V7: Cellular differentiation - development

In developmental biology, **cellular differentiation** is the process where a cell changes its **cell fate** from one cell type to another. Most commonly the cell changes to a **more specialized type**.

Differentiation occurs numerous times during the development of a multicellular organism as it changes from a simple zygote to a complex system of tissues and cell types.

Differentiation continues in **adulthood** as adult stem cells divide and create fully differentiated daughter cells during tissue repair and during normal cell turnover.

Differentiation dramatically changes a cell's size, shape, membrane potential, metabolic activity, and responsiveness to signals.

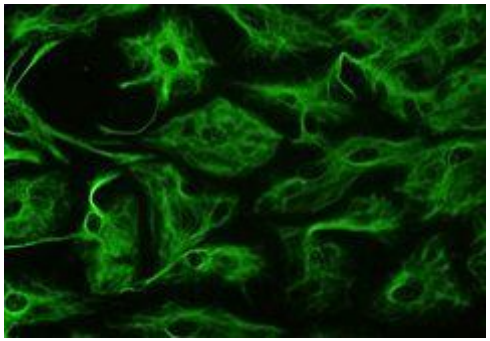
These changes are largely due to highly controlled modifications in gene expression that are often controlled by **epigenetic** effects.

www.wikipedia.org

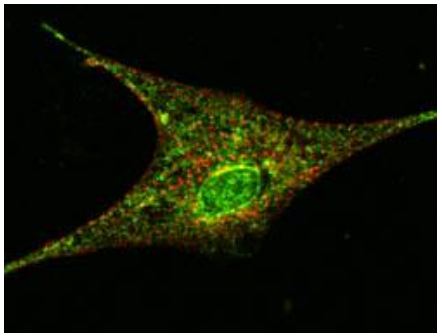
Different cell types

Complex genomes can generate a range of **different cell types** in a highly ordered and reproducable 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

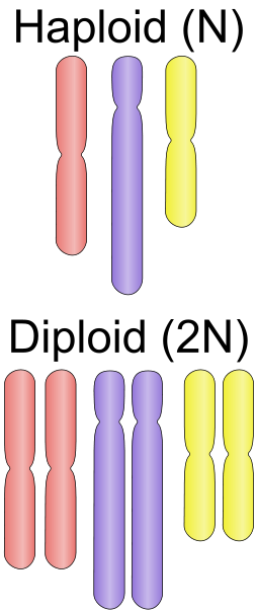
Fibroblast (connective tissue)
(wikipedia.org)

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.

www.wikipedia.org

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.

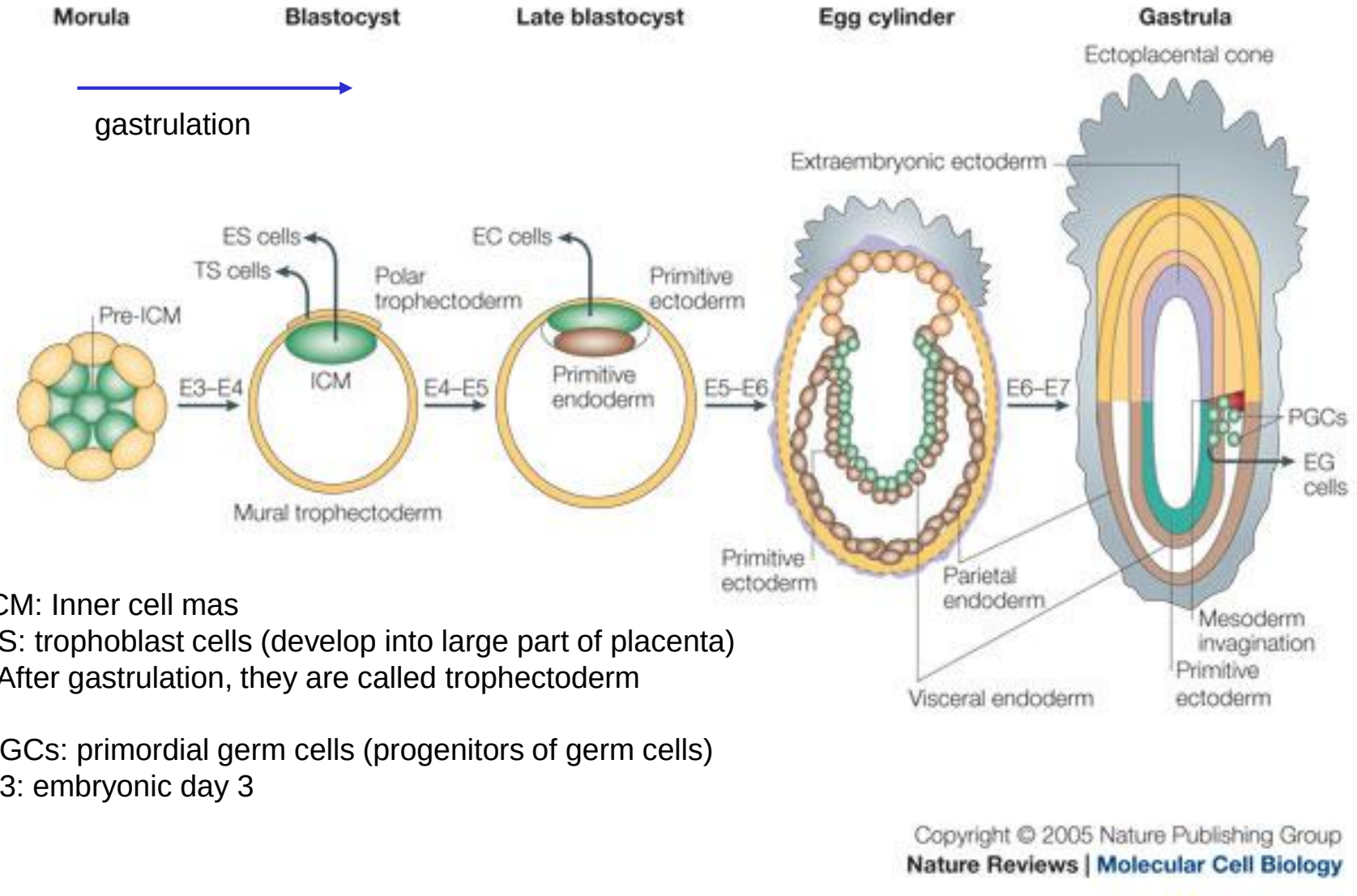
The different cells derived from cleavage are called **blastomeres**
and form a compact mass called the **morula**
(because it resembles a mulberry/ *dt. Maulbeere*).

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

Embryonic development of mouse



ICM: Inner cell mas
TS: trophoblast cells (develop into large part of placenta)
- After gastrulation, they are called trophectoderm

PGCs: primordial germ cells (progenitors of germ cells)
E3: embryonic day 3

Boiani & Schöler, Nat Rev Mol Cell Biol 6, 872 (2005)

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

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 stem 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 stem 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 (neorons, glia), but likely not into liver or muscle cells.

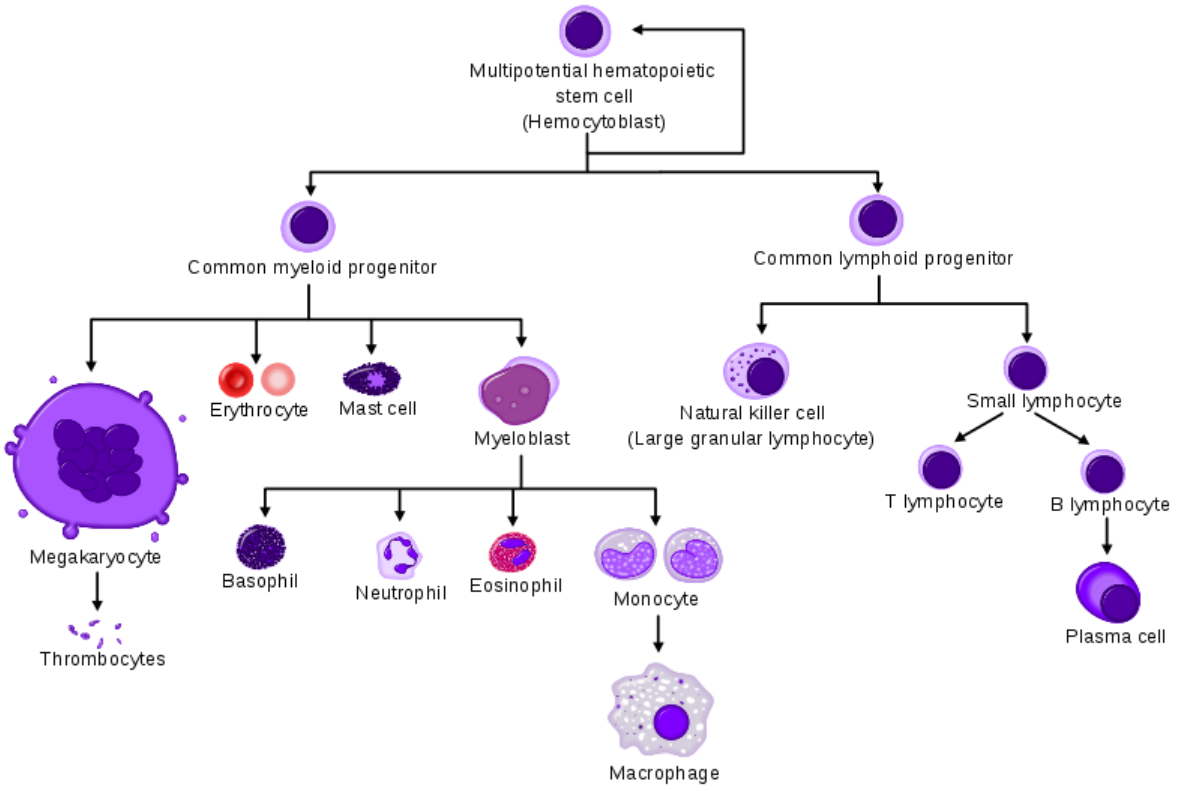
www.wikipedia.org

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

www.wikipedia.org

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.

www.wikipedia.org

Glossary I

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

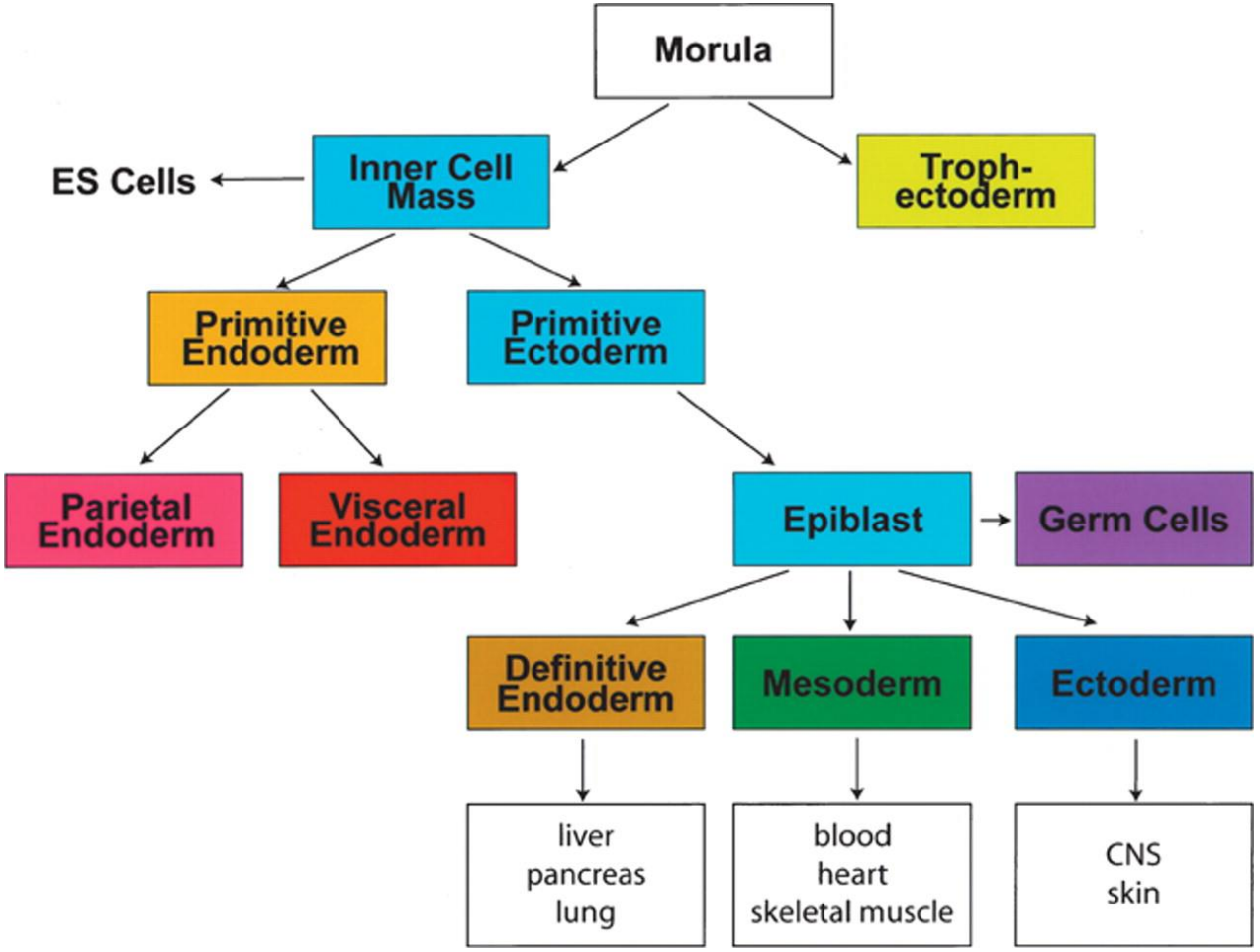
Pluripotency Ability of a cell to give rise to all cells of the embryo.
Cells of the inner cell mass (ICM) and its derivative,
embryonic stem (ES) cells, are pluripotent.

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)

Cell populations in early mouse development



Scheme of **early mouse development** depicting the relationship of early cell populations to the primary germ layers

Keller, Genes & Dev. (2005) 19: 1129-1155

Types of body cells

3 basic categories of cells make up the mammalian body:

germ cells (oocytes and sperm cells)
somatic cells, and
stem cells.

Each of the approximately 100 trillion (10^{14}) cells in an adult human has its own copy or copies of the genome except certain cell types, such as red blood cells, that lack nuclei in their fully differentiated state.

Most cells are **diploid**; they have two copies of each chromosome.

Cells differentiate to specialize for different functions.

Somatic cells make up most of the human body, such as skin and muscle cells.

www.wikipedia.org

Development controlled by transcriptional programs

Embryonic development is a complex process that remains to be understood despite knowledge of the complete genome sequences of many species and rapid advances in genomic technologies.

A fundamental question is how the unique gene expression pattern in each cell type is established and maintained during embryogenesis.

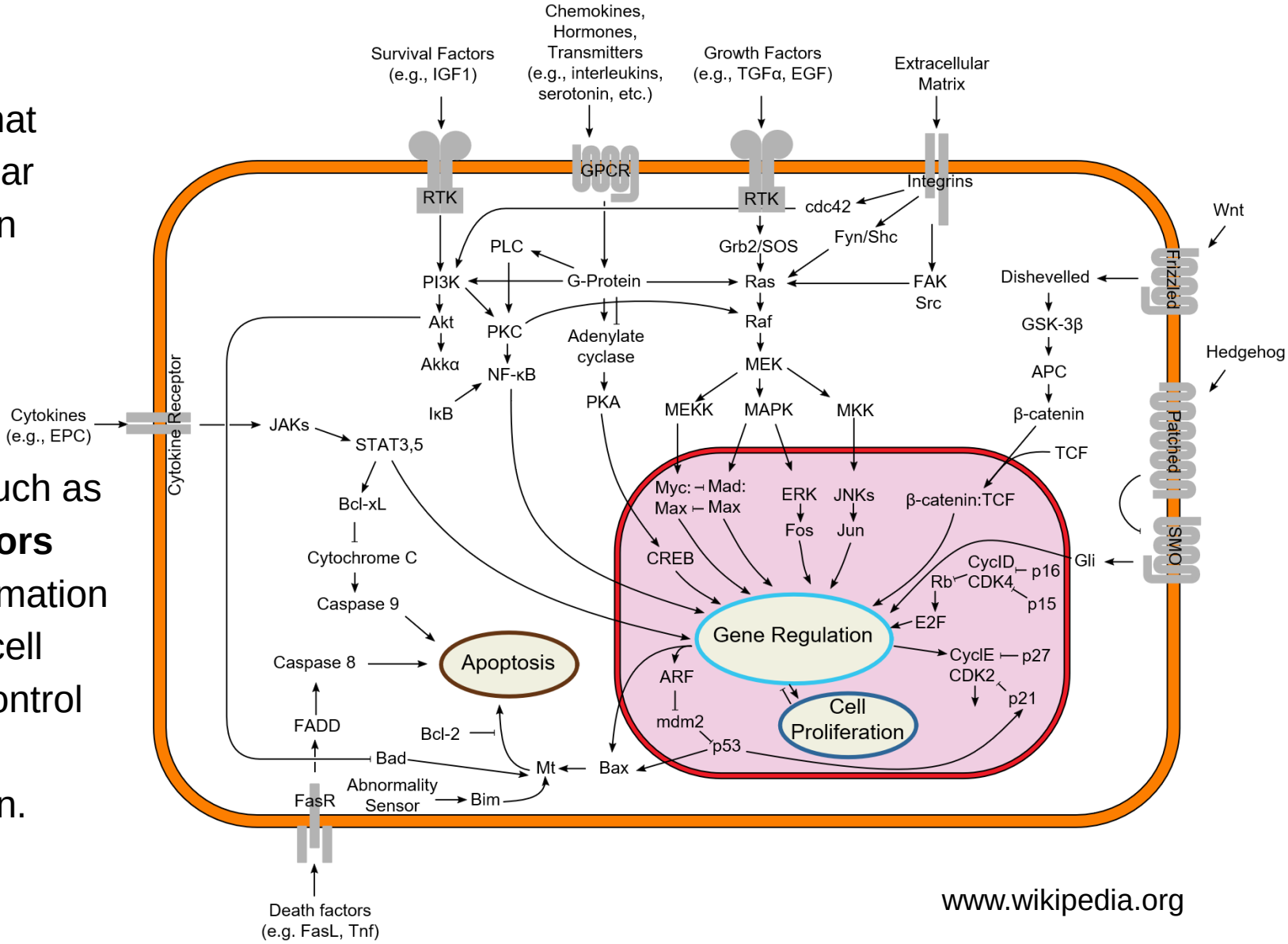
It is well accepted that the gene expression program encoded in the genome is executed by **transcription factors** that bind to cis-regulatory sequences and modulate gene expression in response to **environmental cues**.

Xie et al., Cell 153, 1134-1148 (2013)

Growth factors induce cell differentiation

The major molecular processes that control cellular differentiation involve **cell signaling**.

Signalling molecules such as **growth factors** convey information from cell to cell during the control of cellular differentiation.



www.wikipedia.org

TFs in Core Pluripotency Network

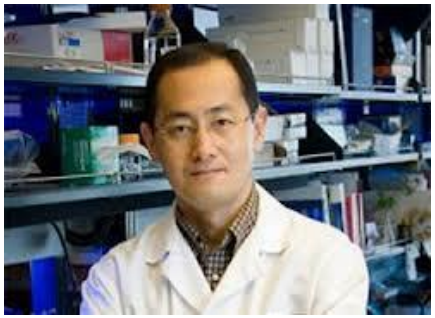
Oct4, encoded by ***Pou5f1***, is a POU domain-containing TF that is essential to ES cells and early embryonic development.

Oct4 binds to **Sox2**, another TF.

Genome-wide mapping of OCT4 and SOX2 sites in human ES cells shows that they **co-target** multiple genes.

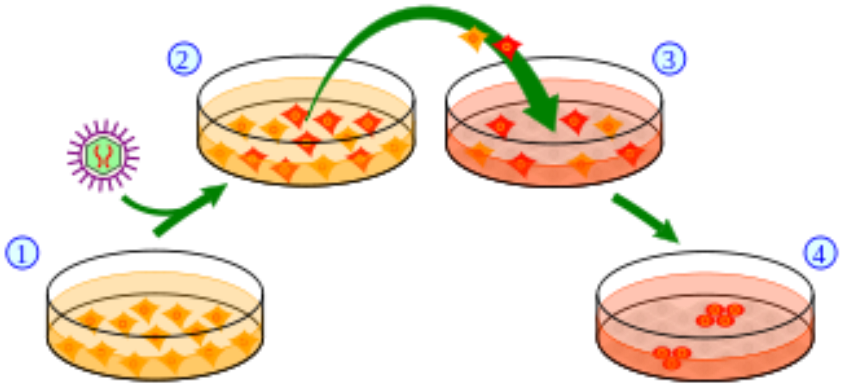
Oct4 and Sox2, along with **c-Myc** and **Klf4**, appear to be sufficient for reprogramming fibroblasts to **induced pluripotent stem cells (iPS),** which are functionally similar to ES cells (→ **Yamanaka factors**).

Shinya Yamanaka
noble price for medicine 2012



Chen et al., Cell 133, 1106-1117 (2008)

How to produce iPSC cells



A scheme of the generation of induced pluripotent stem (IPS) cells.
(1) Isolate and culture donor cells.
(2) Transduce stem cell-associated genes into the cells by viral vectors. Red cells indicate the cells expressing the exogenous genes.
(3) Harvest and culture the cells according to ES cell culture, using mitotically inactivated feeder cells (lightgray).
(4) A small subset of the transfected cells become iPS cells and generate ES-like colonies.

www.wikipedia.org

Paper #7

Single-cell RNA-sequencing of differentiating iPS cells reveals dynamic genetic effects on gene expression

Anna S. E. Cuomo, Daniel D. Seaton, Davis J. McCarthy, Iker Martinez, Marc Jan Bonder, Jose Garcia-Bernardo, Shradha Amatya, Pedro Madrigal, Abigail Isaacson, Florian Buettner, Andrew Knights, Kedar Nath Natarajan, HipSci Consortium, Ludovic Vallier, John C. Marioni, Mariya Chhatriwala **& Oliver Stegle**

Nature Communications 11, 810 (2020)
https://www.nature.com/articles/s41467-020-14457-z

iPSC cells from 125 donors

Aims:
- understand how development varies across individuals
- study influence of common genetic variants during this process

Protocol to induce differentiation into endoderm

Cells were incubated for 24 h (Day 1) in CDM-PVA containing

100 ng/mL Activin A, member of the TGF-beta (transforming growth factor-beta) superfamily of proteins. The encoded preproprotein is proteolytically processed to generate a subunit of the dimeric activin and inhibin protein complexes. These complexes activate and inhibit, respectively, follicle stimulating hormone secretion from the pituitary gland. The encoded protein also plays a role in eye, tooth and testis development.

80 ng/mL FGF2 : fibroblast growth factor 4

10 ng/mL BMP4: bone morphogenetic protein 4

10 µm Ly294002 : PI3 kinase inhibitor - *Activin A Efficiently Specifies Definitive Endoderm from Human Embryonic Stem Cells Only When Phosphatidylinositol 3-Kinase Signaling Is Suppressed* (DOI: 10.1634/stemcells.2006-0219)

3 µM CHIR99201 : glycogen synthase kinase (GSK) 3 inhibitor. GSK3 is a serine/threonine kinase that is a key inhibitor of the WNT pathway; therefore CHIR99021 functions as a WNT activator.

https://www.sciencedirect.com/science/article/pii/S1873506117302477

Protocol to induce differentiation into endoderm

After 24 h, the day 1 media was replaced with CDM-PVA containing
100 ng/mL ActivinA,
80 ng/mL FGF2,
10 ng/mL BMP4, and
10 µm Ly294002 for another 24 h (Day 2).

Day 2 media was then replaced with RPMI/B27 containing
100 ng/mL ActivinA and
80 ng/mL FGF2 for another 24 h (Day 3).

CDM-PVA medium: Iscove's modified Dulbecco's medium (50%) plus F12 NUT-MIX (50%), supplemented with insulin (7 µg/ml), transferrin (15 µg/ml), monothioglycerol (450 µM), and polyvinyl alcohol (PVA).

RPMI-B27 medium: 98% (v/v) RPMI 1640 (Invitrogen), 2% B27 serum supplement (Invitrogen), and 2 mM L-glutamine

https://www.sciencedirect.com/science/article/pii/S1873506117302477
https://discovery.lifemapsc.com/stem-cell-differentiation/in-vitro-cells/inner-cell-mass-homo-sapiens-bi-1-3-university-of-cambridge

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4045647/

iPSC marker genes/proteins

Surface markers such as TRA-1-60, TRA-1-81, SSEA-3 and SSEA-4 are routinely used to identify hPSCs.

TRA-1-60 is a 200-240 kD cell surface antigen expressed by human embryonic stem cells (ESC), embryonic germ cells (EG), and embryonal carcinoma cells. The expression of TRA-1-60 on human ESC is downregulated upon differentiation.

CXCR4 : the markers most commonly used for monitoring endoderm induction from hPSCs are CD184 (CXCR4), CD117 (KIT) and EPCAM. When used in combination, they do provide a reasonable assessment of the efficiency of endoderm development. However, none of these markers is endoderm specific

https://www.sciencedirect.com/science/article/pii/S1873506117302477

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4689216/

eQTLs

Genetic variation in a population is commonly studied through the analysis of single nucleotide polymorphisms (**SNPs**).
SNPs are genetic variants occurring at specific sites in the genome.

Expression quantitative trait loci (**eQTL**) analysis seeks to identify genetic variants that affect the expression of one or more genes.

https://www.sciencedirect.com/science/article/pii/S1873506117302477

Single cell sequencing

Single cell sequencing means that one attempts to determine the genome, epigenome of transcriptome of a single biological cell, not of a large sample.

One reason for this is that tissues usually contain a mixture of cell types, even tumors have a heterogeneous composition.

Another advantage is that differentiation in single cells may proceed at a different speed.

Also, there may be multiple subtypes of a single cell type that show differing expression patterns.

In short, single cell sequencing is a current hype, just like single molecule spectroscopy about 25 years ago.

https://www.sciencedirect.com/science/article/pii/S1873506117302477