Epigenetic events in early embryos

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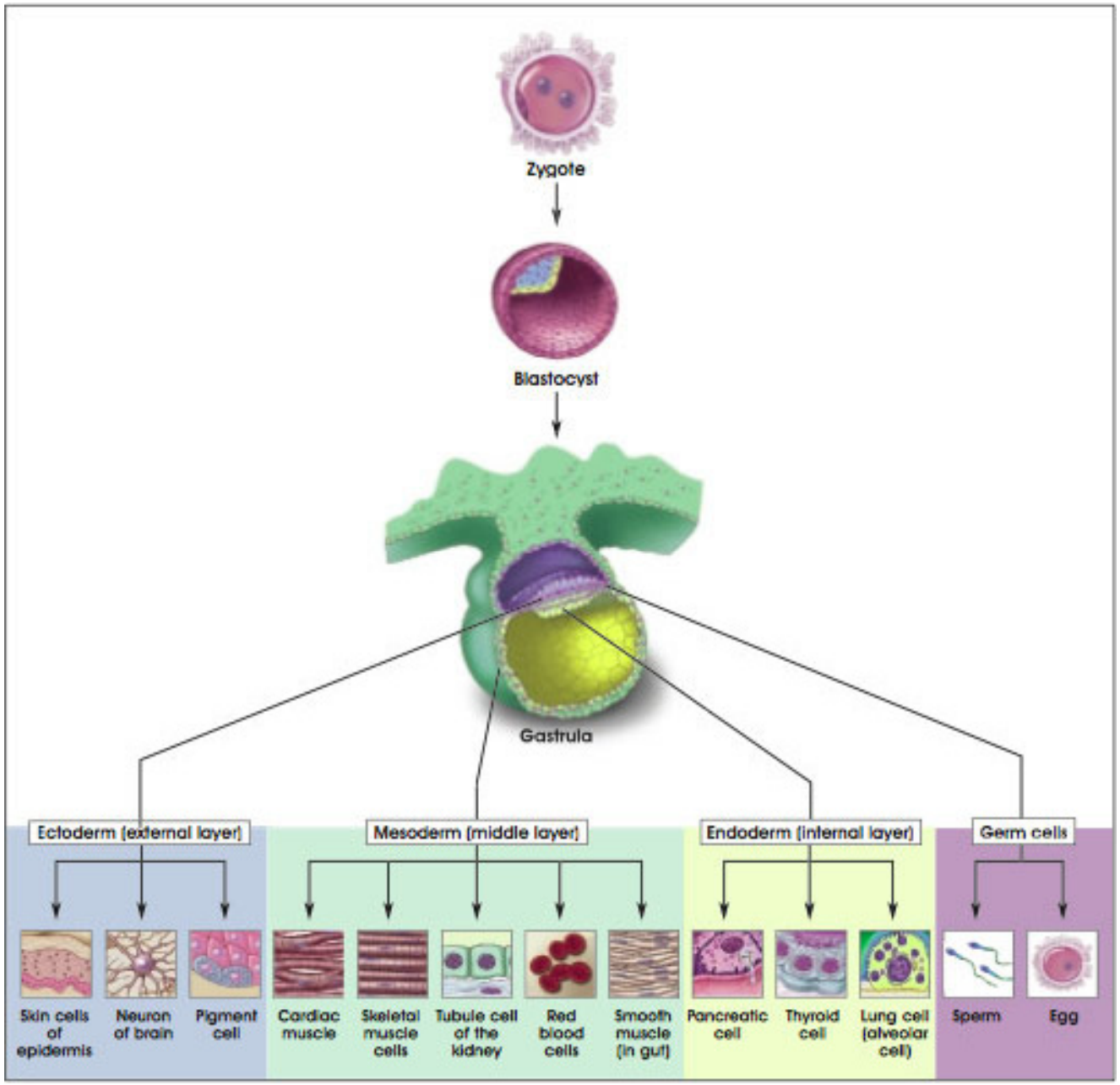
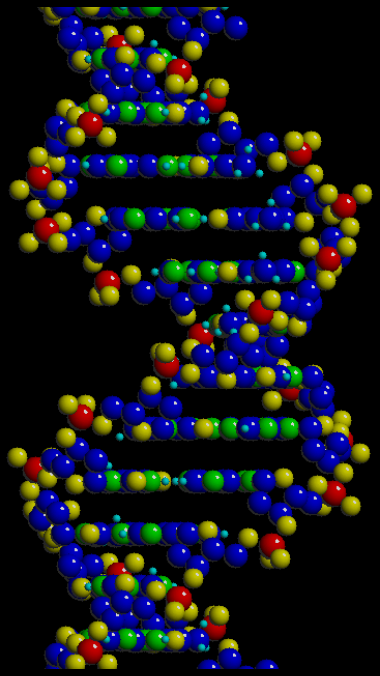
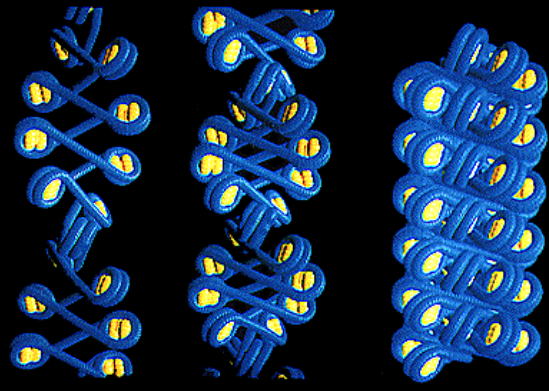


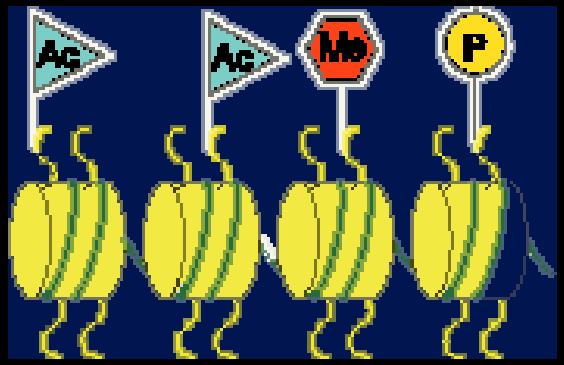
Development
Differentiation
Cell fate





Chromatin structure

Histone modifications



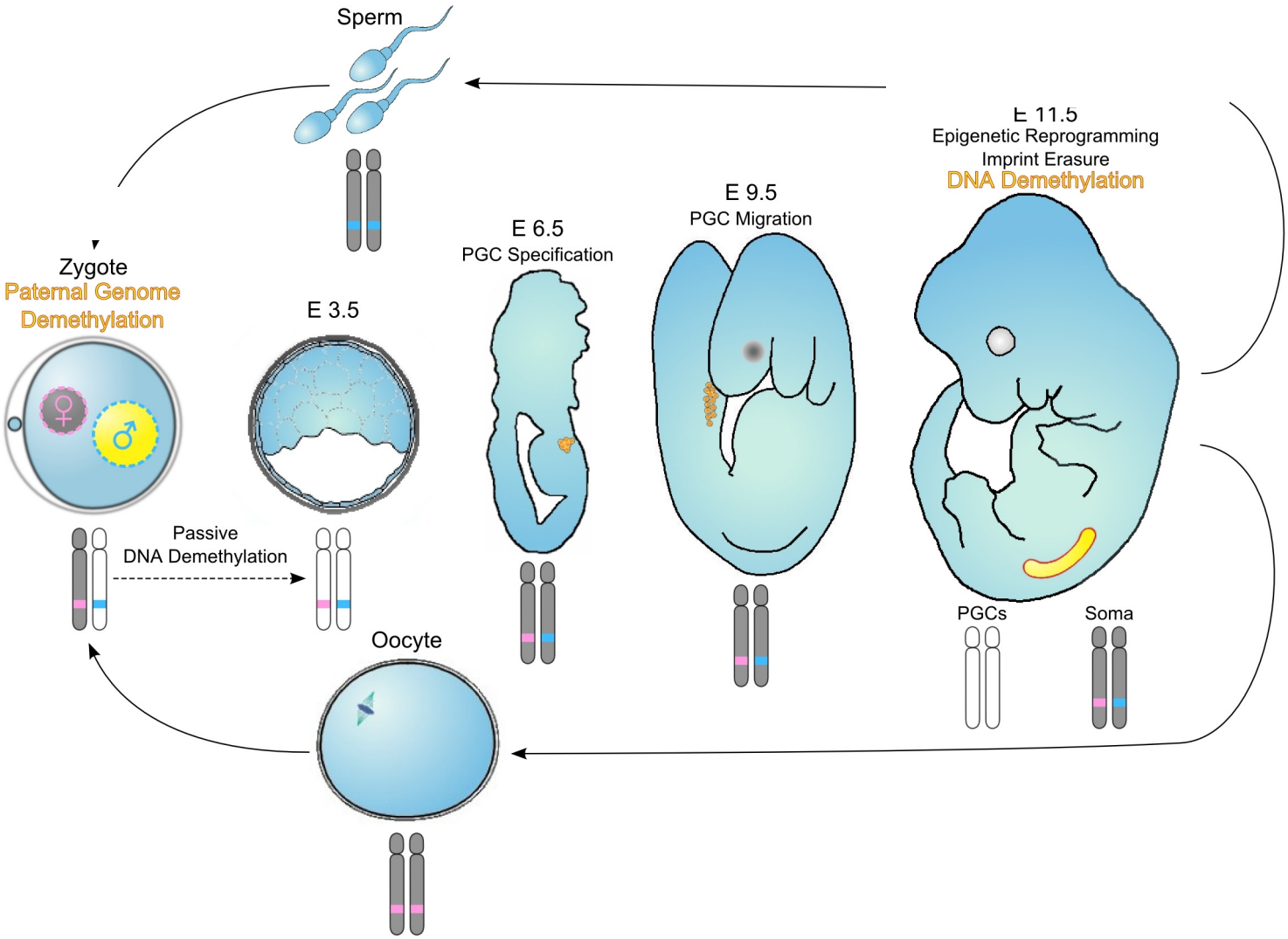
DNA methylation

Epigenetic information

- Not coded for in the DNA (not genetic)
- Propagated through cell divisions (heritable)

- DNA methylation (5mC, 5hmC)
- Histone modifications (methylation, acetylation, phosphorylation, ribosylation…)
- Higher order chromatin structure/ folding (structural information) ?

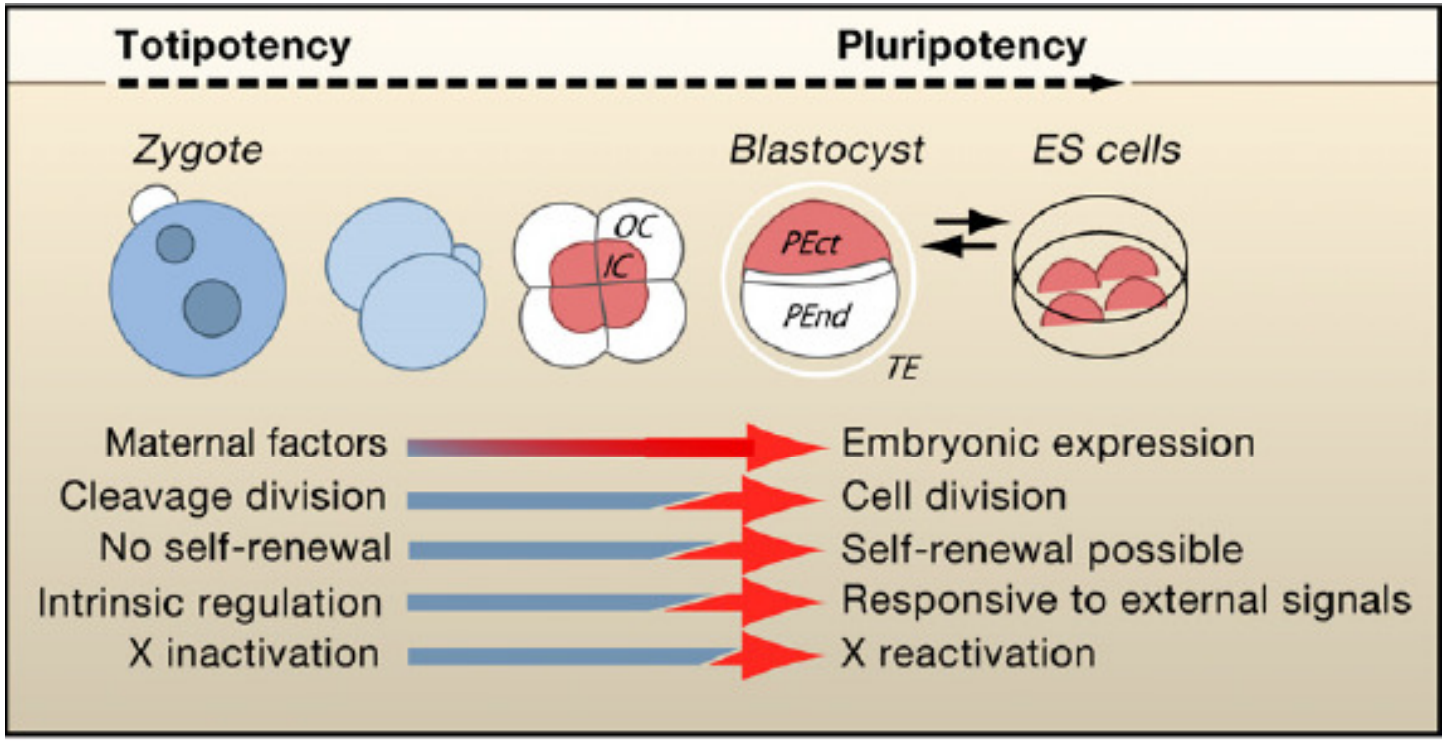
Mouse development – an epigenetic perspective



Surani and Barton, 1983, Barton, Surani, Norris 1984,
McGrath and Solter, 1984, Mayer et al, 2000; Oswald et al, 200

Hajkova et al, 2002;
Lee et al, 2002

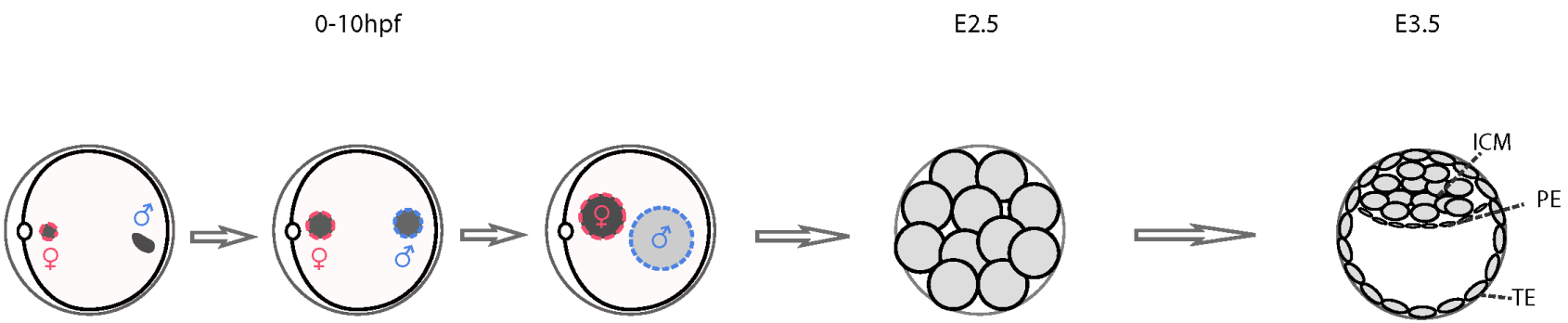
Preimplantation development



Transcriptional networks ⟺ Epigenetic regulation

Surani, Hayashi and Hajkova,2007

Epigenetics of early development - overview



replacement of protamines
with histones
removal of some histone variants
(paternal genome)

DNA demethylation
(active)
(paternal genome)

DNA demethylation
(passive)

Xi reactivation
(ICM, female embryos)

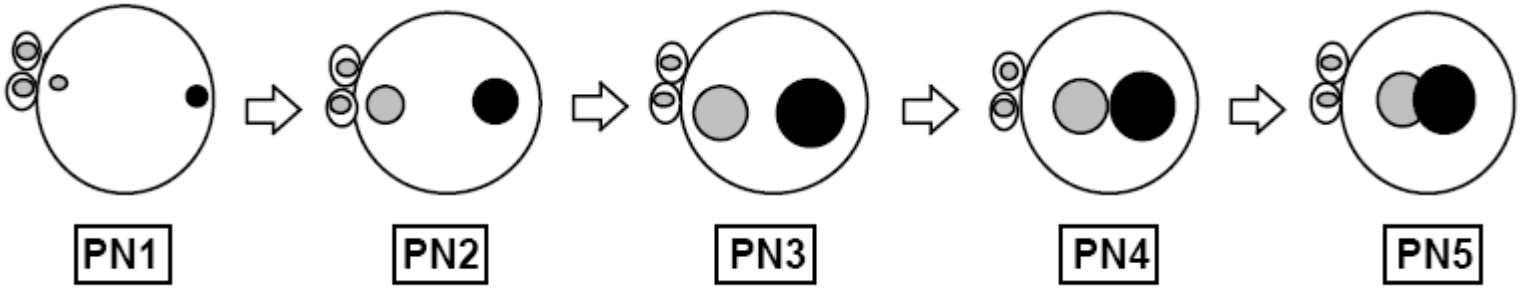
Phase I

Zygotic epigenetic factory

Protamine to histone exchange

DNA demethylation & chromatin assymetry of parental genomes

Schematic representation of pronuclear stages in mouse zygote



♂ protamines

histones

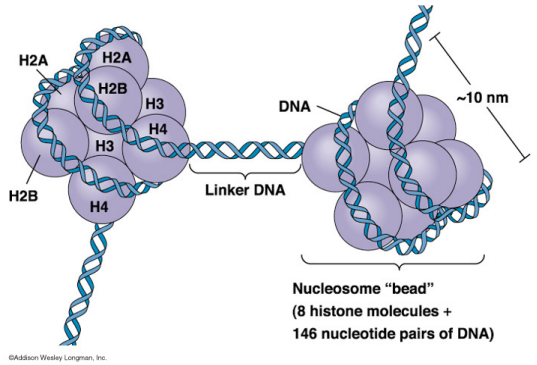
Remodelling sperm genome

Removal of protamines

Deposition of new histones…. Histone chaperones (Hira)

Histone variants:

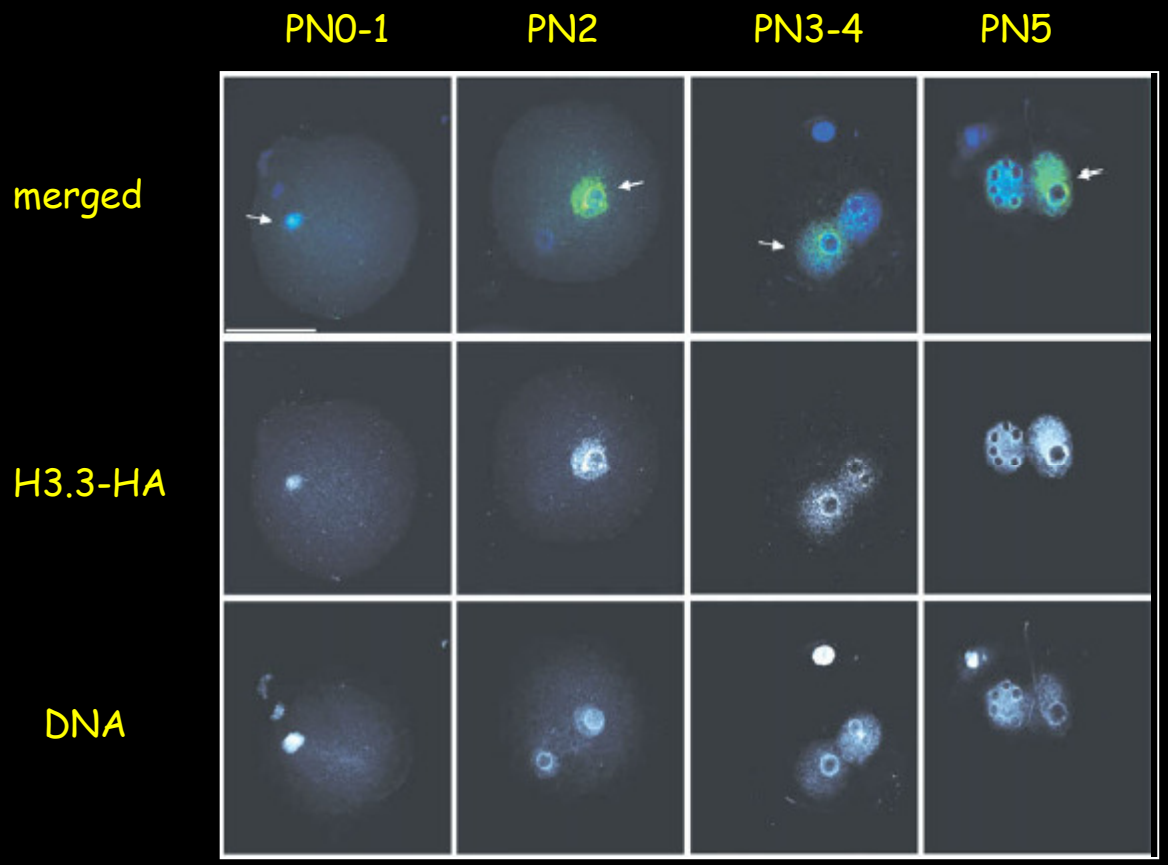
- Incorporated into chromatin outside S phase
- Contain introns, UTRs
- Outside the the “histone cluster” in the genome



Histones	Features
Archaeal histones	Ancestral histone fold proteins without tails found in singly wrapped tetrameric units that comprise nucleosome particles.
H2A, H2B	Canonical core histones encoded by replication-coupled genes.
H2AZ	H2A variant found in nearly all eukaryotes that has a diverged self-interaction domain.
macroH2A	Vertebrate-specific H2A variant with a C-terminal globular domain. Enriched on the mammalian inactive X-chromosome.
H2A-Bbd	Vertebrate-specific H2A variant that is widely distributed. Relatively deficient on the inactive X-chromosome.
H2AX	H2A form with an SQ[E/D] Ø (Ø = hydrophobic) C-terminal motif that becomes serine phosphorylated at sites of double-stranded breaks.
H3, H4	Canonical core histones encoded by replication-coupled genes.
H3.3 (H3.2 in plants)	H3 variant that replaces H3 and differs at position 31 and at a few residues on helix 2 that allow deposition outside of replication.
Packaging histones	Core and linker histone variants adapted for tight packaging of DNA in sperm and pollen in some organisms.

Henikoff and Ahmad, 2005

Assymmetric distribution of histone variant H3.3 in zygotes



Protamines in the paternal genome are replaced by H3.3

Torres-Padilla et al, 2006

Histone based epigenetic inheritance?

5-15% of histones retained in mature spermatozoa

Presence of canonical histones and testes specific histone variants

Histones retained over promoters of some developmentally regulated genes

Potential for inheritance of epigenetic marks (do these histones persist through zygotic reprogramming?)

(Bradley Cairn's lab – Nature 2009
A.Peter's lab – Nat Struct Mol Biol 2010)

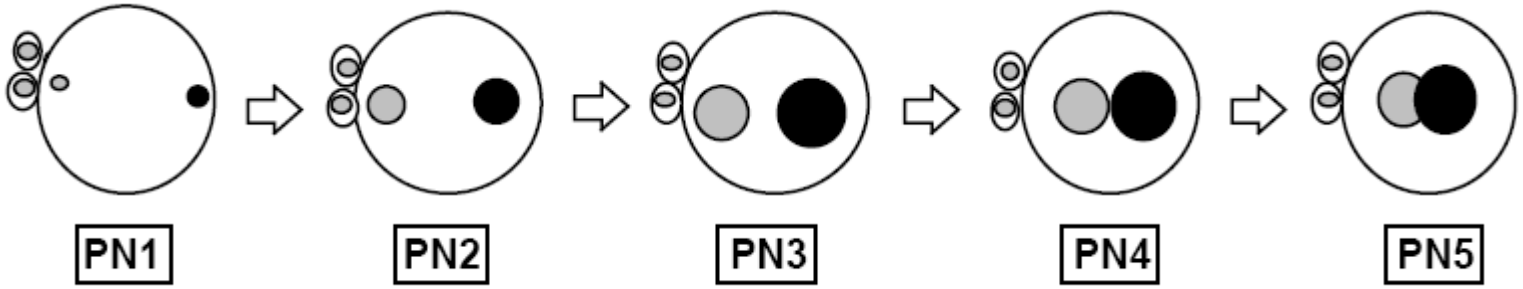
Phase I

Zygotic epigenetic factory

Protamine to histone exchange

DNA demethylation & chromatin asymmetry of parental genomes

Schematic representation of pronuclear stages in mouse zygote

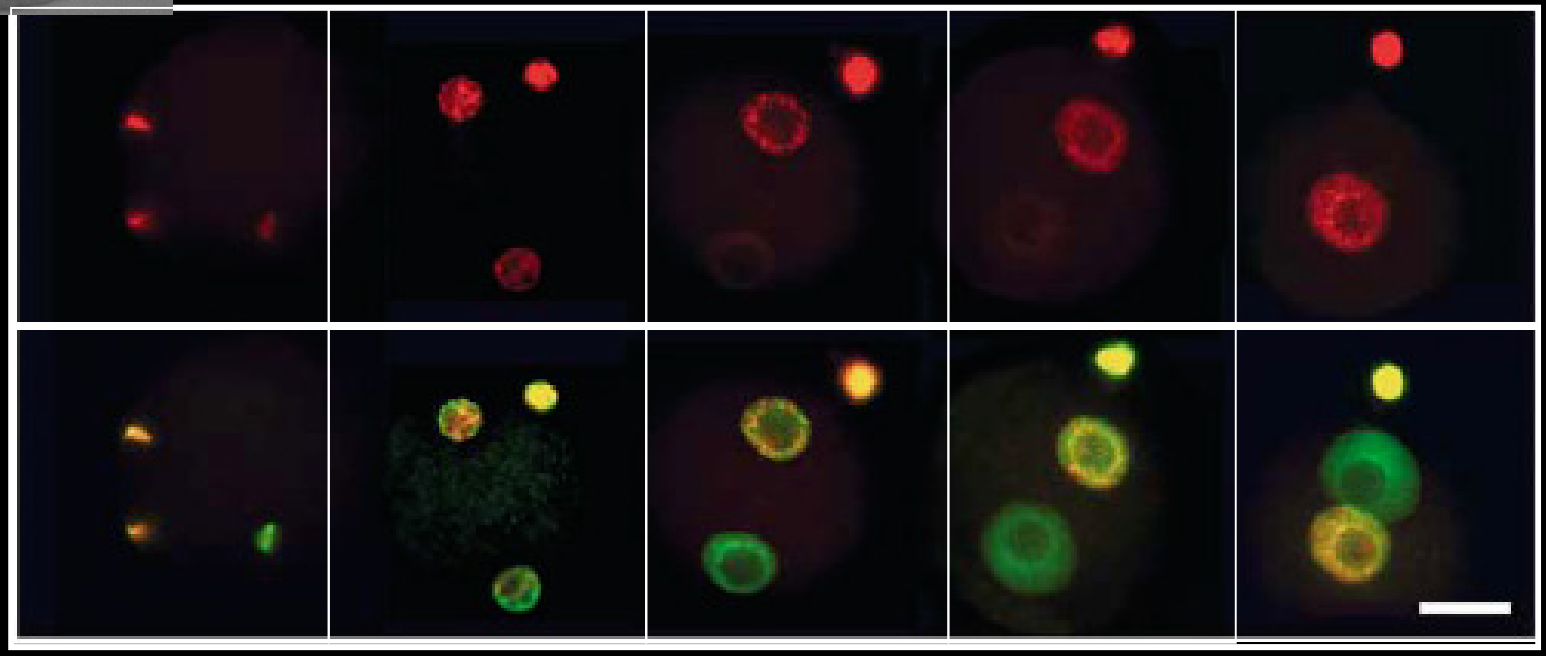


DNA demethylation

♂ protamines

histones

Kinetics of DNA demethylation in mouse zygote



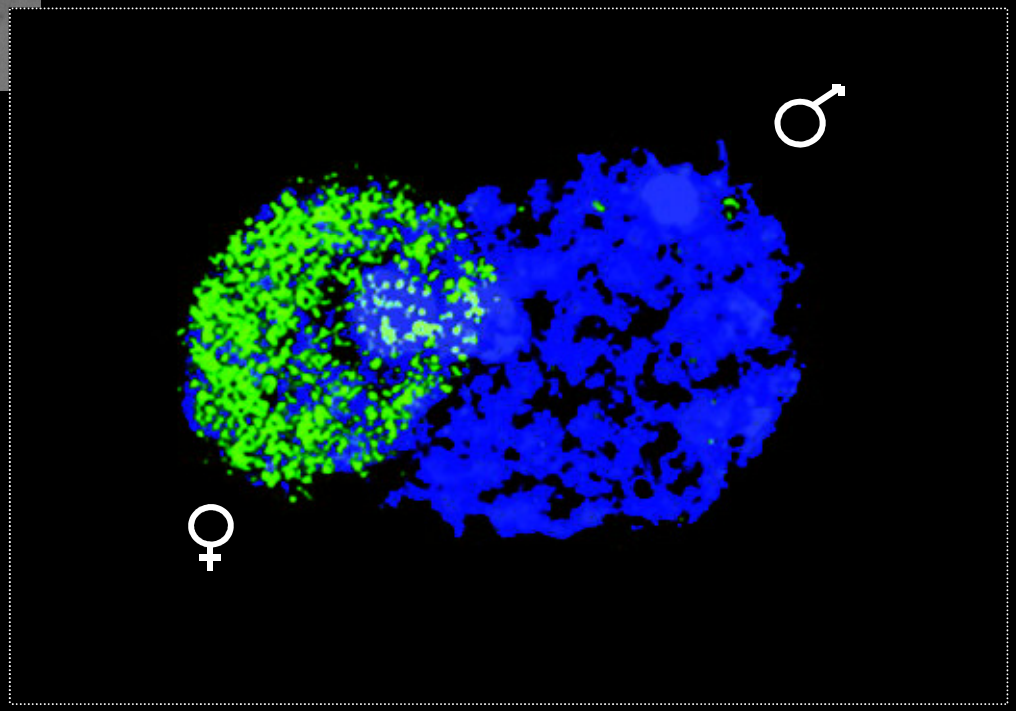
PN0 (3hpf) | PN1 (4hpf) | PN2 (5hpf) | PN3 (6-7hpf) | PN4-5 (8-10hpf)

♂ protamines

histones

Santos and Dean, 2004

Zygotic reprogramming : DNA demethylation

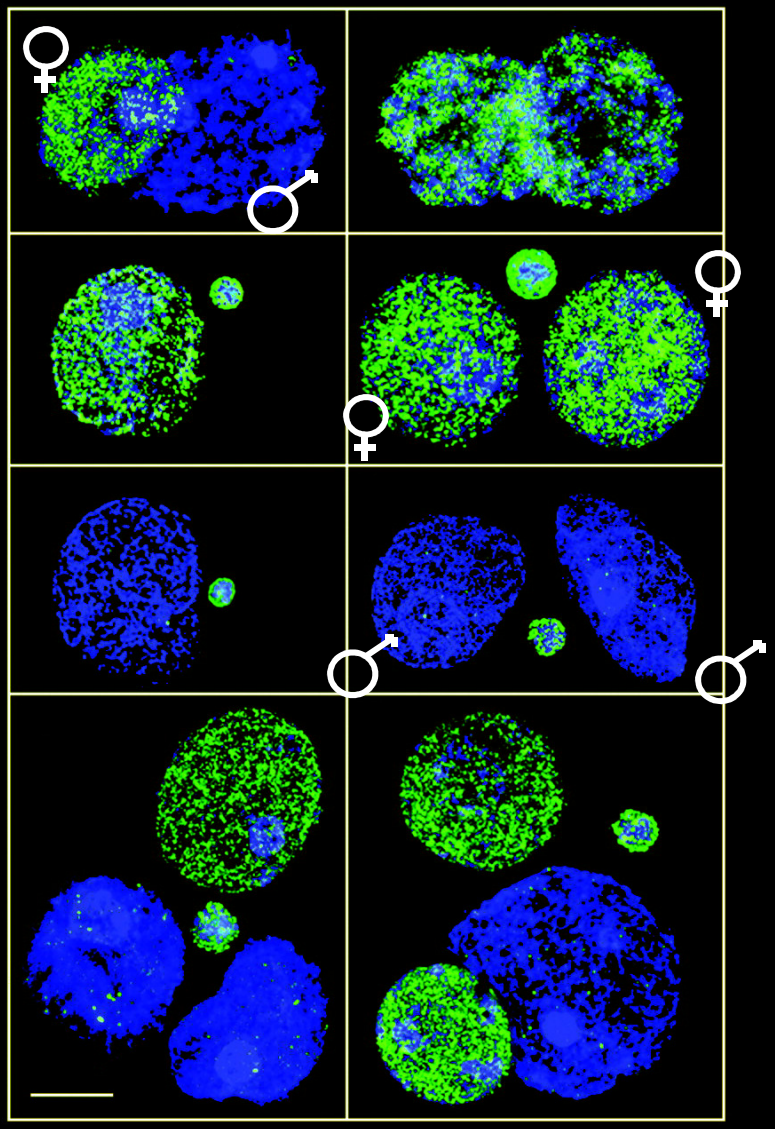


5mC

DAPI

Barton *et al*, 2001

Zygotic DNA demethylation – pronuclear transplantation experiments



5mC

DAPI

Barton *et al*, 2001

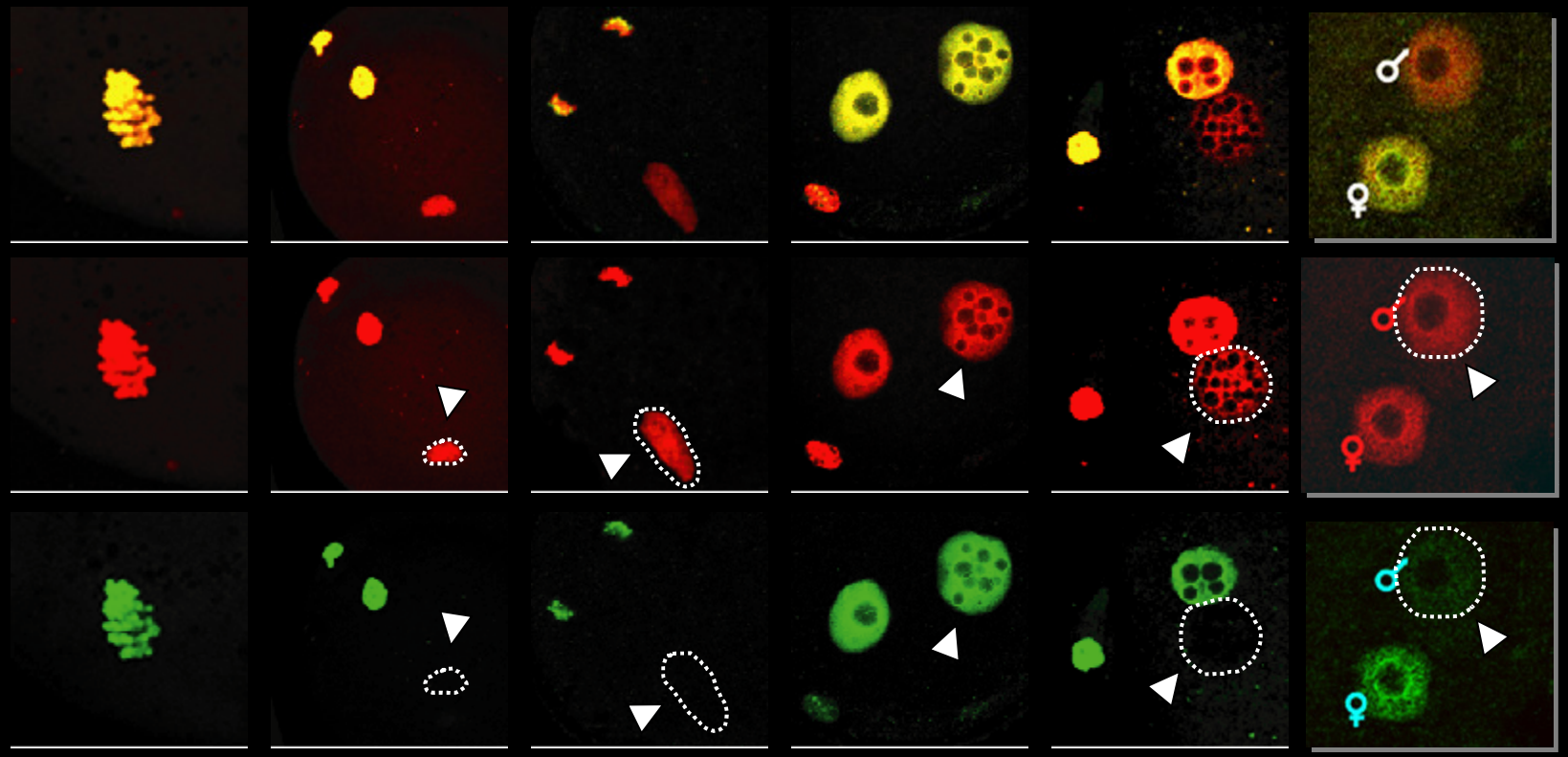
Regulation of epigenetic reprogramming

template or **activity**

Chromatin template

Presence of (de)modification enzymes

Zygotic reprogramming – chromatin assymmetry



Arney *et al*, 2002, Erhardt *et al*, 2003

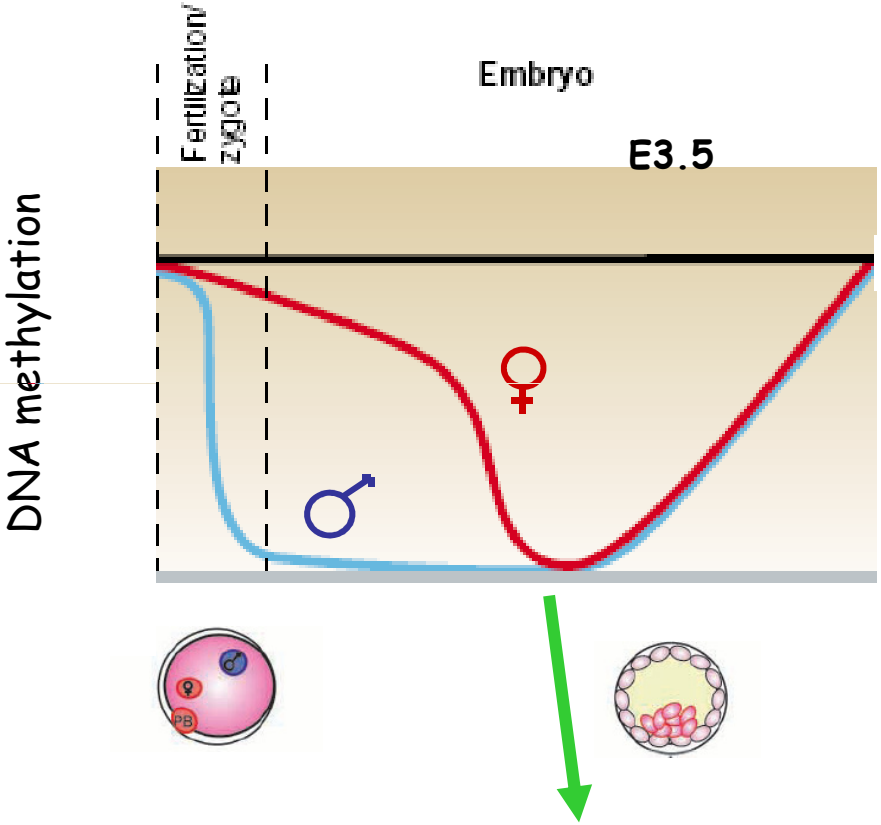
What is the importance of the zygotic DNA demethylation?

Species specific differences (mouse, bovine, human X sheep, rabbit)

Aberrant (does not occur) in ROSI (development proceeds normally till blastocyst)

What about the maternal genome?

Dynamic changes of DNA methylation in early mouse embryos

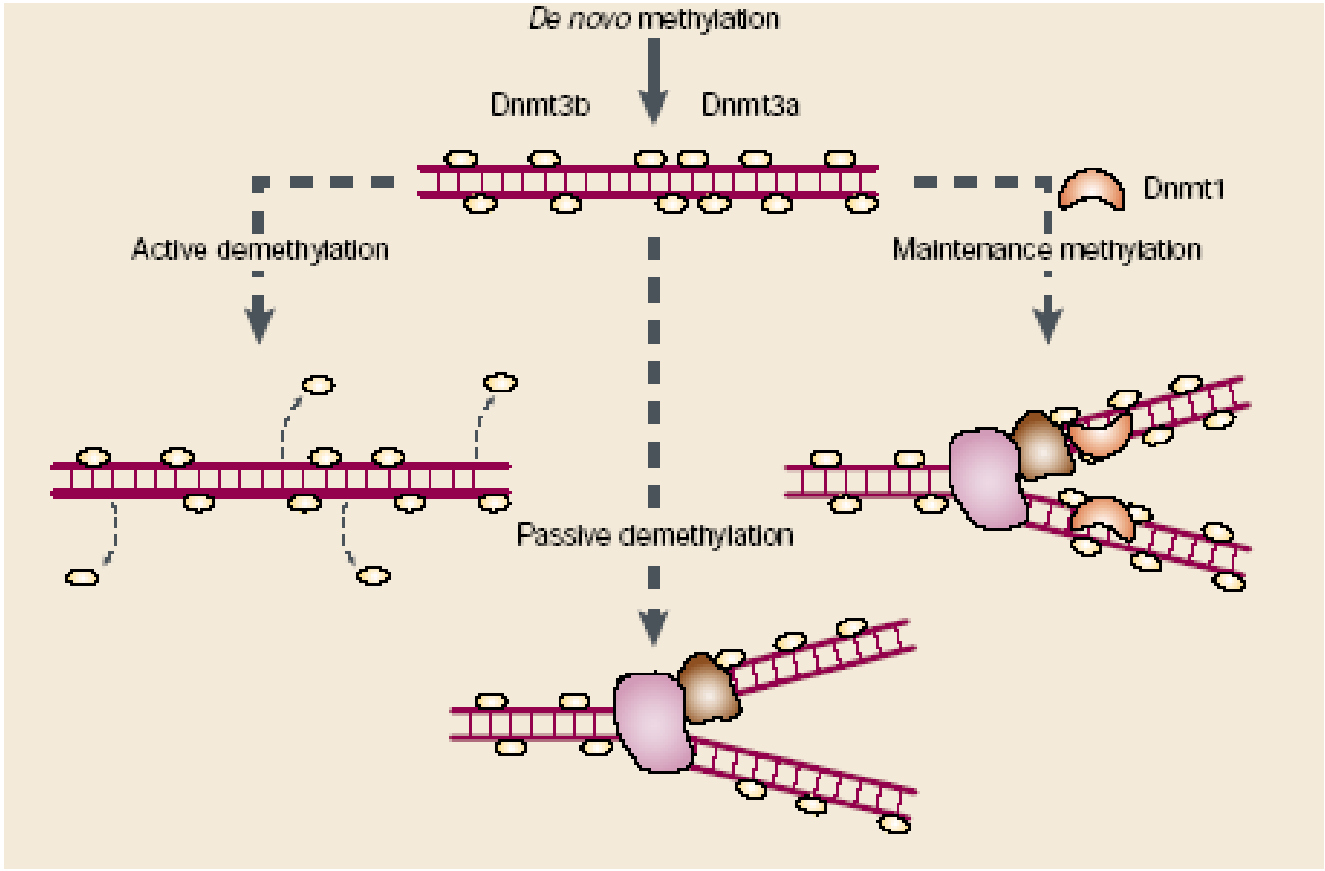


Passive DNA demethylation of <u>maternal genome</u> by exclusion of Dnmt1

Reik and Walter, 2001

Mechanism of DNA demethylation?

Principles of active and passive DNA demethylation



Reik and Walter, 2001

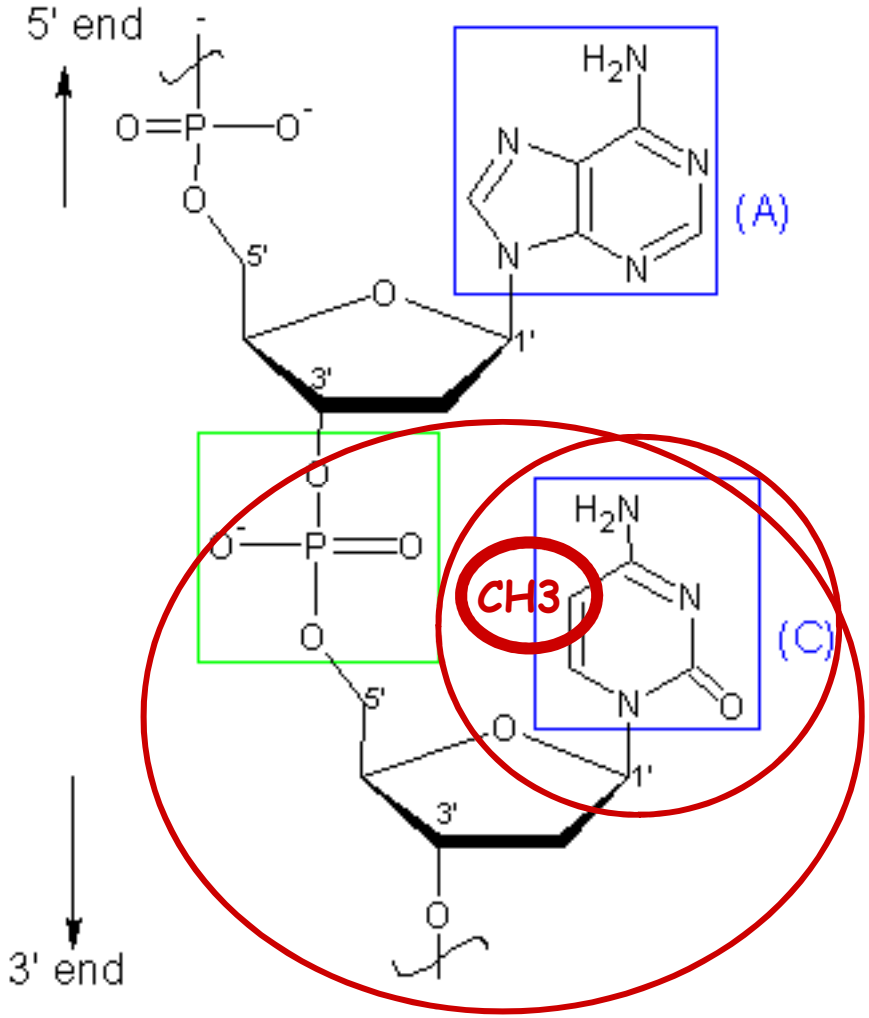
Active DNA demethylation - models….

Direct removal of methyl group

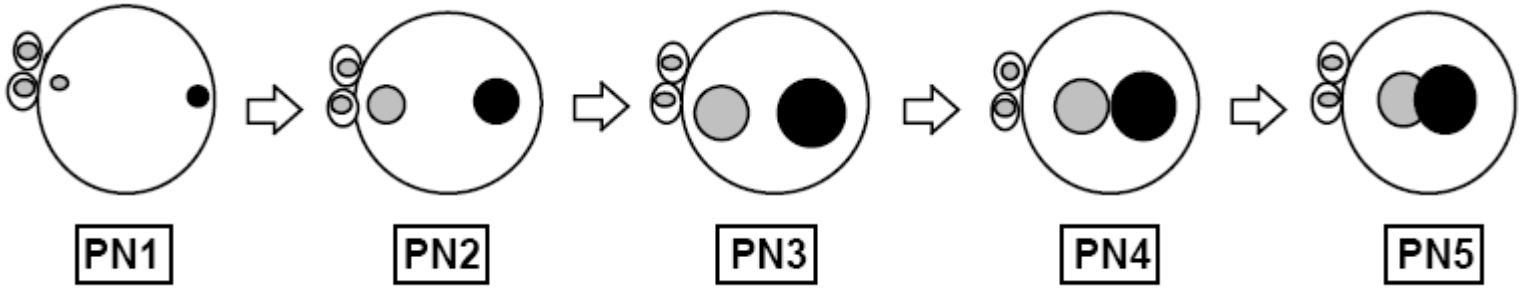
Removal of modified base

(BER)

Removal of nucleotide(s) - NER



Schematic representation of pronuclear stages in mouse zygote

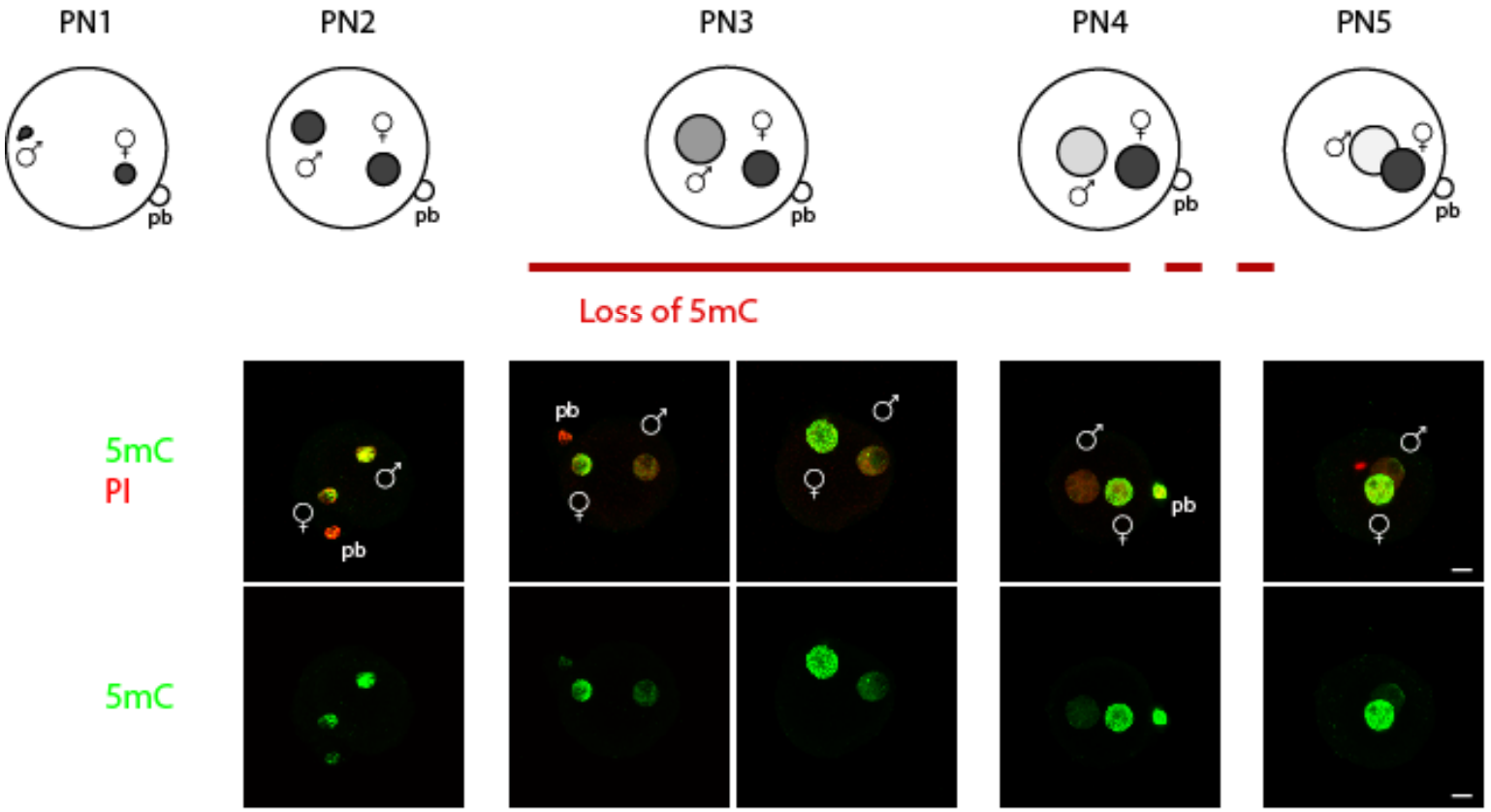


DNA demethylation

♂ protamines

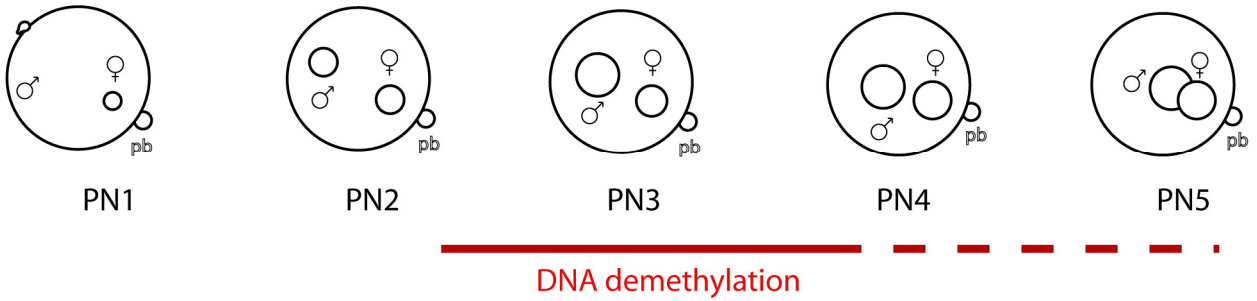
histones

Kinetics of DNA demethylation in mouse zygotes



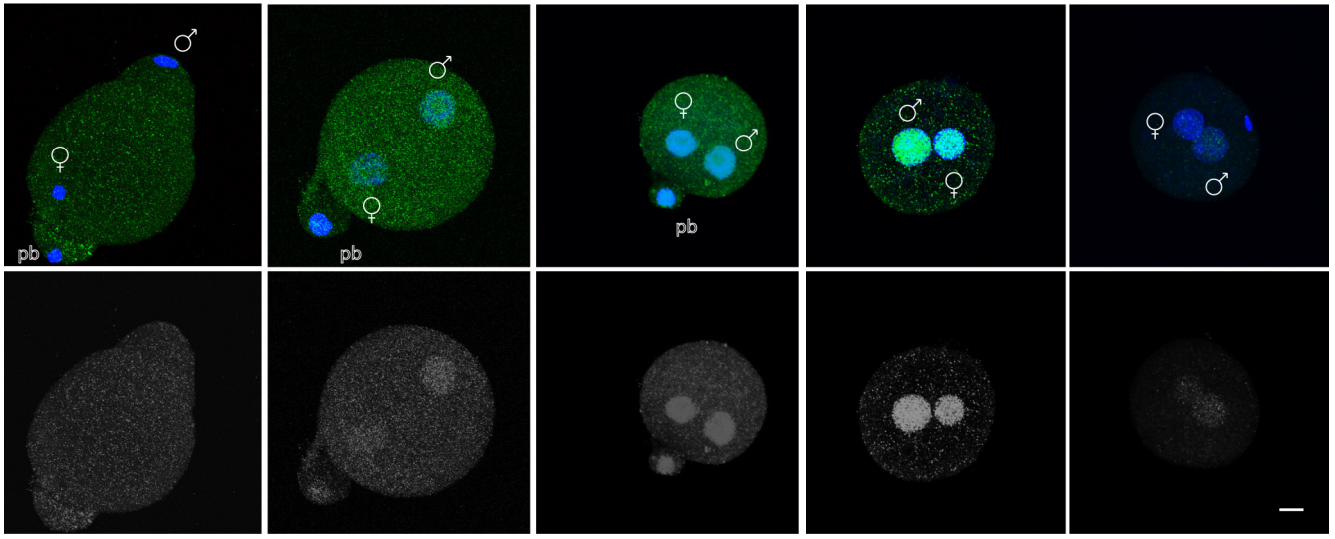
Hajkova et al, 2010

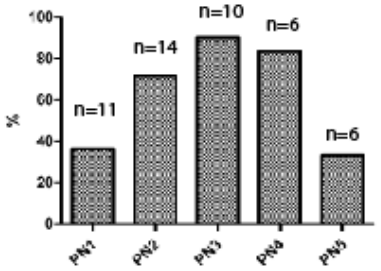
Activation of BER components in zygote



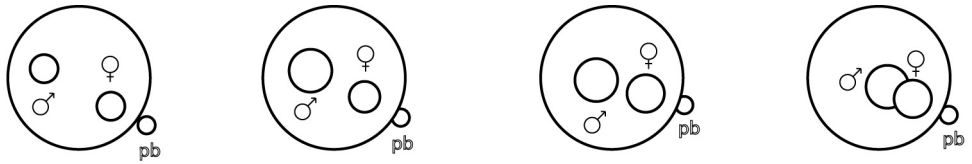
merged

PAR





Hajkova et al, 2010

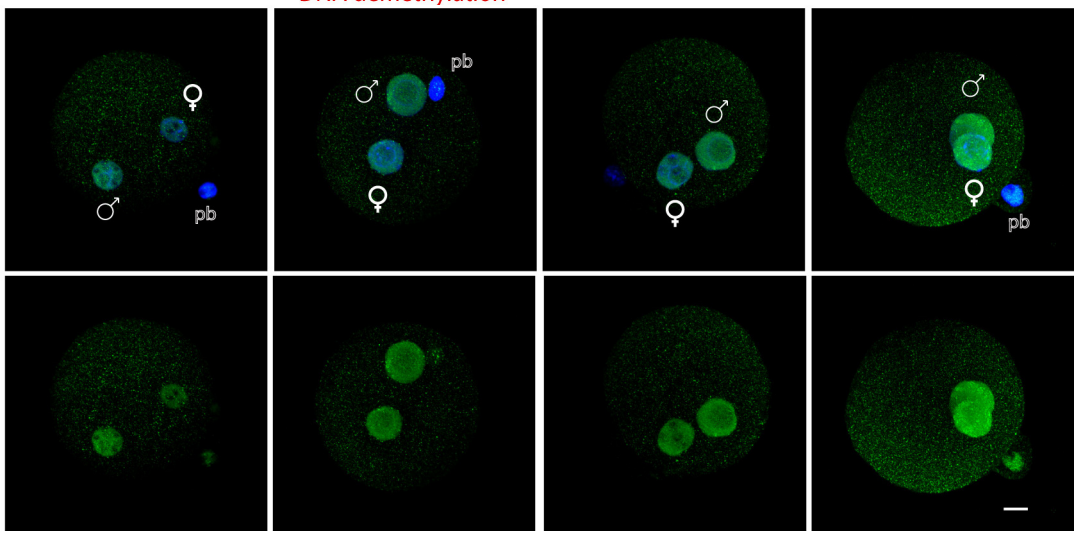


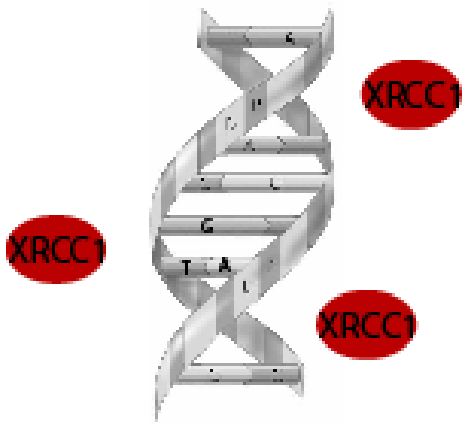
PN2 PN3 PN4 PN5

DNA demethylation

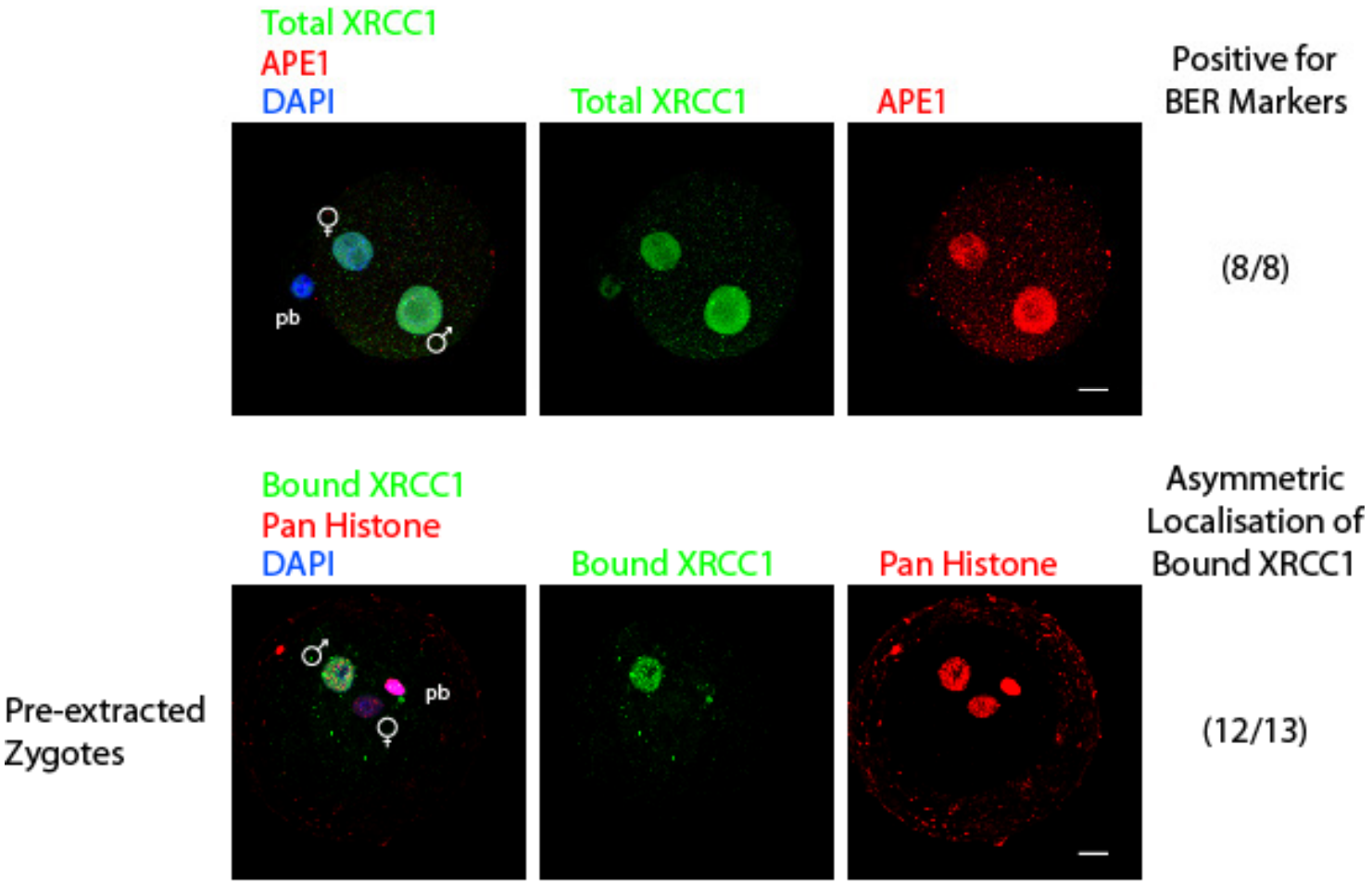
merged

total XRCC1





Active BER in zygotes in the absence of DNA replication

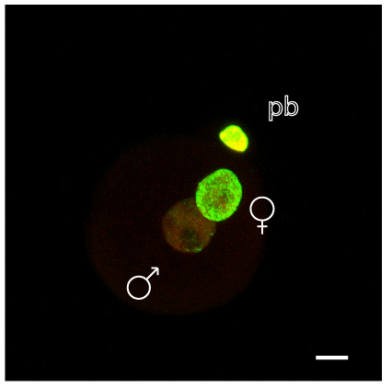


Hajkova et al, 2010

Inhibition of BER pathway perturbes DNA demethylation

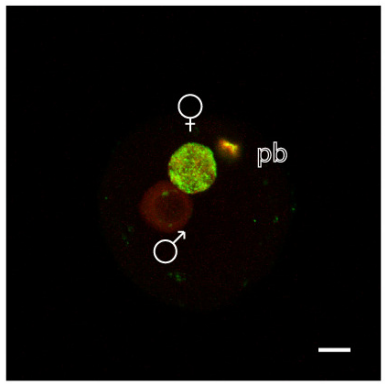
CTRL

5mC
PI



CTRL

5mC
PI

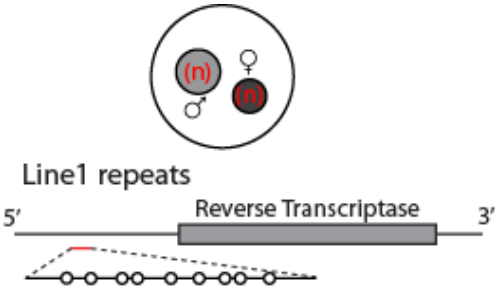


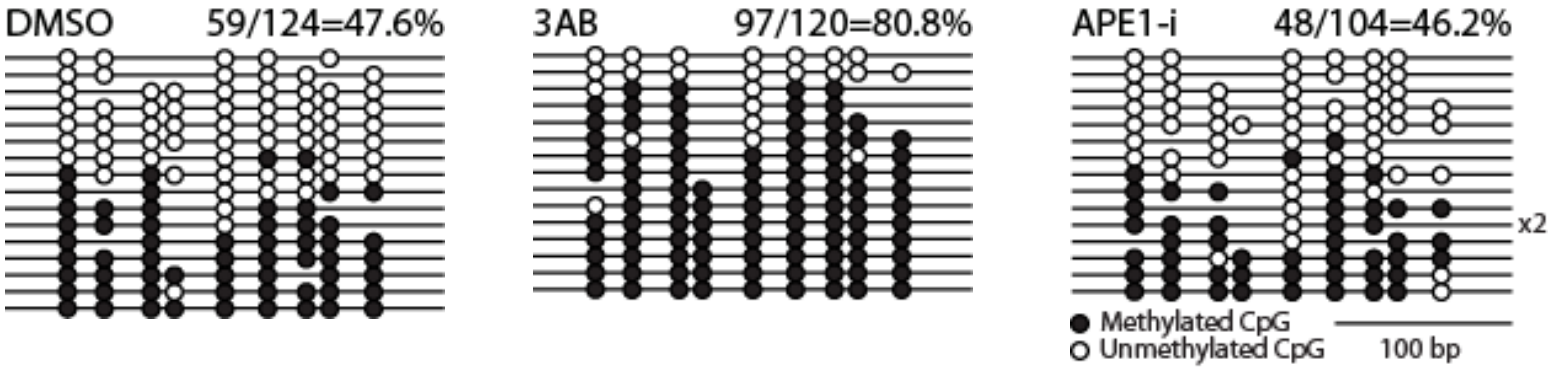
Hajkova et al, 2010

Effect of small molecule inhibitors on DNA demethylation in mouse zygotes

-

Bisulphite sequencing



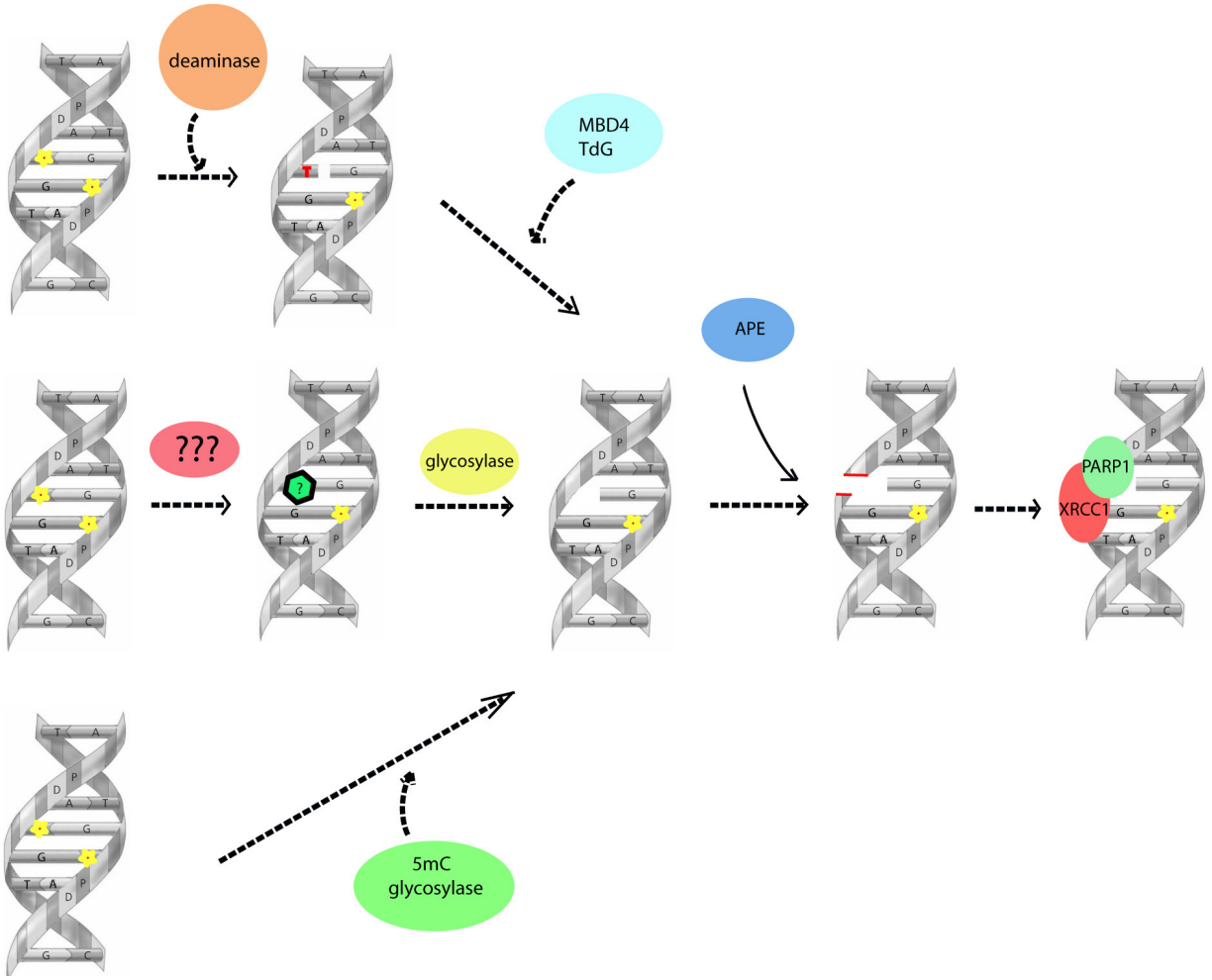


Hajkova et al, 2010

5mC deamination

chemical modification of 5mC

5mC glycosylase



Hajkova et al, 2010

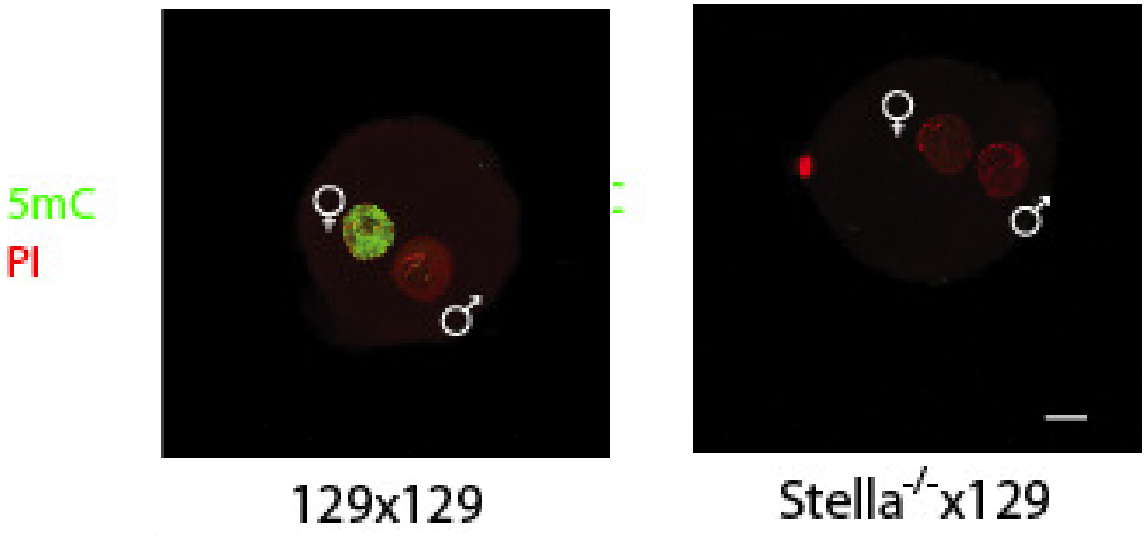
Genetic model:

Nat Cell Biol. 2007 Jan;9(1):64-71. Epub 2006 Dec 3.

PGC7/Stella protects against DNA demethylation in early embryogenesis.

Nakamura T, Arai Y, Umehara H, Masuhara M, Kimura T, Taniguchi H, Sekimoto T, Ikawa M, Yoneda Y, Okabe M, Tanaka S, Shiota K, Nakano T.
Department of Pathology, Graduate School of Medicine and Frontier Biosciences, Osaka University, Osaka 565-0871, Japan. tnakano@patho.med.osaka-u.ac.jp

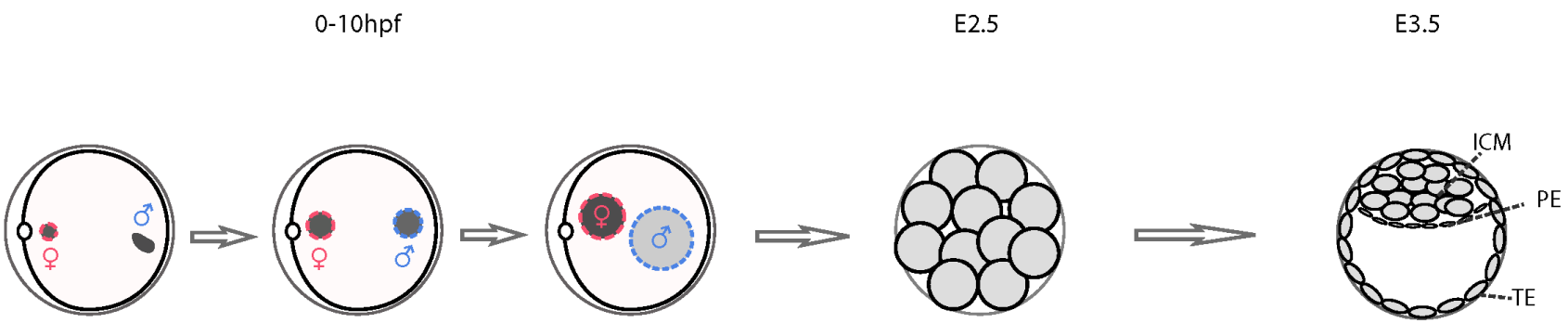
BER is active in both pronuclei in Stella depleted zygotes



Bound XRCC1

Hajkova et al, 2010

Epigenetics of early development - overview



replacement of protamines
with histones
removal of some histone variants
(paternal genome)

DNA demethylation
(active)
(paternal genome)

DNA demethylation
(passive)

Xi reactivation
(ICM, female embryos)

Phase II

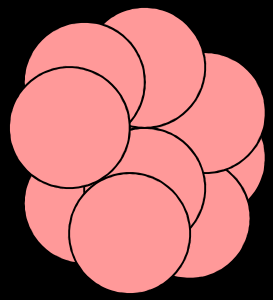
The story of ICM – regaining pluripotency

Re-activation of Xi in the cells of ICM

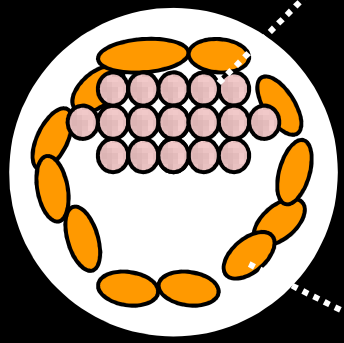
(female embryos)

Morula

Xi Xa



Blastocyst



Xa Xa

Xi Xa

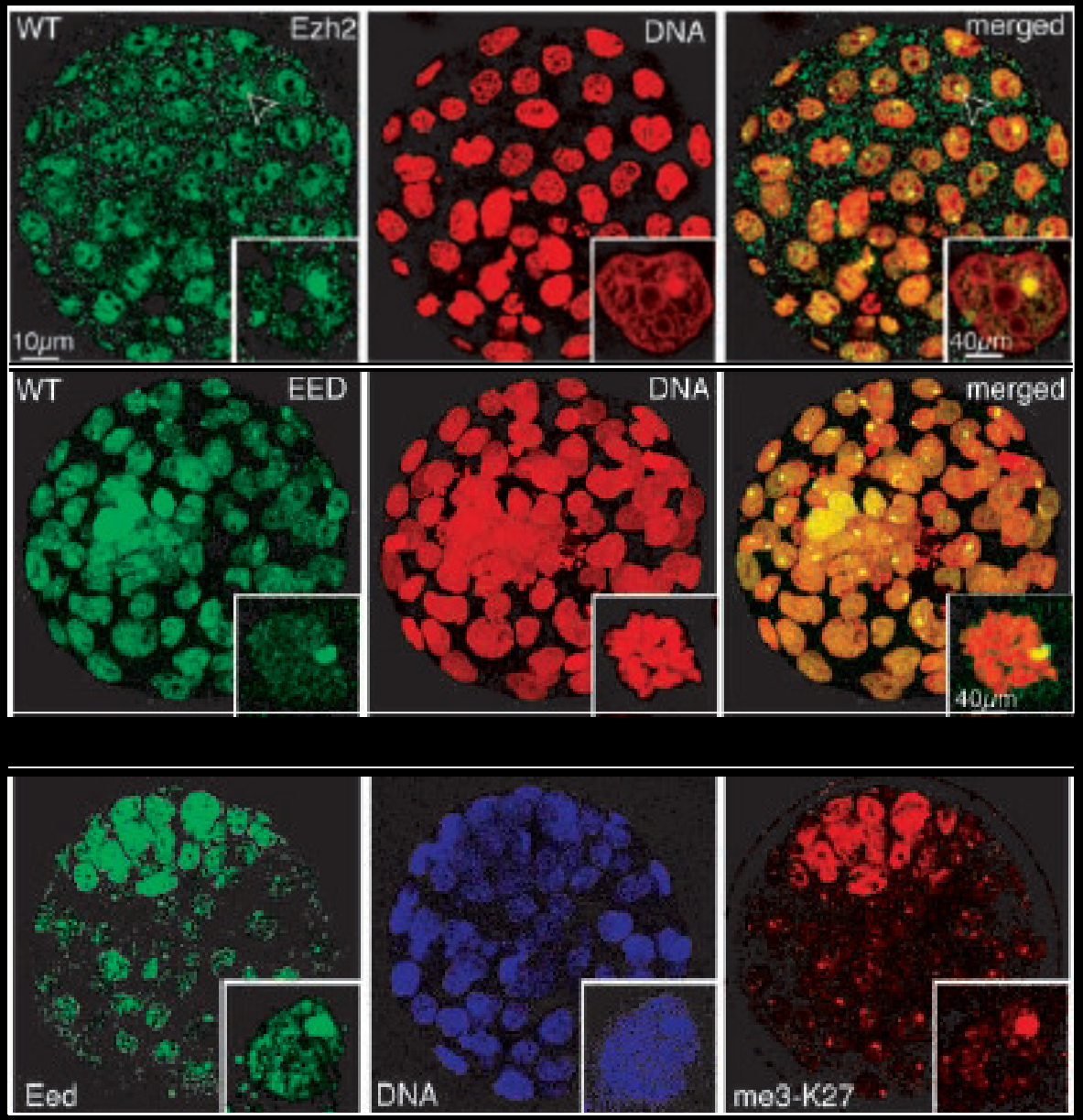
Xist

PRC1(Ring1b)

PRC2 (Ezh2, Eed)

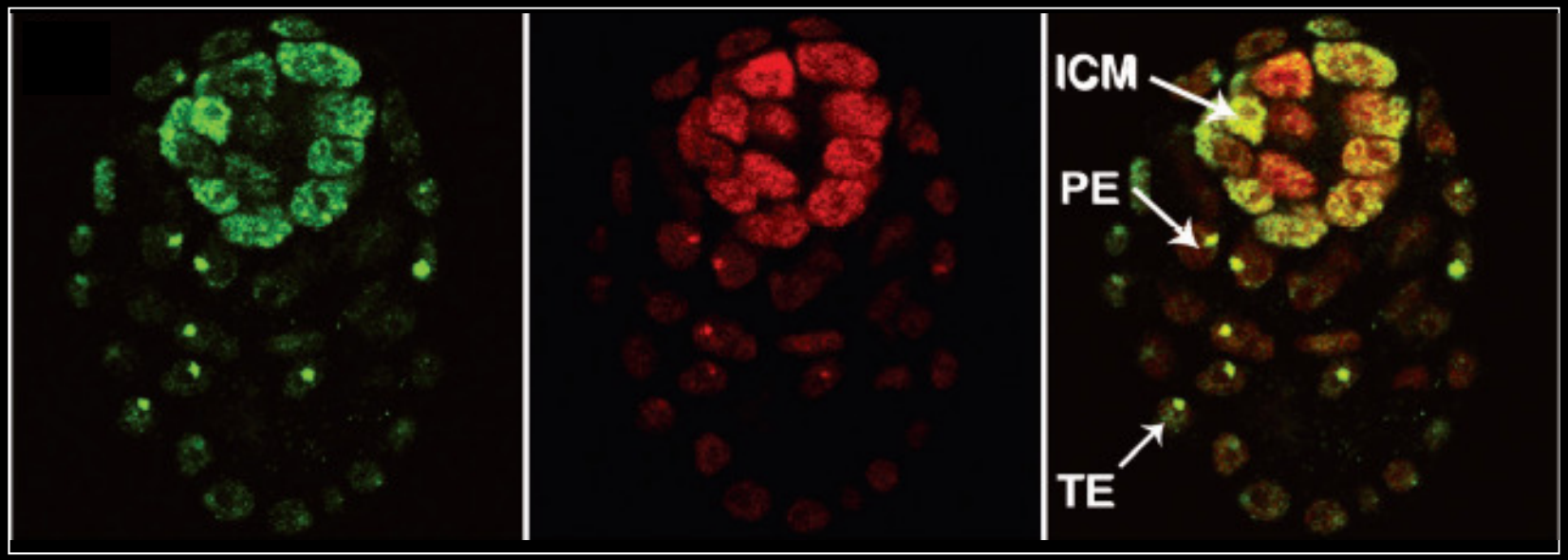
H3k27me3…

Heterochromatin marks of Xi in female pre-implantation embryos



Erhardt et al, 2003

Re-activation of Xi in late blastocyst



Mak et al, 2004

Mouse development

Re-activation of Xi

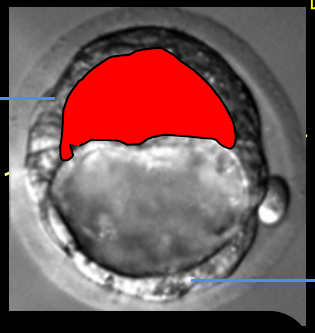
Erasure of polycomb marks

Maintenance of DNA methylation

blastocyst

ICM

TE



E6.5

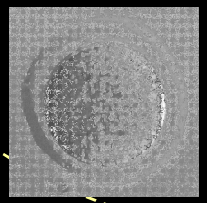
extraembryonic ectoderm

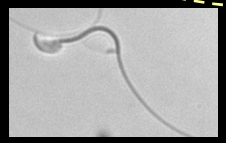
epiblast

zygote

DNA demethylation

Maintenance of some chromatin marks





Mature gametes

E 10.5-11.5

Environmental factors affecting epigenetic processes in early embryos

Effect of culture conditions/ medium composition on genomic imprinting

Effect of small molecule compounds on DNA demethylation in zygote

Effect of manipulation or use of immature/defective gametes (abnormal zygotic DNA demethylation in ROSI generated zygotes)

Acknowledgment

Hajkova's Lab (MRC-CSC ,London)

Rachel Amouroux
Aleksandra Turp
Aditya Sankar

Azim Surani (Gurdon Institute, Cambridge)

Sean Jeffries

Caroline Lee

Kat Arney

Sylvia Erhardt

Sheila Barton

Siqin Bao

Steve Jackson (Gurdon Institute, Cambridge)

MPI Freiburg

Rob Schneider

Thomas Jenuwein

IGBMC Strasbourg

Maria Elena Torres Padilla

MRC Clinical Sciences Centre

Imperial College London

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