Contrasted regulation of pericentromeric hetero chromatin in mouse ground naive and primed pluripotent stem cells

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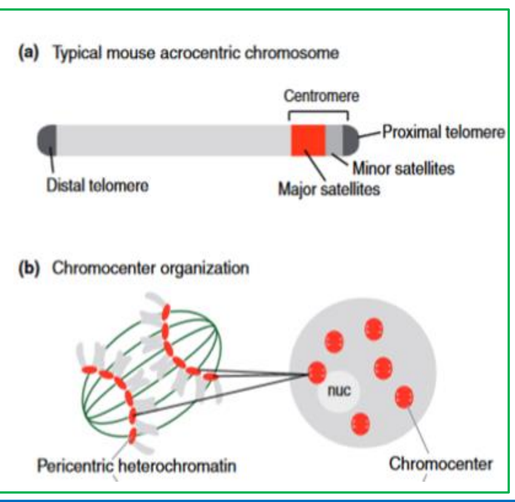
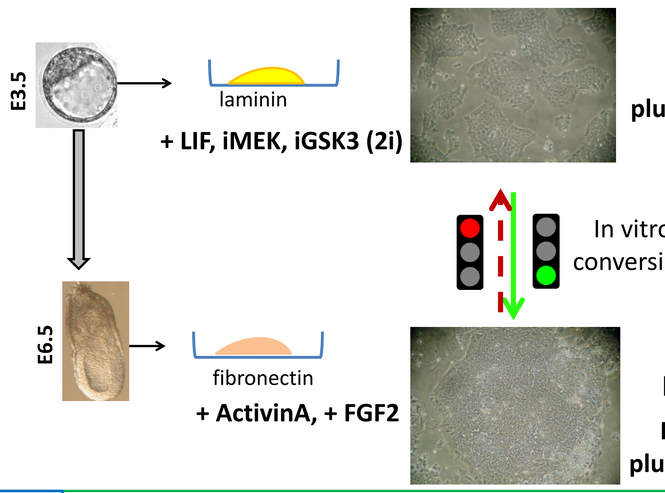
Background

The constitutive heterochromatin compartment:
- composed of telomeres, centromeres and peri-centromeric (PCH) regions
- PCH = Major satellites : 234bp, ~ 10.000 repeats (3% of the mouse genome)
- Compacted and mainly silenced
- Usually enriched in H3K9me3 and DNA methylation

= repressive transcriptionnal environment

Probst, Almouzni et al. TIG 2011





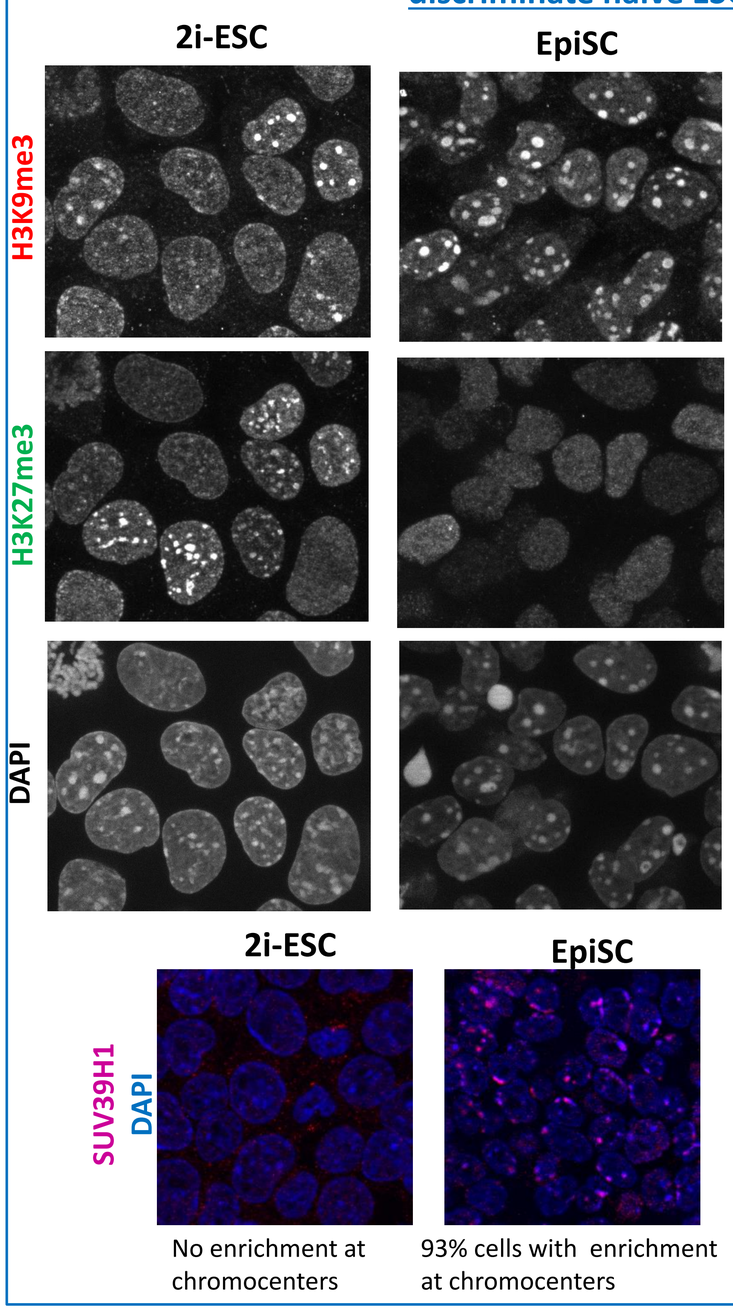
Focus on constitutive heterochromatin:
- *Epigentic constituents?*
- *Role in the epigenetic barrier ?*

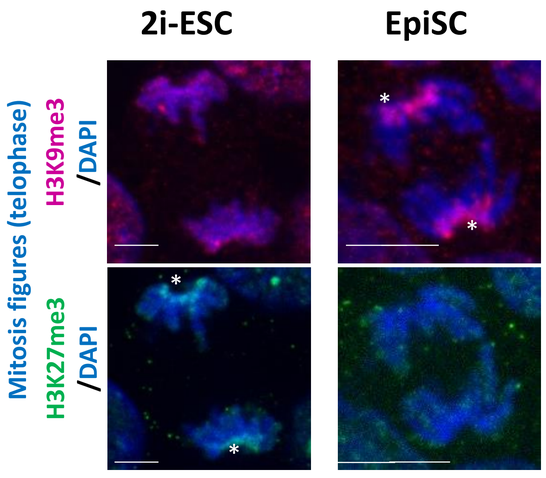
Model: Conversion of WT and mutant ESC into cEpiSC: Suv39hdn :double KO of Suv39h enzymes that deposit H3K9me3 at PCH (Lehnertz et al, Current Biol 2003)

DnmtTKO :dnmt1-/-; dnmt3a-/-; dnmt3b-/- (Tsumura et al, Genes to cell, 2006)

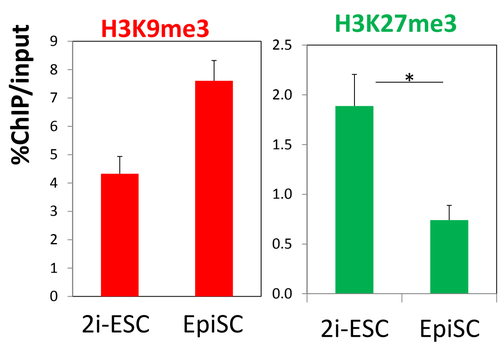
Figure 1

Relative enrichment of H3K27me3 and H3K9me3 discriminate naïve ESC from primed EpiSC





ChIP QPCR on Major satellites



Only 2i-ESC show H33K27me3 enriched chromocenters (in 36% of the cells)

All EpiSC display H3K9me3 enriched chromocenters (vs 30% of 2i-ESC)

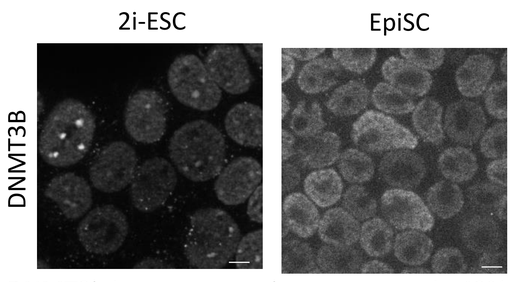
No enrichment at chromocenters

93% cells with enrichment at chromocenters

Control of satellite transcription by epigenetic marks ?

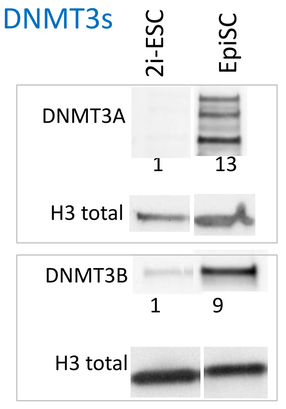
Figure 2

DNA methylation increases between 2i-ESCs and EpiSCs, along with Dnmt3s enzymes

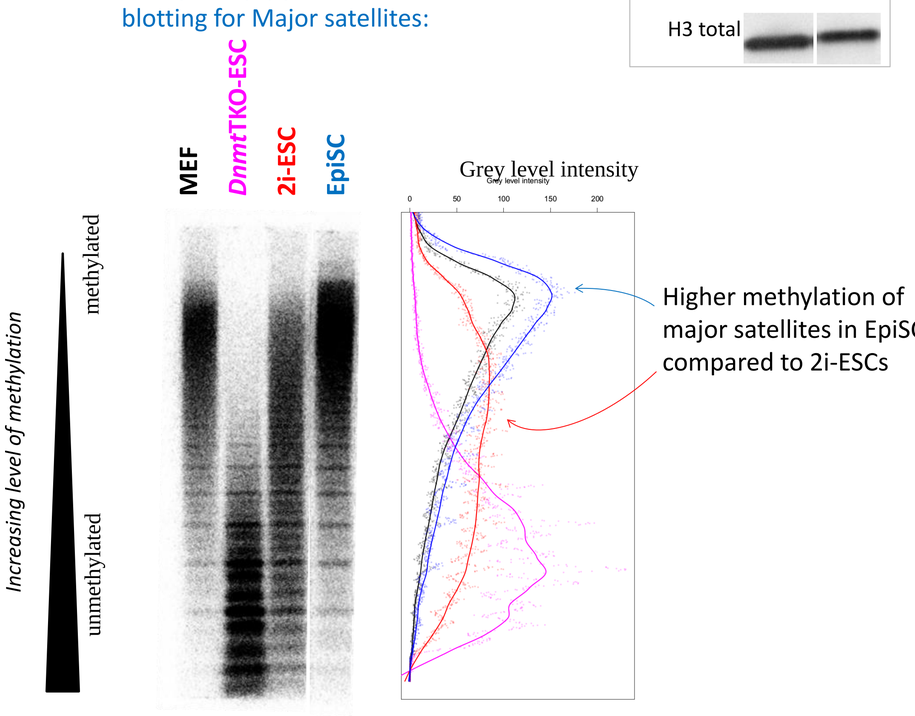


DNMT3b is present at chromocenters in 42% of 2i-ESC and strong but difuse in EpiSC

Western-blot for DNMT3s

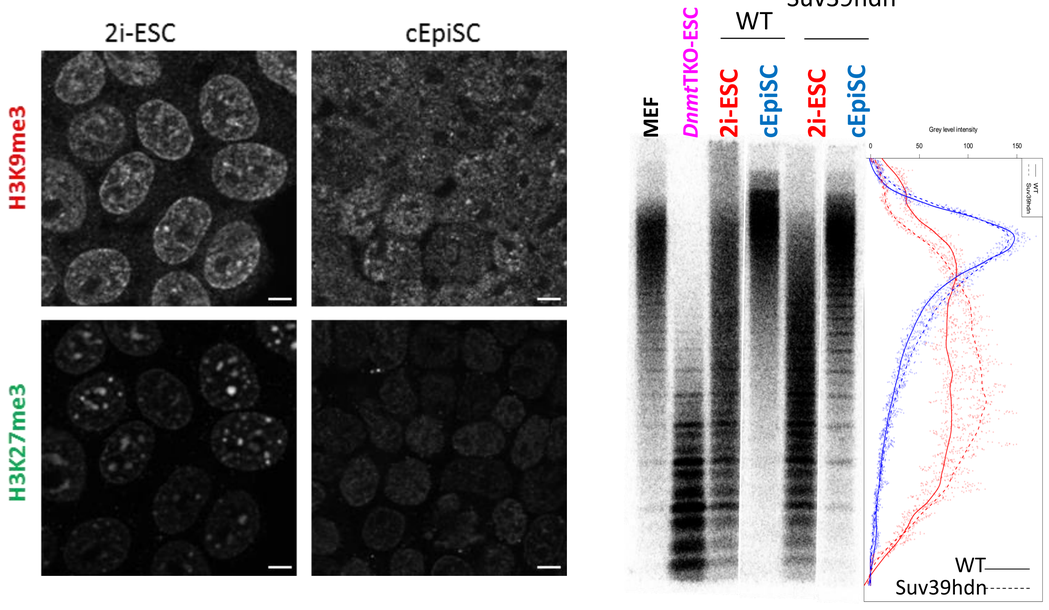


Digestion of genomic DNA with methyl-sensitive enzymes (HpyCH4IV and southern blotting for Major satellites:



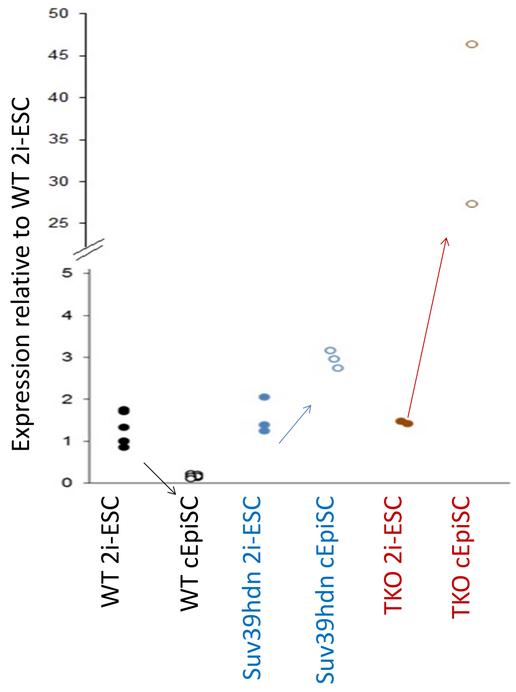
Higher methylation of major satellites in EpiSC compared to 2i-ESCs

Suv39hdn ESC = H3K9me3 at PCH



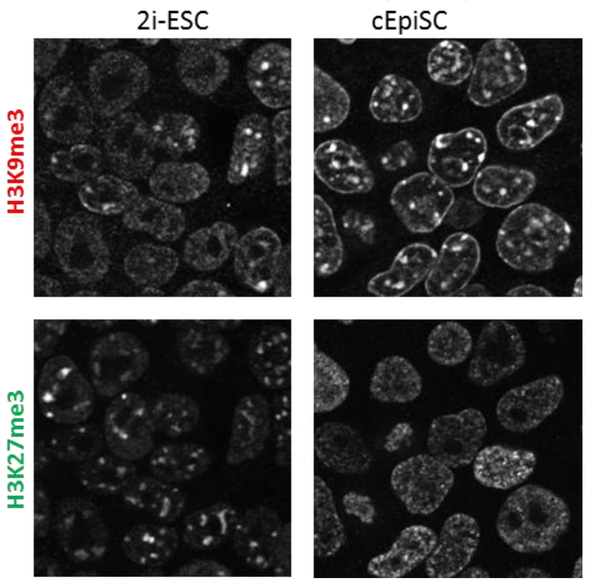
In ESC, gain of H3K27me3 enriched cells, but none in cEpiSC
Gain of DNA methylation in cEpiSC, in spite of the absence of H3K9me3

RNA level of Major satellites transcripts (qRT-PCR)



In absence of either H3K9me3 or DNA methylation at PCH: derepression of satellite transcription during transition from naive to primed cells

*Dnmt*TKO mutant = 5meC



In ESC : gain of H3K27me3 enriched cells, but none in cEpiSC

CONCLUSION :

Transition from naïve to primed state of pluripotency is characterized by a more repressive status of PCH, with a loss of H3K27me3 and a gain of H3K9me3 and 5meC.

Regulation of transcription at PCH in naïve (ground) 2i-ESCs is uncoupled with their epigenetic state, while it is tightly controlled by heterochromatic marks in primed pluripotent cells.

WORKING MODEL