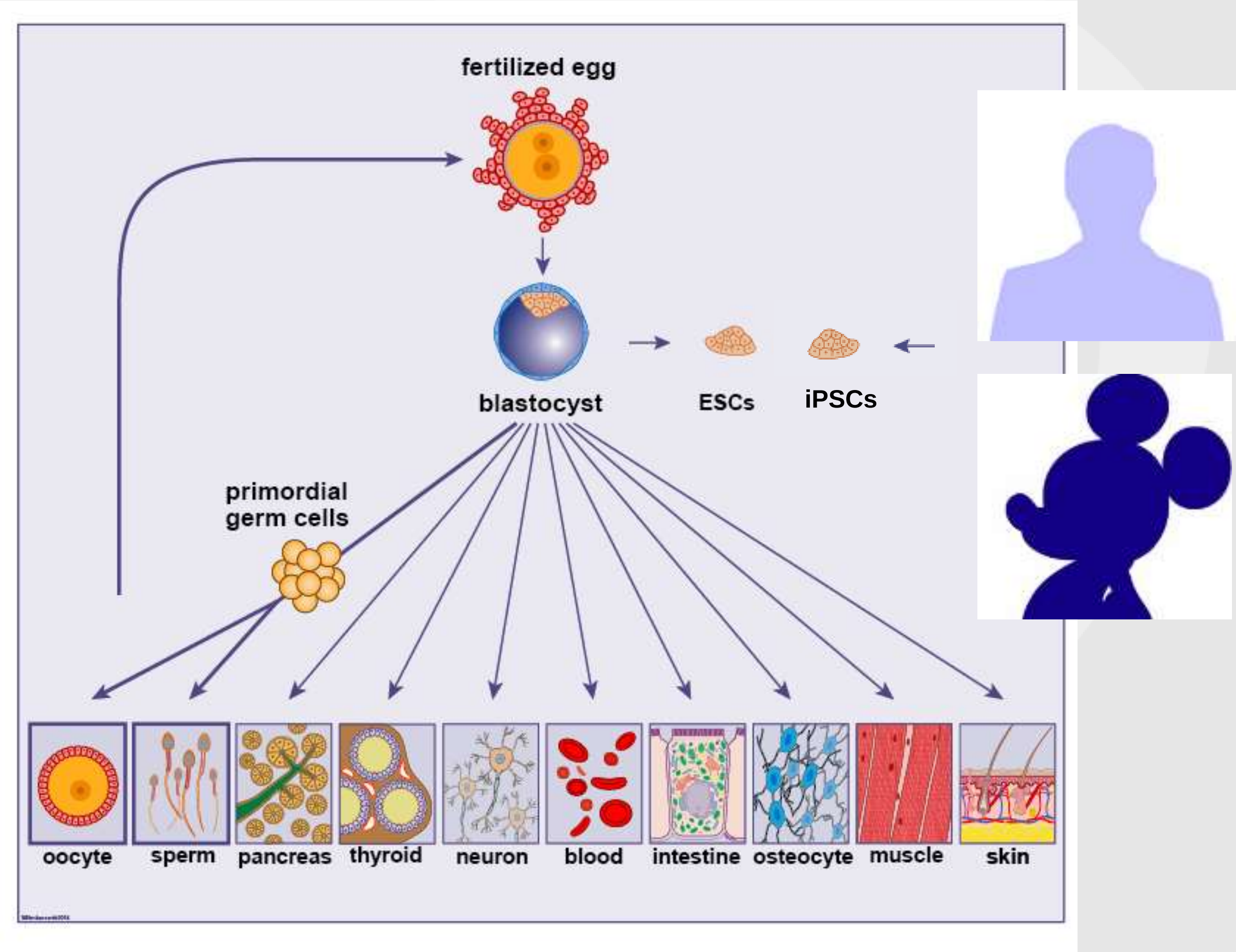
Principles of epigenetics

S.M. Chuva de Sousa Lopes
Leiden University Medical Center
Department of Anatomy and Embryology
Leiden, The Netherlands

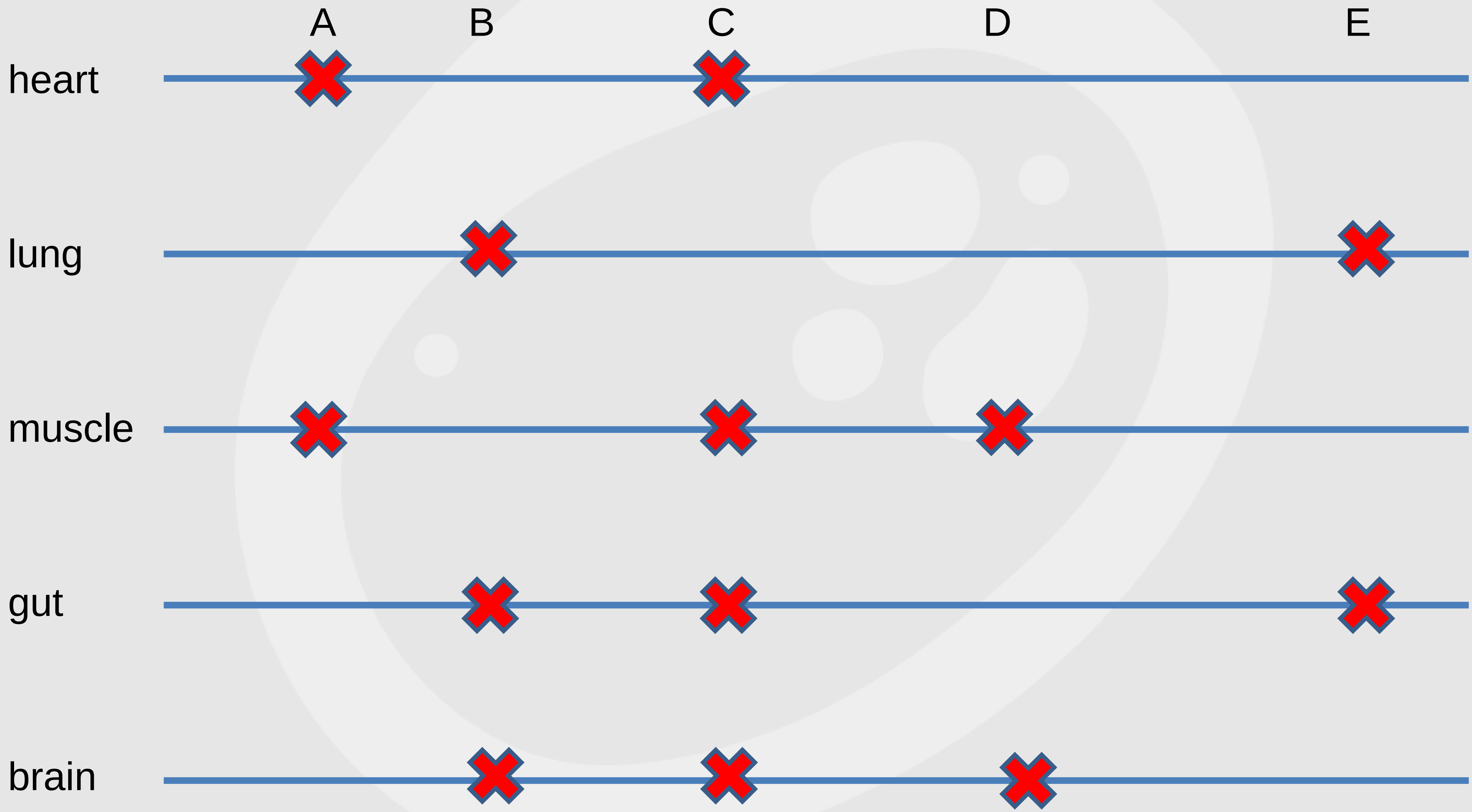
Lopes@Lumc.nl

Where it all begins



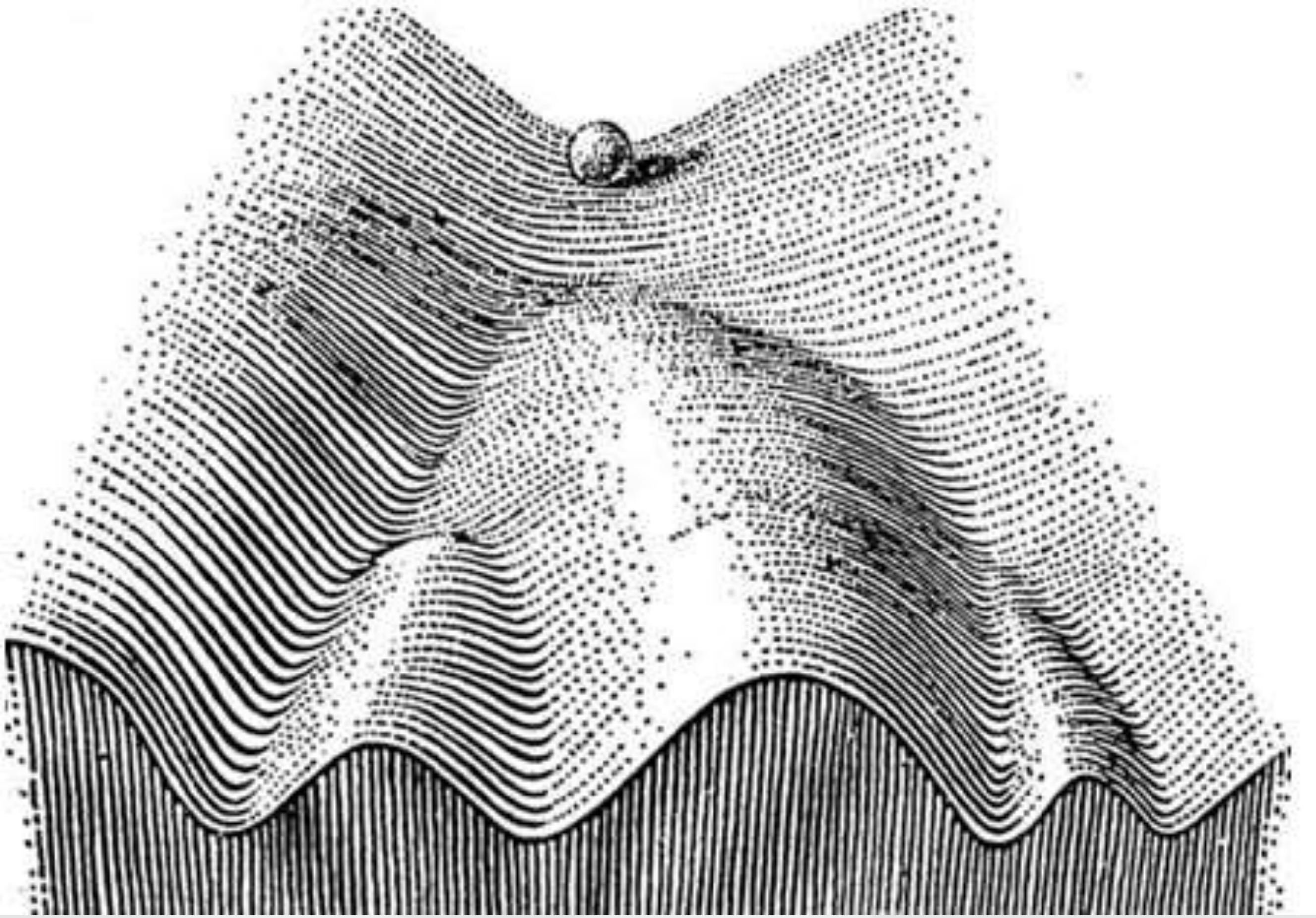
How?

Epigenetic reprogramming: possibility to be transcribed

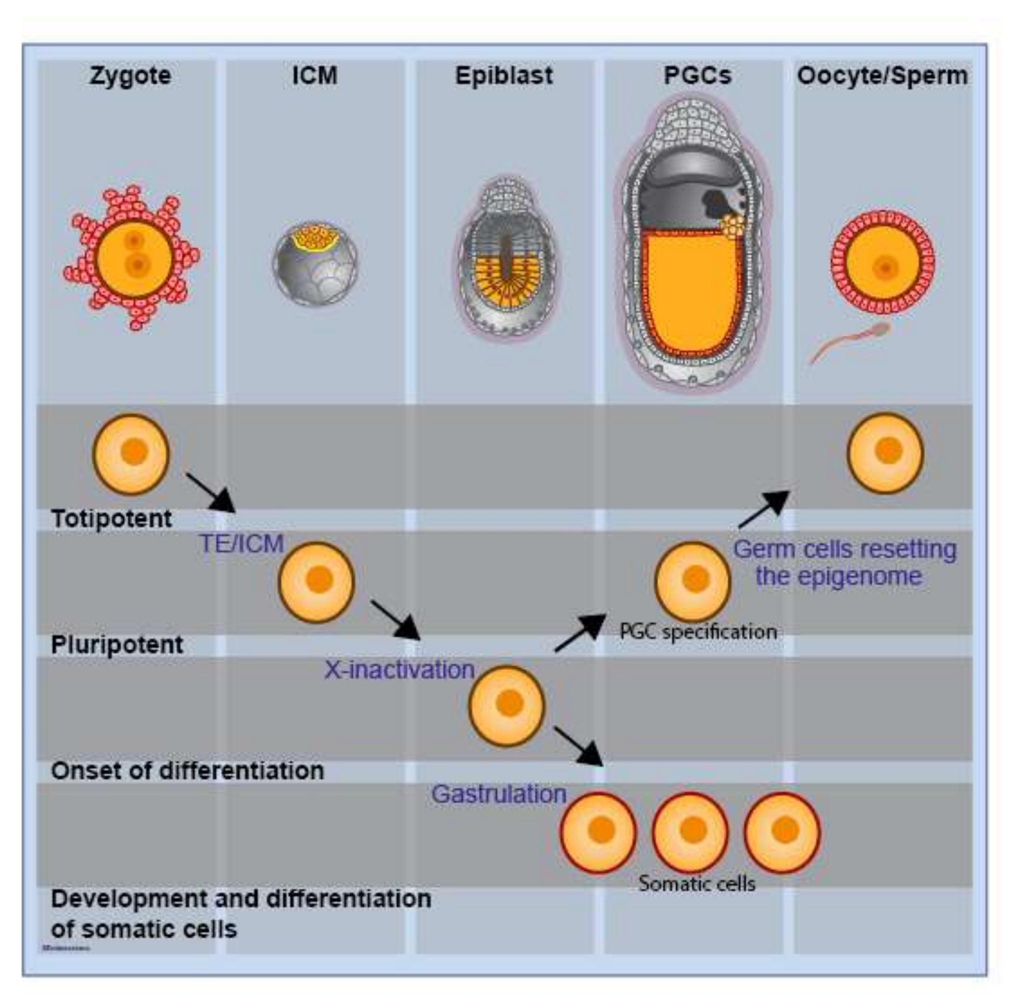


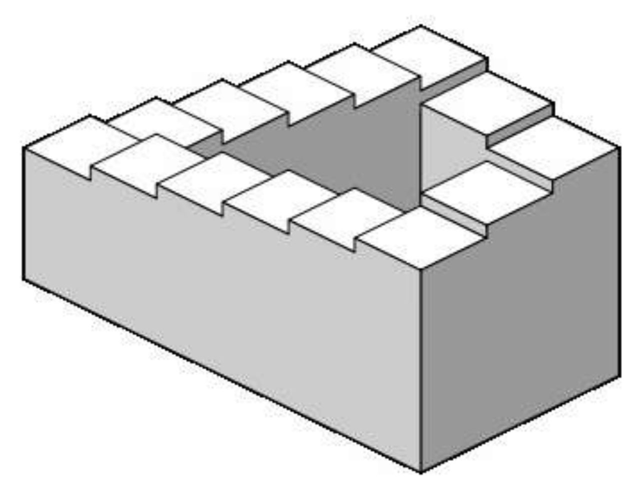
	A	B	C	D	E
heart	✗		✗		
lung		✗			✗
muscle	✗		✗	✗	
gut		✗	✗		✗
brain		✗	✗	✗	

Waddington’s epigenetic landscape



Epigenetics during early development in mice





Epigenetics

Epigenetic regulation

- **accessibility** of genes in a **heritable** fashion

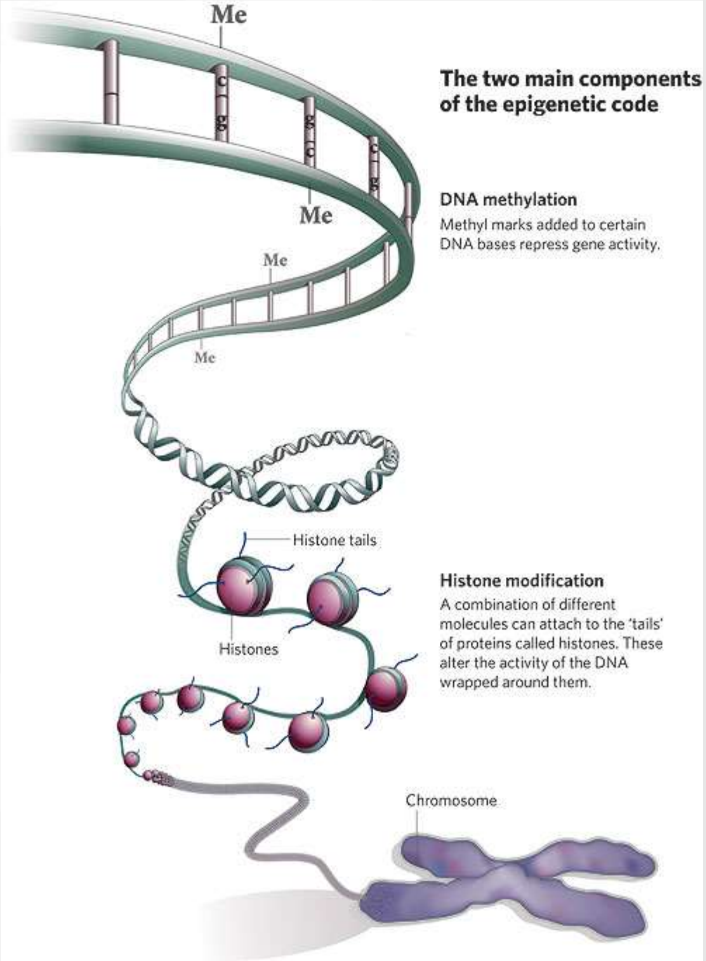
- modification of **chromatin**

    - DNA methylation
    (repetitive DNA, imprinting, X inactivation)

    - histone modifications or
    use of special histone variants

    - association of ATP-dependent
    complexes (polycomb/tritorax proteins)

    - association with non-coding RNAs

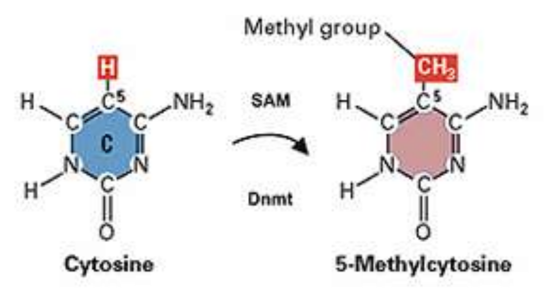


The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

DNA (CpG) methylation in mammals



Methylation

Demethylation

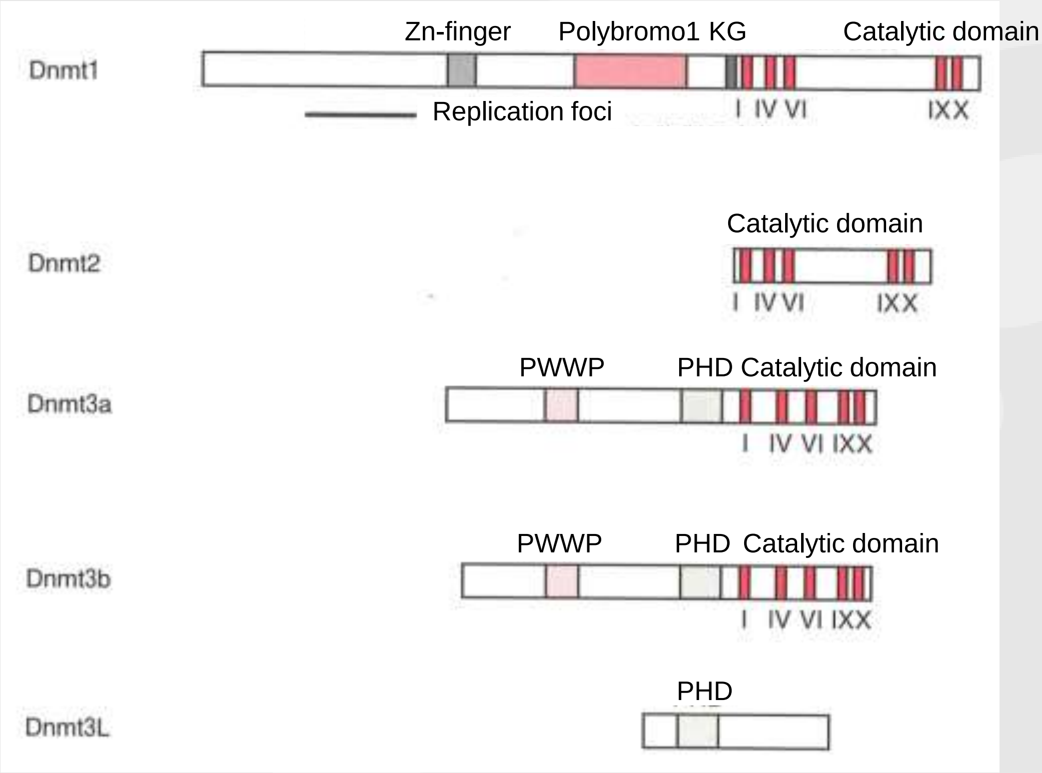
Biological significances of CpG methylation

1. Gene regulation, silencing (cell identity, genomic imprinting, cancer)
2. Suppression of retrotransposons (genome stability)
3. Chromosome organization (chromosome stability)

Distribution of CpG methylation

1. At global level, 80% of CpGs are methylated.
2. CpG islands frequent near promoters
3. Unclear how the methylation enzymes know what sequences to methylate

DNA methyltransferases

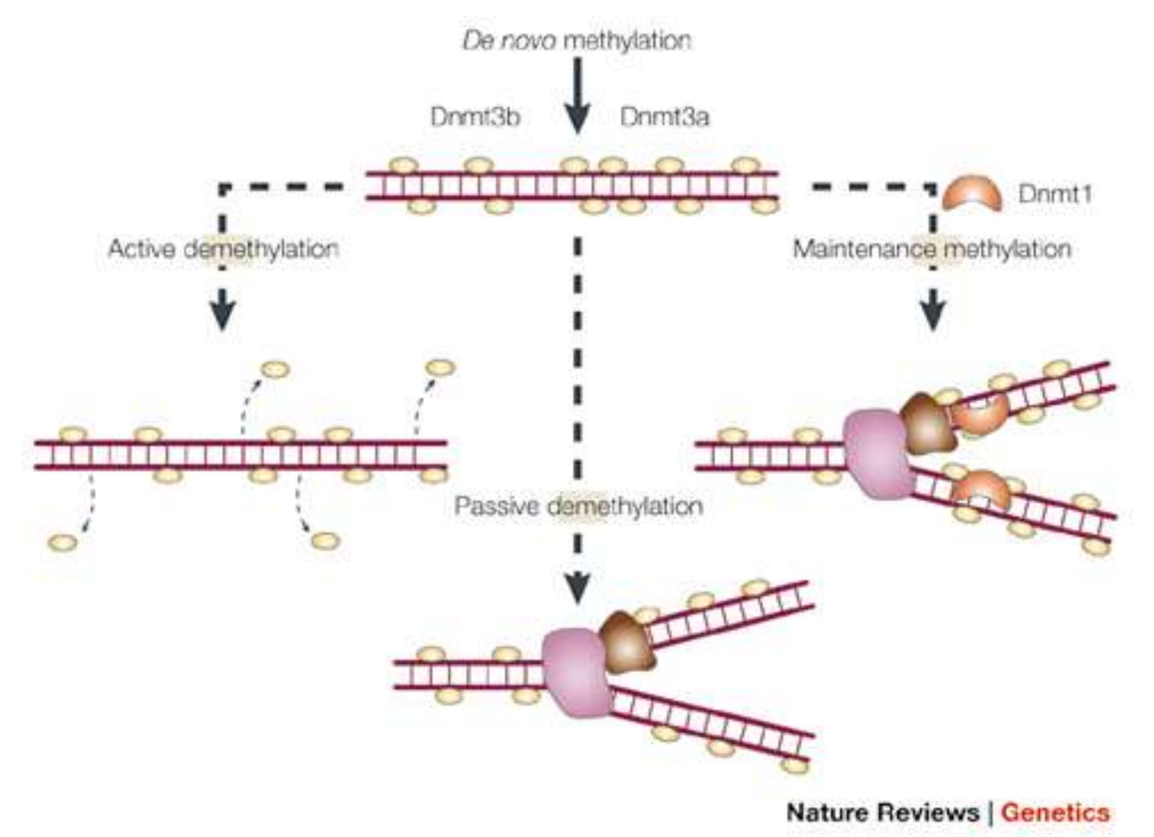


Replication-dependent methyltransferase

De novo methyltransferase

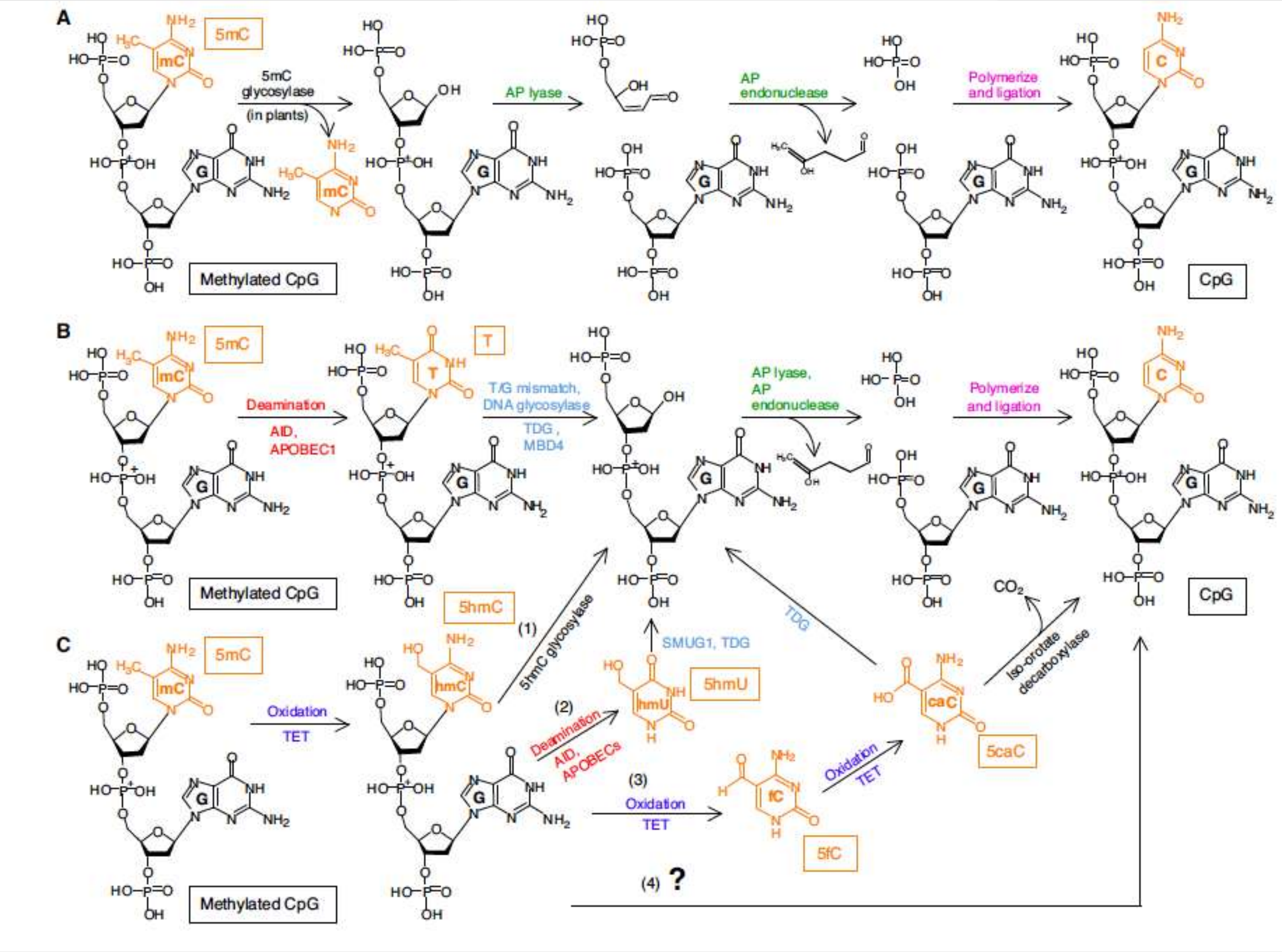
De novo methyltransferase

DNA methylation and DNA demethylation

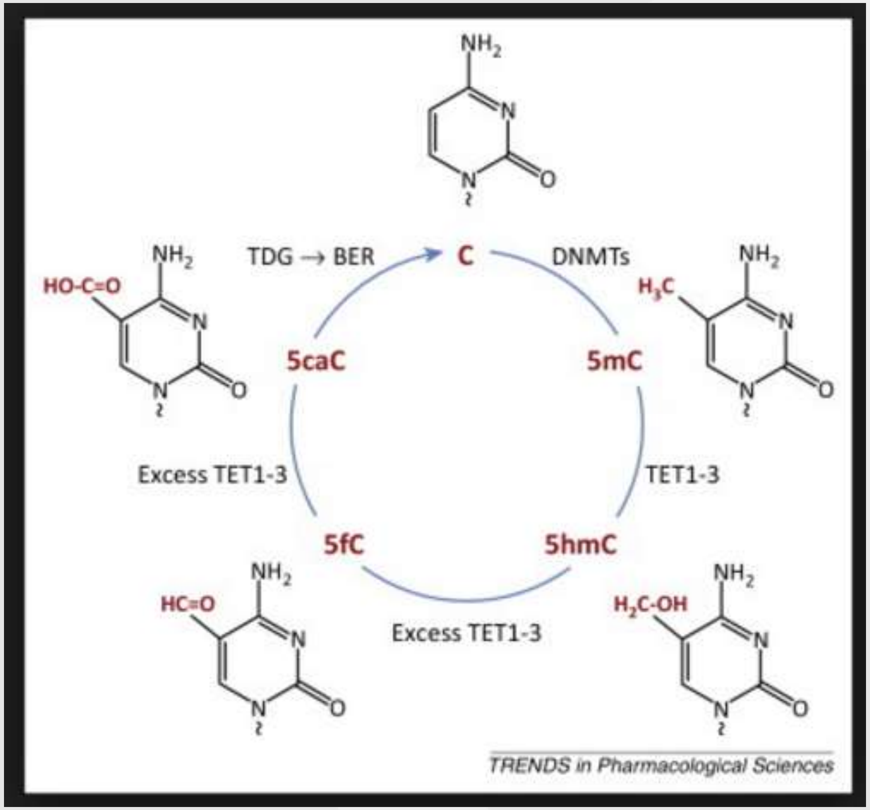


Nature Reviews | Genetics

DNA methylation and DNA demethylation

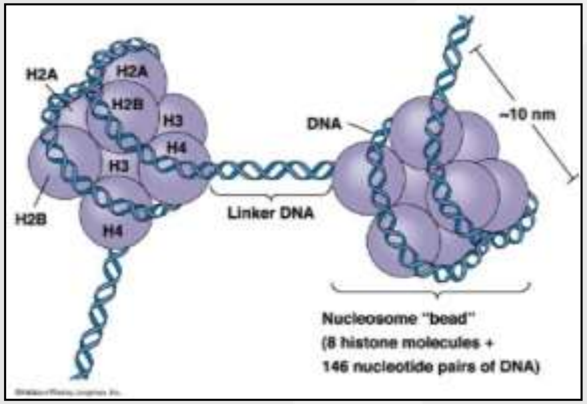


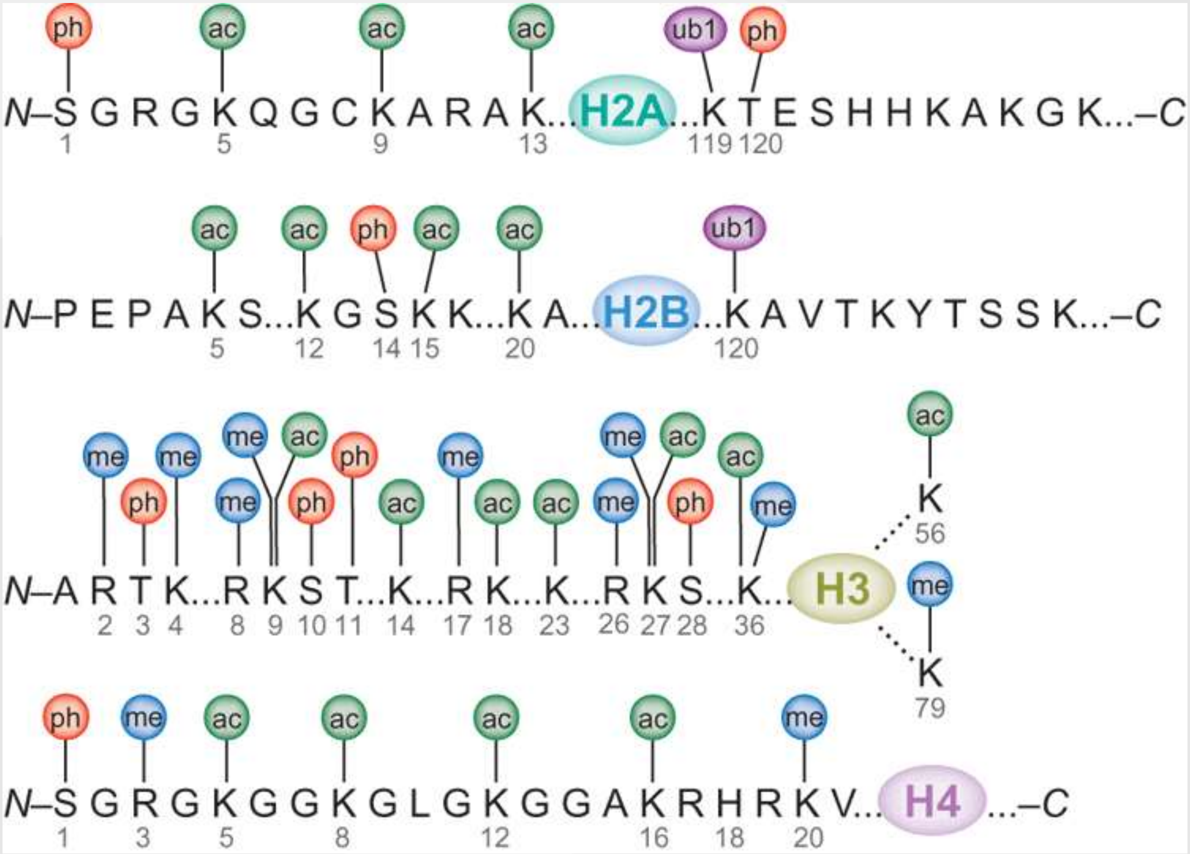
DNA methylation and DNA demethylation



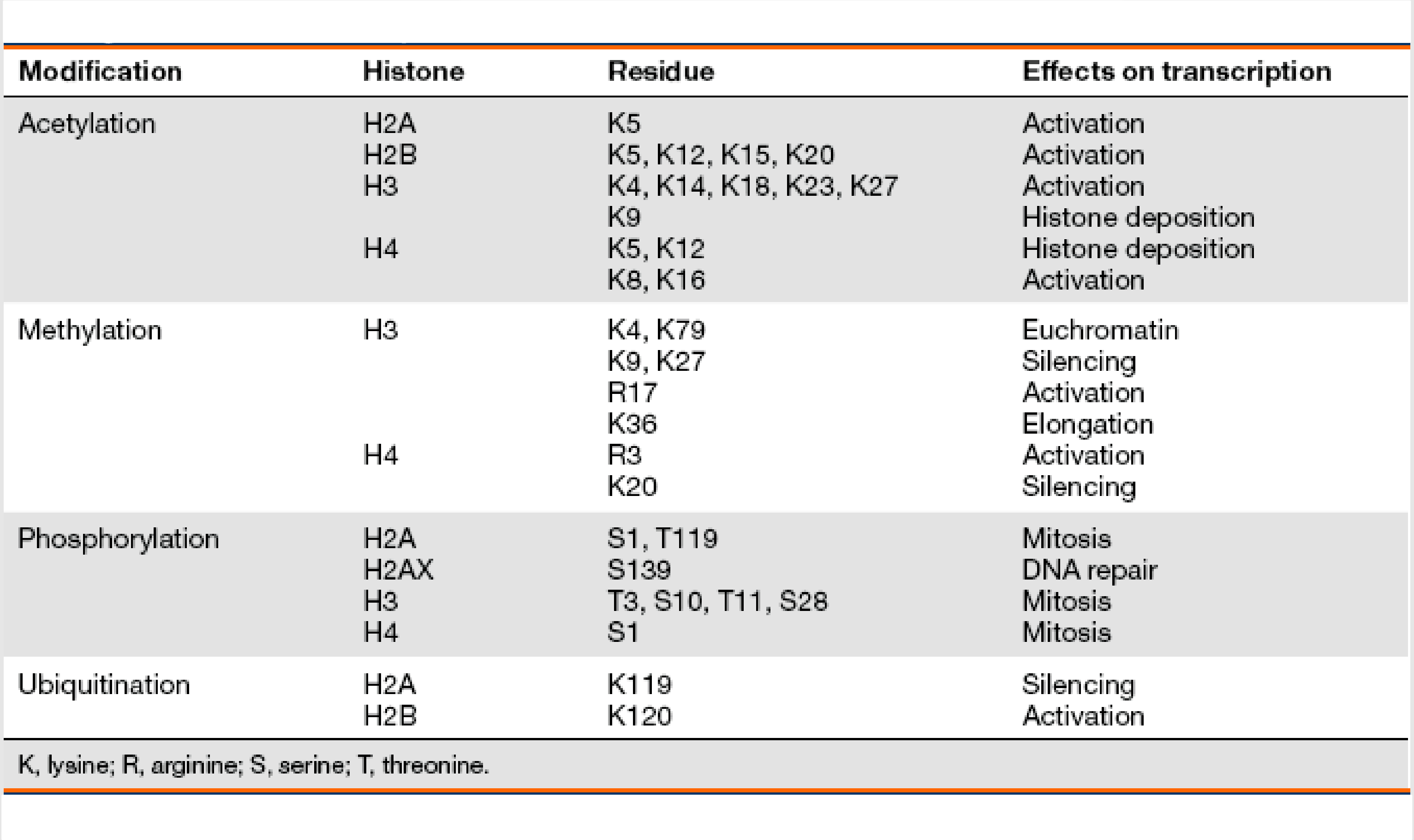
TET: ten-eleven translocation enzymes
TGD: thymide DNA glycosylase
BER: base excision repair

Histone modifications





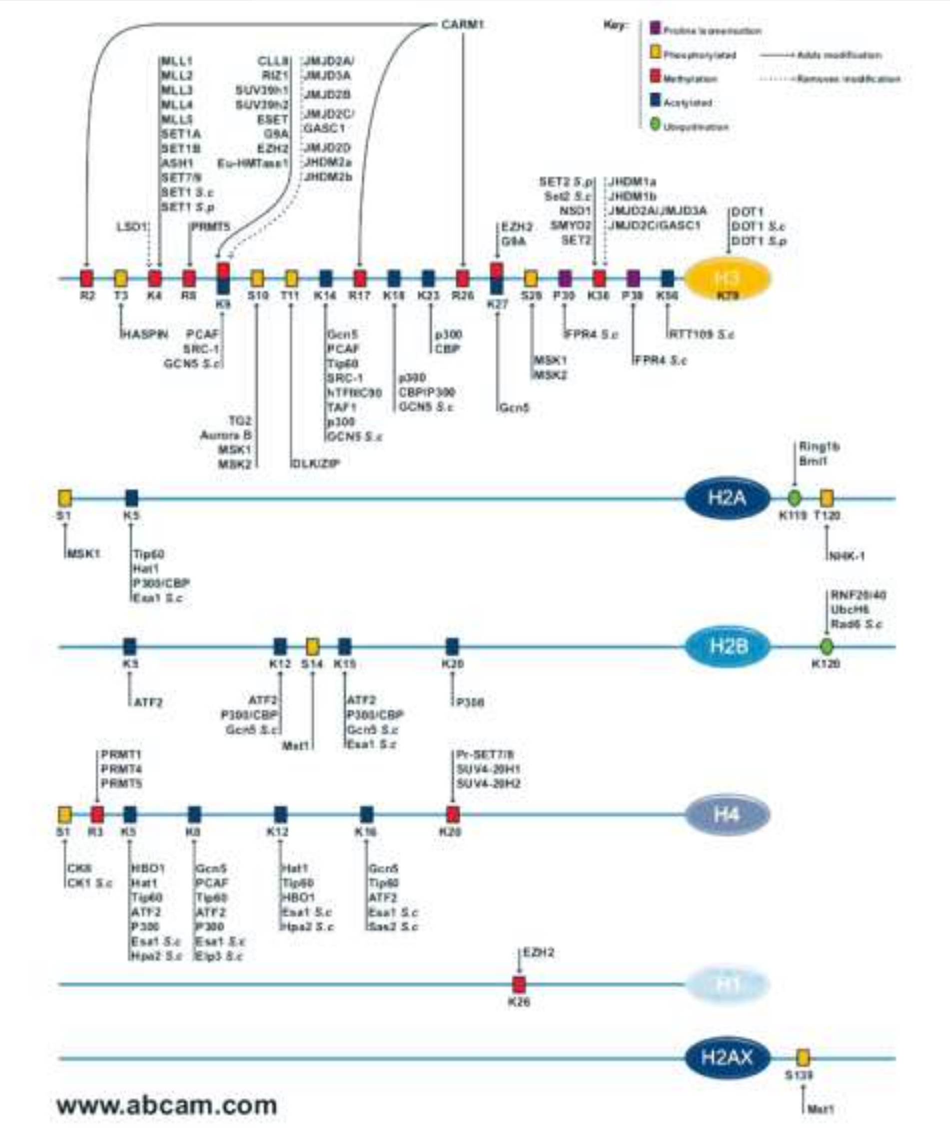
Functional significances of histone modifications



Modification	Histone	Residue	Effects on transcription
Acetylation	H2A	K5	Activation
	H2B	K5, K12, K15, K20	Activation
	H3	K4, K14, K18, K23, K27	Activation
		K9	Histone deposition
	H4	K5, K12	Histone deposition
		K8, K16	Activation
Methylation	H3	K4, K79	Euchromatin
		K9, K27	Silencing
		R17	Activation
		K36	Elongation
	H4	R3	Activation
		K20	Silencing
Phosphorylation	H2A	S1, T119	Mitosis
	H2AX	S139	DNA repair
	H3	T3, S10, T11, S28	Mitosis
	H4	S1	Mitosis
Ubiquitination	H2A	K119	Silencing
	H2B	K120	Activation

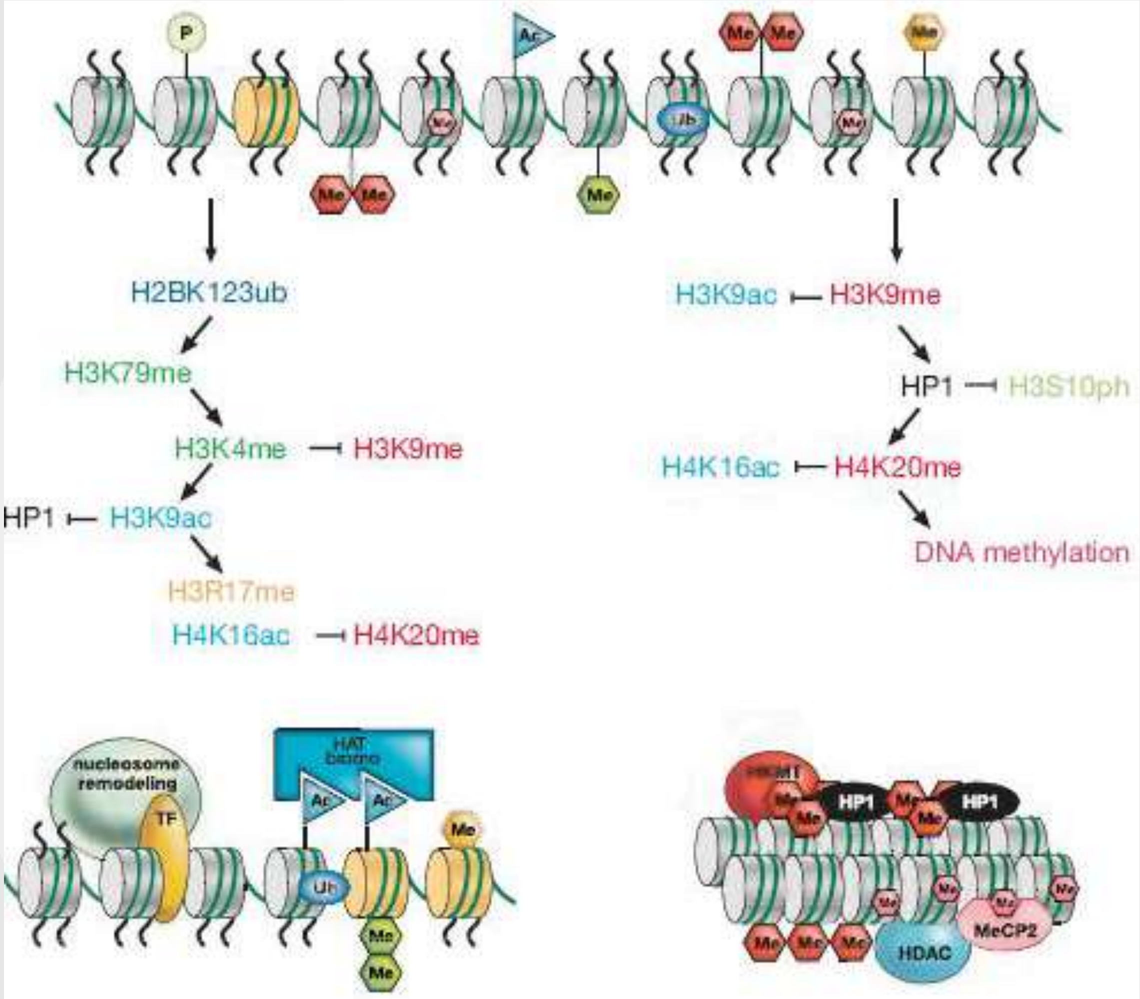
K, lysine; R, arginine; S, serine; T, threonine.

Enzymes regulating histone modifications



www.abcam.com

Euchromatin and Heterochromatin

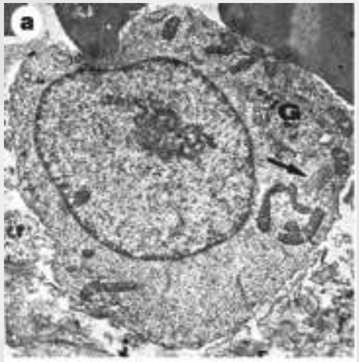


Euchromatin
Gene expression

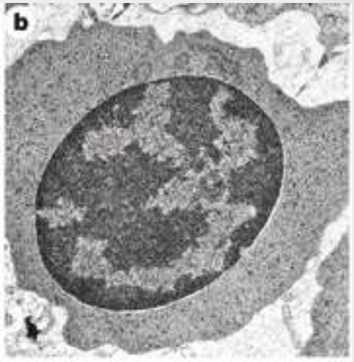
Heterochromatin
Gene silencing

Epigenetic control is critical to control differentiation

Undifferentiated cell



Differentiated cell



Euchromatin	**Large region**	Small region
Heterochromatin	Small region	**Large region**
DNA methylation	Low	High

Differentiation →

← Dedifferentiation
(Reprogramming)

Epigenetics:
X Chr inactivation

What determines sex?

A) Genetics (sex chromosomes)

- birds: female – ZW; male – ZZ
- mammals: female – XX; male – XY

B) Environmental variables (temperature)

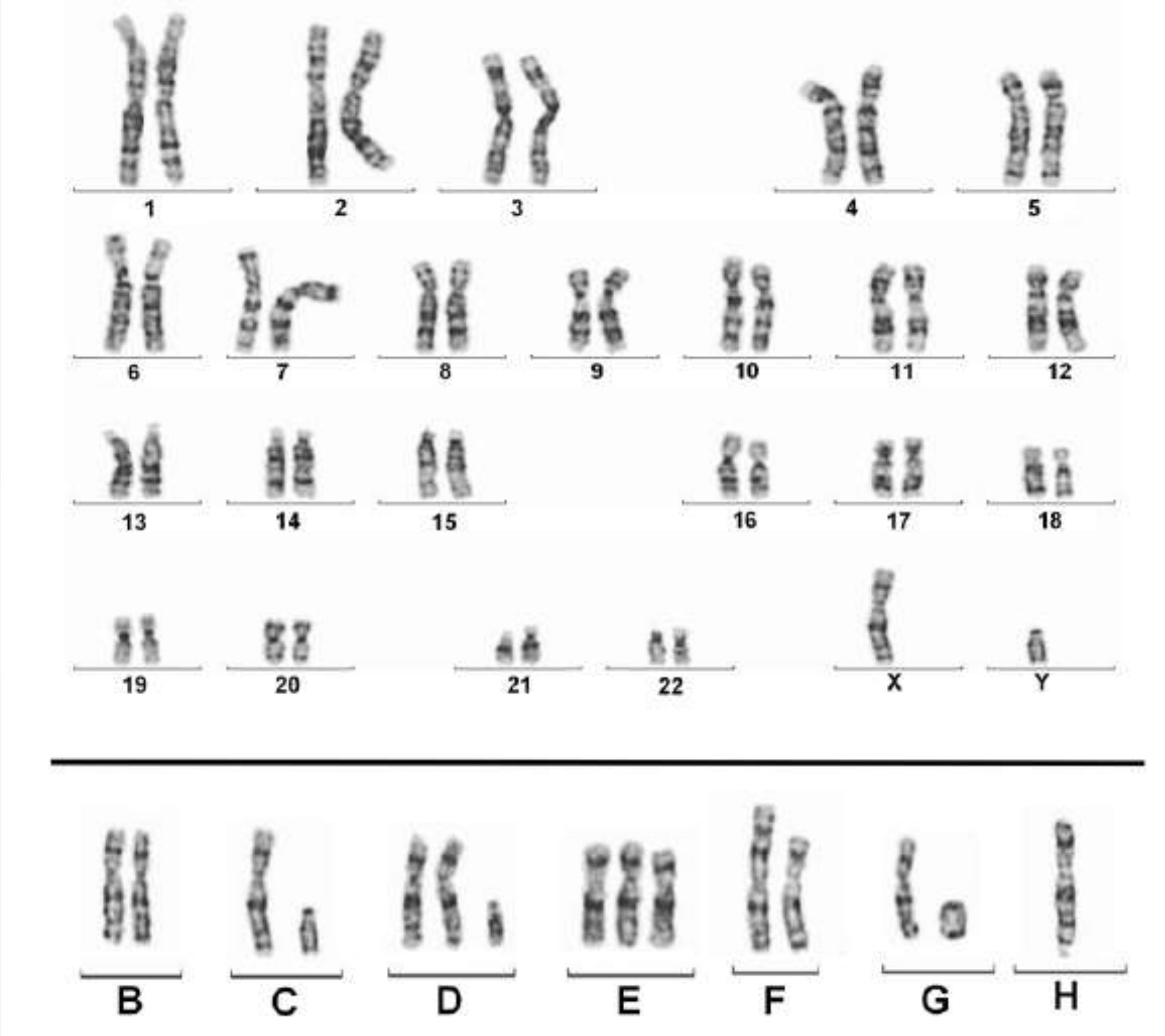
- turtles an crocodiles

C) Social variables (relative size)

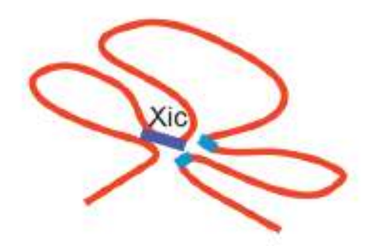
- tropical clown fish – males can become females
- blue wrasse fish - females can become males

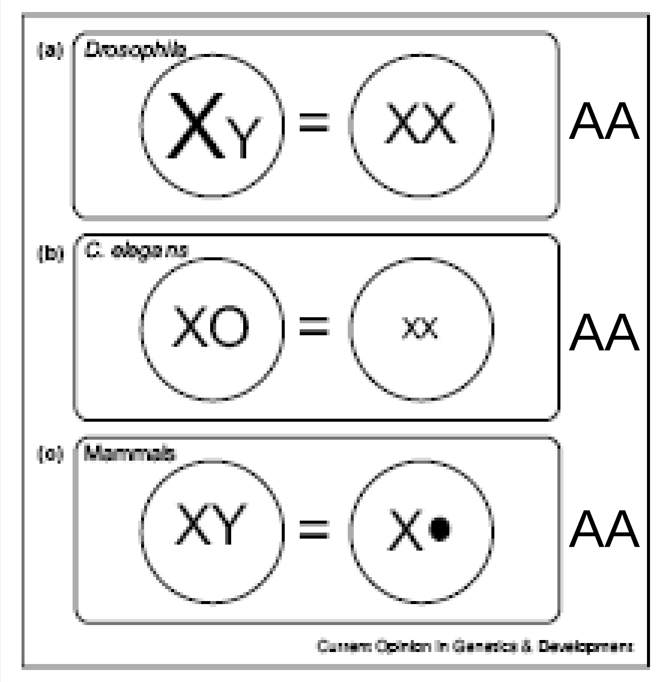


What determines sex?

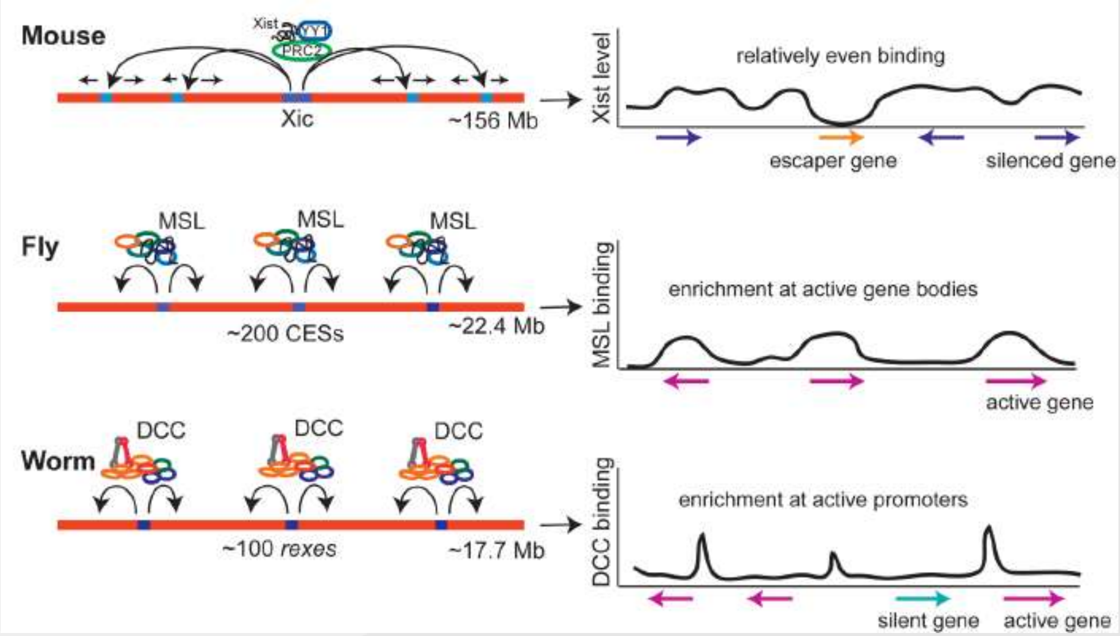


Dosage compensation to equalize X-linked expression



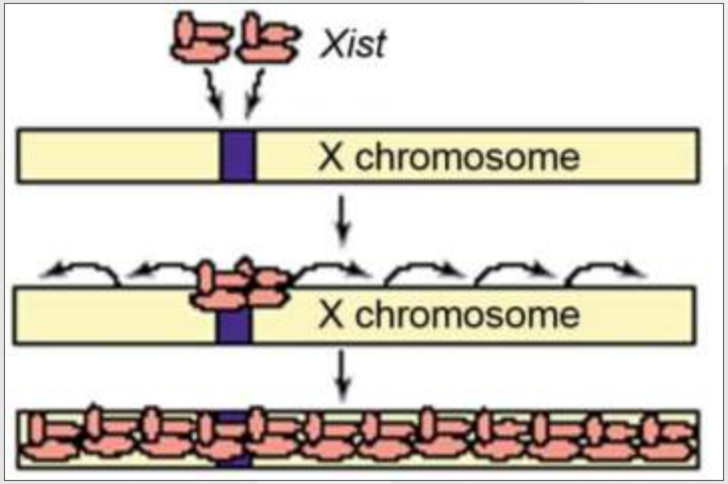


Current Opinion in Genetics & Development

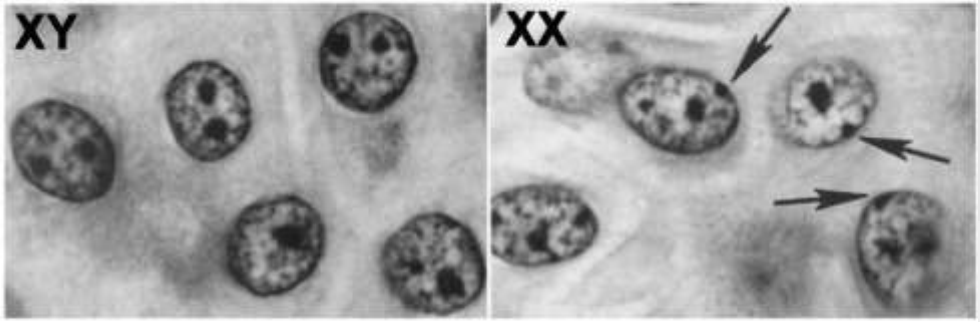


Ercan, J Genomics, 2015

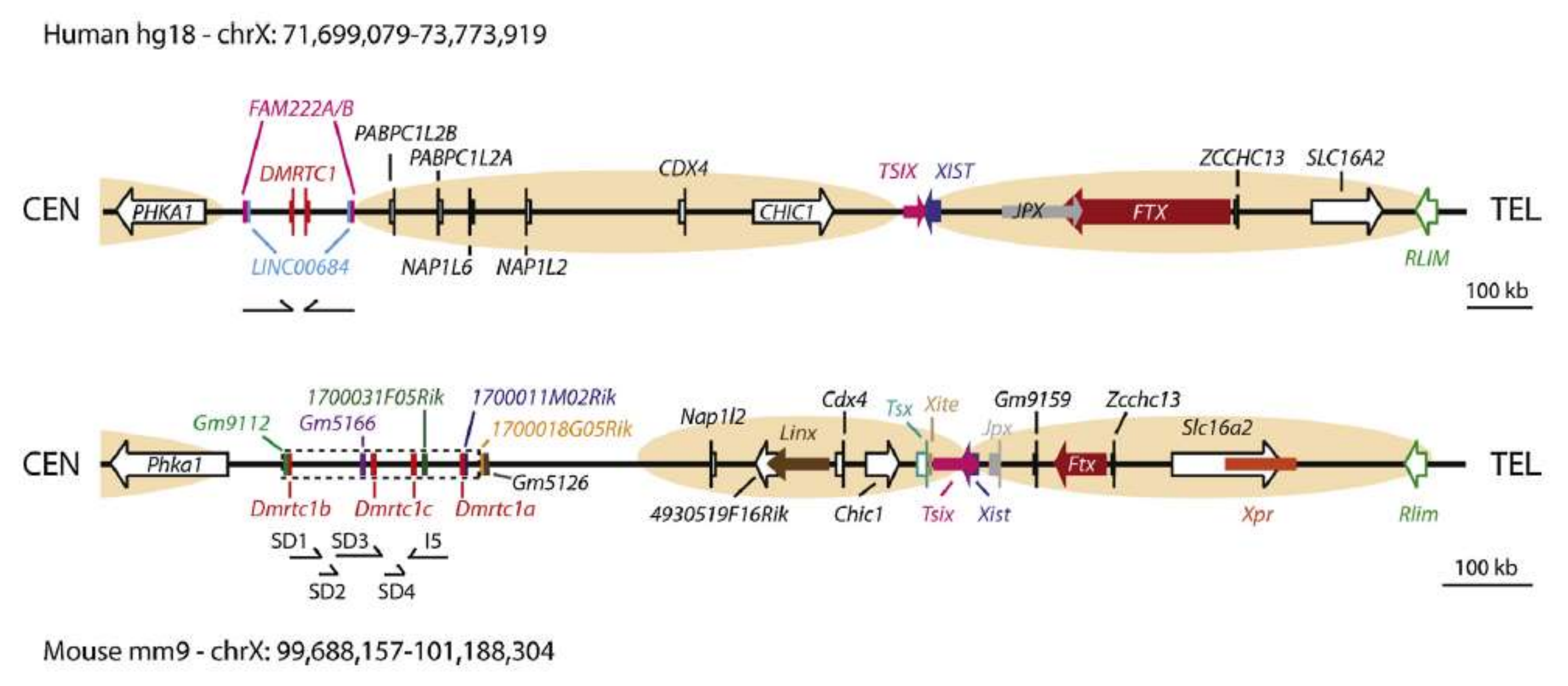
Epigenetic regulation of the X chromosome



Sequence of events:
- XIST (and TSIX)
- Ezh2 polycomb (PRC2)
- H3K27me3
- Histone variants (macroH2A)
- DNA methylation

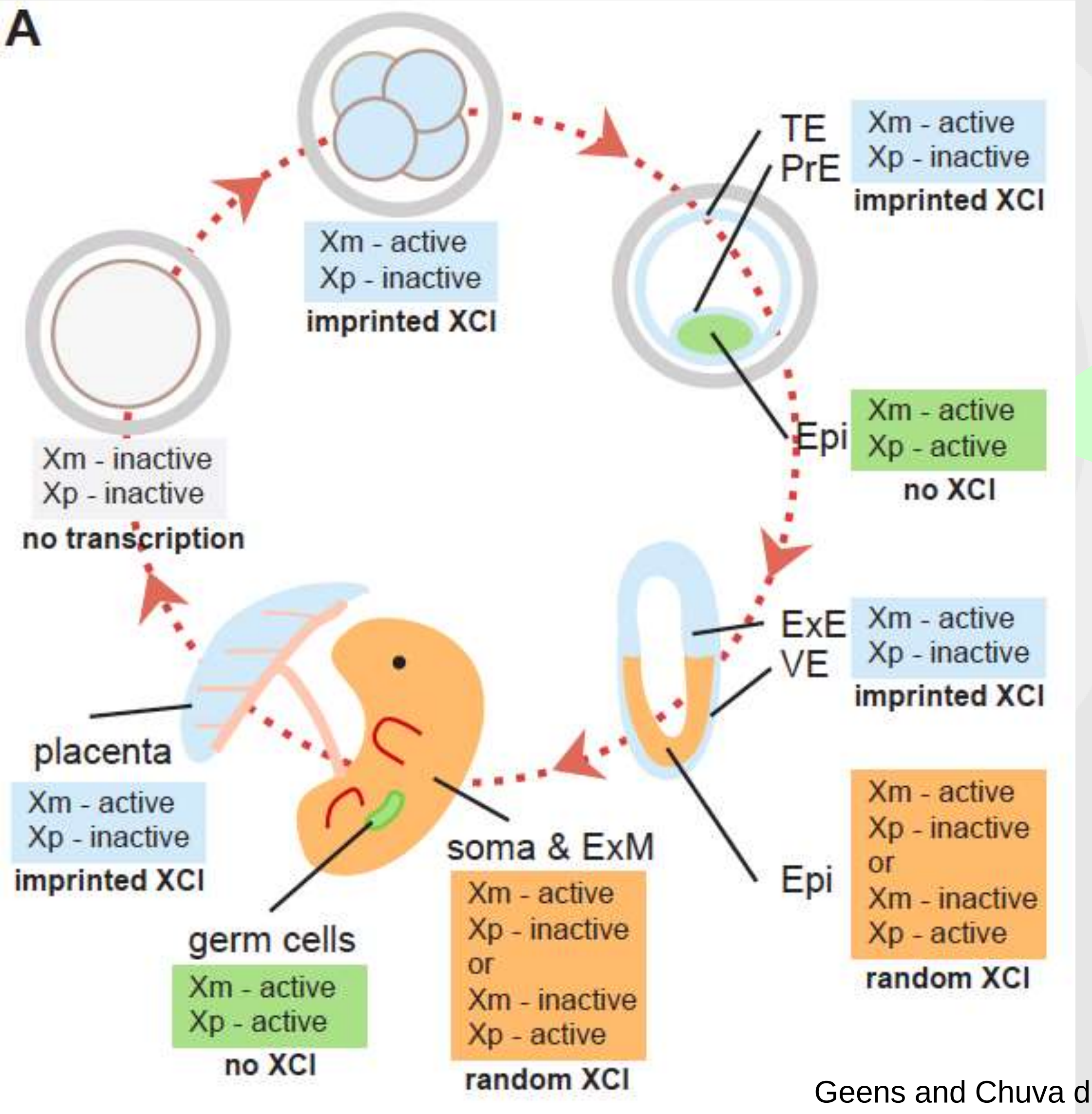


X-inactivation center



Peters, Yang& Brown, SCDB, 2016

Epigenetic regulation – X chromosome inactivation



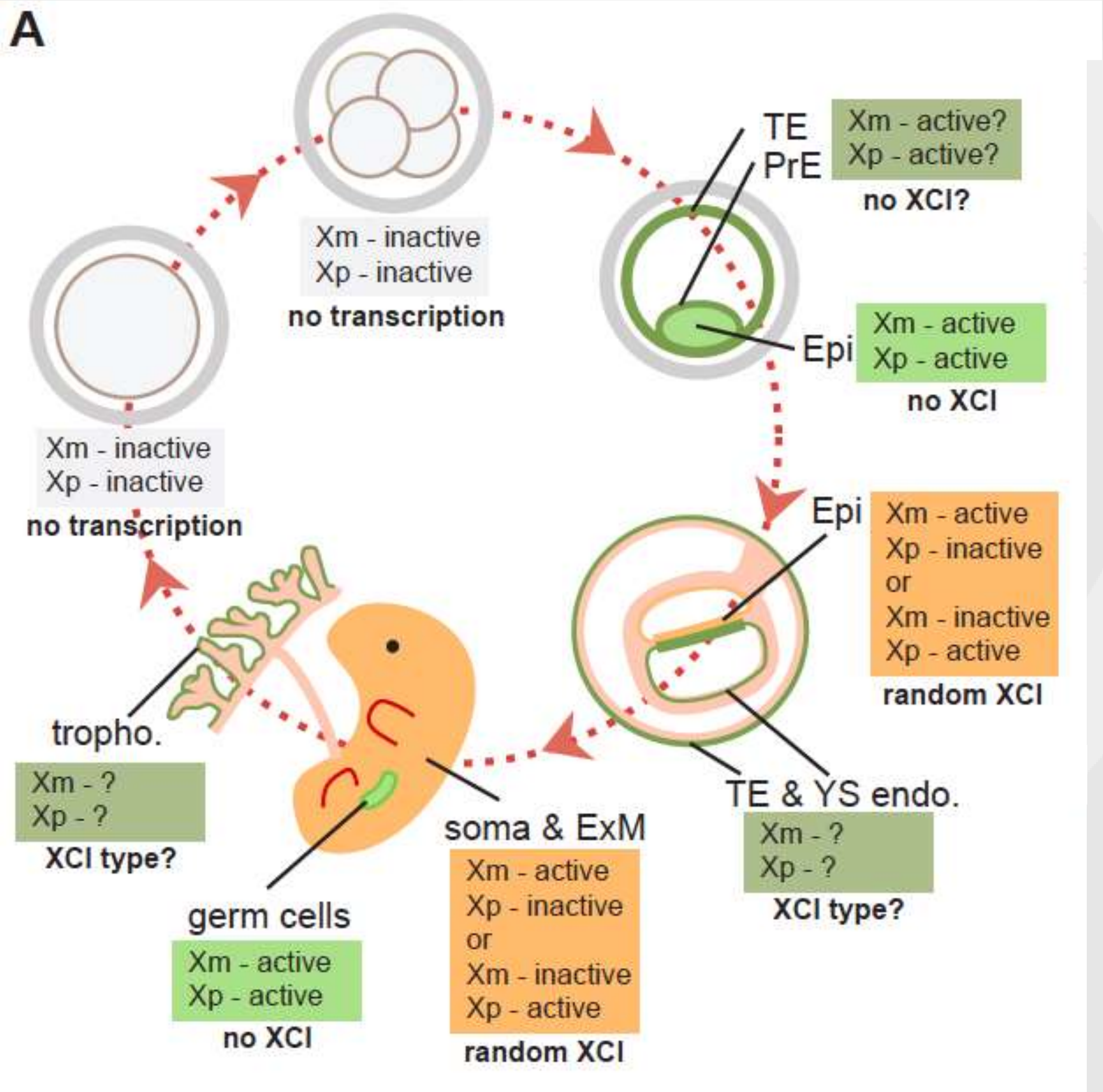
fast

mESC?

slow

Geens and Chuva de Sousa Lopes, HRU, 2017

Epigenetic regulation – X chromosome inactivation



A

Xm - inactive
Xp - inactive
no transcription

Xm - inactive
Xp - inactive
no transcription

TE
PrE
Xm - active?
Xp - active?
no XCI?

Epi
Xm - active
Xp - active
no XCI

Epi
Xm - active
Xp - inactive
or
Xm - inactive
Xp - active
random XCI

TE & YS endo.
Xm - ?
Xp - ?
XCI type?

soma & ExM
Xm - active
Xp - inactive
or
Xm - inactive
Xp - active
random XCI

germ cells
Xm - active
Xp - active
no XCI

tropho.
Xm - ?
Xp - ?
XCI type?

Geens and Chuva de Sousa Lopes, HRU, 2017

Dosage compensation to equalize X-linked expression

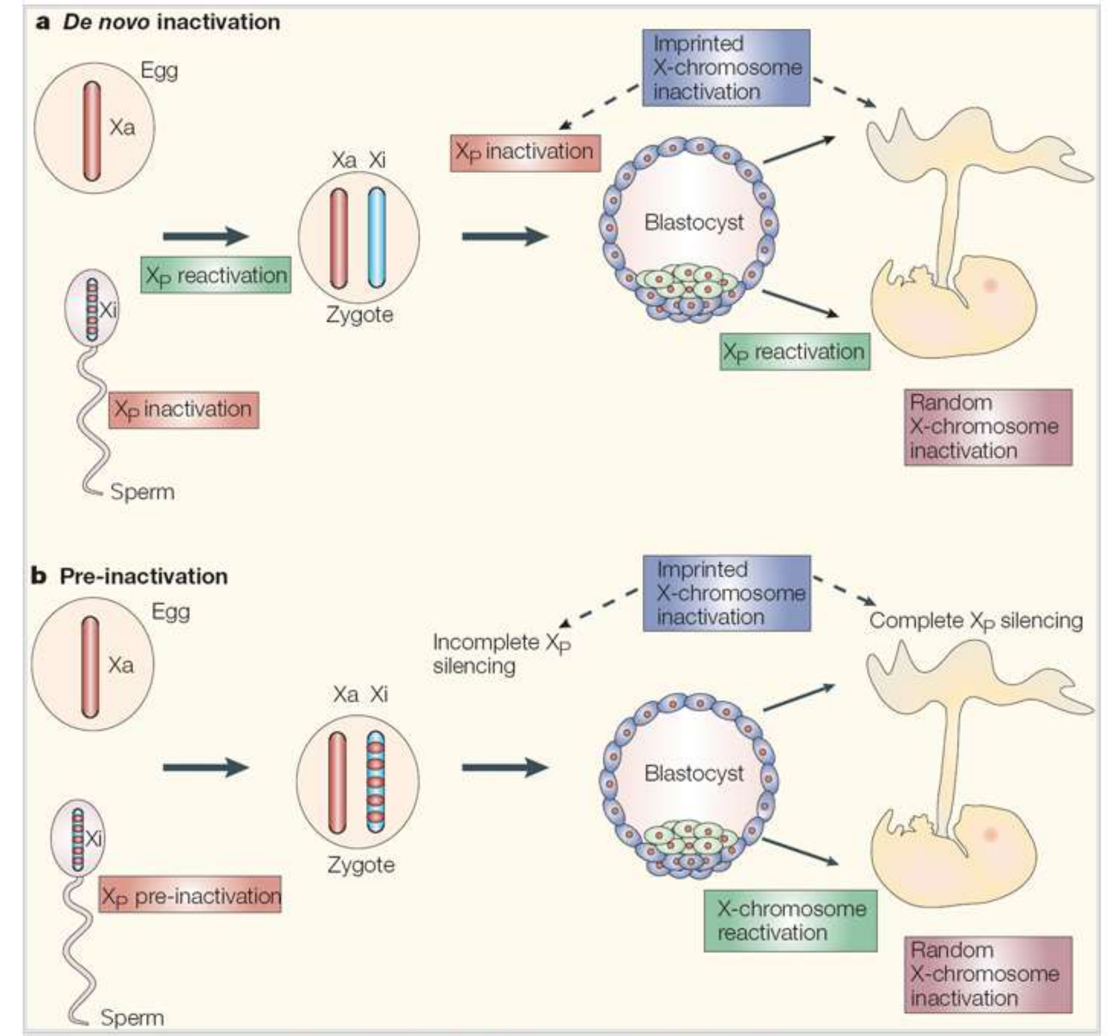


Figure 4: The ontogeny of X-chromosome inactivation in the mouse: two current views.

mES cells
XX active

Differentiation
Random Xi

Recapitulating
embryogenesis

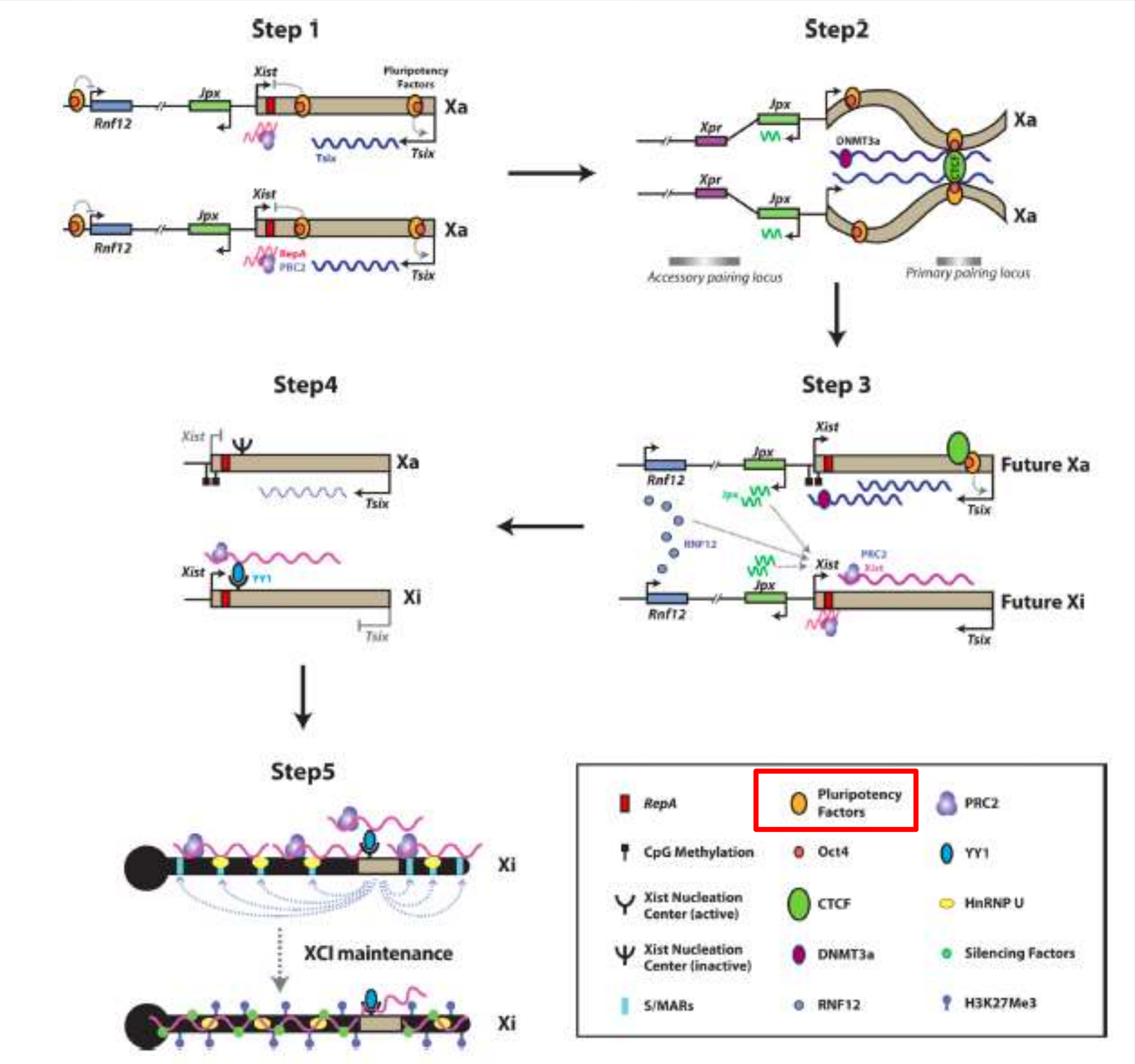
X-inactivation: order of events?

Initiation
-sensing
-counting
-choosing

Silencing
-upregulation XIST
-spreading

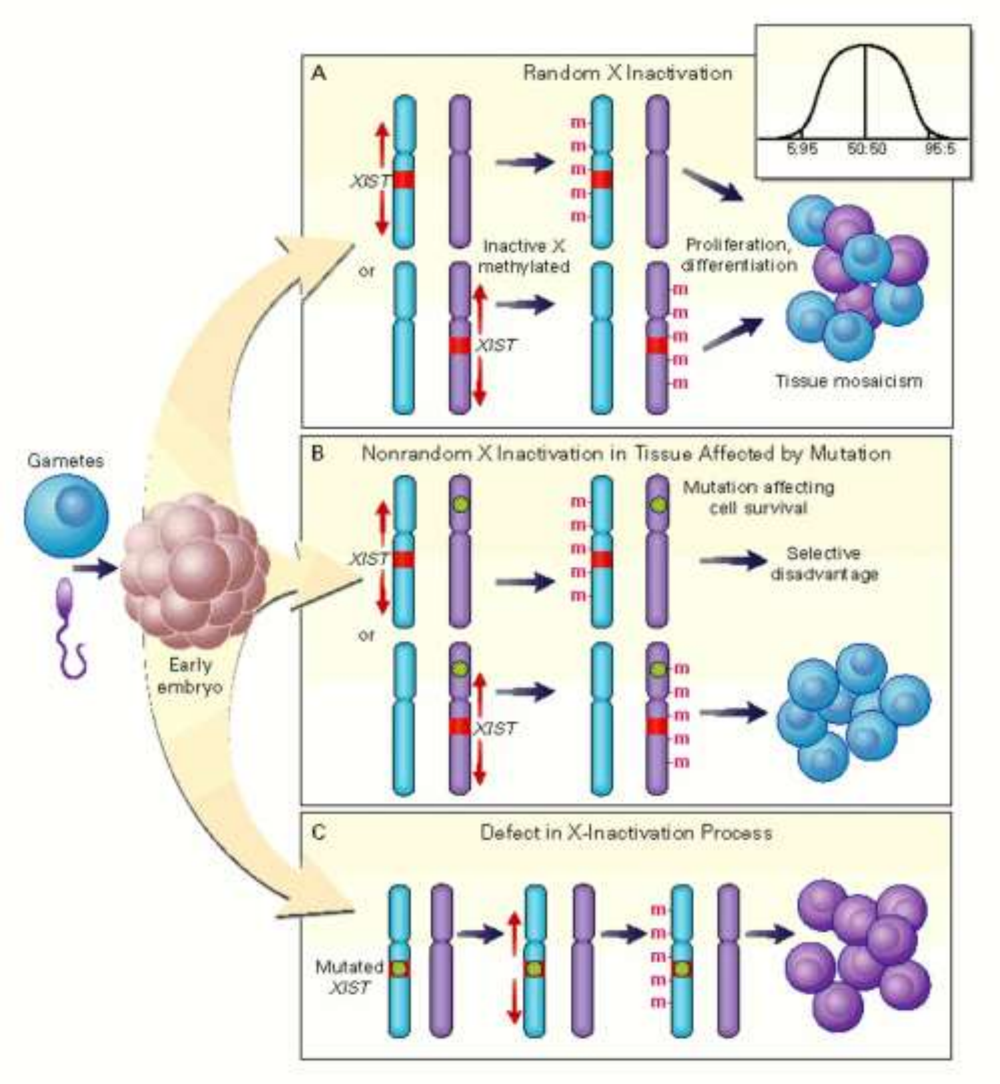
Maintenance

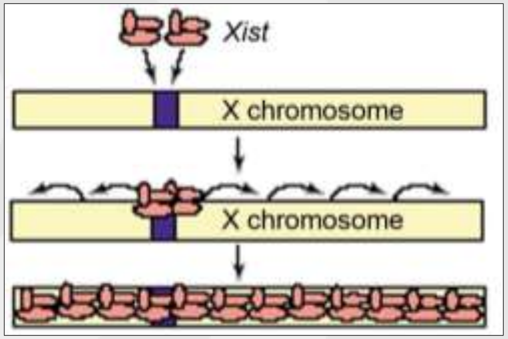
XIST-dependent:
-Random
-Imprinted



Jeon et al, COGD, 2012

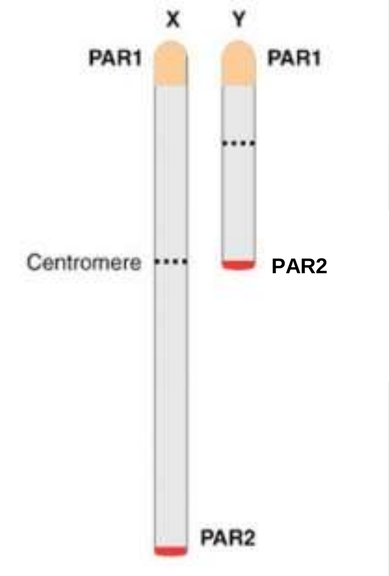
Dosage compensation to equalize X-linked expression



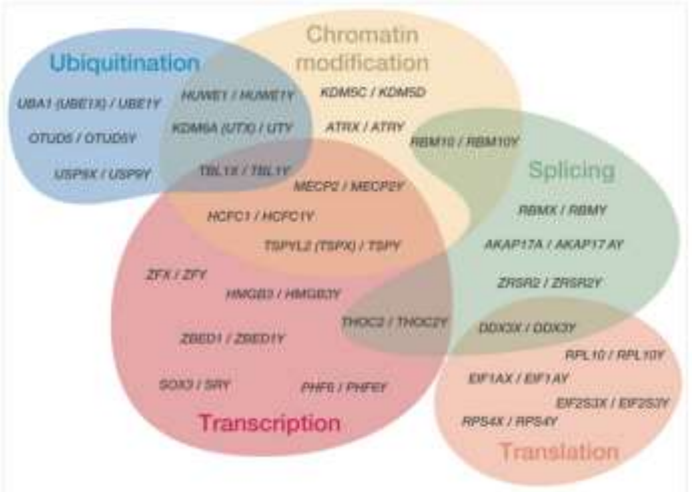


Dosage compensation: XCI-escaping genes (3% vs 15%)

Pseudoautosomal region



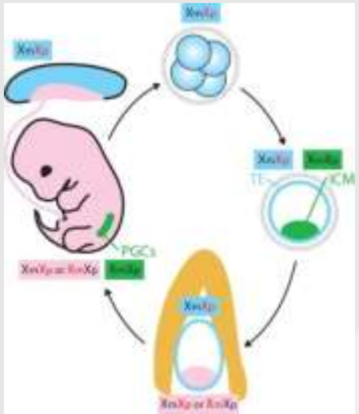
X_Y gene pairs



Venn diagram depicting regulatory functions predicted for selected X–Y pair genes on basis of UniProt annotations of human X-homologue. Common alternatives to official gene symbols in parentheses.

Beliott et al, Nature, 2014

Parental genomes are not equally transcribed



Xm Y
Xm Xp

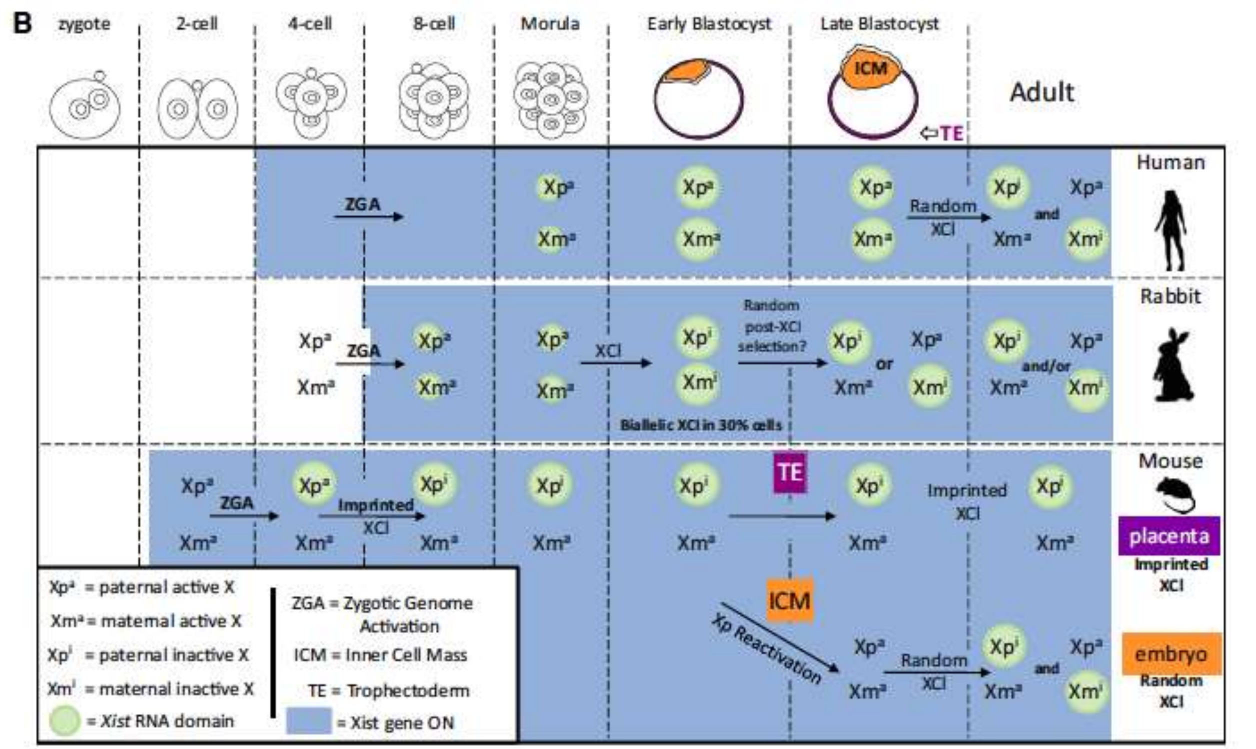
XmXm – not viable
XpXp – not viable (hydatidiform moles in human)
XpY - not viable (hydatidiform moles in human)

Xm0 – viable - Turner syndrome
Xp0 – not viable

XmXmY- Xm refractory to XCI in placenta - Klinefelter syndrome
XmXmXp- Xm refractory to XCI in placenta - Triple X syndrome

XmXpXp - not viable (hydatidiform moles in human)
XmXpY - not viable (hydatidiform moles in human)

X chr in different mammals



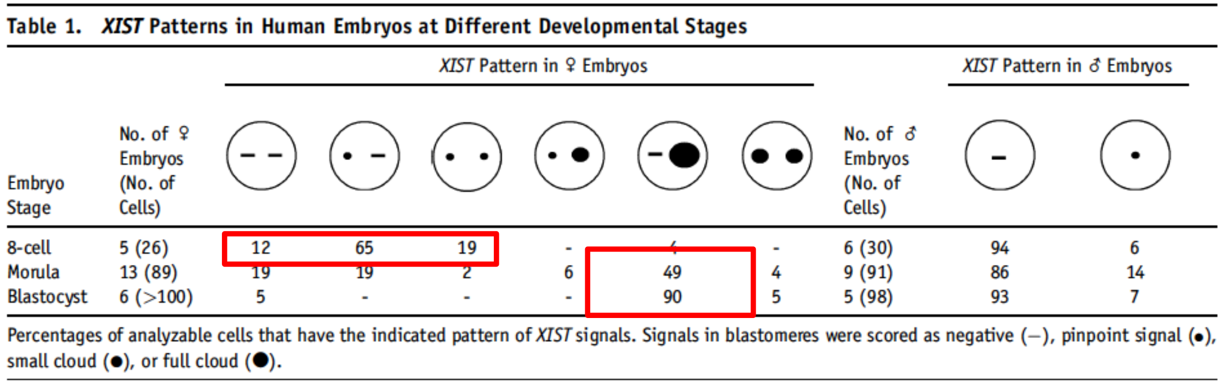
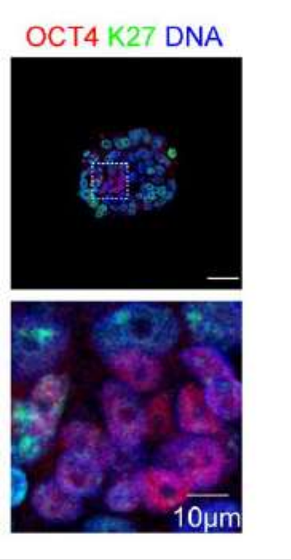


Table 1. ***XIST*** **Patterns in Human Embryos at Different Developmental Stages**

Embryo Stage	No. of ♀ Embryos (No. of Cells)	*XIST* Pattern in ♀ Embryos						No. of ♂ Embryos (No. of Cells)	*XIST* Pattern in ♂ Embryos	
		– –	• –	• •	• ●	– ⬤	● ●		–	•
8-cell	5 (26)	12	65	19	-	4	-	6 (30)	94	6
Morula	13 (89)	19	19	2	6	49	4	9 (91)	86	14
Blastocyst	6 (>100)	5	-	-	-	90	5	5 (98)	93	7

Percentages of analyzable cells that have the indicated pattern of *XIST* signals. Signals in blastomeres were scored as negative (–), pinpoint signal (•), small cloud (●), or full cloud (⬤).

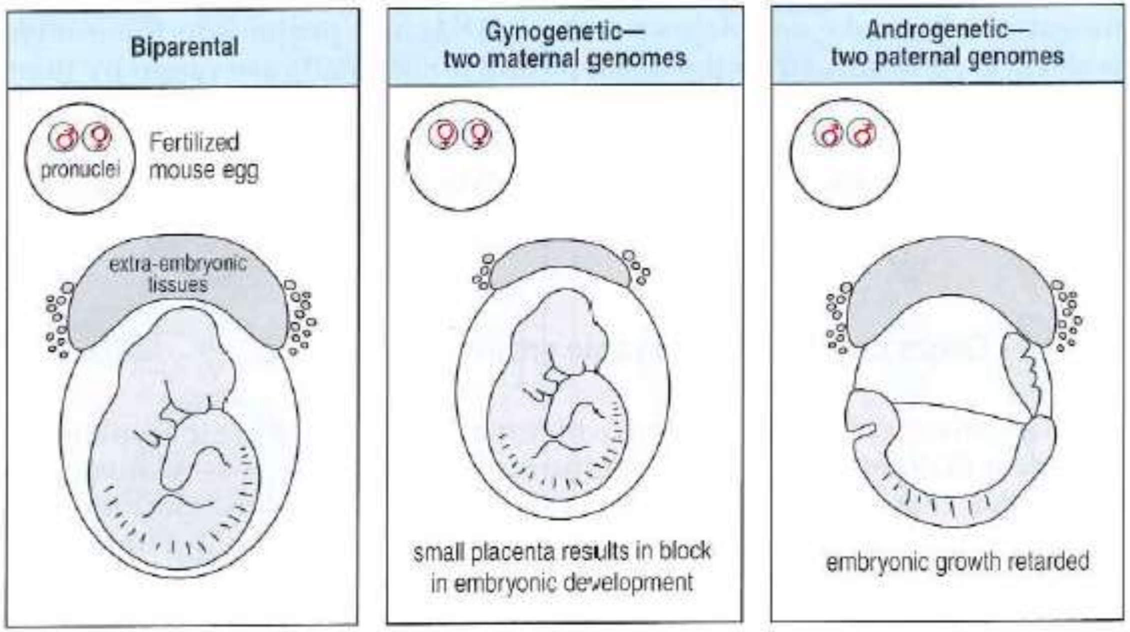
Escamila-del-arenal et al, HG, 2011
Van den Berg et al., AJHG, 2009
Teklenburg et al., Plos One, 2012



Epigenetics:
Genomic imprinting

Parental genome is not equal: **genomic imprinting**

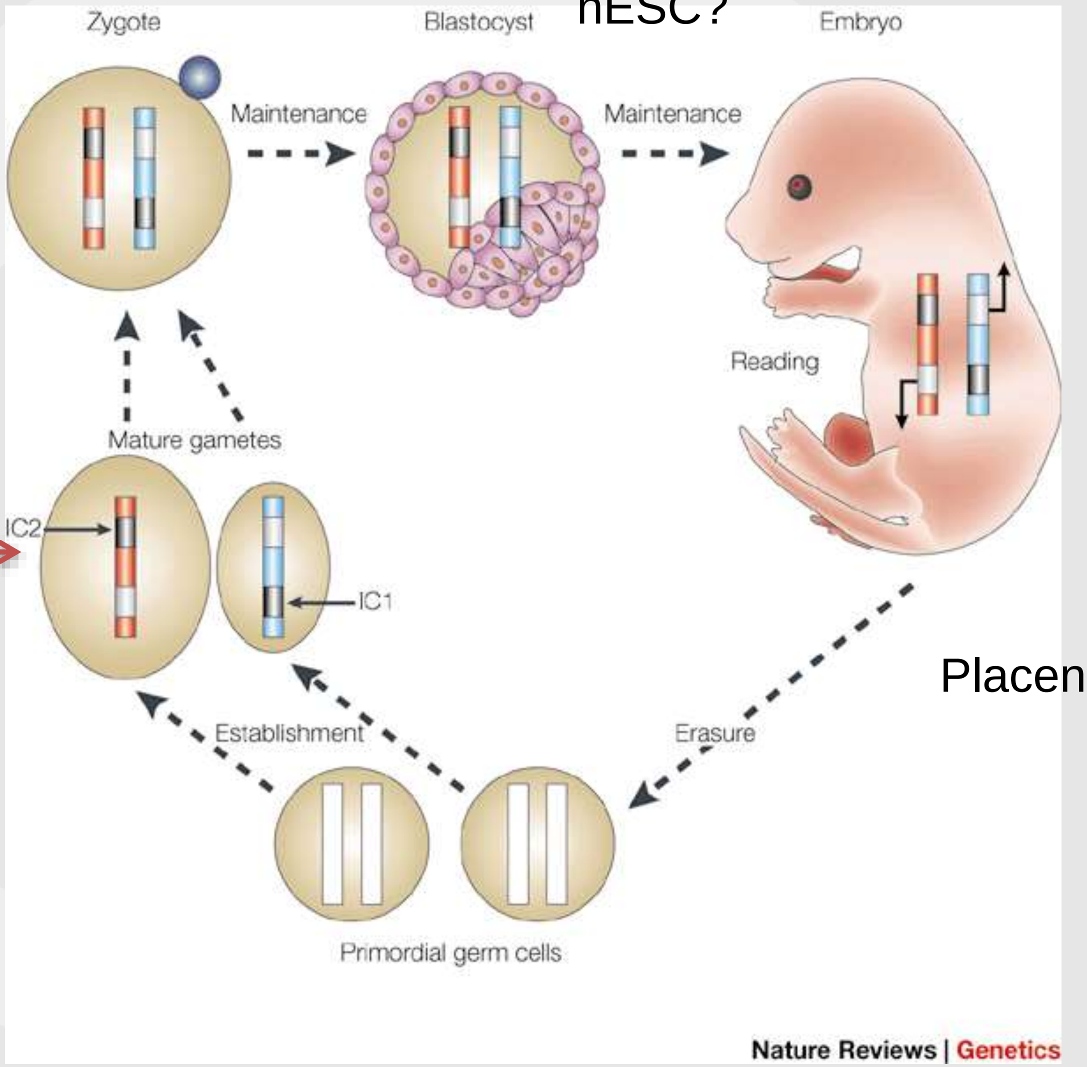
Azim Surani



"Battle of the sexes":
Maternal imprints: smaller placenta
Paternal imprints: smaller embryo

Genomic imprinting: distinguishes female and male gametes

Different manner of establishing imprinting in each sex

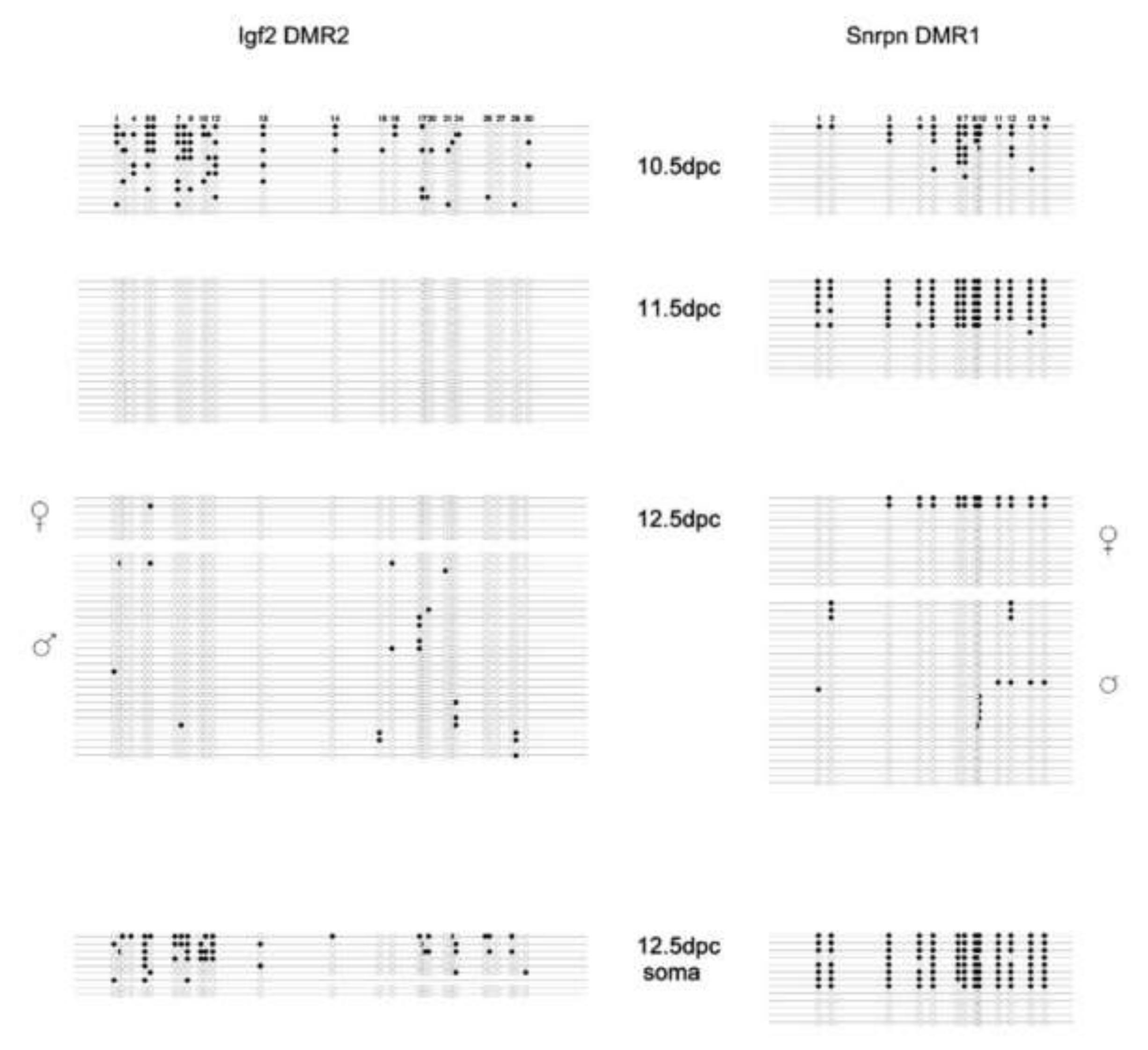


Nature Reviews | Genetics

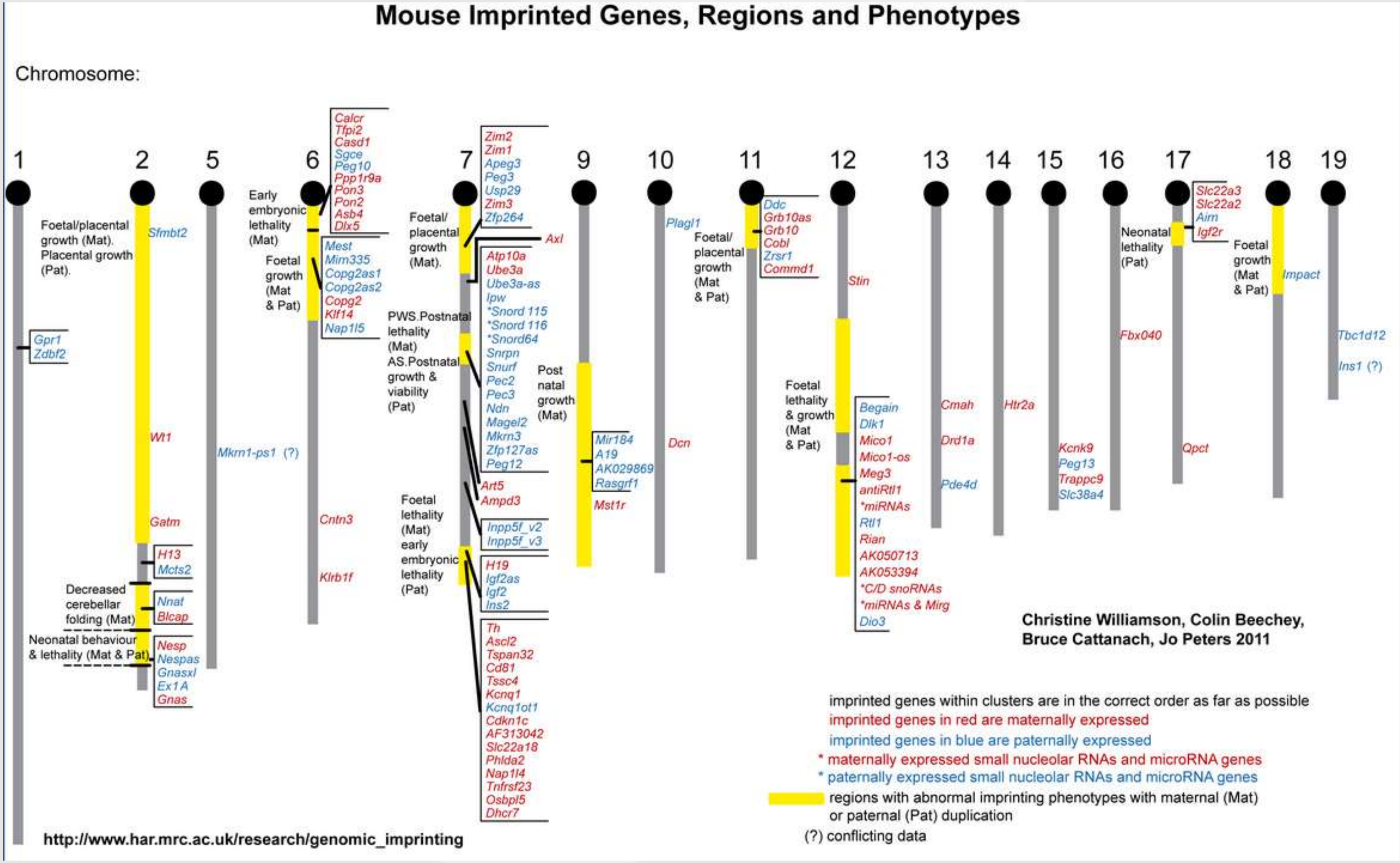
mESC?
hESC?

Placenta?

Examples of erasure of imprinted genes in PGCs



Imprinted genes widely distributed to the genome



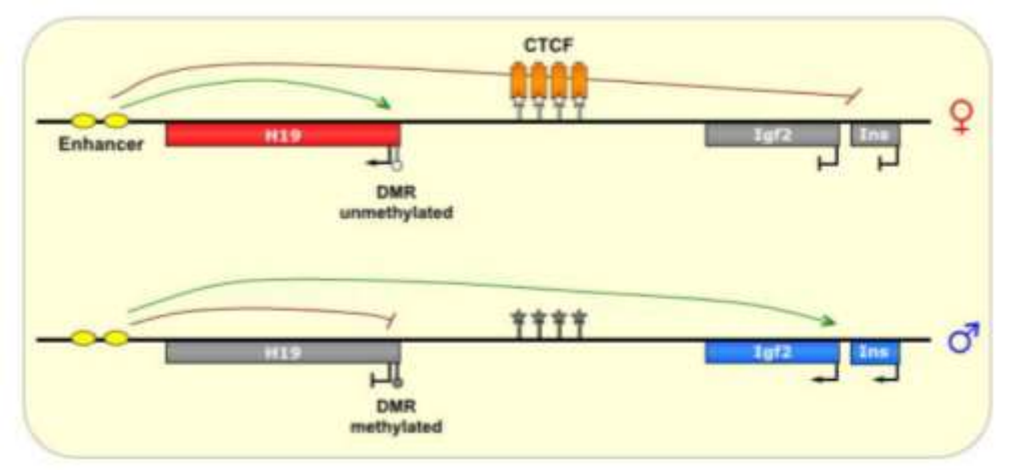
Mouse Imprinted Genes, Regions and Phenotypes

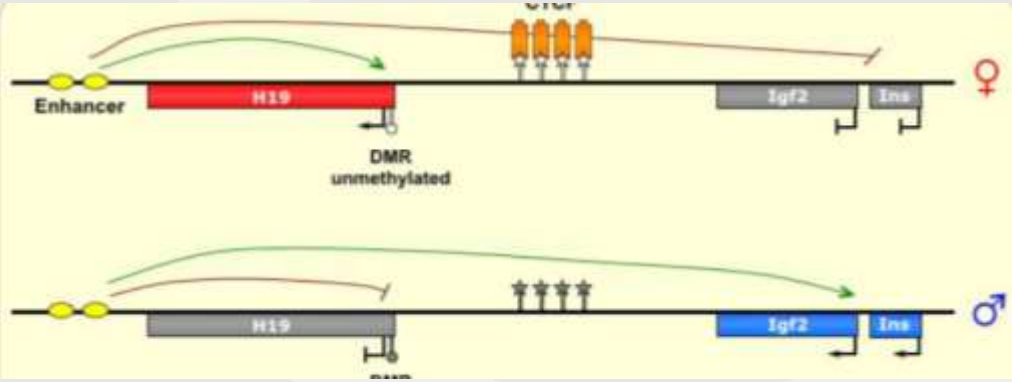
Christine Williamson, Colin Beechey, Bruce Cattanach, Jo Peters 2011

imprinted genes within clusters are in the correct order as far as possible
imprinted genes in red are maternally expressed
imprinted genes in blue are paternally expressed
* maternally expressed small nucleolar RNAs and microRNA genes
* paternally expressed small nucleolar RNAs and microRNA genes
🟨 regions with abnormal imprinting phenotypes with maternal (Mat) or paternal (Pat) duplication
(?) conflicting data

http://www.har.mrc.ac.uk/research/genomic_imprinting

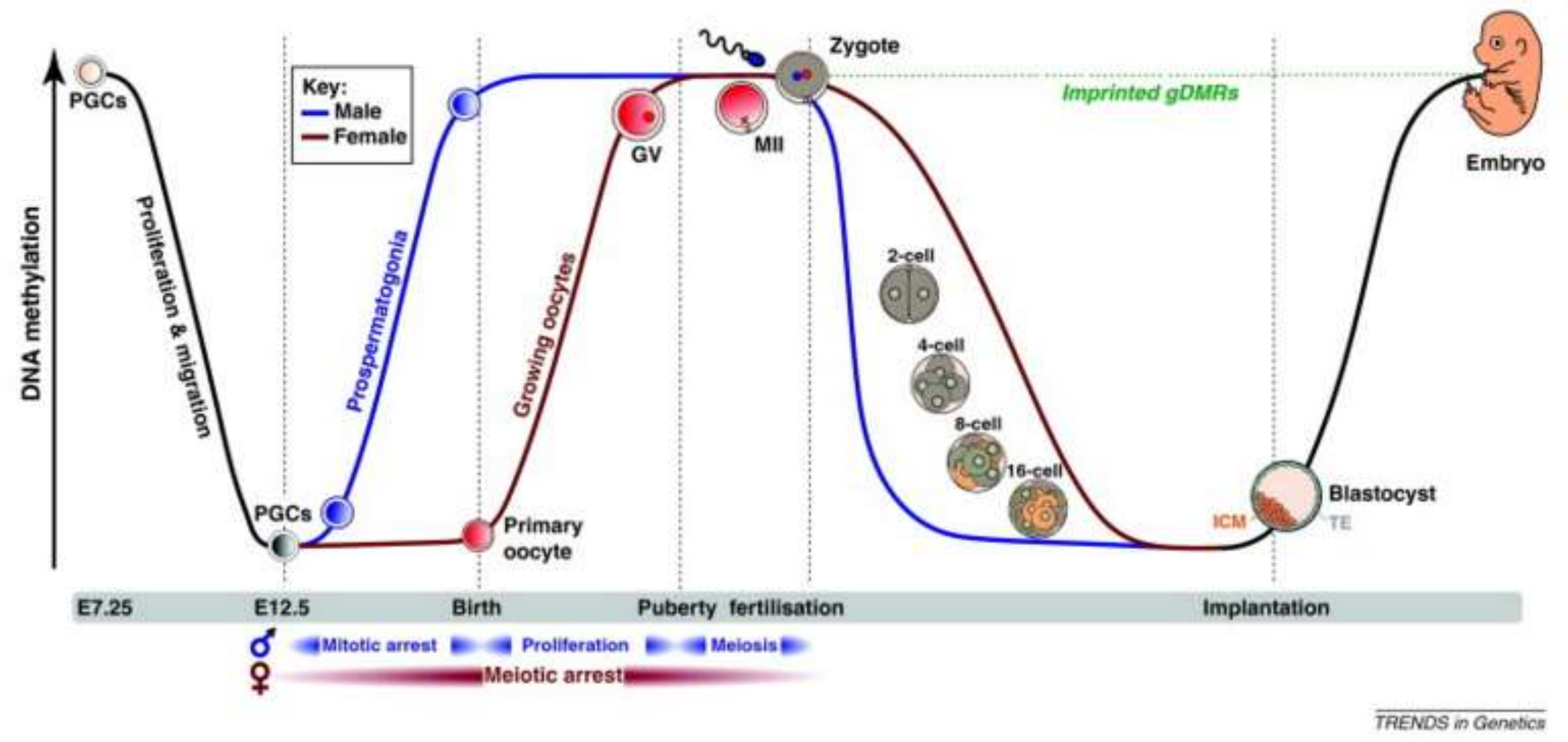
Imprinted control region: paternally and maternally expressed genes



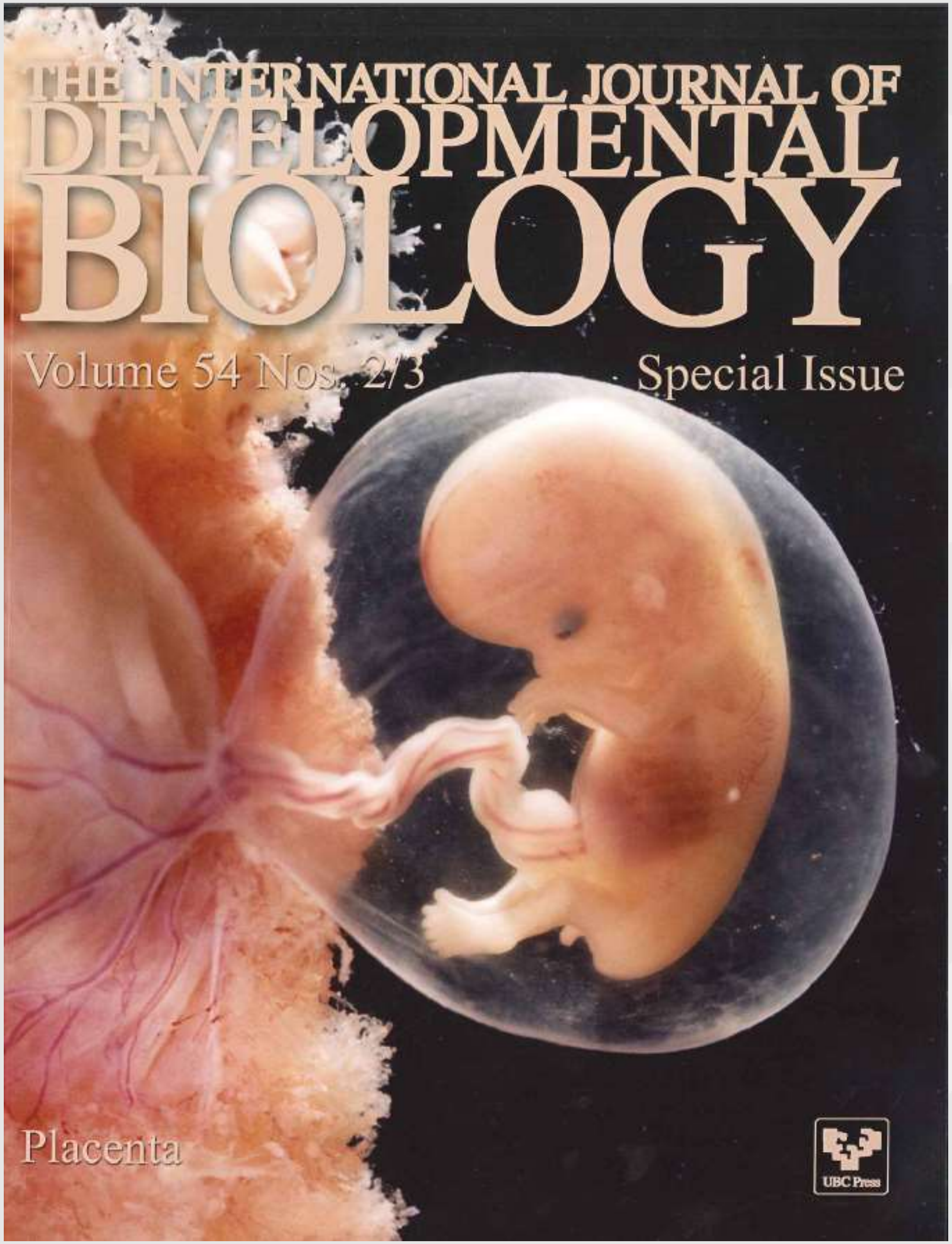


Imprint erasure?

Global levels of DNA methylation



Smallwood and Kelsey, TIGS, 2012



THE INTERNATIONAL JOURNAL OF
DEVELOPMENTAL
BIOLOGY

Volume 54 Nos. 2/3

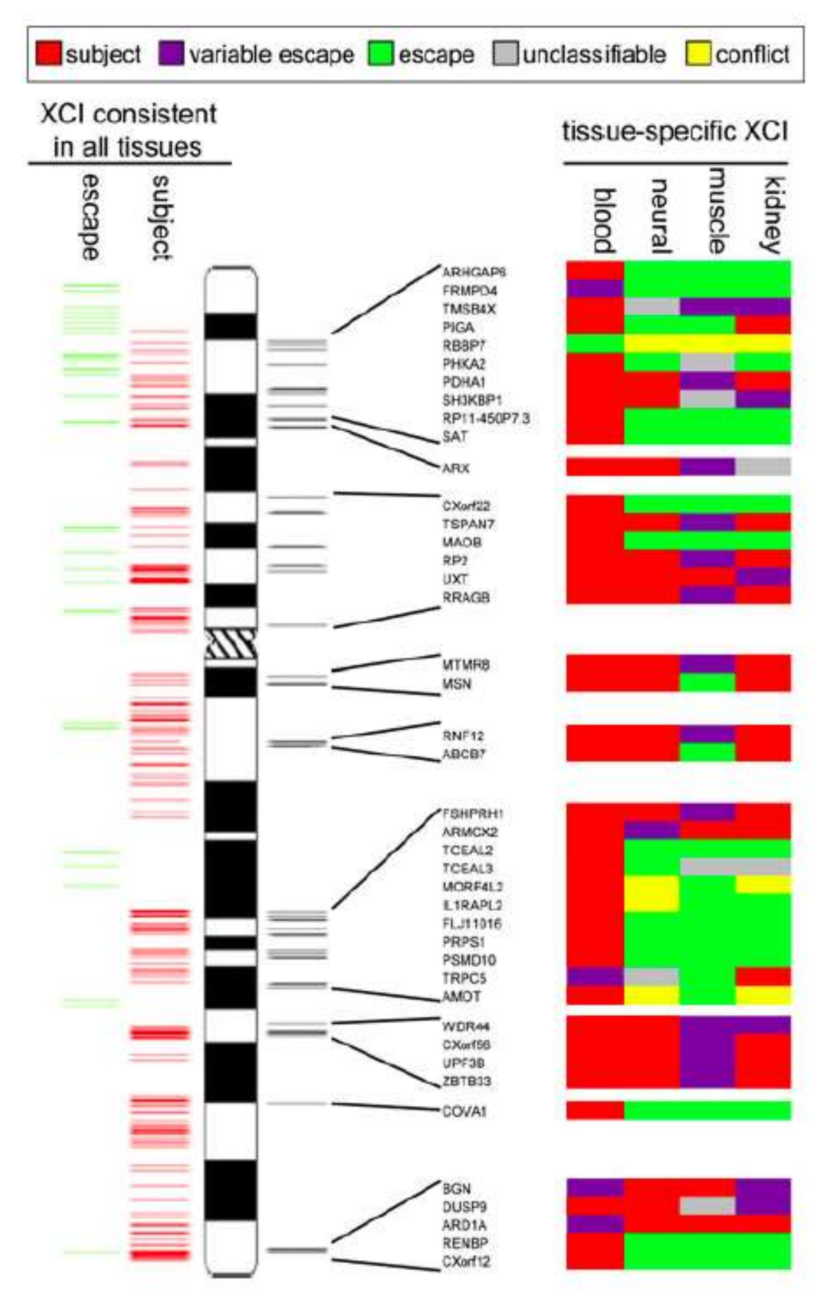
Special Issue

Placenta

UBC Press

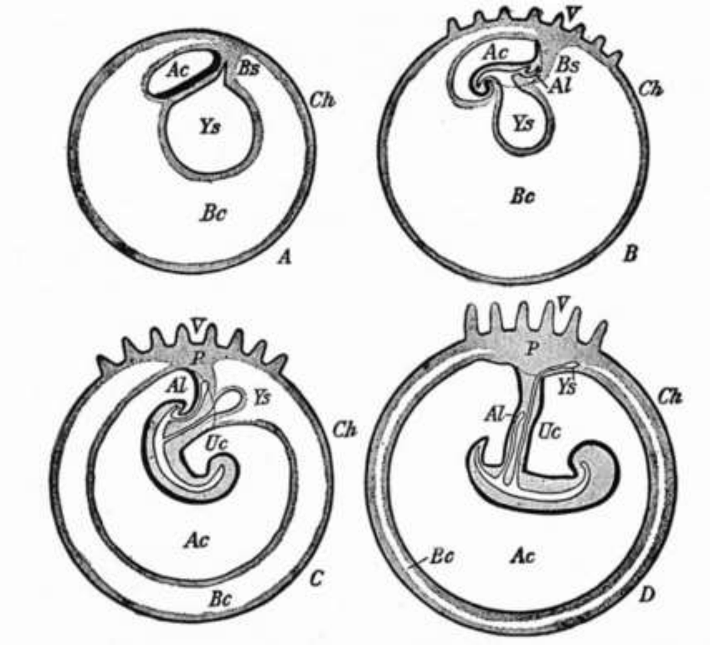
- Somatic
- Germ line
- Extraembr tissues (placenta, amnion, etc)

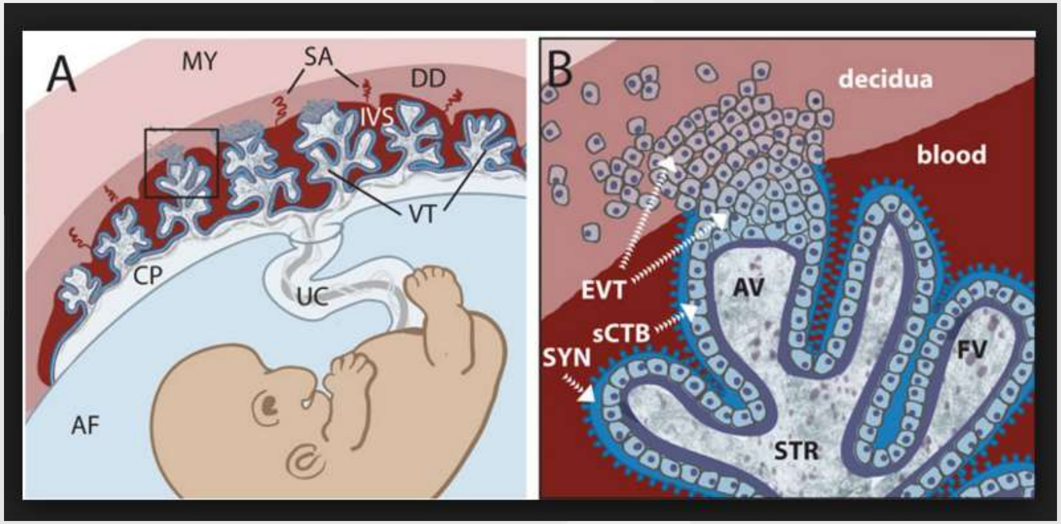
Somatic cells?

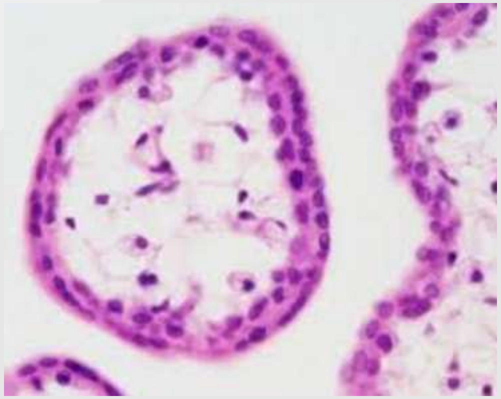


Cotton, HG, 2011

Placenta?

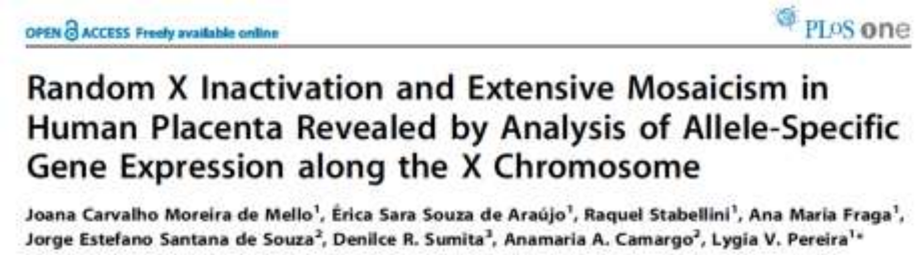






Turner syndrome:
XpO
XmO = 90% births

Placenta?



OPEN ACCESS Freely available online

PLoS one

Random X Inactivation and Extensive Mosaicism in Human Placenta Revealed by Analysis of Allele-Specific Gene Expression along the X Chromosome

Joana Carvalho Moreira de Mello[1], Érica Sara Souza de Araújo[1], Raquel Stabellini[1], Ana Maria Fraga[1], Jorge Estefano Santana de Souza[2], Denilce R. Sumita[3], Anamaria A. Camargo[2], Lygia V. Pereira[1*]

Plos One 5:e10947, 2010

Human Reproduction, Vol.27, No.6 pp. 1745–1753, 2012

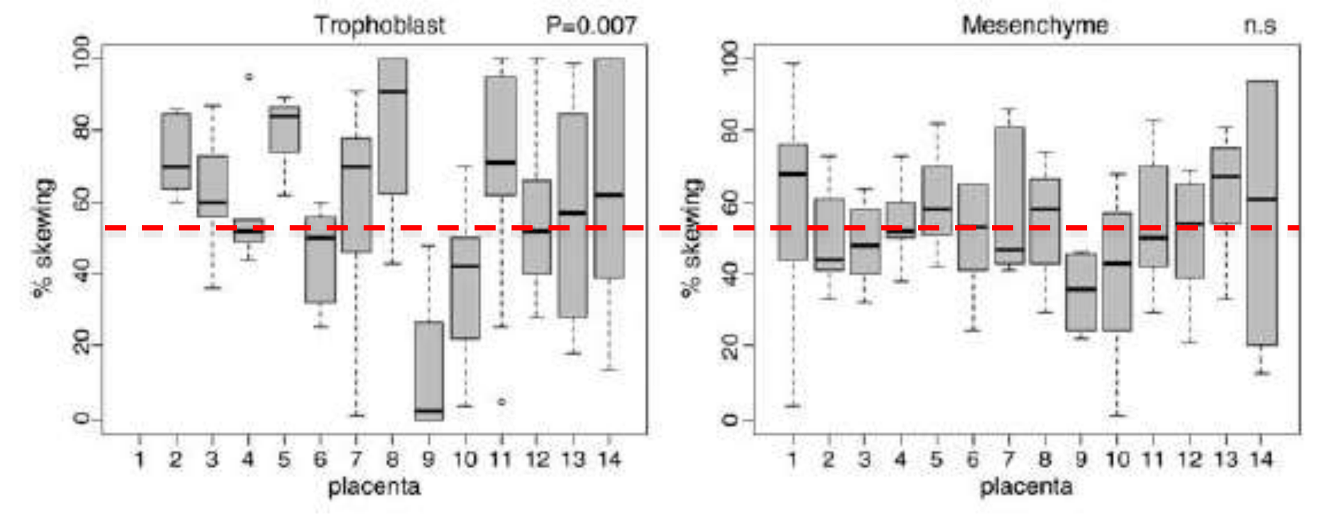
Advanced Access publication on March 19, 2012 doi:10.1093/humrep/des072

human reproduction

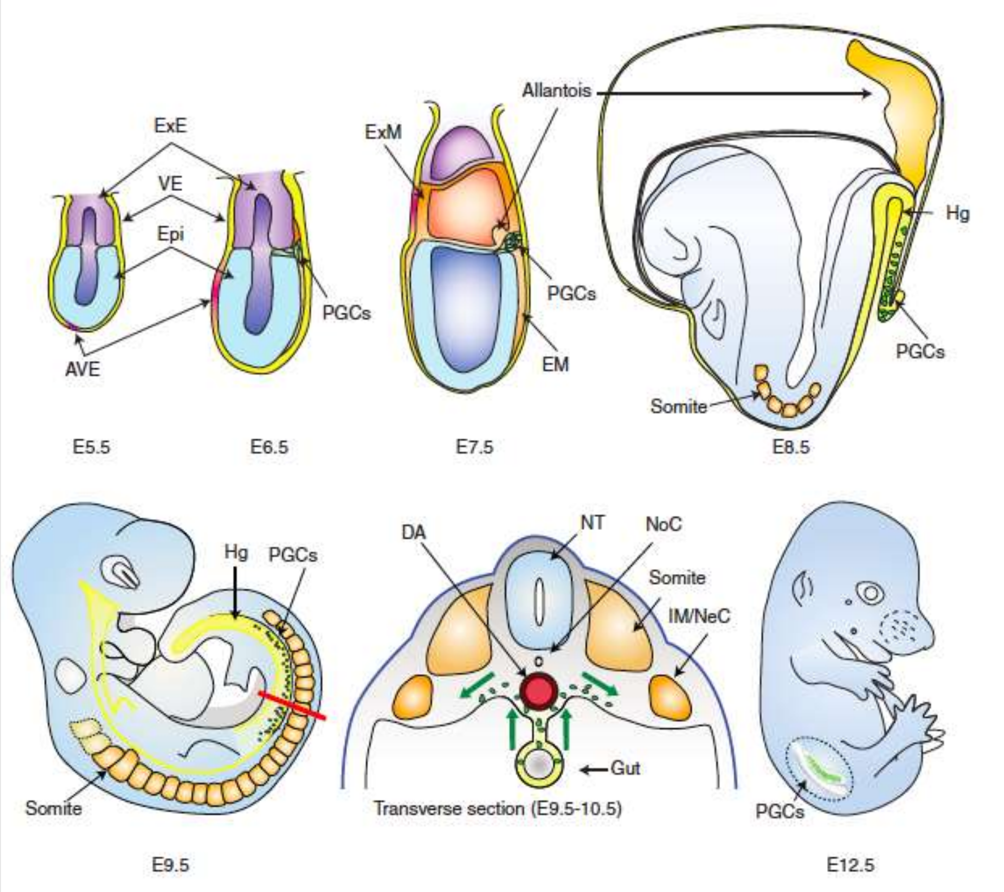
ORIGINAL ARTICLE *Reproductive biology*

Patterns of placental development evaluated by X chromosome inactivation profiling provide a basis to evaluate the origin of epigenetic variation

M.S. Peñaherrera[1,2], R. Jiang[1,2], L. Avila[1,2], R.K.C. Yuen[1,2], C.J. Brown[1], and W.P. Robinson[1,2,*]



Germ cells?



E8.5-E12.5

- X chromosome reactivation
- DNA demethylation
- Imprint erasure
- Histone modifications
- Histone variants (gH2A, H3.3)

XX to male: X reactivation in spermatogenesis is lethal
XY to female: may work more easily (cases of oocytes)

Saitou&Yamaji, CSHP, 2012

Genomic imprinting – same sex couples offspring

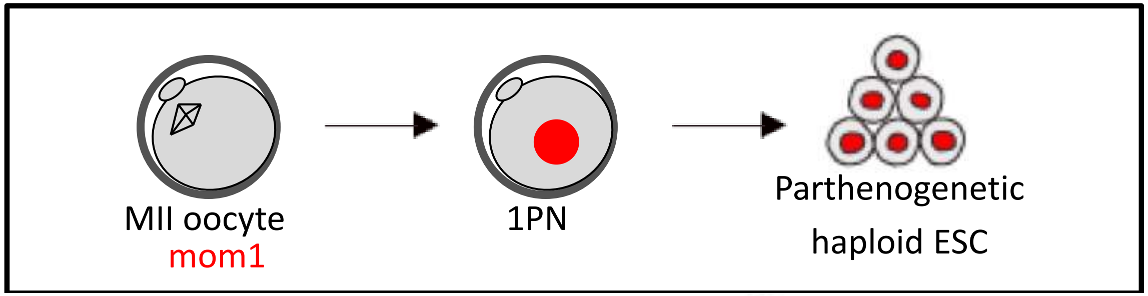
Cell Stem Cell

Article

CellPress

Generation of Bimaternal and Bipaternal Mice from Hypomethylated Haploid ESCs with Imprinting Region Deletions

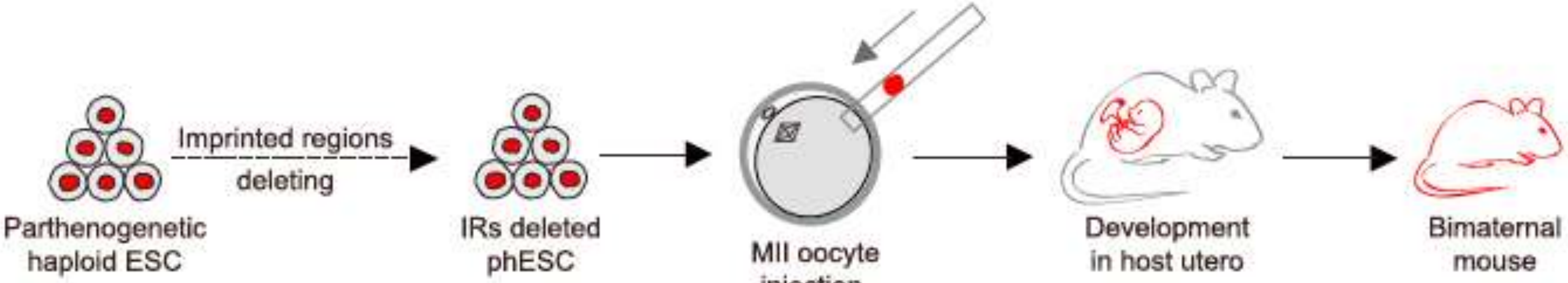
Zhi-Kun Li,[1,2,5] Le-Yun Wang,[1,2,5] Li-Bin Wang,[1,2,3,5] Gui-Hai Feng,[1,2,5] Xue-Wei Yuan,[1,2,4,5] Chao Liu,[1,2,3] Kai Xu,[1,2,3]
Yu-Huan Li,[1,2,3] Hai-Feng Wan,[1,2] Ying Zhang,[1,2] Yu-Fei Li,[1,2,3] Xin Li,[1,2] Wei Li,[1,2,3,*] Qi Zhou,[1,2,3,6,*]
and Bao-Yang Hu[1,2,3,*]



MII oocyte
mom1

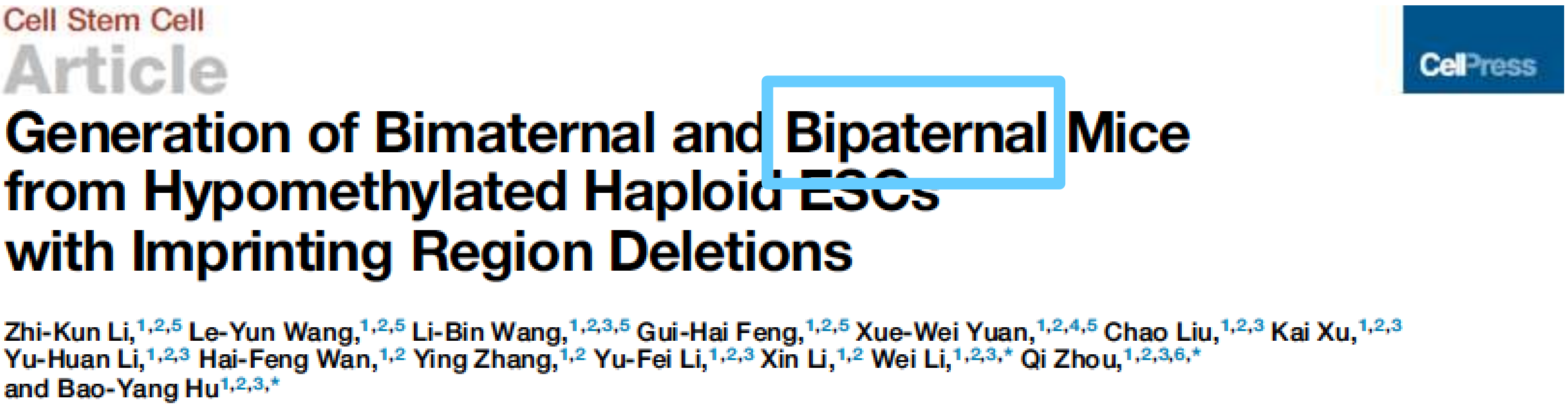
1PN

Parthenogenetic
haploid ESC



mom2

Genomic imprinting – same sex couples offspring

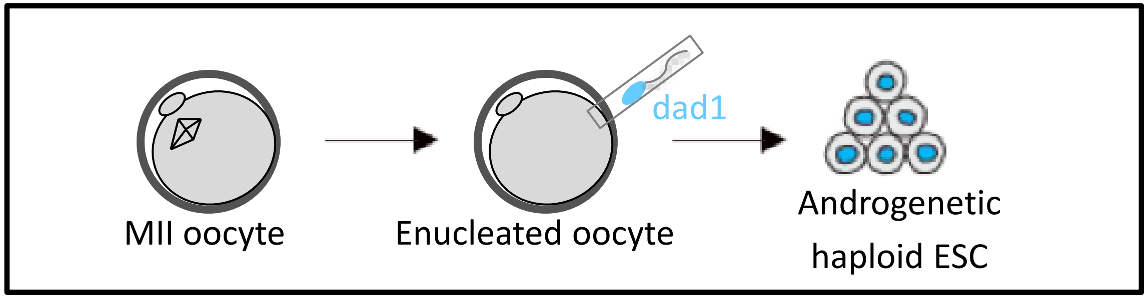


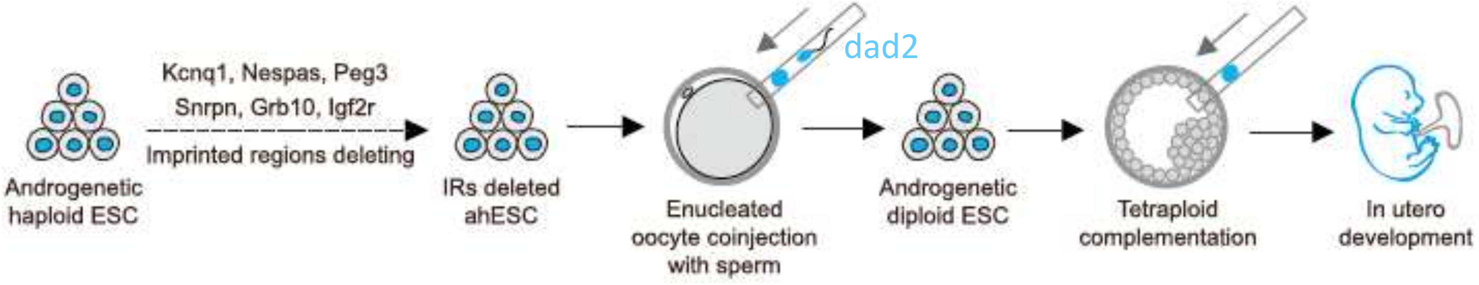
Cell Stem Cell

Article

Generation of Bimaternal and Bipaternal Mice from Hypomethylated Haploid ESCs with Imprinting Region Deletions

Zhi-Kun Li,[1,2,5] Le-Yun Wang,[1,2,5] Li-Bin Wang,[1,2,3,5] Gui-Hai Feng,[1,2,5] Xue-Wei Yuan,[1,2,4,5] Chao Liu,[1,2,3] Kai Xu,[1,2,3] Yu-Huan Li,[1,2,3] Hai-Feng Wan,[1,2] Ying Zhang,[1,2] Yu-Fei Li,[1,2,3] Xin Li,[1,2] Wei Li,[1,2,3,*] Qi Zhou,[1,2,3,6,*] and Bao-Yang Hu[1,2,3,*]





Die E8.5

Learning objectives:

- What is epigenetics?

- What is X inactivation?
- What is imprinting?

- How epigenetics is species-specific/time-specific/tissue-specific