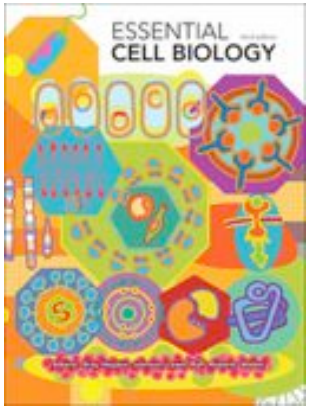
ANAT2341: lecture overview

Stem Cells



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

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ANAT2341: lecture overview

Stem Cell Biology

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

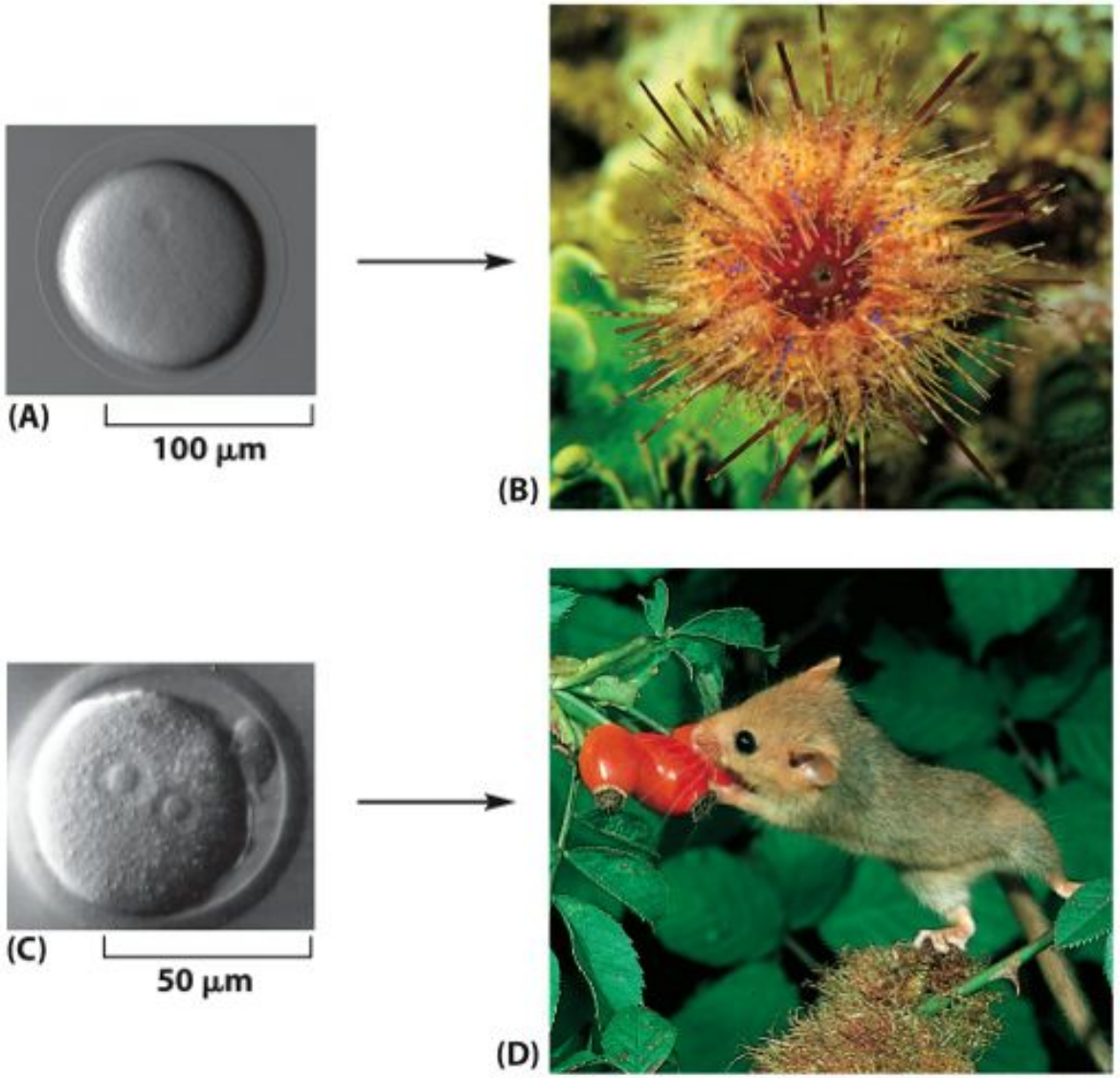
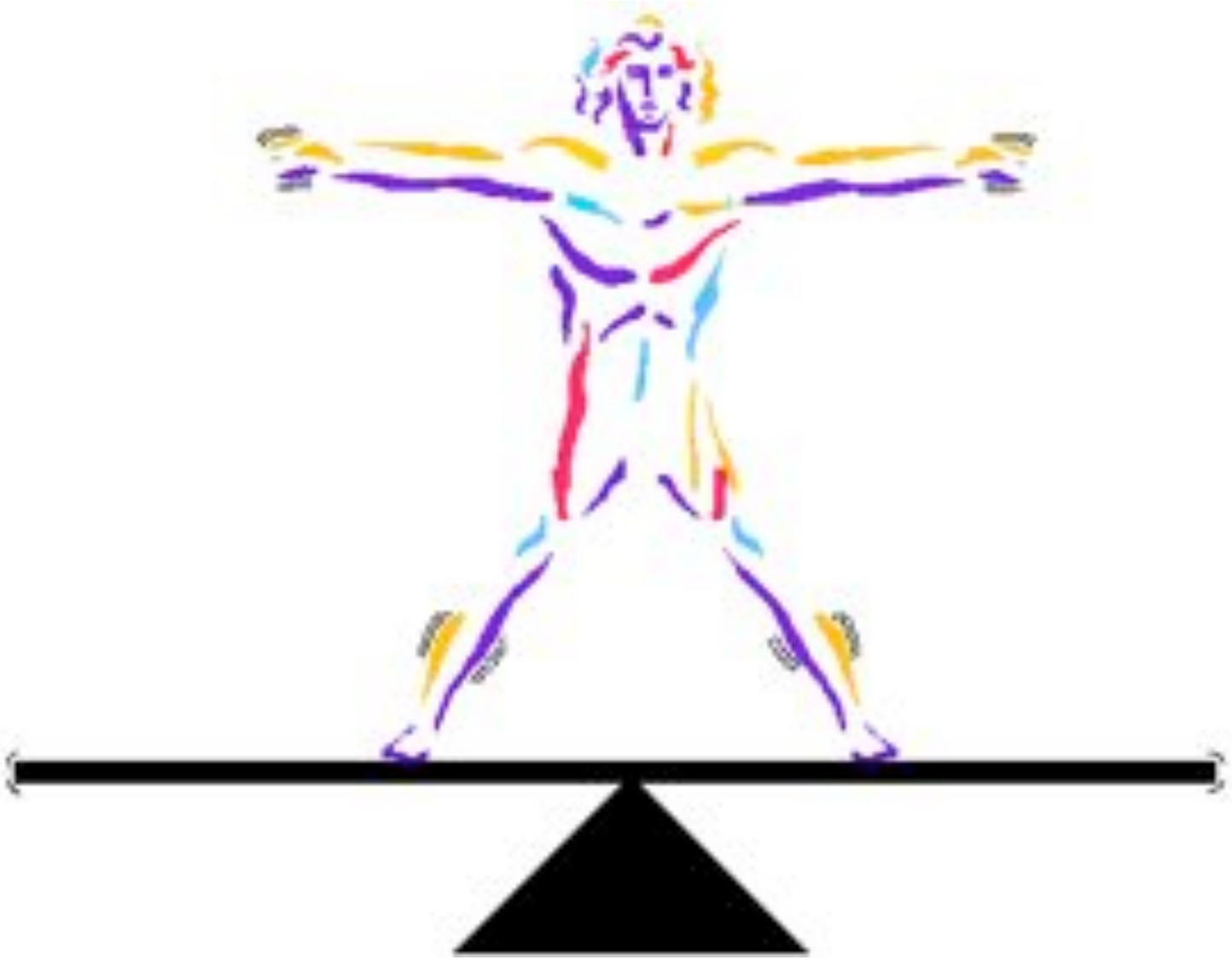
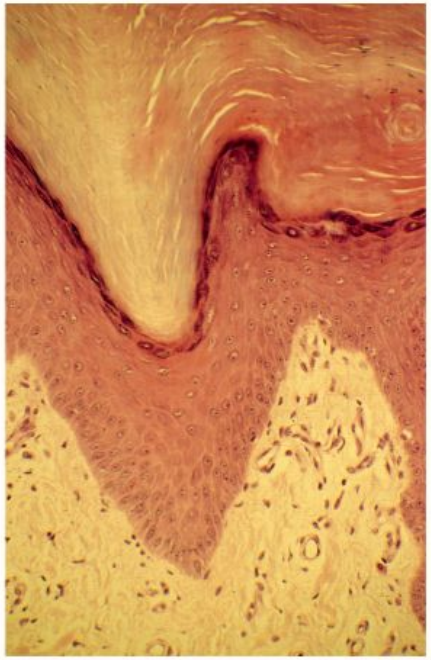


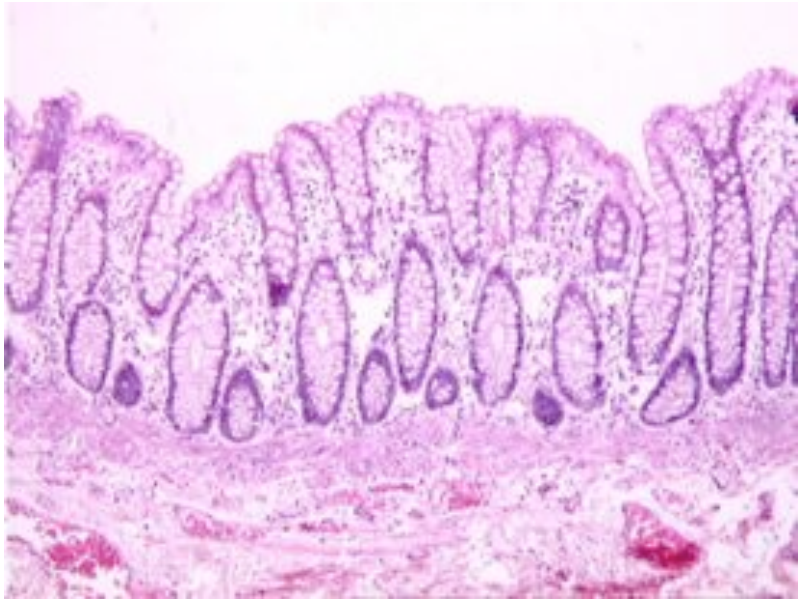
Figure 20-32 Essential Cell Biology 3/e (© Garland Science 2010)

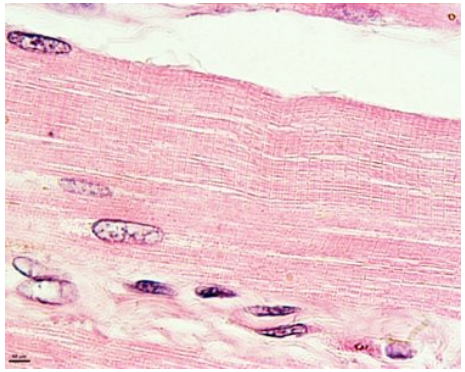
Tissue homeostasis

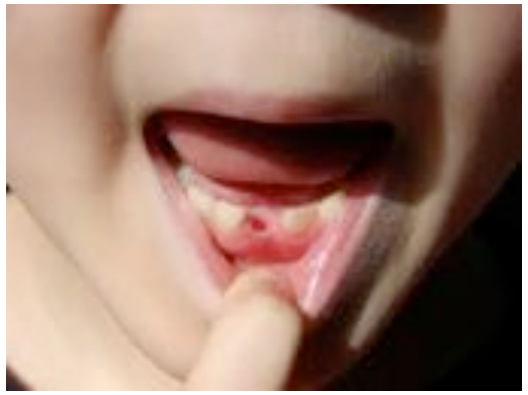


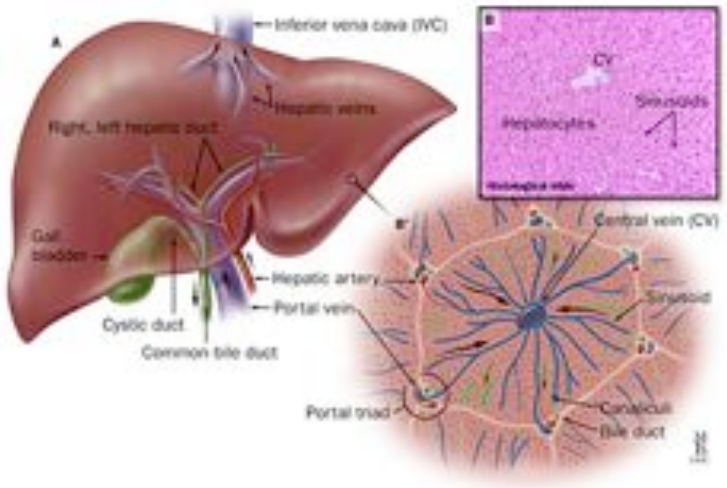
Tissue renewal in higher vertebrates









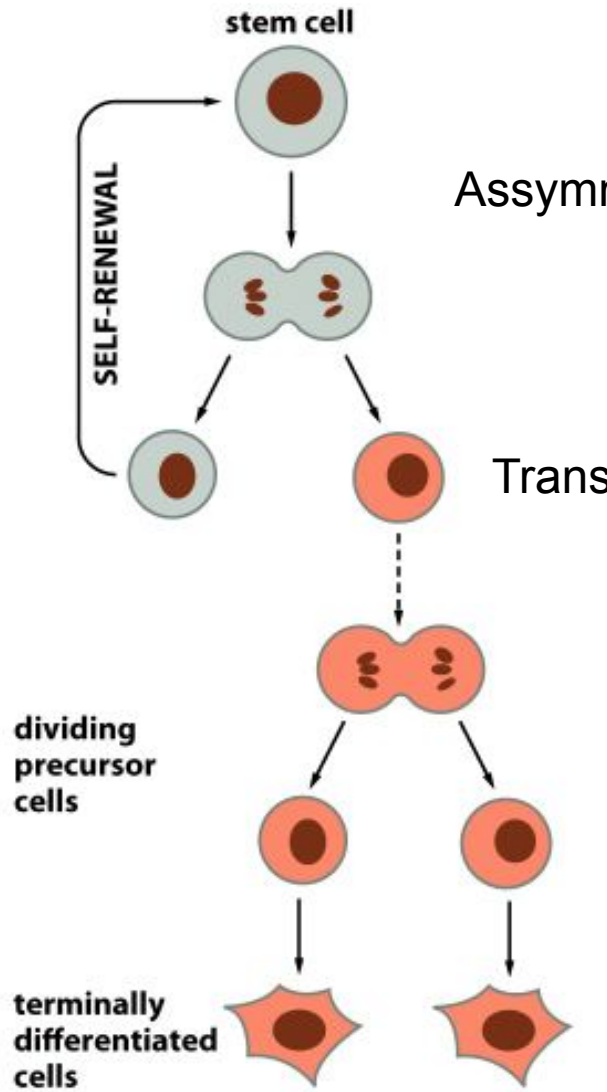


Stem cells divide to self renew and to produce terminally differentiated cell types

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

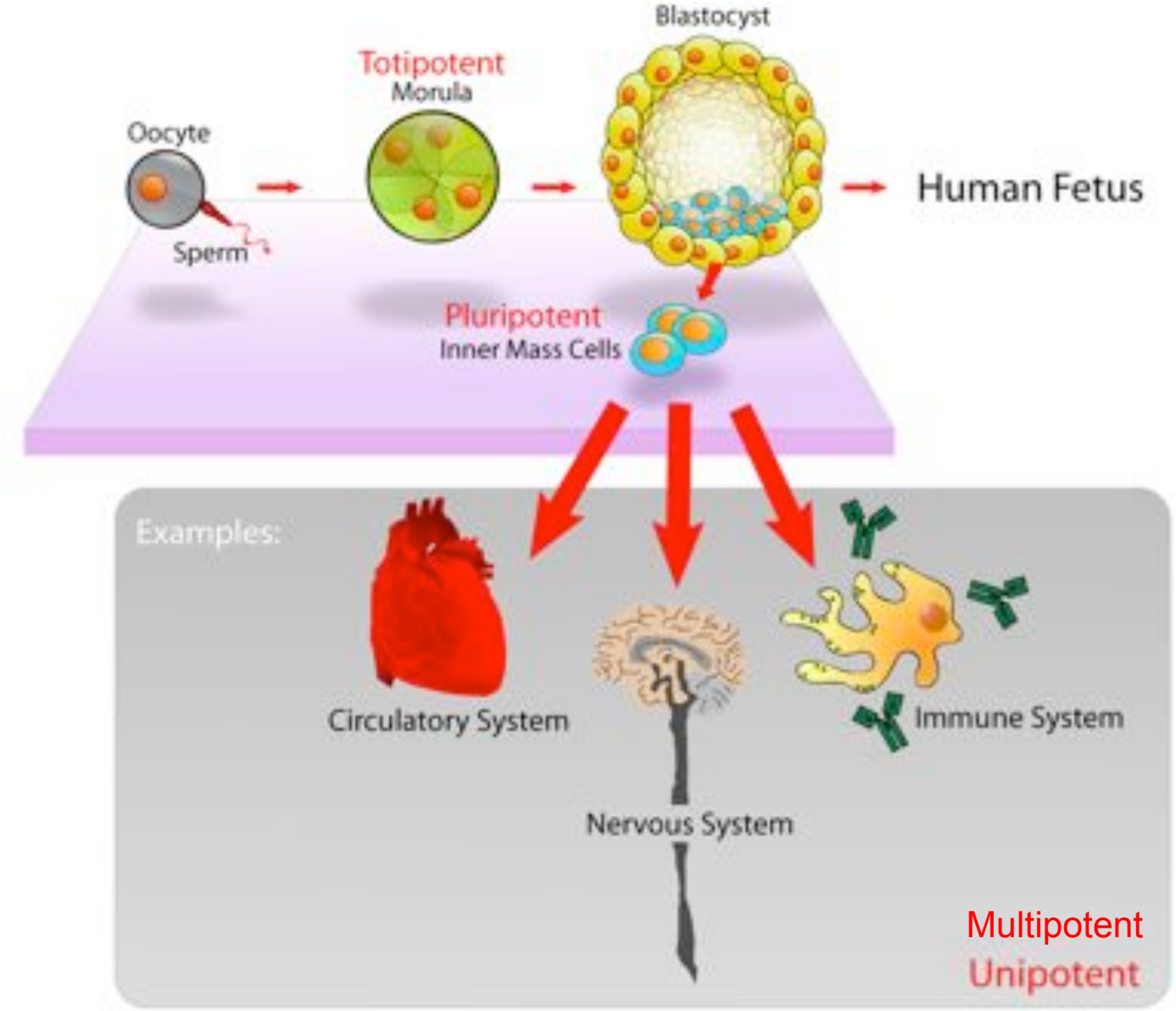
Multipotency:

capacity to give rise to more than 1 cell type

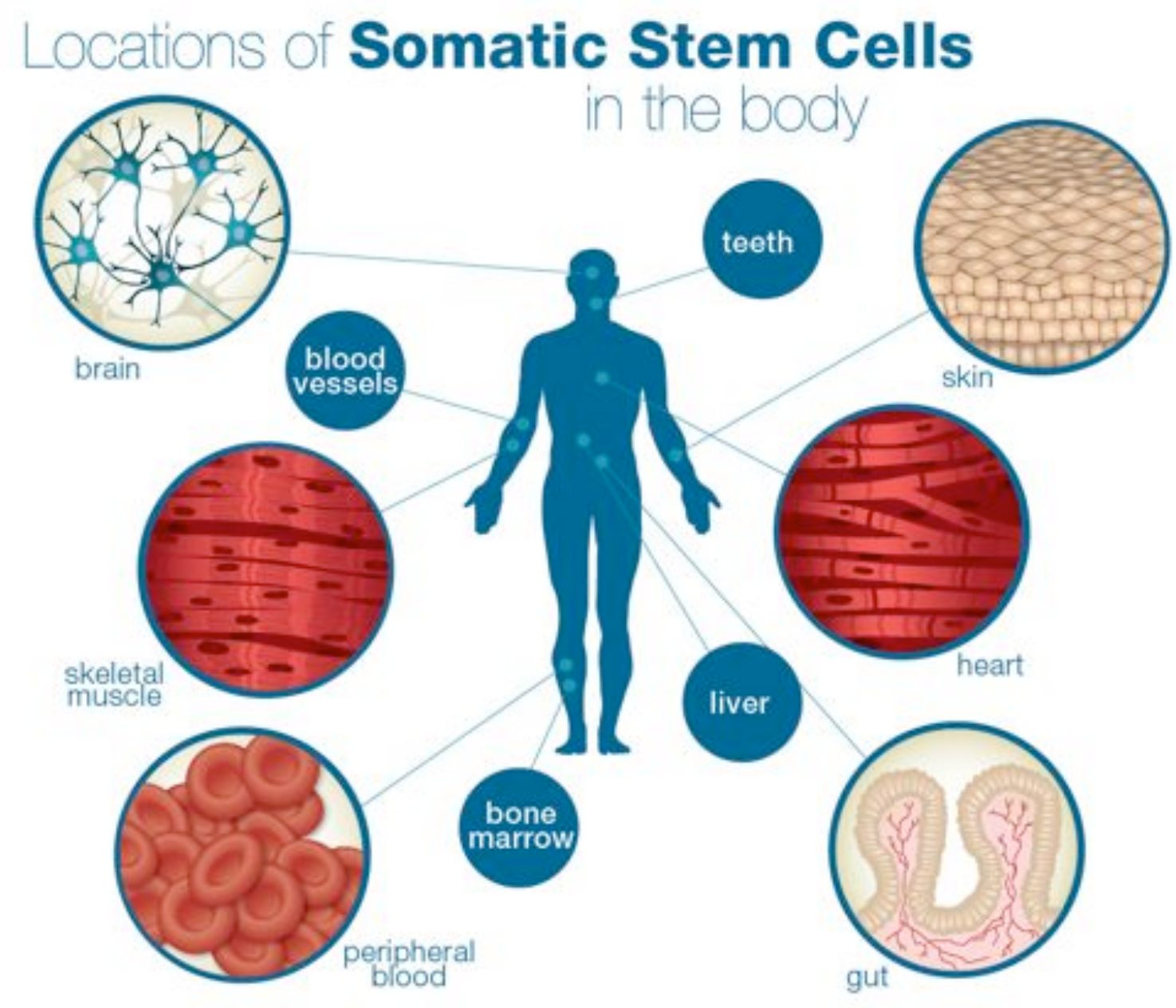
Unipotent stem cell:

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

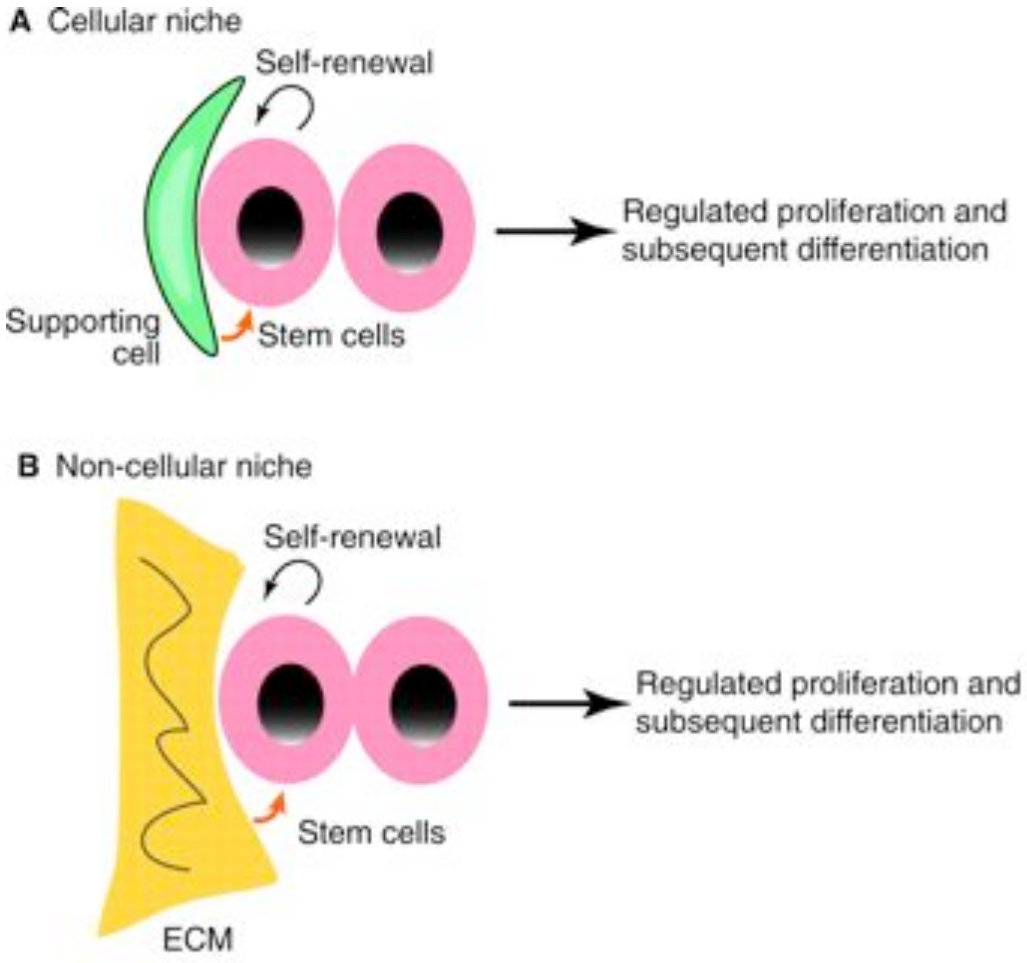
Stem cells potential



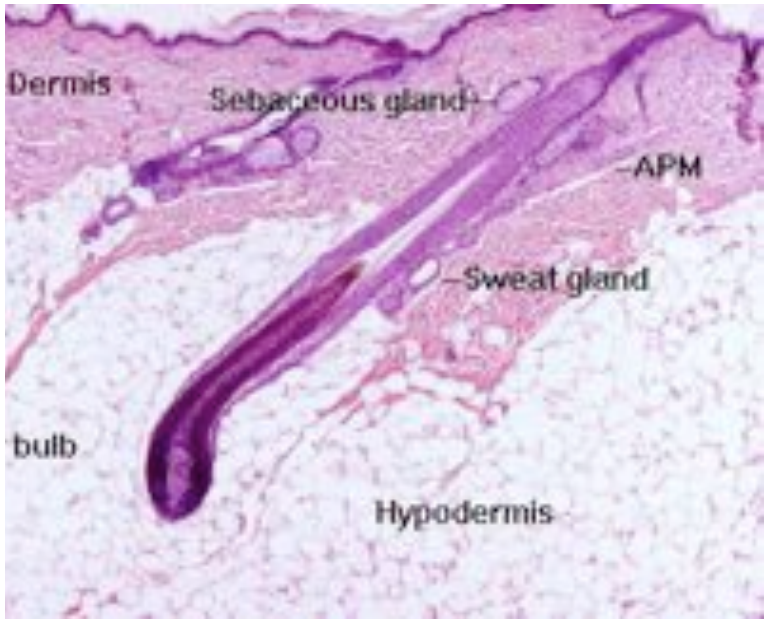
Adult stem cells

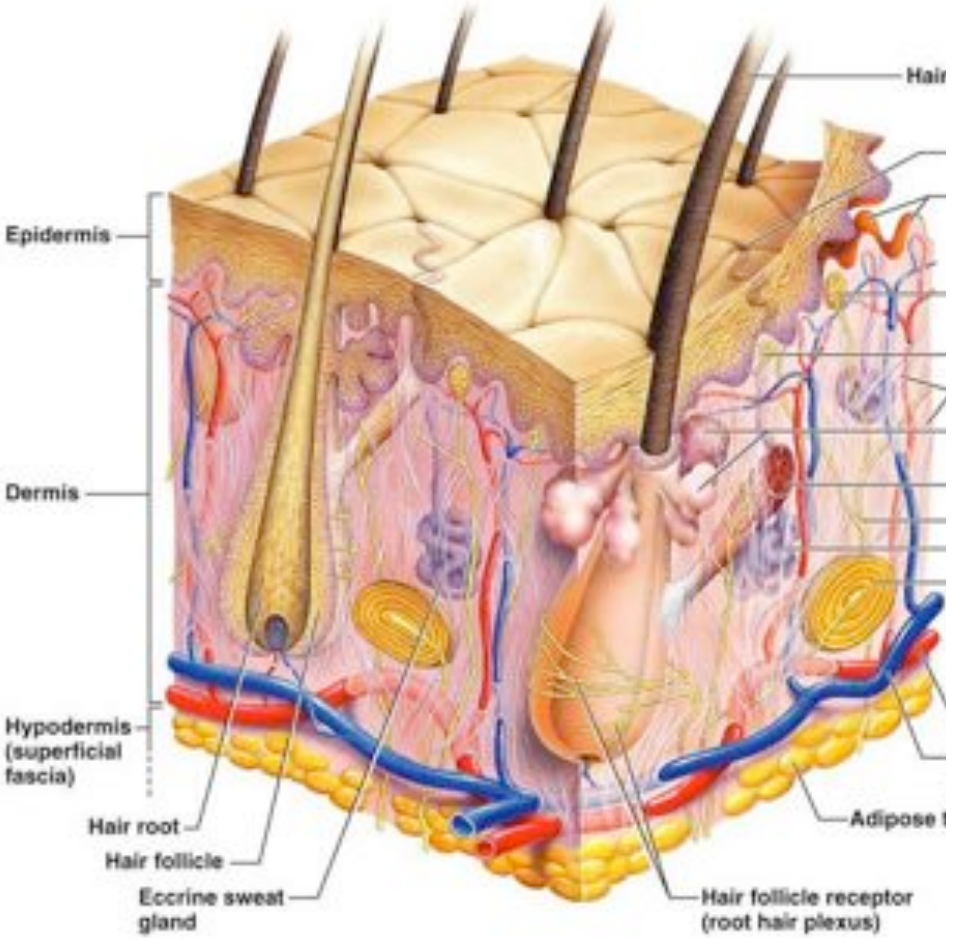


Stem cell niche:
Keeps stem cells in an undifferentiated state



Epidermal Stem Cell Niches





Basal Epidermal Stem Cells

life span basal keratinocyte: 1 month:

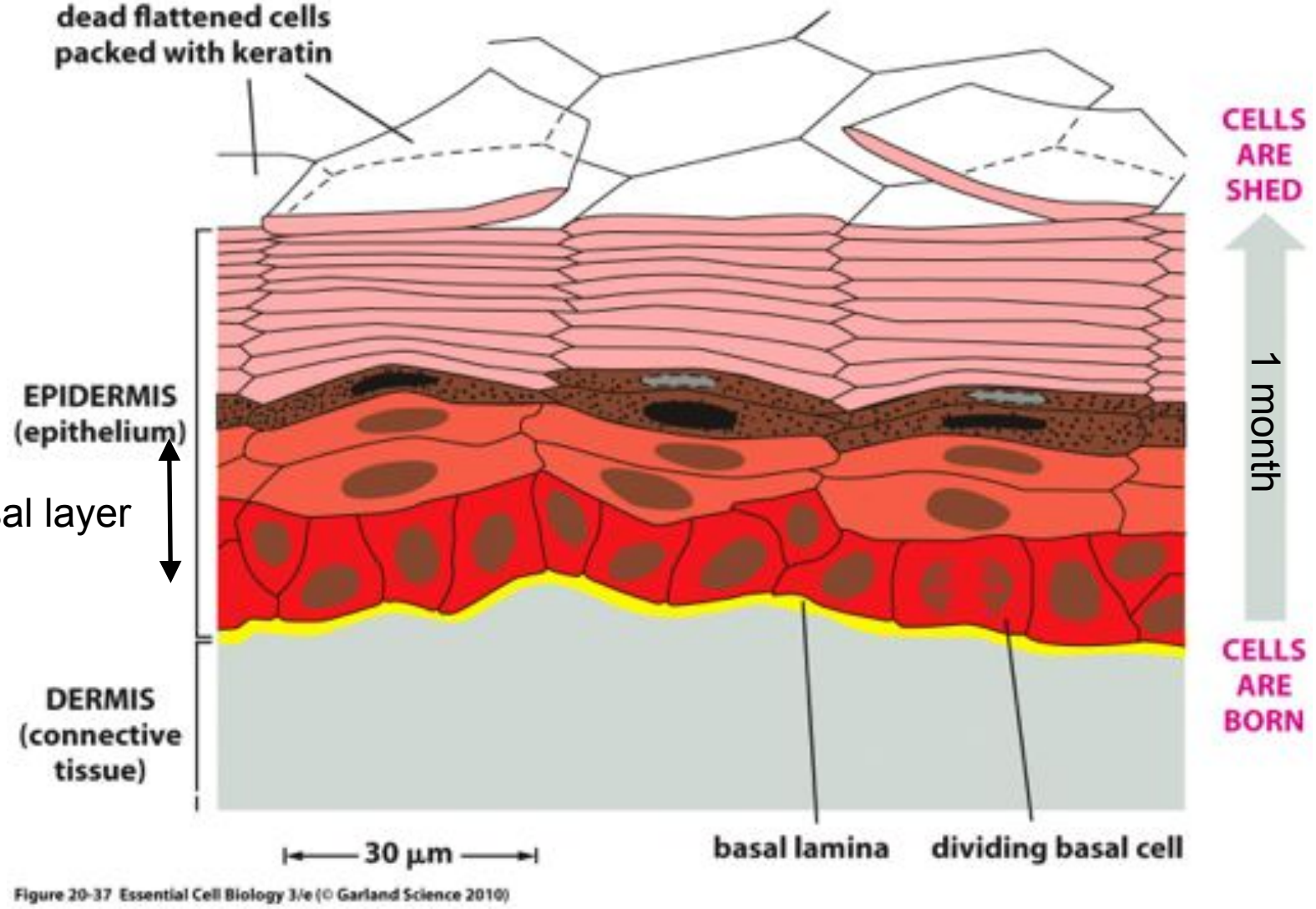
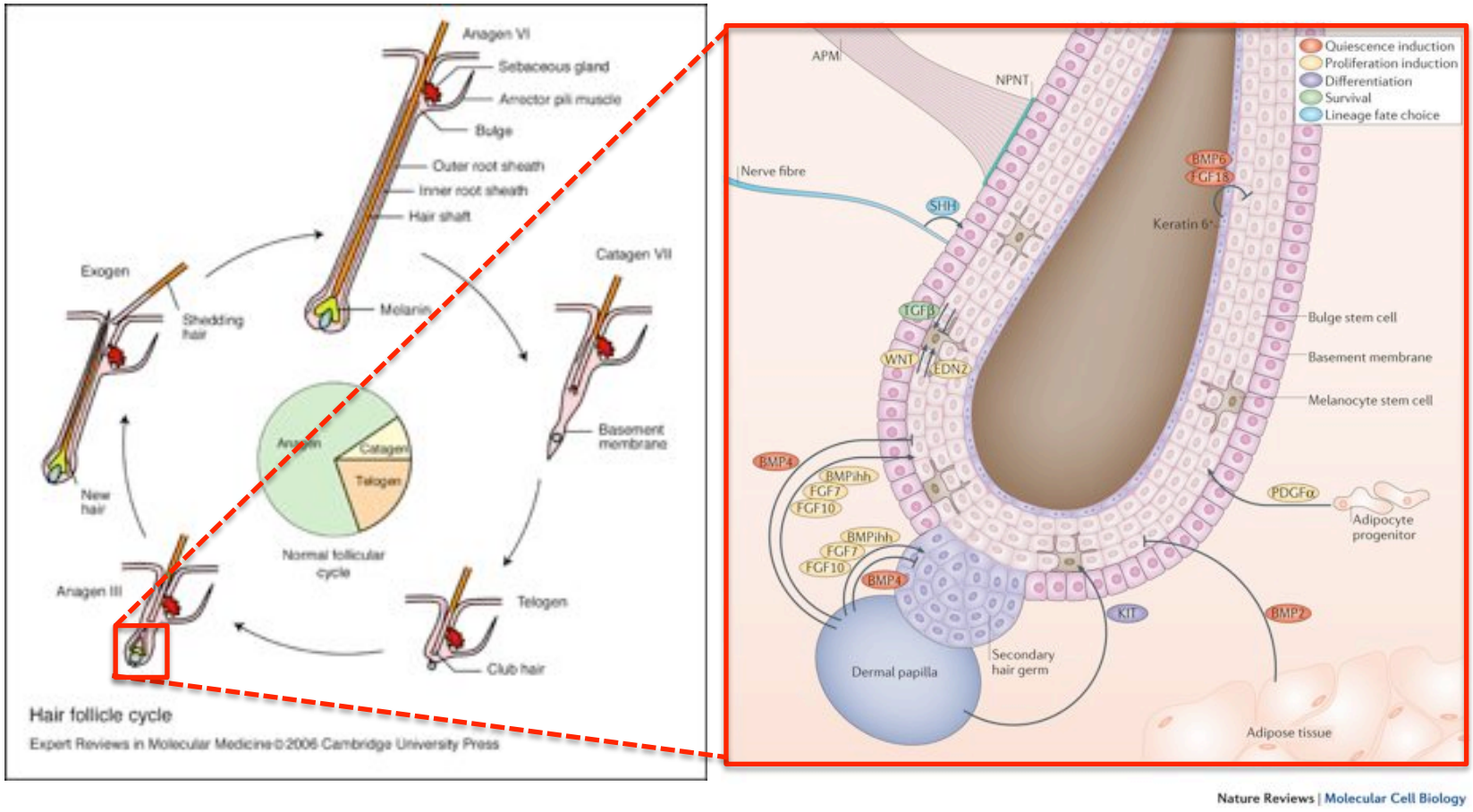


Figure 20-37 Essential Cell Biology 3/e (© Garland Science 2010)

Keratinocytes of the interfollicular epidermis

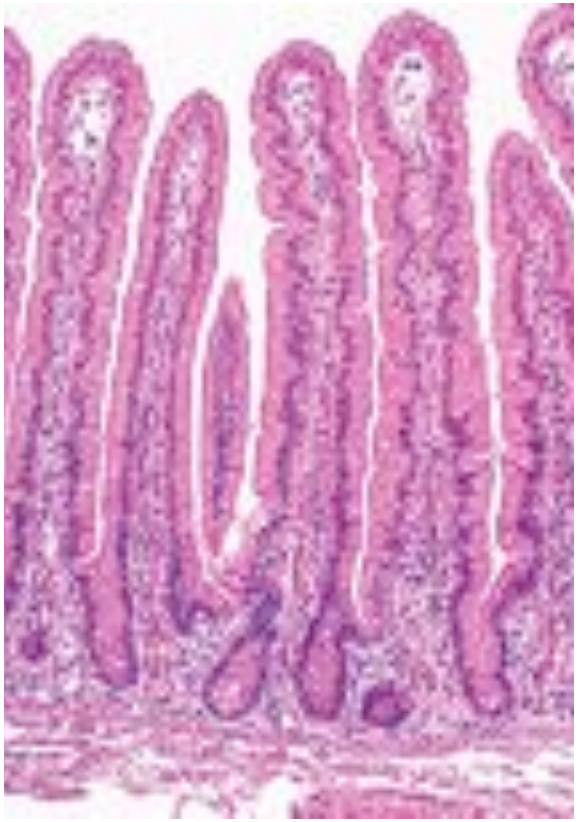
The Hair Follicle Bulge Stem Cell Niche

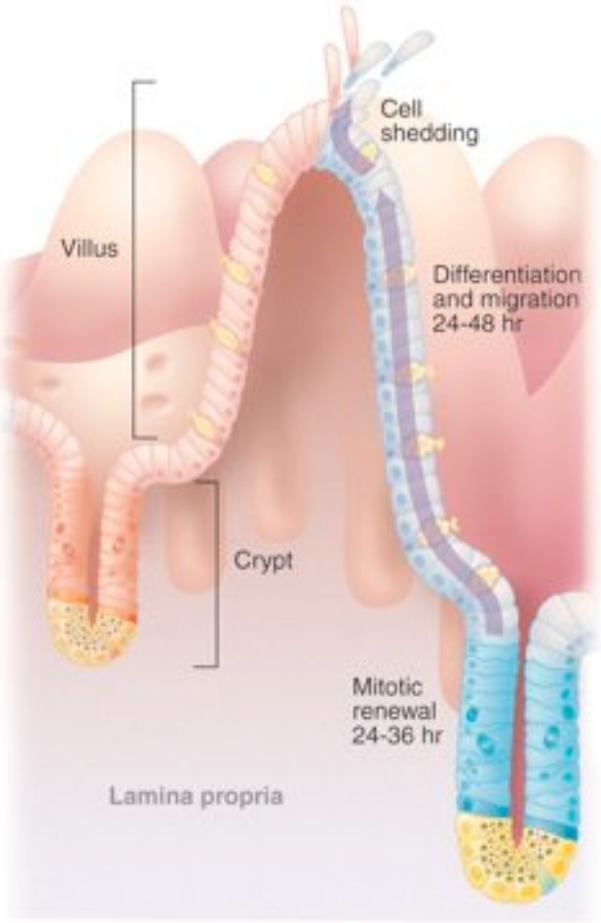


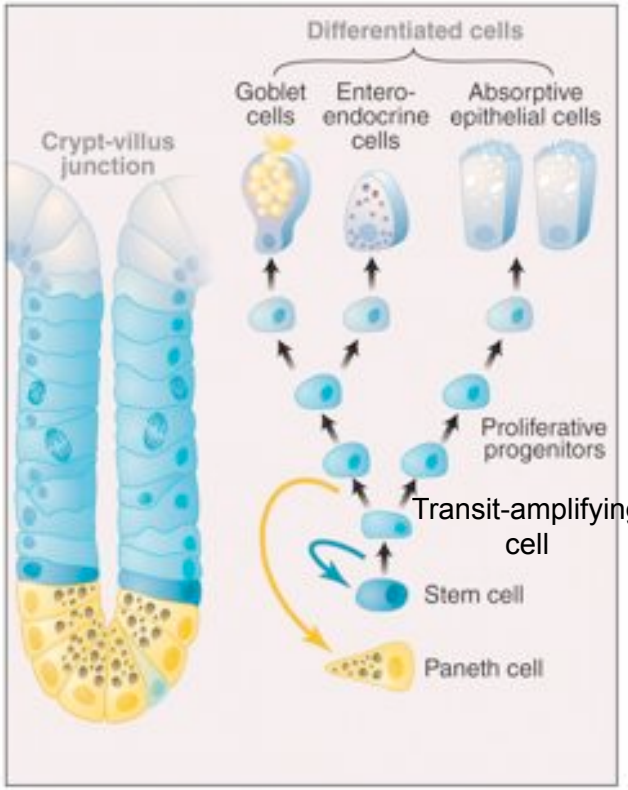
Epidermal bulge stem cells: hair follicle keratinocytes
Melanocyte bulge stem cells: melanocytes

The Intestinal Crypts Stem Cell Niche

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



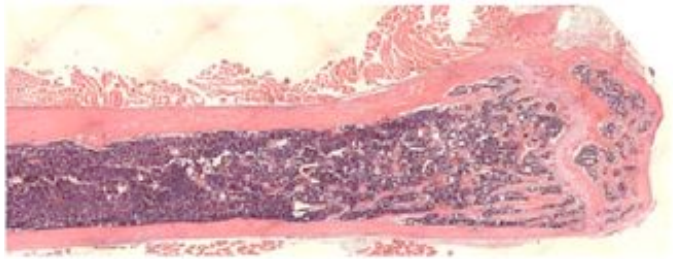


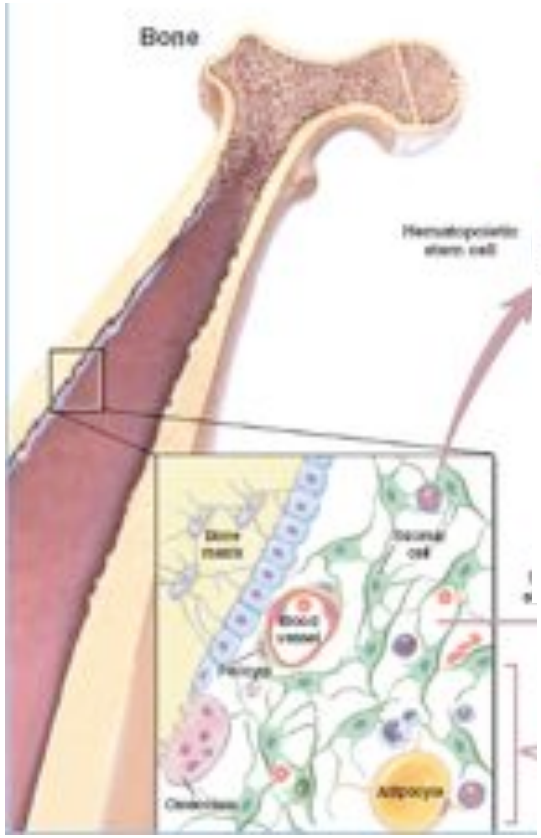


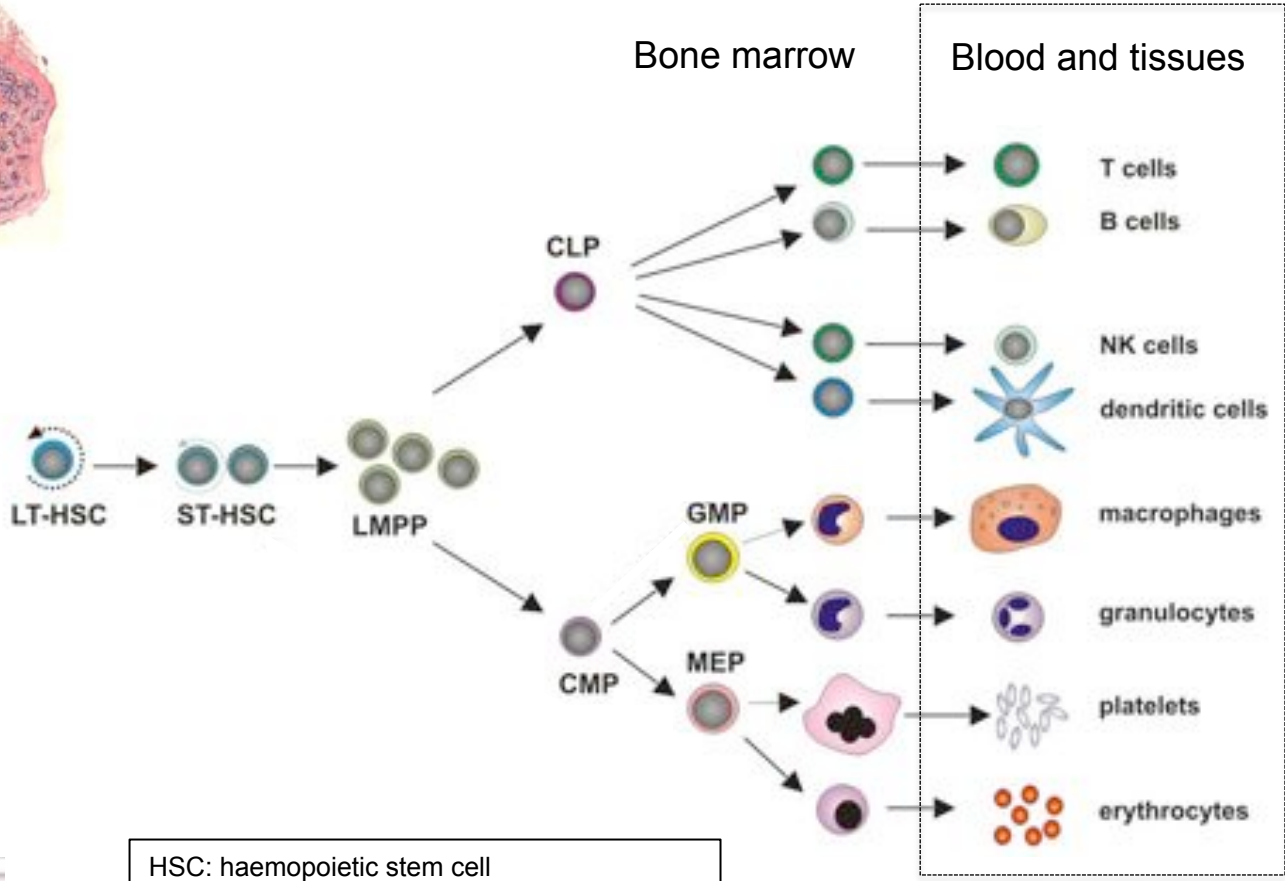
(Radtke and Clevers, Science 2005)

The Haemopoietic Stem Cell Niche

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







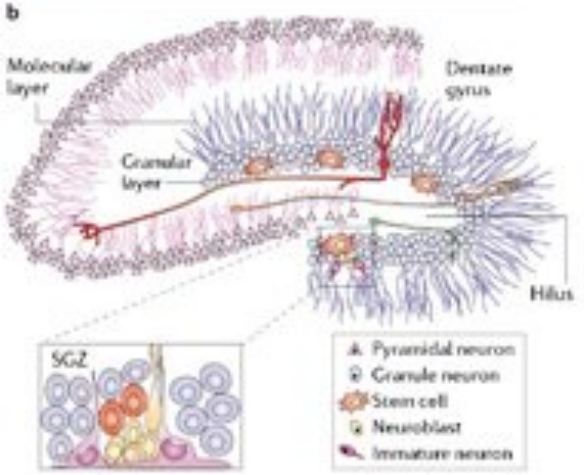
HSC: haemopoietic stem cell
MPP: multipotent progenitor cell
CLP: common lymphoid progenitor
CMP: common myeloid progenitor cell
GMP: granulocyte/monocyte precursor cell
MEP: Megakaryocyte-erythroid precursor cell

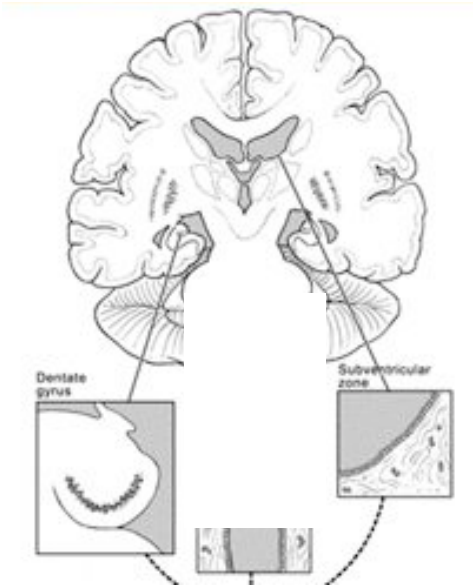
Figure 5.1. Hematopoietic Stem Cell Differentiation (2001 Terese Winslow, Lydia Kibiuk)

Subventricular and Subgranular Zone

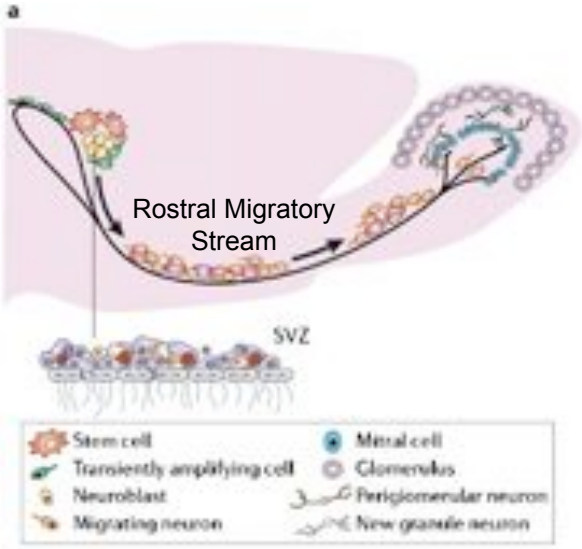
adult neurogenesis

SGZ





SVZ



radial glial cells (type B astrocyte)

↓

TA cells

↓

neuroblasts

↓

post-mitotic excitatory granule cells of dentate gyrus

multipotent type B astrocytes

↓

type C precursors (TA cells)

↓

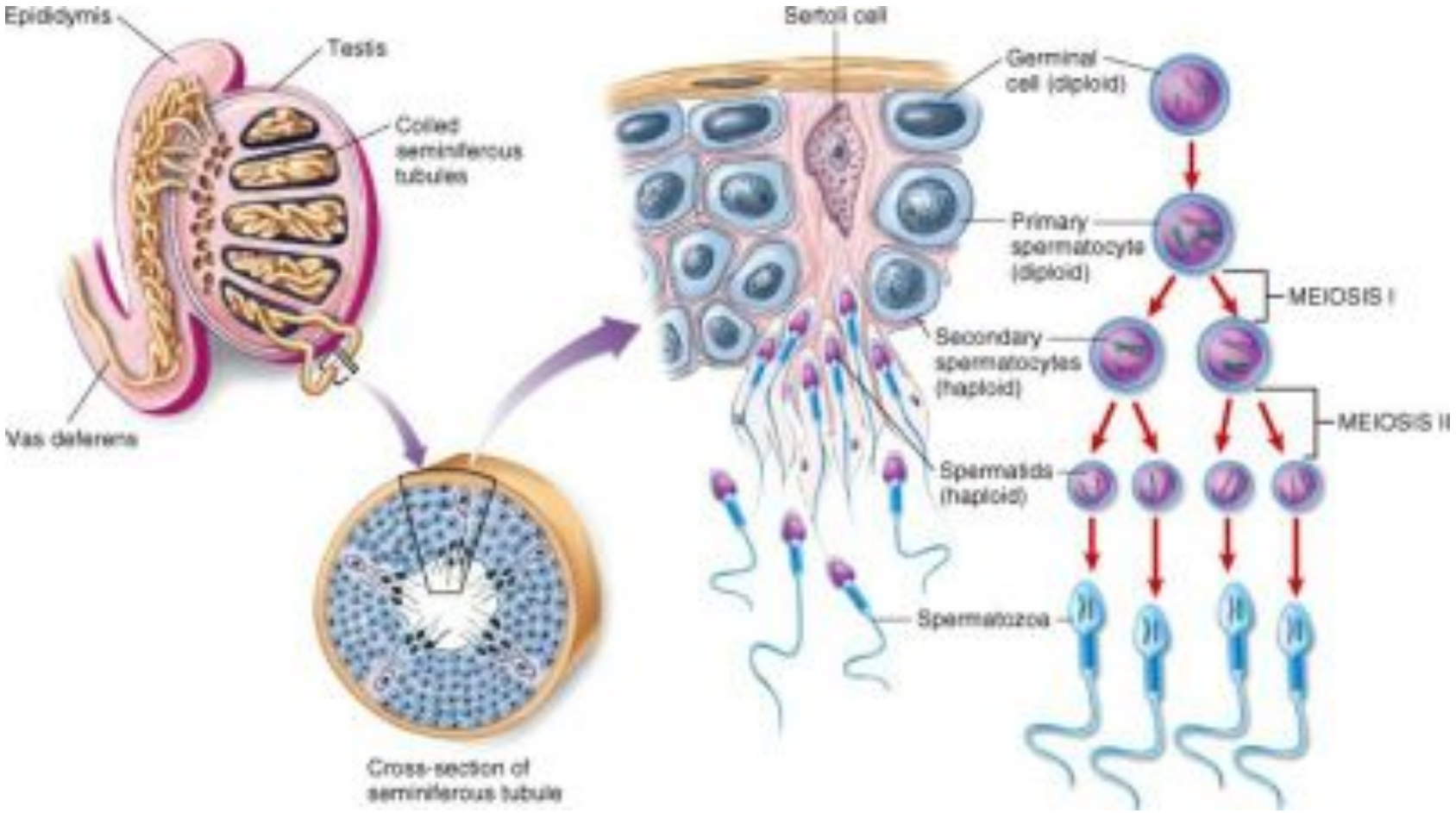
type A neuroblasts (migratory)

↓

interneurons

Seminiferous Tubules

Spermatogenesis: 2 months life span



Regulation of Stem Cells

Should I stay quiescent?

Should I die?

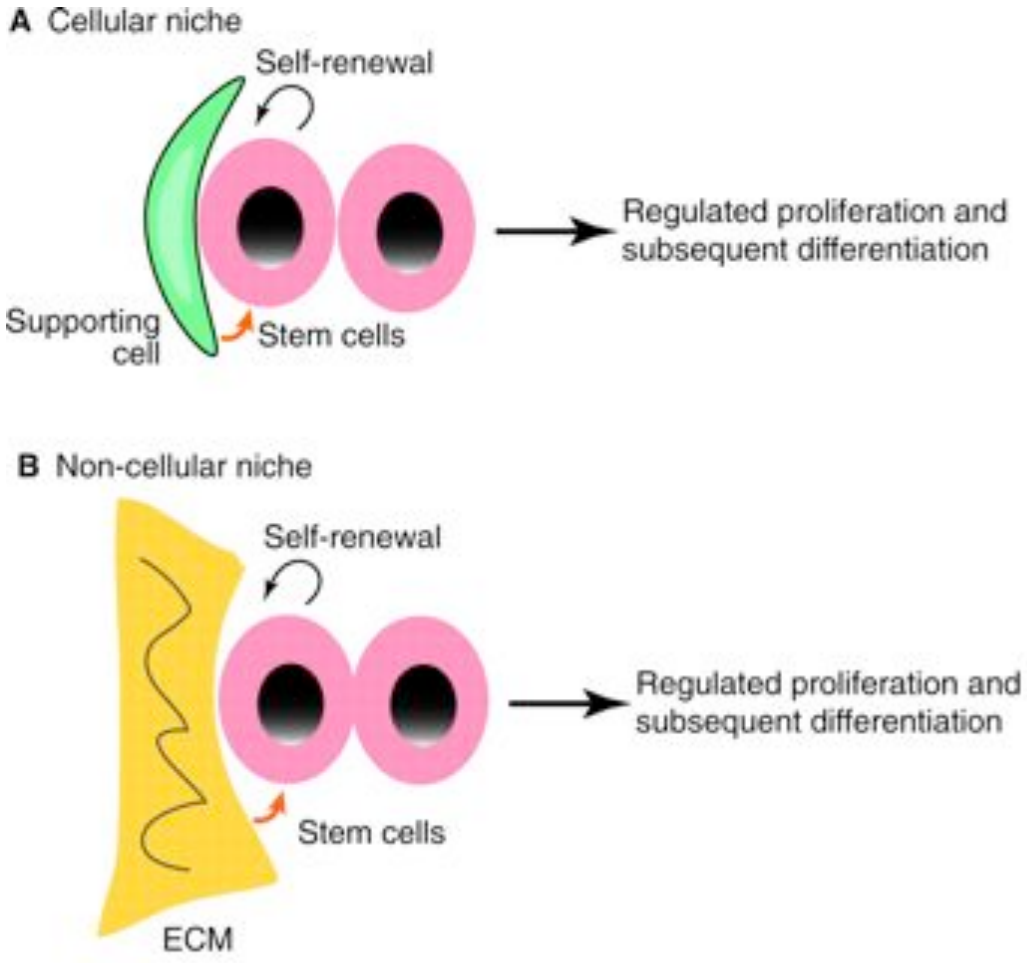
Should I proliferate?

Should I self-renew?

Should I generate transit amplifying cells?

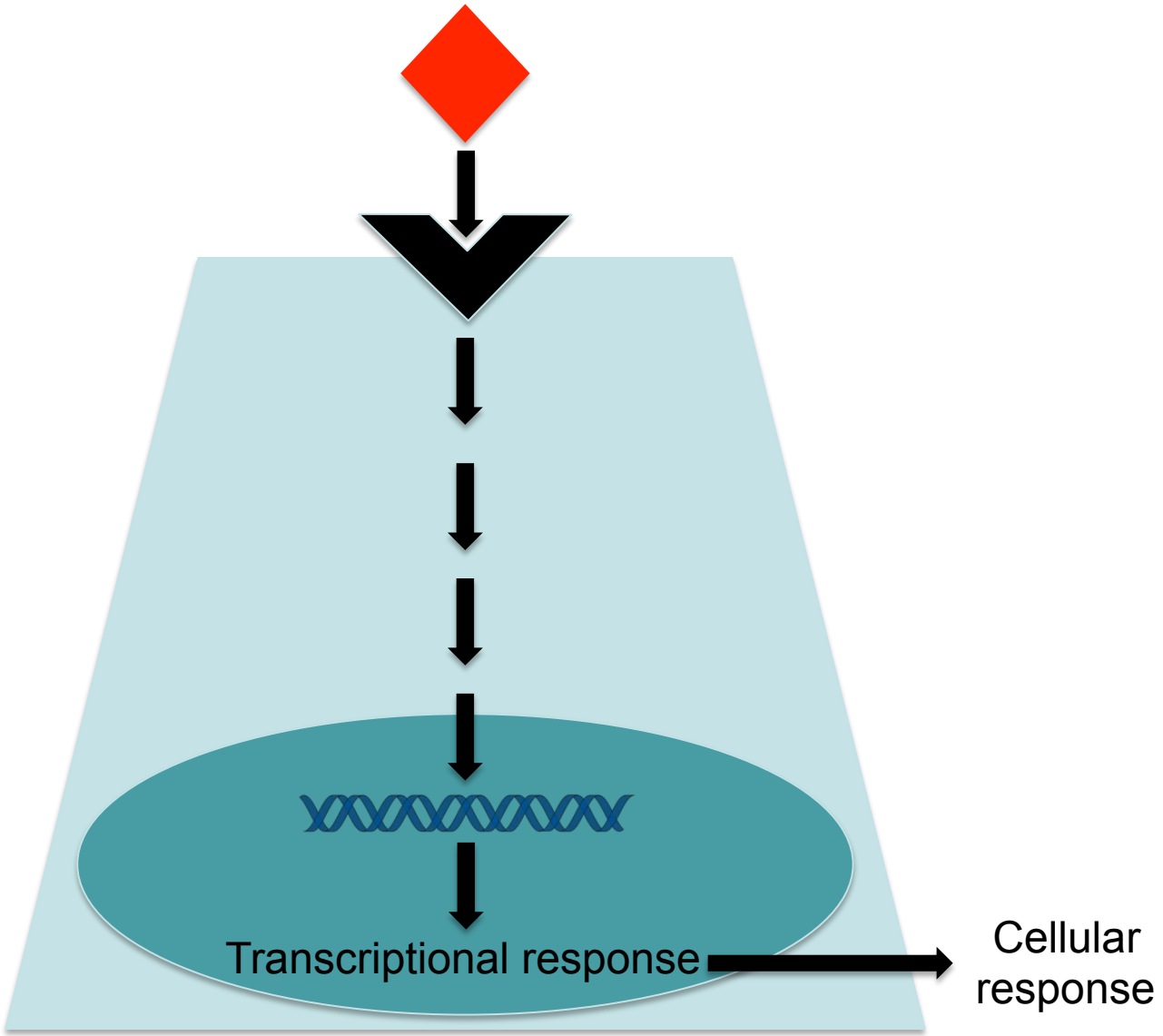
Should I generate differentiating daughter cells?

Stem cell niche:
Keeps stem cells in an undifferentiated state

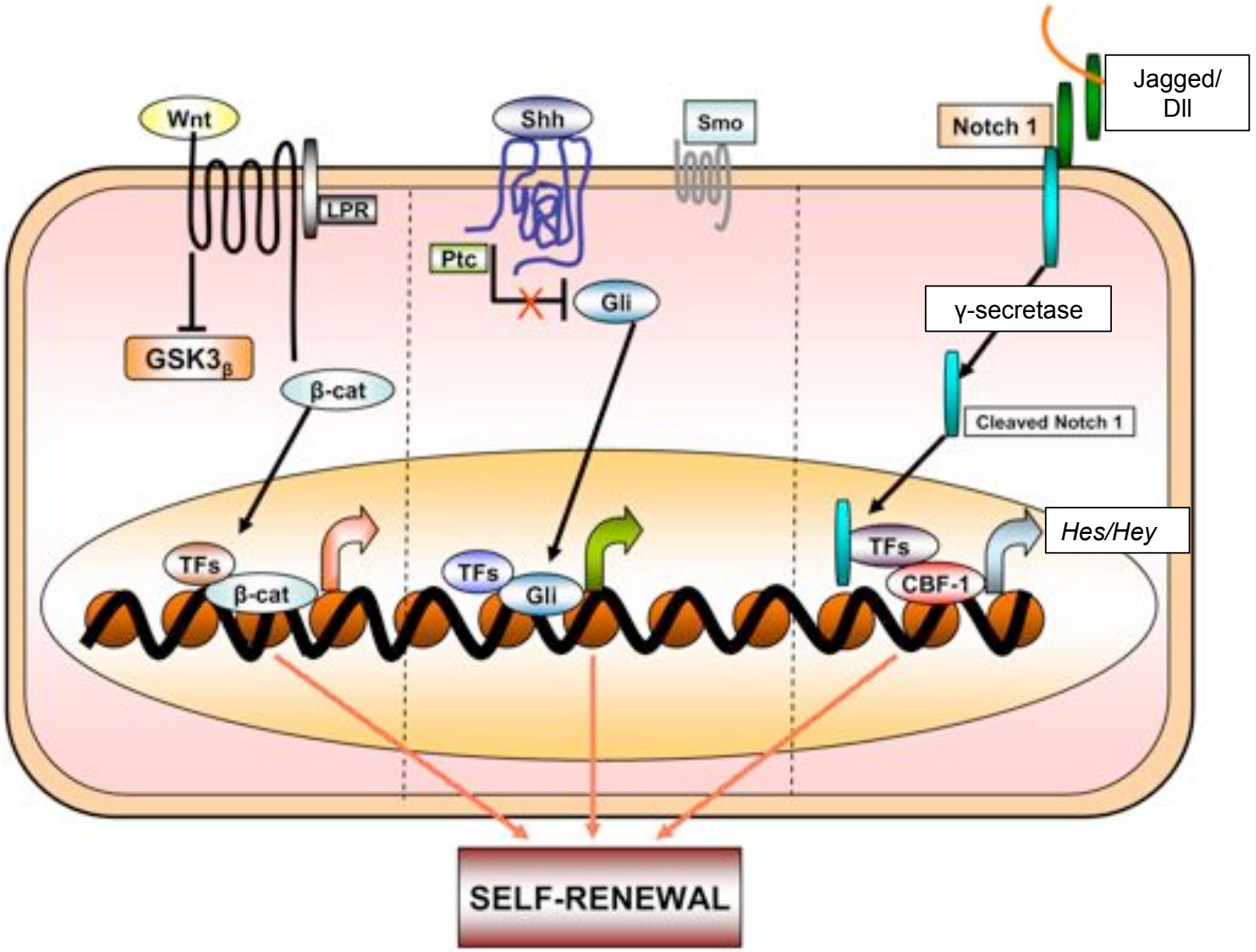


Regulation of Stem Cells

Signalling pathways

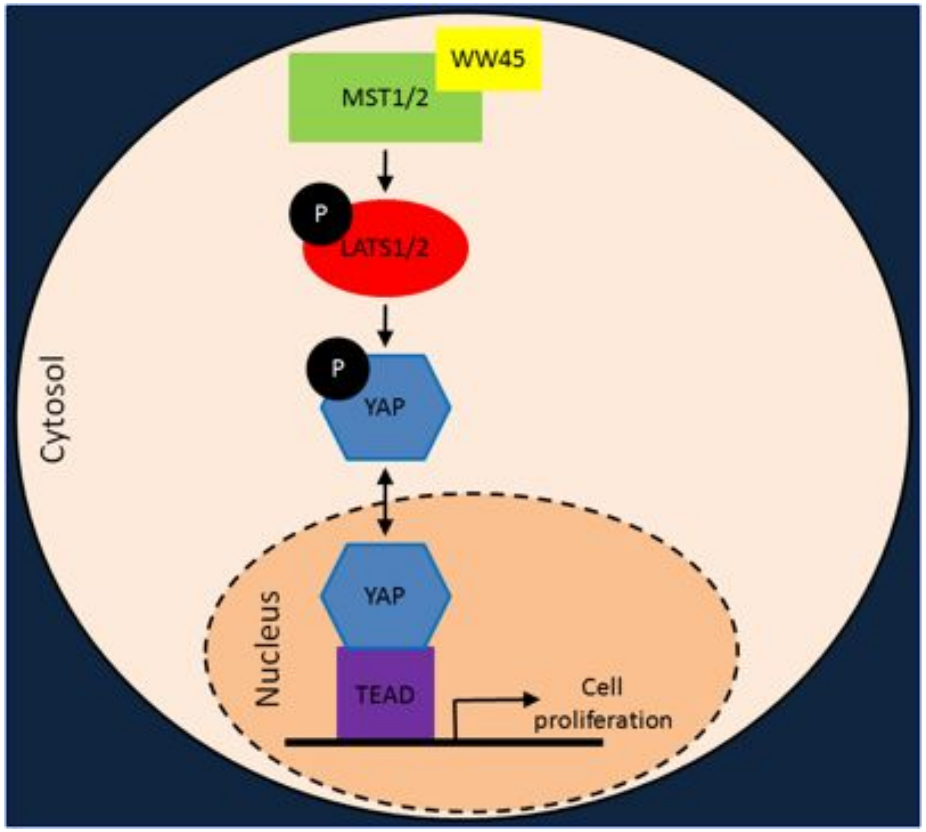


Regulation of Stem Cells

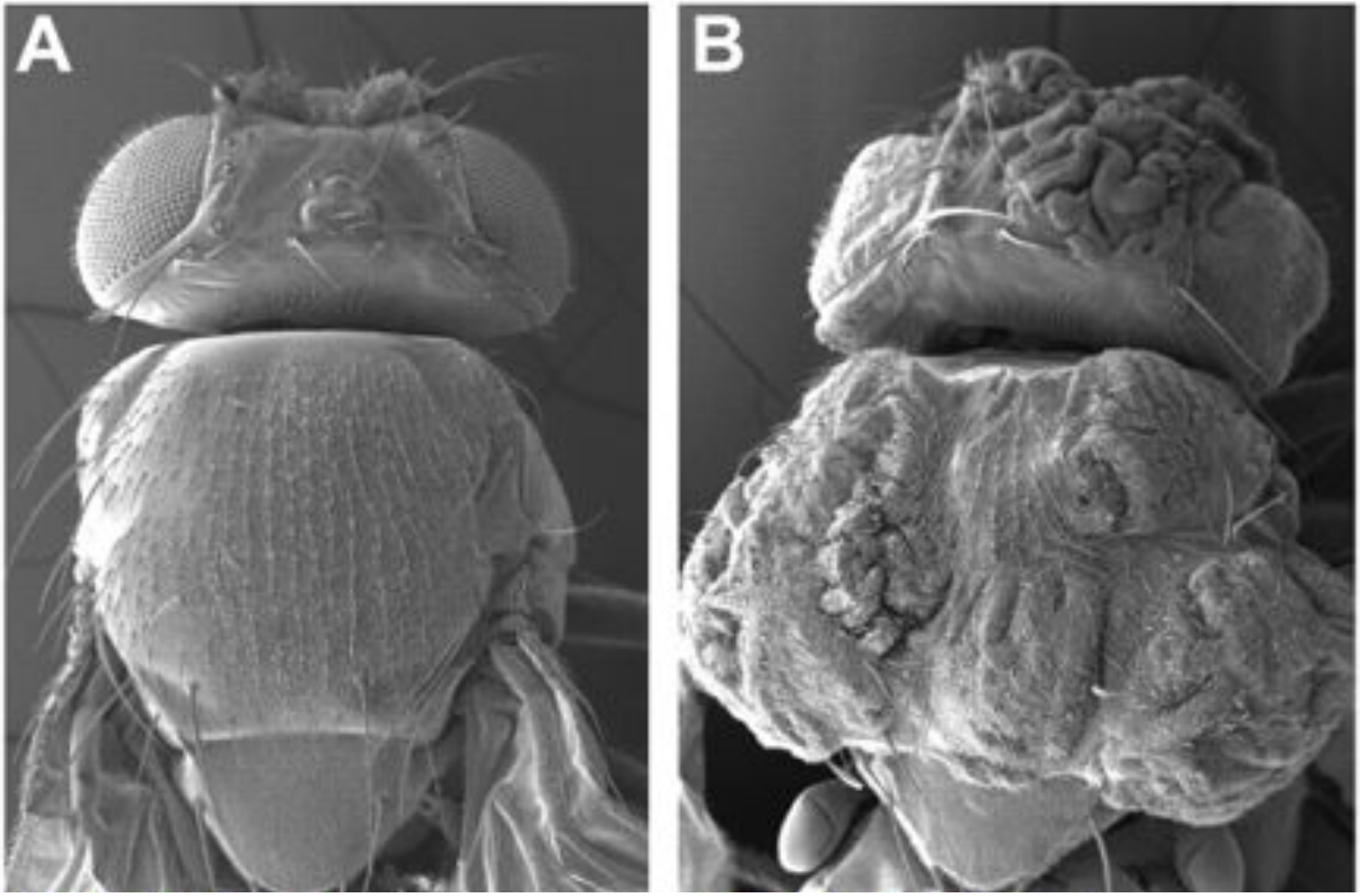


Regulation of Stem Cells

The Hippo Pathway

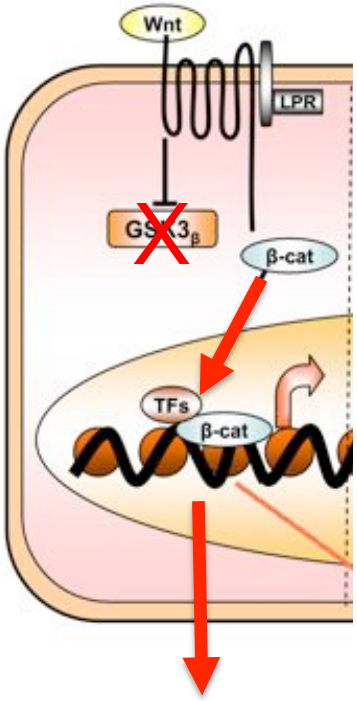


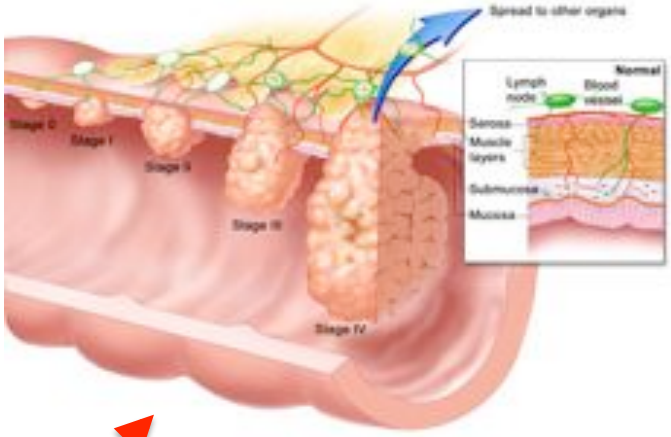
What happens if cell renewal regulation goes wrong?

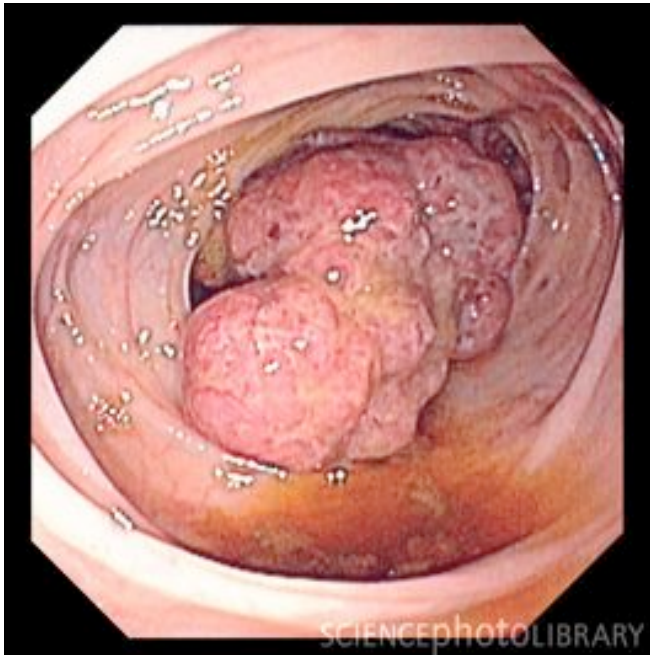


Mutations in Wnt pathway result in Cancer

Adenomatous Polyposis Coli



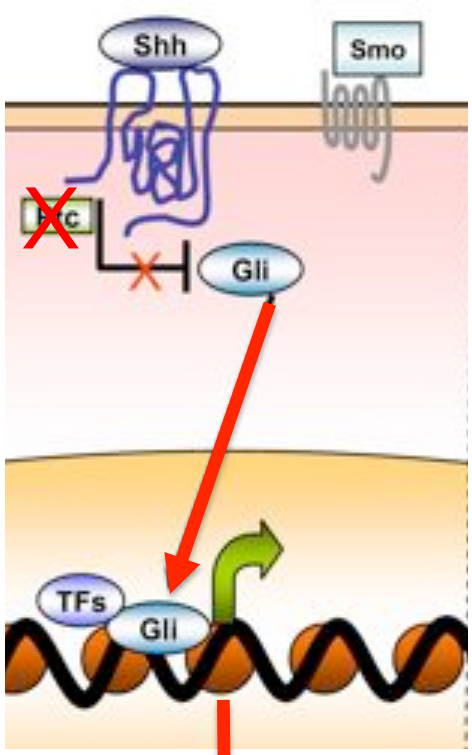




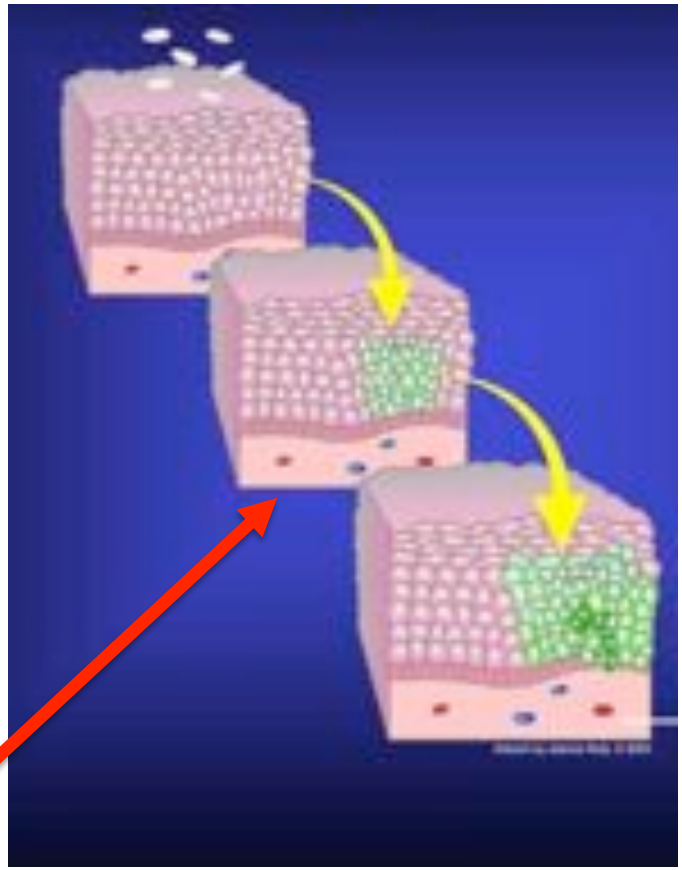
hyperproliferation

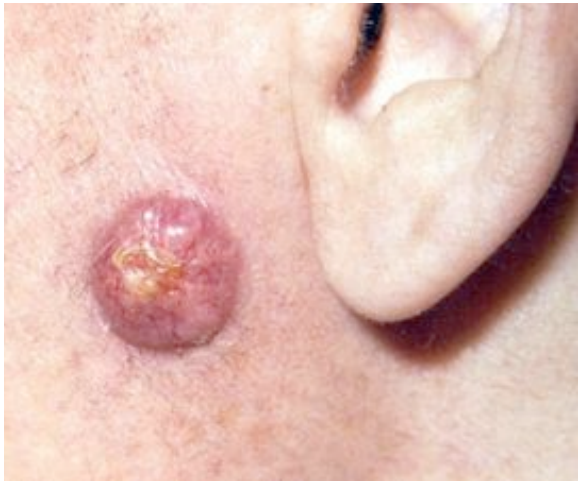
Mutations in Hedgehog pathway result in cancer

Basal Cell Carcinoma



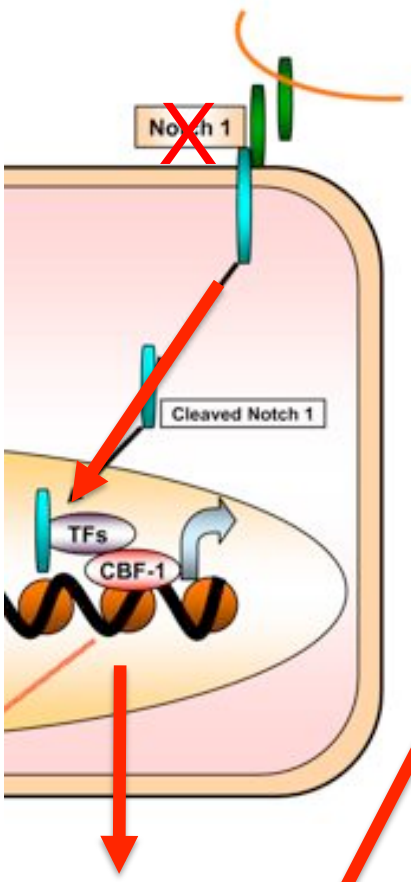
hyperproliferation



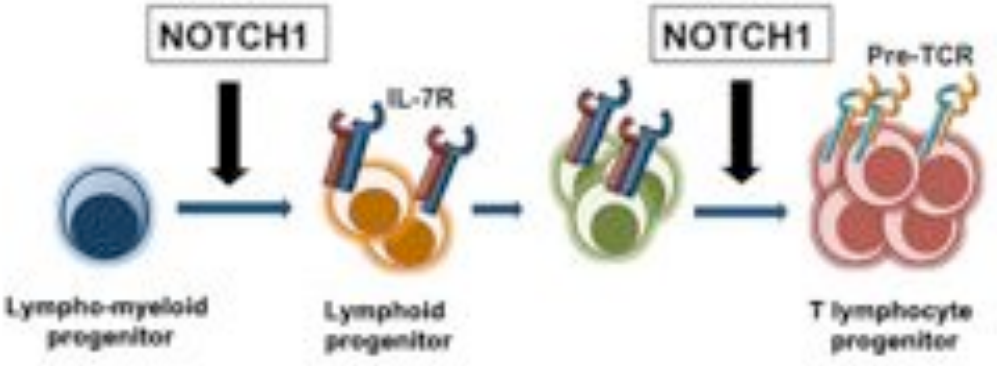


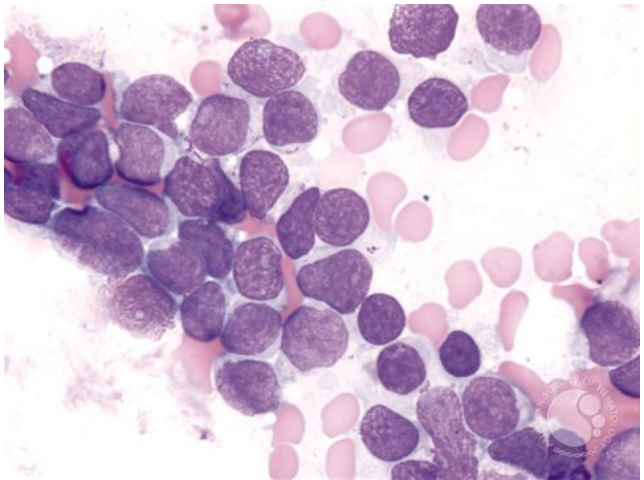
Mutations in Notch pathway result in cancer

T cell acute lymphoblastic leukemia



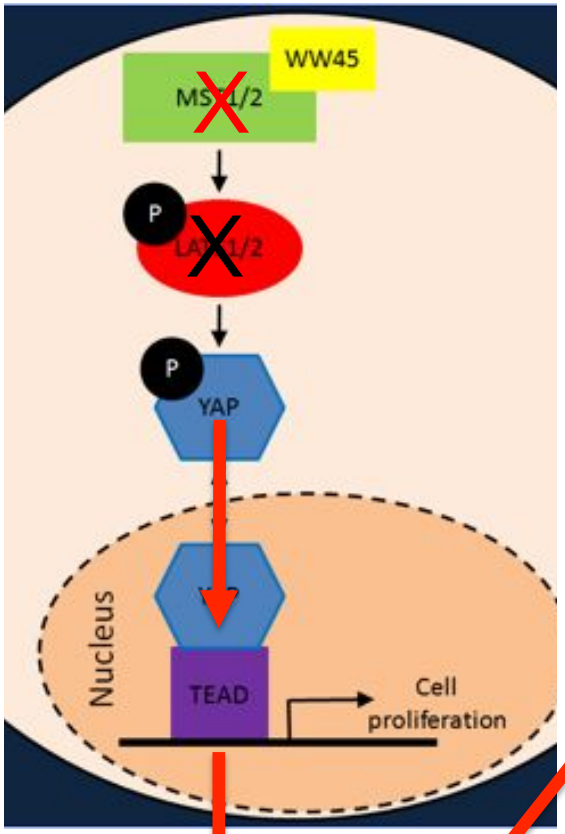
hyperproliferation

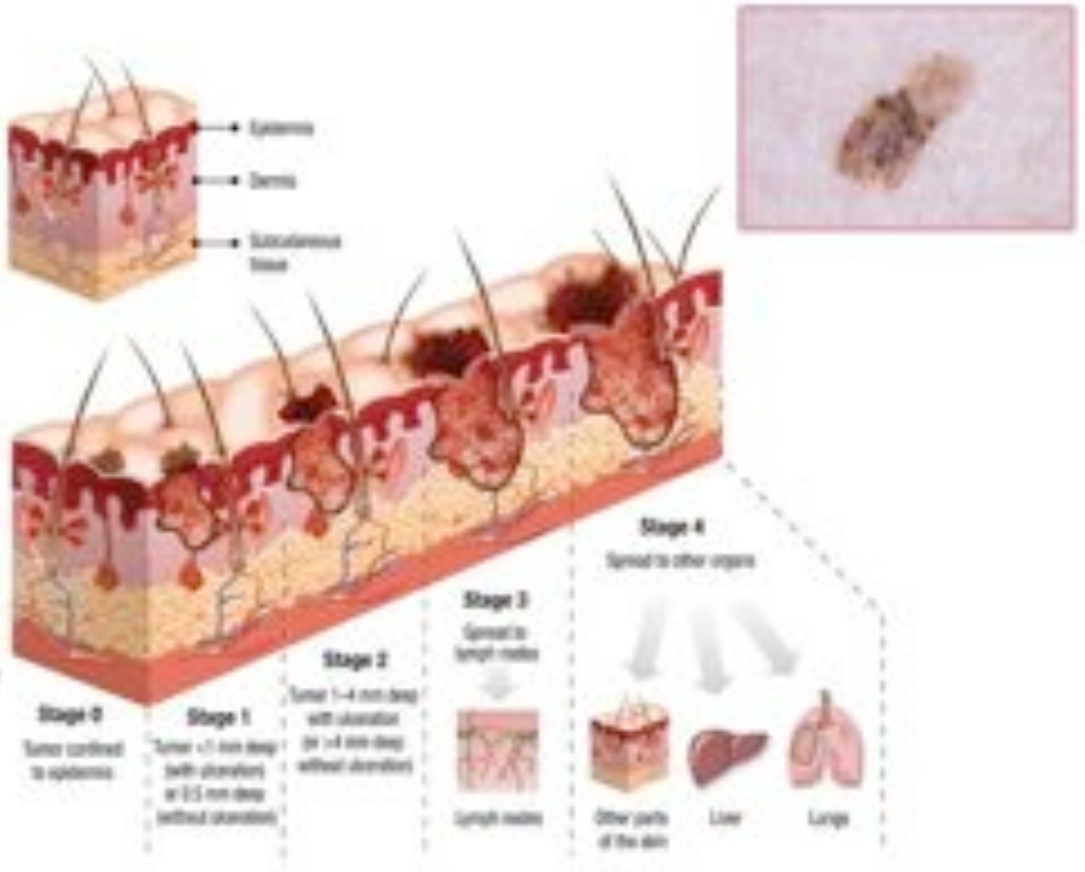




Mutations in Hippo pathway result in cancer

skin cancer

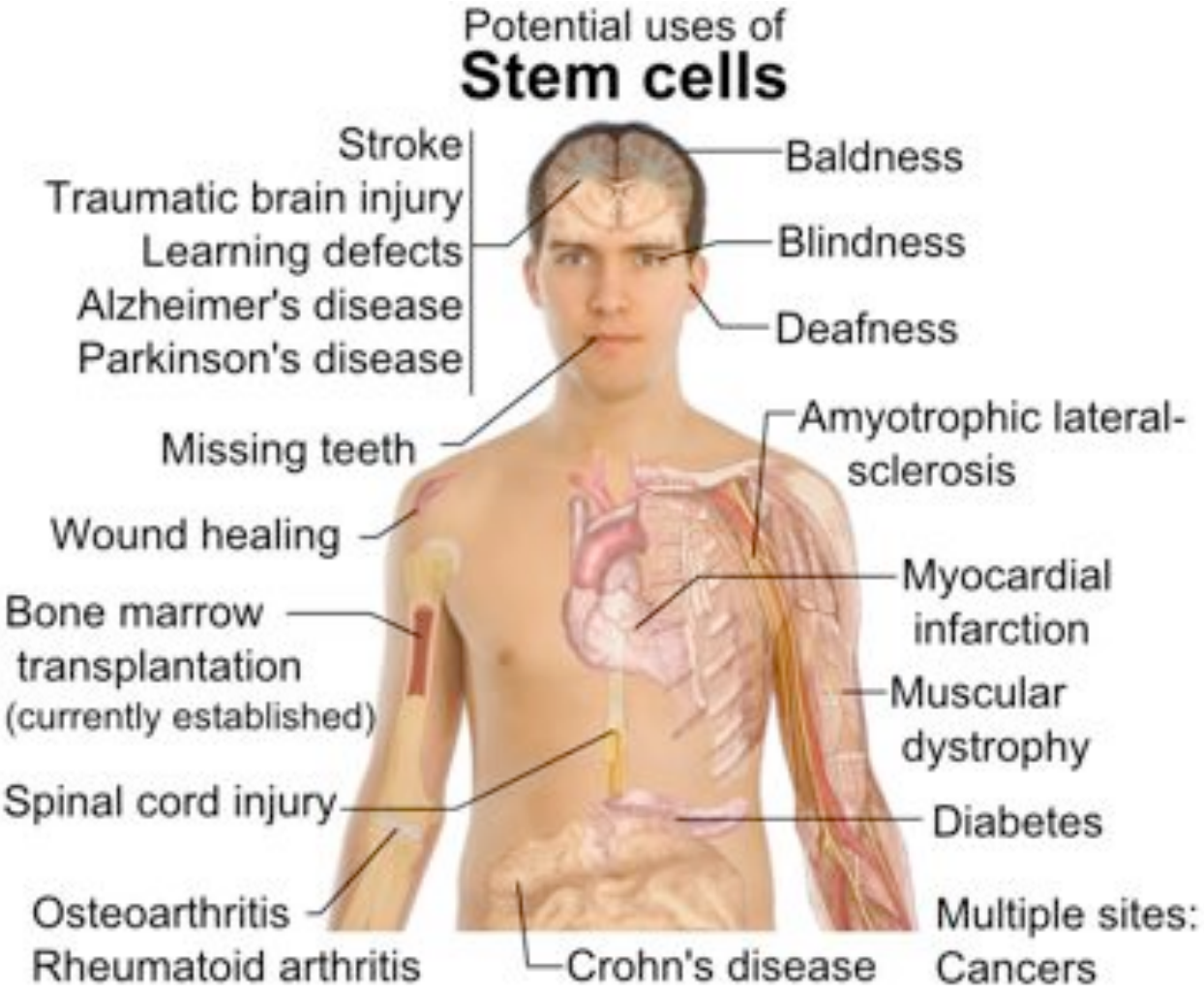




Regenerative medicine

the clinical application of stem cells

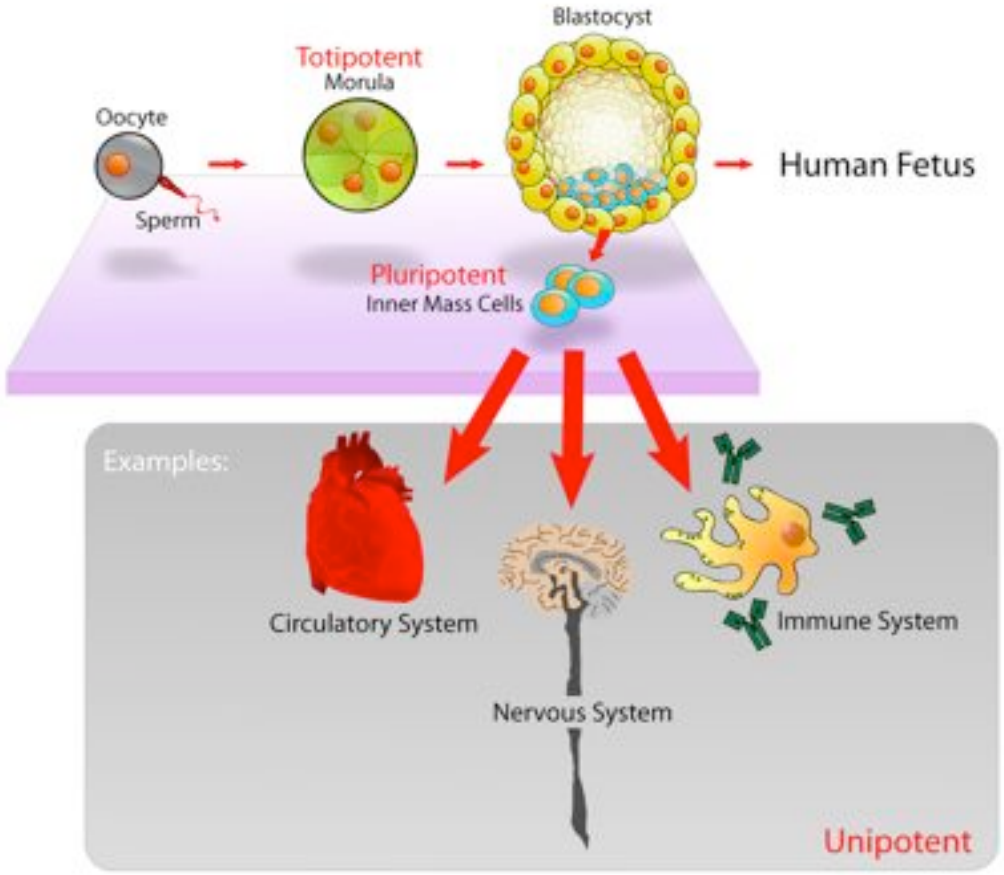
"process of replacing or regenerating human cells, tissues or organs to restore or establish normal function"



Stem Cell Sources for Regenerative Medicine

Stem cells derived from embryos

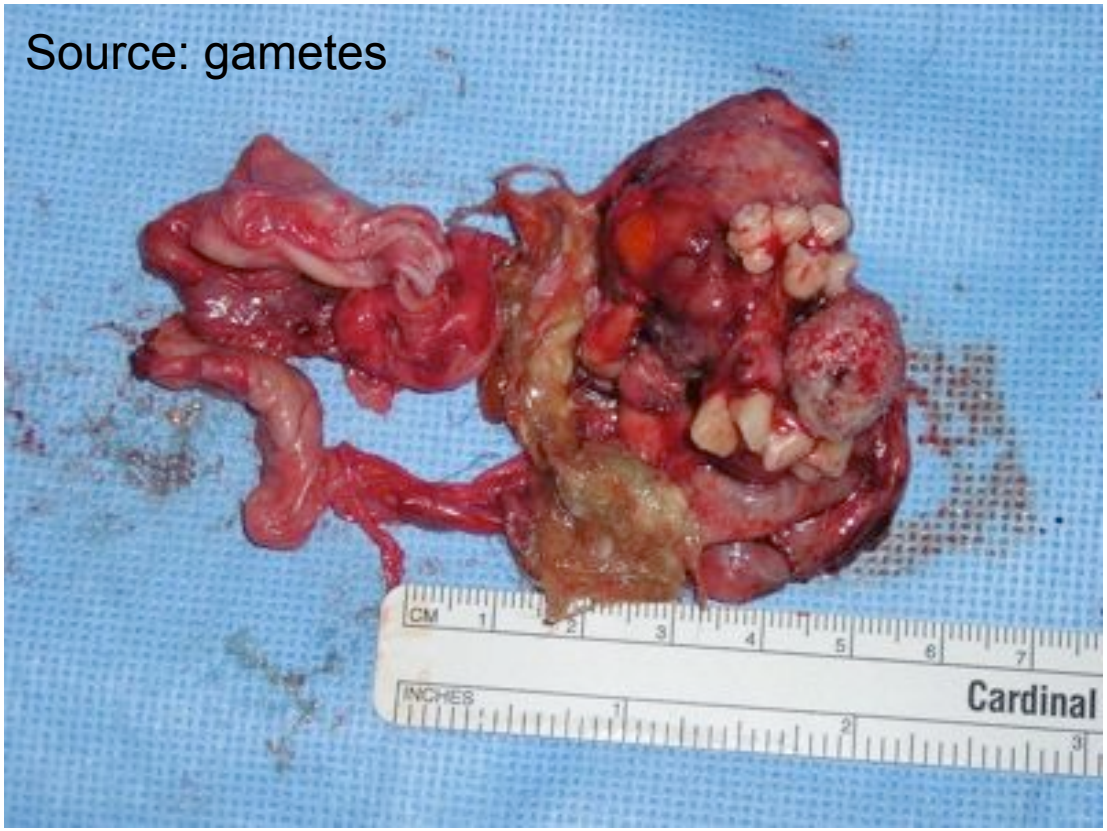
Stem cells derived from adults



Multipotent

Embryonal Carcinoma Cells are pluripotent

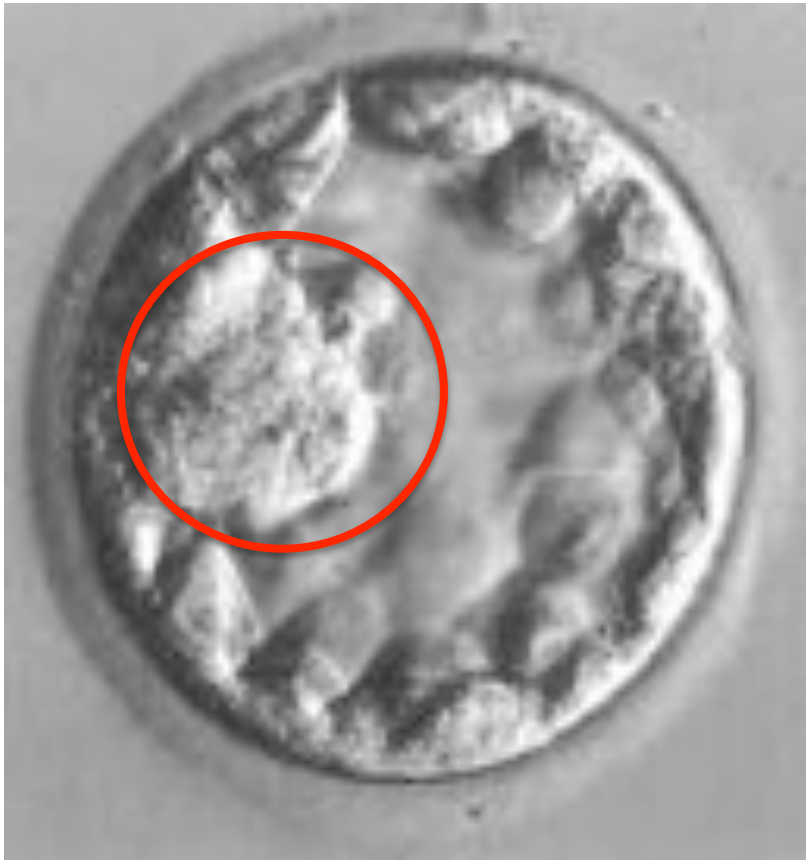
1964 – Pierce and Kleinsmith isolate EC cells from teratocarcinomas



Pluripotent
In vitro culture and expansion
Genetic abnormalities

Embryonic Stem Cells are pluripotent

1981 – Martin Evans, Matthew Kaufman and Gail Martin

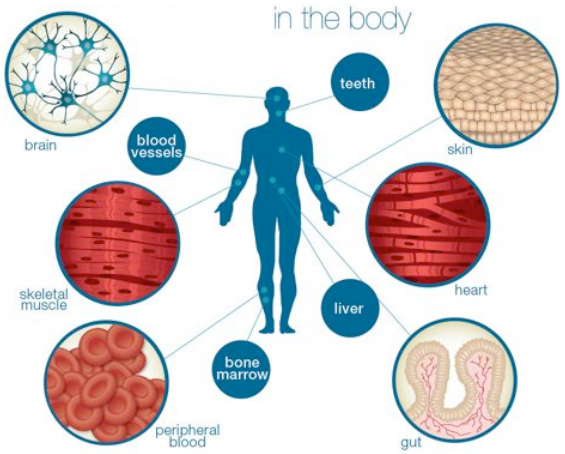


Pluripotent
No genetic abnormalities
In vitro culture and expansion
Ethical issues

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"

- Bone marrow stem cells: haematopoietic stem cells
- Neural stem cells
- Intestinal stem cells
- Skin stem cells
- Umbilical cord stem cells: haematopoietic stem cells

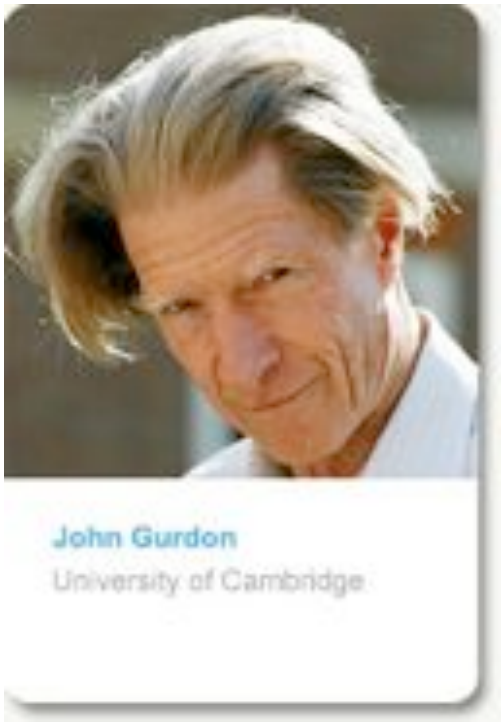


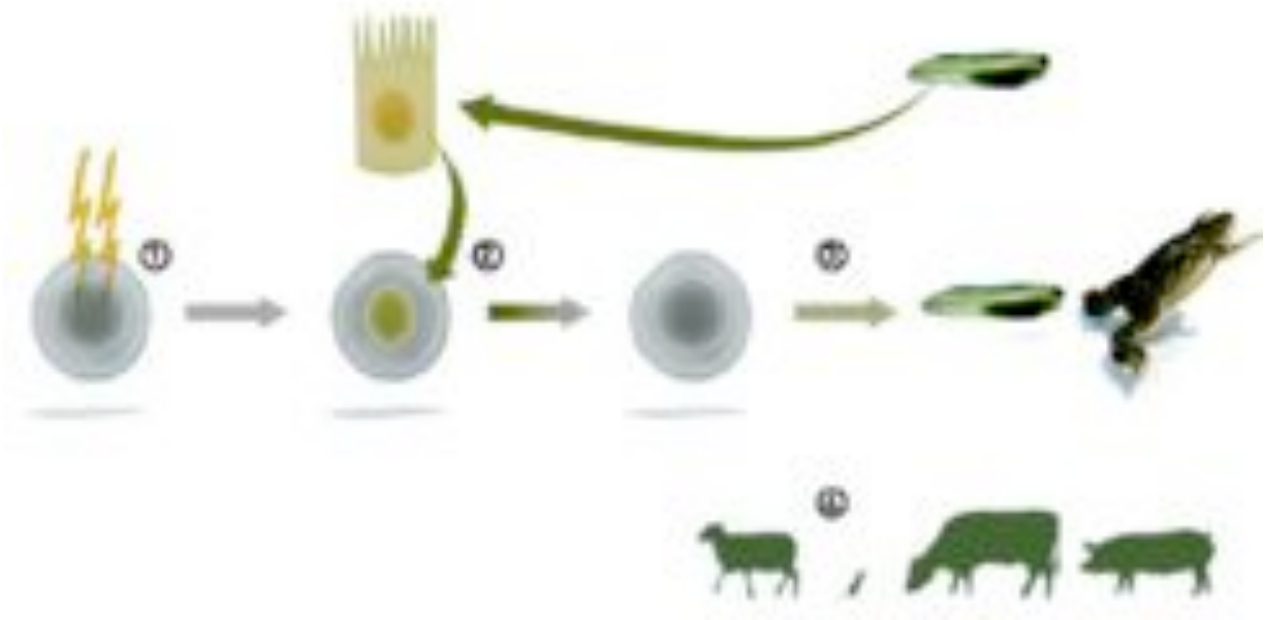
No ethical issues
Restricted plasticity
Limited quantities
Hard to identify

Somatic Cell Nuclear Transfer

John Gurdon, 1958

The developmental potential of nuclei of differentiated cells



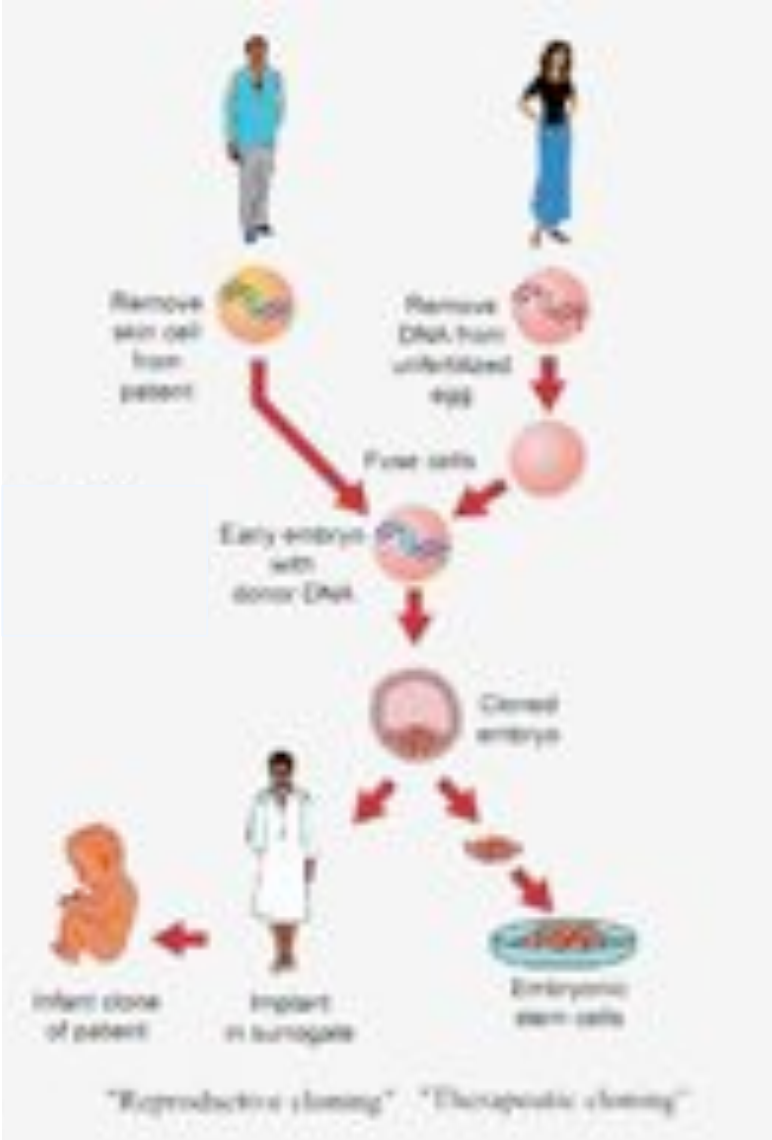


Somatic Cell Nuclear Transfer

"mature, differentiated cells can be reprogrammed to become pluripotent"

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

Reproductive/Therapeutic Cloning

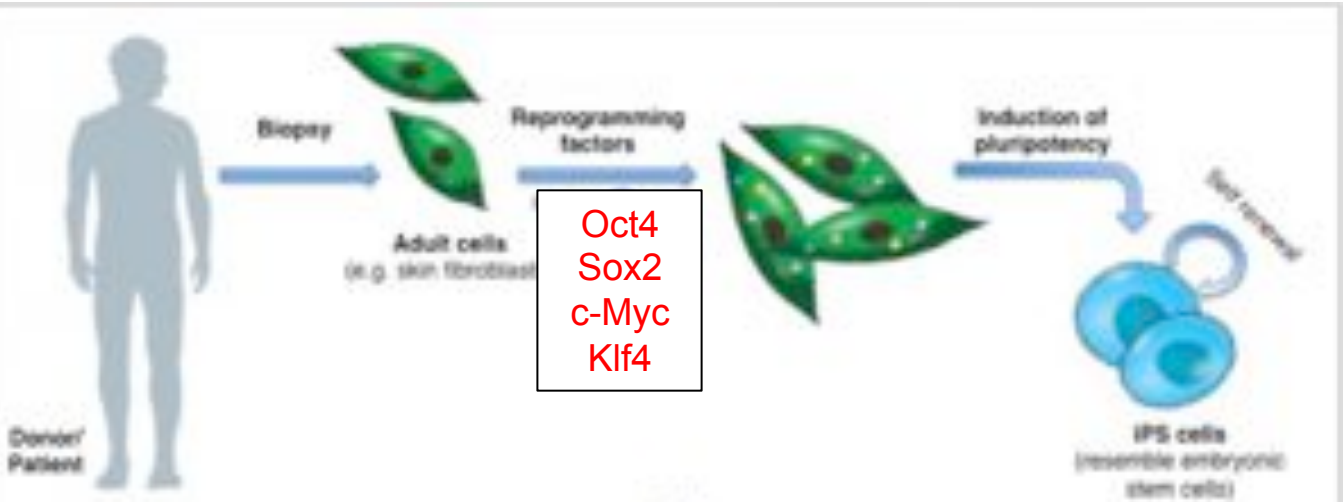


Pluripotent (totipotent?)
Low success rate
Genetic/phenotypic abnormalities
Ethical issues

Nuclear Reprogramming

Induced pluripotency (iPS), Yamanaka, 2006

"mature, differentiated cells can be reprogrammed to become pluripotent"



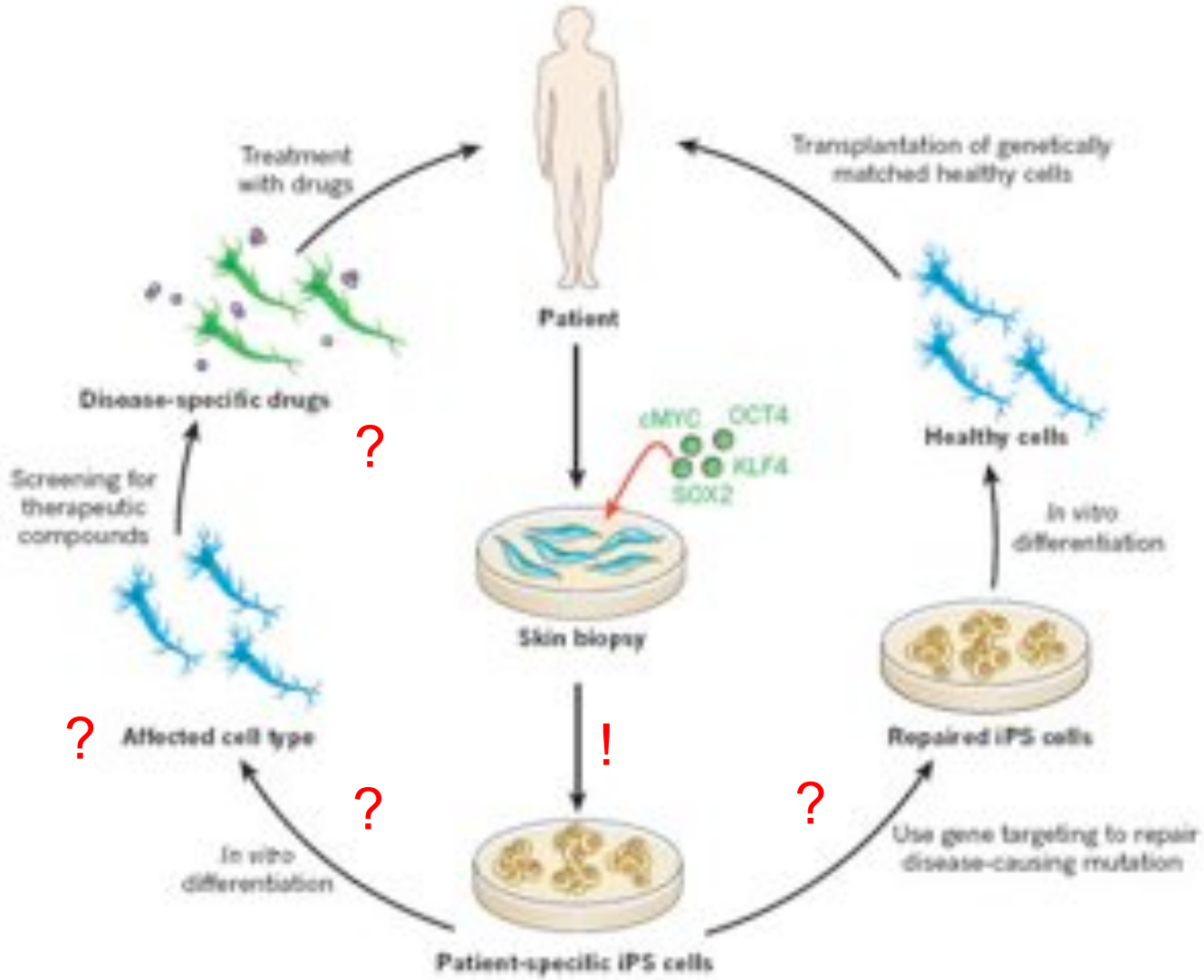
Pluripotent
Good success rates
No need for human embryos
Genetic/phenotypic abnormalities?

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?

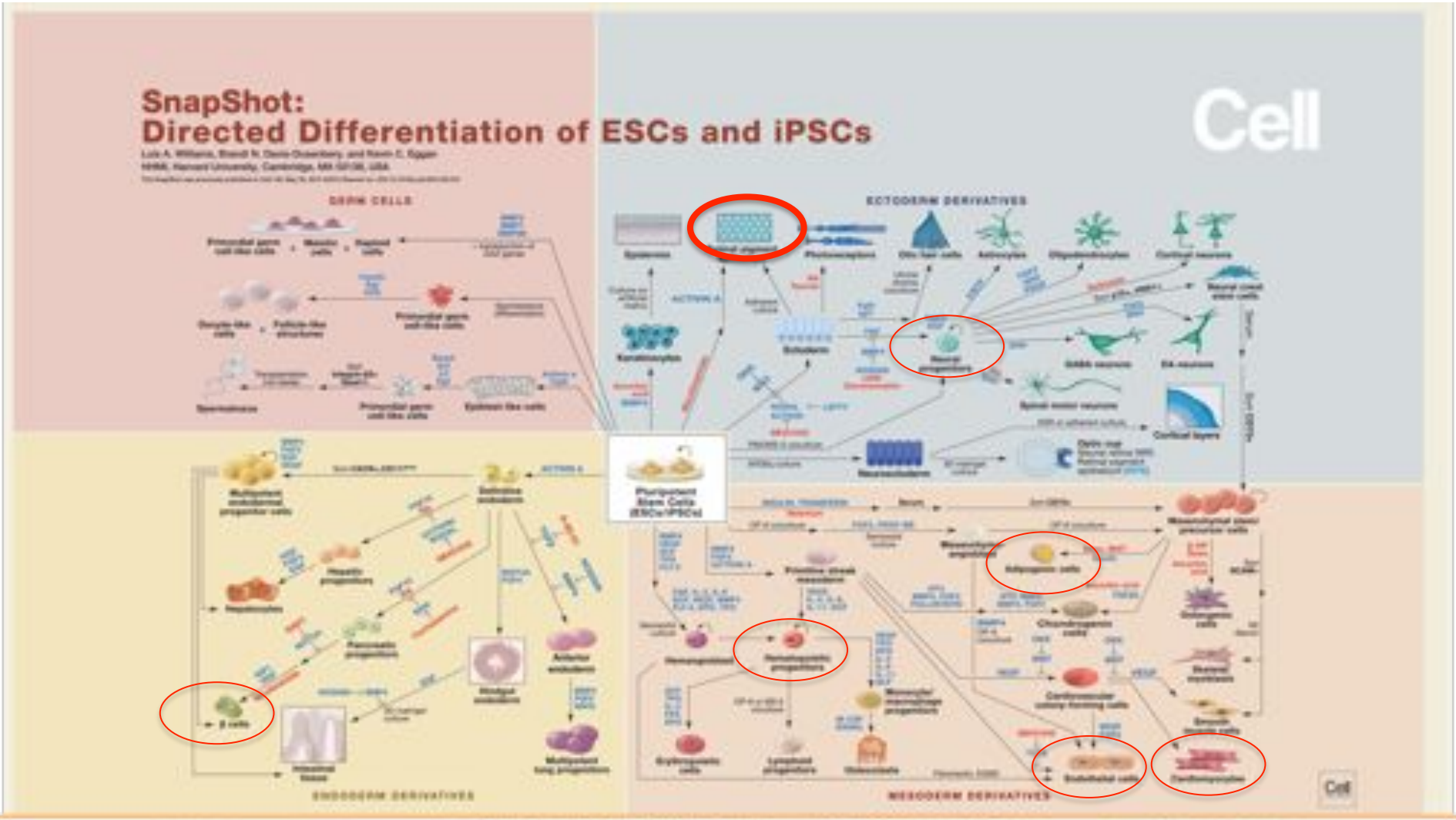
2- how we can direct differentiation?

3- how we can cure diseased cells?

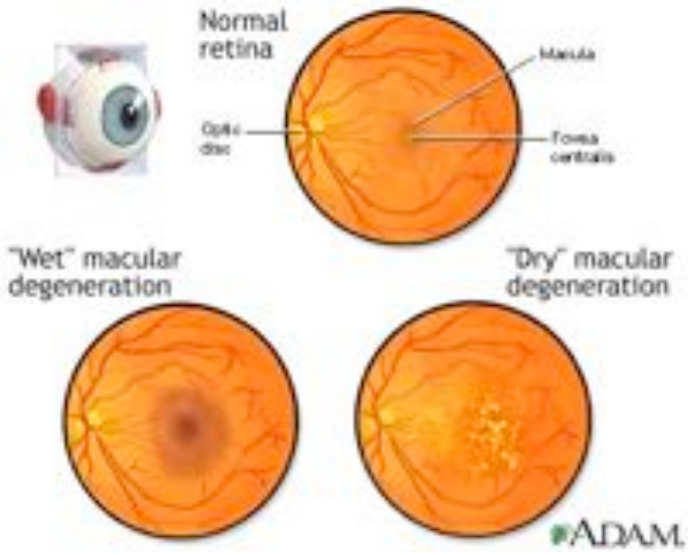
4- how we can repair mutations in cells?

Future of Regenerative Medicine

Directed differentiation of pluripotent stem cells



Stem Cell Therapy
Macular Degeneration



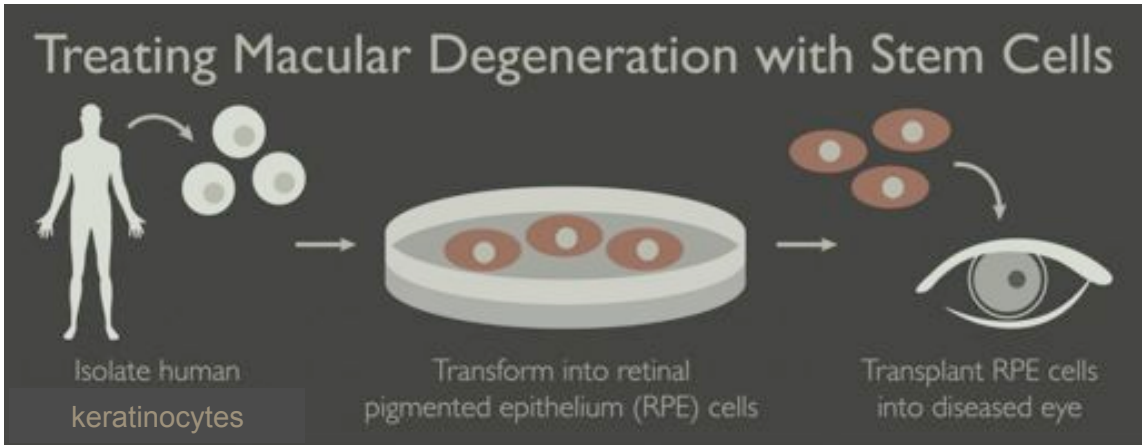
Masayo Takahashi (RIKEN)

iPS on skin cells of patient

Differentiate into retinal pigment epithelium cells

Grow in sheets to transplant in retina

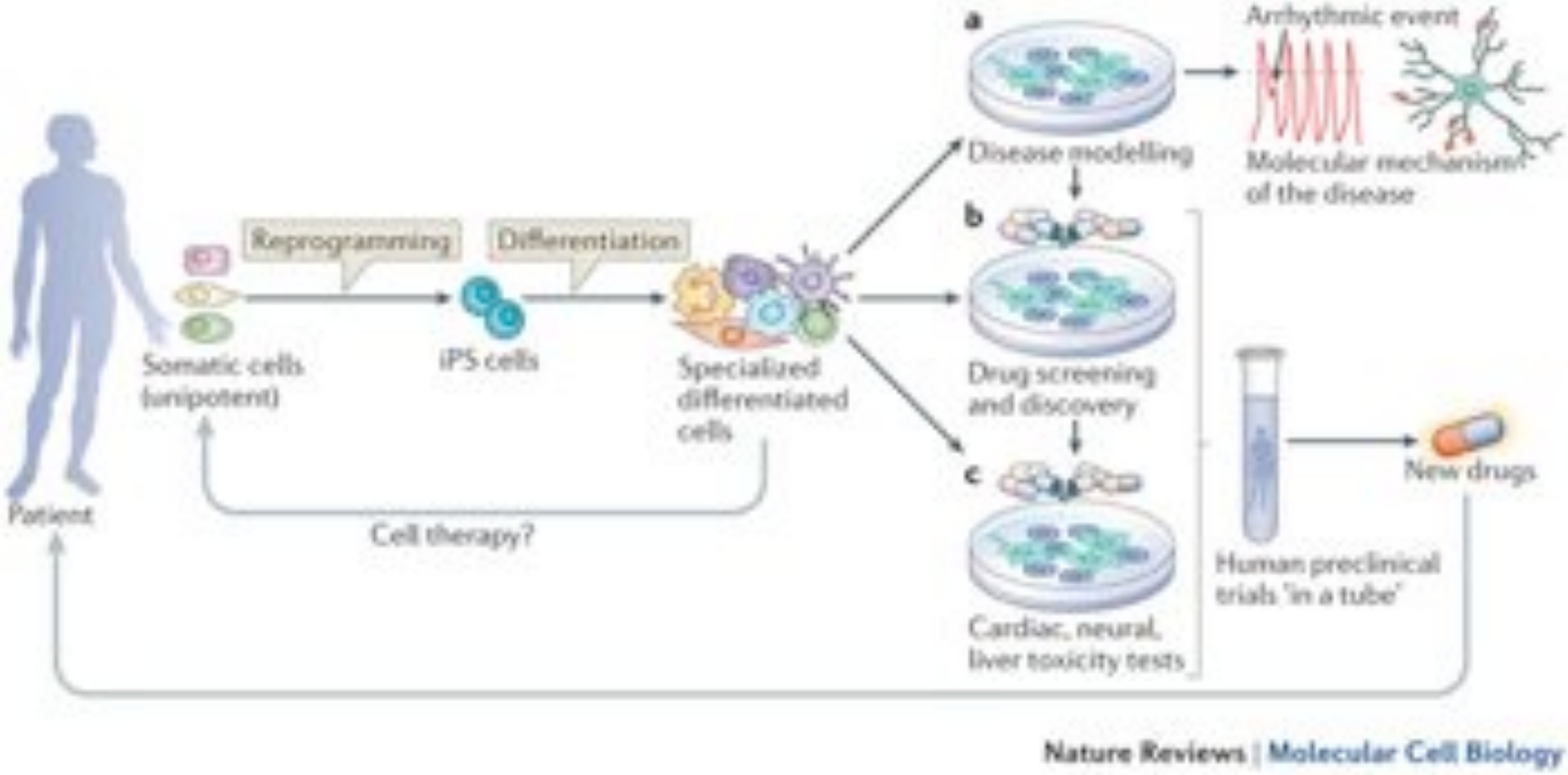
(Surgery on 12 September 2014)



Future of Regenerative Medicine

How can we cure disease?

Disease Modeling and Drug discovery



Future of Regenerative Medicine

Disease Modeling of Spinal Muscular Atrophy

Svensden Lab, 2009

iPS on skin fibroblasts of SMA patient

Differentiate iPS cells into neurons, astrocytes and motor neurons

Selective death of motor neurons after few weeks of culture

Response to drug to increase SMN1 levels in iSMA-motor neurons

http://www.nature.com/nature/journal/v457/n7227/full/nature07677.html

Future of Regenerative Medicine

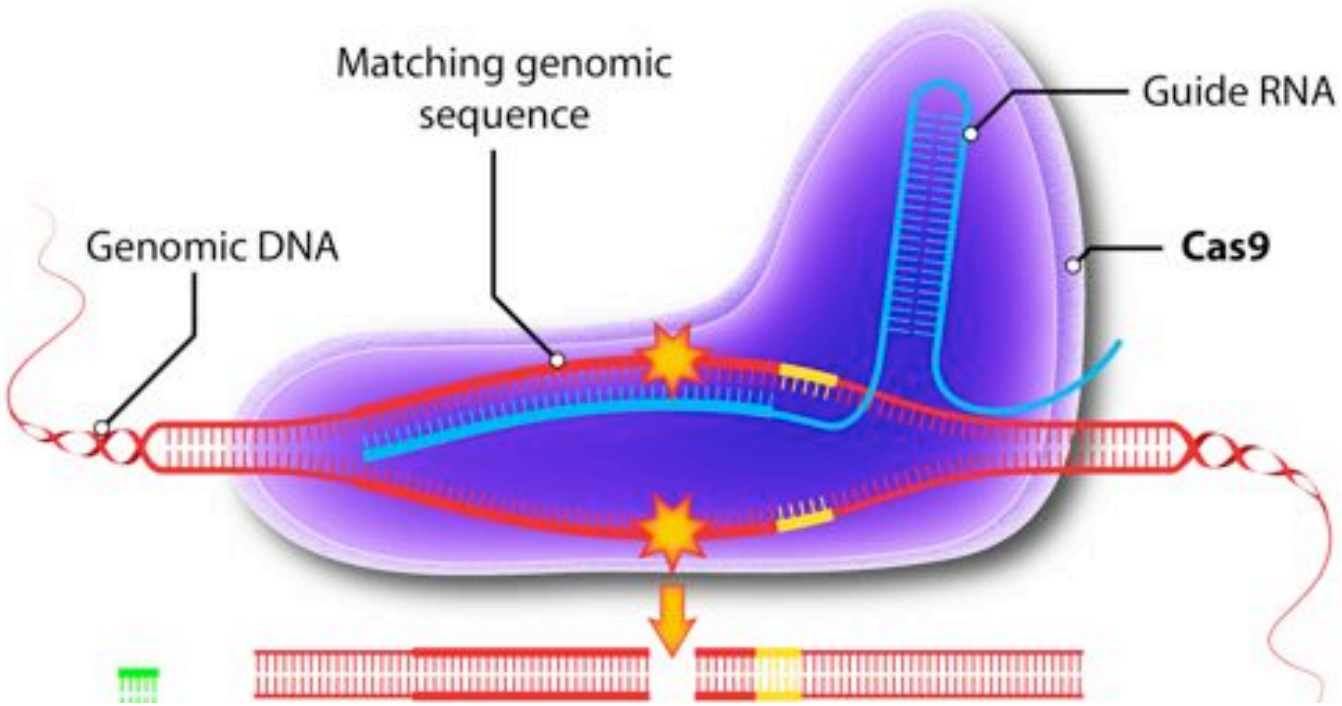
How can we repair mutations in cells?

Gene Therapy:

CRISPR/CAS9 genome editing

CRISPR/Cas9 Genome Engineering

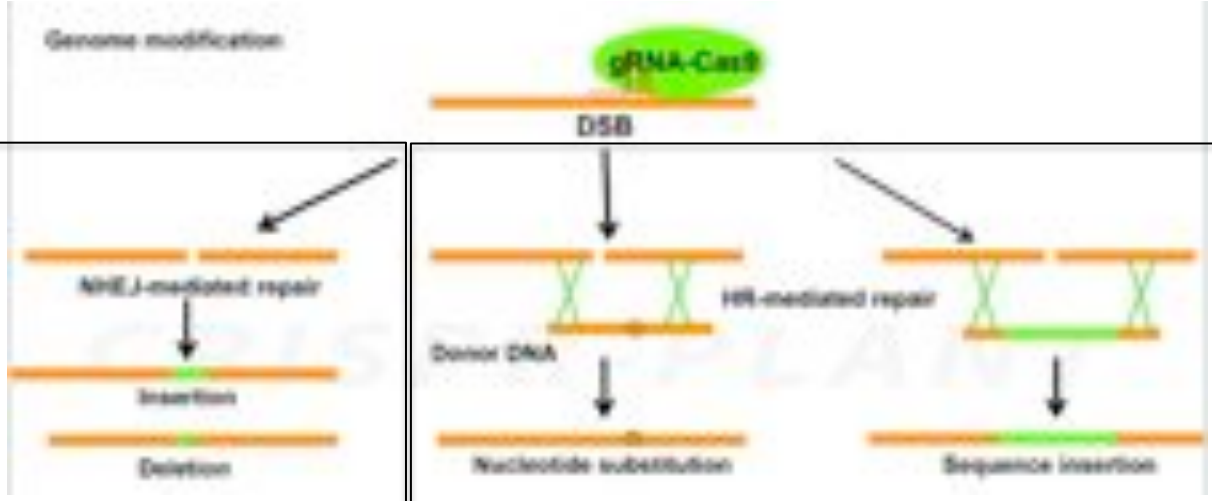
(Clustered Regularly Interspaced Short Palindromic Repeats)
Guide RNA and Cas9



http://www.youtube.com/watch?v=0dRT7slyGhs

CRISPR/Cas9 Genome engineering

Repair

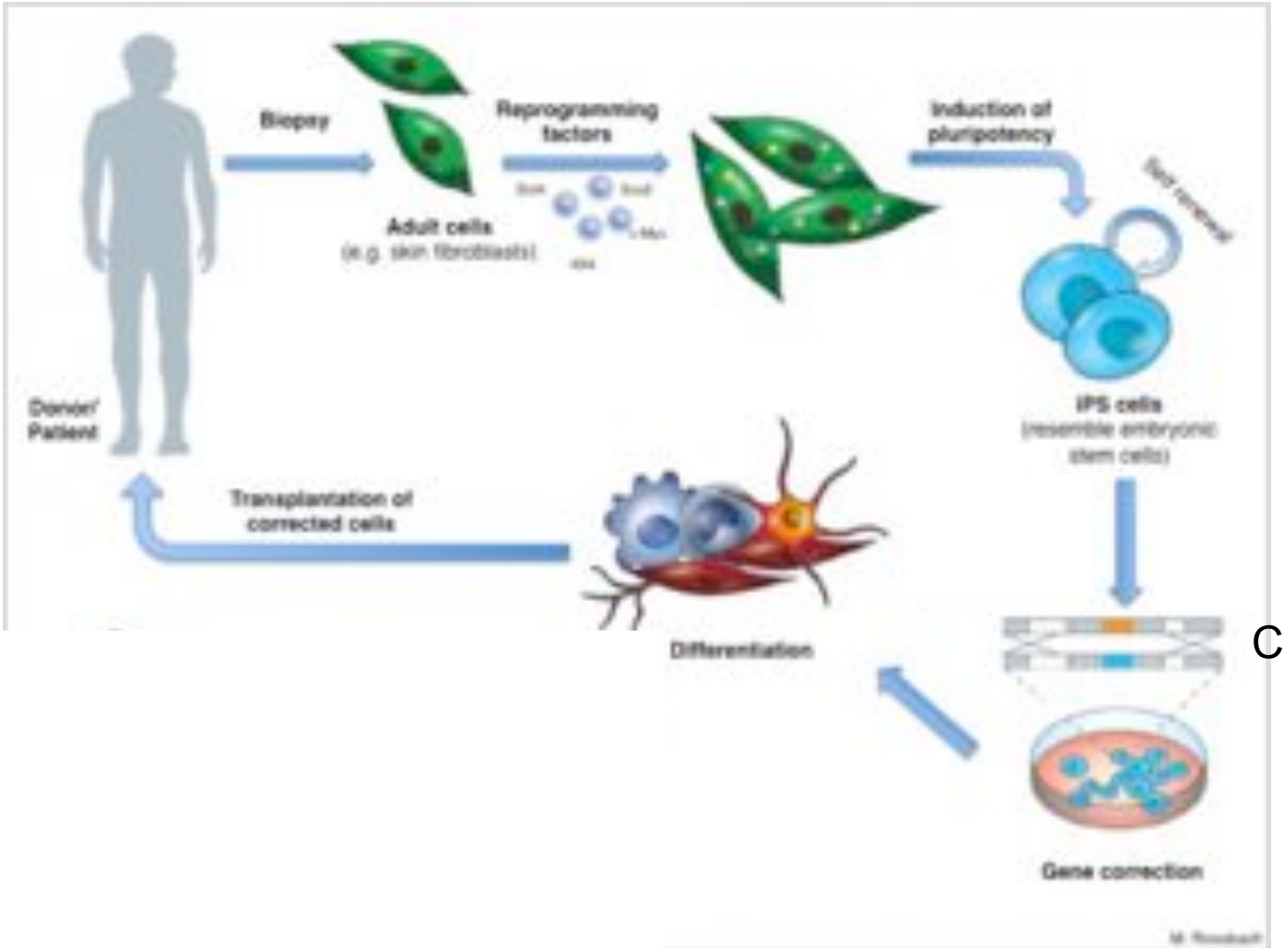


Non-homologous end joining:
Small insertion/deletion
gene disruption
(and occasional errors)

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

CRISPR/Cas9 Genome engineering

Applications in Stem Cells



CRISPR/CAS9

CRISPR/Cas9 Genome Engineering

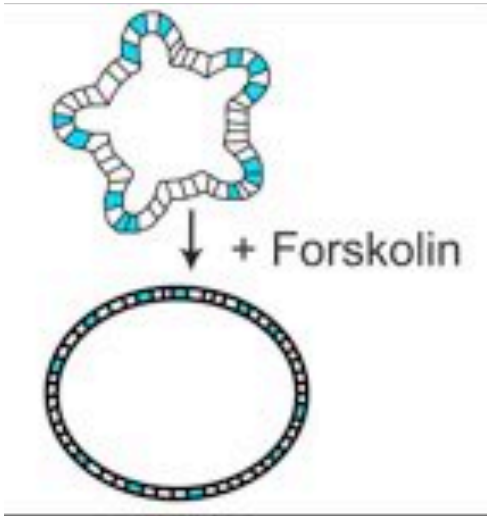
Repair of Cystic Fibrosis Gene CFTR

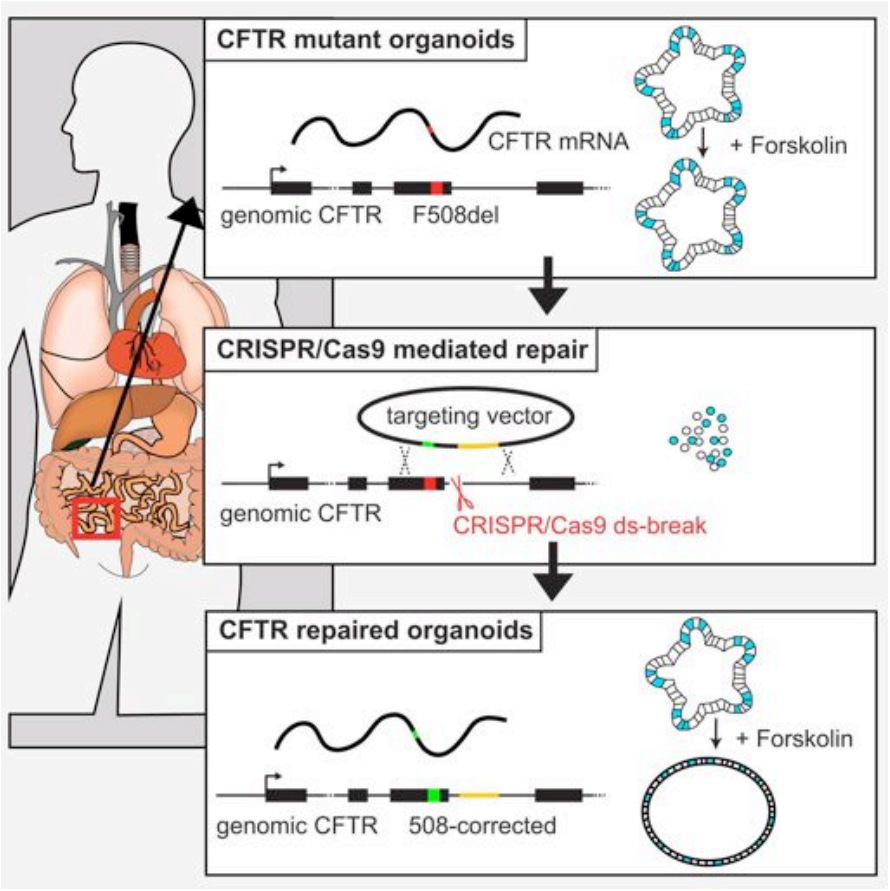
(cystic fibrosis transmembrane conductor receptor)

Lgr5+ intestinal stem cells -> organoids

In vitro assay in intestinal organoids:

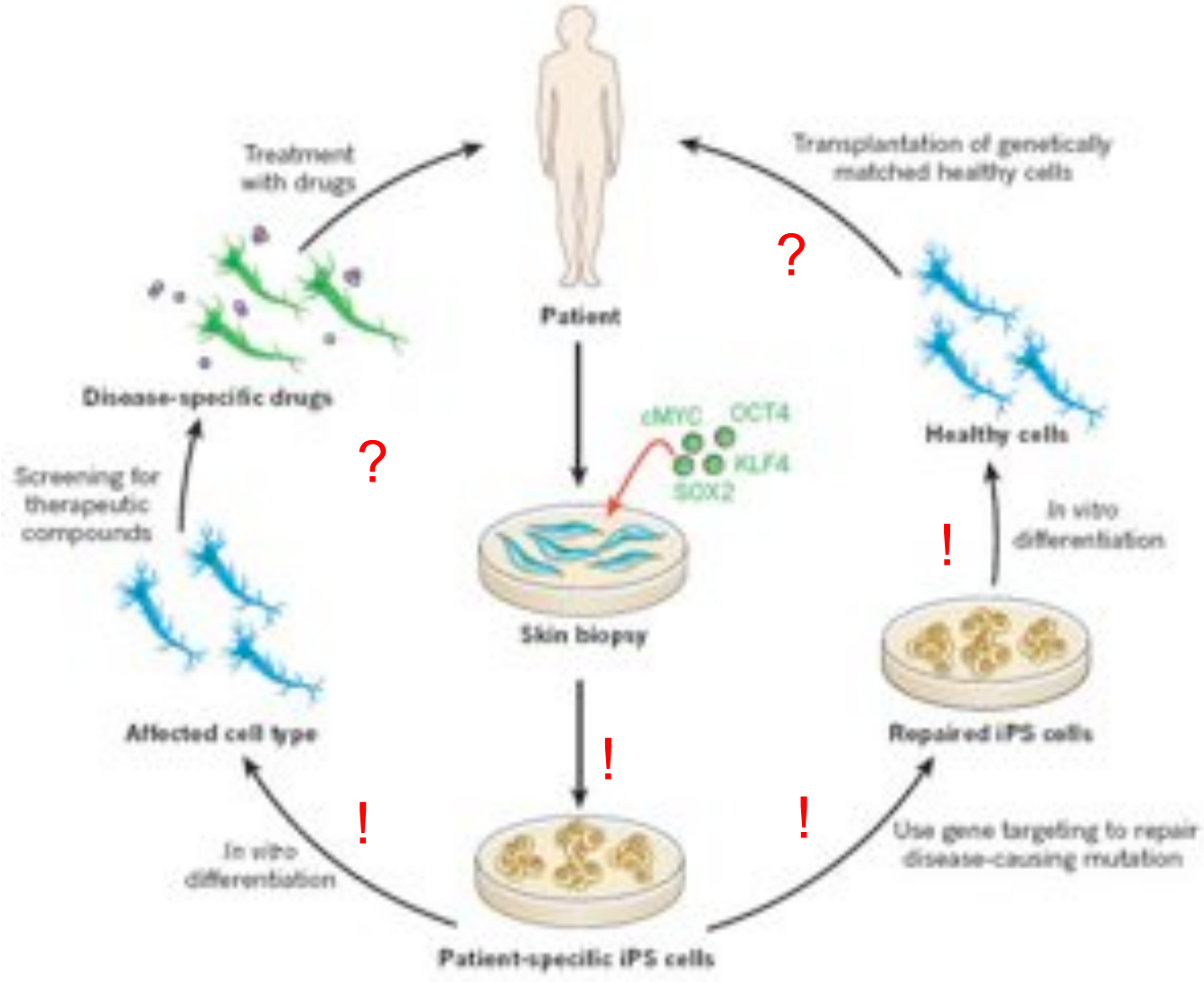
Forskolin -> CFTR -> expansion





Schwank et al., Cell Stem Cell 2013

The Future of Regenerative Medicine



12 September 2014: http://www.nature.com/news/japanese-woman-is-first-recipient-of-next-generation-stem-cells-1.15915

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

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