CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2013-2014 :

“Reprogrammations développementales, induites et pathologiques ”

Cours II

Etapes de la reprogrammation au cours du développement chez les mammifères

17 mars 2014

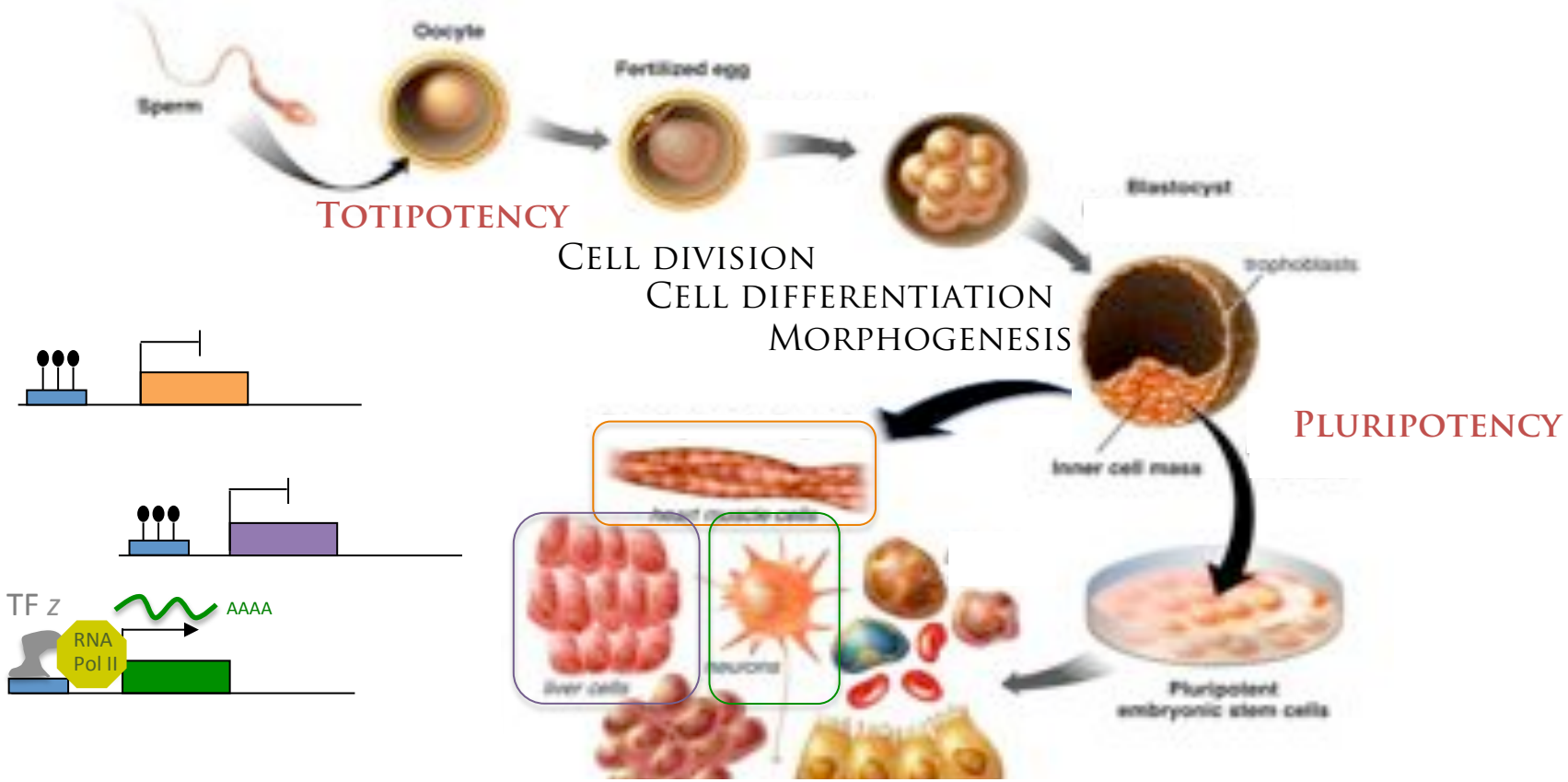
Seminaire:

Prof. Wolf Reik à 17h30

“The Role of DNA Modifications in Epigenetic Reprogramming and Signaling”

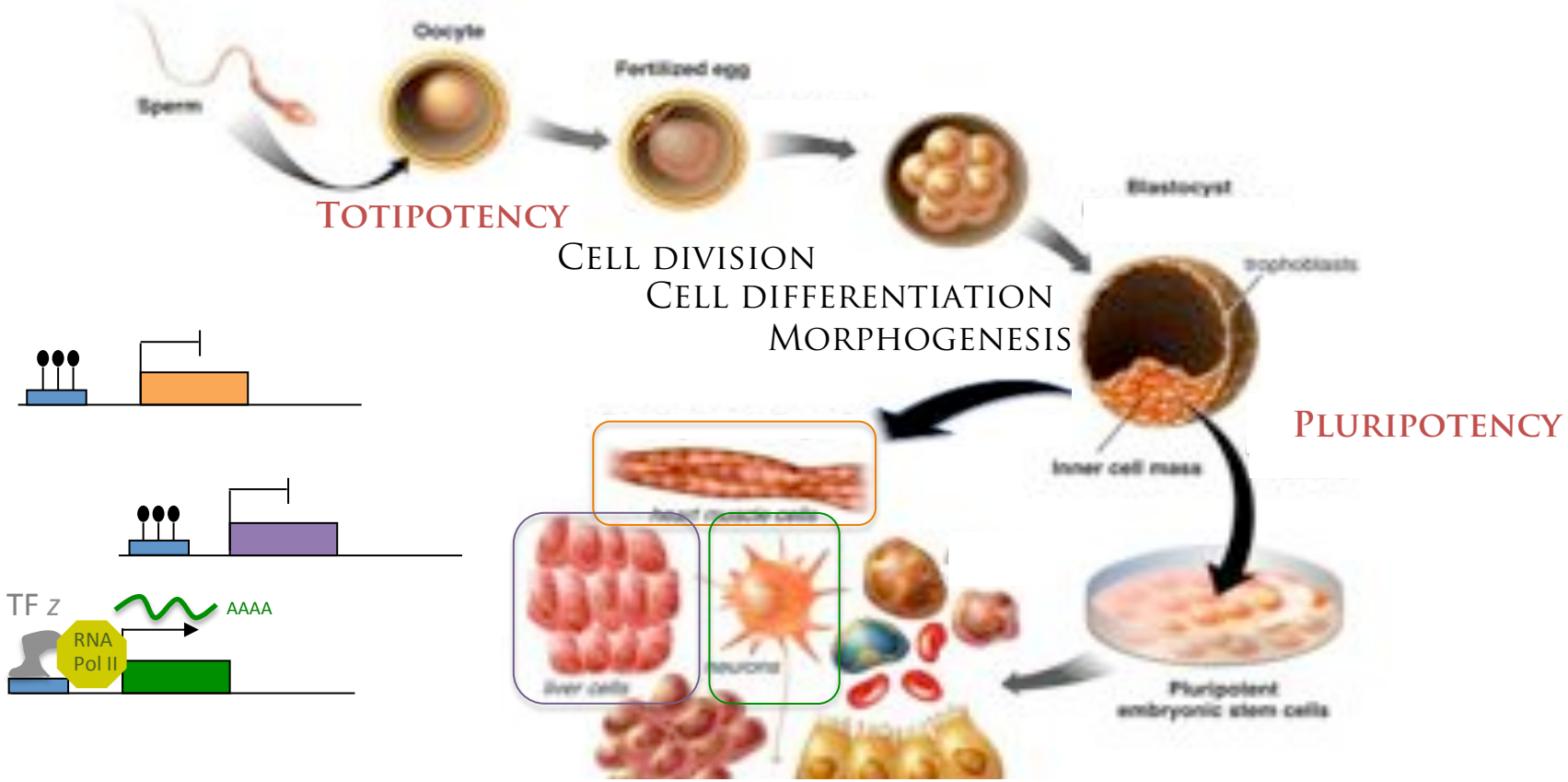
COLLÈGE DE FRANCE 1530

Developmental Programming



1. All cells contain the same genes – cell identities depend on **which** genes are expressed and repressed.
2. These are established by transcription factors via signalling, cell-cell communication, positional information…

Developmental Programming



1. All cells contain the same genes – cell identities depend on **which** genes are expressed and repressed.
2. These are established by transcription factors via signalling, cell-cell communication, positional information…
3. Changes in gene expression patterns become heritable (through mitosis) during development => « **Epigenetics** »

Developmental Programming

Epigenetics: heritable changes in gene function that cannot be explained by changes in DNA sequence.

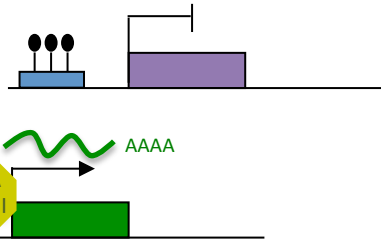
Russo, V.E.A., R.A. Martienssen & A.D. Riggs Eds. (1996) "Epigenetic mechanisms of gene regulation." *CSHL Press*.

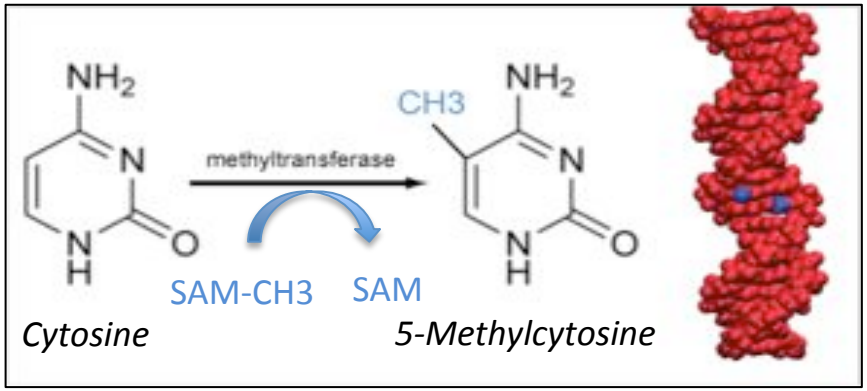
Developmental Programming

Epigenetics: heritable changes in gene function that cannot be explained by changes in DNA sequence.

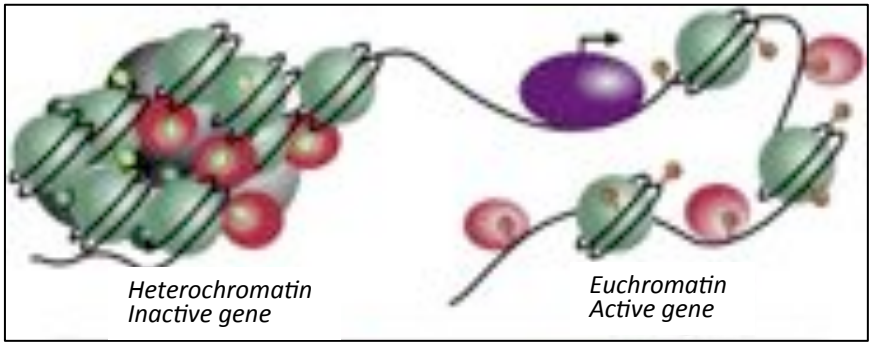
Russo, V.E.A., R.A. Martienssen & A.D. Riggs Eds. (1996) "Epigenetic mechanisms of gene regulation." *CSHL Press*.

Eg **DNA Methylation** – an "epigenetic" modification that can affect gene expression; be propagated over cell division; and "lock in" the silent state
- Important for normal development





Chromatin – histone variants & modifications, and protein complexes such as Polycomb &Trithorax



Okano M, Bell DW, Haber DA, Li E: DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 1999, 99:247-257.

O'Carroll D, Erhardt S, Pagani M, Barton SC, Surani MA, Jenuwein T: The polycomb-group gene Ezh2 is required for early mouse development. *Mol. Cell. Biol*. 2001, 21:4330-4336.

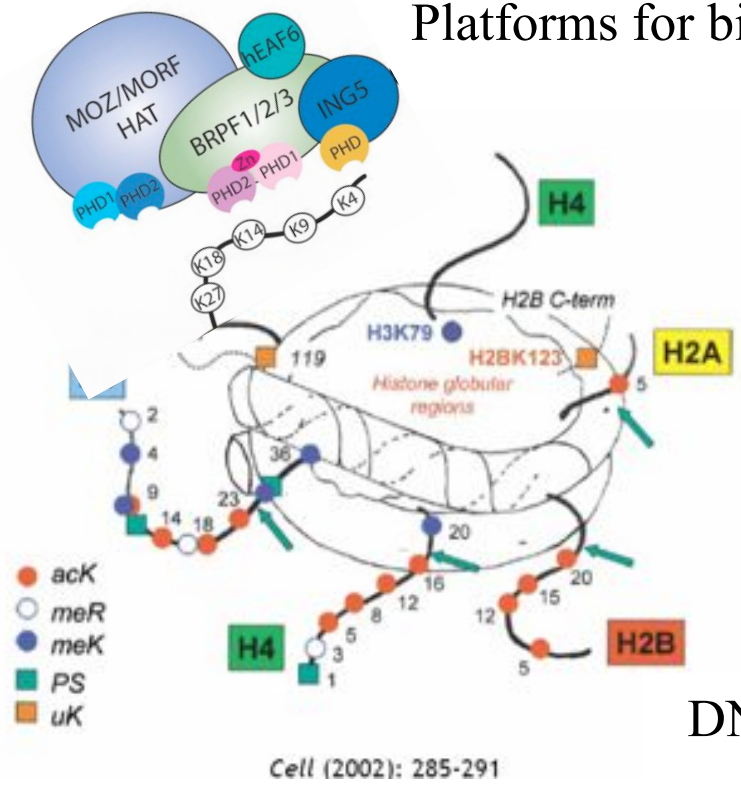
Bledau et al. The H3K4 methyltransferase Setd1a is first required at the epiblast stage, whereas Setd1b becomes essential after gastrulation. *Development* 2014, 141:1022-1035.

Chromatin-based Epigenetic Mechanisms

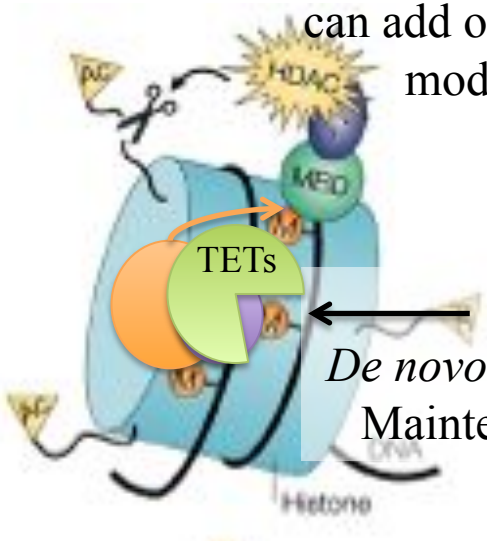
Histone Variants and Histone Modifications are:

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

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)

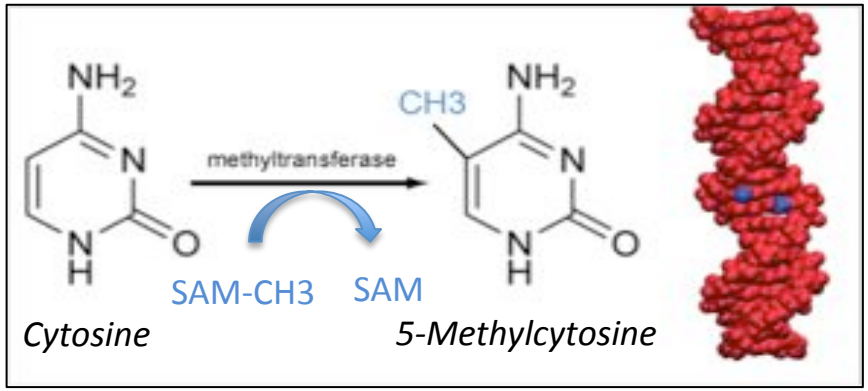
COLLEGE DE FRANCE 1530

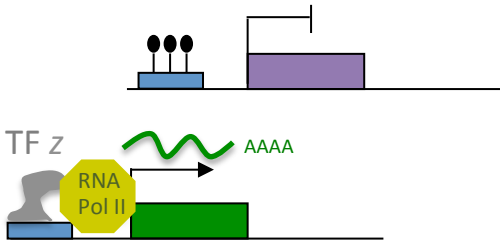
Developmental Programming

Epigenetics: heritable changes in gene function that cannot be explained by changes in DNA sequence.

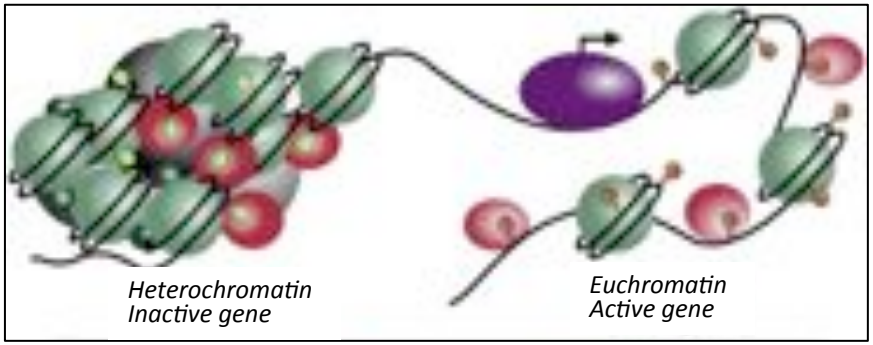
Russo, V.E.A., R.A. Martienssen & A.D. Riggs Eds. (1996) "Epigenetic mechanisms of gene regulation." *CSHL Press*.

Eg **DNA Methylation** – an "epigenetic" modification that can affect gene expression; be propagated over cell division; and "lock in" the silent state





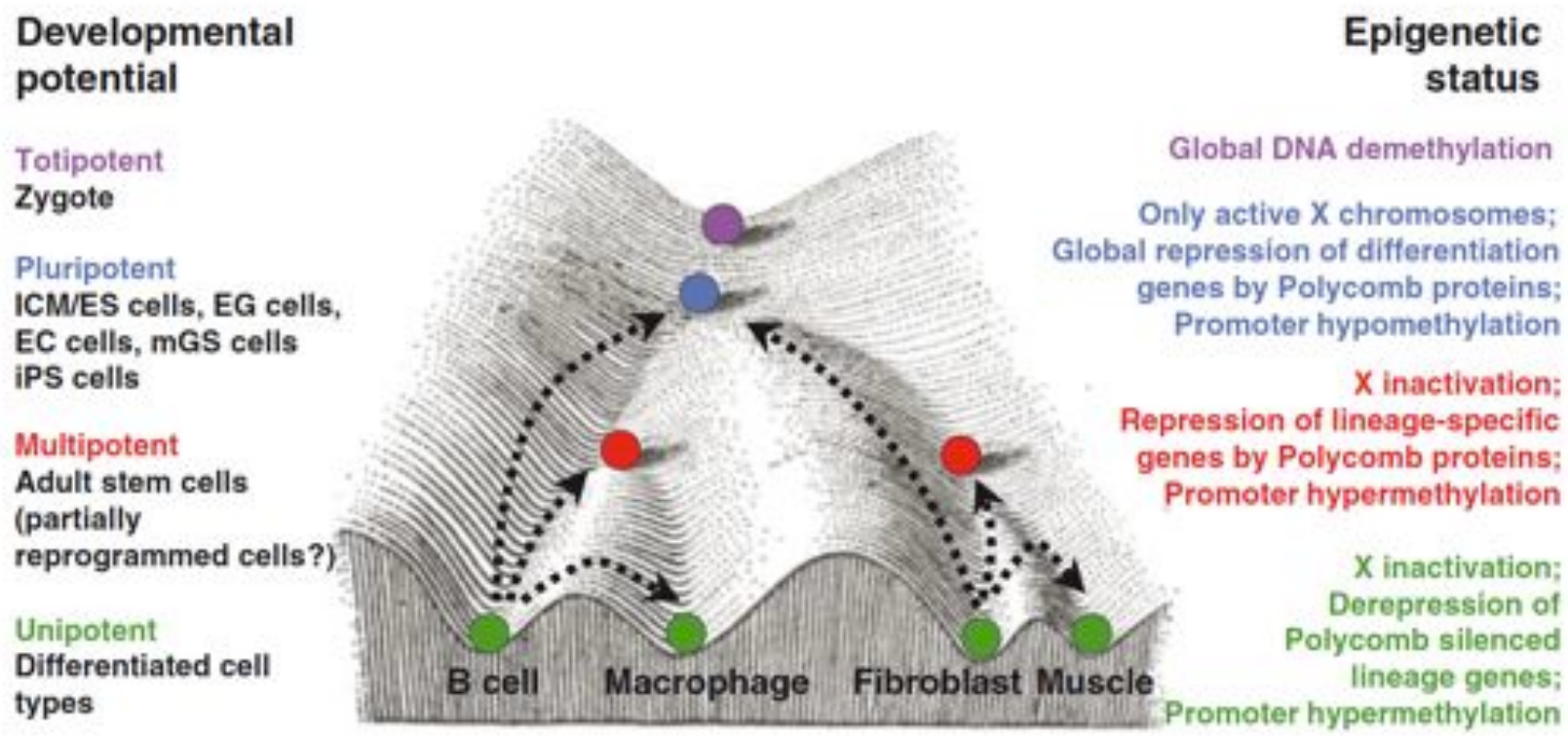
Chromatin – histone variants & modifications, Polycomb &Trithorax complexes



Epigenetic states are **stable** but can be **reversed**: during development, in the germ line, in somatic cells (eg stem cell differentiation), in disease (eg epimutations in cancer), or during nuclear transfer and cloning (reprogramming): in each case **Epigenetic barriers** must be overcome…

Development 2014, 141:1022-1035.

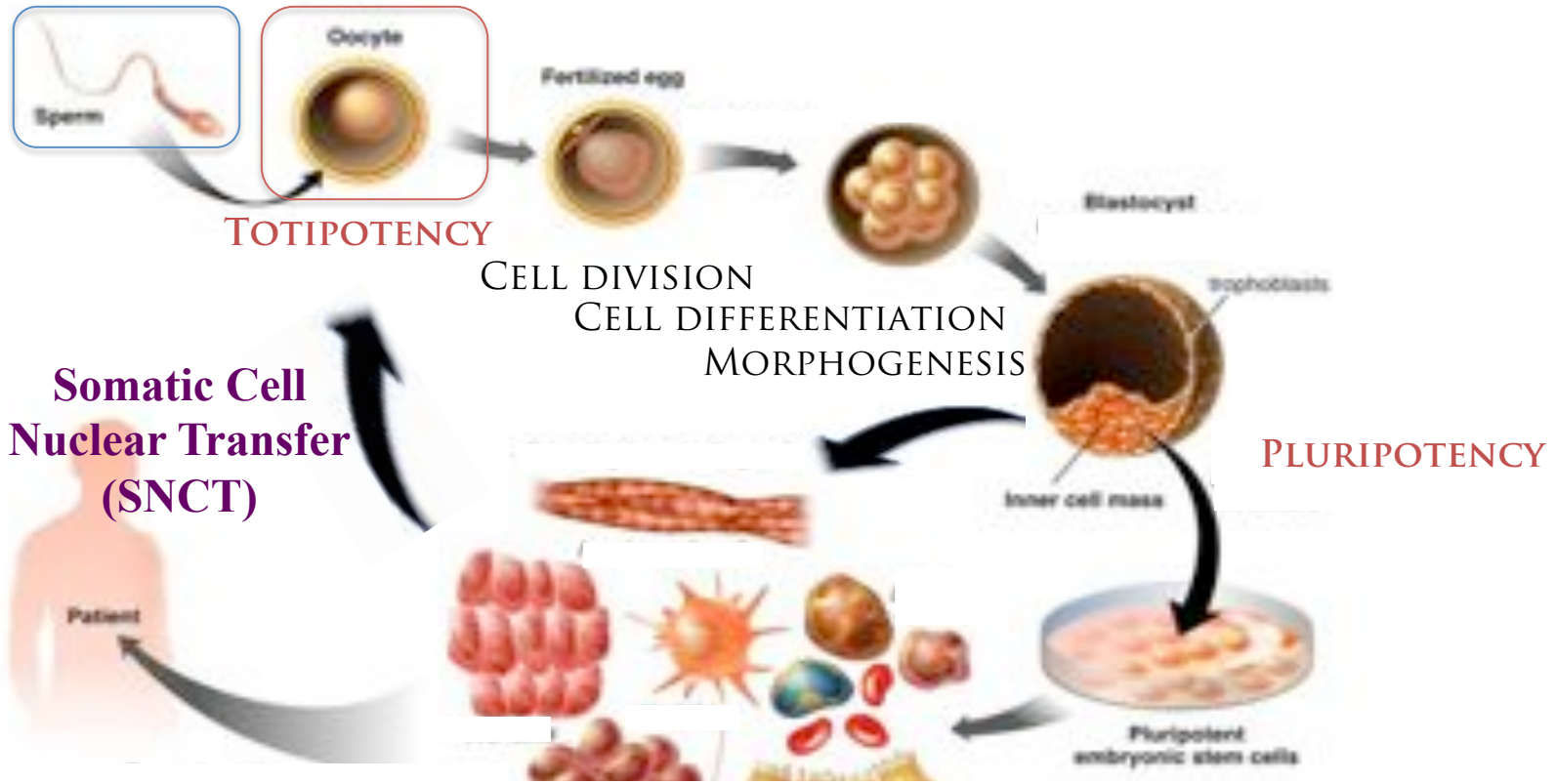
Many types of epigenetic barrier & many ways of overcoming them during development or artificially



Developmental restrictions imposed on the genome during differentiation are due to reversible epigenetic modifications rather than to permanent genetic changes

Epigenetic changes allow the **maintenance of cell identity** but can be overriden by TFs, as well as by active and passive loss

Developmental Reprogramming



The success rate of reproductive cloning is very low compared to natural reproduction

- due to inappropriate expression of somatic genes, inadequate reactivation of developmental genes and other epigenetic errors? (including imprinted genes, X inactivation…)

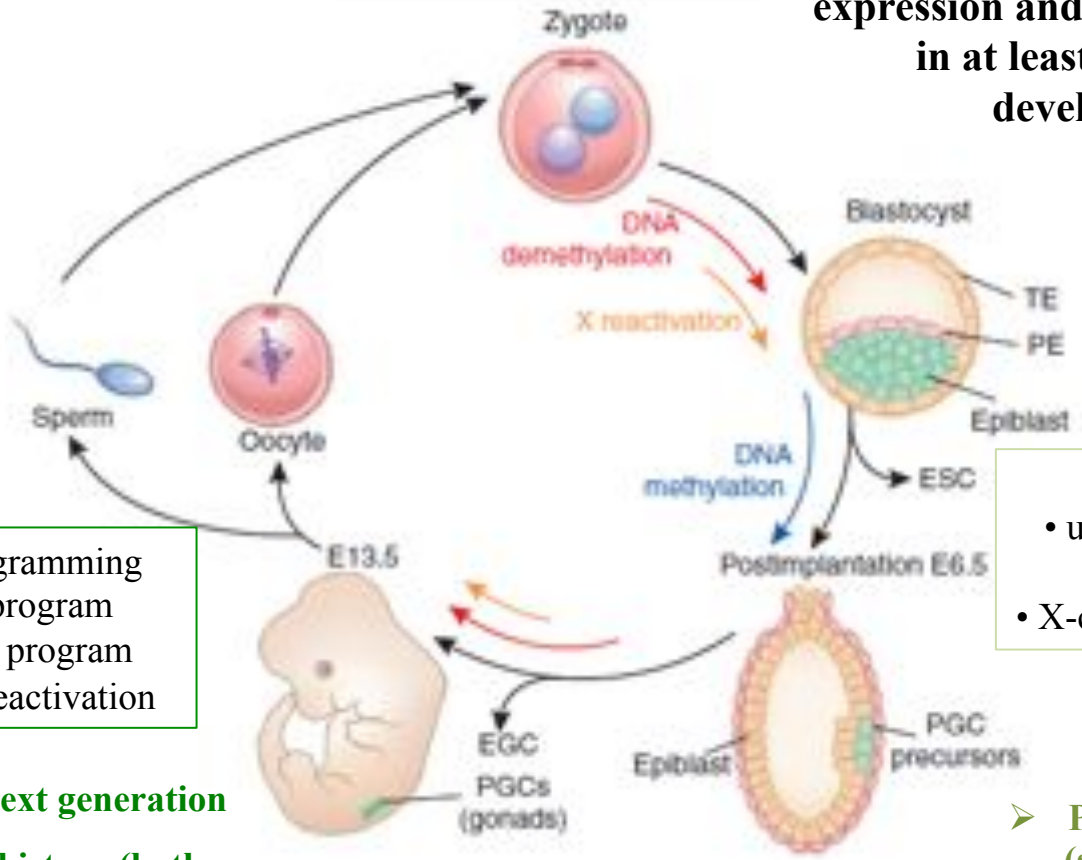
=> The parental genomes inherited from the gametes are <u>more competent</u> to be reprogrammed to totipotency and to support the subsequent changes in cell identity during early embryogenesis?

Developmental Reprogramming

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

Zygotic Reprogramming
• undo gamete programs
• set up totipotency

Dynamic changes in gene expression and epigenetic marks in at least 3 phases of development



Germ Line Reprogramming
• undo somatic program
• set up germ line program
• X-chromosome reactivation

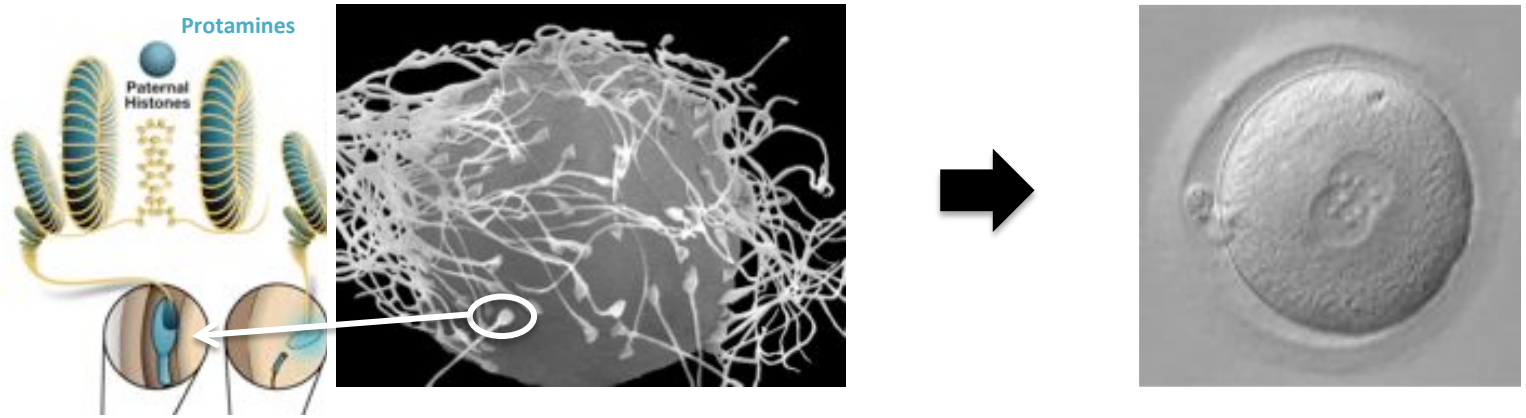
ICM Reprogramming
• undo/prevent TE program
• set up pluripotency
• X-chromosome reactivation

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

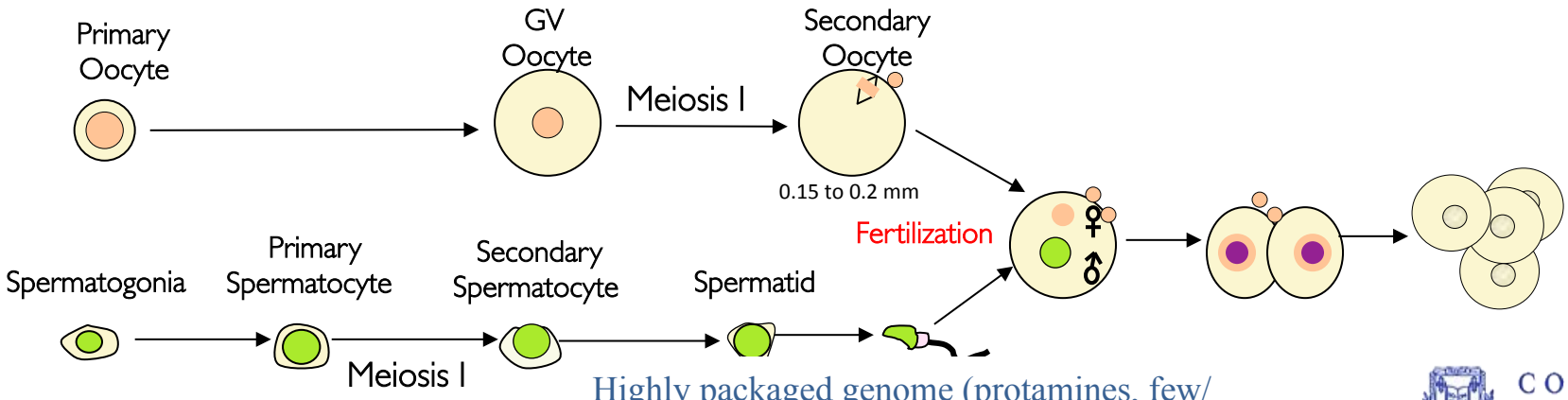
Adapted from Cantone and Fisher, 2013

In the beginning:

Two highly specialized cells, the egg and the sperm, fuse to form a totipotent cell, the zygote



Huge maternal store of proteins, mRNA (to be translated later) to ensure early development, and to enable reprogramming upon fertilization

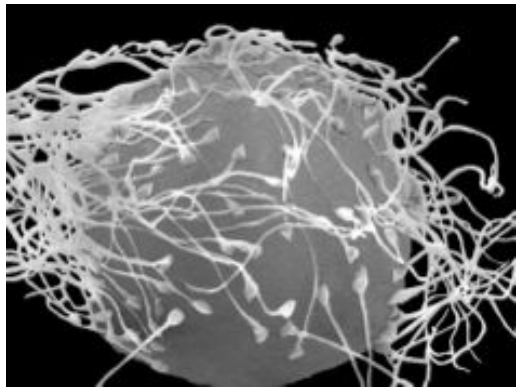


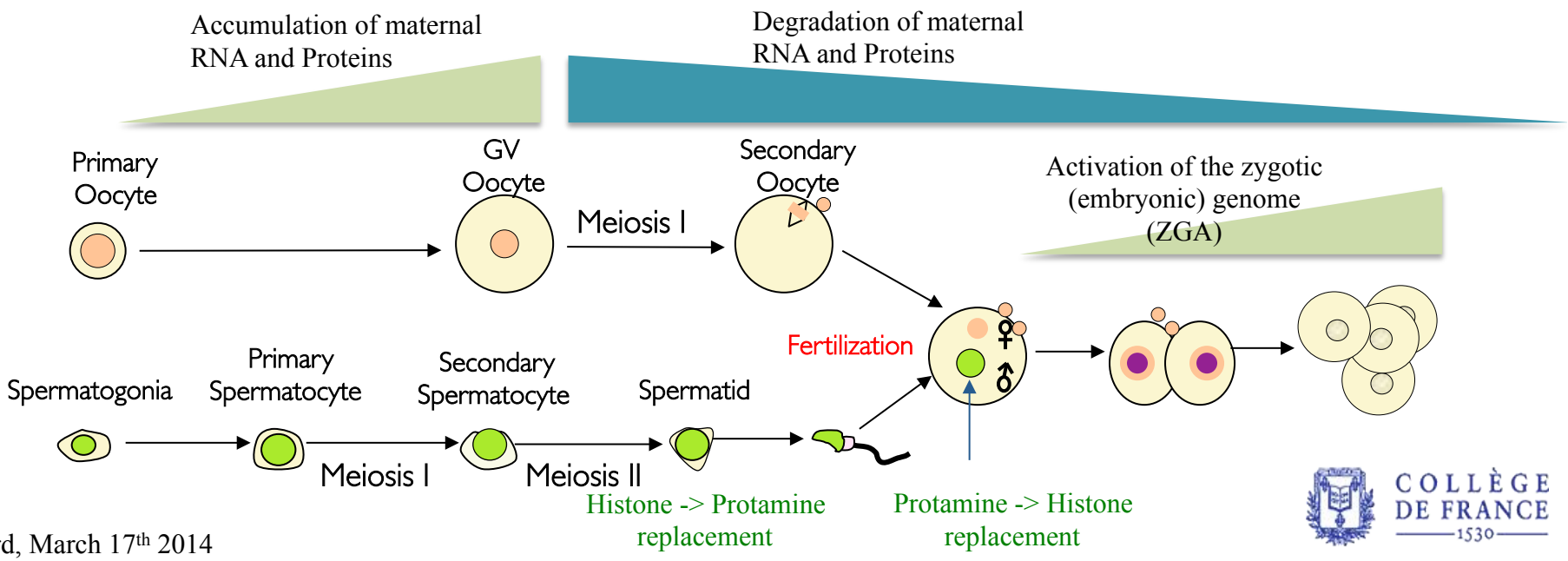
Highly packaged genome (protamines, few/ no histones) and a small amount of RNA

COLLÈGE DE FRANCE 1530

In the beginning:

Two highly specialized cells, the egg and the sperm, fuse to form a totipotent cell, the zygote





Numerous Maternal Factors Required to Orchestrate Reprogramming and appropriate Activation of the Embryonic Genome

A multitude of maternal factors are involved in:

erasing gametic epigenomic landscapes, preparing the embryonic genome for appropriate transcription of developmental genes, protecting some regions from reprogramming and ensuring others are silenced

Table 1. Maternal-effect genes in preimplantation development in mice

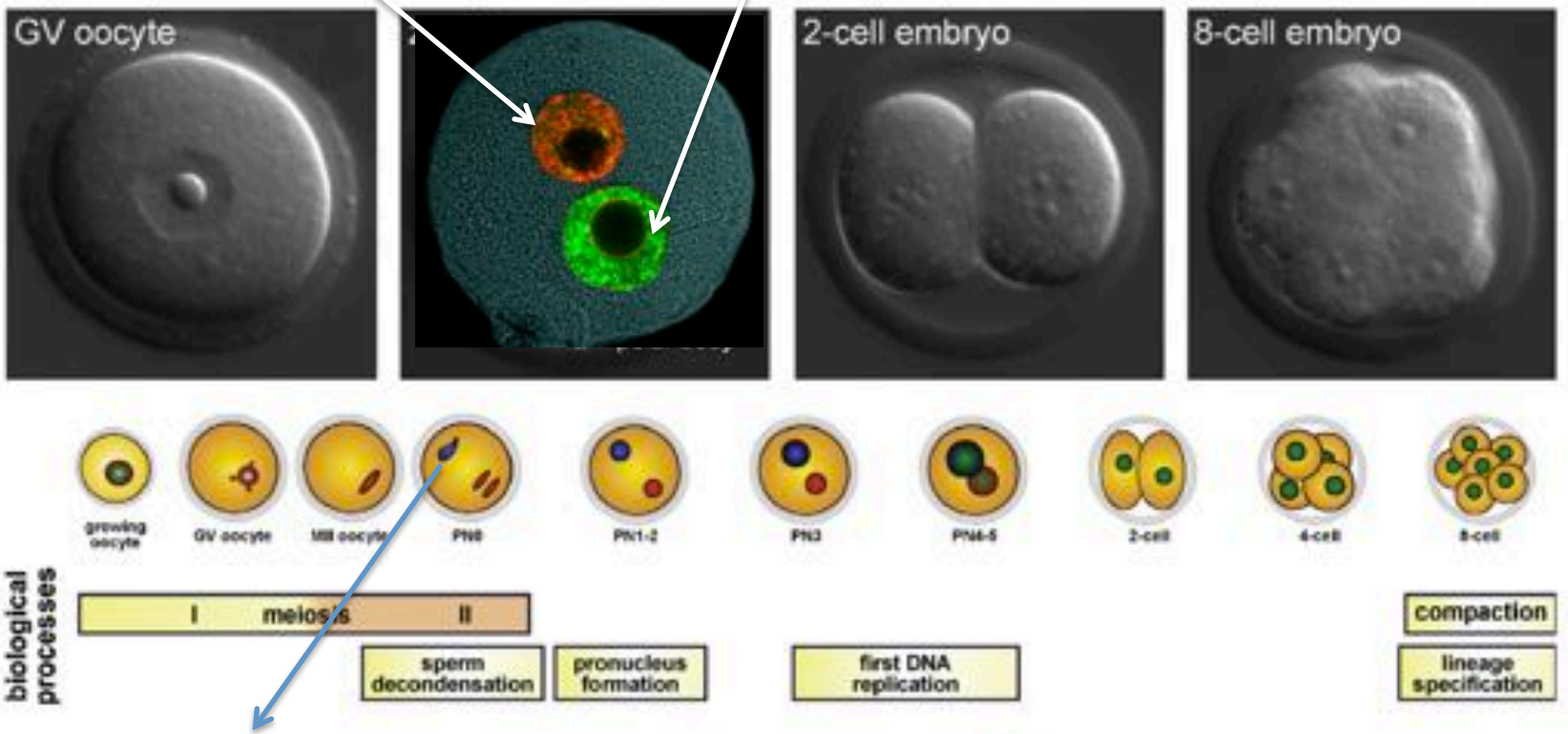
Gene (official symbol)	Protein	Domains and motifs (Pfam)	Human homolog	Null/RNAi
Degrades maternal factors				
Dicer1	Dicer1	DNA/RNA helicase DEAD/DEAH N; PAZ; DNA/RNA helicase C; Dicer dsRNA-binding fold; RNase III	*DICER1*	Murchison et al., 2007
Ago2 (Eif2c2)	Eukaryotic translation initiation factor 2C, 2	DUF1785; PAZ; Piwi	*EIF2C2*	Lykke-Andersen et al., 2008
Zfp36l2	Zinc-finger protein 36, C3H type-like 2	Tis11B N; Znf CCCH	*ZFP36L2*	Ramos et al., 2004
Atg5	Autophagy-related 5	Autophagy protein 5	*ATG5*	Tsukamoto et al., 2008
Chromatin remodelling				
Hr6a (Ube2a)	Ubiquitin-conjugating enzyme E2A, RAD6 homolog	UBQ-conjugat E2	*UBE2A*	Roest et al., 2004
Npm2	Nucleoplasmin 2	Nucleoplasmin	*NPM2*	Burns et al., 2003
Tif1α (Trim24)	Tripartite motif-containing 24	Znf C3HC4 RING-type; Znf B-box; Znf PHD-finger; Bromodomain	*TRIM24*	Torres-Padilla and Zernicka-Goetz, 2006
Brg1 (Smarca4)	SWI/SNF related, matrix associated actin-dependent regulator of chromatin subfamily a, member 4	Gln-Leu-Gln QLQ; HAS; BRK domain; Restrict endonuc I R/III Res; SNF2 N; DNA/RNA helicase C; Bromodomain	*SMARCA4*	Bultman et al., 2006
Brwd1	Bromodomain and WD repeat domain containing 1	WD40 repeat sg; Bromodomain	*BRWD1*	Philipps et al., 2008
Transcription factors				
Hsf1	Heat shock factor 1	HSF DNA bd; Vert HS TF	*HSF1*	Christians et al., 2000
Bnc1	Basonuclin 1	Znf C2H2	*BNC1*	Ma et al., 2006
Ctcf	CCCTC-binding factor	Znf C2H2; AT hook DNA-bd CS	*CTCF*	Wan et al., 2008
Oct4 (Pou5f1)	POU domain, class 5, transcription factor 1	POU specific; Homeobox	*POU5F1*	Foygel et al., 2008
Sox2	SRY-box containing gene 2	HMG HMG1/HMG2	*SOX2*	Avilion et al., 2003
De novo DNA methylation				
Dnmt3a	DNA methyltransferase 3-a	PWWP; CS DNA methylation	*DNMT3A*	Kaneda et al., 2004
Dnmt3l	DNA methyltransferase 3-like	No significant matches	*DNMT3L*	Bourc'his et al., 2001
DNA methylation maintenance				
Dnmt1	DNA methyltransferase 1	DMAP1 bd; Znf CXXC; BAH; CS DNA methylation	*DNMT1*	Howell et al., 2001; Hirasawa et al., 2008
Stella (Dppa3)	Developmental pluripotency-associated 3	No significant matches	*DPPA3*	Payer et al., 2003
Zfp57	Zinc-finger protein 57	Kruppel-associated box; Znf C2H2	*ZFP57*	Li et al., 2008b
Preimplantation development				
Zar1	Zygote arrest 1	No significant matches	*ZAR1*	Wu et al., 2003
Mater (Nlrp5)	Maternal antigen that embryos require	NACHT NTPase; Leu-rich repeat	*NLRP5*	Tong et al., 2000
Floped (Ooep)	Factor located in oocyte permitting development	Atypical KH	*OOEP*	Li et al., 2008a
Padi6	Peptidyl arginine deiminase (PAD), type VI	PAD N; PAD central; PAD C	*PADI6*	Esposito et al., 2007
Tle6	Transducin-like enhancer of split 6	WD40 repeat	*TLE6*	
Filia (2410004A20Rik)	Filia	No significant matches	*FILIA*	Zheng and Dean, 2009
Tcl1	T-cell lymphoma breakpoint 1	TCL1 MTCP1	*TCL1*	Narducci et al., 2002
Uchl1	Ubiquitin carboxyl-terminal hydrolase L1	Peptidase C12	*UCHL1*	Sekiguchi et al., 2006

Fertilization triggers massive reorganization of the paternal and maternal epigenomes (prior to transcription)

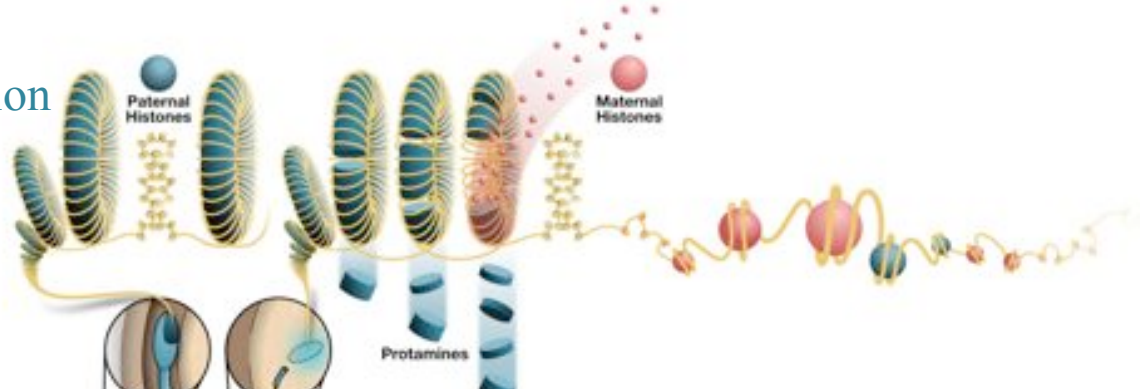
Maternal interphase pronucleus

Paternal interphase pronucleus

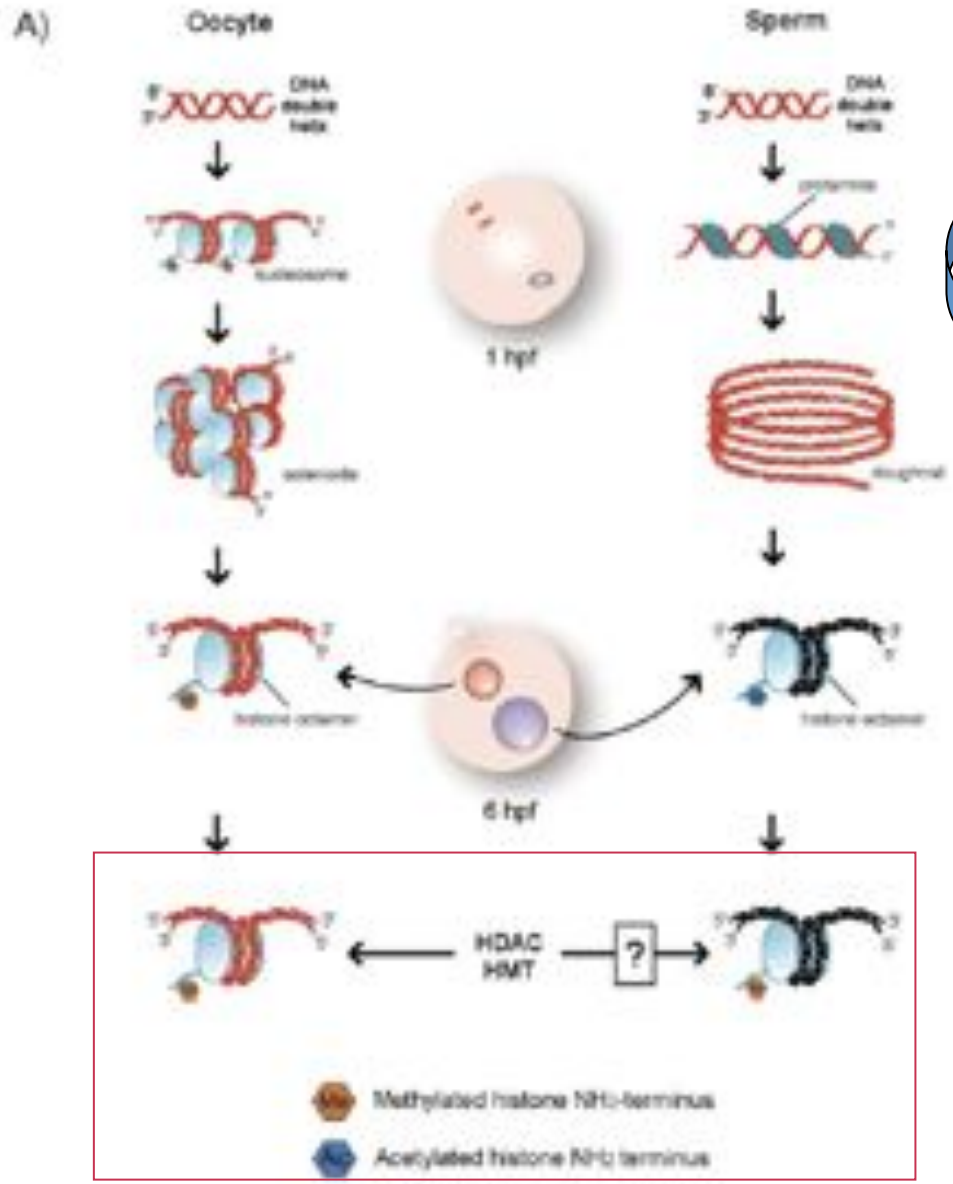
The two parental pronuclei remain separate initially



Protamine eviction,
maternal histone incorporation



Fertilization triggers massive reorganization of the paternal and maternal epigenomes (prior to transcription)



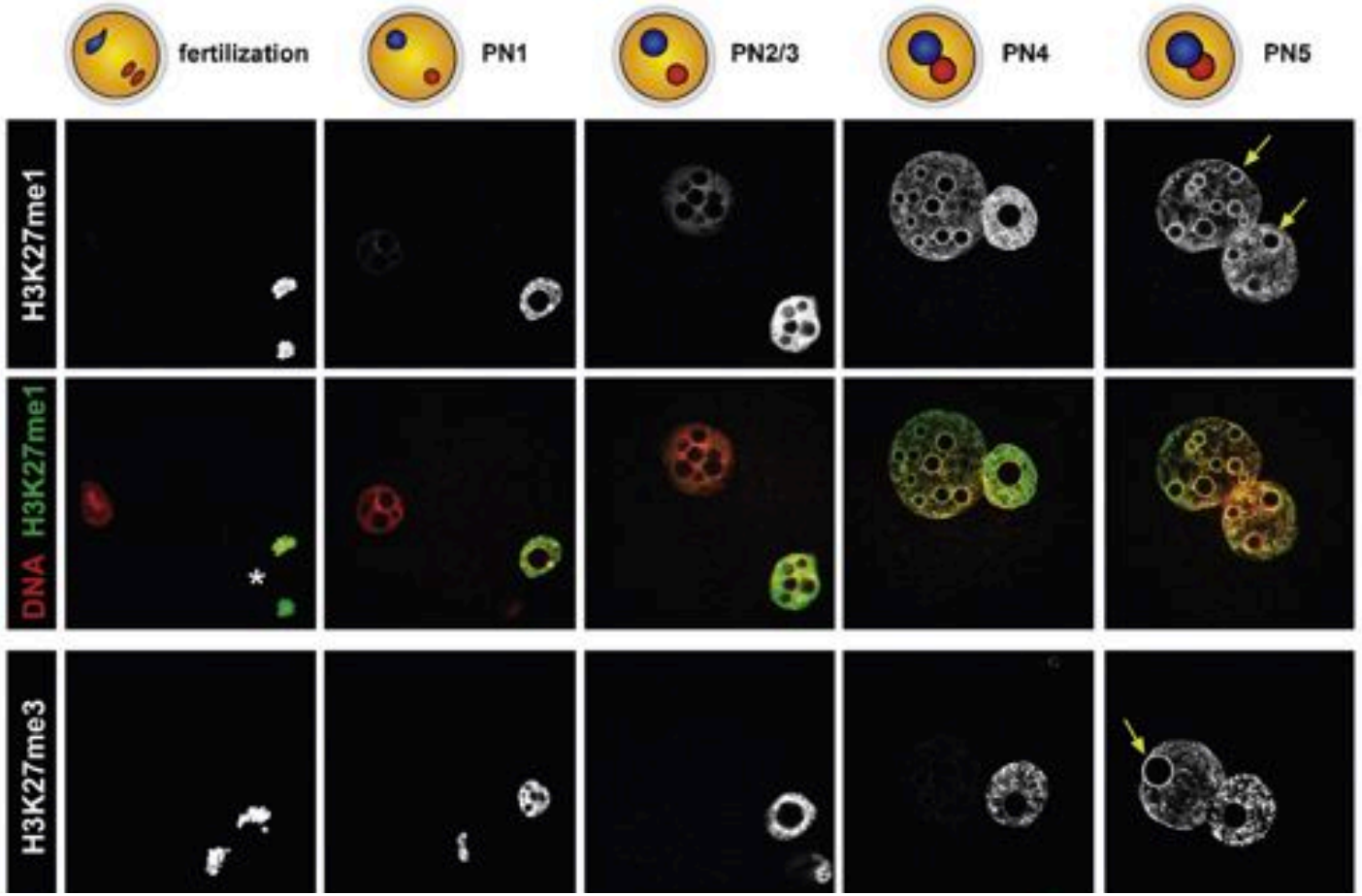
protamine - histone exchange

Asymmetric Chromatin states

from Santos & Dean, *Reproduction*, 2004

COLLÈGE DE FRANCE 1530

Fertilization triggers massive reorganization of the paternal and maternal epigenomes (prior to transcription)



H3.3 lys27 methylation 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).

Fertilization triggers massive reorganization of the paternal and maternal epigenomes (prior to transcription)

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

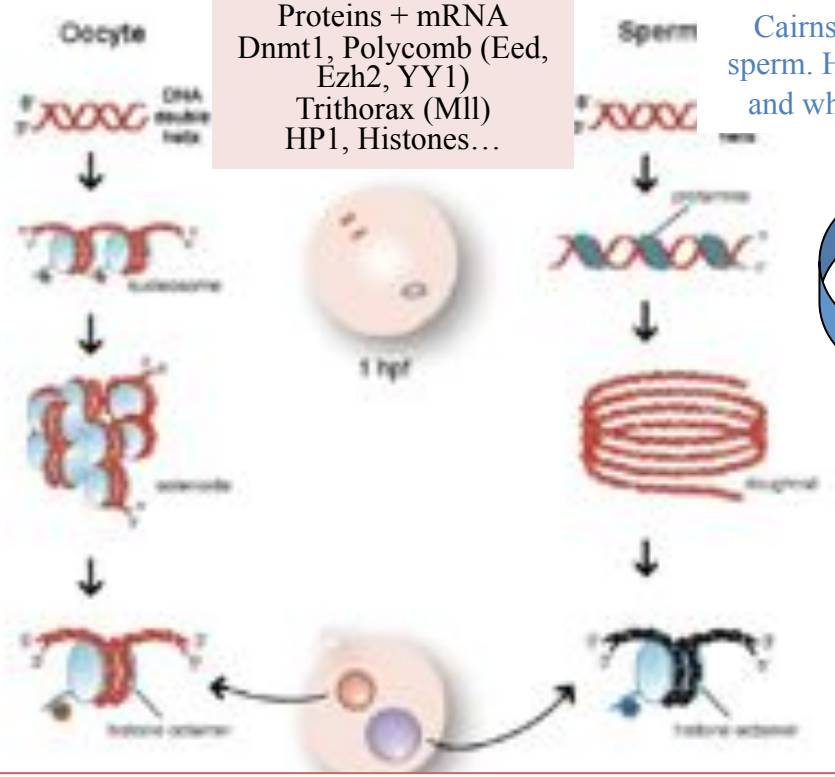
Maternal Pool:
Proteins + mRNA
Dnmt1, Polycomb (Eed, Ezh2, YY1)
Trithorax (Mll)
HP1, Histones…

Paternal chromatin inheritance:
Cairns and Peters groups: 1-15% histones in sperm. How much of this resists reprogramming and what role they might play is still unclear

Maternal H3.3

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



protamine - histone exchange

HIRA
van der Heidjden et al, 2005

H4Ac (++)
H3K9Ac
H3K9me1

Is zygotic chromatin dynamics actually important for subsequent development?
How much is just a consequence of major chromatin remodelling?

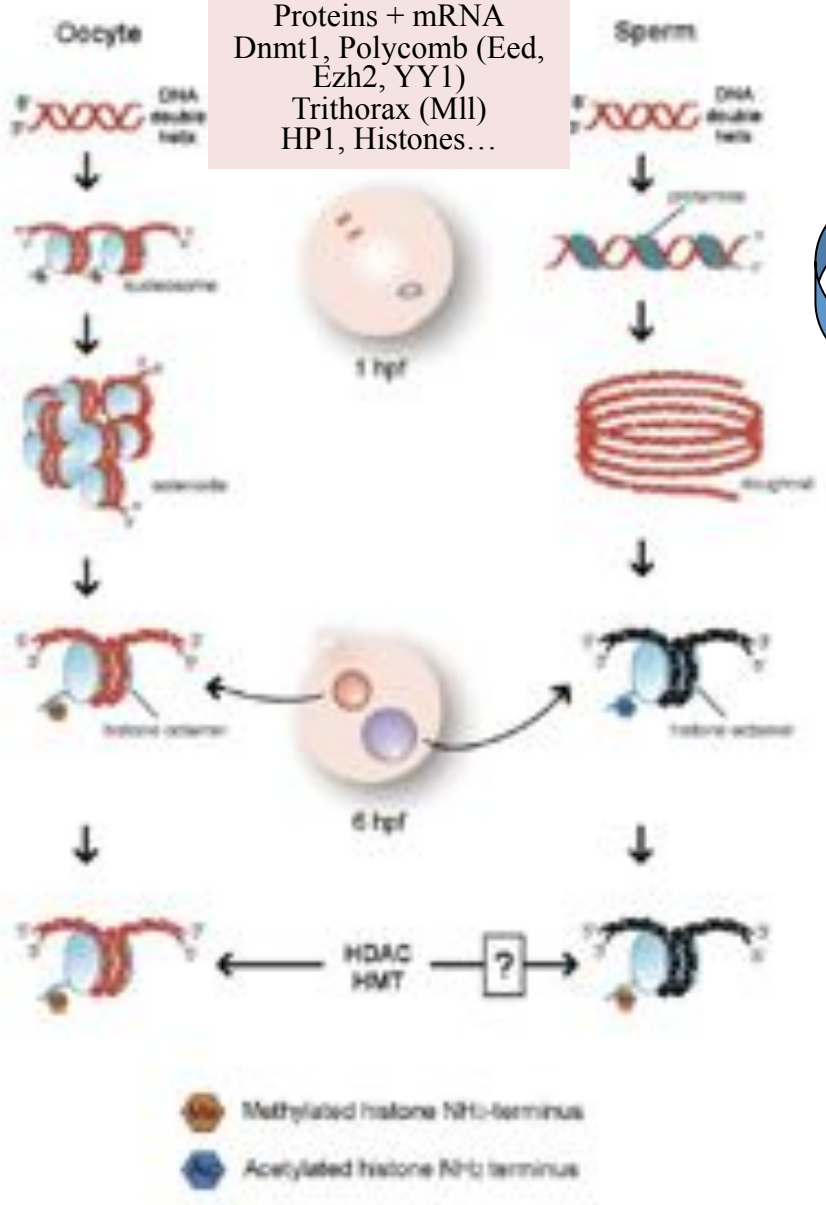
- Maternal Mll2 (TrX) is required for the acquisition and maintenance of H3K4 methylation in the zygote and for normal embyonic gene activation (Andreu-Vieyra et al, 2010)
- In the absence of maternal histone variants TH2A/TH2B, paternal genome activation, which accompanies H3K4me3 and DNA demethylation, is defective. (Shinagawa et al, 2013)

NB TH2A/TH2B also enhances OSKM reprogramming during iPS!

Fertilization triggers massive reorganization of the paternal and maternal epigenomes (prior to transcription)

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

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



H4Ac
H3K4me3
H3K9Ac
H3K9me1
H3K9me2
H3K9me3
H3K4me1
H3K27me1
H4K20me1

DNA Demethylation (passive)

Maternal H3.3

protamine - histone exchange

HIRA
van der Heidjden et al, 2005

DNA demethylation
active (ie before DNA replication)
& passive

H4Ac (++)
H3K9Ac
H3K9me1
H3K4me1
H3K27me1
H4K20me1

H3K4me3

from Santos & Dean, *Reproduction*, 2004

E. Heard, March 17th 2014

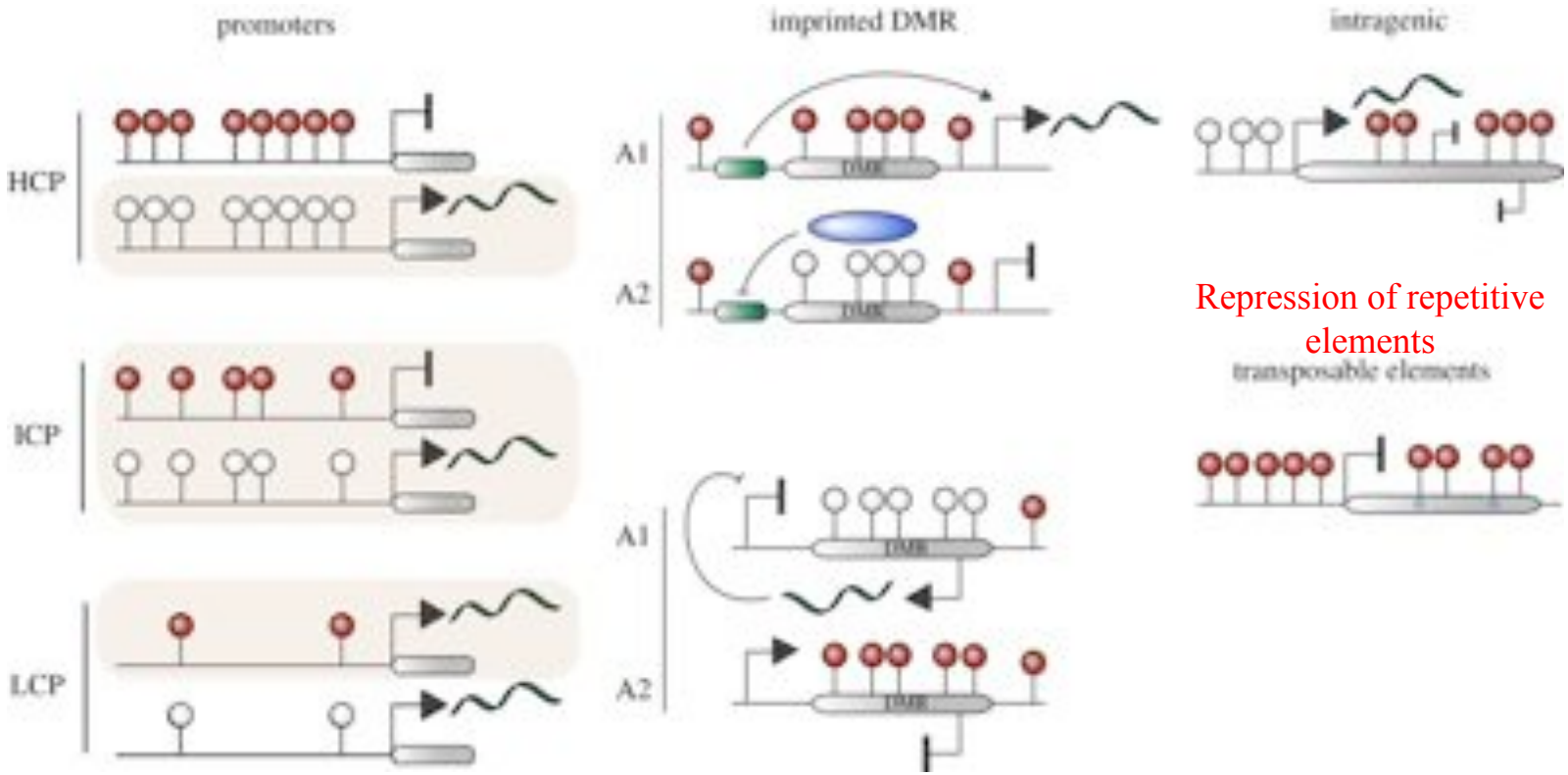
COLLÈGE DE FRANCE 1530

DNA Methylation in the Mammalian Genome

Lineage-specific DNA methylation
Participates in maintaining cellular identity

Parent-of-origin specific
DNA methylation

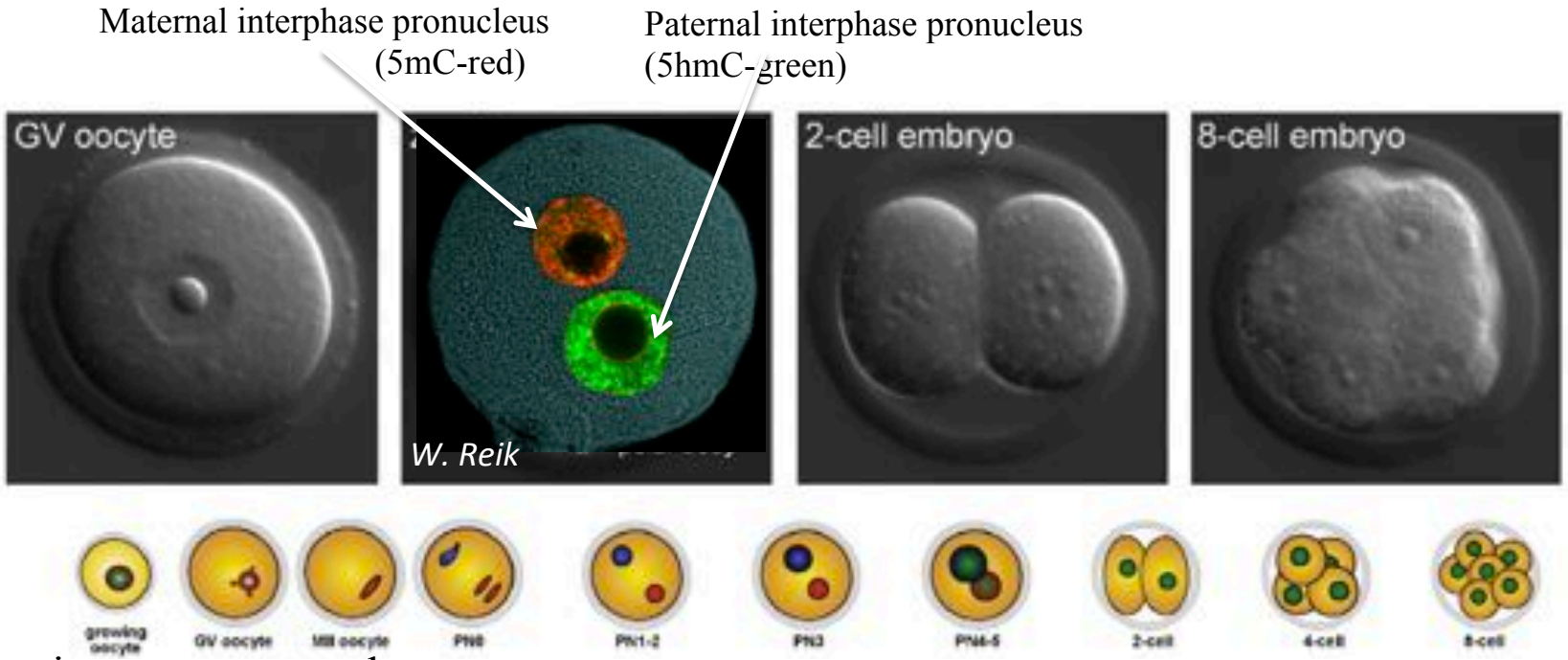
Transcription-associated
DNA methylation of gene bodies



Repression of repetitive elements

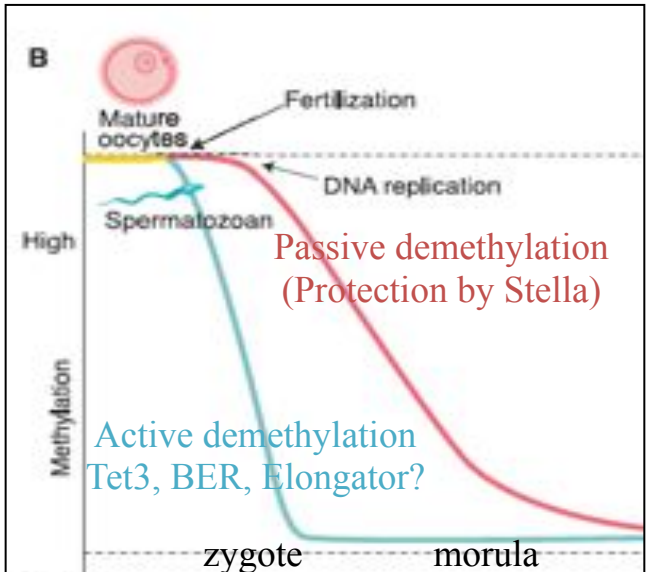
After fertilisation a globally demethylated state is established. Then, a progressively lineage-specific DNA methylome is acquired during pre-implantation development that maintains cellular identity and genomic stability

Developmental Dynamics of DNA Methylation



A few regions are <u>protected</u> from demethylation: DNA sequences that carry parental "imprints"

<u>Parental imprinting:</u>
Paternal or maternal specific gene expression
Essential for normal development
(Cattanach, Surani, Solter)
(see last week)



What about repeats?
Transposons, retrotransposons and endogenous retroviruses….

Control of Repeat Elements after Fertilization?

• Retrotransposons, eg endogenous retroviruses (ERVs), present in mammalian genomes must be controlled – their expression and mobility/reintegration can be deleterious

• In the soma and germ line: repression of repeats is via DNA methylation (& piRNAs in the latter)

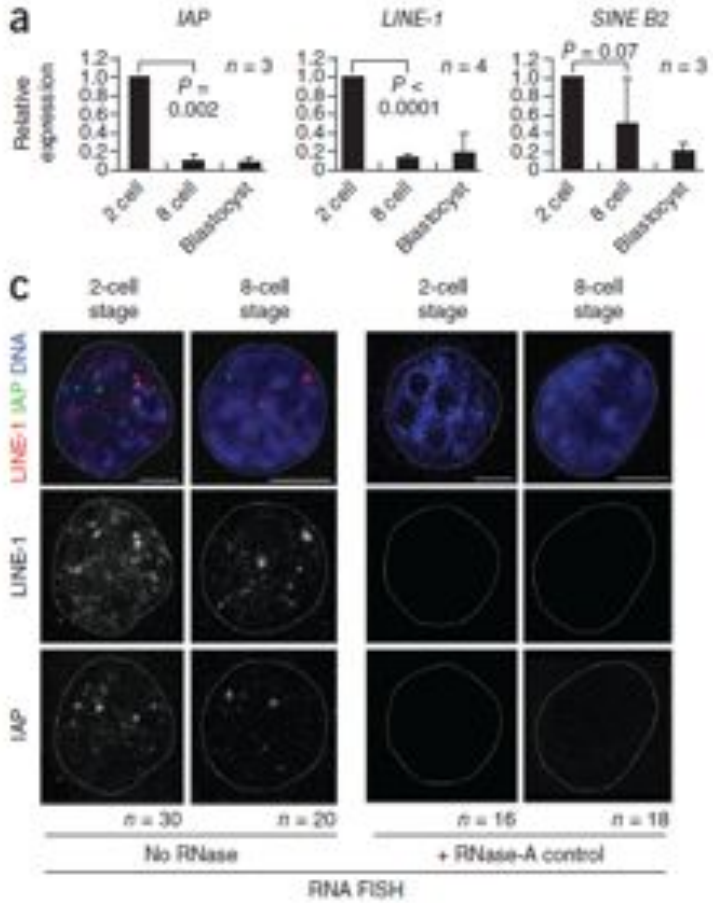
• In early embryo: global DNA hypomethylation and no piRNA machinary mean that repeats can (and some do) become expressed. (Bachvarova, 1988; Efroni et al., 2008; Evsikov et al., 2004; Packer, Manova, & Bachvarova, 1993; Peaston et al., 2004).

➢ Might they play a role(s) in early development?
(Peaston et al. 2004, Beraldi et al, 2006 and others)

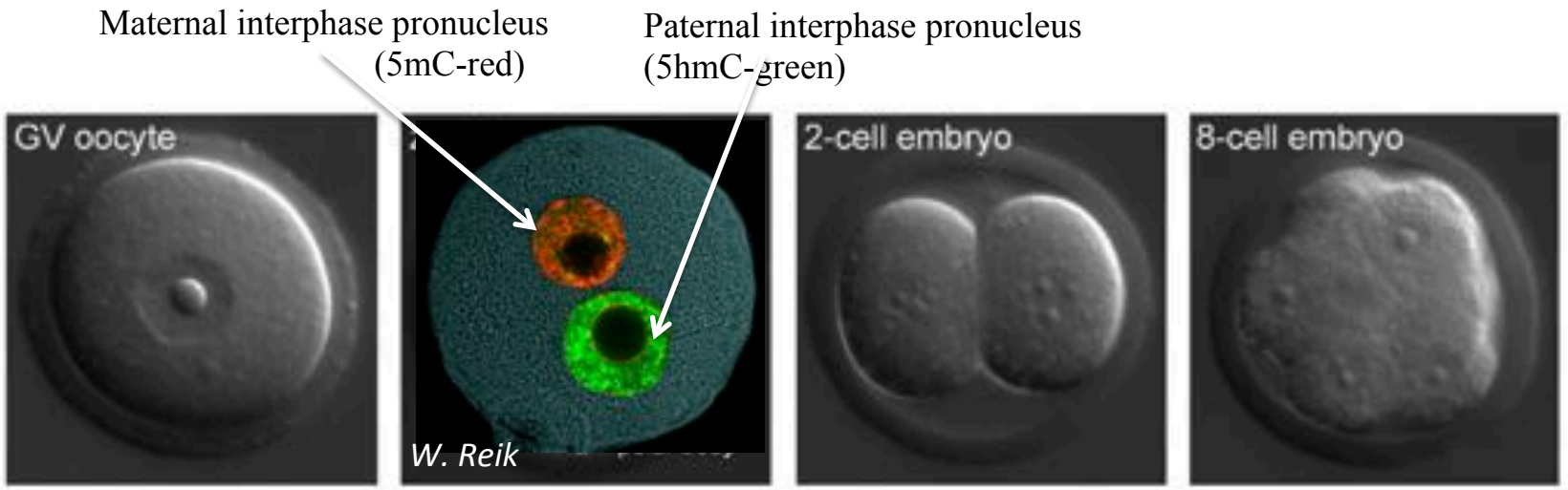
• DNA meth-independent mechanisms, involving histone modifications, may control repeat silencing and heterochromatin formation during pre-implantation development.

Fadloun et al. 2013 « Chromatin signatures and retrotransposon profiling in mouse embryos reveal regulation of LINE-1 by RNA ».

Peters et al 2001. Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. Cell, 107, 323–337.



Reprogramming in the Zygote



Male genome

Does transient epigenetic asymmetry have a role?
Is it simply the result of the different histories of the paternal and maternal (epi)genomes?
Does repeat expression have a role, or is it simply a result of incomplete silencing following reprogramming?

The two parental genomes have different reprogramming requirements
This is <u>essential</u> for subsequent development
At the same time imprints must be preserved….
(Cours IV – Mechanisms)

2013

E. H

Pre-implantation Mouse Development

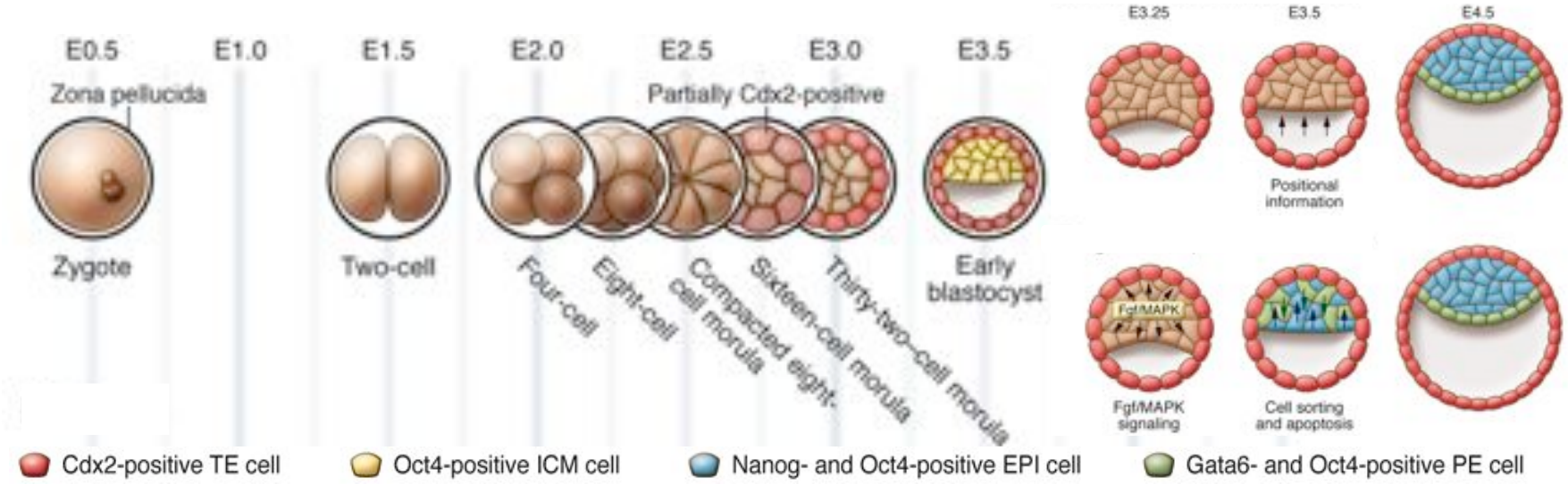
The next steps:

The road to extra-embryonic tissue formation
and to the embryo proper (pluripotency)

Pre-implantation Mouse Development

• Progressive restriction of cellular plasticity from 4-cell stage (Totipotency lost at this stage in mouse)

• Early preimplantation mouse development is highly regulative - cleavage stage blastomeres are developmentally plastic and influenced by cell-cell interactions



First cell lineage to be specified is the **trophectoderm (TE)**

Beginning at 8-16 cell stage (morula compaction), **inside-outside** and/or **cell polarity changes** result in transcription factor (TF) modulation that ultimately translate into **cell fate**

• Cdx2 up-regulation in outer cells (via Tead4/Yap1 and Hippo signalling) lead to TE specification

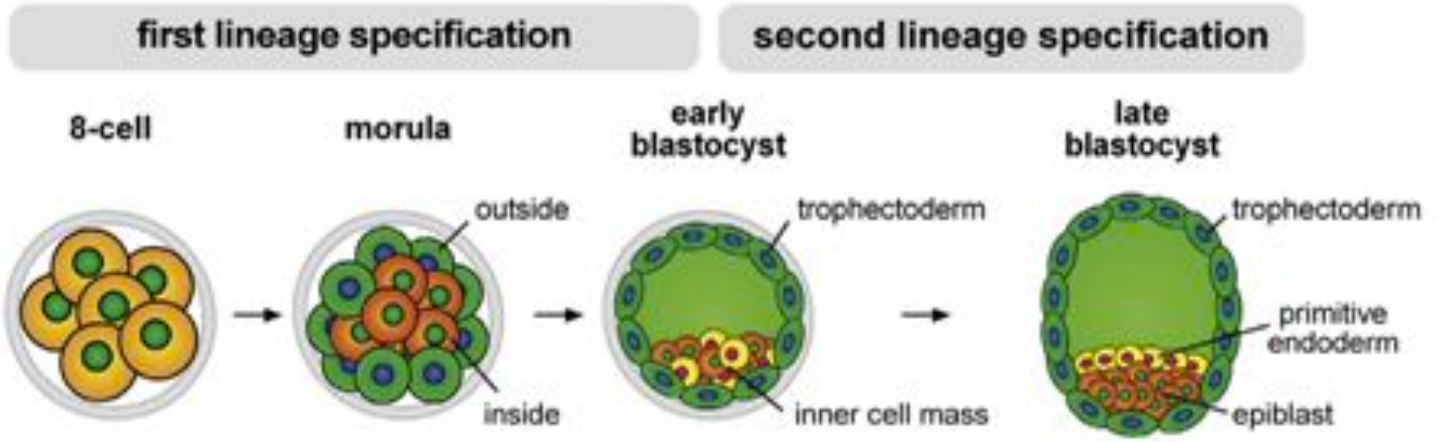
• Pluripotency markers Oct4, Nanog and Sox2 become progressively up-regulated in inner cells, ICM

• Oct4 is essential for ICM and its levels distinguish between TE, ICM, PE (no Oct4->TE; hi Oct4-> PE)

Second cell lineages to be segregated in the **ICM** are primitive endoderm (PE) & epiblast (EPI) Through **position-dependent** and/or **Fgf/MAPK signalling** determination

Adapted from Cockburn and Rossant, JCI, 2010

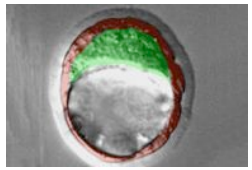
Transcriptional Networks leading to Early Lineage Specification



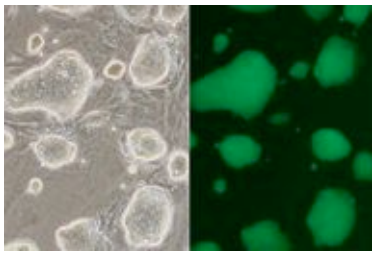
GATA6/Nanog/Oct4 levels and ERK signalling are key for the second Prim Endoderm vs Epiblast decision

⇒ Positional information triggers signalling and TF modulation - for both lineage specifications, and cell fate progressively becomes locked in epigenetically.

Pluripotent "ground state" is transiently present in the pre-epiblast
Oct4+/Nanog+/GATA6- cells = ES cells



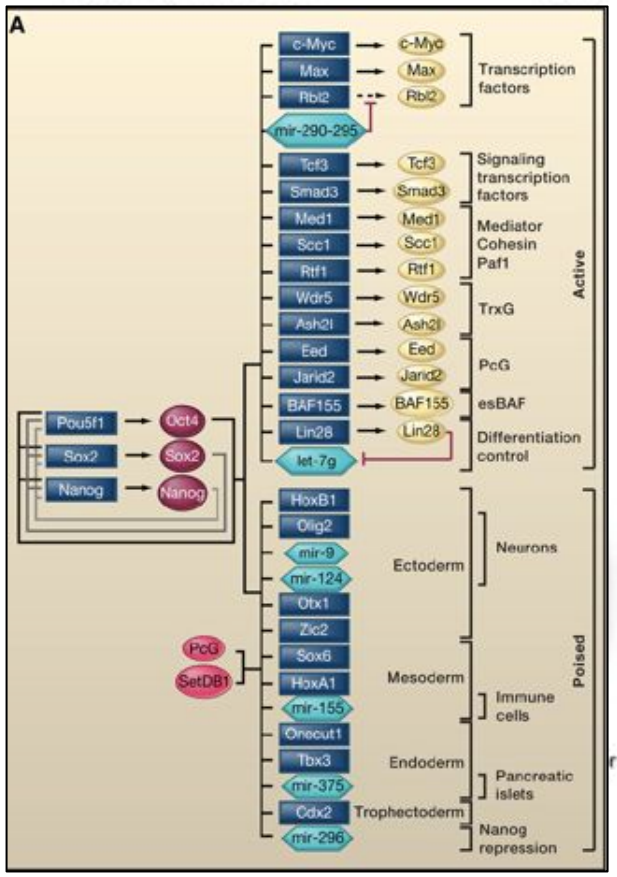
Capable of self-renewal
Pluripotent – can form teratomas
can differentiate into all 3 germ layers



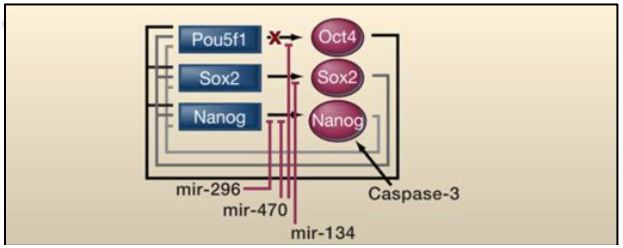
Pluripotency and the Ground State in ES cells

Pluripotency transcriptional network driven by the core transcription factors Oct4, Nanog, Sox2, Klf4 is essential to maintain the undifferentiated state.

This network activates genes that are required for ES cell survival and proliferation while repressing target genes that are activated only during differentiation.



Transcriptional feed-forward loops, specific miRNAs and chromatin states, promote the pluripotency network



Oct4 gene silencing, degradation of Nanog, miRNAmediated reduction in Oct4, Nanog, and Sox2 mRNA levels.

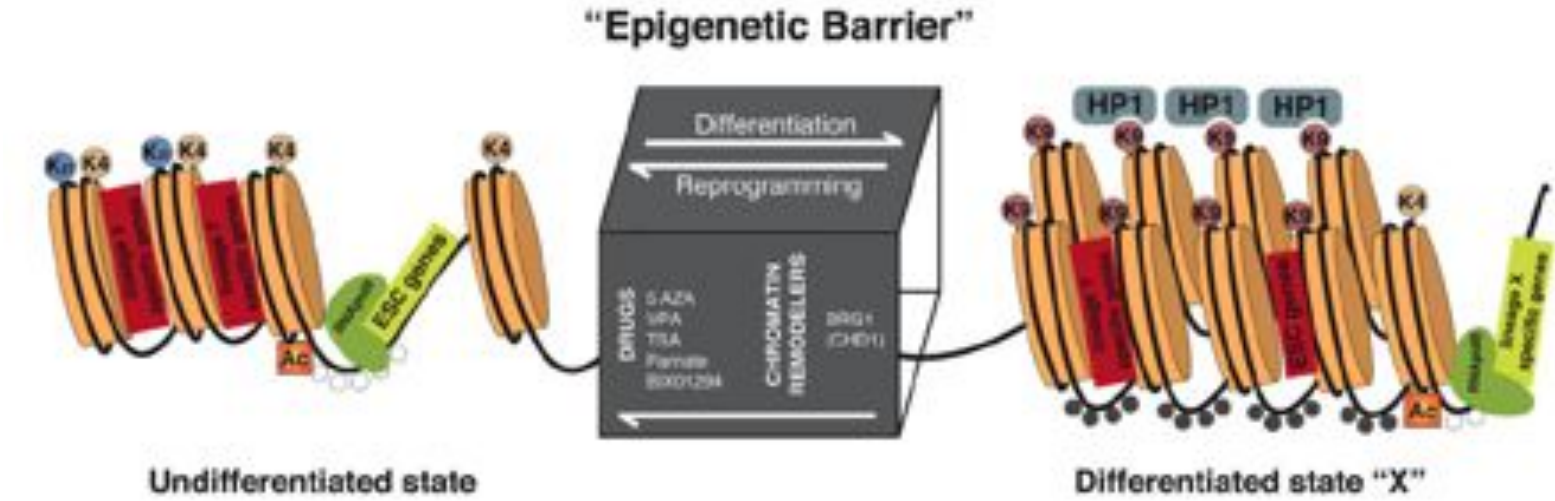
NB Ground state is transient in vivo
The pluripotency network is rapidly dismantled and only re-established in the germ line

COLLÈGE DE FRANCE 1530

Pluripotency and the Ground State in ES cells

Pluripotency transcriptional network driven by the core transcription factors Oct4, Nanog and Sox2, is essential to maintain the undifferentiated state.

This network activates genes that are required for ES cell survival and proliferation while repressing target genes that are activated only during differentiation.



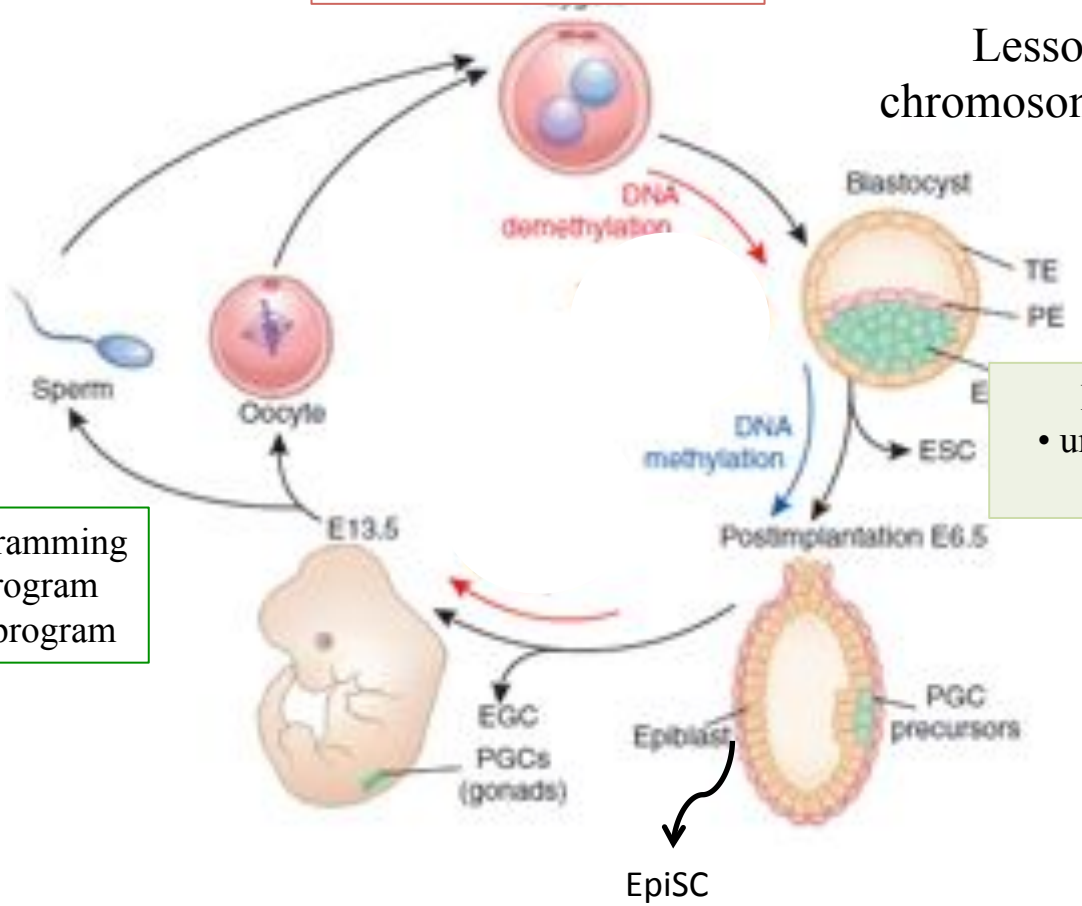
In Primed ESC (*not* Ground state!):
Poised state with « bivalent » domains
H3K27me3+H3K4
Transcription factors, Oct4…

ESC gene silencing: H3K9me3
DNA methylation, PcG

Epigenetic Dynamics during Pre-Implantation Development

Zygotic Reprogramming
• undo gamete programmes
• set up totipotency

Lessons from X-chromosome inactivation?



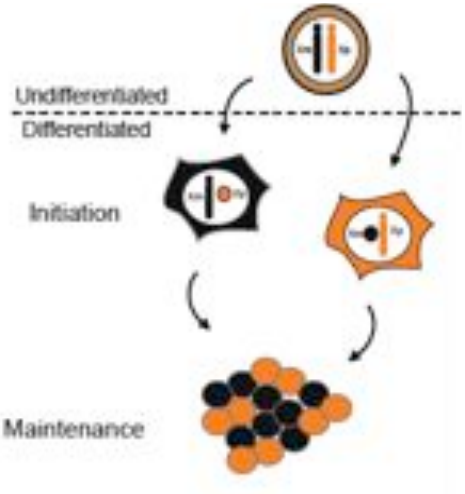
ICM Reprogramming
• undo/prevent TE program
• set up pluripotency

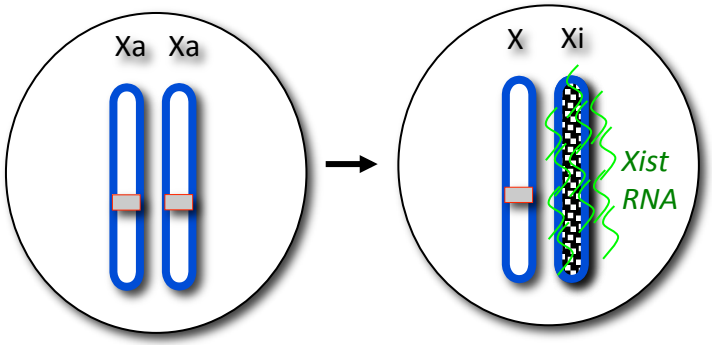
Germ Line Reprogramming
• undo somatic program
• set up germ line program

Cantone & Fisher Nat Struc & Mol Biol revue 2013

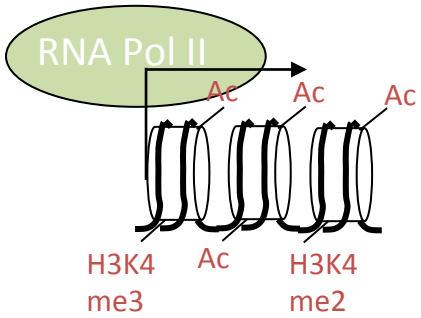
X-Chromosome Inactivation

One of the two X chromosomes must be silenced during early embryogenesis in order for female development to proceed

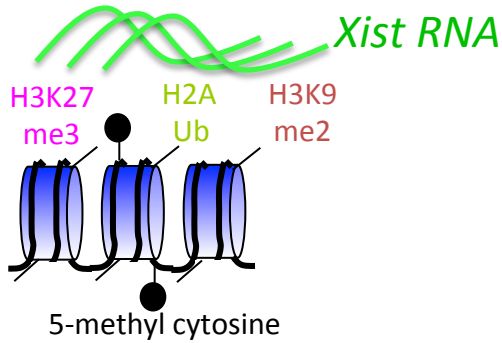




Active X chromosome

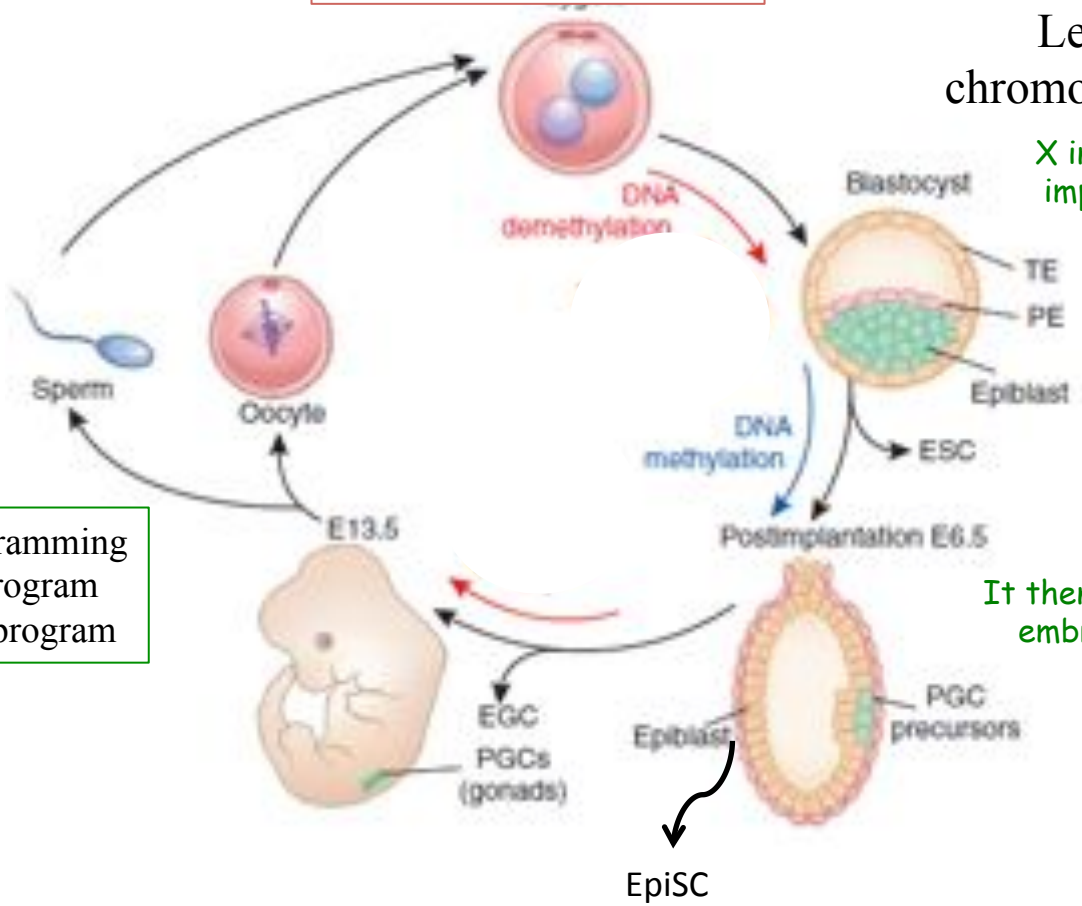


Inactive X chromosome



Developmental Reprogramming in the Inner Cell Mass

Zygotic Reprogramming
• undo gamete programmes
• set up totipotency



Germ Line Reprogramming
• undo somatic program
• set up germ line program

Lessons from X-chromosome inactivation?

X inactivation is initially imprinted in the mouse (paternal X only)

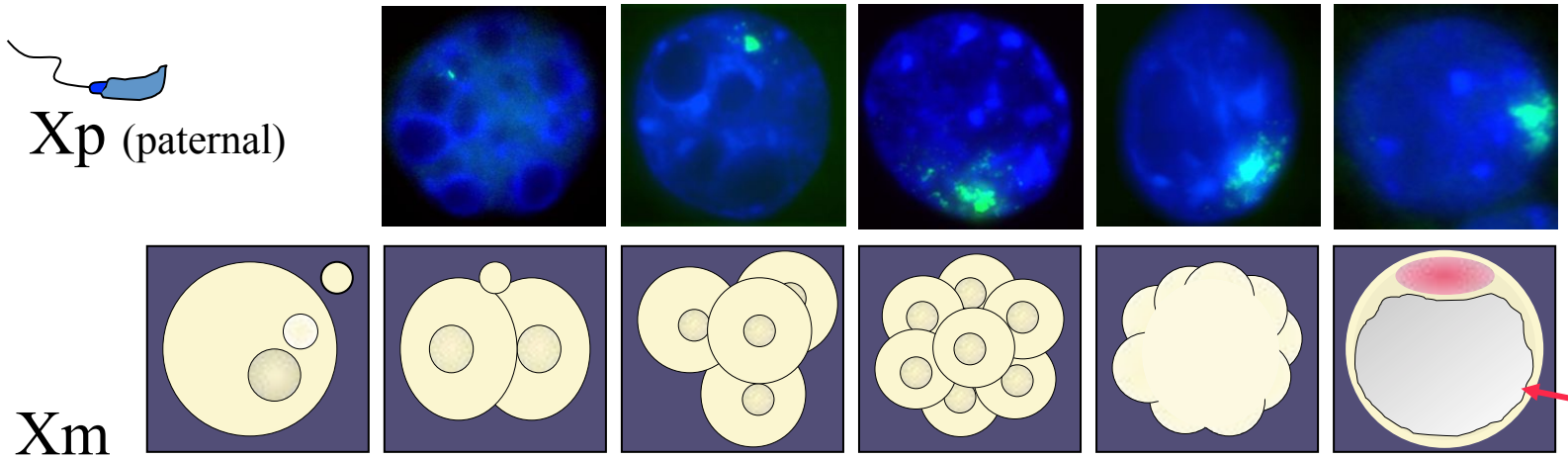
?

It then becomes random in the embryo proper (Xm or Xp)

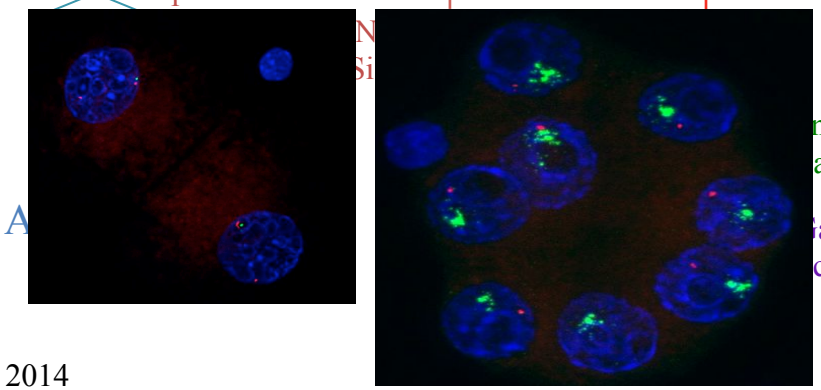
Cantone & Fisher Nat Struc & Mol Biol revue 2013

X-Chromosome Inactivation during Pre-Implantation Development

When is the paternal X silenced?
Are cells set aside with an active Xp that will give rise to the embryo proper?



Kay et al, 1994
Huyhn and Lee, 2003
Okamoto et al, 2004
Mak et al, 2004
Patrat et al, 2009

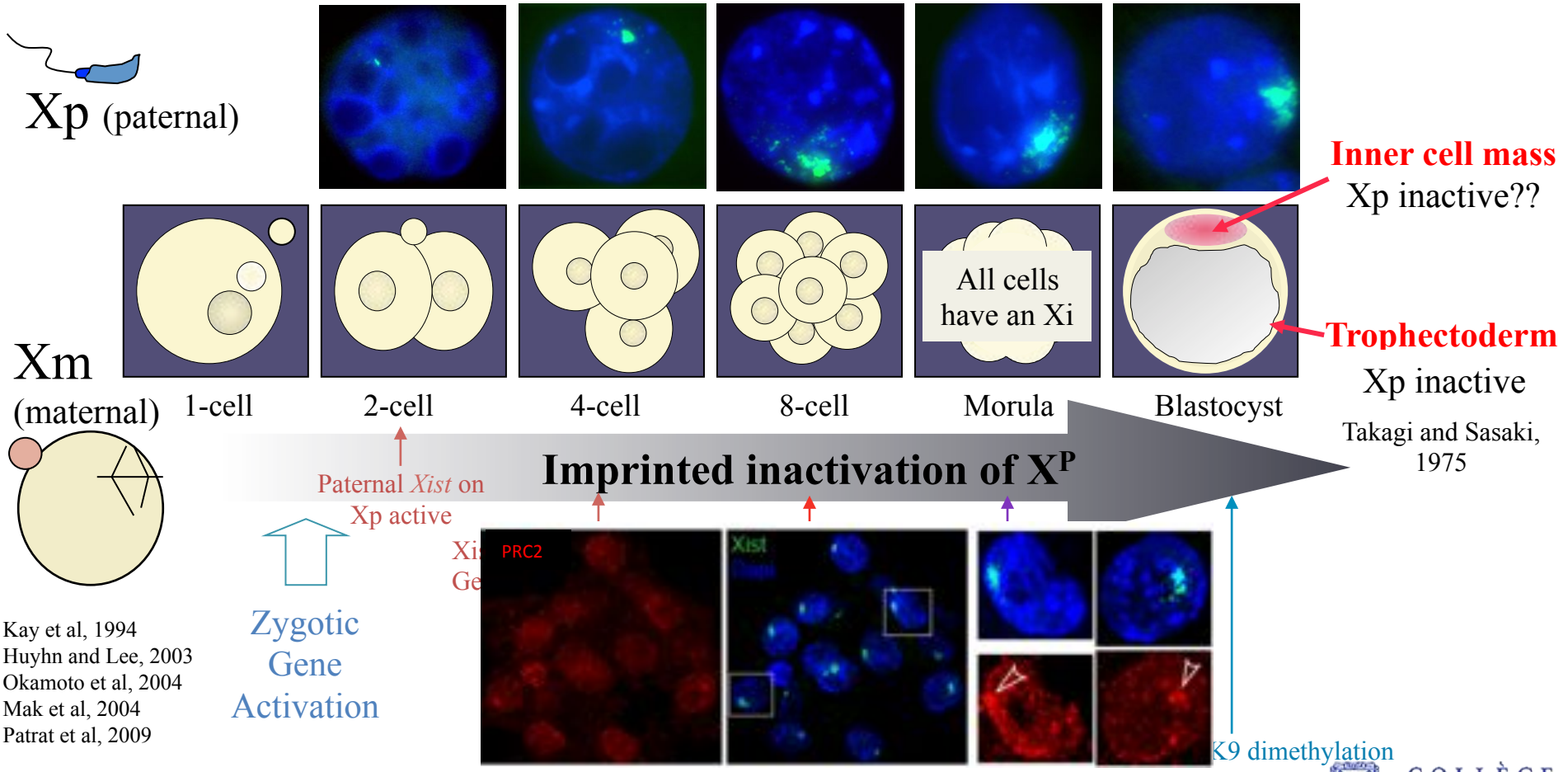


E. Heard, March 17th 2014

COLLÈGE DE FRANCE 1530

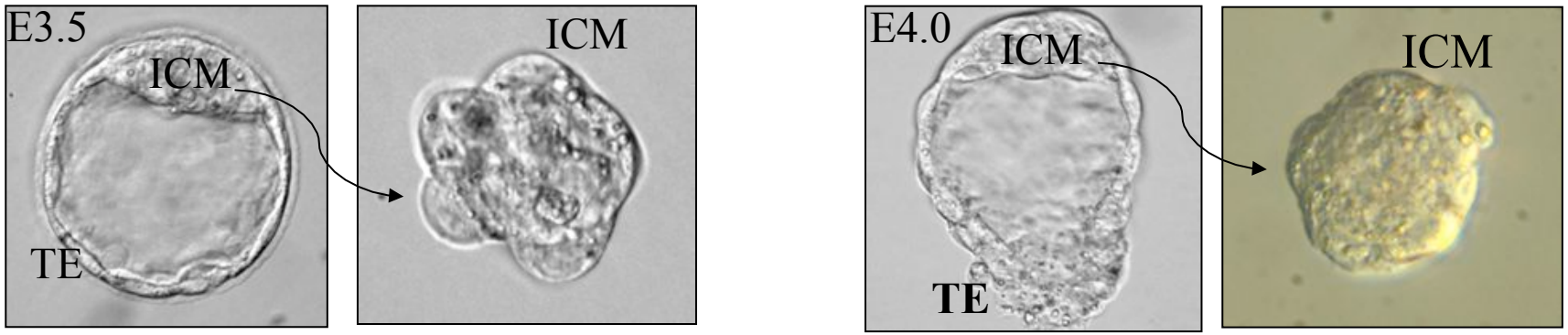
X-Chromosome Inactivation during Pre-Implantation Development

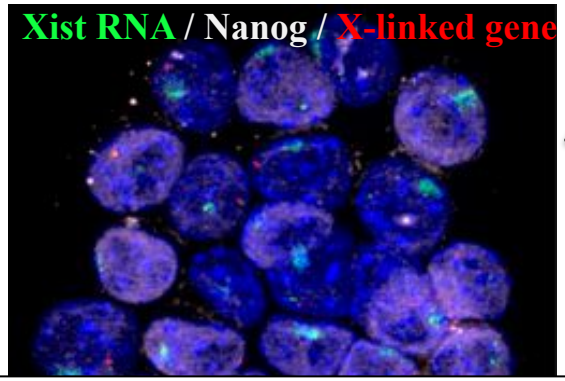
When is the paternal X silenced?
Are cells set aside with an active Xp that will give rise to the embryo proper?



Kay et al, 1994
Huyhn and Lee, 2003
Okamoto et al, 2004
Mak et al, 2004
Patrat et al, 2009

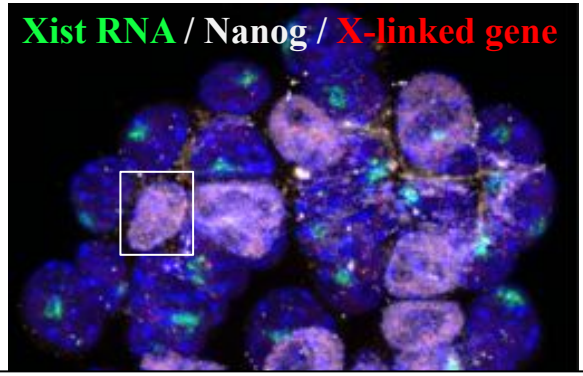
The paternal X is reactivated in the ICM (E3.5-E4.5)





↑Nanog, Oct4, Sox2
Xist repression
Xi reactivation

Okamoto et al, 2004

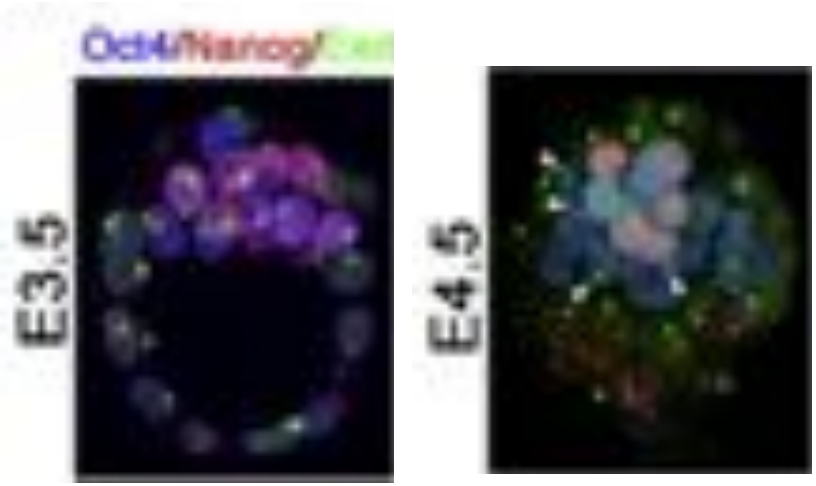


The inactive Xp (PRC1/2+, mH2A+, but *not* DNA me) is reprogrammed in the ICM in epiblast cells within 1-2 cell cycles

First *in vivo* evidence of such epigenetic dynamics in ICM
(Okamoto et al, 2004; Mak et al, 2004).

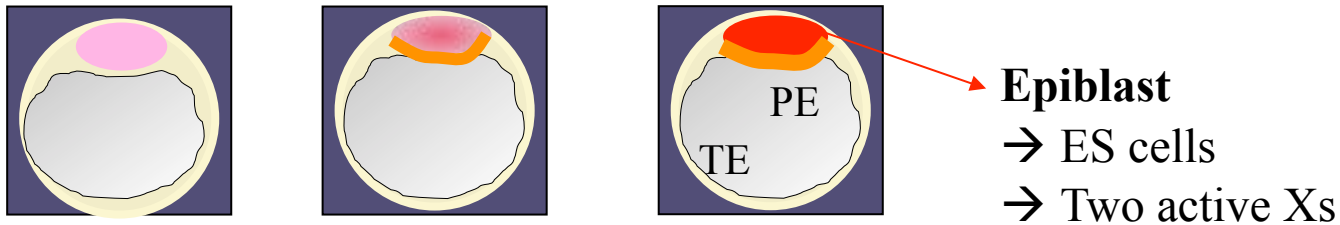
Symptomatic of more global reprogramming?
Resetting pluripotency following lineage restriction to TE?

The Pluripotent State imposes X-chromosome Reactivation



Nanog (+Sox2, Oct4…?) (Silva et al, Cell 2009)

***Xist* repression + Gene reactivation + Chromatin changes**



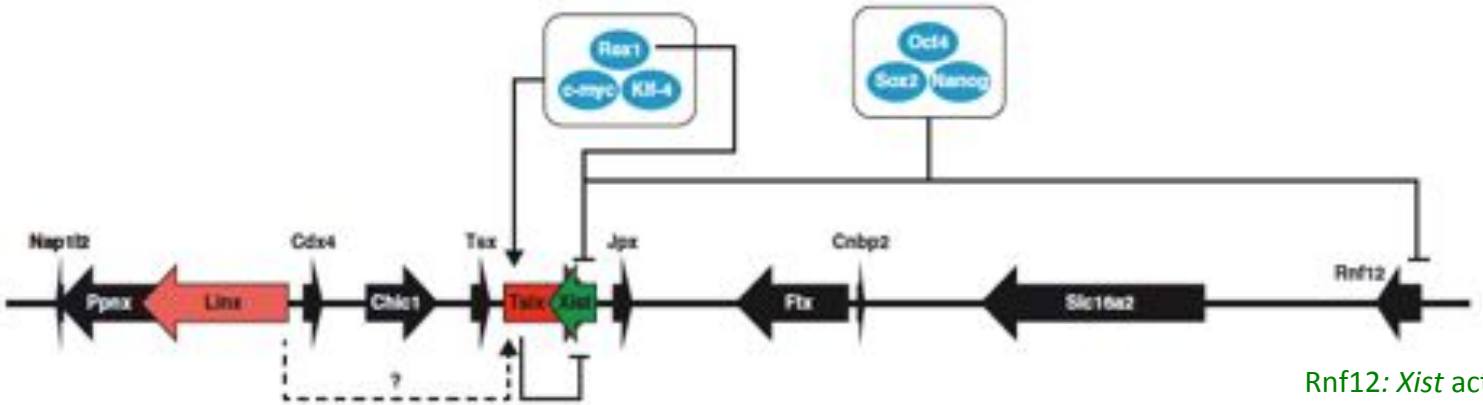
Epiblast
→ ES cells
→ Two active Xs

3.5 dpc 4.0 dpc 4.5 dpc

PE and **TE**
Xp remains inactive

The Pluripotency network controls *Xist*

Xist regulation is controlled (partly) by the pluripotency/stem cell factors network:
***Xist* is repressed** directly/indirectly by pluripotency factors in mouse ESCs
(Navarro et al, 2008, 2010, 2011; Gontan et al, 2012; Minkovsky et al, 2013)



Tsix: Xist repressor
(Avner, Brockdorff, Lee and Rougeulle labs)

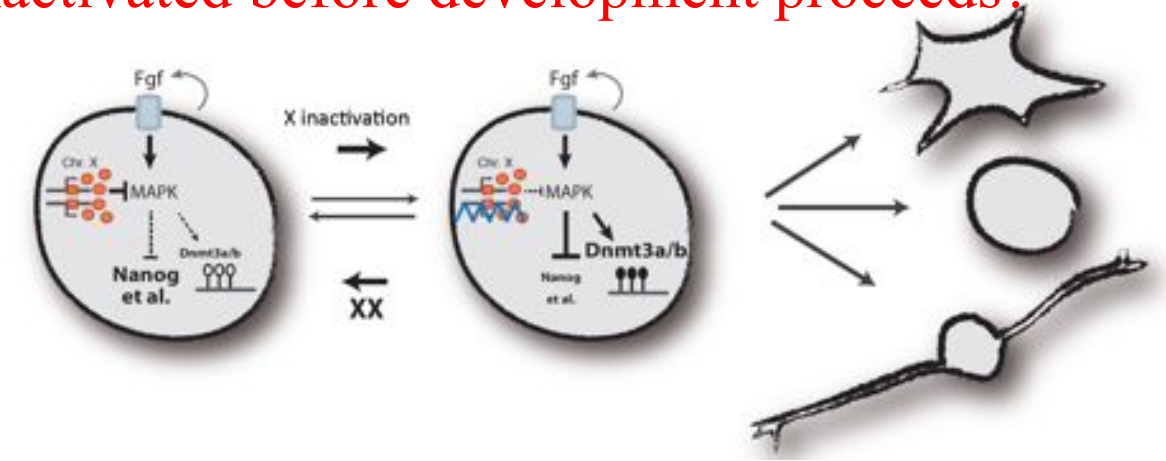
Rnf12*: Xist* activator
(Gribnau lab)

X-inactivation controls the Pluripotency Network

- Two active Xs block exit from stem cell state and delay differentiation
- Double dose of unknown X-linked genes inhibits Fgf/MAPK signaling:
  - reduces DNA methylation levels
  - prevents down-regulation of stem cell factors
- X inactivation overrides this block and allows differentiation to proceed in XX c

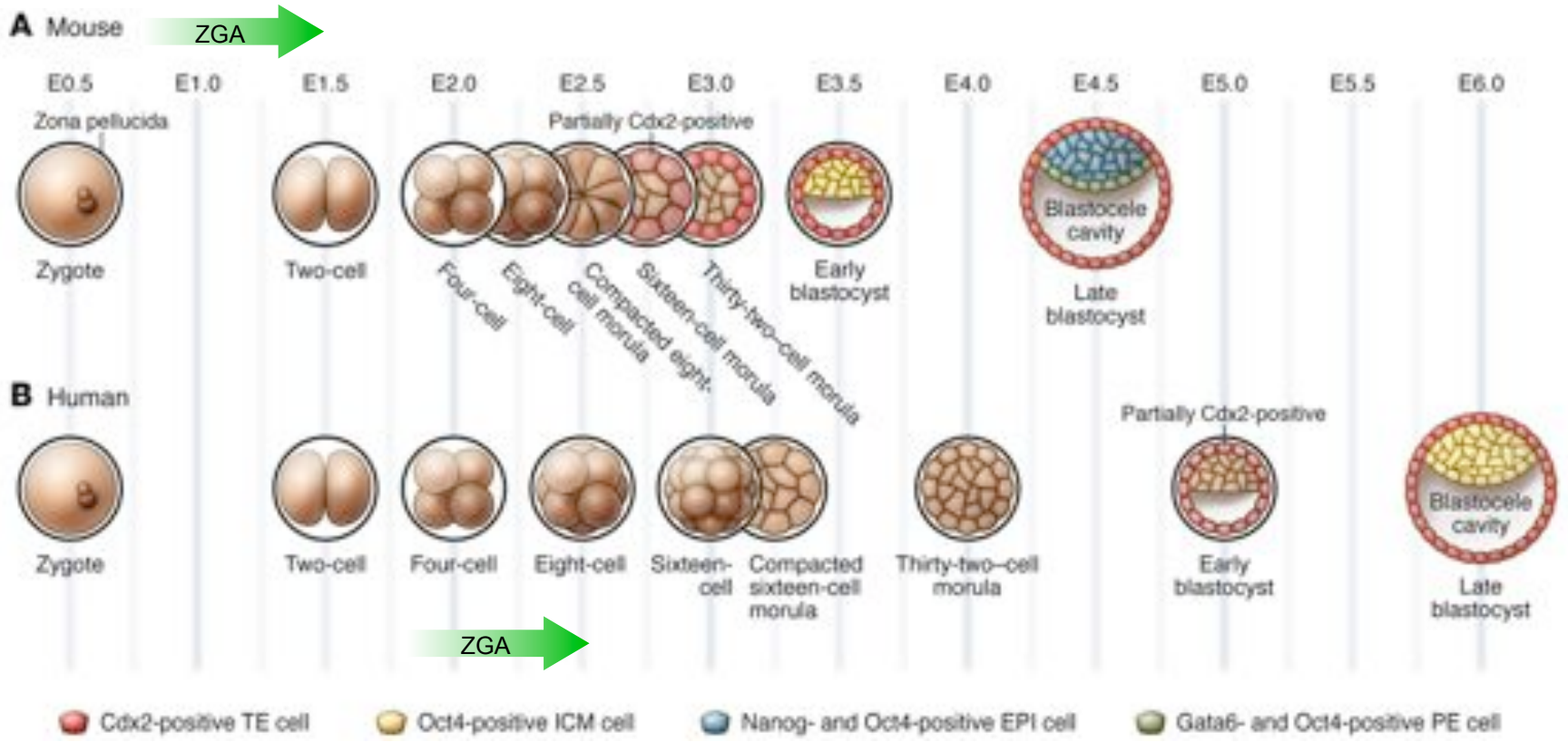
⇒ **XCI is controlled by the pluripotency network via *Xist* repression**
⇒ **And it also enables exit from pluripotency in XX cells**

A developmental checkpoint - to ensure that one X is inactivated before development proceeds?



Schulz et al, Cell Stem Cell, 2014

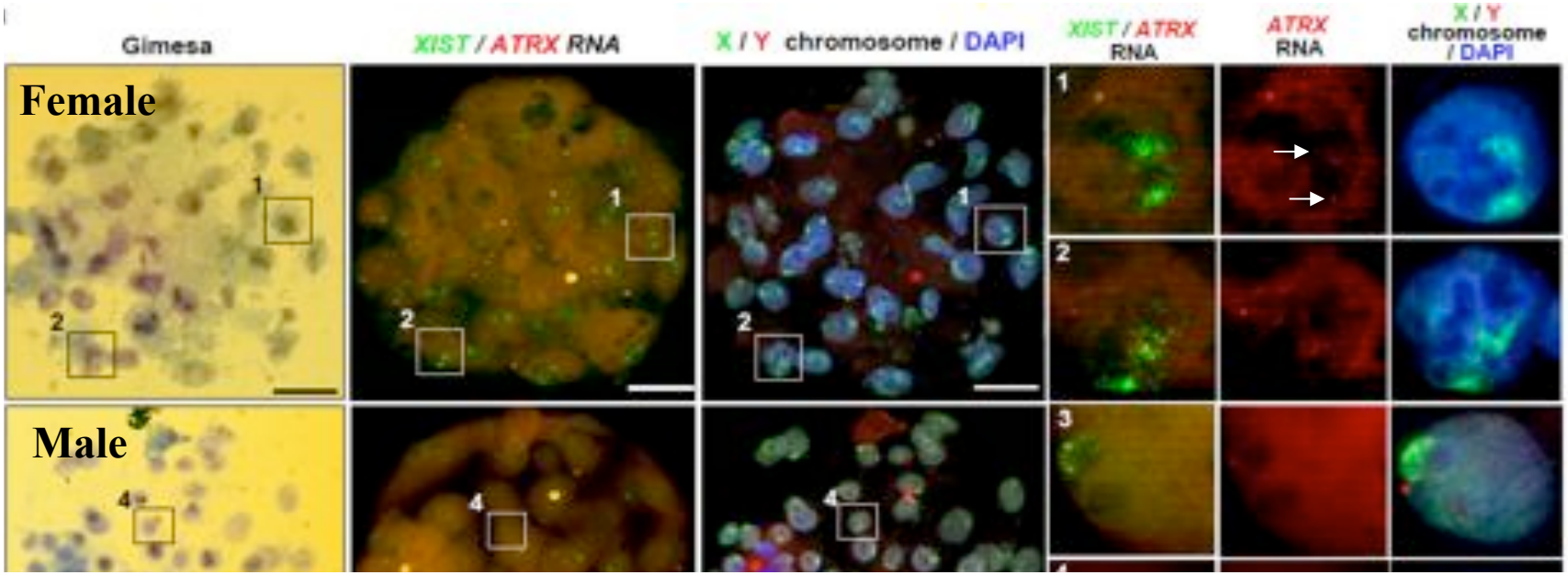
What about Human Pre-implantation Development?



Adapted from Cockburn and Rossant, JCI, 2010

Although stage-specific gene activation seems to be preserved in human and mouse pre-implantation development (eg Xue et al, 2013), there seem to be **major differences** in the **timing** of events (eg ZGA, implantation), and in the **signalling** pathways used to modulate TFs and **control lineage specification** (eg Kuijk et al, 2012)
(see Niakan et al, 2012 for review)

Constitutive XIST RNA up-regulation but no X inactivation during human pre-implantation development



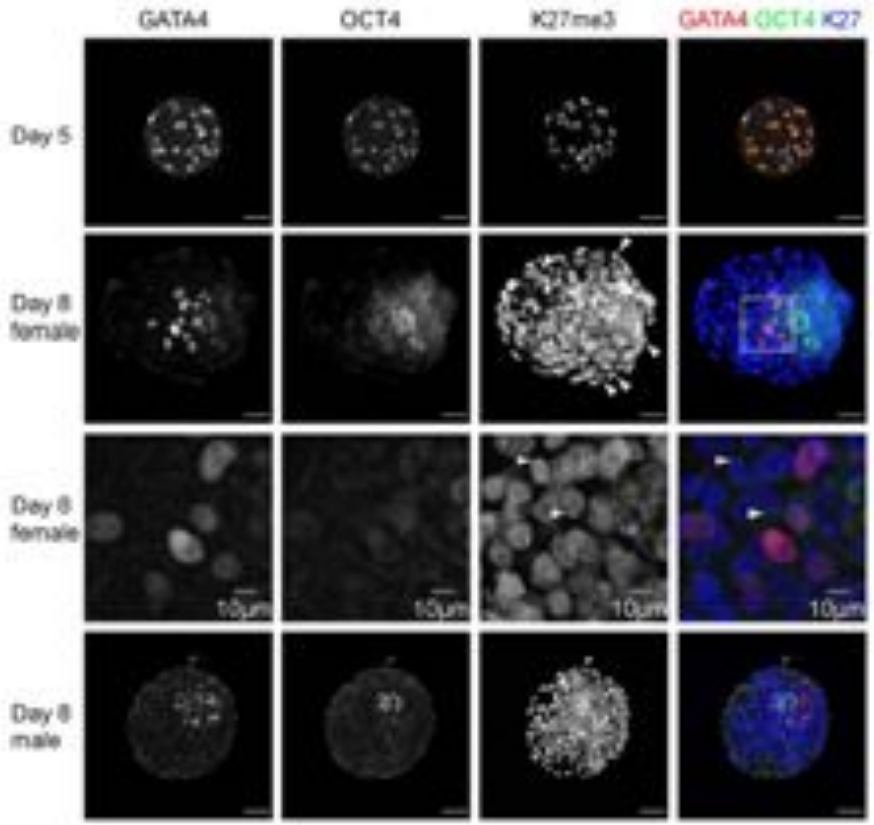
- No X inactivation for the first 7 days of development!
- ICM shows high *XIST* expression despite Nanog/Oct4 expression!

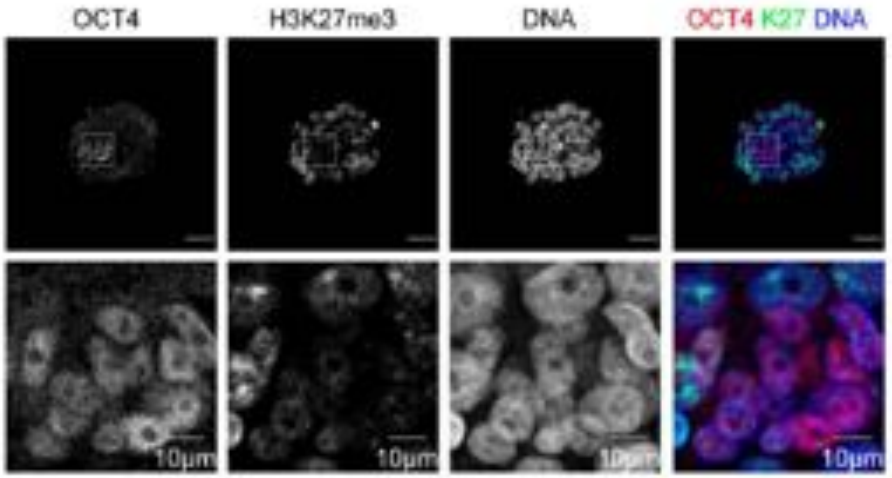
⇒ VERY different timing and regulation to the mouse…

But this is consistent with differences between mESCs and hESCs: hESCs grown in Fgf2/Activin, show fluctuating Xi states…

(=> Claire Rougeulle seminar, March 23rd)

Human pre-implantation embryos: Initiation of X inactivation occurs after day 7





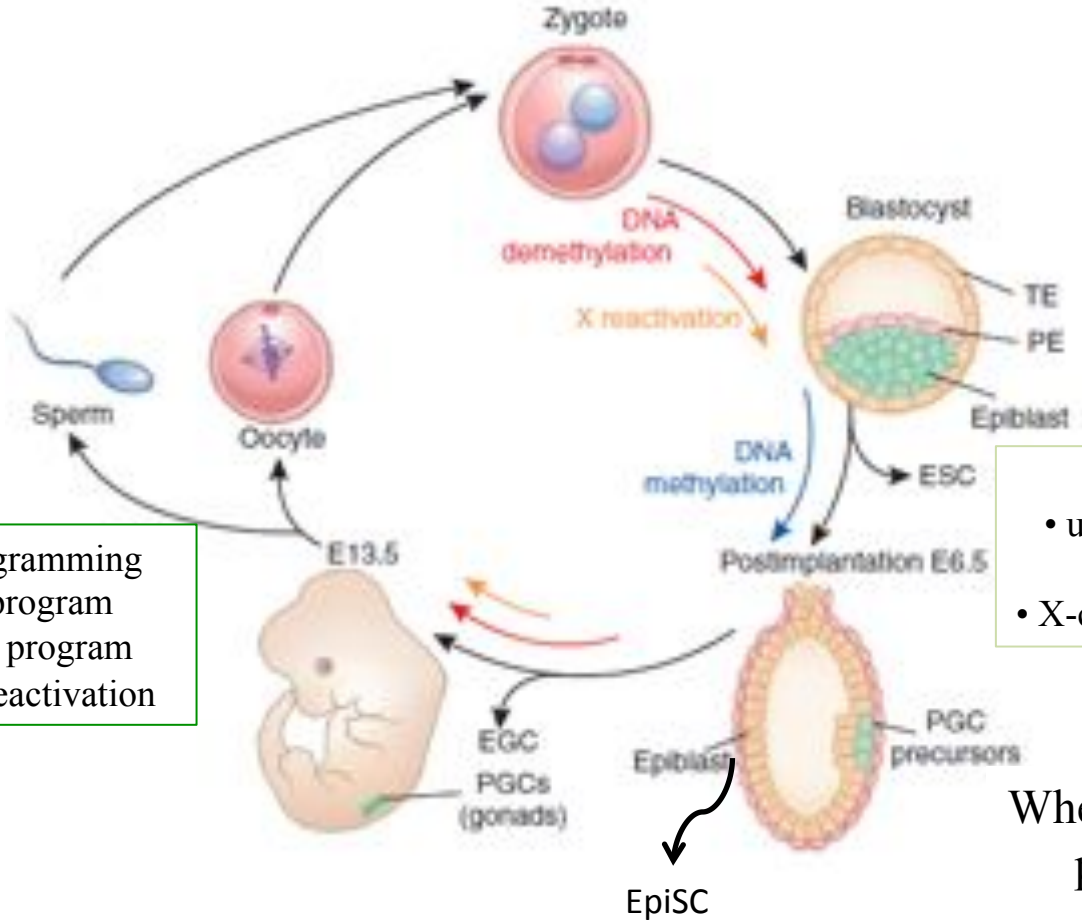
First signs of H3K27me3 accumulation on he X chromosome in human day 8 embryos co-cultured on endometrial cells

Teklenburg et al, PLoS ONE 2012

XIST becomes monoallelic and X inactivation initiates in human embryos from ~ day 8 onwards – around the time of implantation

Developmental Reprogramming

Zygotic Reprogramming
• undo gamete programs
• set up totipotency



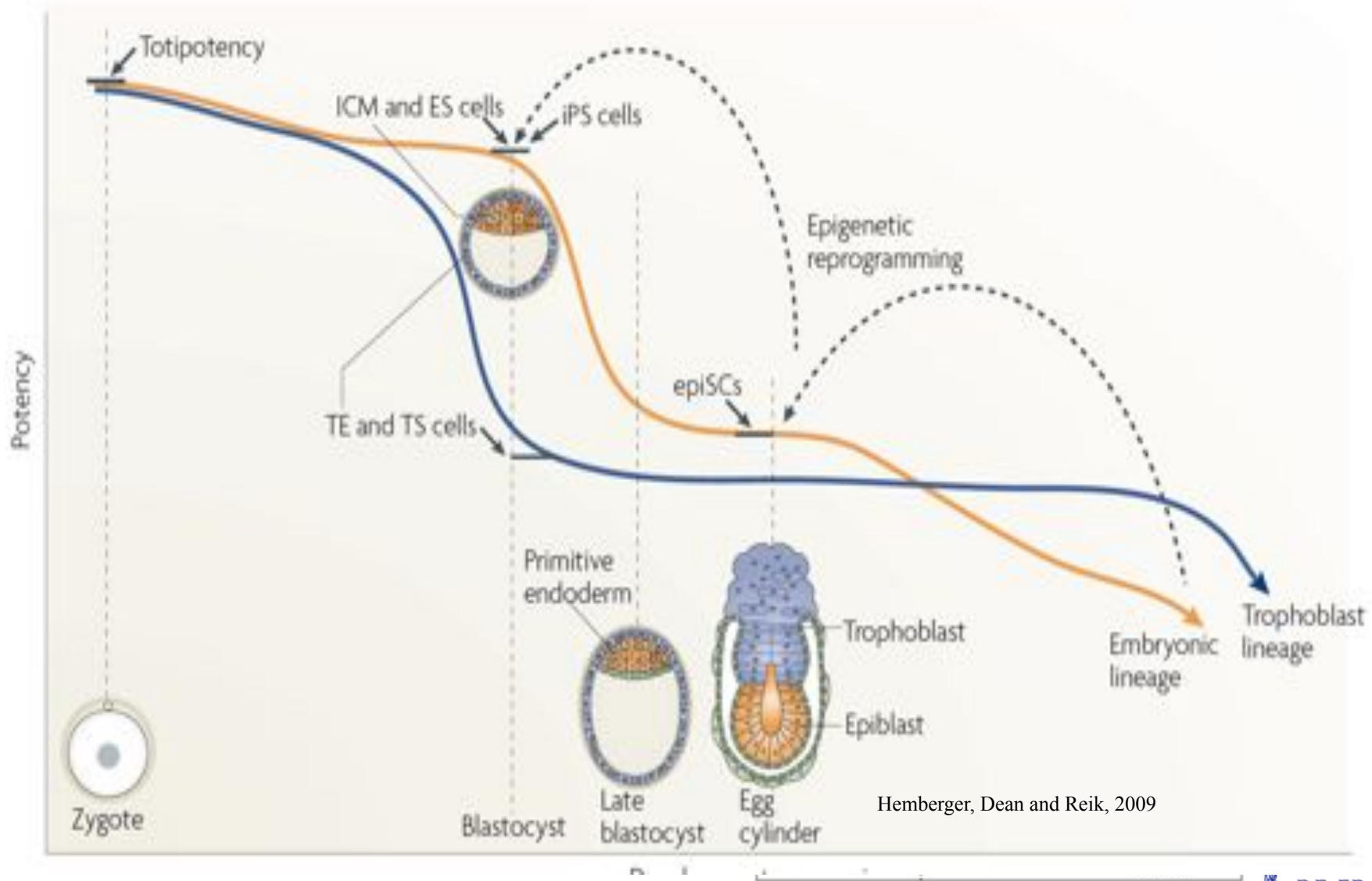
Germ Line Reprogramming
• undo somatic program
• set up germ line program
• X-chromosome reactivation

ICM Reprogramming
• undo/prevent TE program
• set up pluripotency
• X-chromosome reactivation

When is pluripotency lost post-implantation?

Adapted from Cantone and Fisher, 2013

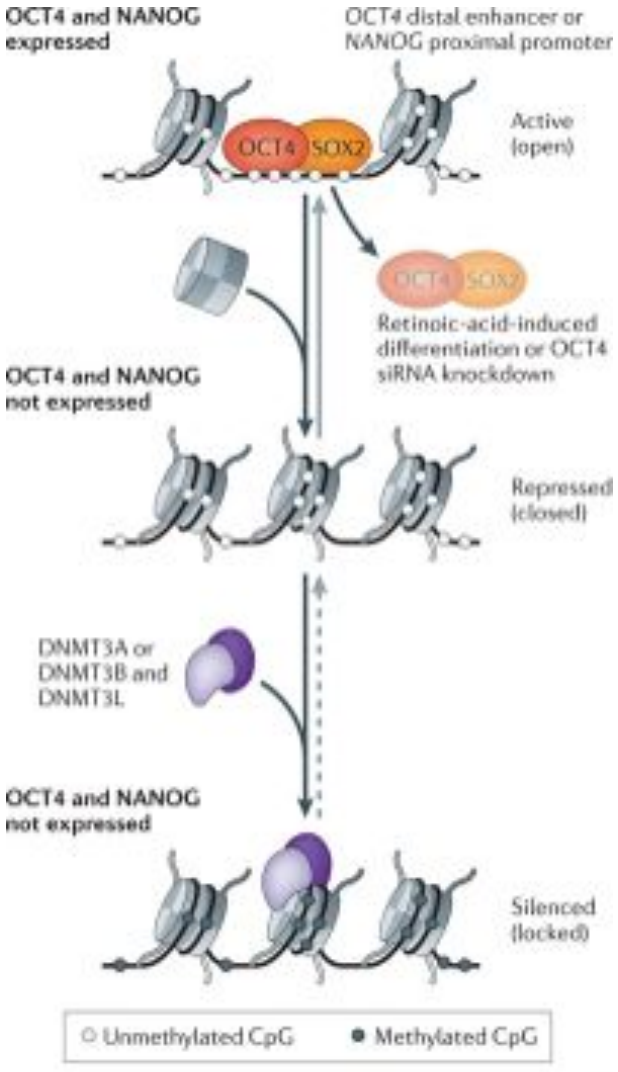
Pluripotency is rapidly lost in the post-implantation Embryo



Hemberger, Dean and Reik, 2009

Pluripotency is rapidly lost in the post-implantation Embryo

Potential Scenario



Active promoters and enhancers have nucleosome-depleted regions (NDRs) that are often occupied by transcription factors and chromatin remodellers.

Loss of factor binding during differentiation — leads to increased nucleosome occupancy of the regulatory region, providing a substrate for *de novo* DNA methylation.

DNA methylation subsequently provides added stability to the silent state and is likely to be a mechanism for more accurate epigenetic inheritance during cell division.

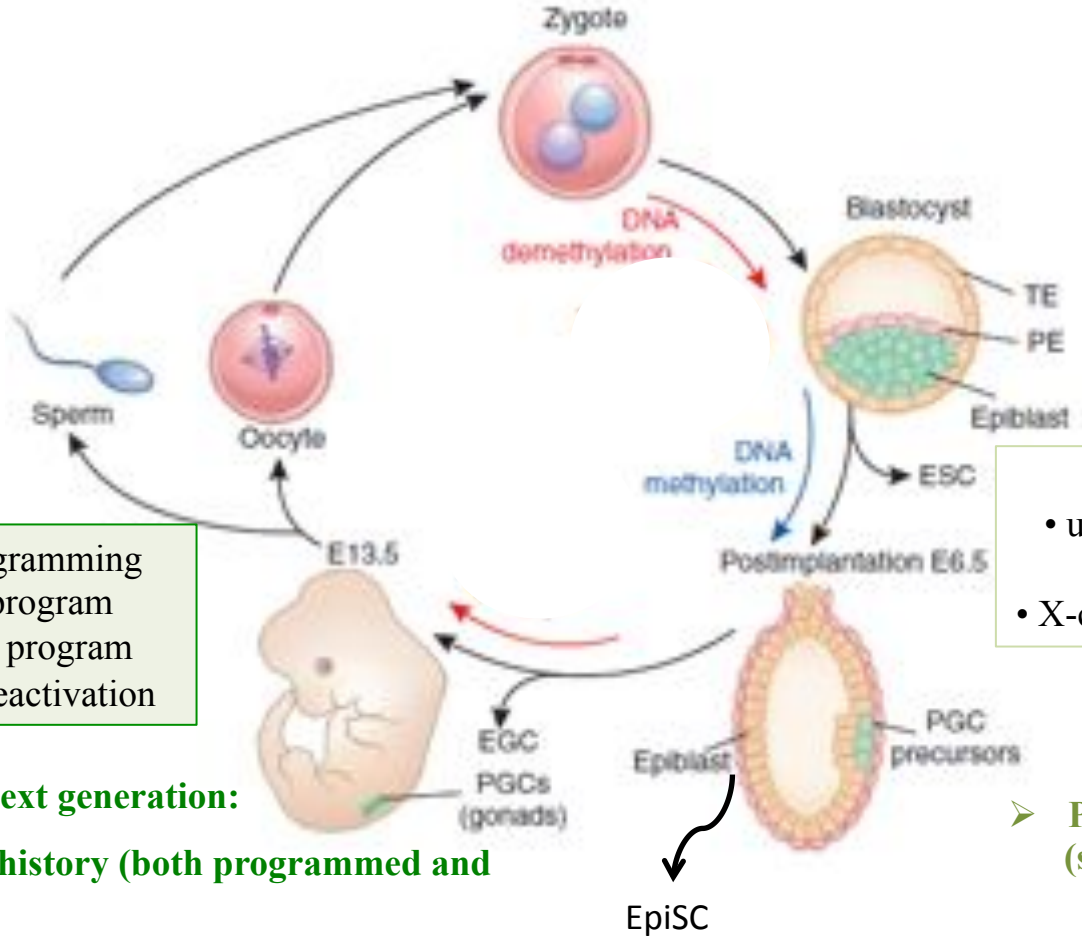
Jones, P. (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond *Nature Rev. Genetics* 13, 484-493

COLLÈGE DE FRANCE 1530

Developmental Reprogramming

- **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
• X-chromosome reactivation

Germ Line Reprogramming
• undo somatic program
• set up germ line program
• X-chromosome reactivation

- **Prepare for the next generation:**
- **Erase epigenetic history (both programmed and accidental)**
- **Establish parent-specific information (imprints)**

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

Adapted from Cantone and Fisher, 2013

PGC specification: Initiating the genetic program for epigenetic reprogramming

Epigenetic Barrier

E4.5

Inner Cell Mass

Pluripotent

Primed Epiblast

E5.75……

Competence

X inactivation
DNA methylation
Repression of some pluripotency genes
Onset of somatic cell differentiation

Somatic cells

Epigenetic Reprogramming

Specification

BMP signaling induces Blimp1 (Blimp1 KO -> somatic program)

Blimp1/Prdm1

Prdm14

Tcfap2c/Ap2g

E7.5

Primordial Germ Cells

Repression of somatic program

Initiation of epigenetic changes

Re-expression of pluripotency genes

X-reactivation

DNA demethylation

[Imprints erased]

→Epigenetic ground state

- *Histone demethylases (Erasure of H3K9me2)*
- *Repression of maintenance & de novo DNA methylation*
- *Tet1/Tet2 (5mC->5hmC)*

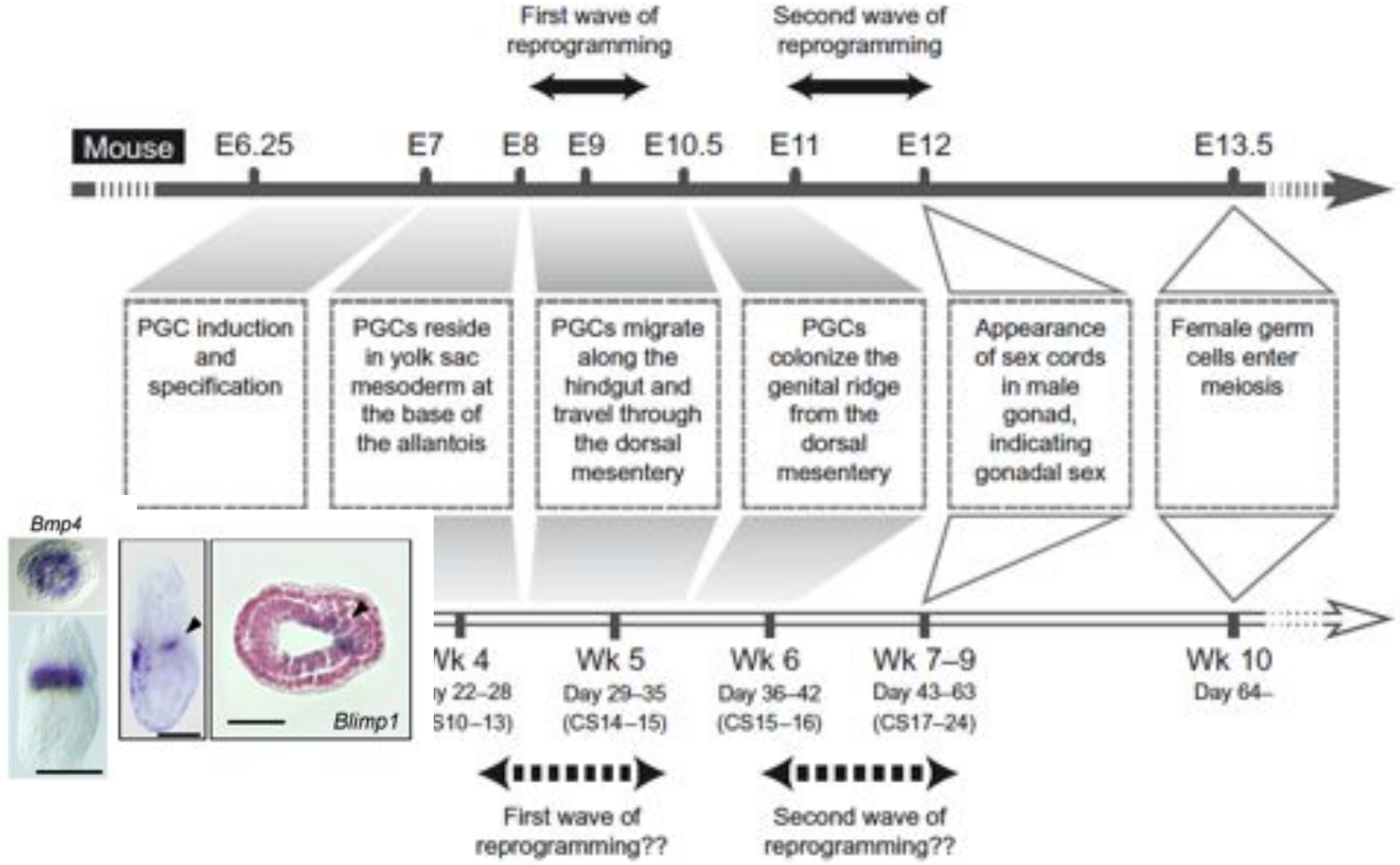
Courtesy of A. Surani

E. Heard, March 17th 2014

COLLÈGE DE FRANCE 1530

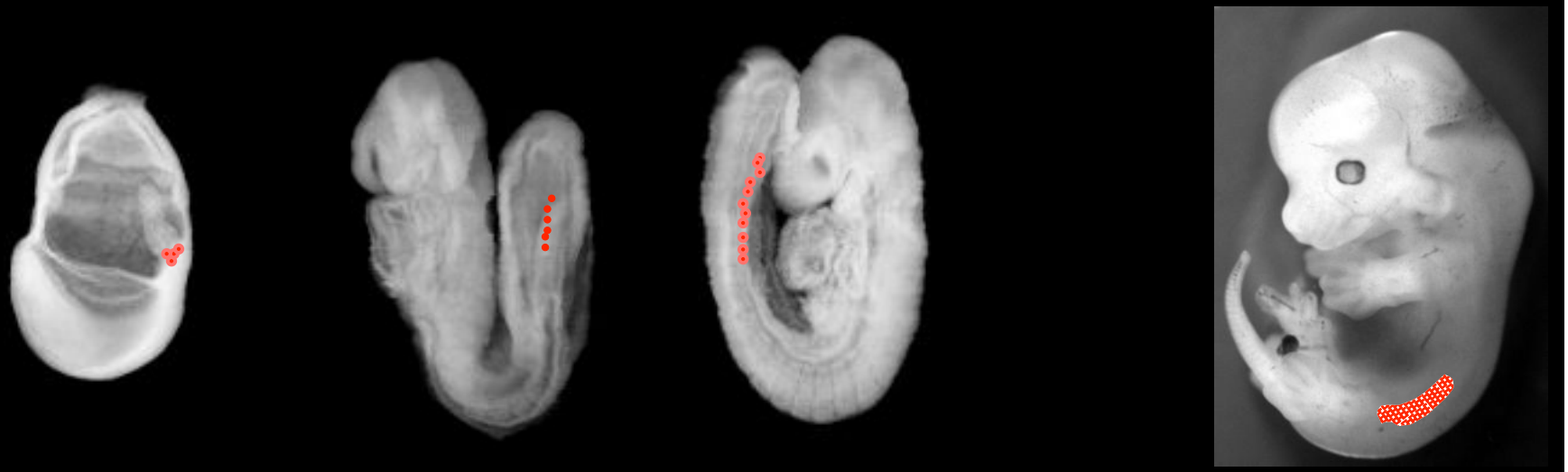
Reprogramming into the Germ Line

Reprogramming to ensure germ line–specific genes are primed and an epigenetic landscape compatible with totipotency to next generation is established. Remodeling is a multistep, coordinated process that requires timely expression of key TFs & appropriate epigenetic modifiers.



Kurimoto, K., Yabuta, Y., Ohinata, Y., Shigeta, M., Yamanaka, K., & Saitou, M. (2008).
Complex genome-wide transcription dynamics orchestrated by Blimp1 for the specification of the germ cell lineage in mice. Genes and Development, 617–1635

Reprogramming of PGCs upon entry into the genital ridge



E7.5 → E8.5 → E9.5 → E11.5 → E12.5

5mC = 12%

Popp et al, 2010

PGC Specification

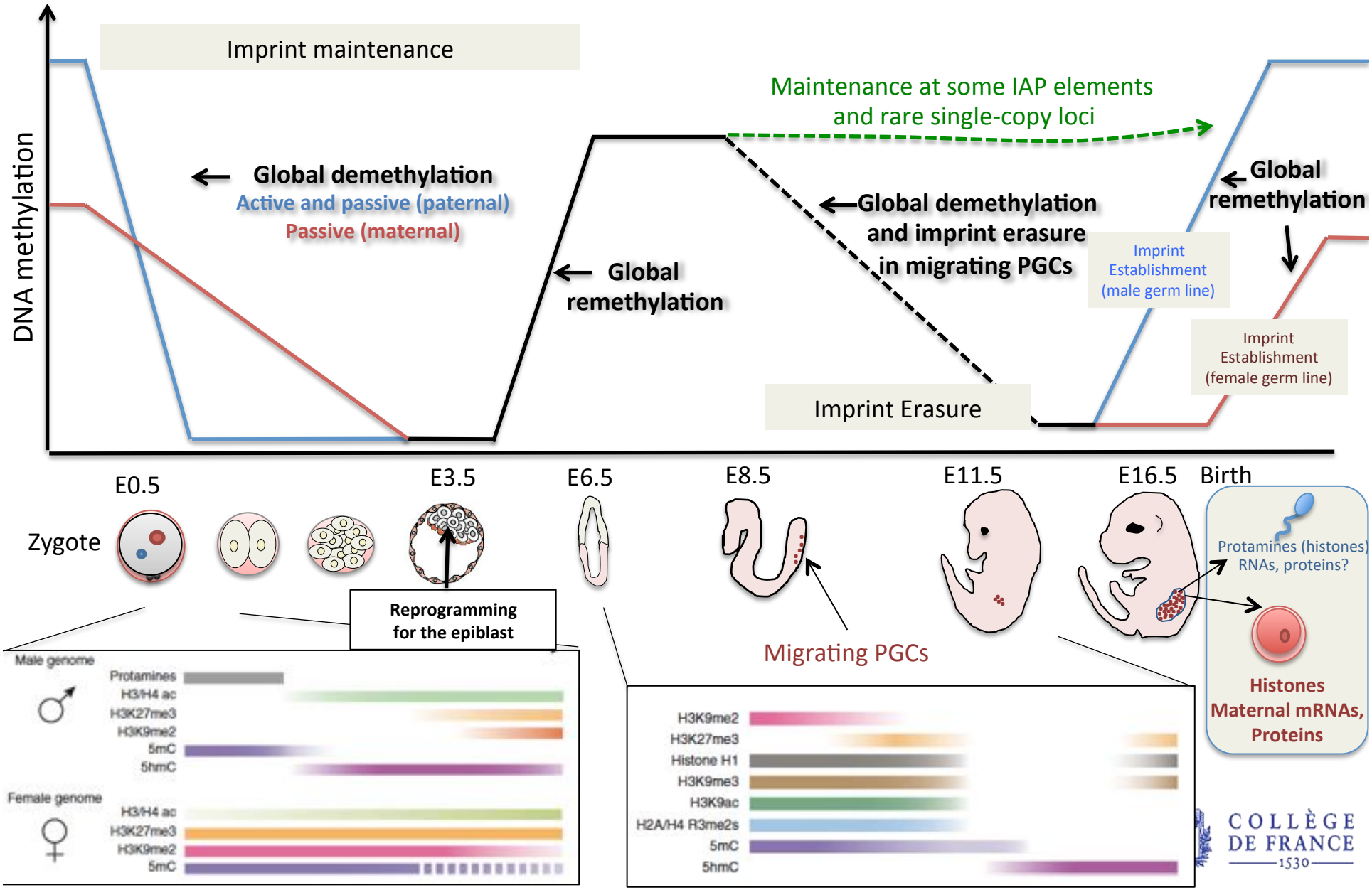
Epigenetic Reprogramming (Step 1)

Migration

Reprogramming

How similar or different are the reprogramming mechanisms in the germ line, to those that take place in the inner cell mass/ESCs or in the zygote, or during induced pluripotency?
(Cours IV)

Epigenetic reprogramming in mammals



“The Role of DNA Modifications in Epigenetic Reprogramming and Signaling”

Professor Wolf Reik
(Babraham Institute, Cambridge, UK)