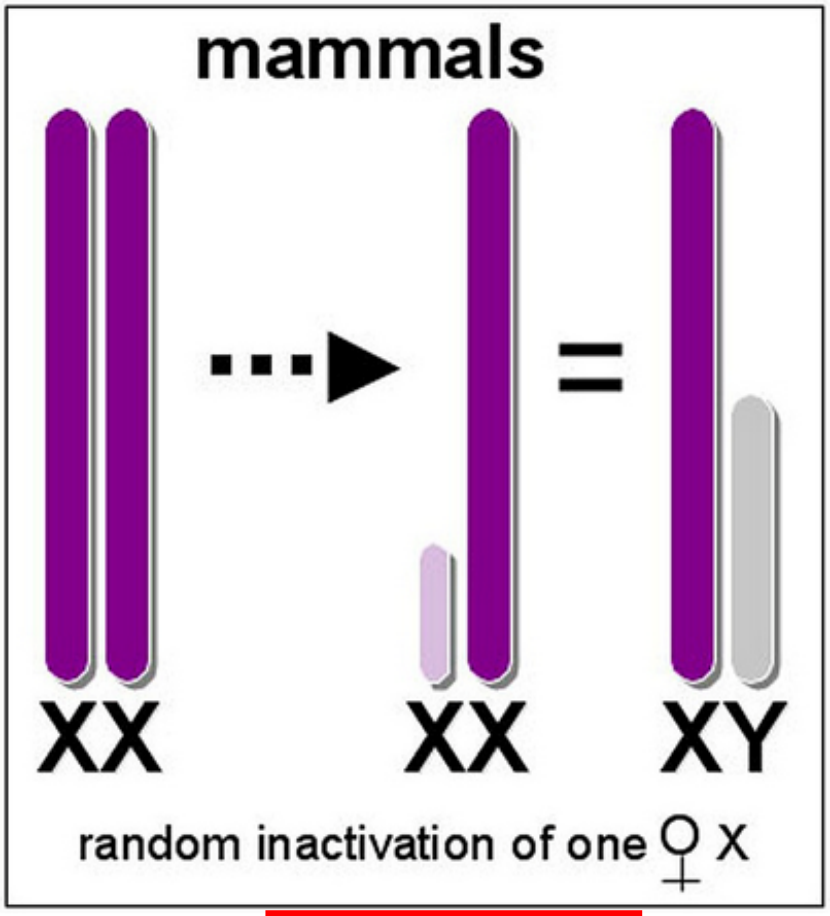
L’inattivazione del cromosoma X

Female cells have double the number of X chromosomes as male cells. Therefore, female cells should express twice the amount of X chromosome genes (n=1300!) than male cells. BUT - they DON'T.

Male and female cells express X chromosome genes at the same level.

XX XY

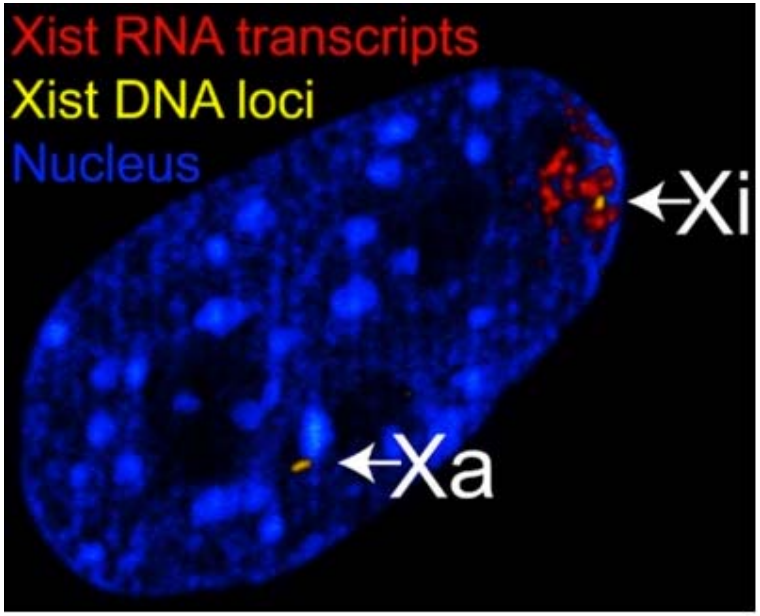
Mechanisms of X chromosome dosage compensation

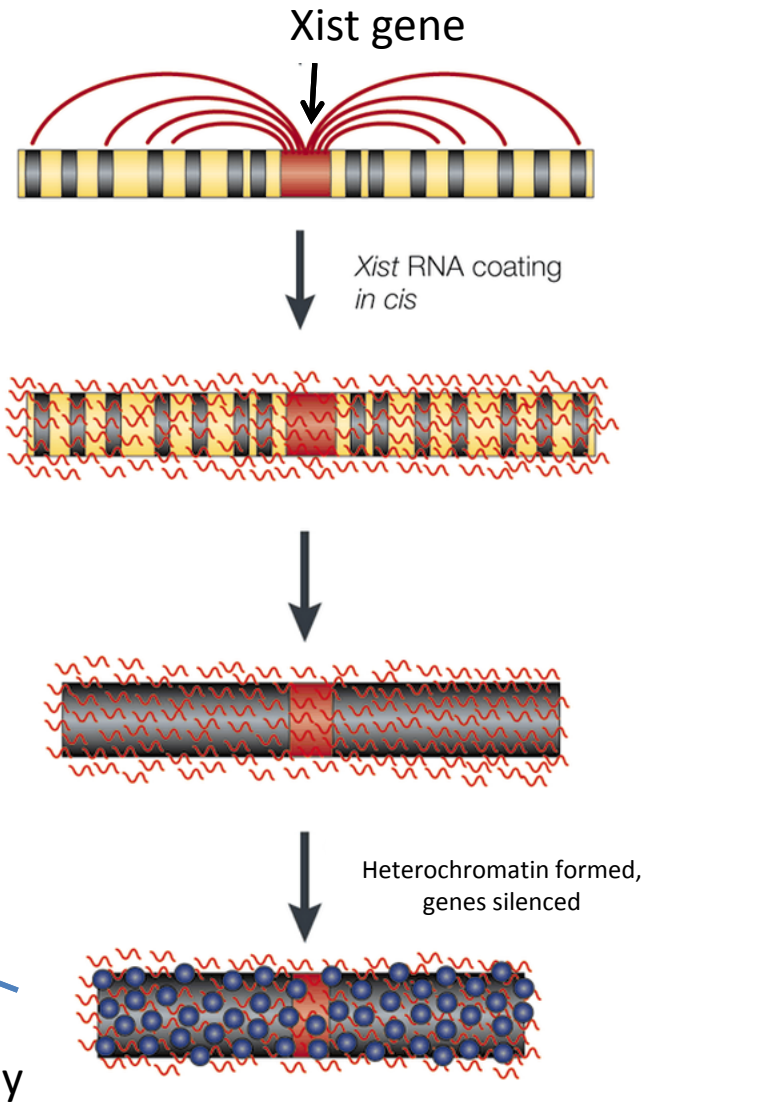


10-15%
mantenuti attivi

wormbook.org

X Chromosome Inactivation





http://embryology.med.unsw.edu.au/embryology/images/thumb/3/3f/X_inactivation_Xist.jpg/400px-X_inactivation_Xist.jpg

lncRNA long non-coding RNA

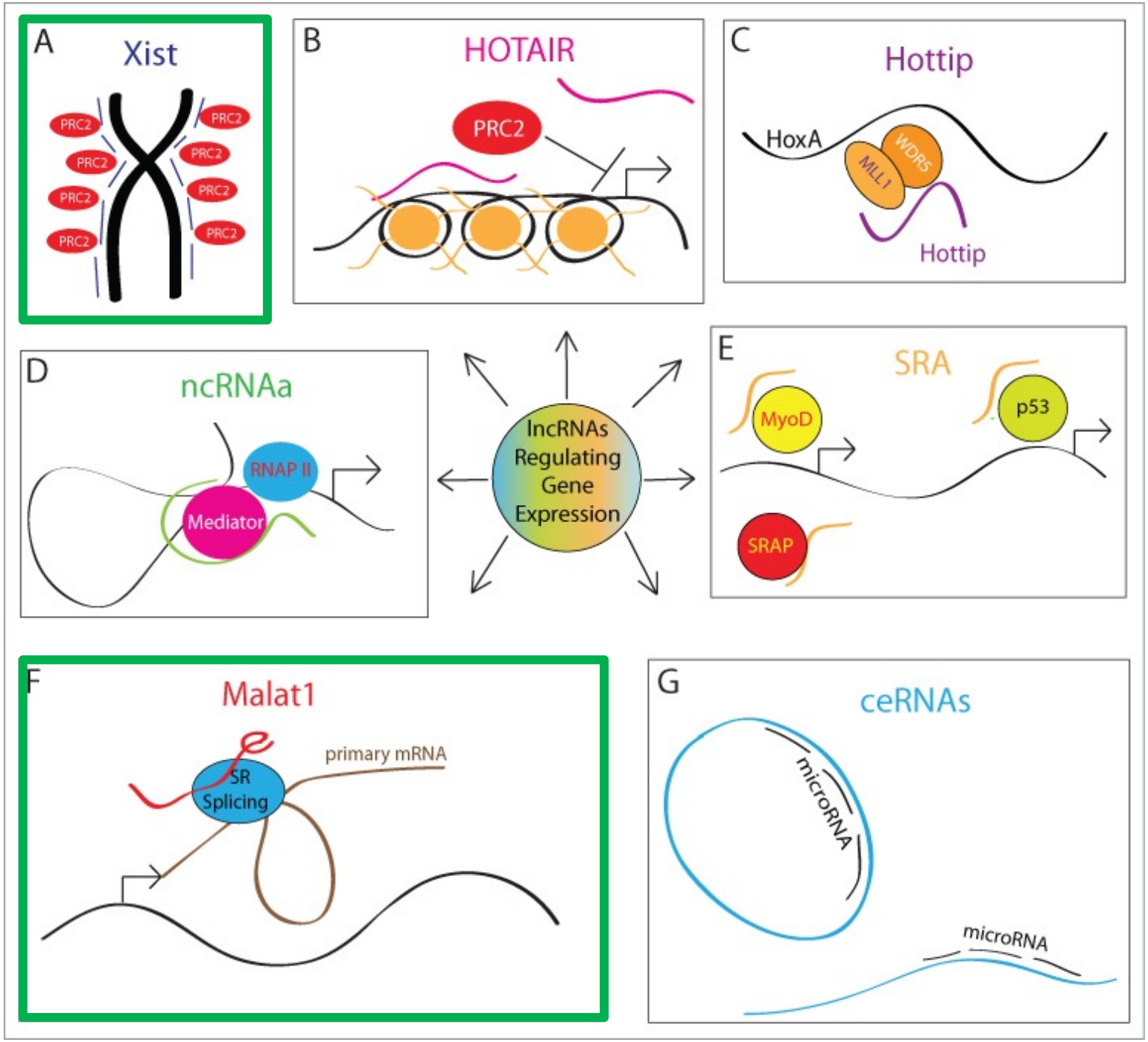
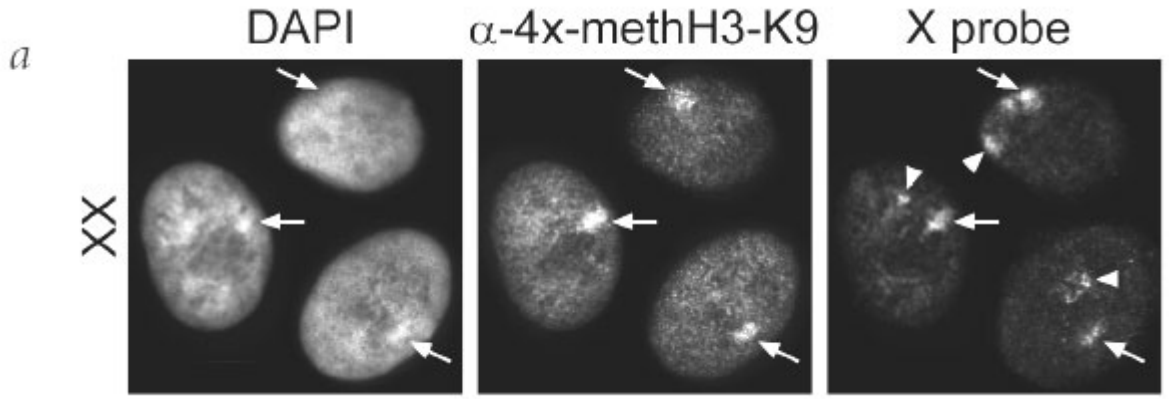

A
Xist
PRC2
PRC2
PRC2
PRC2
PRC2
PRC2
PRC2
PRC2
B
HOTAIR
PRC2
C
Hottip
HoxA
WDR5
MLL1
Hottip
D
ncRNAa
RNAP II
Mediator
lncRNAs
Regulating
Gene
Expression
E
SRA
MyoD
p53
SRAP
F
Malat1
primary mRNA
SR
Splicing
G
ceRNAs
microRNA
microRNA

Table 1. Prominent lncRNAs and their functions in development and disease

lncRNAs	Organisms	Functions	Phenotypes/disease
ANRIL/CDKN2B-AS1	Human	Transcriptional regulation	Prostate cancer, leukemia
Airn, Kcnq1ot1	Mouse	Epigenetic regulation; Embryonic gene activation	Growth defects; breast, colon carcinoma
Malat1	Mouse, Human	Splicing, gene regulation	Tumor; myoblast differentiaiton
HOTAIR	Mouse, Human	Hox gene regulation; Recruitment of PRC2 and LSD1	Tumor formation; cancer metastasis
Hottip	Chicken	HoxA gene regulation	Defect in limb formation
Xist, Tsix	Mouse	Dosage compensation	Loss of function causes embryonic lethality
Fendrr, Braveheart	Mouse, Human	Heart development	Loss of function causes embryonic lethality
Miat, Six3os1, Tug1, Vax2os	Mouse	Retinal development	Defects in retinal specification; photoreceptor differentiation
Dlx1os, Dlx6os1	Mouse	Brain development	Neurological deficit
Megamind	Zebrafish	Brain and eye development	Defects in brain and eye development
H19	Mouse, Human	Posttranscriptional regulation by producing microRNAs	Skeletal muscle differentiation, regeneration, cancer
SRA	Mouse, Human	Transcriptional activity of MyoD and p53	Skeletal muscle differentiation; breast, uterus, ovary tumor
Linc-MD1	Mouse, Human	Sequestration of microRNAs	Myogenic differentiation, Duchenne Muscular Dystrophy
SINE-containing lncRNAs	Mouse	Staufen-mediated mRNA degradation	Myogenic differentiation
bII NAT	Mouse	Suppress MHC IIb transcription	Skeletal muscle development
CE, DRR lncRNAs	Mouse	Transcriptional regulation of MyoD	Skeletal muscle differentiation
YAMS	Mouse	Transcriptional regulation	Myogenic differentiation

X Chromosome Inactivation



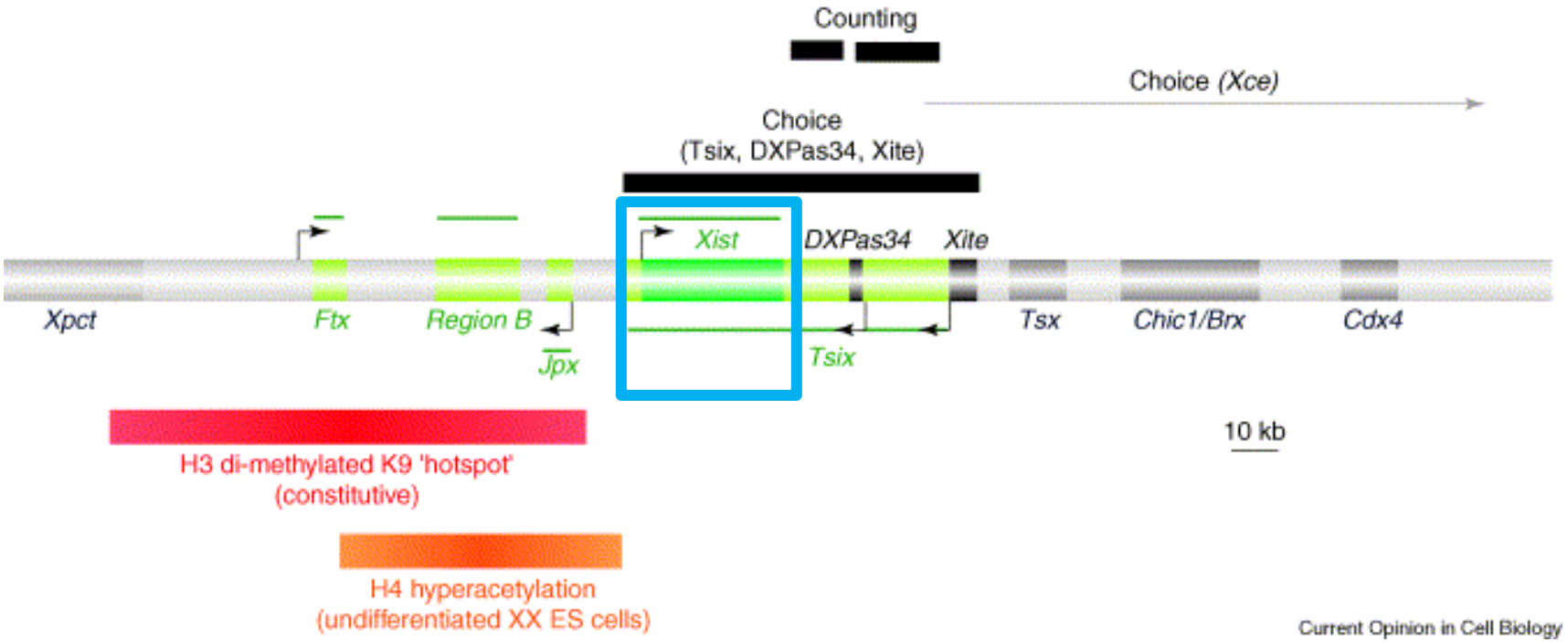
Peters *et al.* *Nature Genetics* **30**, 77 – 80 (2002)

X Chromosome Inactivation

- X chromosome inactivation occurs early during development – around 24 cell

- Thus, females embryos have two active X chromosomes until one is inactivated

What Determines X-chromosome Inactivation?



X Chromosome Inactivation

- Mechanism of X Chromosome inactivation
- XIC – X chromosome Inactivation Center
  - XIC controls expression of the *XIST* gene
  - *XIST*: X-inactive-specific transcript
  - *XIST* produces a non-coding 17 kb RNA molecule
  - “Coats” the entire *local* X-chromosome – *cis*-acting

EMBO Rep. 2007 January; 8(1): 34–39.
doi: 10.1038/sj.embor.7400871.

X Chromosome Inactivation

- Approaches for examining *XIST* biology

1) Knock it out!

Nature, January 1996 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

X Chromosome Inactivation

- Approaches for examining *XIST* biology

2) Knock it in!

Molecular Cell, Vol. 5, 695–705, April, 2000, Copyright ©2000 by Cell Press

A Shift from Reversible to Irreversible X Inactivation Is Triggered during ES Cell Differentiation

Anton Wutz and Rudolf Jaenisch*
Whitehead Institute for Biomedical Research
9 Cambridge Center
Cambridge, Massachusetts 02142

Tet Repressor Model

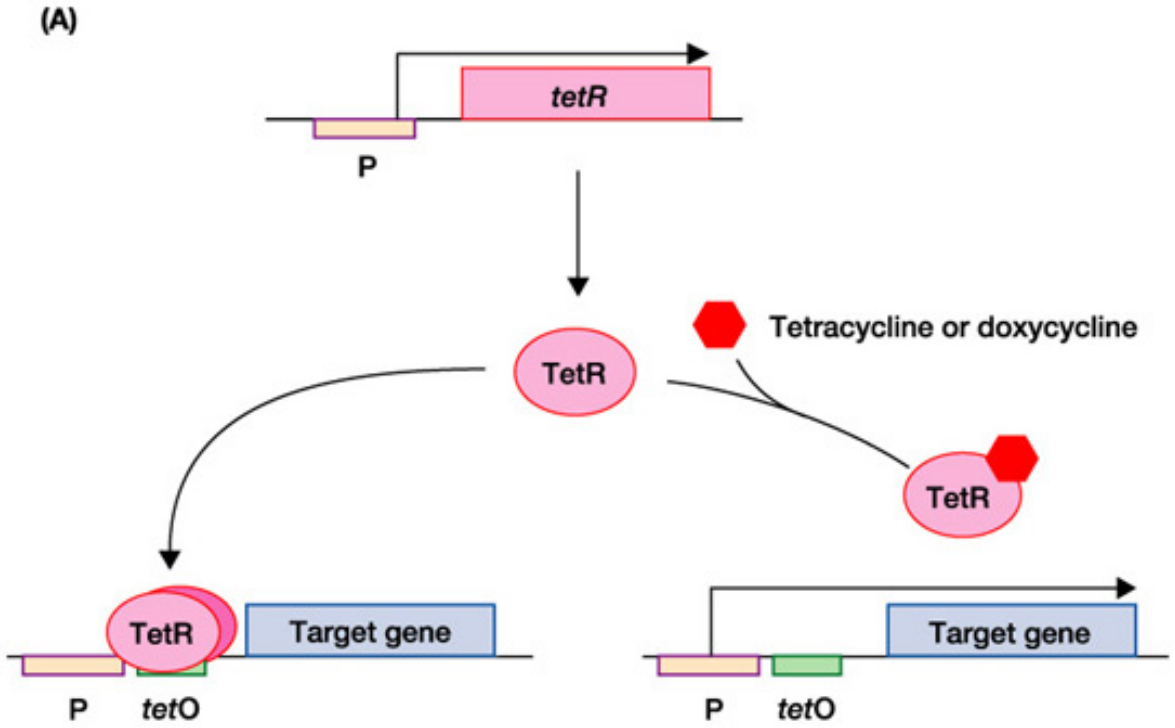
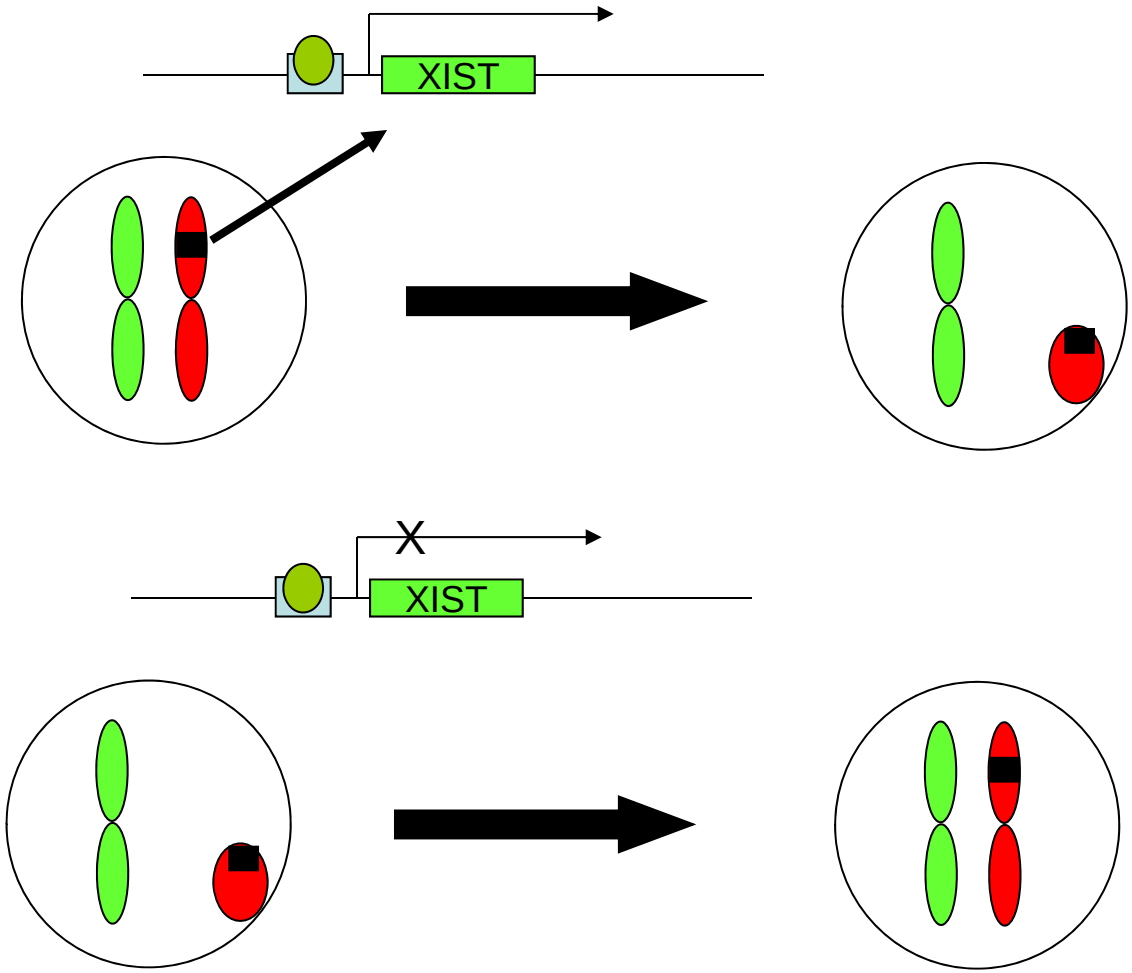
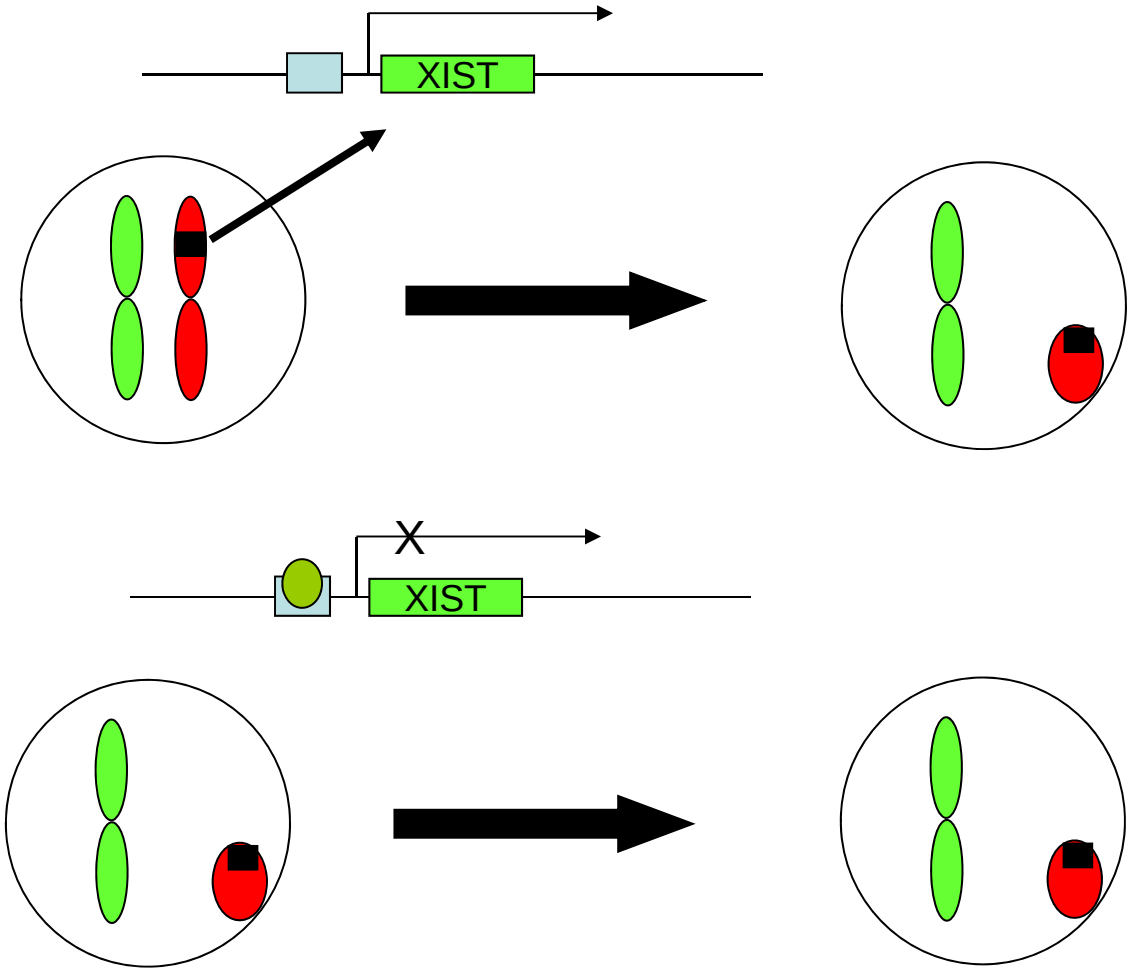


Figure 20-5 part 1 of 2 Human Molecular Genetics, 3/e. (© Garland Science 2004)

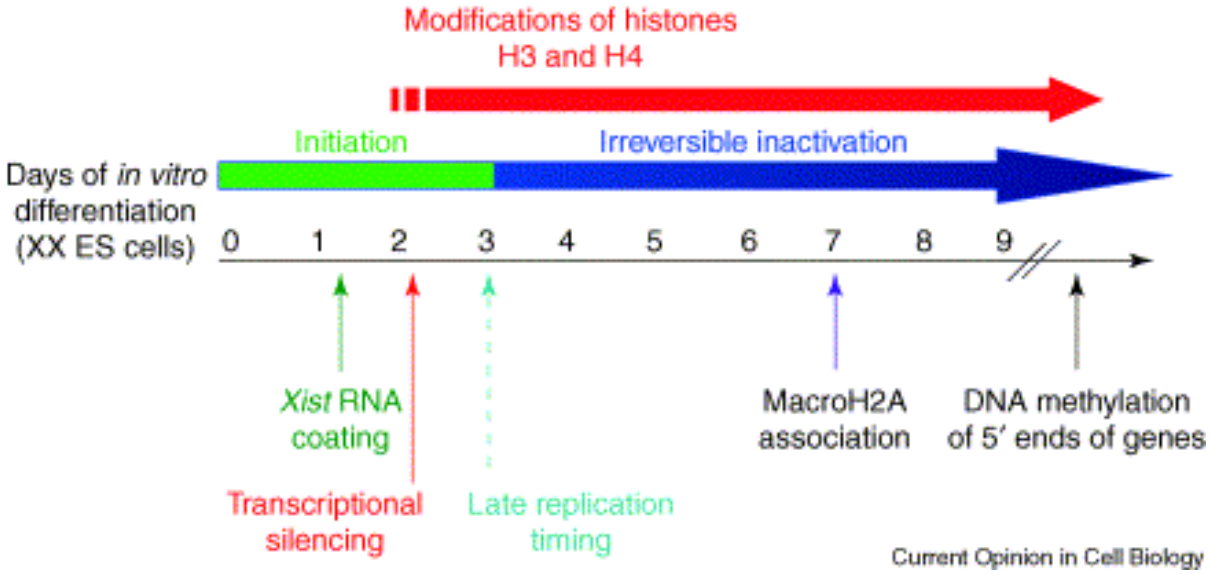
XIST inactivation is Reversible up to 48 hours



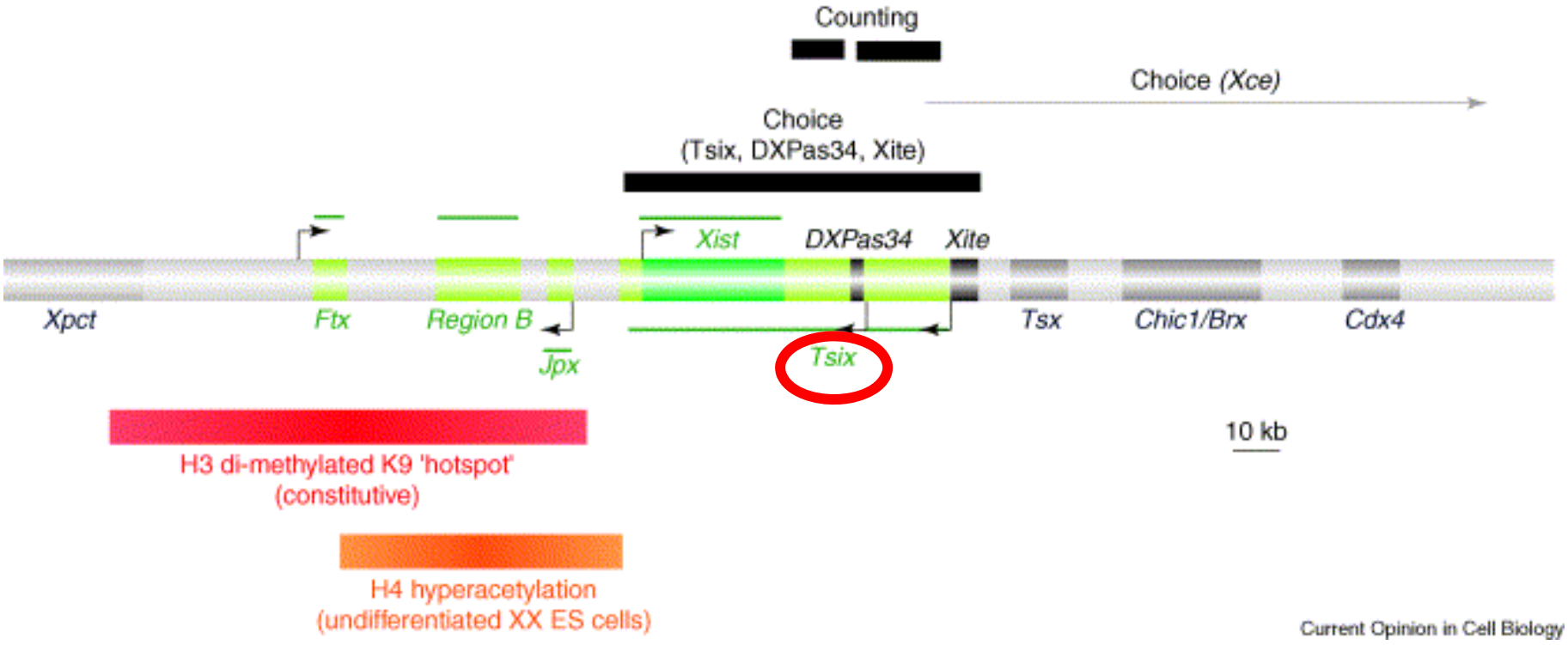
No Choice after 48 hrs



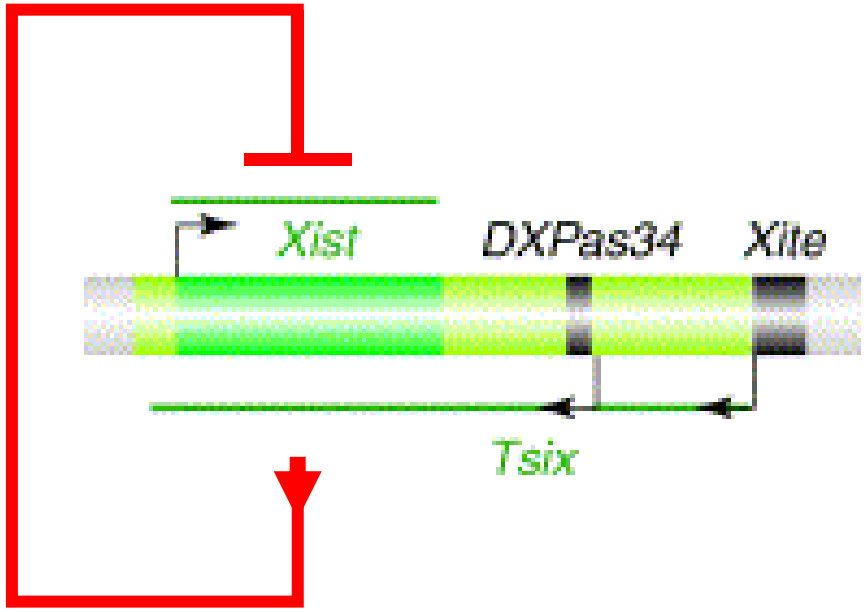
XIST acts Early During Development and is Irreversible



What Controls *XIST* Expression?



TSIX is the Anti-Sense Strand of the *XIST* gene



TSIX is the Anti-Sense Stand of the *XIST* gene

Cell, Vol. 99, 47–57, October 1, 1999, Copyright ©1999 by Cell Press

Targeted Mutagenesis of *Tsix* Leads to Nonrandom X Inactivation

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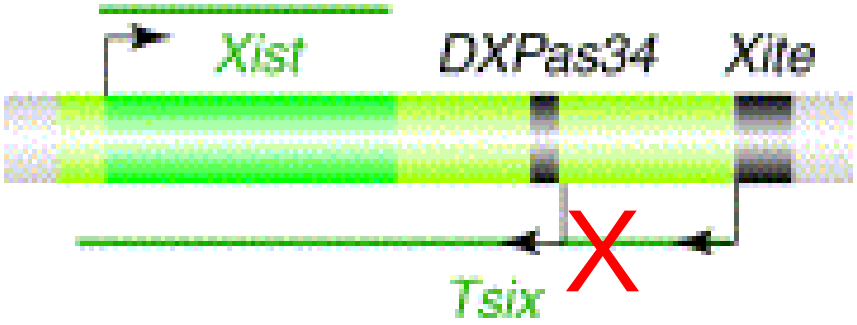
*Department of Molecular Biology
Massachusetts General Hospital
Boston, Massachusetts 02115
†Department of Genetics
Harvard Medical School
Boston, Massachusetts 02114

Knock-down of *TSIX* Causes Skewed X-Chromosome Inactivation



	Pgk1 or Mecp2 RNA colocalization with:			#nuclei
	X^{mut} (%)	X^{wt} (%)	both (%)	n†
Pgk1	3 (1.2%)	259 (98.8%)	0 (0%)	262
Mecp2	0 (0%)	251 (98.4%)	4 (1.6%)	255

† Only cells with high level Xist RNA and detectable Pgk1 or Mecp2 expression were scored.

TSIX Asymmetry Governs *Choice*

- *TSIX must be downregulated for XIST expression on the (future) inactivated X Chromosome*

- *TSIX expression must remain for XIST downregulation on the (future) activated X Chromosome*

Table 1 Factors involved in X chromosome inactivation

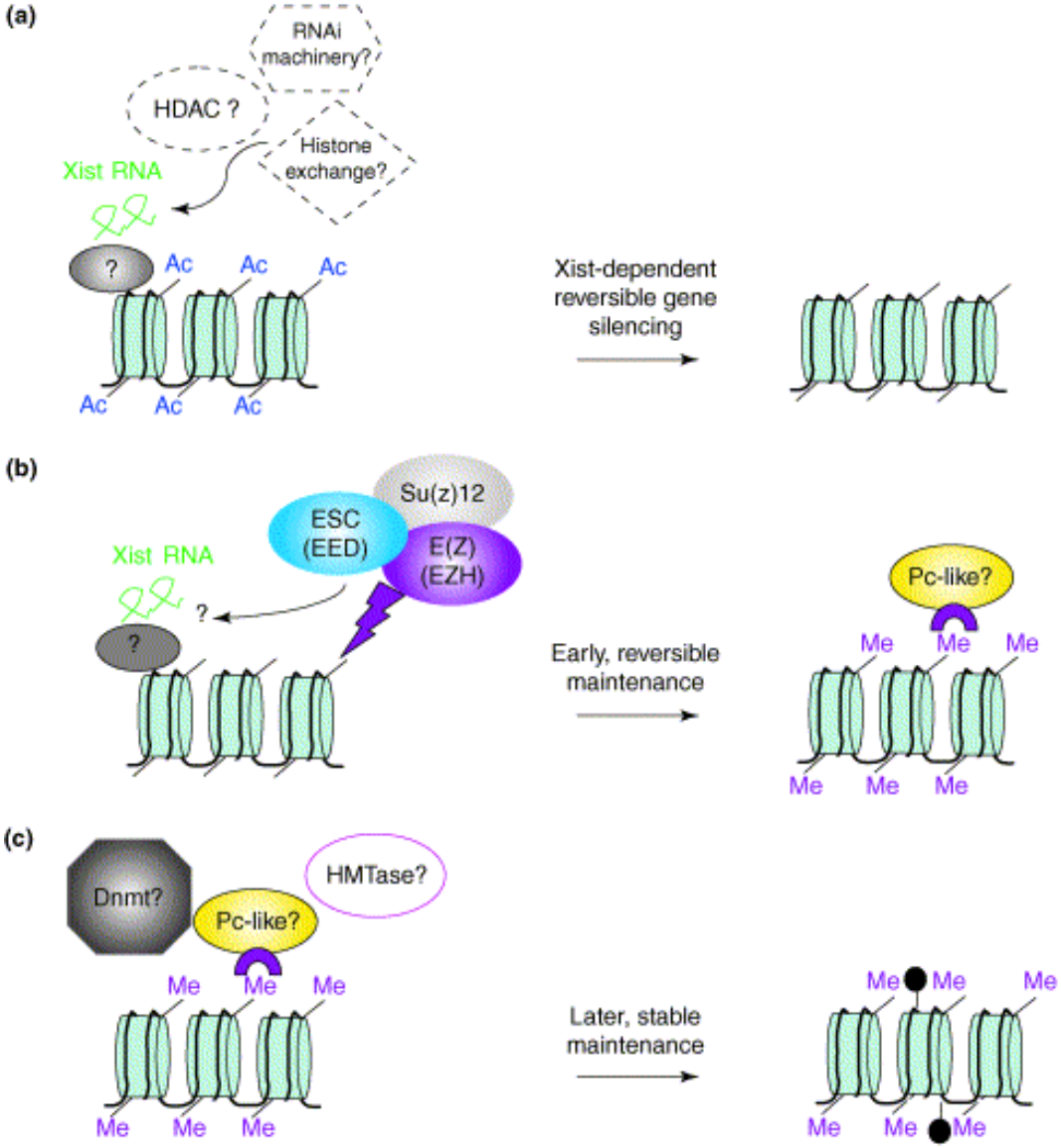
Factors involved in XCI	Function in the context of XCI
Proteins	
PRC2	The polycomb repressive complex 2 (PRC2) is known to be recruited early on the inactive X (Xi) during differentiation of embryonic stem cells (ESCs) and embryonic development and catalyzes methylation of histone H3 at K27 on chromatin
PRC1	The activity of polycomb repressive complex 1 (PRC1) on chromatin reinforces gene silencing by ubiquitylation of histone H2A at K119 and chromatin compaction. The order of recruitment of PRC2 and PRC1 to the Xi is still a matter of debate
Saf-A (HnrnpU)	The Saf-A (HnrnpU) factor directly binds to Xist and mediates its interaction with chromatin through direct interaction with SARS/MARS elements
SHARP (Spen)	SHARP (Spen) directly binds to Xist and mediates the functional interaction between Xist and the NCoR complex
CTCF	The CCCTC-binding factor (CTCF) might work as a genomic insulator. In the context of X chromosome inactivation (XCI), it might serve as a barrier to Xist-induced chromatin reorganization
SATB1	The special AT-rich sequence-binding protein-1 (SATB1) cellular regulator of higher chromatin organization has a role in the initiation of XCI. However, its precise role in XCI is not clear
YY1	Yin-Yang 1 (YY1) is a bivalent protein with DNA-binding and RNA-binding motifs. It might have a role in tethering Xist to chromatin (spreading in cis) as well as a role in the regulation of Xist
SmchD1	The protein structural maintenance of chromosome hinge domain 1 (SmchD1) has a role in maintaining a correct pattern of DNA methylation on the Xi during the maintenance phase of XCI
WTAP	Wilms' tumor-associated protein (WTAP) is a splicing factor and interactor with Xist. It is involved in regulating RNA methylation. It might have a role in the post-transcriptional modification of Xist
LBR	The lamin B receptor (LBR) was recently identified as an Xist-binding protein. It is known to localize with the nuclear lamina and to interact with repressive complexes as well as with lamin B
Rbm15	Rbm15 belongs to the SPEN family of transcriptional repressors and directly binds to Xist RNA
hnRNPK	Heterogeneous nuclear ribonucleoprotein K (hnRNPK) is an RNA-binding protein that interacts with Xist and plays a role in the Xist-mediated recruitment of repressive chromatin marks
Oct4, Sox2, Rex1, Nanog, PRDM14, Klf4	Pluripotency factors and epigenetic regulators that have been shown to control XCI through the regulation of Xist and Tsix
Rnf12	The Rnf12 protein seems to regulate the expression of Xist through degradation of Rex1
Atrx	The protein alpha thalassemia/mental retardation syndrome X-linked (Atrx) is involved in the recruitment of PRC2 on the inactive X chromosome

ncRNAs	
Xist/Tsix	Xist is the master regulator of XCI, and Tsix is its major antagonist. Regulation of the levels of Xist and Tsix regulates the initiation of XCI
Jpx	The Jpx ncRNA seems to act as an activator of Xist
Ftx	The Ftx ncRNA seems to be an Xist activator
Genomic elements	
LINEs	The LINEs class of genomic repeats colocalize with inactive genes in the Xi territory and might have a role in the establishment and maintenance of XCI
SARS/MARS	Facultative scaffold/matrix attachment regions enriched in open chromatin and gene bodies where Xist accumulates

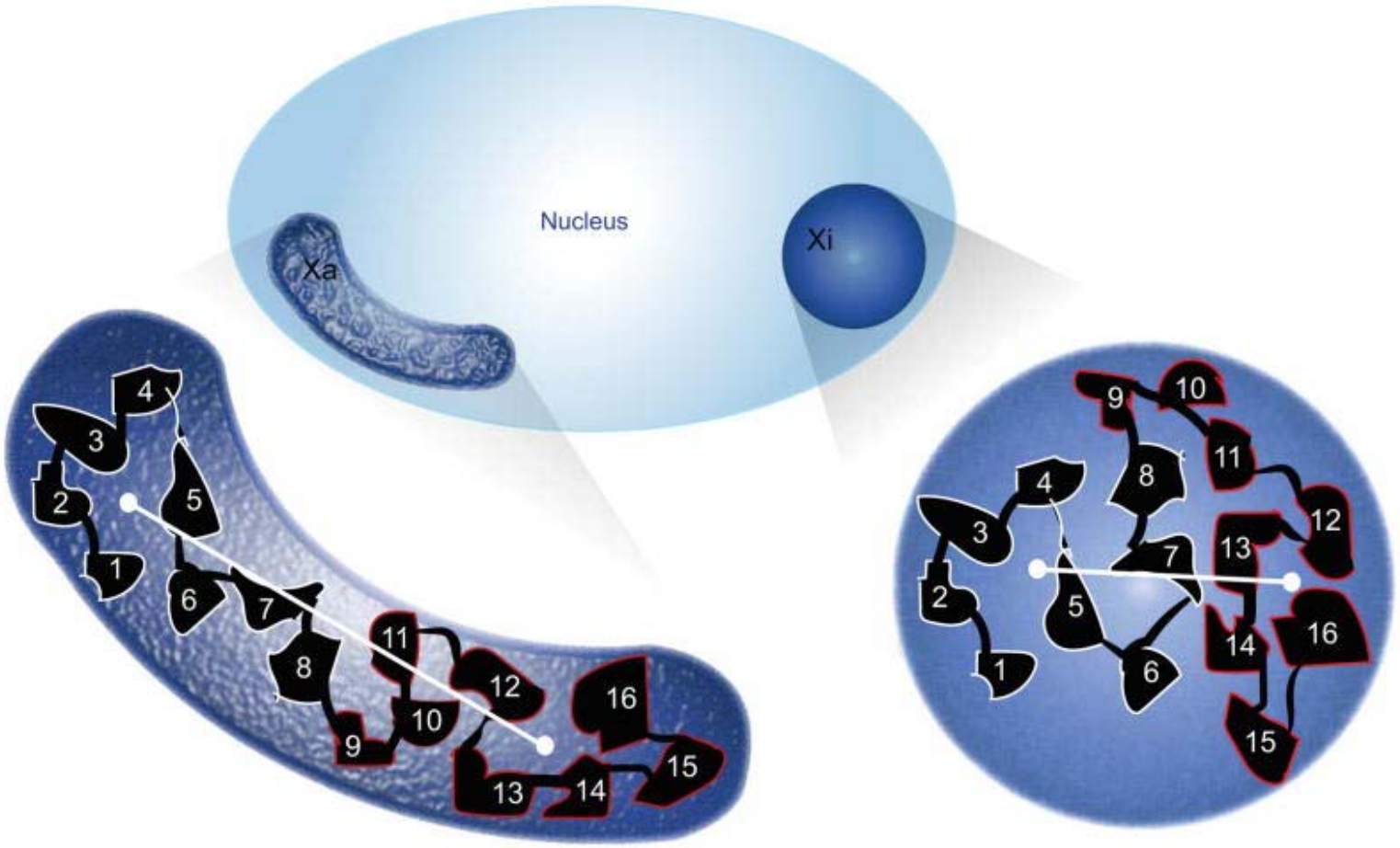
X Chromosome Inactivation

X chromosome inactivation requires:

- Initial *XIST* RNA expression and coating
  - Association of chromatin modifying proteins
  - DNA methylation 5’ of X-chromosome genes
  - Modification of histones by methyltransferases (HMTase)
  - Other chromatin modifying proteins



The shape and chromatin organization of the Xi is distinct from that of the Xa



The Xist RNA cloud is composed of discrete RNA foci distributed throughout the Xi territory

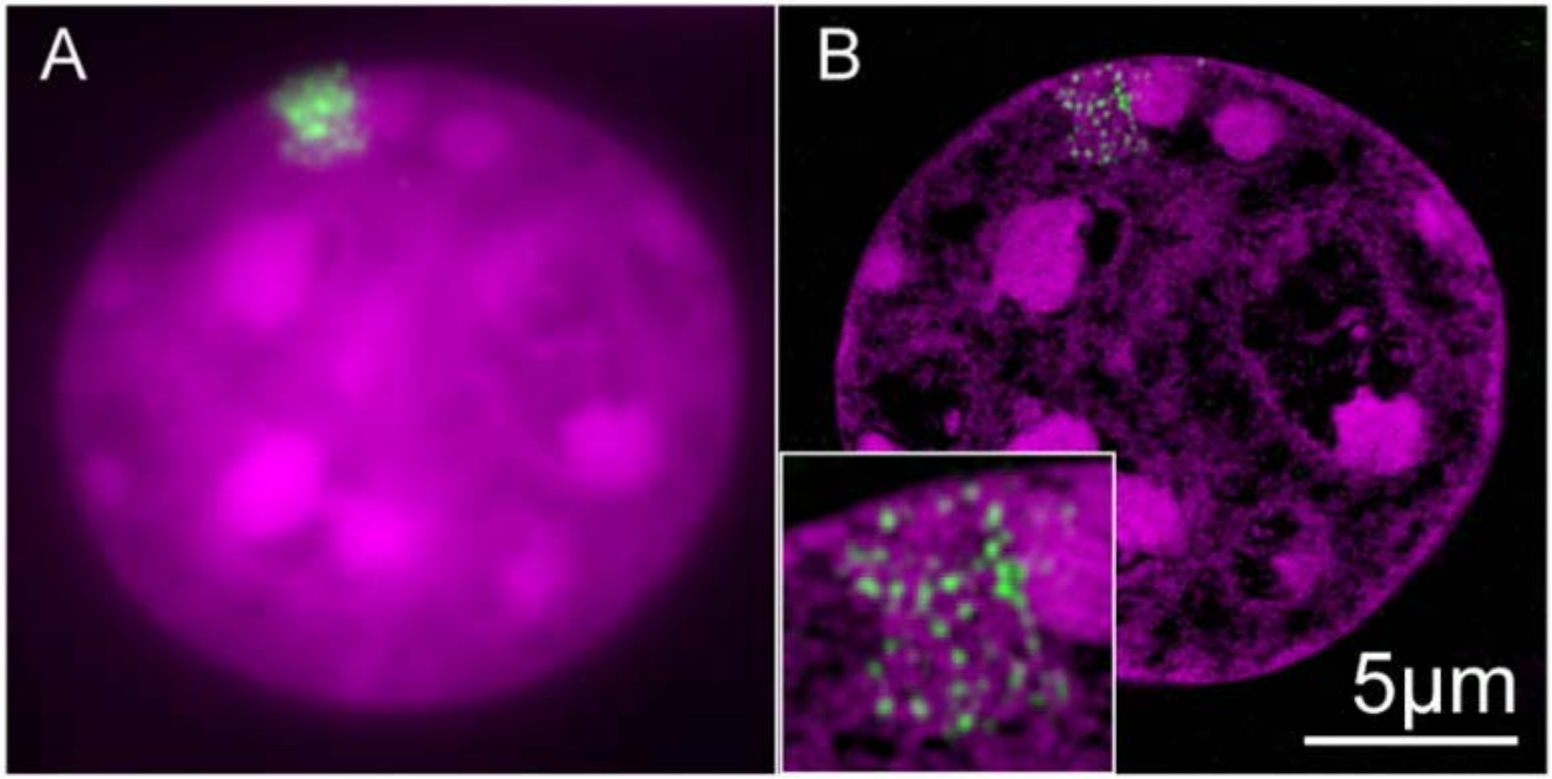
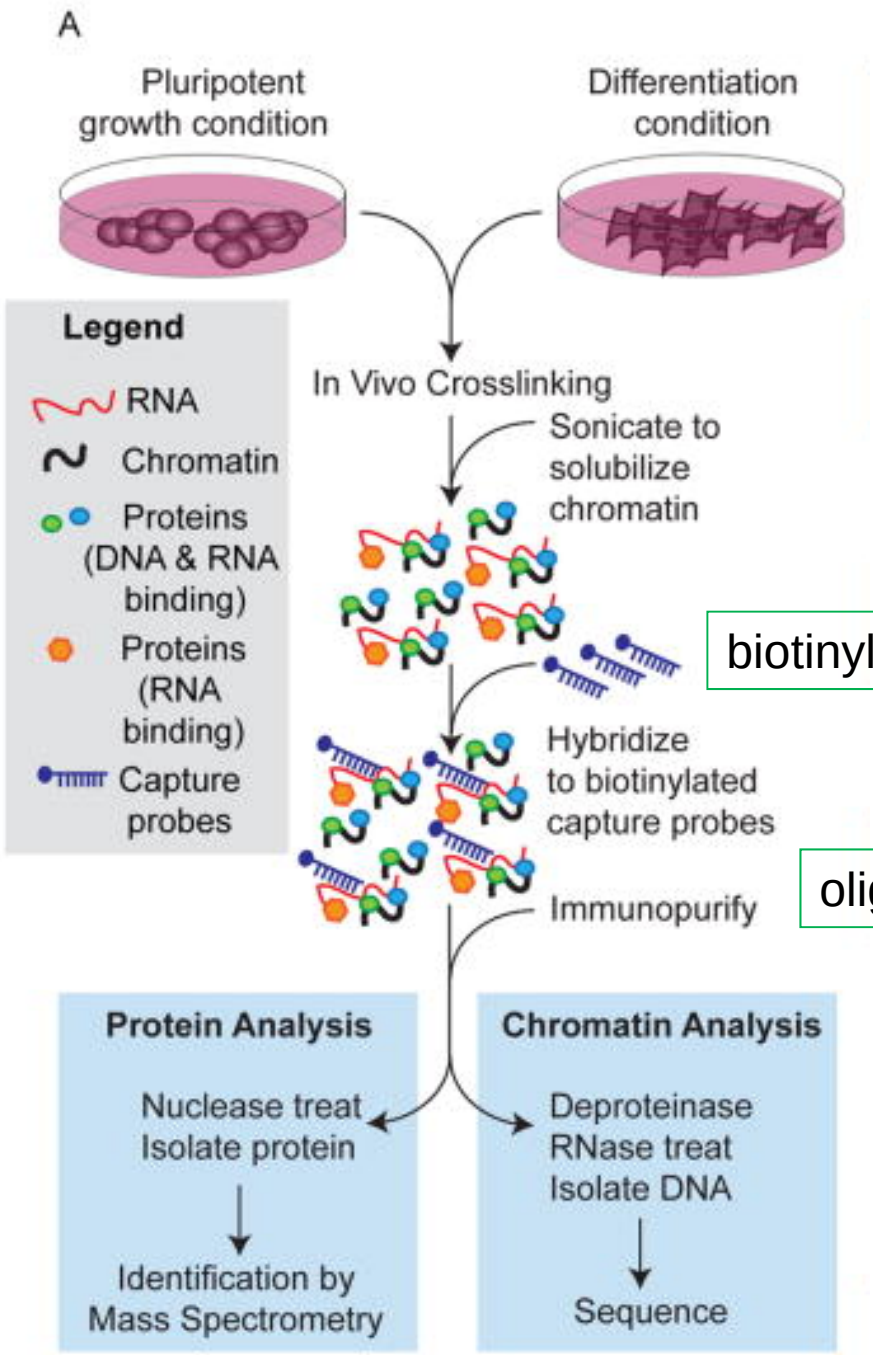


Image courtesy of Dr. Yolanda Markaki

DNA probes against the full length Xist RNA

METODI

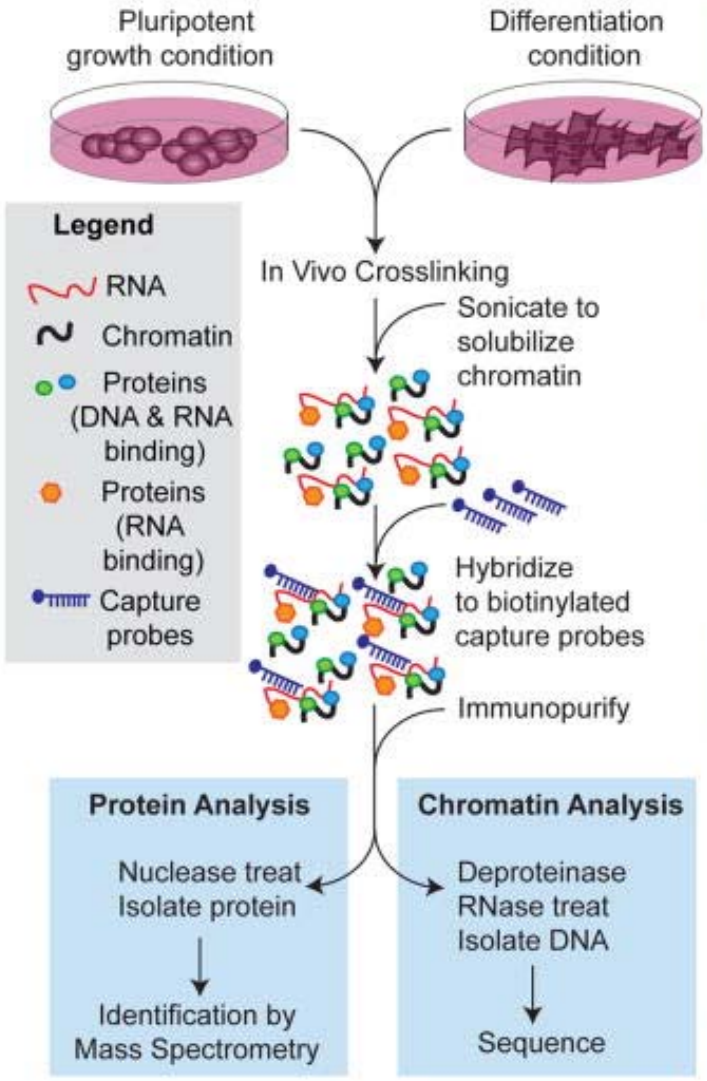


treatment with doxycycline if using cells harboring a tetracycline-inducible Xist transgene

biotinylated oligos antisense to the target RNA

oligos immunopurified using streptavidin

A

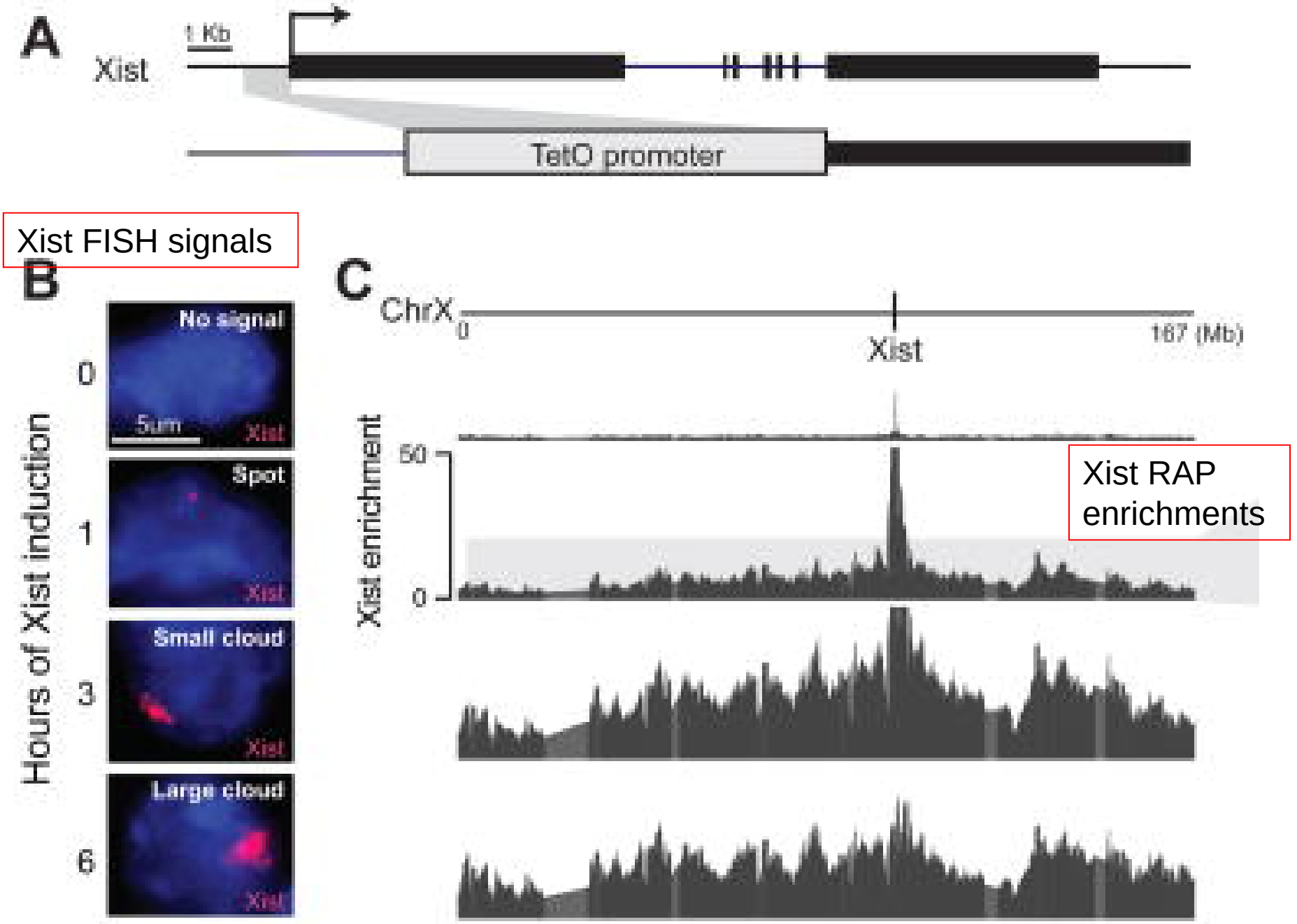


B

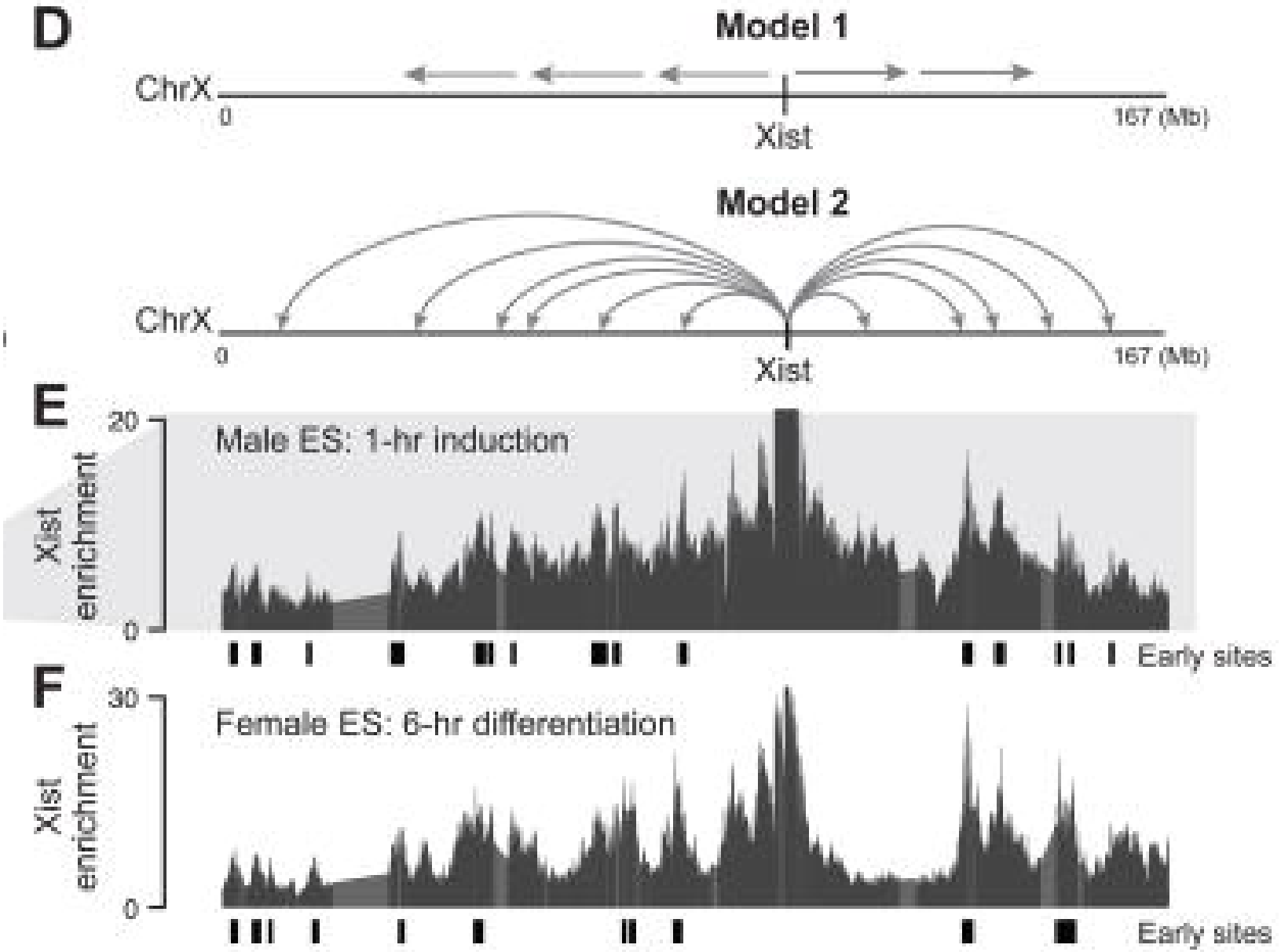
Parameter	RAP-seq	CHART-seq	ChIRP-seq
Fixation	DSG 3% FA	1% FA	1% GA or 3% FA
DNA Fragmentation	Sonication & DNase	Sonication	Sonication
Capture Probe Length (nt)	120	22-28	20
Probe type	RNA or DNA	DNA	DNA
Probe distribution	Tiled with a 15nt overlap	1 probe per 300-500nt	1 probe per 100nt
Targeted region	Complete Xist RNA	Complete Xist RNA	N/A
Hybridization conditions	Tris Buffered solution with: Na Deoxycholate SDS NP40 LiCl Guanidine Iso-thiocyanate	HEPES Buffered solution with: Denhards sol'n SDS Sarkosyl	Tris Buffered solution with: SDS Formamide
Adapted for Mass Spectrometry	Yes	Yes	Yes
Cell types used for *Xist* RNA analysis	- Mouse Lung Fibroblasts - Differentiated female ESCs (6h) - Inducible male ESCs (0-6h dox)	- Mouse Embryonic Fibroblasts - female ESCs differentiated for 3 and 7 days	N/A

RNA-Seq Analysis Pipeline RAP

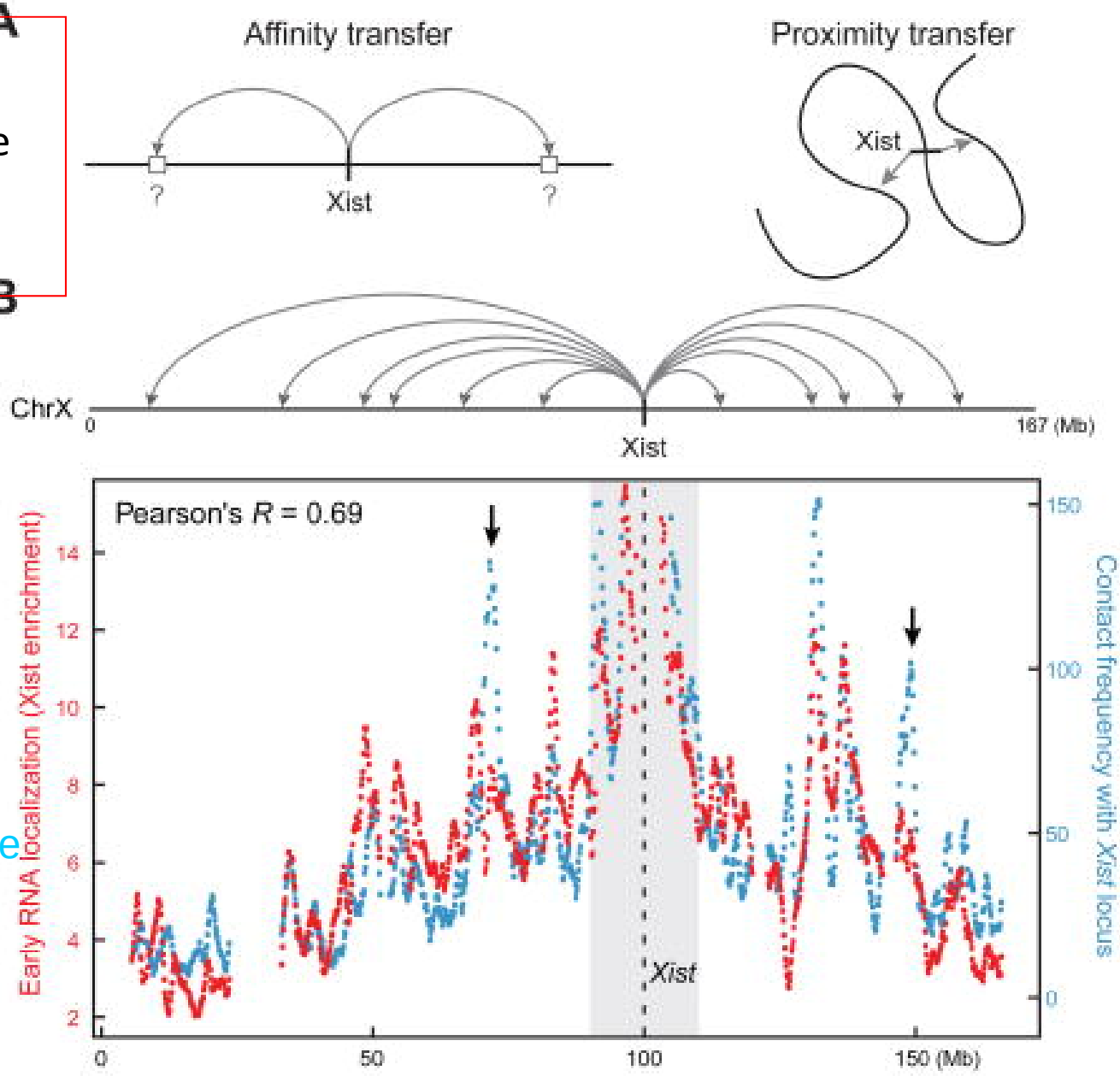
High-resolution view of Xist spreading during initiation of XCI



High-resolution view of Xist spreading during initiation of XCI



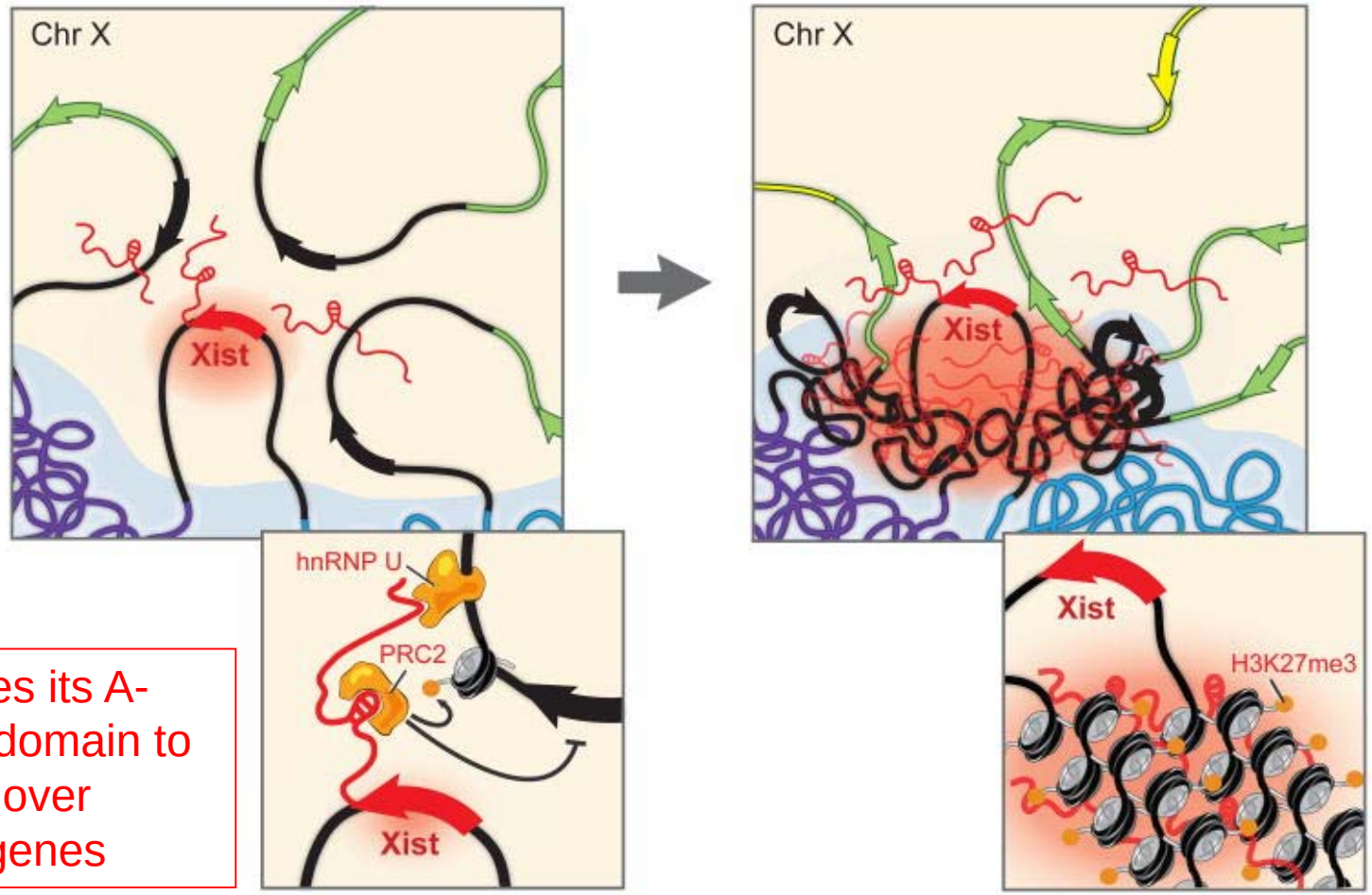
Early Xist localization correlates with the 3-D proximity contacts of the Xist transcription locus



Xist RNA localization (red) after one hour of Xist induction

contact frequencies (blue) between distal sites and the Xist genomic locus

model for how Xist exploits and alters three-dimensional genome architecture to spread across the X-chromosome

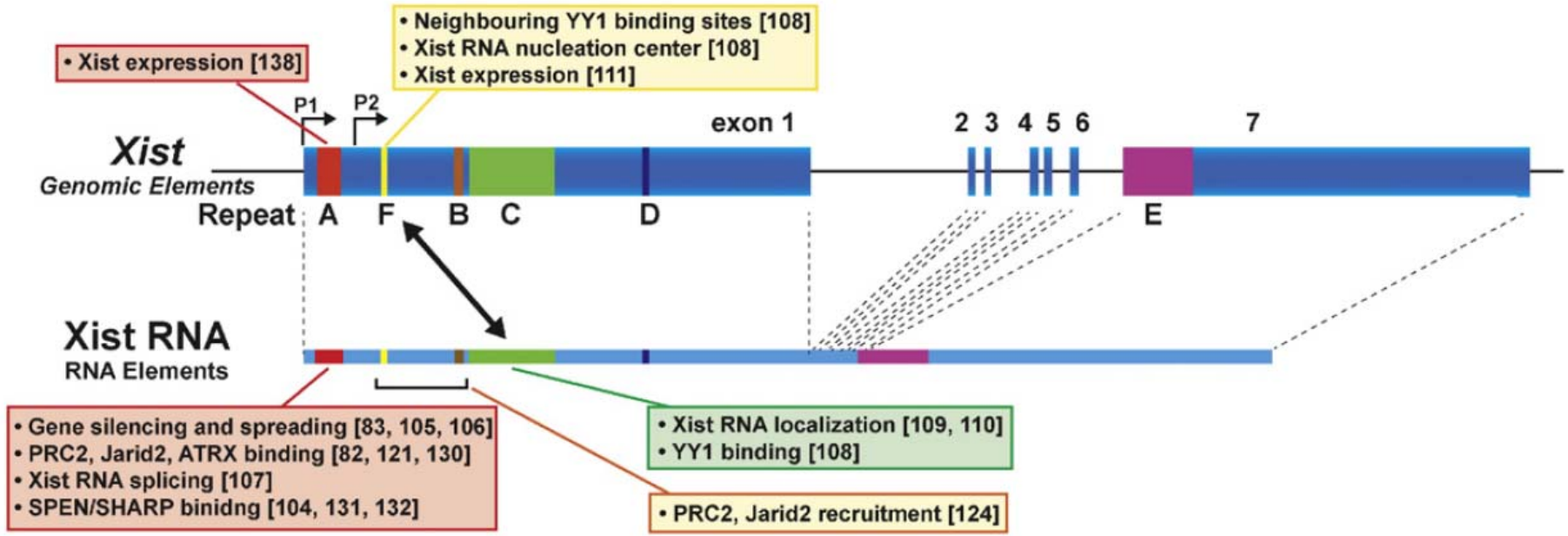


Xist uses its A-repeat domain to spread over active genes

Regolazione XIST vs TSIX

Xist induction and maturation

- Various RNA and protein factors are involved in coordinating the regulation of Xist mono-allelic expression from the Xi.
- + Several lncRNAs within the Xic have been identified (Tsix, Xite, DxPas34, Tsx, Jpx/Enox, Ftx and RepA) which act cooperatively together for the induction of monoallelic Xist expression from the future Xi in order to initiate XCI
- Nascent Xist RNA is processed to ~17 kb-length RNA by splicing, and different polyadenylation sites and alternative splicing patterns contribute to the generation of multiple isoforms of Xist RNA


• Xist expression [138]
• Neighbouring YY1 binding sites [108]
• Xist RNA nucleation center [108]
• Xist expression [111]
P1
P2
exon 1
2 3 4 5 6
7
Xist
Genomic Elements
Repeat
A
F
B
C
D
E
Xist RNA
RNA Elements
• Gene silencing and spreading [83, 105, 106]
• PRC2, Jarid2, ATRX binding [82, 121, 130]
• Xist RNA splicing [107]
• SPEN/SHARP binidng [104, 131, 132]
• Xist RNA localization [109, 110]
• YY1 binding [108]
• PRC2, Jarid2 recruitment [124]

Random X Inactivation Mediated by Xic, Xist, and Tsix

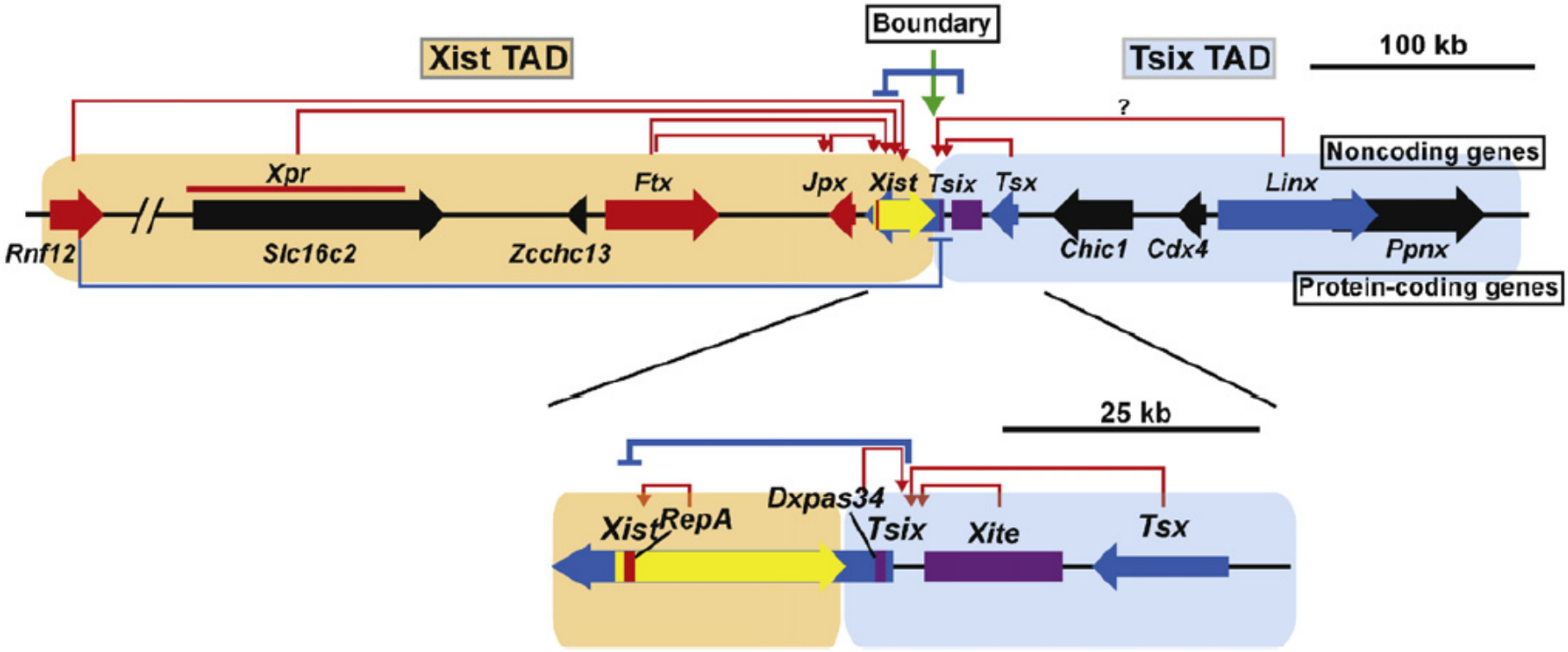
Random X chromosome inactivation mediated by interaction of X inactivation centers (Xics) on two X chromosomes of female cells.

Xic interaction first triggers transcription of Xist and Tsix RNAs from each X chromosome. Xist and Tsix are large RNAs ***without*** coding sequences.

Xist binds to the chromosome from which it was transcribed, and Tsix probably prevents accidental binding to other X chromosome.

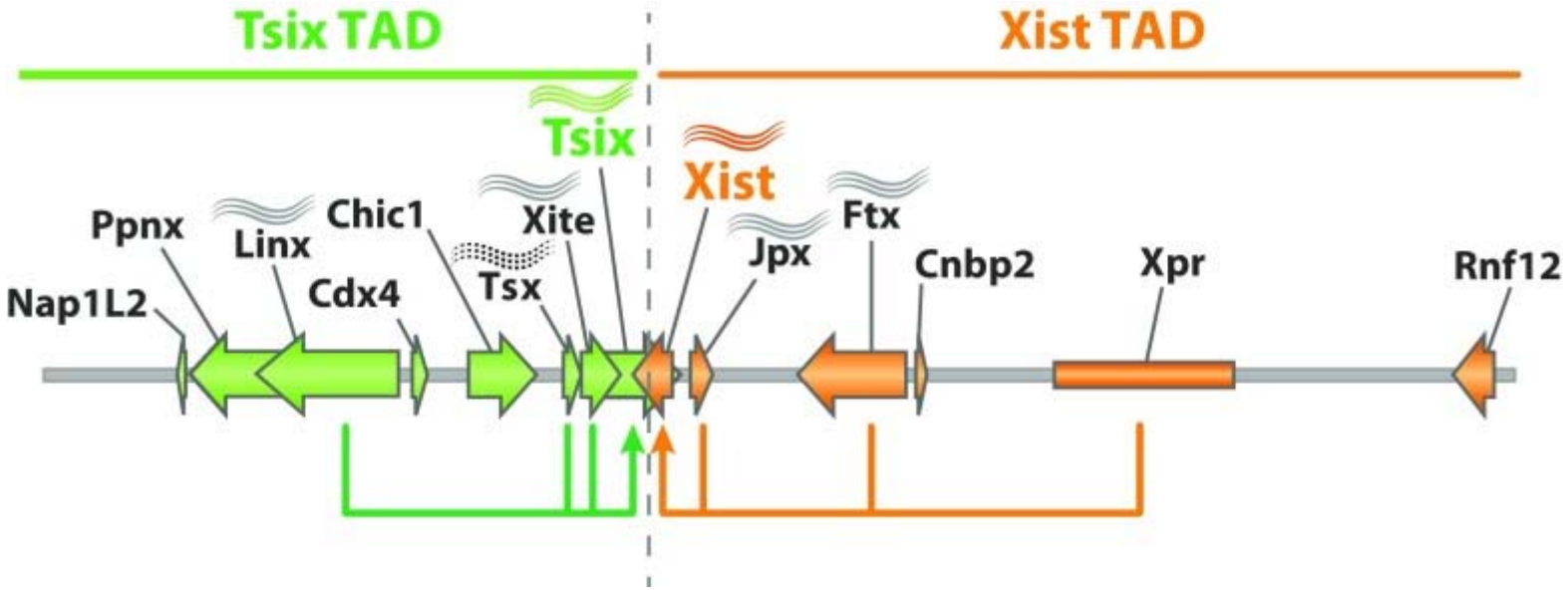
Xist RNA eventually "paints" the entire X chromosome from which it is transcribed, causing inactivation …. By this point, the only gene transcribed from the inactivated chromosome is Xist.

Meanwhile, Tsix paints the active X chromosome, preventing spread of inactivation to the second chromosome.

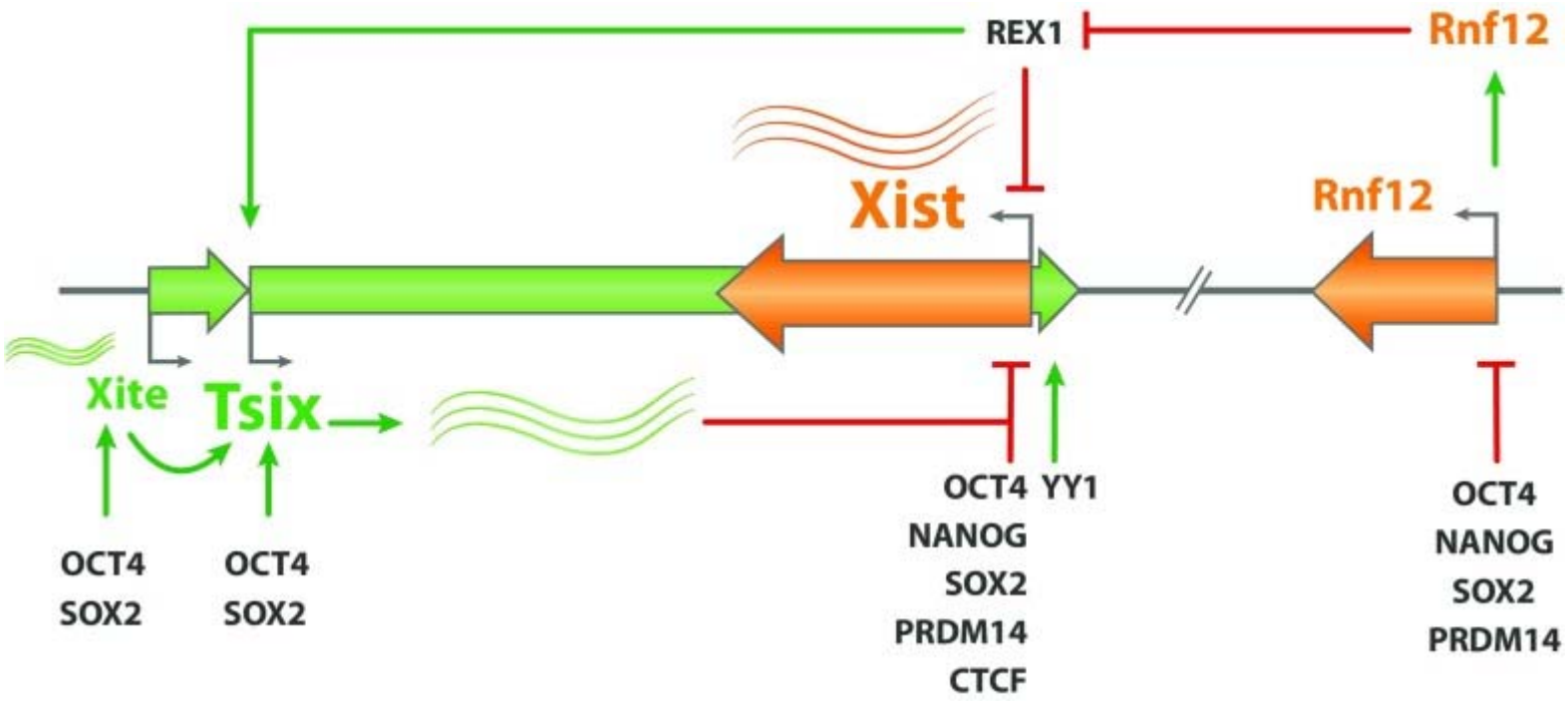


+ Tsix, Xite, DxPas34, Tsx, Jpx, Ftx and RepA

The cis-regulatory environment and its spatial separation



Trans-regulation of the Xic in ES cells



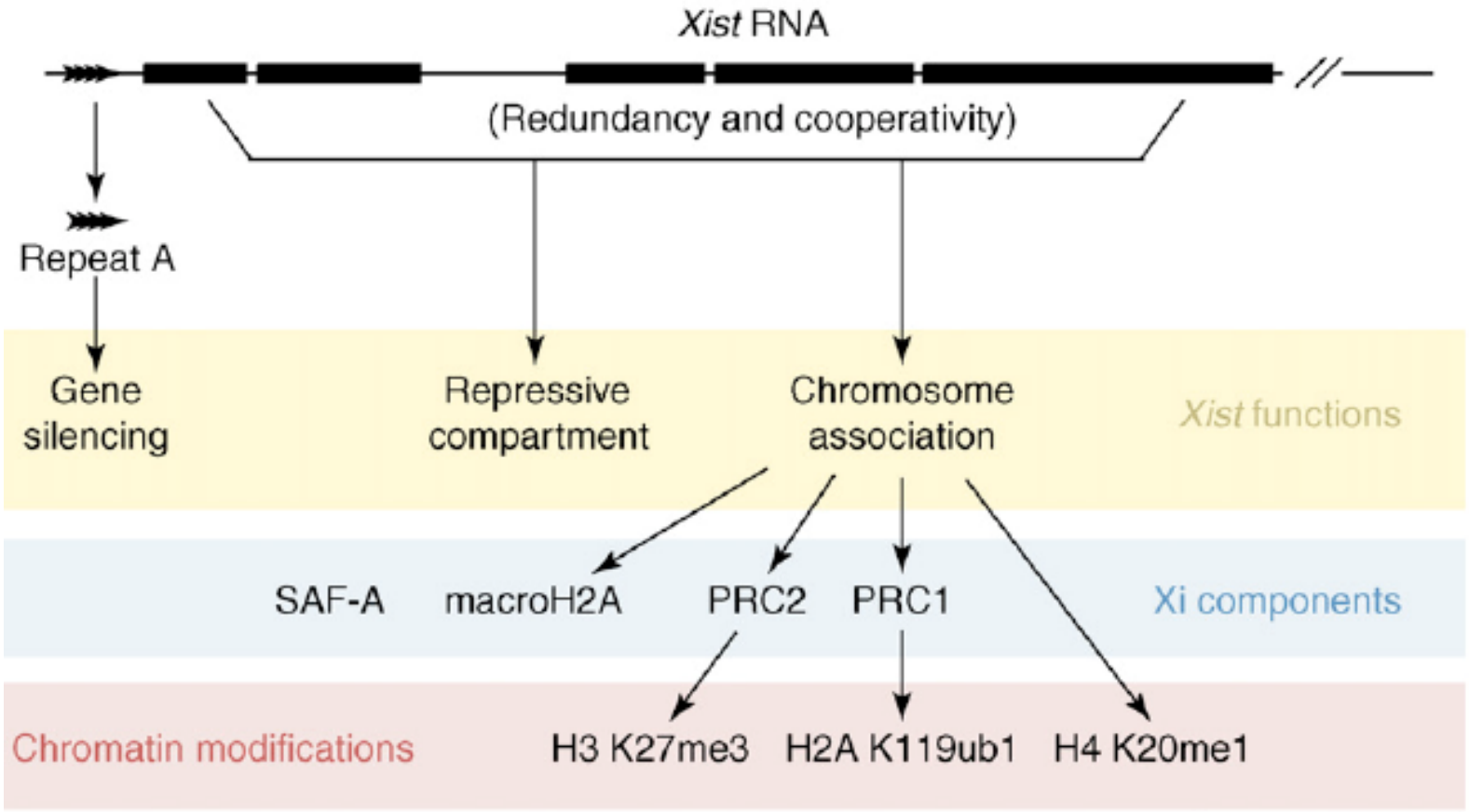
Progressione

- At the onset of random XCI, Tsix expression becomes monoallelic and designates the future Xa;
- consequently, Xist expression is upregulated on the future Xi and repressed on the future Xa.
- Xist lncRNA initiates XCI in cis by covering the entire Xi and recruiting multiple chromatin modifying enzymes for repressive epigenetic modification, including polycomb repressive complex 2 (PRC2) for histone H3 trimethylation at lysine 27 (H3K27me3)
- unique repeat elements make Xist RNA is a hub for a variety of protein factors, which induce multiple layers of repressive modification on the Xi
- PRC2 binds to a limited number of sites (about 1500) frequently associated with H3K4me3/H3K27me3 bivalent domains and CpG islands, which are followed by additional binding sites (about 4000) at intergenic regions and spreading across the Xi as differentiation progresses

Mechanism of Xist-Mediated X Inactivation Still Uncertain

Xist RNA recruits novel proteins to inactivating X chromosome, induces methylation of histone tails, and bears a 5' end essential for gene silencing.

After induction of X-inactivation, Xist is no longer required for its maintenance.



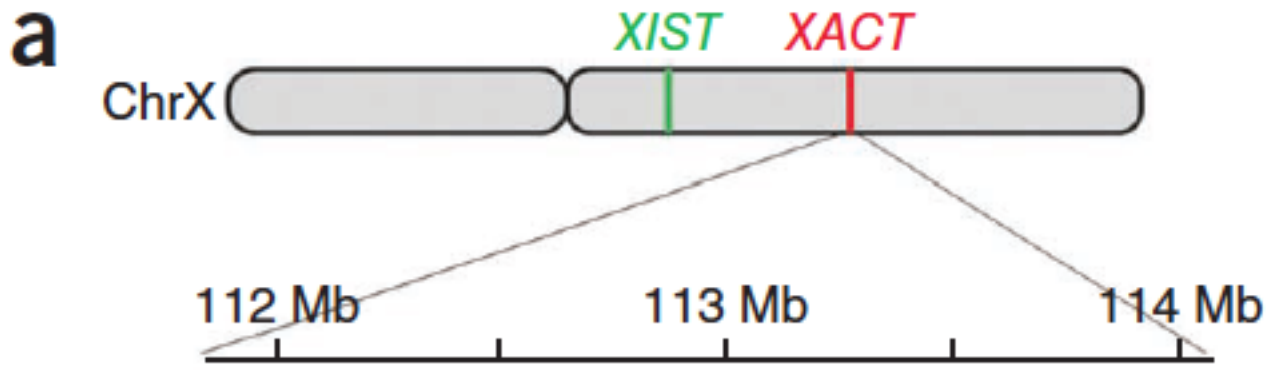
nature genetics

published online 20 January 2013; doi:10.1038/ng.2530

XACT, a long noncoding transcript coating the active X chromosome in human pluripotent cells

Céline Vallot[1,2], Christophe Huret[1,2], Yann Lesecque[3], Alissa Resch[4], Noufissa Oudrhiri[5], Annelise Bennaceur-Griscelli[5], Laurent Duret[3] & Claire Rougeulle[1,2]

X-chromosome inactivation (XCI) in mammals relies on *XIST*, a long noncoding transcript that coats and silences the X chromosome in *cis*. Here we report the discovery of a long noncoding RNA, *XACT*, that is expressed from and coats the active X chromosome specifically in human pluripotent cells. In the absence of *XIST*, *XACT* is expressed from both X chromosomes in humans but not in mice, suggesting a unique role for *XACT* in the control of human XCI initiation.


a
XIST
XACT
ChrX
112 Mb
113 Mb
114 Mb

f