Somatic (“adult”) stem cells are found in many organs:

- Bone marrow
- Pancreas
- Skin
- Dental pulp
- Brain
- Intestine

Other sources include:

- Liver
- Fat
- Muscle
- Amniotic fluid
- Amnion
- Eye
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’

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Lecture outline

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

Braids (nucleosomal arrays)

Untangled hair (DNA strands)

Leonardo da Vinci, Head of Leda

Chromatin compaction in eukaryotic cells
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

DNA methyltransferases
- **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?

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

- **High CpG promoter (HCP)**: ON or OFF (OFF)
- **Low CpG promoter (LCP)**: ON or OFF (ON or OFF)
- **Intermediate CpG promoter (HCP)**: ON OFF (OFF)

**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

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

- Acetylation
- SUMOylation
- Methylation
- Phosphorylation

Core histone:

- H3K4, H3K36, H3K79
- H3K9, H3K27, H4K20
- "Wedging" effect?

(+/− : 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

Methylated genes:
- Pluripotency
- Embryonic development
- Germline development

Changes in DNA methylation during ES Cell differentiation into neurons

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

- Needed now
- Needed soon
- Needed (much) later
- Needed (much) later
Lecture outline

- Introduction to epigenetics
- What provides embryonic stem cells with pluripotent differentiation capacity?
- **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

Sørensen et al., 2010. Mol. Biol. Cell
Differentiation segregates the H3K4me3 and H3K27me3 marks

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')