Stem Cell Epigenetics

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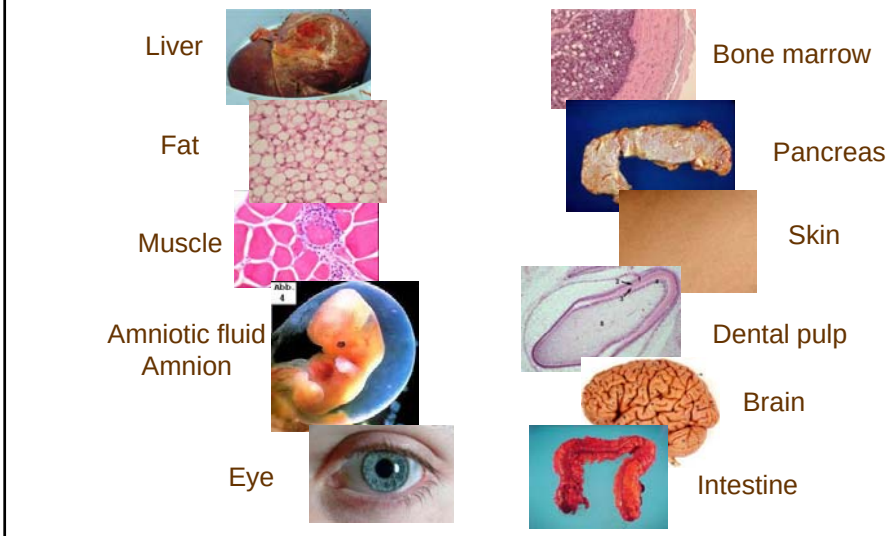
Source of stem cells in the body

Somatic ("adult") stem cells are found in many organs

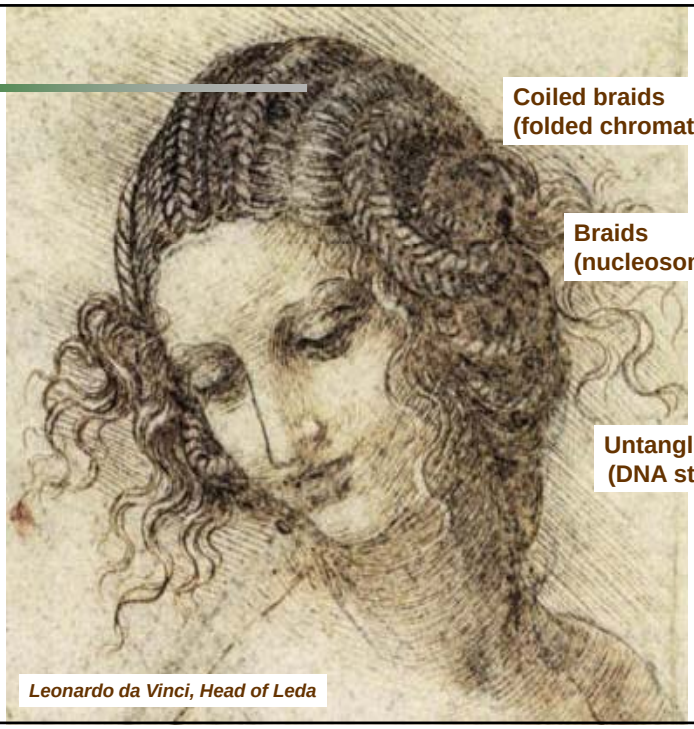


What makes stem cells pluripotent?

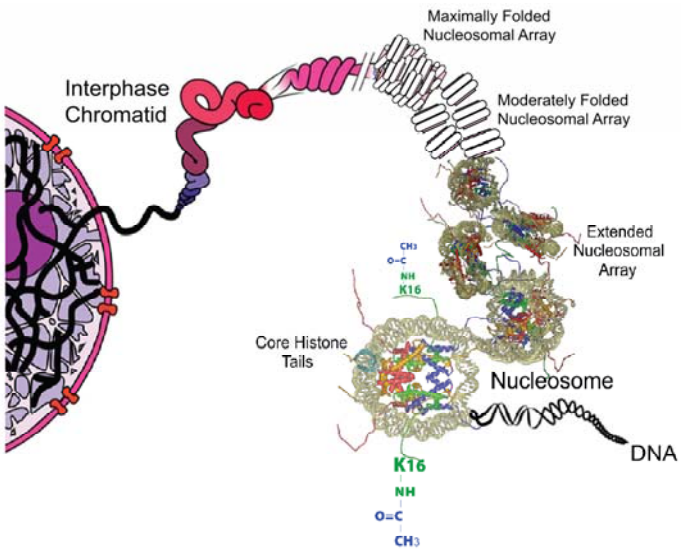
- Receptors on their surface, that make stem cells responsive to signals from their environment (the niche)
- Low level expression of genes normally expressed in many different specific cell types (e.g., bone, fat, neurons, muscle, cartilage, etc)
- How genes are packaged in the cell nucleus
  - **active genes**: 'open' configuration (accessible)
  - **inactive genes**: 'closed' configuration (inaccessible)
  - **inactive genes with a potential for activation**:'open' configuration, but with a 'brake on'

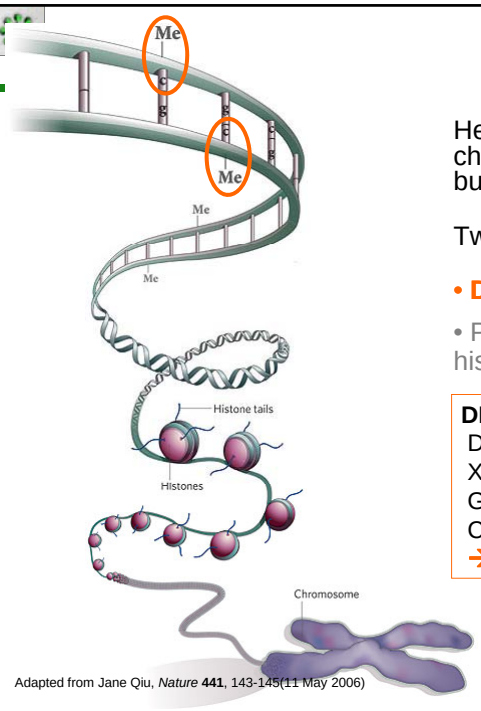
Lecture outline

- **Introduction to epigenetics**
- What provides embryonic stem cells with pluripotent differentiation capacity?
- What about epigenetic states in somatic (adult) stem cells?



Chromatin compaction in eukaryotic cells





Adapted from Jane Qiu, *Nature* **441**, 143-145(11 May 2006)

Epigenetics

Heritable modifications of DNA or chromatin that affect gene function, but not DNA sequence.

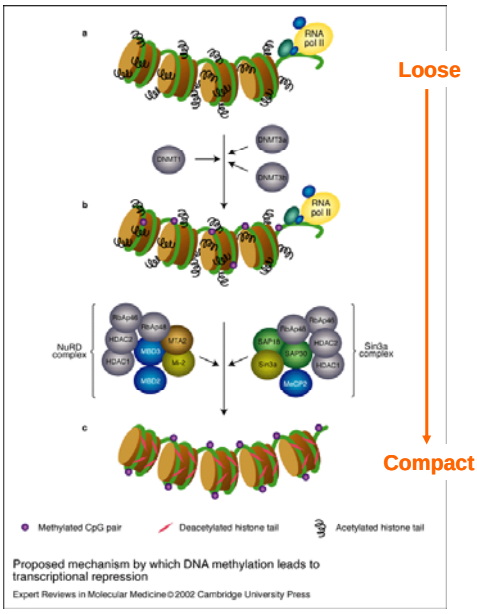
Two main components:

- **DNA methylation**
- Post-translational modifications of histones

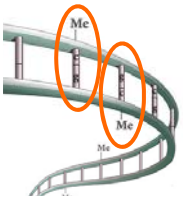
DNA methylation is implicated in:
Development
X chromosome inactivation
Genomic imprinting
Cancer: silencing of tumor suppressors
→ **Long-term gene silencing**

A few facts about DNA methylation

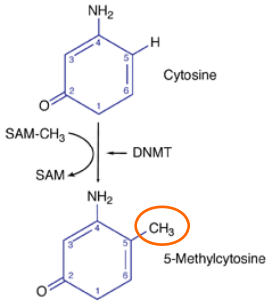
Proposed mechanism by which DNA methylation leads to gene repression



A few facts about DNA methylation



5' – C(m)pG – 3'
3' – GpC(m) – 5'



DNA methyl transferases

- **DNMT1**: maintenance methyltransferase; recognizes hemimethylated DNA after replication; ensures fidelity of methylation in daughter cells after cell division
- **DNMT3a**: de novo methyltransferase (embryo development, differentiation)
- **DNMT3b**: de novo methyltransferase (embryo development, differentiation)
- **DNMT2**: no known DNA methyltransferase activity; methylates RNA?

A few facts about DNA methylation

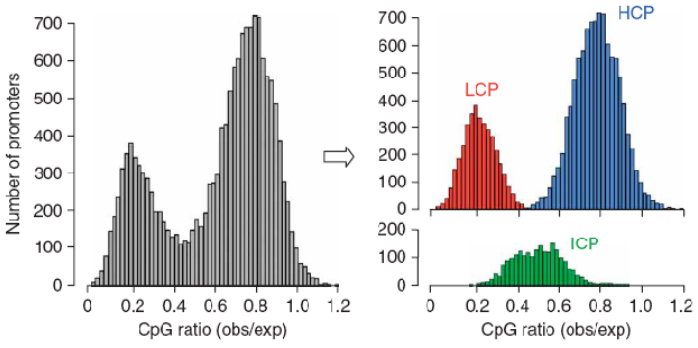
Effect of DNA methylation on promoter activity depends on the density of CpGs in the promoter

Promoter classification based on CpG representation
(Weber et al., 2007. Nat. Genet.)

High CpG promoter (HCP)

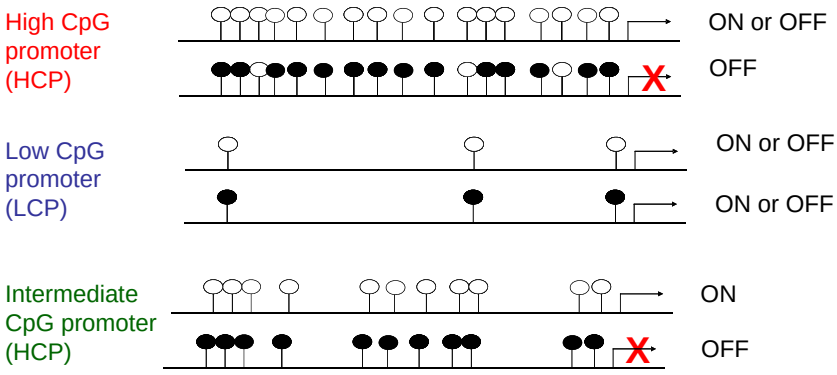
Low CpG promoter (LCP)

Intermediate CpG promoter (HCP)

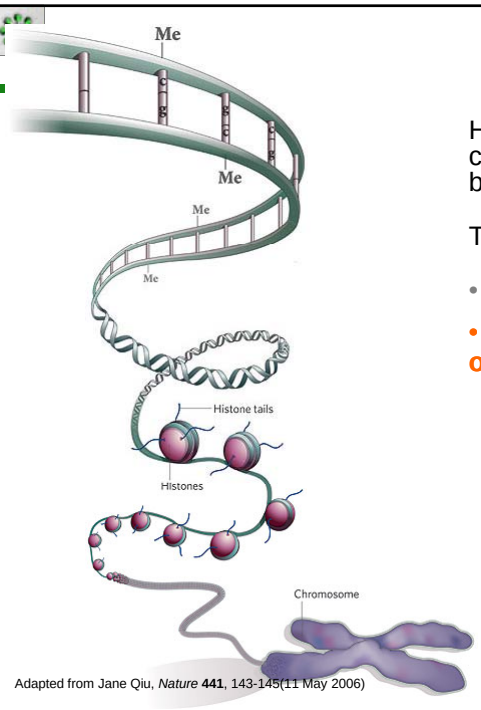


A few facts about DNA methylation

Effect of DNA methylation on promoter activity depends on the number and density of CpGs in the promoter



Epigenetics



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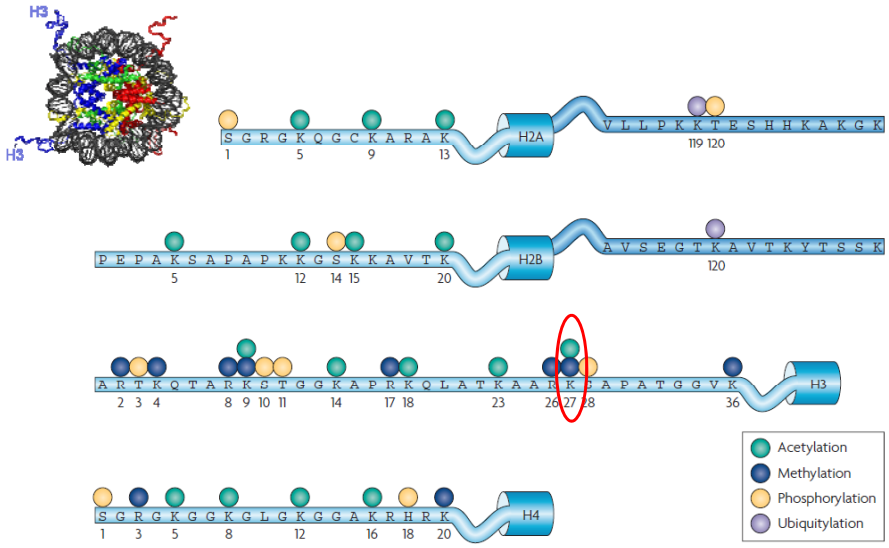
Heritable modifications of DNA or chromatin that affect gene function, but not DNA sequence.

Two main components:

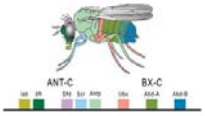
- DNA methylation
- **Post-translational modifications of histones**

Combinations of histone tail modifications make up a 'code'

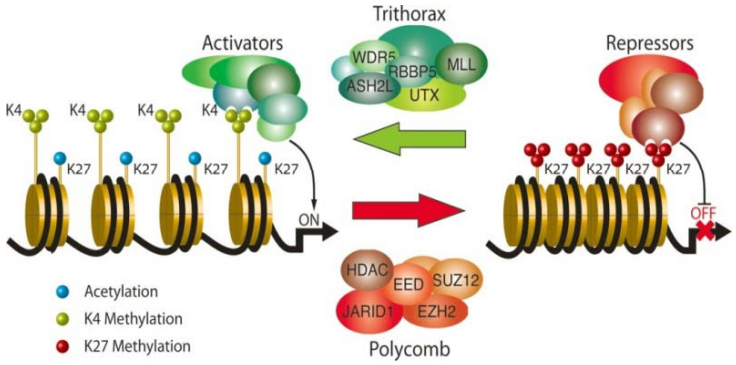


Polycomb group proteins (PcG) are key regulators of cell-fate decisions

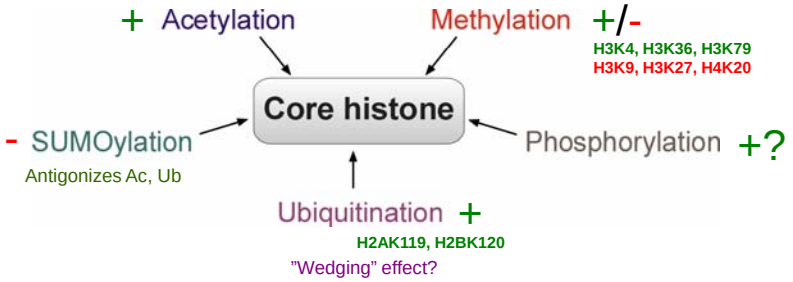
Regulate anterior-posterior axis



Role in chromatin condensation and promoter inactivation



Post-translational modifications of histones



(+/- : effect on gene expression)

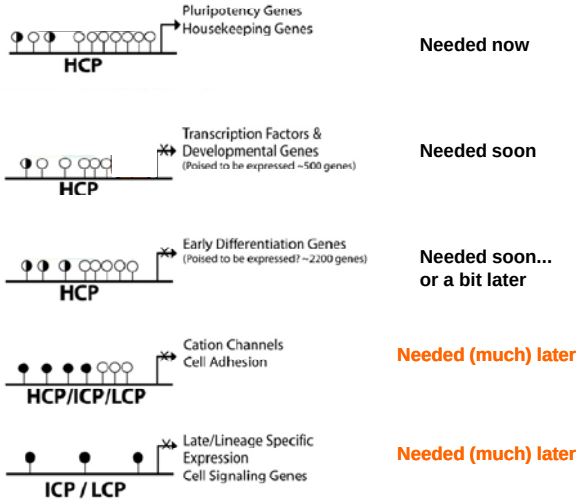
Lecture outline

- Introduction to epigenetics
- **What provides embryonic stem cells with pluripotent differentiation capacity?**
- What about epigenetic states in somatic (adult) stem cells?

DNA methylation in ES cells

- **Overall less DNA methylation than in differentiated cells**
- **But *not* all genes are unmethylated!**

○ Unmethylated CpG
● Methylated CpG



Needed now

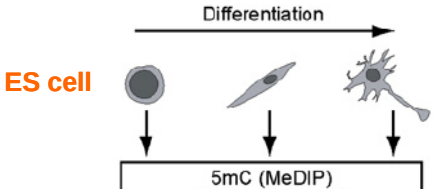
Needed soon

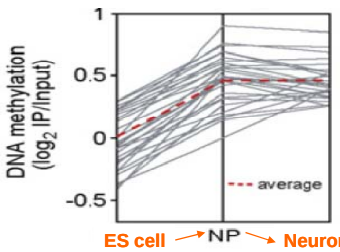
Needed soon...
or a bit later

Needed (much) later

Needed (much) later

Changes in DNA methylation during ES Cell differentiation into neurons





Methylated genes:
- **Pluripotency**
- Embryonic development
- Germline development

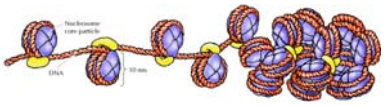
DNA methylation changes **correlate with commitment to a progenitor state**, when ES cells lose pluripotency

Mohn et al., 2008. Mol Cell

A few facts about chromatin in ES cells

A looser and more dynamic chromatin organization than in differentiated cells

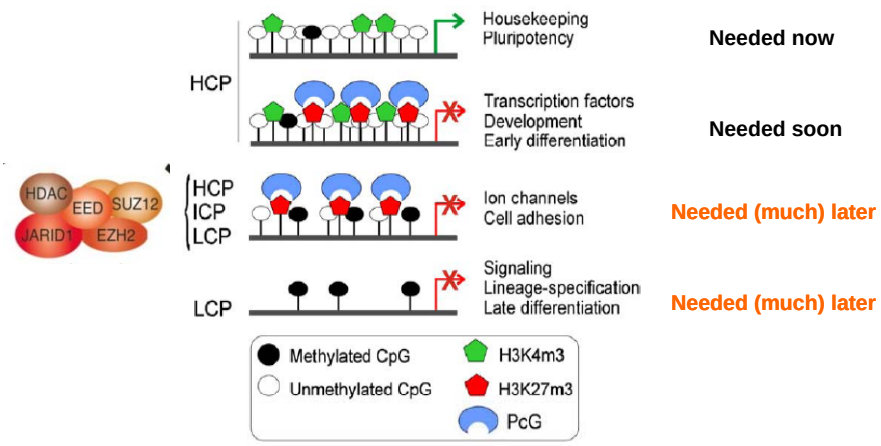
- Overall less DNA methylation than in differentiated cells
- Only one histone H1 molecule per 2 nucleosomes – loosening of chromatin?



- ES cell chromatin is ”hyperdynamic”: histones are more mobile (not as tightly bound to DNA)
- Genes important for development & differentiation are temporarily ”poised” – primed for activation, or repression

Linking DNA methylation & histone modifications in embryonic stem cells

Specific combinations of DNA methylation and histone modifications mark distinct functional classes of genes



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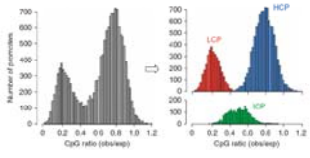
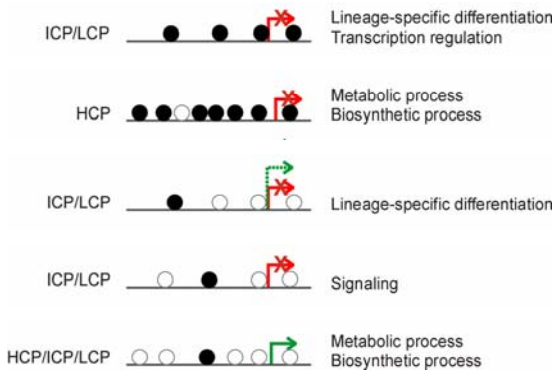
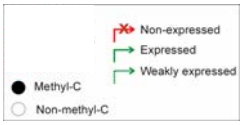
- **What about epigenetic states in somatic (adult) stem cells?**

Functional attributions of methylated and unmethylated promoters in MSCs

Promoter classification based on CpG representation
(Weber et al., 2007. Nat. Genet.)

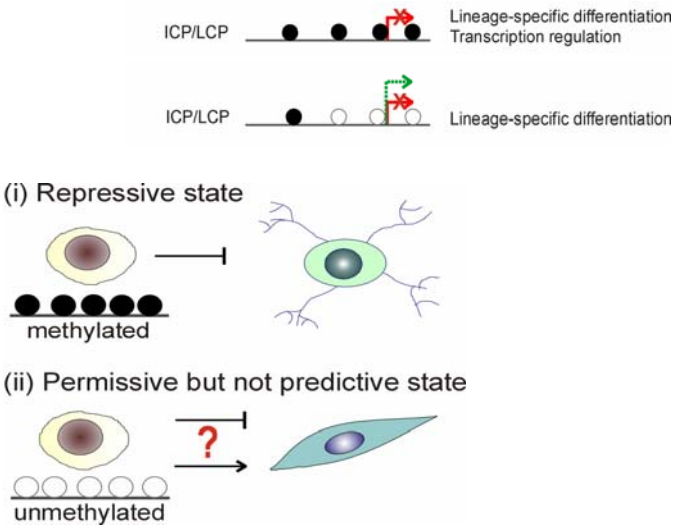




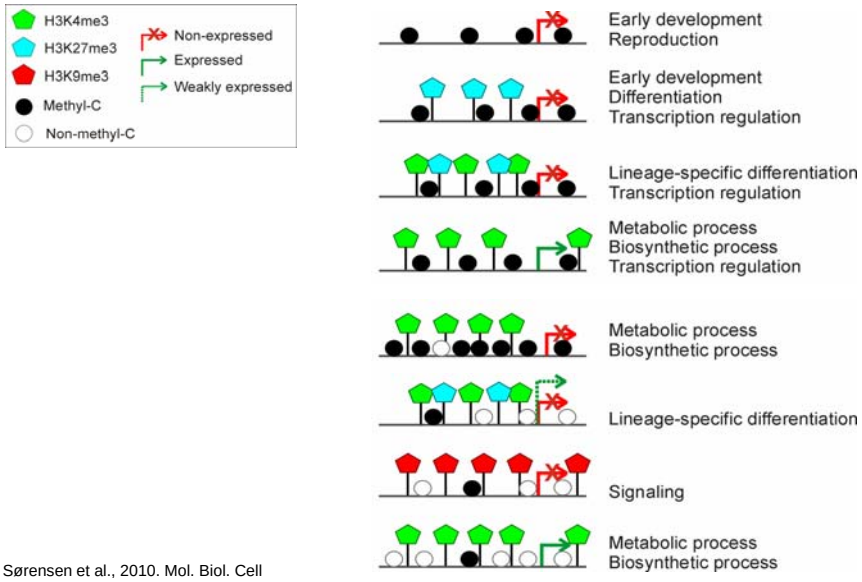


Sørensen et al., 2010. Mol. Biol. Cell

Promoter CpG methylation confers repression, but lack of or weak methylation is not predictive



Combinatorial association of DNA methylation and histone modifications on promoters



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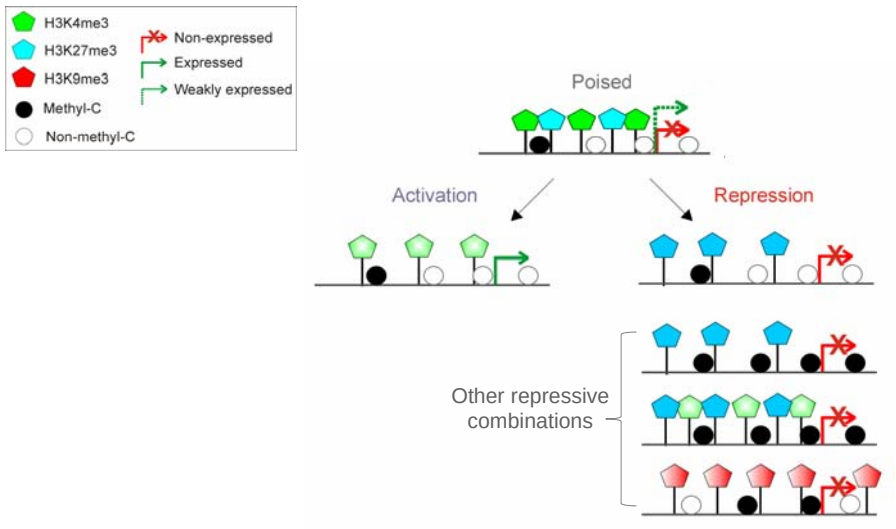
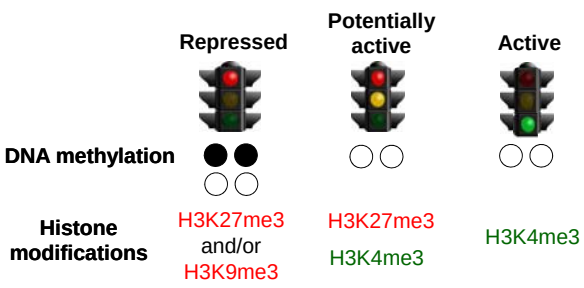
Differentiation segregates the H3K4me3 and H3K27me3 marks



In conclusion...

- Promoter DNA methylation only partly contributes to gene expression potential in stem cells
  - Hypermethylation predicts pathway exclusion
  - Hypomethylation is permissive but not a predictor of differentiation
- A repressed, but *permissive* epigenetic state on lineage-specific promoters is established by a combination of 'repressing' and 'activating' marks on a hypomethylated DNA background



Regulatory levels of gene expression and cell fate decisions ('molecular layers')