19 to 21 April 2012 Stresa Italy

"**7th Workshop Mammalian Folliculogenesis and Oogenesis**",

**Global DNA methylation erasure in primordial germ cells**

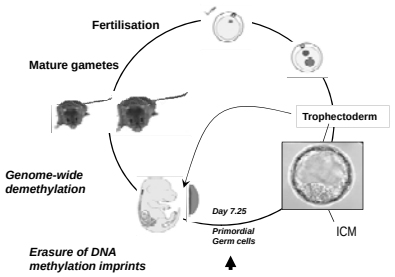
WENDY DEAN

EPIGENETICS PROGRAM

THE BABRAHAM INSTITUTE

CAMBRIDGE UK

# Reprogramming the Genome




## Epigenetic reprogramming

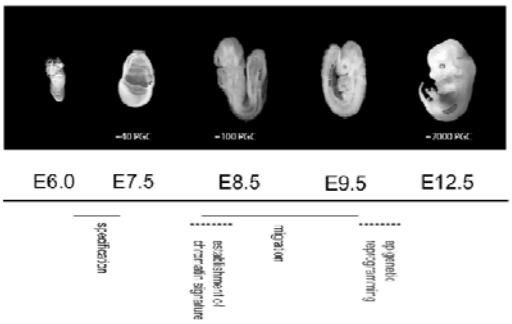
1. Epigenetic reprogramming mechanisms in early development
   (Dynamic chromatin modifications in early embryos)

2. Identifying 'reprogramming factors'
   (Modulation of DNA methylation and the role of DNA deaminases in vivo)

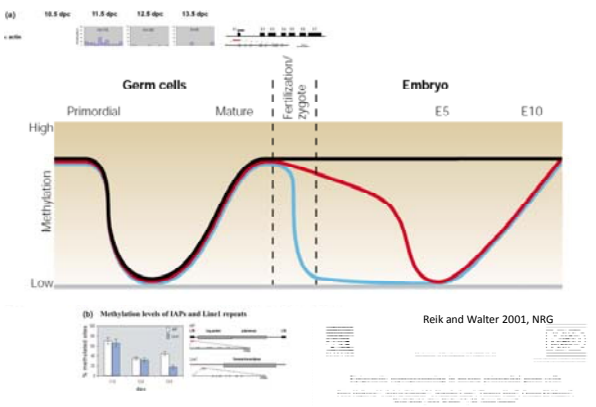
3. Epigenetic regulation of pluripotency genes and early cell lineage determination
   (eg genome-wide screens for promoter DNA methylation using MeDIP)

4. DNA methylation profiling in primordial germ cells using Next Generation Sequencing

Primordial Germ Cell Development

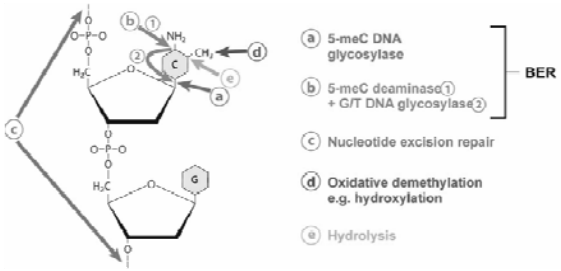



Modified from Hajkova et al., 2008- NATURE



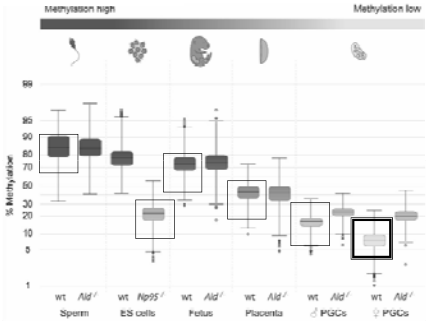

Reik and Walter 2001, NRG

## Models of Active Demethylation




Adapted from Zhu, 2009

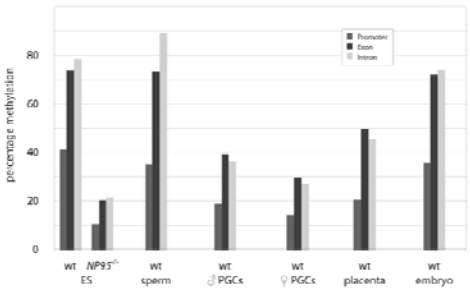
**Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency**




**BS-Seq reveals high levels of genome-wide DNA methylation (i.e. 70-80% of all CpGs are methylated)**

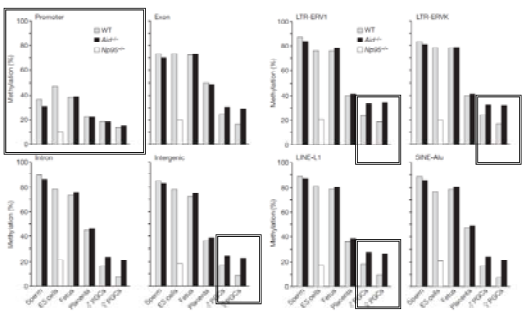
**Genome-wide analysis of methylation in specific genomic features**

Greatest loss of methylation in PGCs occurs in introns, the least in promoters




S Andrews, C Popp

AID deficiency does not effect DNA methylation in promoters regions



## Conclusion

Erasure of DNA methylation in the germ line is a global process, hence limiting the potential for transgenerational epigenetic inheritance.

Aid deficiency interferes with genome-wide erasure of DNA methylation patterns, suggesting that Aid has a critical function in epigenetic reprogramming and **potentially in restricting the inheritance of epimutations** in mammals.

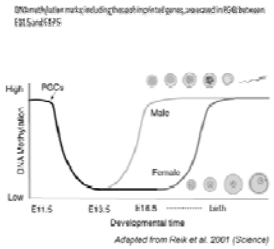
## Unbiased bisulphite Next Generation Sequencing- BSeq

"Wash-up Report"

- One sample per time point
- Input amount between 15ng and 150ng (majority > 50ng)
- GAIIx
- Around 25 million reads per sample
- Duplication rates very high in small input sample
- **Genomic coverage around 0.3%**

Steffi Seisenberger

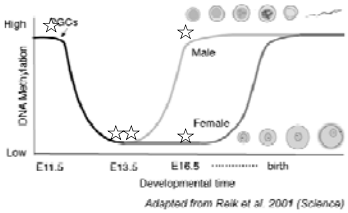
**Epigenetic reprogramming**




*Adapted from Reik et al. 2001 (Science)*

## Epigenetic reprogramming

☆ MeDIP-Seq ☆ BS-Seq




*Adapted from Reik et al. 2001 (Science)*

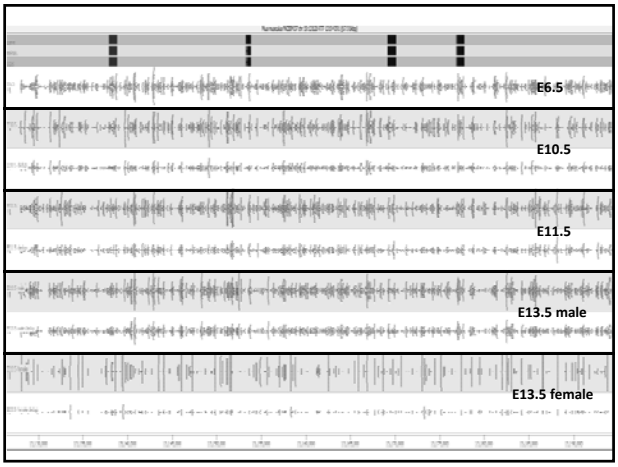
## Unbiased bisulfite Next Generation Sequencing- HiSeq

-Input amounts between 8ng and 100ng (majority < 50ng)

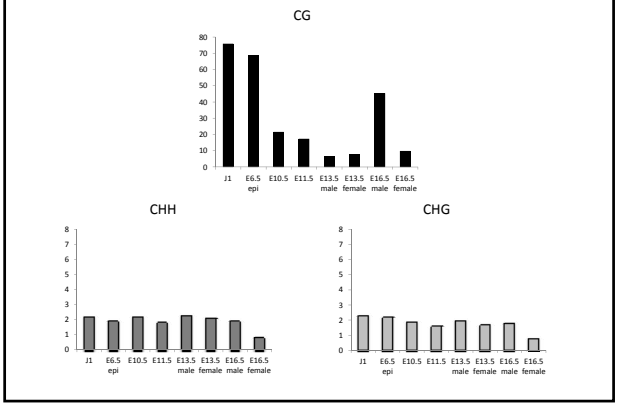
- HiSeq (Sanger, 2x100bp)

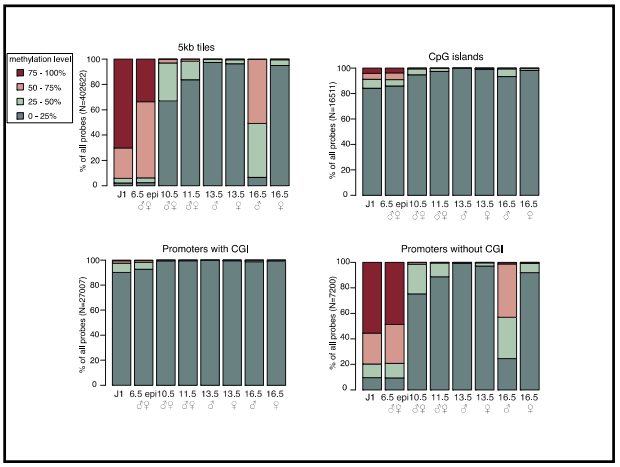
| Sample | Input (ng) | Cycles amp | library concentration (nM) | raw sequences | % mapping efficiency | % duplication | de-duplicated alignments | Coverage |
|---|---|---|---|---|---|---|---|---|
| E6.5 epi | 100 | 14 | 45 | 245586488 | 77.0 | 22.7 | 145460221 | 9.7 |
| E10.5 | <12ng | 18 | 58 | 241241958 | 69.1 | 63.8 | 60031047 | 4.0 |
| E11.5 | 19 | 16 | 30 | 243402414 | 72.3 | 54.6 | 79538927 | 5.3 |
| E13.5 male | 28 | 16 | 30 | 244054365 | 71.8 | 47.3 | 91897781 | 6.1 |
| E13.5 female | 8 | 18 | 5 | 242331031 | 60.4 | 92.3 | 11275162 | 0.8 |
| E16.5 male | 56 | 16 | 80 | 232013149 | 74.3 | 25.6 | 127760318 | 8.5 |
| E16.5 female | 63? | 18 | 12 | 247637095 | 67.5 | 88.5 | 19158063 | 1.3 |
| J1 | 150 | 16 | 33 | 242714563 | 72.9 | 15.5 | 147758679 | 9.9 |

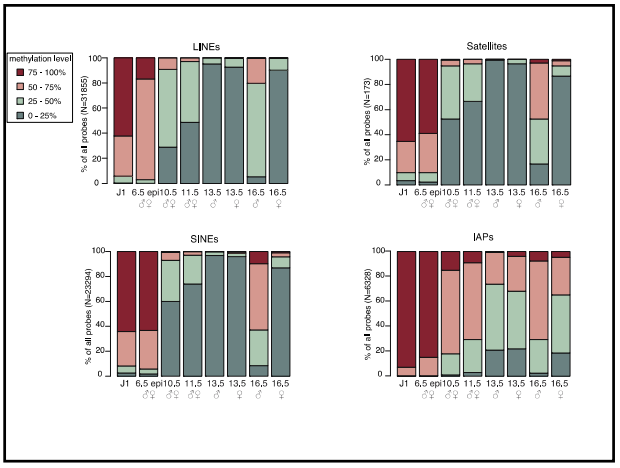
Technical tour de force with 250 million reads per sample

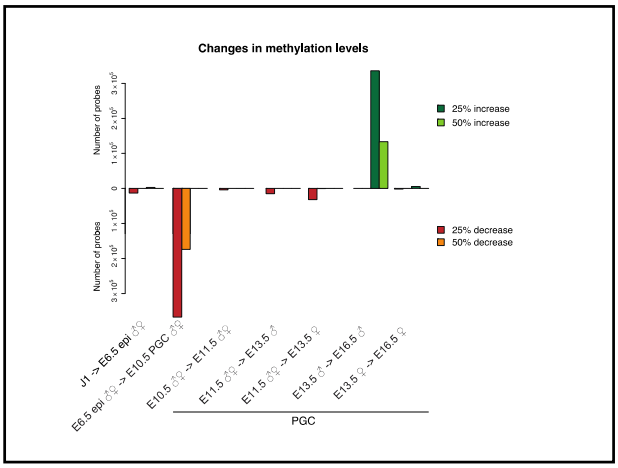


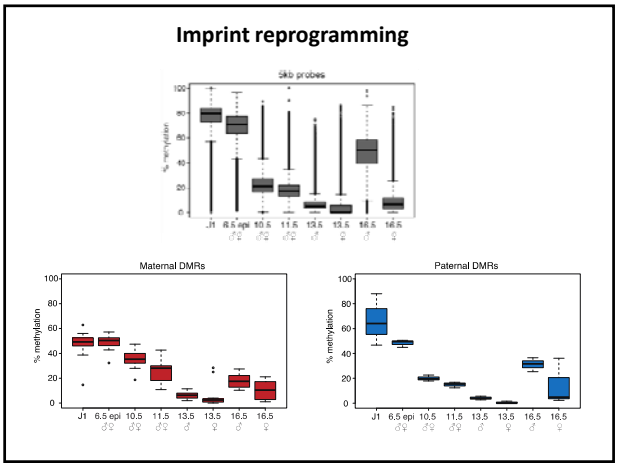
## Global methylation levels

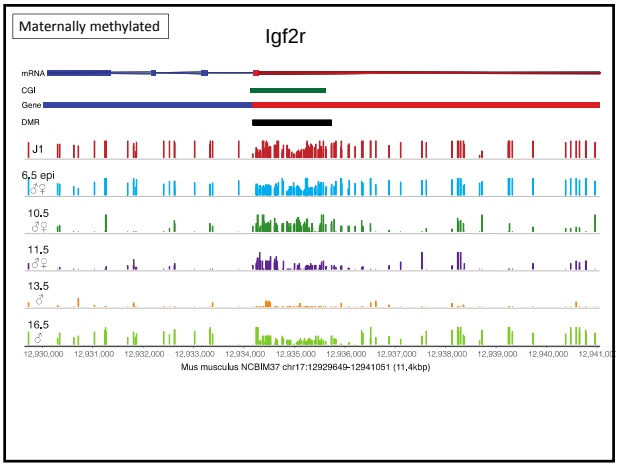


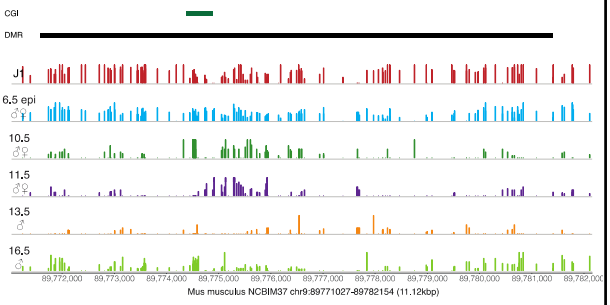

Changes in methylation levels
Number of probes
25% increase
50% increase
25% decrease
50% decrease
J1 -> E6.5 epi ♂♀
E6.5 epi ♂♀ -> E10.5 PGC ♂♀
E10.5 ♂♀ -> E11.5 ♂♀
E11.5 ♂♀ -> E13.5 ♂
E11.5 ♂♀ -> E13.5 ♀
E13.5 ♂ -> E16.5 ♂
E13.5 ♀ -> E16.5 ♀
PGC


Imprint reprogramming
5kb probes
% methylation
Maternal DMRs
Paternal DMRs
J1
6.5 epi
10.5
11.5
13.5
16.5

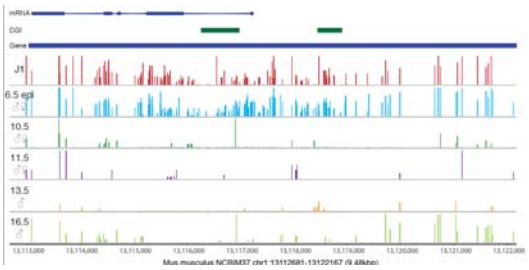

Maternally methylated
Igf2r
mRNA
CGI
Gene
DMR
J1
6,5 epi
10.5
11.5
13.5
16.5
Mus musculus NCBIM37 chr17:12929649-12941051 (11,4kbp)

paternally methylated

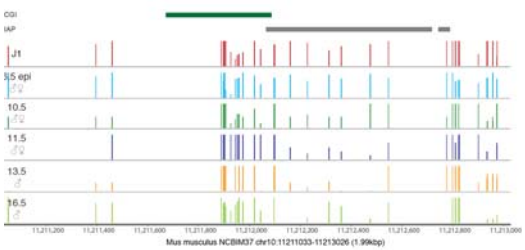
Rasgrf1




Prmd14




**Some portions of the genome are not apparently reprogrammed !**



## What have I shown you

Global erasure of DNA methylation marks occurs earlier than previously anticipated prior to E10.5, at which point the PGC genome is already in a hypomethylated state

Imprint DNA methylation marks are maintained to a large extent during this early demethylation phase and undergo gradual demethylation up to E11.5 followed by complete erasure at E13.5

We find some repeat classes, especially IAPs, to be resistant to demethylation but we also find groups of promoters and CGIs that do not seem to undergo reprogramming and are maintained in a hypermethylated state at all times.

We have base-resolution DNA methylation maps, which we are now analyzing allowing us the identification of the targets of DNA methylation erasure and de novo methylation

## What I haven't shown you

Transcriptional profiles(RNA- Seq )of the same time points indicate that the transcriptional landscape also undergoes drastic reprogramming

DNA methylation and transcription are entirely uncoupled from E11.5 until E16.5 in male PGCs, at which point the well-described correlation between DNA methylation and transcription returns

The temporary uncoupling between DNA methylation and transcriptional regulation facilitates the drastic transcriptional changes observed

**Babraham Institute**
**&**
**University of Cambridge**
**Reik Lab**

Chris
Steffi Se

Babraham Bio

Simon And
Felix Krüge
Laura Biggin

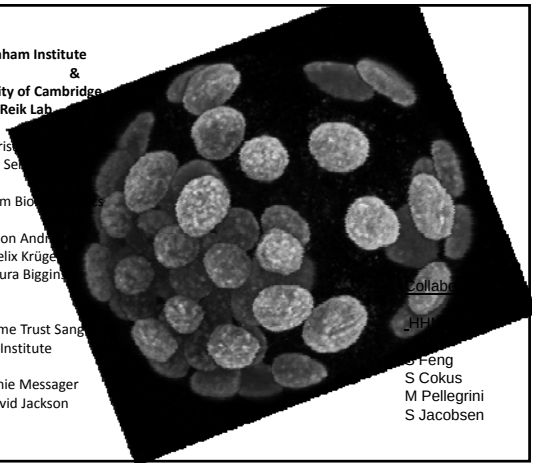
Wellcome Trust Sang
Institute

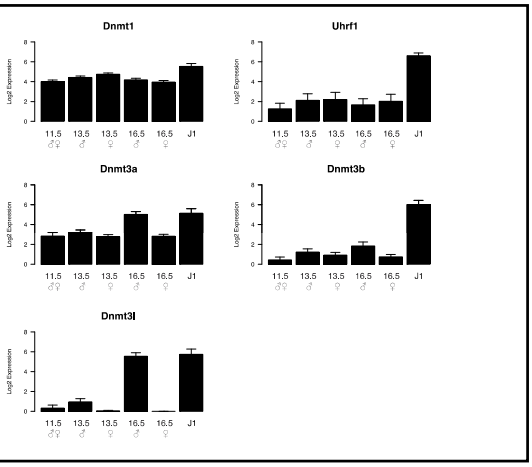
Sophie Messager
David Jackson

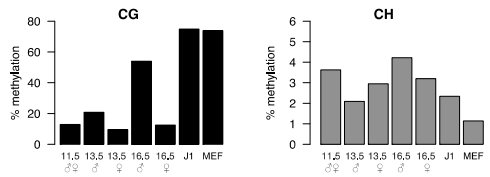
Collabo

HH

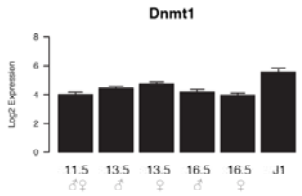
Feng
S Cokus
M Pellegrini
S Jacobsen

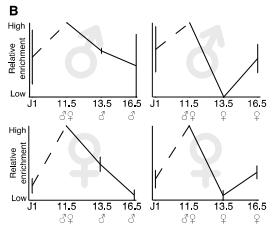




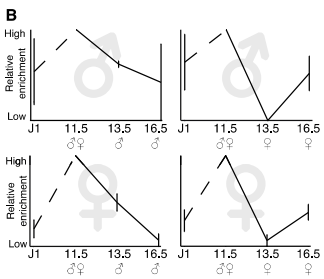

- DNA demethylation occurs earlier than previously anticipated and is completed on a global scale by E11.5 (in CG context)
- Additional demethylation occurs after E11.5 in CH sequence context
- *De novo* methylation occurs in male PGCs at E16.5 in CG and CH sequence context

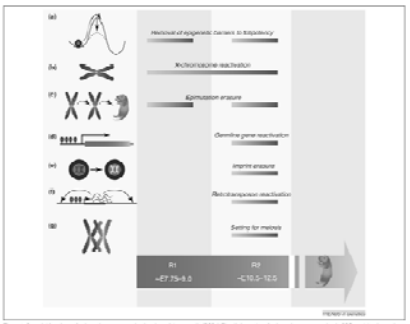



Imprint DNA methylation marks seem to have different reprogramming kinetics than the rest of the genome and undergo specific demethylation starting from around E11.5 whilst the rest of the genome is already hypomethylated at this stage.

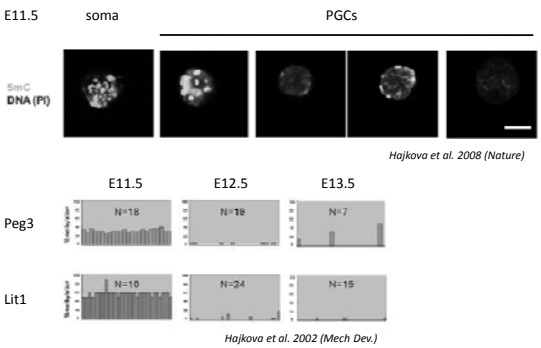
## MeDIP-Seq: imprint reprogramming





Parallel mechanisms of epigenetic reprogramming in the germline

## DNA demethylation in PGCs



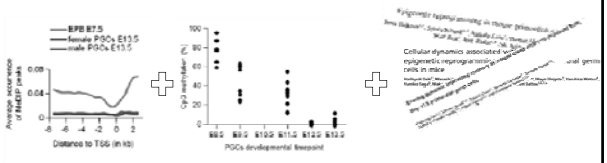

*Hajkova et al. 2008 (Nature)*




*Hajkova et al. 2002 (Mech Dev.)*

## Global profiling of DNA methylation erasure in mouse primordial germ cells

Sylvain Guibert,[1,2] Thierry Forné,[1] and Michael Weber[1,2,3]




(Fig. 3B; Supplemental Fig. 8C). These kinetics of demethylation initiated in early PGC precursors contrast with the demethylation at ICRs that seems to occur mostly during later stages between E10.5 and E12.5 (Hajkova et al. 2002; Sato et al. 2003; Li et al. 2004), which we could also recapitulate in our samples for the *Peg3* and *Igf2r* imprinted loci (Supplemental Fig. 9). We therefore conclude that imprinted and nonimprinted sequences do not follow the same kinetics of demethylation and that most nonimprinted genes initiate progressive erasure of DNA methylation in early PGC precursors around E8.0–E9.0 (Fig. 3C).

## Thank you!

Wolf Reik
Wendy Dean
Christian Popp

Simon Andrews
Felix Krueger
Laura Biggins

Sophie Messager
David Jackson

**LINEs**

| | J1 | E6.5.epi | E10.5 | E11.5 | E13.5 m | E13.5 f | E16.5 m | E16.5 f |
|---|---|---|---|---|---|---|---|---|
| 0-25 | 107 | 19 | 9194 | 15479 | 30284 | 29466 | 1681 | 28725 |
| 25-50 | 1753 | 916 | 19696 | 15408 | 1549 | 2287 | 23690 | 3025 |
| 50-75 | 10168 | 25543 | 2939 | 964 | 22 | 99 | 6379 | 102 |
| 75-100 | 19827 | 5377 | 26 | 4 | 0 | 3 | 105 | 3 |

**Satellites**

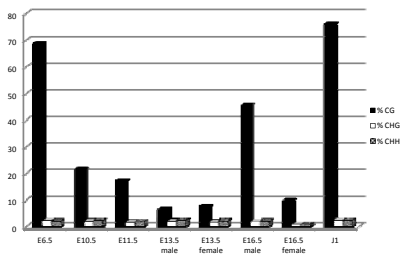
| | J1 | E6.5.epi | E10.5 | E11.5 | E13.5 m | E13.5 f | E16.5 m | E16.5 f |
|---|---|---|---|---|---|---|---|---|
| 0-25 | 6 | 4 | 91 | 115 | 172 | 167 | 29 | 150 |
| 25-50 | 11 | 13 | 73 | 52 | 1 | 6 | 62 | 14 |
| 50-75 | 43 | 54 | 8 | 6 | 0 | 0 | 77 | 7 |
| 75-100 | 113 | 102 | 1 | 0 | 0 | 0 | 5 | 2 |

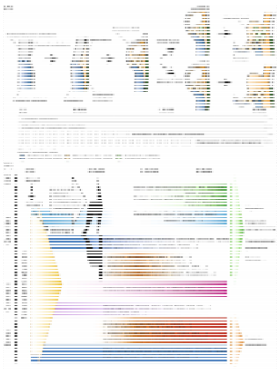
**SINEs**

| | J1 | E6.5.epi | E10.5 | E11.5 | E13.5 m | E13.5 f | E16.5 m | E16.5 f |
|---|---|---|---|---|---|---|---|---|
| 0-25 | 606 | 431 | 13946 | 17209 | 22532 | 22342 | 1983 | 20231 |
| 25-50 | 1309 | 920 | 7688 | 5379 | 690 | 616 | 6647 | 2059 |
| 50-75 | 6436 | 7195 | | 1463<br>648 | 62 | 241 | 12387 | 758 |
| 75-100 | 14943 | 14748 | 197 | 58 | 10 | 95 | 2277 | 246 |

**IAPs**

| | J1 | E6.5.epi | E10.5 | E11.5 | E13.5 m | E13.5 f | E16.5 m | E16.5 f |
|---|---|---|---|---|---|---|---|---|
| 0-25 | 4 | 7 | 70 | 189 | 1324 | 1391 | 155 | 1178 |
| 25-50 | 17 | 20 | 1070 | 1667 | 3350 | 2920 | 1704 | 2948 |
| 50-75 | 432 | 935 | 4234 | 3900 | 1603 | 1767 | 3986 | 1907 |
| 75-100 | 5875 | 5366 | 954 | 572 | 51 | 250 | 483 | 295 |

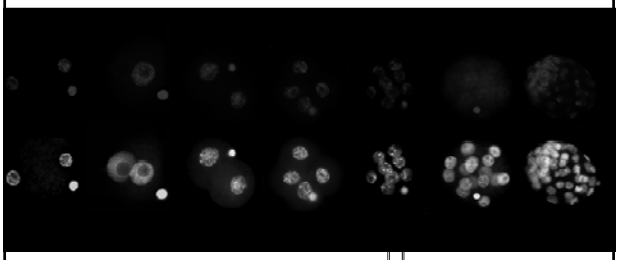




## Extensive methylation reprogramming in preimplantation embryos

Fertilisation — preimplantation development — specification of ICM and TE lineages

active demethylation — passive demethylation (Dnmt1 excluded from nucleus) — de novo methylation in inner cell mass (Dnmt3b?)



F Santos, W Dean

**5kb**

| | J1 | E6.5.epi | E10.5 | E11.5 | E13.5 m | E13.5 f | E16.5 m | E16.5 f |
|---|---|---|---|---|---|---|---|---|
| 0-25 | 8667 | 9622 | 269391 | 336806 | 392142 | 388002 | 26160 | 382750 |
| 25-50 | 14718 | 15419 | 121072 | 59206 | 9522 | 12879 | 171680 | 18144 |
| 50-75 | 96758 | 242348 | 11931 | 6552 | 957 | 1724 | 203794 | 1717 |
| 75-100 | 282479 | 135233 | 228 | 58 | 1 | 17 | 988 | 11 |

**CGIs**

| | J1 | E6.5.epi | E10.5 | E11.5 | E13.5 m | E13.5 f | E16.5 m | E16.5 f |
|---|---|---|---|---|---|---|---|---|
| 0-25 | 13906 | 14192 | 15646 | 16069 | 16478 | 16363 | 15419 | 16191 |
| 25-50 | 1163 | 838 | 771 | 411 | 29 | 110 | 995 | 256 |
| 50-75 | 766 | 865 | 85 | 28 | 4 | 32 | 94 | 56 |
| 75-100 | 676 | 616 | 9 | 3 | 0 | 6 | 3 | 8 |

**Promoters with CGI**

| | J1 | E6.5.epi | E10.5 | E11.5 | E13.5 m | E13.5 f | E16.5 m | E16.5 f |
|---|---|---|---|---|---|---|---|---|
| 0-25 | 24335 | 25018 | 26750 | 26875 | 26996 | 26843 | 26652 | 26754 |
| 25-50 | 2018 | 1484 | 238 | 121 | 9 | 125 | 331 | 227 |
| 50-75 | 499 | 381 | 18 | 11 | 2 | 37 | 22 | 25 |
| 75-100 | 155 | 124 | 1 | 0 | 0 | 2 | 2 | 1 |

**Promoters without CGI**

| | J1 | E6.5.epi | E10.5 | E11.5 | E13.5 m | E13.5 f | E16.5 m | E16.5 f |
|---|---|---|---|---|---|---|---|---|
| 0-25 | 697 | 672 | 5421 | 6384 | 7135 | 6994 | 1766 | 6622 |
| 25-50 | 769 | 831 | 1683 | 777 | 56 | 182 | 2332 | 517 |
| 50-75 | 1742 | 2188 | 93 | 38 | 8 | 21 | 3008 | 60 |
| 75-100 | 3992 | 3509 | 3 | 1 | 1 | 3 | 94 | 1 |