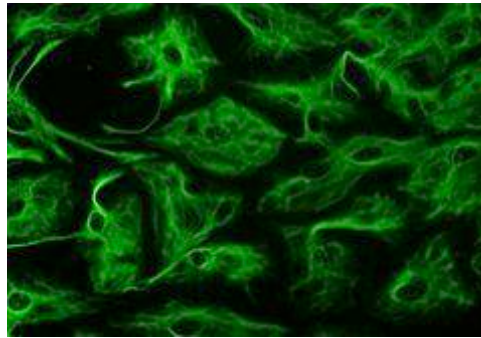


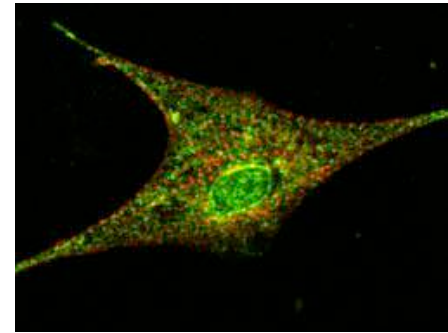
## V5: Cell differentiation

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

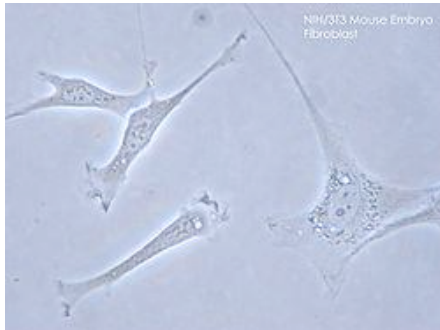
**Epigenetic modifications** are important for 'programming' lineage determination and cellular identity during development.



**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)

# 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. **How?**

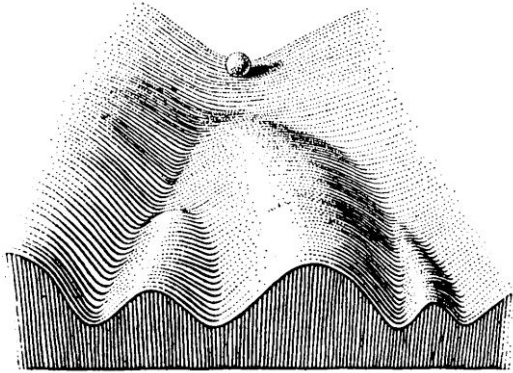
In at least two phases of the life cycle of mammals, epigenetic stability is globally perturbed:

- 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



Nature Reviews | Genetics

Conrad H. Waddington 1956: "Principles of Embryology"; [www.nature.com](http://www.nature.com)

**Developmental potential**

**Epigenetic status**

**Totipotent**

Zygote

**Pluripotent**

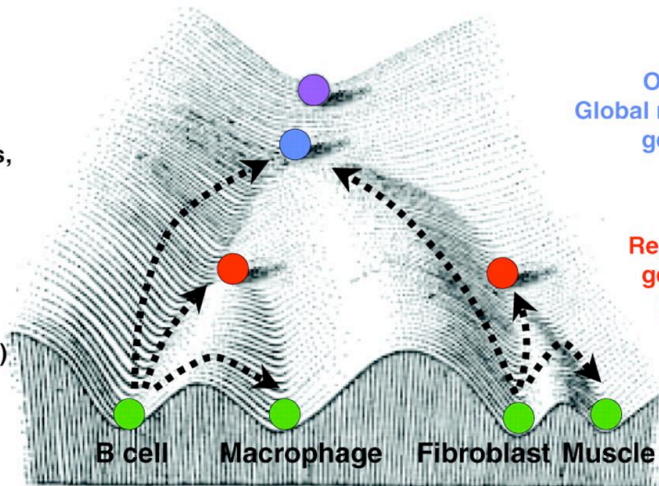
ICM/ES cells, EG cells,  
EC cells, mGS cells  
iPS cells

**Multipotent**

Adult stem cells  
(partially  
reprogrammed cells?)

**Unipotent**

Differentiated cell  
types



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)

# Zygotes - fertilization

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

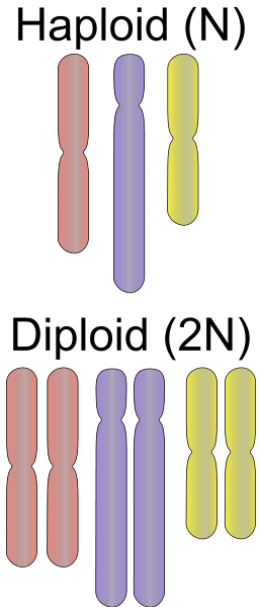
Zygotes are usually produced by a **fertilization** event between two **haploid** cells — an **ovum** from a female and a **sperm** cell from a male—which combine to form the single **diploid** cell.

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.

The haploidity number ( $n=23$  in humans) is the number of chromosomes in a gamete.

A **somatic cell** has twice that many chromosomes ( $2n=46$ ).



## 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](http://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.

Morula

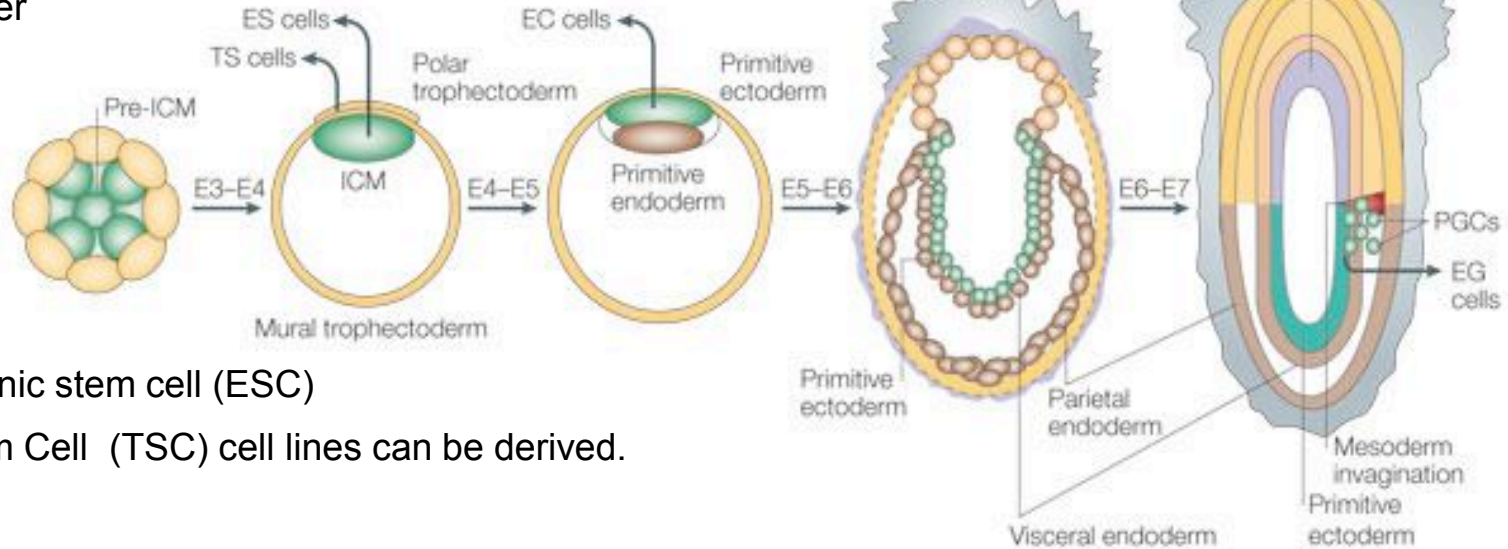
Blastocyst

Late blastocyst

Egg cylinder

Gastrula

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 **trophoblast**. In mammals, the ICM will ultimately form the "embryo proper", while the trophoblast will form the placenta and other extra-embryonic tissues.<sup>1</sup>

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.

[www.wikipedia.org](http://www.wikipedia.org)



# 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.

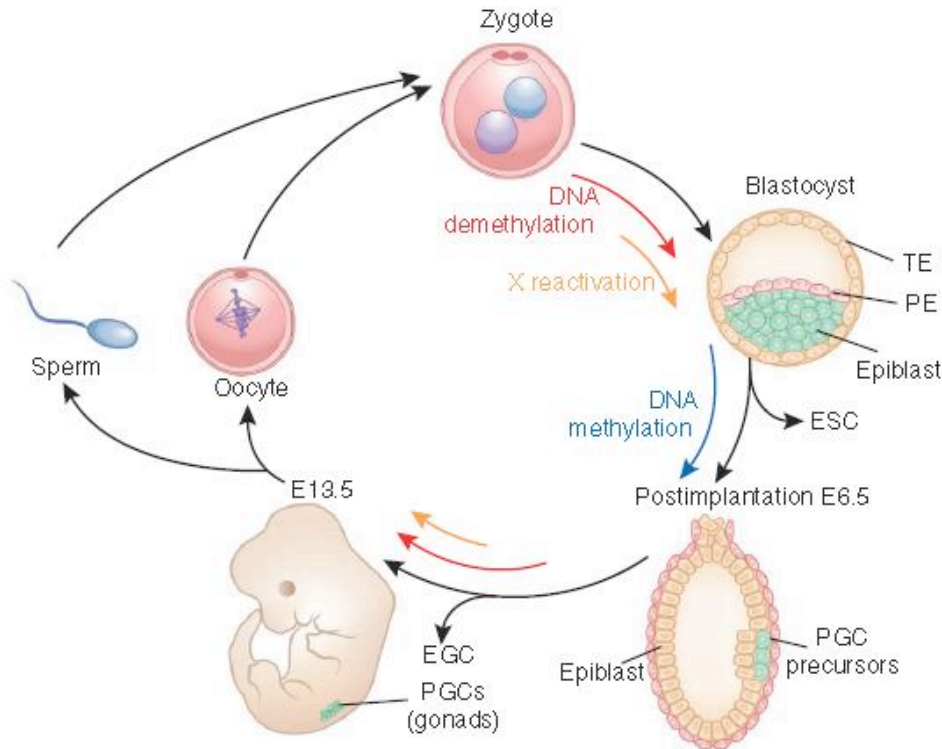
We all possess **adult stem cells**, from which new specialized cells are formed throughout our life time.

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.

# 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.

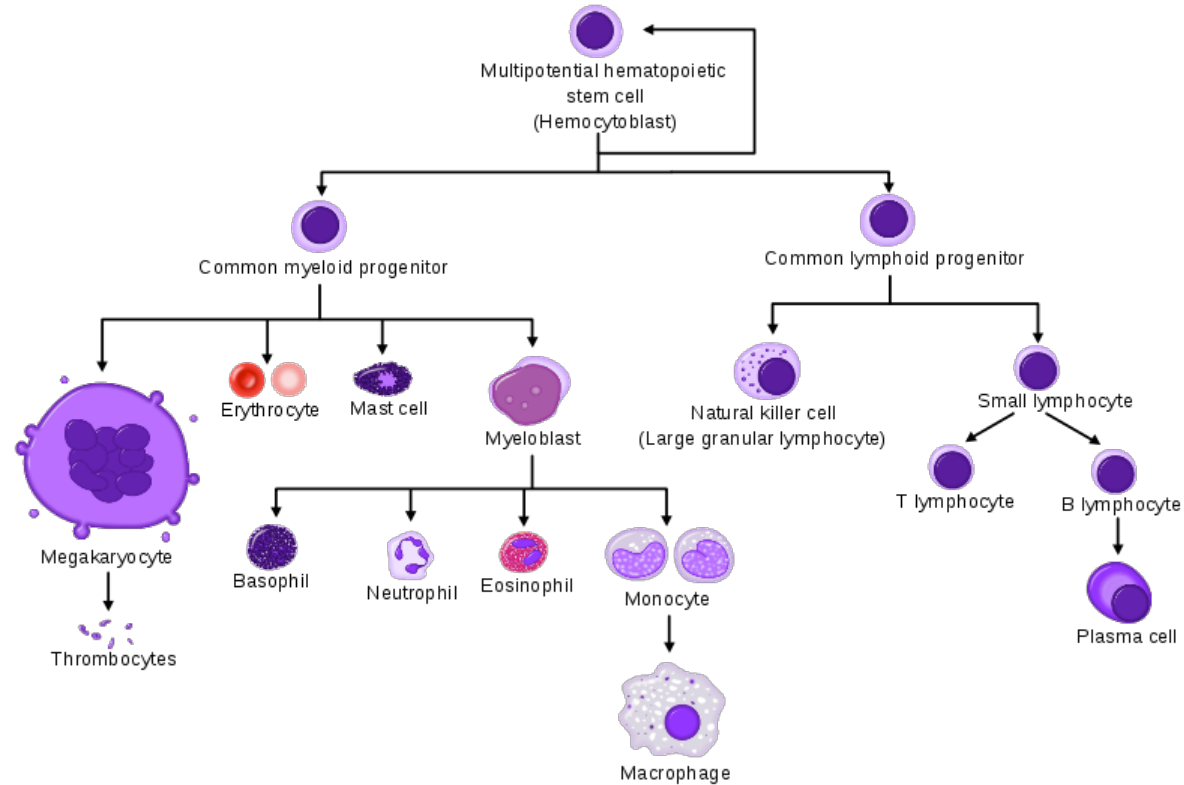
Cantone & Fisher,  
Nature Struct Mol  
Biol. 20, 292 (2013)

# 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

Zygotes 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.

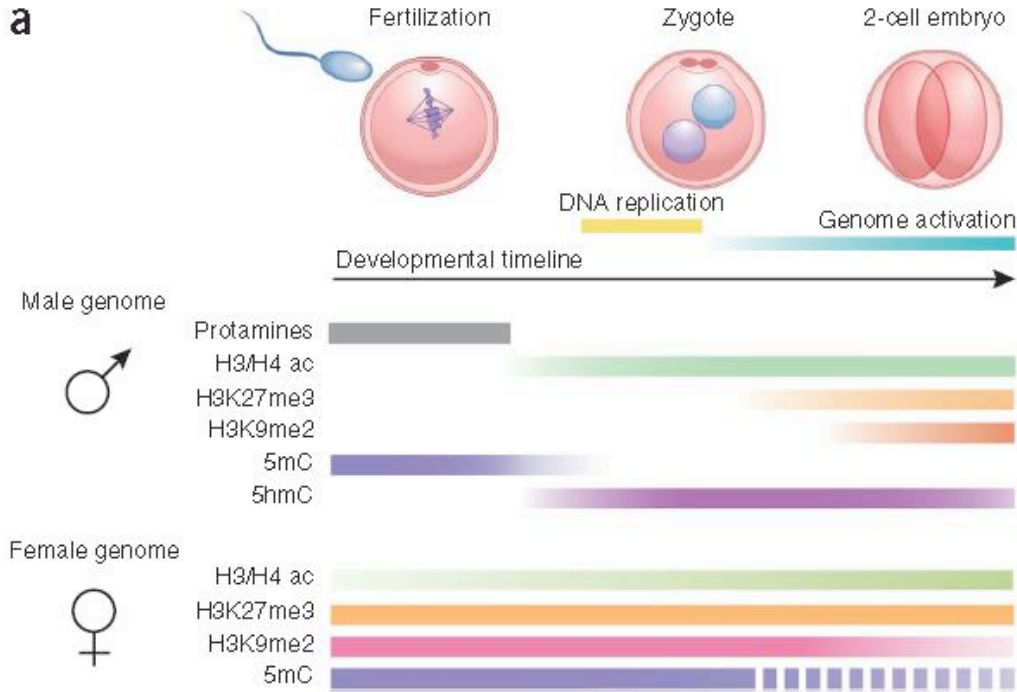
**Multipotency** Ability of a cell to give rise to different cell types of a given cell lineage. These cells include most adult stem cells, such as gut stem cells, skin stem cells, hematopoietic stem cells and neural stem cells.

**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 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.

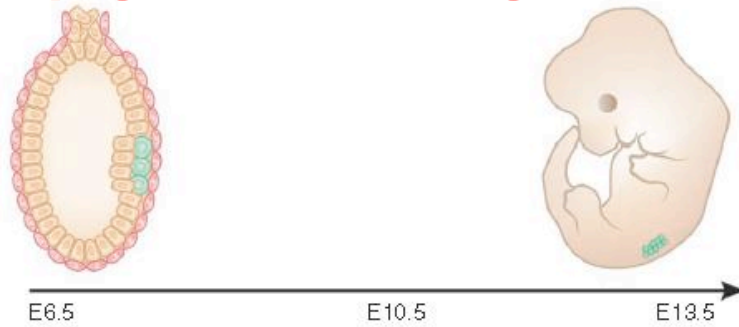
**Protamines** are small, arginine-rich, nuclear proteins that replace histones late in the haploid phase of spermatogenesis and are believed essential for sperm head condensation and DNA stabilization. In humans, 10-15% of the sperm's genome is packaged by histones thought to bind genes that are essential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



# 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?