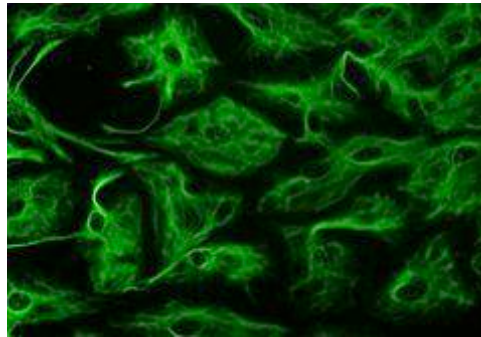


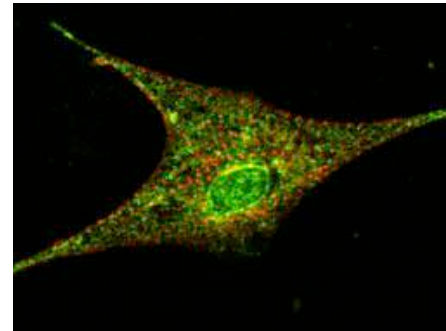
V7: Cell differentiation

Complex genomes can generate a range of **different cell types** in a highly ordered and reproducible manner.

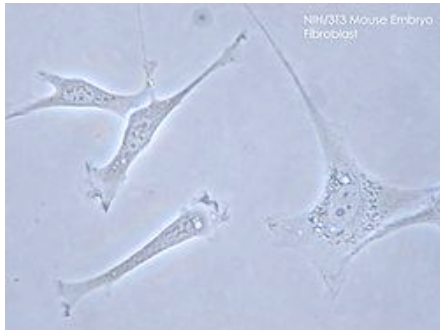
Transcriptional programs and **epigenetic modifications** are important for 'programming' lineage determination and cellular identity during development.



Astrocyte (nerve cell)
(wikipedia.org)



Cardiomyocyte (heart muscle)
(<http://www.kcl.ac.uk/content/1/c6/01/66/46/gautel3.jpeg>)



Fibroblast (connective tissue)
(wikipedia.org)

Cantone & Fisher,
Nature Struct Mol
Biol. 20, 292 (2013)

Zygotes - fertilization

In living organisms that reproduce sexually, development starts from a single cell, the **zygote** (*dt: befruchtete Eizelle*).

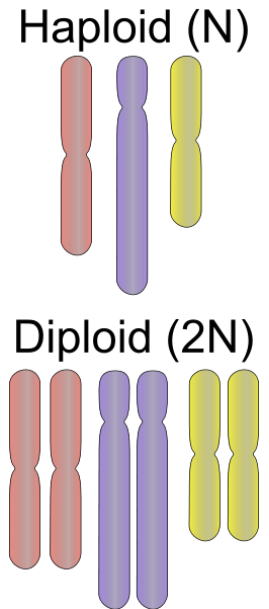
Zygotes are usually produced by a **fertilization** event between two **haploid** cells — an **ovum** from a female and a **sperm** cell from a male—which combine to form the single **diploid** cell.

Human sperm and egg (sex cells) have one complete set of chromosomes from the male or female parent.

Sex cells, also called **gametes**, combine to produce somatic cells. Somatic cells therefore have twice as many chromosomes.

In humans, gametes have 23 chromosomes.

Human **somatic cells** have 46 chromosomes.



some terms from developmental biology

somatic cells = cells forming the body of an organism

germ cells (dt. *Keimzelle*, *Ovolum*) are part of the germline.

germline (dt. *Keimbahn*) = line of germ cells that have genetic material that may be passed to a child/embryo. Germline cells are immortal.

Gametocyte = eukaryotic germ cell; includes spermatocytes (male) and oocytes (female)

primordial germ cells : predecessors of germ cells.

They migrate to the gonadal ridge (precursor of gonads).

They may be detected from expression of *Stella* (gene)

gonad (dt. *Keimdrüse*)

www.wikipedia.org

Germ line development

Germline cells are produced by embryonic cleavage.

Cleavage: division of cells in the early embryo.

The **zygotes** of many species undergo rapid cell cycles with no significant growth.

The different cells derived from cleavage are called **blastomeres** and form a compact mass called the **morula** (because it resembles a mulberry/ *dt. Maulbeere*).

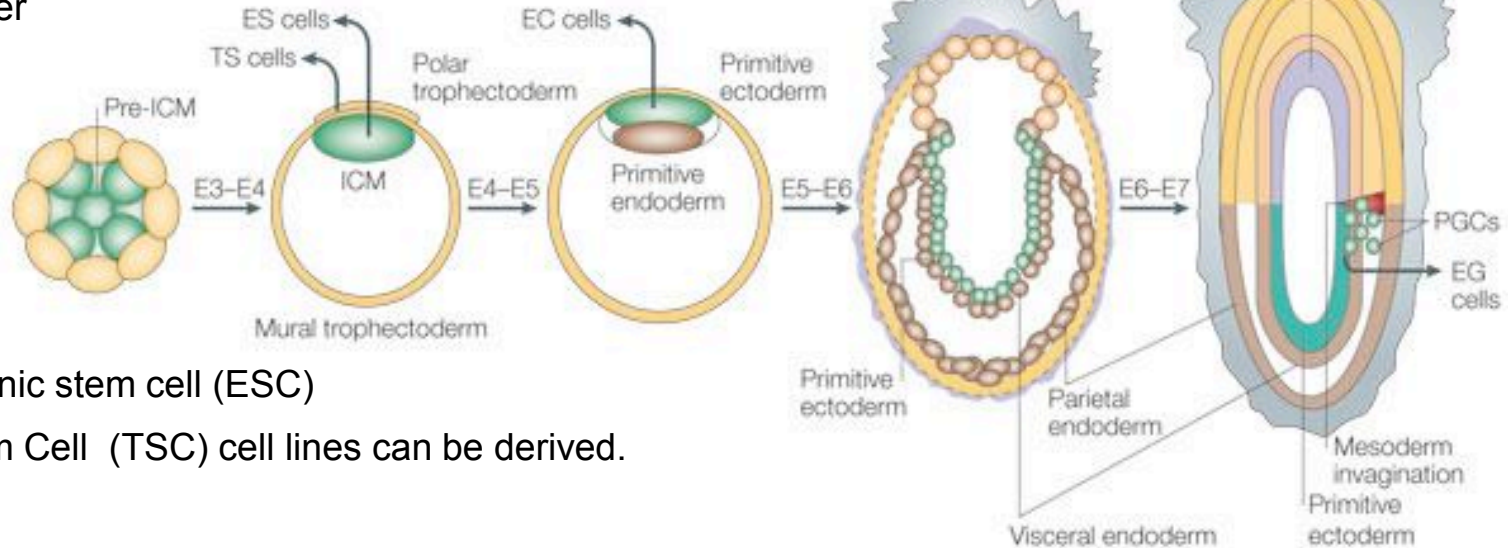
Cleavage ends with the formation of the **blastula**.

Cleavage in **mammals** is slow.

Cell division takes 12 – 24 hours and is asynchronous.

www.wikipedia.org

From left to right, the morula-stage mouse embryo (embryonic day 2.5; E2.5) holds a core of **pre-ICM** (inner cell mass) cells that turn into **ICM** cells at cavitation/blastulation (E3–E4).



At this stage, embryonic stem cell (ESC) and Trophoblast Stem Cell (TSC) cell lines can be derived.

As the blastocyst fully expands, the ICM delaminates giving rise to a primitive **ectoderm** and a primitive **endoderm** layer.

The pluripotent cells of the embryo are tracked in green.

The blastocyst's outer cells are termed **trophoctoderm**. In mammals, the ICM will ultimately form the "embryo proper", while the trophoctoderm will form the placenta and other extra-embryonic tissues.¹

At E6 and subsequently, the embryo will start **gastrulating**. This process involves the formation of a **mesoderm** layer between ectoderm and endoderm, and the formation of the **primordial germ cells (PGCs)**.

3 primary germ cell layers

The **ectoderm** is the **outer layer** of the early embryo.

It emerges first and forms from the outer layer of germ cells.

The ectoderm differentiates to form the nervous system (spine, peripheral nerves and brain), tooth enamel and the epidermis.

It also forms the lining of mouth, anus, nostrils, sweat glands, hair and nails.

The **endoderm** develops at the **inner layer**.

Its cells differentiate to form the gastrointestinal tract, the respiratory tract, endocrine glands and organs, auditory systems, and the urinary system.

The **mesoderm** is the **middle layer**.

It differentiates to give rise to a number of tissues and structures including bone, cartilage (*dt: Knorpel*), muscle, connective tissue (including that of the dermis), the middle layer of the skin, blood vascular, reproductive, excretory and urinogenital systems and contributes to some glands.

Developmental Glossary (I)

Inner cell mass (ICM): Cells of the blastocyst embryo that appear transiently during development and give rise to the three germ layers of the developing embryo.

Embryonic stem (ES) cells:

Pluripotent cell line derived from the ICM upon explantation in culture.

In vitro, ES cells can differentiate into many different lineages and cell types.

Upon injection into blastocysts, ES cells can give rise to all tissues including the germline.

Primordial germ cells (PGCs):

In vivo, PGCs give rise to oocytes and sperm.

When explanted in vitro, PGCs give rise to embryonic germ (EG) cells.

Hochedlinger, Development 136, 509 (2009)

Adult stem cells

Embryonic stem cells only exist in the early embryo.

We all possess **adult stem cells**, from which new specialized cells are formed throughout our life time.

Adult cells exist predominantly in bone marrow (*dt. Knochenmark*), but also in skin, fat tissue, umbilical cord (*dt. Nabelschnur*), brain, liver, and in pancreas (*dt. Bauchspeicheldrüse*).

Adult cells in cell culture have a much reduced ability of self regeneration and a reduced ability for differentiation compared to embryonic stem cells.

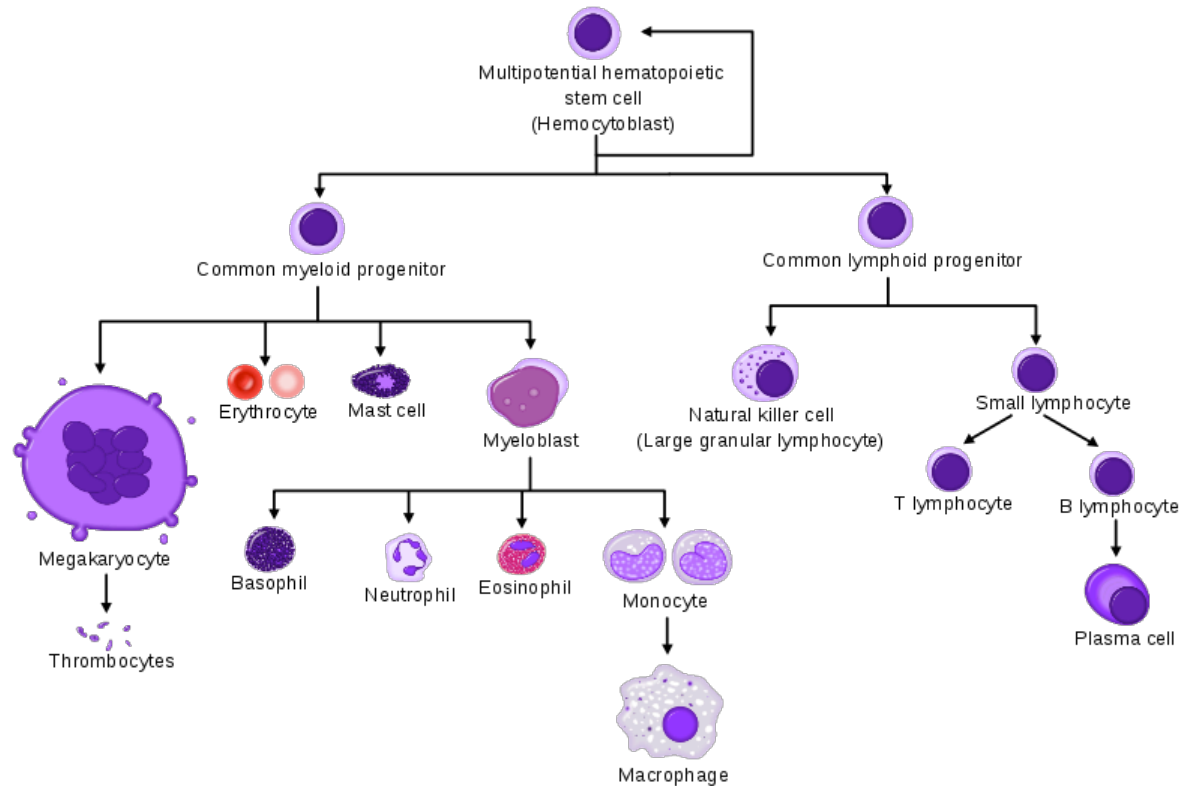
For example, neural stem cells can differentiate to all cell types of neural tissue (neurons, glia), but likely not into liver or muscle cells.

Haematopoiesis

Haematopoiesis (from Ancient Greek: αἷμα, "blood"; ποιεῖν "to make") is the formation of blood cellular components.

All cellular blood components are derived from **haematopoietic stem cells**.

In a healthy adult person, approximately 10^{11} – 10^{12} new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation.



Development of different blood cells from haematopoietic stem cell to mature cells

Differentiation

(Review) A **zygote** is a eukaryotic cell formed by a fertilization event between two gametes.

Zygotes therefore contain DNA derived from both the mother and the father, and this provides all the genetic information necessary to form a new individual.

This property is named „**totipotency**“
(latin: totus – all, potentia – power/ability).

Continuous cell division produces daughter cells that start to specialize on individual functions.

This developmental process of cells and tissue from a less specialized to a more specialized state is called **differentiation** in developmental biology.

Glossary I

Totipotency Ability of a cell to give rise to all cells of an organism, including embryonic and extraembryonic tissues. Zygotes are totipotent.

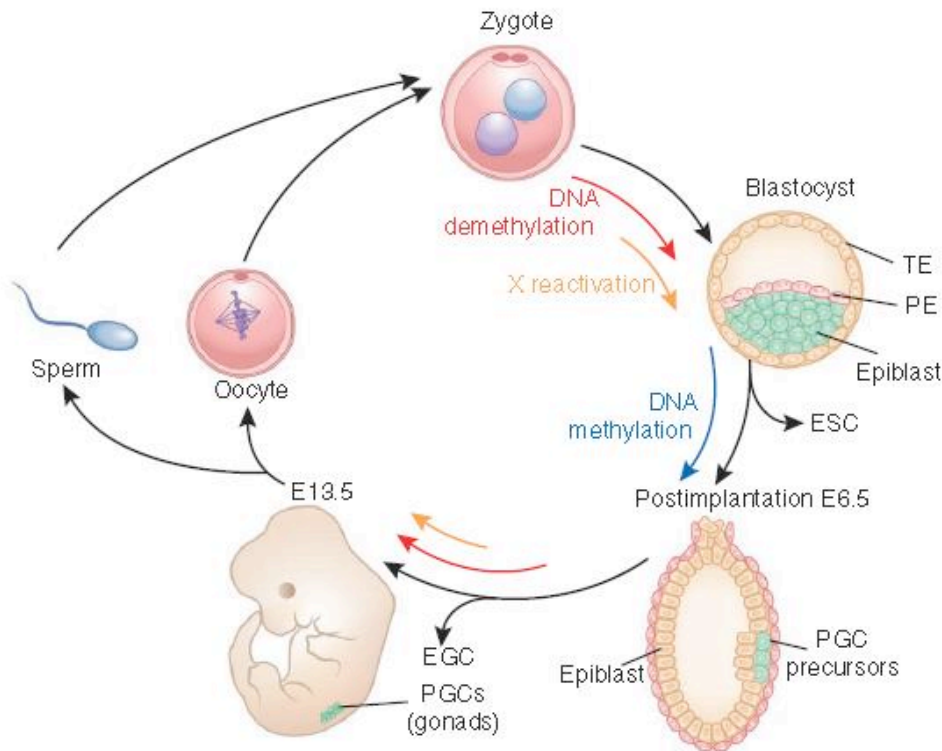
Pluripotency Ability of a cell to give rise to all cells of the embryo. Cells of the inner cell mass (ICM) and its derivative, embryonic stem (ES) cells, are pluripotent.

Multipotency Ability of a cell to give rise to different cell types of a given cell lineage. These cells include most adult stem cells, such as gut stem cells, skin stem cells, hematopoietic stem cells and neural stem cells.

Unipotency Capacity of a cell to sustain only one cell type or cell lineage. Examples are terminally differentiated cells, certain adult stem cells (testis stem cells) and committed progenitors (erythroblasts).

Hochedlinger, Development 136, 509 (2009)

Epigenetic programming and reprogramming during the mouse life cycle.



Two populations of pluripotent cells can be established *ex vivo* within the time window in which extensive epigenetic reprogramming takes place.

These cells are **ESCs and embryonic germ cells (EGCs)** that are derived from the inner cell mass of the blastocyst and from the PGCs at E8.5–E13.5, respectively.

Major remodeling events (e.g. DNA demethylation and X-chromosome reactivation) are highlighted in the figure by colored arrows.

TE, trophoectoderm;

PE primitive endoderm.

Cantone & Fisher,
Nature Struct Mol
Biol. 20, 292 (2013)

What is epigenetics?

Epigenetics refers to **alternate phenotypic states** that are **not based** in **differences in genotype**, and are potentially reversible, but are generally stably maintained during cell division.

Examples: imprinting, twins, cancer vs. normal cells, differentiation, ...

Laird, Hum Mol Gen 14, R65 (2005)

What is epigenetics?

A much more expanded view of epigenetics has recently emerged in which multiple mechanisms interact to collectively establish

- alternate states of chromatin structure (open – packed/condensed),
- **histone modifications**,
- associated protein (e.g. histone) composition,
- transcriptional activity,
- activity of microRNAs, and
- in mammals, **cytosine-5 DNA methylation** at CpG dinucleotides.

Laird, Hum Mol Gen 14, R65 (2005)

Basic principles of epigenetics: DNA methylation and histone modifications

The human genome contains 23 000 genes that must be expressed in specific cells at precise times.

Cells manage gene expression by wrapping DNA around clusters (octamers) of globular **histone** proteins to form **nucleosomes**.

These nucleosomes of DNA and histones are organized into **chromatin**, the building block of a chromosome.

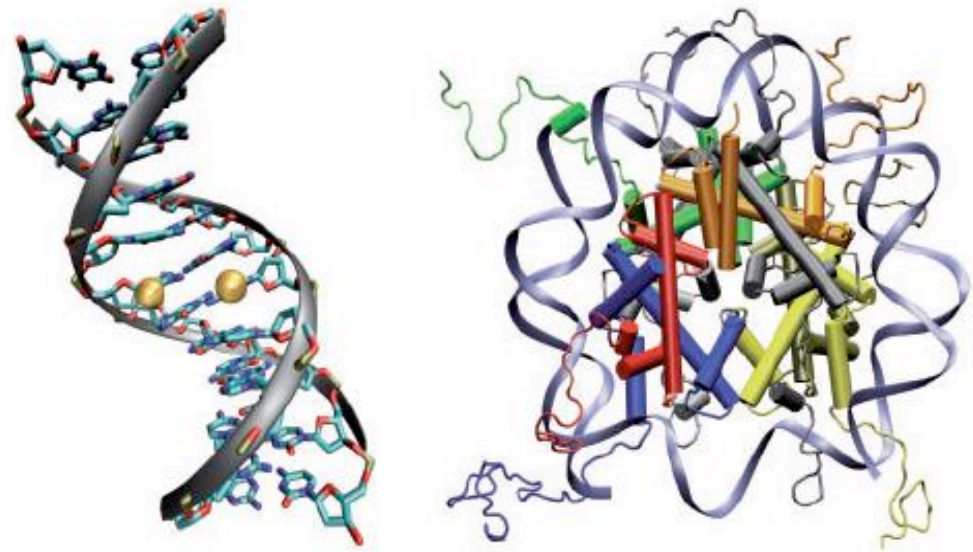


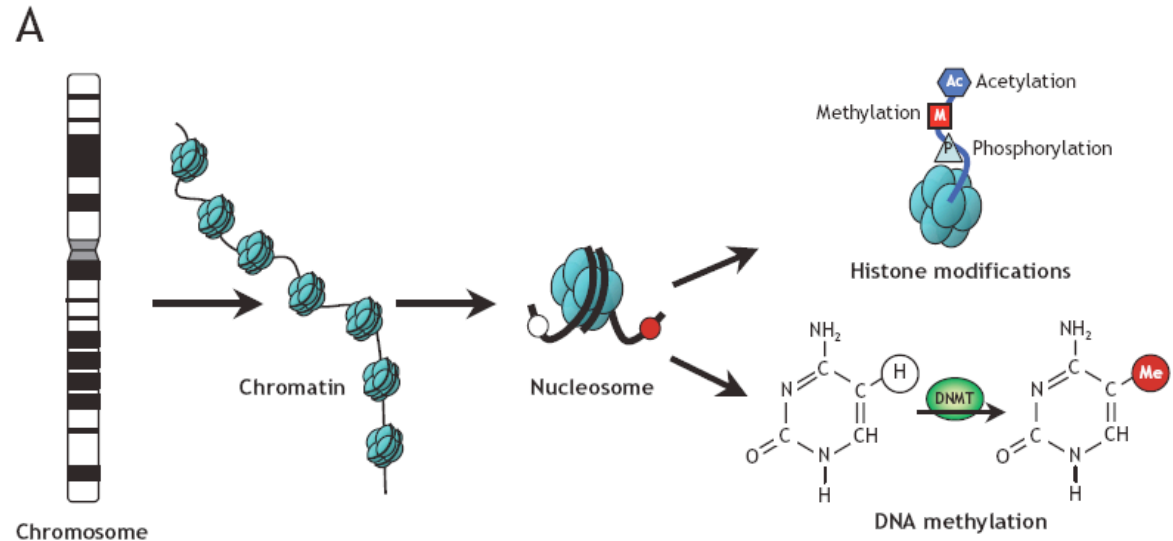
Fig. 1. Carriers of epigenetic information: DNA and nucleosome. The left panel shows a DNA double helix that is methylated symmetrically on both strands (orange spheres) at its center CpG (PDB structure: 329d). DNA methylation is the only epigenetic mechanism that directly targets the DNA. The right panel shows a nucleosome spindle consisting of eight histone proteins (center), around which two loops of DNA are wound (PDB structure: 1KX5). The nucleosome is subject to covalent modifications of its histones and to the binding of non-histone proteins.

Rodenhiser, Mann,
CMAJ 174, 341 (2006)

Bock, Lengauer, Bioinformatics 24, 1 (2008)

Epigenetic modifications

Rodenhiser, Mann,
CMAJ 174, 341 (2006)



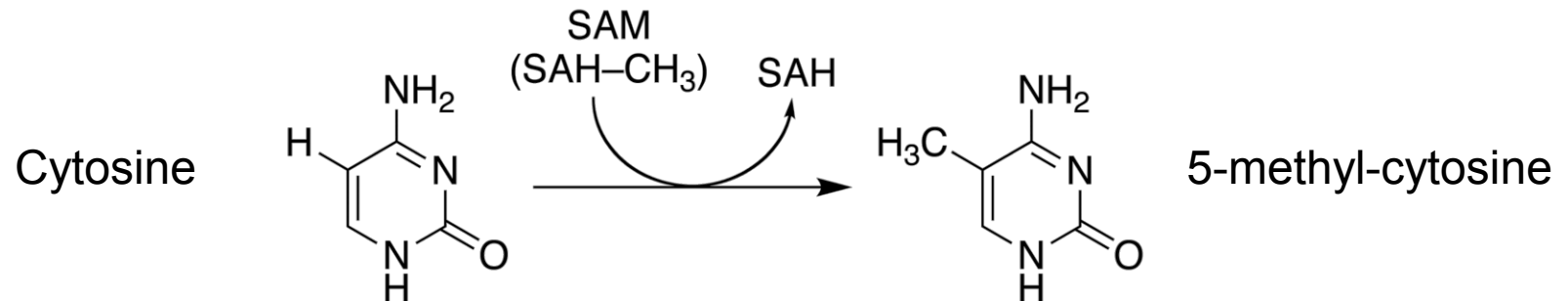
Reversible and site-specific **histone modifications** occur at multiple sites at the unstructured histone tails through **acetylation**, **methylation** and **phosphorylation**.

DNA methylation occurs at 5-position of cytosine residues within CpG pairs in a reaction catalyzed by DNA methyltransferases (DNMTs).

Cytosine methylation

Observation: 3-6 % of all cytosines are methylated in human DNA.

This methylation occurs (almost) exclusively when cytosine is followed by a guanine base -> **CpG dinucleotide**.



Mammalian genomes contain much fewer (only 20-25 %) of the CpG dinucleotide than is expected by the G+C content (we expect $1/16 \approx 6\%$ for any random dinucleotide).

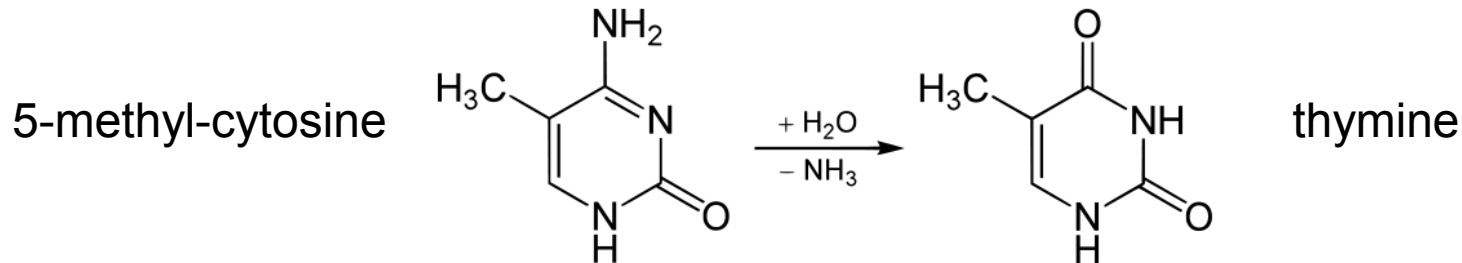
This is typically explained in the following way:

As most CpGs serve as targets of DNA methyltransferases, they are usually methylated.

Esteller, Nat. Rev. Gen. 8, 286 (2007)
www.wikipedia.org

Cytosine methylation

5-Methylcytosine can easily **deaminate** to **thymine**.



If this mutation is not repaired, the affected CpG is permanently converted to TpG (or CpA if the transition occurs on the reverse DNA strand).

Hence, methylCpGs represent **mutational hot spots** in the genome. If such mutations occur in the germ line, they become heritable.

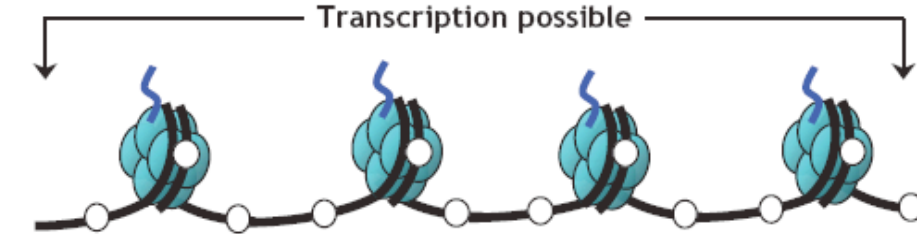
A constant loss of CpGs over thousands of generations can explain the low frequency of this special dinucleotide in the genomes of human and mouse.

effects in chromatin organization affect gene expression

B

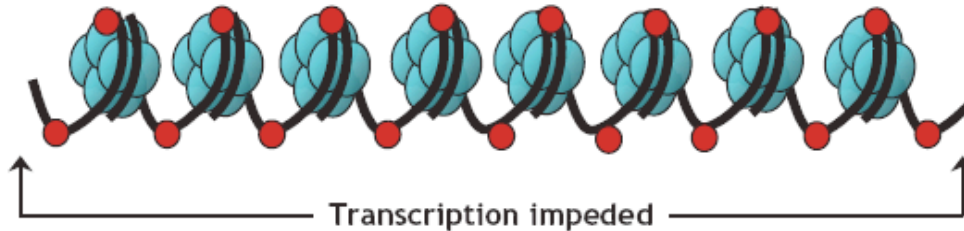
Gene “switched on”

- Active (open) chromatin
- Unmethylated cytosines (white circles)
- Acetylated histones



Gene “switched off”

- Silent (condensed) chromatin
- Methylated cytosines (red circles)
- Deacetylated histones



Schematic of the reversible changes in chromatin organization that influence gene expression:

genes are expressed (switched on) when the chromatin is **open** (active), and they are inactivated (switched off) when the chromatin is **condensed** (silent).

White circles = unmethylated cytosines;

red circles = methylated cytosines.

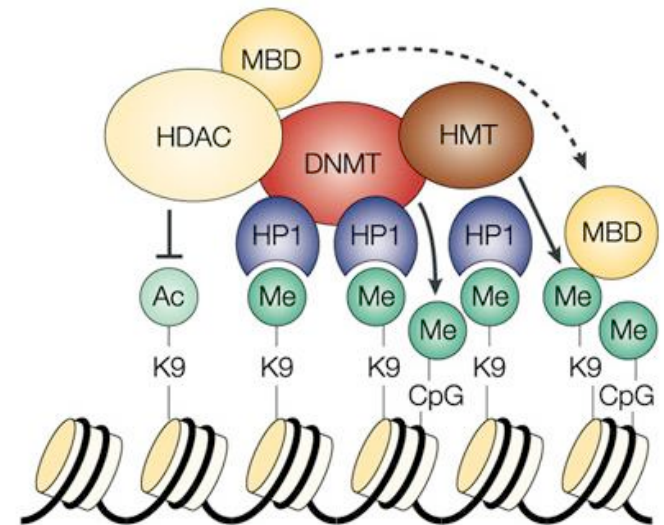
Rodenhiser, Mann, CMAJ 174, 341 (2006)

Enzymes that control DNA methylation and histone modifications

These dynamic chromatin states are controlled by reversible epigenetic patterns of **DNA methylation** and **histone modifications**.

Enzymes involved in this process include

- DNA methyltransferases (DNMTs),
- histone deacetylases (HDACs),
- histone acetylases,
- histone methyltransferases and the
- methyl-binding domain protein MECP2.



For example, **repetitive** genomic sequences (e.g. human endogenous retroviral sequences = HERVs) are **heavily methylated**, which means transcriptionally silenced.

Rodenhiser, Mann, CMAJ 174, 341 (2006)

Feinberg AP & Tycko P (2004) Nature Reviews: 143-153

DNA methylation

Typically, unmethylated clusters of CpG pairs are located in **tissue-specific genes** and in essential **housekeeping genes**.

(House-keeping genes are involved in routine maintenance roles and are expressed in most tissues.)

These clusters, or **CpG islands**, are targets for proteins that bind to unmethylated CpGs and initiate gene transcription.

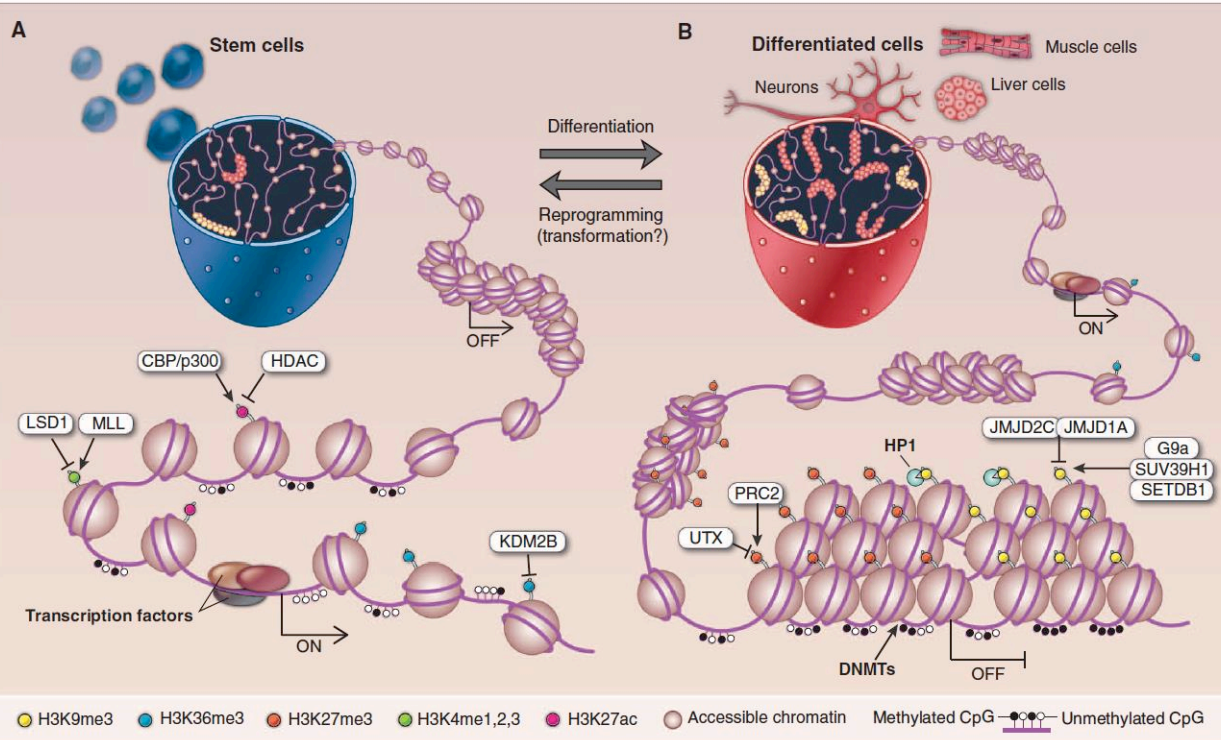
In contrast, **methylated CpGs** are generally associated with silent DNA, can block methylation-sensitive proteins and can be easily mutated.

The loss of normal DNA methylation patterns is the best understood epigenetic cause of disease.

In animal experiments, the removal of genes that encode DNMTs is lethal; in humans, overexpression of these enzymes has been linked to a variety of cancers.

Rodenhiser, Mann, CMAJ 174, 341 (2006)

Differentiation linked to alterations of chromatin structure



(B) Upon differentiation, inactive genomic regions may be sequestered by repressive chromatin enriched for characteristic histone modifications.

(A) In pluripotent cells, chromatin is hyperdynamic and globally accessible.

ML Suva et al. *Science* 2013;
339:1567-1570

Epigenetic stability

In somatic tissues, **CpG islands** at housekeeping or developmental promoters are largely unmethylated, whereas non-regulatory CpGs distributed elsewhere in the genome are largely methylated.

This **DNA methylation landscape** is relatively **static** across all somatic tissues.

Most of methylated CpGs are pre-established and inherited through cell division.

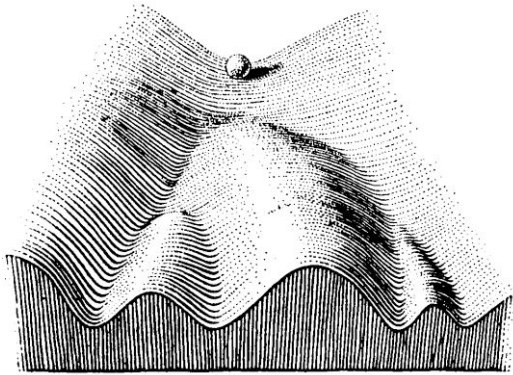
In at least two phases of the life cycle of mammals, epigenetic stability is globally perturbed:

- when gametes fuse to form the zygote and
- when gamete precursors (primordial germ cells; PGCs) develop and migrate in the embryo.

This ***in vivo* 'reprogramming'** of the epigenetic landscape signals the reacquisition of totipotency in the zygote and the formation of the next generation through PGCs.

Cantone & Fisher,
Nature Struct Mol
Biol. 20, 292 (2013)

Waddington: Epigenetic landscape



Conrad H. Waddington 1956: "Principles of Embryology"; www.nature.com

Developmental potential

Totipotent

Zygote

Pluripotent

ICM/ES cells, EG cells,
EC cells, mGS cells
iPS cells

Multipotent

Adult stem cells
(partially
reprogrammed cells?)

Unipotent

Differentiated cell
types

Epigenetic status

Global DNA demethylation

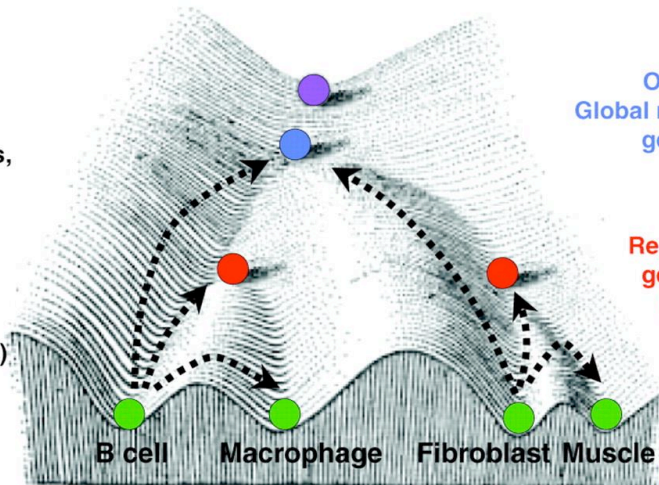
Only active X chromosomes;
Global repression of differentiation
genes by Polycomb proteins;
Promoter hypomethylation

X inactivation;

Repression of lineage-specific
genes by Polycomb proteins;
Promoter hypermethylation

X inactivation;

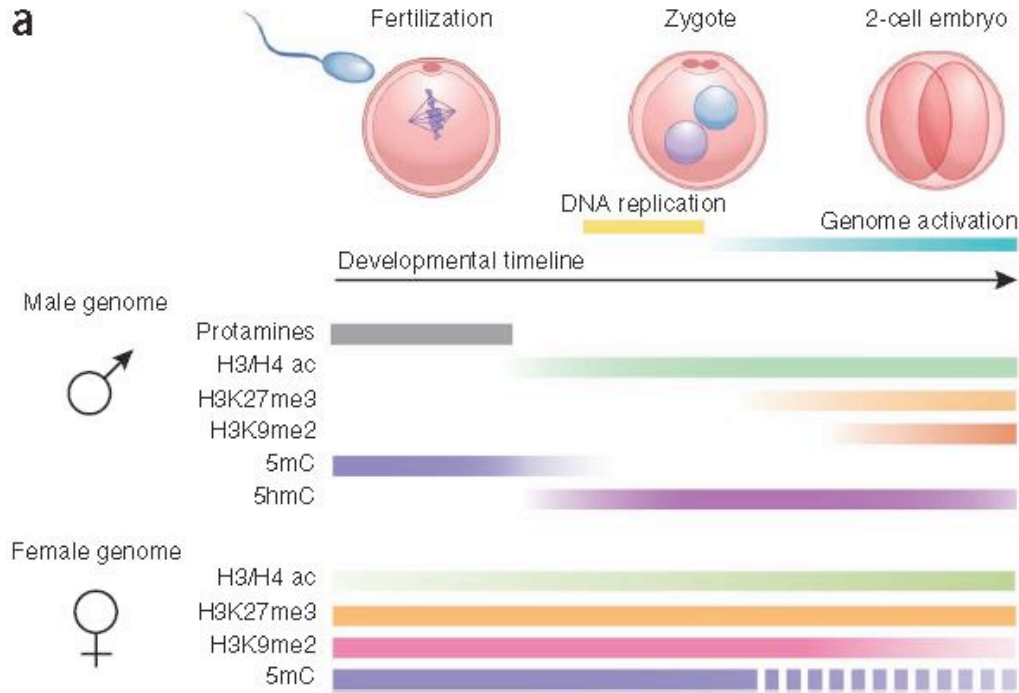
Derepression of
Polycomb silenced
lineage genes;
Promoter hypermethylation



Konrad Hochedlinger and Kathrin Plath,
Development 136, 509-523 (2009)

Epigenetic changes during in vivo reprogramming

a



Global DNA and histone modifications that lead to transcriptional activation of the embryonic genome between the late zygote (paternal genome only) and the 2-cell stage.

Protamines are small, arginine-rich, nuclear proteins that replace histones late in the haploid phase of spermatogenesis and are believed essential for sperm head condensation and DNA stabilization. In humans, 10-15% of the sperm's genome is packaged by histones thought to bind genes that are essential for early embryonic development (www.wikipedia.org).

Gamete genomes undergo different epigenetic programs after fertilization.

The **paternal** genome is mostly subject to epigenetic remodeling at the zygote stage. The **maternal** genome gradually loses repressive modifications during the subsequent cleavage divisions.

Epigenetic changes during germline development

b

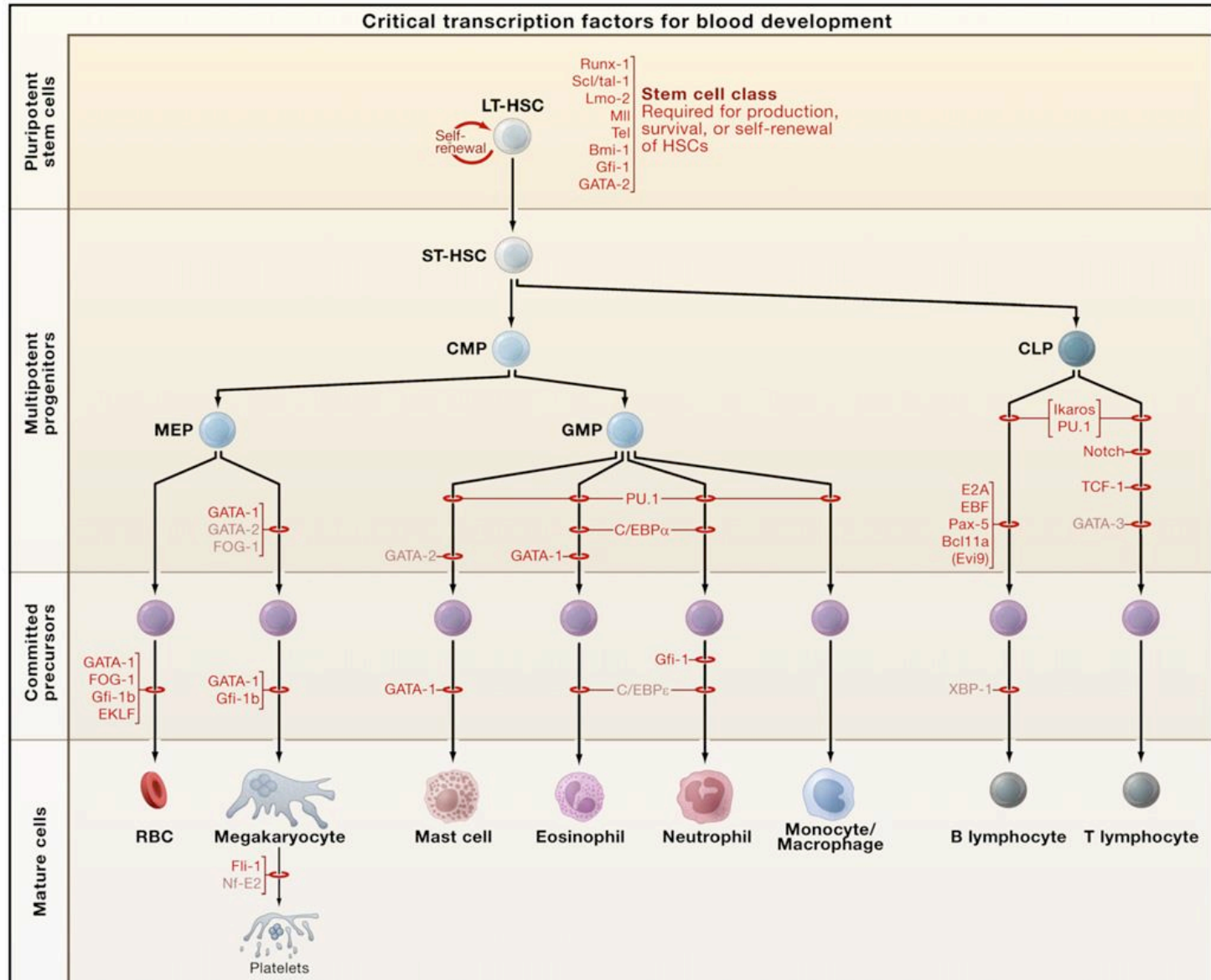


Global epigenetic changes during germline development from PGC specification (E6.5) to the mitotic/meiotic arrest at E13.5.

Two major reprogramming phases can be distinguished during PGC migration toward the genital ridges (E7.5–E10.5) and upon their arrival into the gonads (E10.5–E12.5).

Cantone & Fisher,
Nature Struct Mol
Biol. 20, 292 (2013)

Hematopoiesis: development of blood cells



Orkin & Zon, Cell (2008)
132: 631–644.

Homework

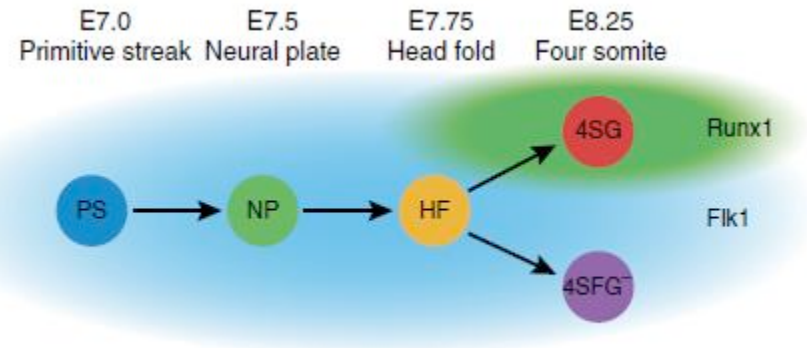
Decoding the regulatory network of early blood development from single-cell gene expression measurements

Nature Biotech 33, 269 (2015)

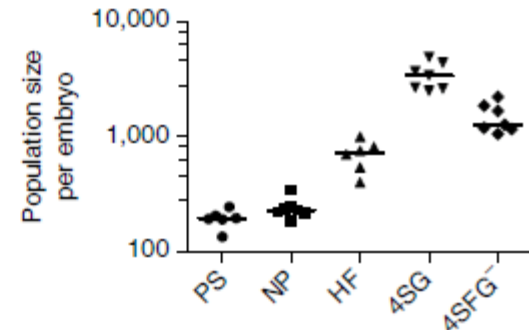
Victoria Moignard^{1,2,11}, Steven Woodhouse^{1,2,11}, Laleh Haghverdi^{3,4}, Andrew J Lilly⁵, Yosuke Tanaka^{1,2,6}, Adam C Wilkinson^{1,2}, Florian Buettner³, Iain C Macaulay⁷, Wajid Jawaid¹, Evangelia Diamanti^{1,2}, Shin-Ichi Nishikawa⁶, Nir Piterman⁸, Valerie Kouskoff⁵, Fabian J Theis^{3,4}, Jasmin Fisher^{9,10} & Berthold Göttgens^{1,2}

The first wave of primitive hematopoiesis originates from Flk1+ mesoderm

Single Flk1+ cells were flow sorted at E7.0 (primitive streak, PS), E7.5 (neural plate, NP) and E7.75 (head fold, HF) stages. We subdivided E8.25 cells into putative blood and endothelial populations by isolating GFP+ cells (four somite, 4SG) and Flk1+GFP-cells (4SFG-), respectively



Cell type	Number of embryos	Cells sorted	Cells retained	Percentage retained
PS	12	725	624	86.1
NP	9	637	552	86.7
HF	8	1,184	1,005	84.9
4SG	3	1,085	983	90.6
4SFG-	4	858	770	89.7
Total	36	4,489	3,934	87.6



Summary

Epigenetic remodelling is responsible for cellular differentiation.

Altering chromatin structure will affect accessibility of genes and, hence, alter the transcriptional program in cells.

Open question:

- which genes/proteins are the **drivers/master regulators**?
- Does epigenetics regulate transcription, or does transcription regulate epigenetics, or are both closely interlinked?
- How can one study such combined epigenetic + gene-regulatory networks by computational modeling?