SAPIENZA
UNIVERSITÀ DI ROMA

DIPARTIMENTO DI
BIOLOGIA E BIOTECNOLOGIA
CHARLES DARWIN

Stem cells and therapeutic applications

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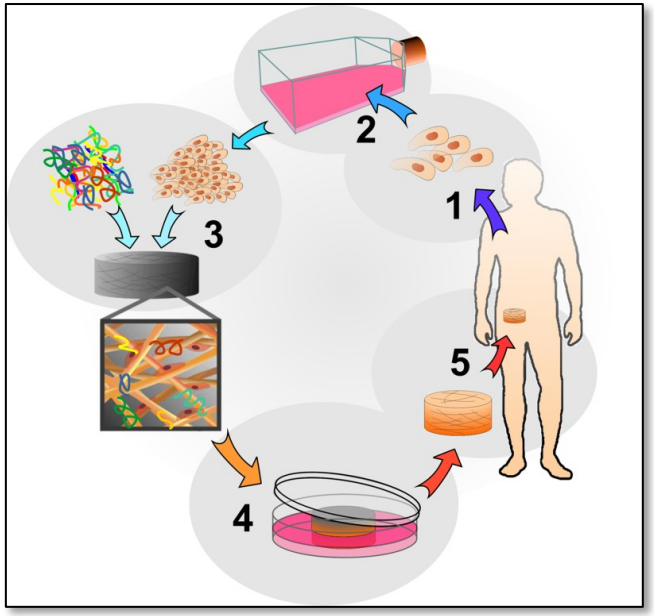
Biologia Molecolare per Fisica
14/5/2015

Using stem cells for therapy

Regenerative medicine is the process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage, or congenital defects.

Cell therapy: treat a disease by introducing new cells into a tissue.

Tissue engineering: combining cells, engineering and materials methods, and suitable biochemical and physio-chemical factors to improve or replace biological functions.



This lesson

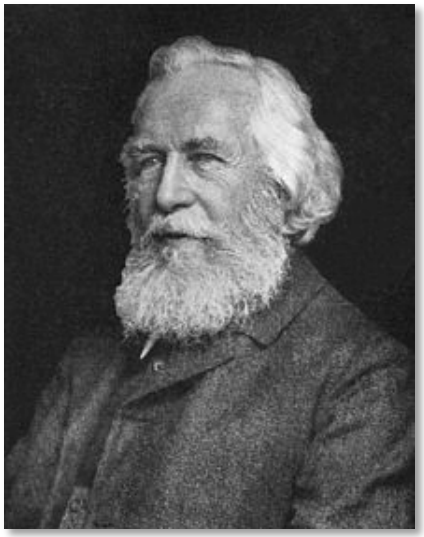
1. What are stem cells? Adult and Embryonic stem cells.

2. Unique properties of pluripotent cells.

3. Generating pluripotent cells from somatic cells through epigenetic reprogramming.

4. Applications of pluripotent cells, from basic research to regenerative medicine.

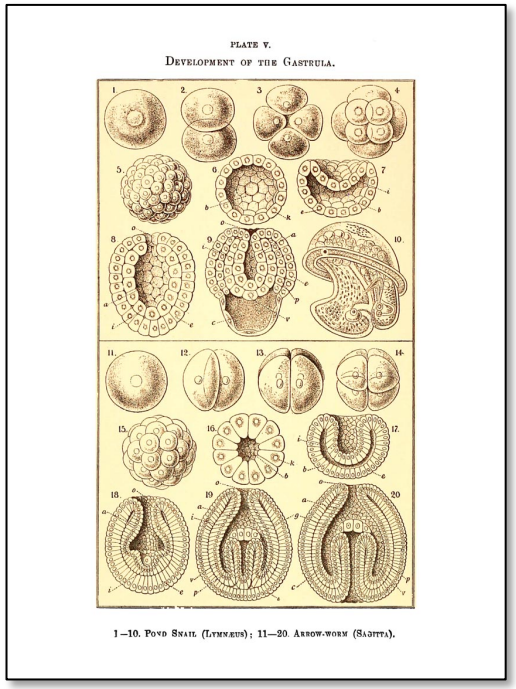
Where does the term "Stem Cell" come from?



Ernst Haeckel (1834-1919)

Stem Cell (1868): "the unicellular ancestor of all multicellular organisms" and "the fertilized egg that gives rise to all cells of the organism".

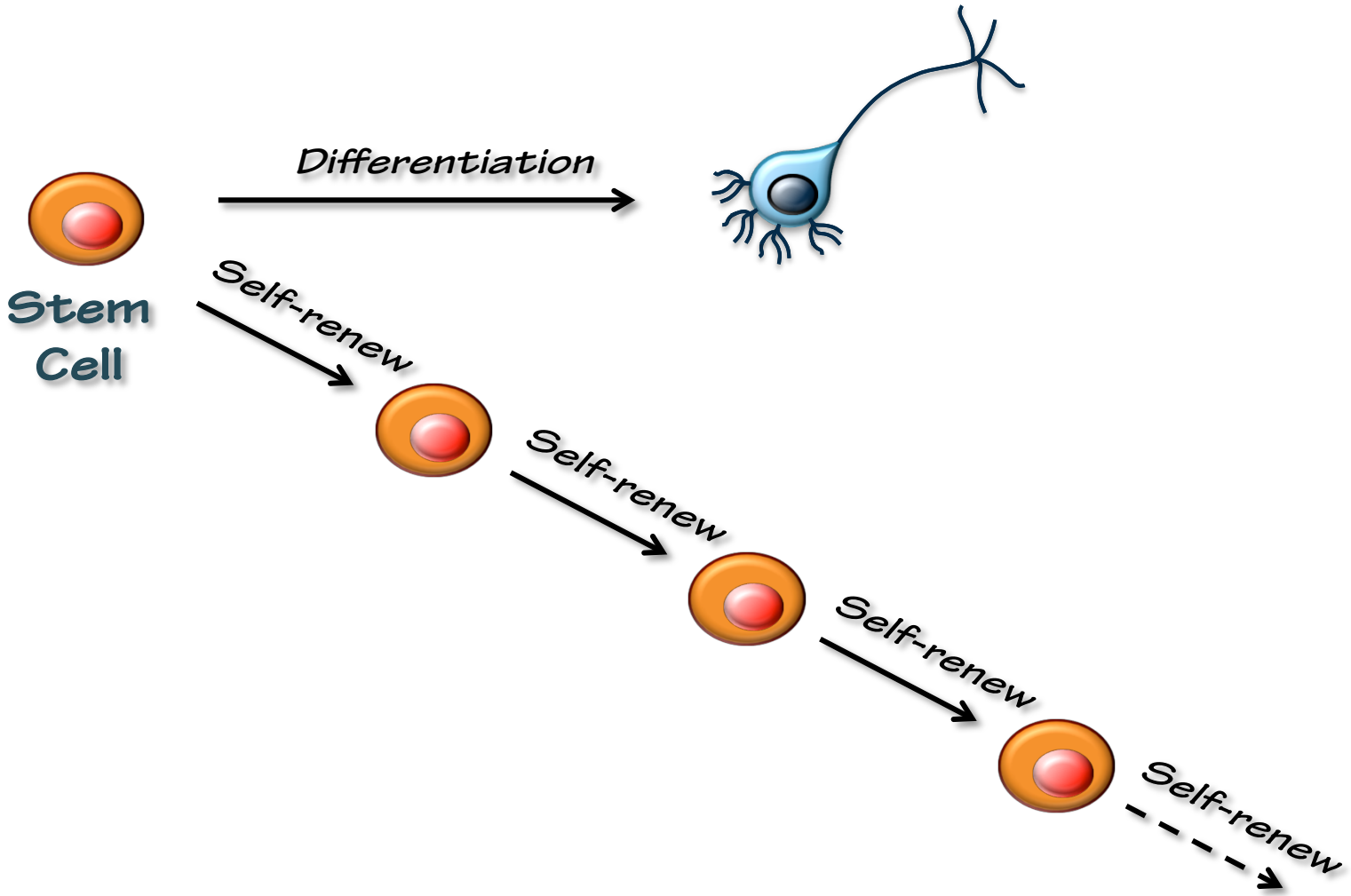
Such definition is in agreement to Haeckel's recapitulation theory ("ontogeny recapitulates phylogeny").



"Stem cell", "Anthropogeny", "Ecology", "Phylum", "Phylogeny" are all terms coined by Haeckel, who was also a major supporter of Darwin's theory.

What does the term Stem mean?

Stamen, staminis (Lat.): "string" (Eng.).



Adult Stem Cells

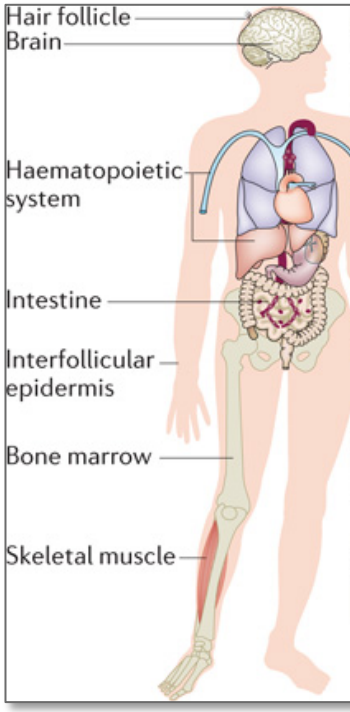
Adult Stem Cells are present in some organs for the entire life of the organism.

They represent immortal populations of cells, able to regenerate the tissues in which they reside.

Prometeus

ἧπαρ (liver) is derived from ἠπάομαι (repair).

Stem Cell Niches



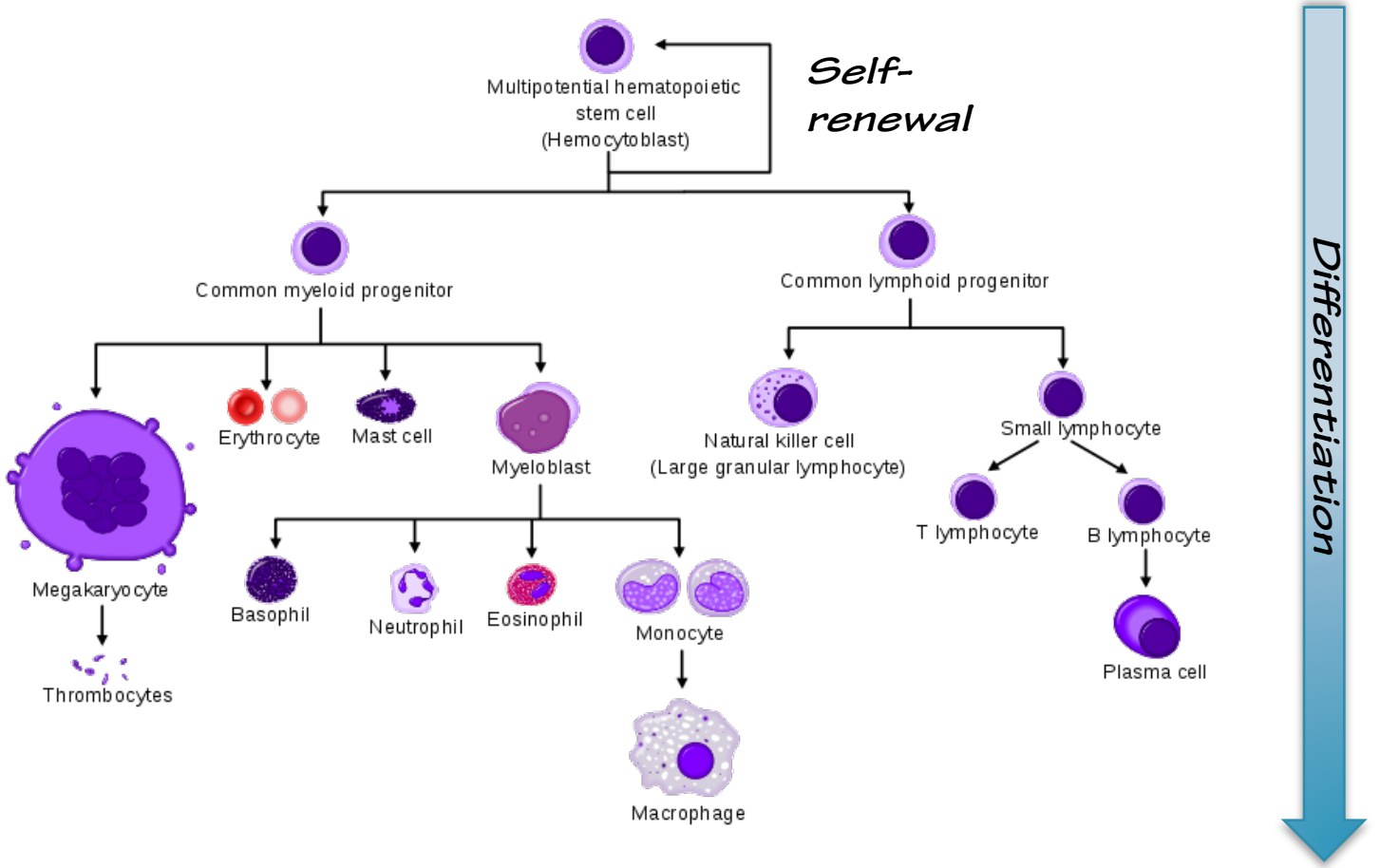
Tissue type	Stem cell location	Niche components
Tissues with constant turnover		
Haematopoietic system	Bone marrow	Macrophages*, T_{Reg} cells*, osteoblasts, adipocytes, nestin$^+$ MSCs, CAR cells, glia
Intestine	Fast-cycling: base of crypt Slow-cycling: '+4 position'	Paneth cells*, mesenchymal cells
Interfollicular epidermis	Basal layer of epidermis	Dermal fibroblasts
Hair follicle	Bulge	K6$^+$ bulge*, dermal papilla, adipocyte precursor cells, subcutaneous fat, dermal fibroblasts
Tissues with low or no turnover		
Brain	Subventricular zone, subgranular zone	Ependymal cells, vasculature
Skeletal muscle	Between the basement membrane and the muscle fibres	Myofibres* (?)

(Hsu and Fuchs, 2012)

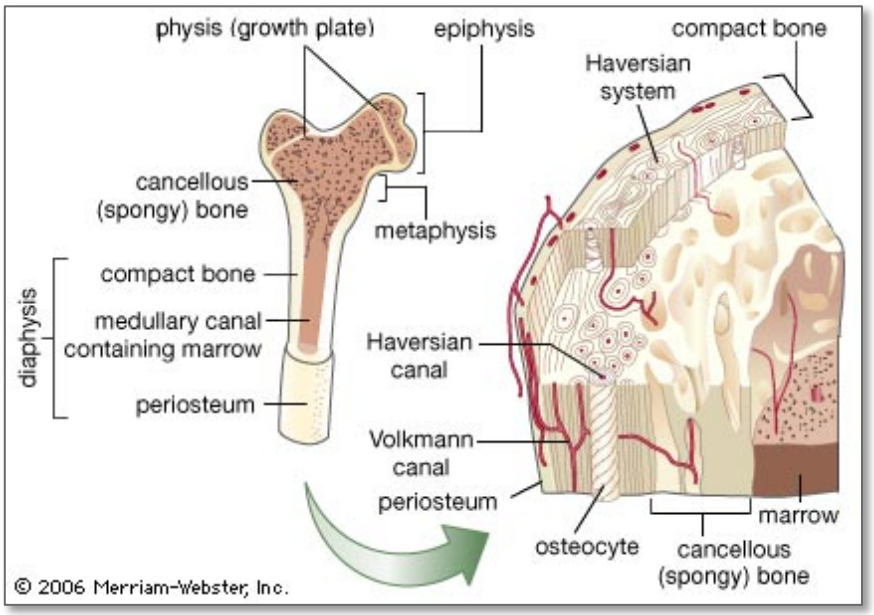
Niche: highly organized microenvironment that controls stem cells homeostasis.

Signals from the niche influence proliferation of stem cells and help to maintain their undifferentiated state.

Hematopoietic Stem Cell



HSC Niche: the Bone Marrow



Unlike most other stem cells, which reside either within their niche or within a relatively limited range of travelling distance surrounding the niche, HSCs are extraordinarily mobile. During homeostasis, HSCs often travel from one bone marrow compartment to another.

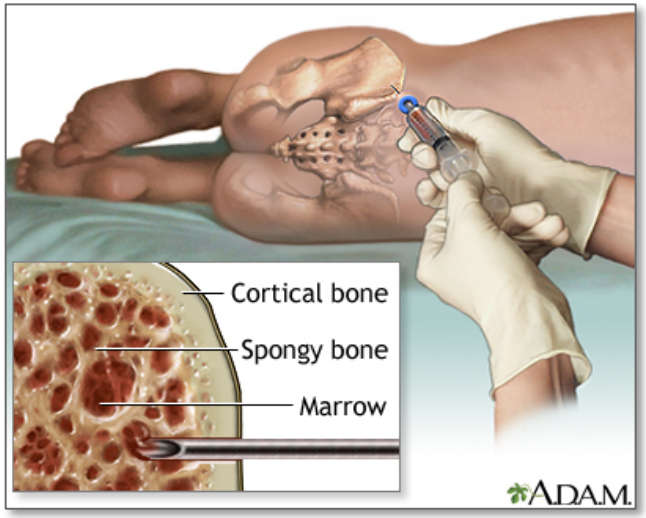
Bone marrow transplant

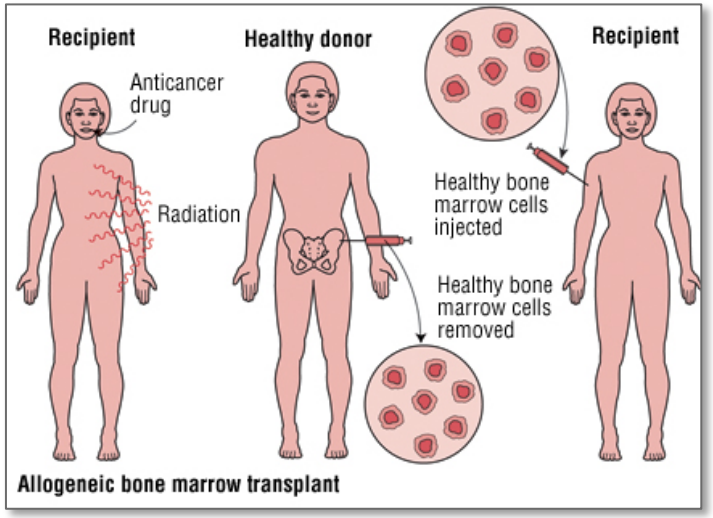
The aim is to replace bone marrow that either is not working properly or has been destroyed by chemotherapy or radiation.

Used to treat:

- Some types of cancer (e.g. leukemia, lymphoma, multiple myeloma)

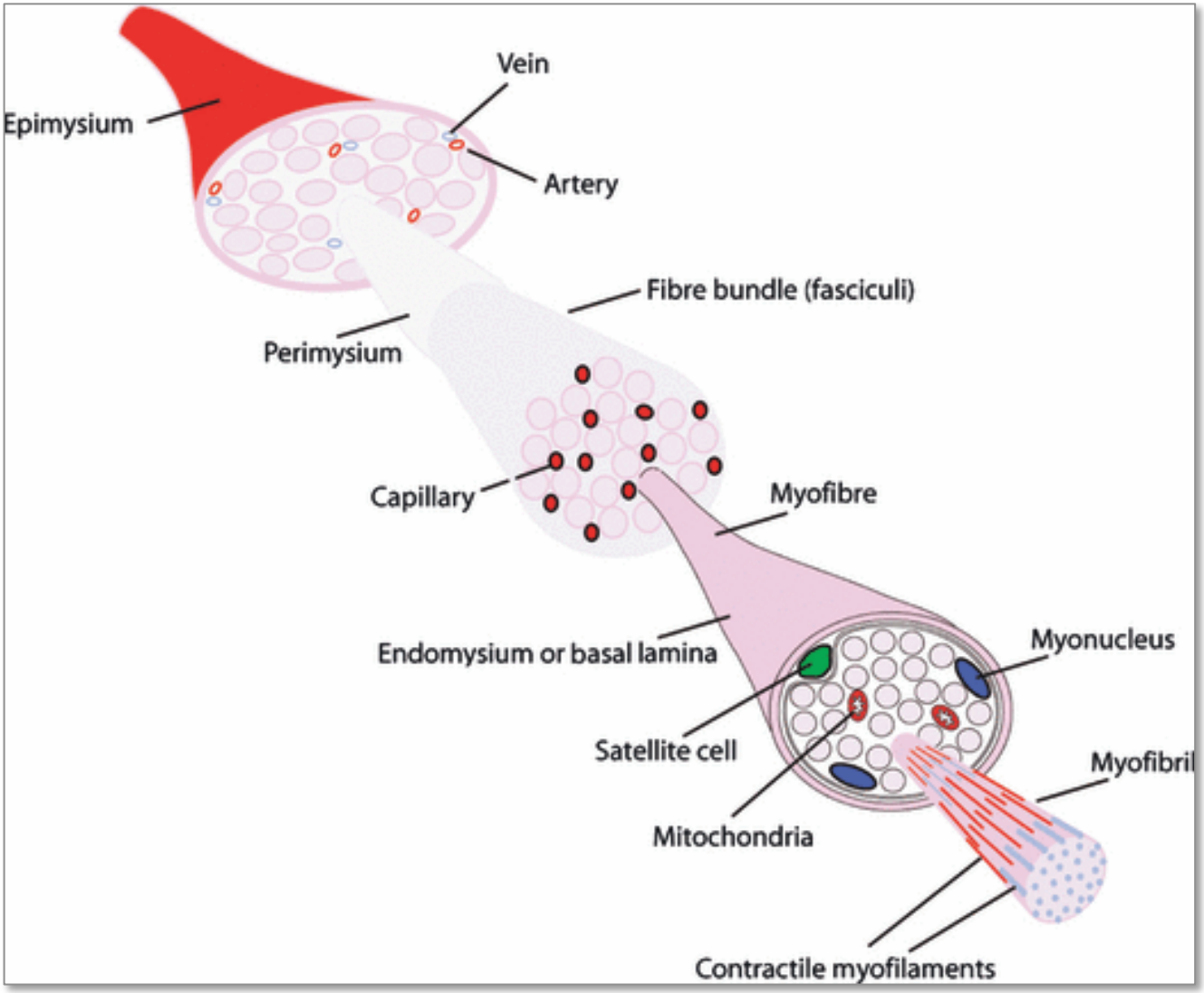
- A disease that affects the production of bone marrow cells (e.g. anemia, immunodeficiency syndromes)





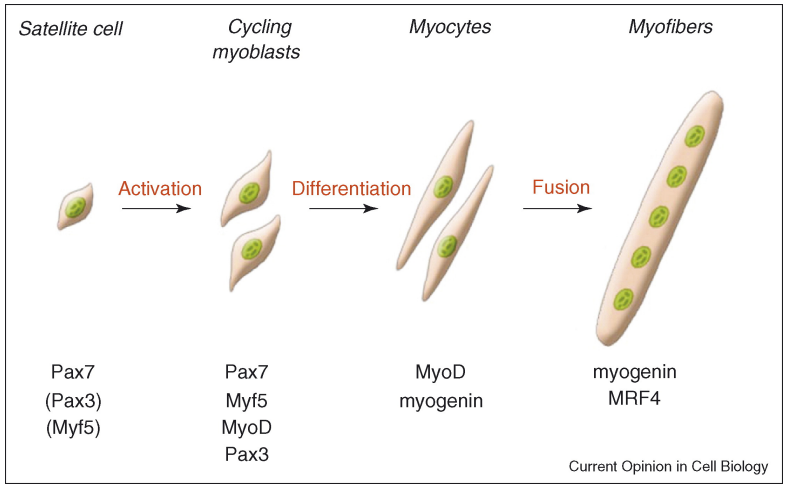
http://gru.edu/cancer/patientcare/services-treatment/bmt/process.php

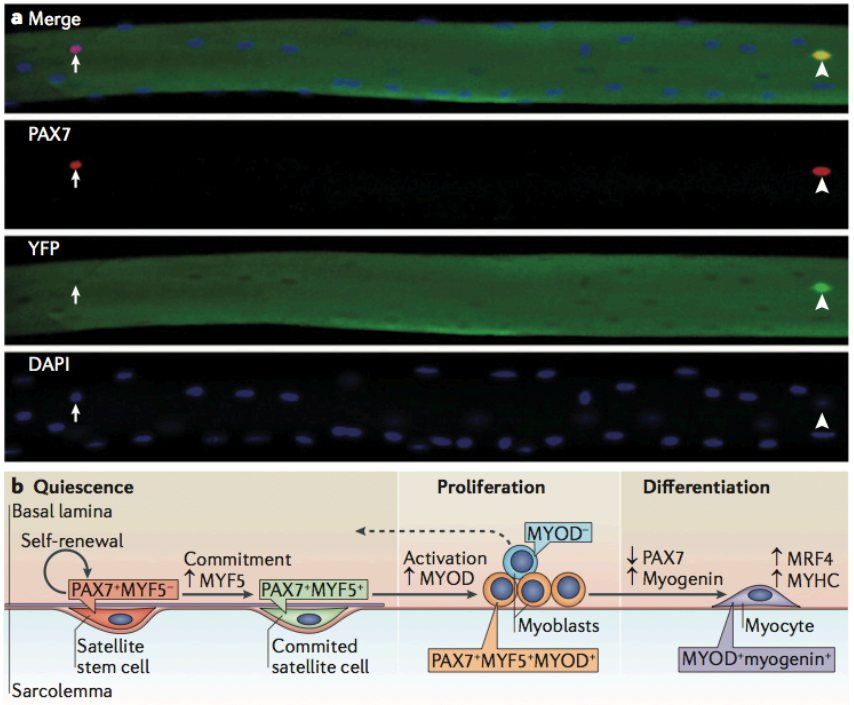
Muscle Stem Cells

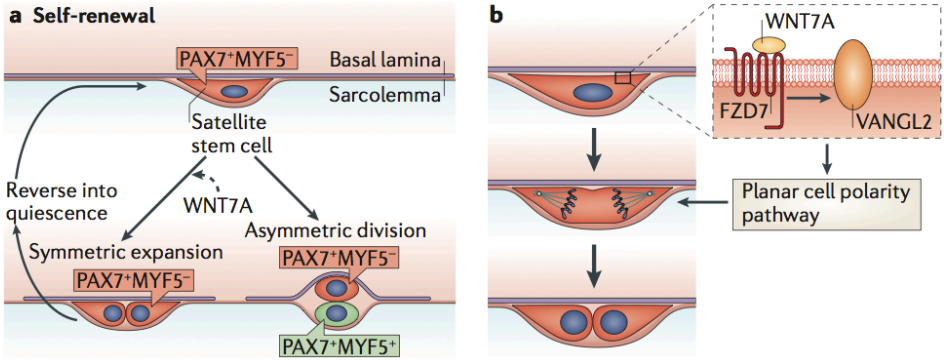


(Otto et al., 2009)

Satellite Cells



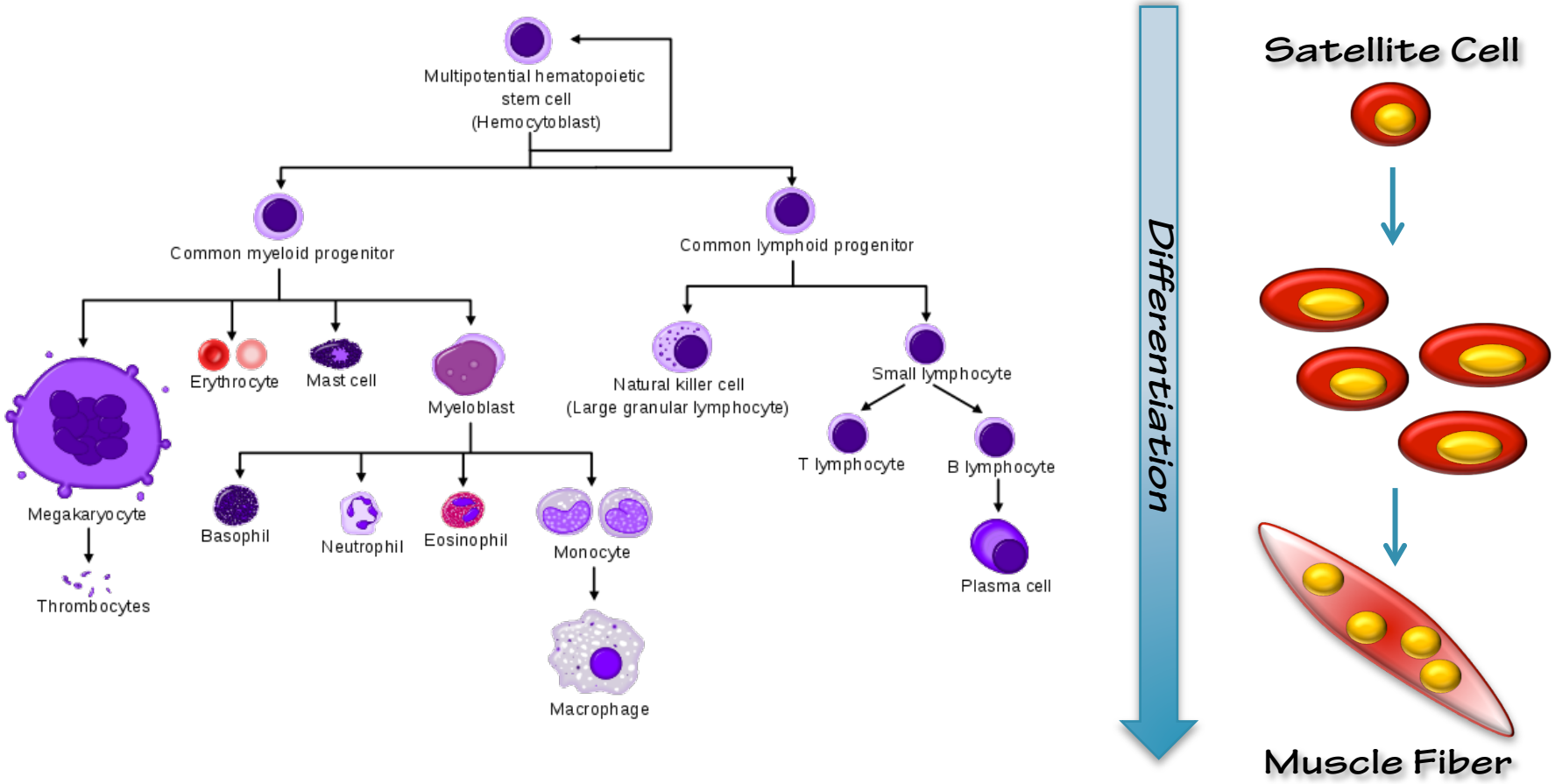




(Wang and Rudnicki, MCB 2012)

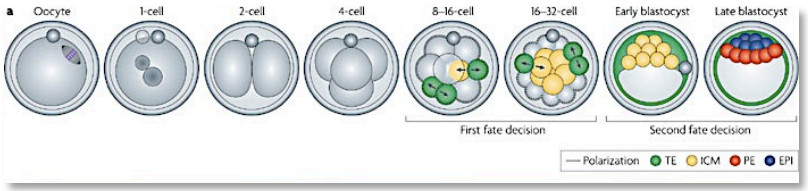
Adult Stem Cells

Adult Stem Cells are multipotent or unipotent.

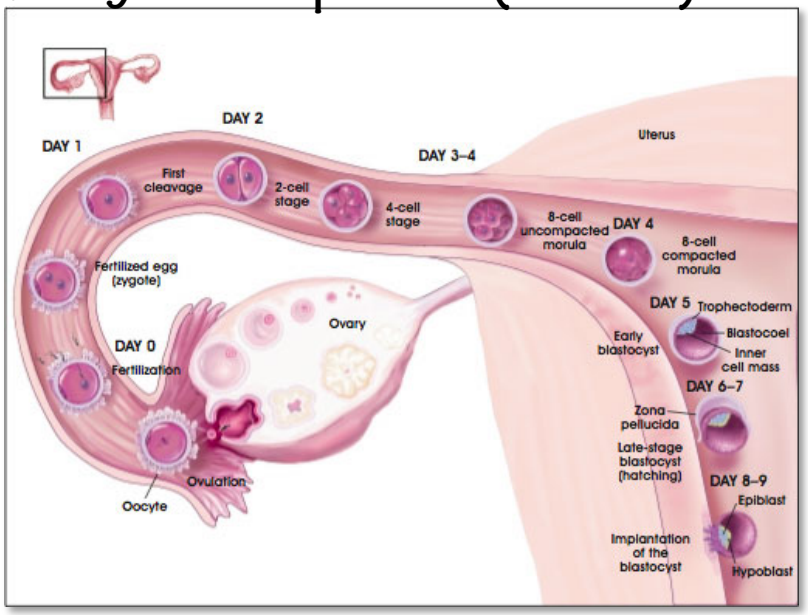


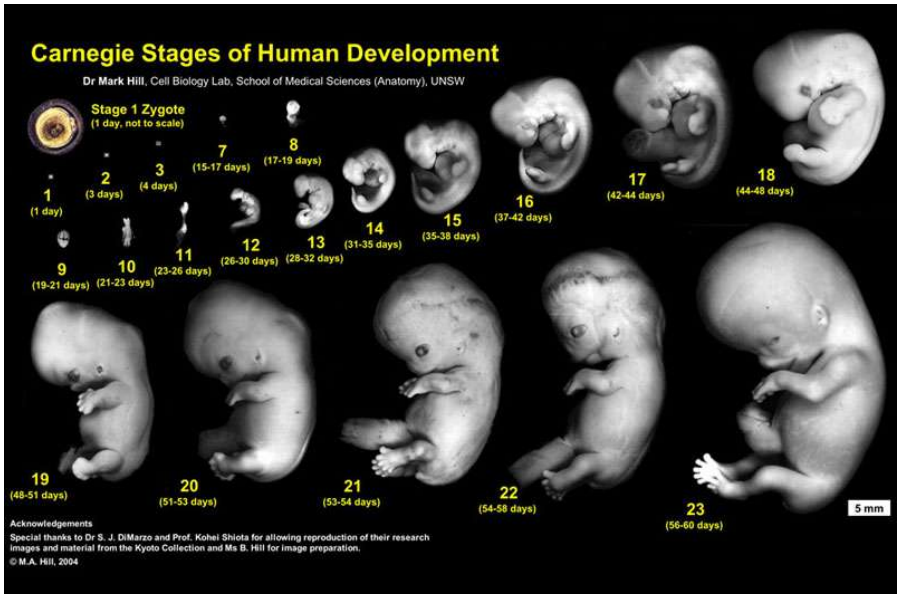
Early embryonic development

Blastocyst formation



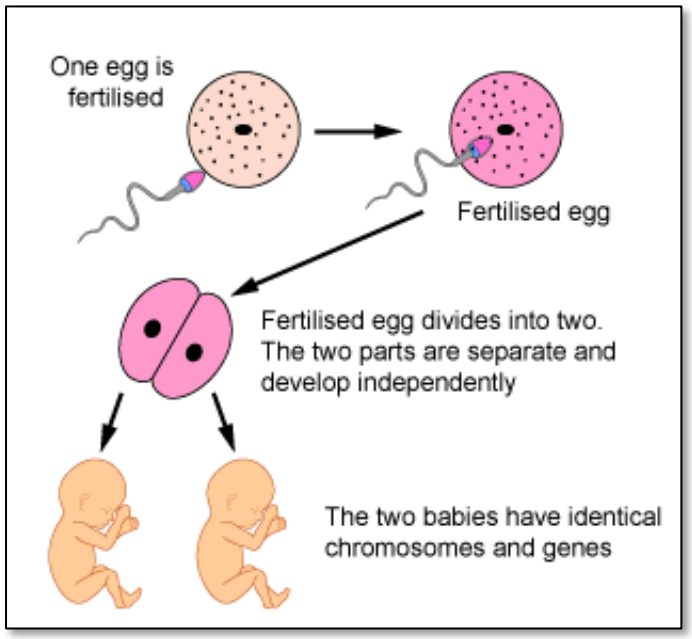
Early development (human)

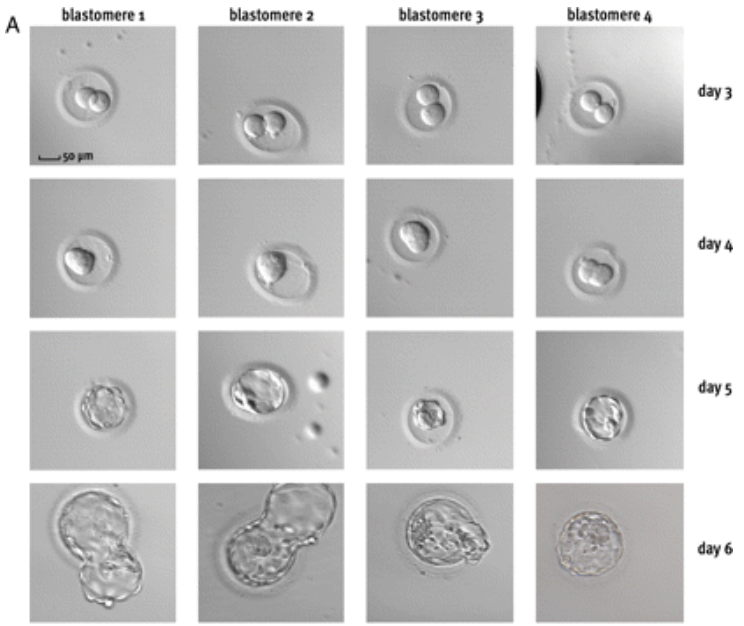




Totipotency and Pluripotency

Totipotency: the ability of a single cell to divide and produce all the differentiated cells in an organism, including extraembryonic tissues. *In mammals: zygote to 4-cell stage embryos.*



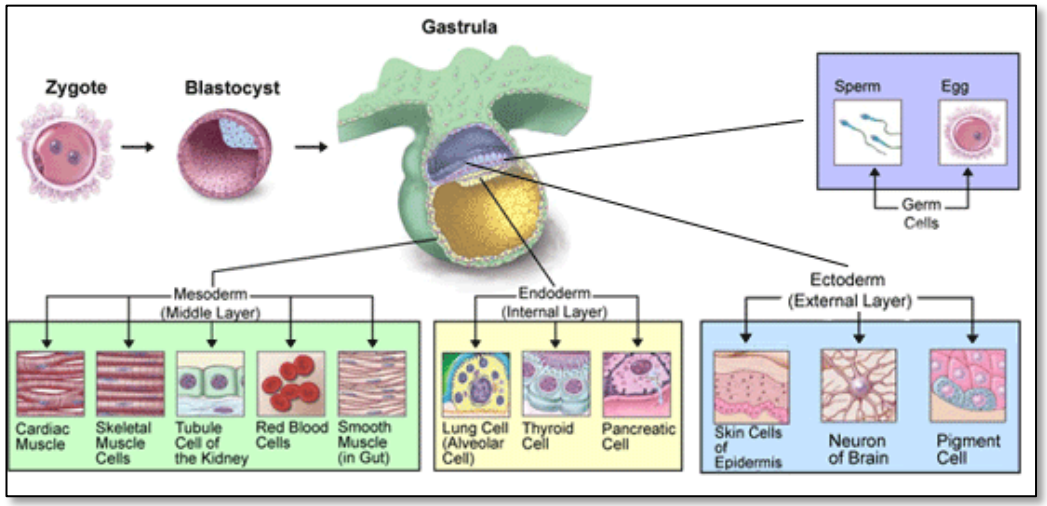


Van de Velde et al., 2008

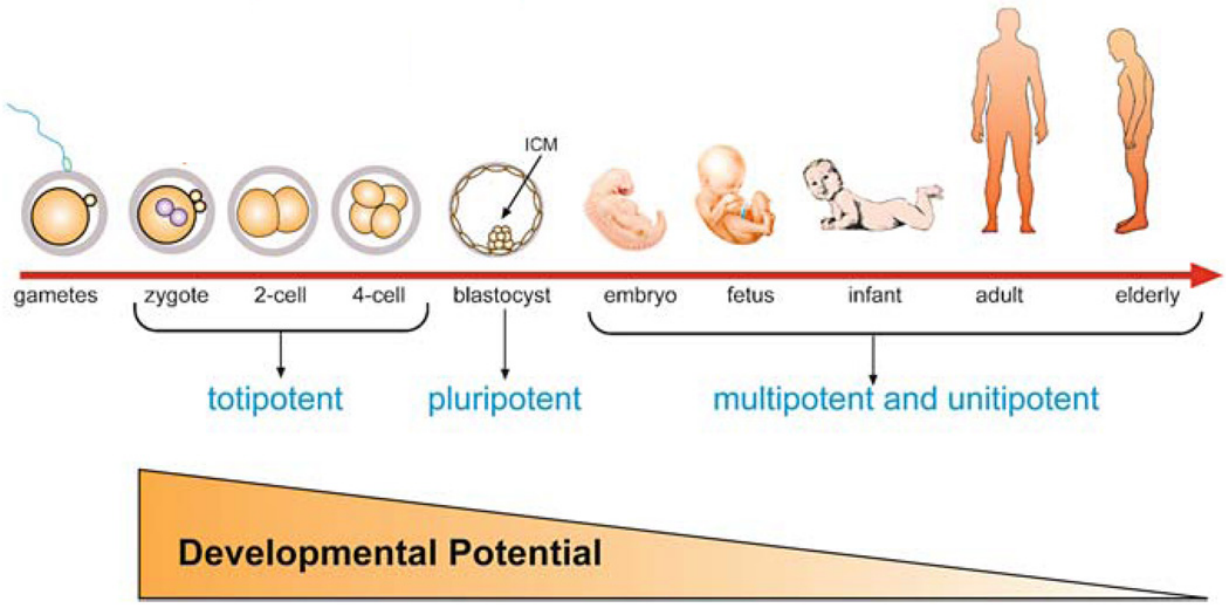
Totipotency and Pluripotency

Totipotency: the ability of a single cell to divide and produce all the differentiated cells in an organism, including extraembryonic tissues. *In mammals: zygote to 4-cell stage embryos.*

Pluripotency: the ability of a cell to differentiate into any of the three germ layers: endoderm, mesoderm or ectoderm. Pluripotent stem cells can give rise to any fetal or adult cell type. However, a single cell or a conglomerate of pluripotent cells cannot develop into a fetal or adult animal because they lack the potential to organize into an embryo. *In mammals: ICM of the blastocyst.*



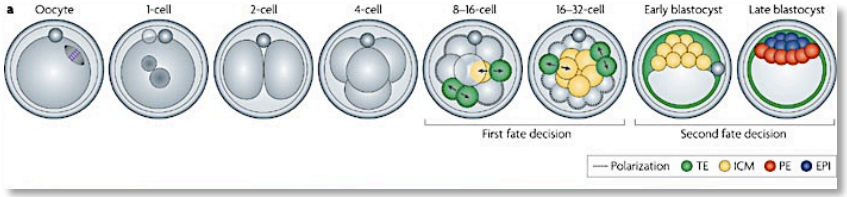
Decrease of developmental potential



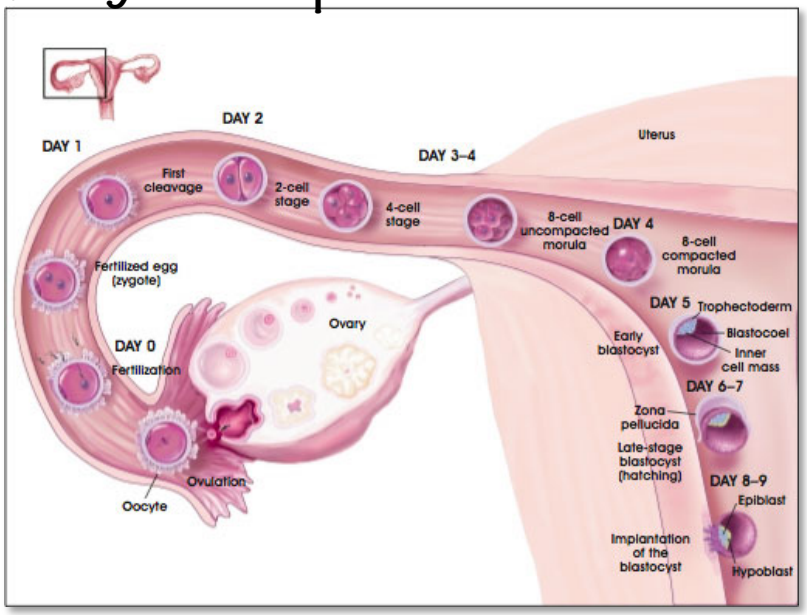
Mitalipov and Wolf, 2009

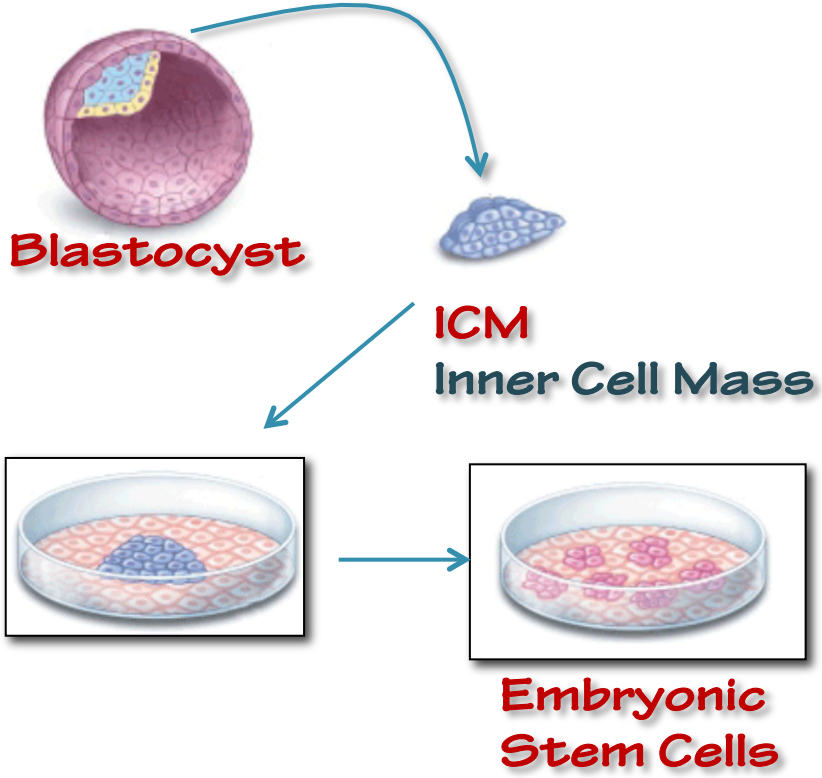
Embryonic Stem Cells

Blastocyst formation

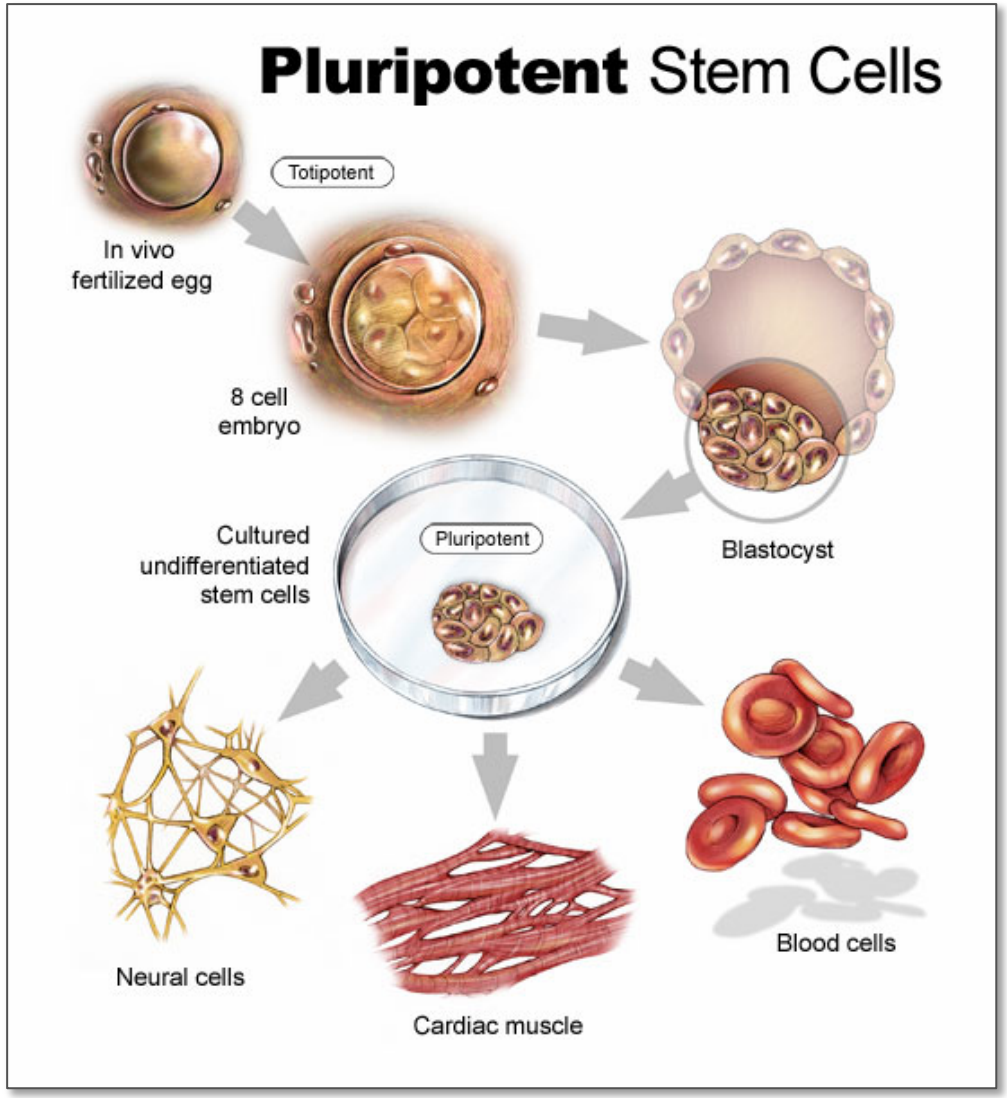


Early development

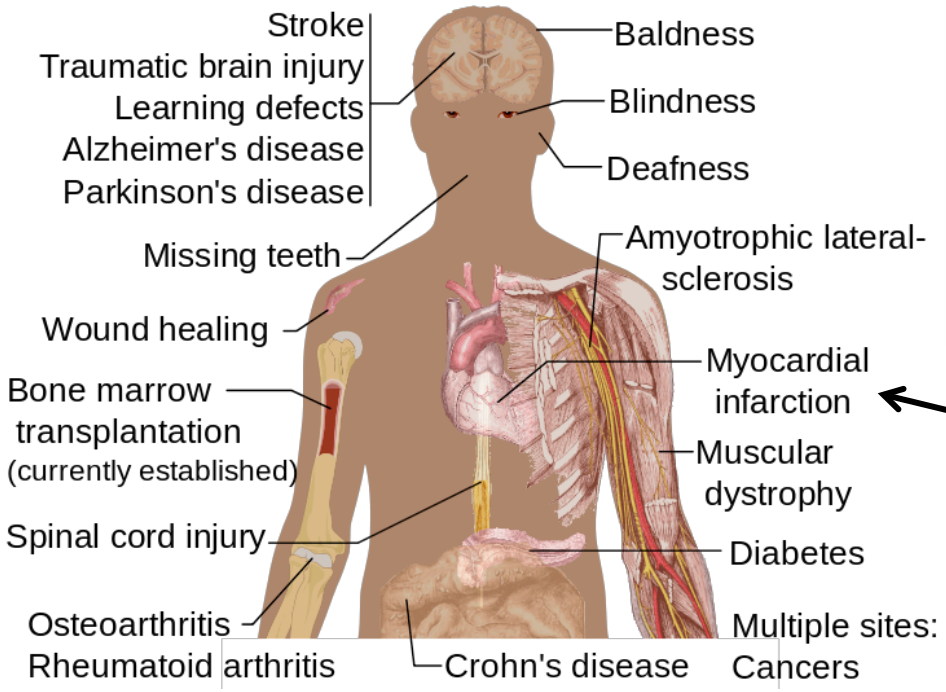


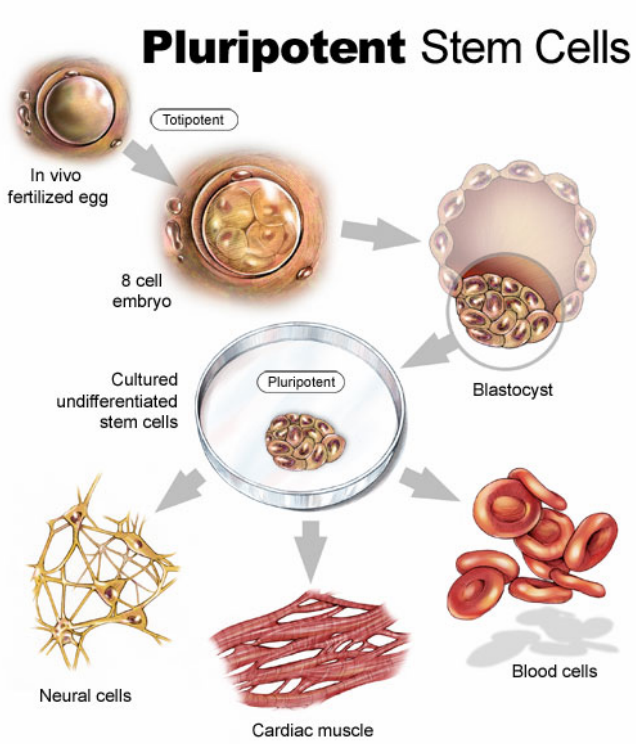


Embryonic Stem Cells are Pluripotent



Differentiation of ESCs for therapy





Embryonic and Adult Stem Cells

Embryonic

In vivo, they exist as a very transient population of cells inside the blastocyst. Their self-renew is limited to a short period of time

In vitro, we can keep them undifferentiated indefinitely (self-renew)

Their developmental potential is: pluripotency

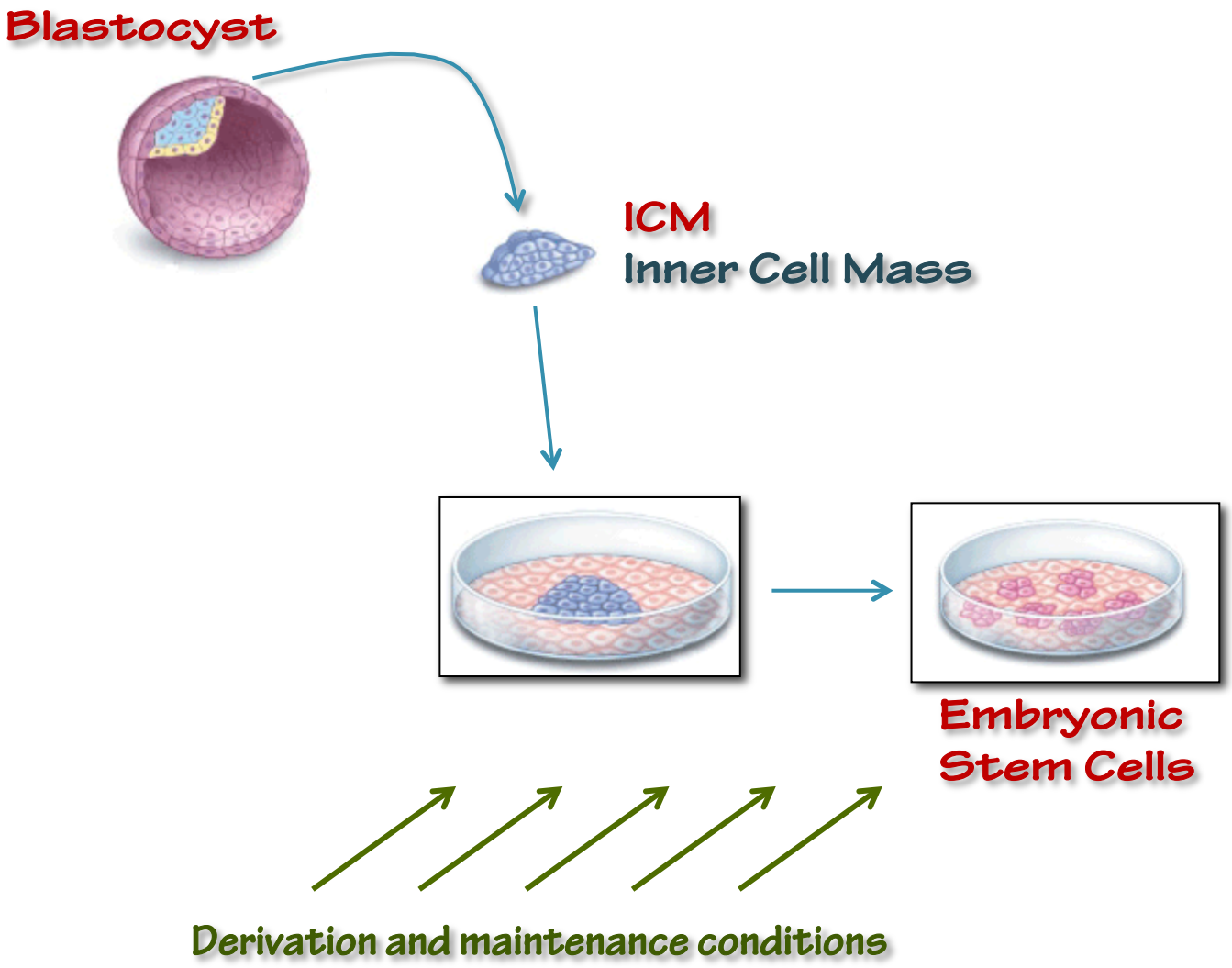
Adult

In vivo, they reside in niches inside adult organs. Their self-renew capacity is virtually unlimited

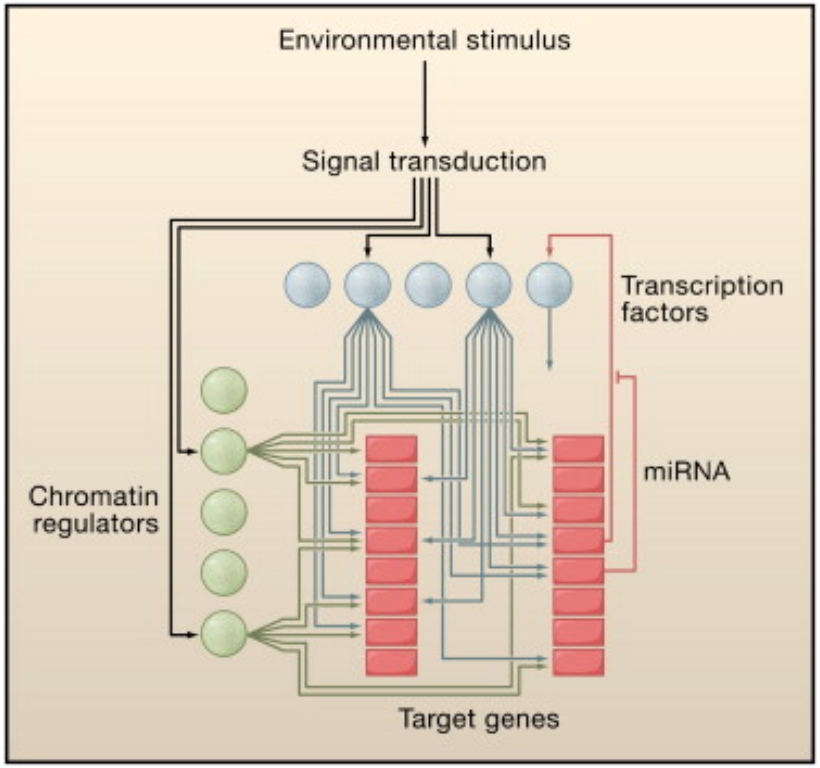
In vitro, not all adult stem cell types can be maintained

Their developmental potential is: multipotency or unipotency

Embryonic Stem Cells



Regulatory circuitries in Embryonic Stem Cells



(Jaenisch and Young, Cell 2008)

Transcription Factors

Chromatin Regulators

Other Genes

Transcriptional circuits in the first cell fate decision

(Zernicka-Goetz et al., Nat Rev Genet 2009)

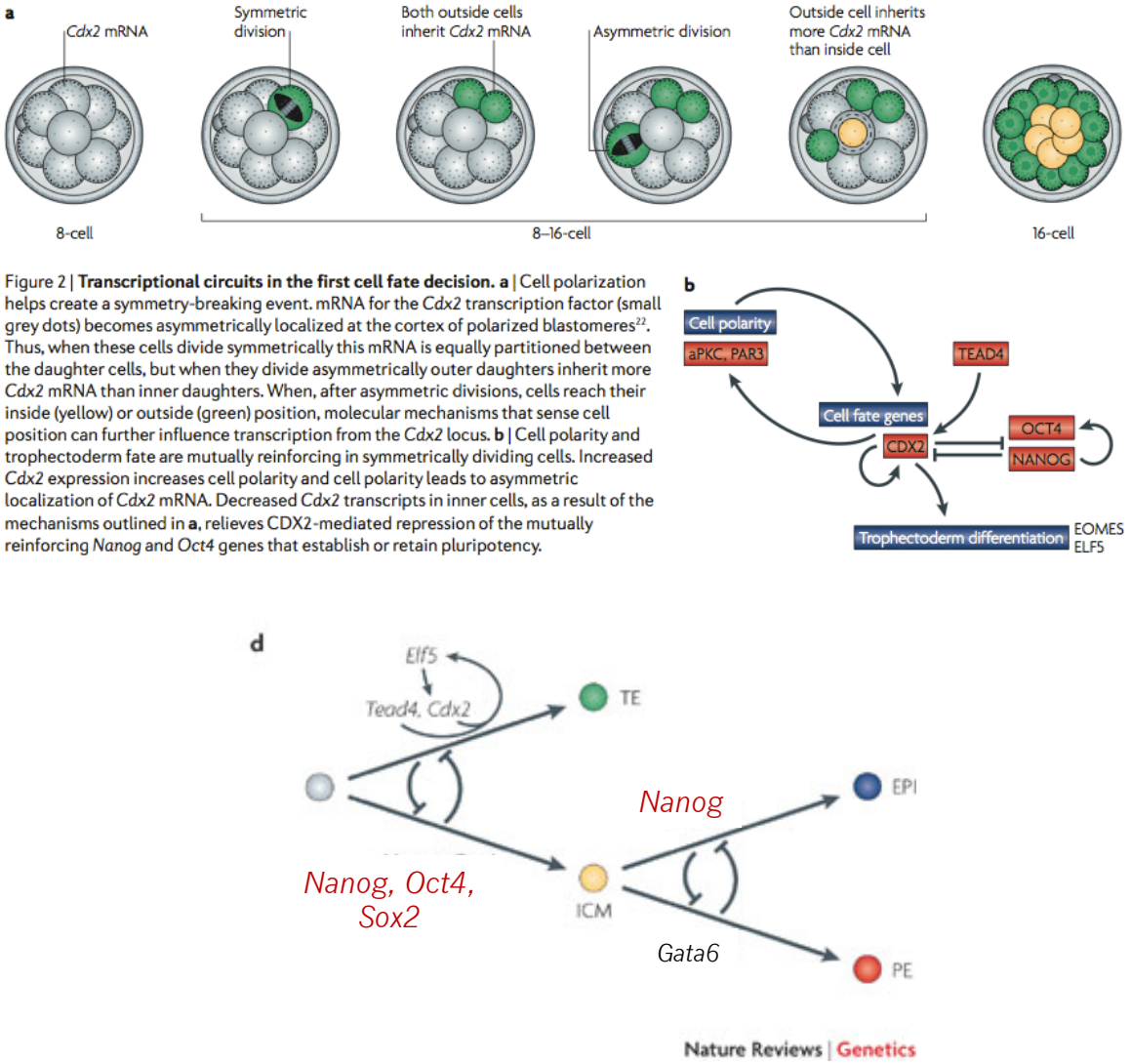
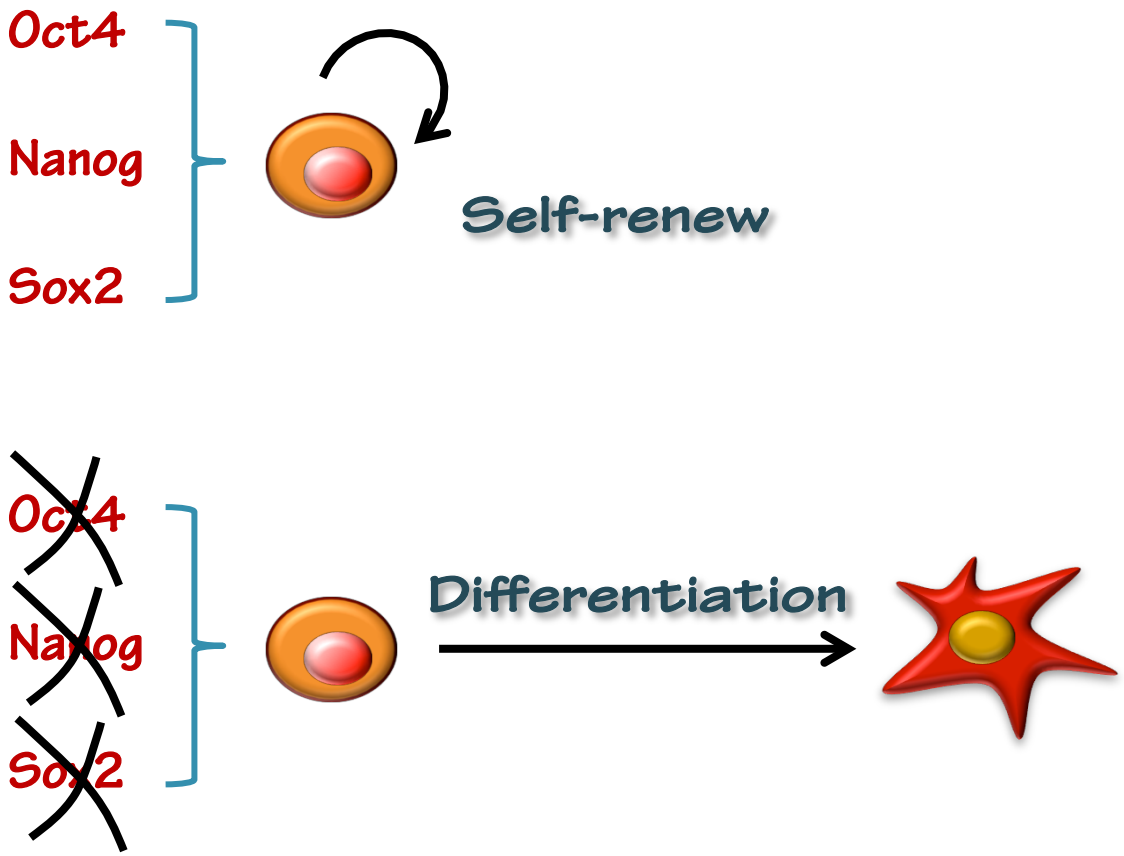
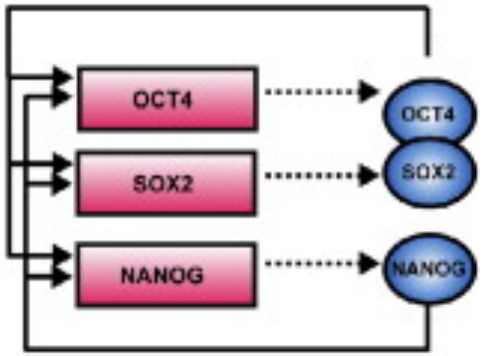


Figure 2 | **Transcriptional circuits in the first cell fate decision. a** | Cell polarization helps create a symmetry-breaking event. mRNA for the *Cdx2* transcription factor (small grey dots) becomes asymmetrically localized at the cortex of polarized blastomeres[22]. Thus, when these cells divide symmetrically this mRNA is equally partitioned between the daughter cells, but when they divide asymmetrically outer daughters inherit more *Cdx2* mRNA than inner daughters. When, after asymmetric divisions, cells reach their inside (yellow) or outside (green) position, molecular mechanisms that sense cell position can further influence transcription from the *Cdx2* locus. **b** | Cell polarity and trophectoderm fate are mutually reinforcing in symmetrically dividing cells. Increased *Cdx2* expression increases cell polarity and cell polarity leads to asymmetric localization of *Cdx2* mRNA. Decreased *Cdx2* transcripts in inner cells, as a result of the mechanisms outlined in **a**, relieves CDX2-mediated repression of the mutually reinforcing *Nanog* and *Oct4* genes that establish or retain pluripotency.

Core TFs are necessary to maintain ESCs pluripotency



Feedforward core loop and regulation of pluripotency

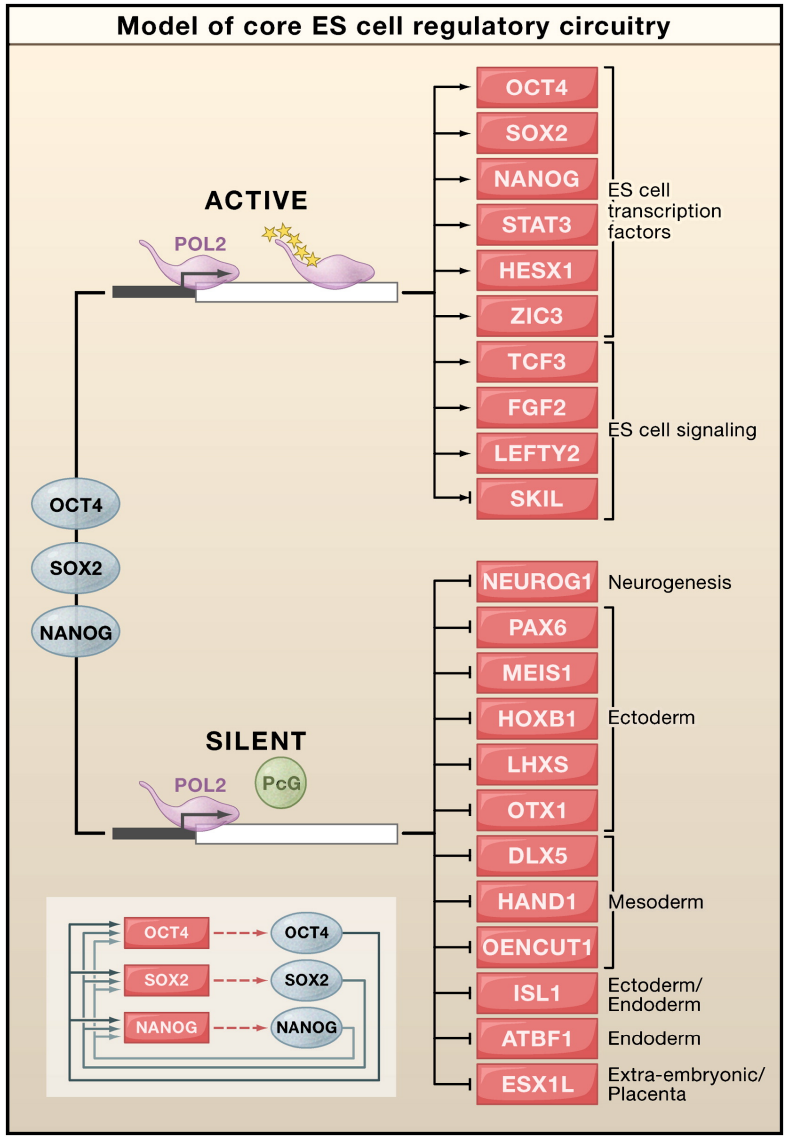


- ✓ Consistent activity that is relatively insensitive to transient changes (stability of gene expression in undifferentiated cells)

- ✓ Reduced response time to environmental stimuli (plasticity; fast activation of differentiation programs)

This autoregulatory and feedforward circuitry provide regulatory mechanisms by which stem cell identity can be robustly maintained yet permit cells to respond appropriately to developmental cues.

Transcriptional Regulation of Stemness



Embryonic stem cells
- key properties -

1. ESCs are pluripotent stem cells: they can self-renew indefinitely and differentiate into derivatives of the 3 germ layers

2. Two states of pluripotency exist: naïve (or ground state) and primed

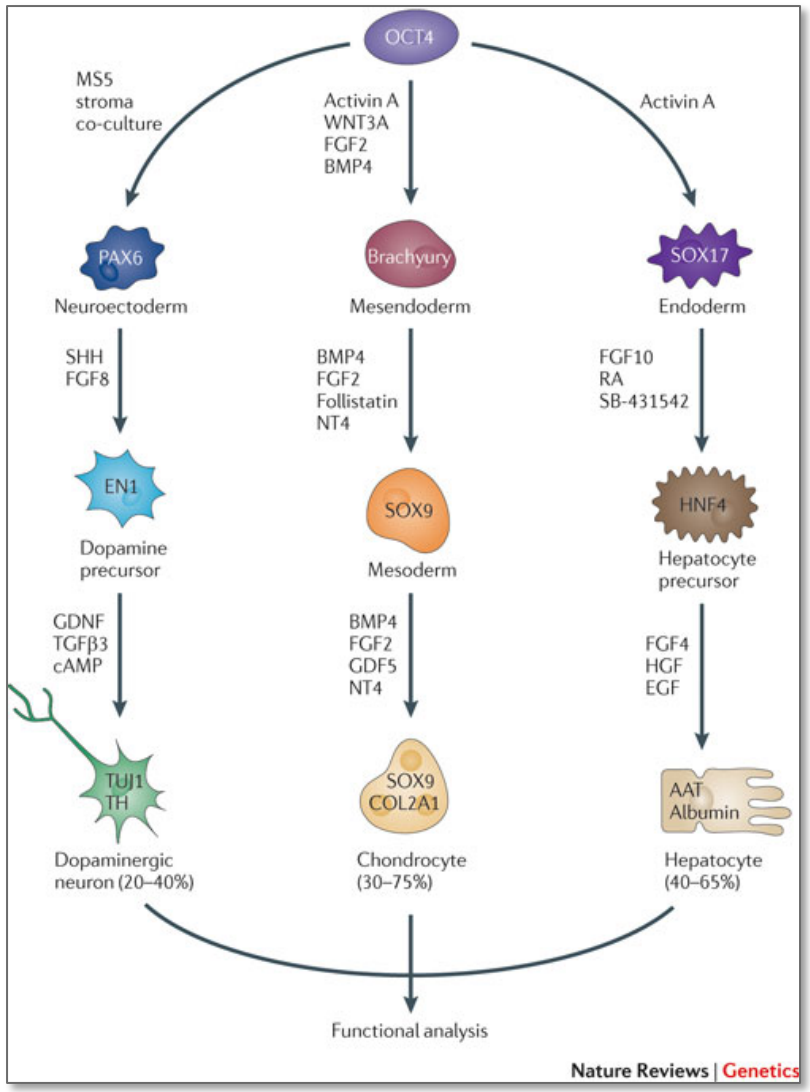
3. A core transcriptional regulatory circuitry plays a central role in ESCs

4. Genes involved in early development present bivalent chromatin domains in ESCs

5. ESCs have a peculiar cell cycle regulated by miRNAs

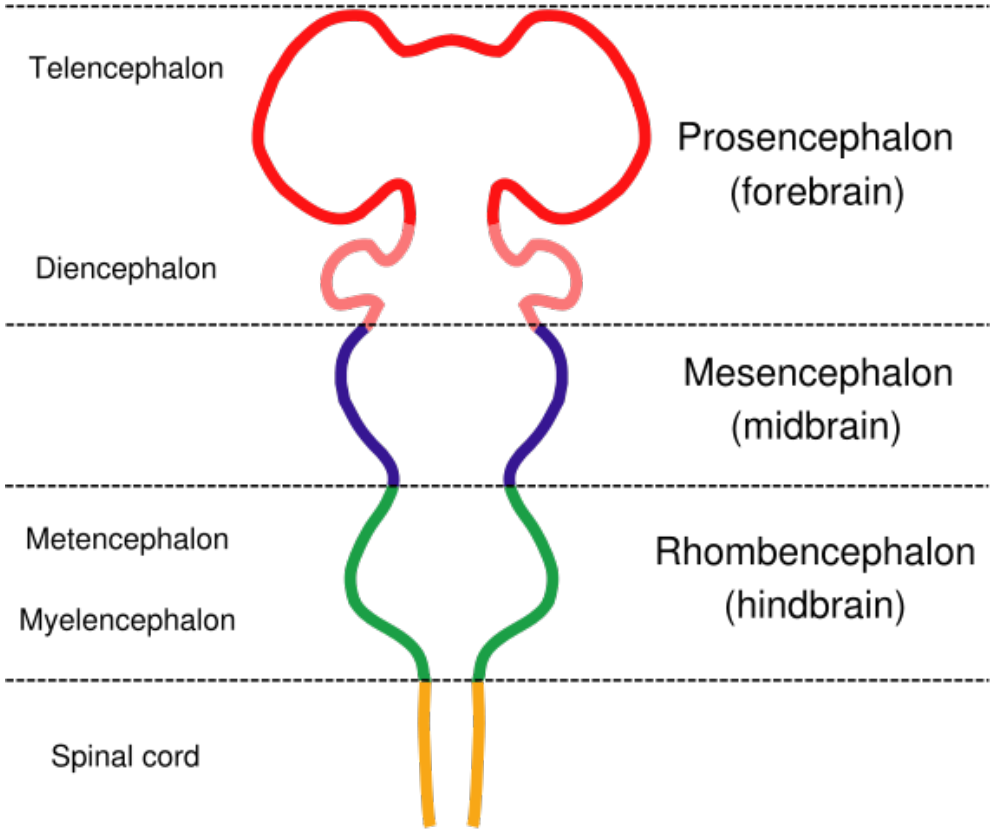
6. miRNAs also regulate differentiation of ESCs

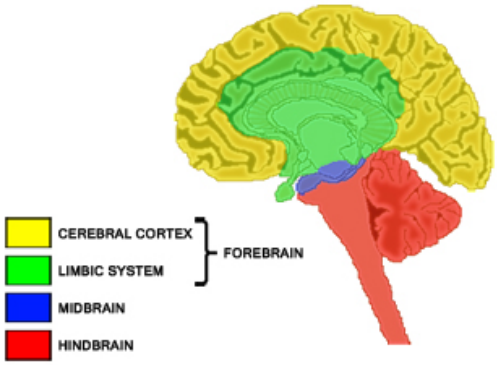
Directing Differentiation of ESCs

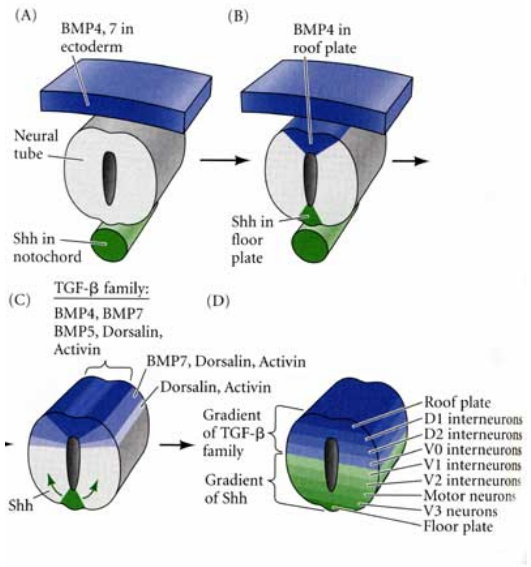


(Cohen and Melton, Nat. Rev. Genet. 2011)

Patterning in the neural tube



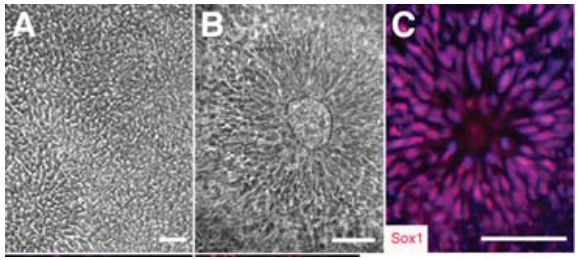




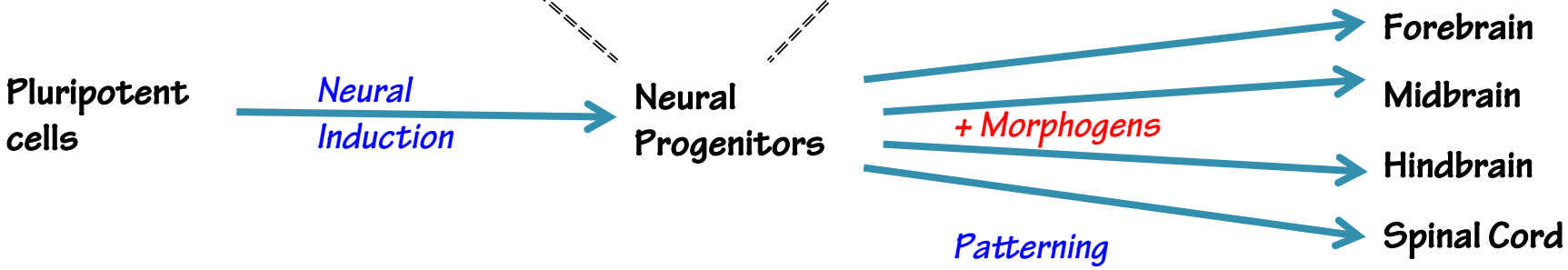
Regional specification of neurons

Depending on the presence of morphogens (e.g., fibroblast growth factors [FGFs], Wnts, and retinoic acid [RA]), the generated neuroepithelial cells are fated to cells of various regional identities including telencephalic, mid-/hindbrain, and spinal cord.

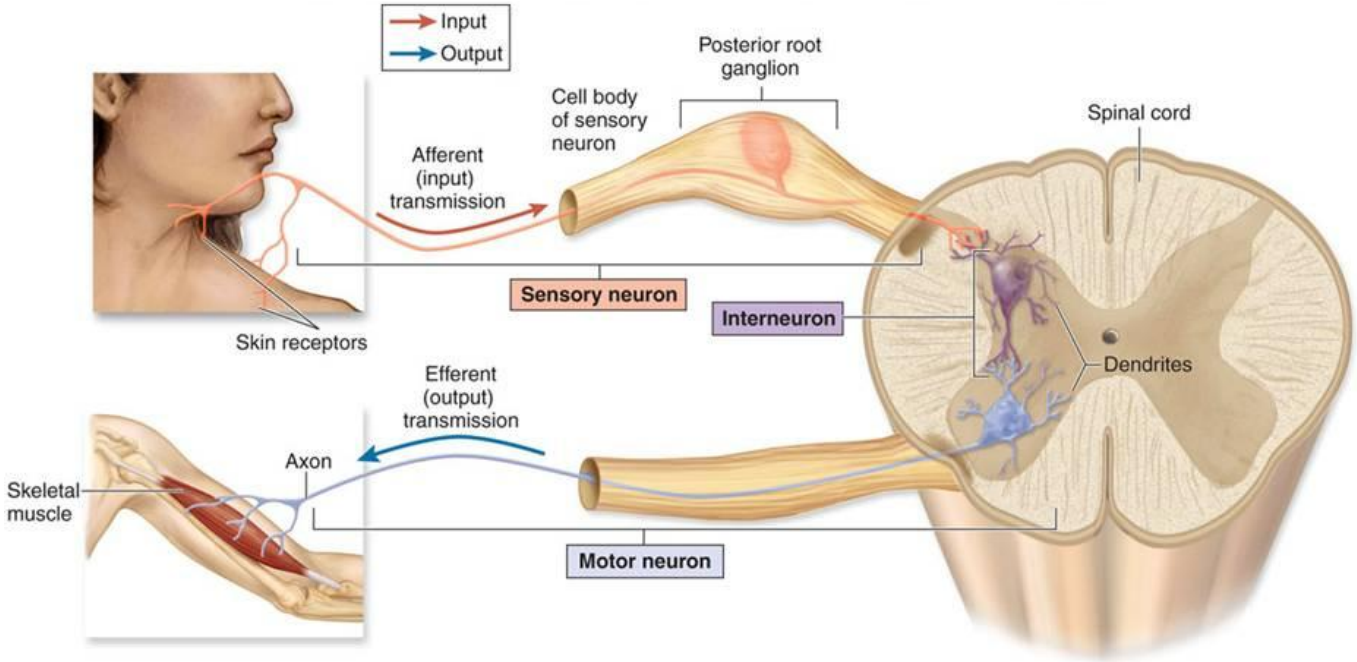
Yan et al., Stem Cells 2005



Neural tube-like rosettes



Differentiation of ESC to obtain motor neurons

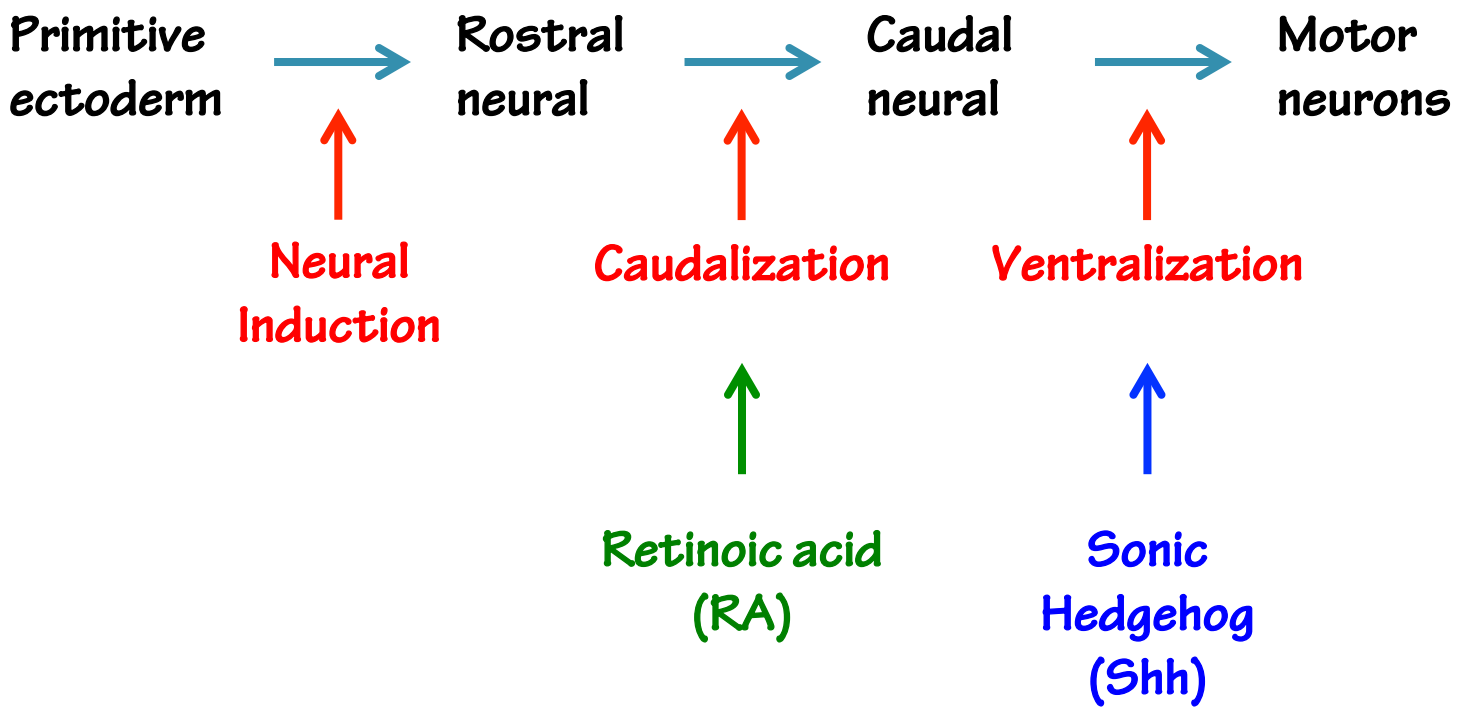


The sensory neurons that process and relay sensory input are found, predominantly, in the dorsal half of the spinal cord.
The motor neurons participate in motor output and are located ventrally.

This positioning reflects, to a large extent, the developmental origin of each individual neuronal subtype.

Differentiation of ESC to obtain motor neurons

Signaling factors involved in normal development of motor neurons in vivo were used to obtain this specific cell type from ESC in vitro.

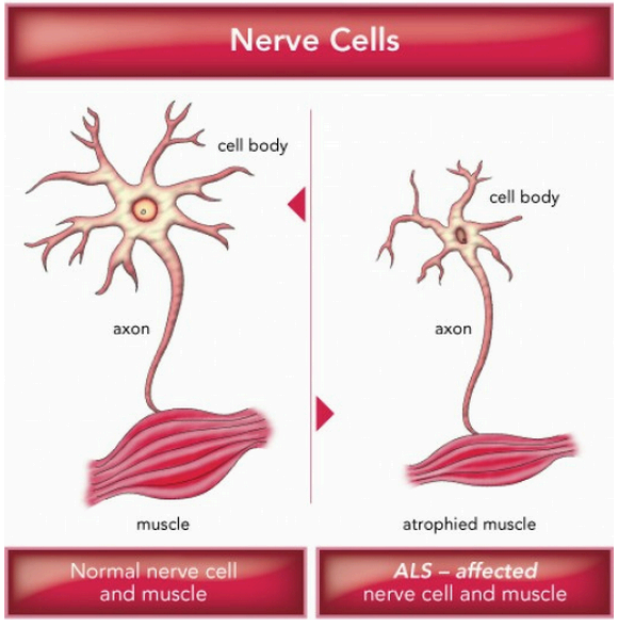


Amyotrophic Lateral Sclerosis (ALS)

• Neurodegenerative disorder caused by loss of motor neurons in the brain and spinal cord, leading to progressive paralysis

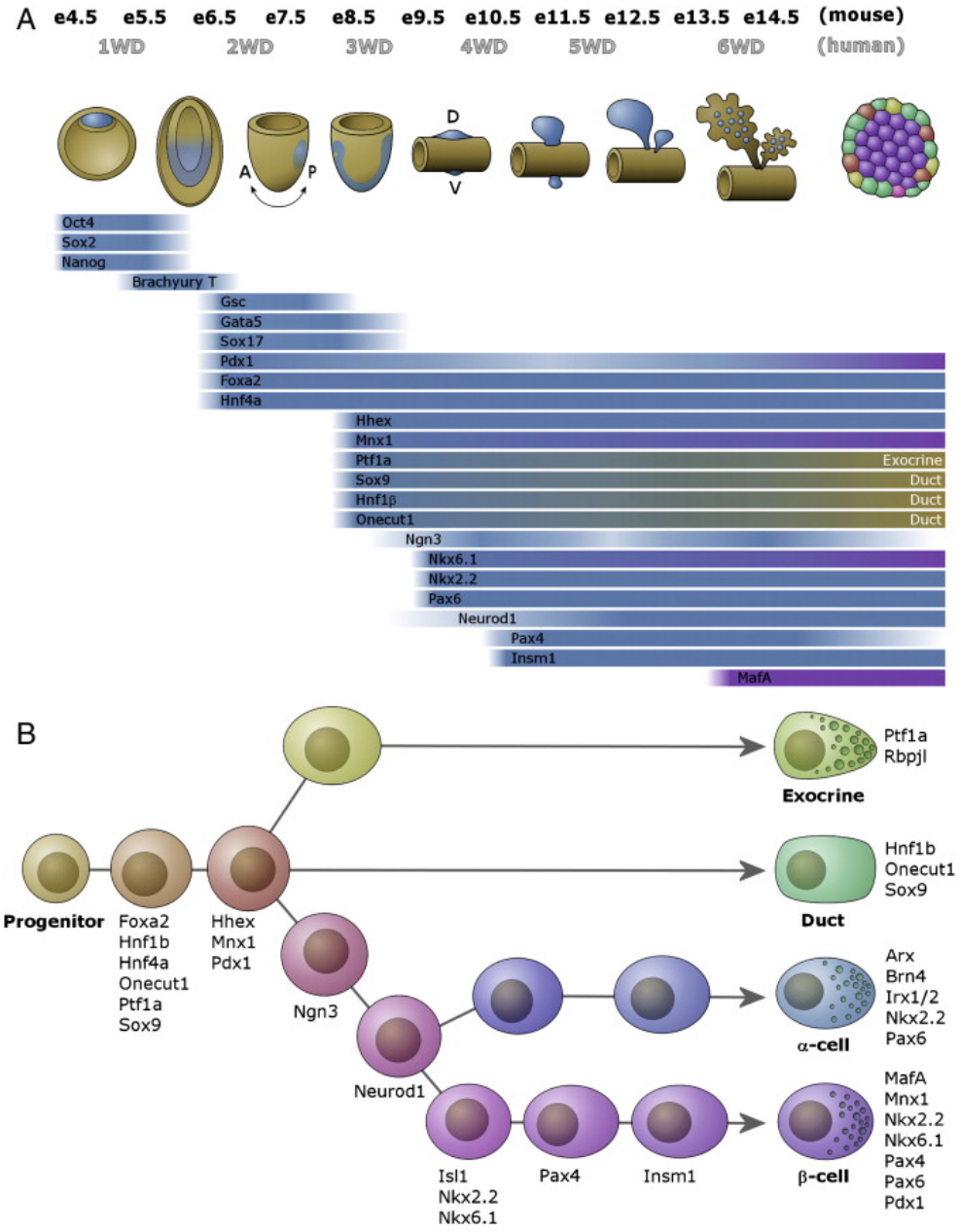
• Frequency: about 6/100.000; typical onset: 40-60 years

• Most ALS patients die from respiratory failure, 3-5 years from the onset of symptoms;
no treatment or cure available



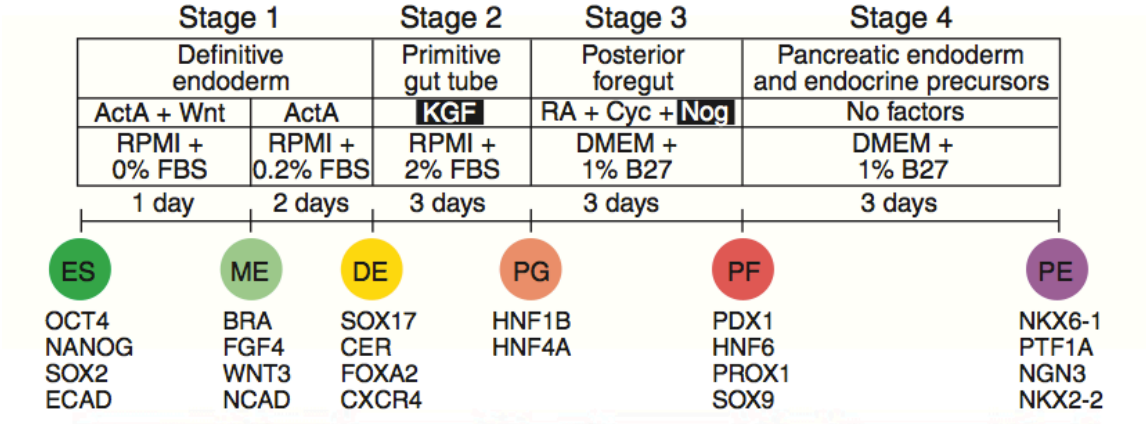
• About 10% of ALS cases are Familial (FALS); the rest are Sporadic (SALS)

Diff. of ESC to obtain insulin-producing cells



(Van Hoof et al., Stem Cell Res. 2009)

Diff. of ESC to obtain insulin-producing cells



(Kroon et al., Nat. Biotech. 2008)

ES: Embryonic Stem Cell

ME: Mesendoderm

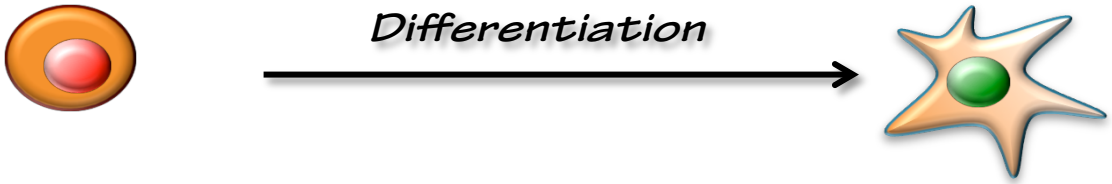
DE: Definitive Endoderm

PG: Primitive Gut

PF: Posterior Foregut

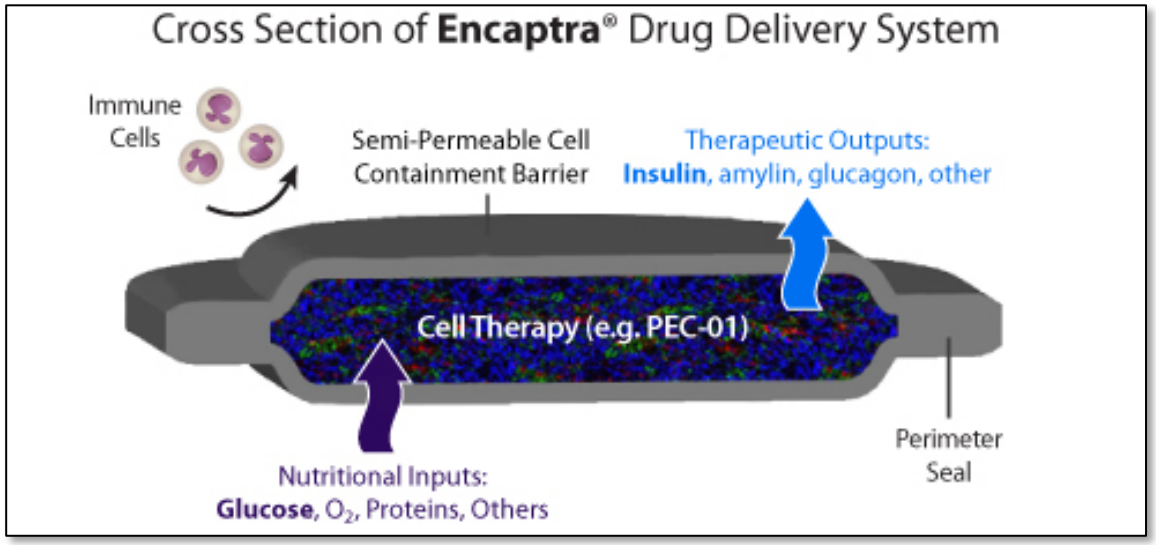
PE: Pancreatic Endoderm

Diff. of ESC to obtain insulin-producing cells



Embryonic Stem Cells

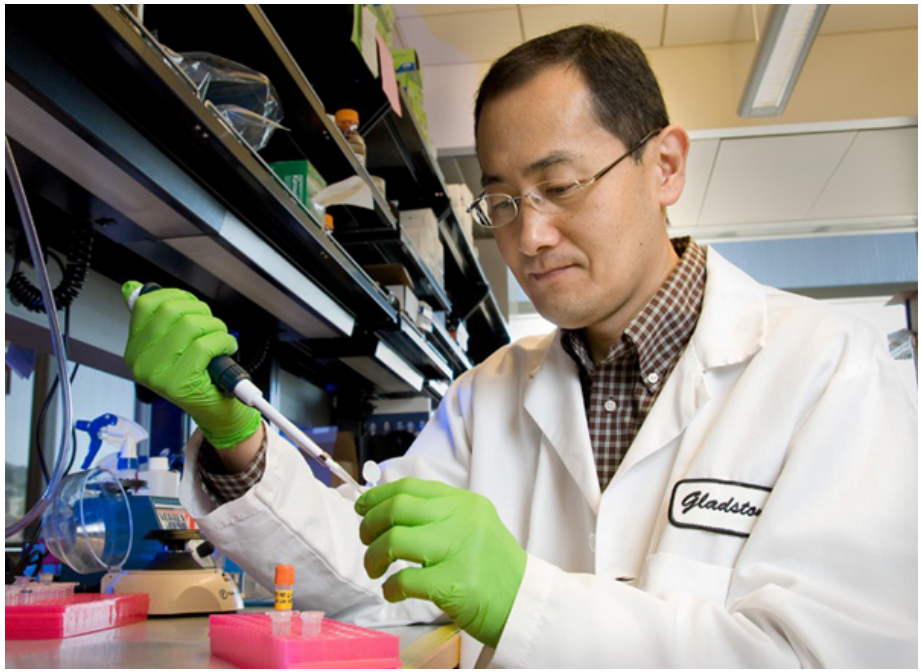
Pancreatic Progenitor Cells (PEC-01)



http://viacyte.com/products/vc-01-diabetes-therapy/

2012 Nobel Prize in Medicine

John Gurdon

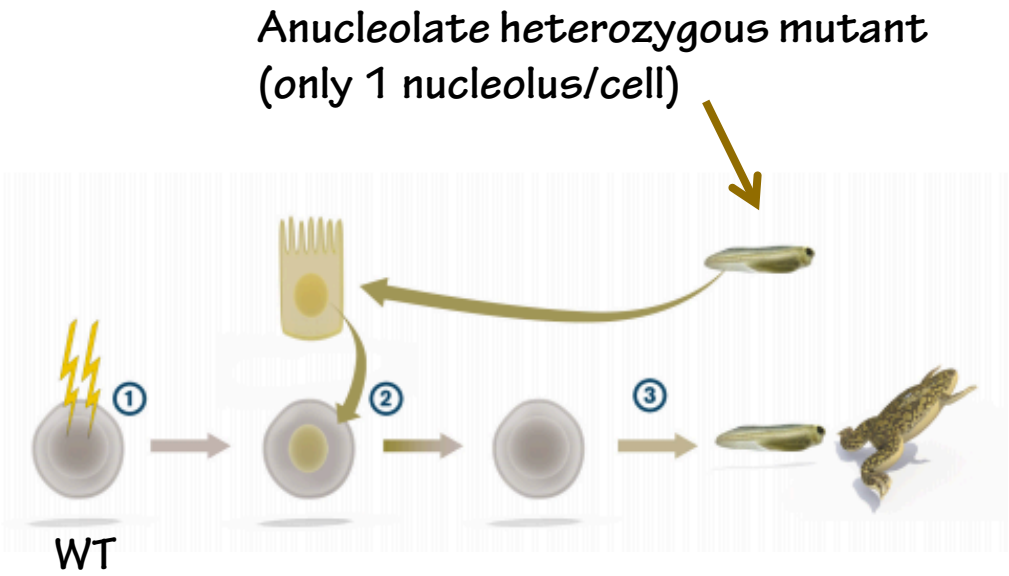


Shinya Yamanaka

Nuclear transfer

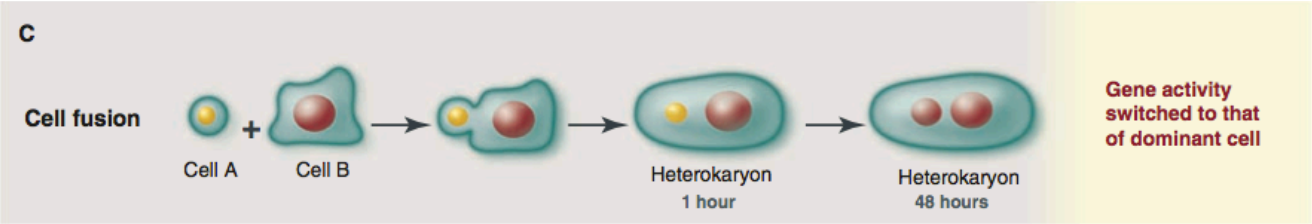
Gurdon 1958-1966 - Nuclear transplantation in *Xenopus laevis.*

John Gurdon



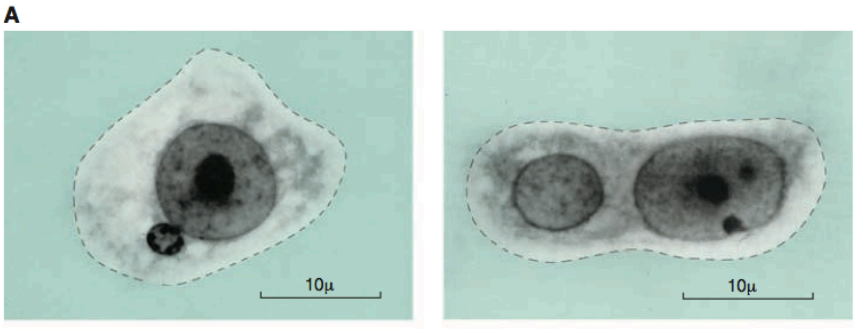
"Fertile intestine nuclei":
Fertile adult male and female frogs, genetically marked as of solely donor origin, were obtained from the serial transplantation of nuclei from intestinal epithelial cells of feeding larvae (success rate ≈1%).

Reprogramming by cell fusion



In the heterokaryon, the dominant cell, usually the larger and more actively dividing partner, imposes its own pattern of gene expression on the other partner.

Examples include the fusion of an erythrocyte with a growing cultured cell or of a human liver cell with a multinucleate muscle cell. If enucleated cytoplasms of one kind of somatic cell (cytoplasts) are fused to another cell, they also impose gene expression of their original cell type on the incoming nucleus.



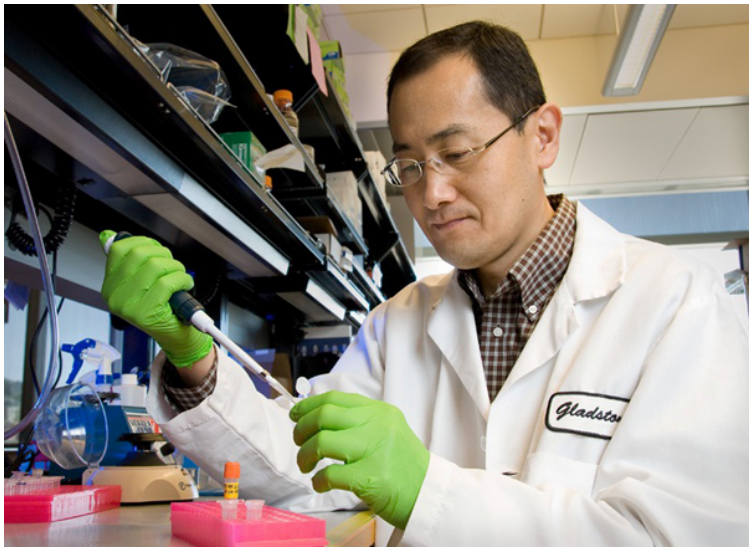
(Gurdon & Melton, Science 2008)

2006

Cell

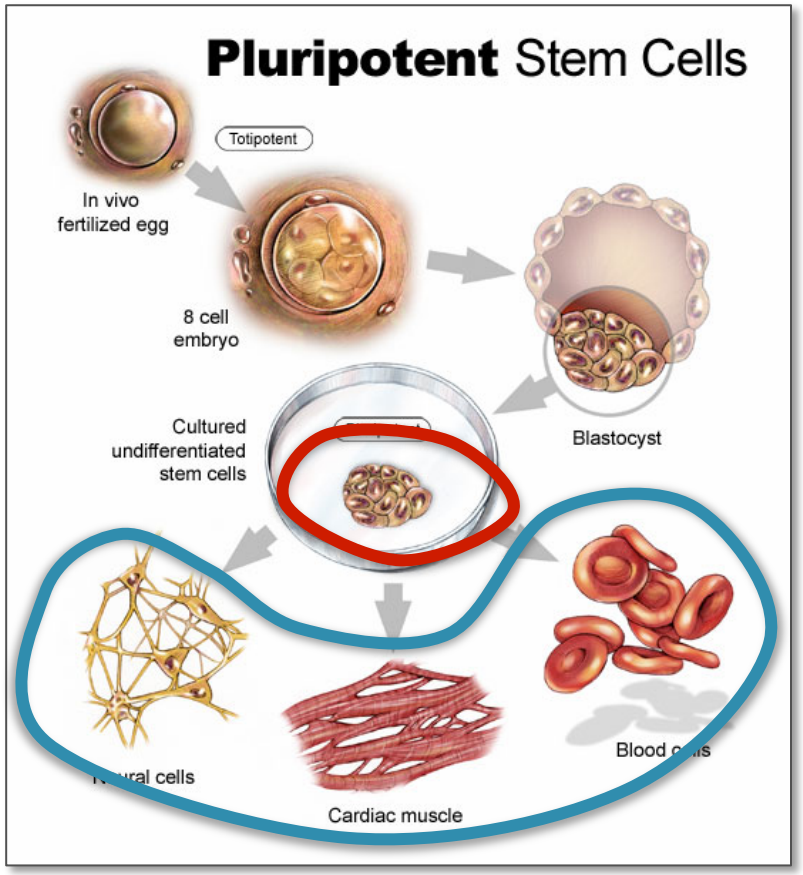
Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]



Shinya Yamanaka

Chasing the Reprogramming Factors: Yamanaka's strategy



Genes expressed in ESC

Vs.

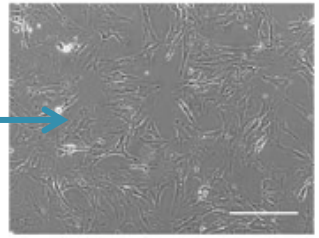
Genes expressed in somatic cells

⇩

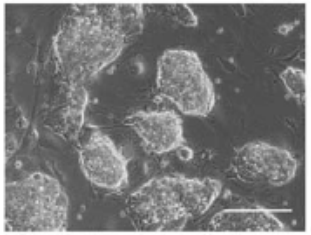
Candidate Reprogramming Factors

Screening the candidates

24 Candidate Reprogramming Factors

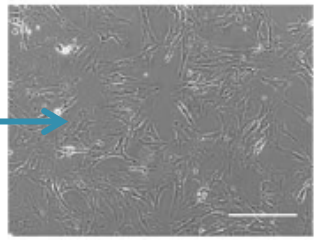


Fibroblasts

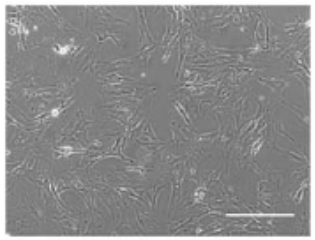


ES-like cells

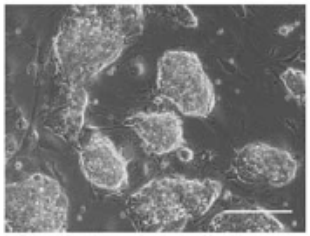
24 Candidates
- Factor 1
  or
- Factor 2
  or
- Factor 3
.....



Fibroblasts



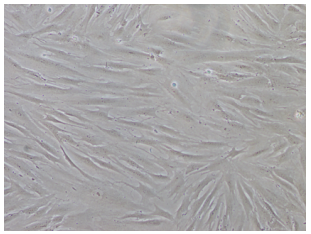
The Factor is essential



The Factor is <u>not</u> essential

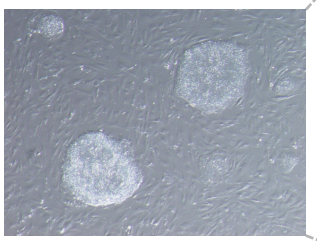
Yamanaka's Reprogramming Factors: The Fantastic Four

Properties of induced Pluripotent Stem Cells (iPSCs)

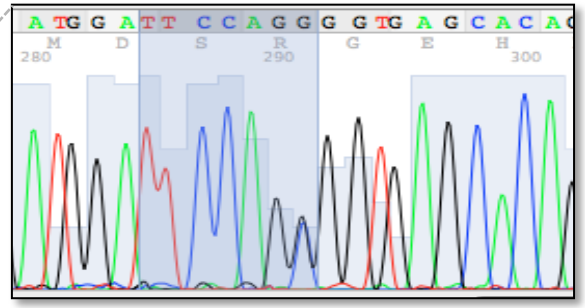


Fibroblasts

Reprogramming Factors (RFs)



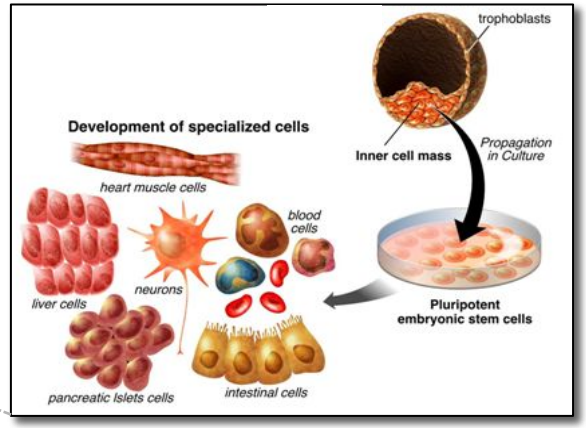
iPS Cells



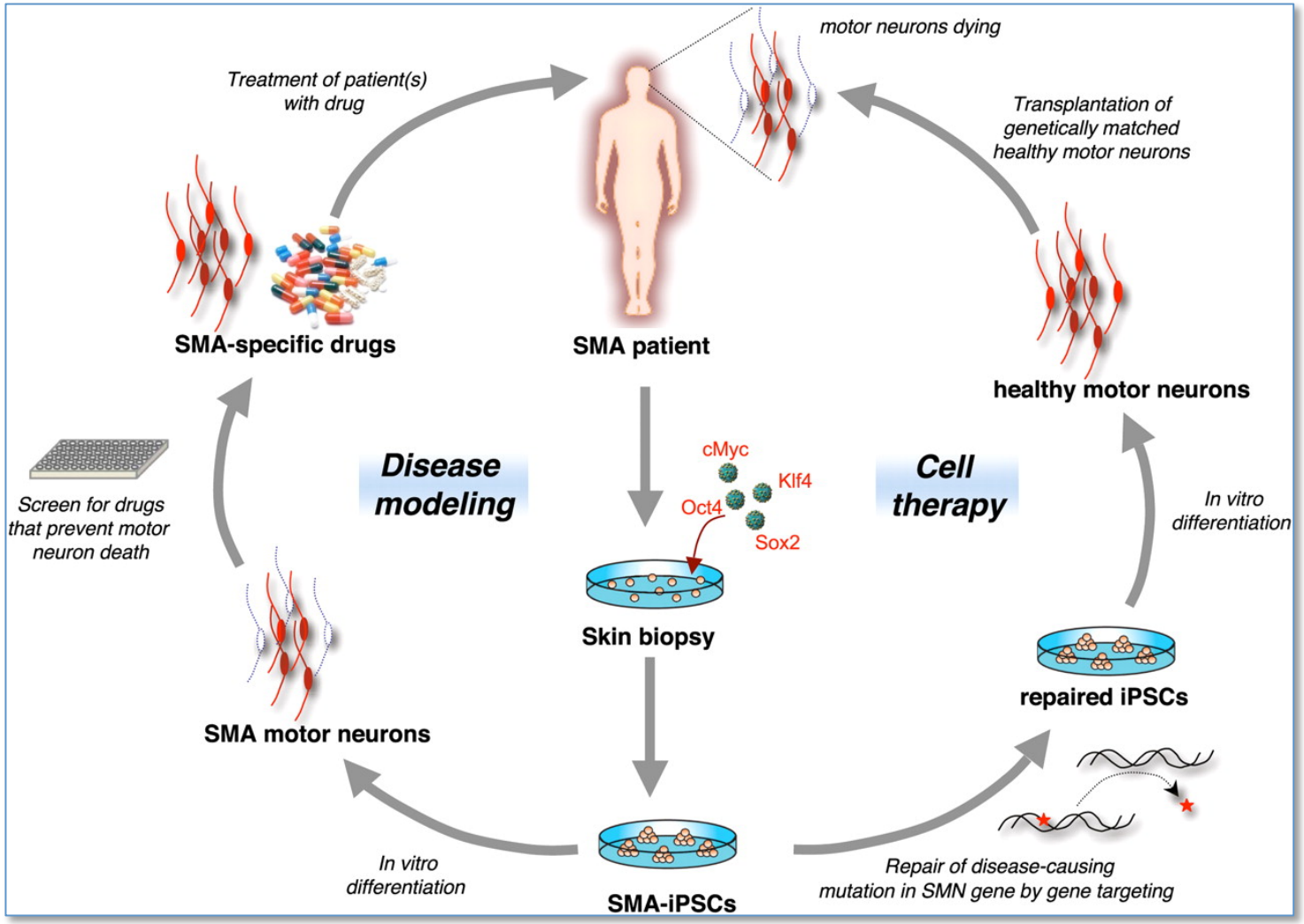
Same Genotype of the Somatic Cells of origin

&

Same Potential of Embryonic Stem Cells

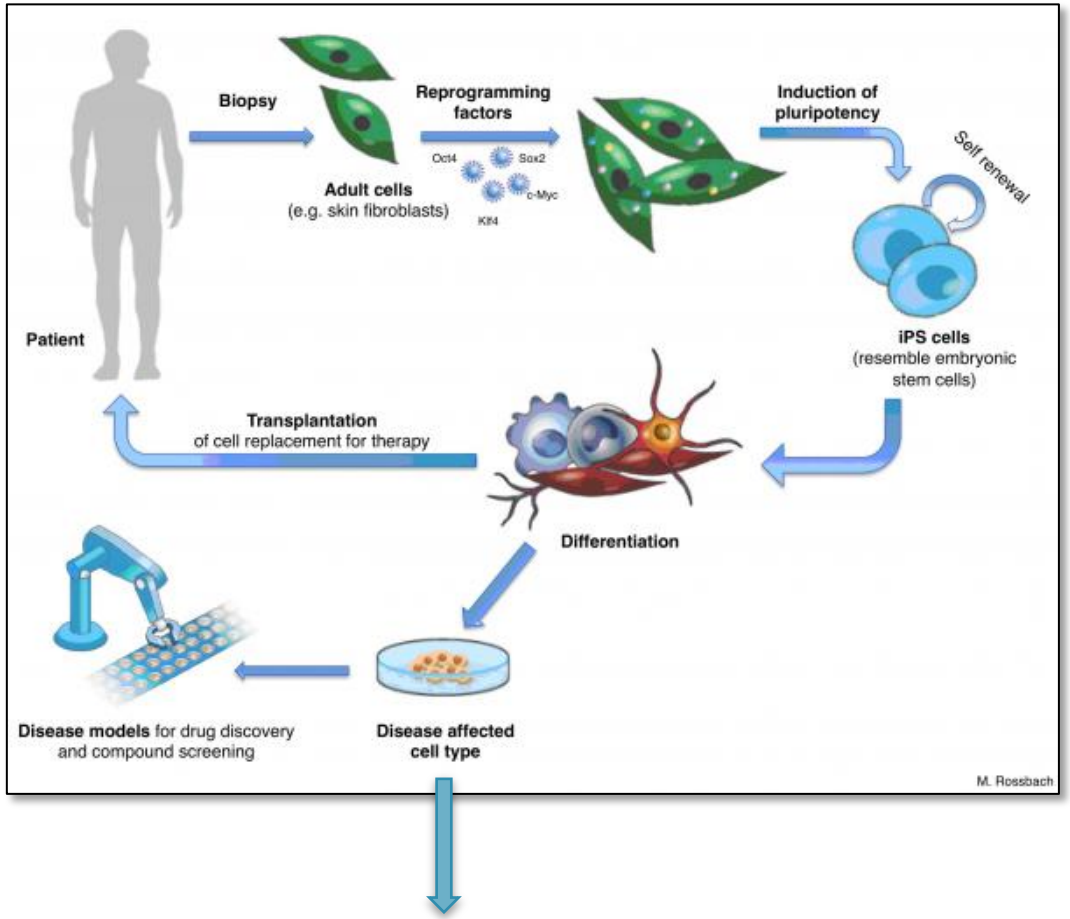


Potential applications of iPSCs



Stadtfeld and Hochedlinger, 2010

iPS cells in research



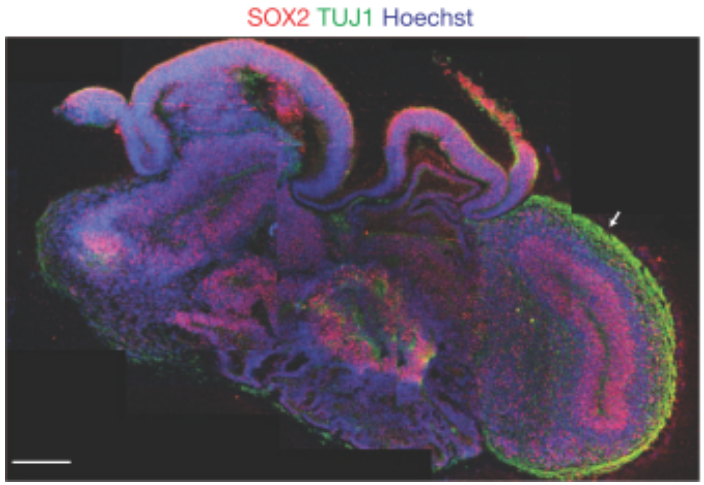
Creation of in vitro model systems :

Compare disease affected cells with their normal counterparts to understand the molecular basis of the disease.

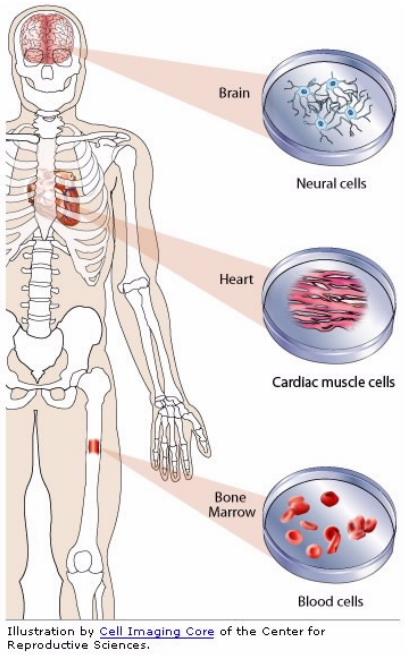
Organoid

Three-dimensional cell mass resembling an organ in some aspects

Model for studying embryogenesis and disease



Regenerative Medicine



Cerebral organoids model human brain development and microcephaly

Madeline A. Lancaster[1], Magdalena Renner[1], Carol-Anne Martin[2], Daniel Wenzel[1], Louise S. Bicknell[2], Matthew E. Hurles[3], Tessa Homfray[4], Josef M. Penninger[1], Andrew P. Jackson[2] & Juergen A. Knoblich[1]

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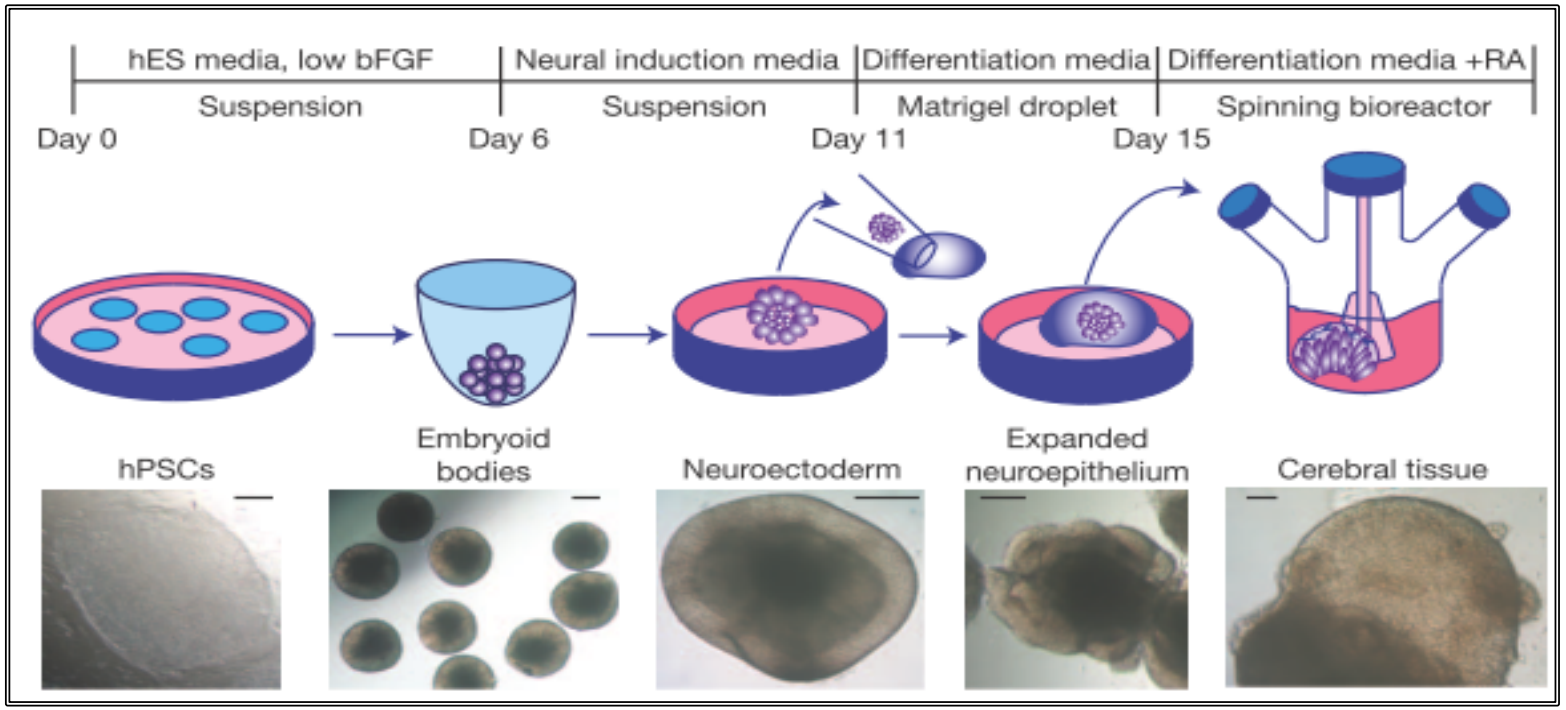

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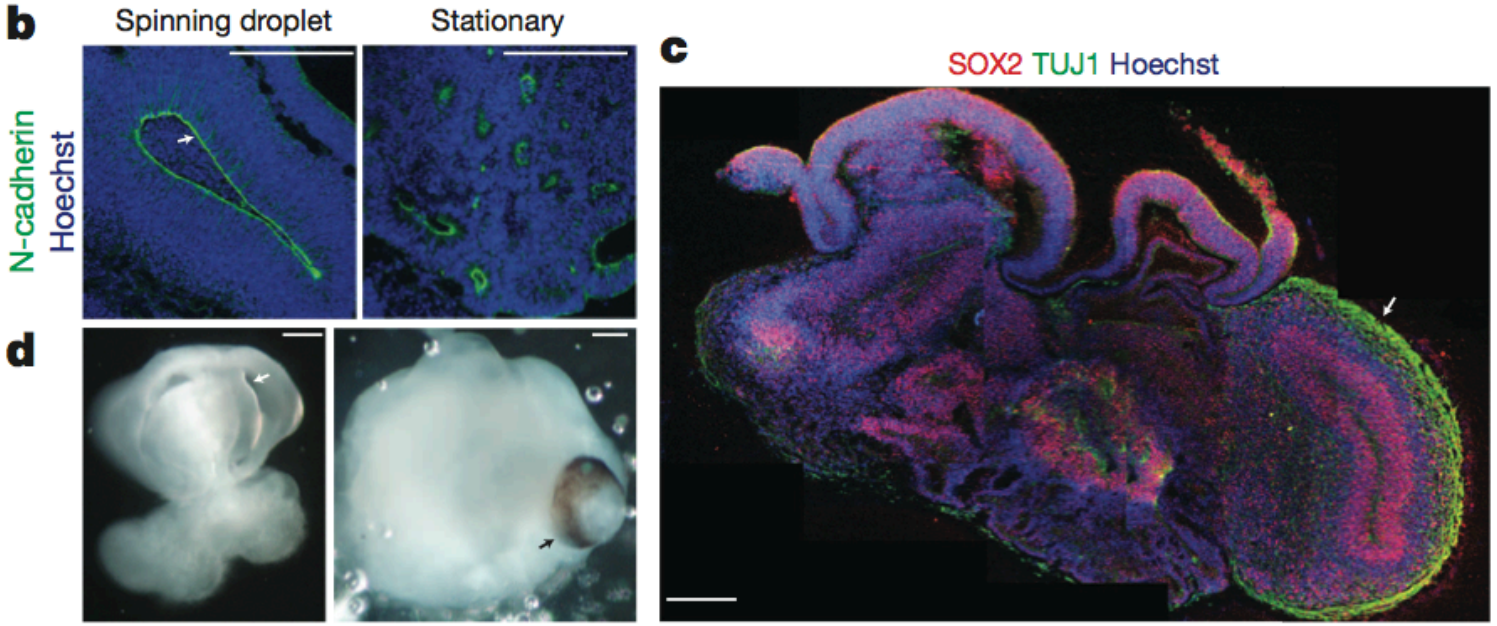

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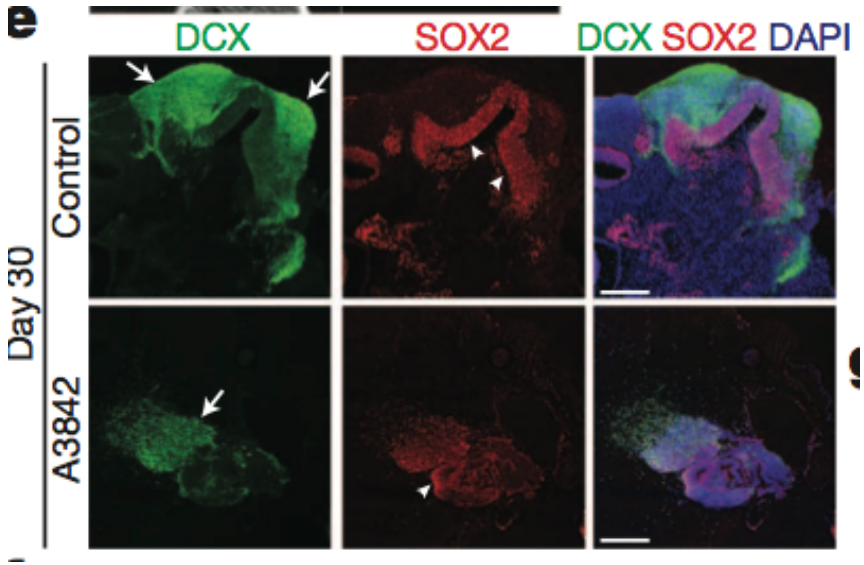
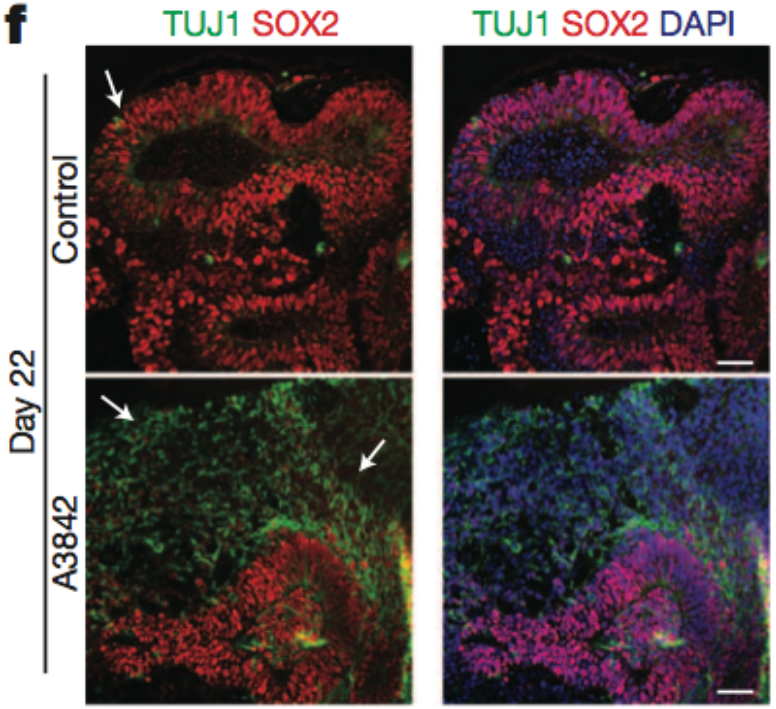
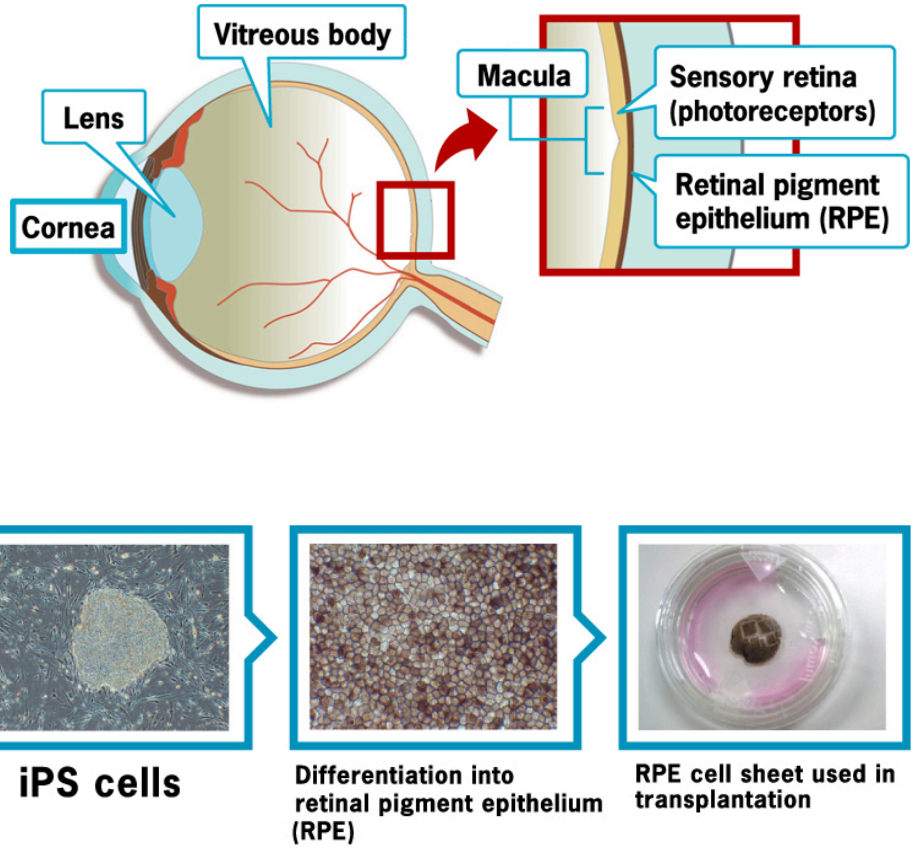
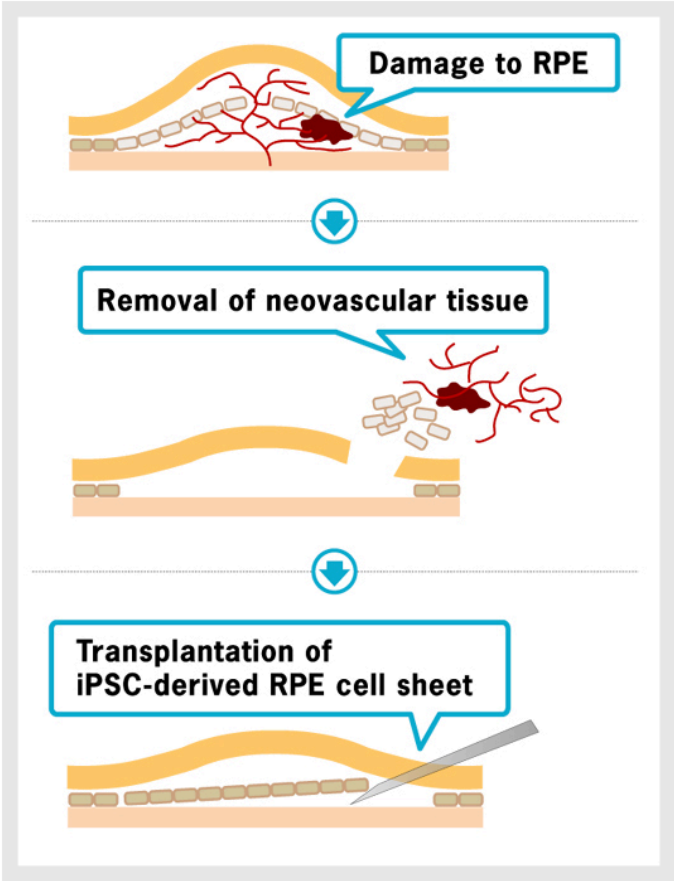
A3842 patient: Microcephaly due to mutation in CDK5RAP2





iPSC pilot safety study for Age-related Macular Degeneration (AMD)





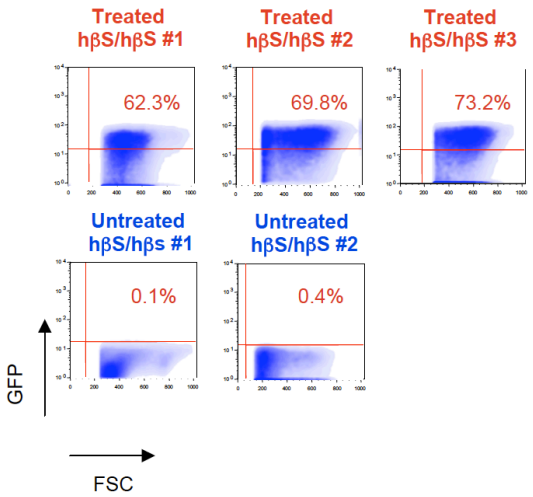
http://www.riken-ibri.jp/AMD/english/research/index.html

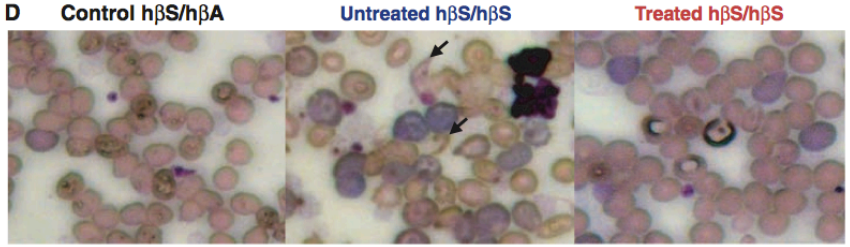
Corrected iPSCs cure anemia in mice

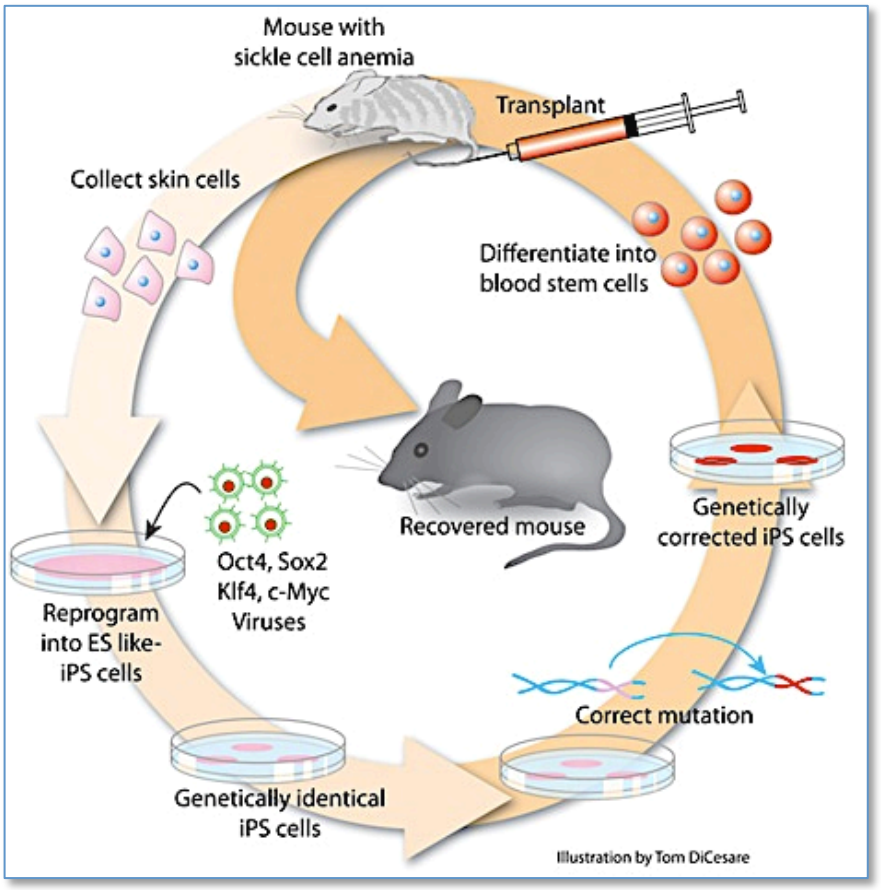
Treatment of Sickle Cell Anemia Mouse Model with iPS Cells Generated from Autologous Skin

Jacob Hanna,[1] Marius Wernig,[1] Styliani Markoulaki,[1] Chiao-Wang Sun,[2] Alexander Meissner,[1] John P. Cassady,[1,3] Caroline Beard,[1] Tobias Brambrink,[1] Li-Chen Wu,[2] Tim M. Townes,[2*] Rudolf Jaenisch[1,3*]

21 DECEMBER 2007 VOL 318 SCIENCE

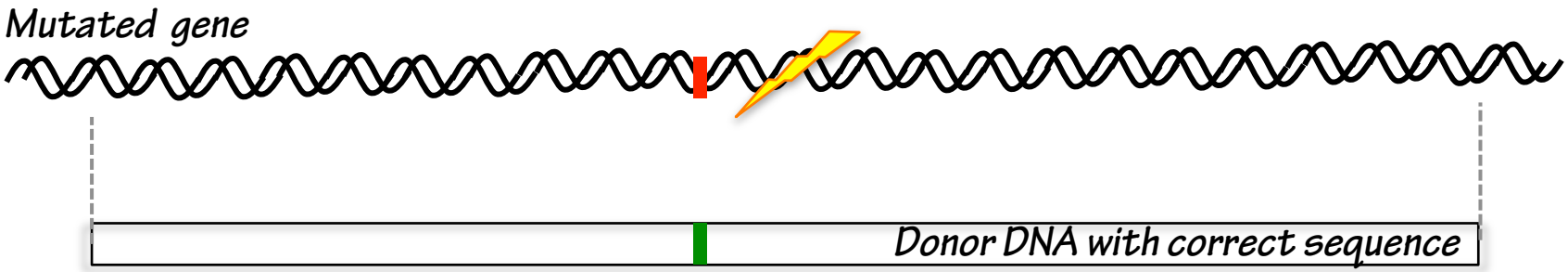


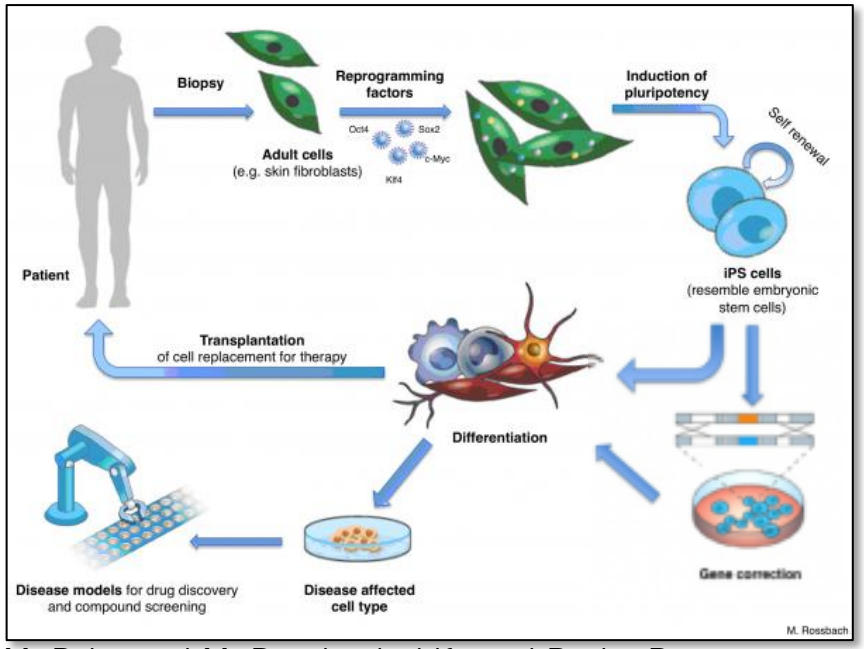




Hanna et al., Science 2007

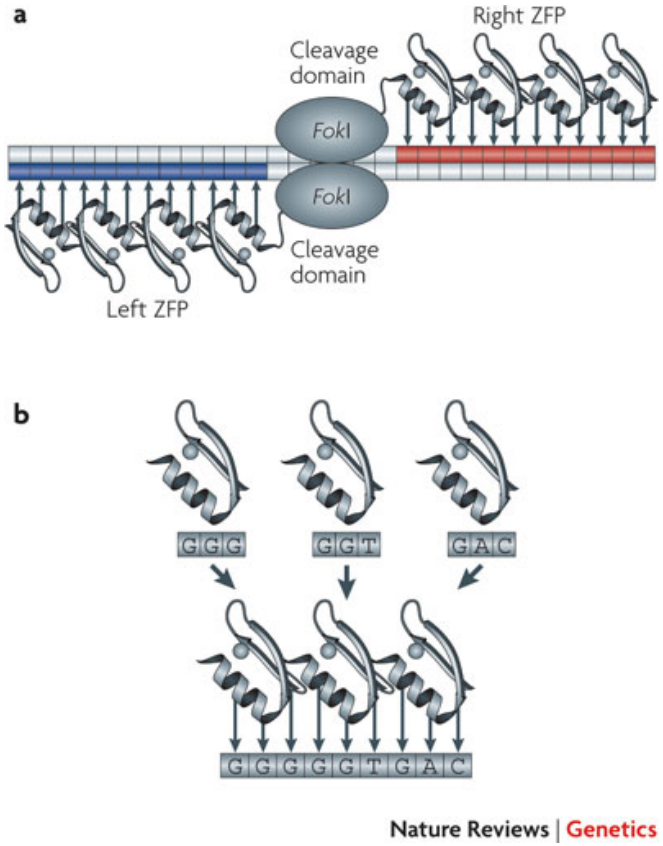
Correction of mutations with genome editing



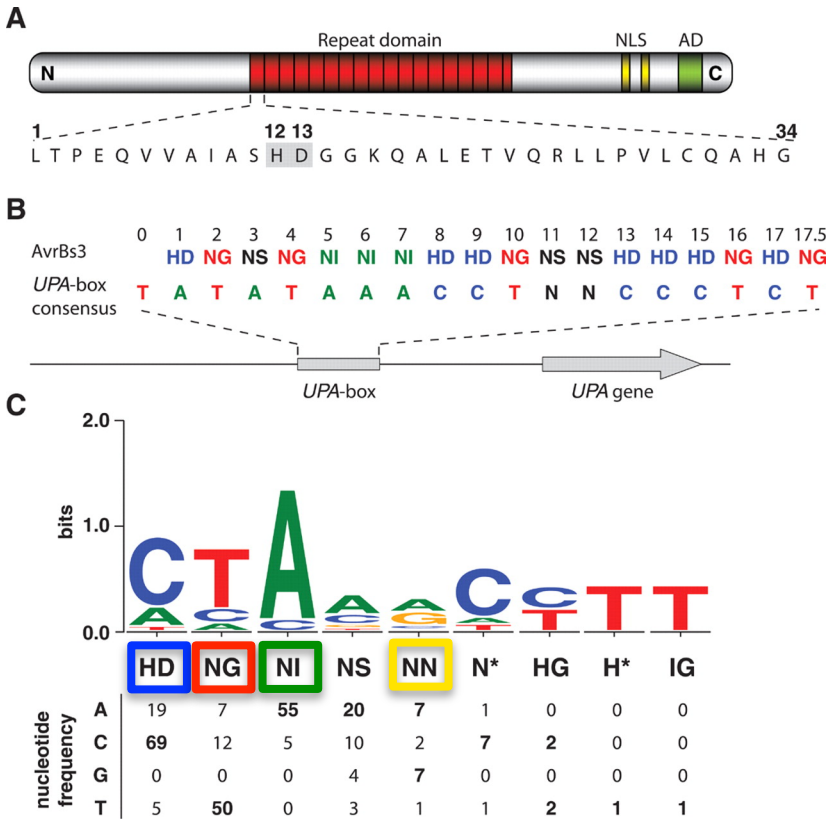


M. Peitz and M. Rossbach, Life and Brain, Bonn

Zinc-finger and TALE nucleases can be directed to specific DNA sequences

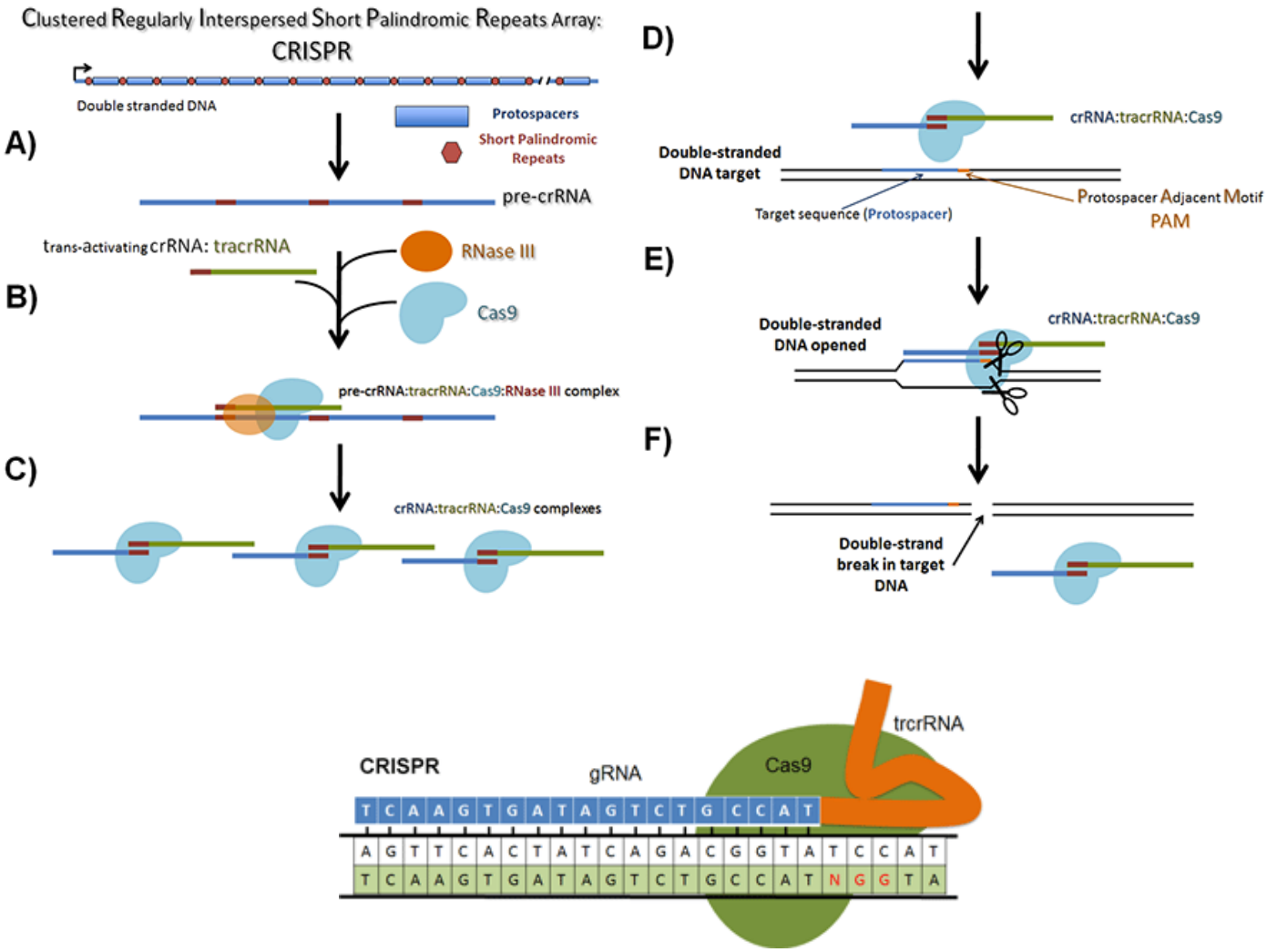


Each zinc-finger recognizes a triplet



Each TALE module recognizes a base

CRISPR



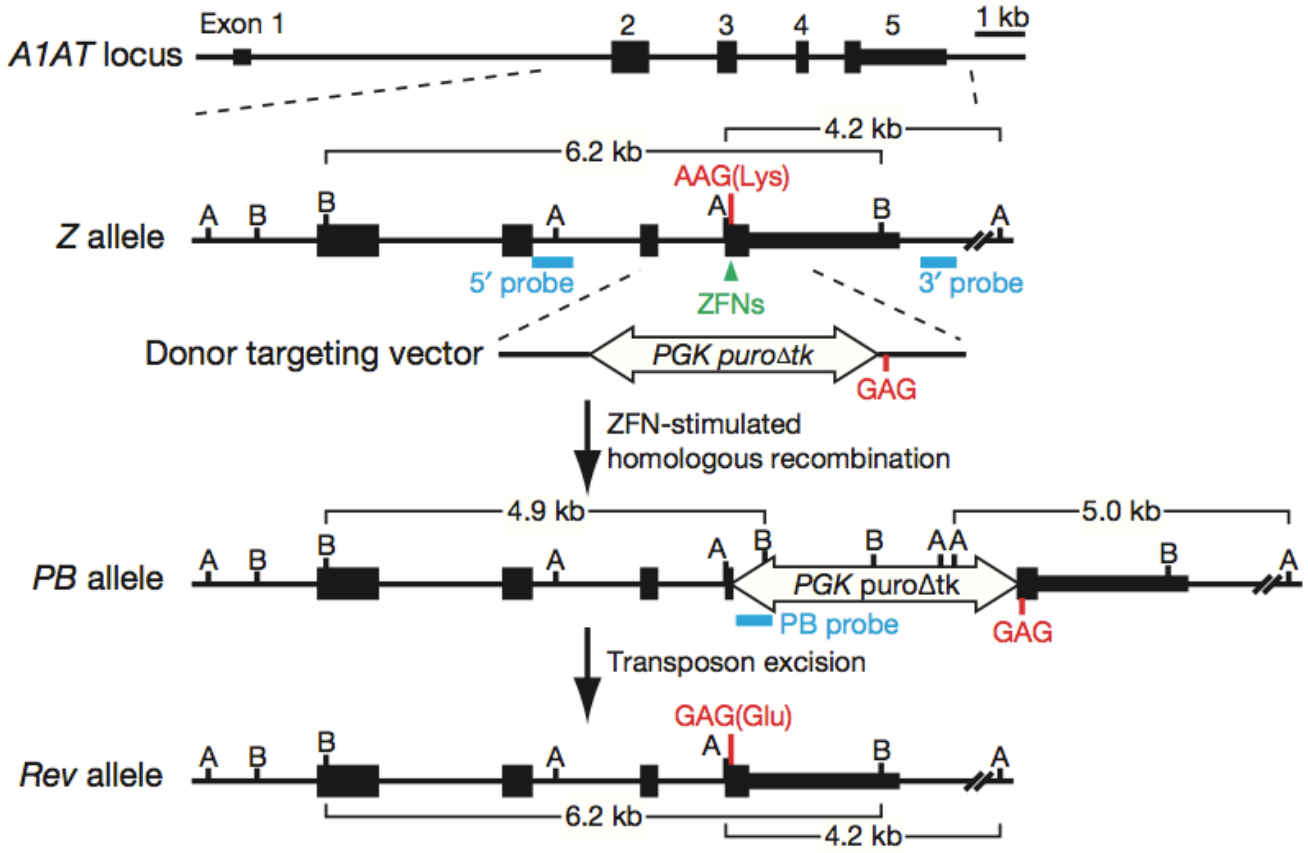
http://www.addgene.org/CRISPR/guide/

CRISPR systems are less specific than TALENs but easier to produce.

Correcting mutations in human iPSCs

Targeted gene correction of α_1-antitrypsin deficiency in induced pluripotent stem cells

Kosuke Yusa[1]*, S. Tamir Rashid[2,3]*, Helene Strick-Marchand[4,5], Ignacio Varela[6], Pei-Qi Liu[7], David E. Paschon[7], Elena Miranda[3,8], Adriana Ordóñez[3], Nicholas R. F. Hannan[2], Foad J. Rouhani[1,2], Sylvie Darche[4,5], Graeme Alexander[9], Stefan J. Marciniak[3], Noemi Fusaki[10,11], Mamoru Hasegawa[10], Michael C. Holmes[7], James P. Di Santo[4,5], David A. Lomas[3]*, Allan Bradley[1]* & Ludovic Vallier[2]*



Nature 2011

Challenges for the Future

- *Cell of origin: skin biopsies are not always available*
  -> reprogramming from blood or hair

- *iPS cells generated with integrating viruses cannot be used in therapy*
  -> non viral methods (Proteins or RNA transfection)

- *Efficient genetic manipulation to correct the mutation*
  -> new tools are now available (ZFN, TALEN, CRISPR)

- *Differentiation to functional disease tissues*
  -> improving differentiation protocols is a major challenge