Epigenetic events in early embryos

Petra Hajkova
Reprogramming and Chromatin Group
MRC Clinical Sciences Centre
London UK
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

Preimplantation development

Transcriptional networks ↔ Epigenetic regulation

Surani, Hayashi and Hajkova, 2007
Epigenetics of early development - overview

0-10hpf

- Replacement of protamines with histones
- Removal of some histone variants (paternal genome)

DNA demethylation (active) (paternal genome)

DNA demethylation (passive)

E2.5

E3.5

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

PN1 (3hpf) → PN2 (4hpf) → PN3 (5hpf) → PN4 (6-7hpf) → PN5 (8-10hpf)

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

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

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

PN1 (3hpf) → PN2 (4hpf) → PN3 (5hpf) → PN4 (6-7hpf) → PN5 (8-10hpf)

protamines

histones

DNA demethylation
Kinetics of DNA demethylation in mouse zygote

Santos and Dean, 2004

5mC

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

DNA

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

protamines
histones

Santos and Dean, 2004
Zygotic reprogramming: DNA demethylation

Barton et al., 2001

5mC

DAPI

Barton et al., 2001
Zygotic DNA demethylation - pronuclear transplantation experiments

Barton et al, 2001
Regulation of epigenetic reprogramming

- Chromatin template
- Presence of (de)modification enzymes

(template or activity)
Zygotic reprogramming - chromatin assymetry

<table>
<thead>
<tr>
<th>Ab</th>
<th>Me₂K9</th>
<th>Me₂K9</th>
<th>HP1β</th>
<th>HP1β</th>
<th>Me₂K9</th>
<th>Me₃K27</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
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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 maternal genome 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

- PN1 (3hpf)
- PN2 (4hpf)
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- PN4 (6-7hpf)
- PN5 (8-10hpf)

protamines

DNA demethylation

histones
Kinetics of DNA demethylation in mouse zygotes

Hajkova et al, 2010
Activation of BER components in zygote

Hajkova et al, 2010
Active BER in zygotes in the absence of DNA replication

Hajkova et al, 2010
Inhibition of BER pathway perturbs DNA demethylation

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:

PGC7/Stella protects against DNA demethylation in early embryogenesis.

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

- **0-10hpf**
  - Replacement of protamines with histones
  - Removal of some histone variants (paternal genome)

- **E2.5**
  - DNA demethylation (active) (paternal genome)
  - DNA demethylation (passive)

- **E3.5**
  - 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

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

Blastocyst

Xa Xa

Xa Xa

Xi Xa
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

- Blastocyst
- ICM
- TE
- Extraembryonic ectoderm
- Epiblast
- DNA demethylation
- Maintenance of some chromatin marks
- Re-activation of Xi
- Erasure of polycomb marks
- Maintenance of DNA methylation

- Zygote
- Mature gametes
- E6.5
- 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)
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