CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2017-2018 :

"Le chromosome X -
paradigme de la génétique et l'épigénetique"

19 février, 2018

Cours III (Fin)

Dynamique de l'hétérochromatine facultative

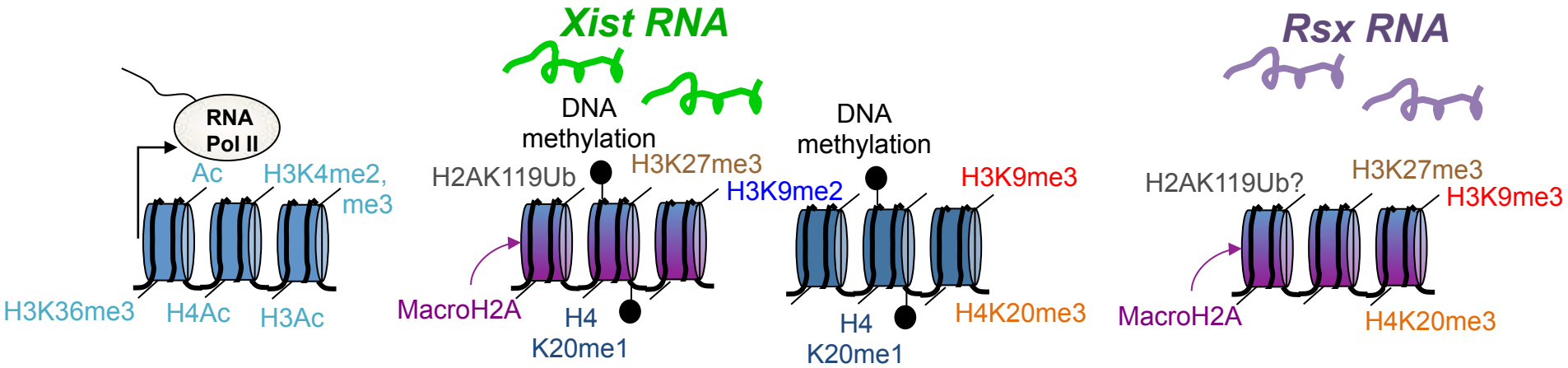
Dynamics of facultative heterochromatin

Cours IV

Les troubles neurologiques liés au chromosome X

Summary of Xi status in Somatic Cells of Mice, Humans and Marsupials

Diversity in heterochromatin marks: facultative heterochromatin is a *means to an end:* to compensate for X-linked gene dose



Active X chromatin

Inactive X chromatin
Barr body
Late replicating

Inactive X chromatin
Barr body
Late replicating

MOUSE

HUMAN

MARSUPIAL

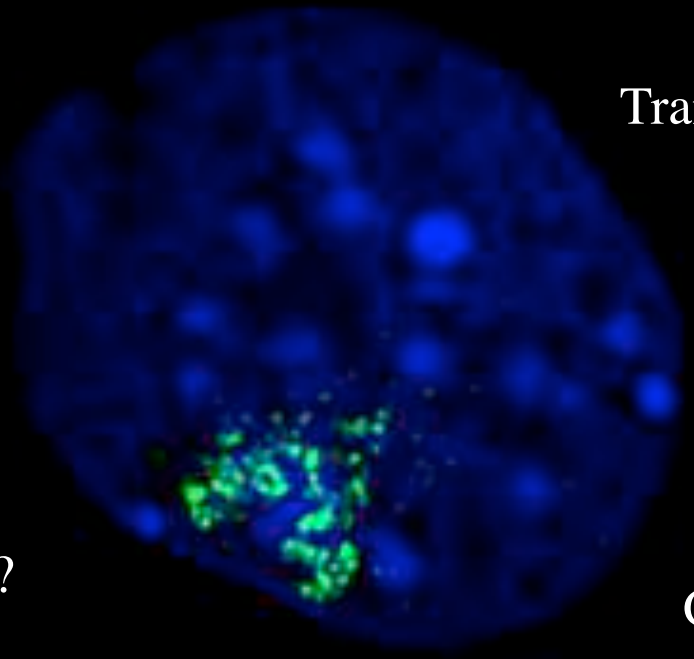
How is Facultative Heterochromatin established? How does XIST work?

(X-Inactive-Specific-Transcript)

Scaffold for repressor recruitment?

Transcriptional interference?

RNA-DNA binding?



Post-transcriptional interference?

Nuclear compartmentalisation ?

Chromatin changes ?

Chromosome 3D organisation

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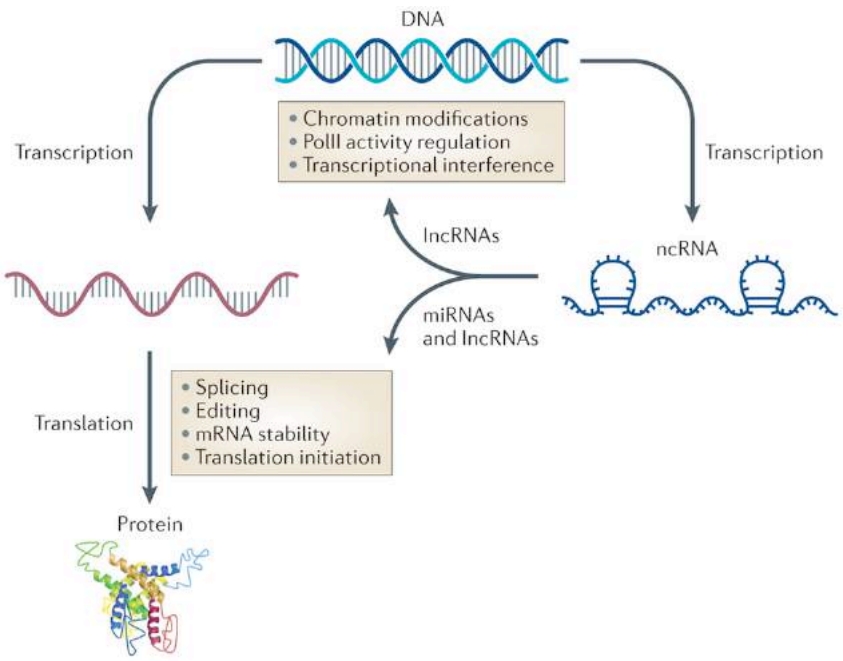
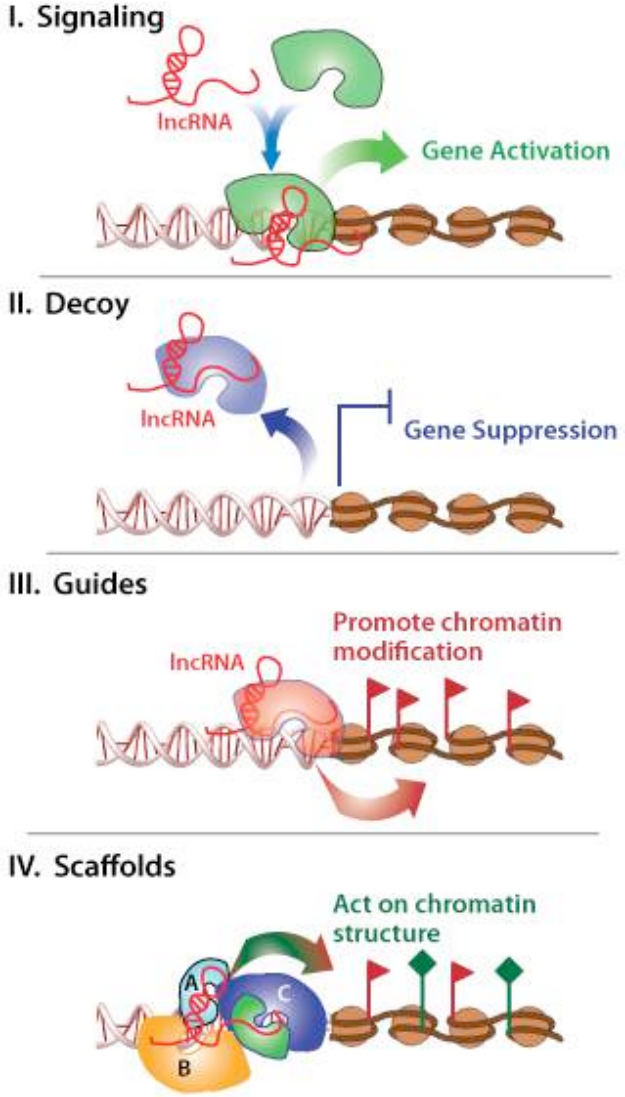
Long non-coding RNAs:
from spurious transcription to functional entities



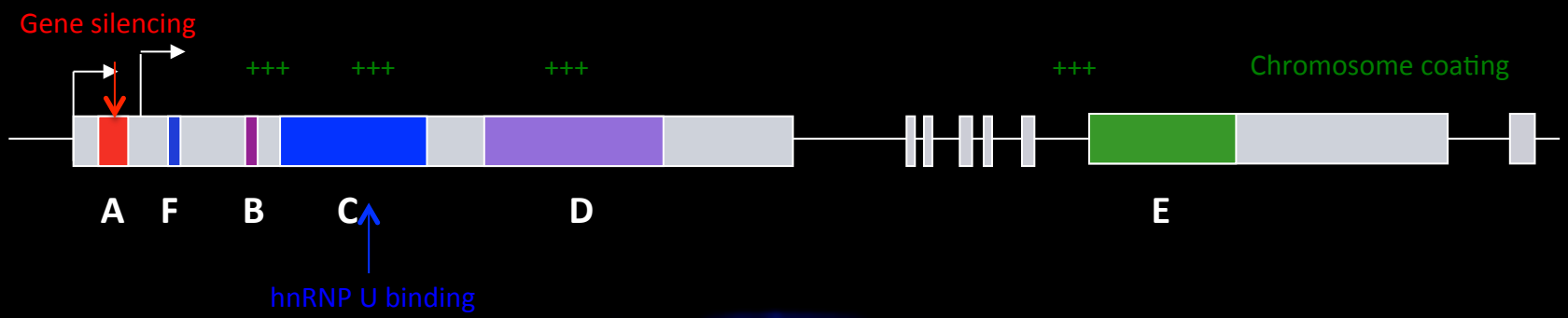
Claes Wahlestedt, 2013

Nature Reviews | Drug Discovery



Adapted from: Wang, KC and Chang HY, Molecular Mechanisms of Long Noncoding RNAs. Mol Cell. 2011 Sep 16;43(6):904-14.

XIST RNA: A Multi-Tasking Molecule

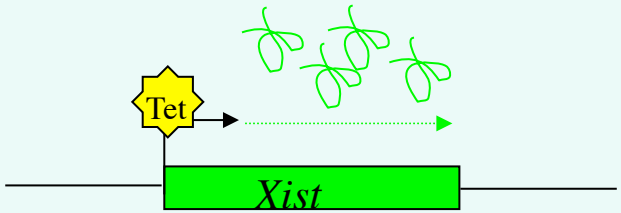


- Poor sequence conservation between mammals - except for repeats A-F
- 17 000 - 19 000 nt, spliced, untranslated, nuclear transcript
- RNA expressed from and "coats" the inactive X chromosome in *cis* (not *trans*)
- *Xist* is **essential** for X inactivation in *cis* (KOs, transgenes in mouse embryos, ES cells)
- Xist can only induce silencing during an early developmental time window
- Xist binds broadly across the X chromosome, exploiting 3D structure for initial binding
- Estimated ~2000 molecules of Xist RNA per nucleus
- Conserved "A" repeats ensure silencing function
- Multiple Xist domains required for coating including C repeats
- Xist RNA reported to recruit chromatin factors eg Polycomb group proteins, macroH2A

When does Xist trigger chromosome-wide silencing?

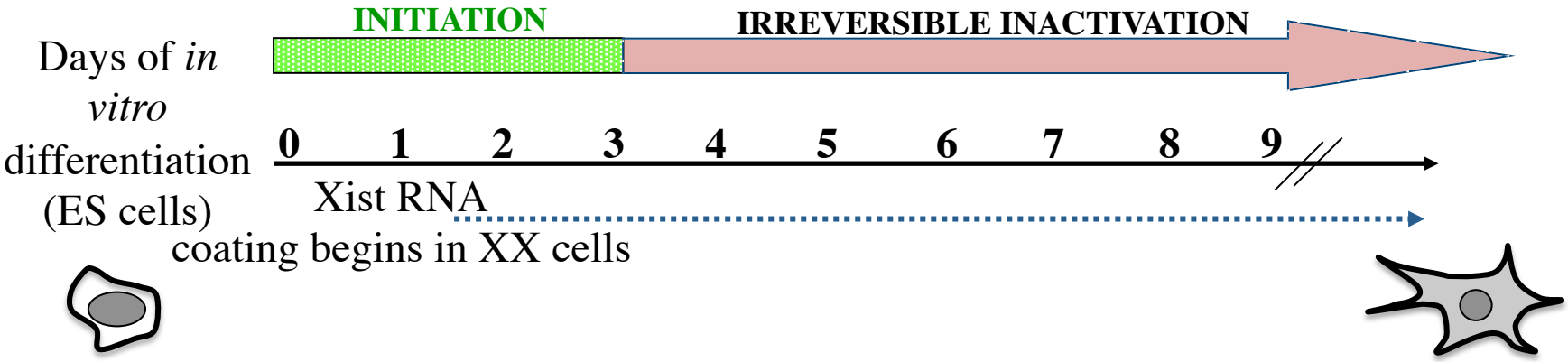
Xist RNA as a trigger for XCI

Inducible *Xist* cDNA :



Cellular Memory?
Epigenetic Marks?

Xist-dependent → Xist-independent

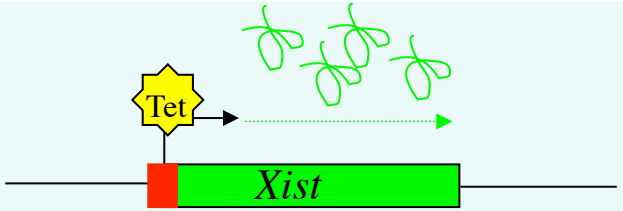


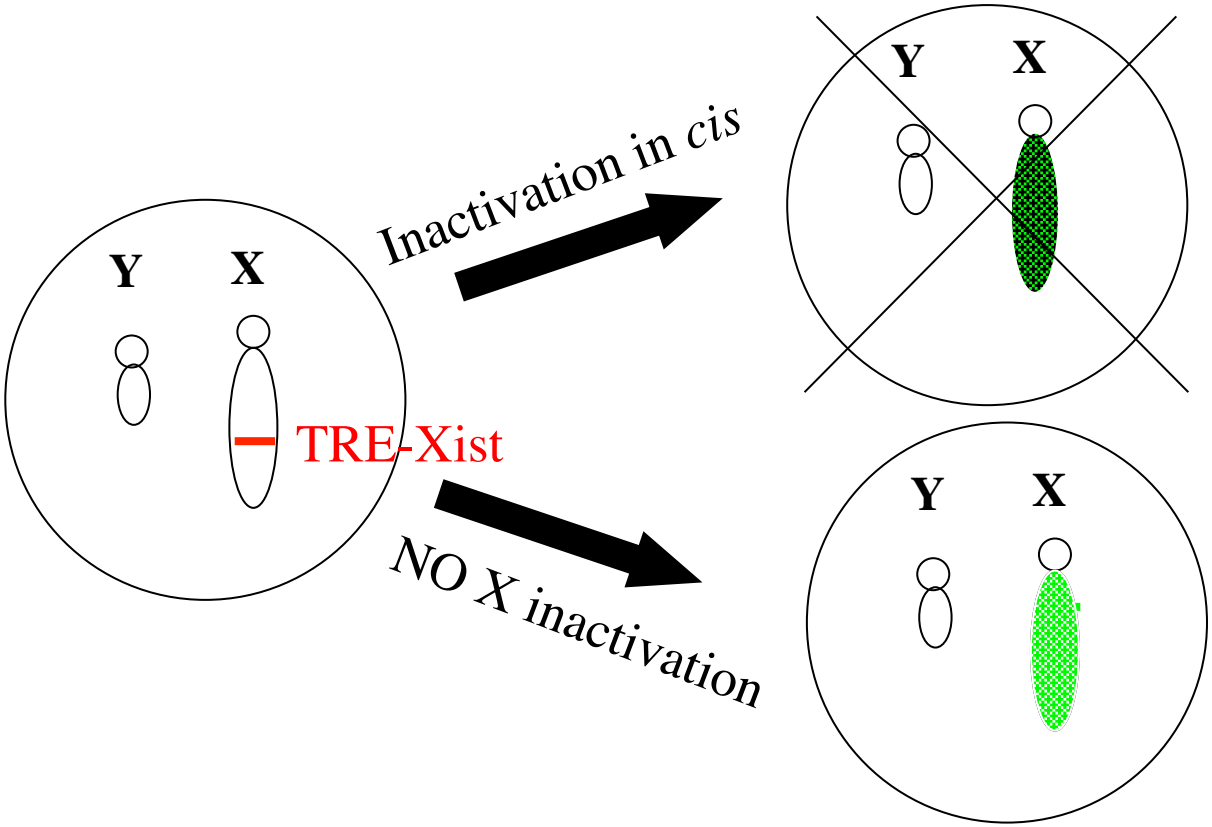
Transcriptional silencing begins
(*Northerns, allele-specific RT PCR, RNA FISH*)

Wutz et al Cell 2001, Nat. Genet. 2002

What are the Functional Domains of Xist RNA?

Inducible *Xist* cDNA :
(Wutz and Jaenisch, 2001)





Functional nullisomy for the X chromosome

↓

Cell death

Gene activity

↓

Survival

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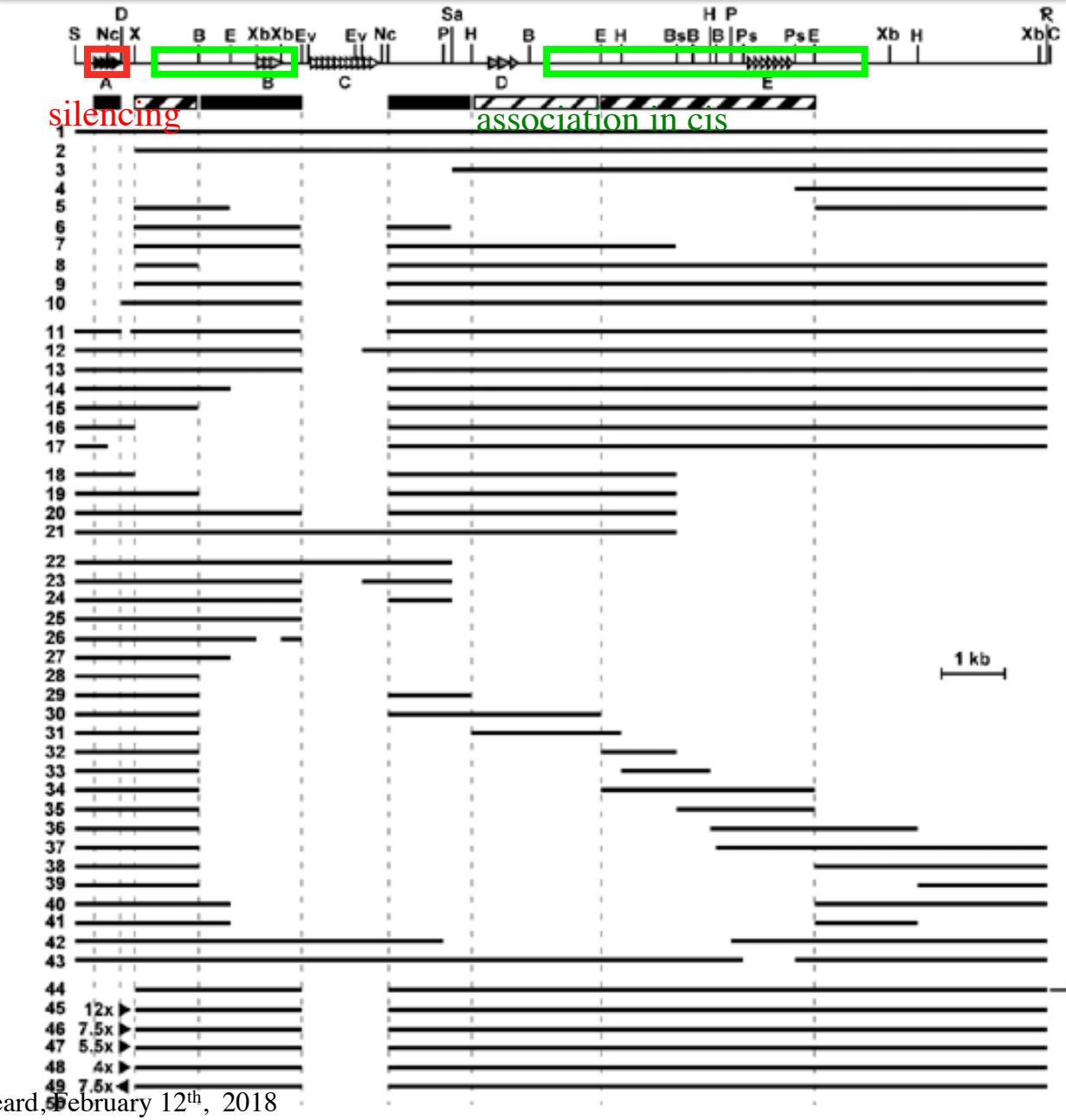
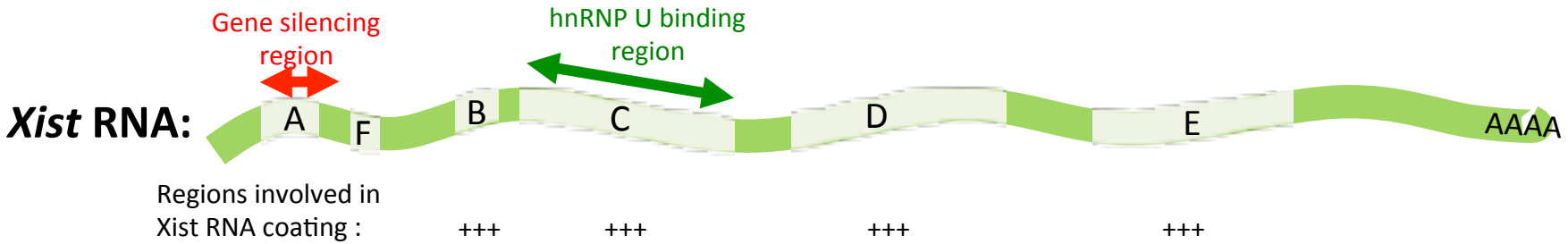
What are the Functional Domains of Xist RNA?



Wutz et al, Nature Genetics 2002

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

What are the Functional Partners of Xist RNA?



Proteomic screens

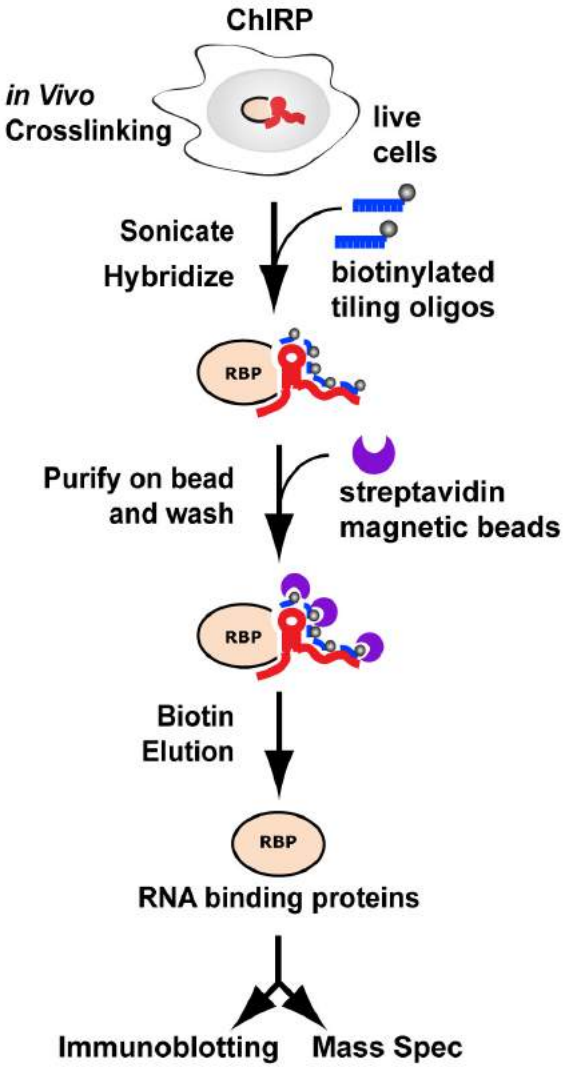
	ChIRP-MS [24]	RAP-MS [25]	iDRiP [26]
Biological samples	*Xist* & ΔA *Xist* inducible mouse ESCs; Mouse XX EpiSCs & TSCs	*Xist* inducible undifferentiated mouse ESCs	Mouse XX immortalized fibroblasts
Crosslinking agent	Formaldehyde	UV	UV
ASOs & *Xist* RNA pull down	43 ASOs of 20nt; 60% *Xist* recovered	142 ASOs of 95 nt 60% *Xist* recovered	9 ASOs of 25 nt 10% *Xist* recovered
MS approaches	Free-label	SILAC	Free-label
Cut-off	Ten-fold over background	Three-fold *Xist* vs U1 snRNA	Three or two-fold over background
Number of proteins identified	81 ΔA *Xist* - all except 3	10	Three-fold: 62 Two-fold: 203

Forward genetic screens

	shRNA screen [34]	Gene trap screen [35]
Biological systems	XY ESCs with inducible *Xist* TG & GFP reporter on chr17	Haploid ESCs with inducible promoter at *Xist* locus
Screening strategy	shRNA library genes for nuclear proteins & in ubiquitylation/ sumolyation pathways	Viral gene trap mutagenesis followed by NGS mapping of insertions
Read-out	GFP reporter activation	Cell survival due to absence of silencing of the single X-chr
Number of genes identified	225 nuclear proteins 34 of ubiquitylation/ sumolyation pathways	6 (5-fold) 21 (2-fold)

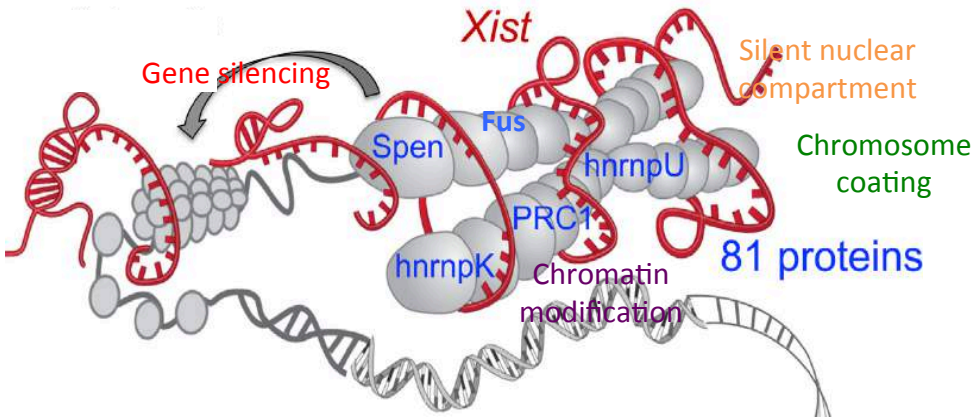
Systematic Discovery of Xist RNA Binding Proteins

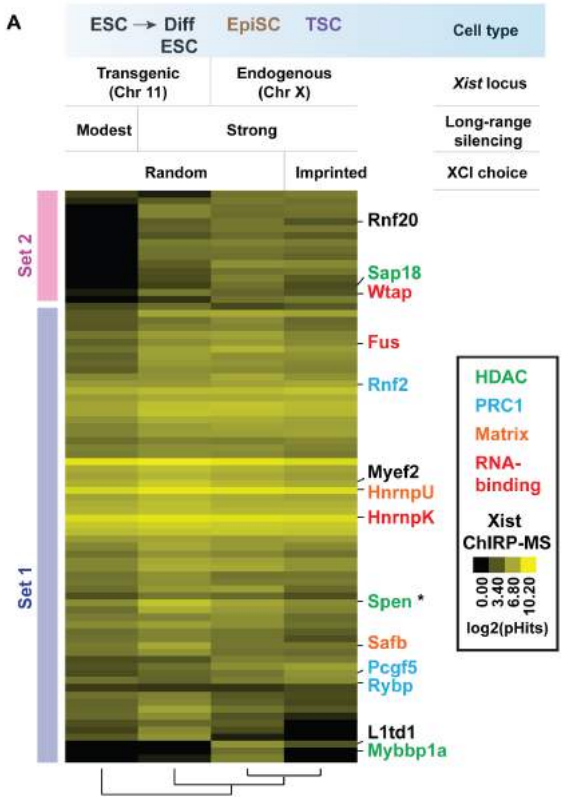
Ci Chu,[1,2] Qiangfeng Cliff Zhang,[1] Simão Teixeira da Rocha,[3] Ryan A. Flynn,[1] Maheetha Bharadwaj,[1] J. Mauro Calabrese,[4] Terry Magnuson,[5] Edith Heard,[3] and Howard Y. Chang[1,*]

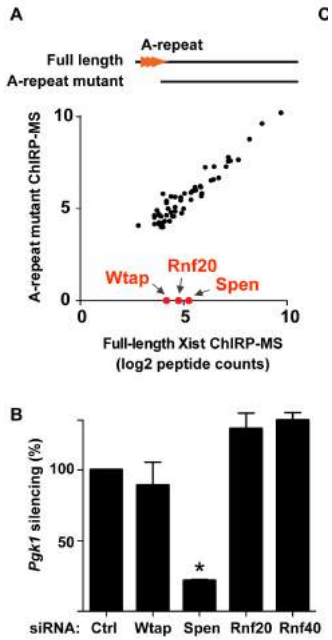


Chu et al, Cell 2015

Xist RNA Functional Partners







Chu et al, Cell 2015

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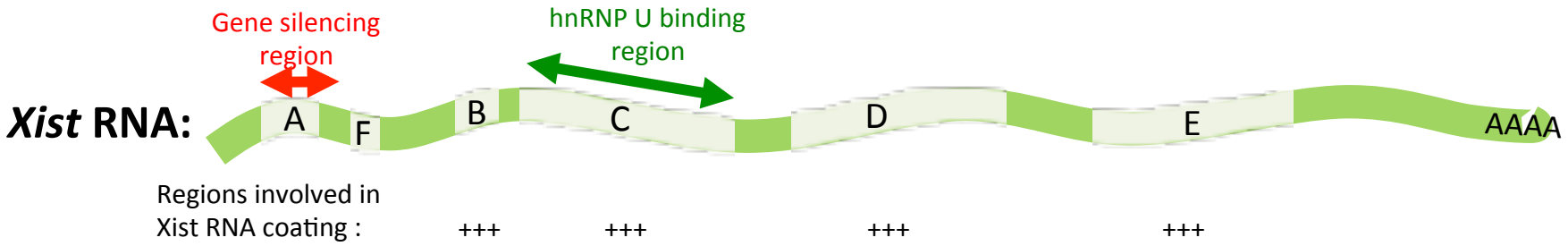
Xist RNA Functional Partners: a few examples



• **HnrnpU (SAF-A)** is required for Xist localisation (as previously shown)

• **Spen** (Drosophila Split ends homolog) interacts via the A-repeat domain of Xist and is required for gene silencing

• **Wtap** – RNA methylation machinery

• Polycomb PRC1 factors **Pcg5, Rybp** – but *no* PRC2 factors

• **HnrnpK**, participates in Xist-mediated gene silencing and recruitment of non-canonical polycomb PRC1 complex but not Xist localization

• LBR – **Lamin B receptor – nuclear organisation?**

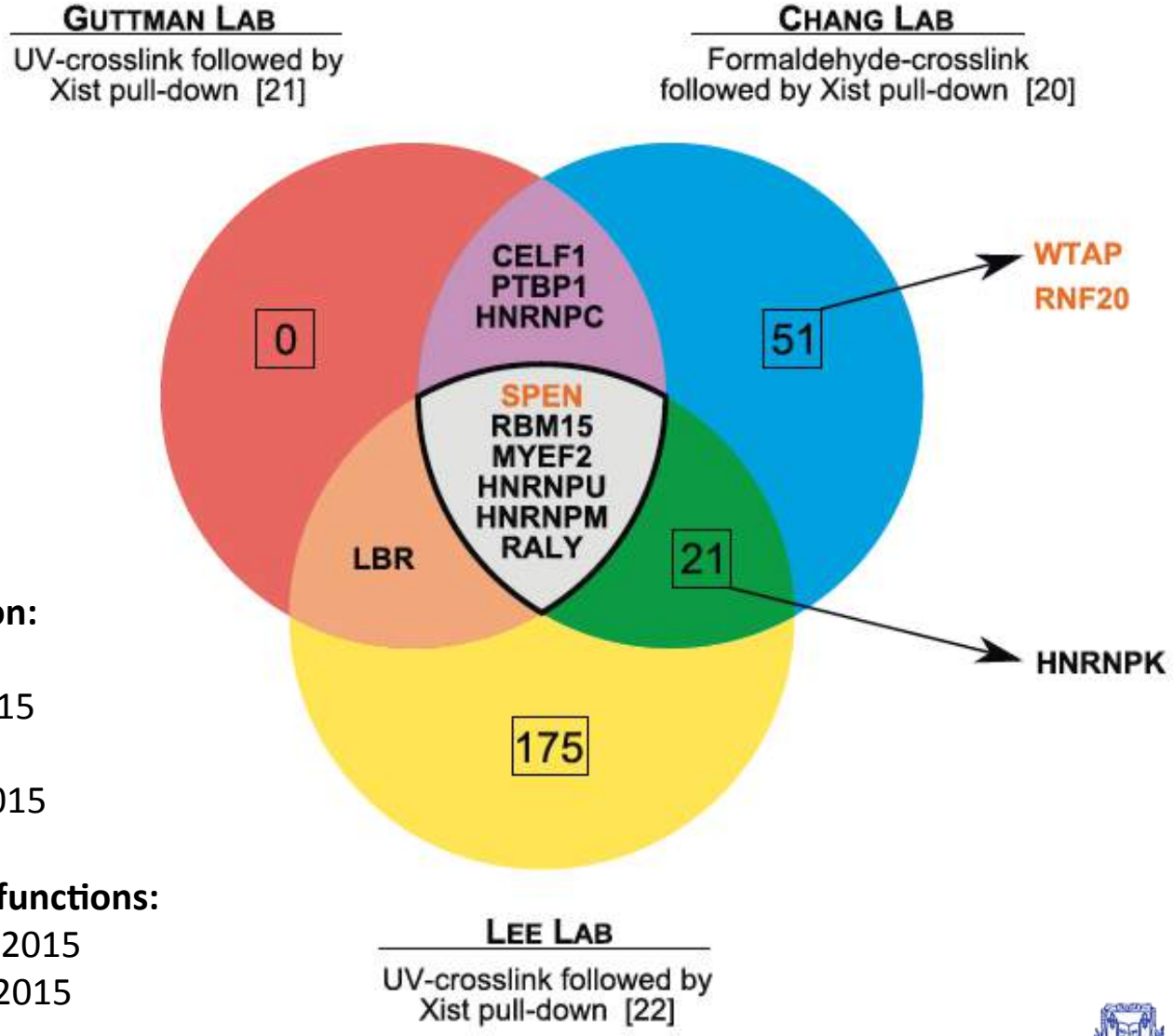
Chu et al, Cell 2015

Identification of the Protein Partners of Xist RNA and the Factors that are implicated in Xist-mediated Silencing

Holy Grail
Or Pandora's box?

Xist RNA partner isolation:
Chu et al, Cell 2015
McHugh et al, Nature 2015
Chen et al Science 2016
Minajigi et al, Science 2015

Genetic screens for Xist functions:
Moindrot et al, Cell Rep. 2015
Monfort et al, Cell Rep. 2015



E. Heard, February 12th, 2018

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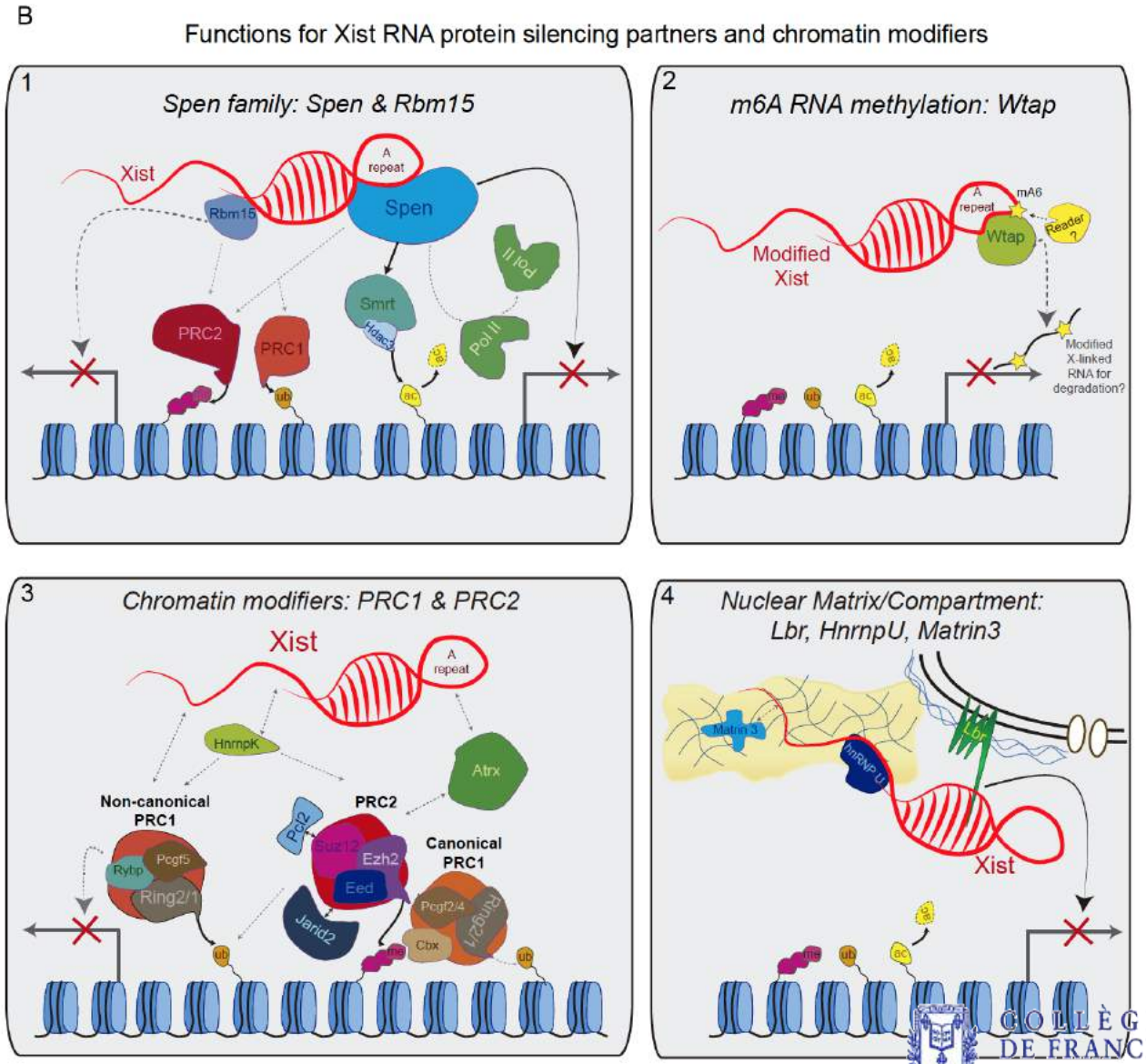
Molecular investigation of Xist RNA function
A new era of X-inactivation research!

Xist RNA partner isolation:
Chu et al, Cell 2015
McHugh et al, Nature 2015
Chen et al Science 2016
Minajigi et al, Science 2015

Genetic screens for Xist functions:
Moindrot et al, Cell Rep. 2015
Monfort et al, Cell Rep. 2015



Simao Teixeira da Rocha

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SUMMARY

Xist RNA and the initiation of X inactivation

- Xist non-coding RNA is a multi-tasking molecule essential for initiation of XCI

- It induces gene silencing, spatial reorganisation of the X chromosome and chromatin changes

- Mass-spec analysis of proteins bound to Xist RNA provide the first molecular handle for exploring its functions

- The first regions Xist targets contain the first genes silenced

- Subsequent spreading due to « relay » elements, or chromatin proteins, or spatial dynamics?

SUMMARY

Xist RNA and the initiation of X inactivation

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- Subsequent spreading due to « relay » elements, or chromatin proteins, or spatial dynamics?

Xist RNA exploits 3D genome architecture to spread across the X chromosome

First regions of the X chromosome that associate with Xist RNA contain some of the first genes silenced during XCI

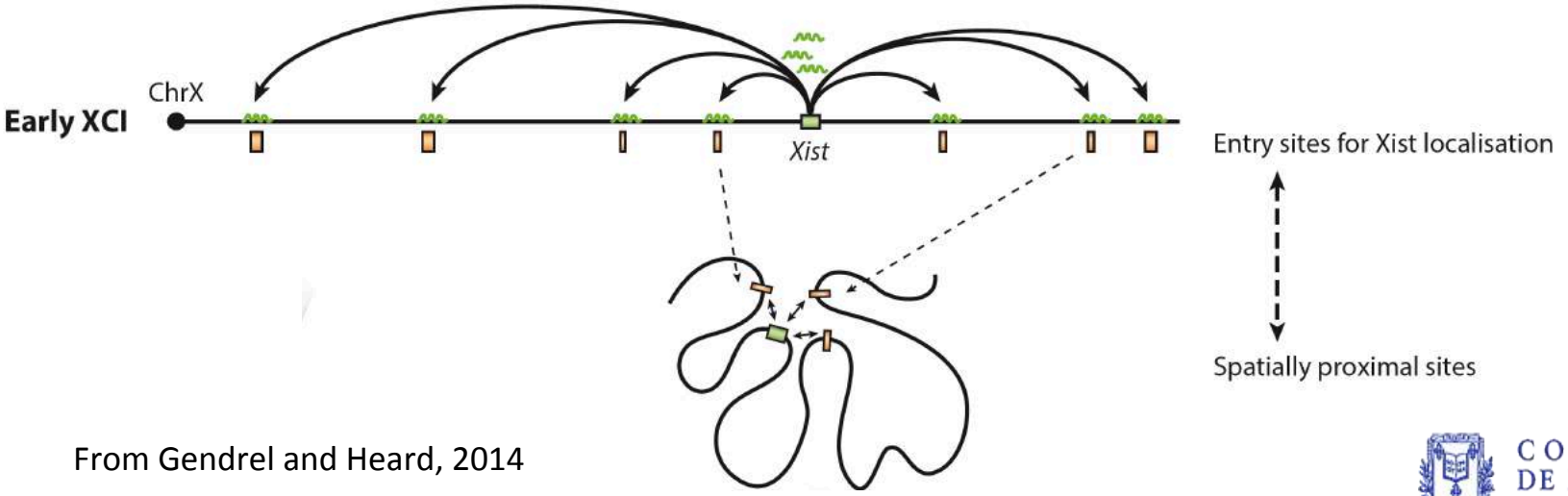
nature structural & molecular biology

Engreitz et al, 2013
RNA Antisense Purification (RAP): Mapping of Xist lncRNA interactions with chromatin

Xist-dependent imprinted X inactivation and the early developmental consequences of its failure

Maud Borensztein[1,5], Laurène Syx[1,2], Katia Ancelin[1], Patricia Diabangouaya[1], Christel Picard[1], Tao Liu[3], Jun-Bin Liang[3], Ivaylo Vassilev[1,2], Rafael Galupa[1], Nicolas Servant[2], Emmanuel Barillot[2], Azim Surani[4], Chong-Jian Chen[3] & Edith Heard[1]

Lieberman-Aiden et al, 2009
Comprehensive mapping of long-range interactions reveals folding principles of the human genome



From Gendrel and Heard, 2014

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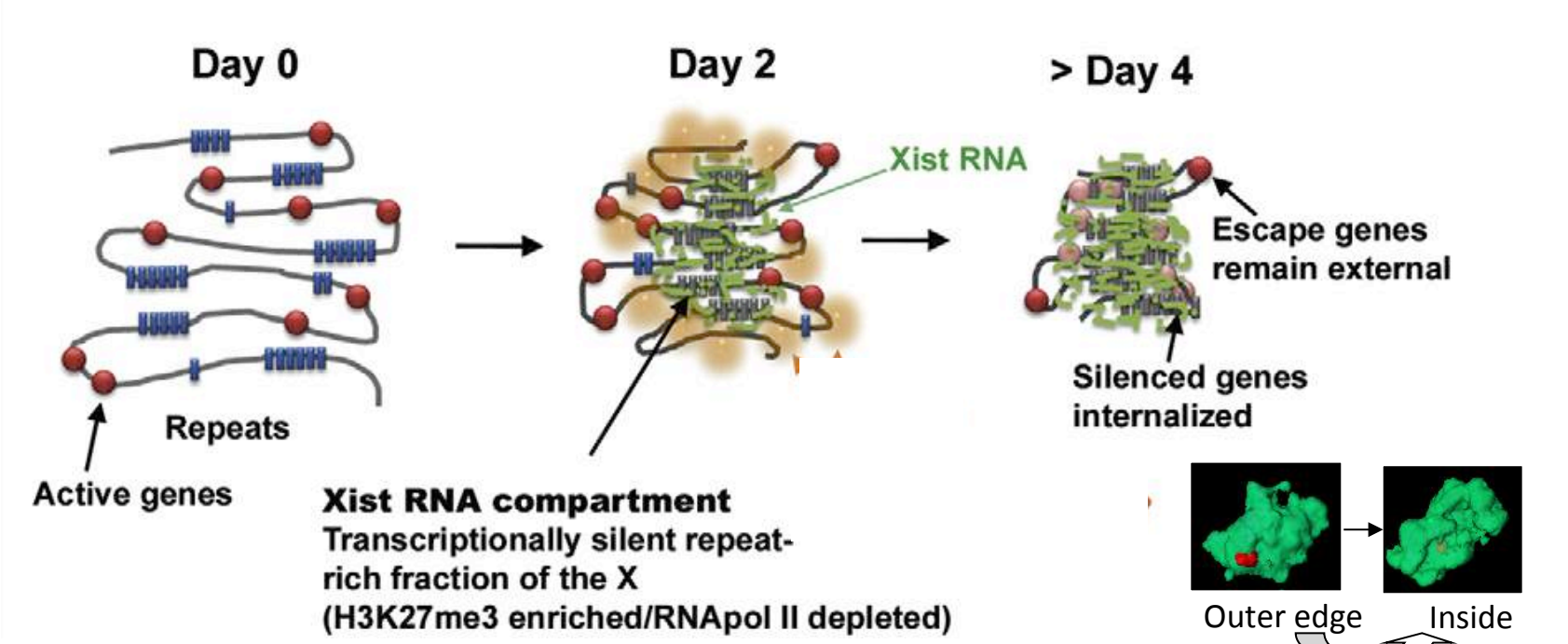
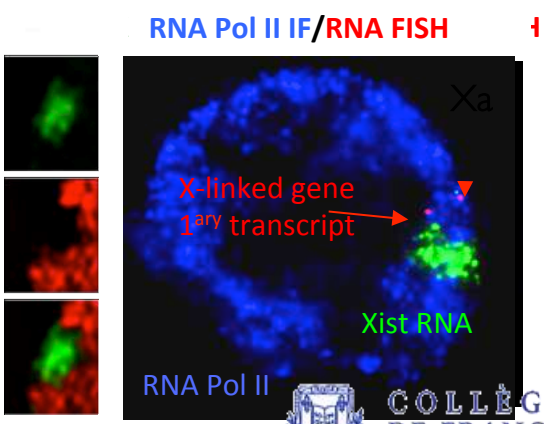
Xist RNA forms a silent nuclear compartment and triggers spatial reorganisation of the Xi during XCI

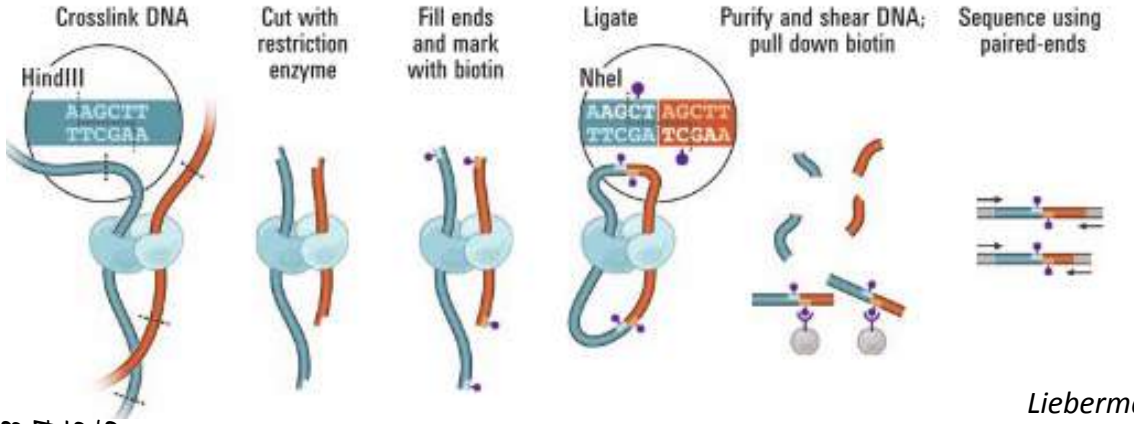




Genes undergoing inactivation are internalised into the Xist RNA compartment, expressed genes (escapees) remain external

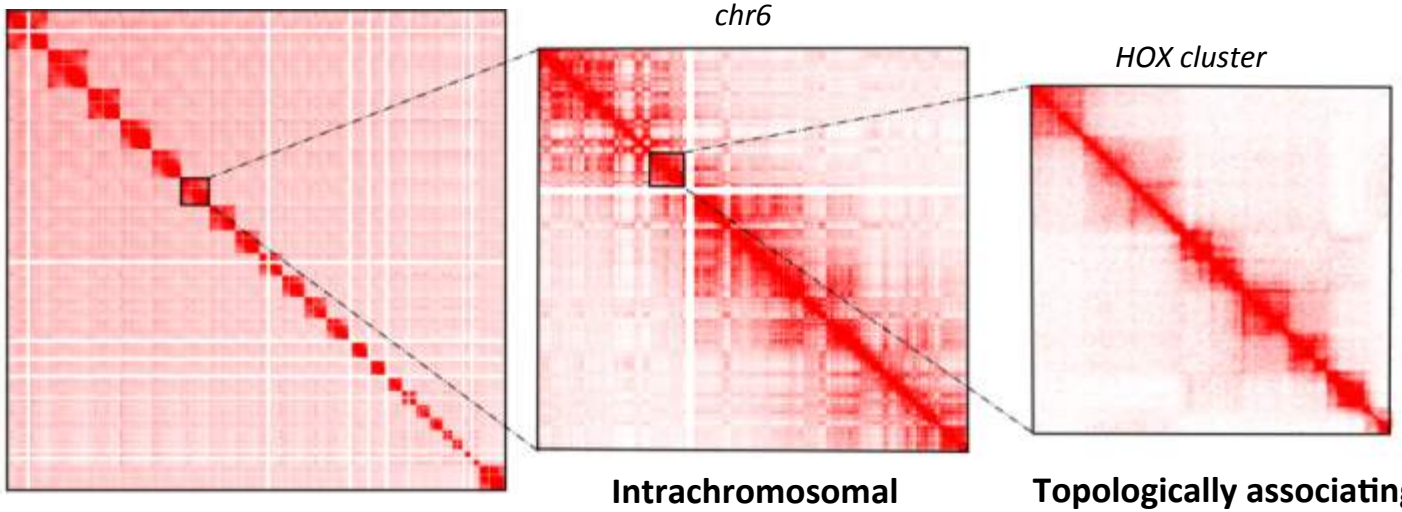
Chaumeil et al, Genes Dev. 2006; Chow et al, Cell 2010

Investigating the molecular architecture of the active and inactive X chromosomes using Hi-C



Lieberman-Aiden et al. 2009

chr1 chr2 chr3 chr4 chr5 chr6 …



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

Job Dekker

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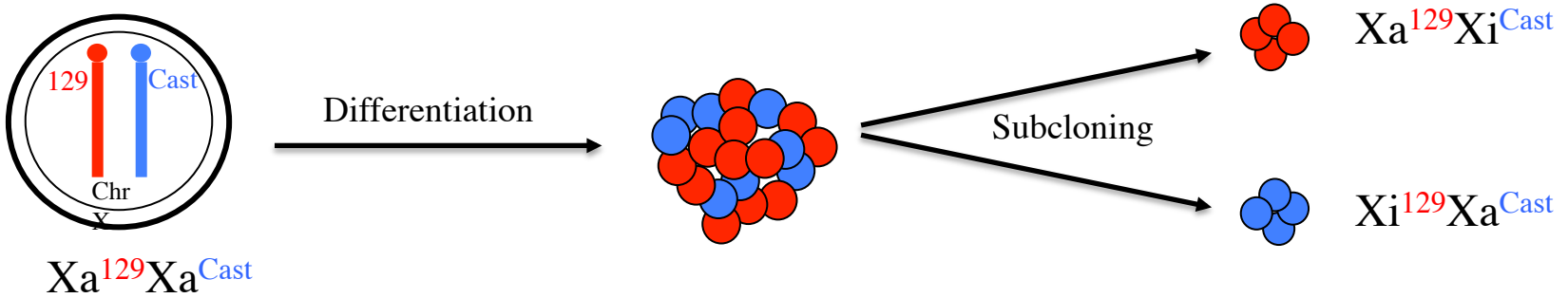
Allele-specific RNA seq and Hi-C in clonal F1 129/Cast ESCs and NPCs

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

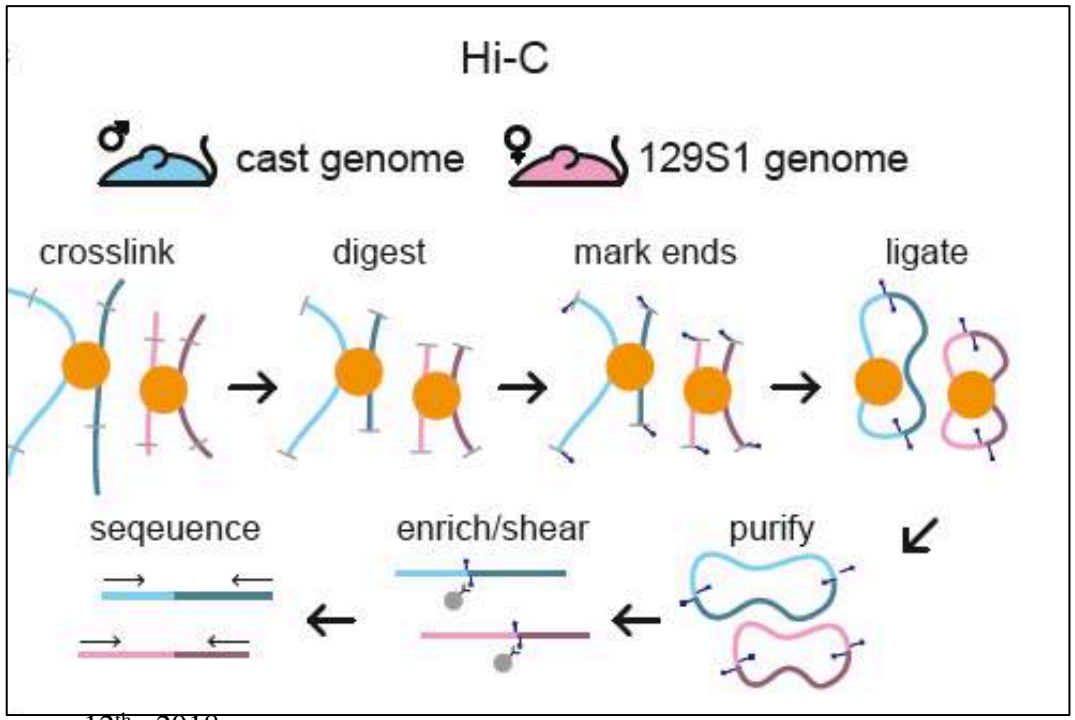
Neural Progenitor cells (NPC)
Random X chromosome inactivation (XCI)

Isolate NPC clones
100% cells with 129 or cast Xi



Xa^{129}Xa^{Cast}

(Gendrel et al, Dev. Cell 2014)



Dekker Lab:
Ye Zhan, Bryan Lajoie
Heard Lab:
Mikael Attia, Luca Giorgetti

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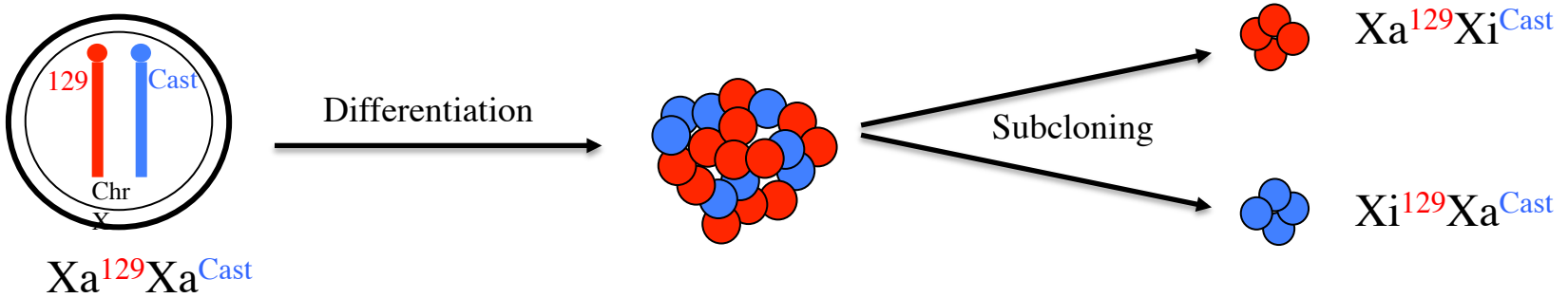
Allele-specific analysis in clonal, polymorphic embryonic stem cells & neural progenitor cells

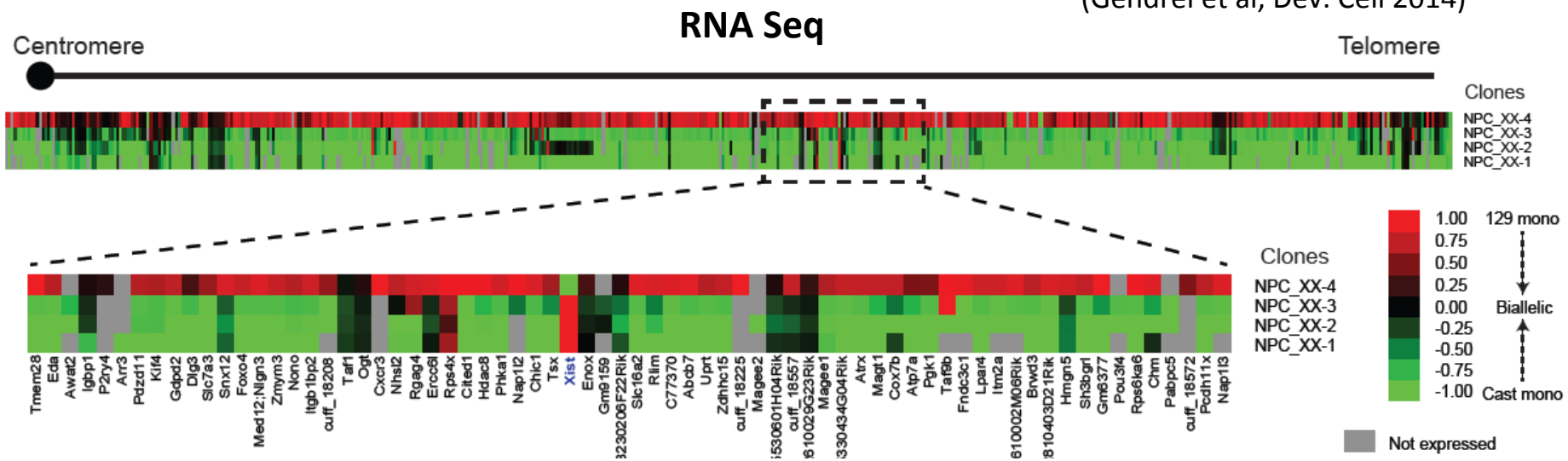
F1 Hybrid ES cell line
(129xCast => 1 SNP /~100bp)

Neural Progenitor cells (NPC)
Random X chromosome inactivation (XCI)

Isolate NPC clones
100% cells with 129 or cast Xi



(Gendrel et al, Dev. Cell 2014)



- Global silencing of one X chromosome but escape from XCI in multiple regions of the X
- Clusters of facultative escapees in NPCs (Gendrel et al, 2014) – vary between different clones
- Also seen in Trophoblast Giant Cells at E8.0 (*in vivo*) (Catherine Corbel)

E.

Unique Chromosome Organisation of the inactive X

Structural organization of the inactive X chromosome in the mouse

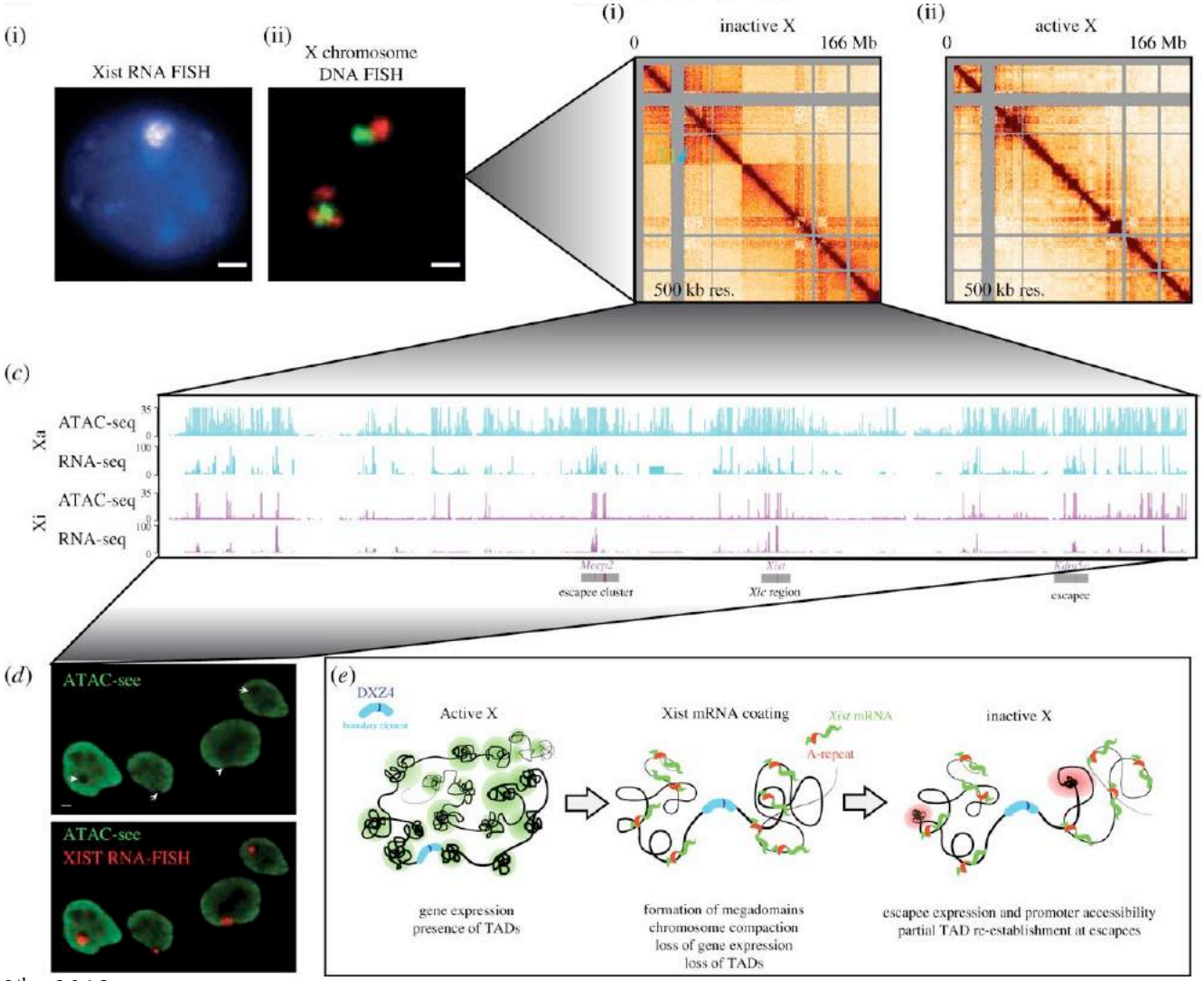
Luca Giorgetti[1*†], Bryan R. Lajoie[2*], Ava C. Carter[3*], Mikael Attia[1*], Ye Zhan[2], Jin Xu[3], Chong Jian Chen[1], Noam Kapla
Howard Y. Chang[3], Edith Heard[1,4] & Job Dekker[2,5]

Giorgetti et al, 2016

Spatial partitioning of the regulatory landscape of the X-inactivation centre

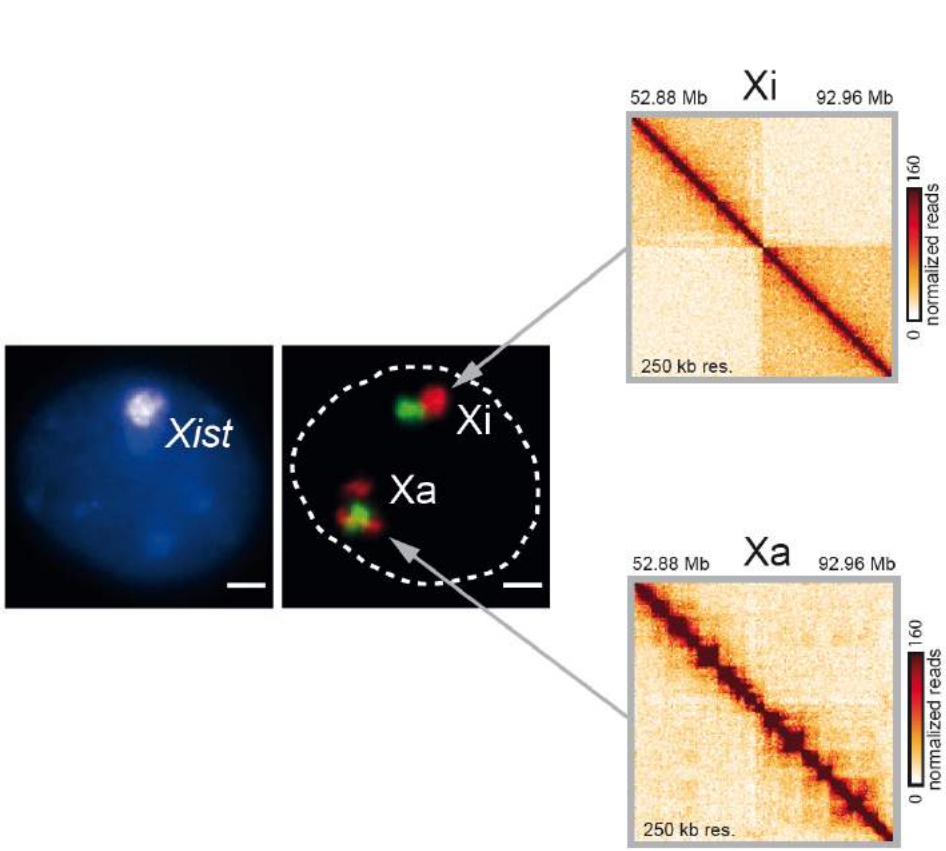
Elphège P. Nora[1,2,3], Bryan R. Lajoie[4*], Edda G. Schulz[1,2,3*], Luca Giorgetti[1,2,3*], Ikuhiro Okamoto[1,2,3], Nicolas Servant[1,5,6], Tristan Piolot[1,2,3], Nynke L. van Berkum[4], Johannes Meisig[7], John Sedat[8], Joost Gribnau[9], Emmanuel Barillot[1,5,6], Nils Blüthgen[7], Job Dekker[4] & Edith Heard[1,2,3]

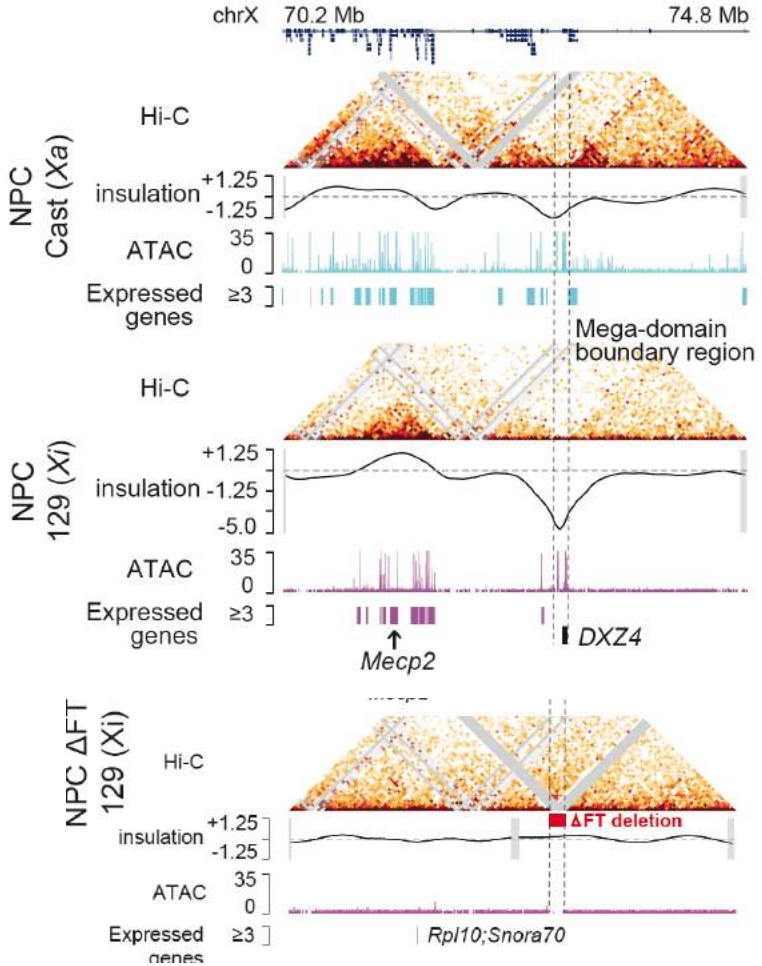
Nora et al, Nature 2012



Unique Chromosome Organisation of the inactive X

Two super-domains and global absence of topological domains
Facultative escape correlates with local 3D-organisation (TADs)
And is influenced by the unusual DXZ4 region…

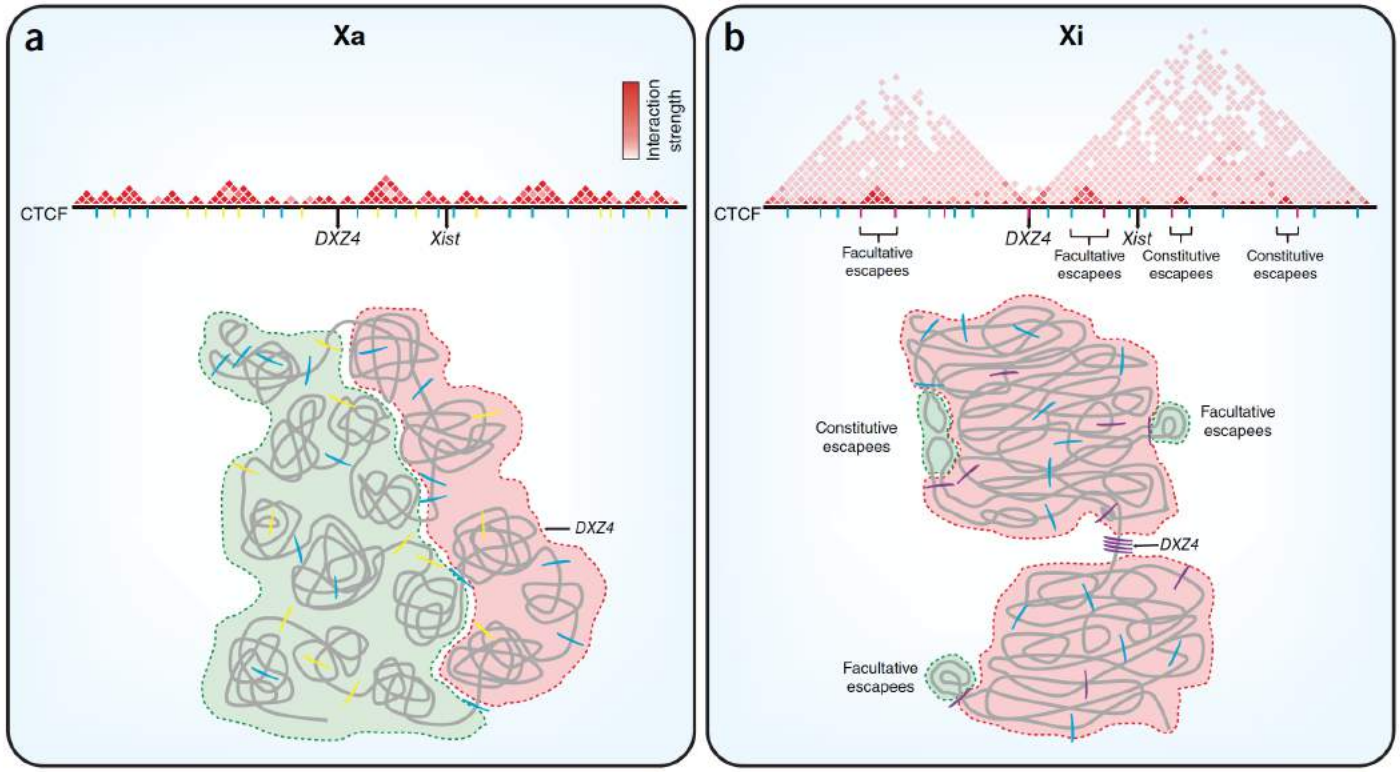




Giorgetti L et al. "Structural organization of the inactive X chromosome in the mouse". *Nature* 535:575-9 (2016)

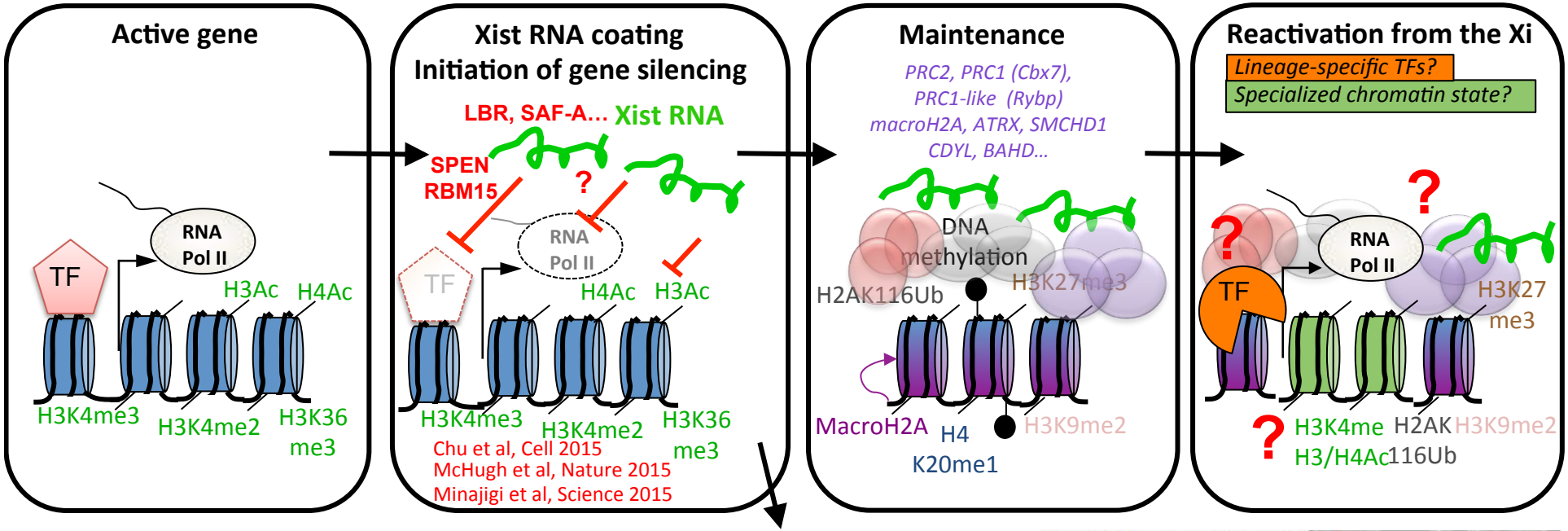
Unique Chromosome Organisation of the inactive X

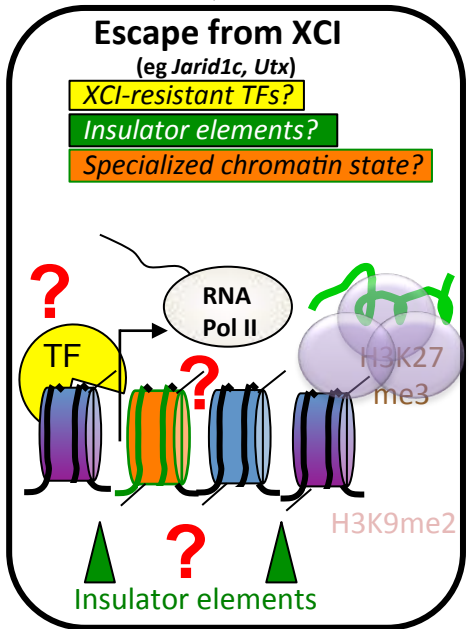
Facultative escape correlates with local 3D-organisation of the chromatin fibre and is influenced by the DXZ4 macrosatellite?



From Rocha and Heard 2017

X-inactivation events: gene silencing and escape





Some genes escape XCI constitutively & autonomously

(eg *Utx*, *Jarid1c*, Li and Carrel, *PNAS* 2008)

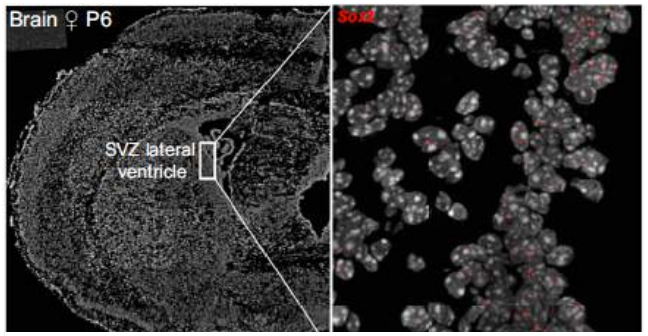
Some genes escape in a lineage or tissue-specific fashion

(eg *Atrx*, Patrat et al, *PNAS* 2009)

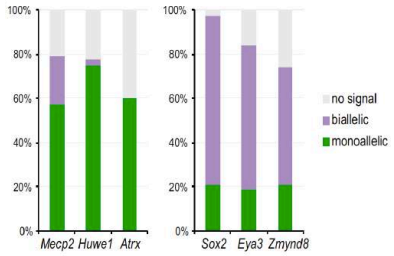
Escapees are often involved in chromatin-associated functions

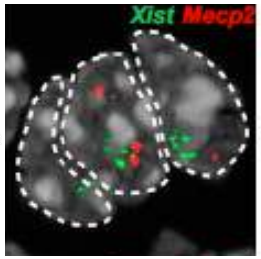
eg Jarid1c, Utx = Histone demethylases

Atrx = chromatin remodeller



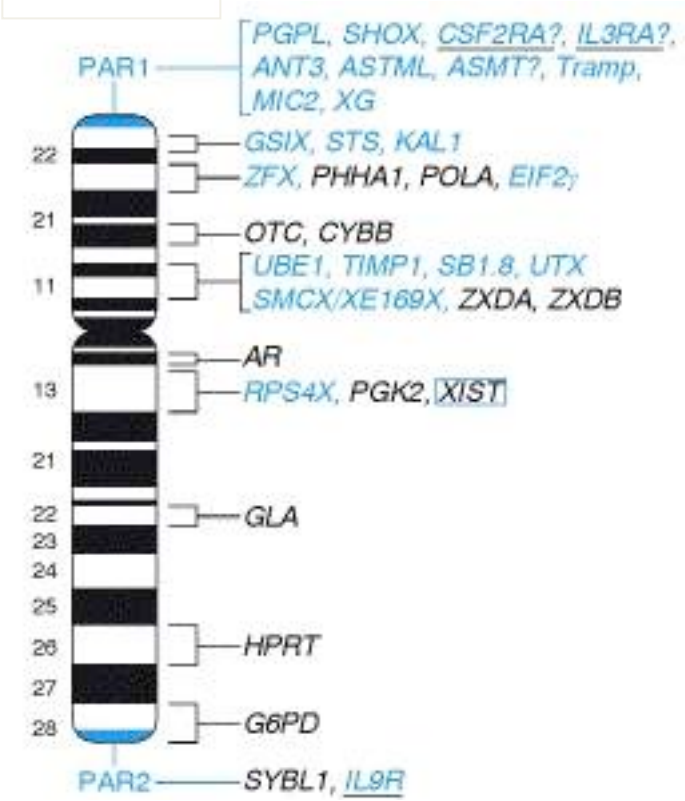
Eg *Mecp2* escapes XCI in neural stem cells in the SVZ



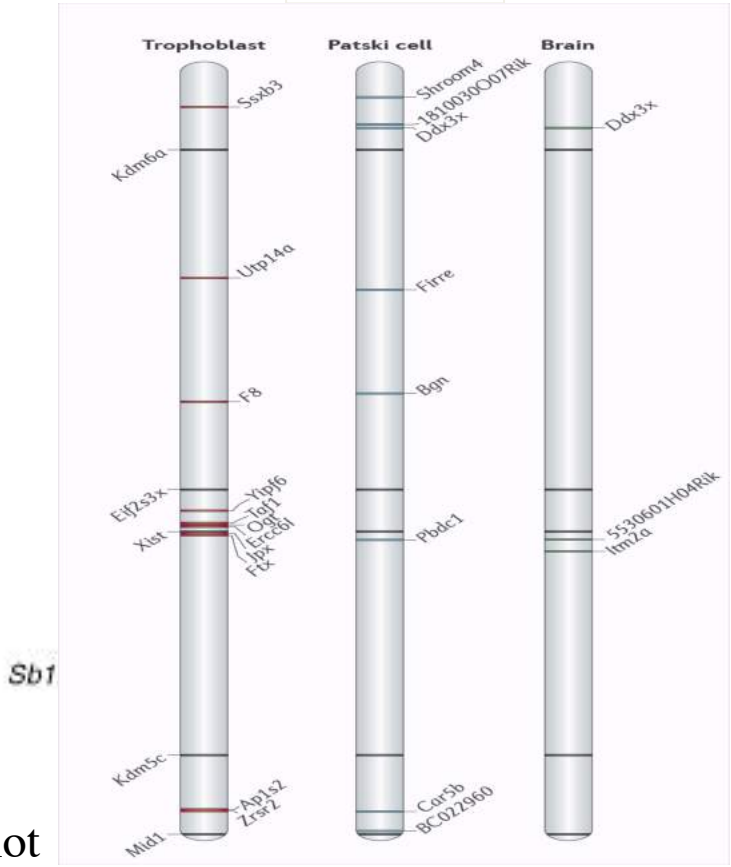


Genes that can *escape* from X inactivation

Human X



Mouse X



- A few escapees have Y-linked homologs, most do not
- Escape may be *accidental* (epigenetic instability) or *purposeful* (requirement of a double dosage in XX)
- Escape may underlie some sex chromosome dosage effects on several sex-biased metabolic, immune and neurological phenotypes **(MORE NEXT WEEK)**

X-inactivation: gene silencing and escape Variability across Human Tissues

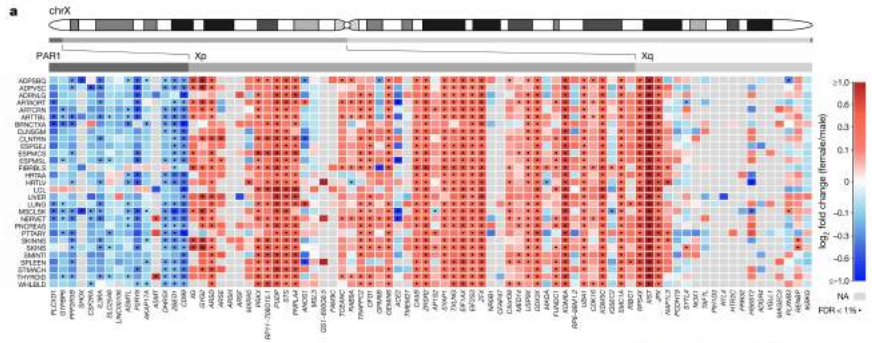
LETTER

OPEN

doi:10.1038/nature24265

Landscape of X chromosome inactivation across human tissues

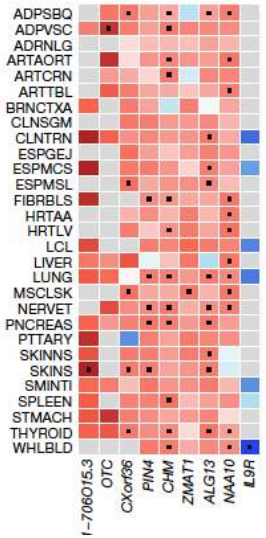
Taru Tukiainen[1,2], Alexandra-Chloé Villani[2,3], Angela Yen[2,4], Manuel A. Rivas[1,2,5], Jamie L. Marshall[1,2], Rahul Satija[2,6,7], Matt Aguirre[1,2], Laura Gauthier[1,2], Mark Fleharty[2], Andrew Kirby[1,2], Beryl B. Cummings[1,2], Stephane E. Castel[6,8], Konrad J. Karczewski[1,2], François Aguet[2], Andrea Byrnes[1,2], GTEx Consortium†, Tuuli Lappalainen[6,8], Aviv Regev[2,9], Kristin G. Ardlie[2], Nir Hacohen[2,3] & Daniel G. MacArthur[1,2]

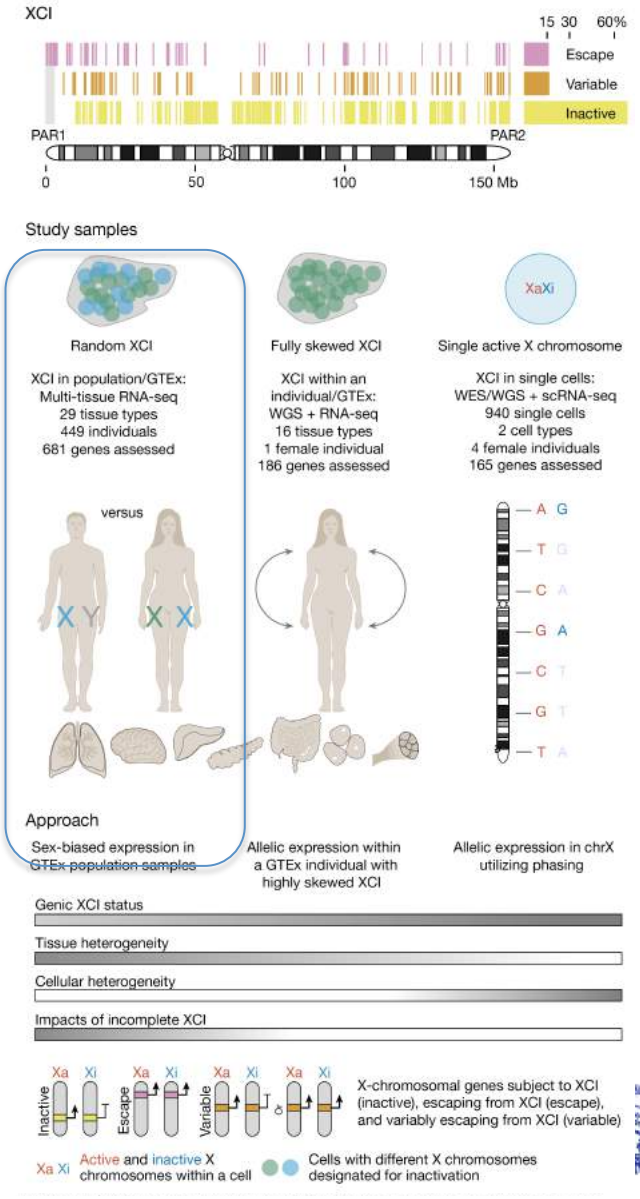


Genes in the pseudoautosomal region show higher expression in XY males than in XX females => lower activity of PAR region on Xi?

Multiple regions show female bias: ie escape from XCI – this is variable between tissues and individuals

Sex bias pattern of nine genes not classified as full escape genes that follow a similar profile to established escape genes

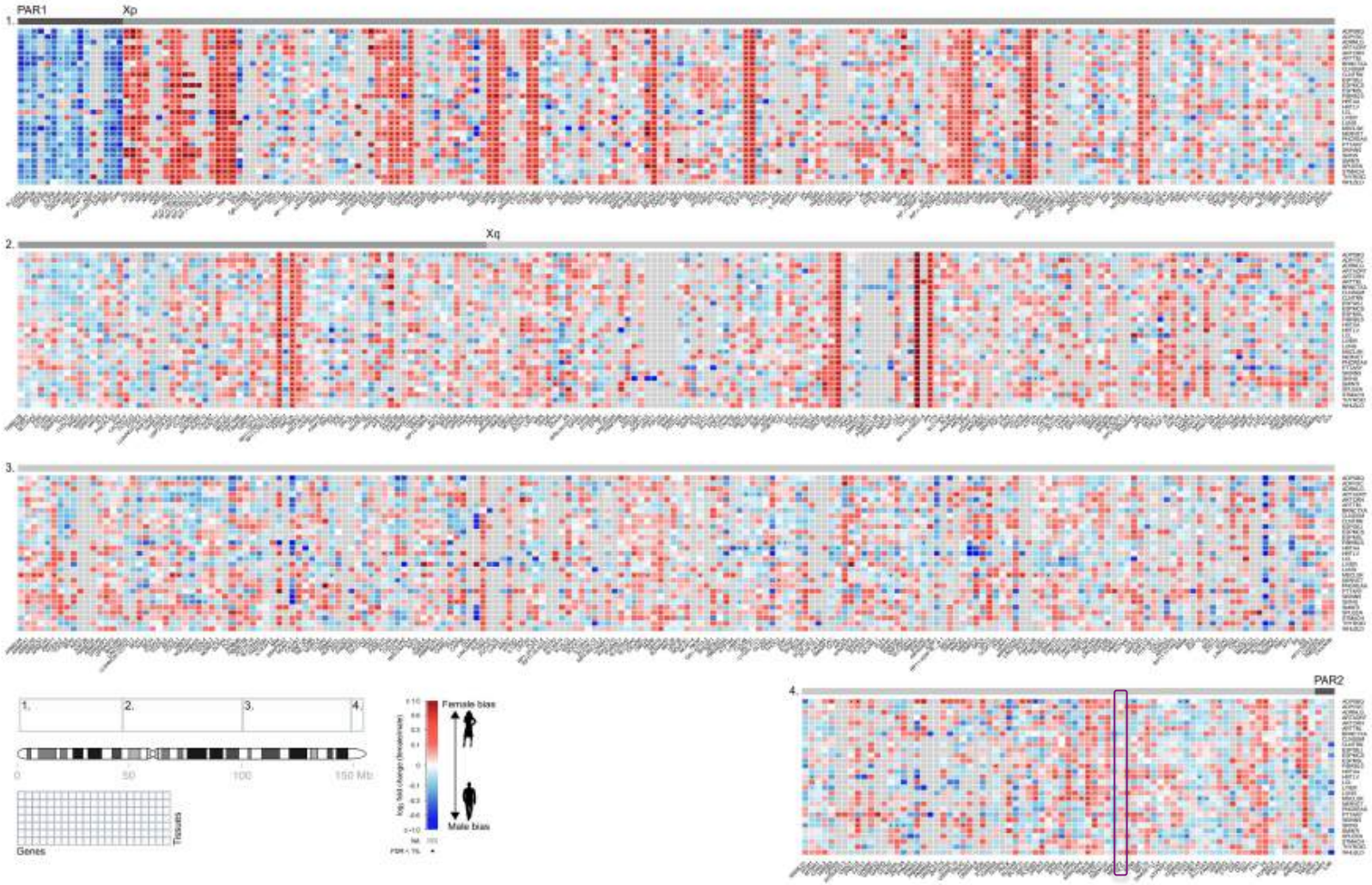




COLLÈGE DE FRANCE 1530

E. Heard, February 19th, 2018

X-inactivation: gene silencing and escape Variability across Human Tissues



Extended Data Figure 4 | Heat map representation of male–female expression differences in all assessed X-chromosomal genes ($n = 681$) across 29 GTEx tissues. The colour scale displays the direction of sex bias, with red colour indicating higher female expression. Genes that were too weakly expressed to be assessed in a given tissue type in the sex bias analysis are coloured grey. Dots mark the observations where sex bias was significant at FDR < 1%.

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Variable escape from XCI in different tissues: How does this relate to epigenetic status of the Xi?

Are the different epigenomic landscapes (H3K27me3/Pc vs H3K9me3/HP1) linked to the cell type specific differences?

Or is the difference gene-specific or gene cluster-specific?

Is it related to looping/TAD formation on the Xi?

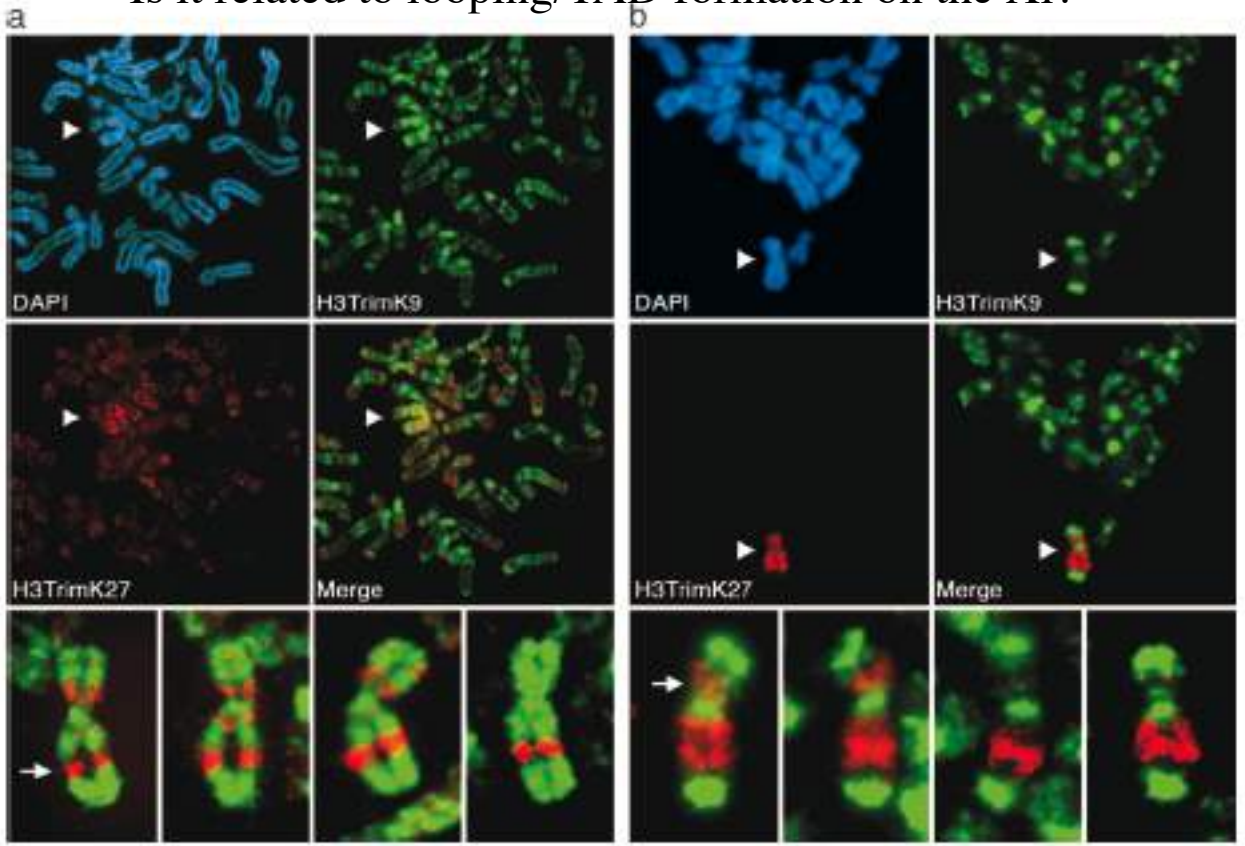


Fig. 1. Spatial relationship of two major Xi heterochromatin types at metaphase. Images represent typical distributions obtained from three independent female cell lines. (*a*) Partial metaphase spread of RPE1 cells showing the spatial distribution of H3TrimK9 (green, FITC) and H3TrimK27 (red, rhodamine) and additional higher-magnification images of the Xi showing the merged H3TrimK9 and H3TrimK27 distributions. The white arrow indicates the major H3Tri band centered at Xq23. (*b*) Distributions of H3TrimK9 and H3TrimK27 in HME1 cells. The location of the Xi in the partial metaphase spreads is indicated by both white and the white arrow indicates the major H3TrimK27 band centered at Xp11. All images were obtained by indirect immunofluorescence.

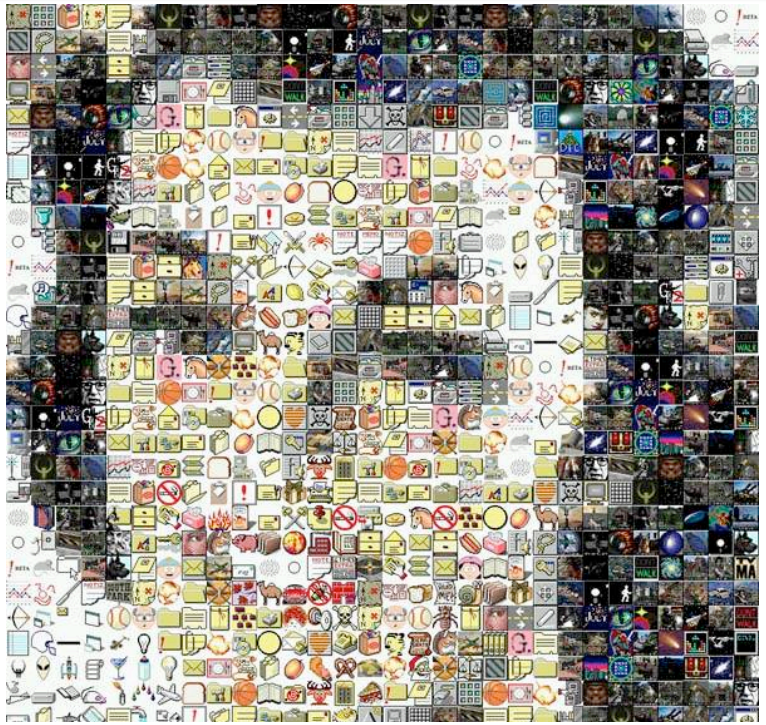
Variable escape from X inactivation leads to even greater female cellular mosiacism

In humans, up to 25% of X-linked genes can escape from X inactivation (ie are biallelic)!

10% of these escape constitutively
15% of these genes show **variability between individuals** – and **tissue specificity**

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

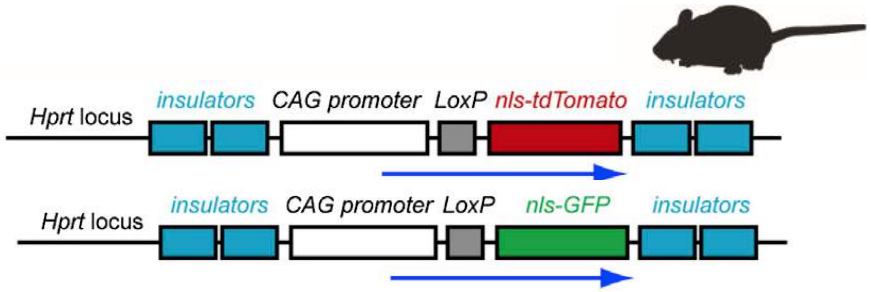
Carrel and Willard (2005) *Nature* 434, 400-404

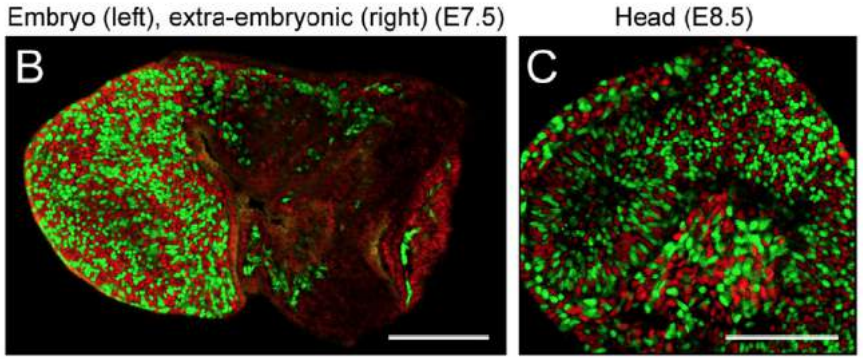


Consequences on physiology, behaviour, disease?

Huntington Willard – 2005:
"Genetically speaking, if you've met one man, you've met them all.
We are, I hate to say it, predictable. You can't say that about women.
Men and women are farther apart than we ever knew. It's not Mars or Venus.
It's Mars or Venus, Pluto, Jupiter and who knows what other planets."

No two females, not even twins, are identical when it comes to X-linked traits



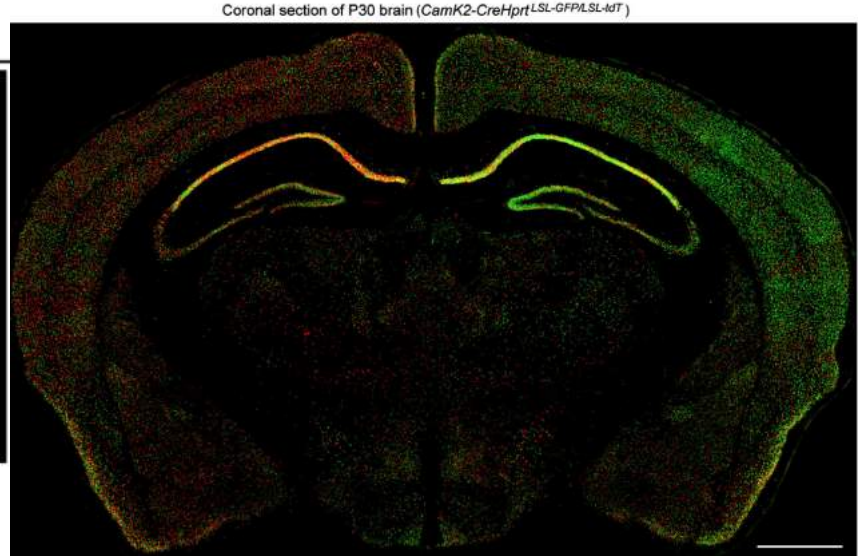


Neuron
Article

Cellular Resolution Maps of X Chromosome Inactivation: Implications for Neural Development, Function, and Disease

Hao Wu,[1,7] Junjie Luo,[2] Huimin Yu,[1] Amir Rattner,[1] Alisa Mo,[3] Yanshu Wang,[1,7] Philip M. Smallwood,[1,7] Bracha Erlanger,[4] Sarah J. Wheelan,[4,6] and Jeremy Nathans[1,3,5,7,*]

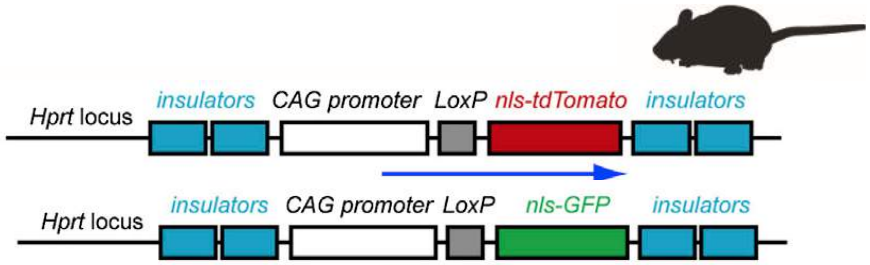
Coronal section of P30 brain (*CamK2-Cre*$Hprt^{LSL\text{-}GFP/LSL\text{-}tdT}$)



XCI mosaicism creates diversity between and within individuals.

Within an individual, inhomogeneities in the XCI mosaic in any particular tissue reflect the interplay of : (1) the ratio of founder cells to adult cells; (2) the timing and extent of cell migration during development; (3) alleles that may be counter-selected in specific cell types

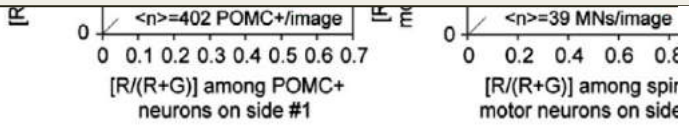
No two females, not even twins, are identical when it comes to X-linked traits

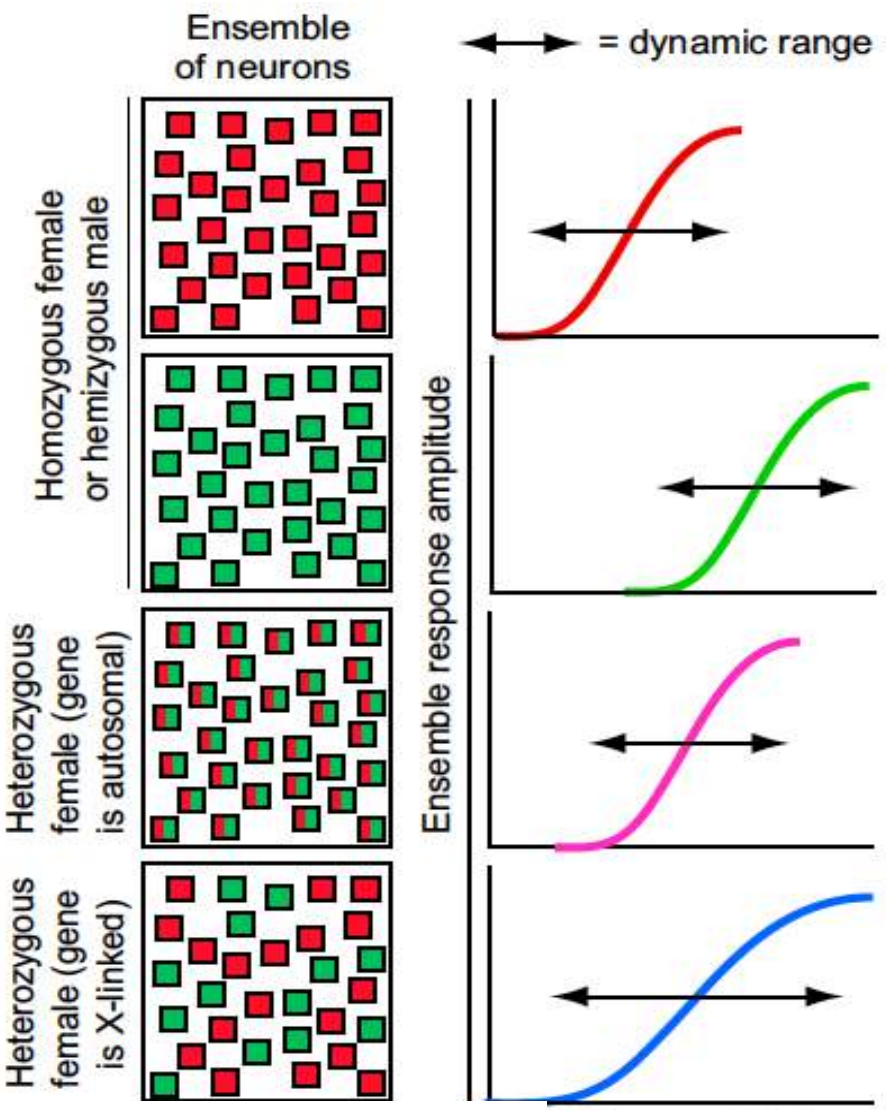


In the Central Nervous System:
Cell-type differences in XCI mosaicism in the cerebral cortex; mosaicism in CNS Vasculature

A diversity-generating mechanism such as XCI, which operates on all cells within the CNS and creates diversity on a spatial scale that encompasses the scale of local circuits, has the potential to *add functionally relevant capabilities*.

Because XCI affects 50% of the individuals in every species of eutherian mammal and because it epigenetically silences one allele among 4% of genes, it may represent one of the more significant mechanisms by which individual differences in CNS function are generated.

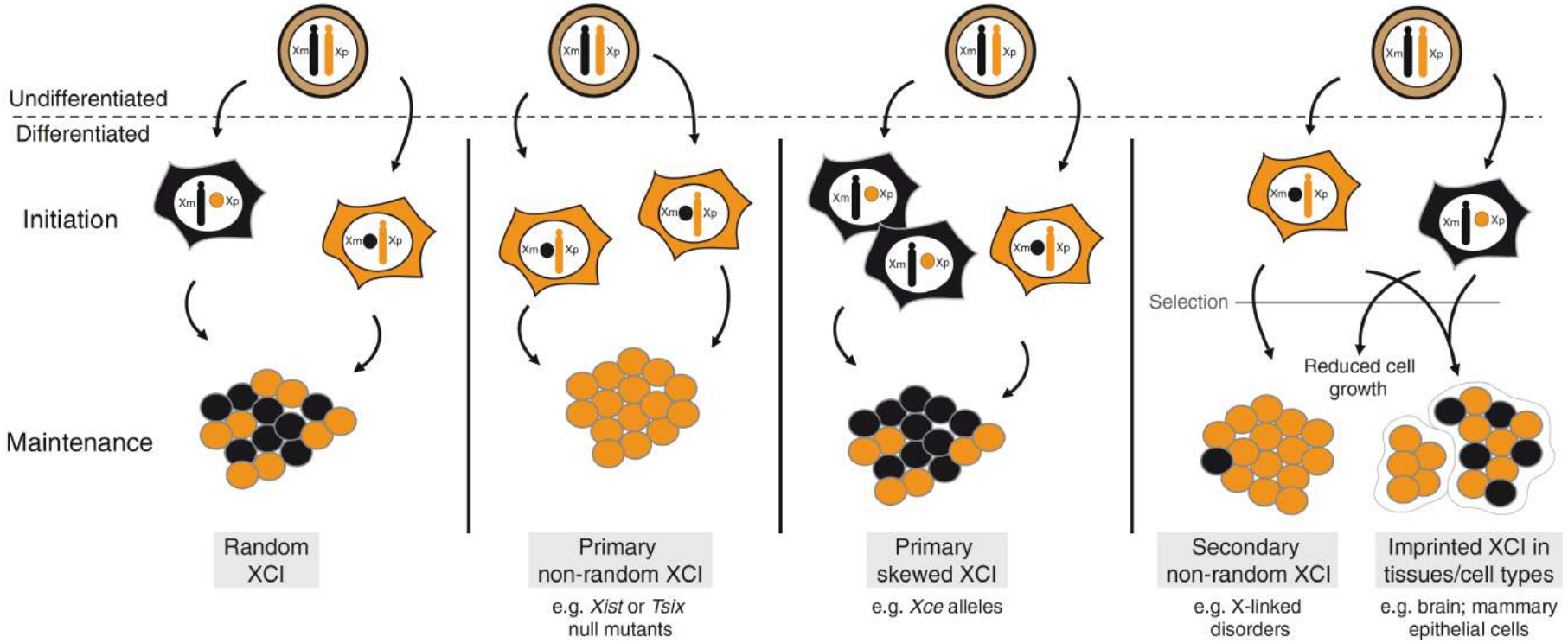




Two biochemically distinct types of neurons => the ensemble's dynamic range is likely to be expanded along the stimulus axis.

Females are Mosaics

However, X-chromosome activity can be **skewed** for different reasons



Different mutations or genetic variants can influence the degree of mosaicism in an individual

Within individuals, different organs can have different degrees of mosaicism if selection for/against cells with a particular allele expressed can occur

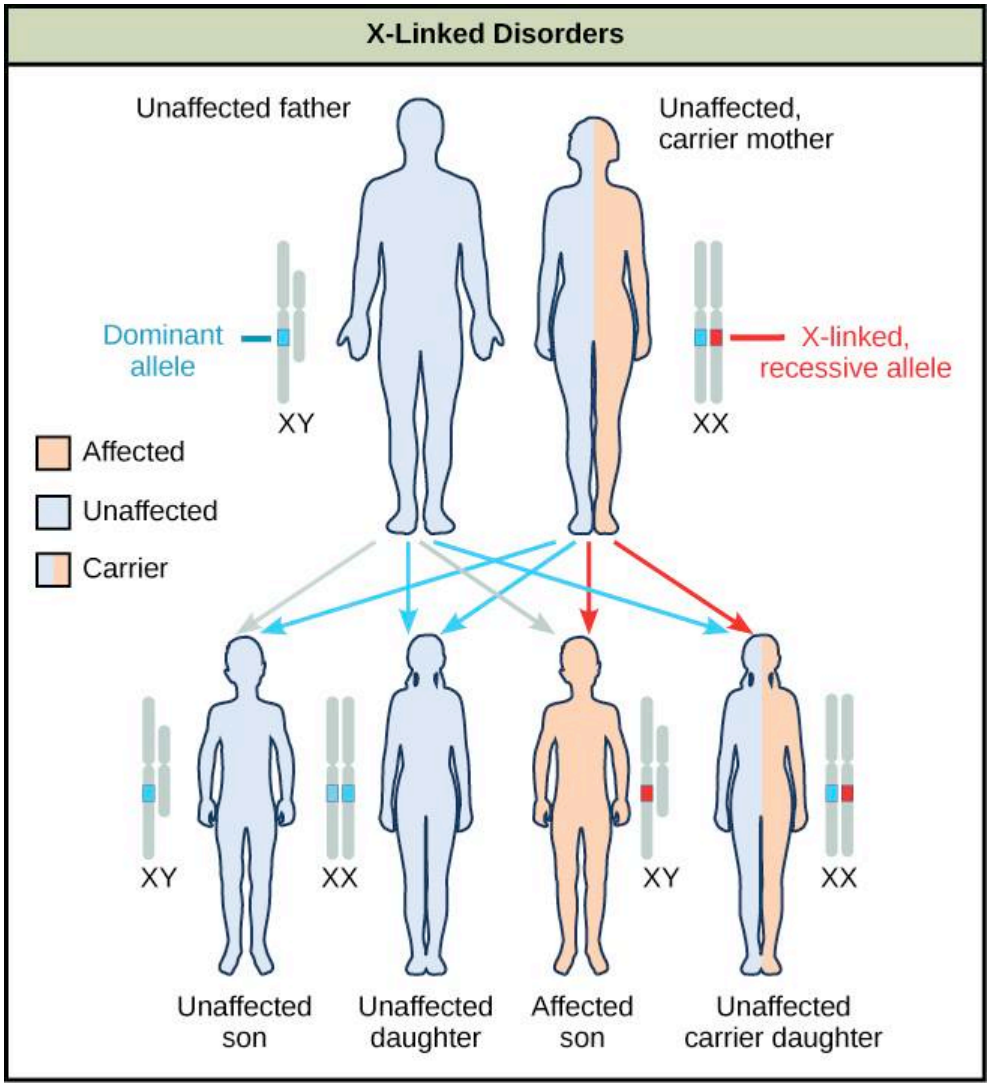
With age the inactive X chromosome can be lost in some cells…

From Gendrel and Heard, Development 2011

Implications of X inactivation for X-linked Diseases

Severe phenotypes or lethality in males
Variable and sometimes no phenotypes in females

Eg Fragile X syndrome, Haemophilia, muscular dystrophy, Incontinentia pigmenti



X-linked intellectual disability(XLID)

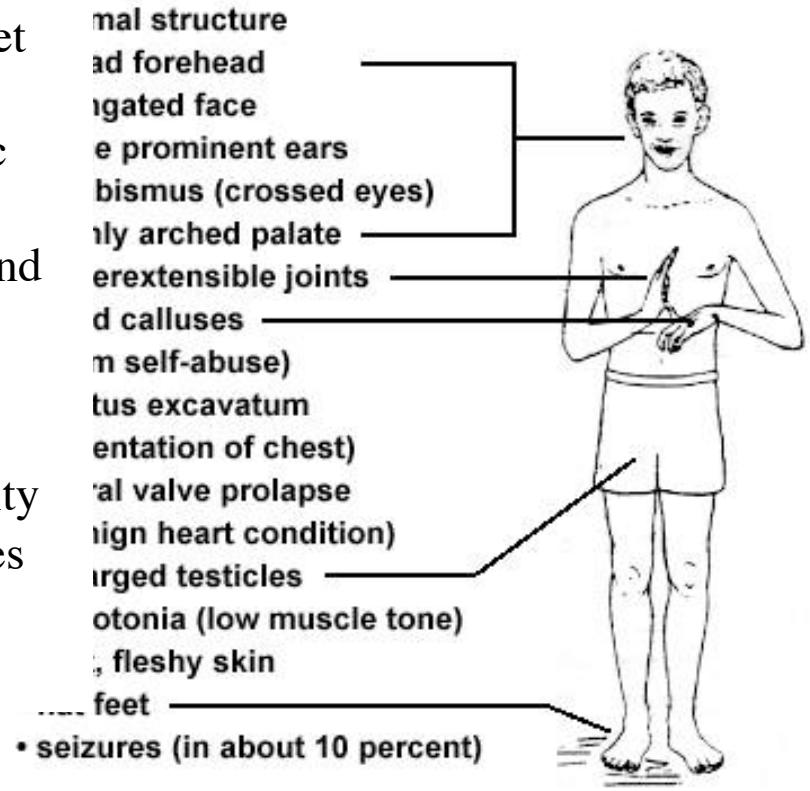
déficience intellectuelle

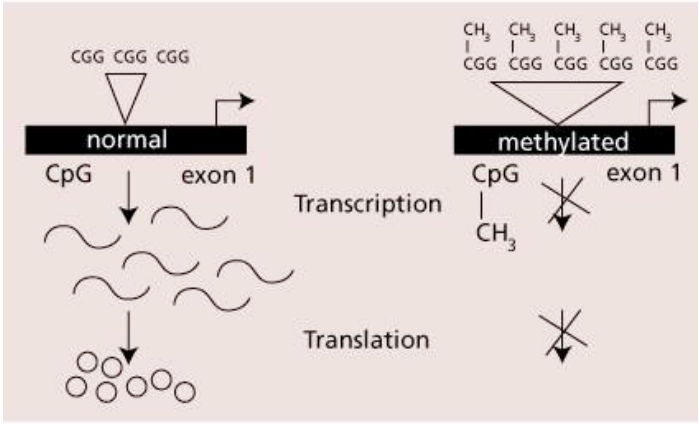
X-linked intellectual disability(XLID)

déficience intellectuelle

Fragile X Syndrome (FXS):

- Mutation or silencing upon methylation of triplet expansions of FMR1 gene at Xq27 lead to dendritic spine abnormalities, impaired synaptic plasticity and mental retardation.
- FMR1 knockout mice show subtle behavioral and visual-spatial difficulties
- FMRP loss leads to dysregulated protein translation at synapses
- Pathophysiological basis of cognitive inflexibility in FXS? Invariant tuning of single-cell responses and inadequate discharge coordination within neural ensembles (Talbot et al, Neuron 2018)





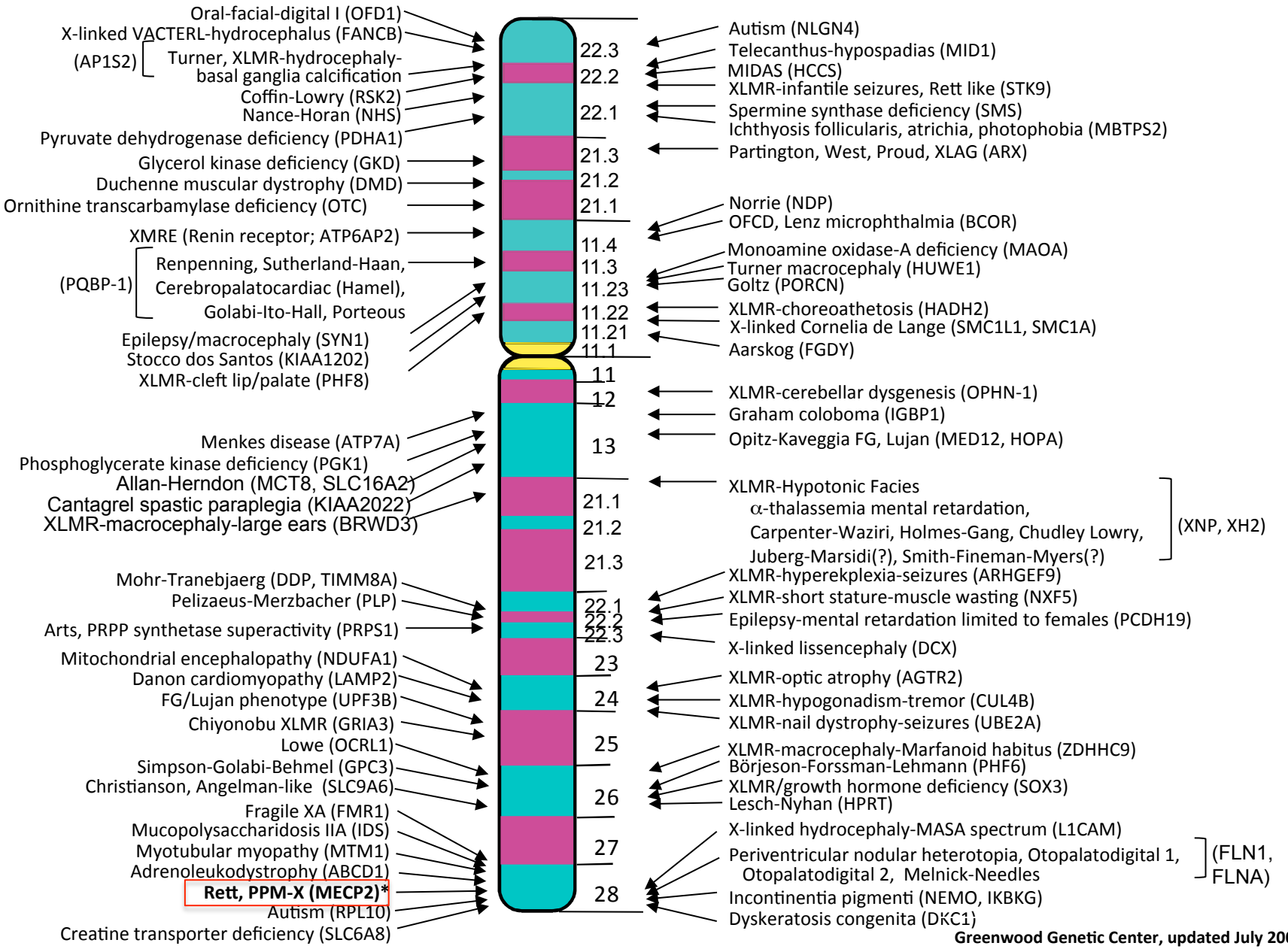
Research Article

Instability of a 550–Base Pair DNA Segment and Abnormal Methylation in Fragile X Syndrome

I. Oberlé, F. Rousseau, D. Heitz, C. Kretz, D. Devys, A. Hanauer, J. Boué, M. F. Bertheas, J. L. Mandel*

X-linked intellectual disability(XLID)

déficience intellectuelle

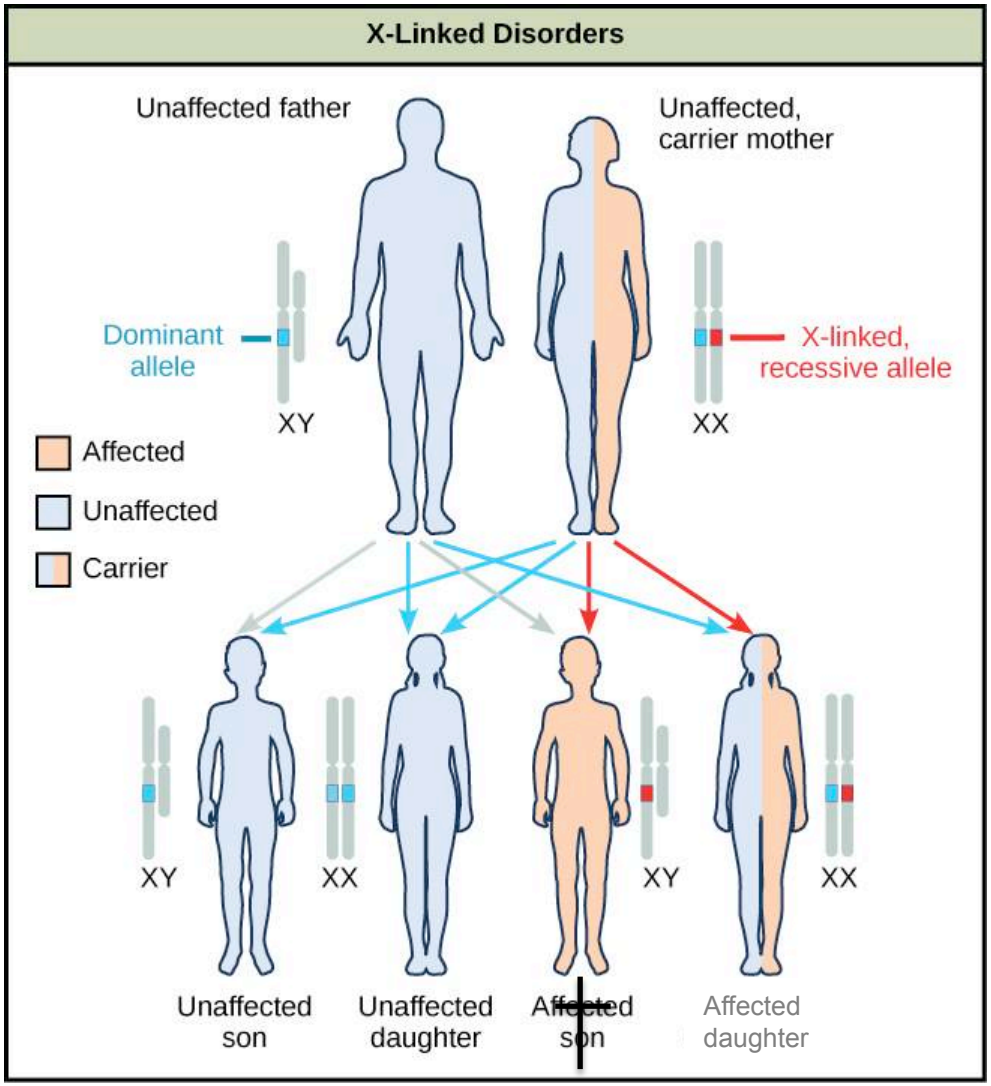


Greenwood Genetic Center, updated July 2009

Implications of X inactivation for X-linked Diseases

Lethality in males
Severe and variable phenotypes in females

Rett Syndrome (MECP2) – affects 1/10 000 girls – severe form of autism



Rett Syndrome

letter

Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl-CpG-binding protein 2

Ruthie E. Amir[1], Ignatia B. Van den Veyver[2,3], Mimi Wan[5], Charles Q. Tran[3], Uta Francke[5,6] & Huda Y. Zoghbi[1,2,4]

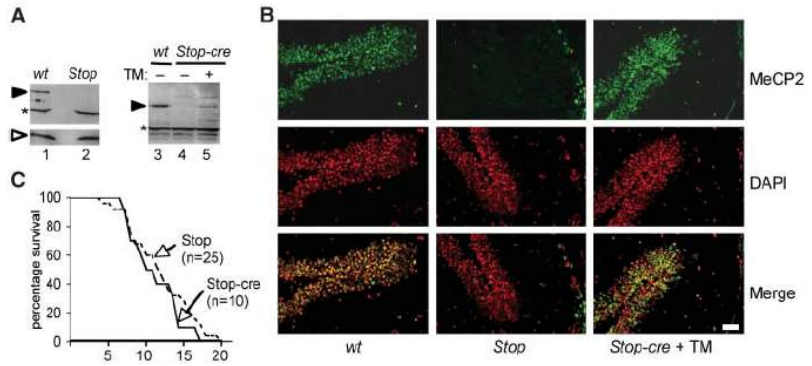
- Rett syndrome (RTT) : progressive neurodevelopmental disorder (Andreas Rett, 1966)
- One of the most common causes of mental retardation in females (incidence 1 in ~10,000)
- Patients with classic RTT appear to develop normally until 6–18 months of age, then gradually lose speech and purposeful hand use, and develop microcephaly, seizures, autism, ataxia, intermittent hyperventilation and stereotypic hand movements.
- After initial regression, the condition stabilizes and patients usually survive into adulthood.
- RTT occurs almost exclusively in females
- *Mecp2* was identified in 1999 as the gene responsible for Rett's syndrome.

Mecp2 re-expression can reverse neurological defects observed in Rett Syndrome mouse model

Reversal of Neurological Defects in a Mouse Model of Rett Syndrome

Jacky Guy,[1] Jian Gan,[2] Jim Selfridge,[1] Stuart Cobb,[2] Adrian Bird[1]*

Rett syndrome is an autism spectrum disorder caused by mosaic expression of mutant copies of the X-linked *MECP2* gene in neurons. However, neurons do not die, which suggests that this is not a neurodegenerative disorder. An important question for future therapeutic approaches to this and related disorders concerns phenotypic reversibility. Can viable but defective neurons be repaired, or is the damage done during development without normal MeCP2 irrevocable? Using a mouse model, we demonstrate robust phenotypic reversal, as activation of MeCP2 expression leads to striking loss of advanced neurological symptoms in both immature and mature adult animals.



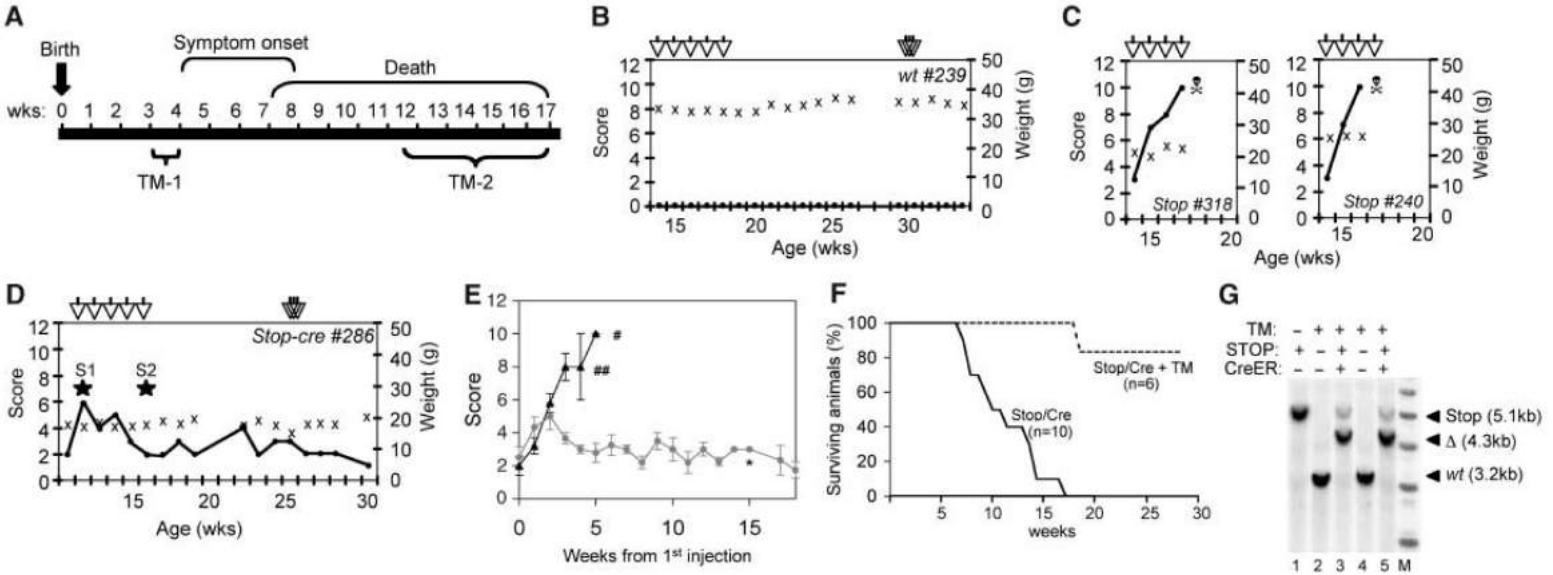


Fig. 2. Reversal of the neurological phenotype by activation of the *Mecp2* gene in *Stop/y,cre* males. (**A**) Time course of the *Stop/y* phenotype. (**B**, **C**, and **D**) Plots of the phenotypic scores (●) and weights (x) of individual wild-type (*wt*) (B), *Stop/y* (*Stop*) (C), and *Mecp2*$^{lox\text{-}Stop/y}$,*cre-ER* (*Stop-cre*) (D) animals after TM injections (vertical arrows). (See also fig. S2.) Stars in (D) indicate when the clips shown in movies S1 and S2 were recorded. (**E**) Aggregate symptom score profiles following TM injection of *Stop/y,cre* (○, n = 3 to 6, except *, which was a single animal) and *Stop/y* (▲, n = 4 to 5; except ## and #, which are 2 and 1 data points, respectively) mice. (**F**) Survival profiles of TM-treated *Stop/y,cre* mice and control *Stop/y* mice. (**G**) Southern blot showing deletion of the lox-Stop cassette (lanes 3 and 5) after a weekly TM injection regime + booster injections.

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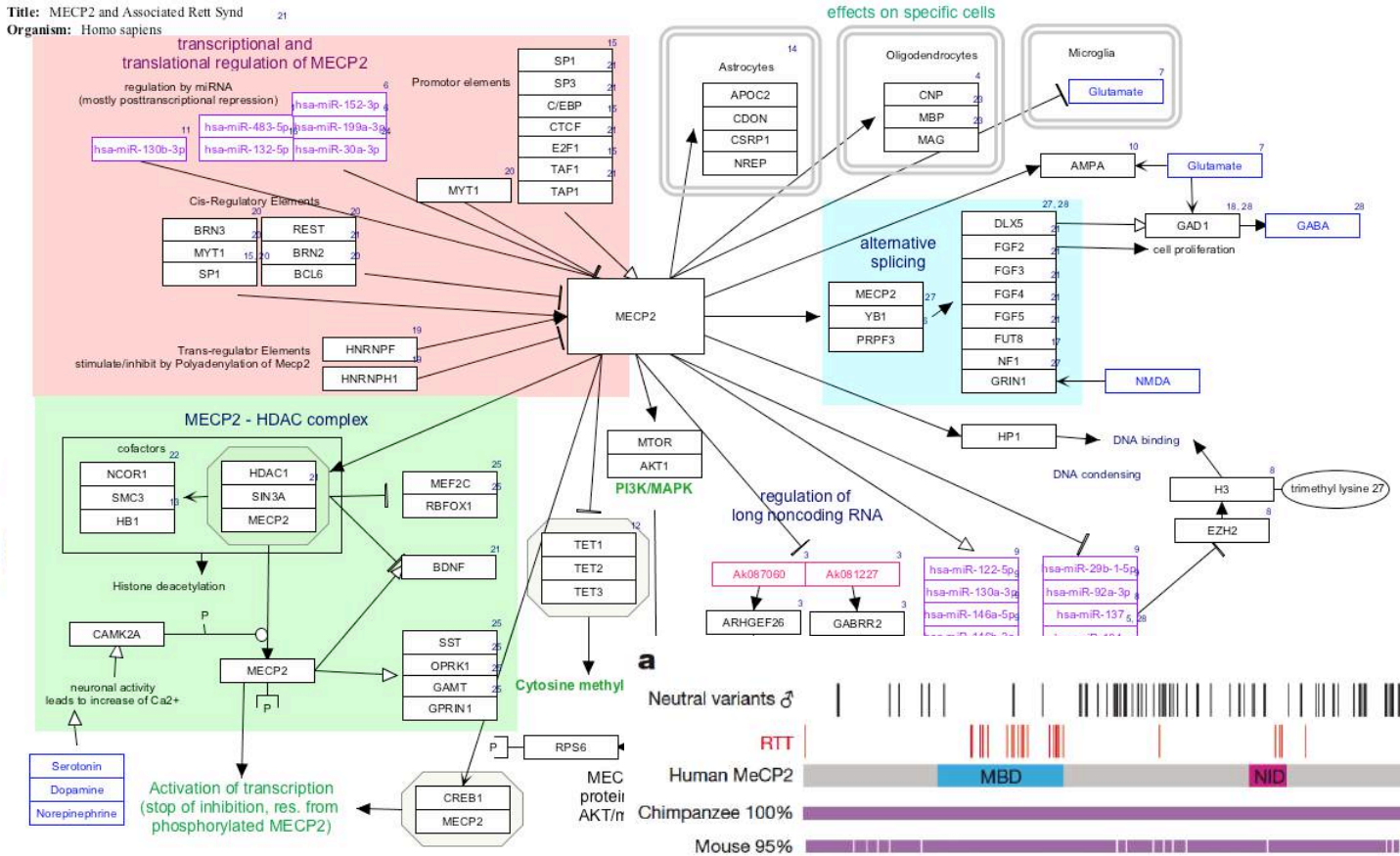
Functions of Mecp2 protein and role(s) in Rett Syndrome?



LETTER

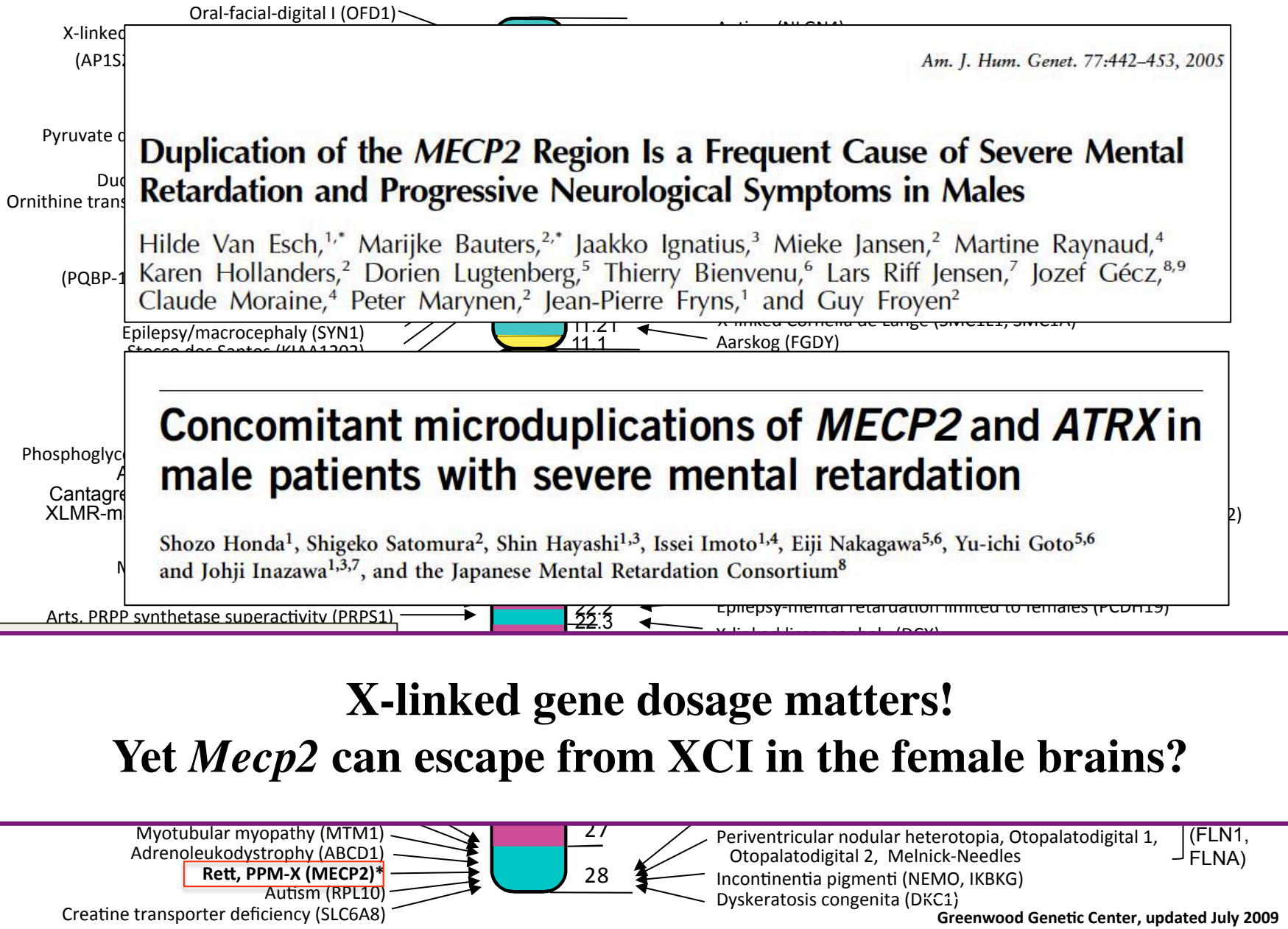
doi:10.1038/nature24058

Radically truncated MeCP2 rescues Rett syndrome–like neurological defects

Rebekah Tillotson[1], Jim Selfridge[1], Martha V. Koerner[1], Kamal K. E. Gadalla[2,3], Jacky Guy[1], Dina De Sousa[1], Ralph D. Hector[2], Stuart R. Cobb[2] & Adrian Bird[1]

X-linked intellectual disability(XLID)

déficience intellectuelle



Am. J. Hum. Genet. 77:442–453, 2005

Duplication of the *MECP2* Region Is a Frequent Cause of Severe Mental Retardation and Progressive Neurological Symptoms in Males

Hilde Van Esch,[1,*] Marijke Bauters,[2,*] Jaakko Ignatius,[3] Mieke Jansen,[2] Martine Raynaud,[4] Karen Hollanders,[2] Dorien Lugtenberg,[5] Thierry Bienvenu,[6] Lars Riff Jensen,[7] Jozef Gécz,[8,9] Claude Moraine,[4] Peter Marynen,[2] Jean-Pierre Fryns,[1] and Guy Froyen[2]

Concomitant microduplications of *MECP2* and *ATRX* in male patients with severe mental retardation

Shozo Honda[1], Shigeko Satomura[2], Shin Hayashi[1,3], Issei Imoto[1,4], Eiji Nakagawa[5,6], Yu-ichi Goto[5,6] and Johji Inazawa[1,3,7], and the Japanese Mental Retardation Consortium[8]

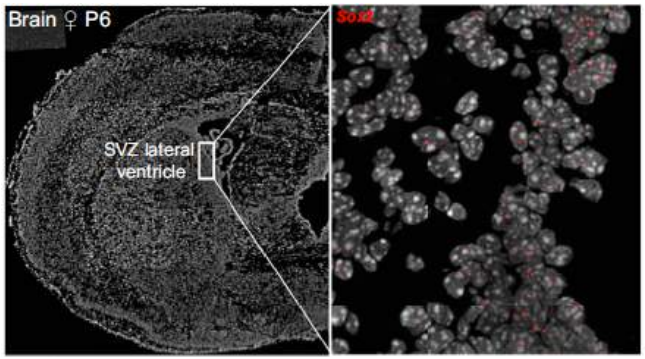
X-linked gene dosage matters!

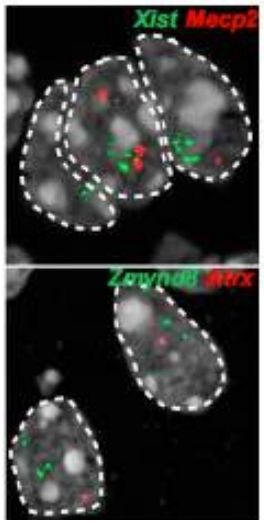
Yet *Mecp2* can escape from XCI in the female brains?

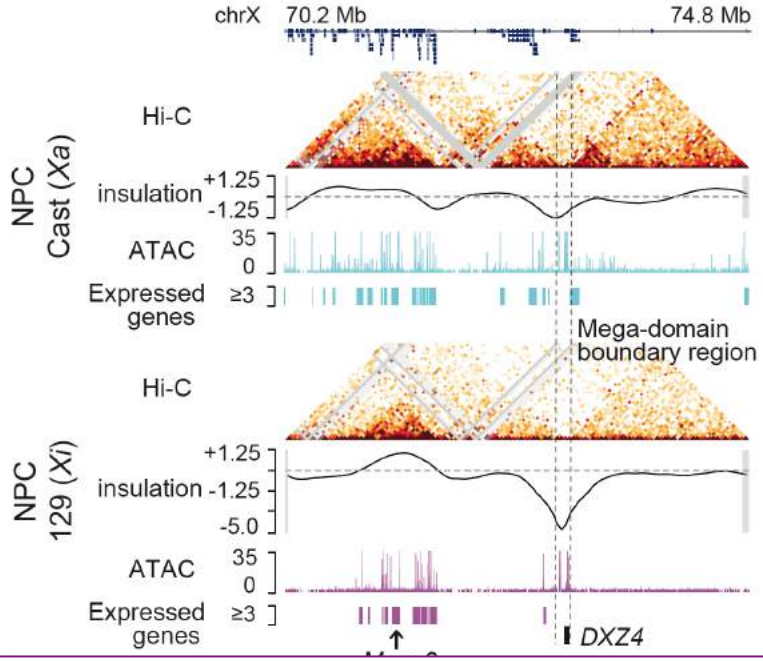
Mecp2 escape from X-inactivation in a subset of cells in the brain *in vivo* (*Where, When, How, Why)*

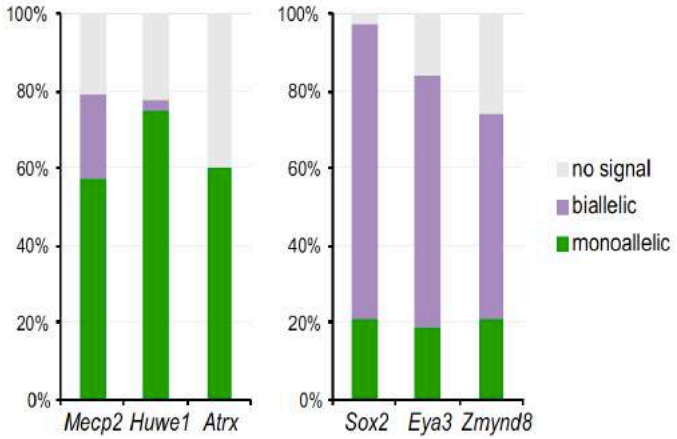
Early post natal (P6) brain:
Mecp2 escape was only seen in SVZ – in ~20% *Sox2* positive (neural stem?) cells

Nascent RNA-FISH on adult brain sections





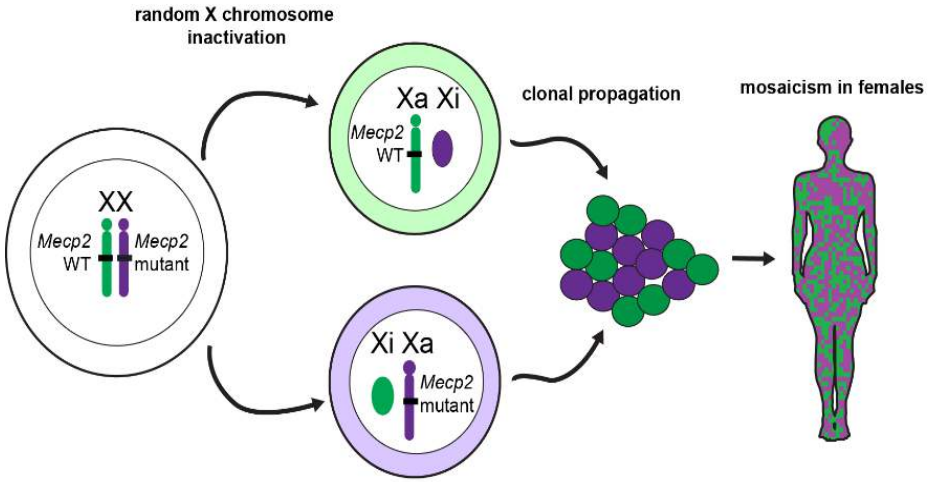




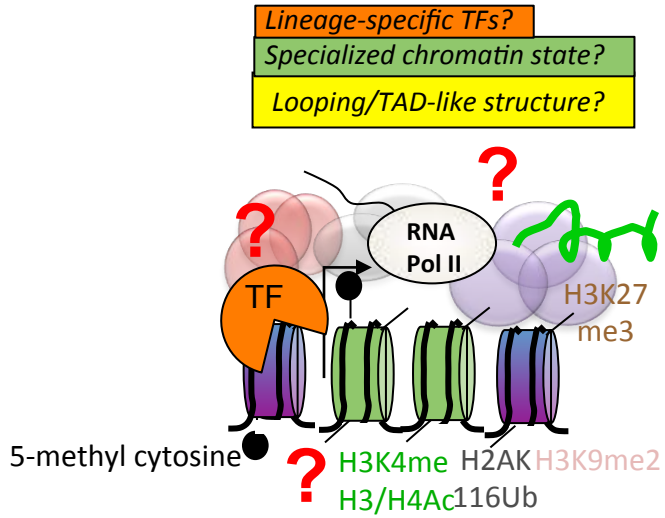
Gendrel et al, Dev. Cell 2014

Formation of TAD accompanies *Mecp2* escape on the Xi in NPCs
Which cells escape and why *in vivo*?
What is the chromatin and topological state of Mecp2 escapee cells *in vivo*?
Therapeutic targeting strategy for *Mecp2* reactivation in Rett's syndrome?

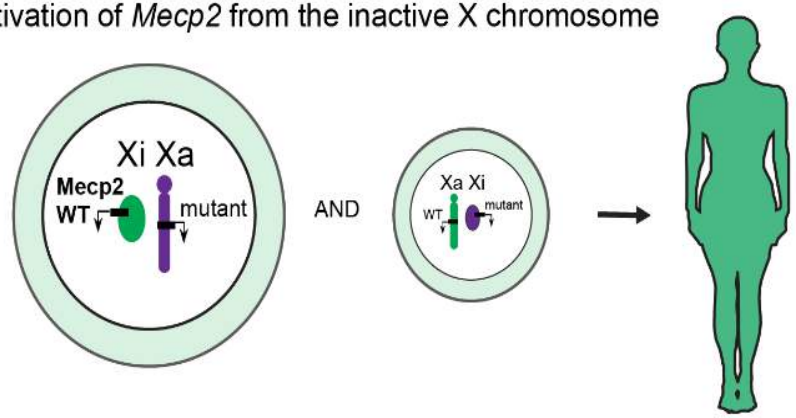
Reversing *Mecp2* silencing on the inactive X chromosome



Reactivation from the Xi



Reactivation of *Mecp2* from the inactive X chromosome



- Epigenetic drugs? (but non-specific effects)
- CRISPR/dCas9 delivery of transcriptional activators to the silent *Mecp2* allele
- Or of architectural proteins to promote TAD formation

Precise timing and cellular context of *Mecp2* reactivation will be critical
(NB dosage sensitivity of Mecp2 during development: *Mecp2* duplication syndrome)

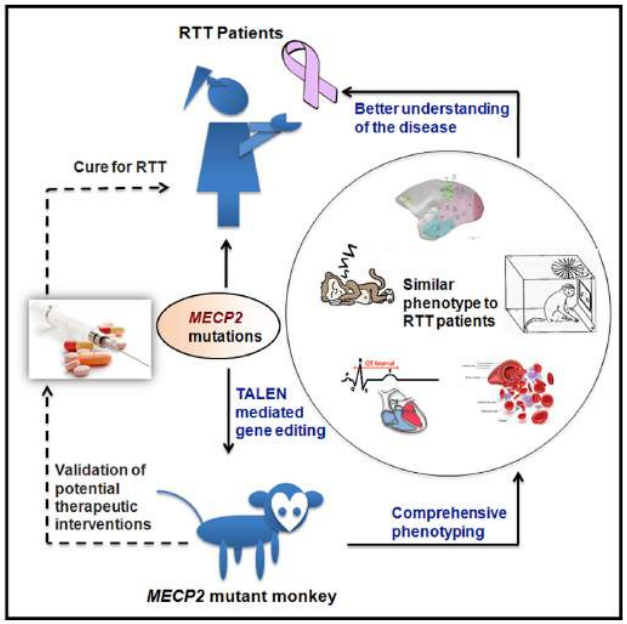
Modeling Rett syndrome in CynomolgusMonkeys

Article

Cell

Modeling Rett Syndrome Using TALEN-Edited *MECP2* Mutant Cynomolgus Monkeys

Graphical Abstract



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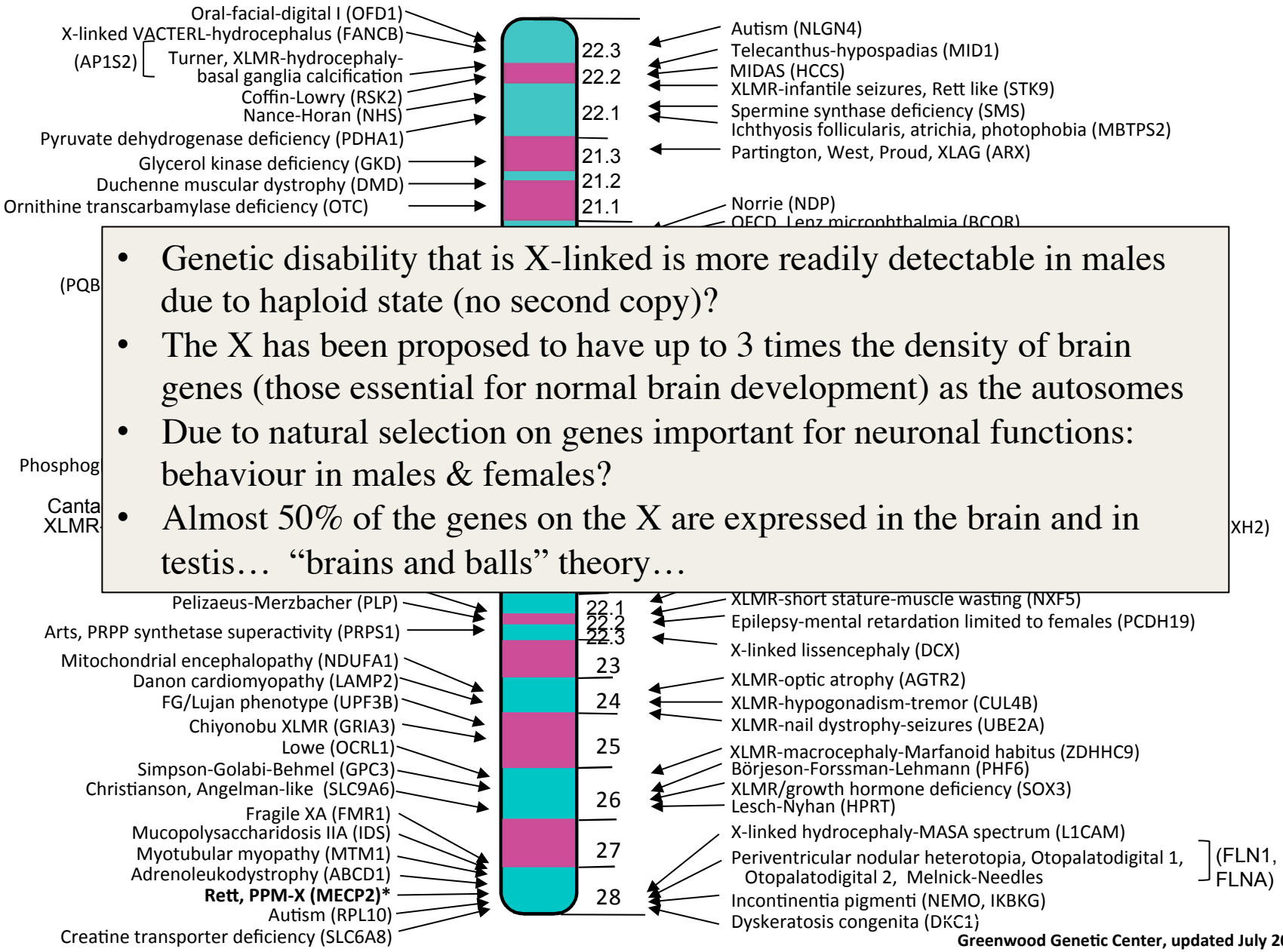
In Brief

TALEN-edited MeCP2 mutant monkeys share phenotypes with Rett syndrome patients, providing a valuable model for studying disease mechanisms and for the development of potential therapeutics.

Highlights

- Modeling Rett syndrome using TALEN-edited MECP2 mutant cynomolgus monkeys
- MECP2 mutations induce male lethality and females resembling RTT patients
- Phenotypes include brain developmental and complex behavioral abnormalities
- The model will facilitate the deciphering of RTT mechanisms and development of new interventions

X-linked intellectual disability(XLID) genes: Why so Many?



- Genetic disability that is X-linked is more readily detectable in males due to haploid state (no second copy)?
- The X has been proposed to have up to 3 times the density of brain genes (those essential for normal brain development) as the autosomes
- Due to natural selection on genes important for neuronal functions: behaviour in males & females?
- Almost 50% of the genes on the X are expressed in the brain and in testis… "brains and balls" theory…

Greenwood Genetic Center, updated July 2009

X-linked Genes and their proposed roles in "Intelligence"?

J Med Genet 1991; 28: 429–432

LETTERS TO THE EDITOR

Genes for intelligence on the X chromosome

Some 20 years ago Robert Lehrke, a psychologist from Minnesota working in a state hospital for the mentally retarded, suggested that genes that determine the major intellectual traits are carried on the X chromosome.[1 2] At that time Lehrke was severely criticised on the grounds that his hypothesis was inherently improbable,[3] and that the evidence was meagre and could be interpreted in other ways.[4 5] Since then more medical evidence has accumulated to support two of the steps in Lehrke's argument.

(1) *"The well documented excess of males among the mentally retarded (25–50%)"*. Two further studies[6 7] have shown that this male excess results from mutations on the X chromosome, using as evidence the excess of affected brothers over affected sisters and calculating this as a gene frequency for X linked forms of mental retardation.

(2) *"A review of families published at that time with mental retardation showing an X linked pattern of inheritance—which only numbered 5, together with 5 new families that he had identified"*. In the former group three were shown later to have the fragile X syndrome and this we now know is very common. A further two had specific features, one spasticity and the other obesity, and in the remainder, as best as can be judged, the clinical description fell into the non-specific group. As we can see in this issue, this is the most common form. There are now three separate gene localisations, *MRX1*, *MRX2*, and *MRX3*, and it seems likely that more loci will be defined in the future. His suggestion, therefore, that X linkage may be important, is being cemented by fact.

Lehrke's two other arguments were the lesser variability and reduced extremes of intelligence in the female when compared with the male, which he suggested resulted from the averaging out of the effects at different alleles through Lyonisation. He also noted that mental retardation was transmitted more often from mother to child than from father to child.

If there are genes which directly determine intellectual traits, then one would expect that mutations of such genes would produce phenotypes showing only effects on intelligence, perhaps with secondary effects on behaviour and personality. If so, there should also be no somatic changes, no recognisable metabolic abnormalities, no other neurological signs, and no progression with age, although the effects of the mutations would be less obvious in infancy than in childhood when intellectual thought becomes evident. This is the clinical picture of non-specific mental retardation. Clinical descriptions of autosomal dominant and recessive forms of non-specific mental retardation are rare, ill defined, and found mainly in older publications. The X linked forms are common and are now being mapped on the X chromosome. We would like to reawaken Lehrke's hypothesis and suggest that the mutations that we are now locating associated with non-specific mental retardation are those that have determined the higher intelligence of homo sapiens.

Why should intelligence be coded primarily on the X chromosome? Although, as Ohno[8] and others have stressed, genes on the X chromosome have been conserved throughout mammalian evolution we have to suppose that, in man, additional genes for intelligence have arisen there. Once they had appeared their advantage in a hunter-gatherer society would assure male dominance and rapid dissemination throughout the group.[9] In recent correspondence on this subject Ohno philosophised: "Most mammalian species, including our own, are noticeably sexually dimorphic. As a rule such species practice the polygamous, more precisely the polygynous, mating system; after exhaustive combat between adult males, only the victor gains possession of a large number of females. Is it not ironic if the reward of a victor has been to transmit his intelligence only to his daughters and never to his sons. If the main genetic source of intelligence resides on the X chromosome, man, at least, should have organised the matriarchial society with the polyandrous mating system. Perhaps we are still paying for the mistake of organising the patriarchal society of kings and dukes."

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X linked complicated spastic paraplegia, MASA syndrome, and X linked hydrocephalus owing to congenital stenosis of the aqueduct of Sylvius: variable expression of the same mutation at Xq28

Hereditary 'pure' spastic paraplegia is a disorder characterised by progressive spasticity of the legs in otherwise normal subjects. In the majority of families pedigree data are in accordance with autosomal dominant inheritance, but X linked recessive transmission has also been documented.[1] In the 'complicated' form the spasticity may be combined with a variety of one or more symptoms, such as mental retardation, micro- and macrocephaly, epilepsy, and ocular symptoms.[2 3]

In 1974 Blanchine and Lewis[4] delineated, on the basis of clinical and

PERSPECTIVES ON PSYCHOLOGICAL SCIENCE

A Role for the *X* Chromosome in Sex Differences in Variability in General Intelligence?

Wendy Johnson,[1,2] Andrew Carothers,[3] and Ian J. Deary[1]

[1]University of Edinburgh, United Kingdom, [2]University of Minnesota–Twin Cities, and [3]Public Health Sciences, University of Edinburgh Medical School, United Kingdom

Commentary on "A Role for the *X* Chromosome in Sex Differences in Variability in General Intelligence?" (Johnson et al., 2009)

Ian W. Craig, Claire M.A. Haworth, and Robert Plomin

Social, Genetic, and Developmental Psychiatry Centre, Institute of Psychiatry, London, United Kingdom

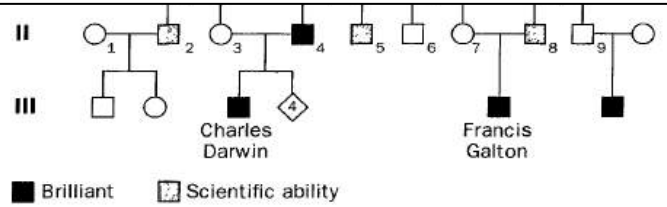


Figure: **Abridged pedigree of the Wedgwood, Darwin, Galton family tree[15]**

X-linked Genes and their proposed roles in "Intelligence"?

1. Many genetic defects affecting general intelligence are located on the X chromosome and hence are observed more often in males.

XLID affects 30%–50% more males than females, and a large number of X-linked pedigrees exist for this condition. However, autosomal genes associated with XLID are likely to be underrepresented - more difficult to detect them;

2. Some of these loci are implicated in "special abilities" affecting general intelligence, both throughout the distribution and at the extremes, and that, for at least some features of general intelligence, males show greater variance than females (ie extreme alleles: "genius" genes)

Although analysis of XLID mutations and sex chromosome anueploidies supports the contribution of X-linked genes to a wide range of developmental features relevant to the brain and cognition, there is **no simple way** to predict whether the 'wild type" allele of a XLID gene will enhance IQ, even though they may be necessary for 'normal" brain development!

3. Analysis of two data sets leads to conclusion that genes involved in general intelligence are overrepresented on the X chromosome

4. Propose that epigenetic and genetic regulatory mechanisms and genetic and co-evolutionary processes that underpin sex differences in patterns and differences in ability and that these provide opportunities for rapid evolutionary response to changing circumstance.

- Genes that arise with male advantage, in comparison with those that arise with female disadvantage, will benefit from being X linked because their immediate expression in males is not masked by the presence of a second X.
- Evidence for accumulation of "male advantageous" genes on the X in mice: 10 of the 12 spermatogenesis genes detected were found to be located on the X (Wang, Page, 2001) & in Humans (Ross et al, 2005) remarkable concentration of X-linked testis-associated genes.
- Might X-linked genes that impact on intelligence *promote* reproductive success in males (sexual selection)?
- Might X-linked genes that escape from XCI confer a reproductive advantage to females?
- Much more difficult to prove!

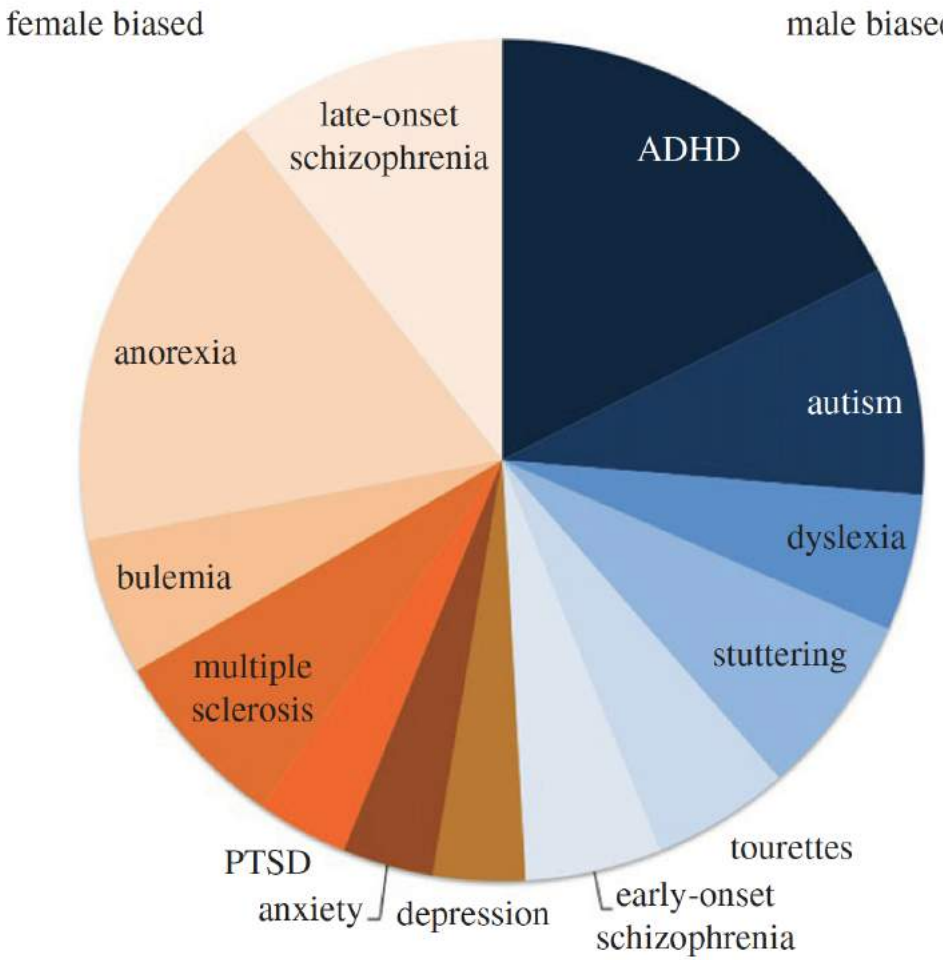
Are genes the escape X inactivation under selection?

Genes That Escape X-Inactivation in Humans Have High Intraspecific Variability in Expression, Are Associated with Mental Impairment but Are Not Slow Evolving

Yuchao Zhang,[1,2] Atahualpa Castillo-Morales,[3] Min Jiang,[1] Yufei Zhu,[1] Landian Hu,[1] Araxi O. Urrutia,[3] Xiangyin Kong,[*,1] and Laurence D. Hurst[*,3]

Sex Differences in Neurological functions?

- Pervasive gender bias in the frequency of diagnosis of numerous disorders.
- Identification of biological variables provides diagnostic value and insight into disease aetiology.
- Hormonal effects versus sex chromosome complement? (ie XY, XX, escapees etc)



- Autism spectrum disorder is diagnosed in boys four to five times more often than girls
- Schizophrenia manifests differently in men and women across lifespan
- Unipolar depression and PTSD are up to twice as frequent in women and girls. This may be skewed by social factors such as willingness to seek treatment.
- Differences in drug and alcohol abuse in men and women are speculated to be based in sex differences in risk-seeking and reward systems.

More Next Week

CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2017-2018 :

"Le chromosome X -
paradigme de la génétique et l'épigénetique"

26 février, 2018

Cours V

Le chromosome X et les maladies autoimmunes