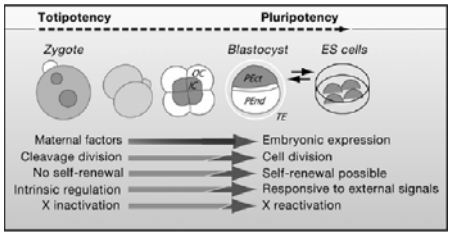
Epigenetic events in early embryos

Petra Hajkova
Wellcome Trust / CR UK Gurdon Institute
Cambridge UK

Gurdon INSTITUTE

Preimplantation development

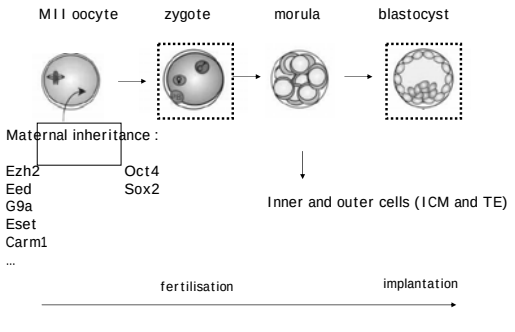


Transcriptional networks ⟺ Epigenetic regulation

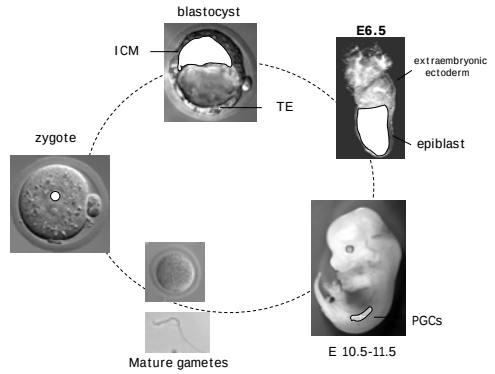
Surani, Hayashi and Hajkova,2007

Maternally inherited factors

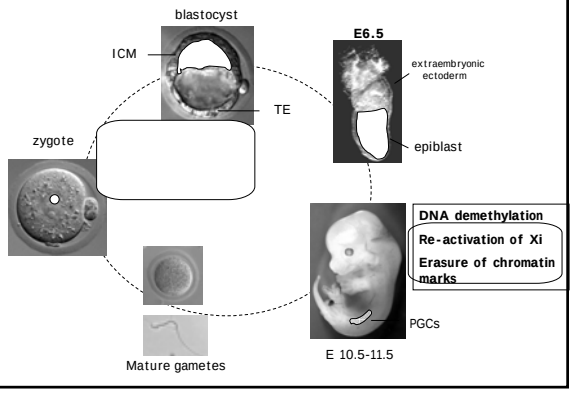
(transcription factors and epigenetic modifiers)



Mouse development



Mouse development

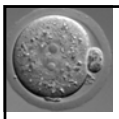


Phase I

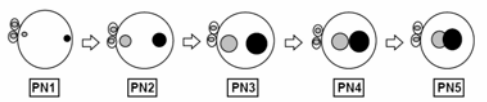
Zygotic epigenetic factory

DNA demethylation

Protamine to histone exchange



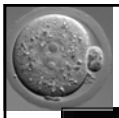
Schematic representation of pronuclear stages in mouse zygote



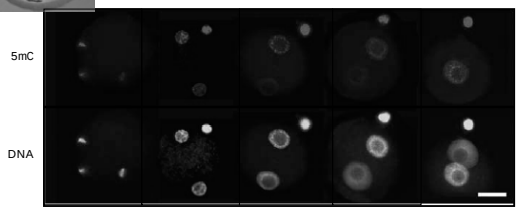
(3hpf) (4hpf) (5hpf) (6-7hpf) (8-10hpf)

♂ protamines

histones



Kinetics of DNA demethylation in mouse zygote



PN0 PN1 PN2 PN3 PN4-5

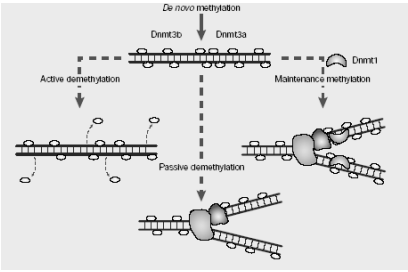
(3hpf) (4hpf) (5hpf) (6-7hpf) (8-10hpf)

♂ protamines

histones

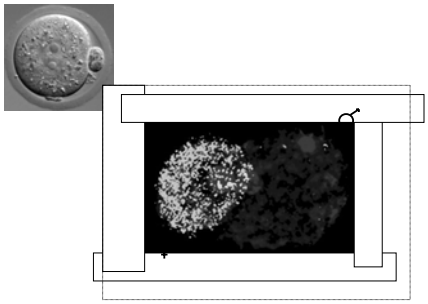
Santos and Dean, 2004

Principles of active and passive DNA demethylation



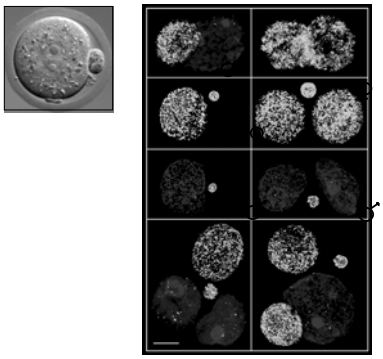
Reik and Walter, 2001

Zygotic reprogramming : DNA demethylation



Barton *et al*, 2001

Zygotic DNA demethylation – pronuclear transplantation experiments



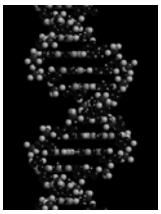
Barton *et al*, 2001

Regulation of epigenetic reprogramming

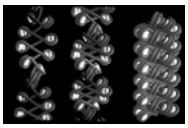
template or **activity**

Chromatin template

Presence of (de)modification enzymes



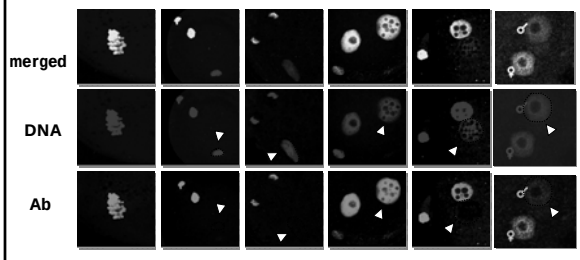
DNA methylation



Chromatin structure

Histone modifications

Zygotic reprogramming – chromatin assymmetry



Me_2K9 Me_2K9 HP1β HP1β Me_2K9 Me_3K27

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

Similarity between chromatin configuration during the reprogramming in the zygote and the early germ line

	pronucleus/zygote	germline (at the time of DNA demethylation)
absence of me2(H3)K9	✓	✓
absence of me3(H3)K27	✓	✓
absence of HP1	✓	✓
absence of linker histones	?	✓

Phase I

Zygotic epigenetic factory

DNA demethylation

Protamine to histone exchange

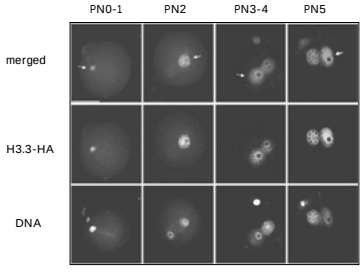
Histone variants:

- 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

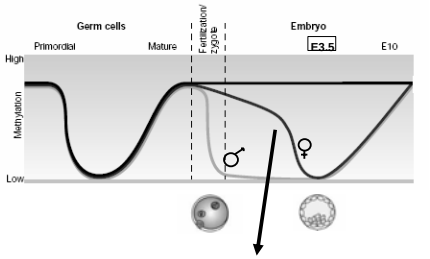
Assymmetric distribution of histone variant H3.3 in zygotes



Protamines in the paternal genome are replaced by H3.3

Torres-Padilla et al, 2006

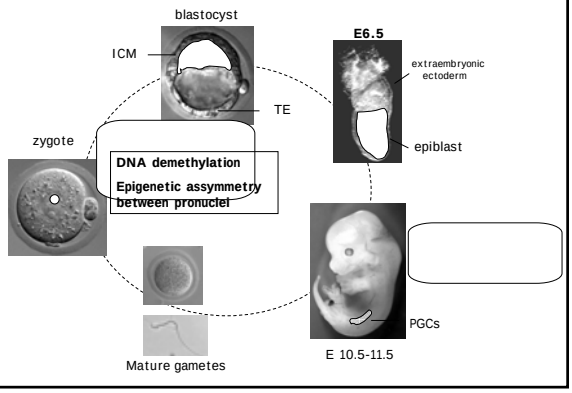
Dynamic changes of DNA methylation in early mouse embryos

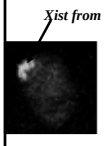


Passive DNA demethylation of maternal genome by exclusion of Dnmt1

Reik and Walter, 2001

Mouse development

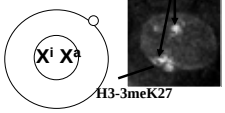




Reprogramming X chromosomes during SCNT *Xist from Xi and Xa*

Somatic cell

Female somatic nucleus



Xi : Xist expression and histone H3-3meK27

Xa : DNA methylation of *Xist* locus

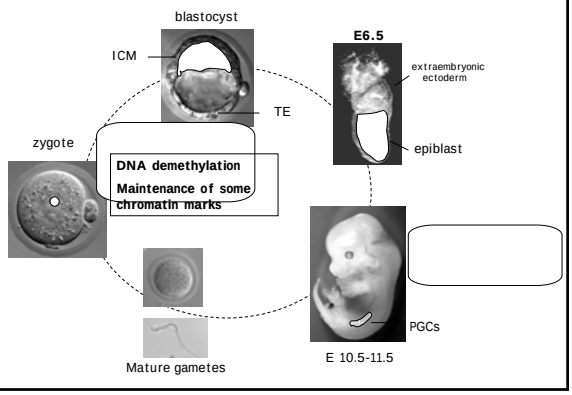
Xi retains the histone H3-3meK27 methylation mark
Xist on Xa is activated

Preferential inactivation of Xi in extraembryonic tissues

Histone lysine methylation is 'erased' later in the epiblast cells

Bao et al 05

Mouse development



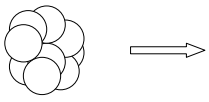
Phase II

The story of ICM

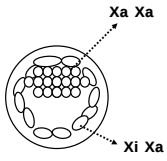
Re-activation of Xi in the cells of ICM
(female embryos)

Morula

Xi Xa

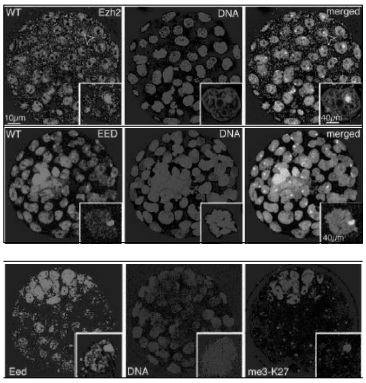


Blastocyst



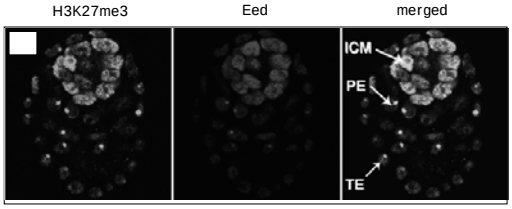
Xist
PRC1(Ring1b)
PRC2 (Ezh2, Eed)
H3k27me3…

Heterochromatin marks of Xi in female pre-implantation embryos



Erhardt et al, 2003

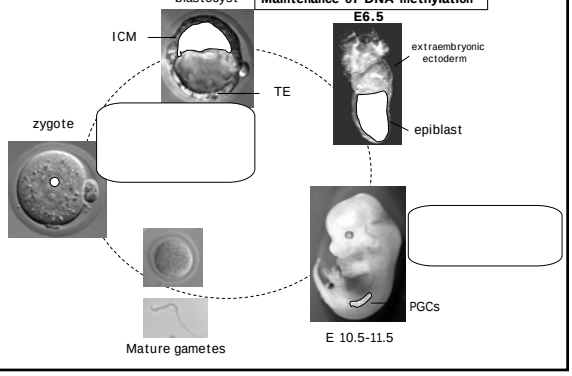
Re-activation of Xi in late blastocyst

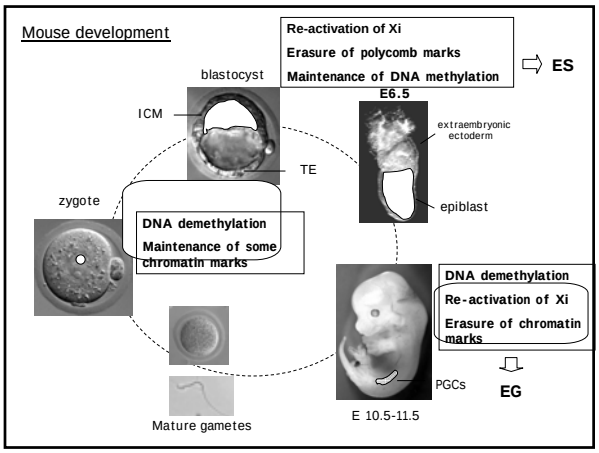


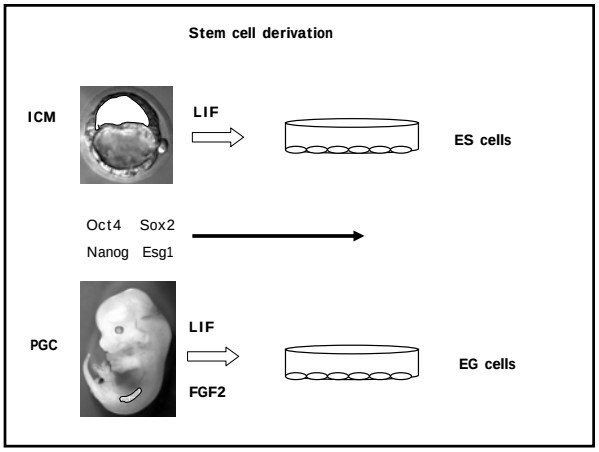
Mak et al, 2004

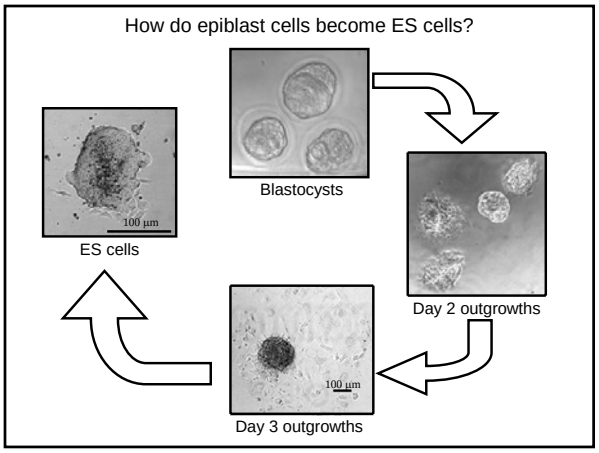
Mouse development

Re-activation of Xi
Erasure of polycomb marks
Maintenance of DNA methylation

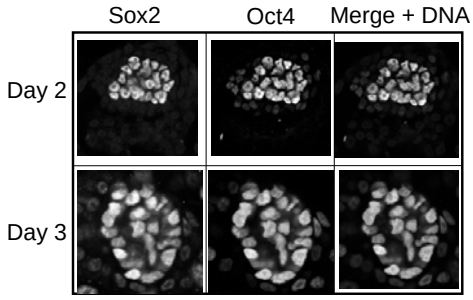



Mouse development
Re-activation of Xi
Erasure of polycomb marks
Maintenance of DNA methylation
ES
blastocyst
ICM
TE
E6.5
extraembryonic ectoderm
epiblast
zygote
DNA demethylation
Maintenance of some chromatin marks
DNA demethylation
Re-activation of Xi
Erasure of chromatin marks
PGCs
EG
E 10.5-11.5
Mature gametes

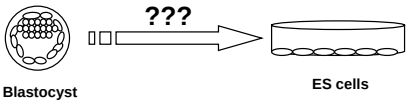

Stem cell derivation
ICM
LIF
ES cells
Oct4 Sox2
Nanog Esg1
PGC
LIF
FGF2
EG cells

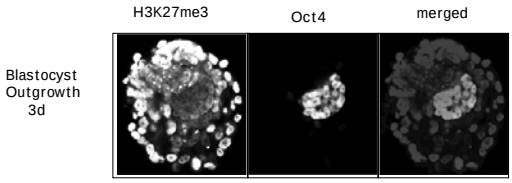

How do epiblast cells become ES cells?
Blastocysts
Day 2 outgrowths
Day 3 outgrowths
ES cells
100 µm
100 µm

Maintenance of pluripotency markers expression

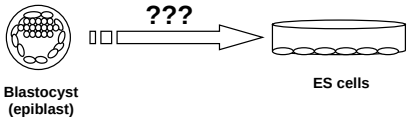


J.Maldonado-Saldivia



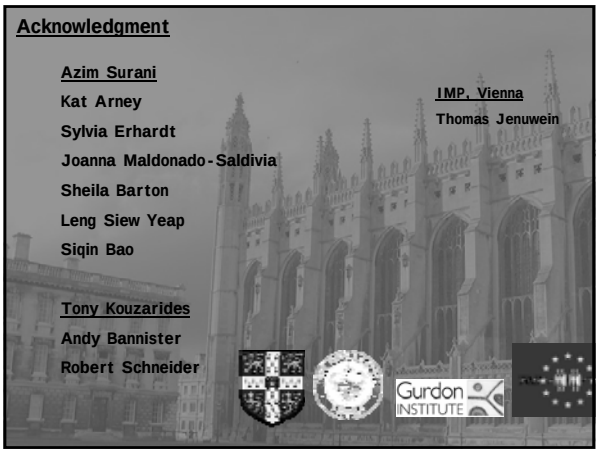


J.Maldonado-Saldivia



Expression of pluripotency associated factors (oct4, sox2, nanog, esg1) →

Epigenetic configuration epigenome →X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