CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2014-2015 :
"Chromatine et Mémoire cellulaire"

23 Février, 2015

Cours IV

"La mémoire chromatinienne au cours du développement et à travers les générations"

Seminaire:
Dr. Deborah BOURC'HIS (Institut Curie, Paris)
"Rôle de la méthylation de l'ADN dans la préservation du paysage chromatinien méiotique"

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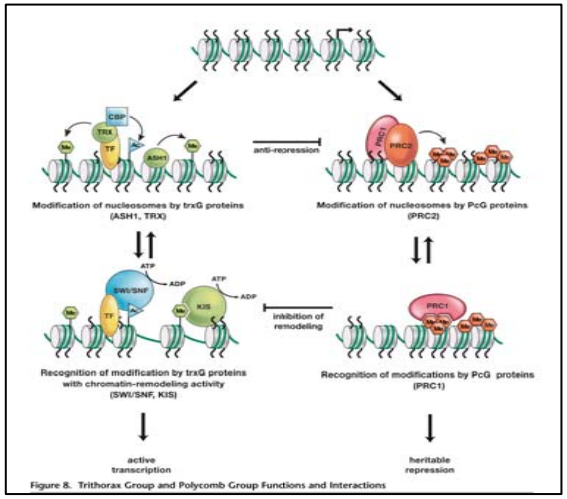
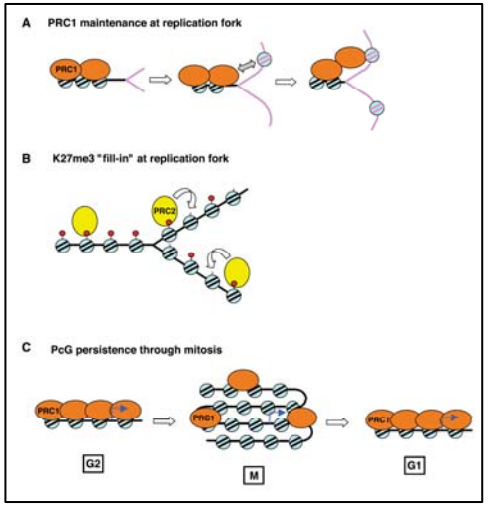
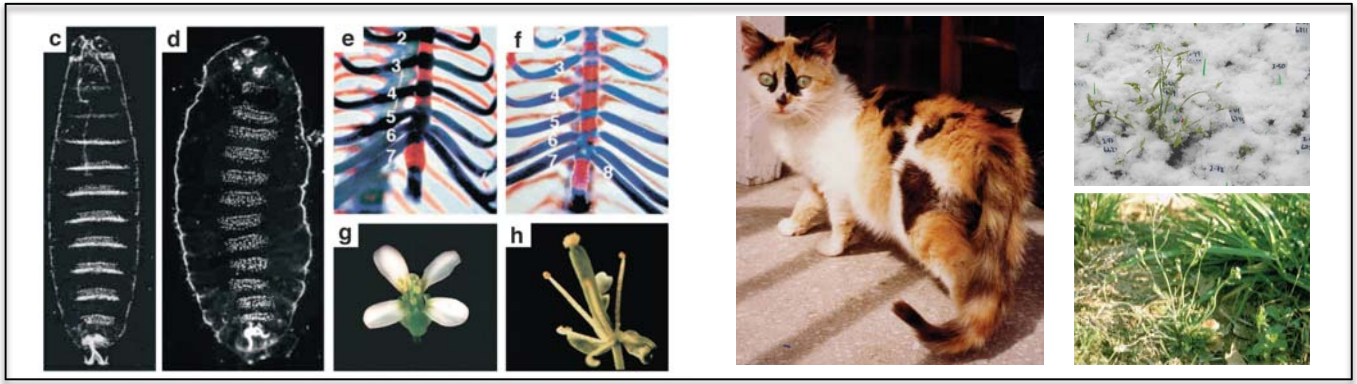
Summary of Last Week:

Chromatin memory: PcG and trxG protein complexes

- Polycomb and Trithorax proteins maintain developmental decisions & ensure transitions
- Roles of PcG proteins in maintenance of developmentally or environmentally programmed expression states (X inactivation, vernalization)
- Mechanisms of PcG and trxG transmission during DNA replication and mitosis
- Aberrant PcG reprogramming can leads to inter-generational transmission…







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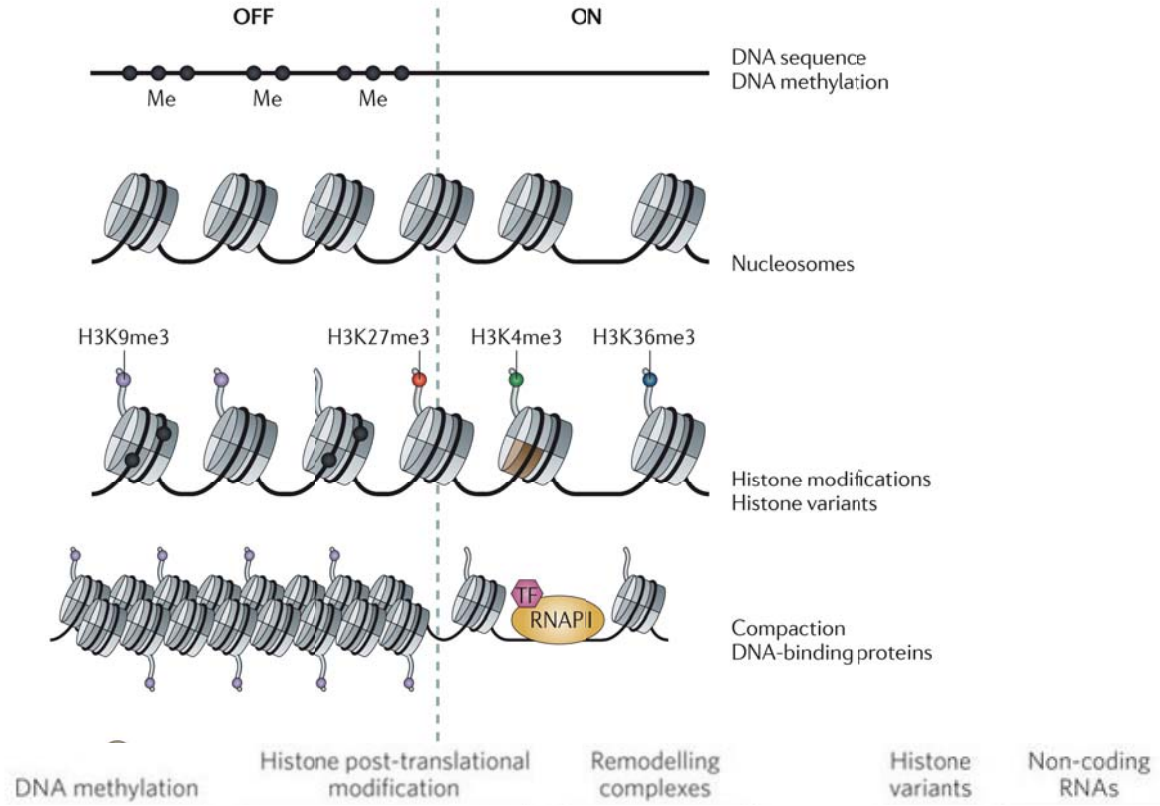
Chromatin: the physiological template of the genome and as a carrier of cellular memory



• Chromatin acts as an epigenetic barrier

• Chromatin memory can be dynamic (plasticity versus stability)

• Chromatin states are established and reprogrammed during development

• Is *all* chromatin memory erased at every generation?

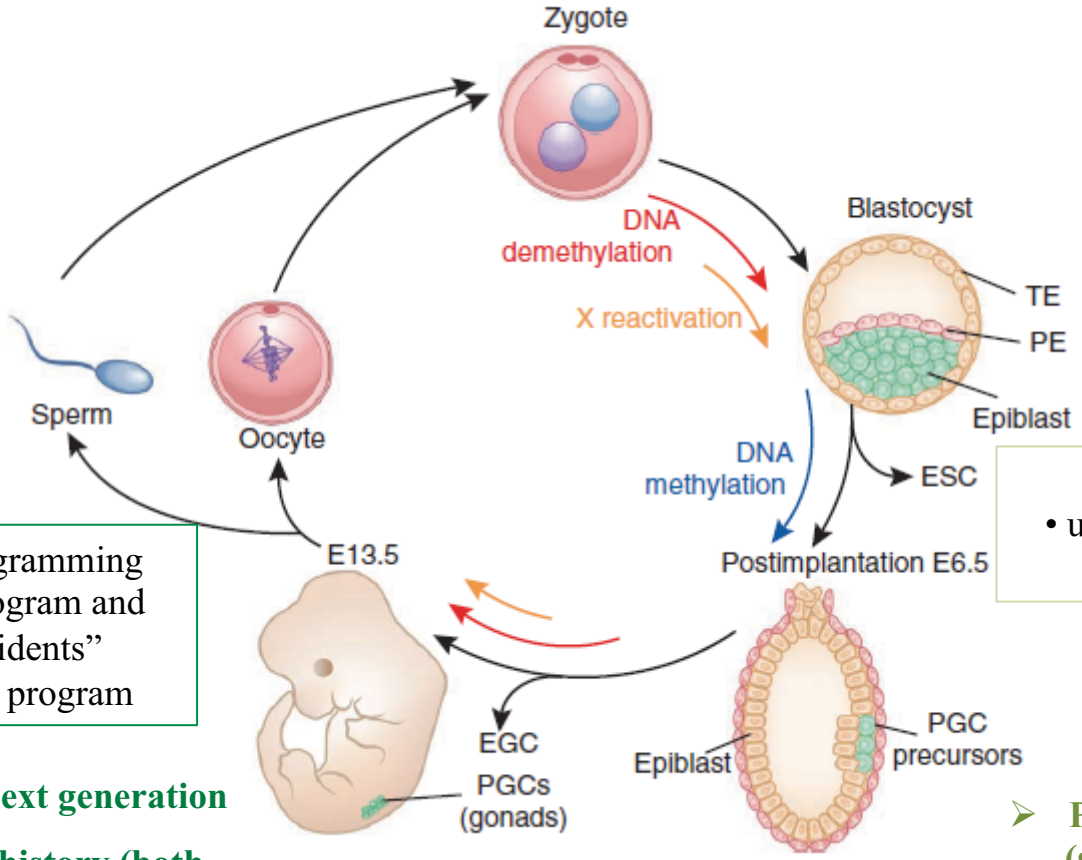
If not, which epigenetic marks can be transmitted across generations?

E

Chromatin inheritance and reprogramming during development

- **Prepare for development (epigenesis)**
- **Preserve some epigenetic marks (parental imprints), erase others**

Zygotic Reprogramming
• undo gamete programs
• set up totipotency



ICM Reprogramming
• undo/prevent TE program
• set up pluripotency

Germ Line Reprogramming
• undo somatic program and epigenetic "accidents"
• set up germ line program

- **Prepare for the next generation**
- **Erase epigenetic history (both programmed and accidental)**

- **Prepare for the epiblast (soma and germ line)**

Adapted from Cantone and Fisher, 2013

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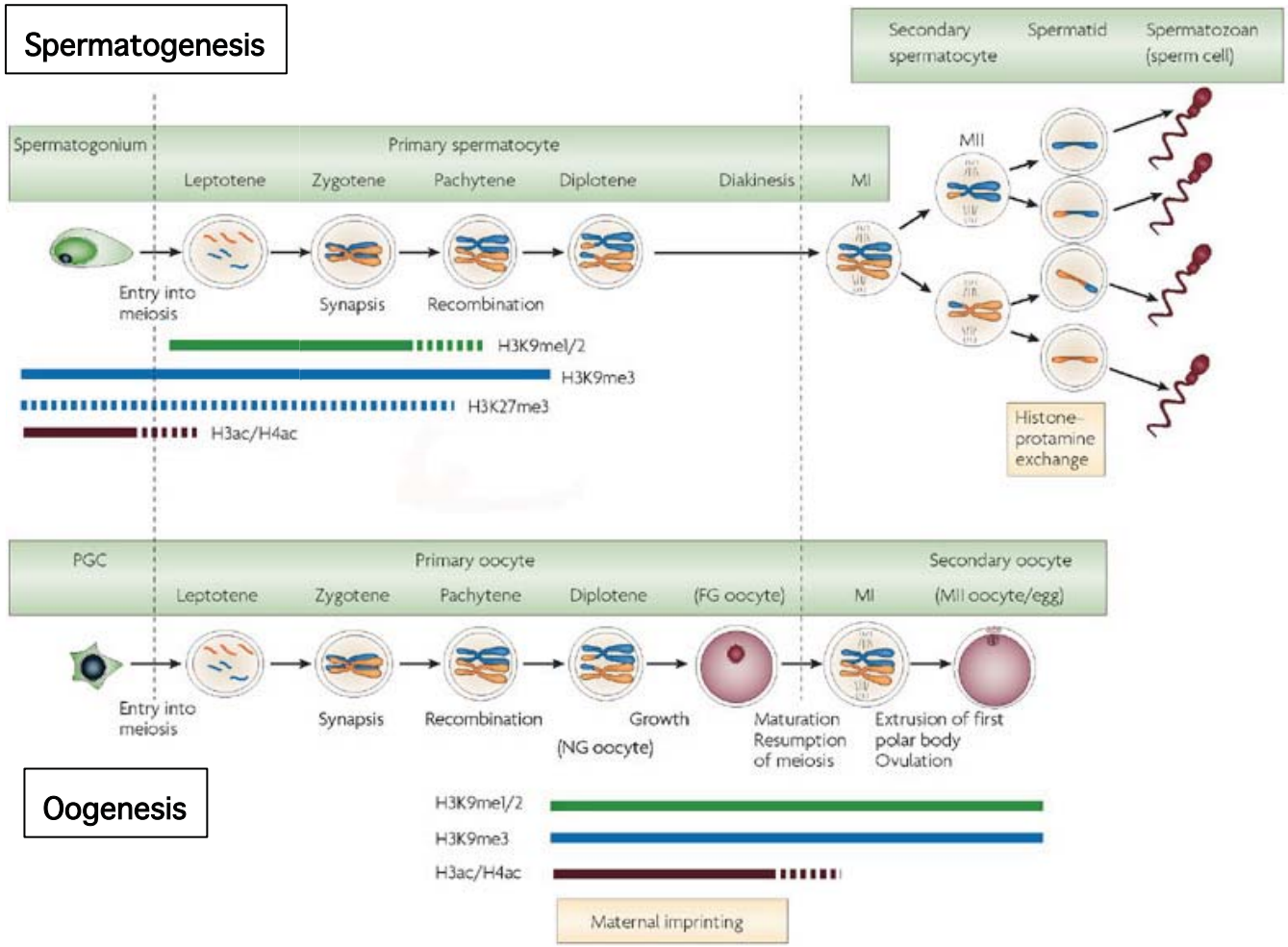
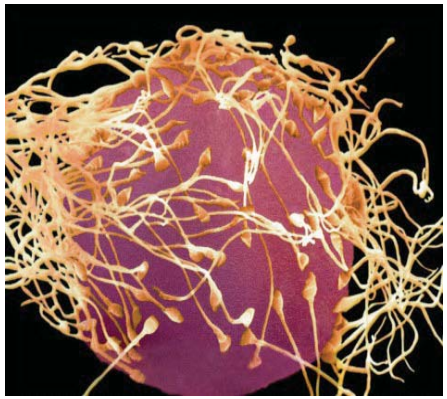
Chromatin inheritance and Zygotic Reprogramming

The germ cells are highly differentiated with very specialised chromatin states that must be reprogrammed in preparation for totipotency





Nature Reviews | Genetics

From Sasaki, H. & Matsui, Y, 2008

Spermatogenesis involves dynamic chromatin changes to package, protect (and mark?) the genome

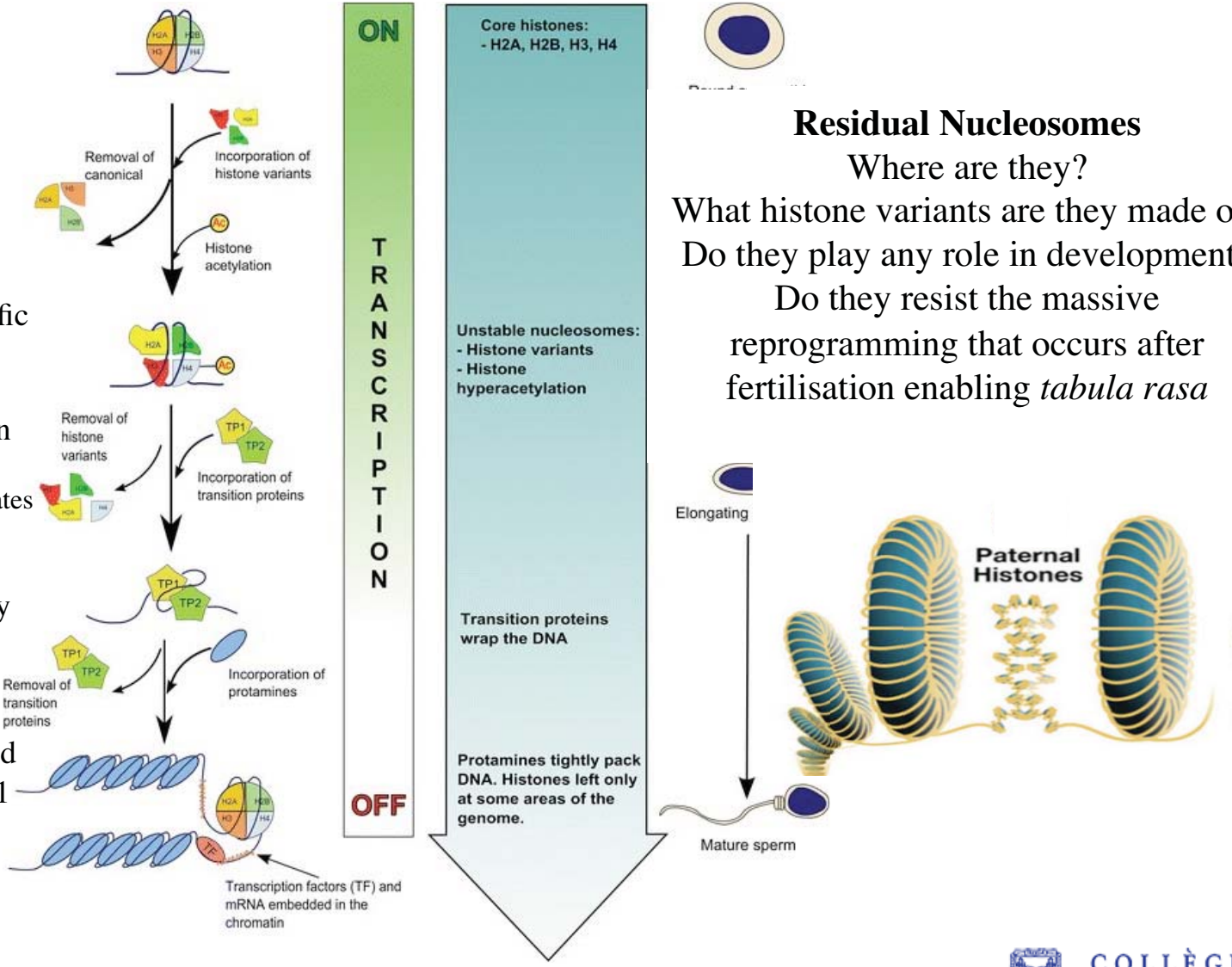
Removal of core histones (with their modifications)

Incorporation of testis-specific histone variants

Wave of histone acetylation (to enable binding of BRDT bromodomain protein that facilitates histone eviction)

Replacement of histones by Transition Proteins (TPs)

Protamine incorporation and removal of TPs – via CAF1 chaperone



Residual Nucleosomes

Where are they?

What histone variants are they made of?

Do they play any role in development?

Do they resist the massive reprogramming that occurs after fertilisation enabling *tabula rasa*

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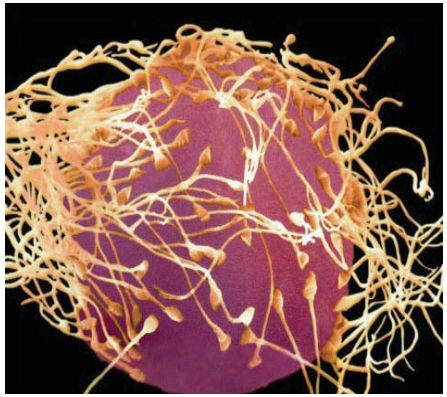
Chromatin inheritance and Zygotic Reprogramming

Spermatogenesis

Highly packaged paternal genome: DNA + protamines (1-15% histones; small amount of RNA; other factors?)

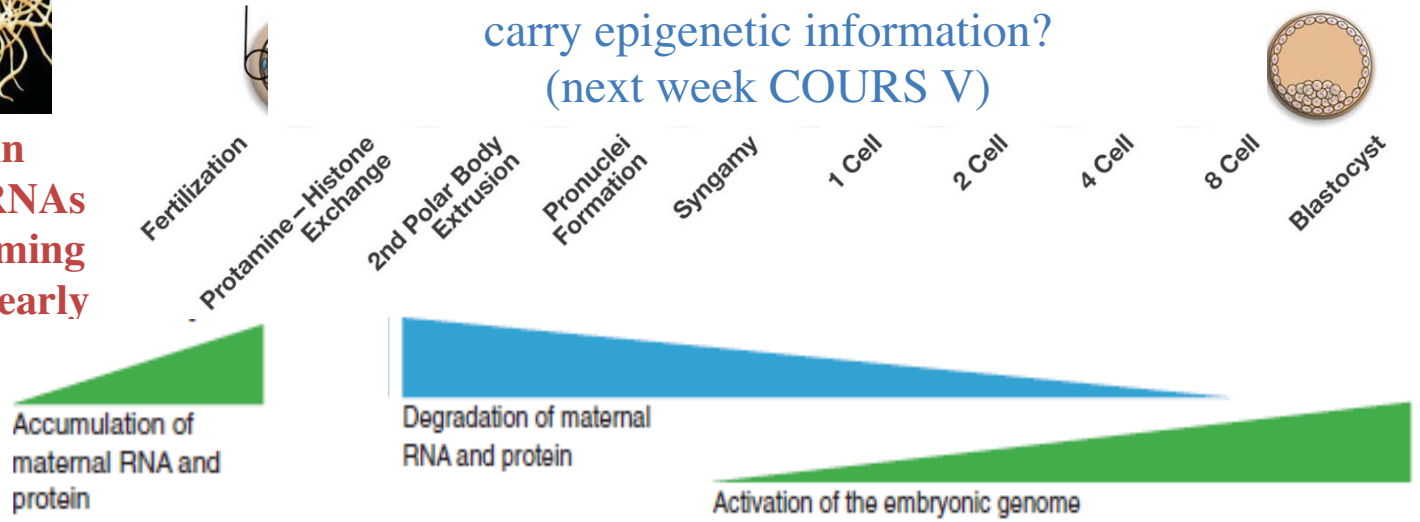


Maternal chromatin maternal proteins, mRNAs - to enable reprogramming upon fertilization and early development

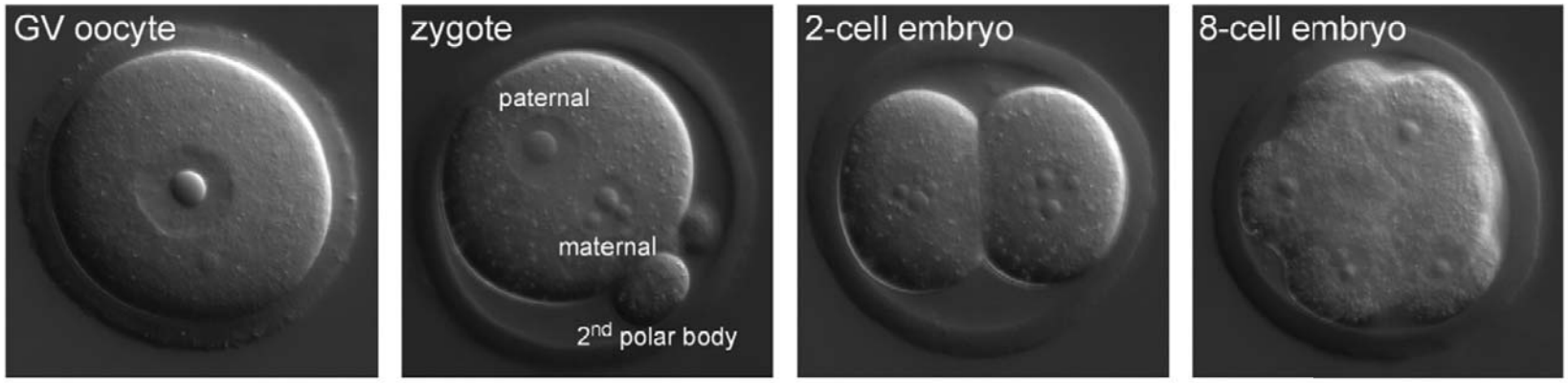
Oogenesis

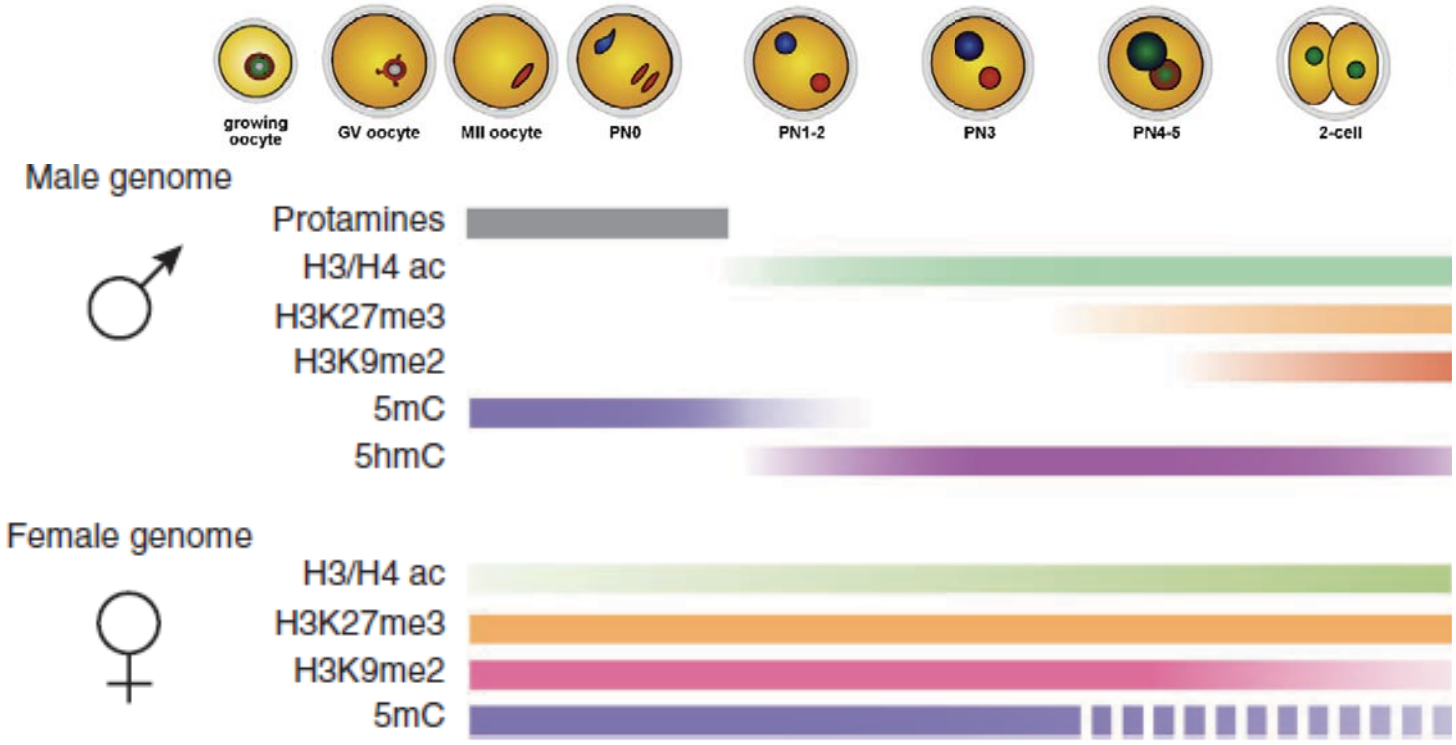
At fertilization the sperm genome is largely "repackaged" with maternal histones

Remarkably, the few **paternal** nucleosomes may resist this dramatic remodeling and potentially carry epigenetic information? (next week COURS V)



Initial parental chromatin asymmetry followed by dynamic changes in early pre-implantation development





Adapted from Cantone and Fisher, 2013

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Fertilization triggers massive reorganization of the paternal and maternal chromatin

Maternal histones
H3.1, H3.3
TH2A, TH2B

Maternal Pool:
Proteins + mRNA
Dnmt1, Polycomb (Eed, Ezh2, YY1, Ring1, Rnf2), Trithorax (Mll), HP1, Histones…

1-15% Paternal histones persist in 1-cell embryo?

van der Heijden et al. (2006)
van der Heijden et al. (2008)

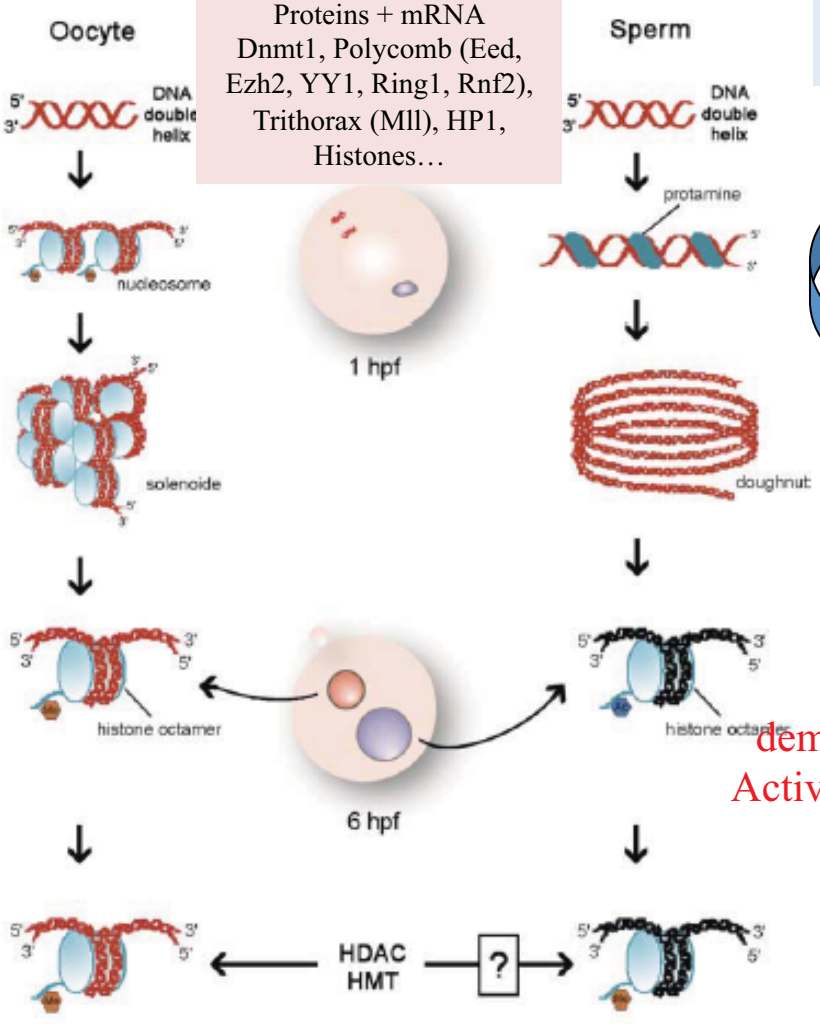
HIRA
protamine - histone exchange
van der Heidjden et al, 2005

Maternal H3.3 incorporation

Maternal chromatin inheritance:
Some chromatin marks are laid down during oogenesis and are essential for early heterochromatin establishment

H4Ac
H3K4me3
H3K9Ac
H3K9me1
H3K9me2
H3K9me3
H3K4me1
H3K27me1
H4K20me1

DNA Demethylation (passive)



DNA demethylation Active & passive

H4Ac (++)
H3K9Ac
H3K9me1
H3K4me1
H3K27me1
H4K20me1

H3K4me3

from Santos & Dean, *Reproduction*, 2004

E. Heard, February 23rd, 2015

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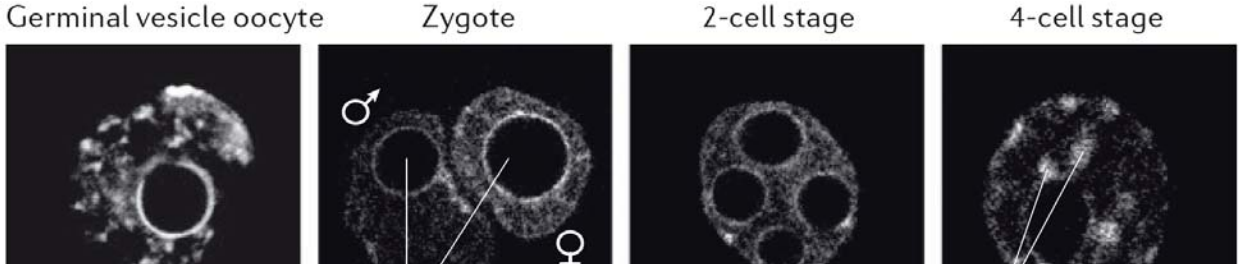
Setting up new chromatin states?

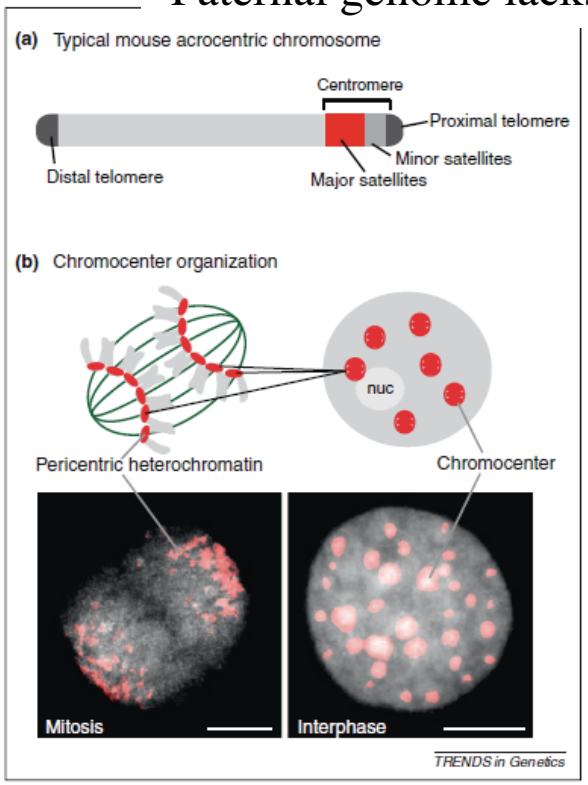
Constitutive Heterochromatin

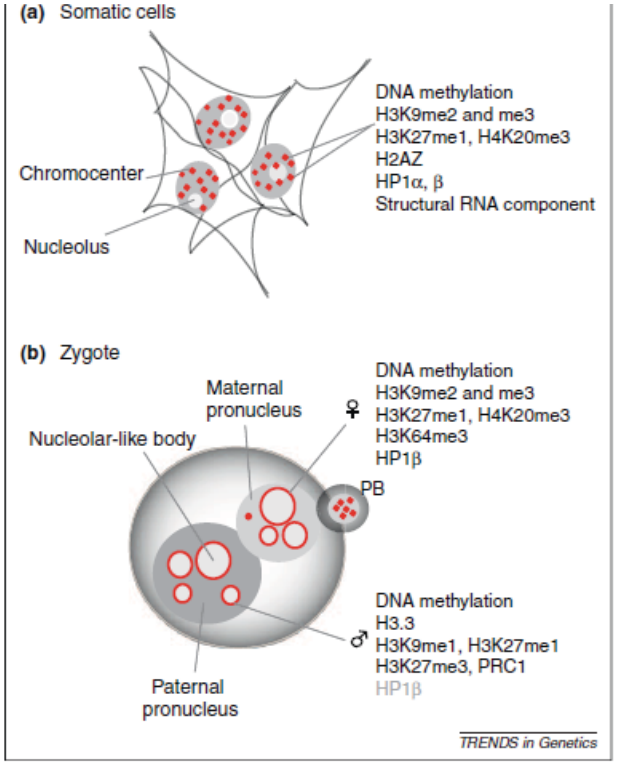
Importance of the H3.3 Histone variant and expression of repeat (satellite) sequences

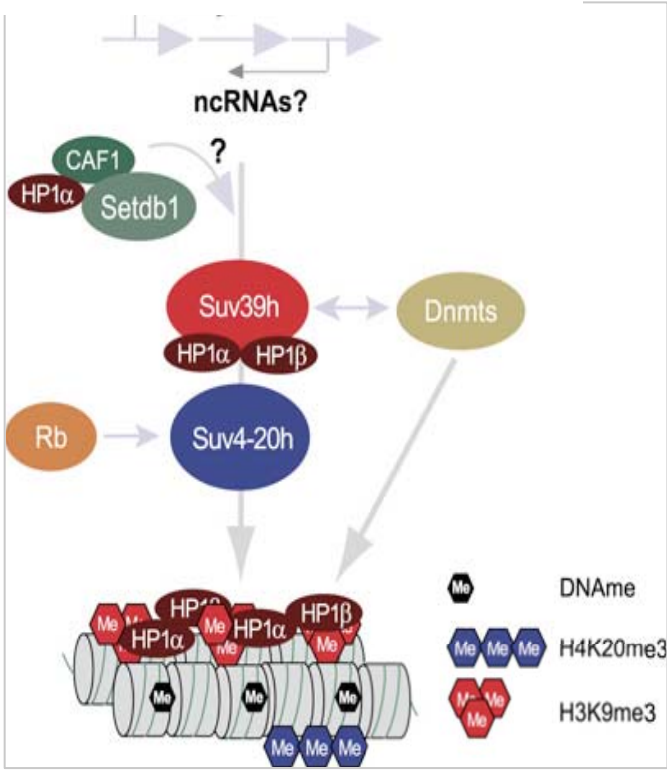


At fertilzation, maternal genome is associated with H3K9me3

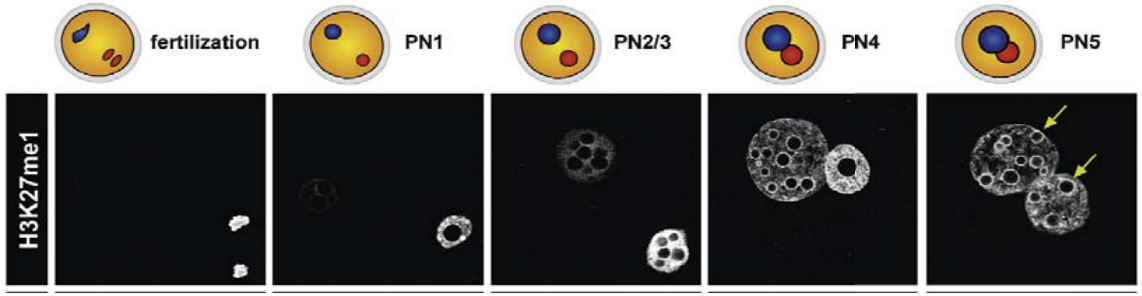
Paternal genome lacks this – establish constitutive heterochromatin state *de novo*…?







Chromatin factors are critical for reorganizing the paternal and maternal epigenomes & preparing the zygotic genome for transcription



• **Maternal PRC1 factors Ring1 and Rnf2 required for early embryonic development beyond the two-cell stage** (Posfai et al, 2012)

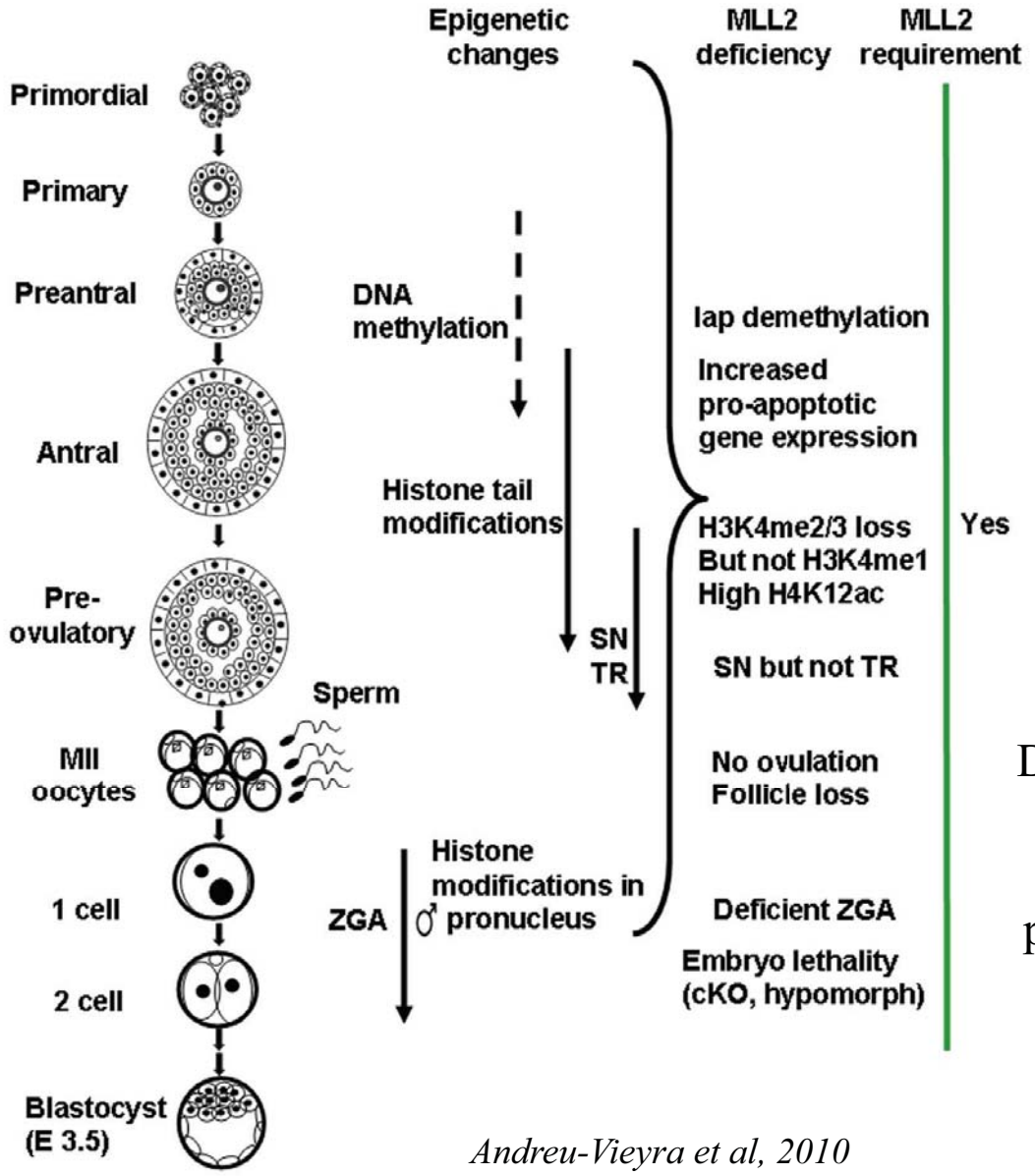
• **Maternal histone variants TH2A/TH2B required for paternal genome activation, which accompanies H3K4me3 and DNA demethylation** (Shinagawa et al, 2013)

• **Maternal Mll2 (TrxG) required for the acquisition and maintenance of H3K4 methylation in the zygote and for normal embyonic gene activation** (Andreu-Vieyra et al, 2010)

• **Maternal PRC2-mediated H3.3 lys27me has a role in remodeling heterochromatin after fertilization**

- Incorporation of H3.3 into **paternal pericentric heterochromatin** is important for the initial establishment of pericentromeric heterochromatin through lysine 27. (Akiyama, Suzuki, Matsuda, & Aoki, 2011; Santenard et al., 2010).
- Mutation of Histone H3.3 lysine K27 to alanine results in a missegregation of chromosomes, developmental arrest and mislocalization of HP1. Same mutation in H3.1 – no effect on HP1 localization or development. (Santenard et al., 2010).

Mll2 (TrxG) is required for the acquisition and maintenance of H3K4 methylation in the zygote and for normal embryonic gene activation



Andreu-Vieyra et al, 2010

Different stage and tissue-specific functions of Mll protein complexes throughout life

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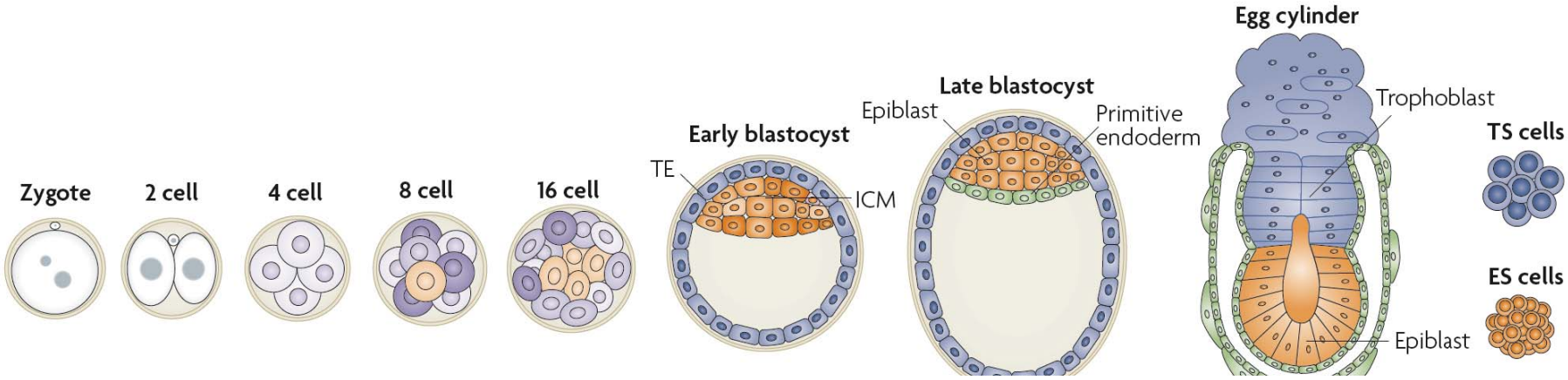
Establishing and maintaining early lineage decisions during Mouse development

Totipotency in the mouse embryo up to ~4-cell stage

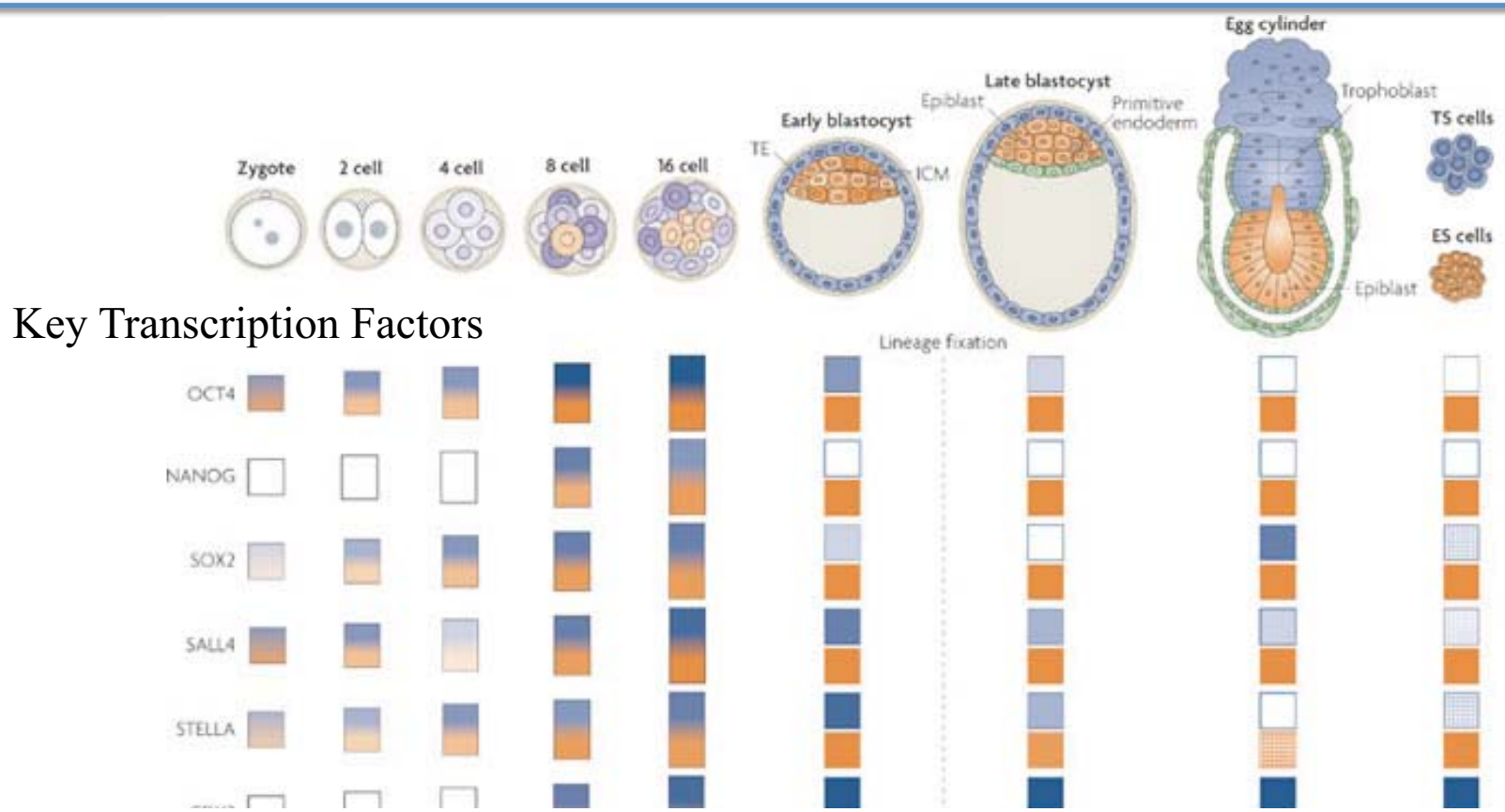


- Progressive restriction of cellular plasticity from 4-cell stage
- Positional cues start to play a role at ~8-16 cell morula stage:
  - **inner cells** tend to form inner cell mass (epiblast = soma + germ line; primitive endoderm)

    embryo-proper extra-embryonic

    pluripotent ES Cells
  - **outer cells** tend to form trophectoderm TE (extra-embryonic tissues)
- Key transcription factors are essential to determine cell fate and establish cell lineages of the early embryo – chromatin factors are also important (eg histone modifiers CARM1, SETDB1, PRC2 etc) to impose permissive (or non-permissive) environment for cell fate, and to predispose a cell towards a particular lineage.
- Chromatin marks and DNA methylation also progressively lock in active and inactive states

Establishing and maintaining early lineage decisions during Mouse development



• First, permissive chromatin states for lineage determination are established by histone modifications in a tight interplay with transcriptional regulators, including OCT4, NANOG, SOX2, SALL4 as well as TEAD4, CDX2 and EOMES.

• Once lineages have been specified, DNA methylation of key loci, eg Elf5 and Stella, is then required to restrict their differentiation potential and to establish lineage-committed cell populations, the fate allocation of which is stably inherited by all descendants

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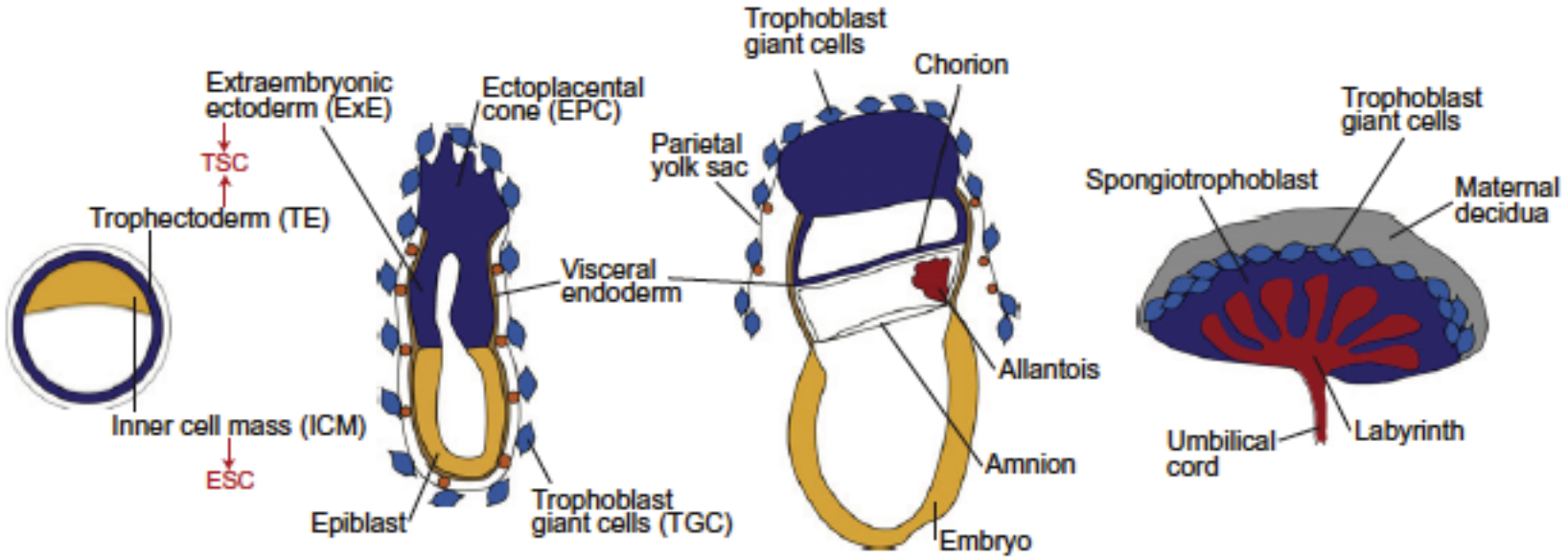
Multiple chromatin factors required for the establishment and maintenance of the extra-embryonic lineages



Establishment of trophoblast lineage — Chorioallantoic attachment — Trophoblast specialisation

E3.5 → E6.5 → E7.5 → E12.5 → E19.5

Eset (HMT)
Carm1? (HMT)
Tet1? (5hmC)

Prmt1 (HMT)
Ezh2 (HMT)

Dnmt1 (5mC)
Dnmt3a/3b (5mC)
Dnmt3l (5mC)
G9a (HMT)
Suz12 (HMT)
Rnf2 (HMT)

Hdac1 (HDAC)
Myst2 (HAT)
Eed (HMT)
Tet1? (5hmC)

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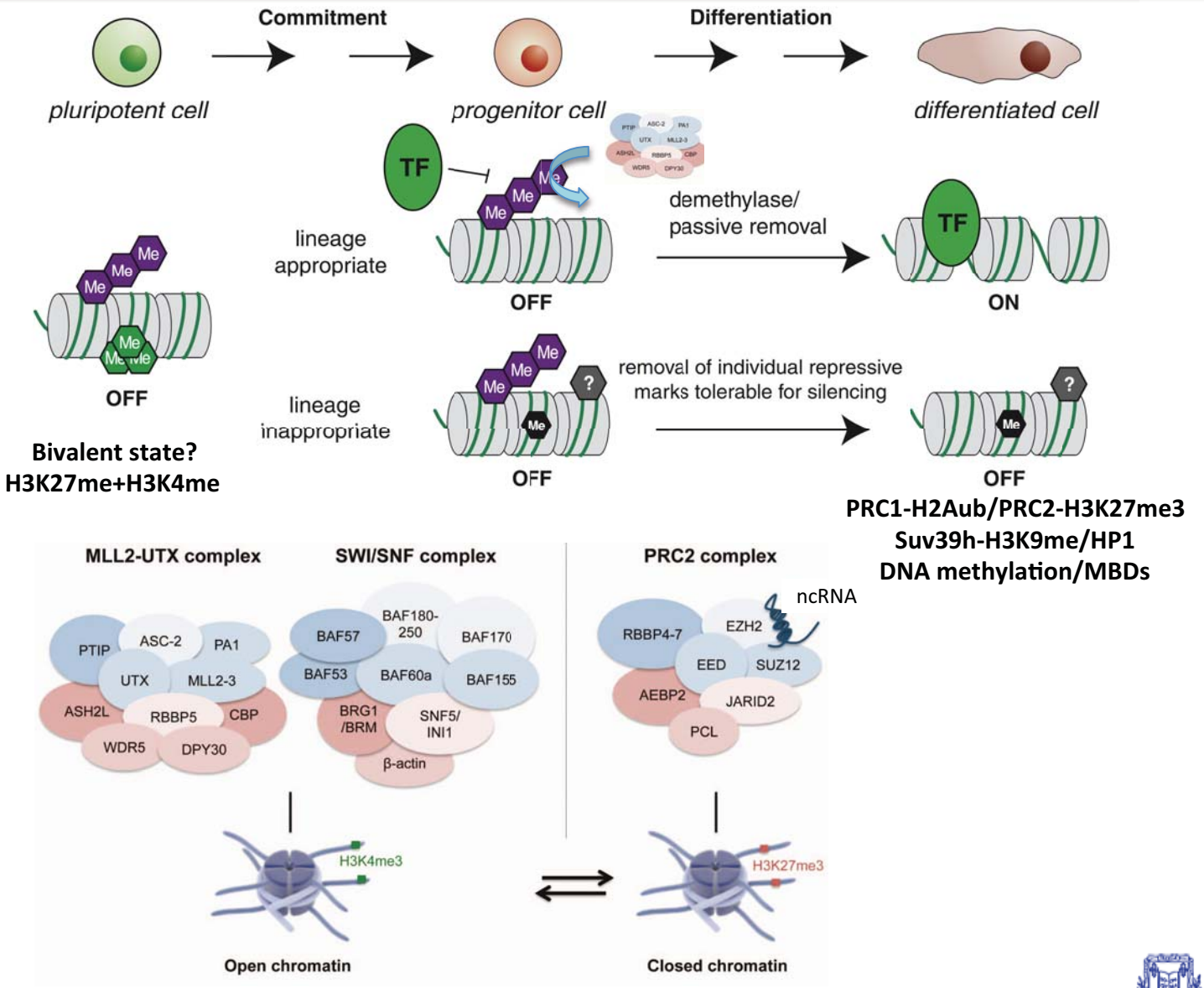
Developmental phenotypes due to mutation of chromatin modifiers : many have one or more roles in development

	Modifier	Function	Mutant Phenotype	Maternally Inherited	ES Cell Derivation	Reference
	Histone Modifications					
H3K9me pathways	Glp/Ehmt1	HMTase	Severe growth retardation and lethality at E9.5; reduction of H3K9me1 and H3K9me2 in embryos	ND	yes	Tachibana et al. (2005)
	G9a/Ehmt2	HMTase	Loss of H3K9 methylation in euchromatin; developmental and growth arrest at E8.5	yes	yes	Tachibana et al. (2002)
	Eset/ SETDB1	HMTase	Peri-implantation lethality (between E3.5 and E5.5); defects in ICM outgrowth	yes	no	Dodge et al. (2004)
	Suv39h1 Suv39h2	HMTase	Double knockout shows loss of H3K9 methylation in heterochromatin; polyploidy in MEF cells; chromosome pairing defects during spermatogenesis; male sterility and death of some double-mutant embryos at E14.5	ND	yes	Peters et al. (2001)
H3K27me pathways	Ezh2/ Enx-1	HMTase PRC2 complex	Growth defect of the primitive ectoderm; peri-implantation lethality	yes	no	O'Carroll et al. (2001)
	Eed	PRC2/3 complex	Defective gastrulation; failure to maintain inactive X in trophoblast cells	yes	yes	Shumacher et al. (1996)
	Suz12	PRC2/3 complex	Early postimplantation lethality; gastrulation defects	yes	ND	Pasini et al. (2004)
	YY1	PRC2/3 interaction	Defects in epiblast cell growth/survival; peri-implantation lethality	yes	no	Donohoe et al. (1999)
	Ring1b/ Rnf2	Ubiquitin ligase PRC1 complex	Gastrulation defects; lethality by E9.5	yes	ES viable	Voncken et al. (2003)
	DNA Methylation					
DNA methylation pathways	Dnmt1	DNA MTase	Genome-wide demethylation; developmental arrest at E8.5	yes	yes	Li et al. (1992)
	Dnmt3a	DNA MTase	Malfunction of gut; spermatogenesis defects; postnatal lethality (~4 weeks of age)	yes	yes	Okano et al. (1999)
	Dnmt3b	DNA MTase	Demethylation of minor satellite DNA; mild neural tube defects; embryonic lethality at E14.5–E18.5	yes	yes	Okano et al. (1999)

Chromatin: enabling developmental transitions and memorising activity states?



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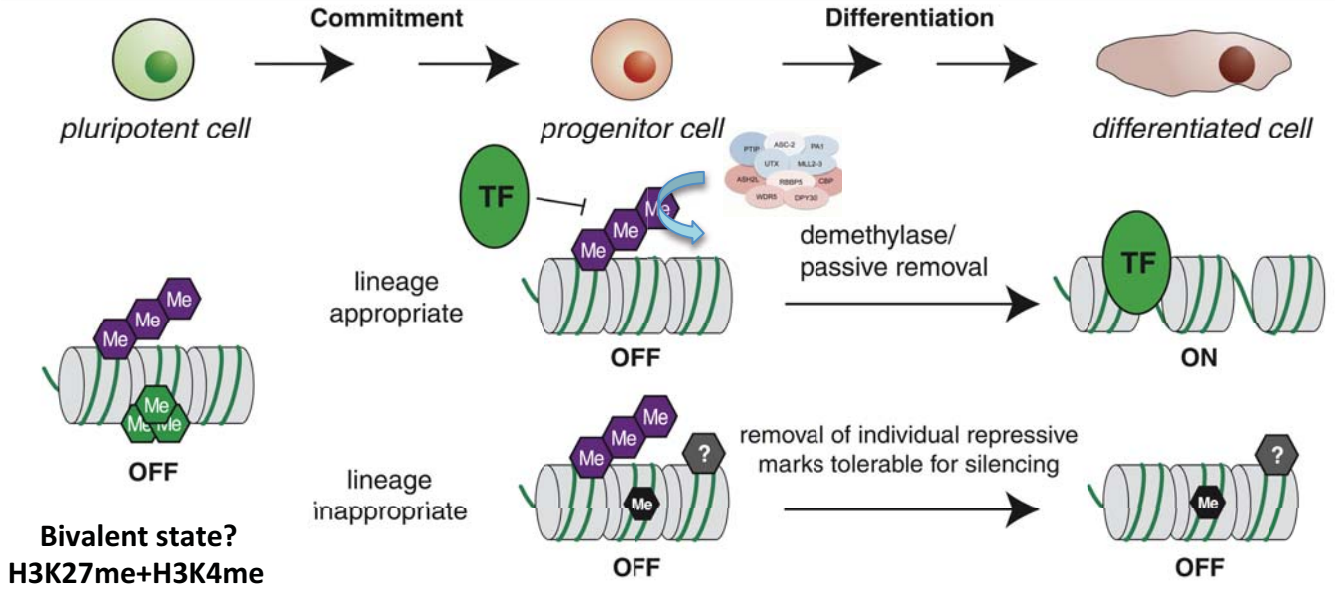
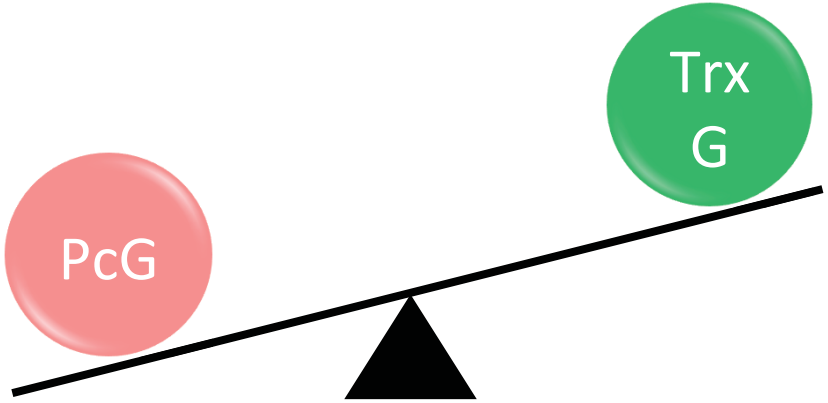
Chromatin: enabling developmental transitions and memorising activity states – balancing acts



PcG proteins keep genes off in tissues where they should not normally be expressed. TrxG complexes with histone demethylases, together with chromatin remodeling complexes counteract PcG, to activate genes (in collaboration with TFs) in appropriate lineages.



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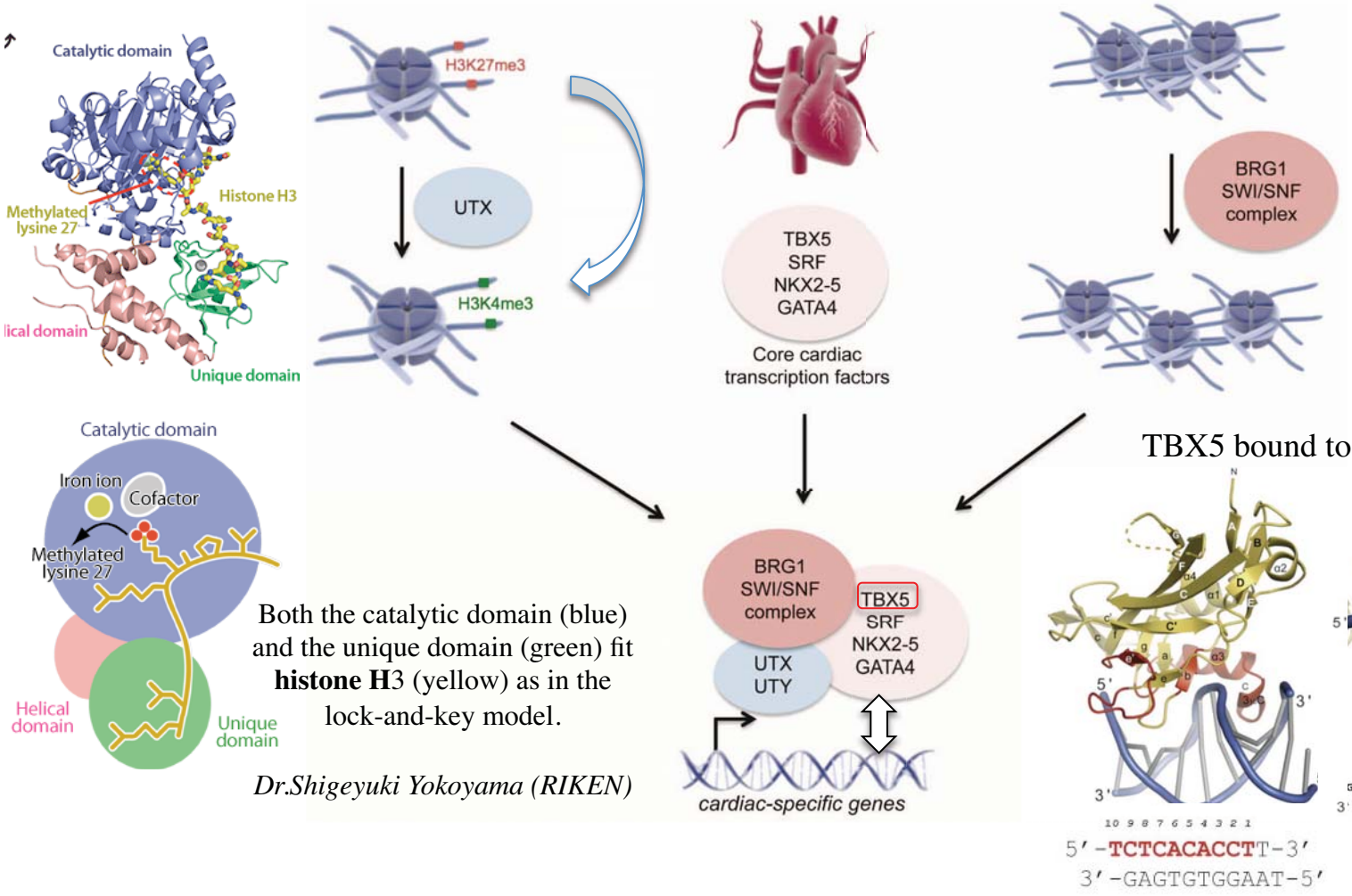
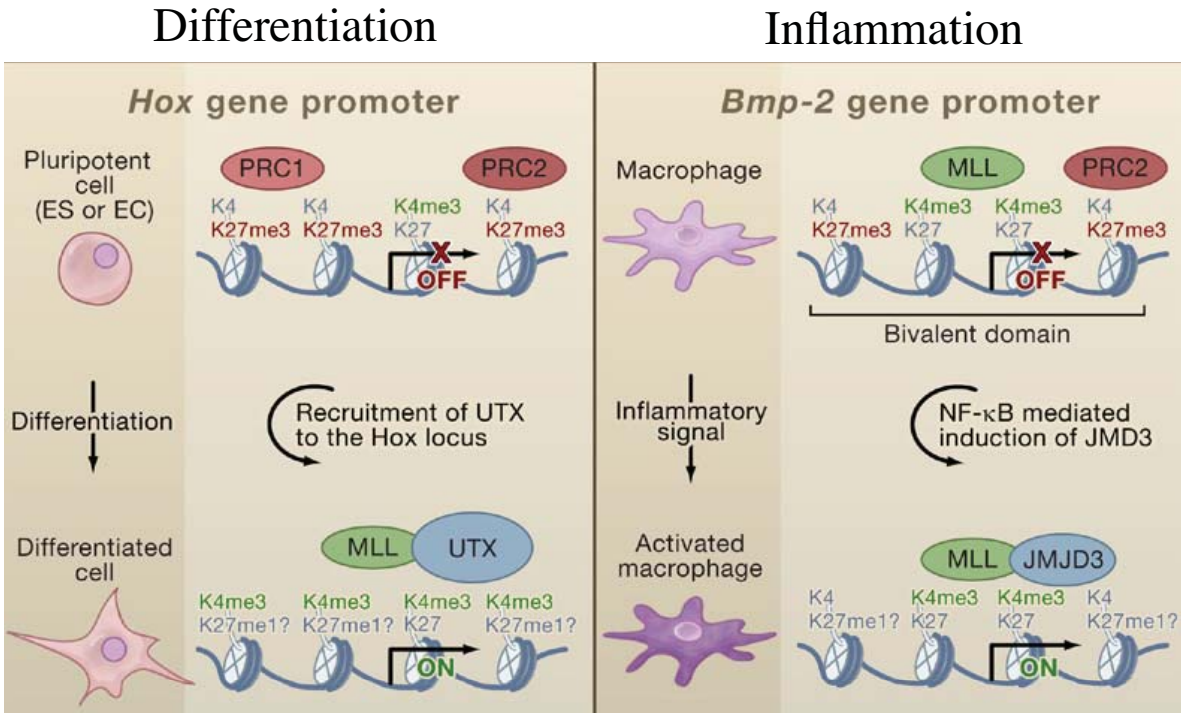
Dynamic chromatin memory during development: removal of PRC-marked chromatin by UTX demethylase



During cardiac development the UTX and BRG1 complexes are guided by the core cardiac Transcription Factors NKX2-5, TBX5, GATA4 and SRF, to promote specific gene activation of cardiac-specific genes (demethylation of H3K27me2/3 at promoter regions)

Dynamic chromatin memory during development and in response to signals such as inflammation



Stepwise recruiting model in which a H3K4 methyltransferase core complex devoid of H3K27 demethylase activity is recruited first and subsequently exchanged by UTX/ JMJD3- containing Mll complexes?

Lee, M. G., Villa R., Trojer P., Norman J., Yan K. P., Reinberg D., Di Croce L. and Shiekhattar R., Demethylation of H3K27 regulates Polycomb recruitment and H2A ubiquitination, *Science* 2007.

Dou, Y., Milne T. A., Ruthenburg A. J., Lee S., Lee J. W., Verdine G. L., Allis C. D. and Roeder R. G., Regulation of MLL1 H3K4 methyltransferase activity by its core components, *Nat Struct Mol Biol* 13 (8), 713–719, 2006.

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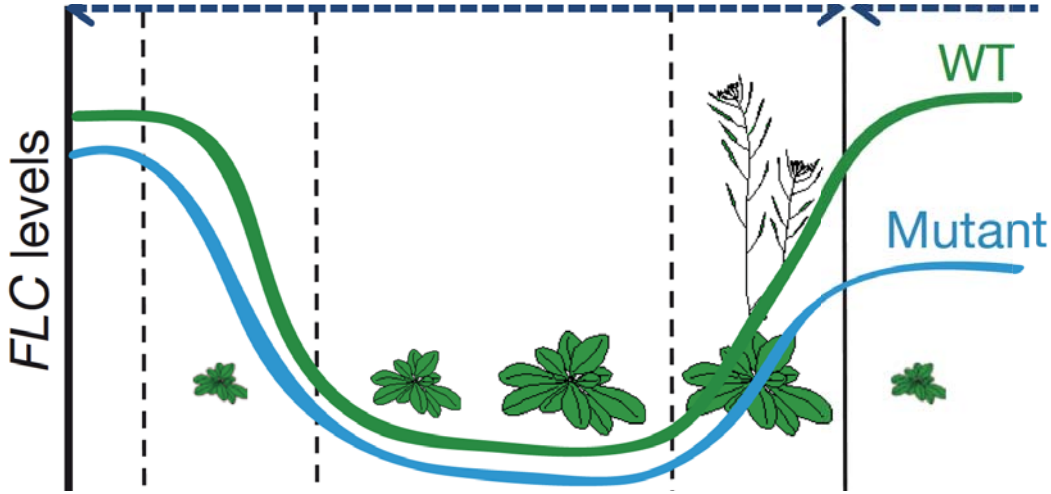
Dynamic chromatin memory following vernalisation

Cold-induced Polycomb repression of the *FLC* locus (a repressor of flowering)

A strategy to allow proper coordination of plant development and appropriate flowering time



Partial resetting

Transmission to next generation…?

- ELF6 has H3K27me3 demethylase activity – a single nucleotide mutation at a highly conserved amino-acid reduced ELF6 enzymatic activity
- In next generation of mutant plants, H3K27me3 levels at the *FLC* locus stayed higher, & *FLC* expression remained lower, than in the wild type.
- Early flowering phenotype was stable for at least three generations following vernalisation but was not enhanced by a second vernalisation treatment in later generations.

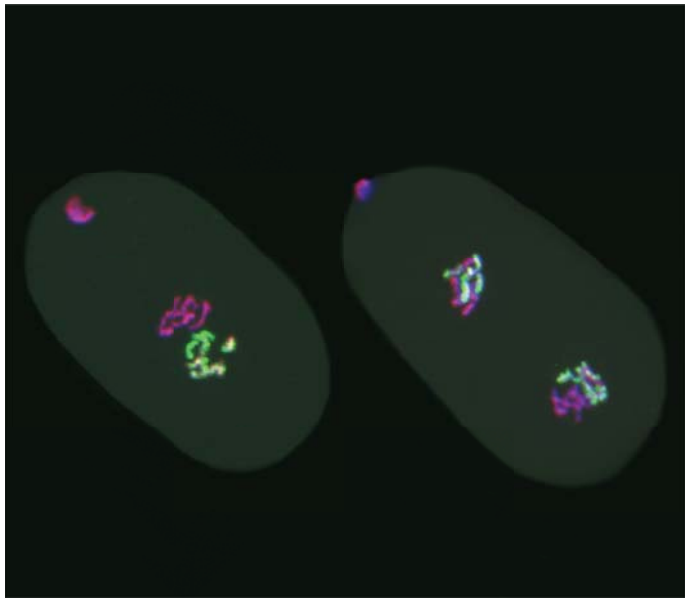
Role for H3K27 demethylation in the reprogramming of epigenetic states in embryos, to prevent transgenerational inheritance of "acquired traits"

Chromatin memory across generations?

Inter and trans-generational memory:

Can chromatin retain any memory
(somatic, germ cell or environmental)
and resist developmental and germ line
reprogramming?

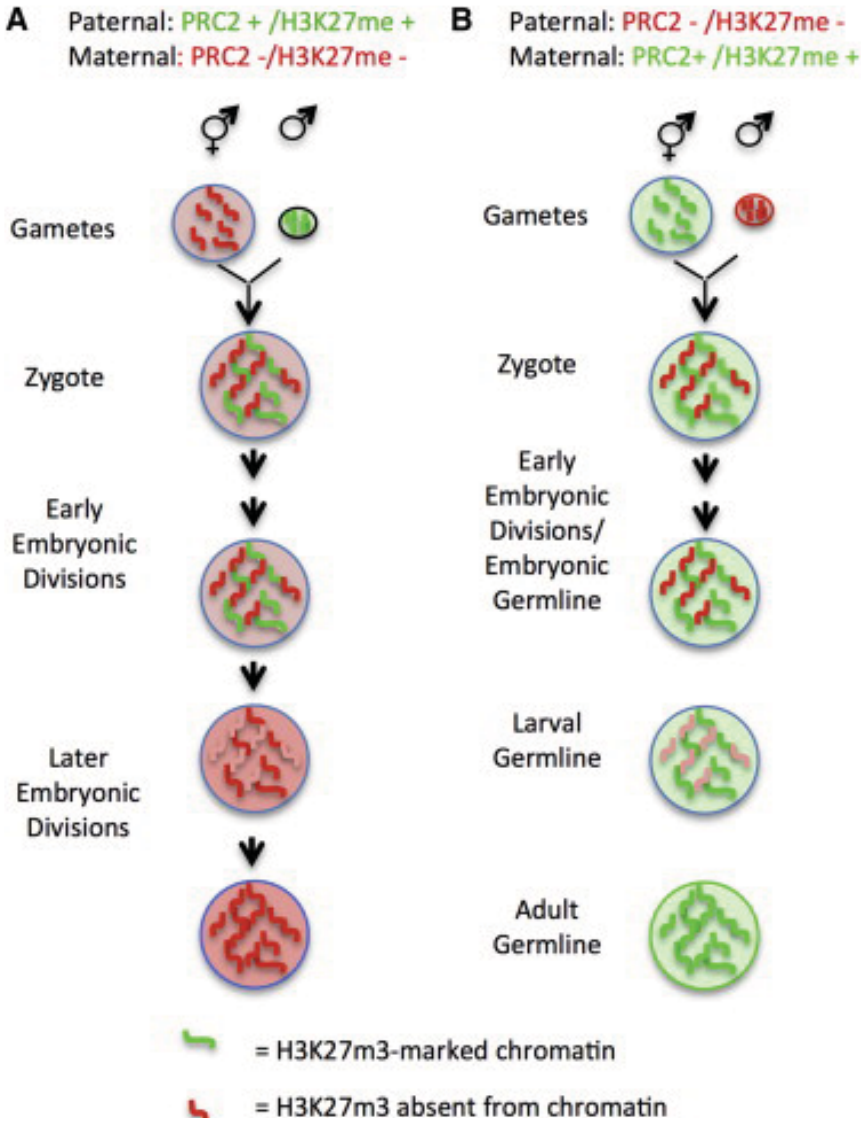
In worms, H3K27me3 can be transmitted in the absence of PRC2 through cell division and even across generations



Inheritance and transmission of H3K27me3 in *C. elegans*. The 1-cell embryo (left) shows H3K27me3 (green) inherited on the sperm chromosomes but not on the oocyte chromosomes (pink) contributed by a *PRC2 mutant mother*.

The 2-cell embryo (right) shows transmission of H3K27me3 on the sperm-derived chromosomes in each nucleus.

Gaydos et al, 2014

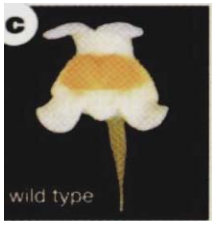


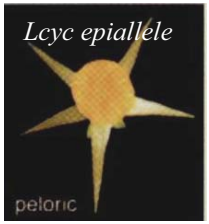
H3K27me3 Persistence and Pattern Maintenance in *C. elegans* Embryos and Germline Gametes originating from germlines with (green-filled) or without (red-filled) MES-3 activity, an essential PRC2 component in *C..elegans*

E. Heard, February 23rd, 2015

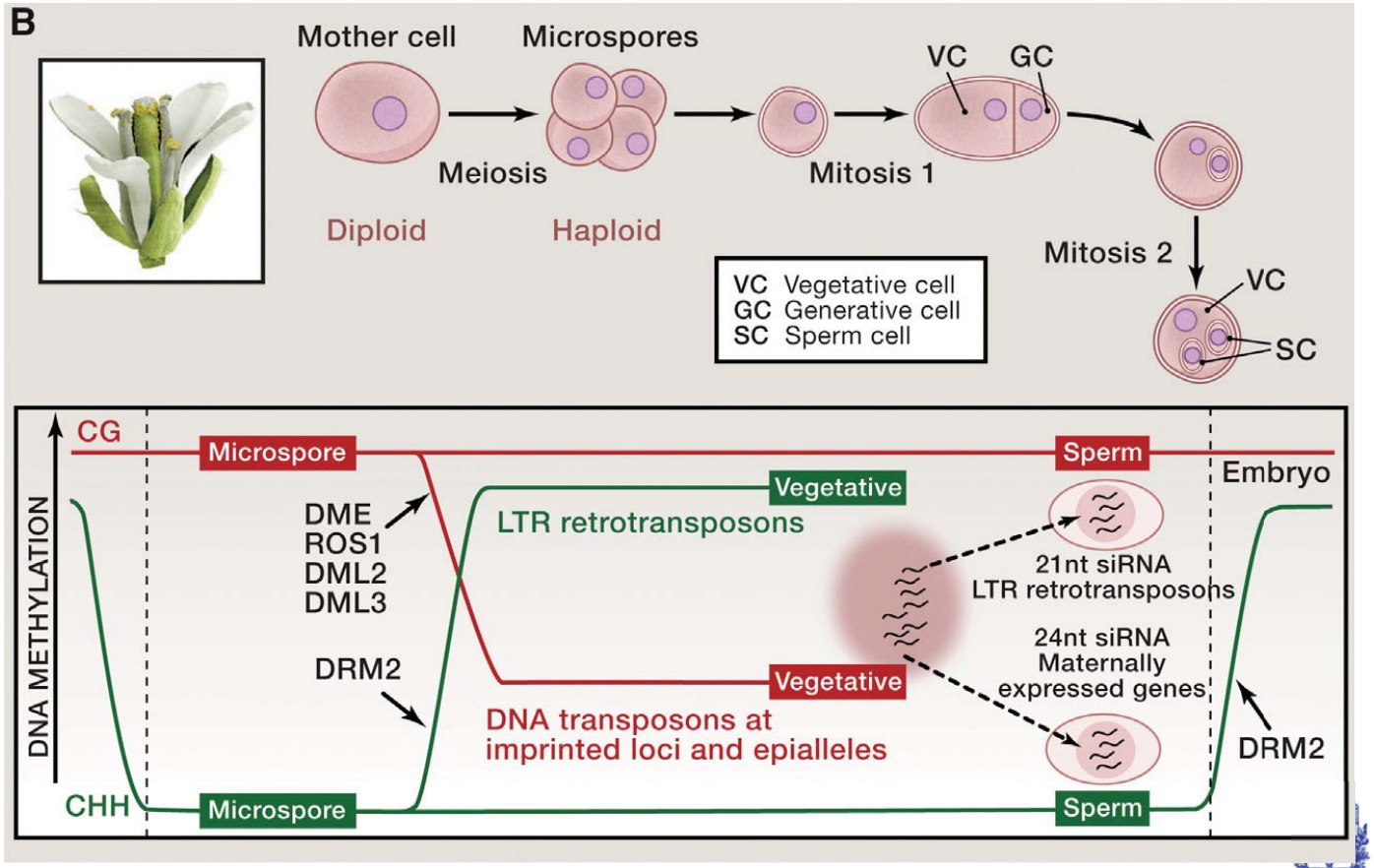
Chromatin memory across generations?

- In plants, unlike animals, there is no early separation of germline and soma thus epigenetic marks acquired throughout their lifetime can be included in the gametes e.g. *Peloric* (*Lcyc* CpG me).
- Most plant developmental genes involve ***non*-CpG** DNA methylation which requires a continuous remethylation cue and as such is continually reprogrammed
- Transposable elements (CpG methylation) are probably key targets for trans-generational effects





From Heard and Martienssen, Cell 2014



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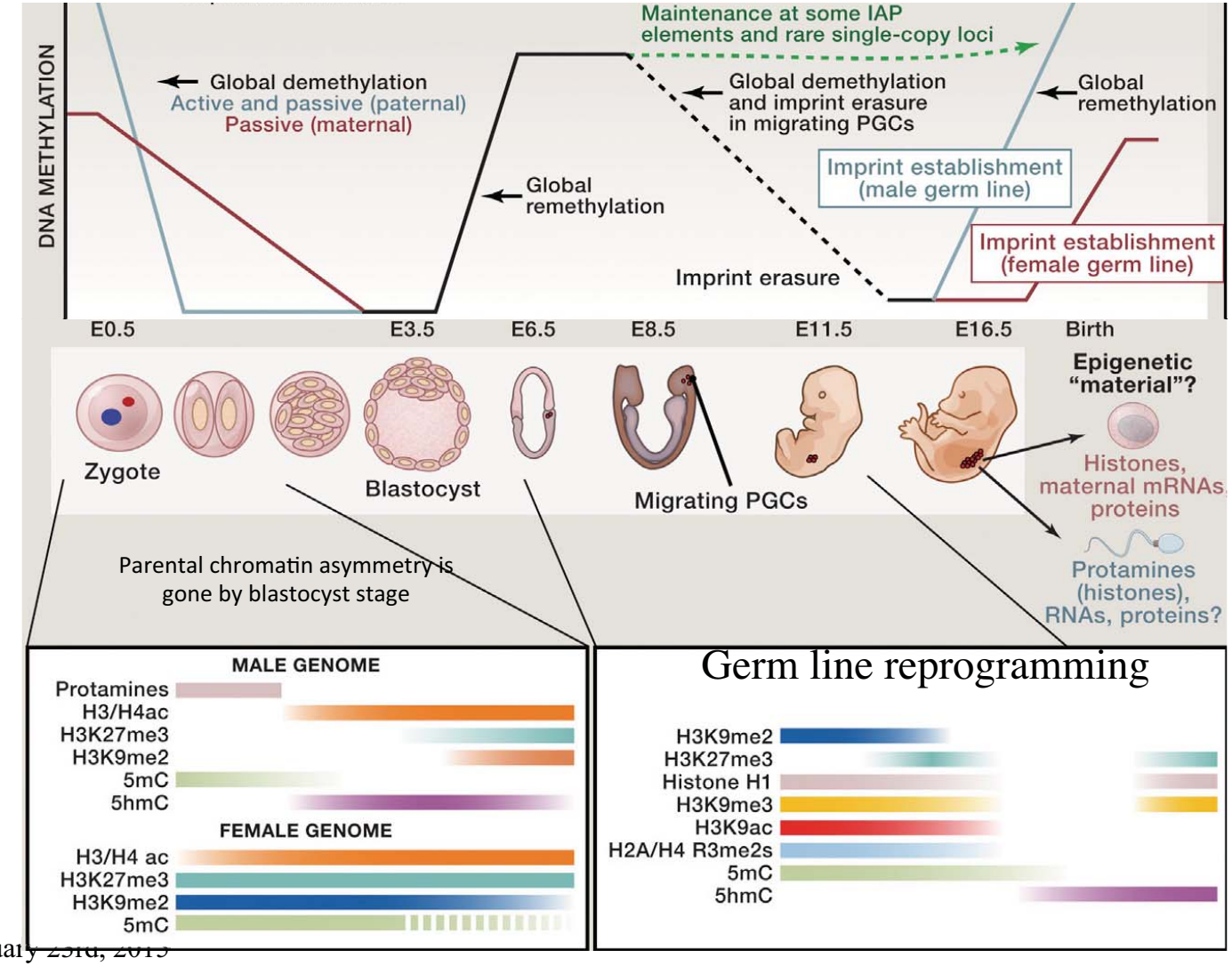
Chromatin memory across generations?

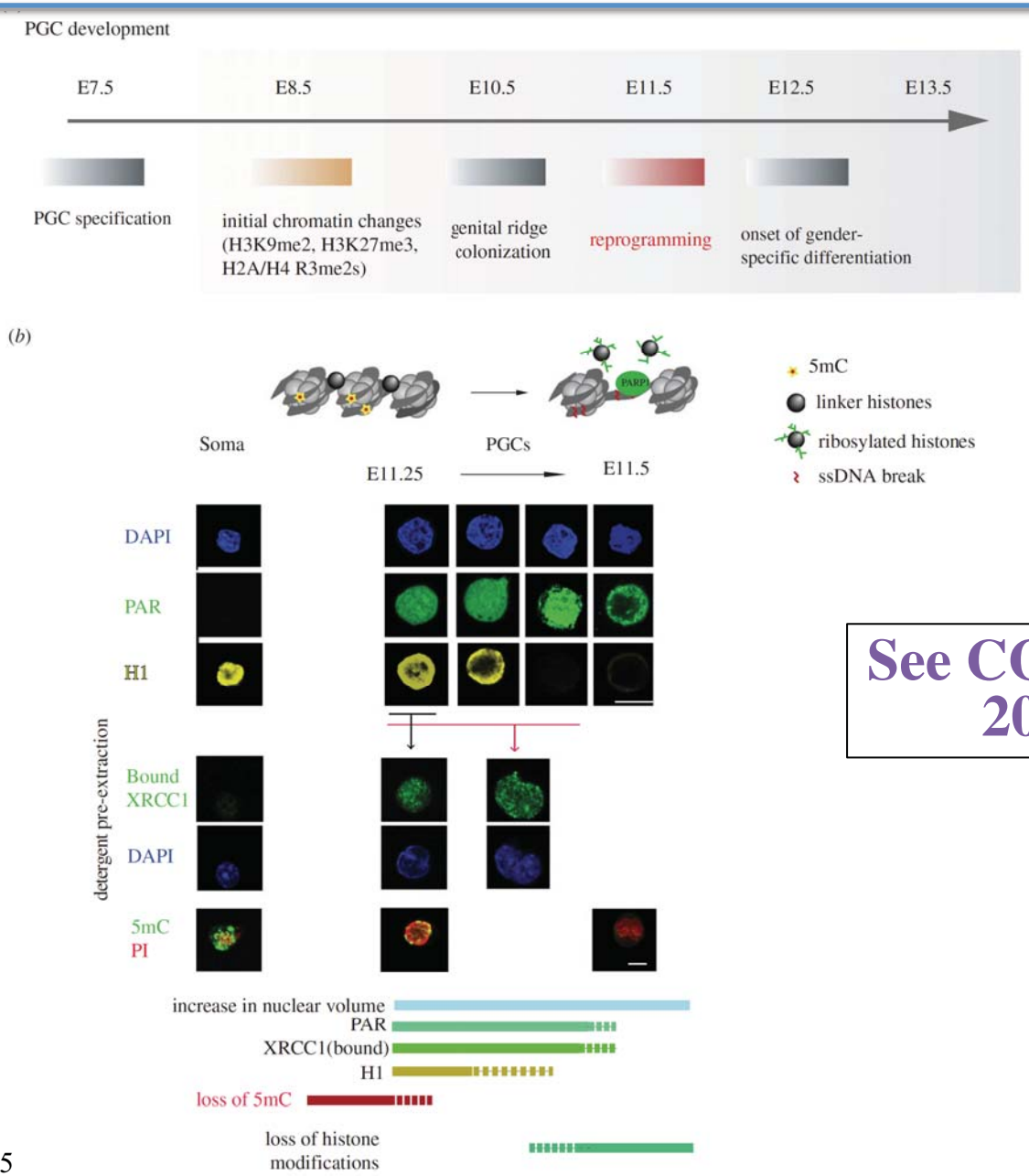
Mammals: chromatin state are reprogrammed in the germ line (somatic marks, inactive X, imprints) and during early development (after fertilisation and in the blastocyst)

Most epigenetic marks are erased at each generation. Except a few….?



From Heard and Martienssen, Cell 2014

Massive Reprogramming occurs in the Mammalian Germ Line



See COURS 2 2014

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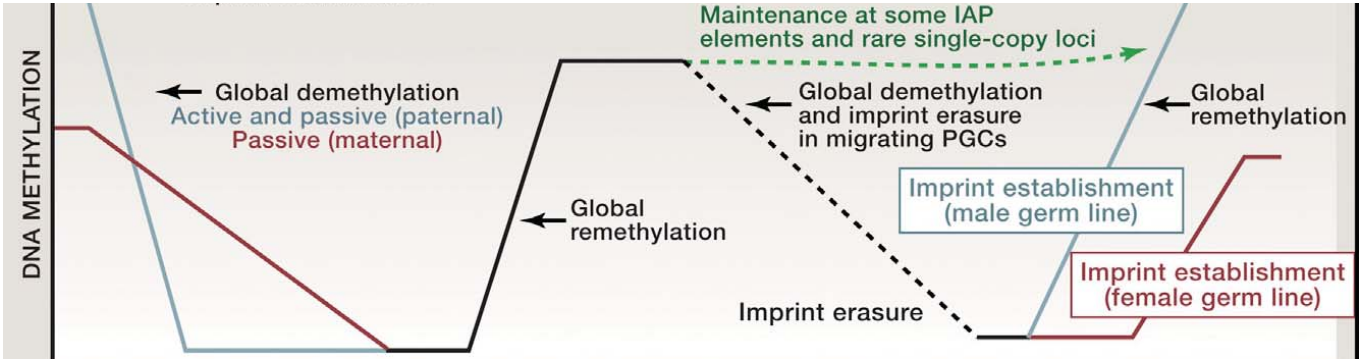
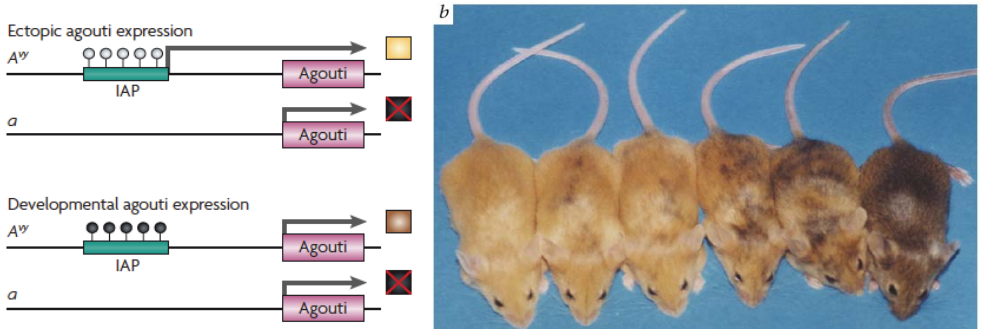


Resistance of retrotransposons to reprogramming may lead to trans-generational epigenetic effects in mammals?



Epigenetic control of transposons is critical in the germ line (seminar D. Bourc'his)

Chromatin memory across generations?

Some organisms transmit epigenetic states between generations (plants, worms, flies…)
(future course!)

Evidence in mammals that chromatin may retain *limited* memory after developmental and germ line reprogramming

=> Any contribution to phenotypic variation…?

Can changes in environmental conditions
such as nutrition, exposure to environmental pollutants
(e.g., endocrine disruptors, smoking)
or even parental care during early postnatal life

- induce chromatin changes
- affect development, physiology and fitness of subsequent generations?

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Can the environment influence epigenomes *and* phenotypes and can this then be transmitted to future generations?

Are we enslaved to our genes that we get from our parents, or can we break free with epigenetic change?

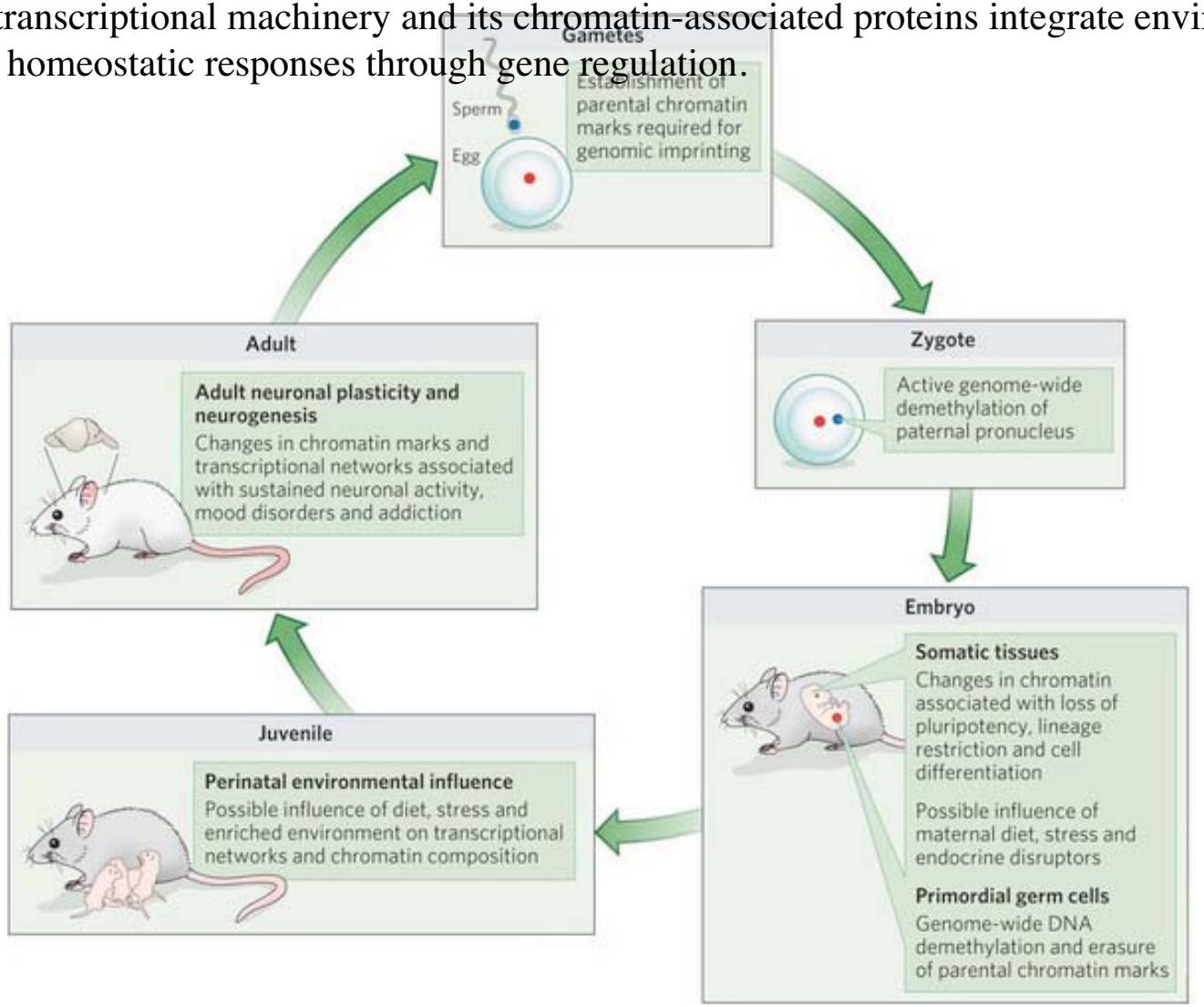
Hauke Dressler/LOOK/-Getty Images

Can we influence our epigenomes and those of our descendents?

Is heredity "nothing but stored environment"?
L. Burbank

Chromatin modifications can occur all through life

- Living organisms and individual cells continuously adapt to changes in their environment.
- Changes are particularly sensitive to fluctuations in the availability of energy substrates.
- Cellular transcriptional machinery and its chromatin-associated proteins integrate environmental inputs to mediate homeostatic responses through gene regulation.



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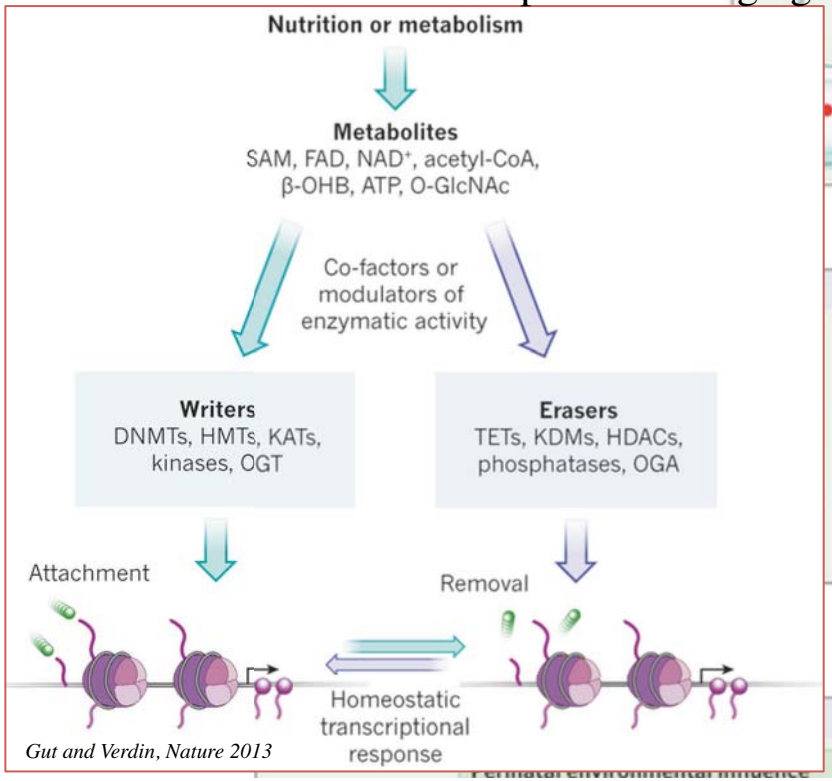
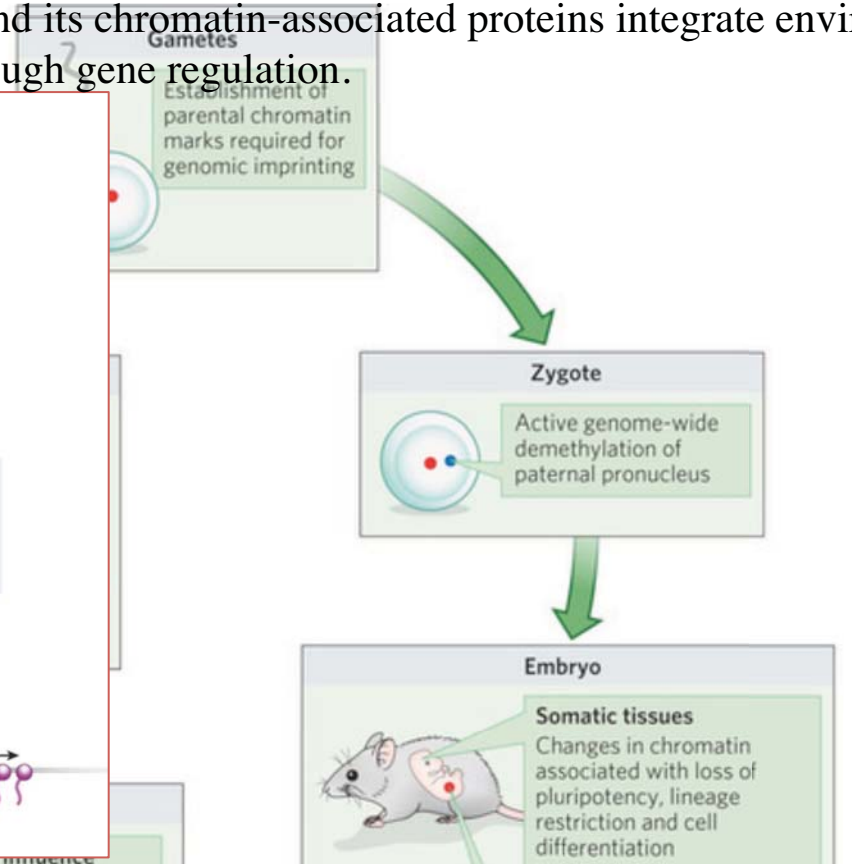




Can nutritional stress- induced chromatin changes be inter-generationally inherited?

Is chromatin involved?

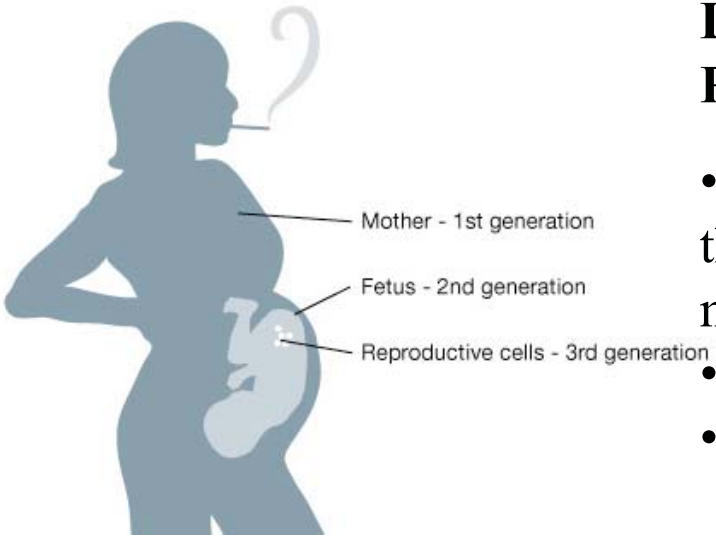
NEXT WEEK

Can the environment influence chromatin states and phenotypes and can this then be transmitted to future generations?

Can the environment ***induce*** germ line heritable epialleles (inter- or trans)generational)

- Very few well-controlled examples in plants or mammals
- Some recent examples, for example in *Drosophila*, *C. elegans*

Can the environment influence the **propagation** across generations of pre-existing epialleles?



Substantial evidence for Inter-generational effects
Less evidence for Transgenerational effects (>F3)
Proof of trans-generational inheritance?

• Rule out direct exposure: epigenetic effect must pass through sufficient generations (4th generation from mother, 3rd generation via father)
• Rule out the possibility of DNA sequence changes
• Rule out the possibility of behavioral/cultural effects

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CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2014-2015 :
"Chromatine et Mémoire cellulaire"

2 mars, 2015

Cours V

"Stabilité versus plasticité chromatinienne en réponse aux stress"

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