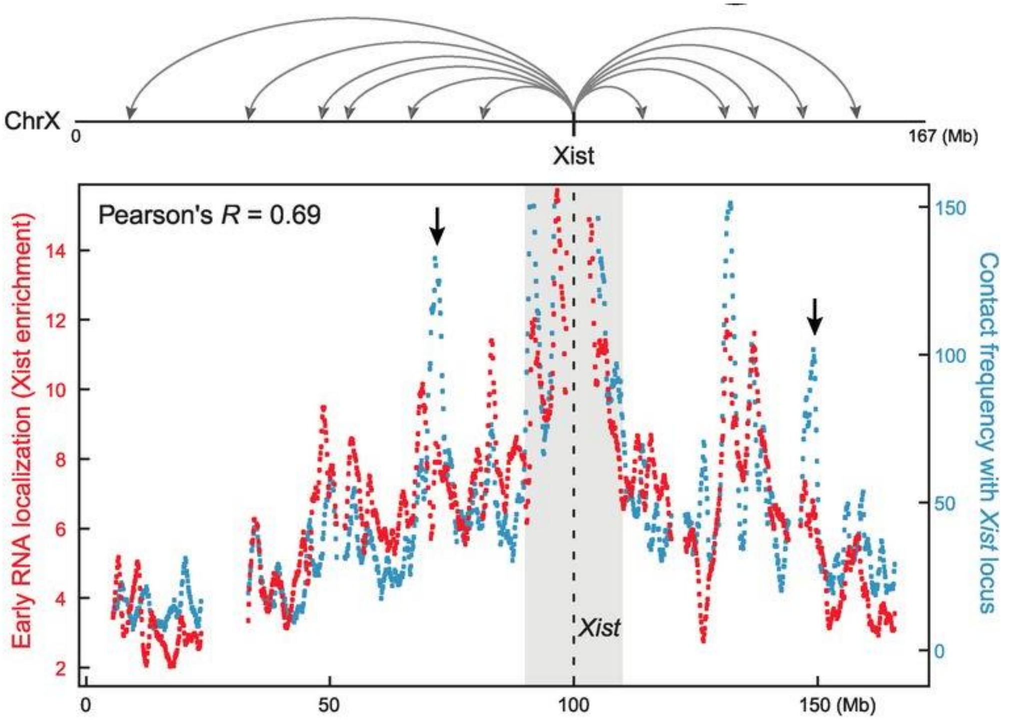


Comparison RAP vs Hi-C

RAP peaks: DNA sequences where Xist RNA locates first

Hi-C peaks: DNA sequences close to the *Xist* gene

- Strong correlation between Xist RNA localization along the X,
- and the frequency at which distal sites contact the Xist genomic locus.

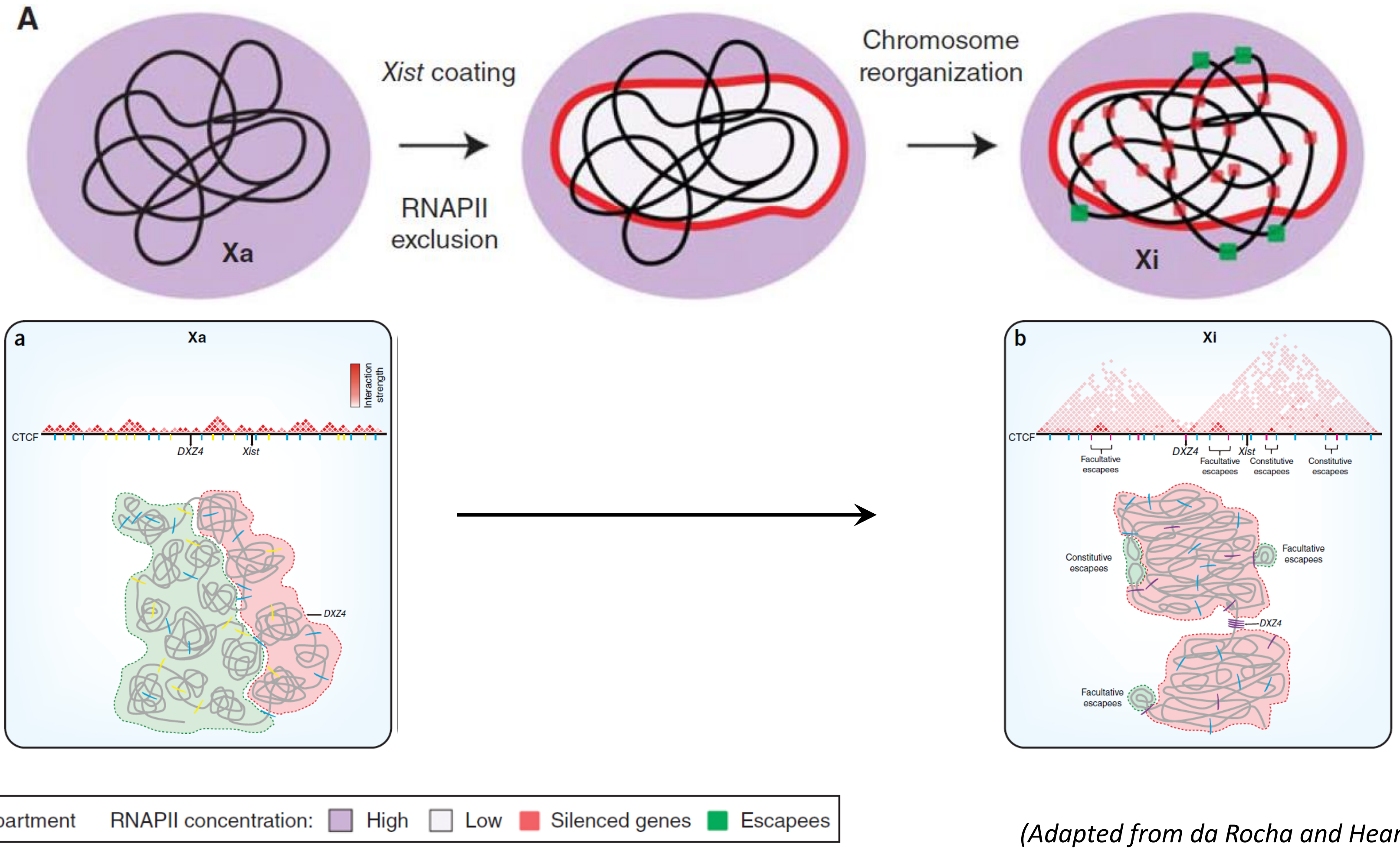
-> Spreading follows a "3D-PROXIMITY MODEL"

Model : Xist coats the X by searching in three dimensions, modifying chromosome structure, and spreading to newly accessible locations.

Engreitz, et al , Science, 2013

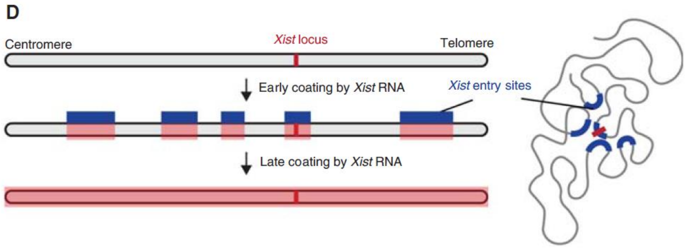
B. Xist as a nuclear and chromosome organizer

- ✓ The Xa shows the typical TAD-organization clustered in active (A) and repressive (B) compartments.
- ✓ **The Xi shows a special organization into 2 mega-domains** with unspecific interactions.
- ✓ Few TAD-like structures loop out from the repressive core of the Xi territory : escaping genes.
- ✓ **Xist RNA is involved in this reorganization** as its deletion is able to restore a TAD-like structure.

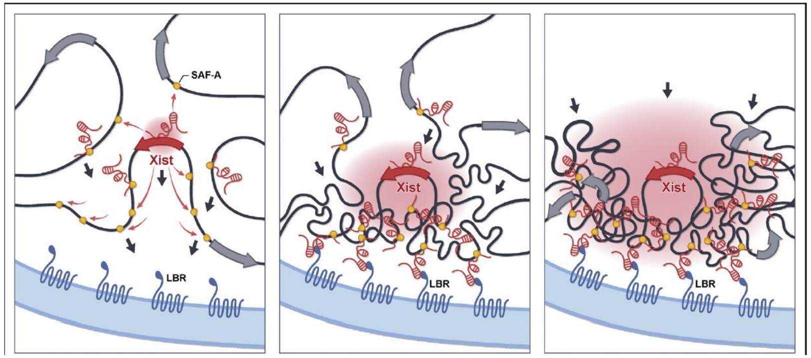


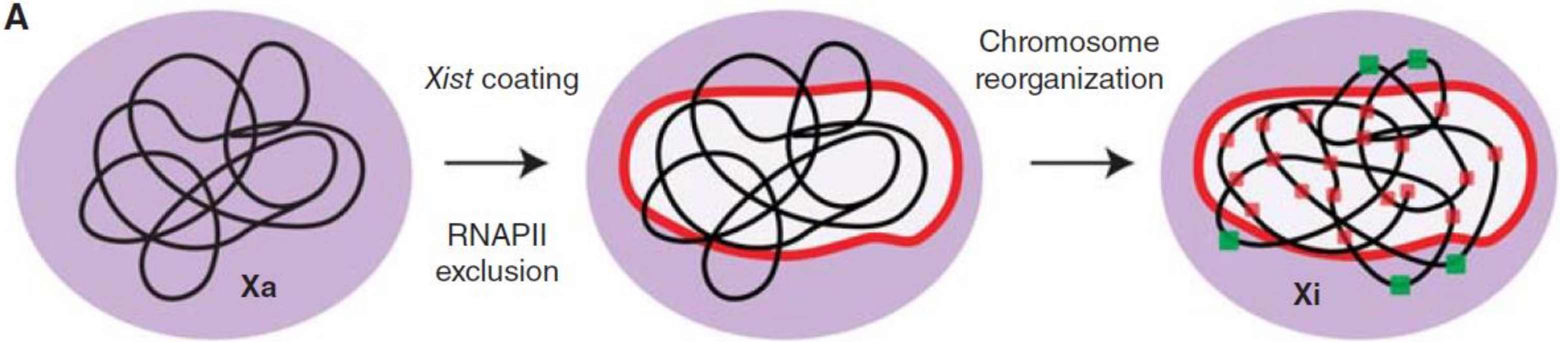
(Adapted from da Rocha and Heard, 2017; Dossin and Heard 2021)

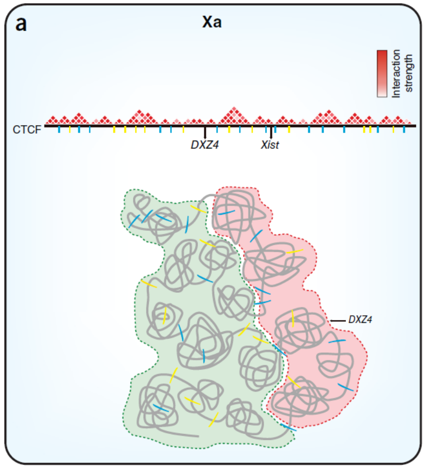
Xist as a nuclear and chromosome organizer



Xist is sequestered by SAF-A and LBR?

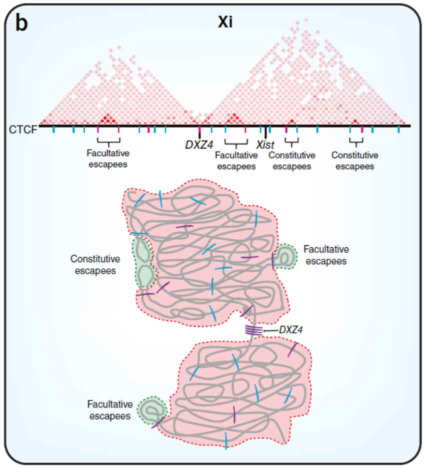






Interplay between Xist spreading along the X and X chromosome change in conformation/gene reorganization

X chromosome reorganization through Cohesin eviction? SAF-A? LBR?



⭕ *Xist* compartment RNAPII concentration: 🟪 High ☐ Low 🟥 Silenced genes 🟩 Escapees

(Adapted from da Rocha and Heard, 2017; Strehle and Guttman, 2020; Dossin and Heard 2021)

1. **Introduction on X Chromosome Inactivation**

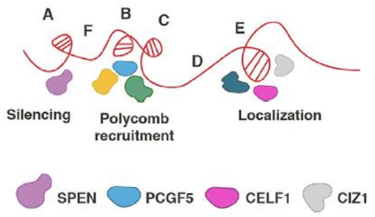
2. **Initiation of XCI: the Xist RNA**

3. **Roles of Xist:**
    - **A. Plaform to recruit silencing factors**
    - **B. Platform to recruit maintenance factors**
    - **C. Nuclear and chromosome organizer**

Roles for the Xist long-non coding RNA

✓Mediated by different regions of the transcript

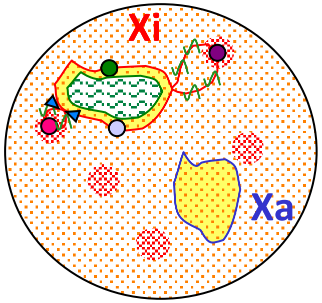
✓Acting at different stages of inactivation (initiation / maintenance)



Transcriptional shutdown

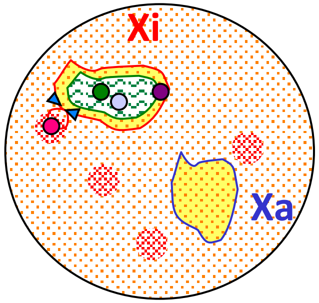
Lock-in of the inactive state

Repressive compartment



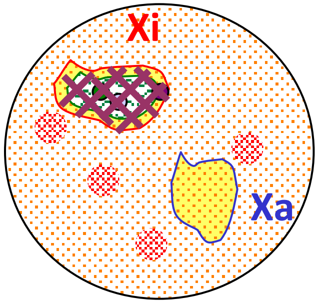
-> Exclusion of Pol II: via Spen/HDAC3/NuRD?

3D organization of genes and Xi



-> SAF-A
-> LBR?
-> SMCHD1 / cohesin eviction (loss of TAD)

Recruitment of repressive marks



-> HDAC3: via Spen
-> PRC1: via hnRNPK/PCGF3/5
-> PRC2: via PRC1/Jarid2

Relationship between expression and gene organization

Role of non-coding RNA in gene regulation through nuclear and chromatin organization

(Chu et al, 2015; McHugh et al, 2015; Minajigi et al, 2015; Moindrot et al, 2015; Monfort et al, 2015; Chen et al, 2016; Jansz et al, 2017; Almeida et al, 2017; Pintacuda et al, 2017;Dossin et al, 2020…..)

Roles for the Xist long-non coding RNA

1) Xist recruits the silencing factor SPEN (A-repeats):
=> X-linked gene silencing

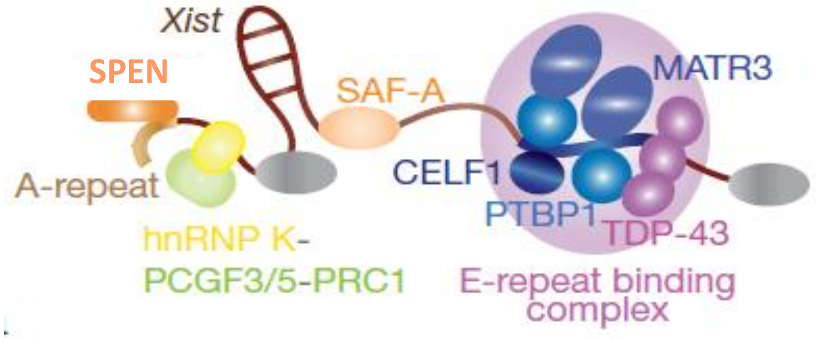
2) Xist recruits hnRNPK (B-repeats):
=> Maintenance of the XCI

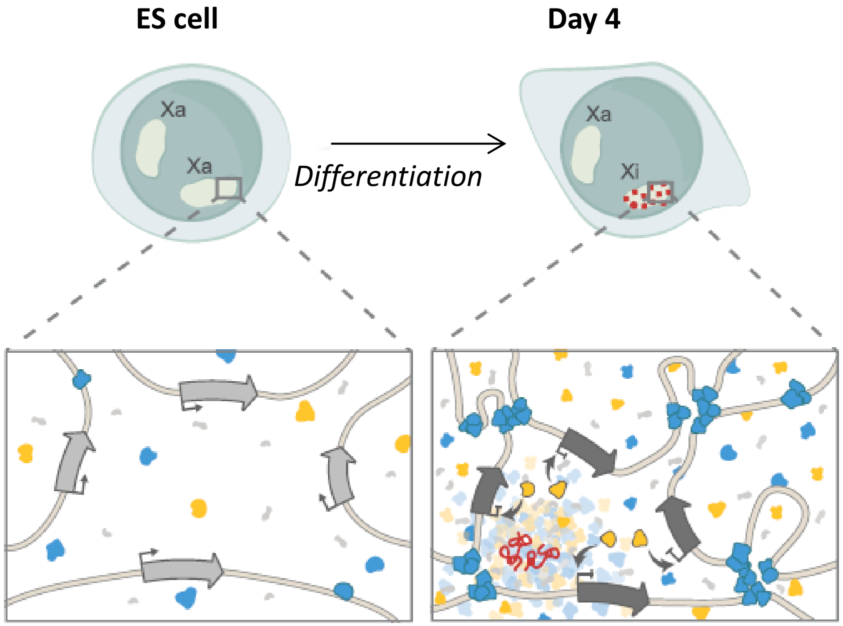
3) Xist and SPEN recruit RBM15/WTAP:
=> Involved in Xist stability? Gene silencing?

4) Xist recruits 4 RNA-binding proteins (E-repeats):
=> Gene silencing / Anchor of Xist to the Xi

5) Xist binds SAF-A and Lamin-B Receptor (LBR):
=> Sequestration for silencing?

=> Creation of a repressive compartment where genes are relocated when silenced





Increased molecular crowding
Higher-order chromatin changes
Chromosome-wide silencing

(Pandya-Jones et al, 2020; Markaki et al, 2021)
(From Dossin and Heard 2021)

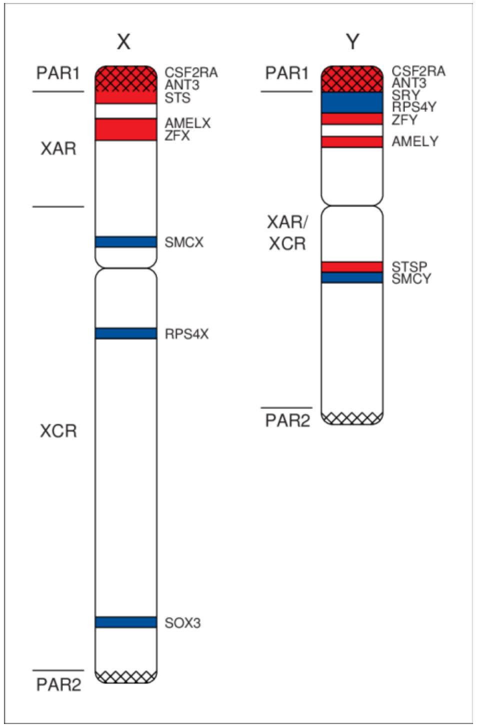
1. Introduction on X Chromosome Inactivation

2. Initiation of XCI: the Xist RNA

3. Roles of Xist:

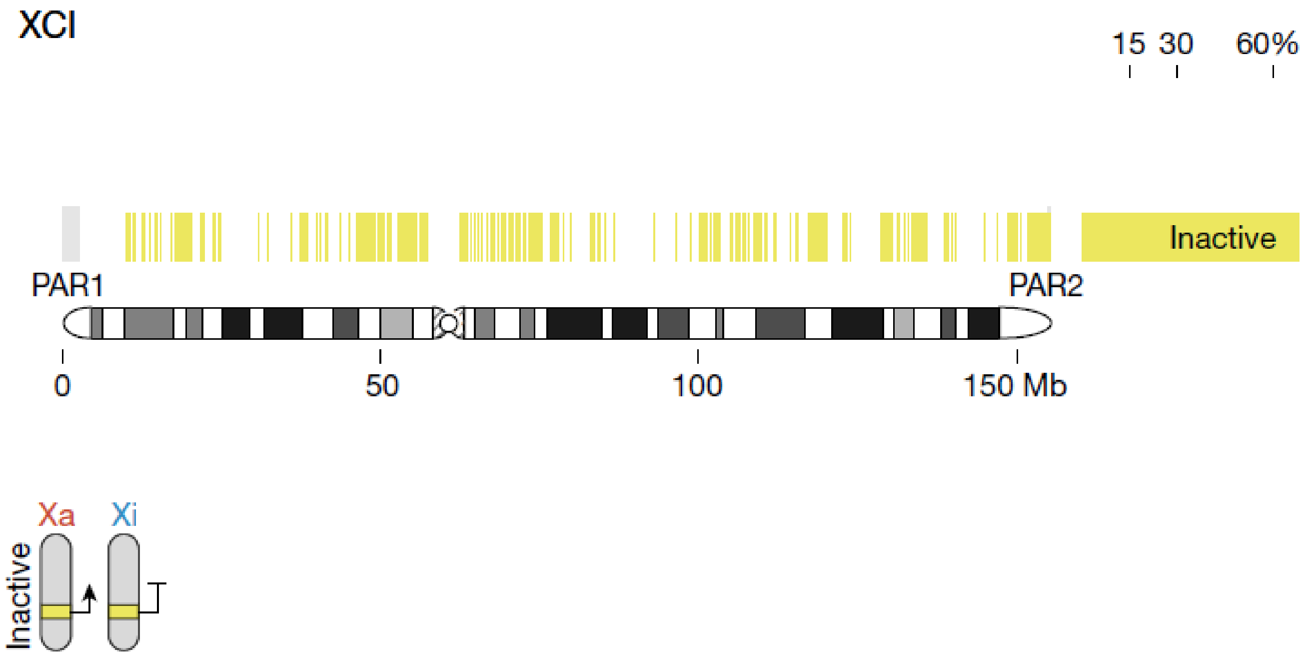
  A. Plaform to recruit silencing factors
  B. Platform to recruit maintenance factors
  C. Nuclear and chromosome organizer

4. Escaping XCI



X chromosome inactivation in female cells

=> Most X-linked genes are stably silenced on 1 of the 2 X chromosomes…

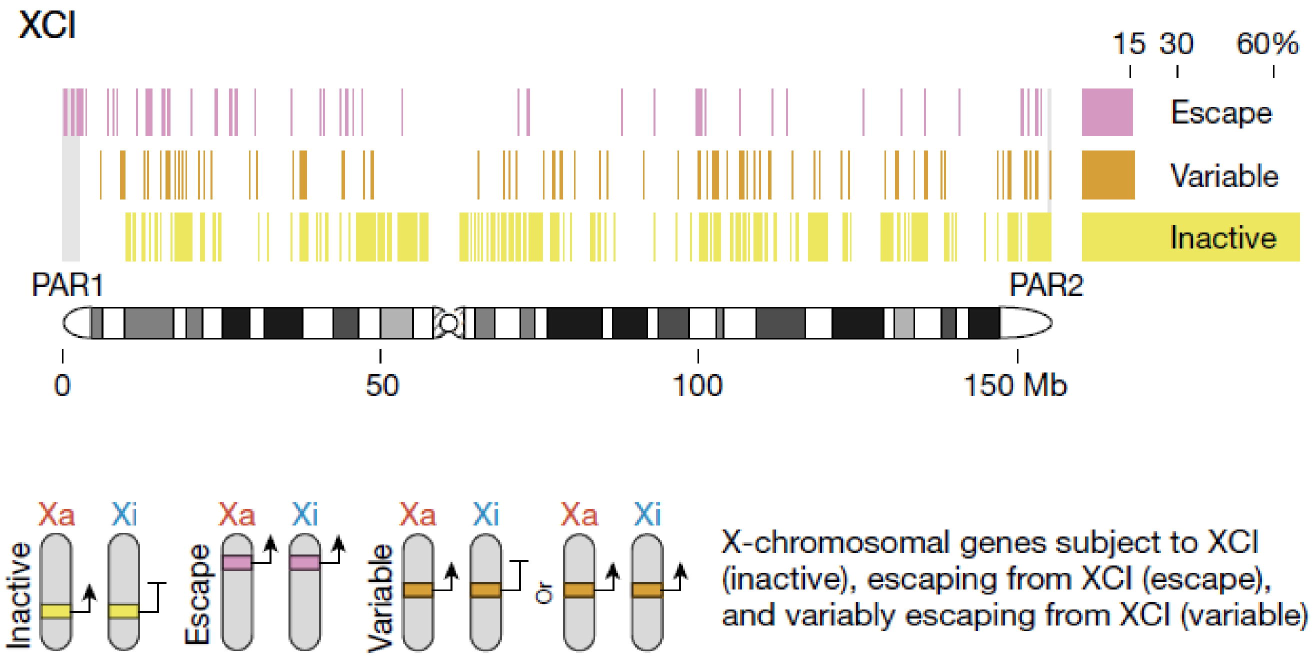


(Tukiainen et al, 2017)

(Other work including: Carrel and Willard, 2005; Cotton et al, 2013; Navarro-Cobos et al, 2020…)

X chromosome inactivation in female cells

=> … However, ~20% of X-linked genes escape XCI in women



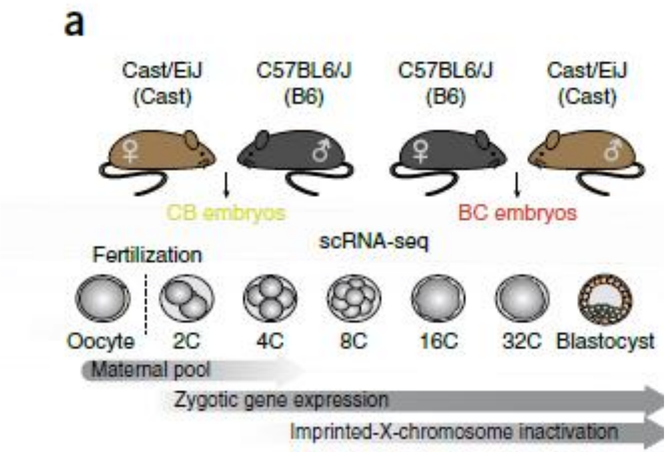
X-chromosomal genes subject to XCI (inactive), escaping from XCI (escape), and variably escaping from XCI (variable)

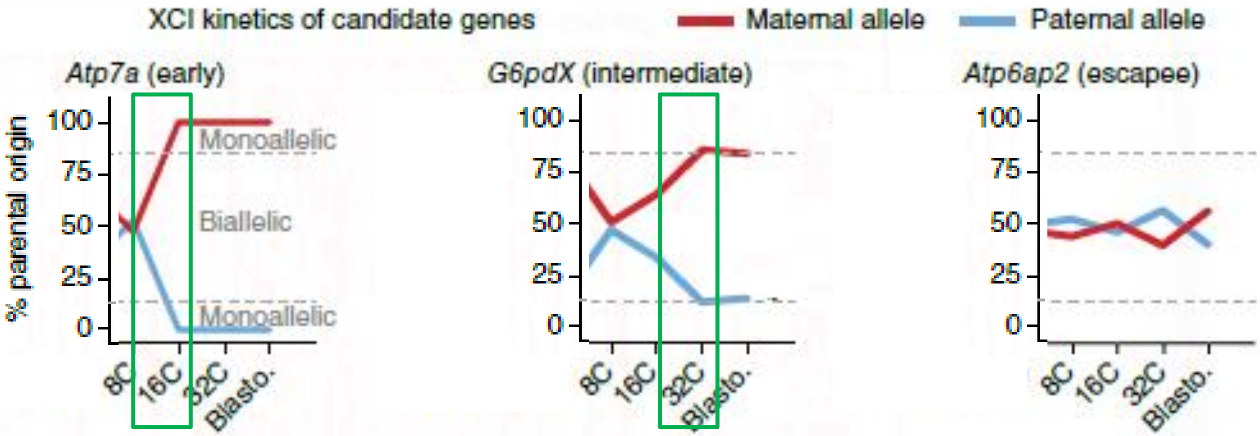
(Tukiainen et al, 2017)

(Other work including: Carrel and Willard, 2005; Cotton et al, 2013; Navarro-Cobos et al, 2020…)

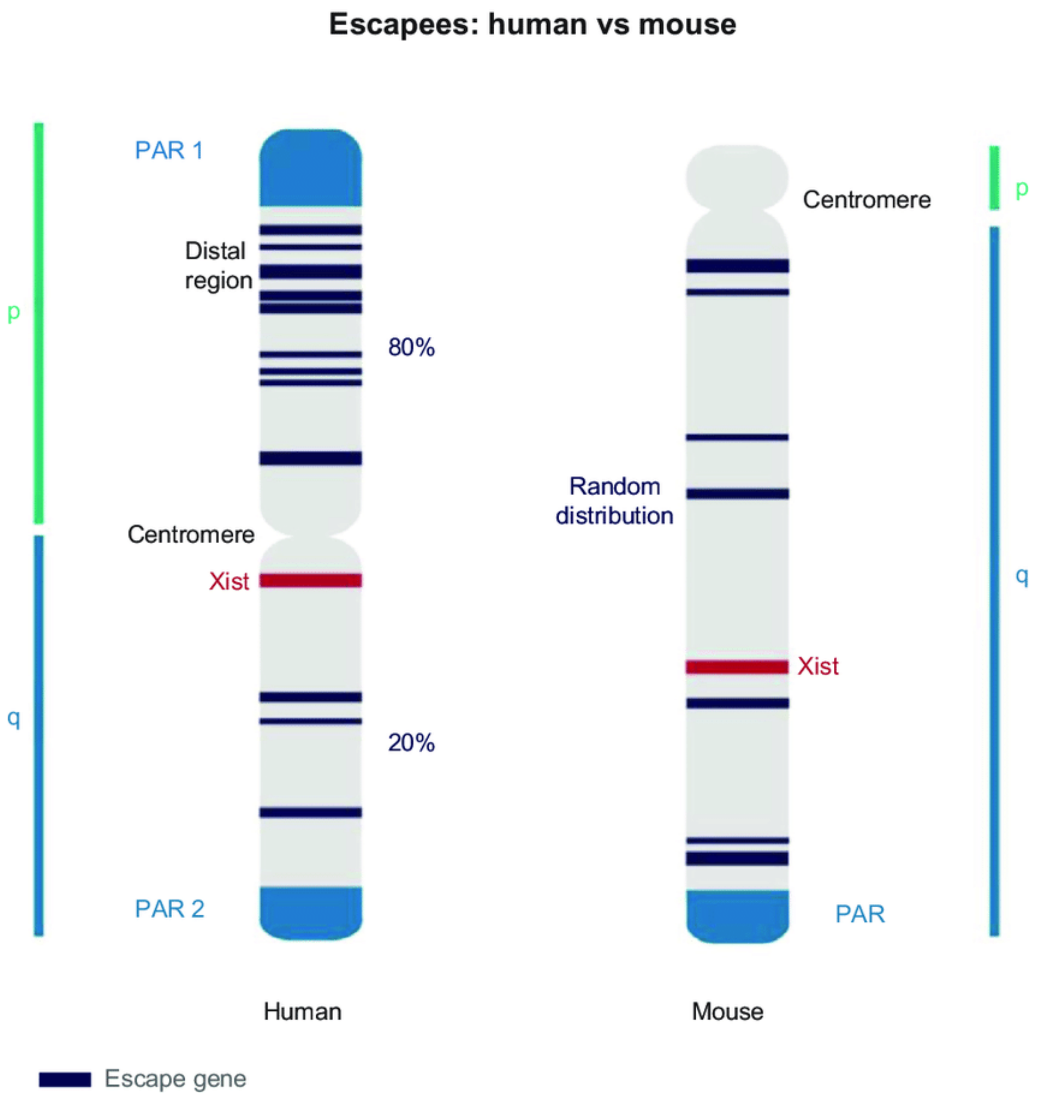
Different kinetics of gene silencing during early mouse development

Single cell RNA-seq in
F1 hybrid embryos

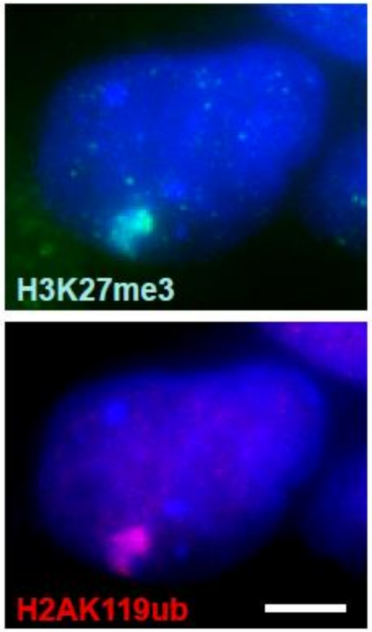


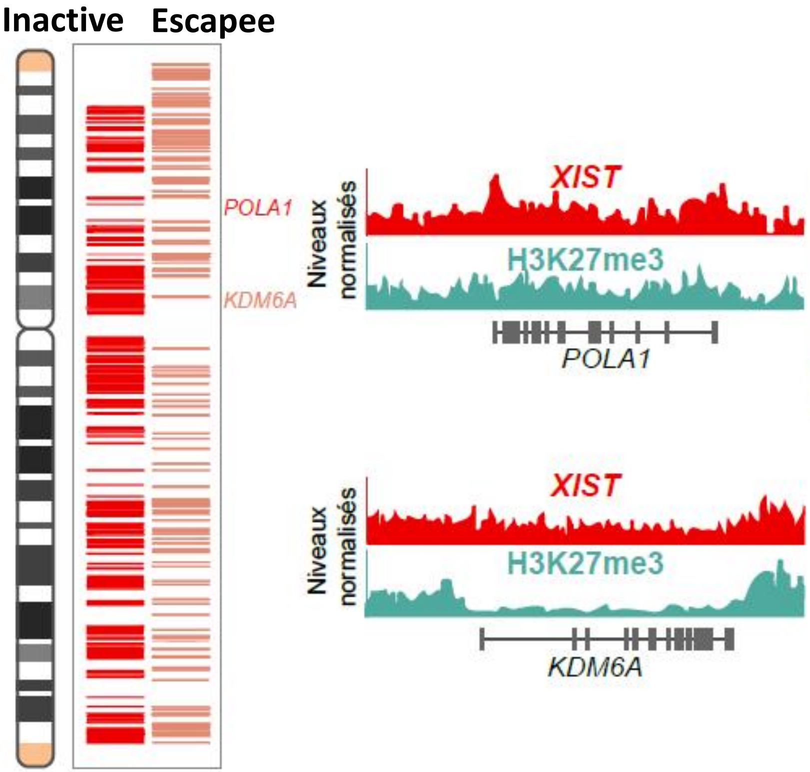


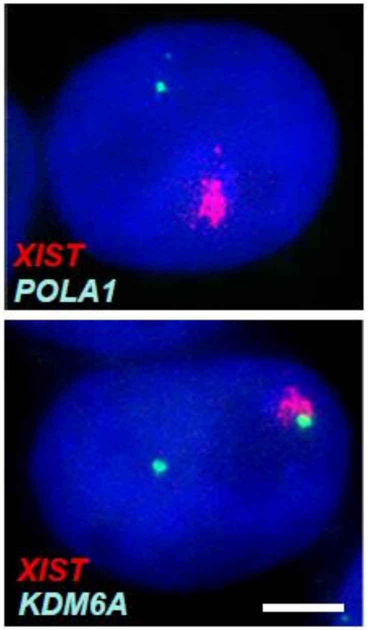
Borensztein et al, 2017


Escapees: human vs mouse
p
q
PAR 1
Distal region
80%
Centromere
Xist
20%
PAR 2
Human
Centromere
p
Random distribution
q
Xist
PAR
Mouse
▬ Escape gene

Xi-specific epigenetic marks







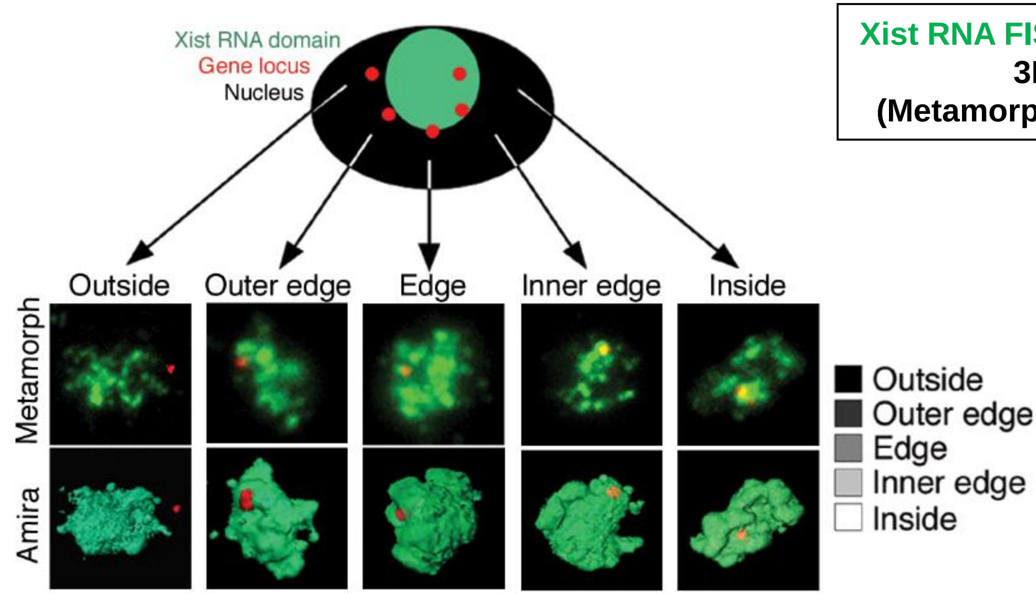
Silenced X-linked genes are enriched in repressive histone marks EXCEPT escapees

(From Chaumeil and Morey, *in press Medecine/Science*)

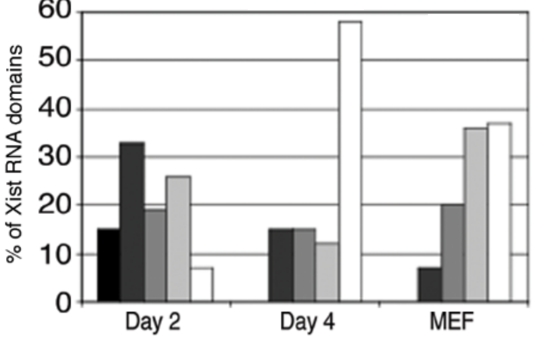
B. Xist as a nuclear and chromosome organizer

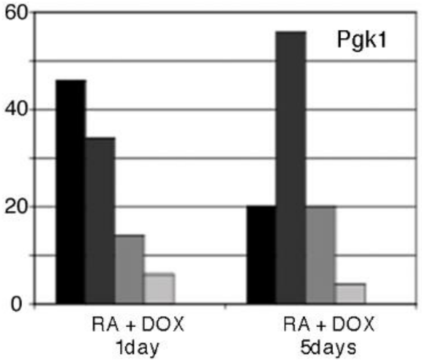
Where are the genes after their silencing?

Xist RNA FISH / Gene DNA FISH
3D analysis
(Metamorph Imaging – Amira)

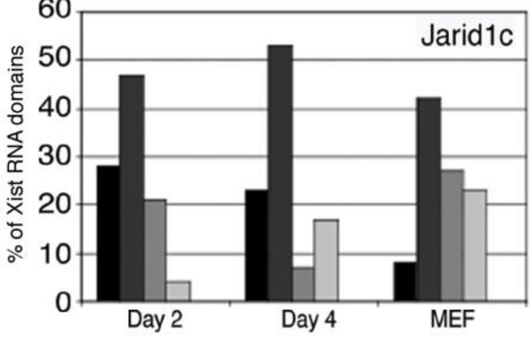


Pgk1





Escapee –> *Jarid1c*



Chaumeil et al, Genes & Dev, 2006

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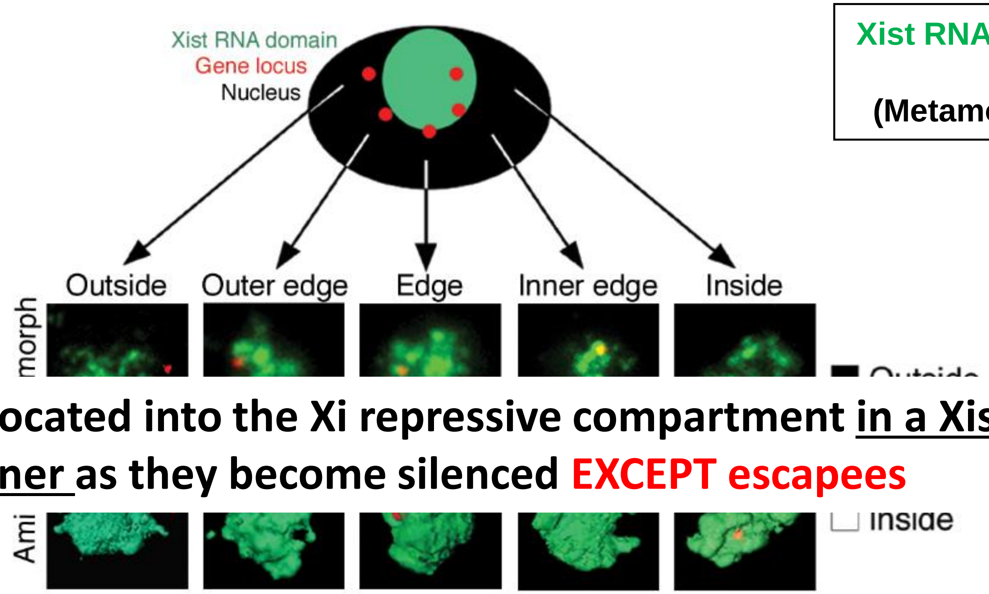
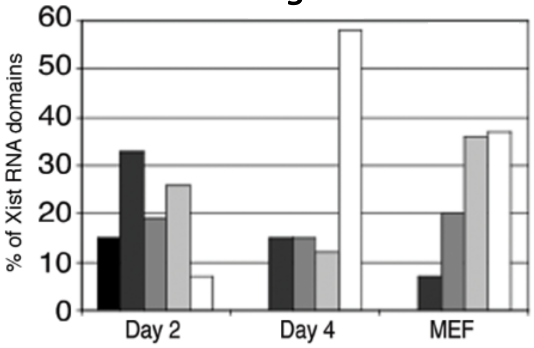
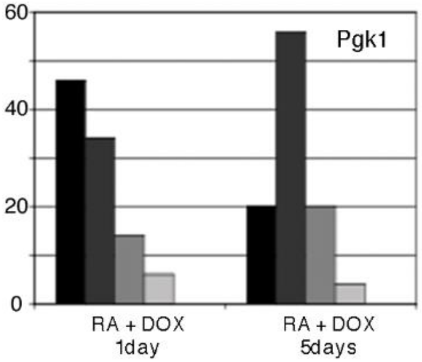


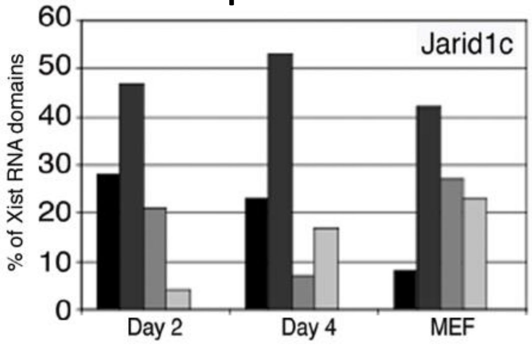
X-linked genes are relocated into the Xi repressive compartment <u>in a Xist-dependent manner</u> as they become silenced EXCEPT escapees

Pgk1





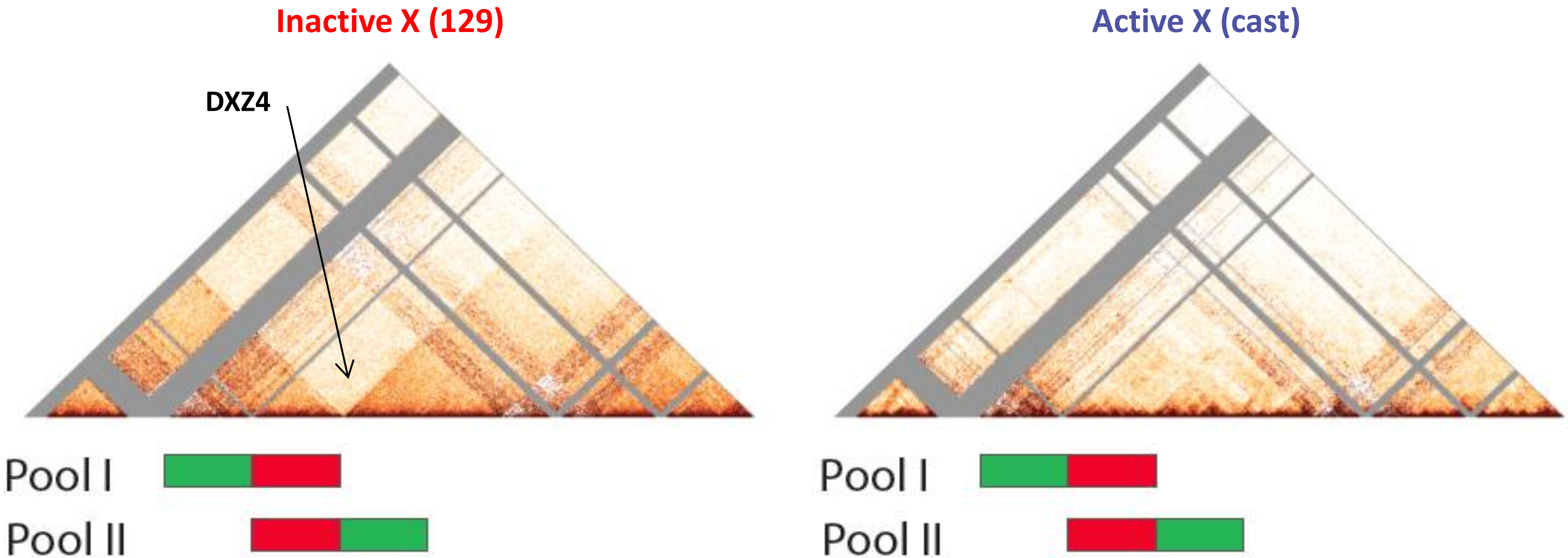
Escapee –> *Jarid1c*

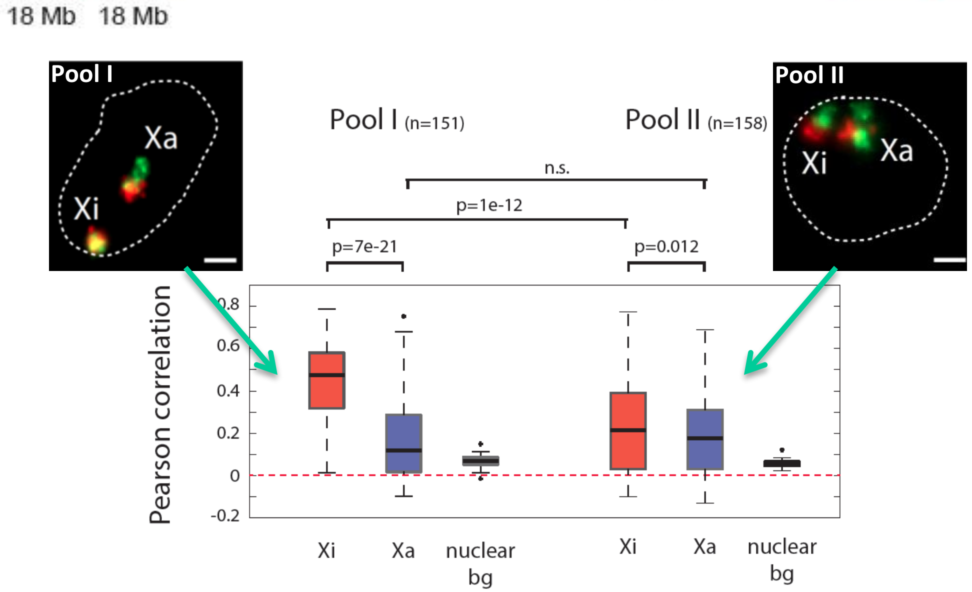


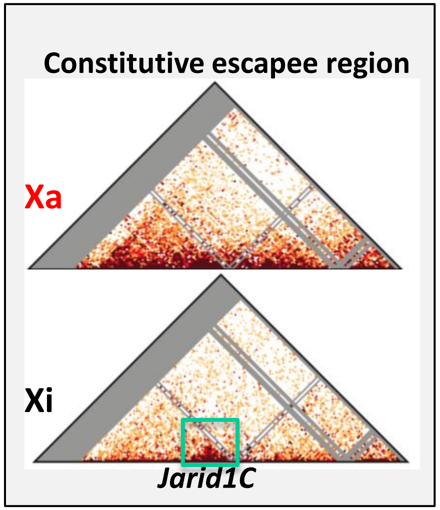
Chaumeil et al, Genes & Dev, 2006

3. Roles of Xist

B. Xist as a nuclear and chromosome organizer







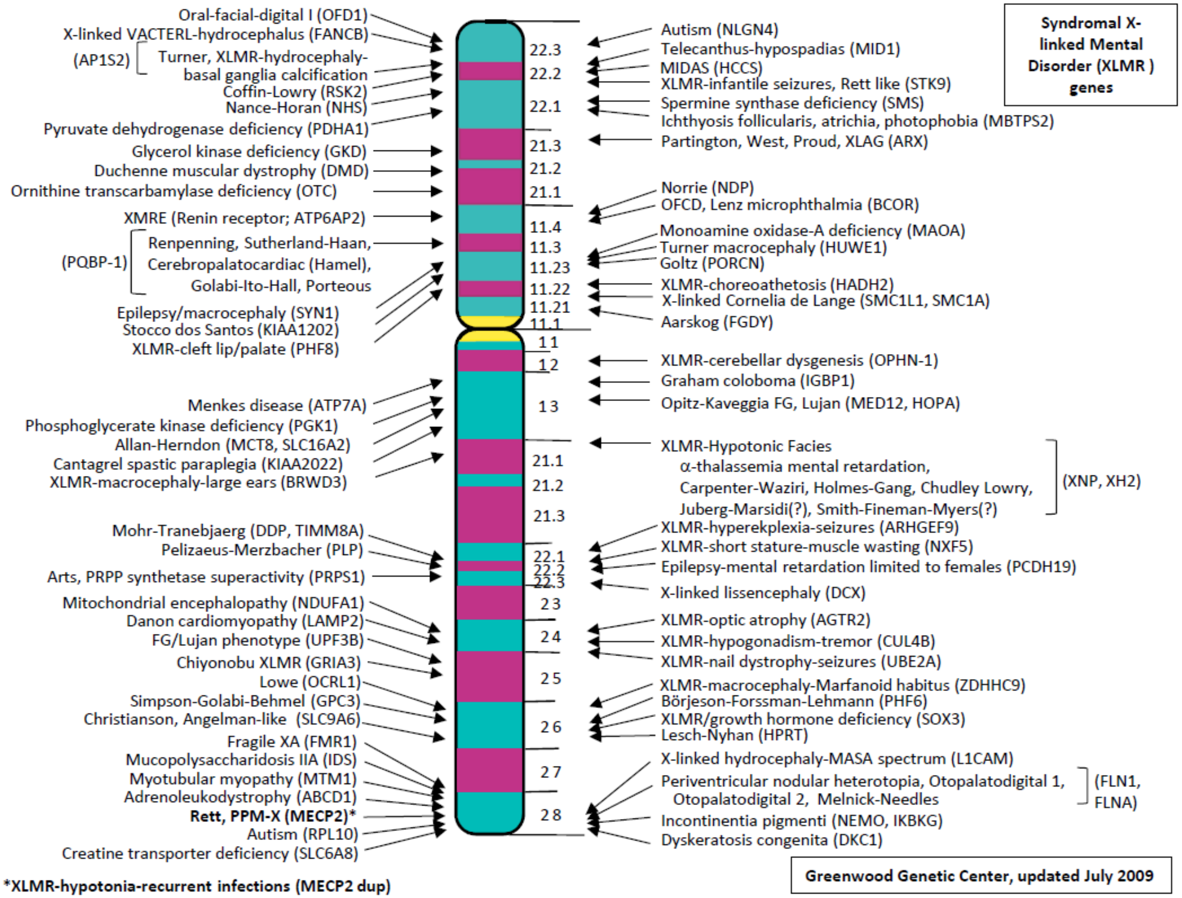
- ✓ **Global loss of TAD on the Xi <u>except in few regions (escaping genes)</u>**
- ✓ **The Xi is divided into 2 Megadomains separated by a boundary (DXZ4 macro satellite repeat)**

(Giorgetti et al, Nature, 2016)

Implication of X inactivation in human diseases

Severe phenotypes / lethality in men
Variable phenotypes / no phenotype in women

But escape from XCI can also gives rise to female-prone diseases …



Syndromal X-linked Mental Disorder (XLMR) genes

Oral-facial-digital I (OFD1)
X-linked VACTERL-hydrocephalus (FANCB)
(AP1S2) Turner, XLMR-hydrocephaly-basal ganglia calcification
Coffin-Lowry (RSK2)
Nance-Horan (NHS)
Pyruvate dehydrogenase deficiency (PDHA1)
Glycerol kinase deficiency (GKD)
Duchenne muscular dystrophy (DMD)
Ornithine transcarbamylase deficiency (OTC)
XMRE (Renin receptor; ATP6AP2)
(PQBP-1) Renpenning, Sutherland-Haan, Cerebropalatocardiac (Hamel), Golabi-Ito-Hall, Porteous
Epilepsy/macrocephaly (SYN1)
Stocco dos Santos (KIAA1202)
XLMR-cleft lip/palate (PHF8)
Menkes disease (ATP7A)
Phosphoglycerate kinase deficiency (PGK1)
Allan-Herndon (MCT8, SLC16A2)
Cantagrel spastic paraplegia (KIAA2022)
XLMR-macrocephaly-large ears (BRWD3)
Mohr-Tranebjaerg (DDP, TIMM8A)
Pelizaeus-Merzbacher (PLP)
Arts, PRPP synthetase superactivity (PRPS1)
Mitochondrial encephalopathy (NDUFA1)
Danon cardiomyopathy (LAMP2)
FG/Lujan phenotype (UPF3B)
Chiyonobu XLMR (GRIA3)
Lowe (OCRL1)
Simpson-Golabi-Behmel (GPC3)
Christianson, Angelman-like (SLC9A6)
Fragile XA (FMR1)
Mucopolysaccharidosis IIA (IDS)
Myotubular myopathy (MTM1)
Adrenoleukodystrophy (ABCD1)
Rett, PPM-X (MECP2)*
Autism (RPL10)
Creatine transporter deficiency (SLC6A8)

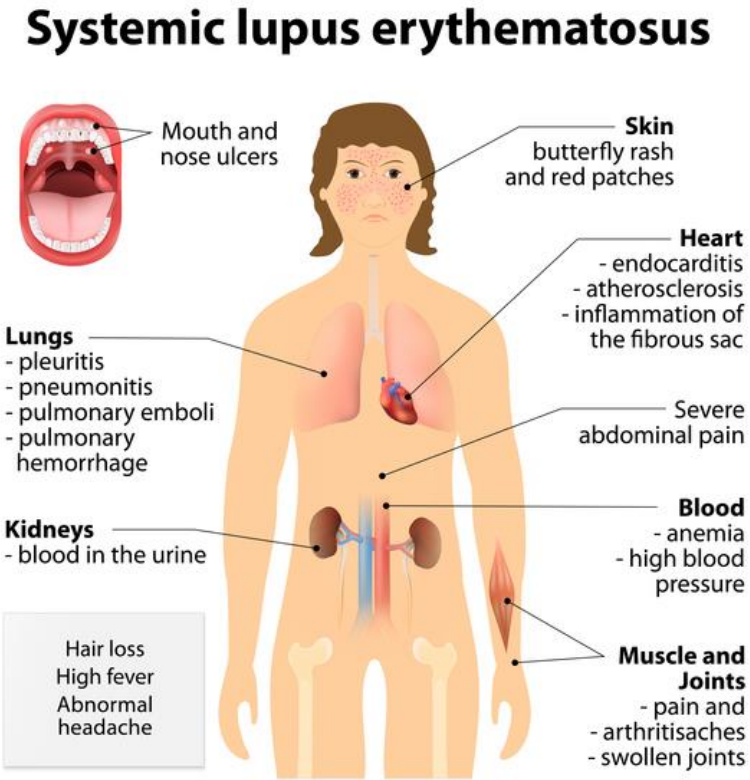
***XLMR-hypotonia-recurrent infections (MECP2 dup)**

Autism (NLGN4)
Telecanthus-hypospadias (MID1)
MIDAS (HCCS)
XLMR-infantile seizures, Rett like (STK9)
Spermine synthase deficiency (SMS)
Ichthyosis follicularis, atrichia, photophobia (MBTPS2)
Partington, West, Proud, XLAG (ARX)
Norrie (NDP)
OFCD, Lenz microphthalmia (BCOR)
Monoamine oxidase-A deficiency (MAOA)
Turner macrocephaly (HUWE1)
Goltz (PORCN)
XLMR-choreoathetosis (HADH2)
X-linked Cornelia de Lange (SMC1L1, SMC1A)
Aarskog (FGDY)
XLMR-cerebellar dysgenesis (OPHN-1)
Graham coloboma (IGBP1)
Opitz-Kaveggia FG, Lujan (MED12, HOPA)
XLMR-Hypotonic Facies, α-thalassemia mental retardation, Carpenter-Waziri, Holmes-Gang, Chudley Lowry, Juberg-Marsidi(?), Smith-Fineman-Myers(?) (XNP, XH2)
XLMR-hyperekplexia-seizures (ARHGEF9)
XLMR-short stature-muscle wasting (NXF5)
Epilepsy-mental retardation limited to females (PCDH19)
X-linked lissencephaly (DCX)
XLMR-optic atrophy (AGTR2)
XLMR-hypogonadism-tremor (CUL4B)
XLMR-nail dystrophy-seizures (UBE2A)
XLMR-macrocephaly-Marfanoid habitus (ZDHHC9)
Börjeson-Forssman-Lehmann (PHF6)
XLMR/growth hormone deficiency (SOX3)
Lesch-Nyhan (HPRT)
X-linked hydrocephaly-MASA spectrum (L1CAM)
Periventricular nodular heterotopia, Otopalatodigital 1, Otopalatodigital 2, Melnick-Needles (FLN1, FLNA)
Incontinentia pigmenti (NEMO, IKBKG)
Dyskeratosis congenita (DKC1)

Greenwood Genetic Center, updated July 2009

Sex bias in the immune compartment

- ✓ **Women are able to mount a more vigorous immune response to infections and to better respond to vaccination than men.**
- ✓ **However, women are more a risk for many autoimmune diseases, including Systemic Lupus Erythrematosus (SLE) and scleroderma.**



(From NIH Medline plus)

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and to better respond to vaccination than men.

✓ However, women are more a risk for many autoimmune diseases, including Systemic Lupus Erythrematosus (SLE) and scleroderma.

Sex hormones (estrogens) contribute to the sex bias in SLE...

✓ SLE incidence ~9 time higher in women after puberty - remission at menopause

✓ Estradiol exacerbates / ERa-deficiency in B cell attenuates disease in female lupus-prone mice

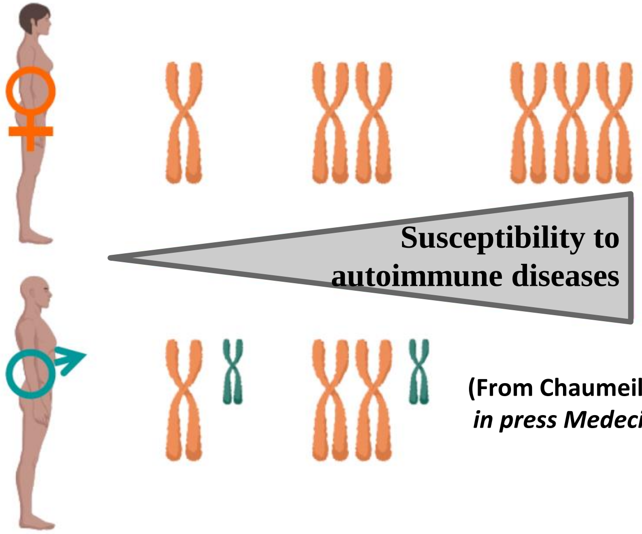
✓ Estrogens modulate immune cells (B cells, pDCs)

=> But not only…

... But the number of X chromosomes also contribute.

✓ Sex bias also observed before puberty (> 3/1)

✓ Similar SLE susceptibility in 47,XXY KS men (

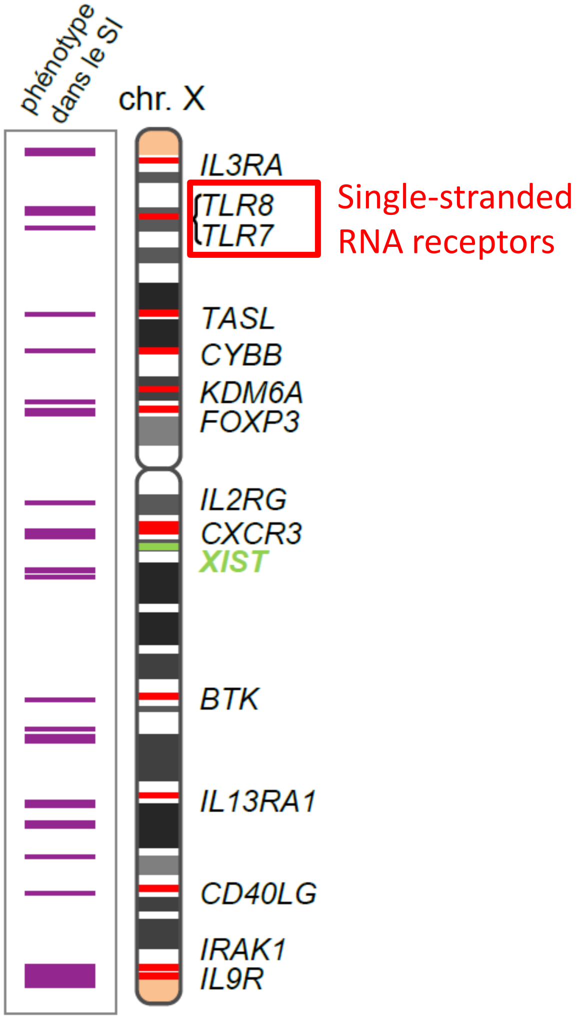


(From Chaumeil and Morey, *in press Medecine/Science*)

(Scofield et al., 2008; Seminog et al. , 2015; Harris et al., 2016; Liu et al., 2016…)

Sex bias in the immune compartment

- ✓ **Many genes involved in immune functions are located on the X chromosome**



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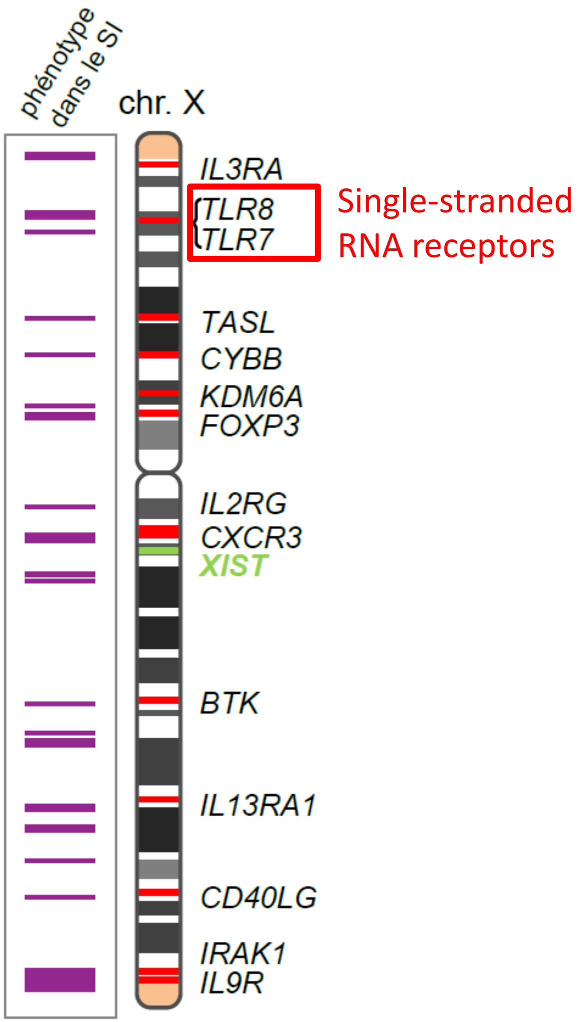

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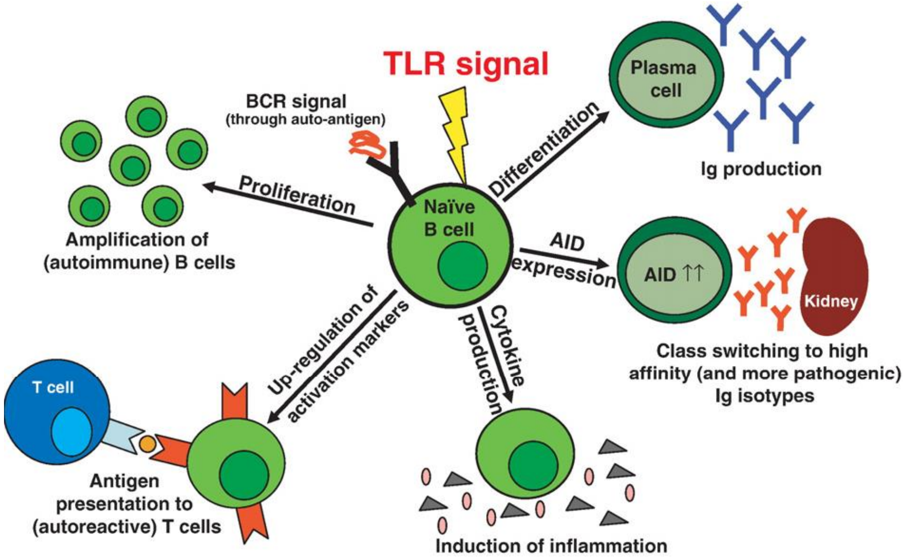


TLR7 and TLR8 **gene dosage plays a crucial role in autoimmunity :**

-> Expression of 2 copies of the *Tlr7* or *Tlr8* genes in mice,
-> **E**xpression of a gain of function mutant of human *TLR7* with enhanced signaling potential,
=> enough to induce full blown autoimmunity

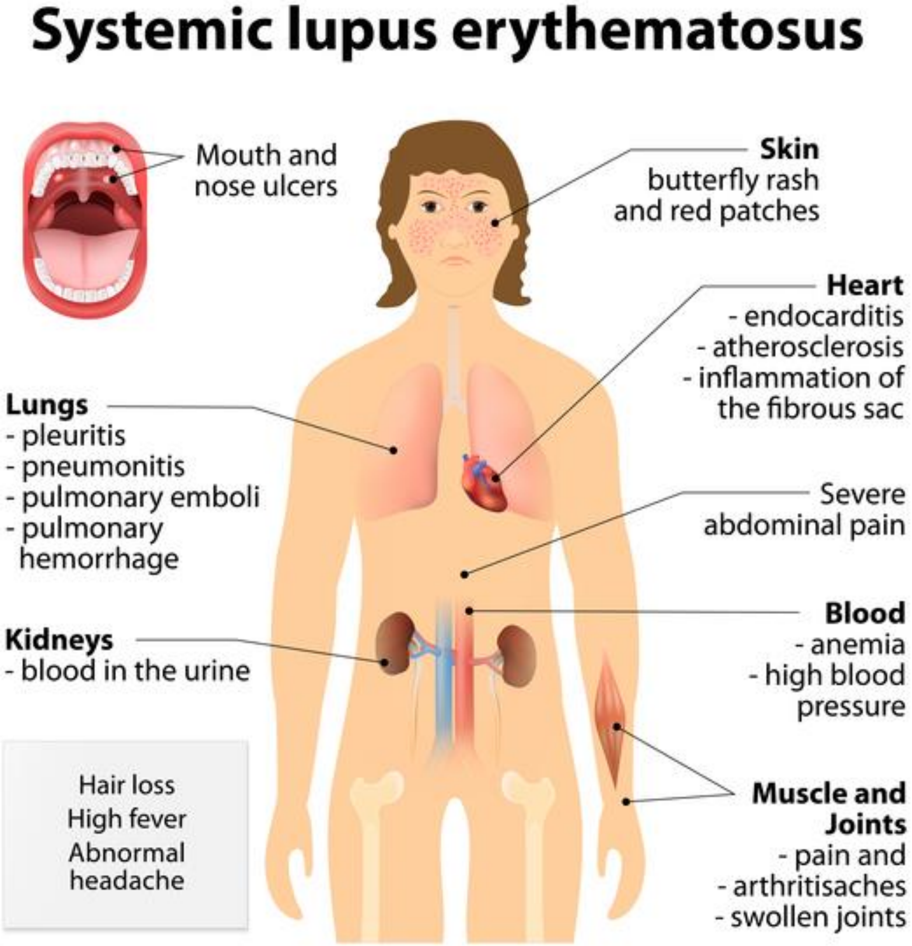
=> Incomplete X inactivation in the immune compartment?

(Christensen et al, 2006; Pisitkun et al, 2006; Deane et al, 2007; Walsh et al, 2012; Guiducci et al, 2013; Jackson et al., 2014; Soni et al, 2014; Brown et al., 2022)

Most autoimmune diseases occur in women

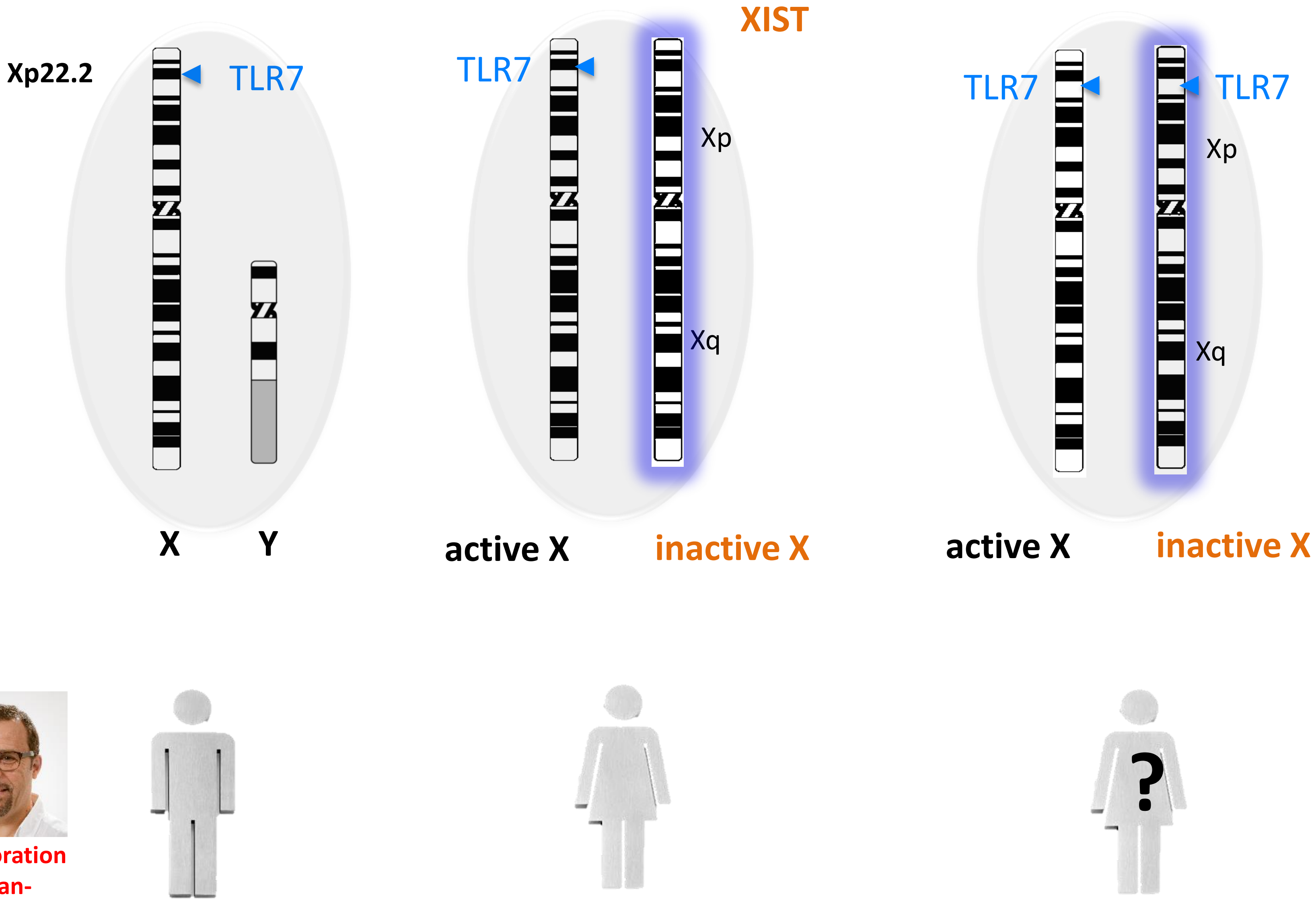
SLE: Systemic Lupus Erythematosus

- ✓ Red rash (face)
- ✓ Swollen joints and lymph nodes
- ✓ Ulcers (mouth, nose)
- ✓ Chest pain
- ✓ Anemia
- ✓ Fever
- ✓ Hair loss



Collaboration with Jean-Charles Guéry (Toulouse)

Does *TLR7* escape from X chromosome inactivation?

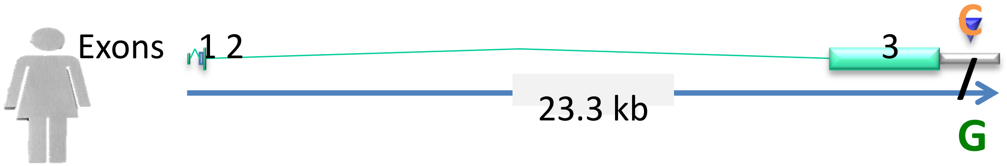


Collaboration with Jean-Charles Guéry (Toulouse)

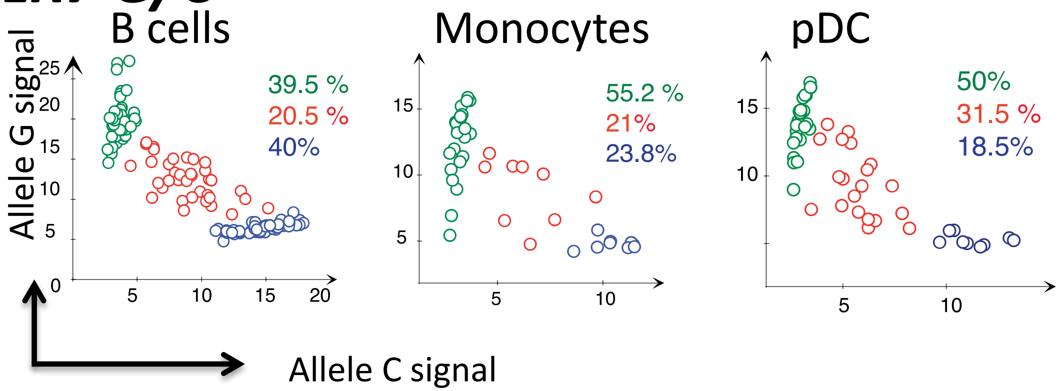
Souyris M, …, Chaumeil J, Mejia, Guéry. *Science immunol.* 2018

TLR7 escapes XCI in all immune cells of all tested donors

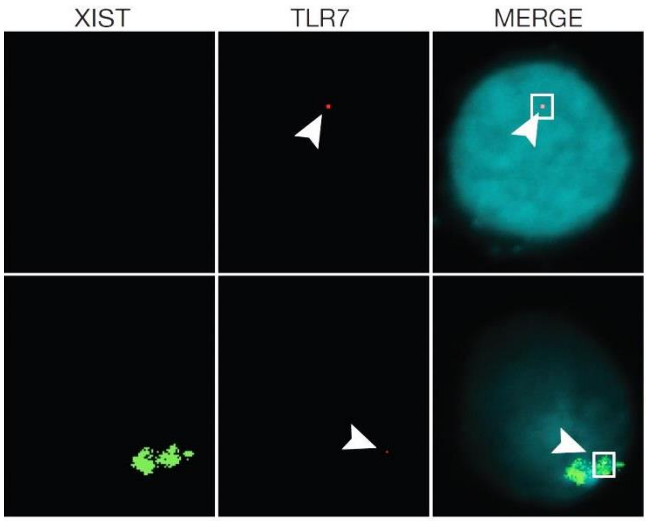
TLR7 single cell allelic expression analysis (KASP-PCR)

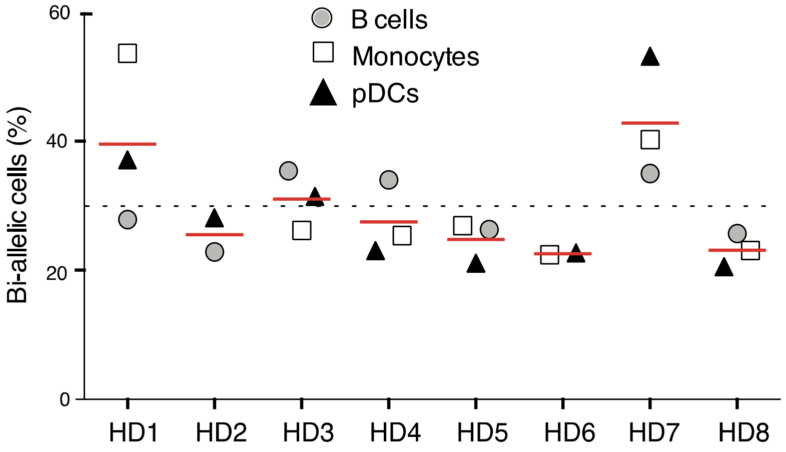


TLR7 G/C



RNA FISH

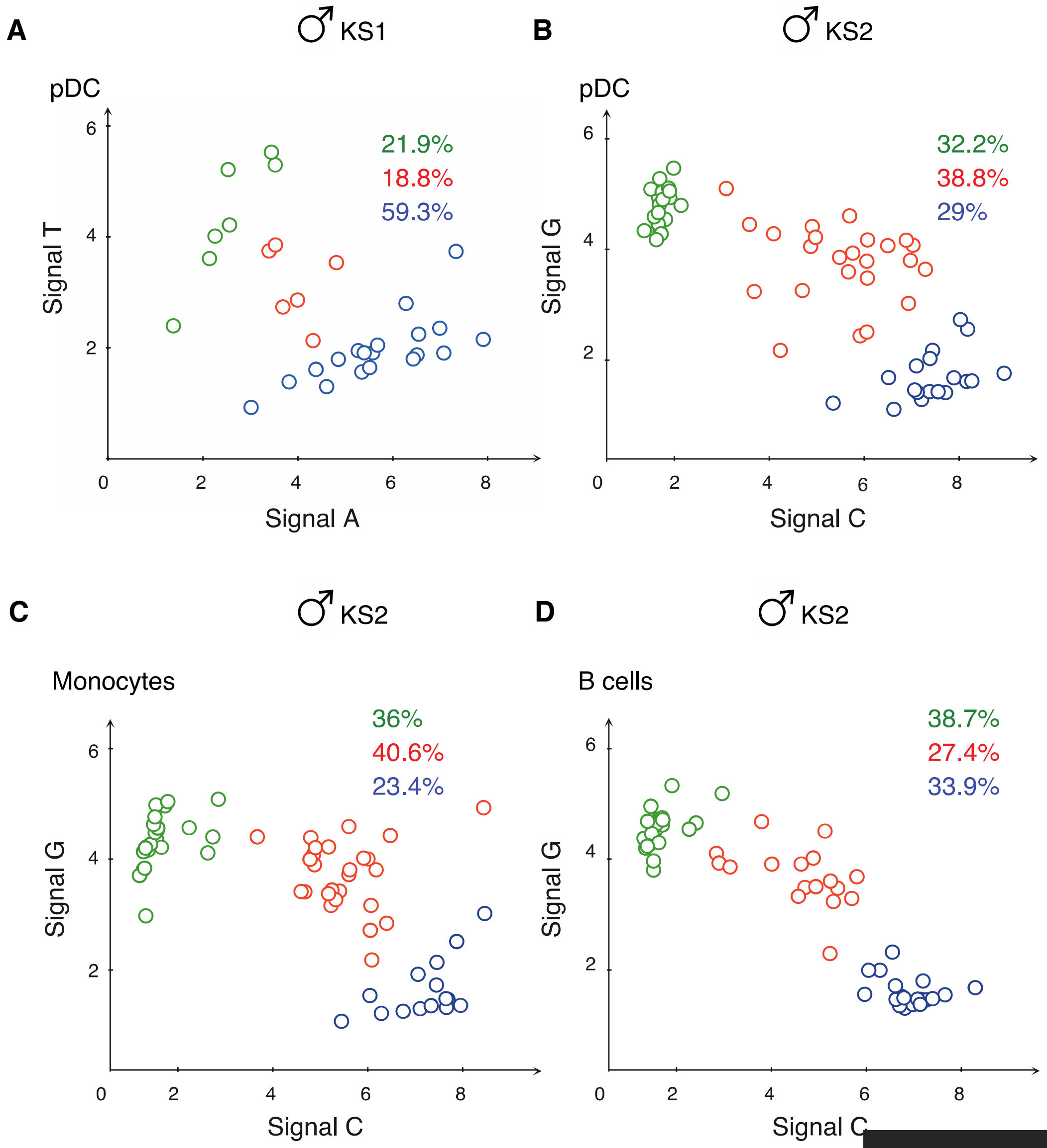




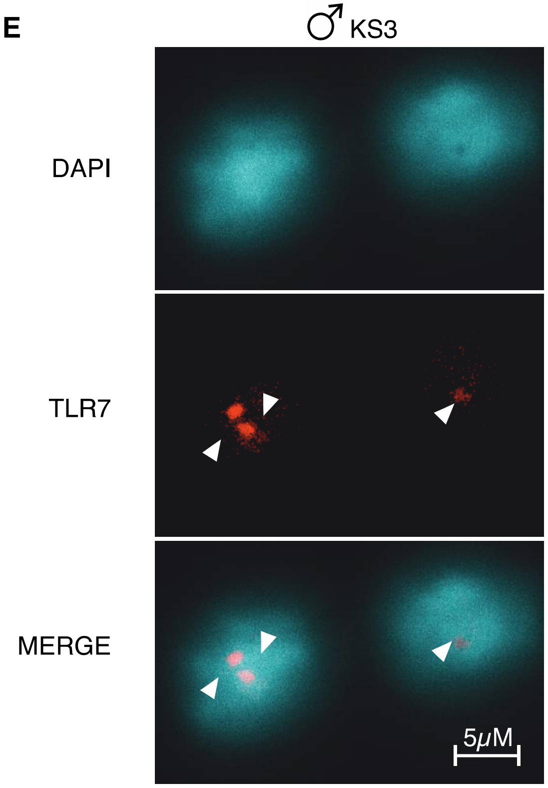
***TLR7* escapes X chromosome inactivation in immune cells**

Souyris M, …, Chaumeil J, Mejia, Guéry. *Science immunol.* 2018

TLR7 escapes XCI in males with Klinefelter's syndrome

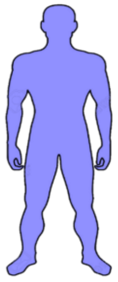


B cells stim TLR7/IFNß



% TLR7 biallelic cells

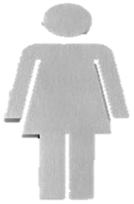
KS#3: 58%
KS#4: 43%



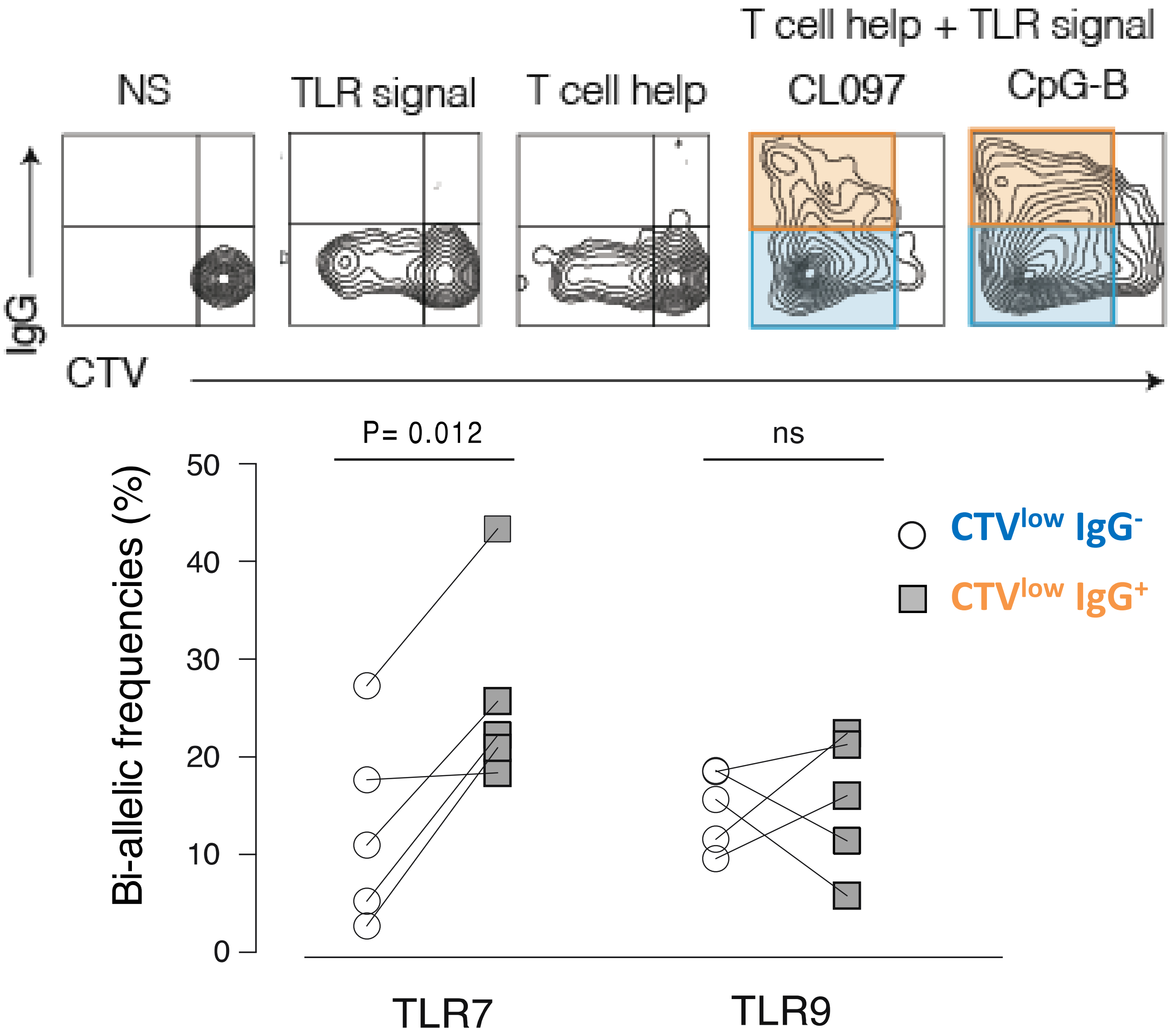
XXY

Souyris M, …, **Chaumeil J**, Mejia, Guéry. *Science immunol.* 2018

Bi-allelic naïve B cells have an advantage to differentiate into IgG+ plasma cells in presence of TLR7 ligand



TLR7 G/C

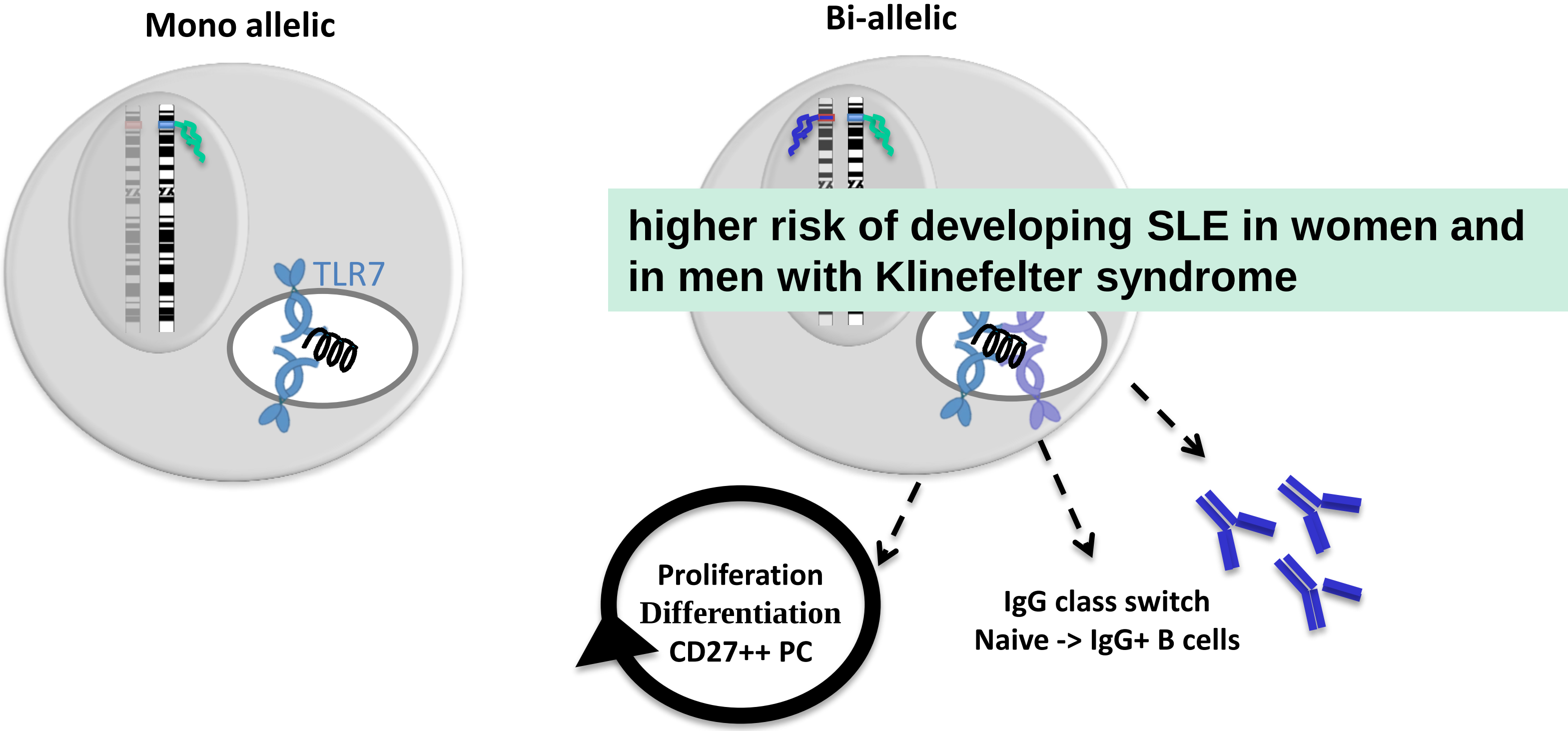


Souyris M, …, Chaumeil J, Mejia, Guéry. *Science immunol.* 2018

Summary

- *TLR7* escapes from X inactivation:
  - in all immune cells tested (pDC, B cells and monocytes)
  - in women and in 47,XXY Klinefelter men

- *TLR7* escape from X inactivation endows the B cell compartment with added responsiveness to TLR7 ligands.



higher risk of developing SLE in women and in men with Klinefelter syndrome

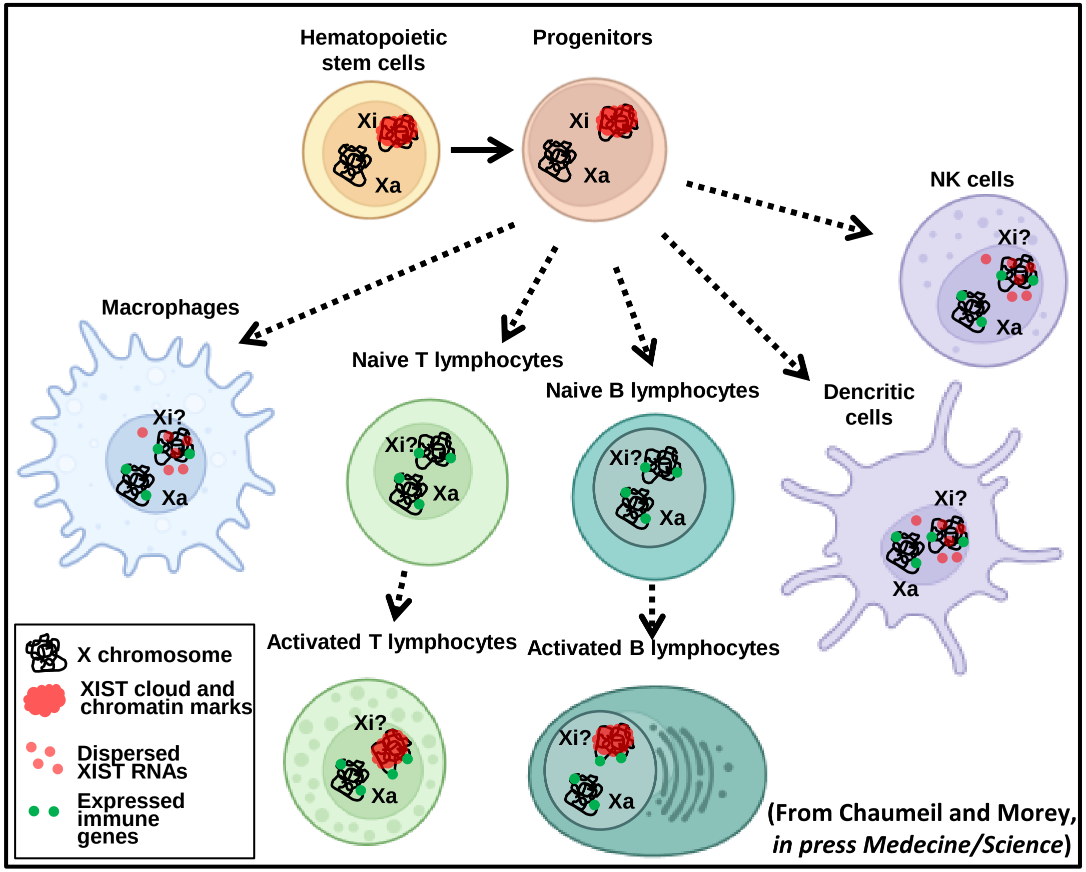
Souyris M, ..., Chaumeil J, Mejia, Guéry. *Science immunol.* 2018

Specific features of X inactivation in the immune compartment?

⇒ Erosion of the canonical Xi features in the hematopoietic / immune compartment:

- Loss of consistant *XIST* RNA cloud
- Loss of repressive histone marks (H3K27me3, H2AK119ub…)
- Change in the XIST partners (B-cell specific cofactor TRIM28)

(Work from the Chang lab: Yu et al, Cell, 2021 / Work from the Anguera lab: for reviews Sierra & Anguera, 2019; Jiwrajka & Anguera, 2023; Chaumeil & Morey, in press)



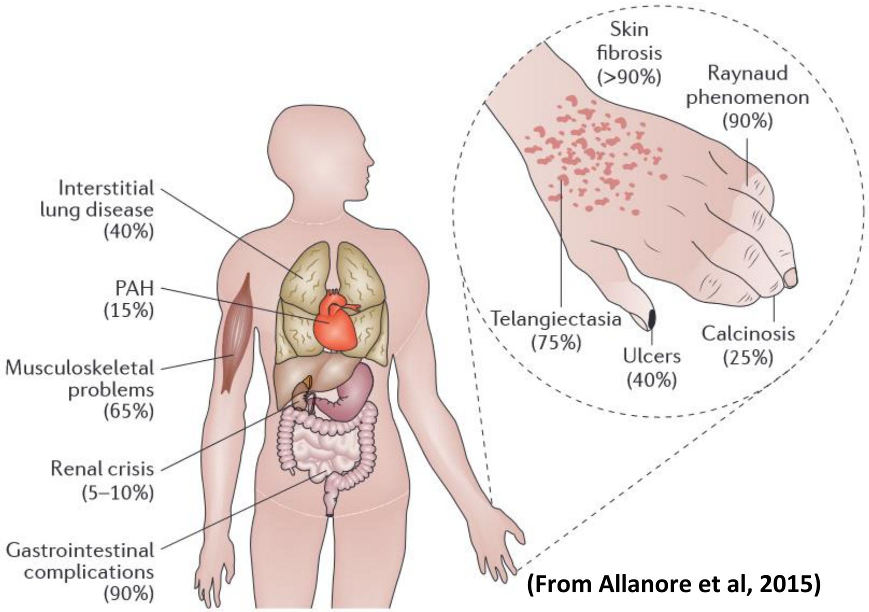
(From Chaumeil and Morey, *in press Medecine/Science*)

⇒ Reduced expression of *Xist* in *Ftx*-deficient mice:

- Reactivation of X-linked genes (including *Tlr7*)
- Development of inflammatory signs typical of lupus (autoantibodies, ↗ age-associated and GC B cells, ↗ macrophages and DC)

(Work from the Rougeulle lab: Huret et al, 2024)

TLR7/8, X chromosome inactivation and systemic sclerosis



(From Allanore et al, 2015)

Systemic sclerosis (SSc):

- elevated autoantibody production,
- vasculopathy and fibrosis of the skin and internal organs
- high morbidity and mortality
- female predominance of about 4:1
- X-linked TLR7 and TLR8 can induce type I IFN by plasmacytoid Dendritic Cells from SSc patients which can promote fibrosis.

- Less pDCs in the circulation in SSc patients but infiltrate fibrotic skin
- Chronic activation of SSc and SLE pDCs linked to the dysregulation of the metabolic response
- *TLR8* ectopically expressed in SSc pDCs and induces IFN-I.

⇒ Heterogeneity of the pDC subsets in patients with SSc?

⇒ Escape of *TLR7* and *TLR8* from XCI? Erosion of XCI?

Guéry and Barrat labs

(Du et al, in revision JEM)

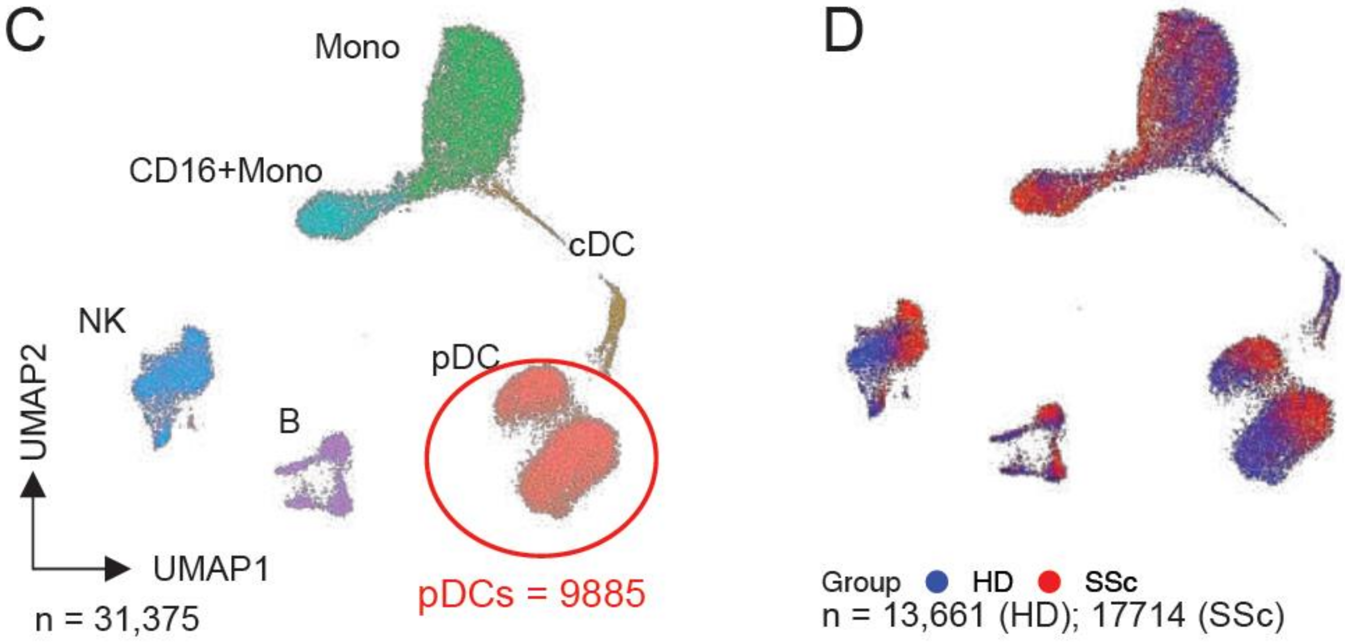
TLR7/8, X chromosome inactivation and systemic sclerosis

Diversity of pDCs and contribution to the IFN signature and pathogenesis of SSc?

-> scRNA-seq analysis on PBMC from 4 female SSc patients and 4 female HDs:

31,375 cells: 13,661 HD and 17,714 SSc cells / 9,885 pDCs + monocytes, B cells, NK cells

⇒ **Most different cell types: NK cells and pDCs**



(Du et al, in revision JEM)

TLR7/8, X chromosome inactivation and systemic sclerosis

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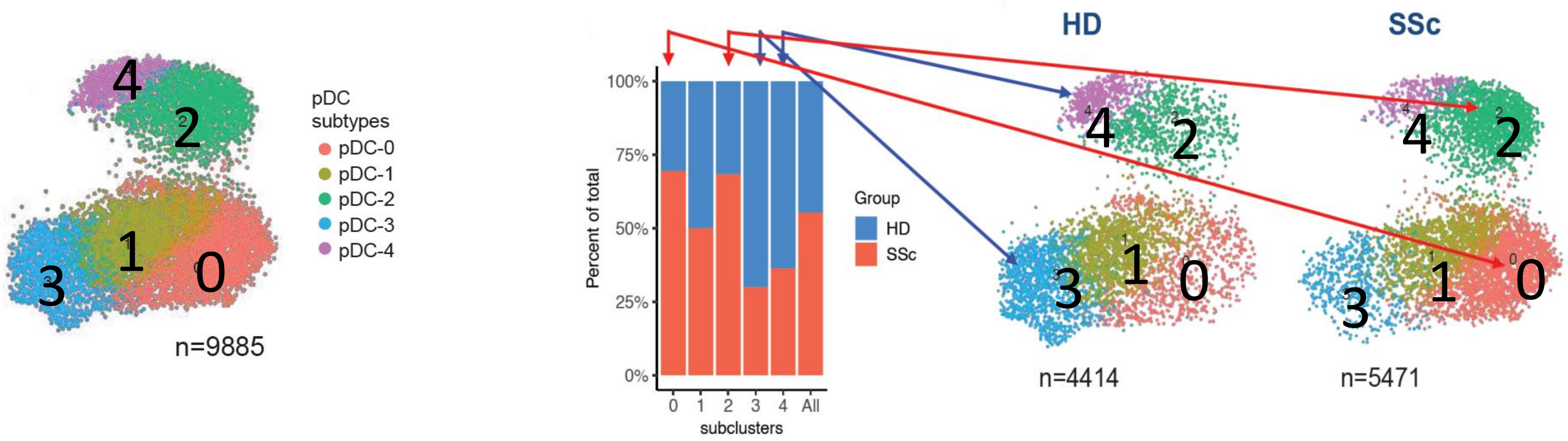
-> Sub-cluster analysis to further investigate differences between SSc versus HD pDCs

⇒ UMAP plot of 9,885 pDCs: 5 sub-clusters (pDC-0 to -4)

⇒ Unbalanced distribution: pDC-0/-2 mostly in SSc - pDC-3/-4 mostly in HD



(Du et al, in revision JEM)

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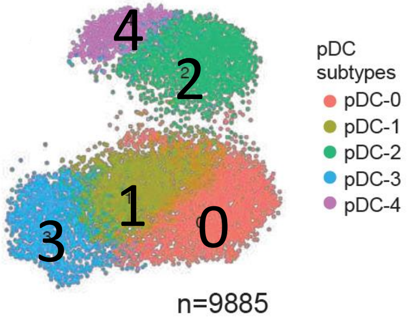

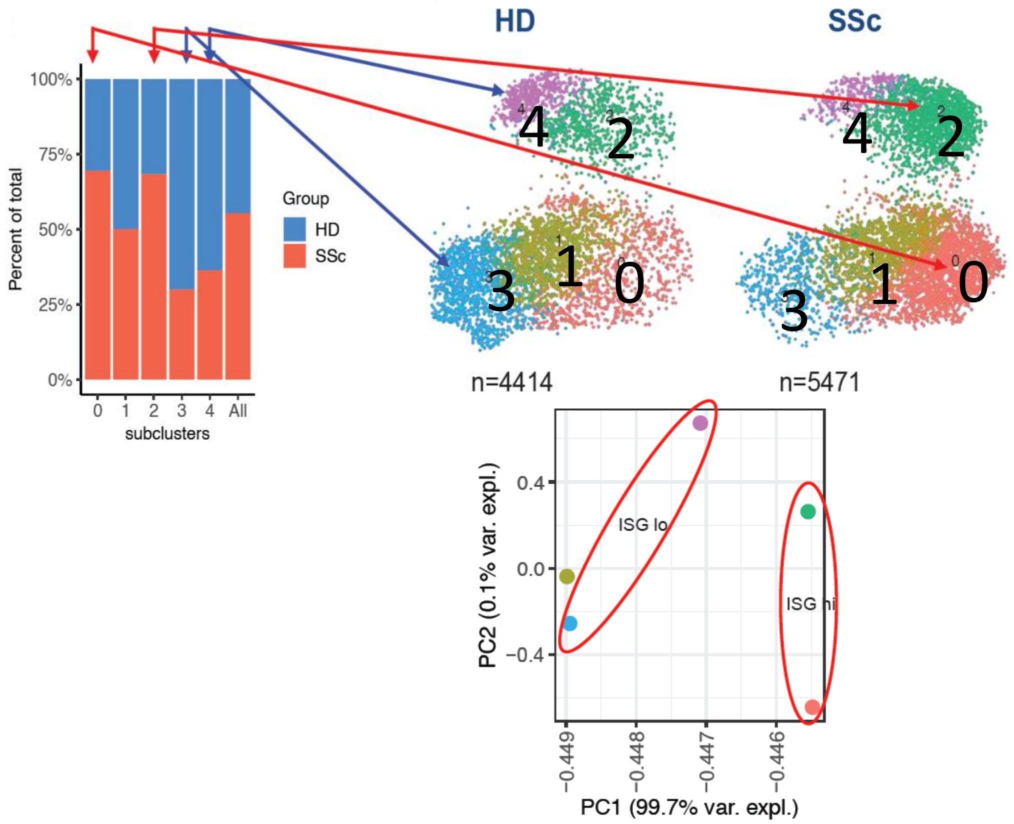

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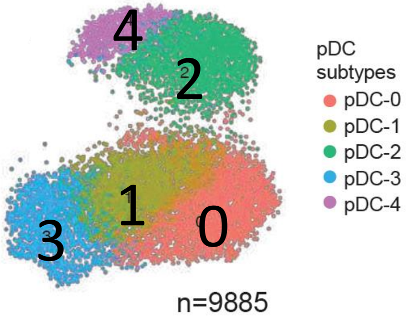
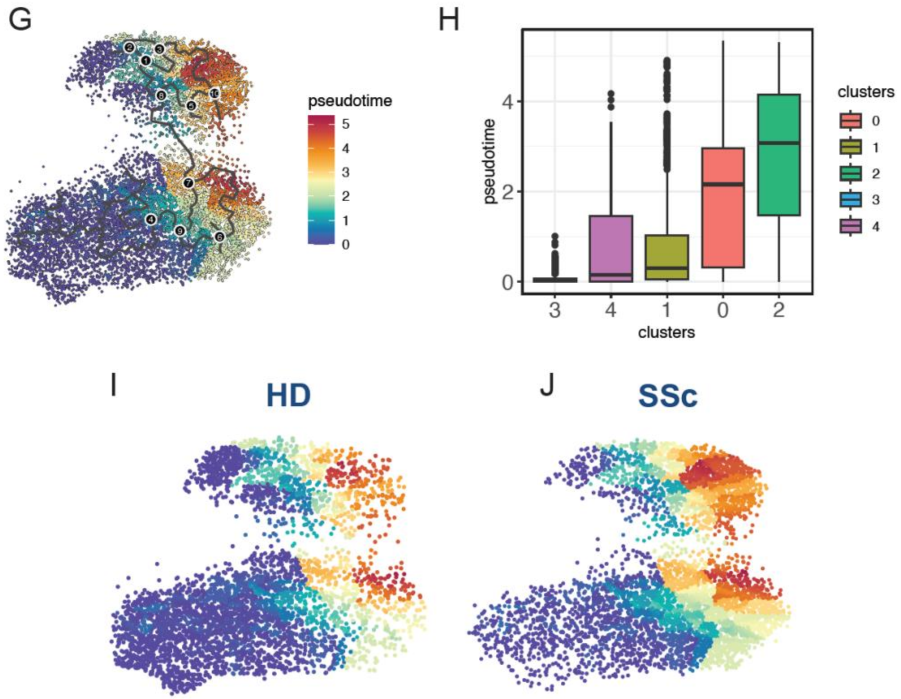
- ⇒ Mapping of IFN-I stimulated genes (ISGs) : pDC-0 and pDC-2 associated with high IFN-I response
- ⇒ Trajectory analysis: pDC-3 - pDC-4 - pDC-1 -> naive cells / pDC-0 - pDC-2 -> activated cells

=> Activated/differentiated cells are enriched in SSc patients





(Du et al, in revision JEM)

TLR7/8, X chromosome inactivation and systemic sclerosis

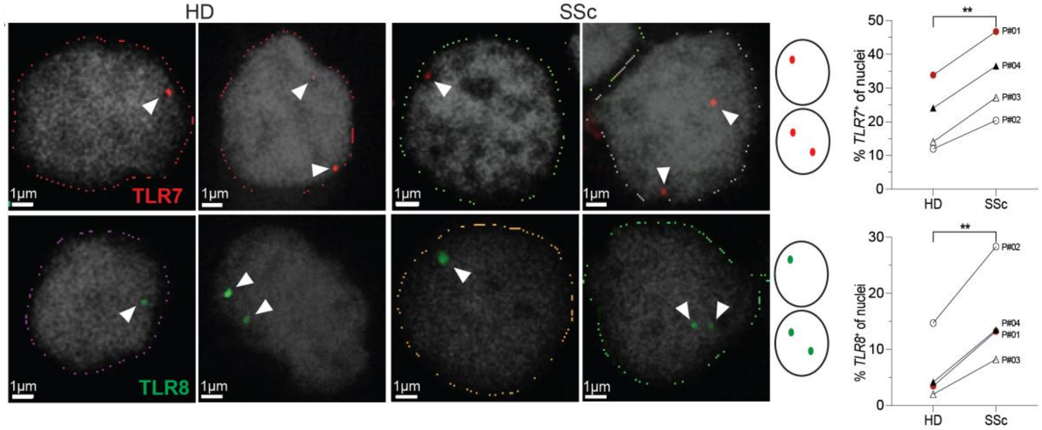
Aberrant expression of the TLR7/8 locus from the inactive X may occur in primary SSc pDCs?

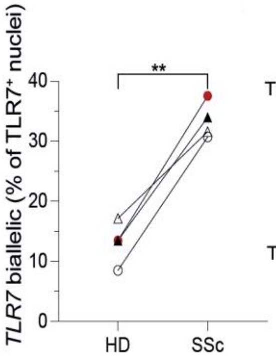
-> RNA FISH, single-cell RT-qPCR

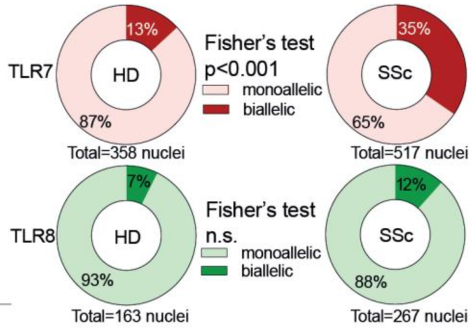
⇒ *TLR7* and *TLR8* RNA signals ↗ in SSc pDCs than in age-matched HD

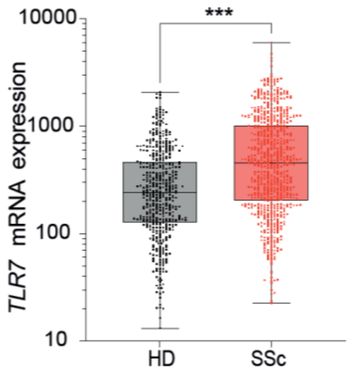
⇒ *TLR7* biallelic RNA signals ↗ in SSc pDCs (slight increase also for *TLR8*)

⇒ *TLR7* mRNA transcripts ↗ in SSc pDCs (1.85-fold increase)









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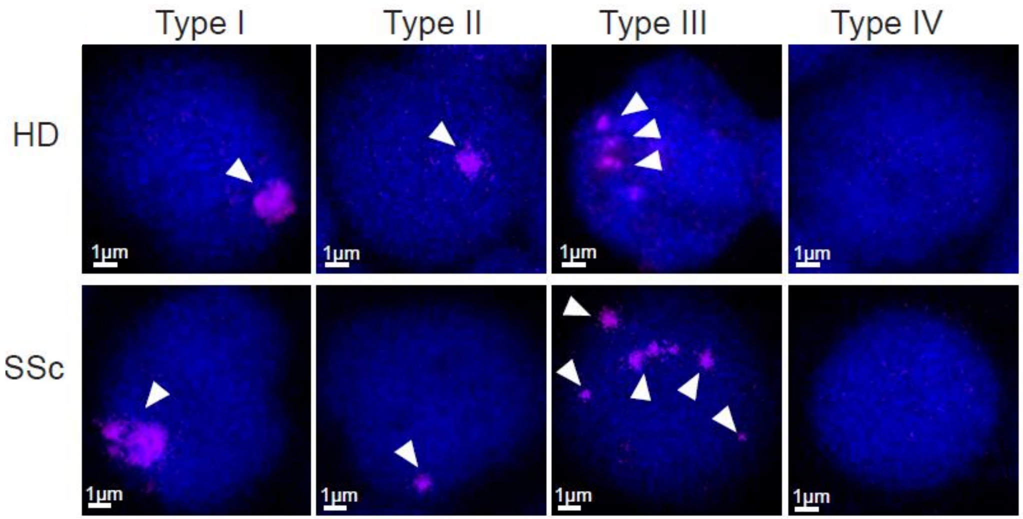
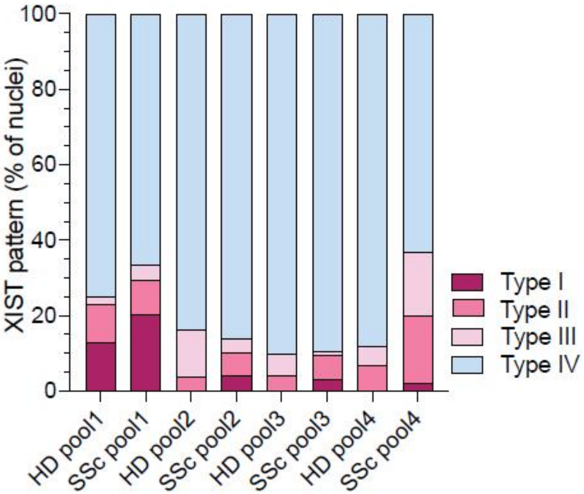
⇒ *TLR7* biallelic RNA signals ↗ in SSc pDCs (slight increase also for *TLR8*)
⇒ *TLR7* mRNA transcripts ↗ in SSc pDCs (1.85-fold increase)
⇒ Most of pDCs lack robust *XIST* RNA cloud (types I and II)





(Du et al, in revision JEM)

TLR7/8, X chromosome inactivation and systemic sclerosis

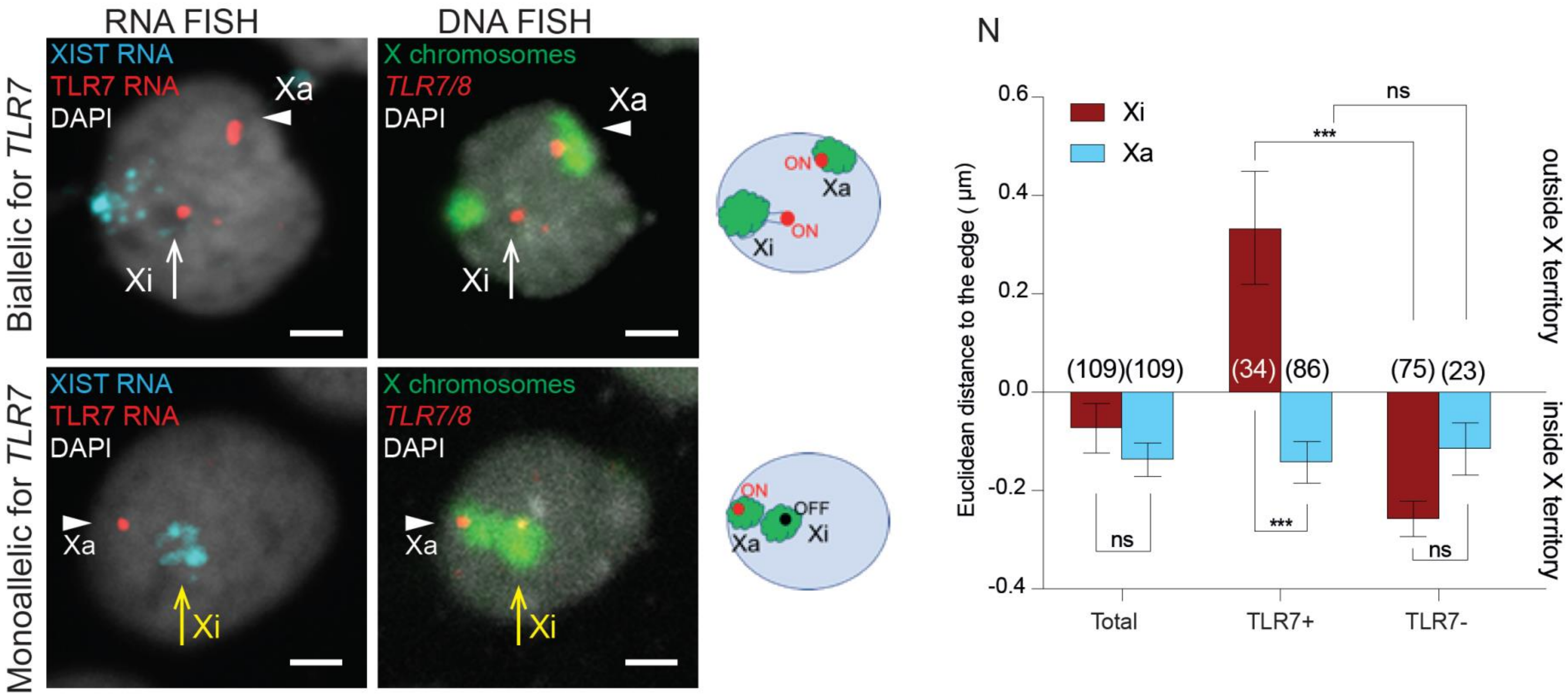
Organization of the inactive X chromosome territory and localization of the TLR7/8 region?

-> Sequential RNA / DNA FISH

⇒ On the active X: *TLR7/8* tends to be located inside the X territory, regarless of *TLR7* expression

⇒ On the inactive X:

- When *TLR7* is silenced: *TLR7/8* tends to be located inside of the X territory
- When TLR7 is expressed: *TLR7/8* tends to be located <u>outside</u> of the X territory



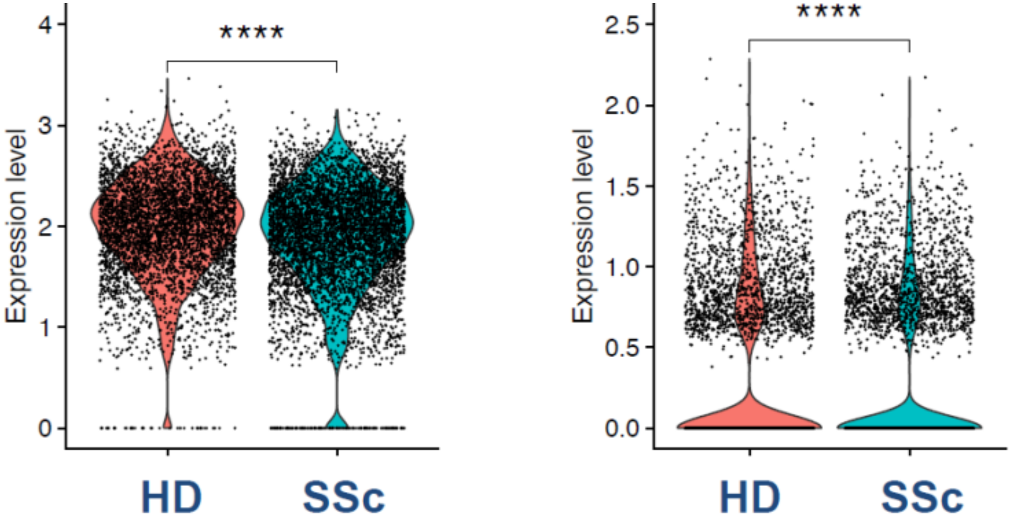
=> Expression of *TLR7* from the inactive X correlates with a chromatin looping of the *TLR7/8* genomic region outside the Xi chromosome territory: may increase the accessibility to the transcription machinery.

(Du et al, in revision JEM)

TLR7/8, X chromosome inactivation and systemic sclerosis

Presence of IFN-I-associated pDC clusters + defect in XCI at the TLR7/8 locus => mechanism involved ?
-> sc RNA-seq

⇒ ↘ *XIST* expression in SSs pDCs
⇒ ↘ *SPEN* expression in SSs pDCs

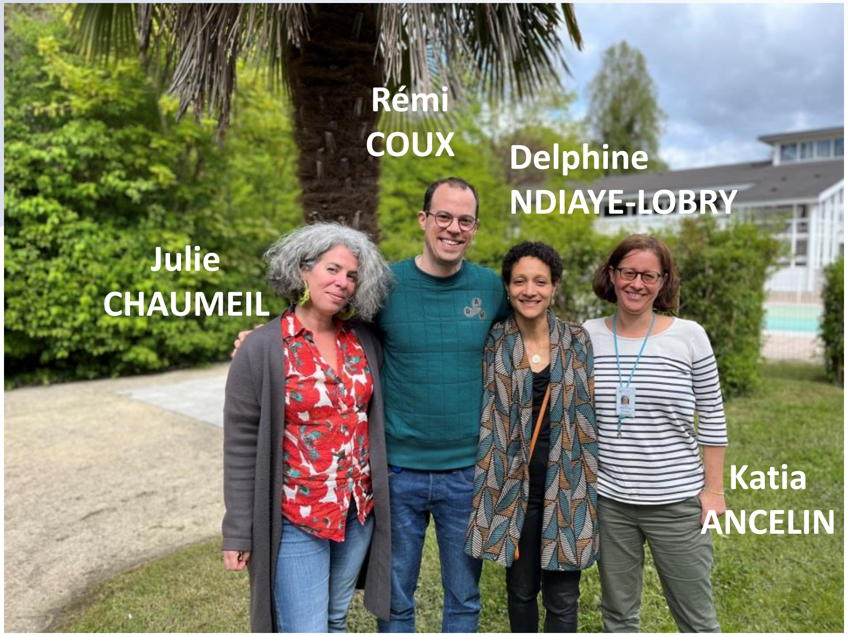


=> Enrichment of pDCs with high expression of IFN-I stimulate genes, with increased *TLR7* and *TLR8* expression and with increased presence of *TLR7*-biallelic cells is related to the dysregulation of key players of the XCI machinery in SSc patients.

⇒ Heterogeneity of pDCs is associated with altered XCI of the *TLR7/8* locus in SSc patients

⇒ Mechanisms of *TLR7* escape from XCI?

(Du et al, in revision JEM)



Collaborations:

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- Dr C. LOBRY *(IGR, Villejuif)*
- Dr T. MERCHER *(IGR, Villejuif)*
- Dr J-P. de VILLARTAY *(Imagine, Paris)*
- Dr R. DI MICCO *(Milan, Italy)*