CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2017-2018 :

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

12 février, 2018

Cours III

Dynamique de l'hétérochromatine facultative

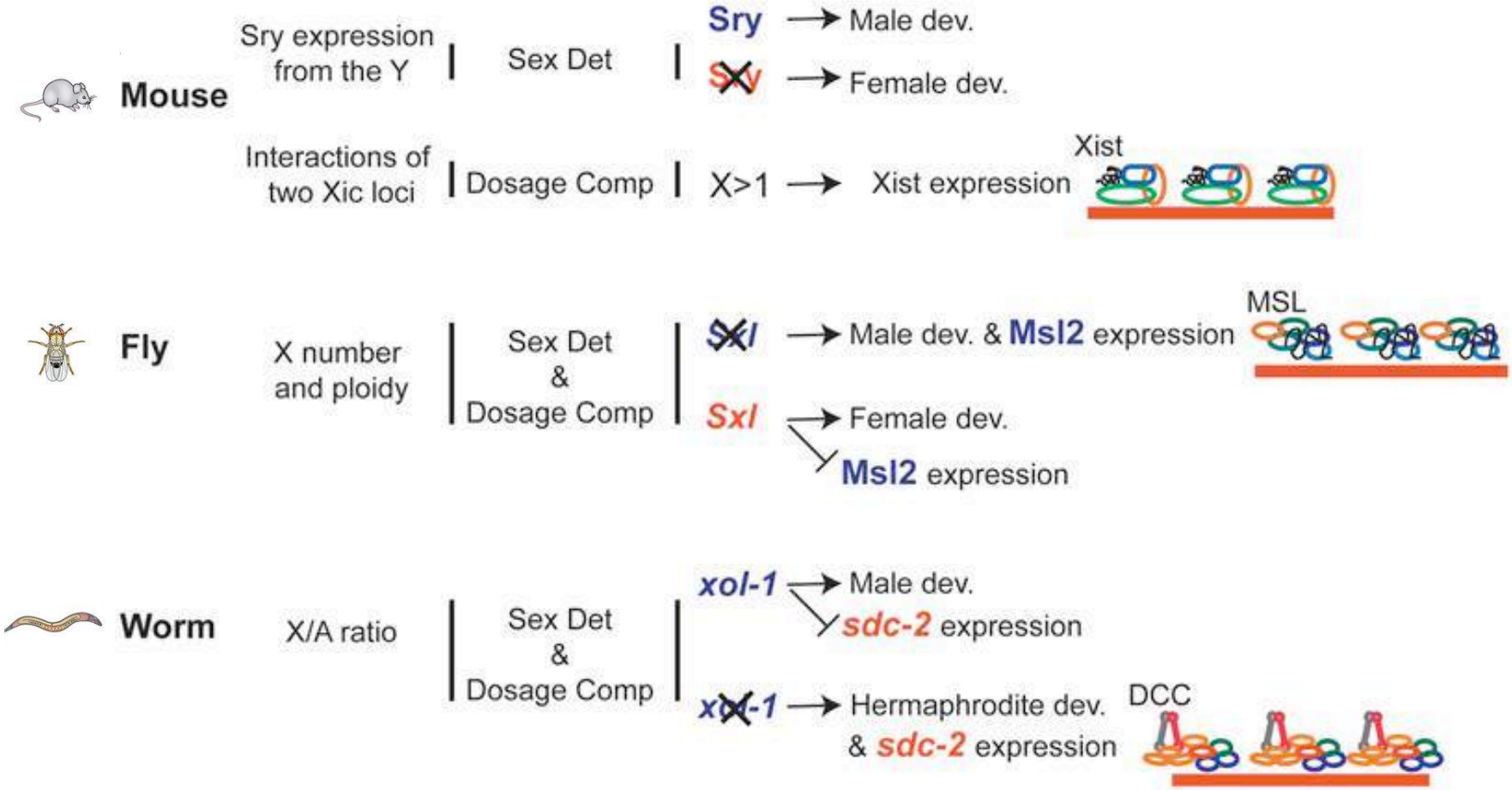
Dynamics of facultative heterochromatin

SUMMARY of last week

- **Very diverse strategies used to achieve dosage equality between the sexes**! Observed in many XX and XY (or XO) animals (flies, worms, placental mammals) but not others
- **Dosage does matter**- failure to establish sex chromosome dosage compensate is lethal during mouse development: perturbs mbryonic and extraembryonic development
- **Dosage compensation is not necessarily chromosome-wide in all organisms**
- **Some genes are more dosage sensitive than others** –this may vary between individuals and in different tissues
- X-chromosome up-regulation of the single X relative to autosomes (Ohno's Hypothesis) is **not** a universal principle –applies to just **some dosage sensitive genes** in humans for ex.
- Sex determination and dosage compensation are triggered by the same pathway in Drosophila and C. elegans -but not in mammals (XX dosage for XCI, Sry for sex)
- Targeting / modulating dosage compensation factors to the sex chromosomes involves DNA elements (C. elegans , Drosophila) and non-coding RNAs (Drosophila, mammals)
- And diverse chromatin and chromosomal complexes

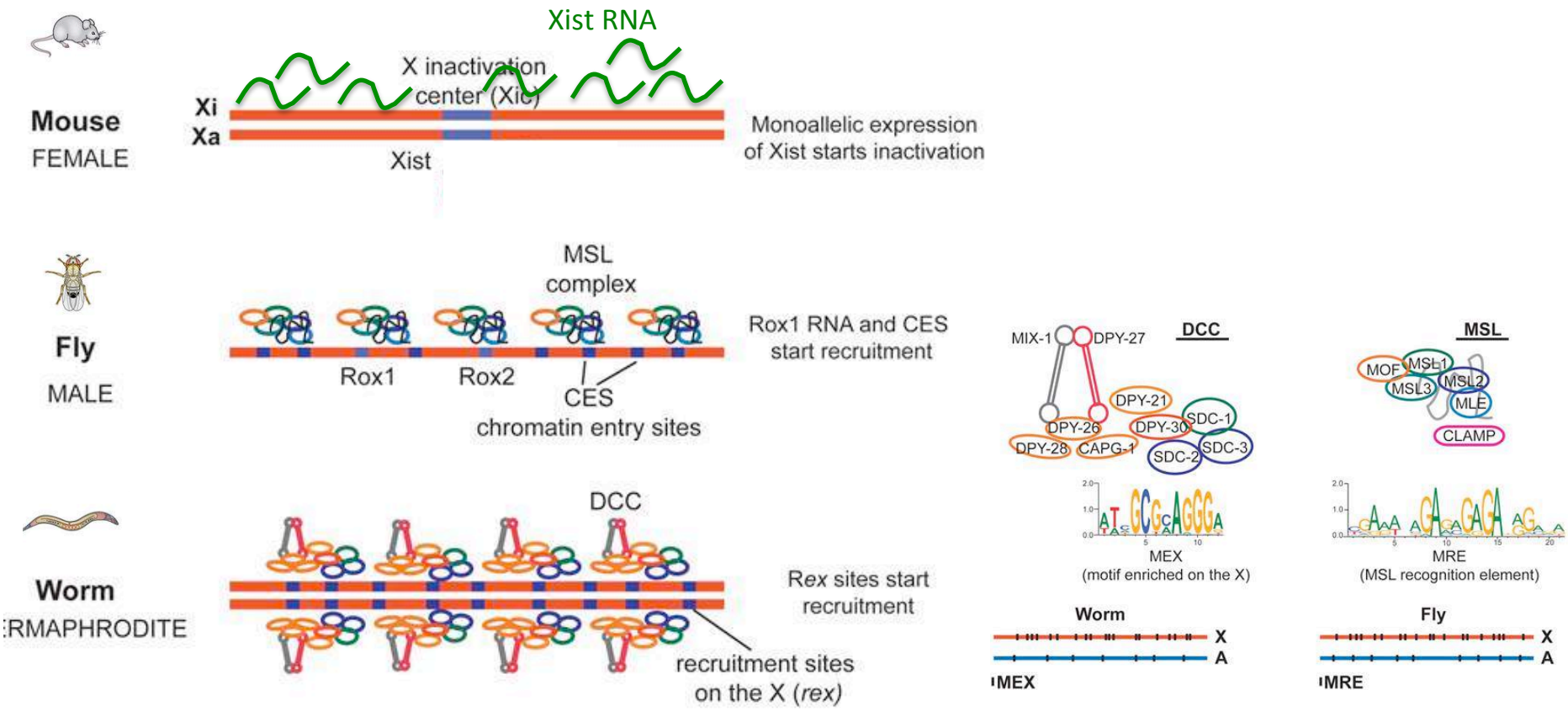
Facultative heterochromatin in dosage compensation

Sex Chromosome Dosage Compensation : Triggering Differential Gene Expression & Chromatin



S. Ercan, J Genomics 2015; 3:1-19. doi:10.7150/jgen.10404

Sex Chromosome Dosage Compensation : Triggering Differential Gene Expression & Chromatin



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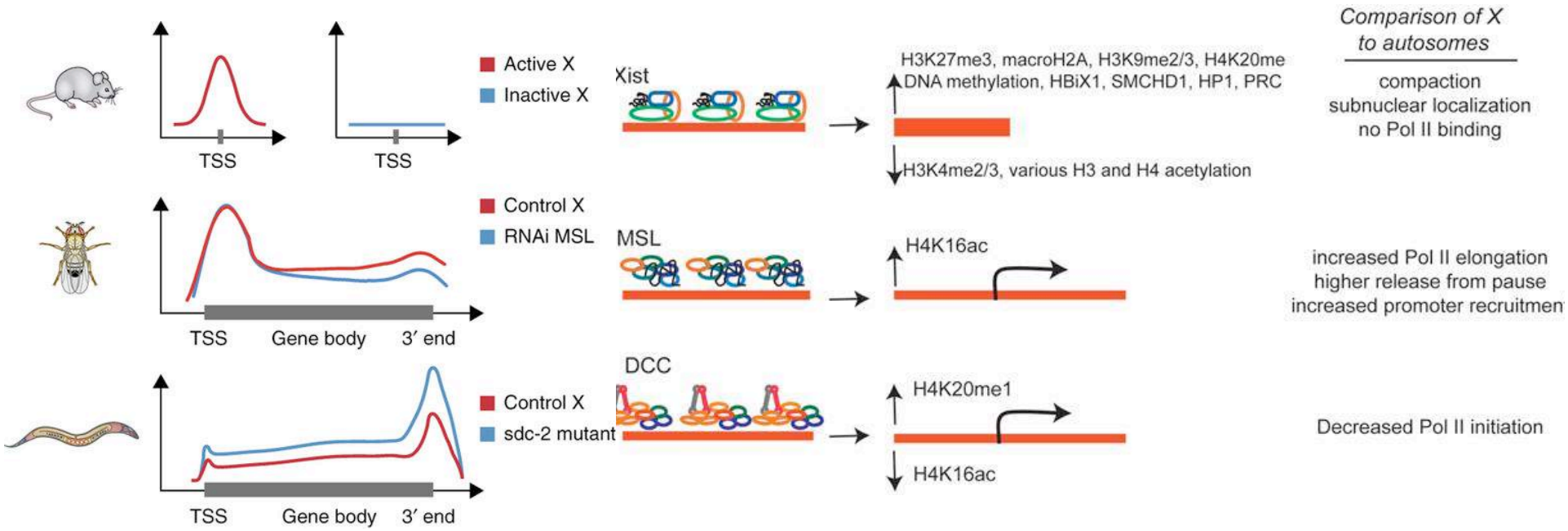
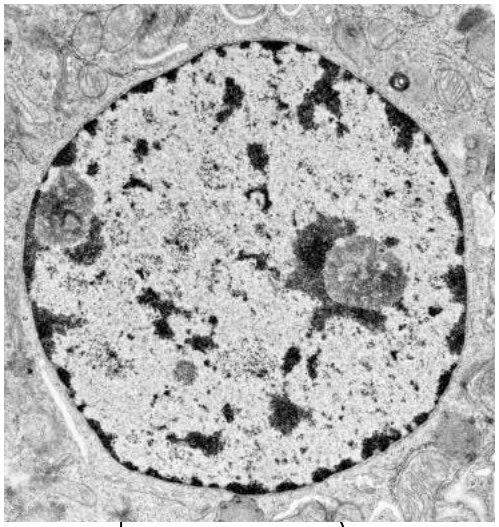
Sex Chromosome Dosage Compensation : Triggering Differential Gene Expression & Chromatin



Maintaining differential activity states requires chromatin changes in all three systems

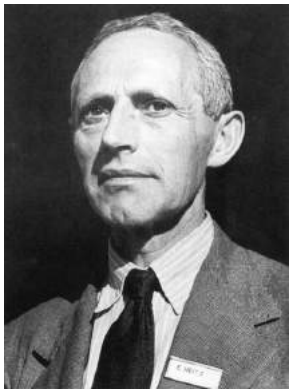
S. Ercan, J Genomics 2015; 3:1-19. doi:10.7150/jgen.10404

Differential States of Chromatin



Euchromatin Heterochroma

http://medcell.med.yale.edu/histology



Emile Heitz
(1892-1965)

"The Heterochromatin of Moss"
"*…heterochromatin refers to a part of a chromosome that remains heteropyknotic after telophase and thus behaves opposite to euchromatin*" Heitz (1928)

Constitutive heterochromatin tends to be non-coding and repetitive (Pontecorvo, 1944)

Facultative heterochromatin
("facultas" = opportunity) can be either active or inactive (eg Barr body, 1949; Lyon, 1961)

Facultative Heterochromatin:
Potential for gene expression at some point in development and can be either **condensed** or **decondensed** depending on cell type
It is reversible, and only appears depending on the stage of development or the cell type examined.

The Inactive X chromosome
A classic examples of facultative heterochromatin

From M. Lyon, 1974

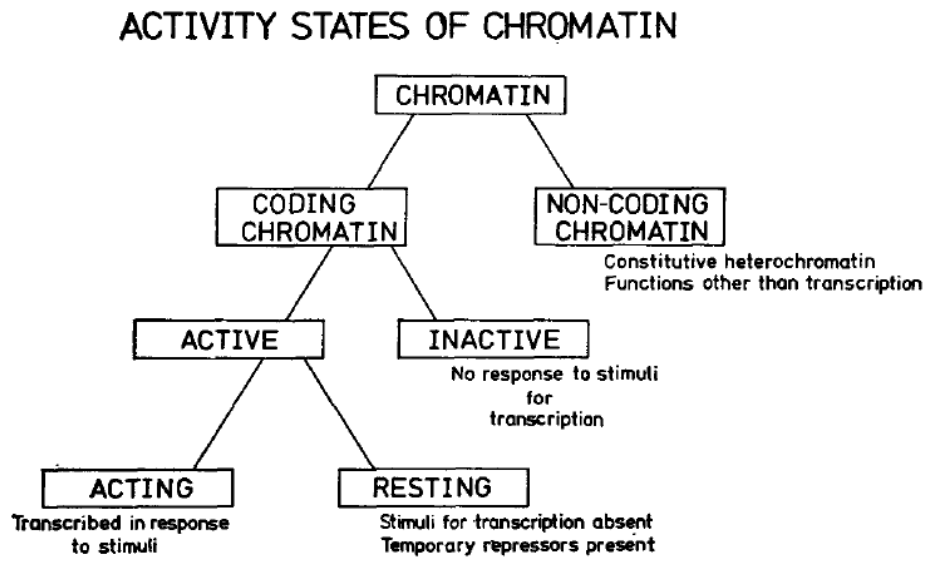


FIGURE 1.—Classification of eukaryote chromatin according to its functional state.

Genetics 78: 305–309 September, 1974.

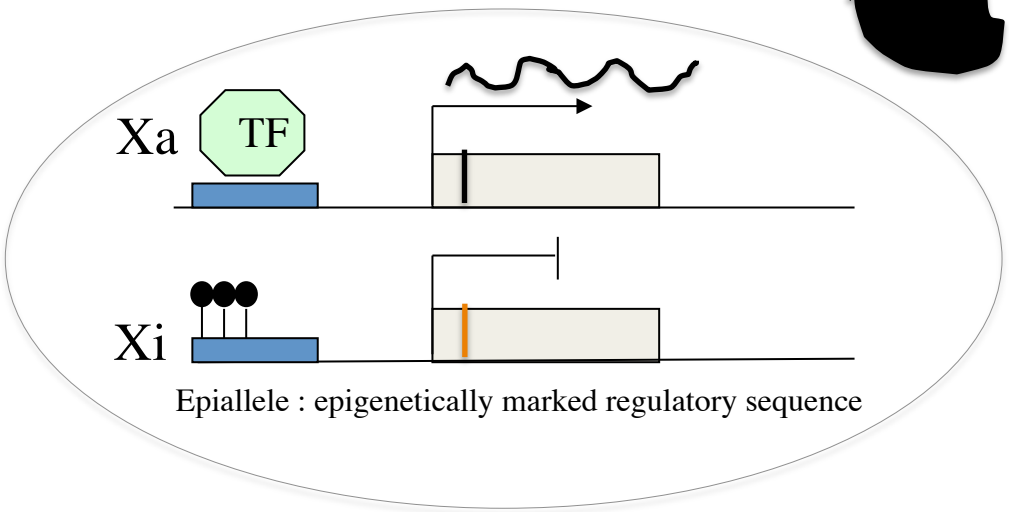
Facultative Heterochromatization

Heterochromatin may be studied least ambiguously when formed *de novo* during development, and we may contrast the two kinds in this regard. In *constitutive* heterochromatization both the homologous chromosomes, one maternal, the other paternal, respond in the same way during development. In *facultative* heterochromatization, the two homologous chromosomes differ; one becomes heterochromatic during development, and the other remains euchromatic. Thus facultative heterochromatization provides an unparalleled opportunity for studying the same genes in the two different states.

"*Changes in quantity, quality or structural organization of heterochromatic elements may well alter the kind and/or degree of particular exchanges that occur, and in this way control the chromosome organization and the kind and the relative effectiveness of genic action*" (McClintock, 1950). (see **COURS 2017**)

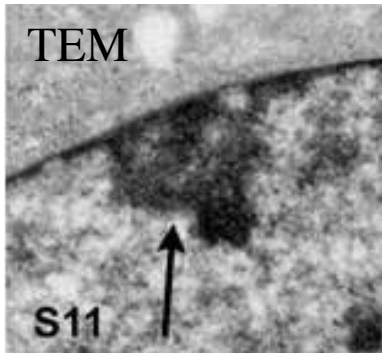
The Inactive X chromosome
A classic examples of facultative heterochromatin

Identical (or almost) DNA sequences
Differential gene activity states
Heritable through cell divisions



Epiallele : epigenetically marked regulatory sequence

Barr body
(Bertram and Barr, 1949)



Rego et al, 2008

The Inactive X chromosome
A classic examples of facultative heterochromatin

Identical (or almost) DNA sequences
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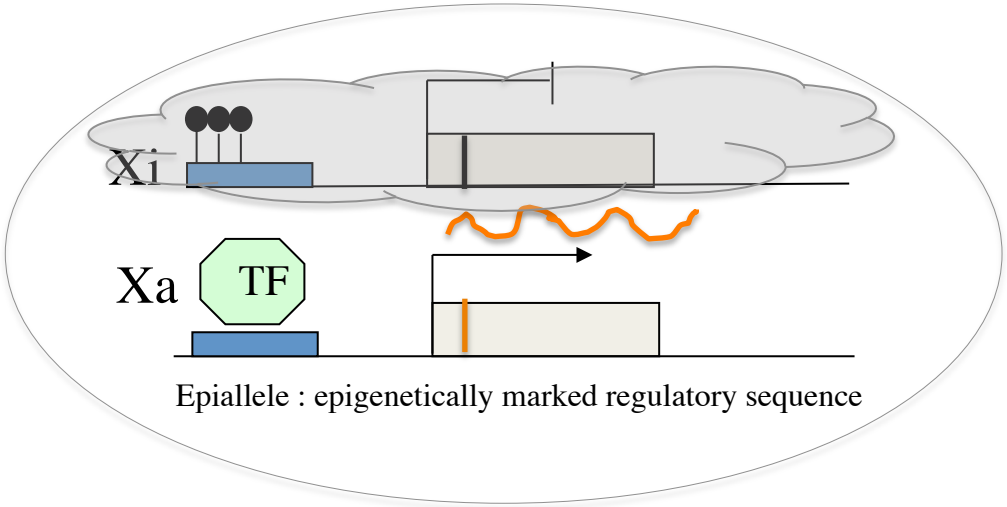

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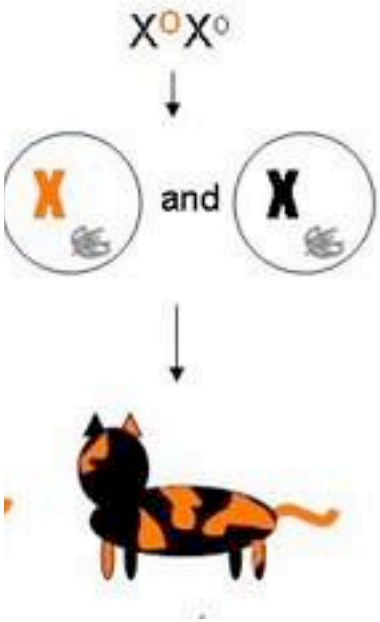

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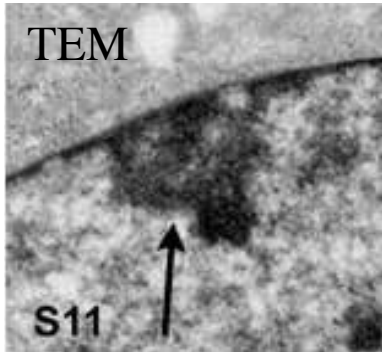
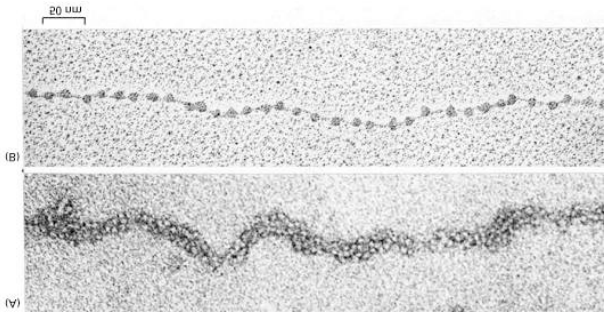


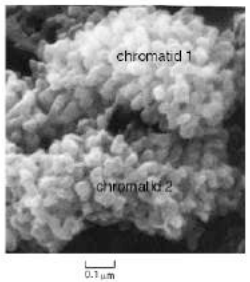
Rego et al, 2008

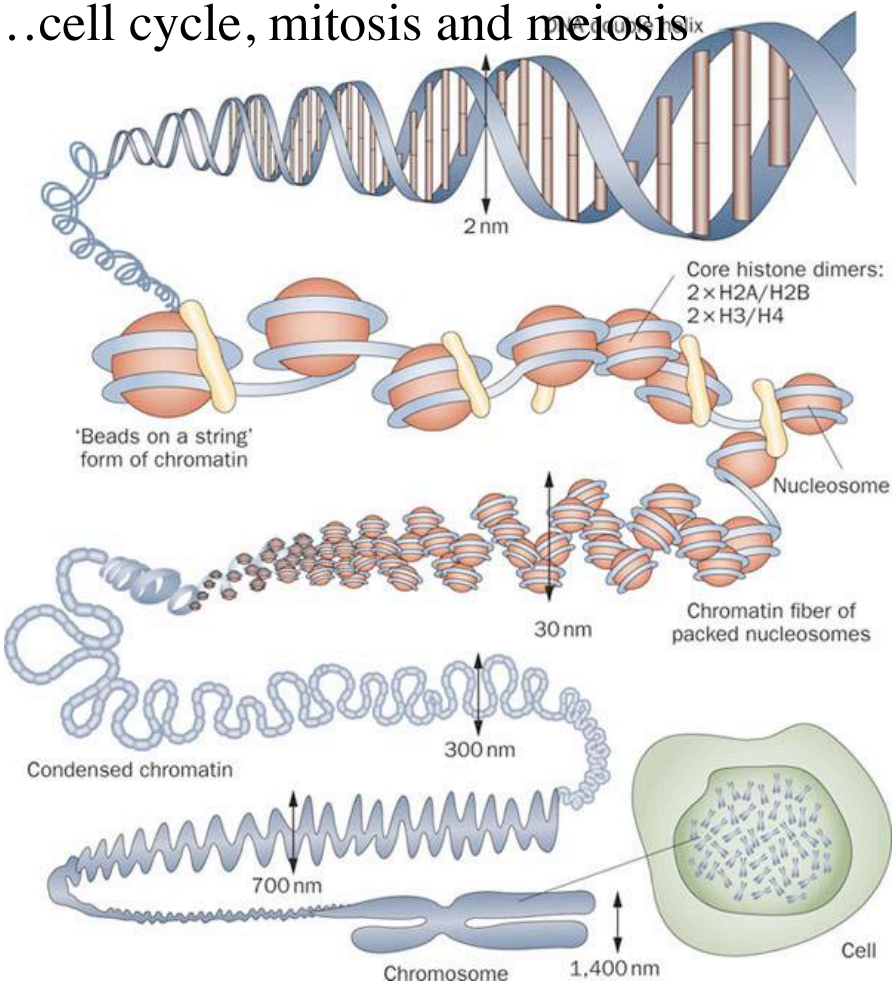
Heritable through cell divisions
Thanks to heterochromatic state…

Reminder: What are the Roles of Chromatin?

- The physiological template of the genome
- Packaging of the the genome in interphase and during mitosis
- Barrier to aberrant gene expression and reprogramming
- Facilitator of gene regulation and genome function
- Integration of environmental signals
- Carrier of cellular memory…cell cycle, mitosis and meiosis



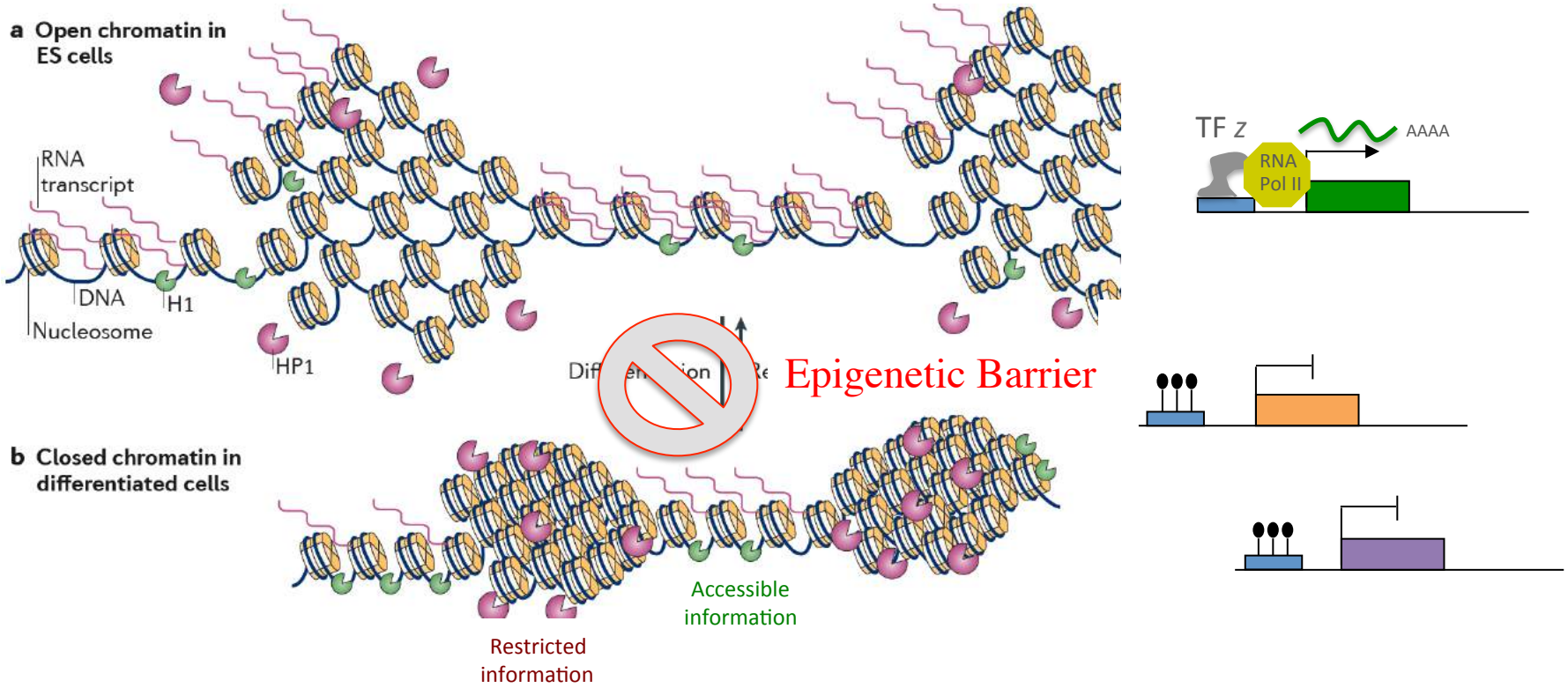




COLLÈGE DE FRANCE 1530

Stable Differential Gene Expression States

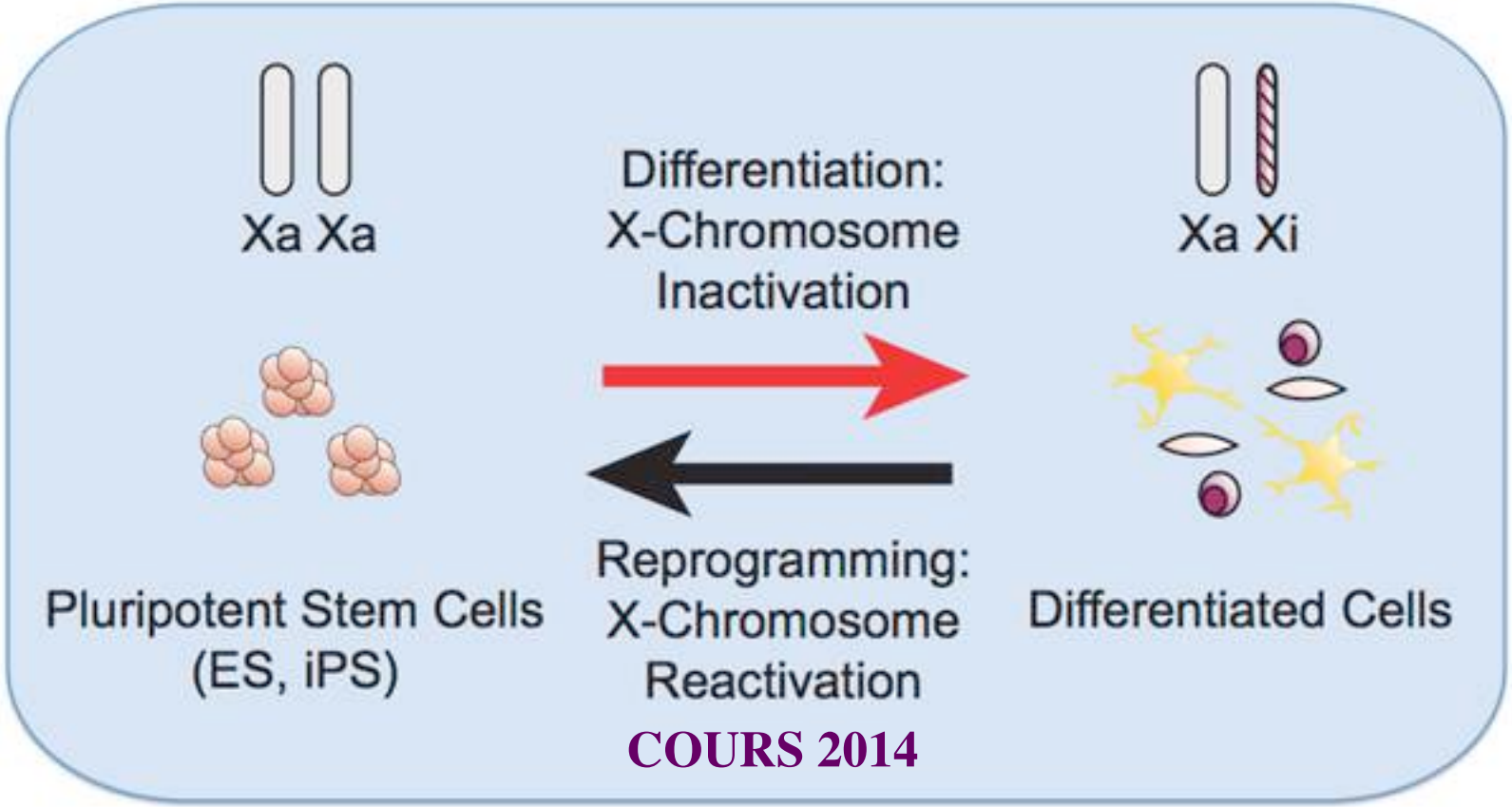
Transitions between euchromatin and heterochromatin



Chromatin states and chromatin compaction change during development and processes such as X-chromosome dosage compensation.

How many types of epigenetic change, or chromatin states?
What enables heritability and reversibility?

Transitions between euchromatin and heterochromatin



Chromatin states and chromatin compaction change during development and processes such as X inactivation.

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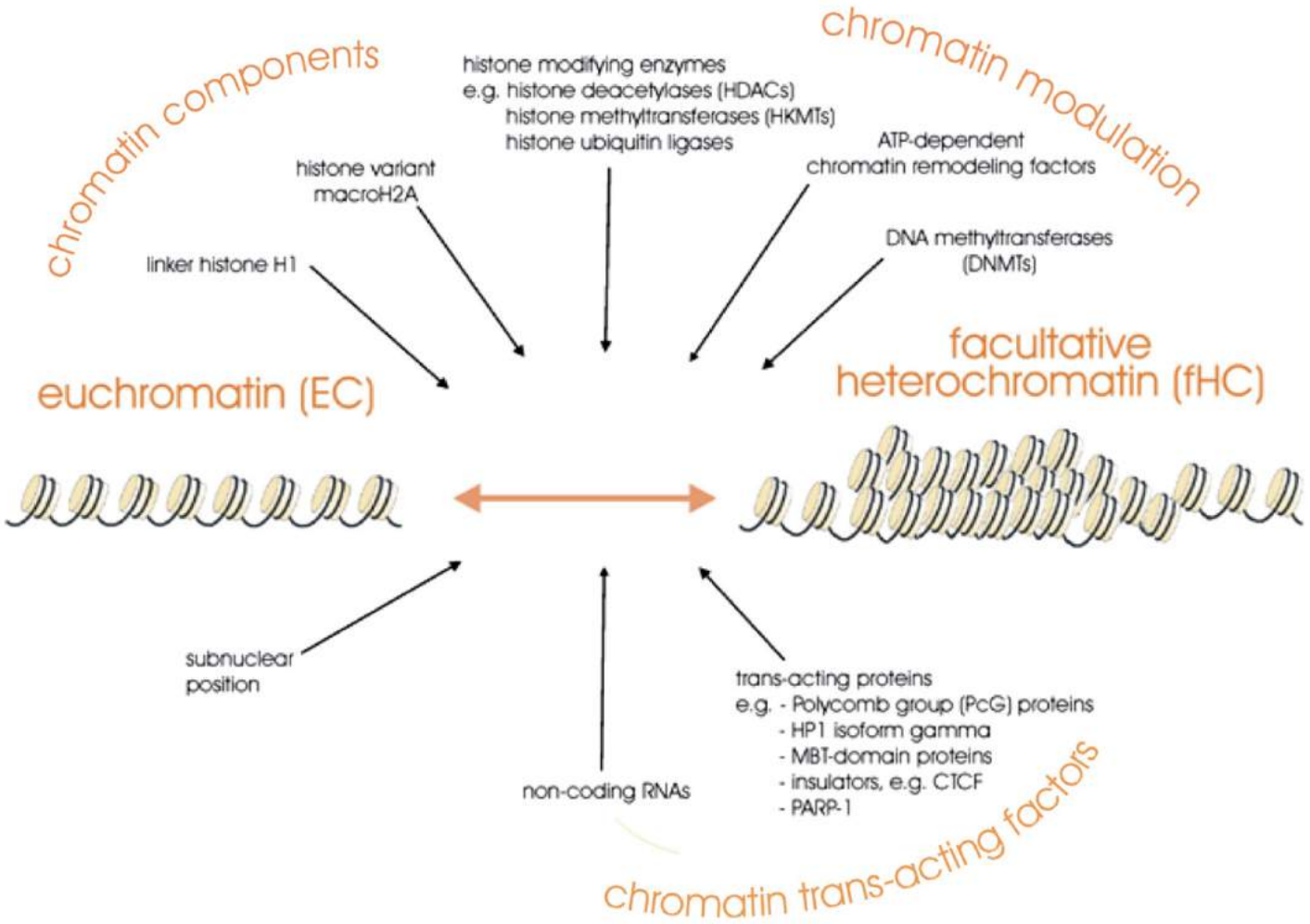
Facultative Heterochromatin



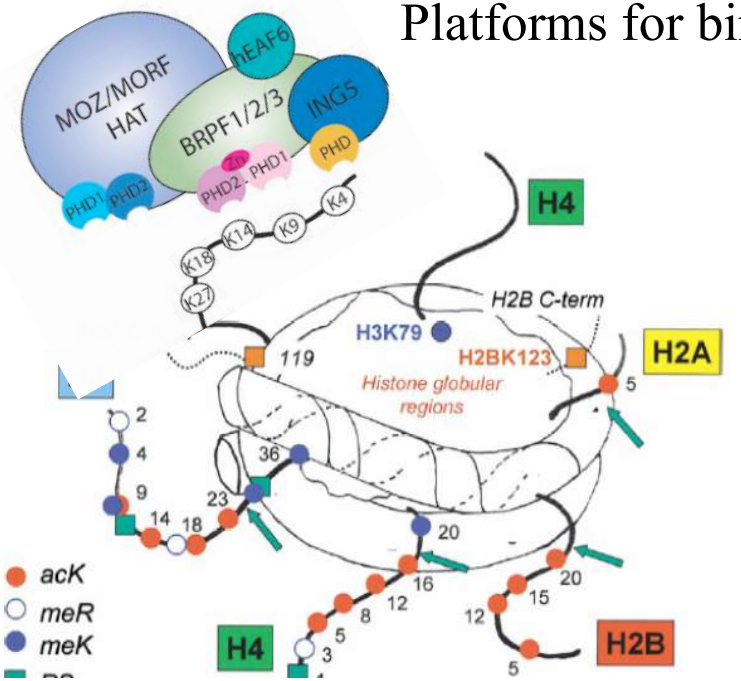
Trojer and Reinberg, 2007

Chromatin-based States and Partners

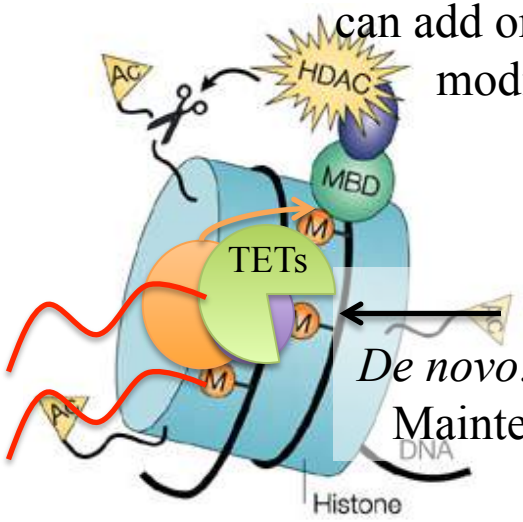
Histone Variants and Histone Modifications are:

Mediators of chromatin accessibility

Platforms for binding proteins



Histone modifying enzymes can add or remove these modifications



Dnmts

De novo: Dnmt3a,3b, 3L

Maintenance: Dnmt1

RNAs are important players:
lncRNAs or small interfering RNAs
(in heterochromatin)

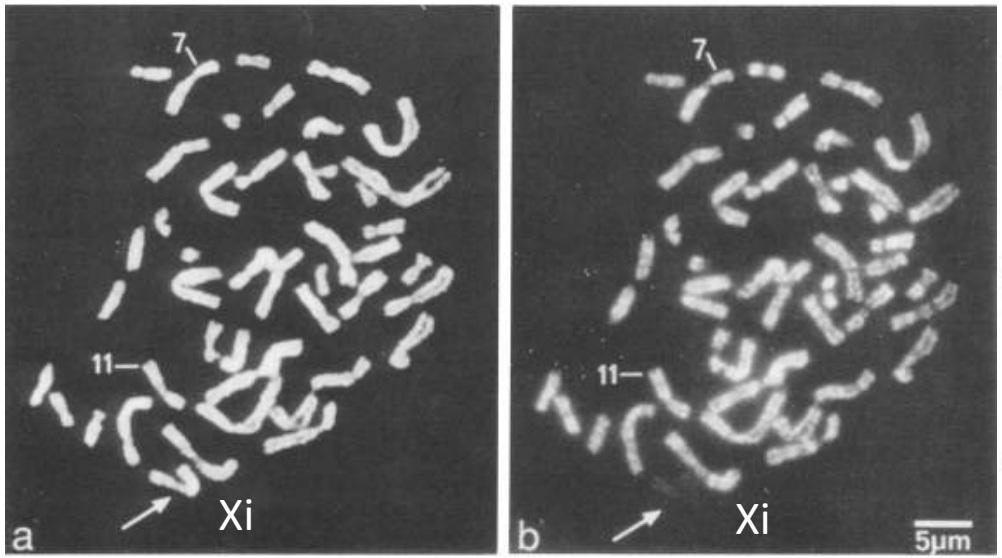
DNA methylation associated with repressed state of some genes, repeats:
Self-templating, stable - but can be removed
(**actively** eg Tet-induced conversion to 5hme;
passively during DNA replication)

Detecting Chromatin Marks using Antibodies

Bryan Turner

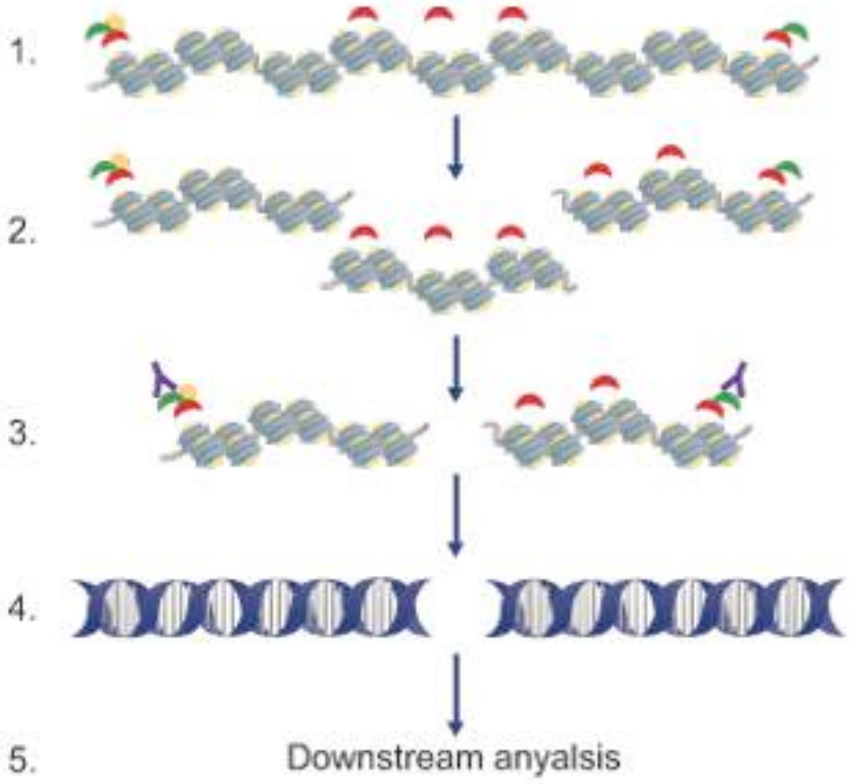
In the 1990's, highly specific **antibodies** raised - discriminating between chemically modified histones at specific amino acids, => histone modifications could be detected by **immunofluorescence** (IF) and **chromatin immunoprecitipation (ChIP).**

Bryan Turner, Dave Allis, Thomas Jenuwein



DNA

Anti-H4K12Ac



Unique tools to explore the differential states of chromatin and generating Epigenomic maps

E.

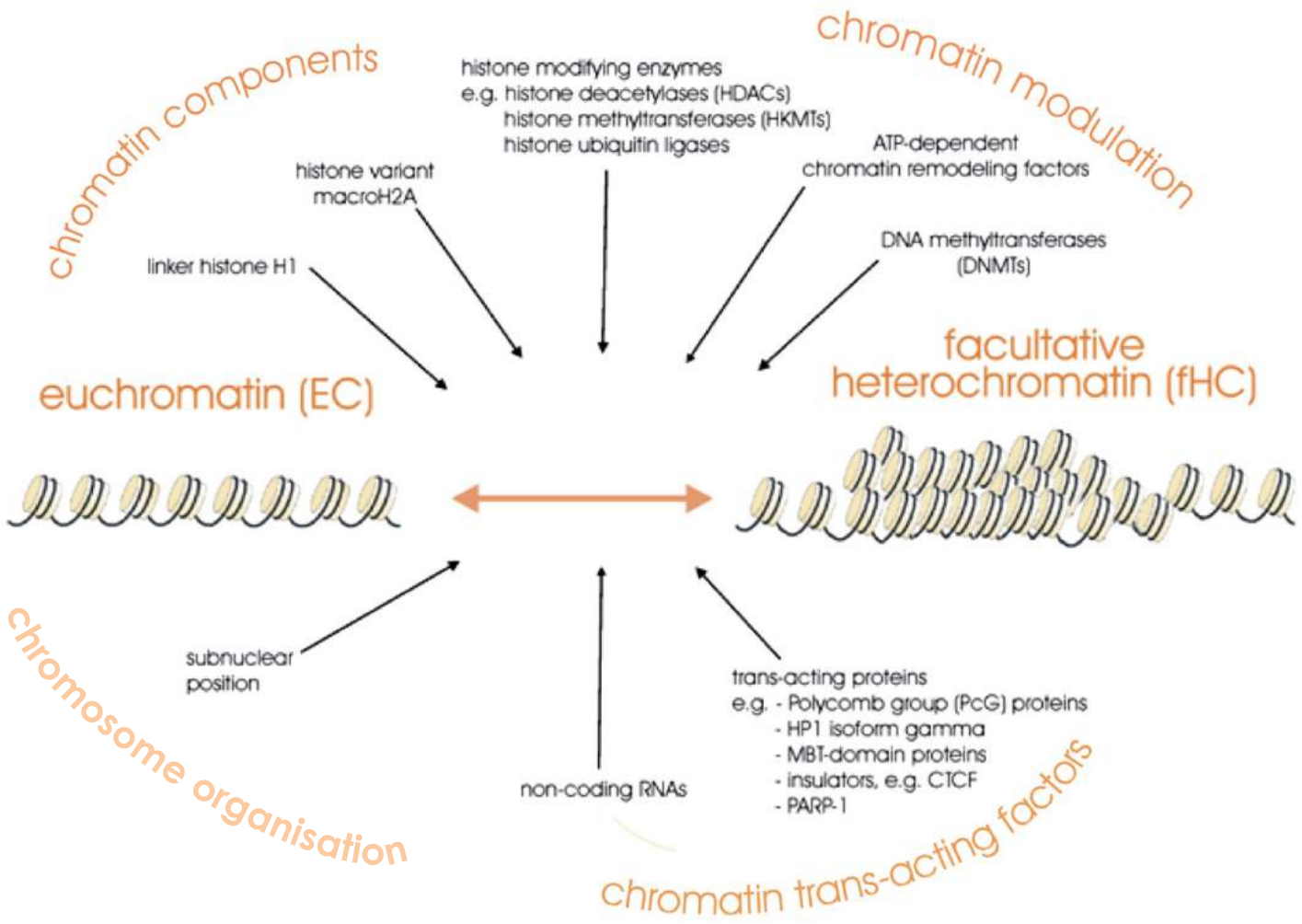
Facultative Heterochromatin

Table 1. Molecular Features of Various Genomic Regions of Facultative Heterochromatin

Facultative Heterochromatin (fHC)	Chromatin Organization	Molecular Features of Various Areas of fHC: DNA Methylation	Histone Modification States	RNA Component	Chromatin Components and *Trans*-Acting Factors
Inactive X chromosome (Xi)	Locally compacted 11 nm fiber, variations of 30 nm fiber and higher-order chromatin	+	Hypoacetylation, H4K20me1, H3K9me2, H3K27me3, H2AK119ub1	+	PRC1,[e] PRC2,[f] other PcG proteins,[g] macroH2A, CULLIN3/SPOP
Autosomal imprinted genomic loci		+[a]	Hypoacetylation, H3K9me2/3, H3K27me3, H4K20me3	+[b]	MacroH2A, CTCF, PRC2[f]
Long-range silencing (e.g., HOX gene clusters)		+	Hypoacetylation, H3K27me2/3, H2AK119ub1, H4K20me3[d]	+[c]	PRC1,[e] PRC2,[f] other PcG proteins[g]
Local gene silencing		?	Hypoacetylation, H3K9me2, H4K20me1, H2AK119ub1	?	PRC1,[e] PRC2,[f] other PcG proteins,[g] HP1γ, MBT proteins
Euchromatin (EC)	11 nm fiber	–	Hyperacetylation, H3K4me2/3, H3K36me3	–	ATP-dependent chromatin remodelers, H3.3, H2A.Z
Constitutive heterochromatin (cHC)	≥30 nm fiber	+	Hypoacetylation, H3K9me3, H4K20me3	+	HP1α/β

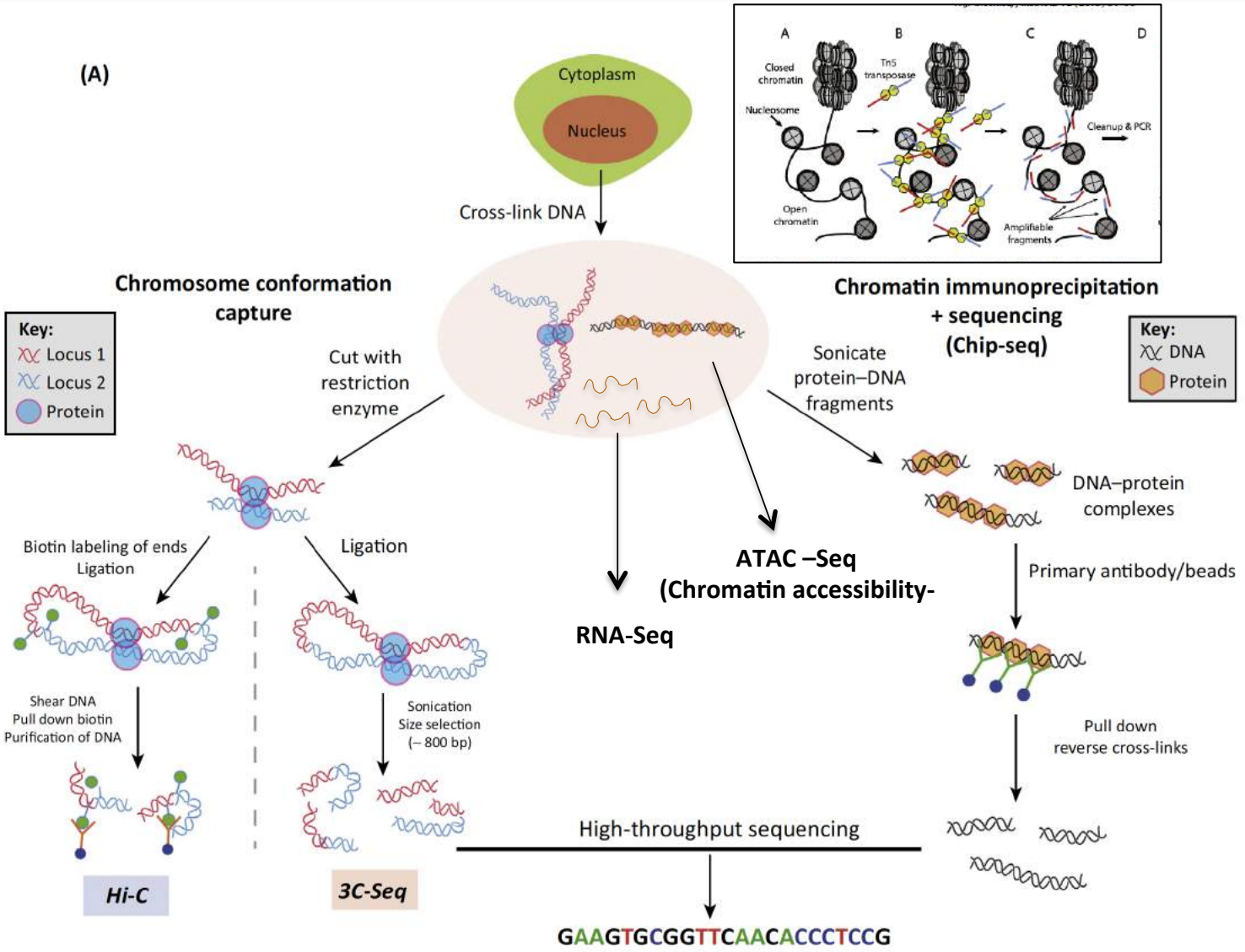
Trojer and Reinberg, 2007

Facultative Heterochromatin



Trojer and Reinberg, 2007

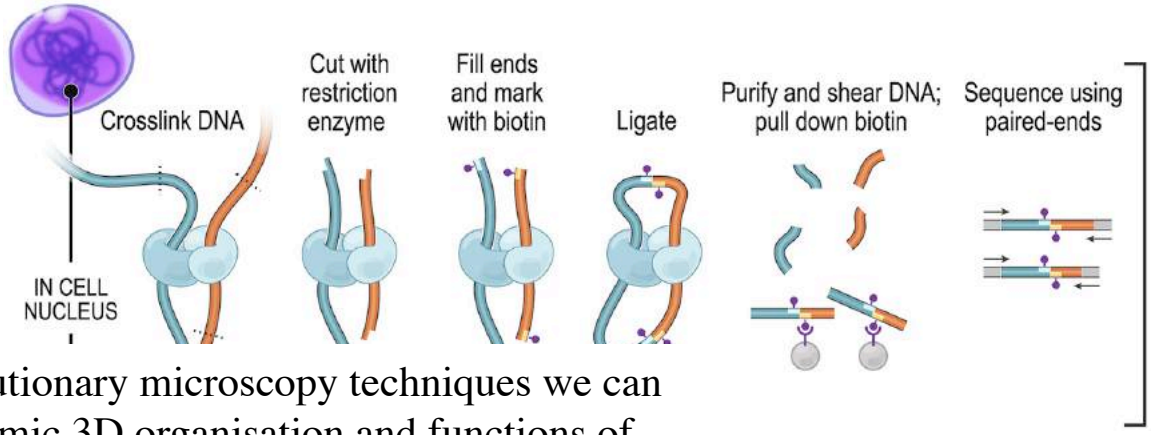
Molecular Characterization of Facultative Heterochromatin



Adapted from Cattoni et al, 2015

New insights into the molecular 3D architecture of chromosomes using Chromosome Conformation Capture (« C ») techniques

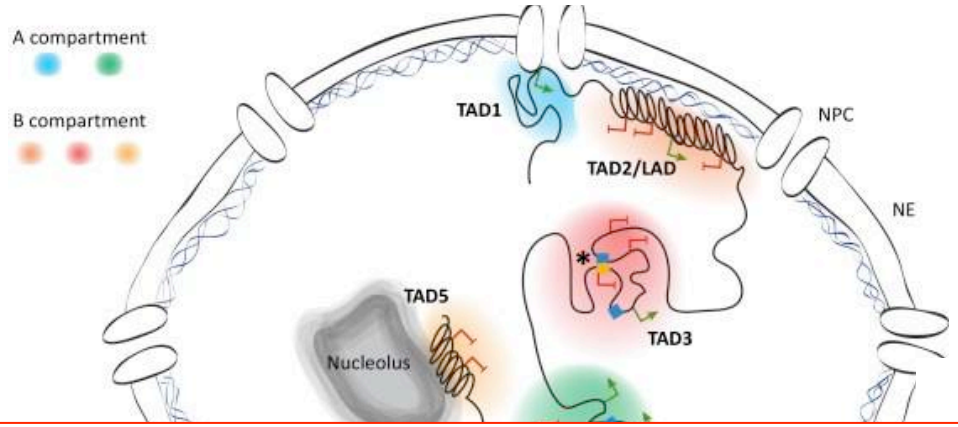
Job Dekker
(UMass)



"Hi-C"
Whole Genome

Combined with revolutionary microscopy techniques we can now observe the dynamic 3D organisation and functions of the genome :

Lieberman-Aiden et al. 2009



Compartments

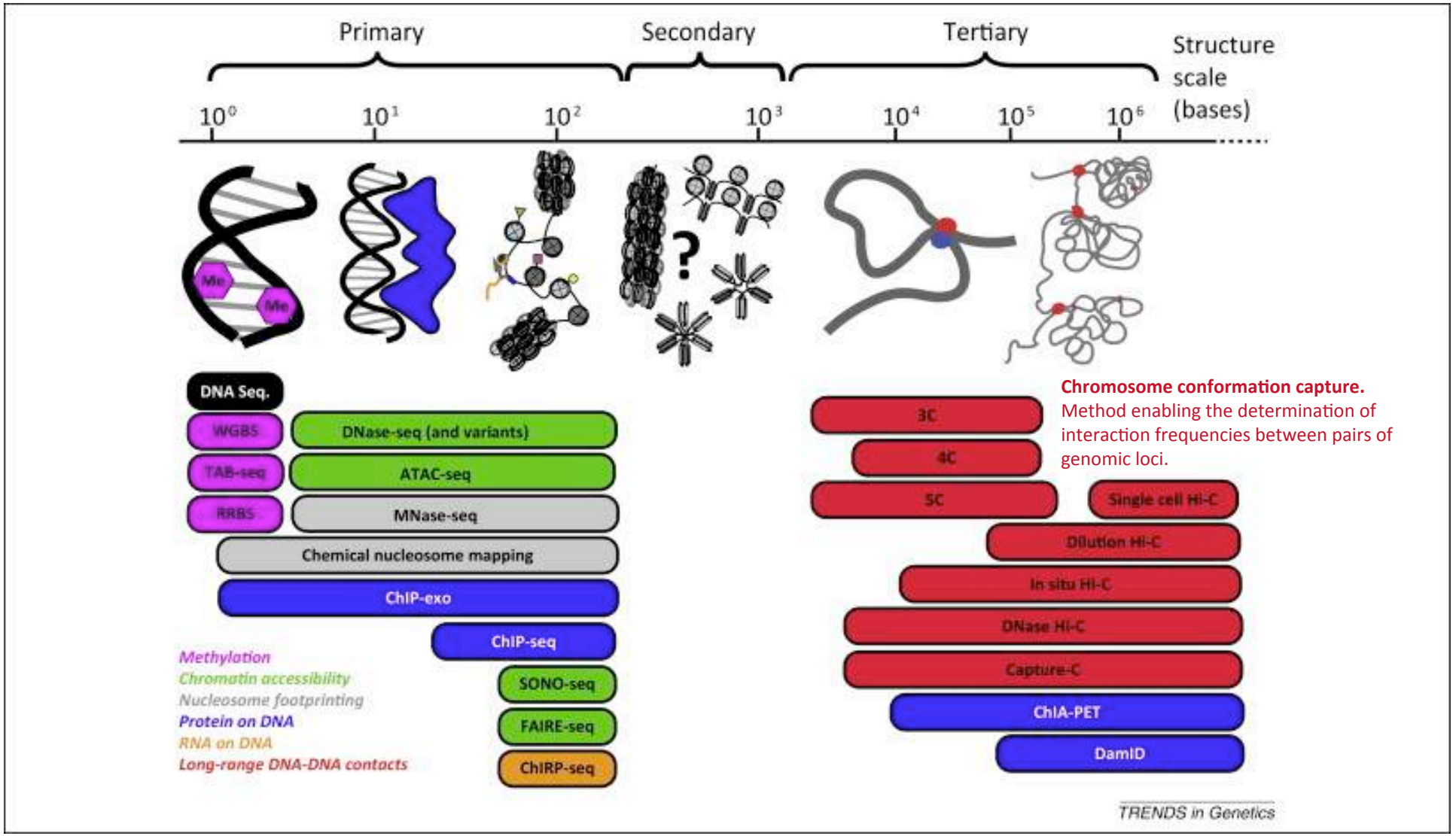
"Active" or "inactive"
Distinctive epigenomic features
Vary between tissues
Cell-type specific
Lieberman-Aiden et al. 2009

TADs (100kb-1Mb scale)

nvariant (almost) between tissues
Nora et al, 2012
Conserved (man/mouse)
Dixon et al, 2012
Also in other species?
Sexton et al, 2012

And ask questions about how facultative heterochromatin is organised over developmental time

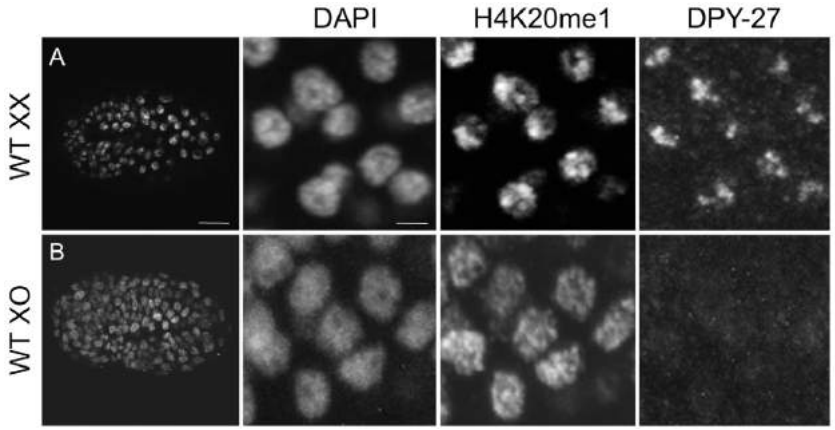
Molecular Characterization of Facultative Heterochromatin



Chromosome changes in Worm Dosage Compensation

H4K20me1 Contributes to Downregulation of X-Linked Genes for *C. elegans* Dosage Compensation

Anne Vielle[1¶], Jackie Lang[2¶], Yan Dong[1], Sevinc Ercan[3,4], Chitra Kotwaliwale[5,6], Andreas Rechtsteiner[2], Alex Appert[1], Q. Brent Chen[3], Andrea Dose[5,6], Thea Egelhofer[2], Hiroshi Kimura[7], Przemyslaw Stempor[1], Abby Dernburg[5,6], Jason D. Lieb[3], Susan Strome[2], Julie Ahringer[1*]

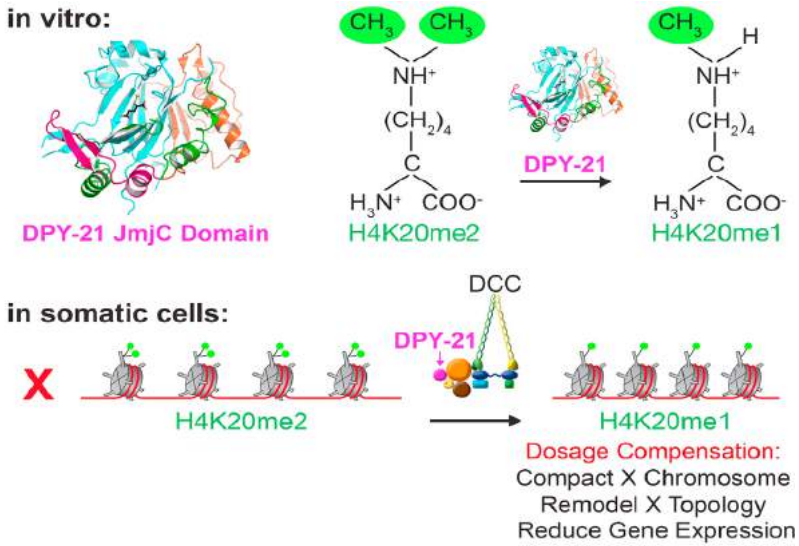


- DC proteins lead to higher levels of histone H4 lysine 20 monomethyl (H4K20me1) on hermaphrodite Xs
- H4K20me1 functions in repressing X-chromosome gene expression (by studying mutants SET1=HMTase)
- Therefore, histone modification is an important aspect of the mechanism of dosage compensation.
- H4K20me1 thought to impact chromatin structure regulation,
- => **Dosage compensation may lower gene expression on hermaphrodite X chromosomes by compacting them?**

(Vielle et al, Plos Gen. 2011)

Dynamic Control of X Chromosome Conformation and Repression by a Histone H4K20 Demethylase

Katjuša Brejc,[1,3] Qian Bian,[1,3] Satoru Uzawa,[1] Bayly S. Wheeler,[1,4] Erika C. Anderson,[1] David S. King,[2] Philip J. Kranzusch,[1,5,6] Christine G. Preston,[1,7] and Barbara J. Meyer[1,8,*]

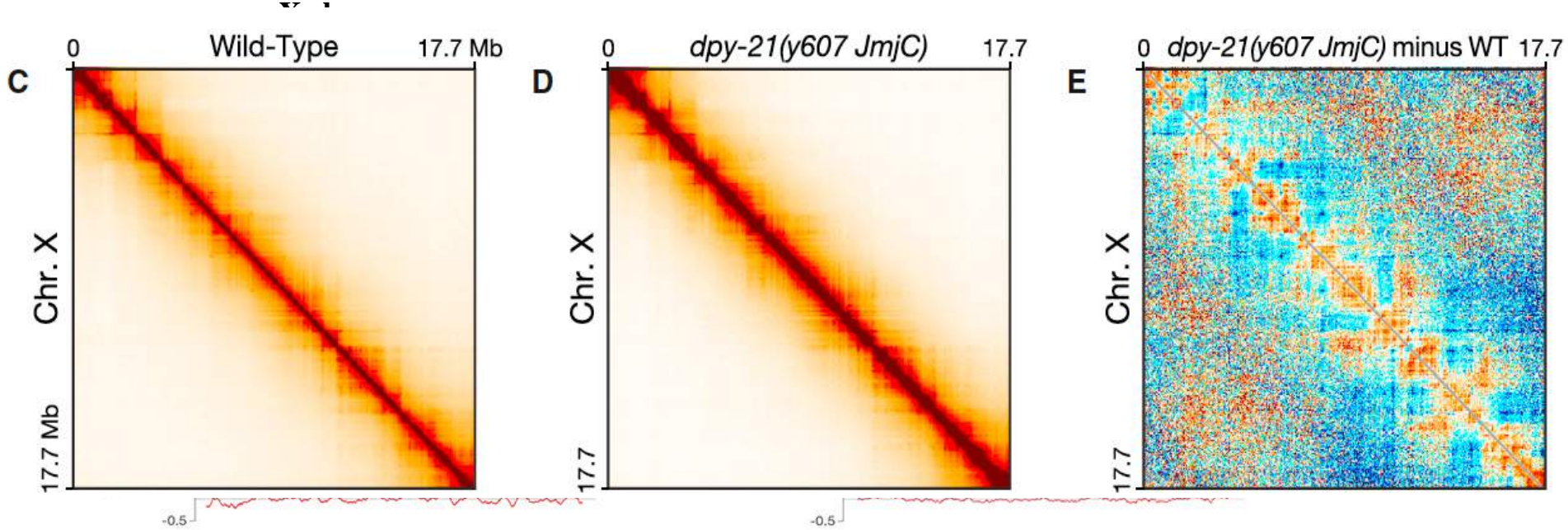


- H4K20me2 JmjC demethylase subfamily revealed by DPY-21 structure and activity
- In somatic cells, DPY-21 enriches H4K20me1 on X chromosomes to repress gene expression
- H4K20me1 enrichment controls the higher-order structure of X chromosomes
- In germ cells, DPY-21 enriches H4K20me1 on autosomes to promote chromosome compaction.

(Brejc et al, Cell 2017)

Chromosome changes in Worm Dosage Compensation

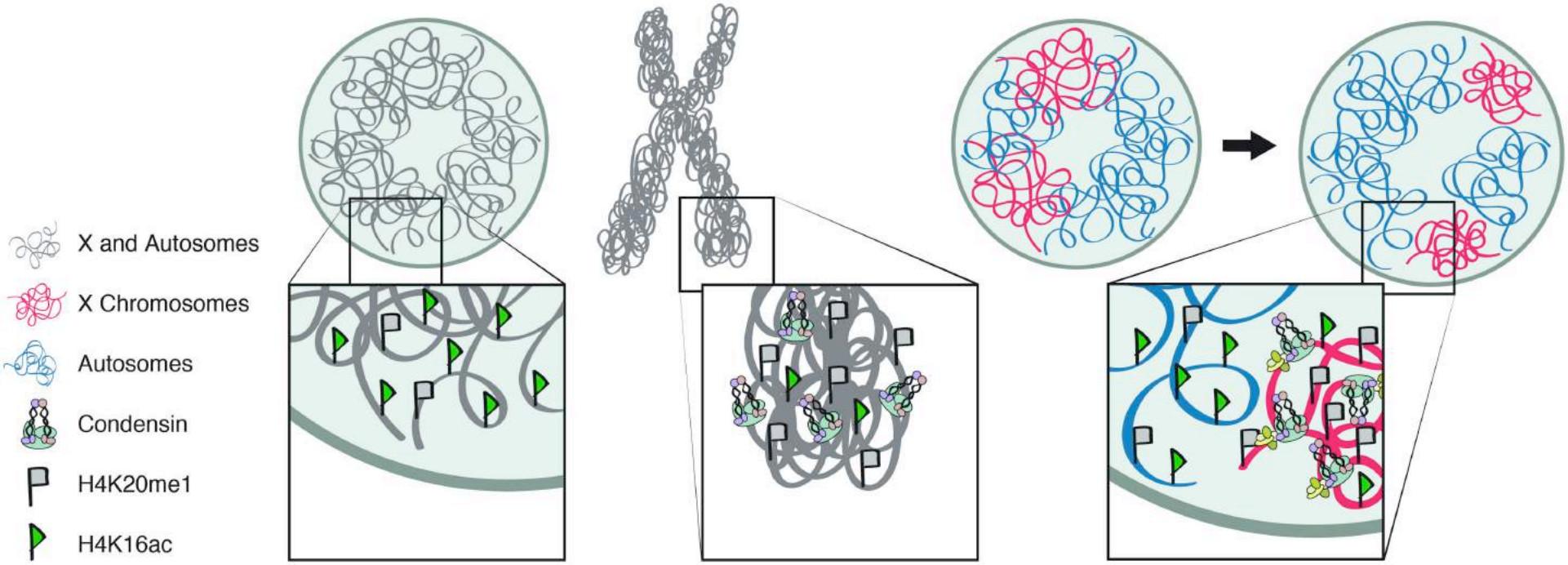
Condensin-driven remodeling of X-chromosome topology during dosage compensation



- Dosage-compensated X chromosomes consist of self-interacting domains (~ 1 Mb) resembling mammalian Topologically Associating Domains (TADs).
- TADs on X have stronger boundaries and more regular spacing than those on autosomes.
- Many TAD boundaries on X coincide with the highest-affinity **rex** sites, and these boundaries become diminished or lost in mutants lacking DCC binding, causing the structure of X to resemble that of autosomes.
- Loss of H4K20me1 (in dpy-21 mutants) leads to reduced TAD boundaries, and greater long range interactions (H4K20me1 reinforces the strength of these boundaries?)

Chromosome changes in Worm Dosage Compensation

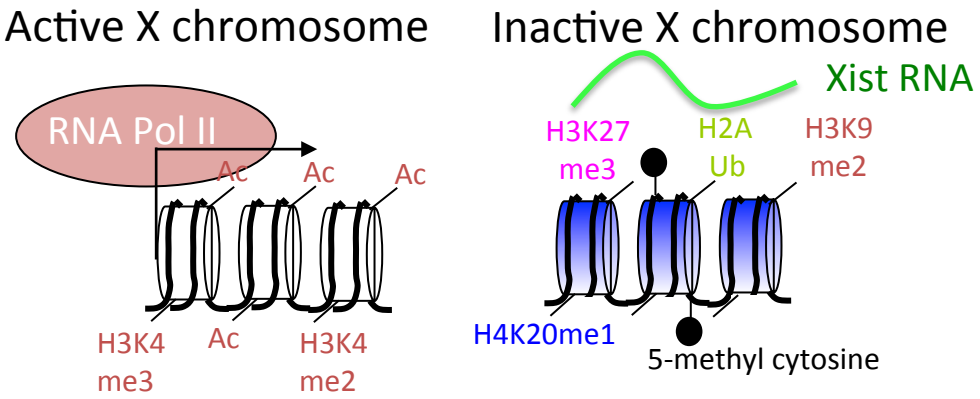
Condensin-driven remodeling of X-chromosome topology during dosage compensation and during mitosis: First insights into the molecular architecture of facultative heterochromatin

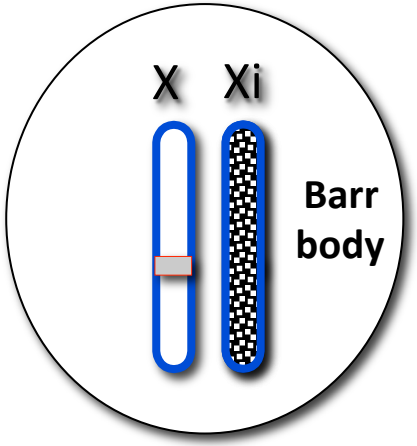


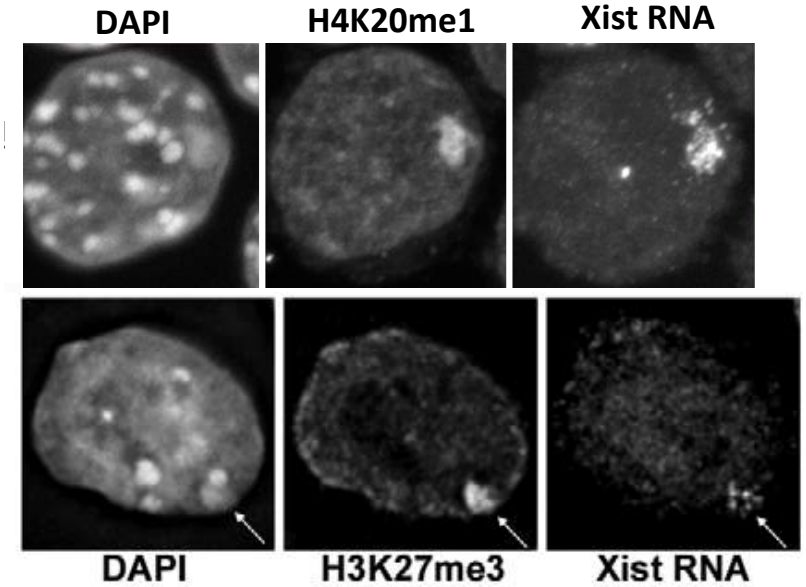
- Condensin and H4K20me1 are also found associated with **mitotic** chromosomes in many organisms
- H4K20me1 enrichment is also associated with the inactive X in mammals
- Are these condensin-driven domains (TADs?) on the C. elegans X chromosomes a cause or simply a consequence of DCC-induced down regulation?
- Is the impact on gene down-regulation of H4K20me1 via chromosome structural changes or rather through local effects on the chromatin?

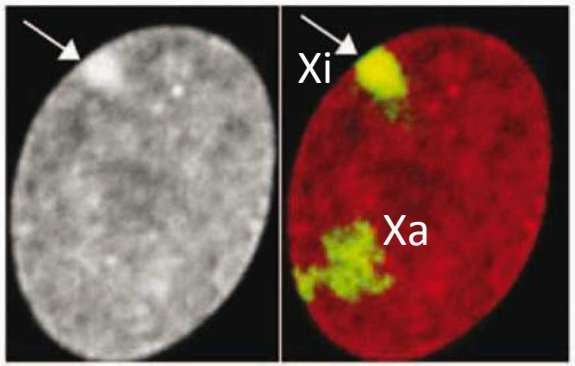
Facultative Heterochromatin of the inactive X

Differential treatment of identical DNA sequences in the same nucleoplasm



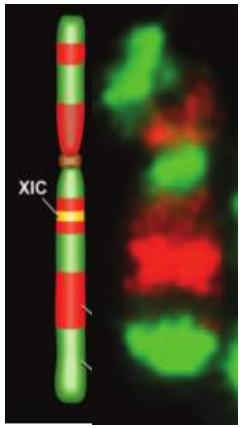






Teller et al, Nucleus, 2013

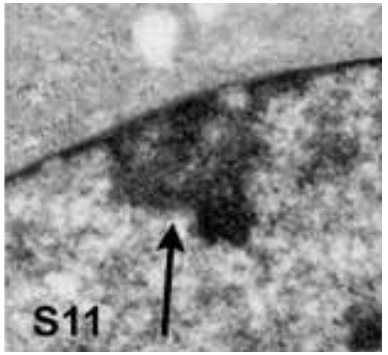
At least Two Flavors of Heterochromatin on the Xi in Normal Human Cells



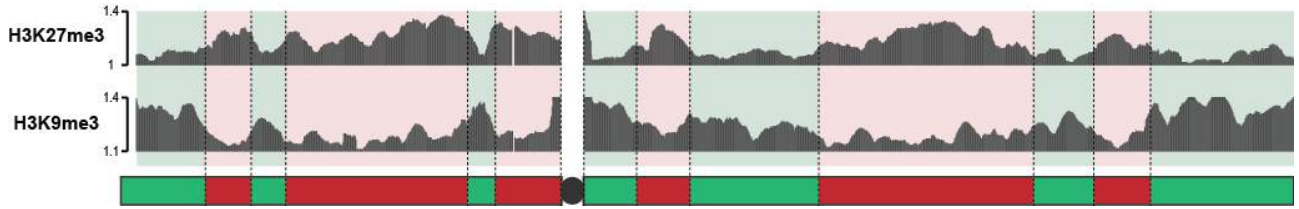
Chadwick et al, 2004

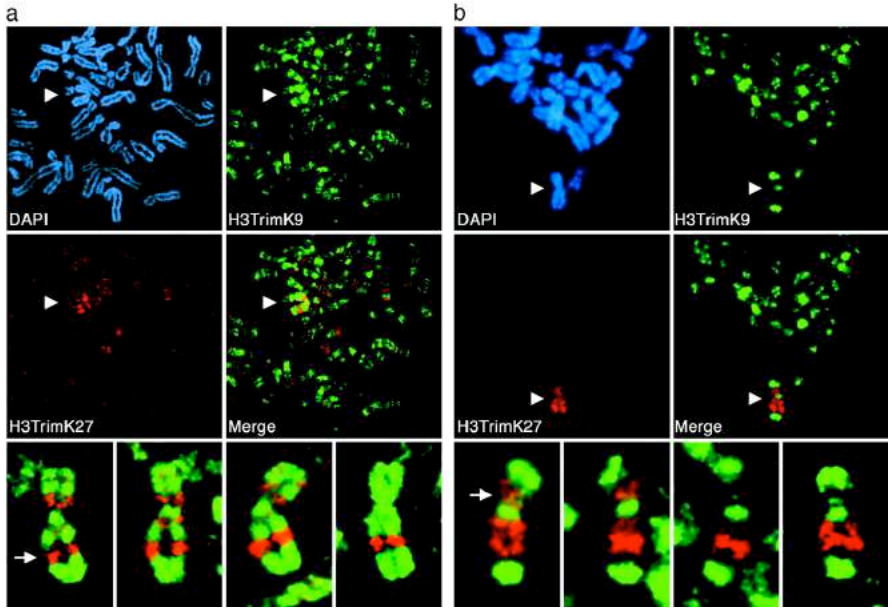
H3K27me3 enriched Xi domains

H3K9me3 enriched Xi domains



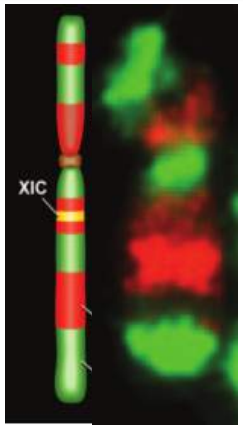
ChIP-seq on
Normal cells (HMEC)





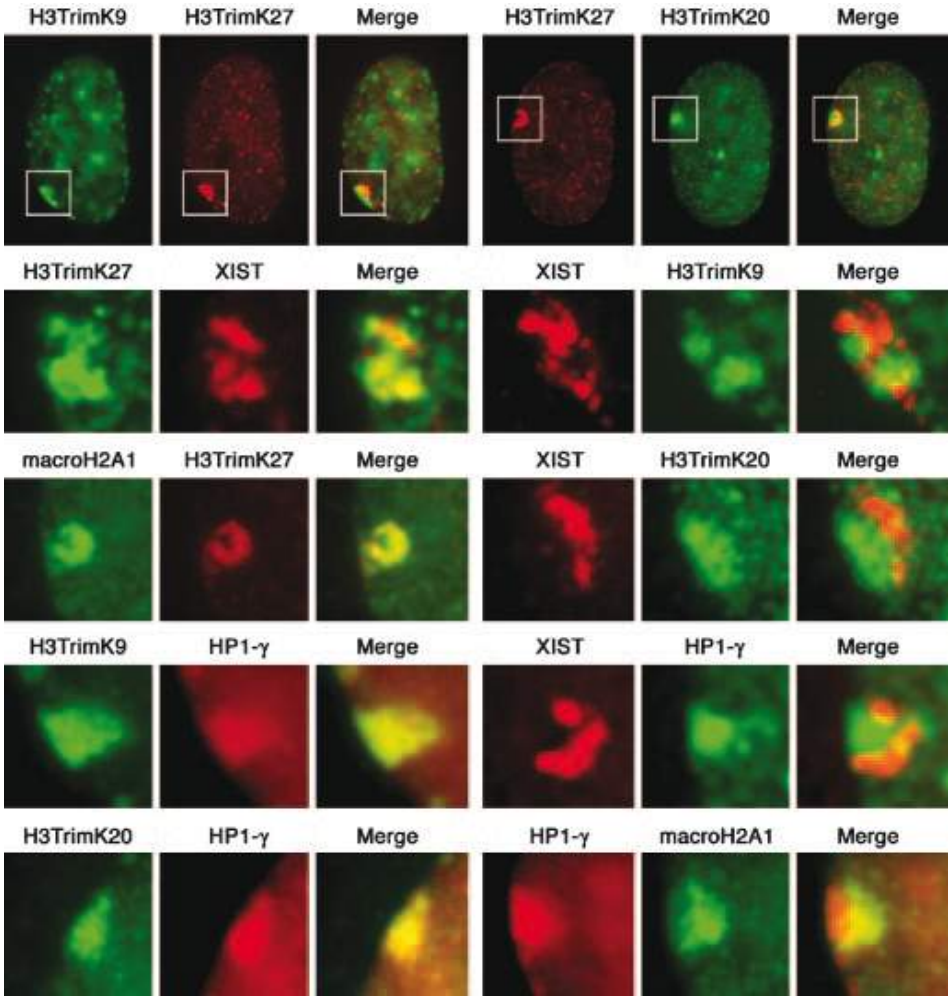
Different human cell types
Have different distributions of
H3K9 or H3K27me3 enriched
heterochromatin

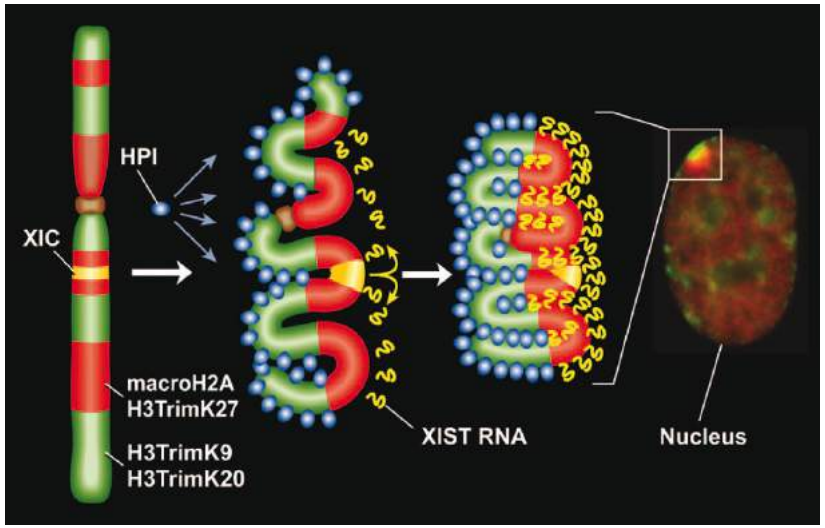
Chromatin Signature of Facultative Heterochromatin on the human inactive X



Chadwick et al, 2004

H3K27me3 enriched Xi domains
H3K9me3 enriched Xi domains





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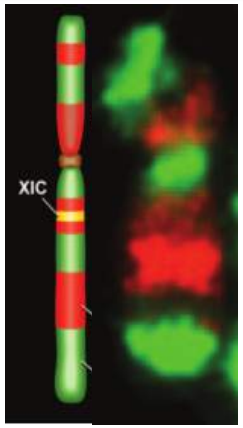
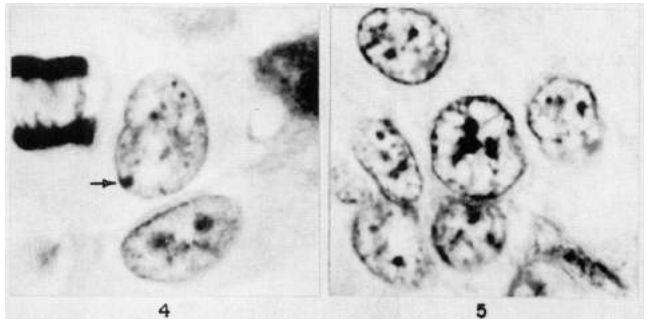
Facultative heterochromatin on the Xi is disrupted in human cancer cells

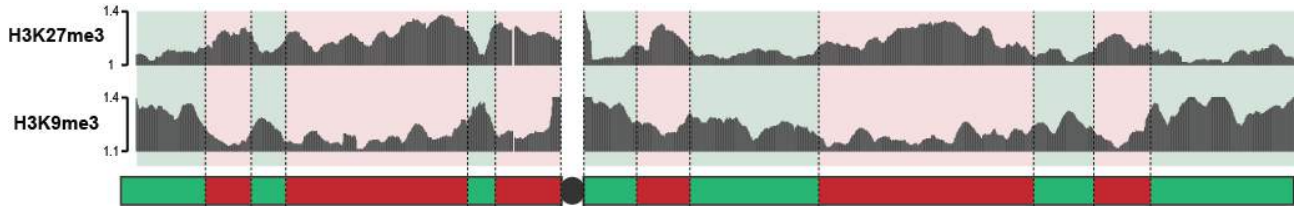


Chadwick et al, 2004

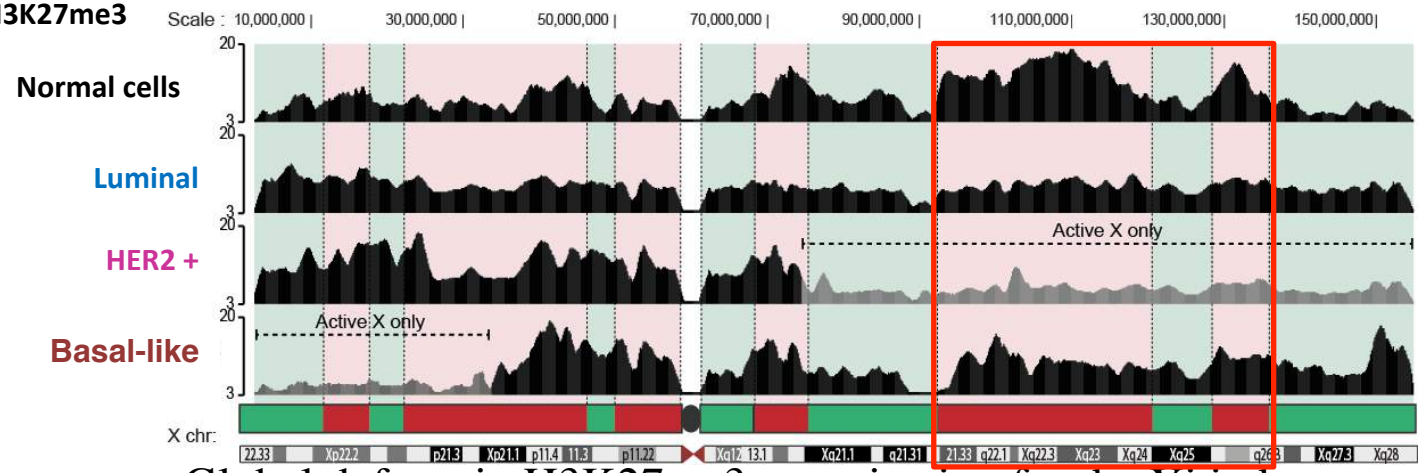
The Sex Chromatin in Human Malignant Tissues
K. L. Moore and M. L. Barr, 1957



ChIP-seq on normal cells (HMEC)



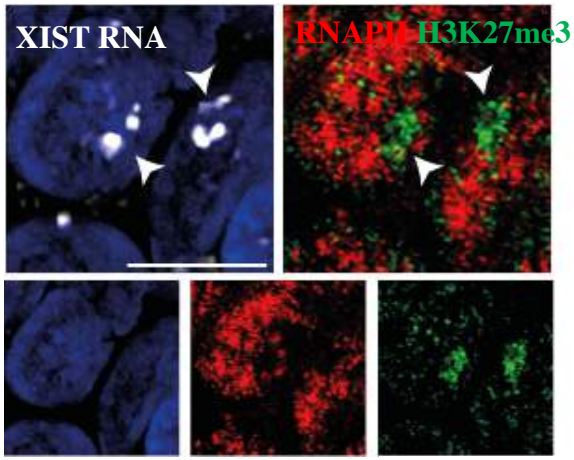
ChIP-seq : H3K27me3



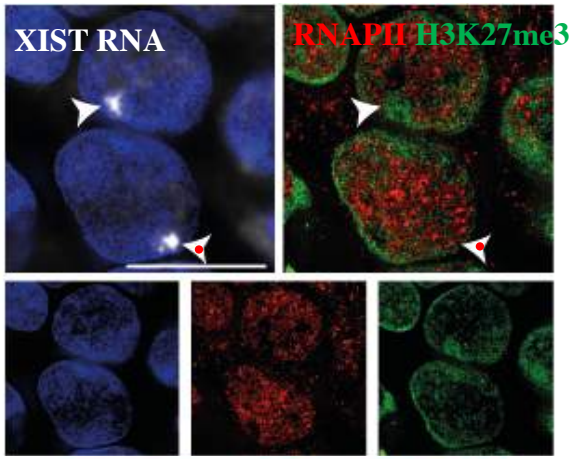
Global defects in H3K27me3 organization for the Xi in breast cancer cell lines (consistent with "loss" of Barr body)
Chaligné et al, Genome Res. 2015

Facultative heterochromatin on the Xi is disrupted in human cancer cells

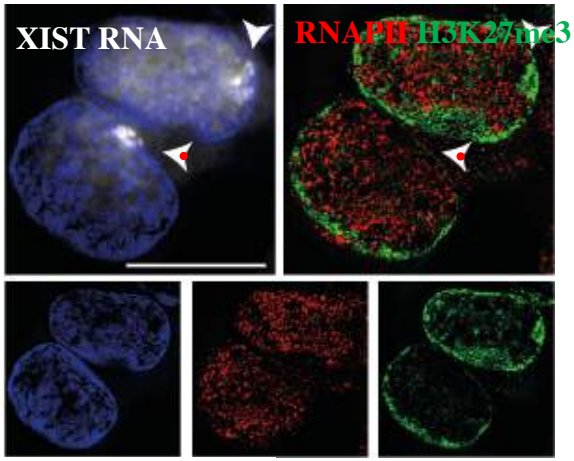
Healthy breast tissue



Luminal A / IDC Grade II
T1

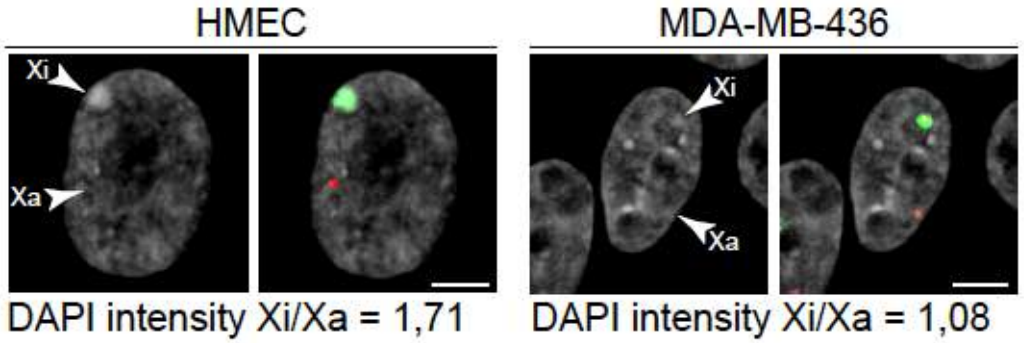


Luminal A / IDC Grade II
T2



E DAPI, XIST RNA, HDAC8 RNA

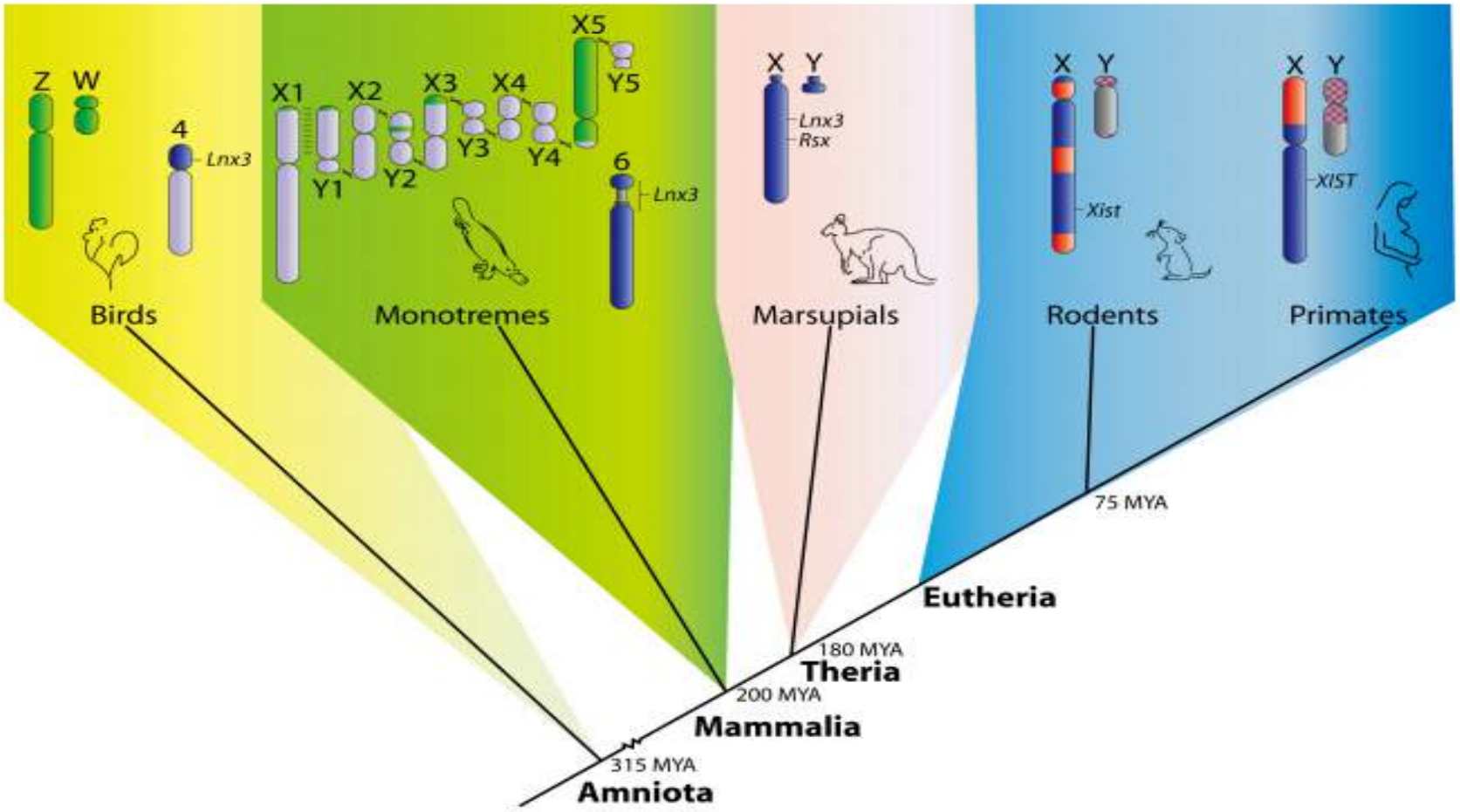
In total, 17 tumors (50%) show escape of ≥1 of the three X-linked genes studied (11/24 luminal, 2/3 HER2 and 4/8 TN)



Global defects in chromatin organization for the Xi in breast cancers are accompanied by aberrant reactivation of X-linked genes

Chaligné et al, Genome Res. 2015

Chromatin Signatures of Facultative Heterochromatin on the inactive X in different mammals



Shafagh A. Waters, Paul D. Waters

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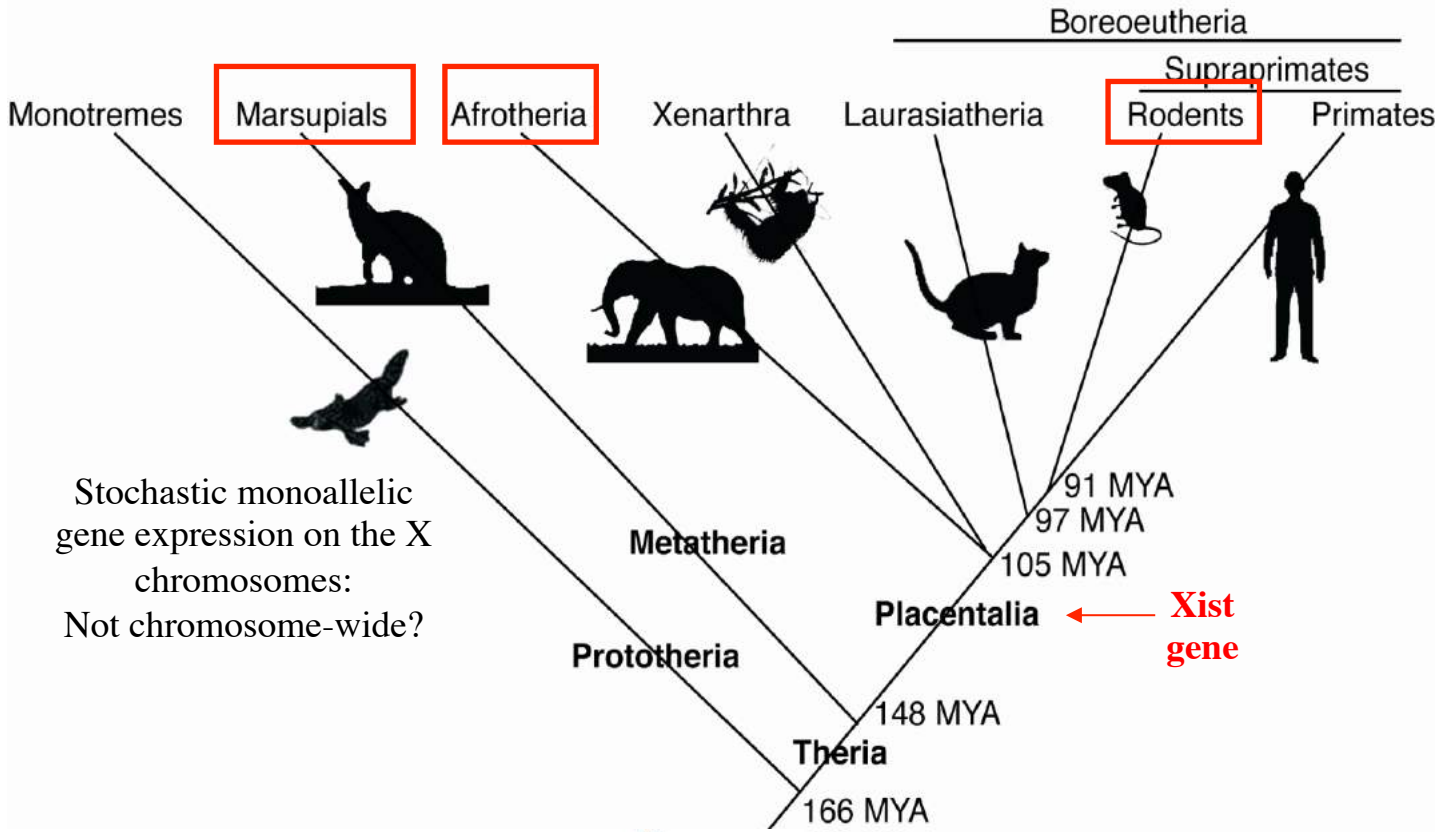
Chromatin Signatures of Facultative Heterochromatin on the inactive X in different mammals



OPEN ACCESS Freely available online PLoS one

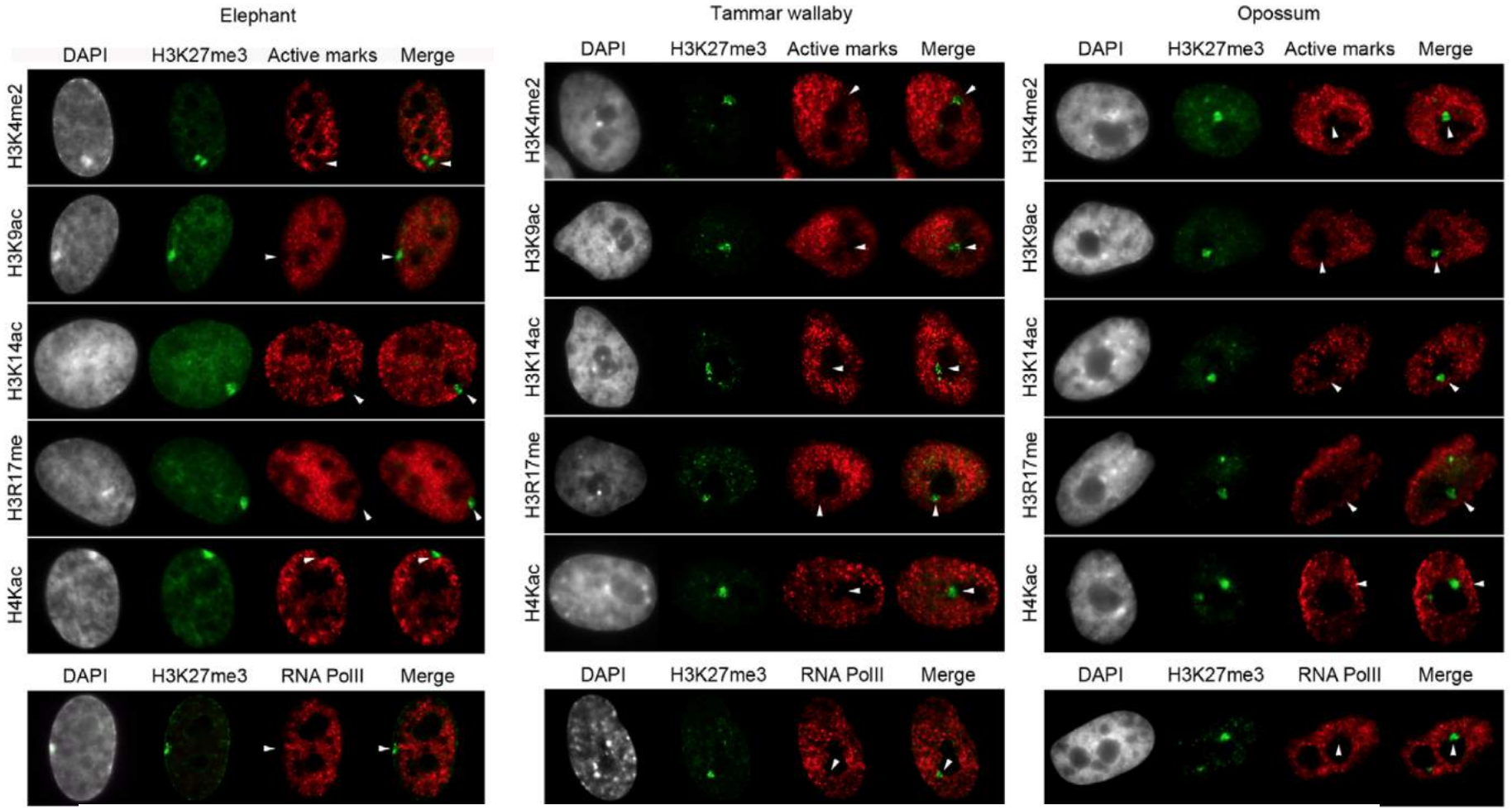
Evolution from XIST-Independent to XIST-Controlled X-Chromosome Inactivation: Epigenetic Modifications in Distantly Related Mammals

Julie Chaumeil[1*¤a], Paul D. Waters[1*¤], Edda Koina[1], Clément Gilbert[2¤b], Terence J. Robinson[2], Jennifer A. Marshall Graves[1]

Courtesy of Julie Chaumeil (Institut Cochin)

Chromatin Signatures of Facultative Heterochromatin on the inactive X in different mammals

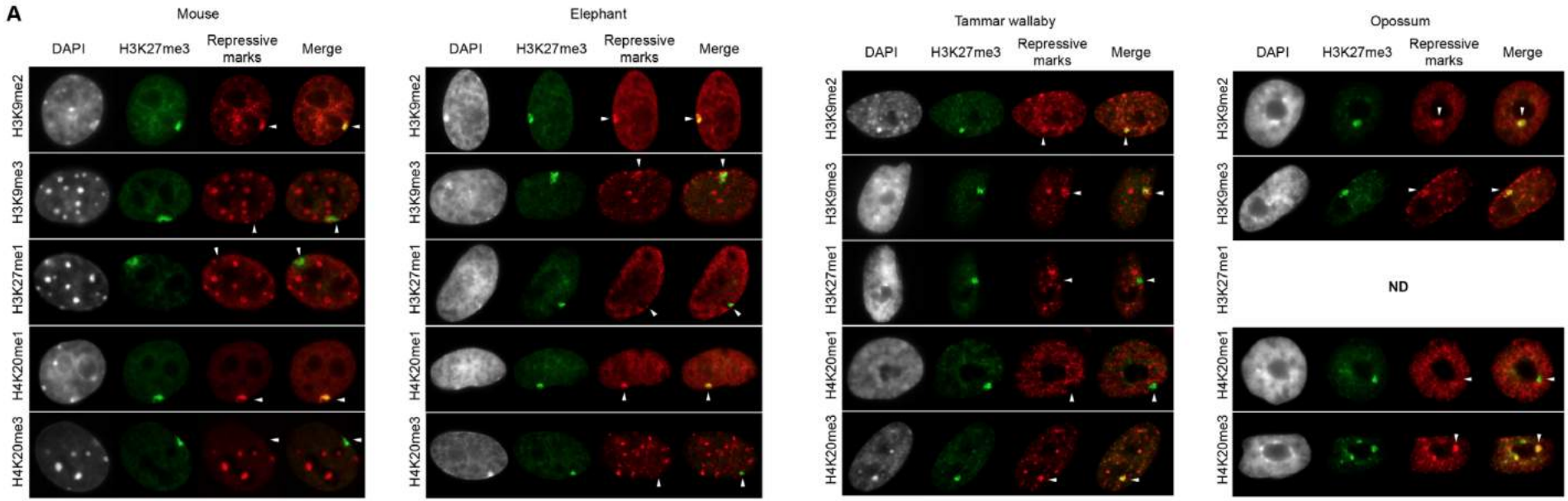
Immunofluorescence with Antibodies against different Histone Modifications



- In the 3 groups, the Xi shows an enrichment in H3K27me3 and a **lack** of RNA PolII and active marks (H3K9Ac, H4Ac, H3R17me)

Chaumeil et al, 2011

Chromatin Signatures of Facultative Heterochromatin on the inactive X in different mammals



- In the 3 groups, the Xi shows an enrichment in H3K27me3 and H3K9me2.
- In elephant and mouse, the Xi shows also an accumulation of H4K20me1.
- The marsupial Xi shows **no enrichment in H4K20me1** but an accumulation of H3K9me3 and H4K20me3 (common features of constitutive heterochromatin at centromeres).
- The marsupial Xi also shows no DNA methylation at promoters
- Reported to have « leaky » XCI (ie frequent escape from XCI)?
- BOTH SIMILARITIES AND DIFFERENCES BETWEEN SPECIES

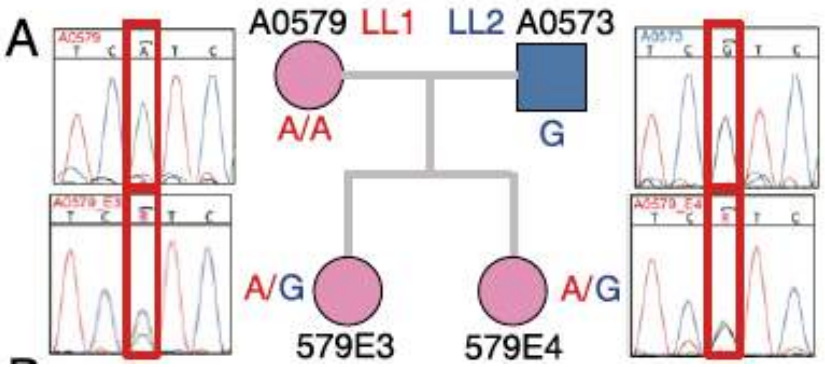
Chaumeil et al, 2011

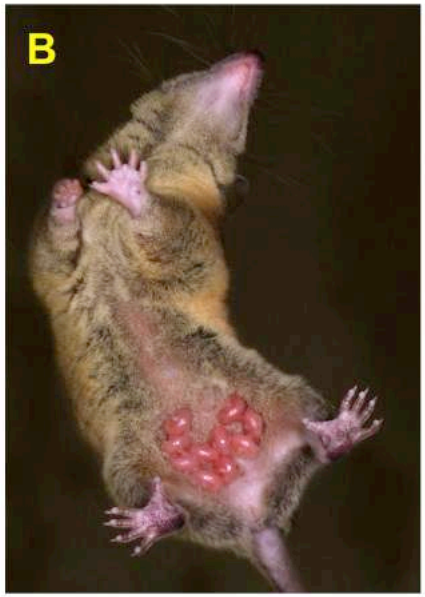
Expression and Chromatin status of the inactive X in Marsupials

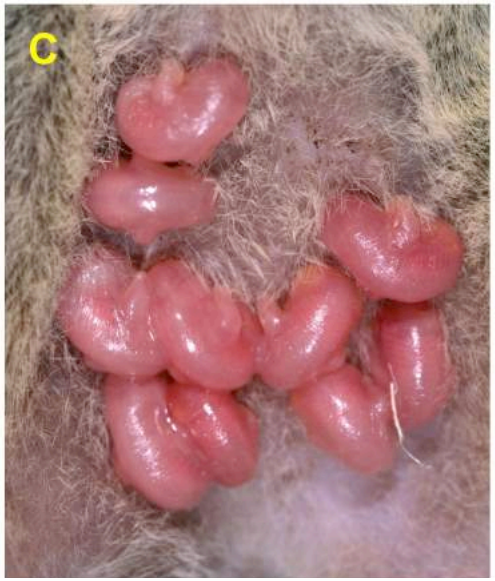
Opposum: *Monodelphis domestica*

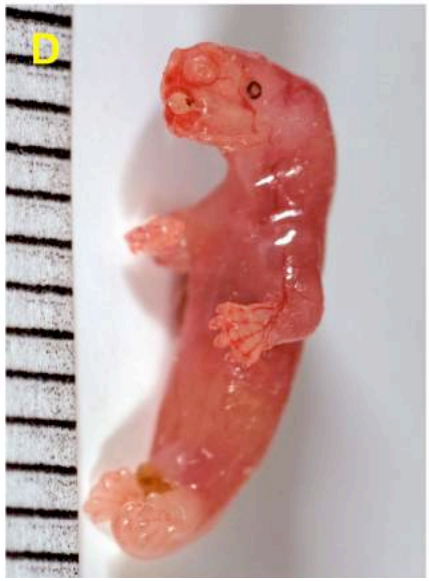
Chromosome-wide profiling of X-chromosome inactivation and epigenetic states in fetal brain and placenta of the opossum, *Monodelphis domestica*

Xu Wang,[1,2,5] Kory C. Douglas,[3,5] John L. VandeBerg,[4] Andrew G. Clark,[1,2] and Paul B. Samollow[3,6]





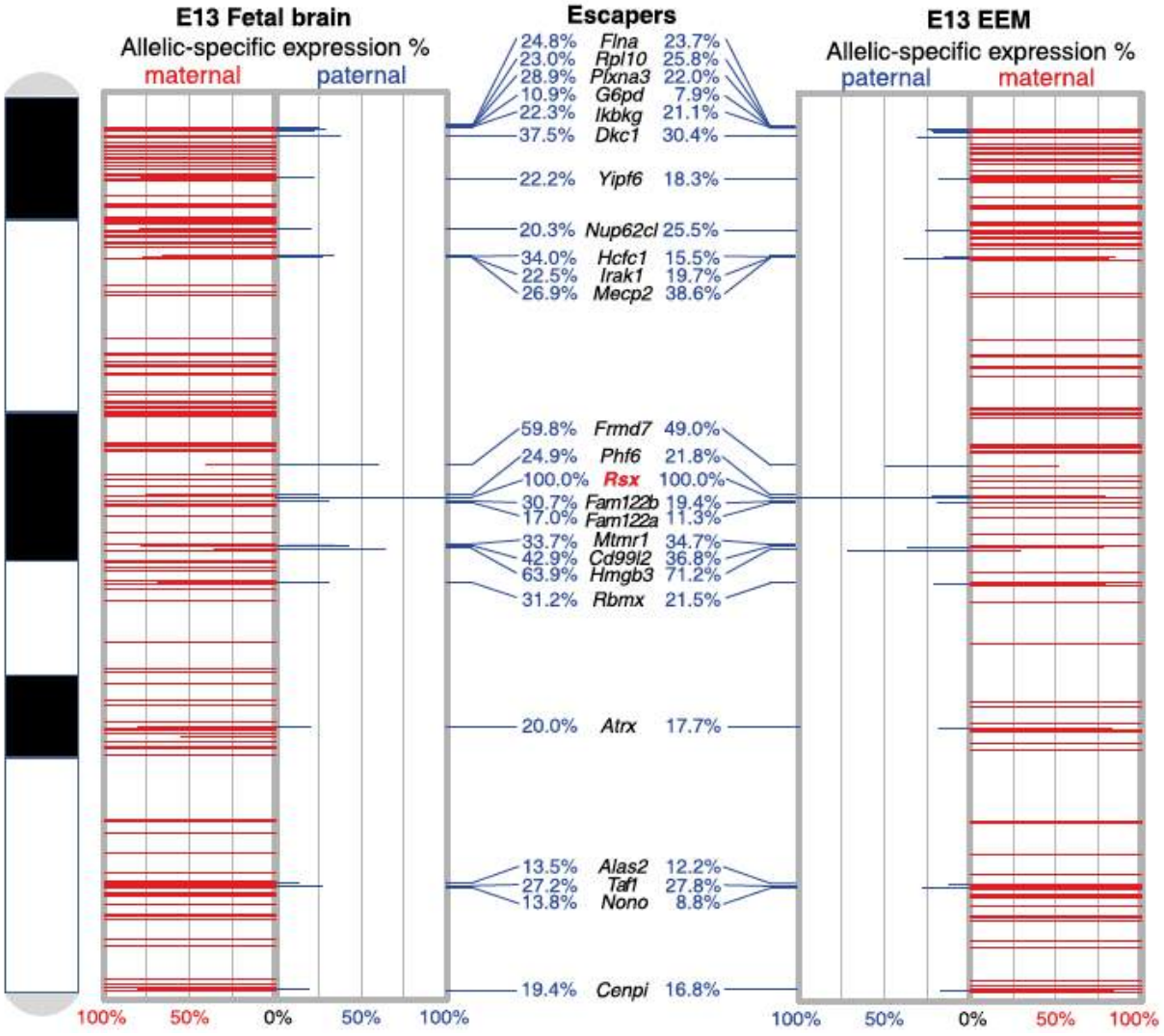




E. Heard, February 12th, 2018

Expression and Chromatin status of the inactive X in Marsupials

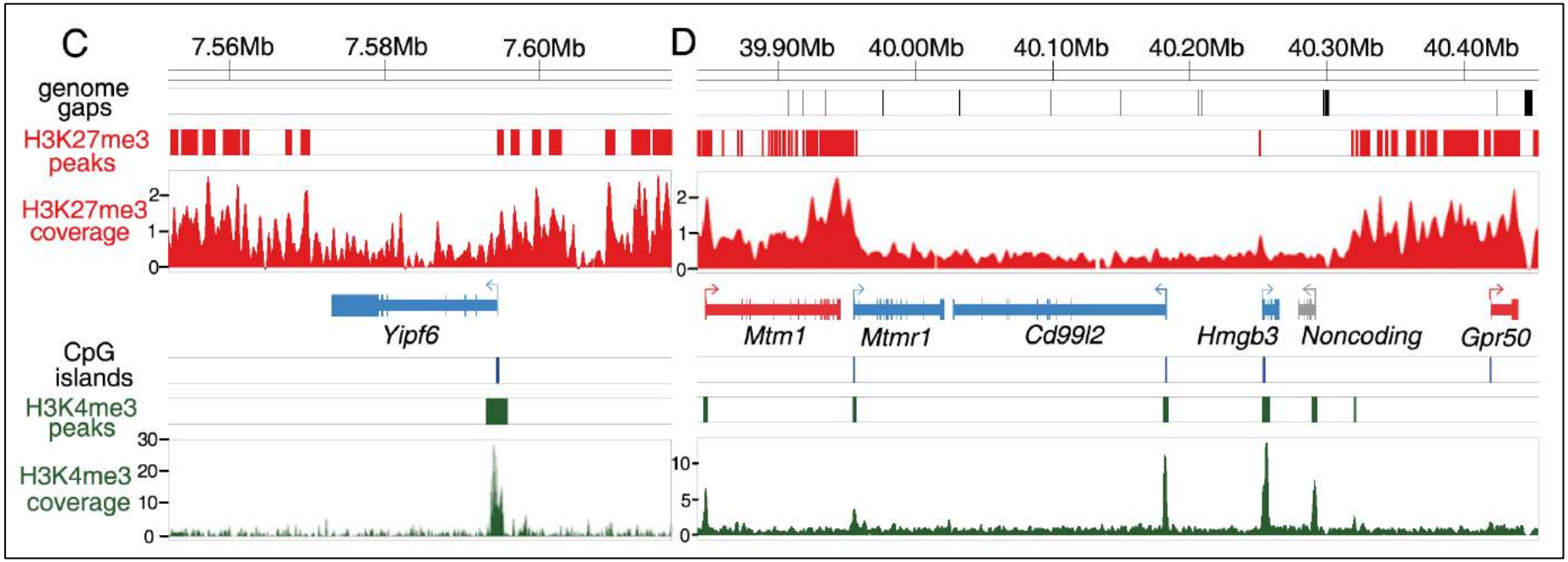
Opposum: *Monodelphis domestica*



Wang et al, 2014

Expression and Chromatin status of the inactive X in Marsupials

Opposum: *Monodelphis domestica*



- Parent-of-origin allele-specific expression, DNA methylation, and histone modifications in fetal brain and extra-embryonic membranes in the opossum (Monodelphis domestica).
- Most X-linked genes (152 of 176 genes with trackable SNP variants) had paternally imprinted silencing, with nearly 100% of transcripts derived from the maternal allele
- 24 loci (14%) escaped inactivation, showing varying levels of biallelic expression.
- No association between X-linked gene expression and promoter DNA methylation,

Wang et al, 2014

Summary of Chromatin Marks

Table 1. Comparison of repressive and active epigenetic marks on the inactive X chromosome in eutherian (mouse, human) and marsupials (Opossum).

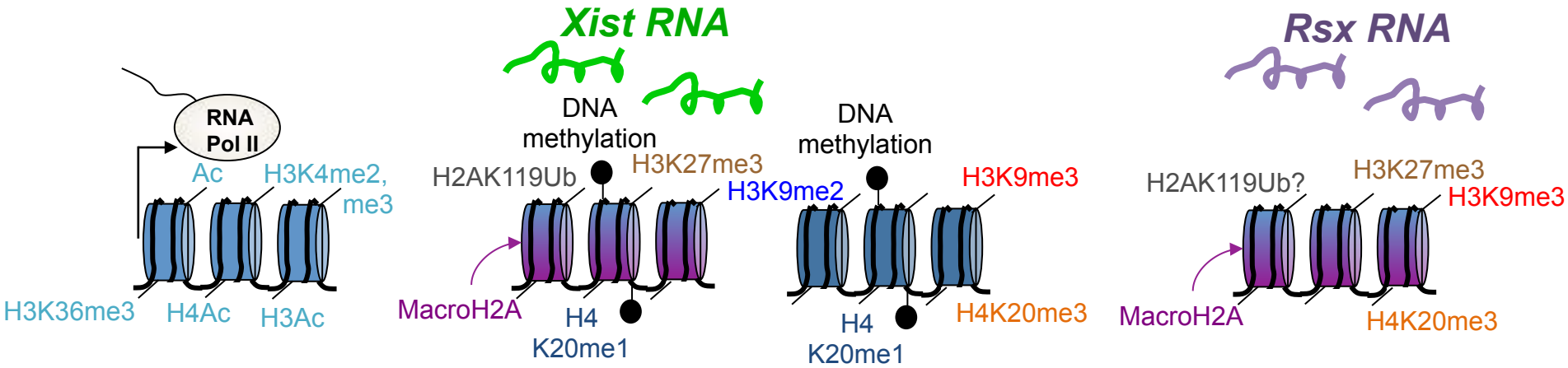
		Eutherian Xi			Marsupial Xi
		Mouse		Human	Opossum
		Somatic cells, or embryonic stem cells	Extra-embryonic cells	Somatic cells, or embryonic stem cells	Somatic cells
		Random XCI	Imprinted XCI	Random XCI	Imprinted XCI
Active marks	H3ac	O	O	O	O
	H4ac	O	O	O	O
	H3K4me2	O	O	O	O
Repressive marks	H3K9me2	★	★⌛	★	★1
	H3K9me3	O	★	★	★
	H3K27me3	★	★⌛	★	★2
	H4K20me1	★	★	?	O
	H4K20me3	O	★	★	★
	Macro-H2A	★⌛	★	★	?
	Promoter CpG hyper-methylation	¶⌛	¶⌛	¶	┤

★ = enriched on Xi, O = excluded from Xi, ¶ = present, ┤ = absent, ? = not determined, 1 = cell cycle specific, 2 = tissue specific;
⌛ = late event

From Waters and Waters, 2015

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

Diversity in heterochromatin marks: facultative heterochromatin is a means to an end – the need to dosage compensate



Active X chromatin

Inactive X chromatin
Barr body
Late replicating

Inactive X chromatin
Barr body
Late replicating

MOUSE

HUMAN

MARSUPIAL

Long Non-Coding RNAs trigger X inactivation in Mice, Humans and Marsupials?

ARTICLES

Requirement for *Xist* in X chromosome inactivation

Graeme D. Penny, Graham F. Kay*, Steven A. Sheardown, Sohaila Rastan* & Neil Brockdorff†

Section of Comparative Biology, MRC Clinical Sciences Centre, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

The *Xist* gene has been proposed as a candidate for the X inactivation centre, the master regulatory switch locus that controls X chromosome inactivation. So far this hypothesis has been supported solely by indirect evidence. Here we describe gene targeting of *Xist*, and provide evidence for its absolute requirement in the process of X chromosome inactivation.

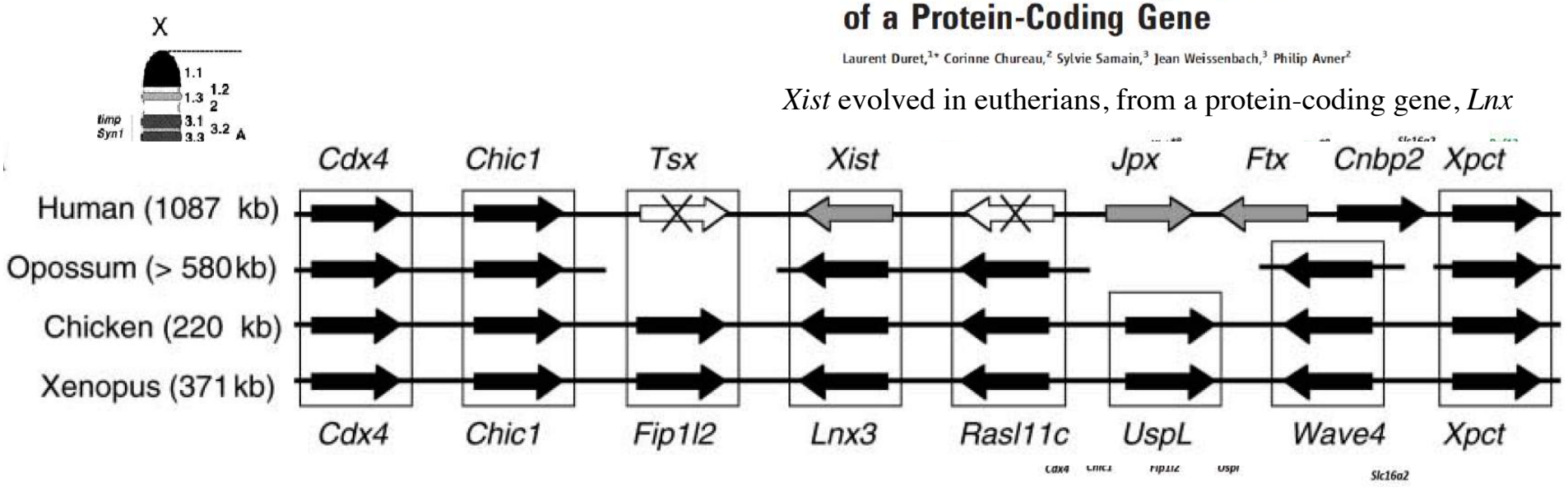
Brown et al, (1991) A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. *Nature* 349, 38-44

Brown et al (1992) The human XIST gene: analysis of a 17kn inactive X-specific RNA that contains conserved repeats and is highly localised within the nucleus. *Cell*, 71, 527-542.

The *Xist* RNA Gene Evolved in Eutherians by Pseudogenization of a Protein-Coding Gene

Laurent Duret,[1*] Corinne Chureau,[2] Sylvie Samain,[3] Jean Weissenbach,[3] Philip Avner[2]

Xist evolved in eutherians, from a protein-coding gene, *Lnx*



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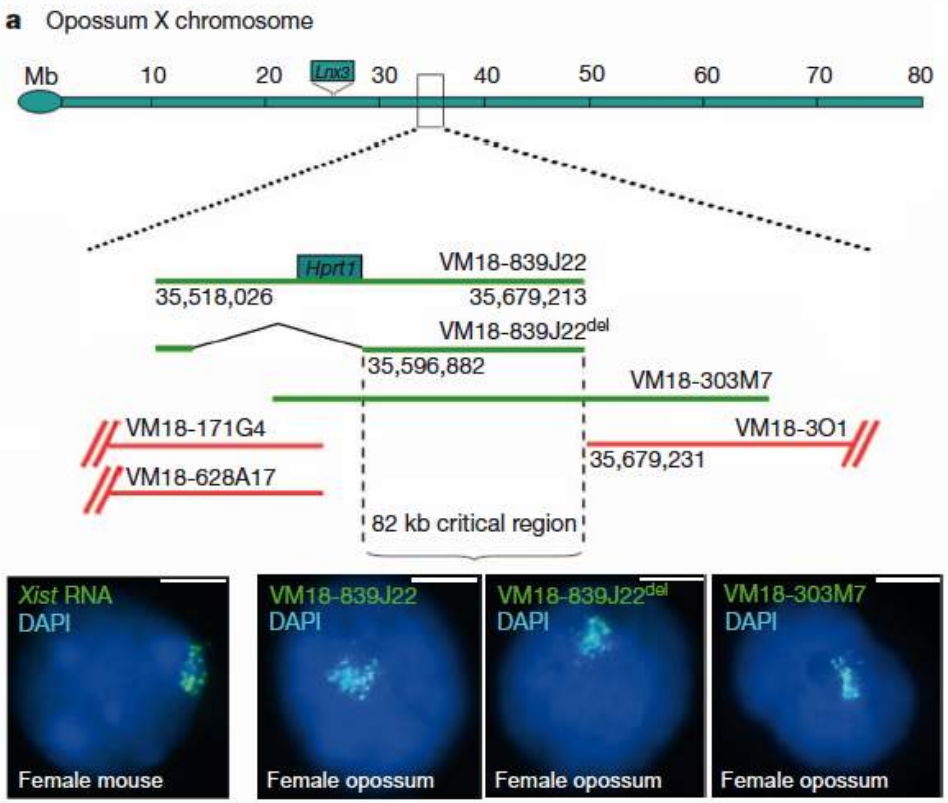
Long Non-Coding RNAs trigger X inactivation in Mice, Humans and Marsupials?

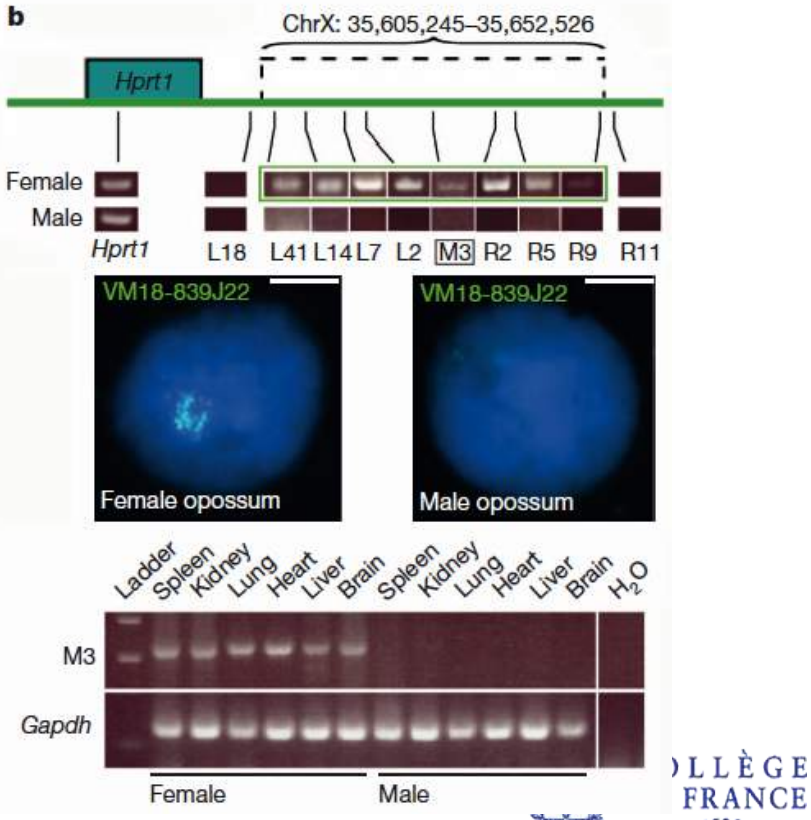
LETTER

doi:10.1038/nature11171

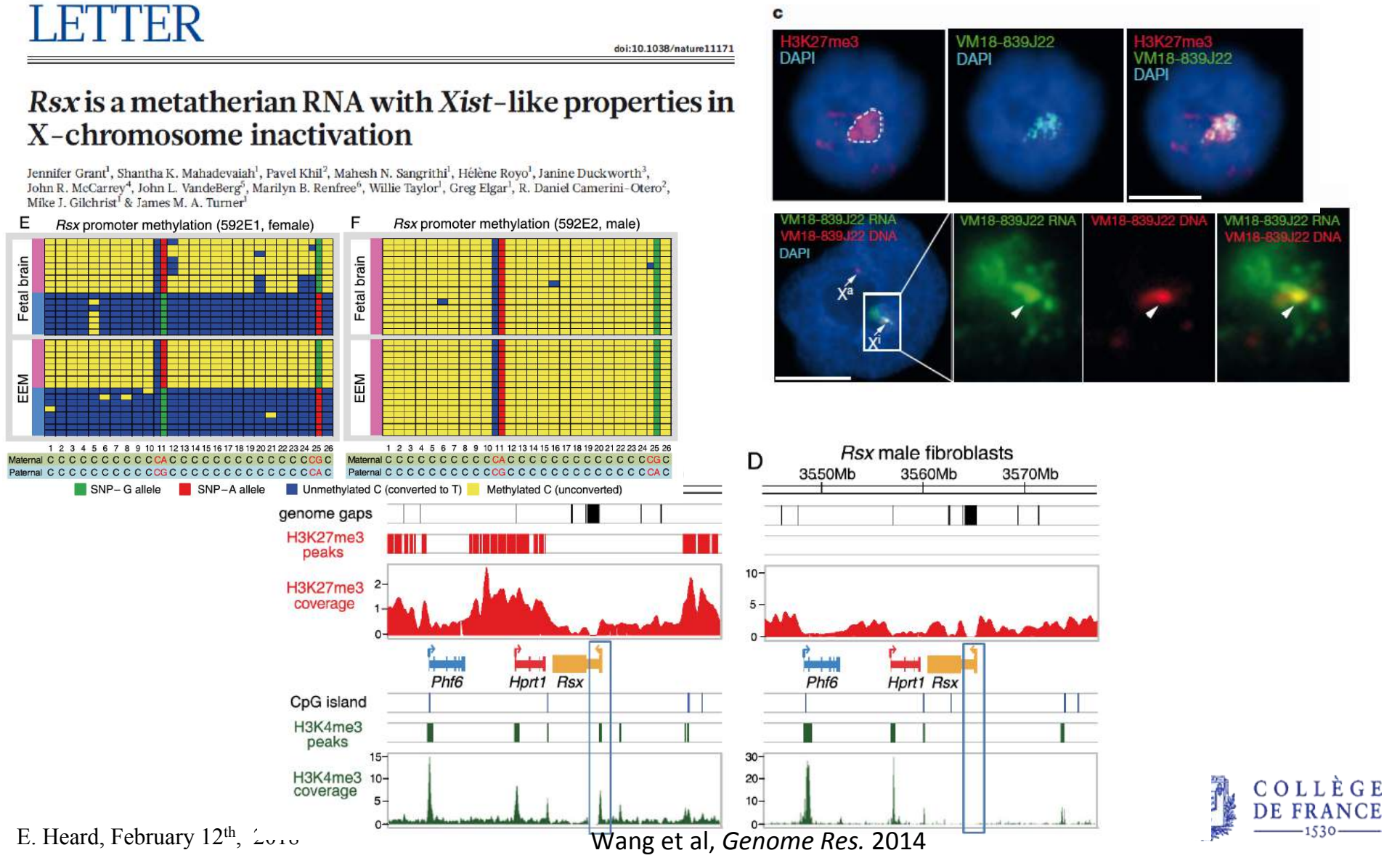
Rsx is a metatherian RNA with *Xist*-like properties in X-chromosome inactivation

Jennifer Grant[1], Shantha K. Mahadevaiah[1], Pavel Khil[2], Mahesh N. Sangrithi[1], Hélène Royo[1], Janine Duckworth[3], John R. McCarrey[4], John L. VandeBerg[5], Marilyn B. Renfree[6], Willie Taylor[1], Greg Elgar[1], R. Daniel Camerini-Otero[2], Mike J. Gilchrist[1] & James M. A. Turner[1]



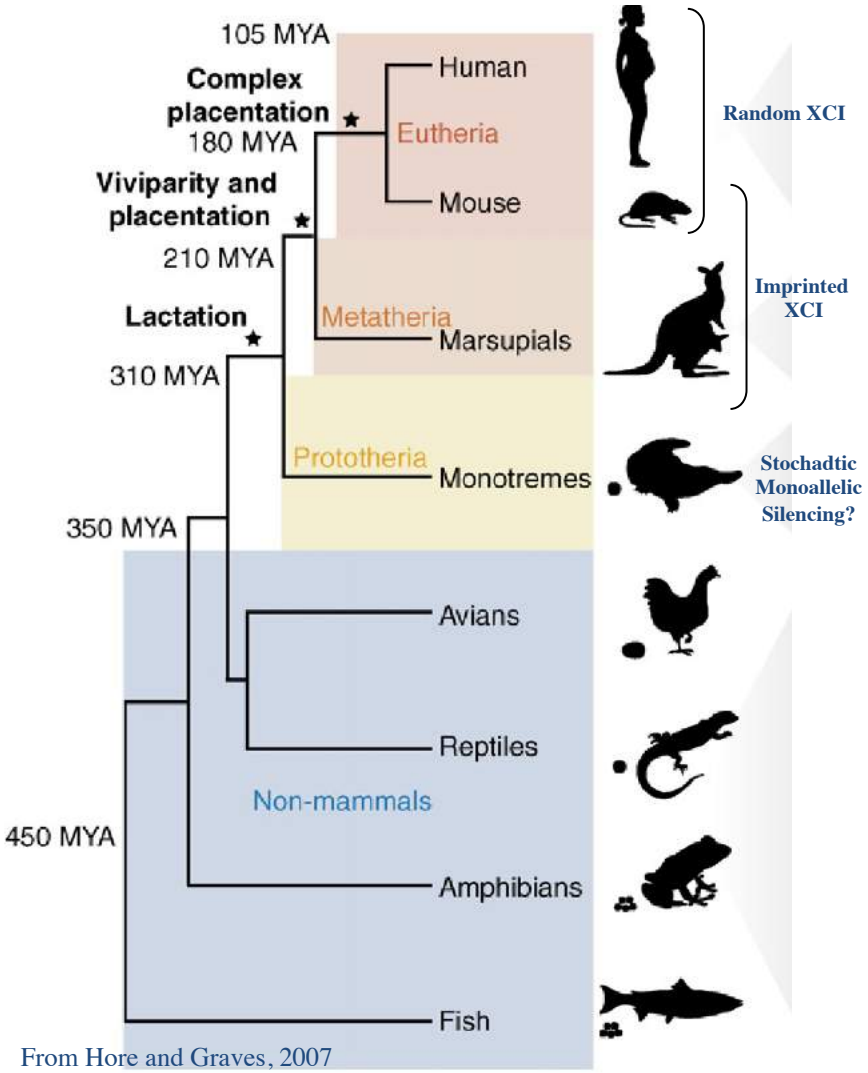


Long Non-Coding Rsx RNA triggers X inactivation in Marsupials?



Wang et al, *Genome Res.* 2014

Sex chromosome Dosage Compensation in Mammals



From Hore and Graves, 2007

Random XCI
(*XIST*)

Imprinted &
Random XCI
(*Xist*)

Imprinted XCI
(*Rsx*)

5X / 5Y
Partial dosage
Compensation?
(no *Rsx*, no *Xist*)

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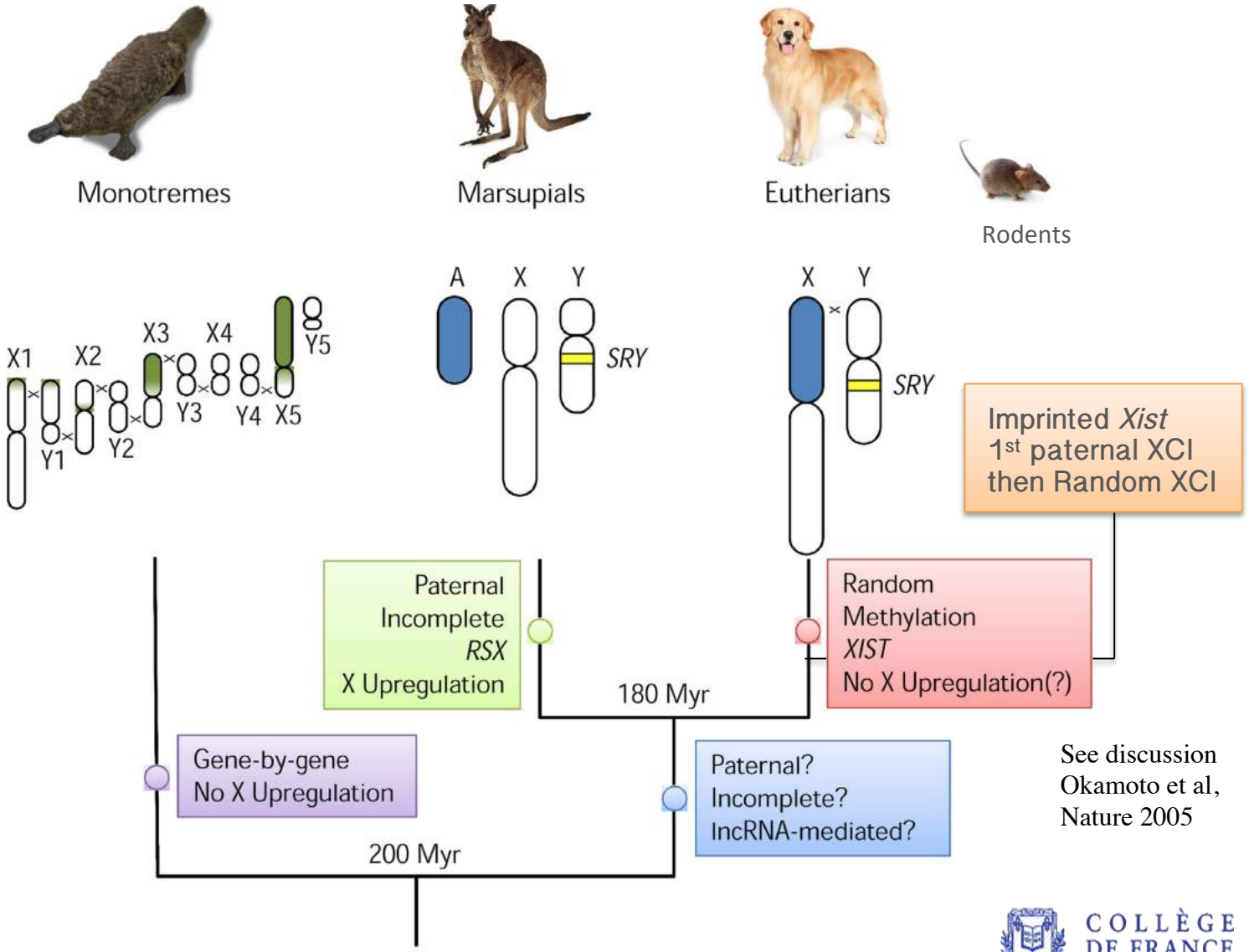
Sex chromosome Dosage Compensation in Mammals

An imprinted form of X inactivation may have evolved more than once



See discussion
Okamoto et al,
Nature 2005

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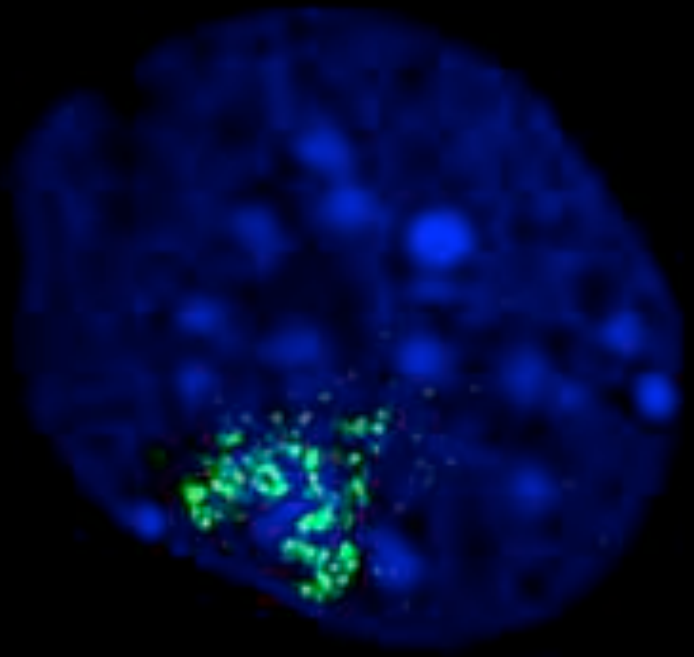
How does XIST work?
(X-Inactive-Specific-Transcript)

A Multi-Tasking Molecule

Scaffold for repressor recruitment?

RNA-DNA binding?



Chromatin changes ?

Nuclear compartmentalisation ?

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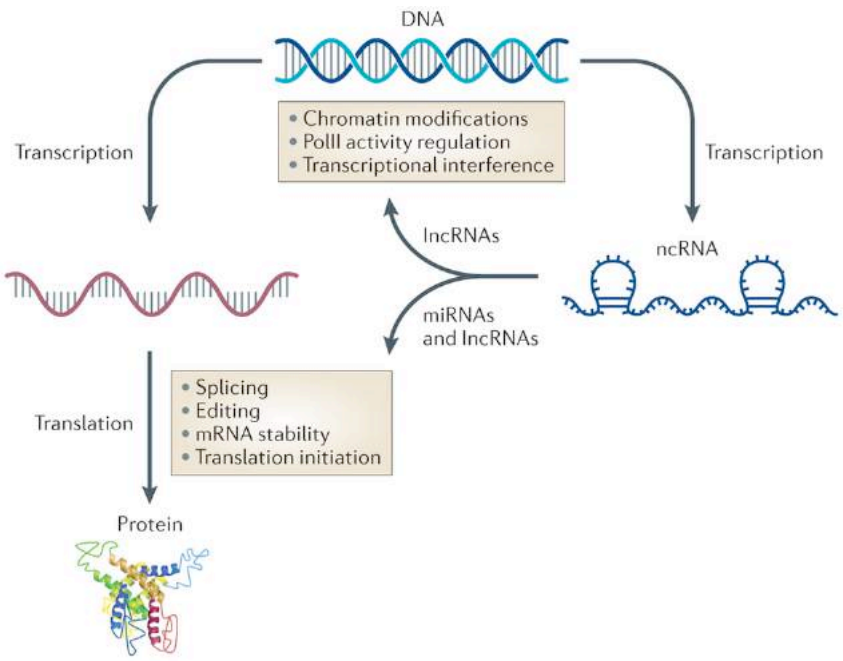
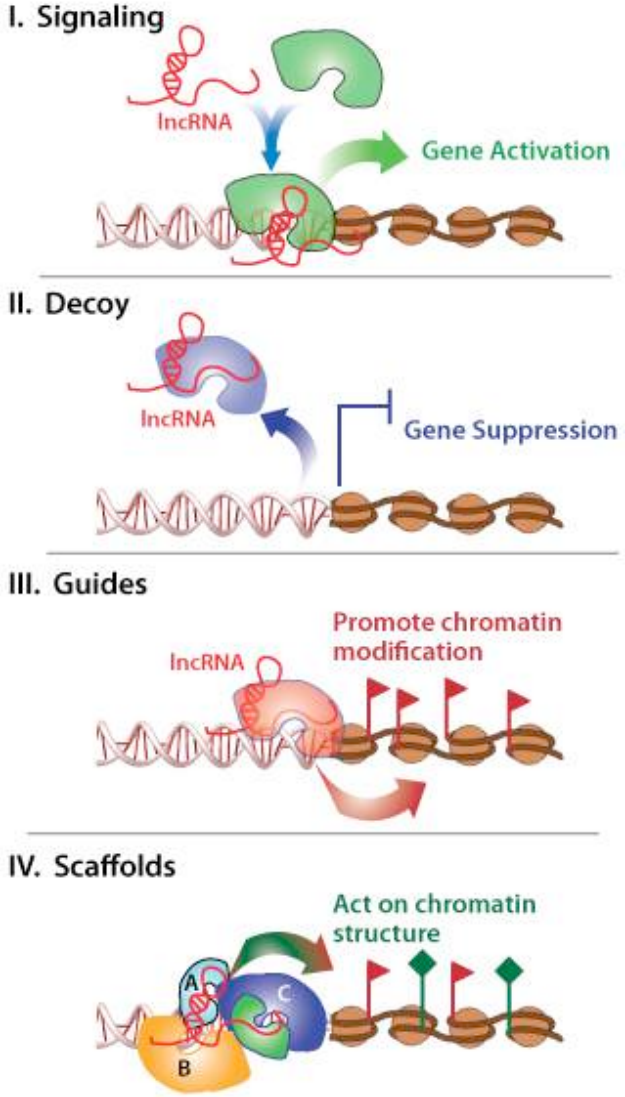
Long non-coding RNAs:
from spurious transcription to functional entities



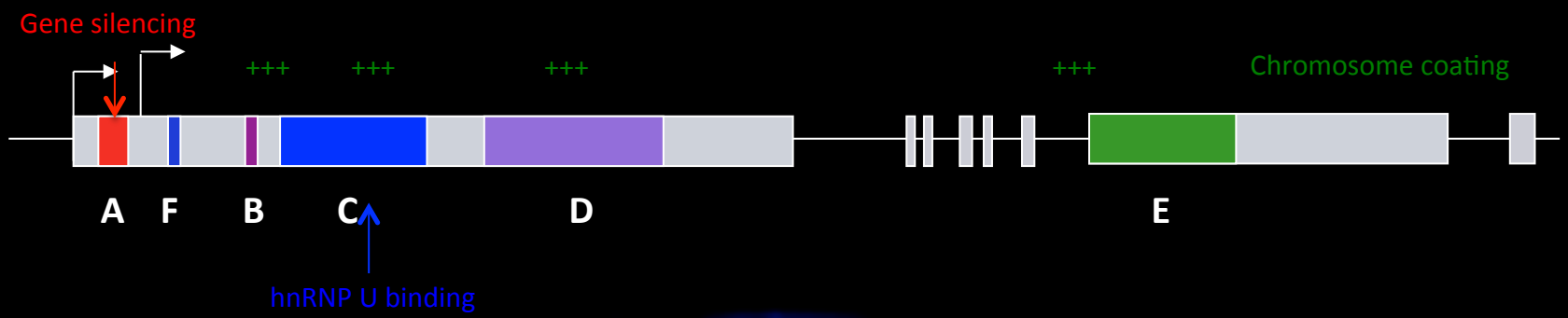
Claes Wahlestedt, 2013

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.

How does XIST work?
(X-Inactive-Specific-Transcript)

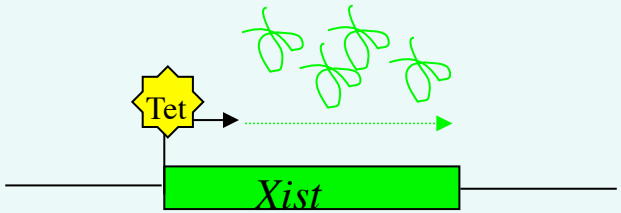


- Poor sequence conservation between mammals - except for repeats A-F
- 17 000 - 19 000 nt, spliced, untranslated, nuclear transcript
- RNA expressed from and "coats" the inactive X chromosome in *cis* (not *trans*)
- *Xist* is **essential** for X inactivation in *cis* (KOs, transgenes in mouse embryos, 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?

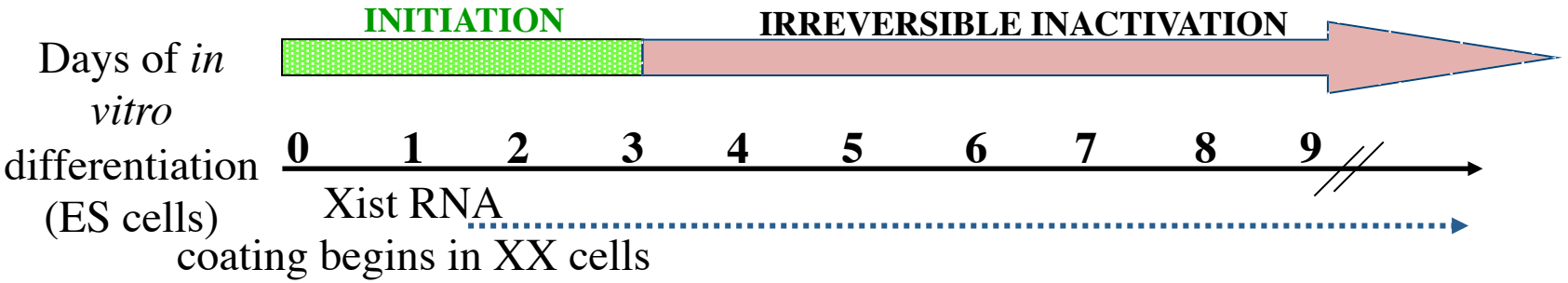
Xist RNA as a trigger for XCI

Inducible *Xist* cDNA :



Cellular Memory?
Epigenetic Marks?

Xist-dependent → Xist-independent

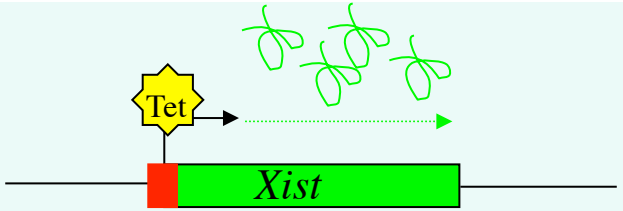


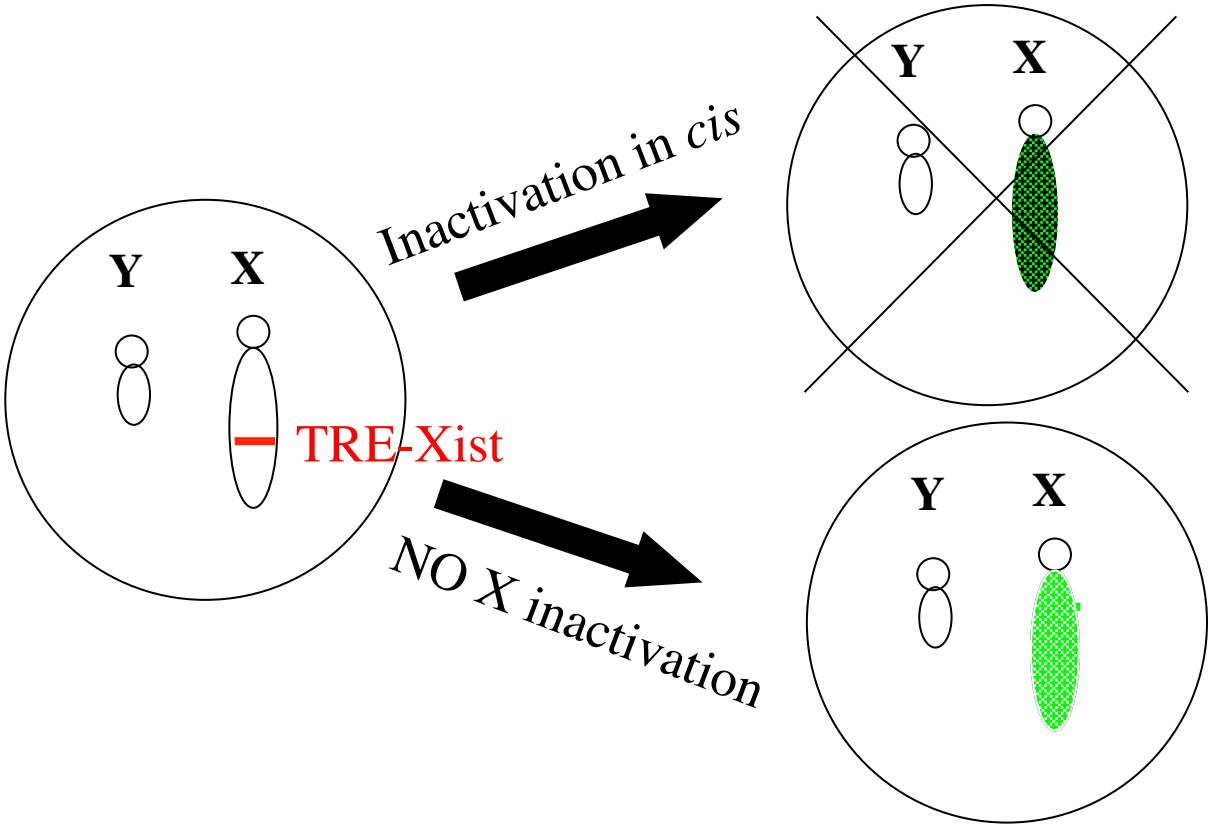
Transcriptional silencing begins
(*Northerns, allele-specific RT PCR, RNA FISH*)

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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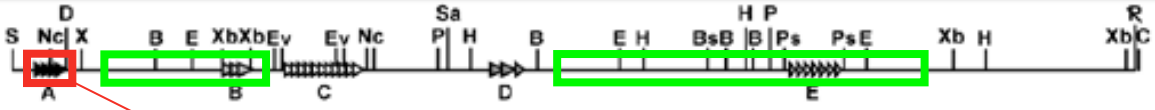
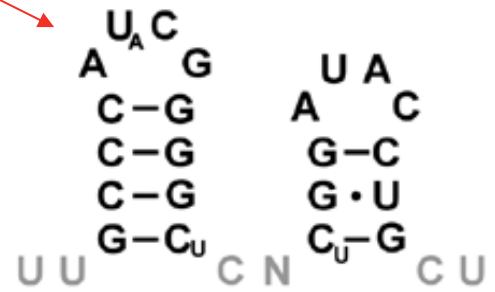
What are the Functional Domains of Xist RNA?



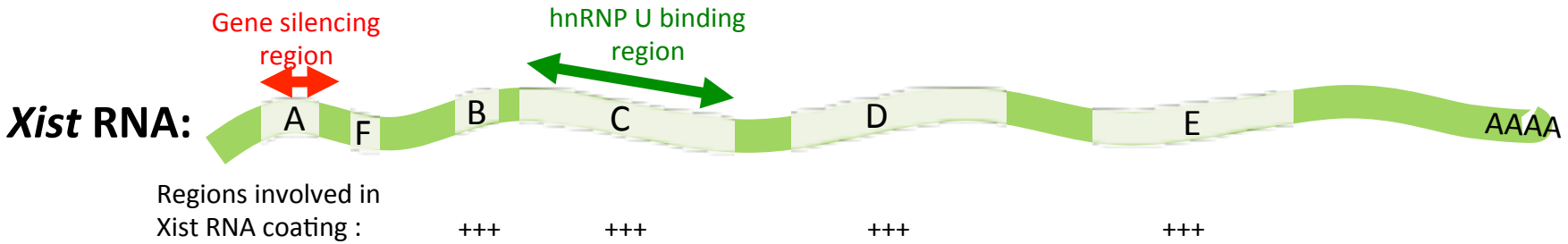
Wutz et al, Nature Genetics 2002



Xist A-repeats are required for gene silencing but NOT for chromosome coating, mH2A and PcG protein recruitment…

Associated proteins ?
Mechanism of action ?

What are the Functional Partners of Xist RNA?



Proteomic screens

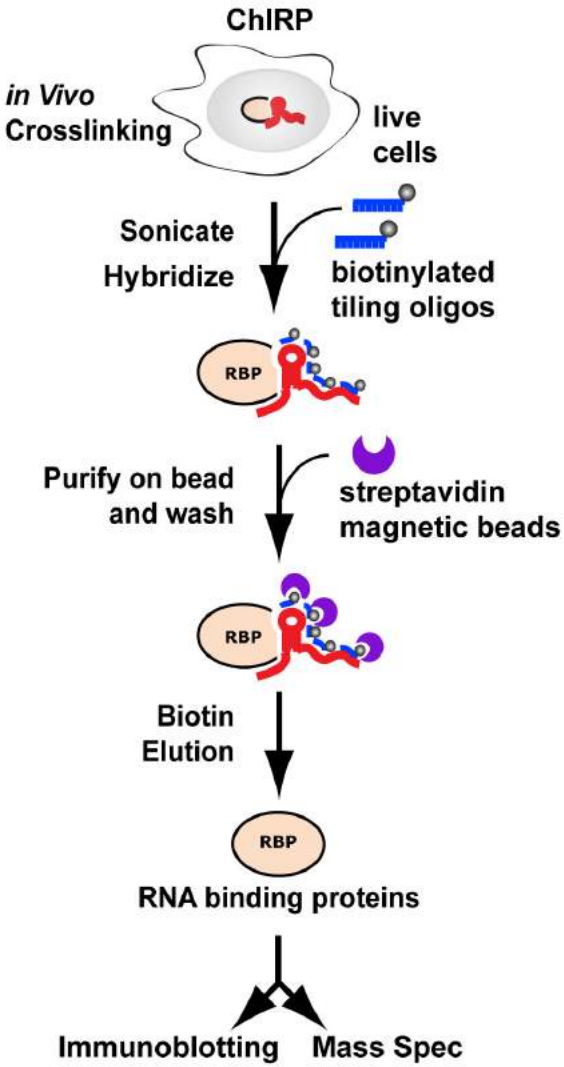
	ChIRP-MS [24]	RAP-MS [25]	iDRiP [26]
Biological samples	*Xist* & ΔA *Xist* inducible mouse ESCs; Mouse XX EpiSCs & TSCs	*Xist* inducible undifferentiated mouse ESCs	Mouse XX immortalized fibroblasts
Crosslinking agent	Formaldehyde	UV	UV
ASOs & *Xist* RNA pull down	43 ASOs of 20nt; 60% *Xist* recovered	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)

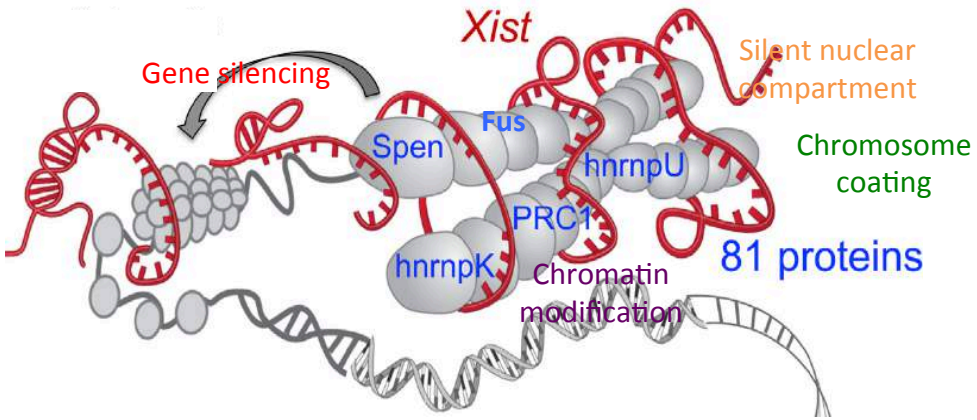
Systematic Discovery of Xist RNA Binding Proteins

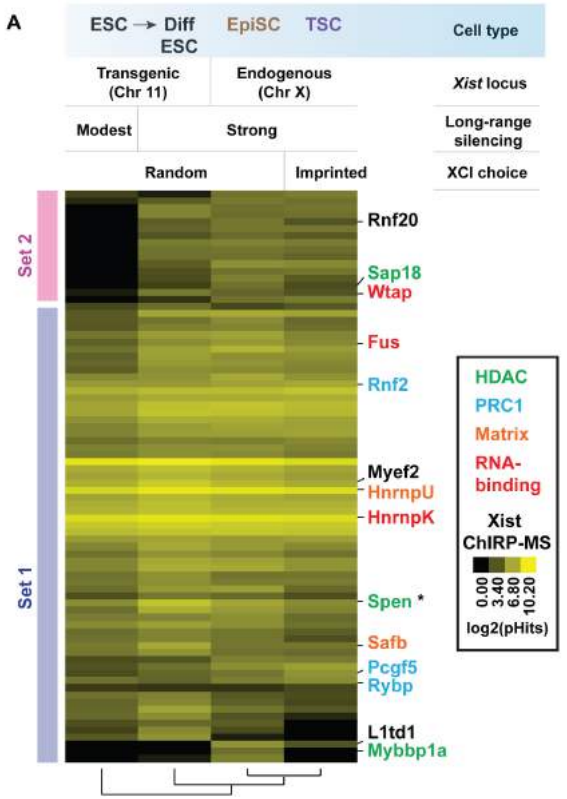
Ci Chu,[1,2] Qiangfeng Cliff Zhang,[1] Simão Teixeira da Rocha,[3] Ryan A. Flynn,[1] Maheetha Bharadwaj,[1] J. Mauro Calabrese,[4] Terry Magnuson,[5] Edith Heard,[3] and Howard Y. Chang[1,*]

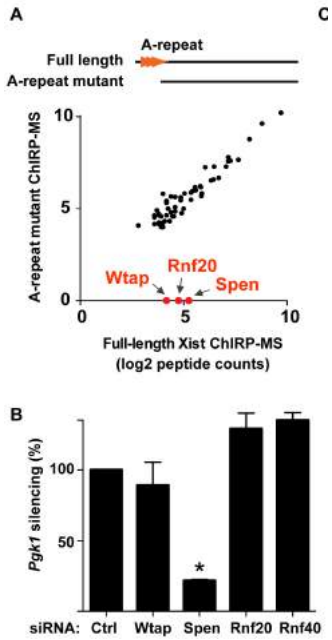


Chu et al, Cell 2015

Xist RNA Functional Partners







Chu et al, Cell 2015

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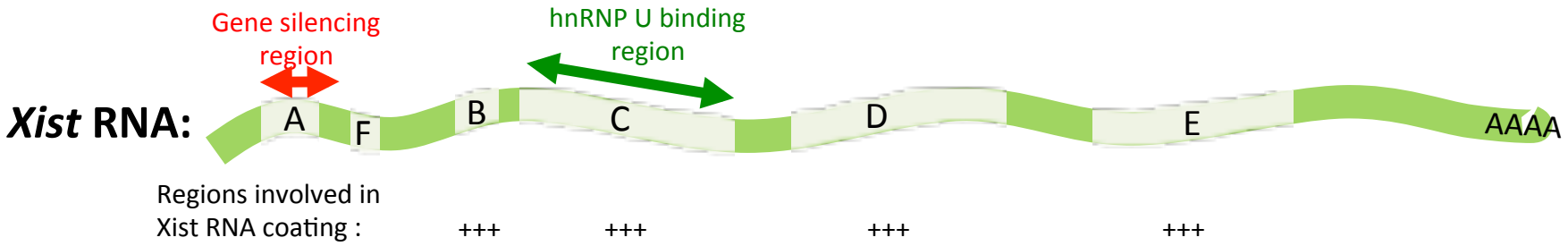
Xist RNA Functional Partners: a few examples



• **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

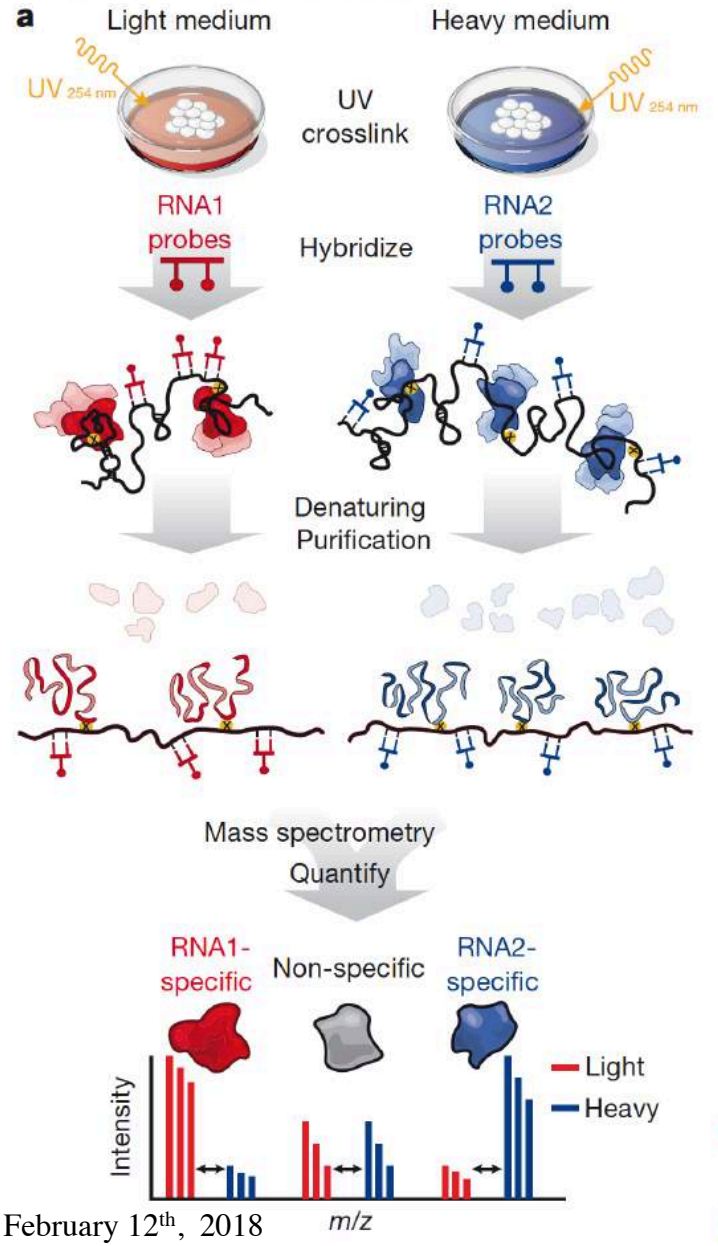
• **HnrnpK**, participates in Xist-mediated gene silencing and recruitment of non-canonical polycomb PRC1 complex but not Xist localization

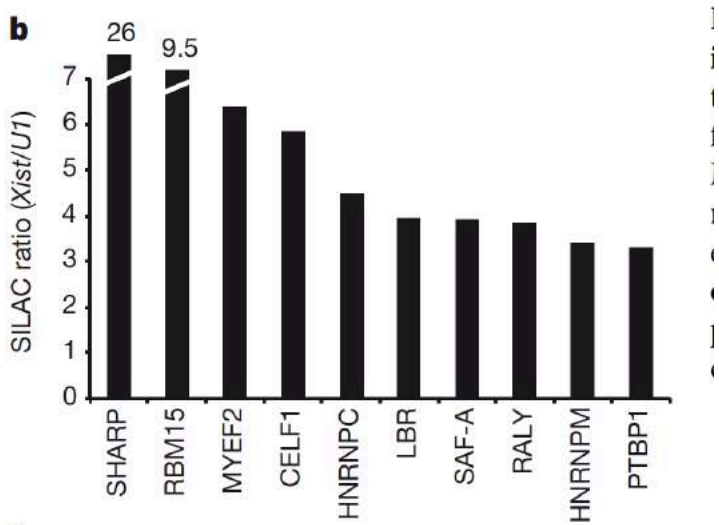
• LBR – **Lamin B receptor – nuclear organisation?**

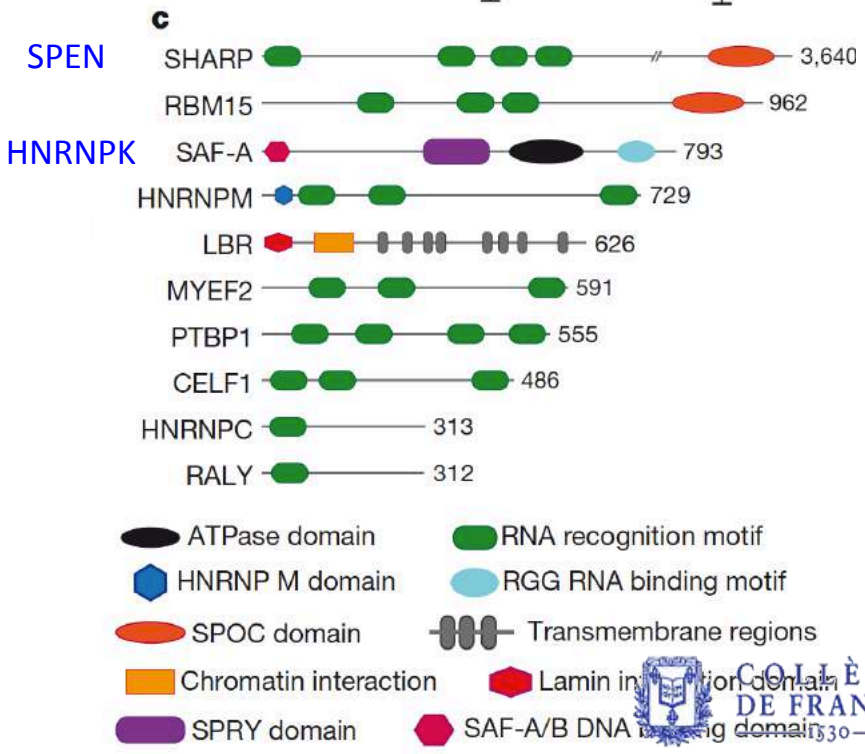
Chu et al, Cell 2015

The *Xist* lncRNA interacts directly with SHARP to silence transcription through HDAC3

Colleen A. McHugh[1]*, Chun-Kan Chen[1]*, Amy Chow[1], Christine F. Surka[1], Christina Tran[1], Patrick McDonel[2], Amy Pandya-Jones[3,4], Mario Blanco[1], Christina Burghard[1], Annie Moradian[5], Michael J. Sweredoski[5], Alexander A. Shishkin[1], Julia Su[1], Eric S. Lander[2], Sonja Hess[5], Kathrin Plath[3,4] & Mitchell Guttman[1]





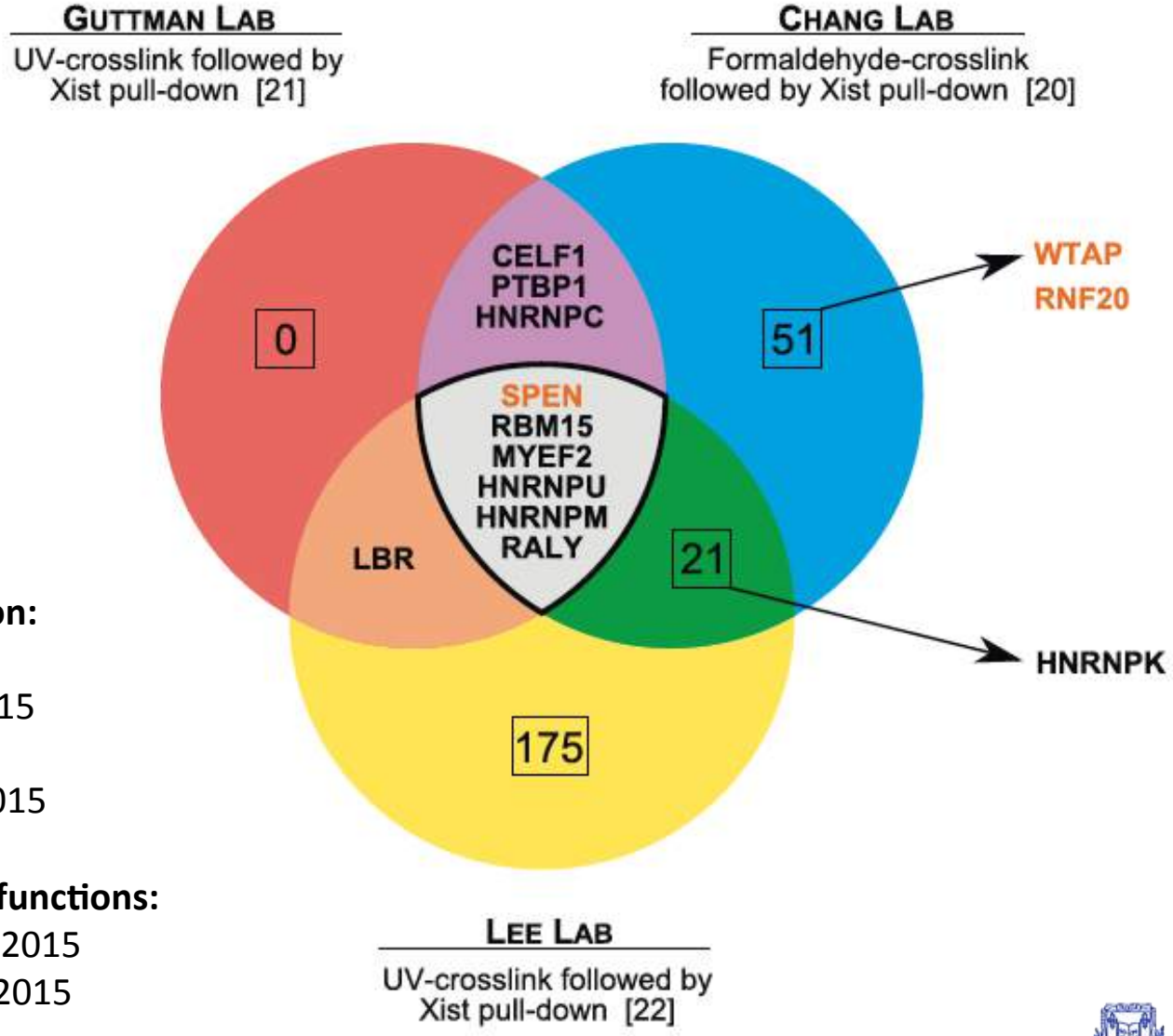


Identification of the Protein Partners of Xist RNA and the Factors that are implicated in Xist-mediated Silencing

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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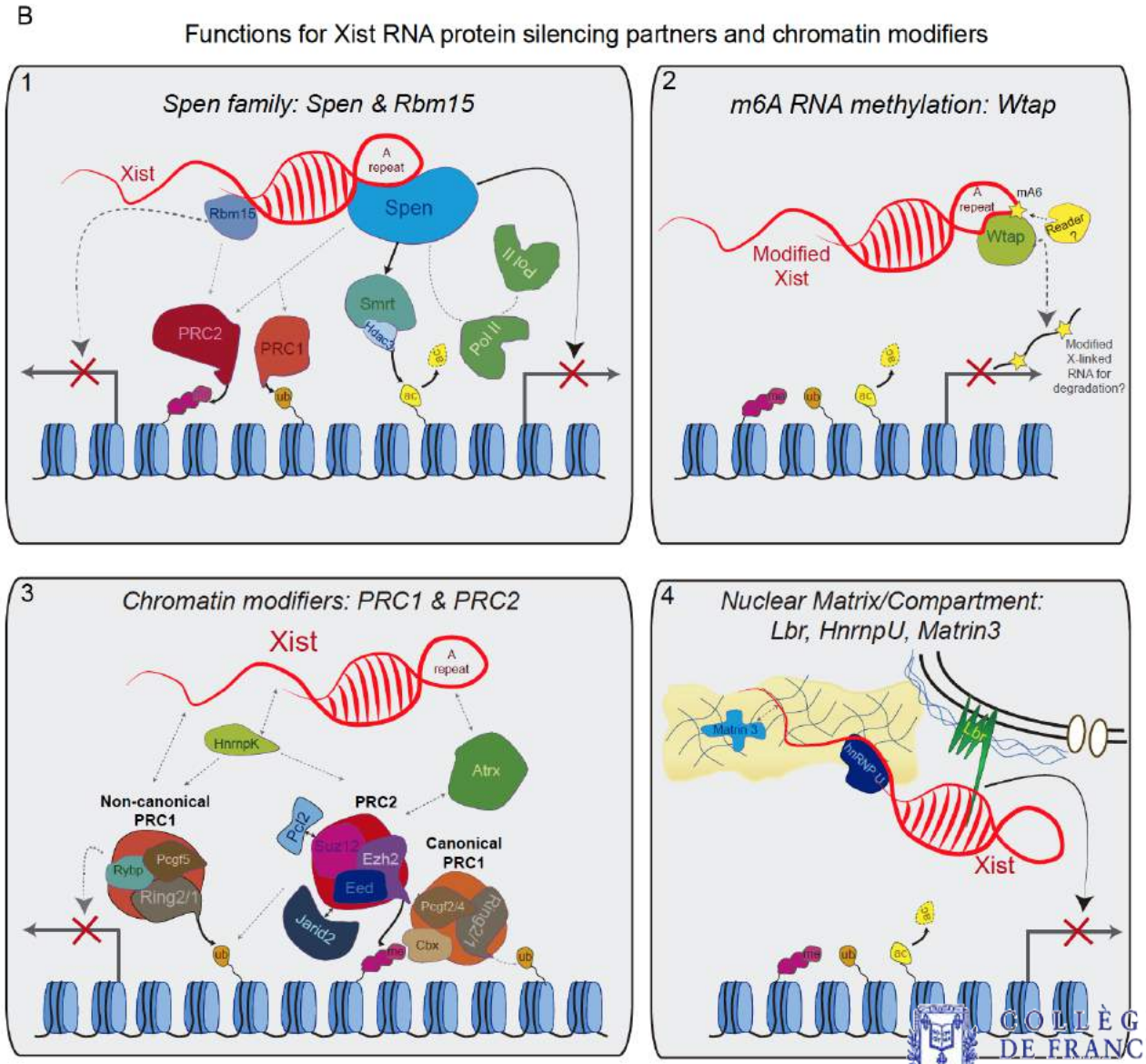
The first molecular handles for Xist RNA function herald a a new era of Xist RNA and X-inactivation research



Xist RNA partner isolation:
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McHugh et al, Nature 2015
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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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Does Xist RNA silence genes via nuclear reorganisation?

Science

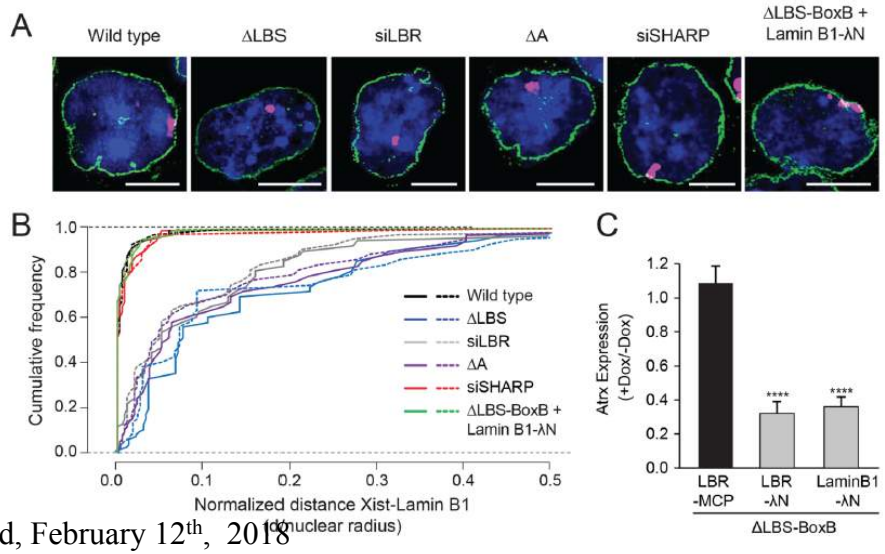
REPORTS

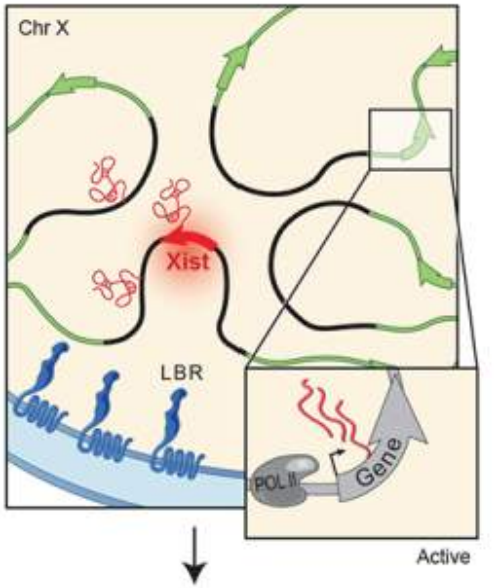
Cite as: C.-K. Chen *et al.*, *Science* 10.1126/science.aae0047 (2016).

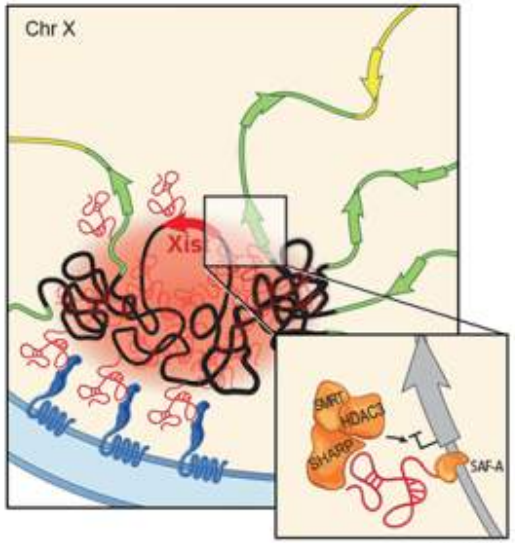
Xist recruits the X chromosome to the nuclear lamina to enable chromosome-wide silencing

Chun-Kan Chen,[1] Mario Blanco,[1] Constanza Jackson,[1] Erik Aznauryan,[1] Noah Ollikainen,[1] Christine Surka,[1] Amy Chow,[1] Andrea Cerase,[2] Patrick McDonel,[3] Mitchell Guttman[1]*

The Xist lncRNA orchestrates X chromosome inactivation, a process that entails silencing and remodeling of the 3-dimensional structure of the X chromosome. Y whether these changes in nuclear structure are mediated by Xist and whether the silencing. Here we show that Xist directly interacts with the Lamin B Receptor (LI component of the nuclear lamina, and that this interaction is required for Xist-me recruiting the inactive X to the nuclear lamina and by doing so enables Xist to spr transcribed genes across the X. Our results demonstrate that lamina recruitment dimensional structure of DNA thereby enabling Xist, and its silencing proteins, to silence transcription.







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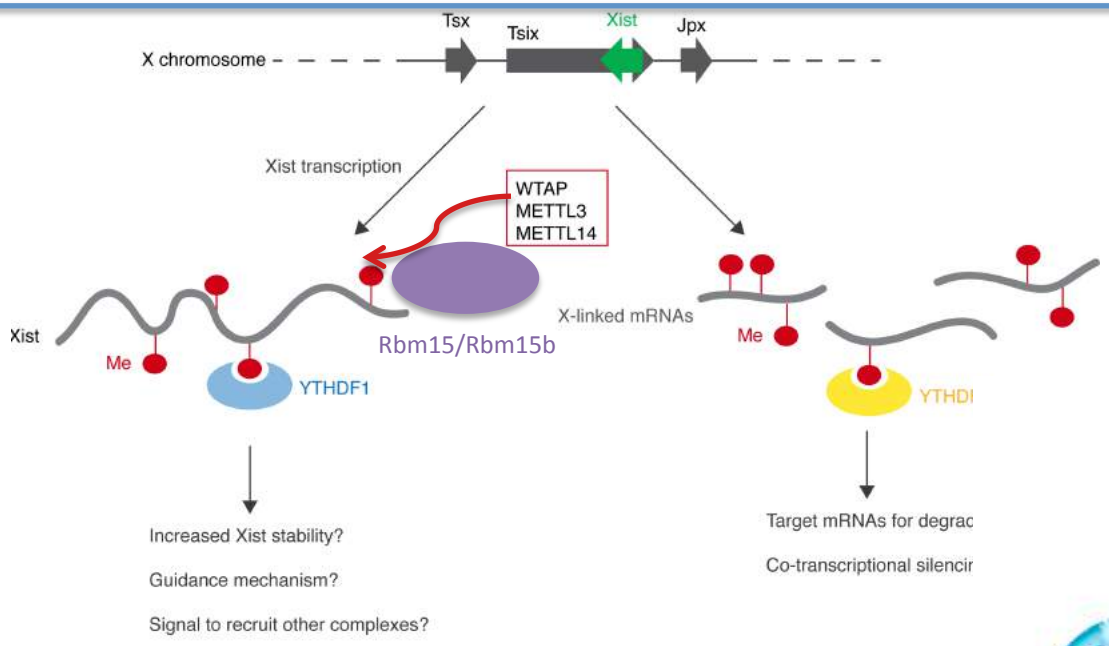
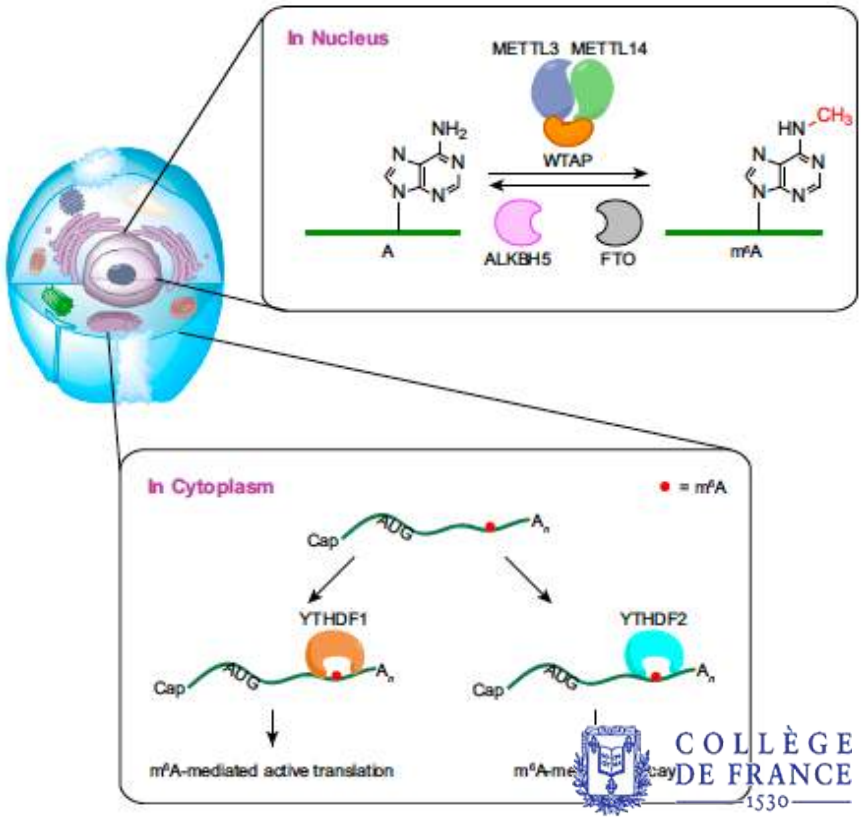
Does Xist RNA silence genes via RNA methylation?





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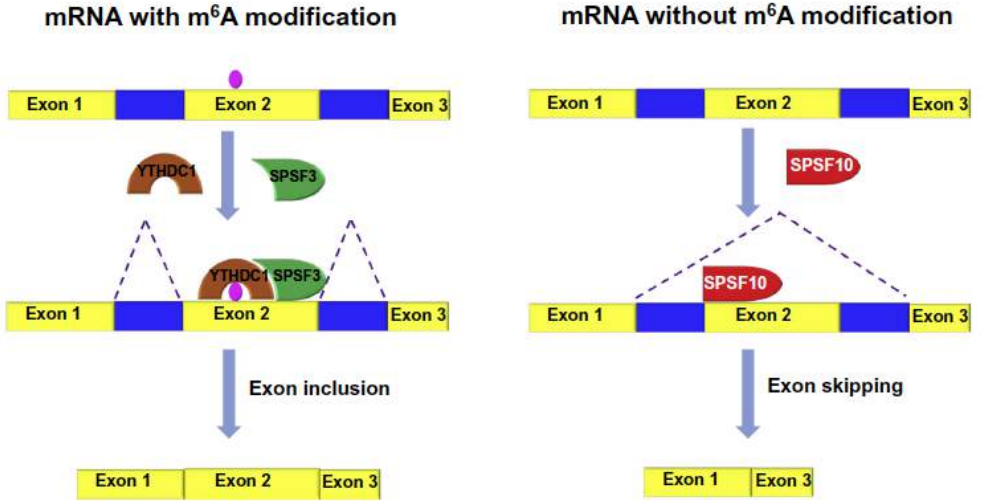
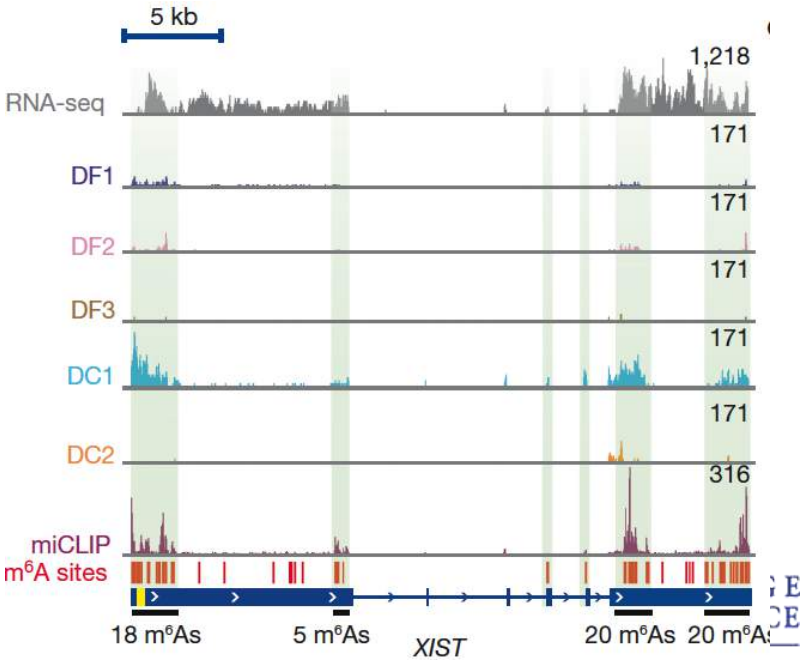
Does Xist RNA silence genes via RNA methylation?

m^{6}A RNA methylation promotes *XIST*-mediated transcriptional repression

Deepak P. Patil[1], Chun-Kan Chen[2], Brian F. Pickering[1], Amy Chow[2], Constanza Jackson[2], Mitchell Guttman[2] & Samie R. Jaffrey[1]

The long non-coding RNA X-inactive specific transcript (*XIST*) mediates the transcriptional silencing of genes on the X chromosome. Here we show that, in human cells, *XIST* is highly methylated with at least 78 N^6-methyladenosine (m^{6}A) residues—a reversible base modification of unknown function in long non-coding RNAs. We show that m^{6}A formation in *XIST*, as well as in cellular mRNAs, is mediated by RNA-binding motif protein 15 (RBM15) and its paralogue RBM15B, which bind the m^{6}A-methylation complex and recruit it to specific sites in RNA. This results in the methylation of adenosine nucleotides in adjacent m^{6}A consensus motifs. Furthermore, we show that knockdown of *RBM15* and *RBM15B*, or knockdown of methyltransferase like 3 (*METTL3*), an m^{6}A methyltransferase, impairs *XIST*-mediated gene silencing. A systematic comparison of m^{6}A-binding proteins shows that YTH domain containing 1 (YTHDC1) preferentially recognizes m^{6}A residues on *XIST* and is required for *XIST* function. Additionally, artificial tethering of YTHDC1 to *XIST* rescues *XIST*-mediated silencing upon loss of m^{6}A. These data reveal a pathway of m^{6}A formation and recognition required for *XIST*-mediated transcriptional repression.





E. Heard, February 12th, 2018

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

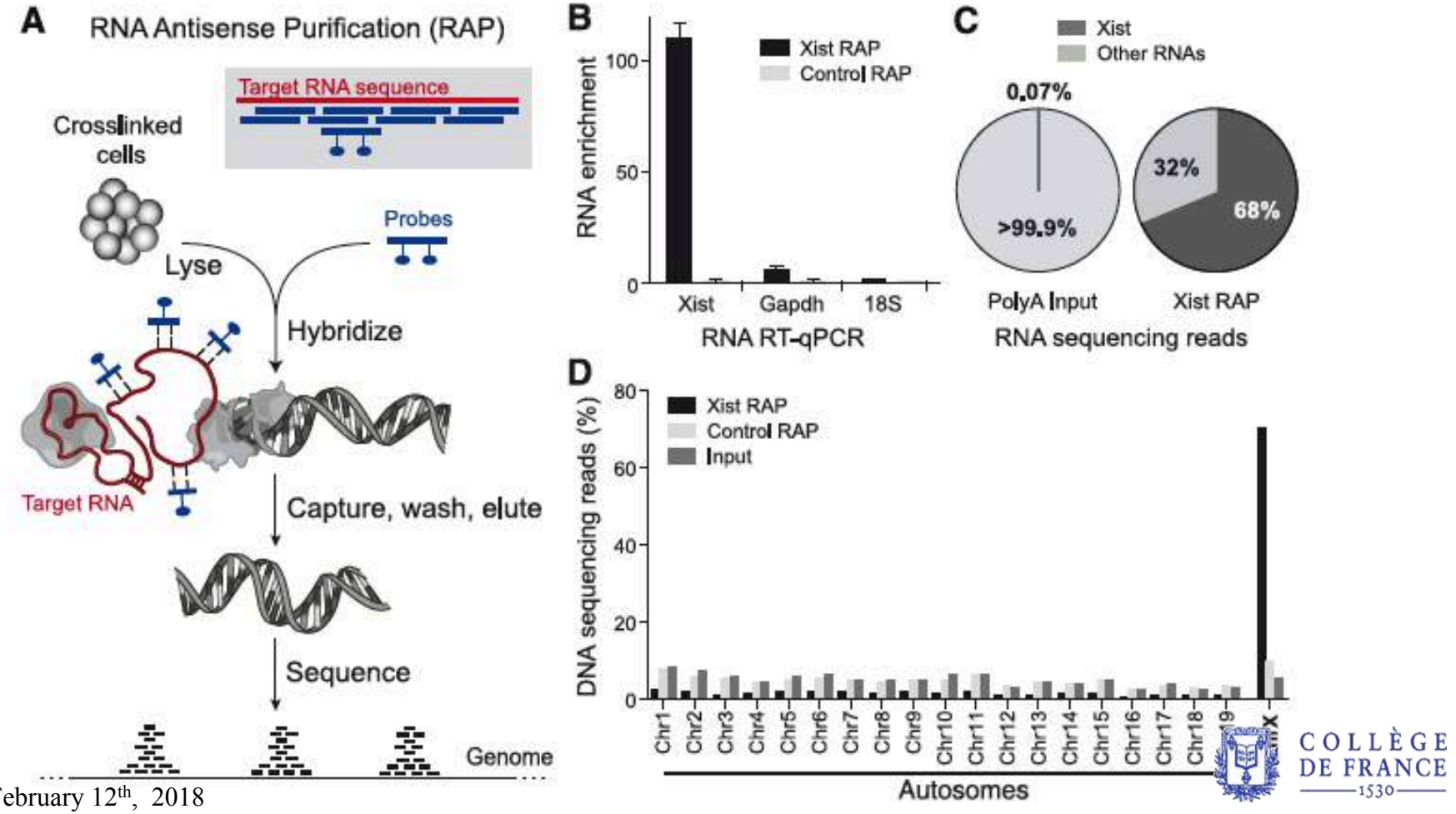
- The first regions Xist targets contain the first genes silenced

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

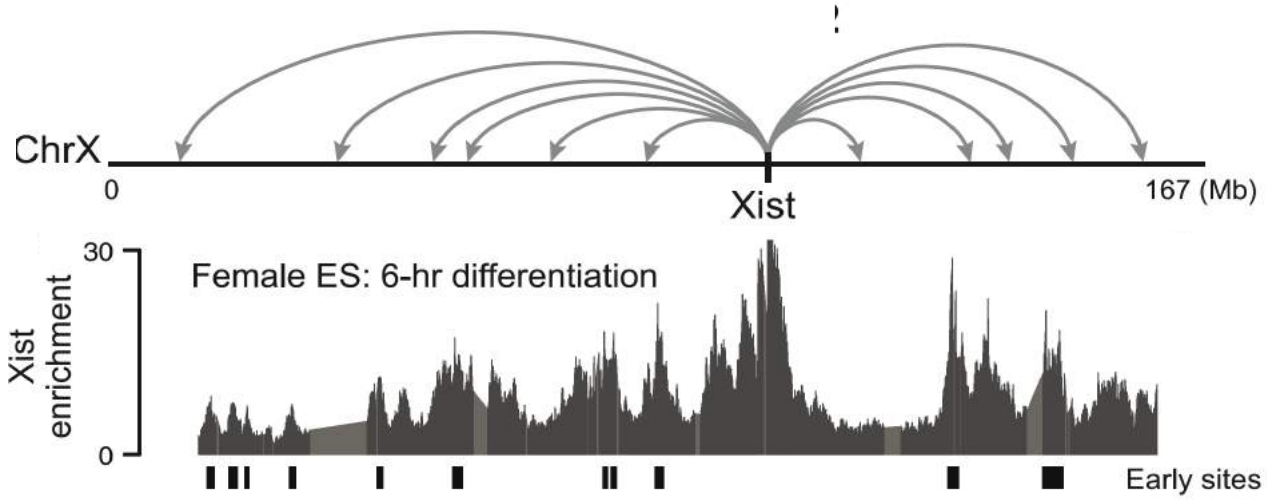
Where does Xist RNA bind on the X chromosome?

The Xist lncRNA Exploits Three-Dimensional Genome Architecture to Spread Across the X Chromosome

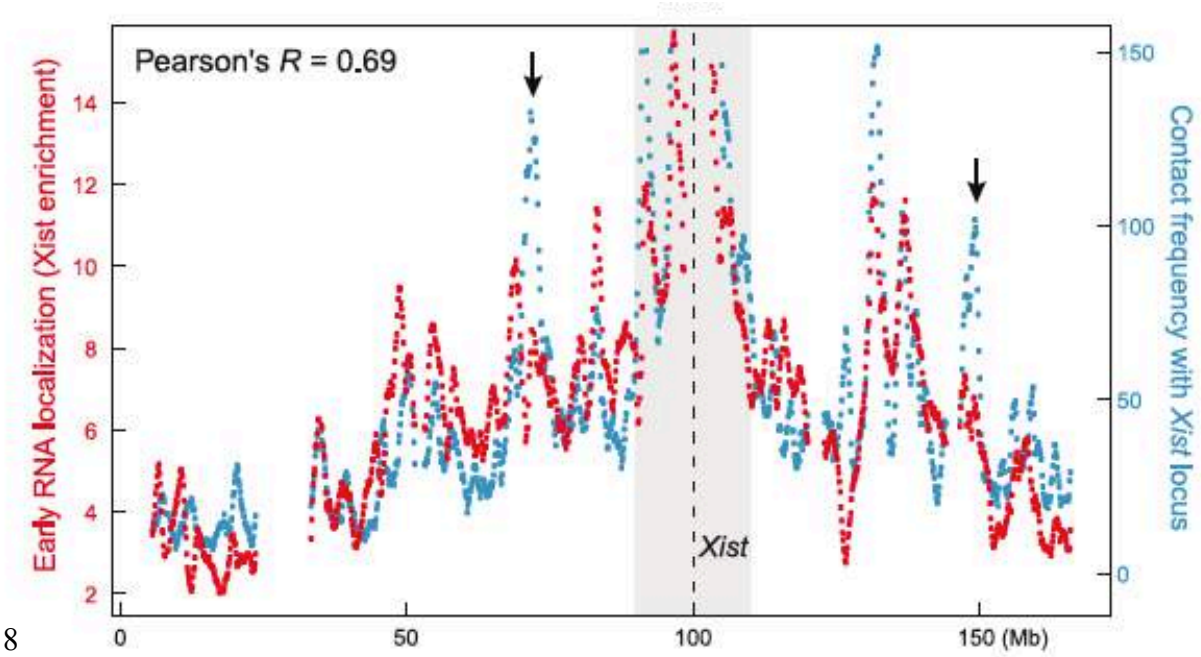
Jesse M. Engreitz,[1,2] Amy Pandya-Jones,[3] Patrick McDonel,[1] Alexander Shishkin,[1] Klara Sirokman,[1] Christine Surka,[1] Sabah Kadri,[1] Jeffrey Xing,[1] Alon Goren,[1] Eric S. Lander,[1,4,5]* Kathrin Plath,[3]* Mitchell Guttman[1]*†



Where does Xist RNA bind on the X chromosome?



The Xist lncRNA Exploits Three-Dimensional Genome Architecture to Spread Across the X Chromosome
Jesse M. Engreitz et al. Science 341, (2013);



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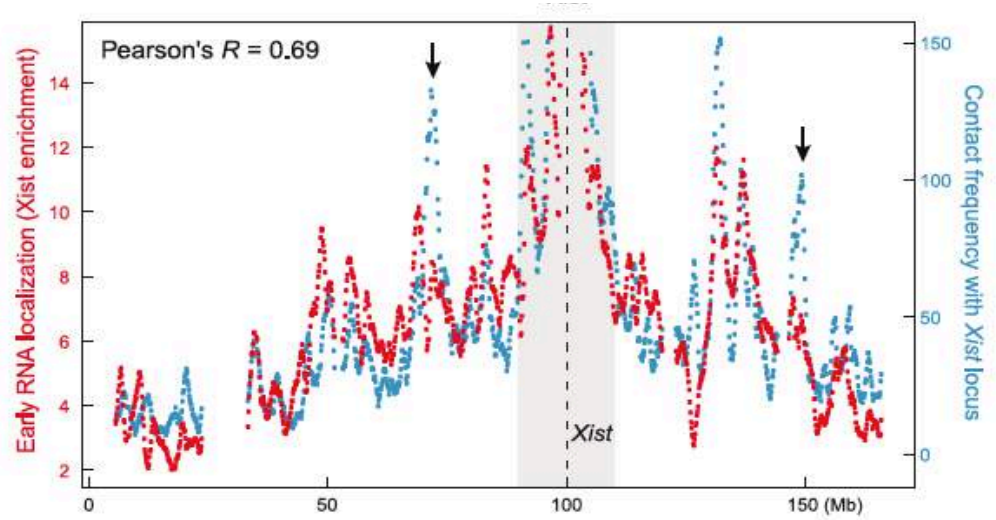
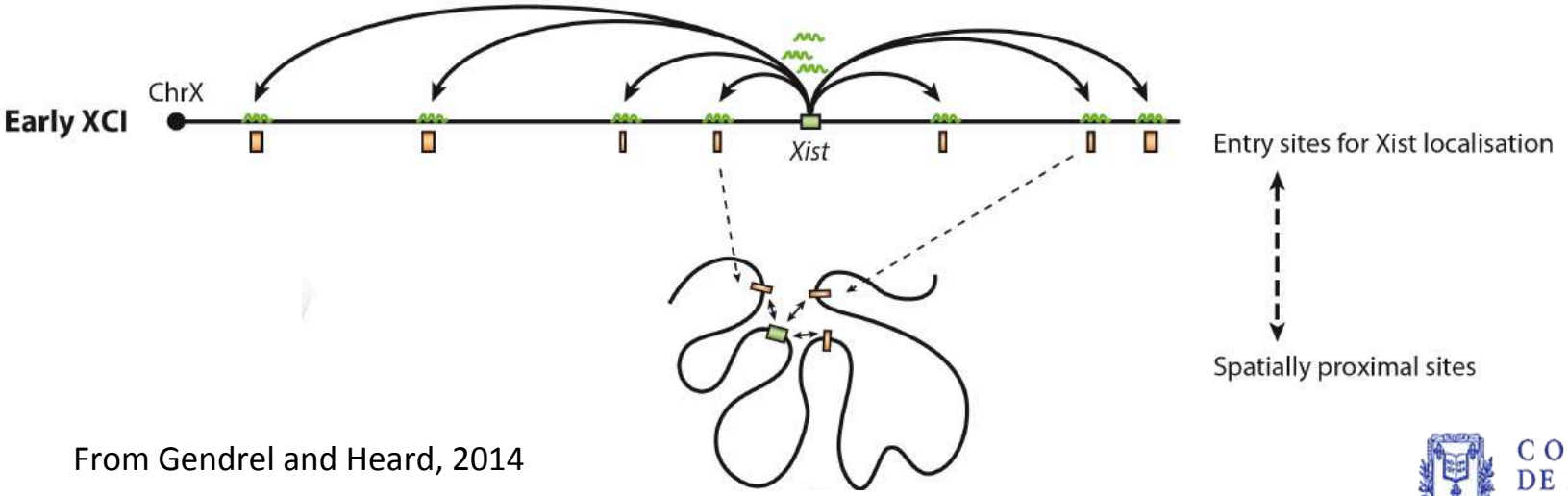
Xist RNA exploits 3D genome architecture to spread across the X chromosome



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

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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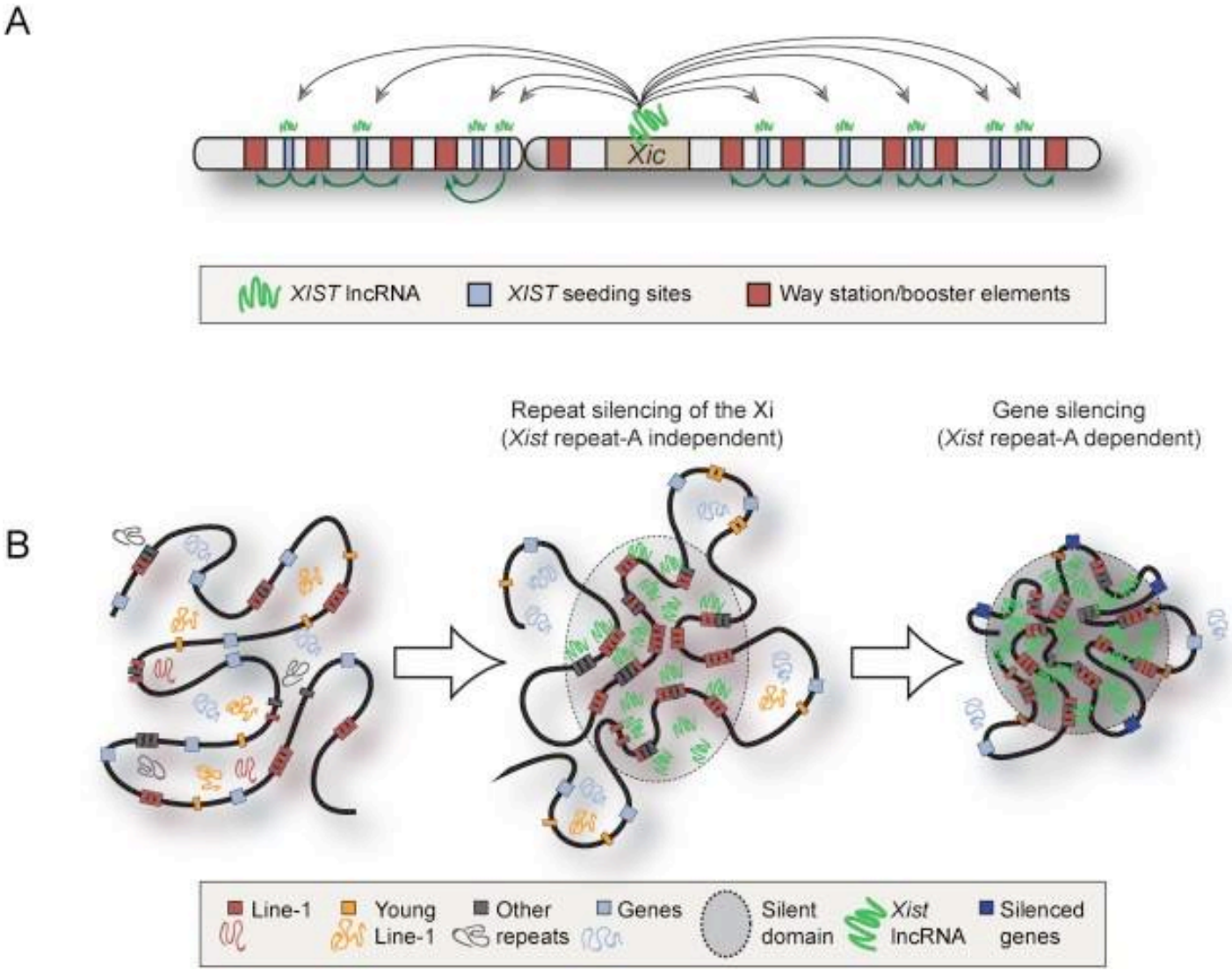
Nuclear Organisation of the Inactive X



Enlightening the contribution of the dark matter to the X chromosome inactivation process in mammals
Author links open overlay panelMiguelCasanovaTharvesh

E. Heard, February 12th, 2018

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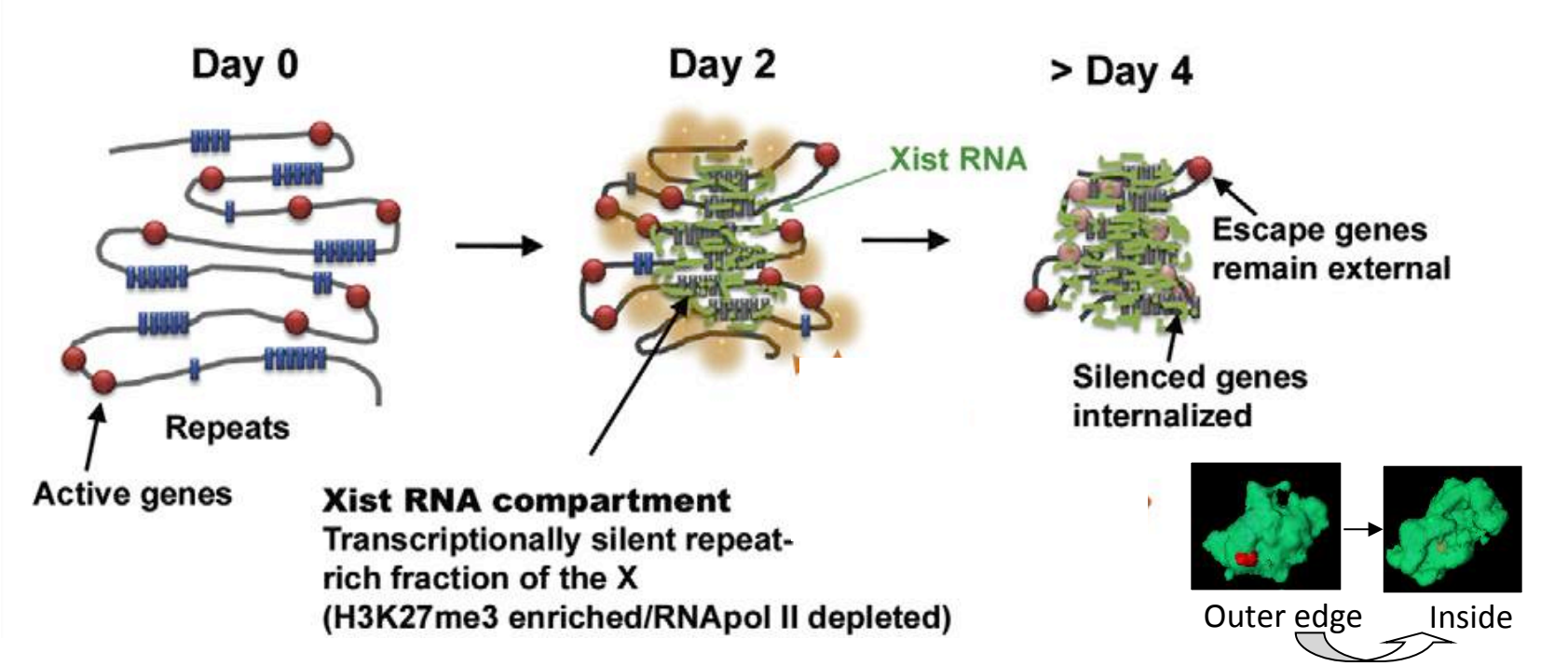
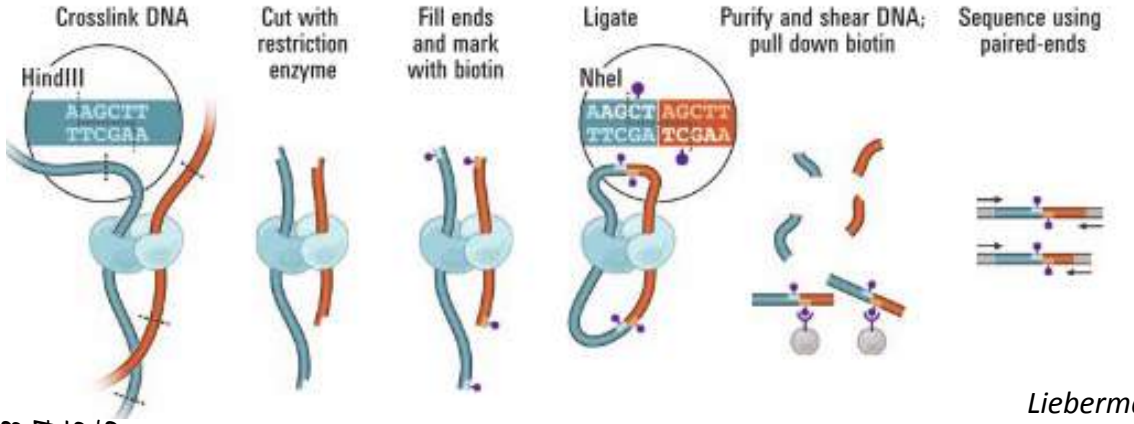
Xist RNA forms a silent nuclear compartment and triggers spatial reorganisation of the Xi during XCI



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

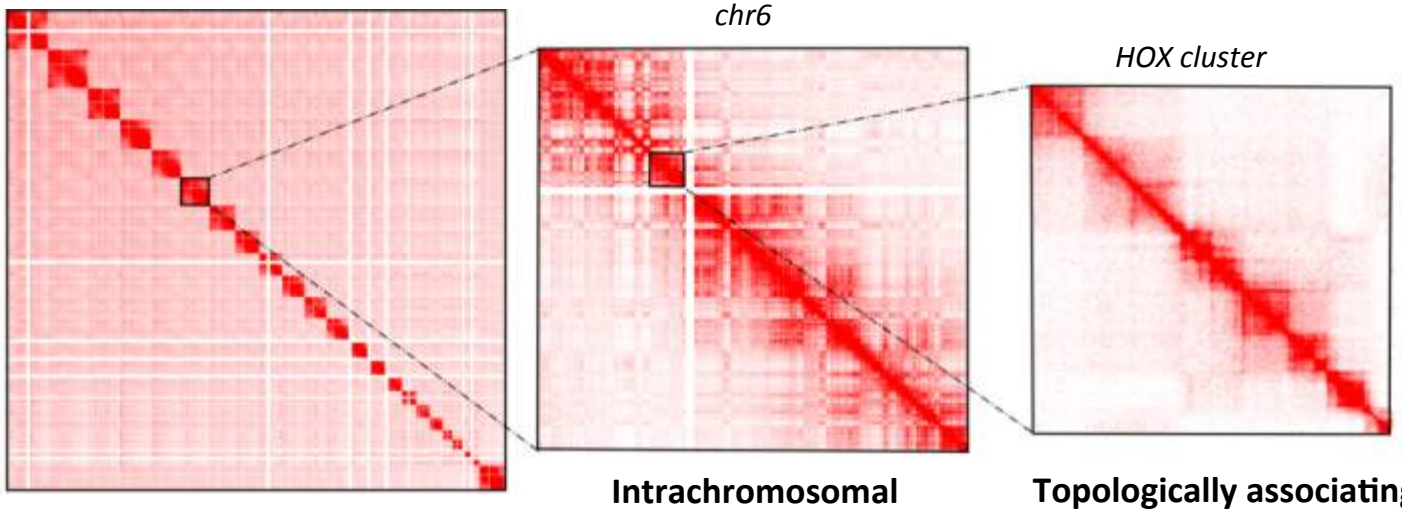
Chaumeil et al, Genes Dev. 2006; Chow et al, Cell 2010

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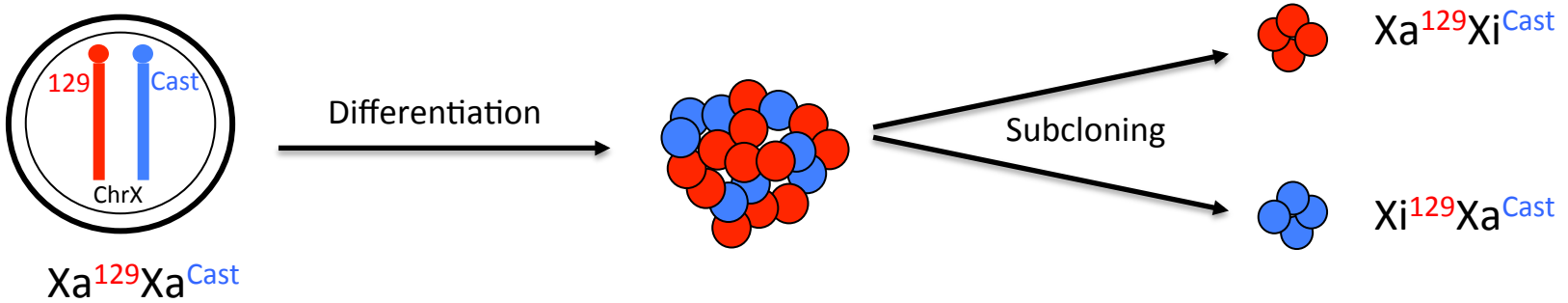
Allele-specific RNA seq and Hi-C in clonal F1 129/Cast ESCs and NPCs

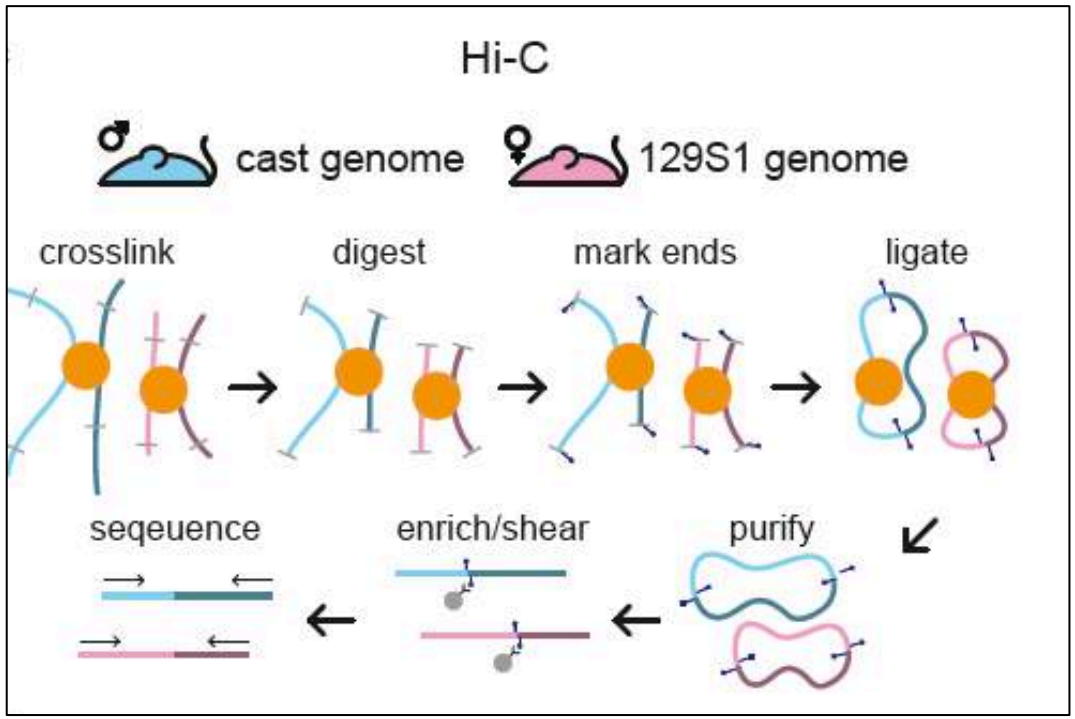
F1 Hybrid ES cell line
(129xCast => 1 SNP /~100bp)

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

Isolate NPC clones
100% cells with 129 or cast Xi

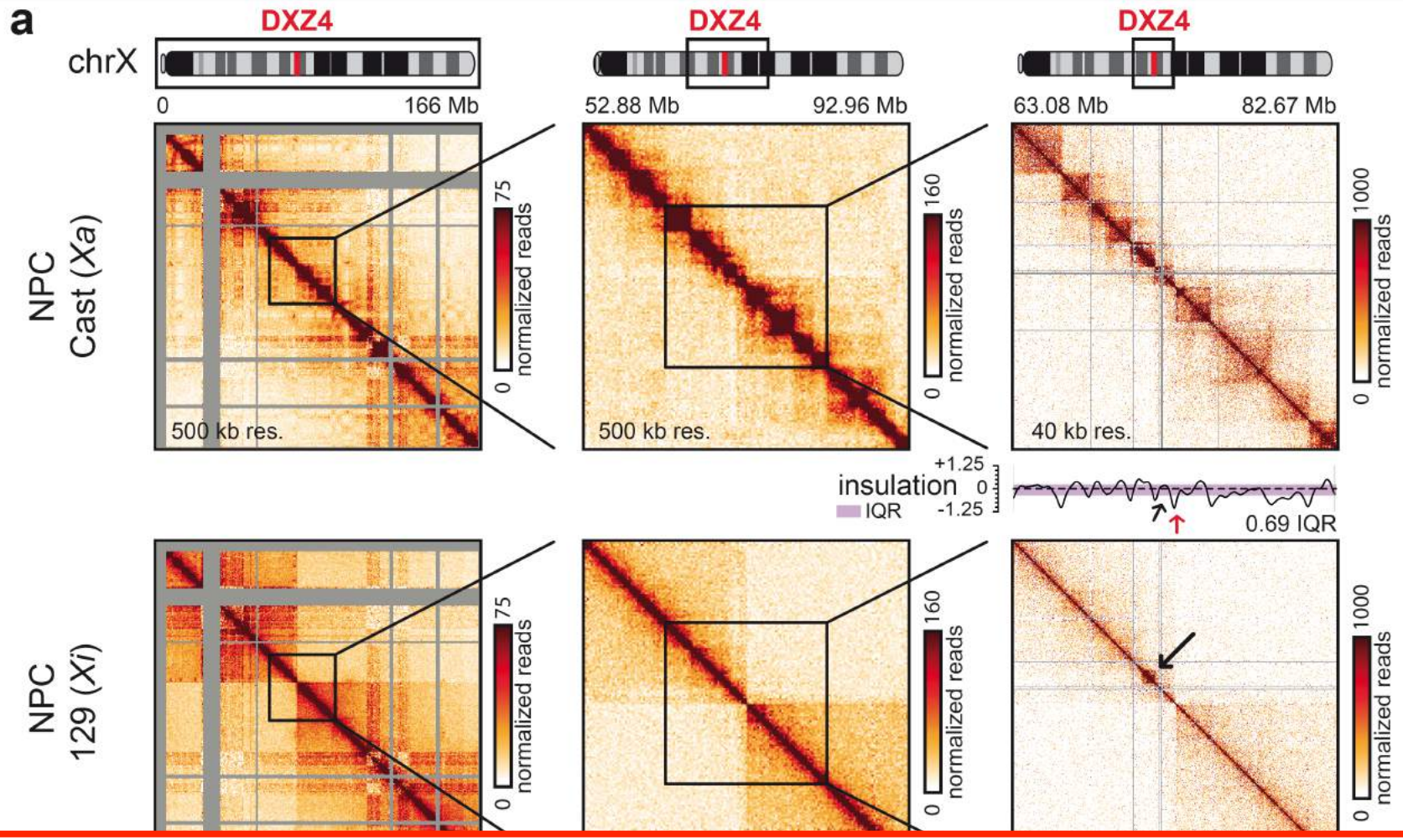


(Gendrel et al, Dev. Cell 2014)



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

Allele-specific HiC analysis of the active and inactive X chromosomes



- The Xi is divided into two megadomains separated by a 200kb boundary region including the CTCF-rich, DXZ4 macrosatellite (Deng et al, 2015; Rao et al, 2014, Giorgetti et al, 2016)
- Only escapee regions show long range interactions in trans (Splinter et al, 2011)
- Xi is devoid of compartments and TADs (Minajigi et al, 2015), except at a few regions…

Unique Chromosome Organisation of the inactive X

Structural organization of the inactive X chromosome in the mouse

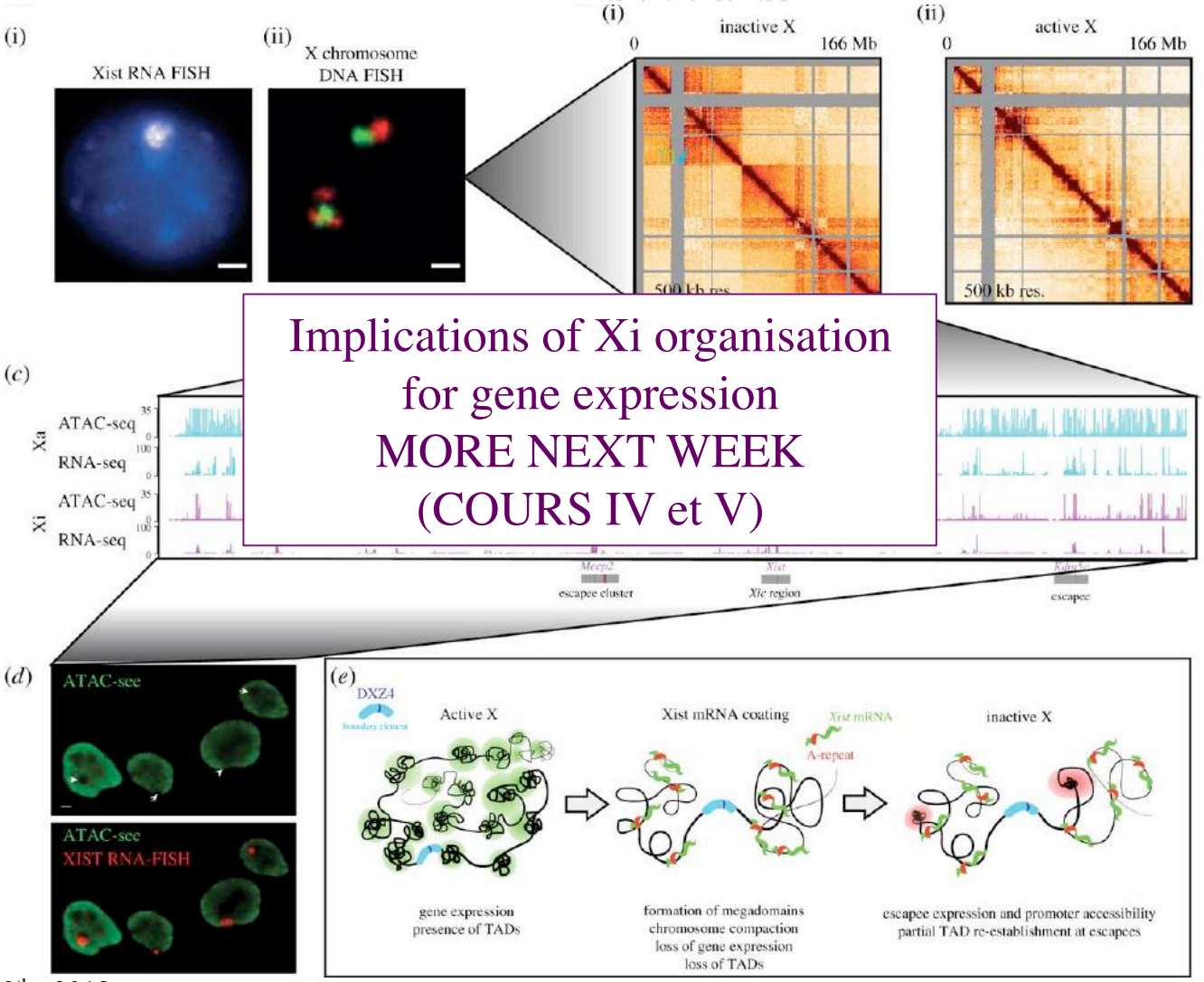
Luca Giorgetti[1*†], Bryan R. Lajoie[2*], Ava C. Carter[3*], Mikael Attia[1*], Ye Zhan[2], Jin Xu[3], Chong Jian Chen[1], Noam Kapla
Howard Y. Chang[3], Edith Heard[1,4] & Job Dekker[2,5]

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



Implications of Xi organisation for gene expression
MORE NEXT WEEK
(COURS IV et V)

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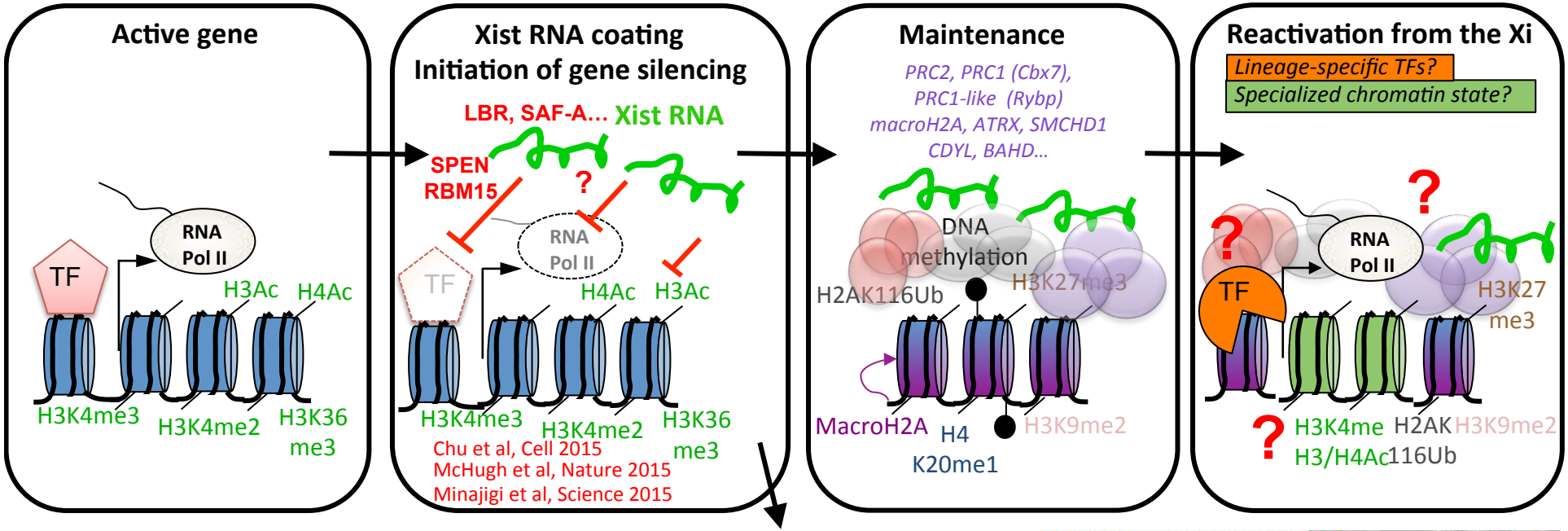
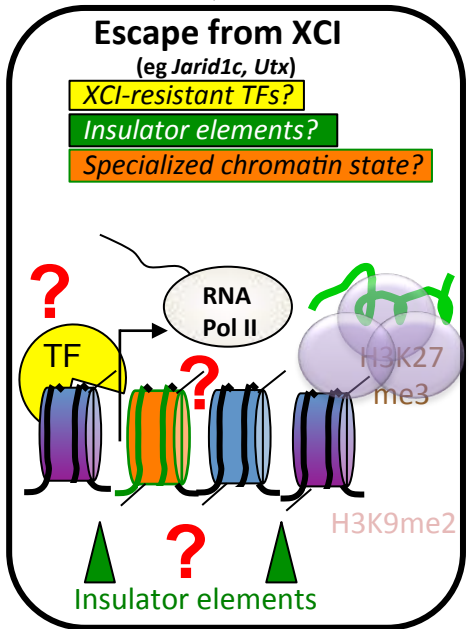
Steps in X-chromosome inactivation





Some genes escape XCI constitutively & autonomously

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

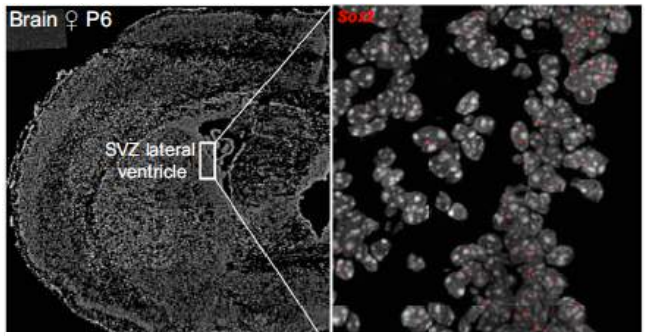
Some genes escape in a lineage or tissue-specific fashion

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

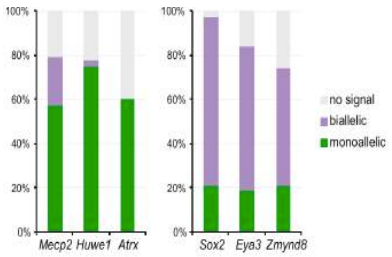
Escapees are often involved in chromatin-associated functions

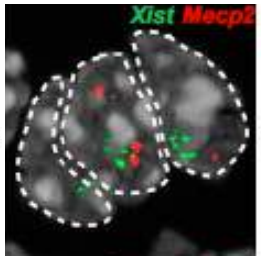
eg Jarid1c, Utx = Histone demethylases

Atrx = chromatin remodeller

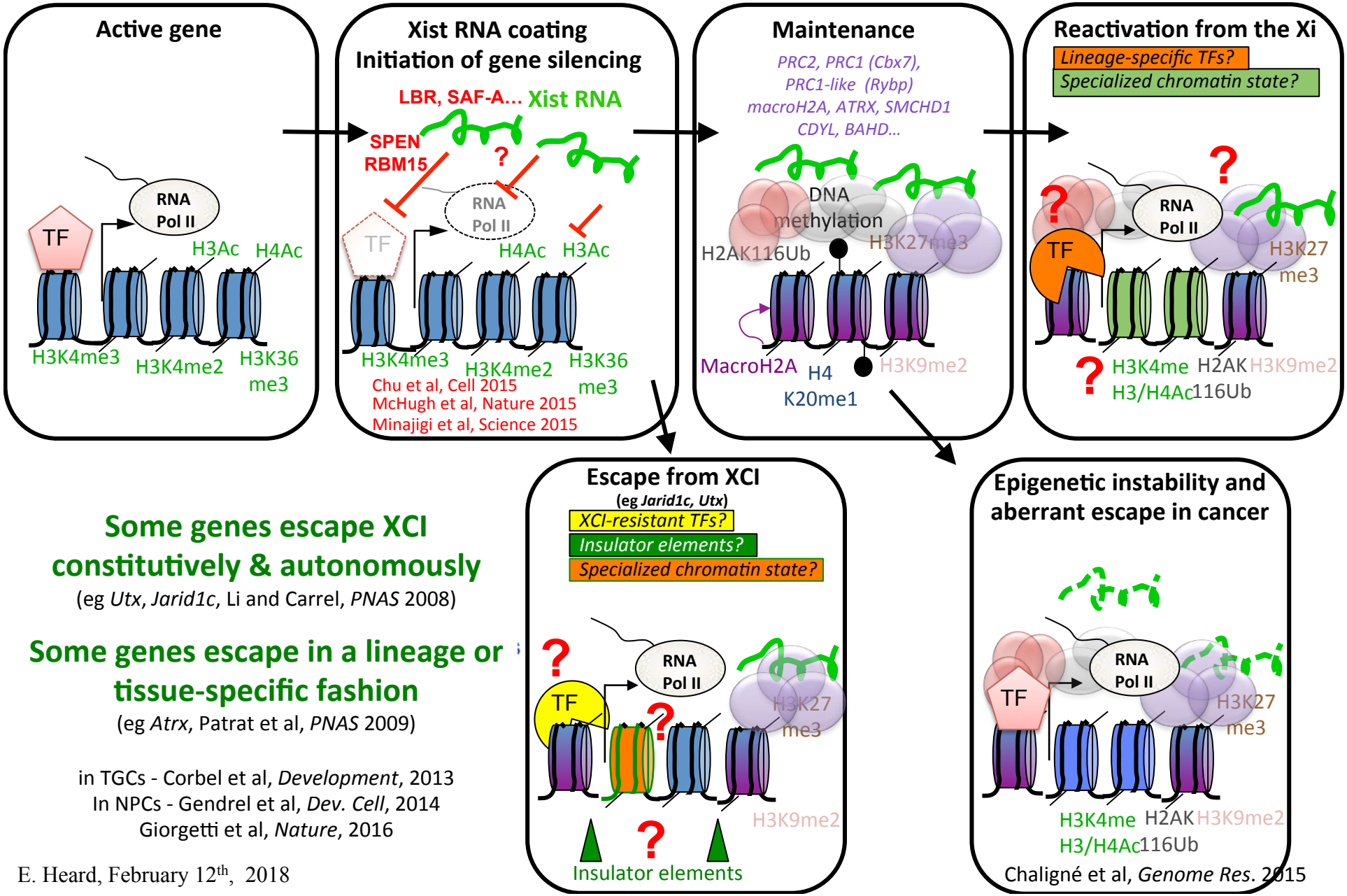


Eg *Mecp2* escapes XCI in neural stem cells in the SVZ





Steps in X-chromosome inactivation



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)

in TGCs - Corbel et al, *Development*, 2013
In NPCs - Gendrel et al, *Dev. Cell*, 2014
Giorgetti et al, *Nature*, 2016

Genes that can *escape* from X inactivation

- 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

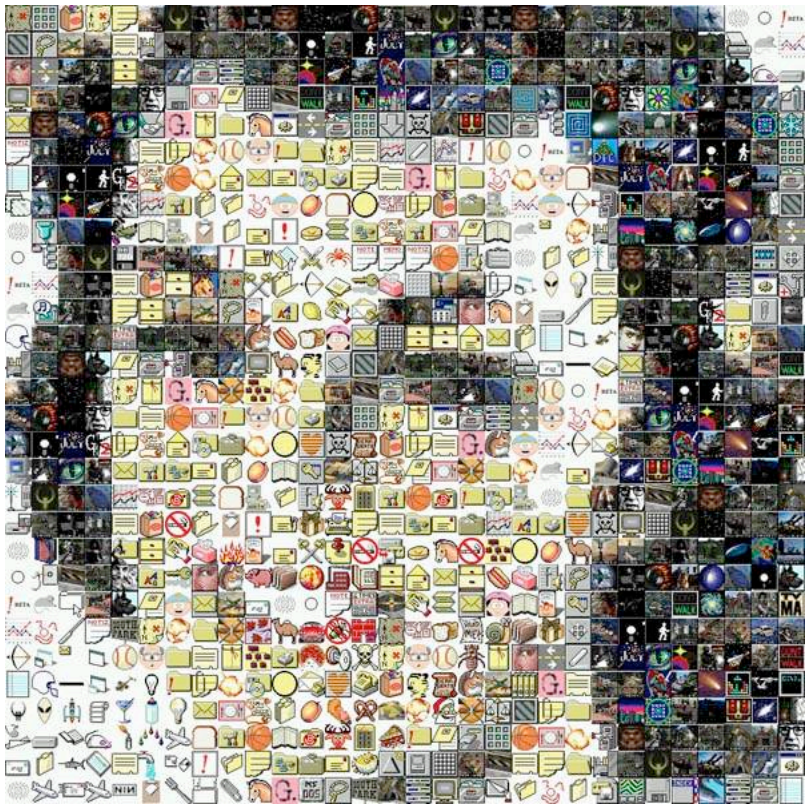
Variable escape from X inactivation leads to even greater female cellular mosiacism

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?
COURS IV and V

CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2017-2018 :

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

19 février, 2018

Cours IV

Les troubles neurologiques liés au chromosome X