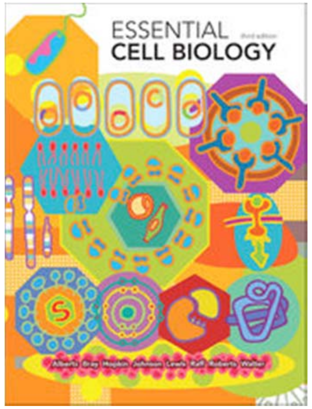
ANAT2341: lecture overview

Stem Cells



Resources:
http://php.med.unsw.edu.au/cell biology/
Essential Cell Biology – 3rd edition Alberts

Dr Annemiek Beverdam – School of Medical Sciences, UNSW
Wallace Wurth Building Room 234 – A.Beverdam@unsw.edu.au

ANAT2341: lecture overview

<u>Stem Cell Biology</u>

Tissue development and regeneration
Stem cell biology
Stem cell niches
Stem cell regulation
Stem cells and cancer
Regenerative medicine
Stem cell sources
Future of regenerative medicine

Dr Annemiek Beverdam – School of Medical Sciences, UNSW
Wallace Wurth Building Room 234 – A.Beverdam@unsw.edu.au

Prenatal development

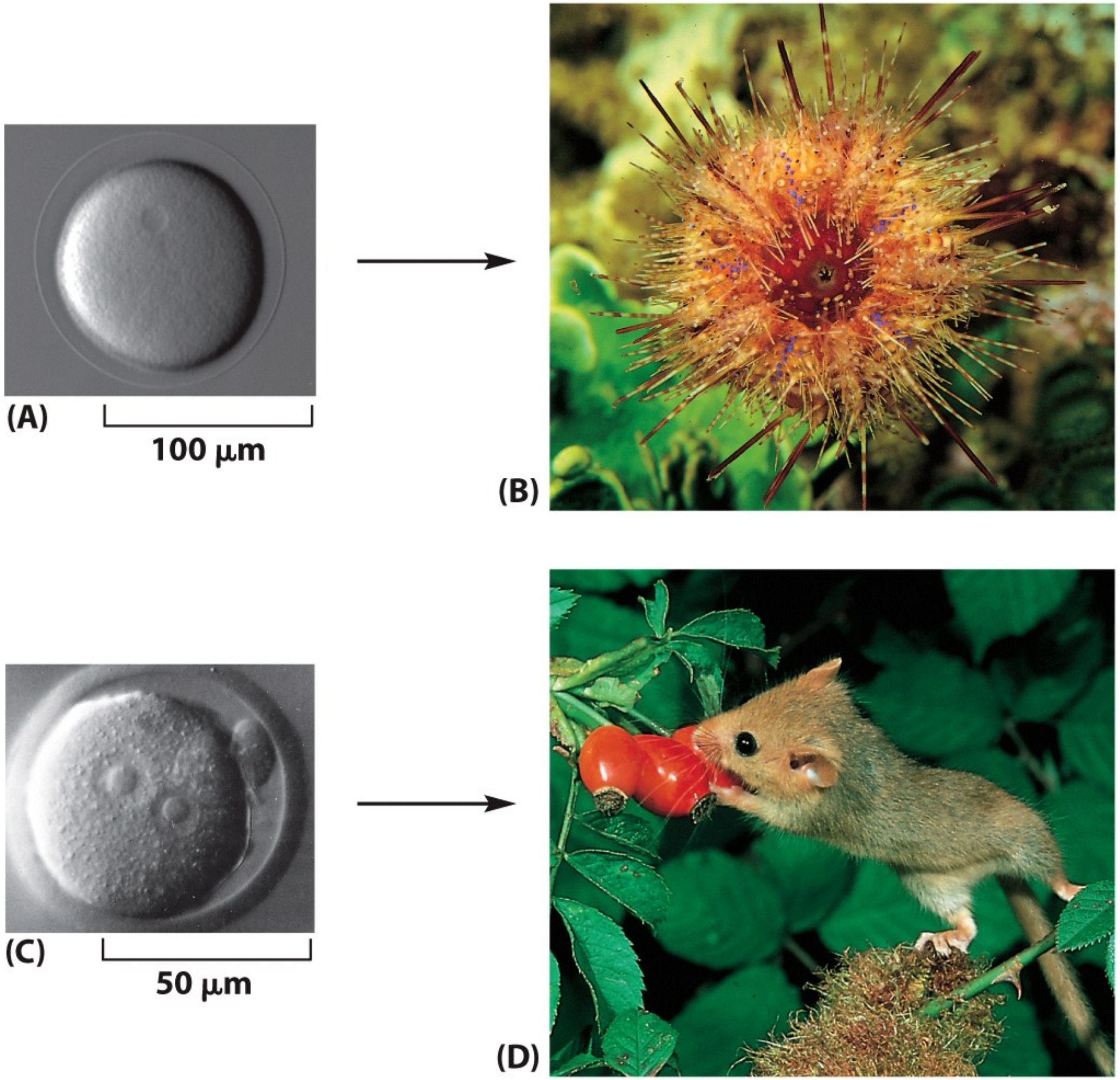
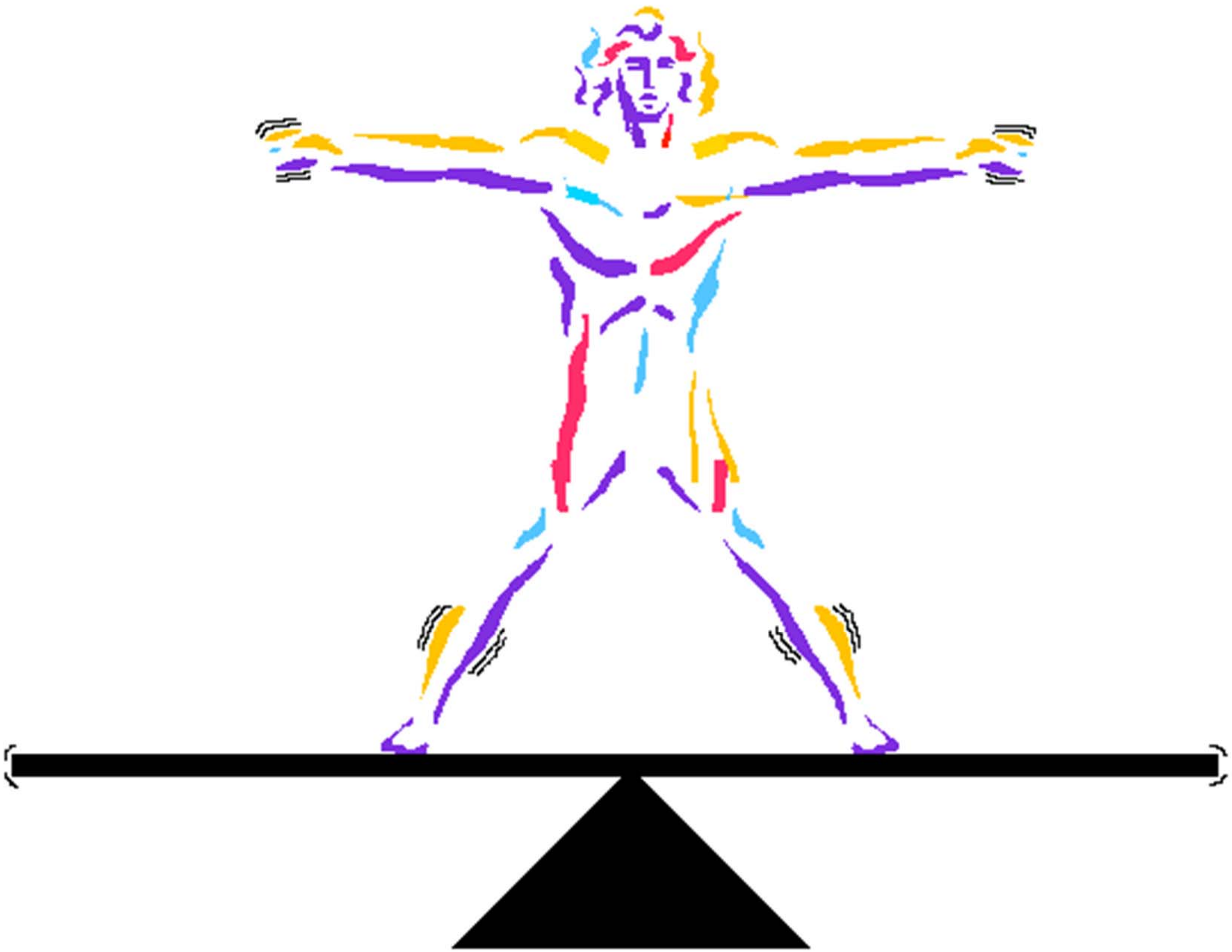


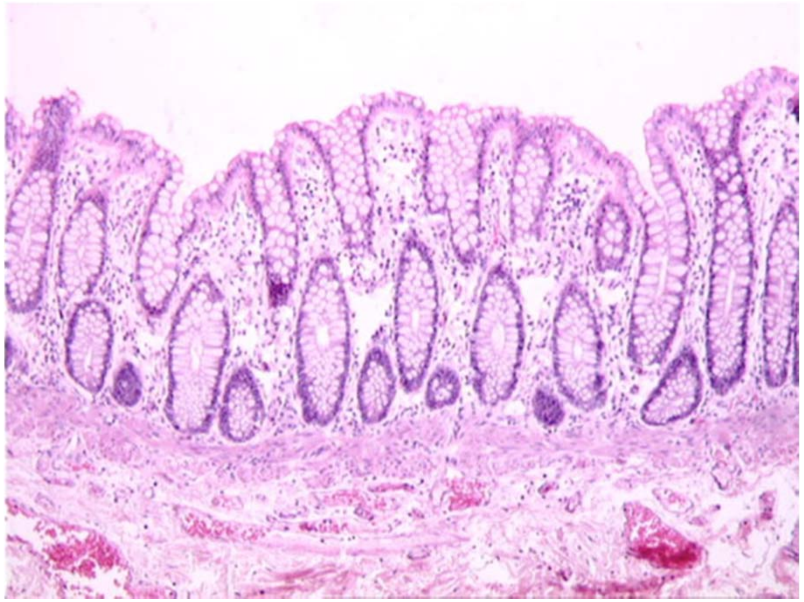
Figure 20-32 Essential Cell Biology 3/e (© Garland Science 2010)

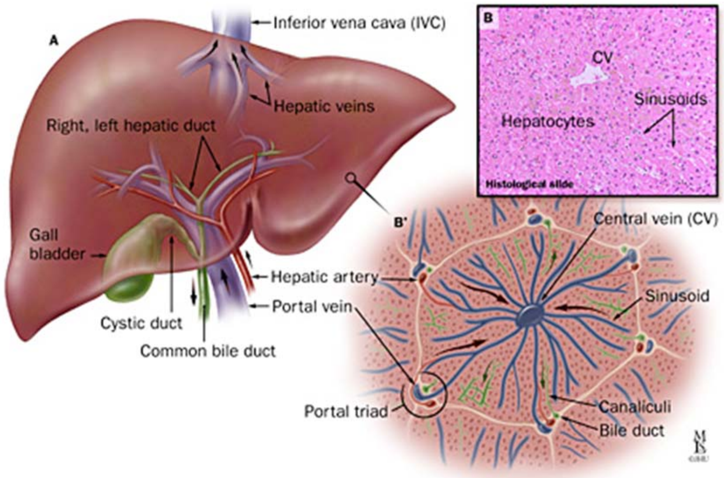
Tissue homeostasis

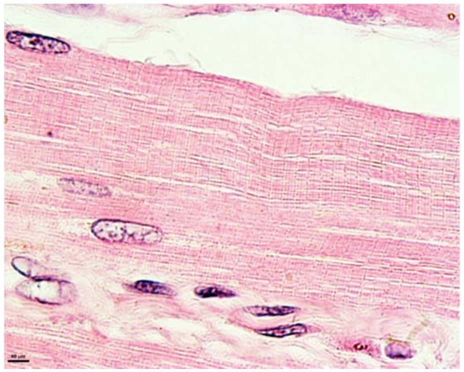
Maintenance of the organism’s internal environment in response to internal and external conditions



Tissue renewal in higher vertebrates







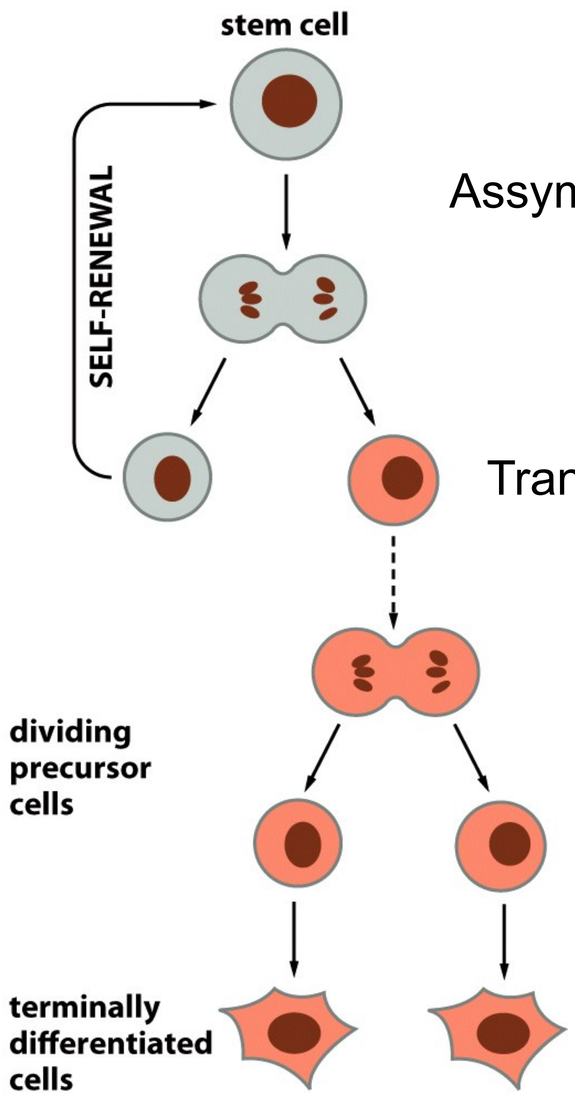
Stem cells divide to self renew and to produce terminally differentiated cell types

Mostly quiescent

High proliferation rates
No function

↓

Limited proliferation
Highly functional cell



Assymmetric cell division

Transit-amplifying cell

Figure 20-35 Essential Cell Biology 3/e (© Garland Science 2010)

Stem cells potential

Totipotency:

capacity to generate all cell types within the body + extraembryonic tissue

Pluripotency:

capacity to generate all cell types within the body

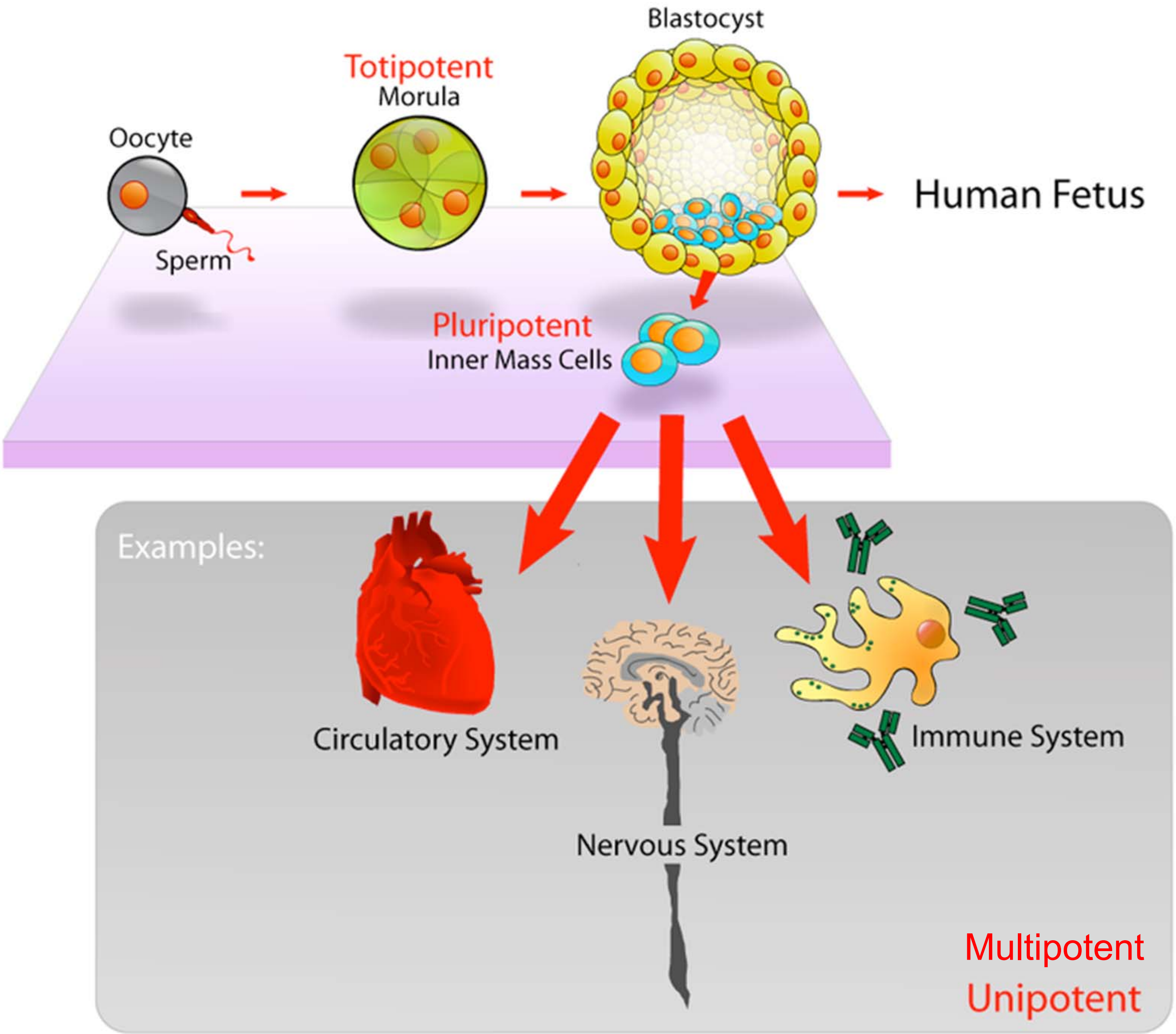
Multipotency:

capacity to give rise to more than 1 cell type

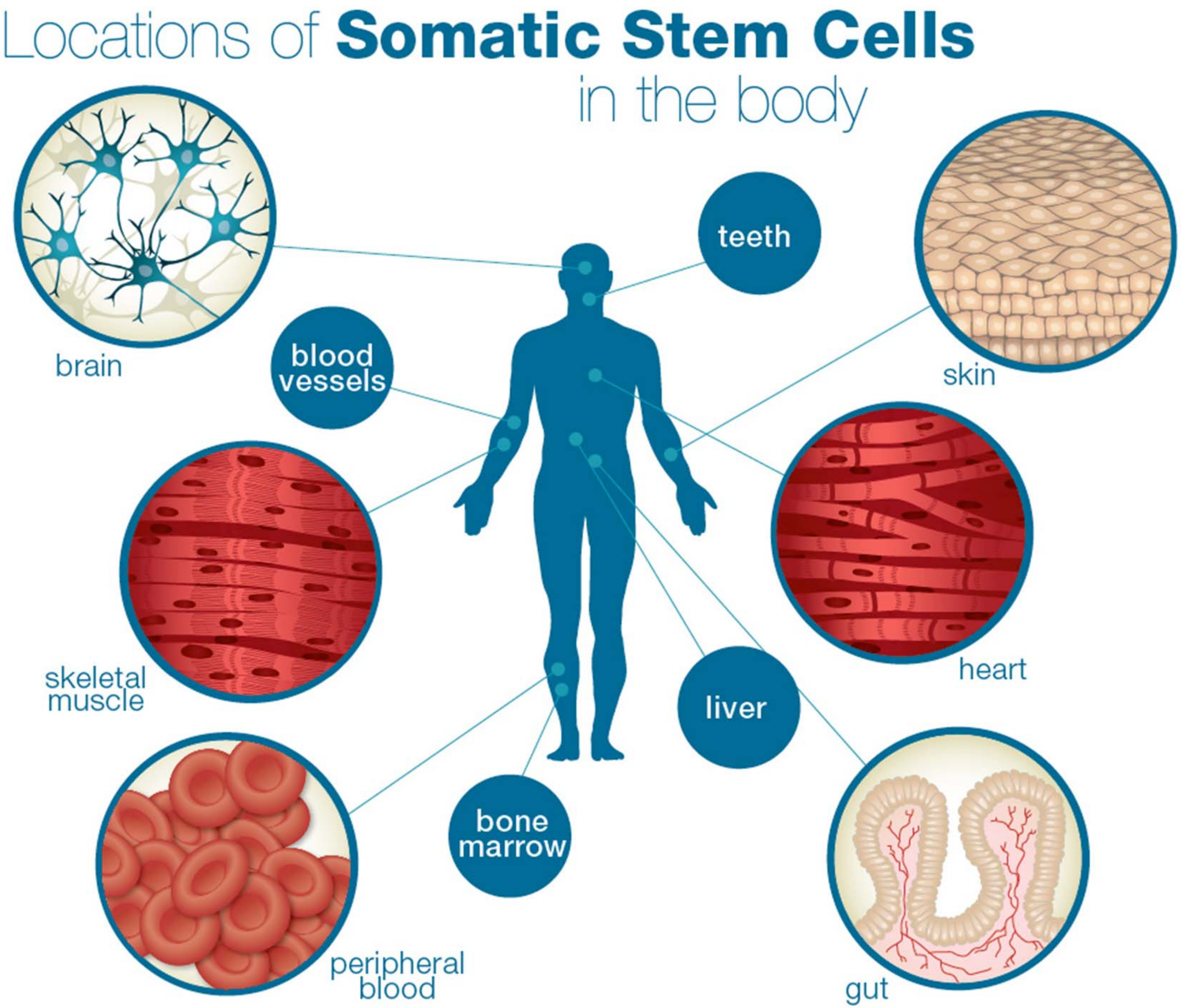
Unipotent stem cell:

tissue precursor cells, capacity to give rise to one cell type only

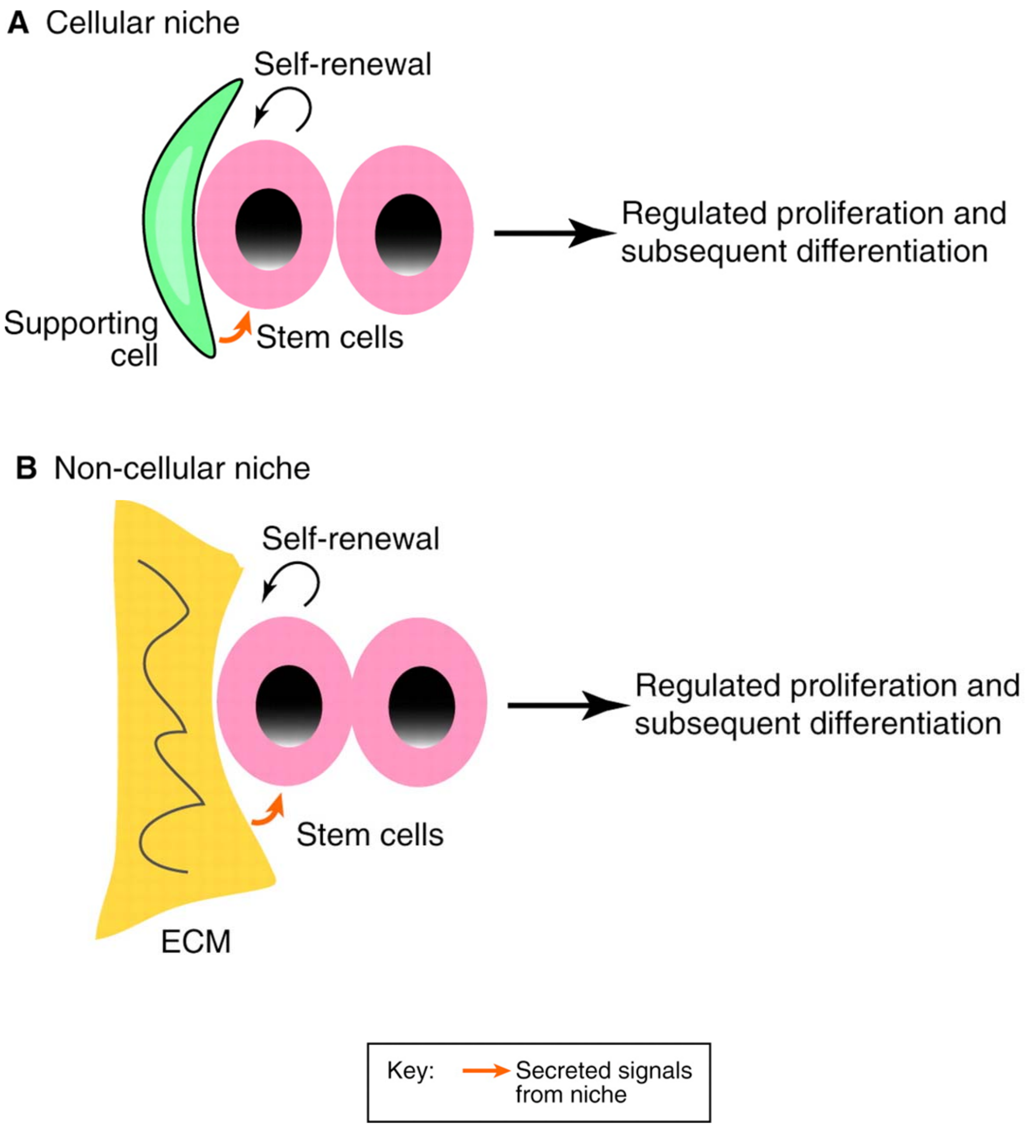
Stem cells potential



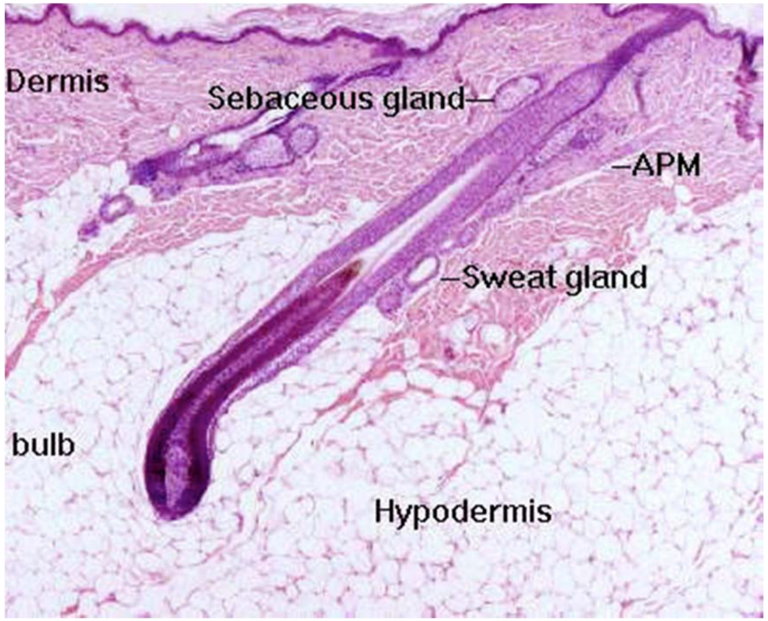
Adult stem cells

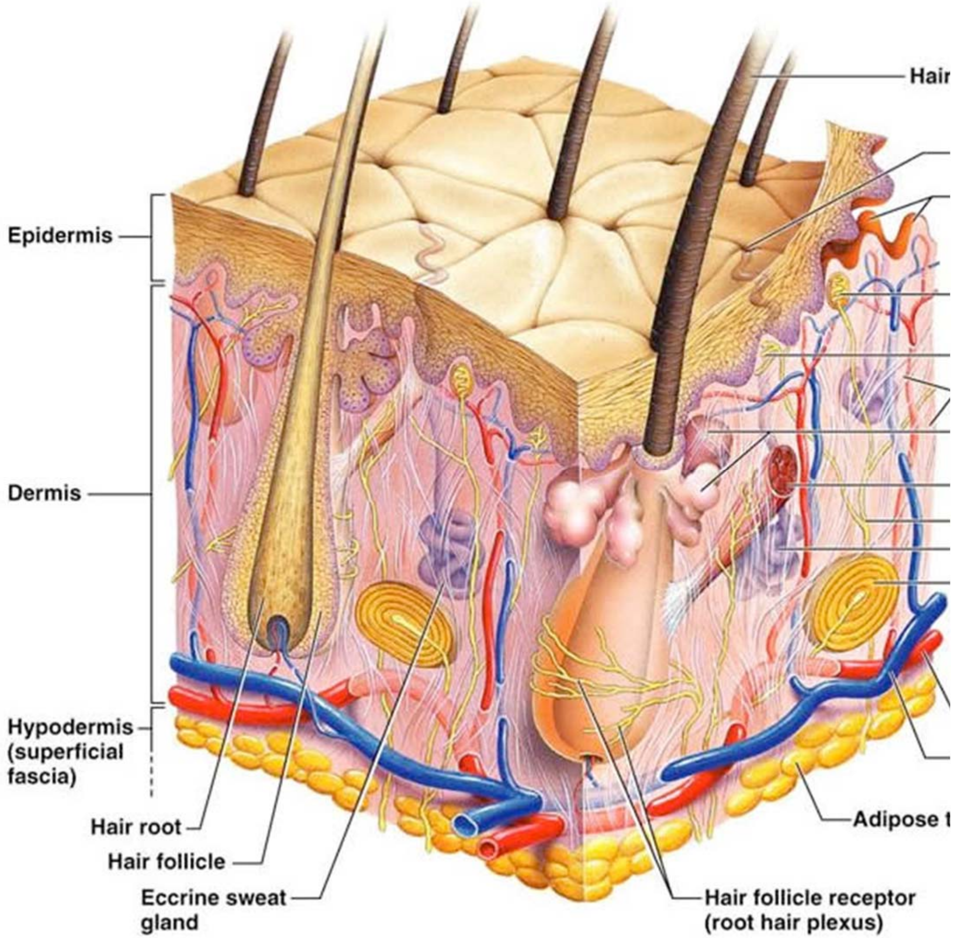


Stem cell niche:
Keeps stem cells in an undifferentiated state



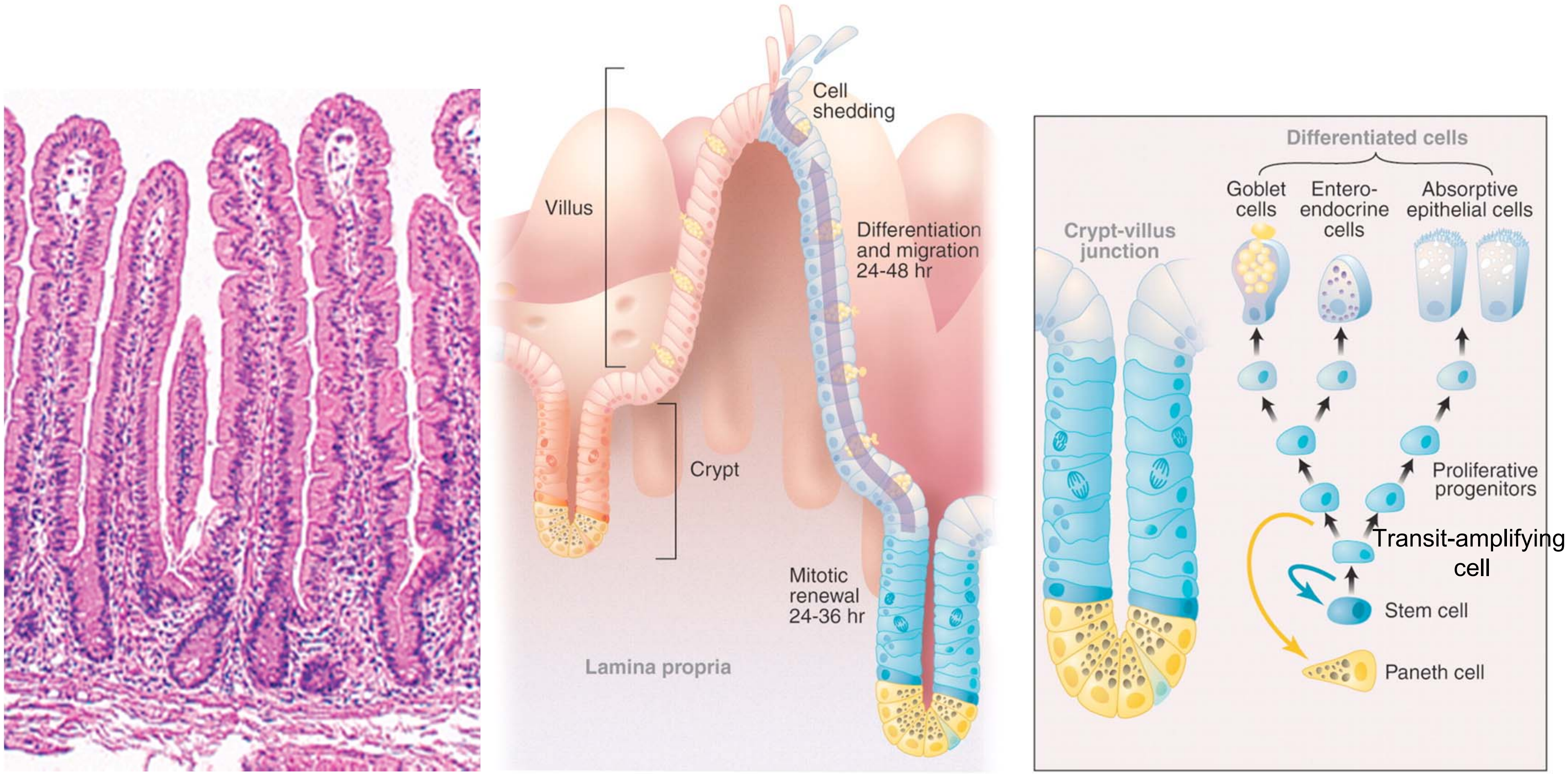
Epidermal Stem Cell Niches





The Intestinal Crypts Stem Cell Niche

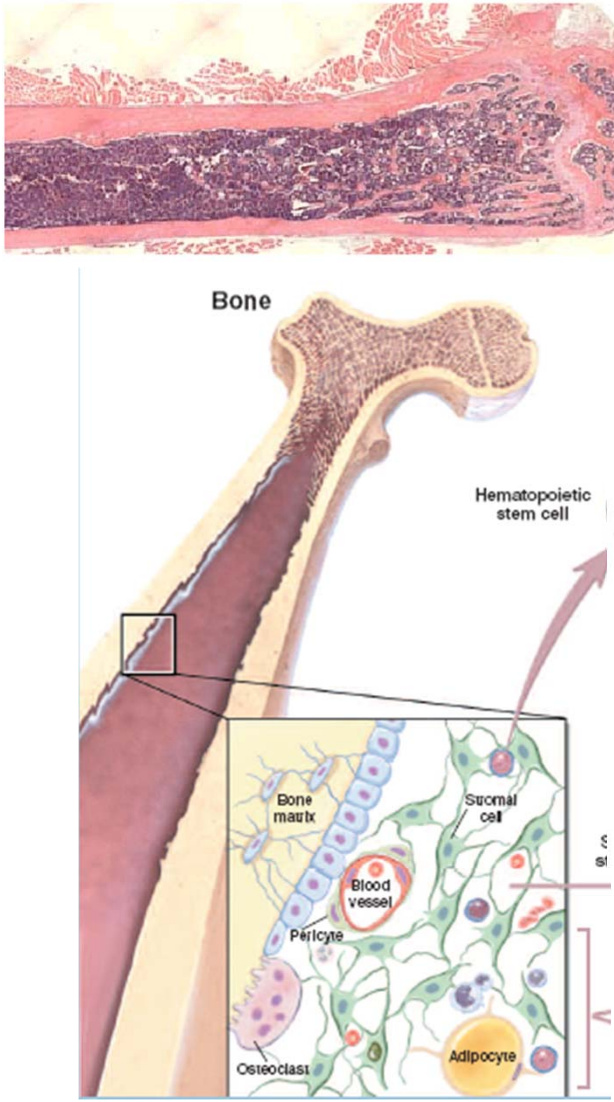
Descendants of Crypt Base Columnar Stem Cells live up to 48-72 hours

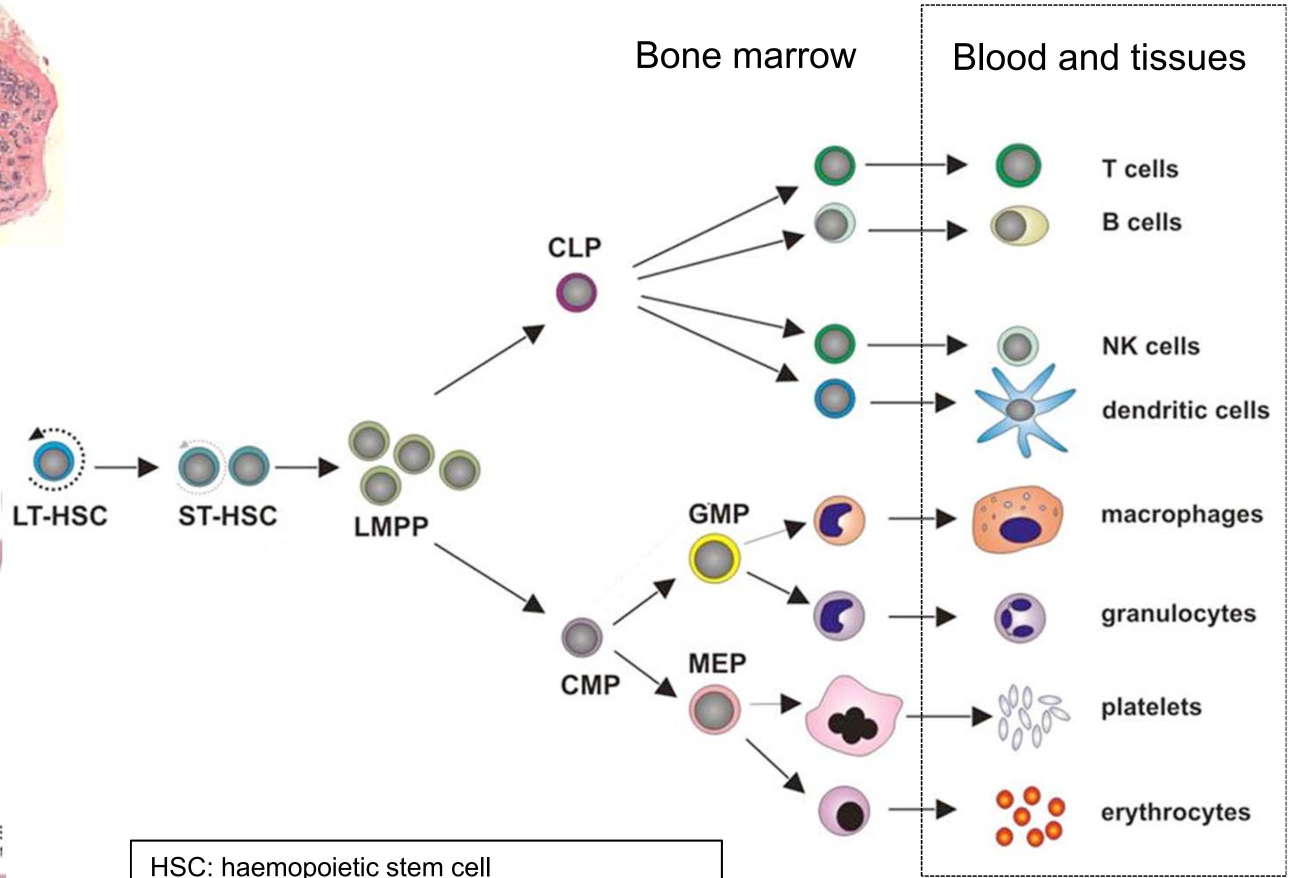


(Radtke and Clevers, Science 2005)

The Haemopoietic Stem Cell Niche

approximately 10^{11}–10^{12} new blood cells are produced daily



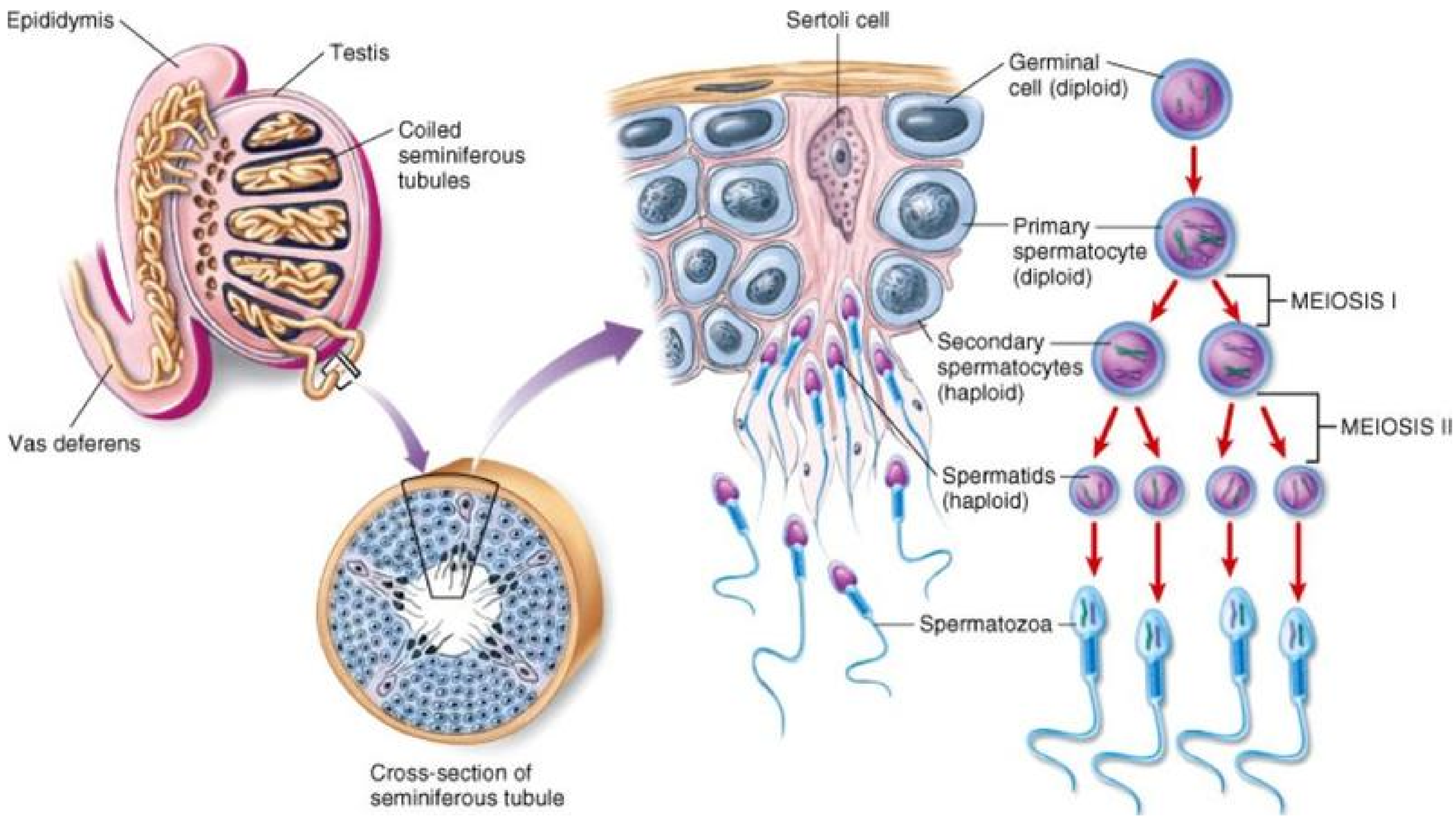


HSC: haemopoietic stem cell
MPP: multipotent progenitor cell
CLP: common lymphoid progenitor
CMP: common myeloid progenitor cell
GMP: granulocyte/monocyte precursor cell
MEP: Megakaryocyte-erythroid precursor cell

Figure 5.1. Hematopoietic Stem Cell Differentiation (2001 Terese Winslow, Lydia Kibiuk)

Seminiferous Tubules

Spermatogenesis: 2 months life span



Regulation of Stem Cells

Should I stay quiescent?

Should I die?

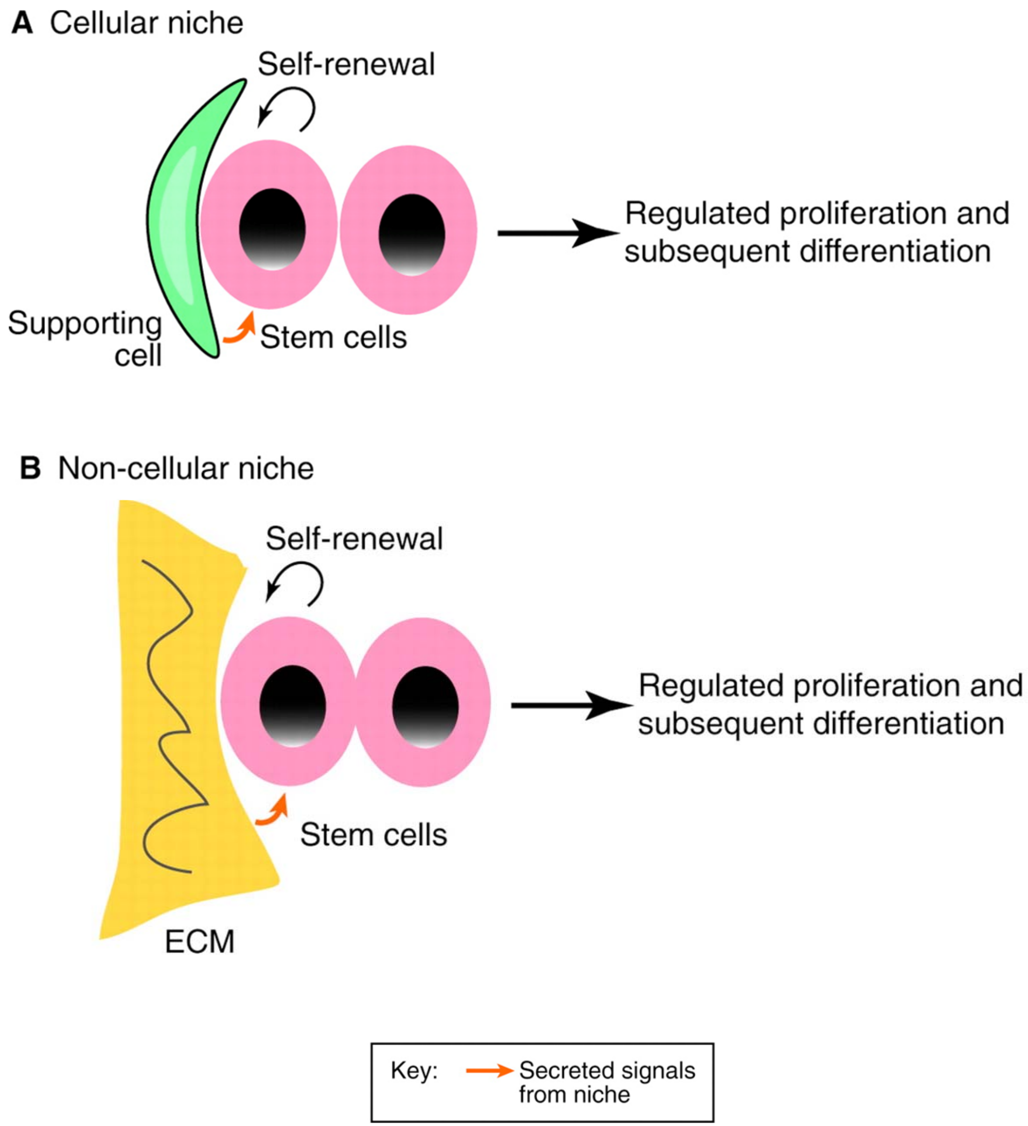
Should I proliferate?

Should I self-renew?

Should I generate transit amplifying cells?

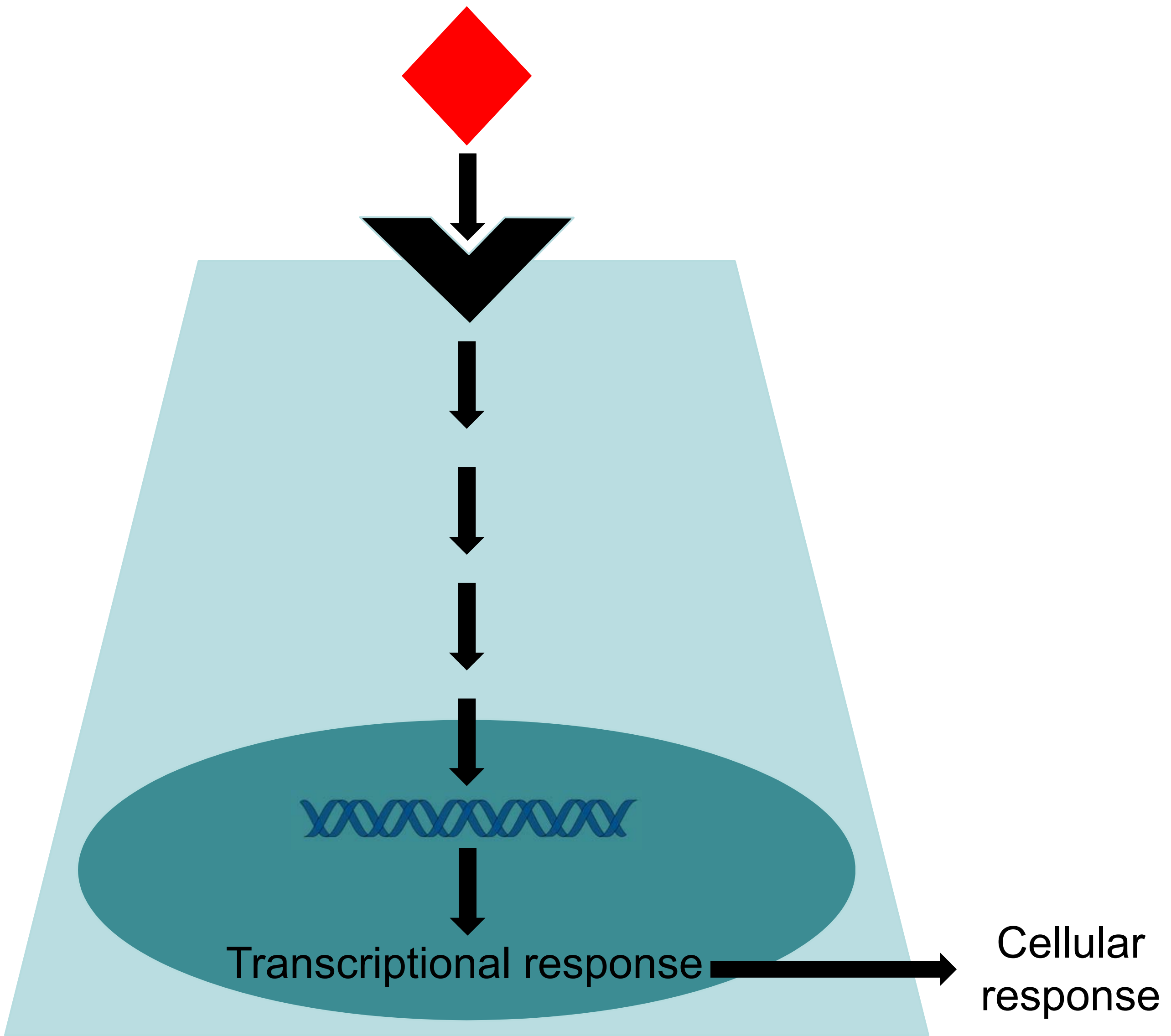
Should I generate differentiating daughter cells?

Stem cell niche:
Keeps stem cells in an undifferentiated state

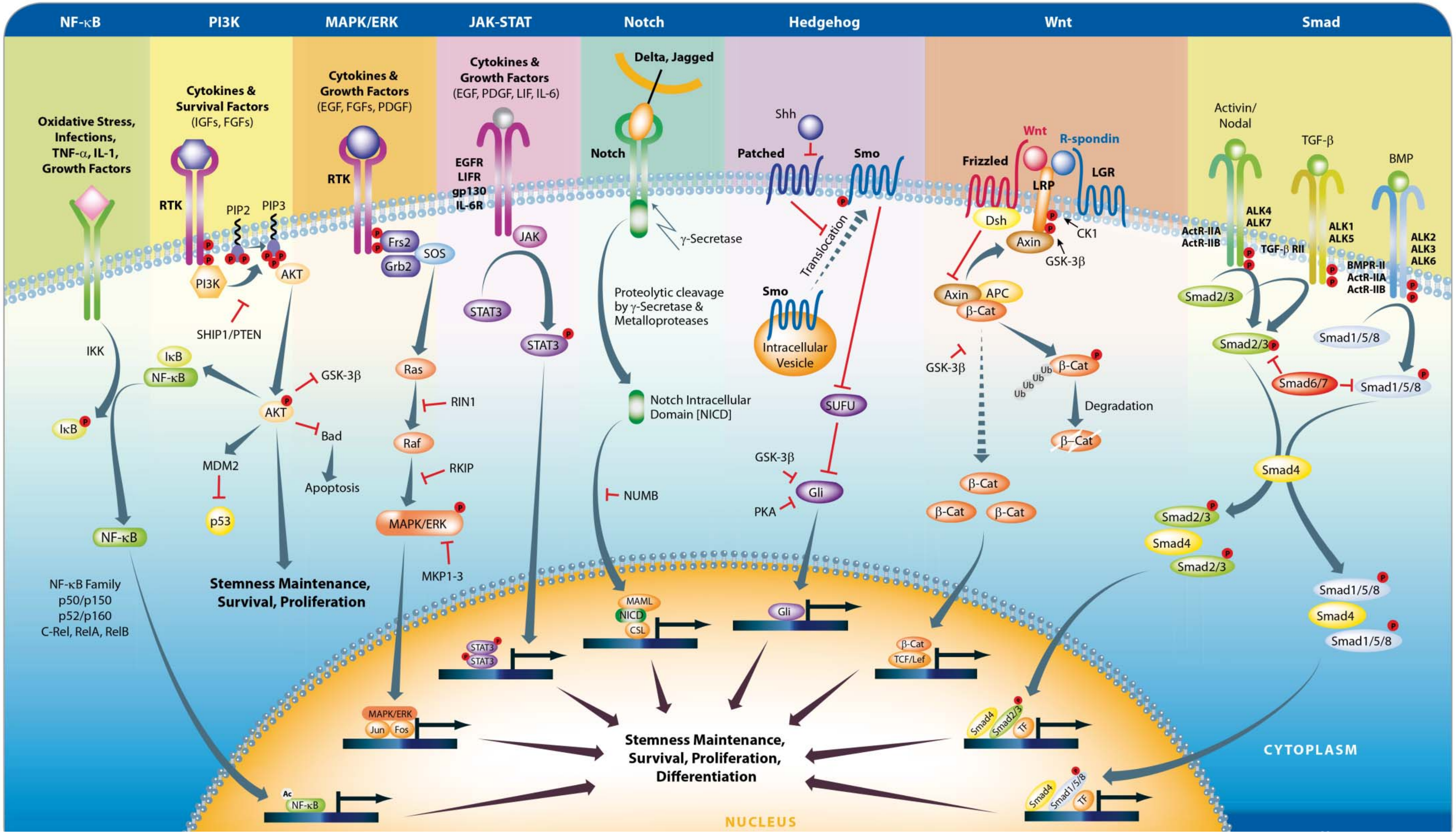


Regulation of Stem Cells

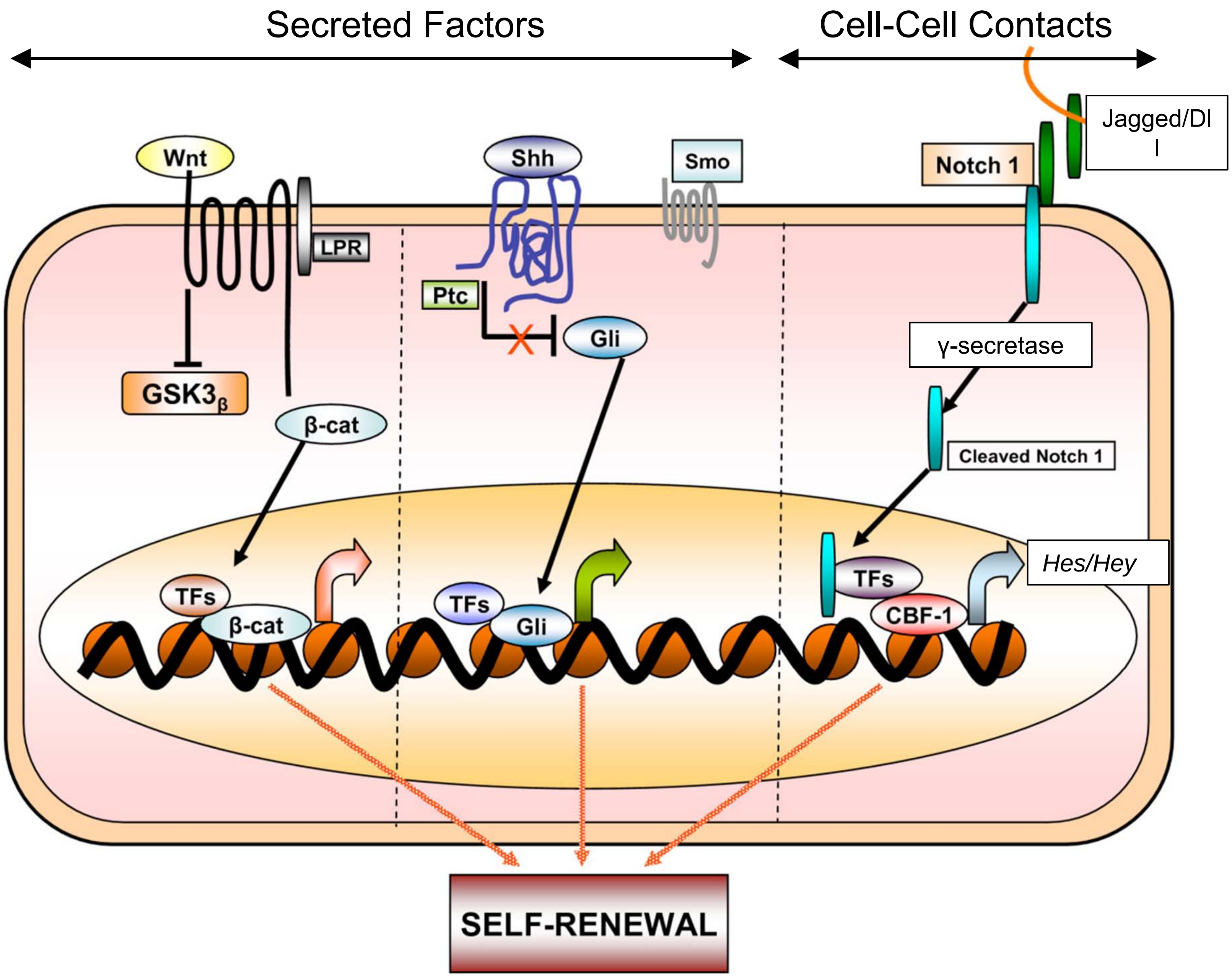
Signalling pathways



Regulation of Stem Cells

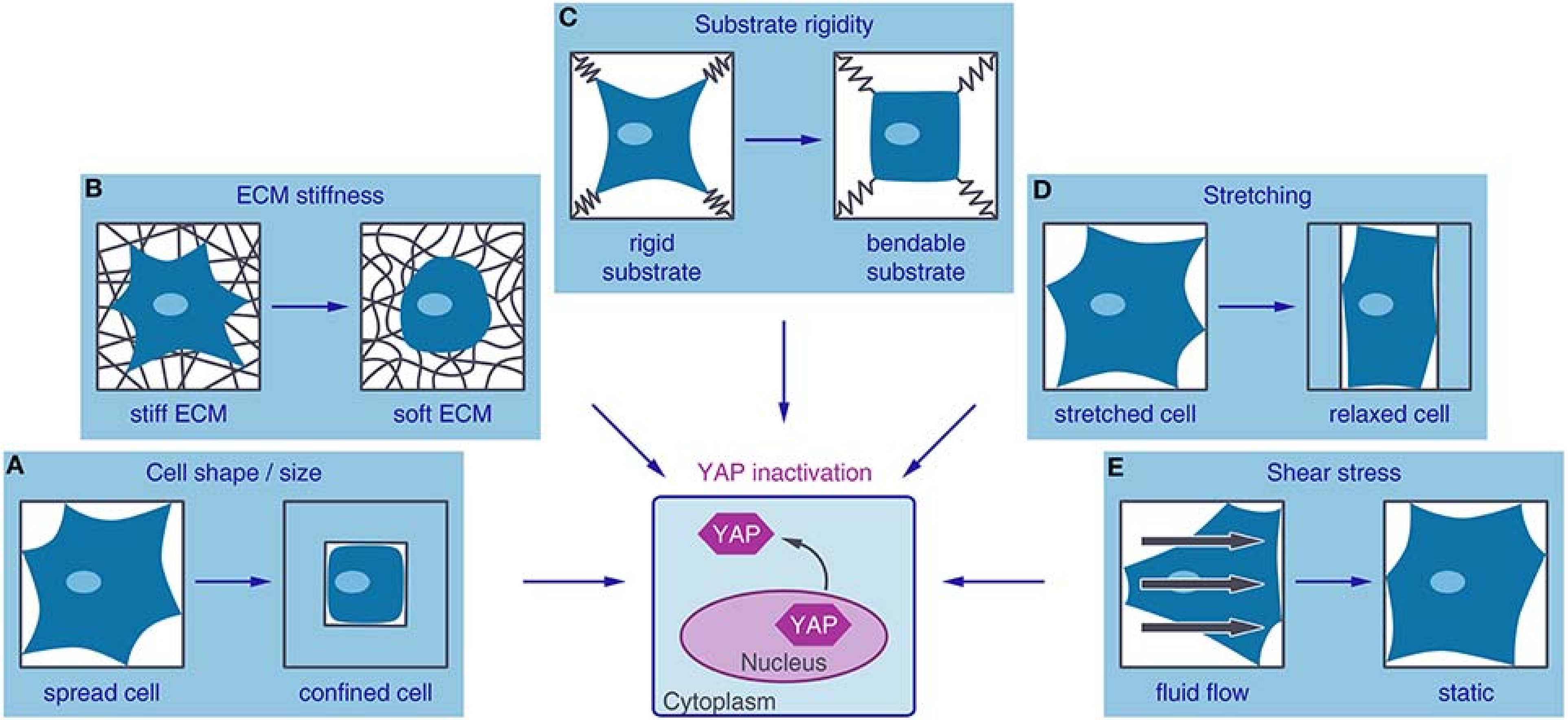


Regulation of Stem Cells



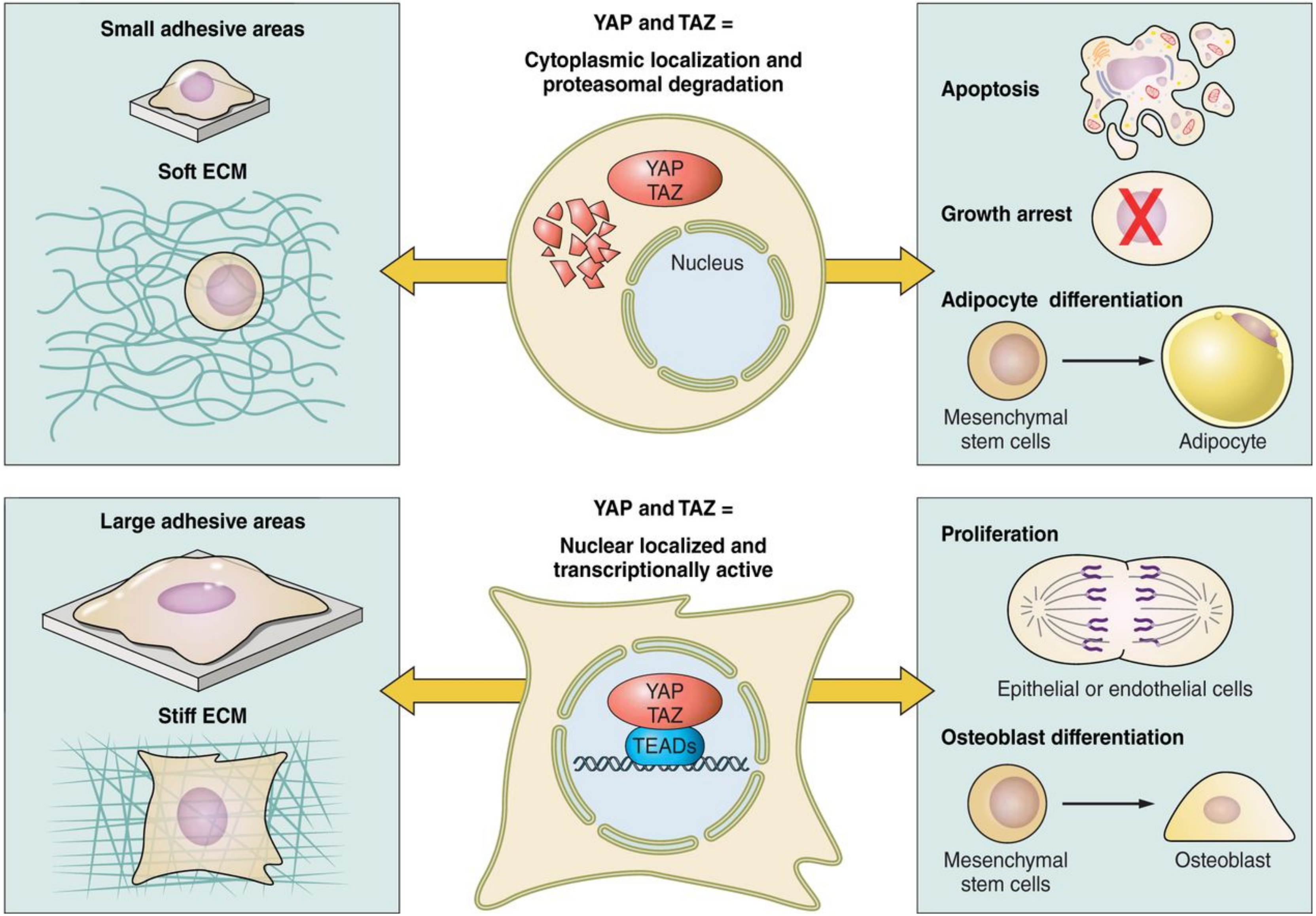
Regulation of Stem Cells

The Mechanosensor YAP

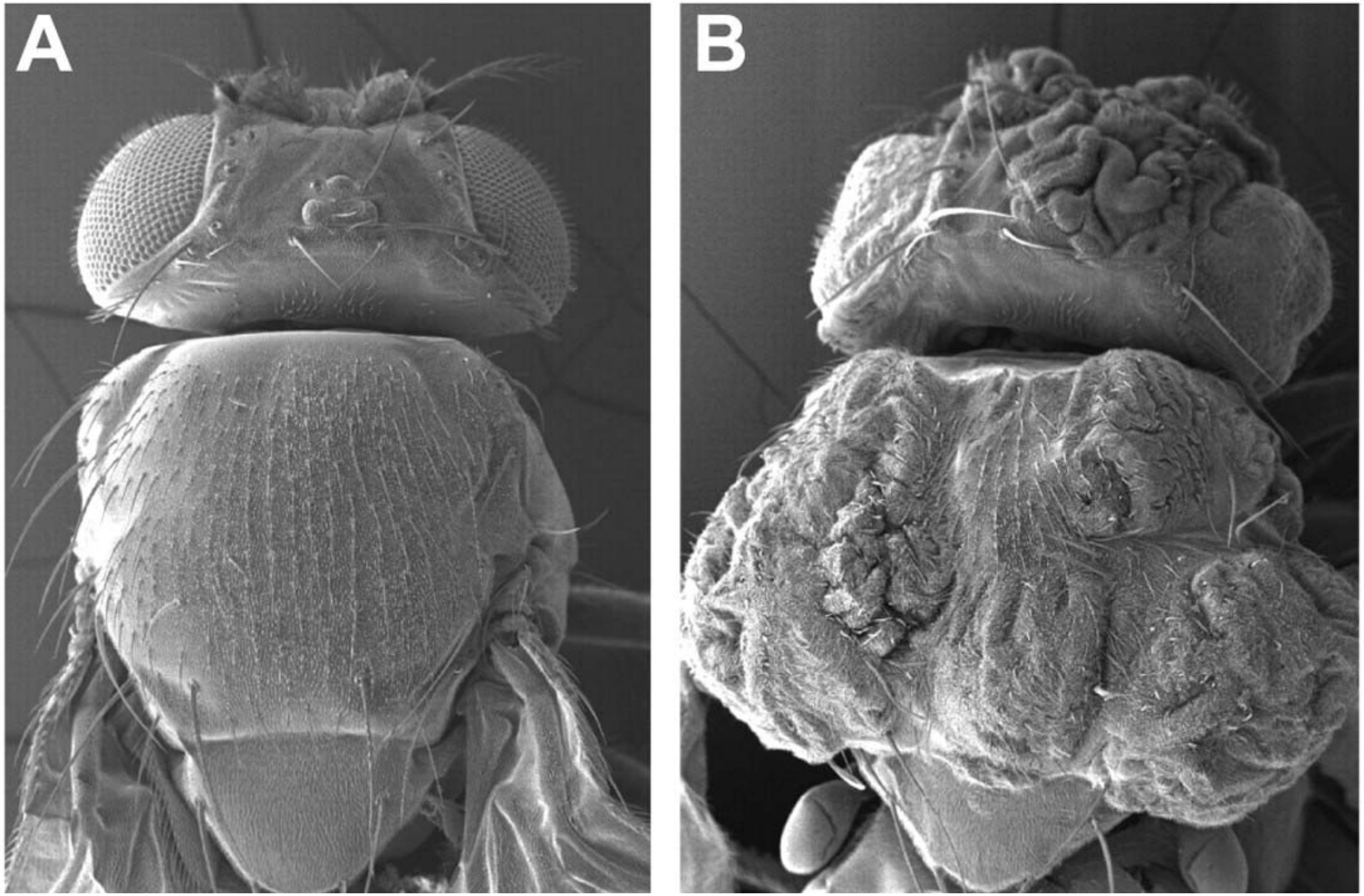


Regulation of Stem Cells

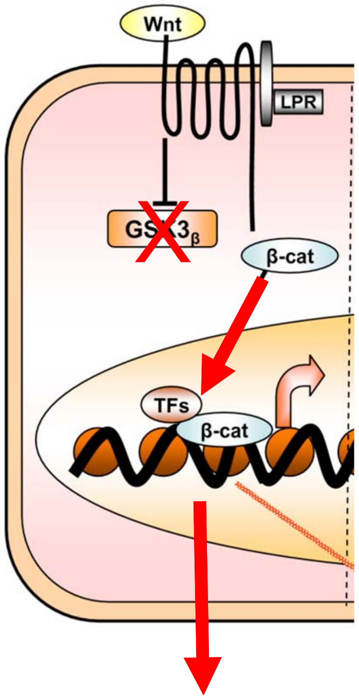
The Mechanosensor YAP



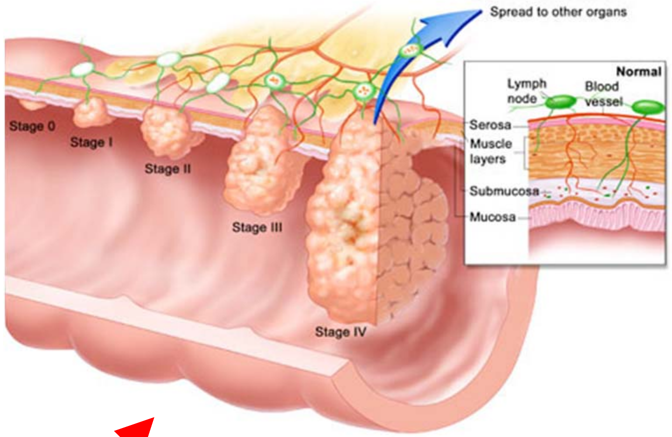
What happens if cell renewal regulation goes wrong?

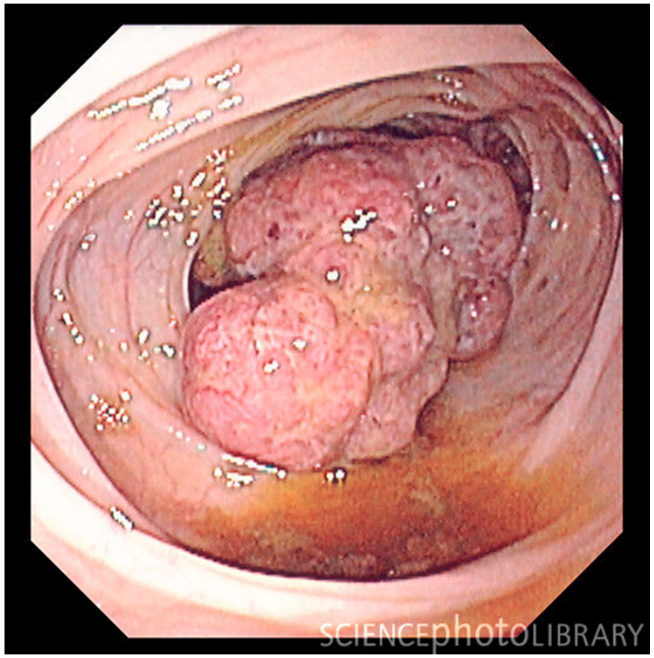


Mutations in Wnt pathway result in Cancer
Adenomatous Polyposis Coli

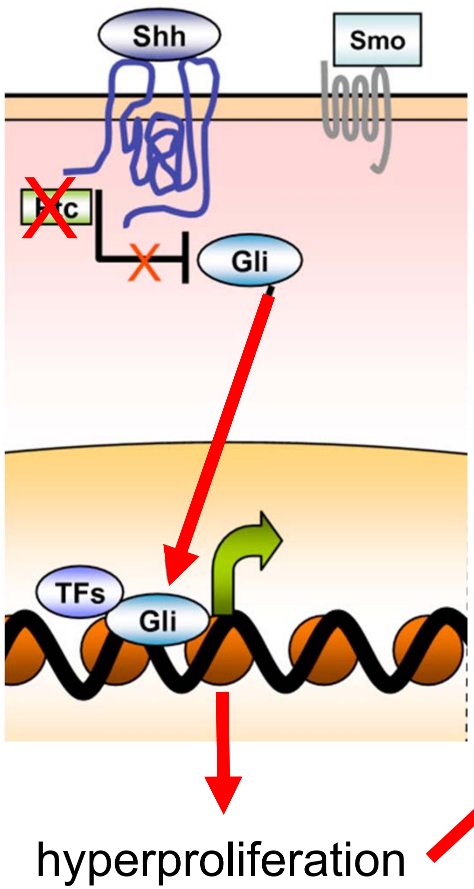


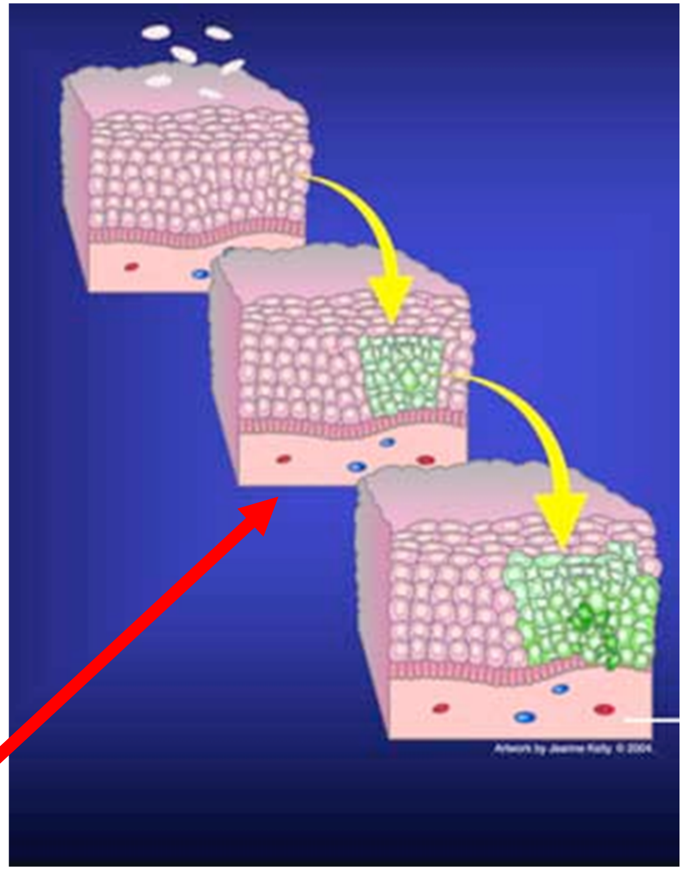
hyperproliferation





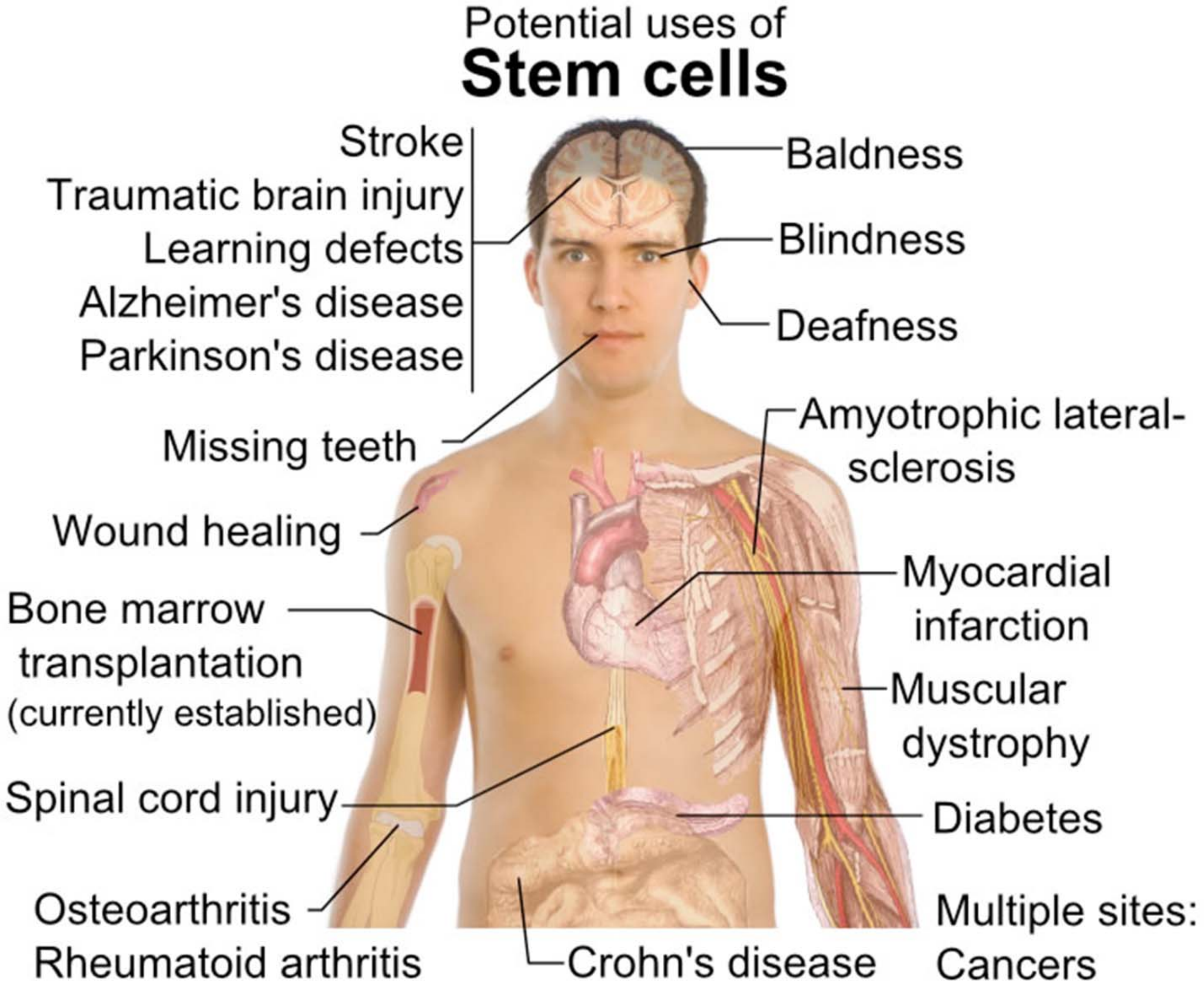
Mutations in Hedgehog pathway result in cancer
Basal Cell Carcinoma





Regenerative medicine
the clinical application of stem cells

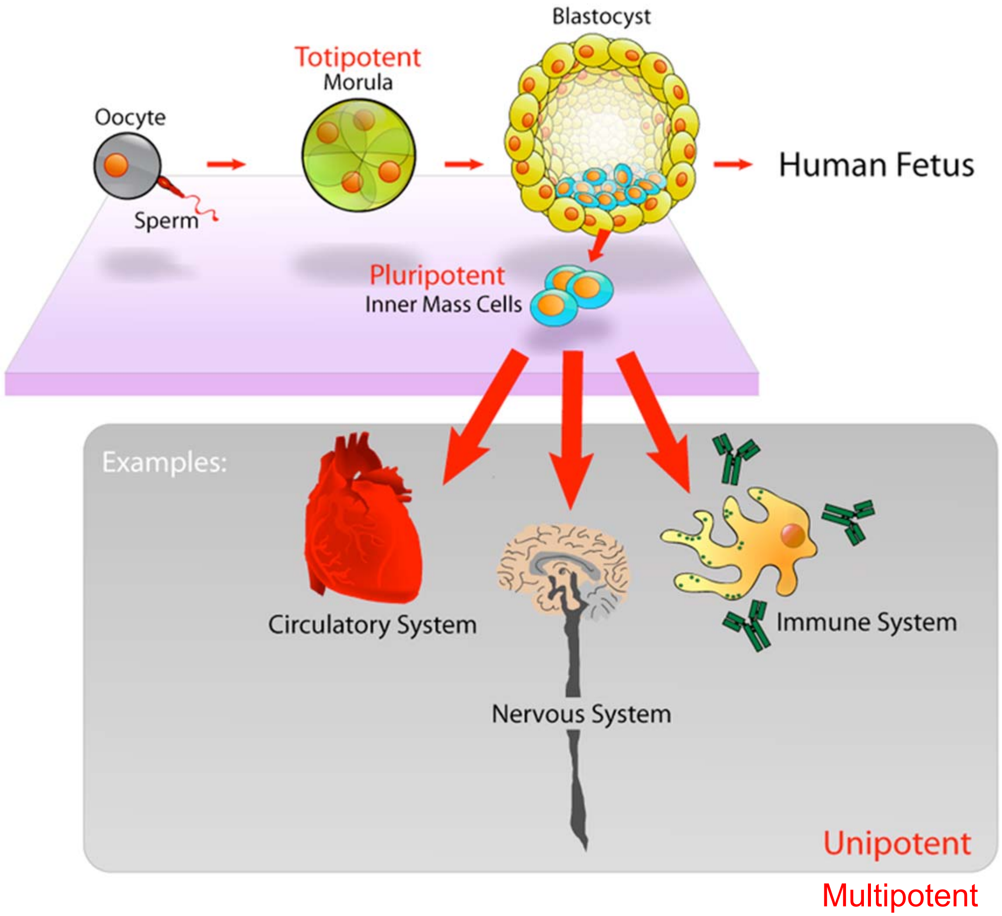
"process of replacing or regenerating human cells, tissues or organs to restore or establish normal function"



Stem Cell Sources for Regenerative Medicine

Stem cells derived
from embryos

Stem cells derived
from adults



Embryonal Carcinoma Cells are pluripotent

1964 – Pierce and Kleinsmith isolate EC cells from teratocarcinomas

Pluripotent
In vitro culture and expansion
Genetic abnormalities

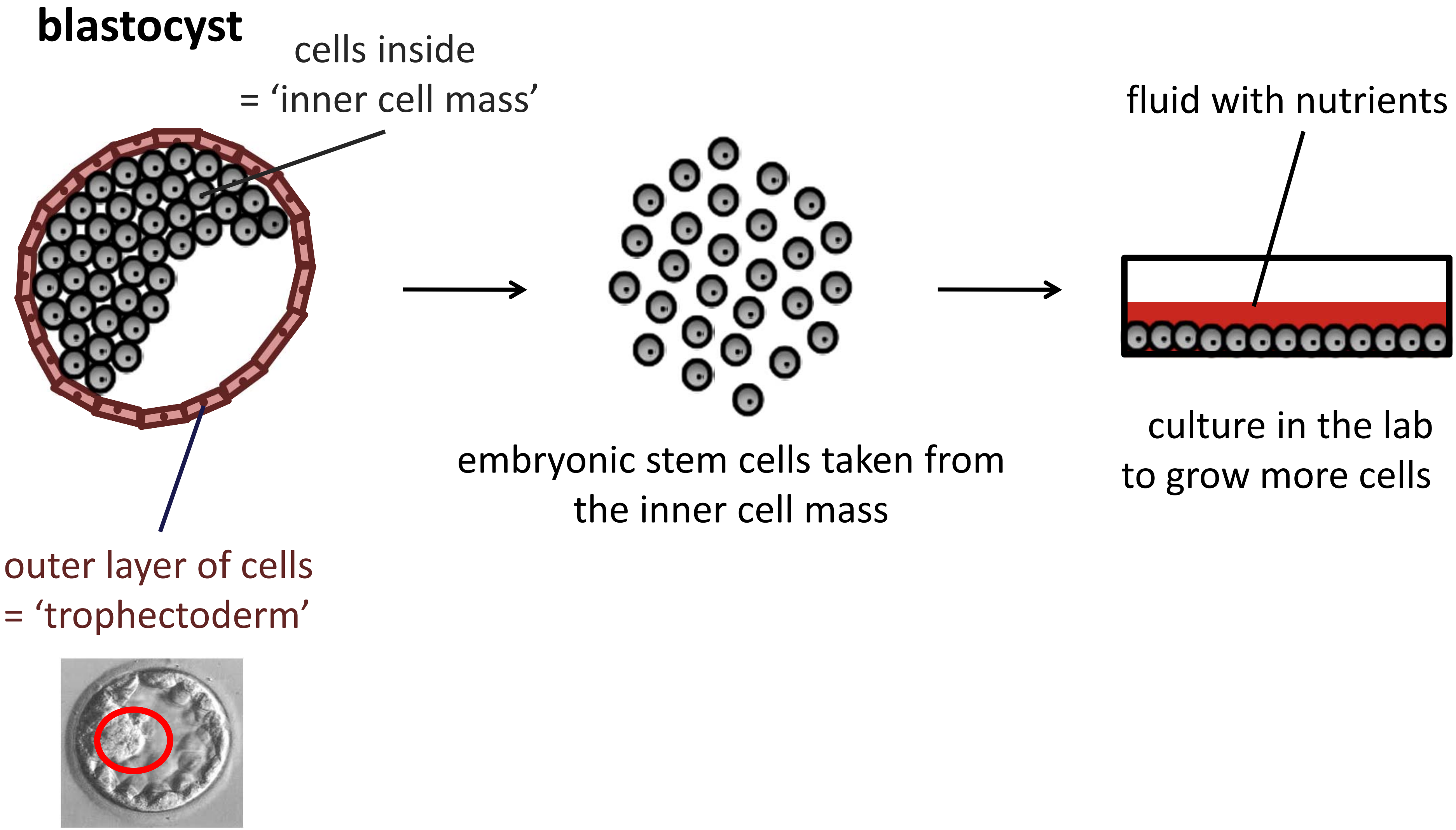
Embryonic Stem Cells are pluripotent

1981 – Martin Evans, Matthew Kaufman and Gail Martin

Pluripotent
No genetic abnormalities
In vitro culture and expansion
Ethical issues

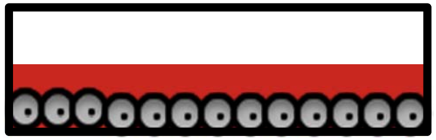
Embryonic Stem Cells are pluripotent

1981 – Martin Evans, Matthew Kaufman and Gail Martin



Embryonic Stem Cells are pluripotent

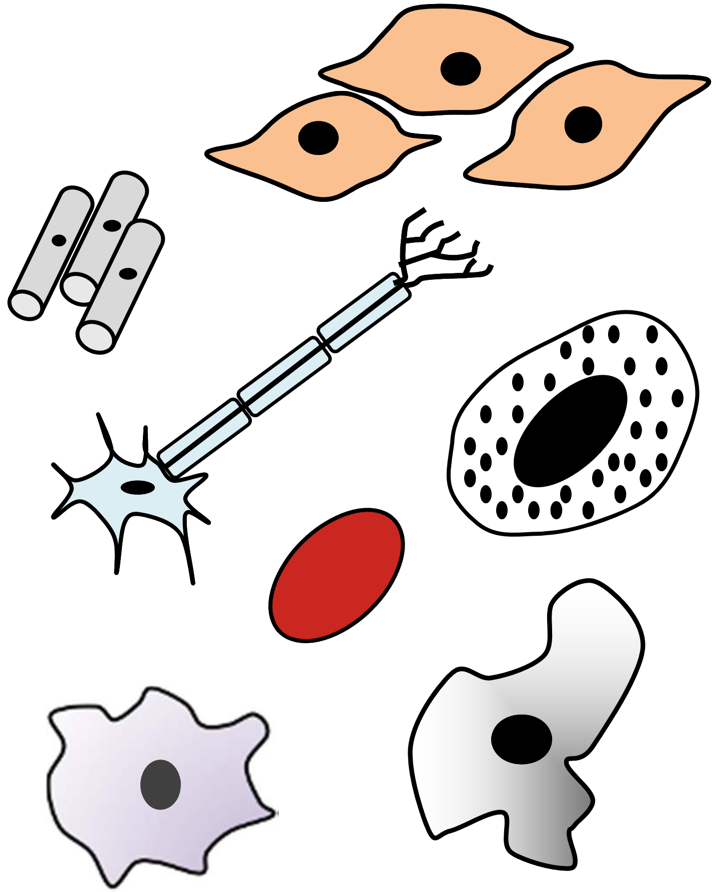
1981 – Martin Evans, Matthew Kaufman and Gail Martin



embryonic stem cells

differentiation

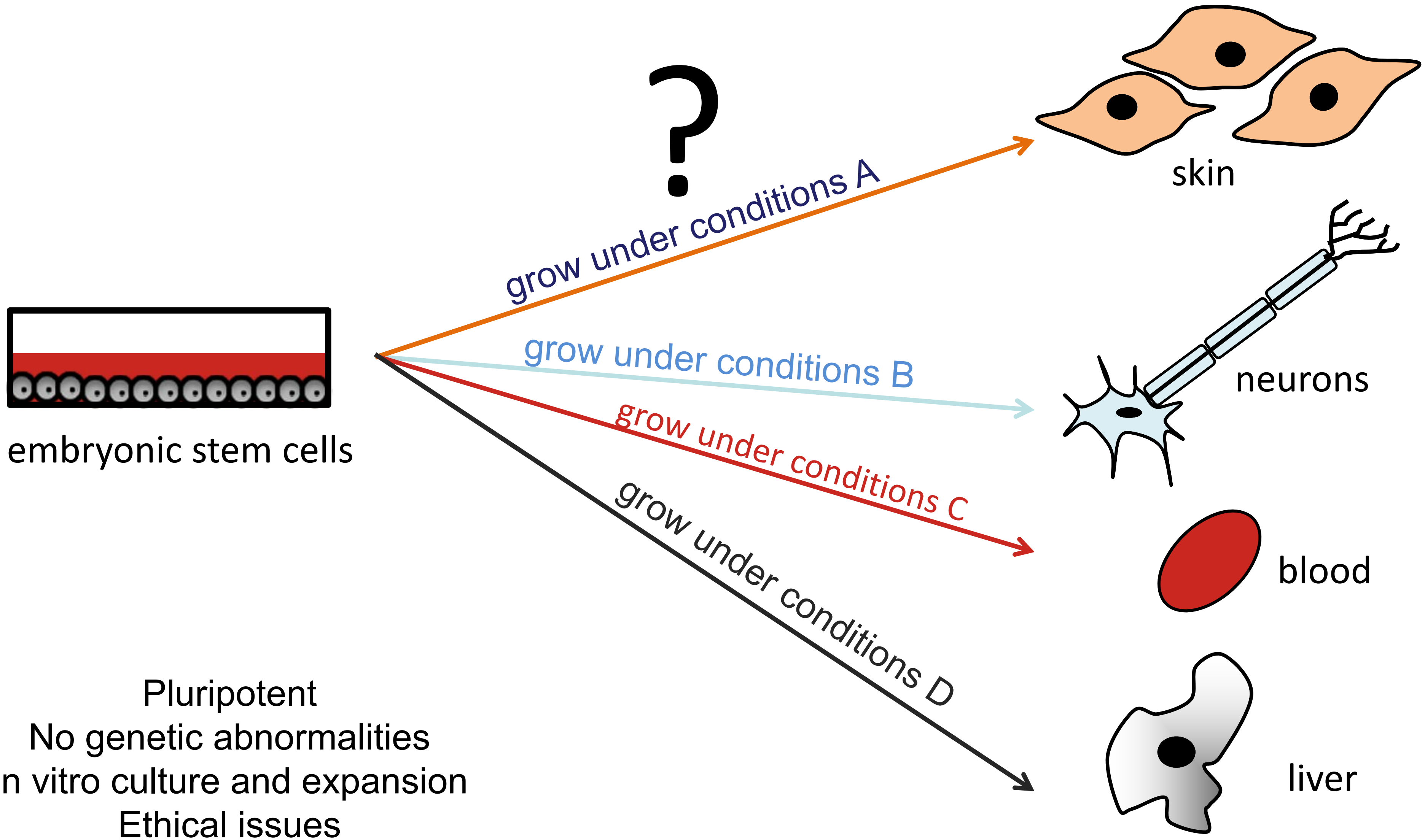
PLURIPOTENT



all possible types of specialized cells

Embryonic Stem Cells are pluripotent

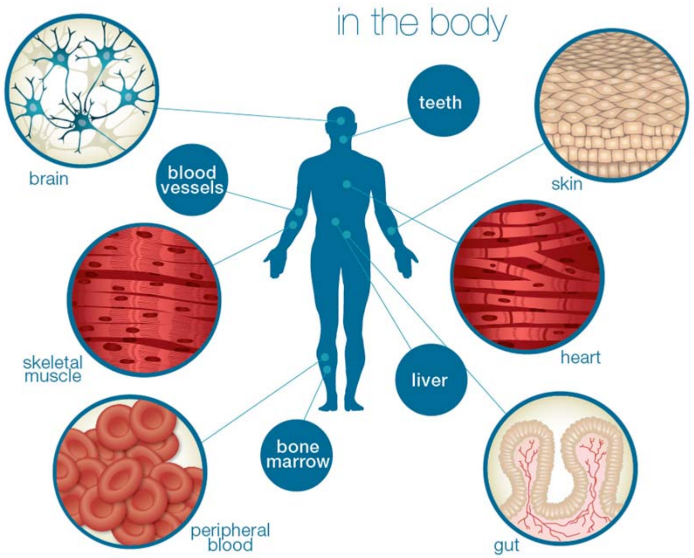
1981 – Martin Evans, Matthew Kaufman and Gail Martin



Pluripotent
No genetic abnormalities
In vitro culture and expansion
Ethical issues

Adult stem cells

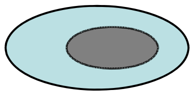
"An undifferentiated cell, found among differentiated cells in a tissue or organ that can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ"



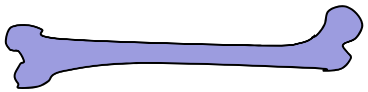
No ethical issues
Restricted plasticity
Limited quantities
Hard to identify

Adult stem cells

Haematopoietic Stem Cells



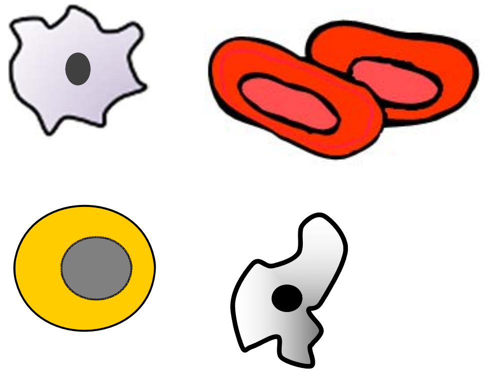
blood stem cell



found in
bone marrow

MULTIPOTENT

differentiation



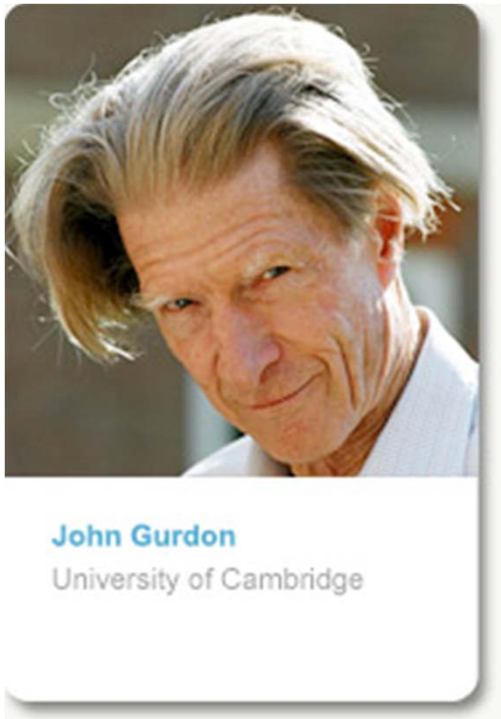
only specialized types of blood cell:
red blood cells, white blood cells,
platelets

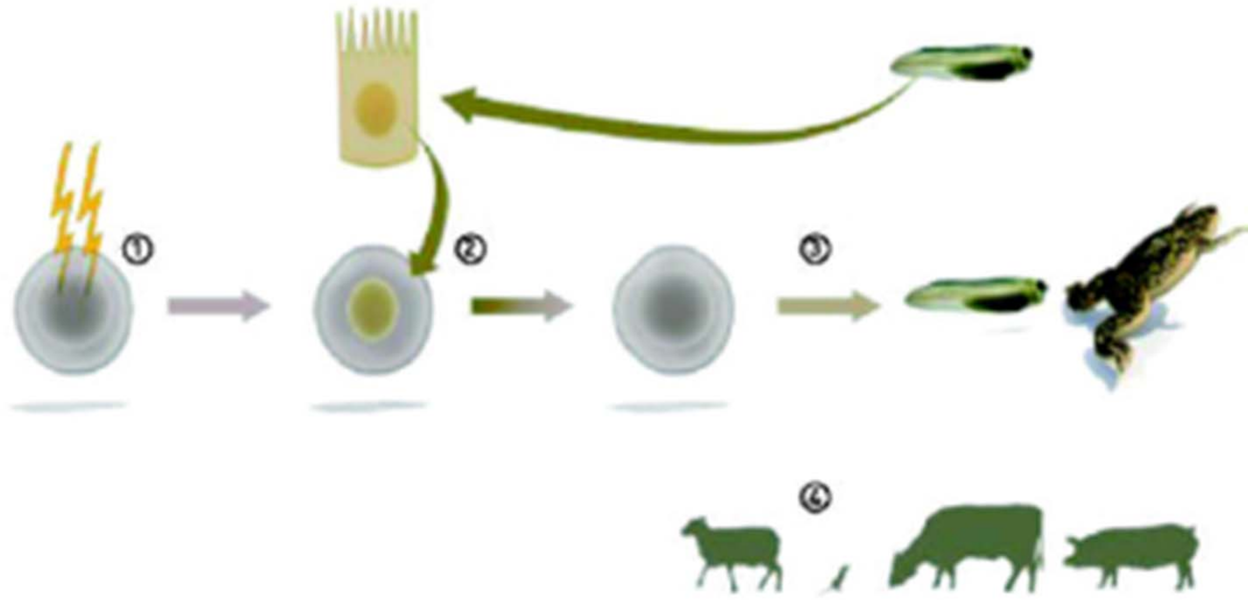
No ethical issues
Restricted plasticity
Limited quantities
Hard to identify

Somatic Cell Nuclear Transfer

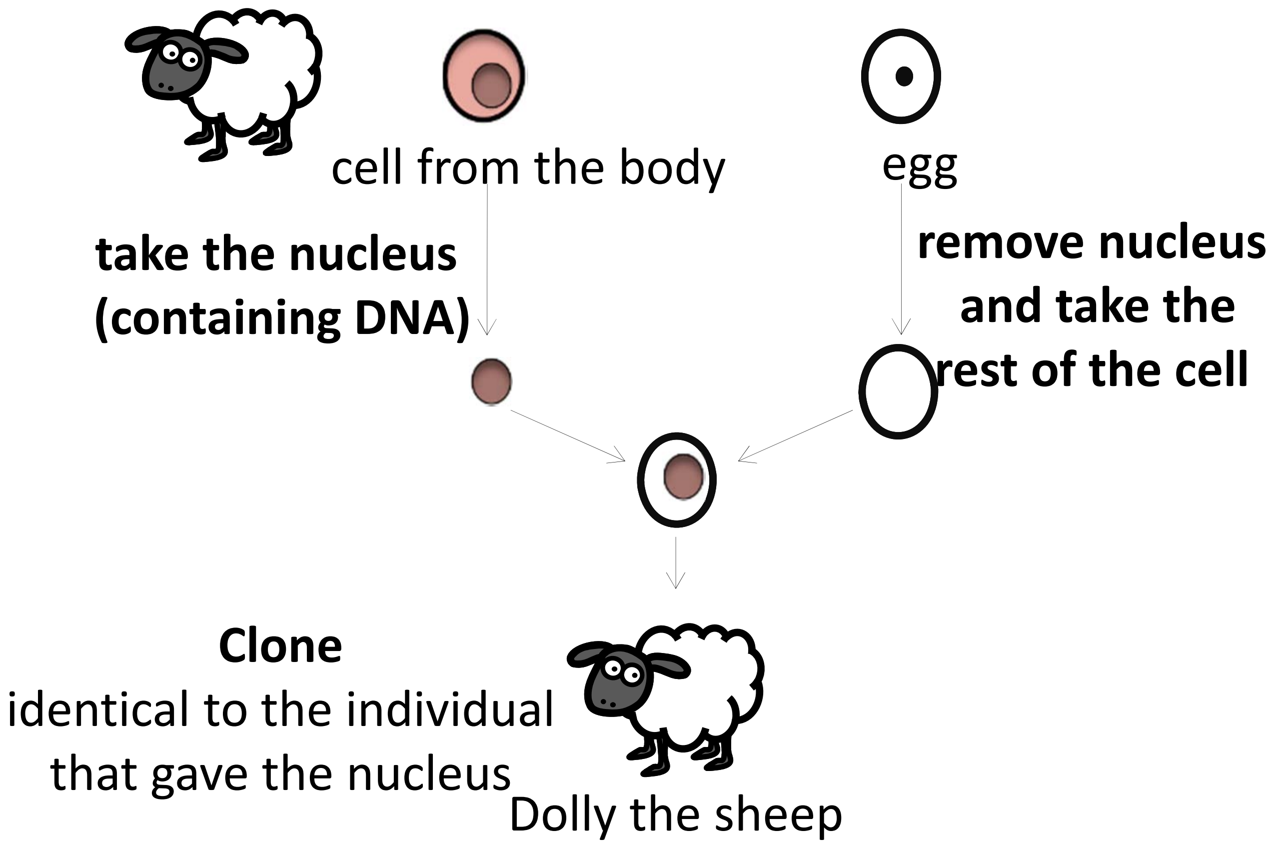
John Gurdon, 1958

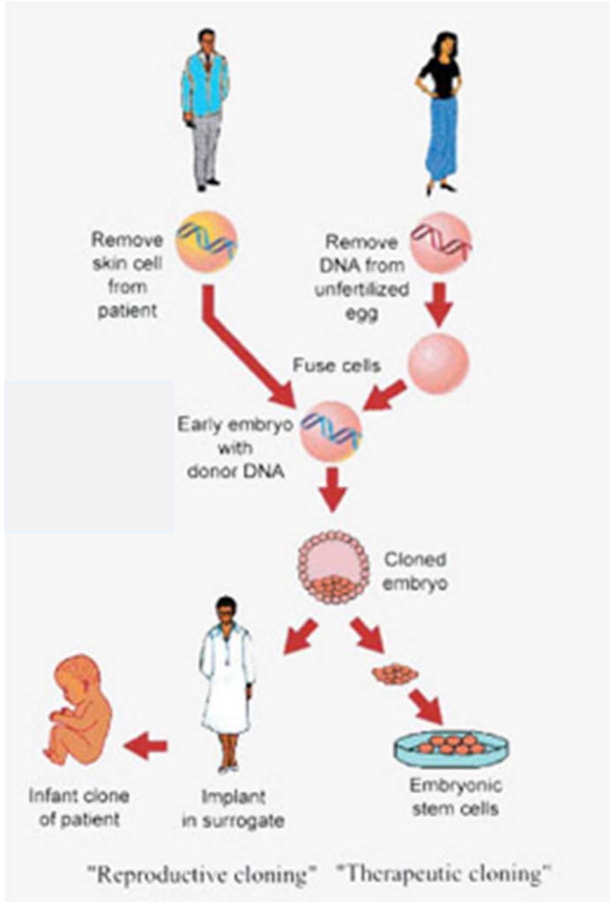
The developmental potential of nuclei of differentiated cells





Reproductive/Therapeutic Cloning





Pluripotent (totipotent?)
Low success rate
Genetic/phenotypic abnormalities
Ethical issues

Somatic Cell Nuclear Transfer

“mature, differentiated cells can be reprogrammed to become pluripotent”

Nuclear Reprogramming

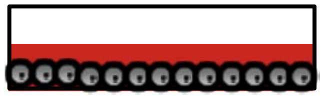
Induced pluripotency (iPS), Yamanaka, 2006

“mature, differentiated cells can be reprogrammed to become pluripotent”

cell from the body

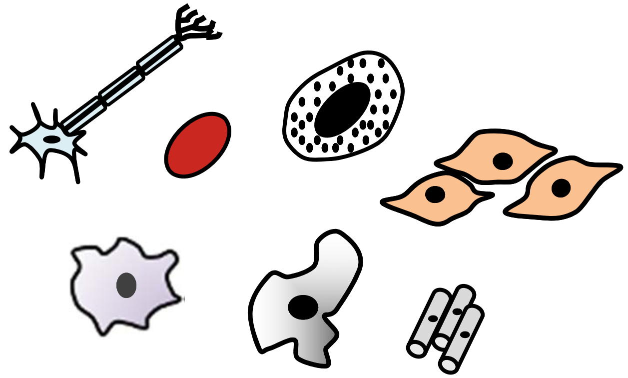
‘genetic reprogramming’
= add certain genes to the cell

induced pluripotent stem (iPS) cell
behaves like an embryonic stem cell



culture iPS cells in the lab

differentiation



all possible types of specialized cells

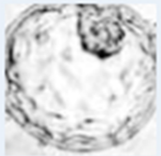
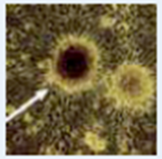
Advantage: no need for embryos!

Nuclear Reprogramming

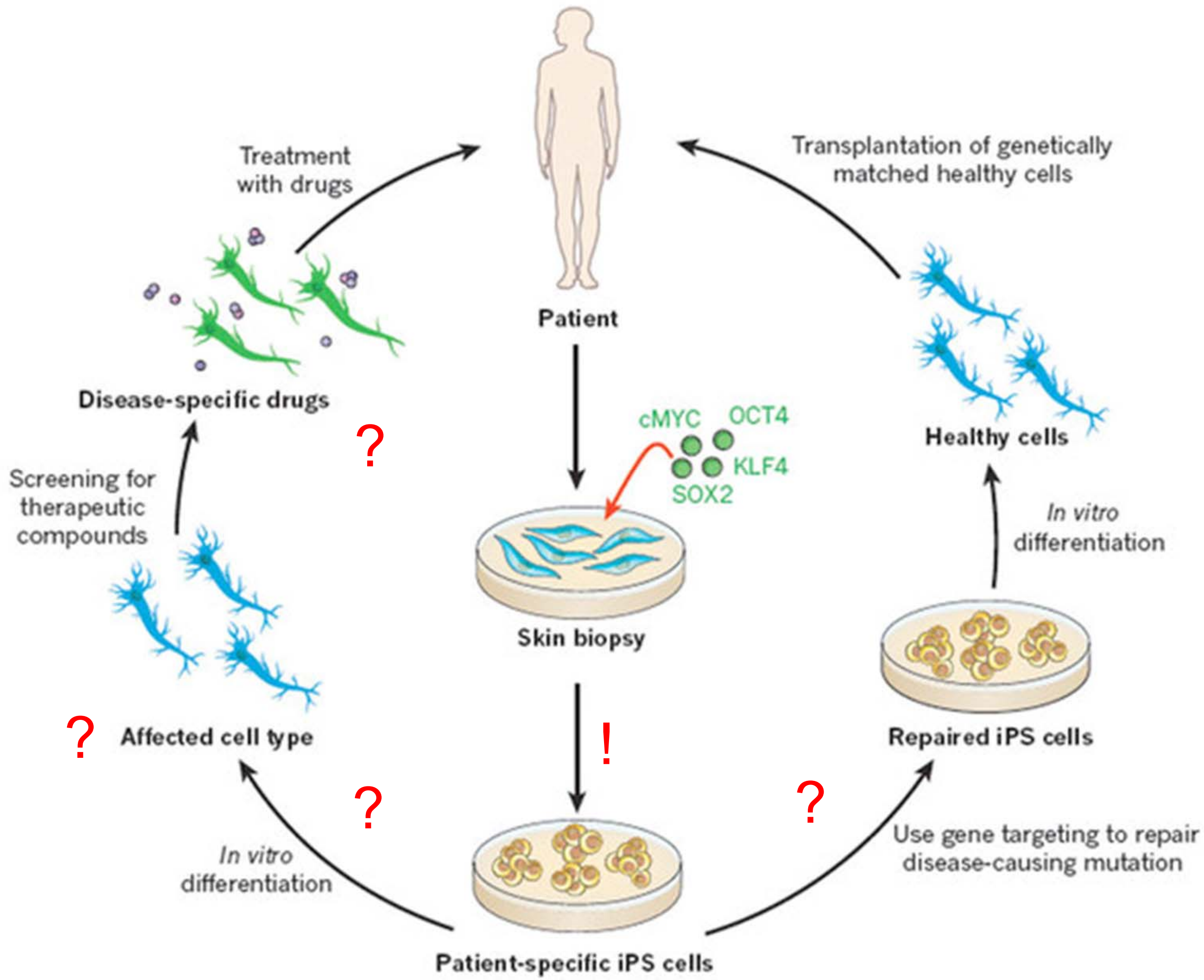
2012 Nobel Prize

Stem Cell Sources

Embryonic vs Adult Stem Cells

COMPARISON OF THE DIFFERENT SOURCES OF STEM CELLS				
	Embryonic Stem Cells		Adult Stem Cells	iPS Cells
				
	In Vitro Fertilization	**Nuclear Transfer**	**Adult Tissues**	
Attributes	• can produce all cell types • relatively easy to identify, isolate, maintain, and grow in the laboratory • large source of "excess" blastocysts from IVF clinics	• can produce all cell types • relatively easy to identify, isolate, maintain, and grow in the laboratory • stem cells may be genetically matched to patient	• demonstrated success in some treatments • stem cells may be genetically matched to patient	- Can generate any cell type - Easy to generate, maintain and grow in lab - Perfect genetic match to patient
Limitations	• limited number of cell lines available for federally funded research	• risk of creating teratomas (tumors) from implanting undifferentiated stem cells	• produce limited number of cell types • not found in all tissues • difficult to identify, isolate, maintain, and grow in the laboratory	- May retain age of parental cell - Inheritance of mutations: teratomas
Ethical Concerns	• destruction of human blastocysts • donation of blastocysts requires informed consent	• destruction of human blastocysts • donation of eggs requires informed consent • concern about misapplication for reproductive cloning	• no major ethical concerns have been raised	- No major ethical concerns

The Future of Regenerative Medicine



Future of Regenerative Medicine

1- how we can induce and maintain pluripotency?

2- how we can direct differentiation?

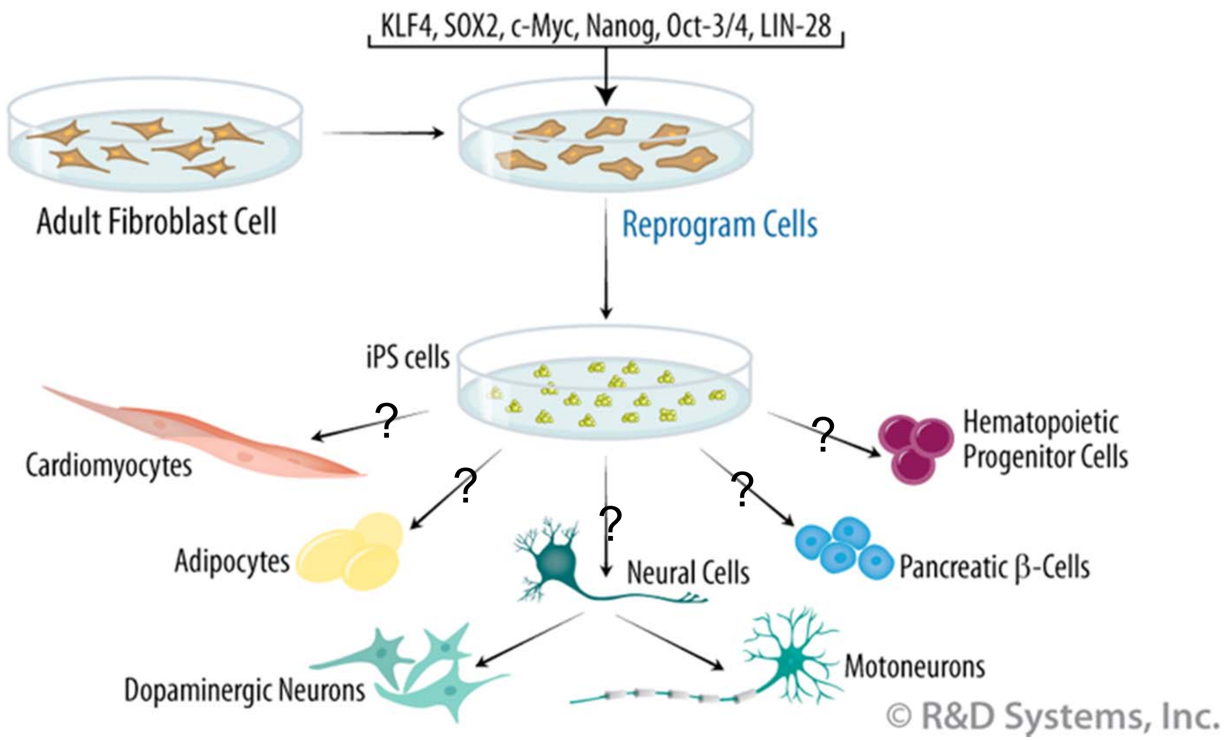
3- how we can cure diseased cells?

4- how we can repair mutations in cells?

Future Stem Cell Technologies

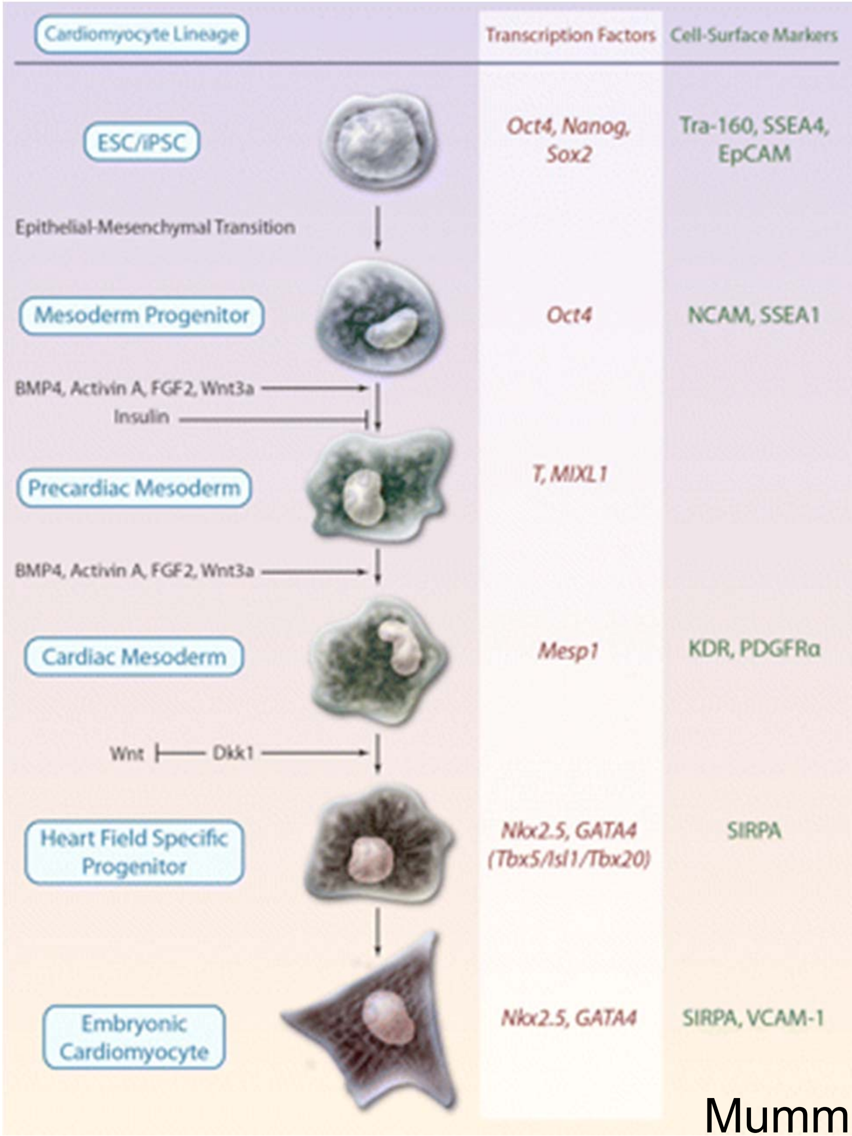
<u>How can we direct differentiation?</u>

- Uncontrolled differentiation
- Directed differentiation



Future Stem Cell Technologies

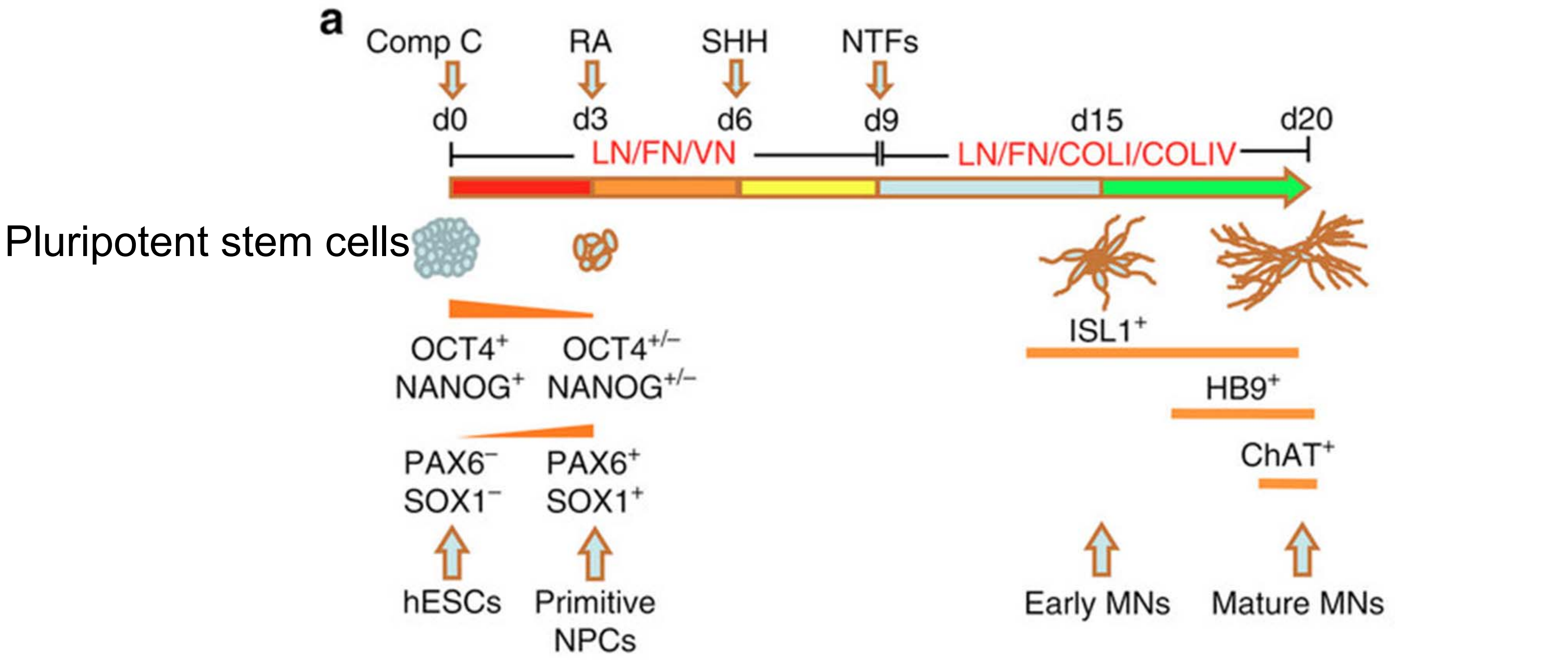
Directed differentiation of cardiomyocytes



Mummery et al., Circ Res 2012

Future Stem Cell Technologies

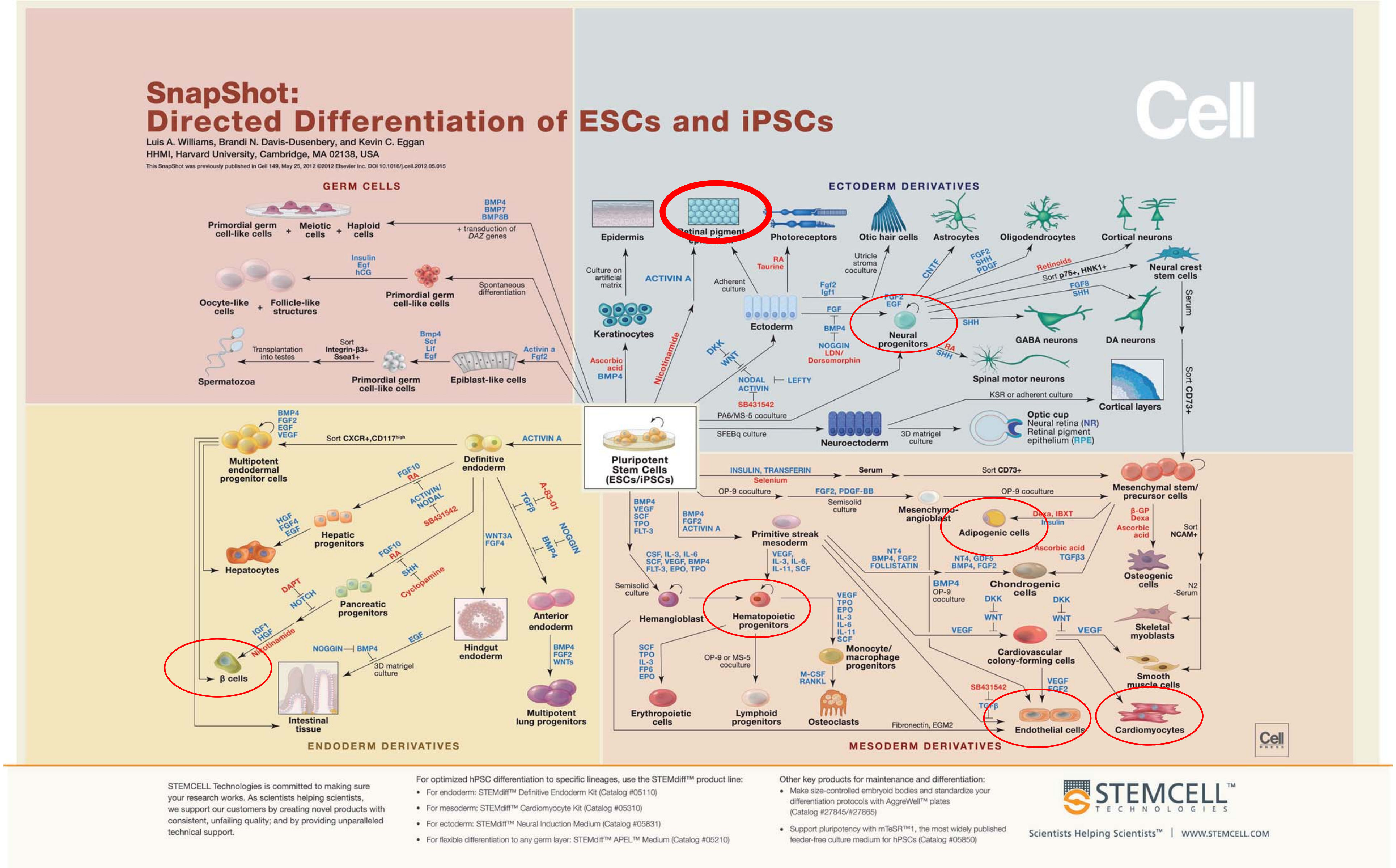
Directed differentiation of motor neurons



Dong et al., Nature 2014

Future of Regenerative Medicine

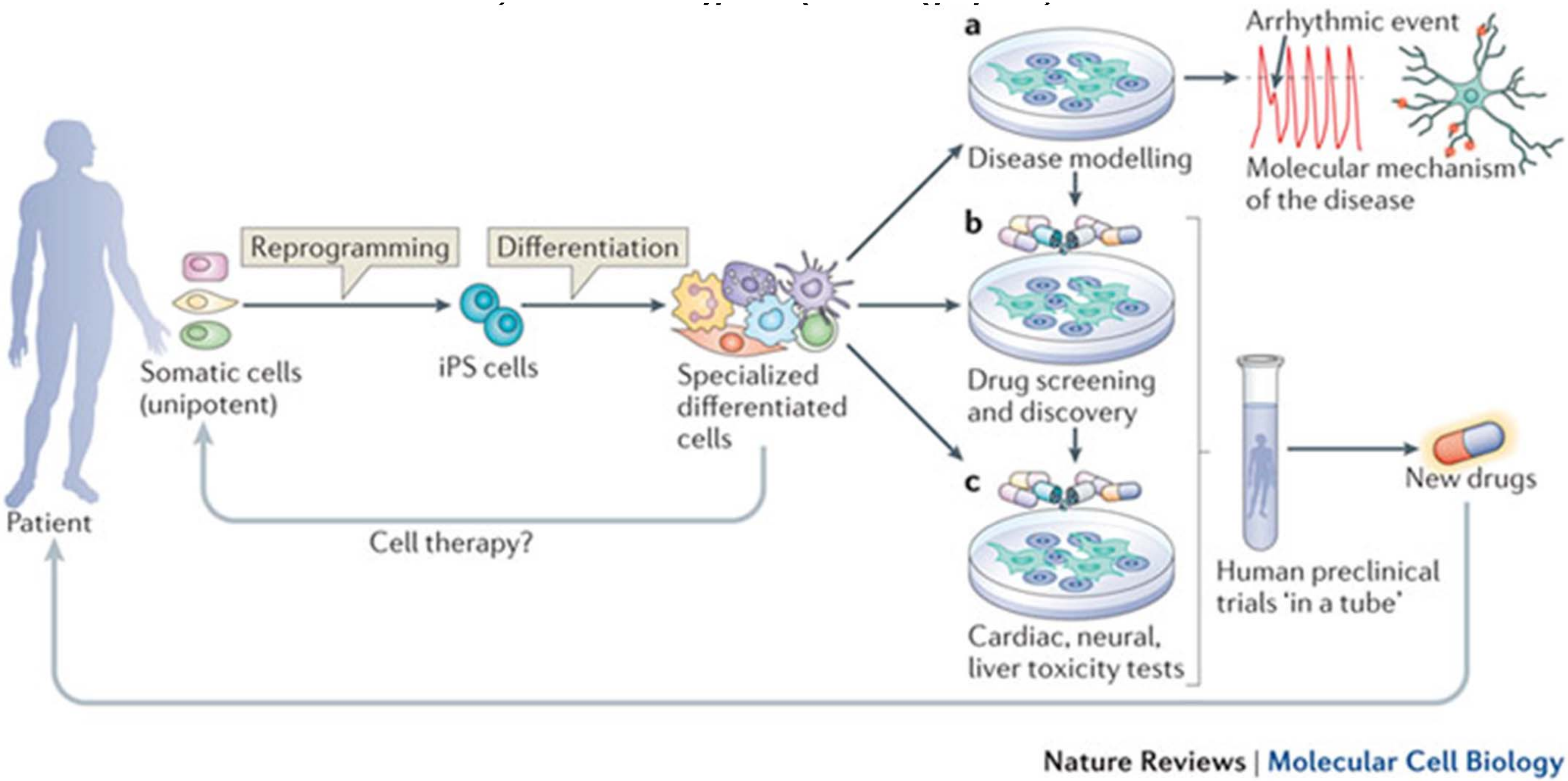
Directed differentiation of pluripotent stem cells



Future of Regenerative Medicine

<u>How can we cure disease?</u>

Disease Modeling and Drug discovery



Future of Regenerative Medicine

<u>How can we repair mutations in cells?</u>

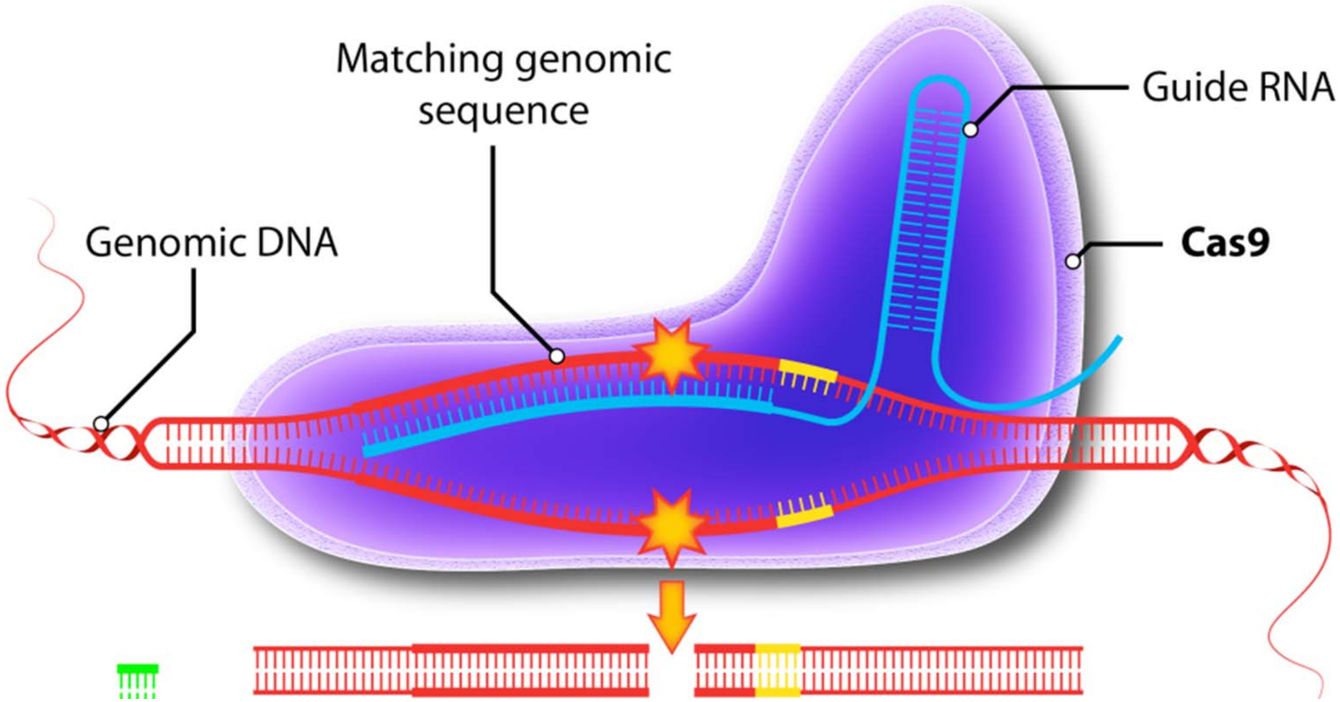
<u>Gene Therapy:</u>

CRISPR/CAS9 genome editing

CRISPR/Cas9 Genome Engineering

(Clustered Regularly Interspaced Short Palindromic Repeats)

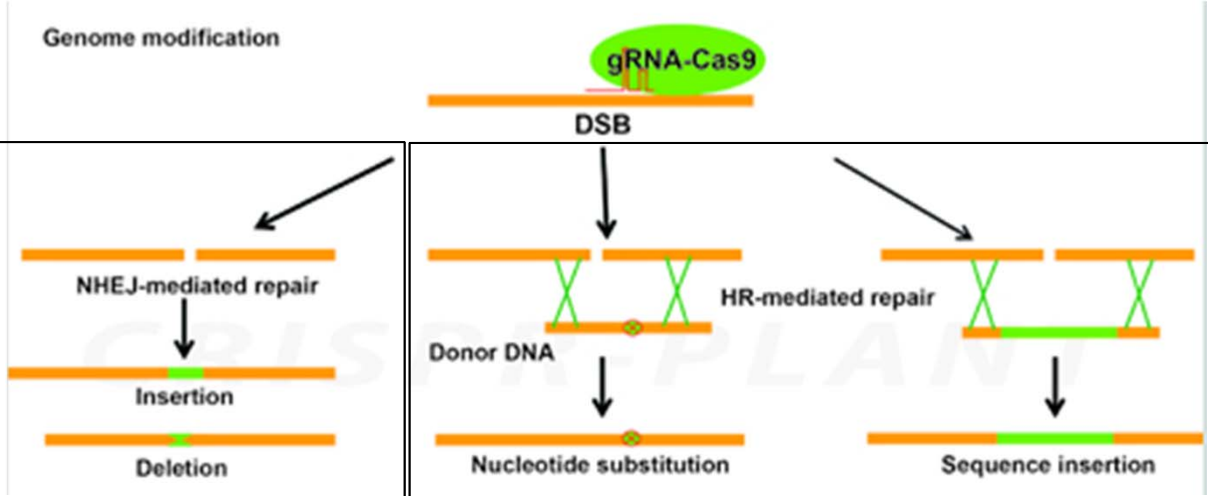
Guide RNA and Cas9



http://www.youtube.com/watch?v=Edx9L0Sasoc

CRISPR/Cas9 Genome engineering

Repair

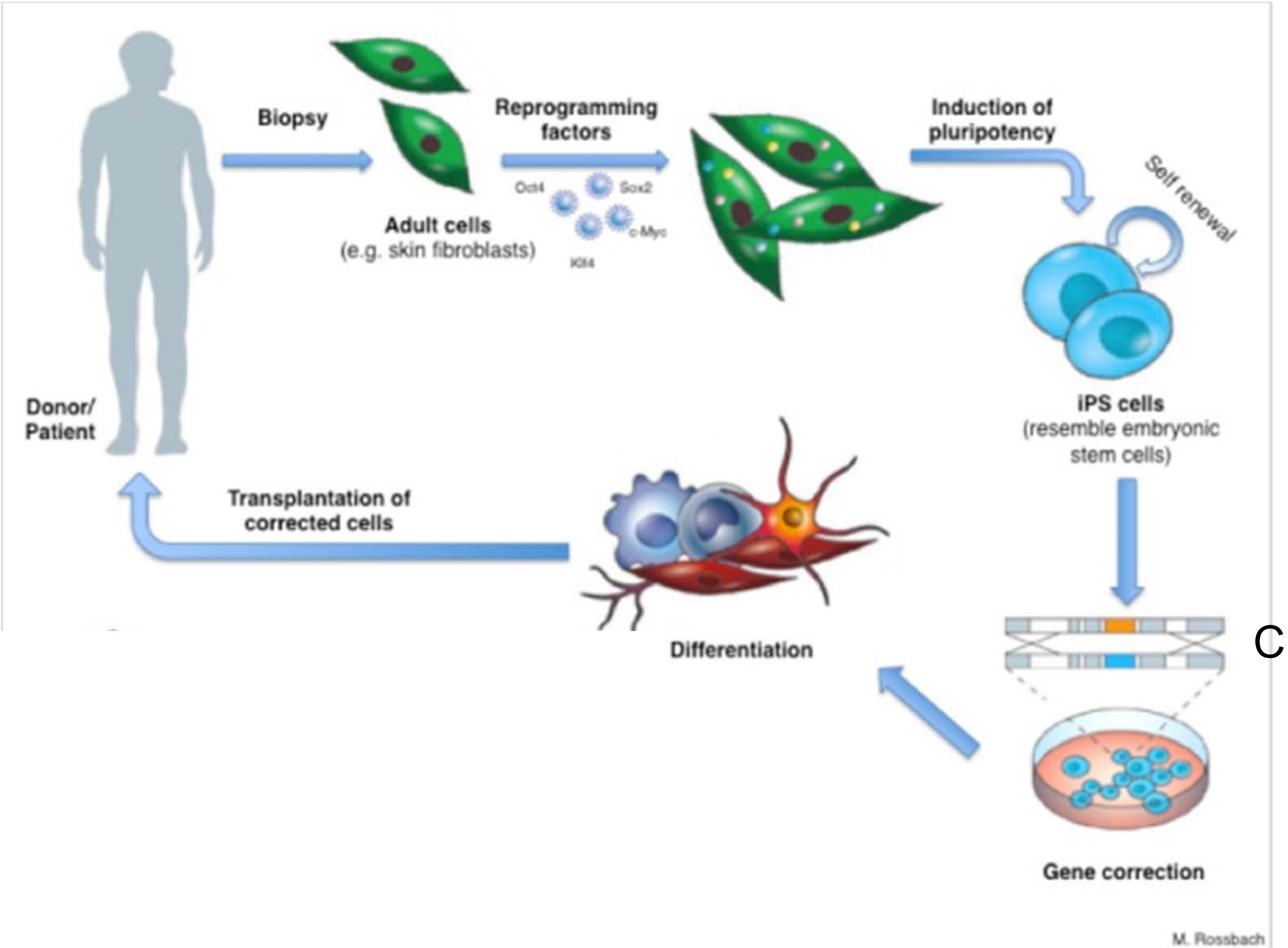


<u>Non-homologous end joining:</u>
Small insertion/deletion
gene disruption
(and occasional errors)

<u>Homology-directed repair:</u>
Provide donor template with homology arms
Gene mutation/correction/addition
(Cas9 D10A mutant)

CRISPR/Cas9 Genome engineering

Applications in Stem Cells



CRISPR/CAS9

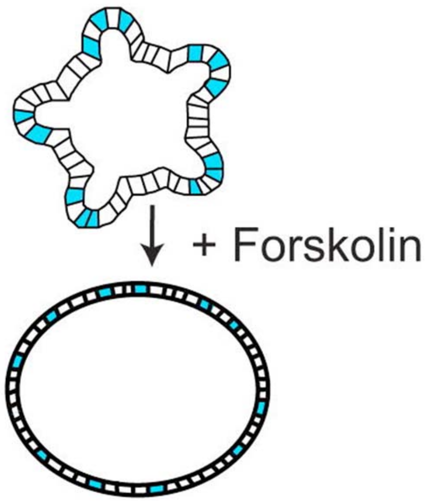
Repair of Cystic Fibrosis Gene CFTR by CRISP/CAS9 (cystic fibrosis transmembrane conductor receptor)

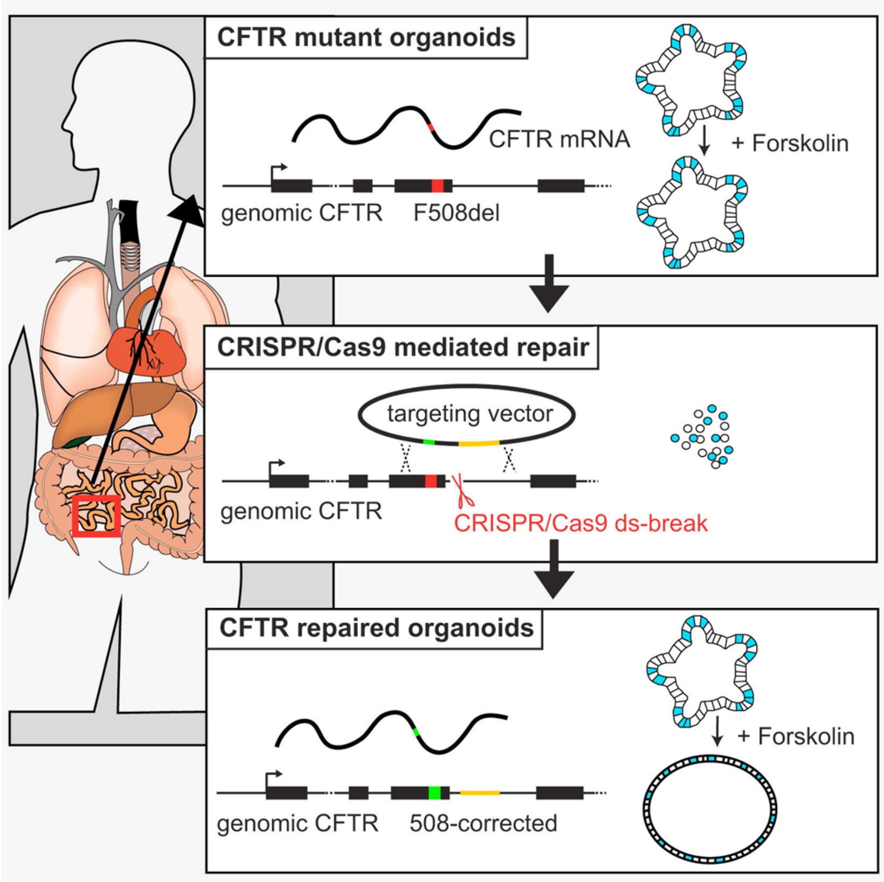
CF: accumulation of viscous mucus in pulmonary and gastrointestinal tract
Life expectancy: 40 years
F508del in CFTR (anion channel essential for fluid and electrolyte homeostasis of epithelia)

Lgr5+ intestinal stem cells -> organoids

In vitro assay in intestinal organoids:

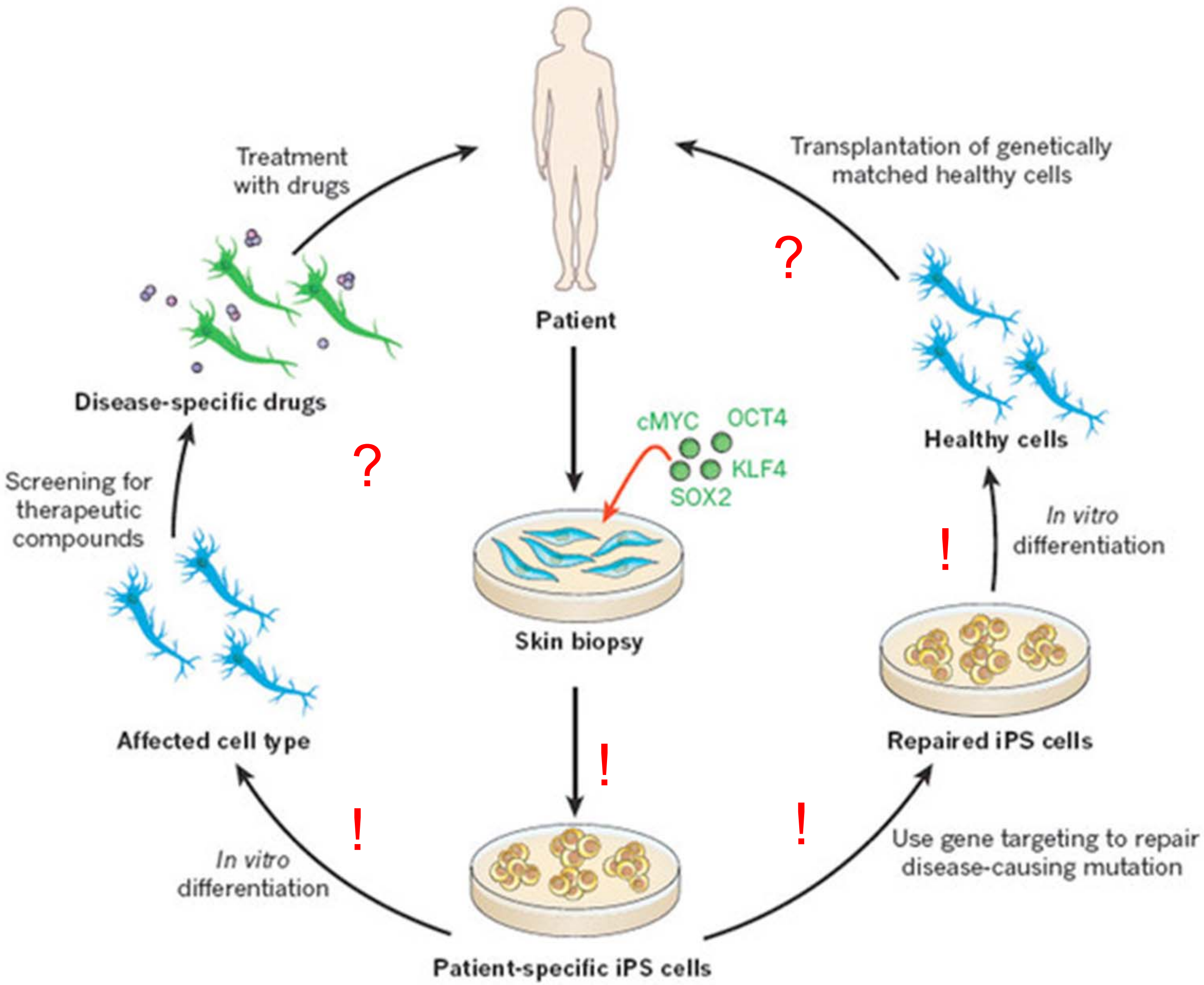
Forskolin -> CFTR -> expansion





Schwank et al., Cell Stem Cell 2013

The Future of Regenerative Medicine



The Future of Regenerative Medicine

Very hopeful and promising,

but are we there yet?

http://www.sbs.com.au/news/insight/tvepisode/stem-cells

http://iview.abc.net.au/programs/head-first/DO1333V001S00

ANAT2341: lecture overview

Stem Cell Biology

Tissue homeostasis and regeneration
Stem cell biology
Stem cell niches
Stem cell regulation
Stem cells and cancer
Regenerative medicine
Stem cell sources
Future of regenerative medicine

Dr Annemiek Beverdam – School of Medical Sciences, UNSW
Wallace Wurth Building Room 234 – A.Beverdam@unsw.edu.au

ANAT2341: Stem Cell Lab

Stem cell generation: Orvin Atthi
Stem cell differentiation: James Isaac, Tony Wang
Regenerative Medicine: Anuj Chavan, Elisa Gill

Group Marianne Daher?

Duration: 15 minutes max!
Do not discuss M&M in detail
Do not improvise your lines, take time to rehearse