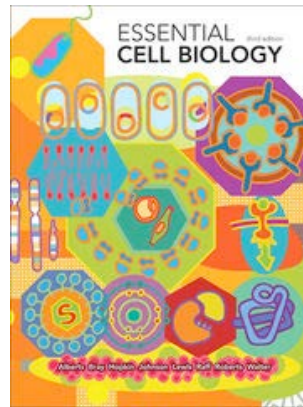


ANAT2341: lecture overview

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

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



Prenatal development

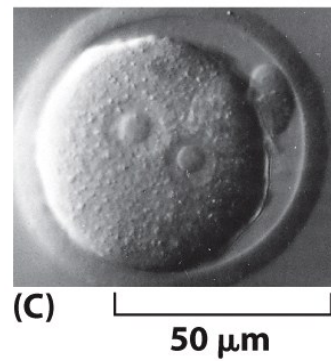
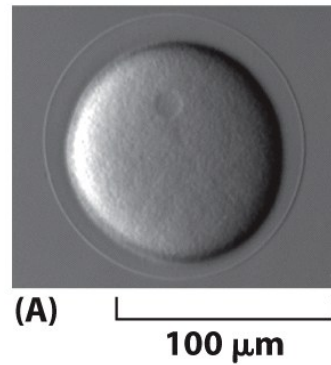
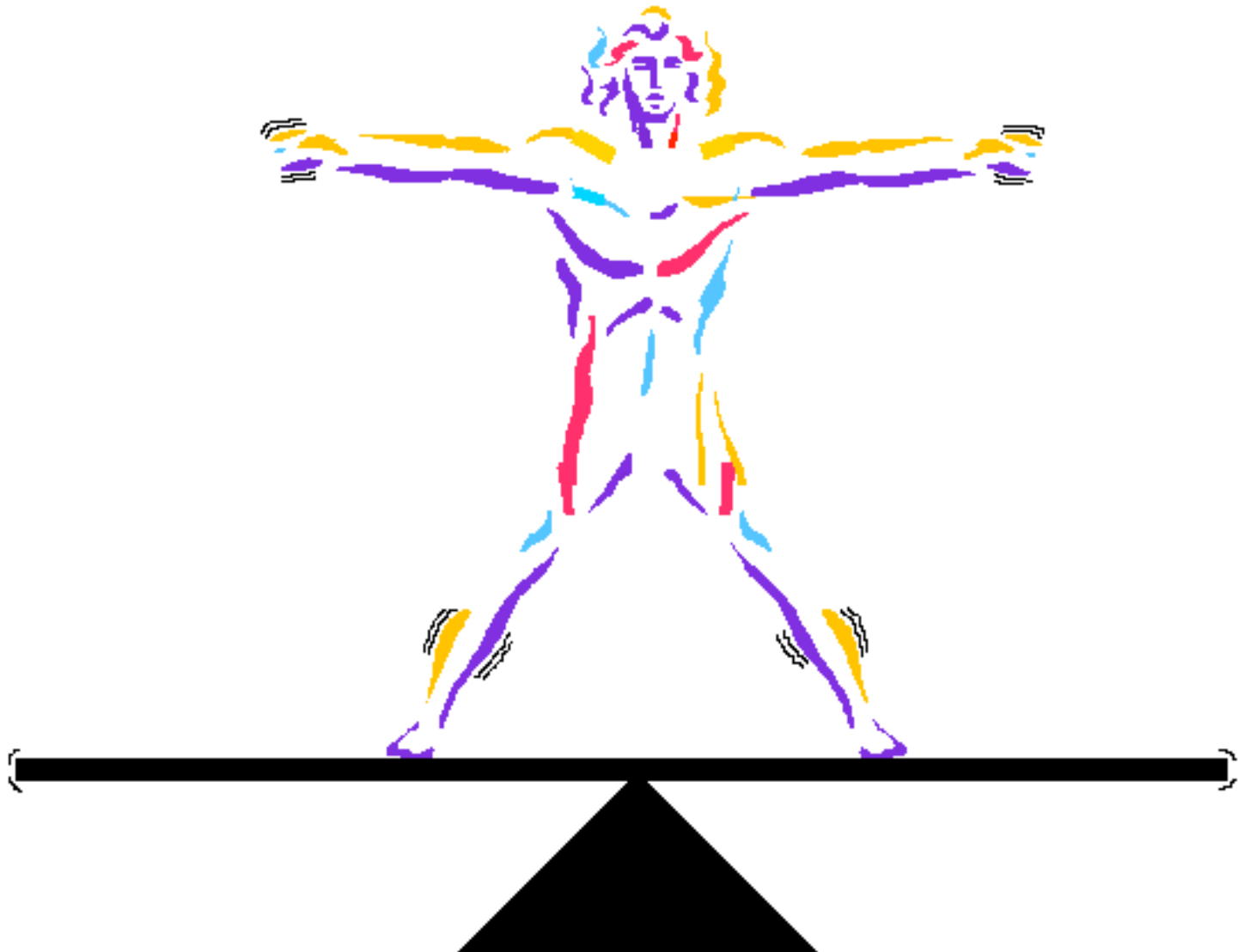


Figure 20-32 Essential Cell Biology 3/e (© Garland Science 2010)

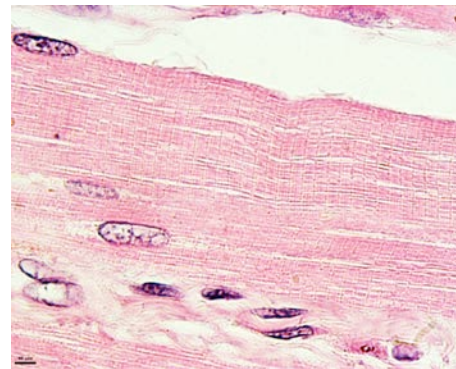
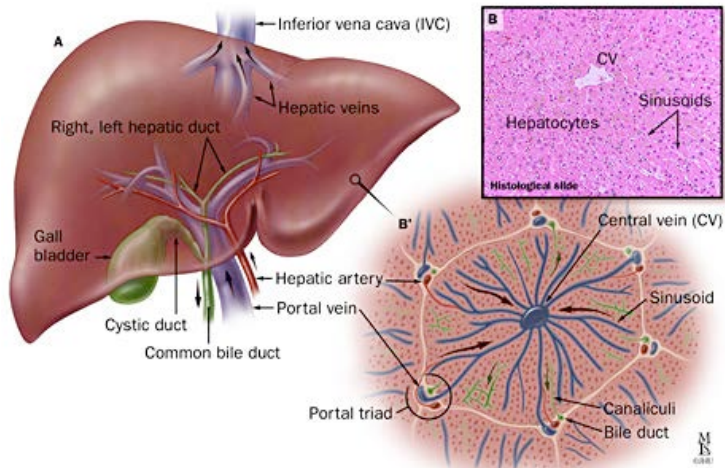
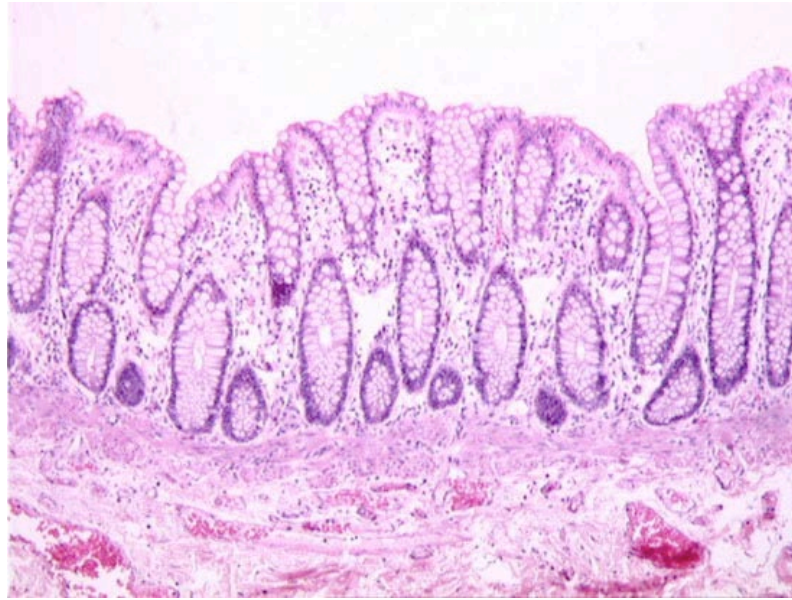
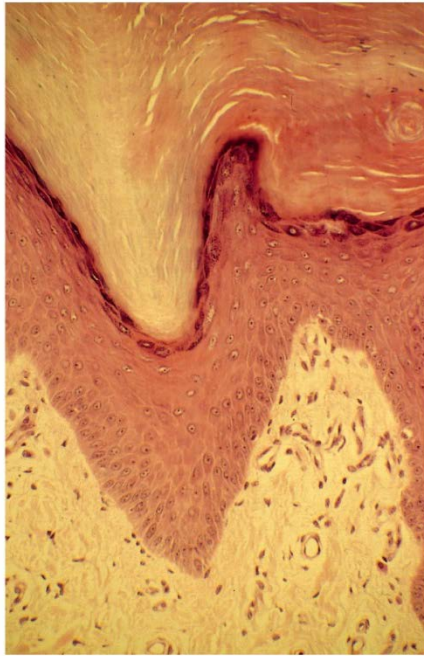


Tissue homeostasis

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

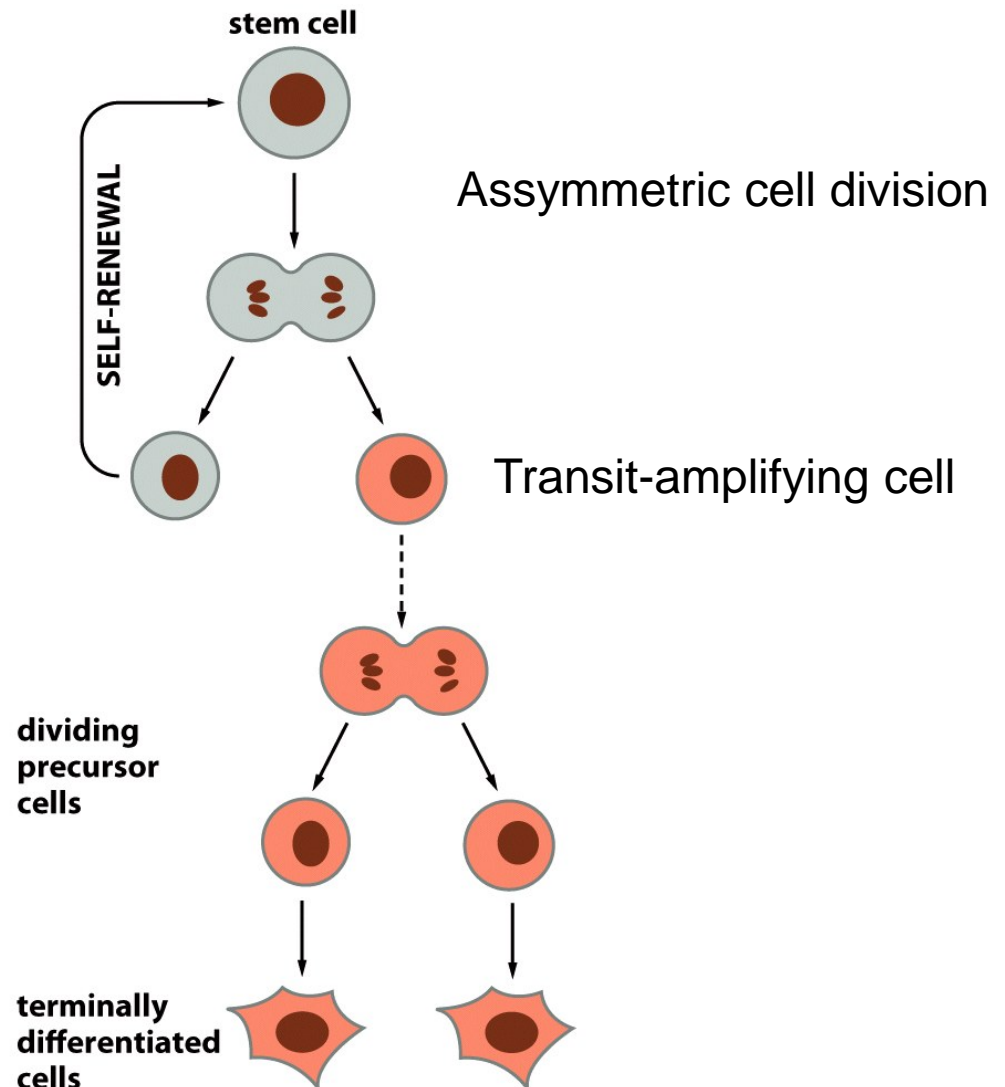
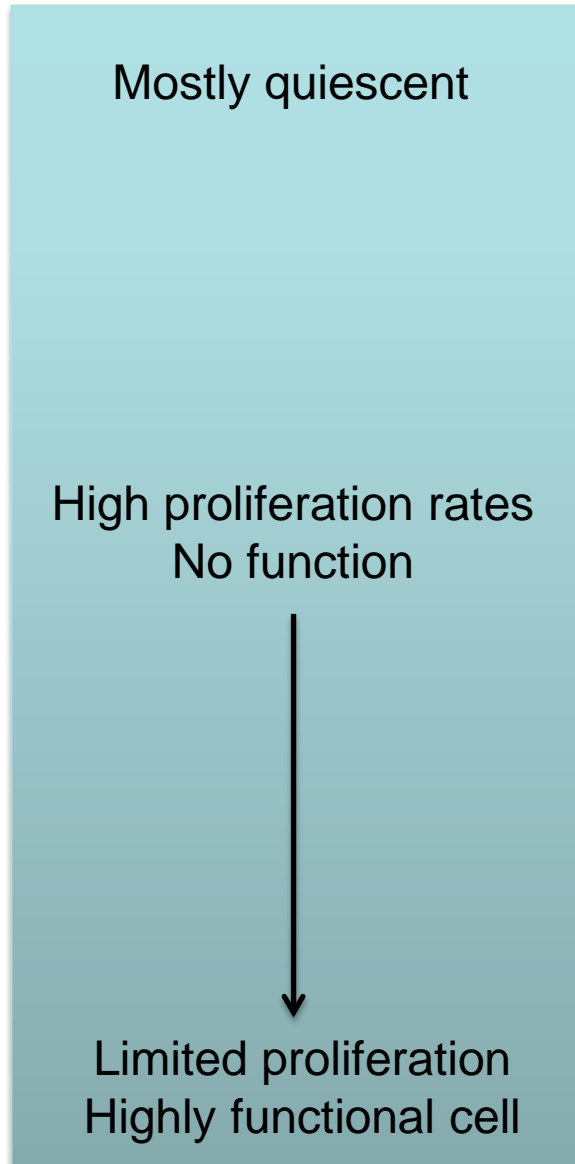


Figure 20-35 Essential Cell Biology 3/e (© Garland Science 2010)



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

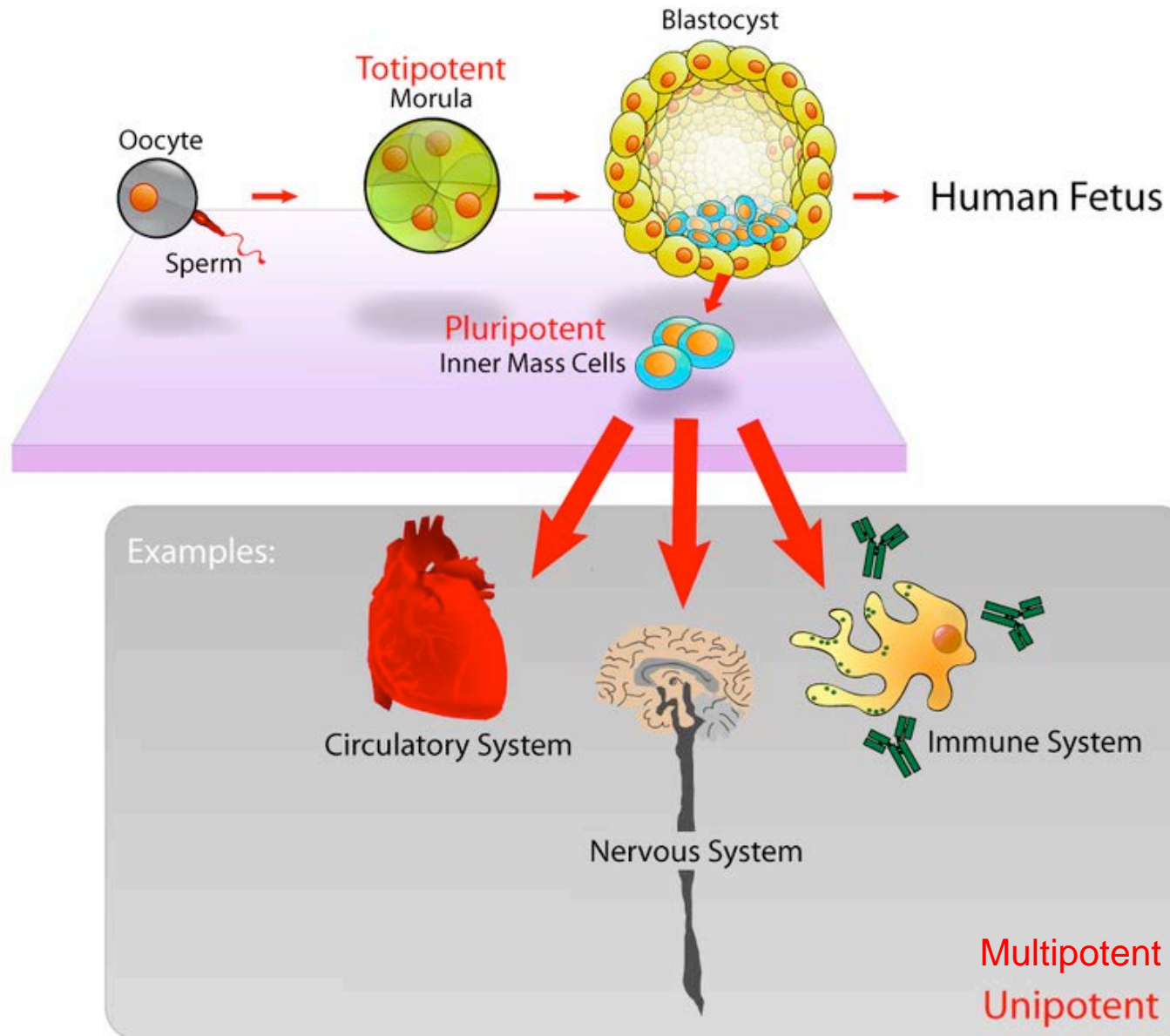
Multipotency:

capacity to give rise to more than 1 cell type

Unipotent stem cell:

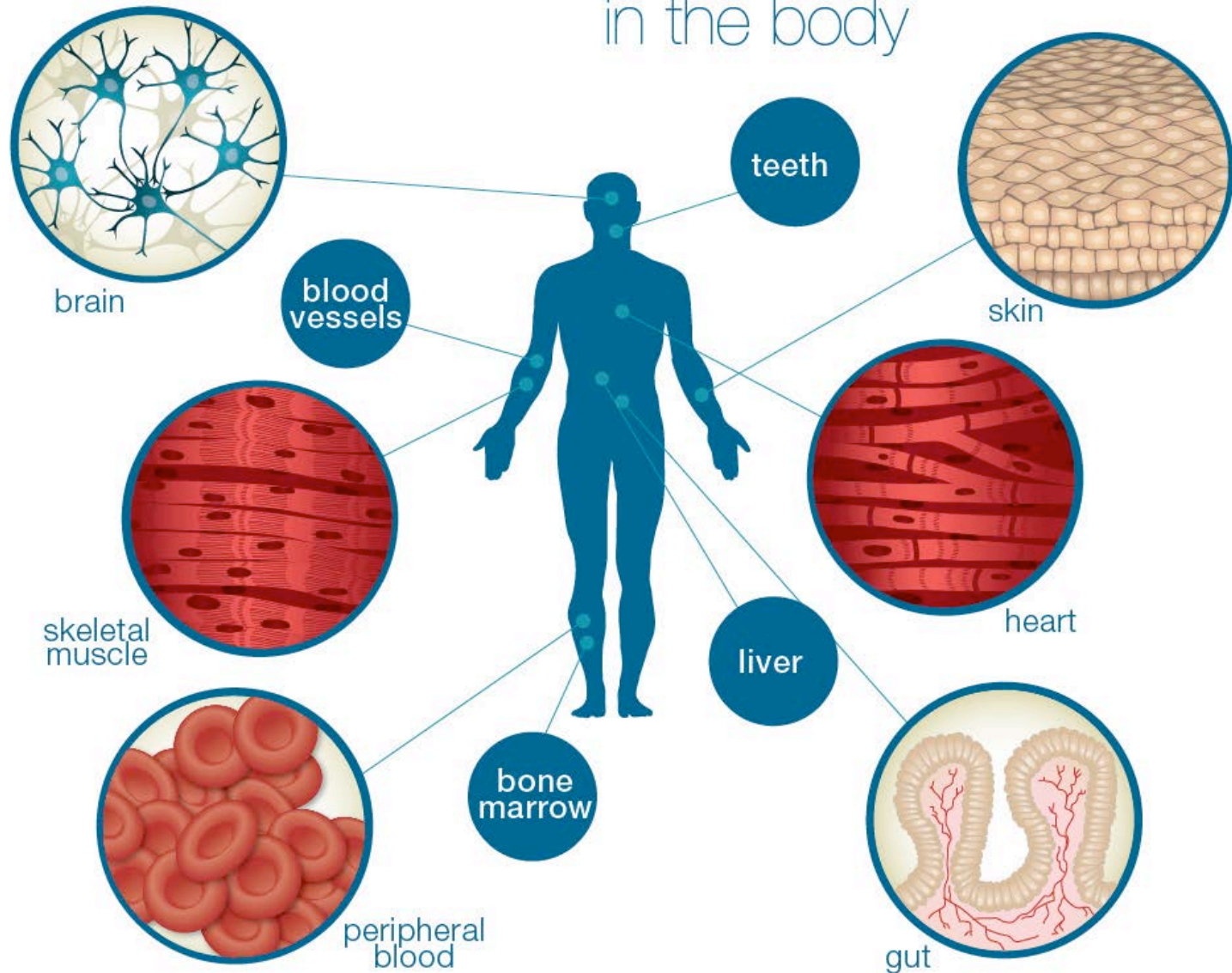
tissue precursor cells, capacity to give rise to one cell type only

Stem cells potential



Adult stem cells

Locations of **Somatic Stem Cells** in the body

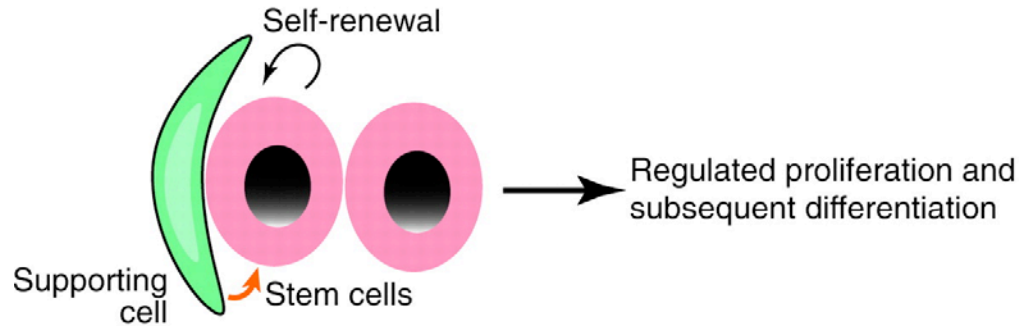




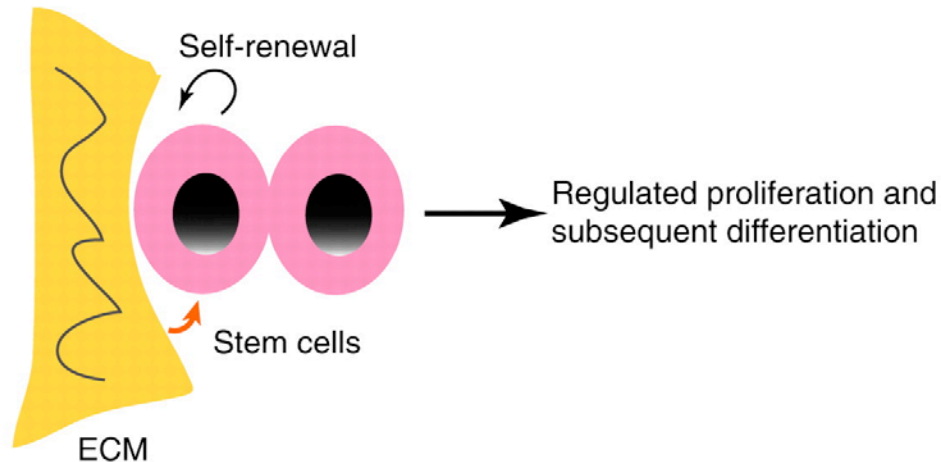
Stem cell niche:

Keeps stem cells in an undifferentiated state

A Cellular niche

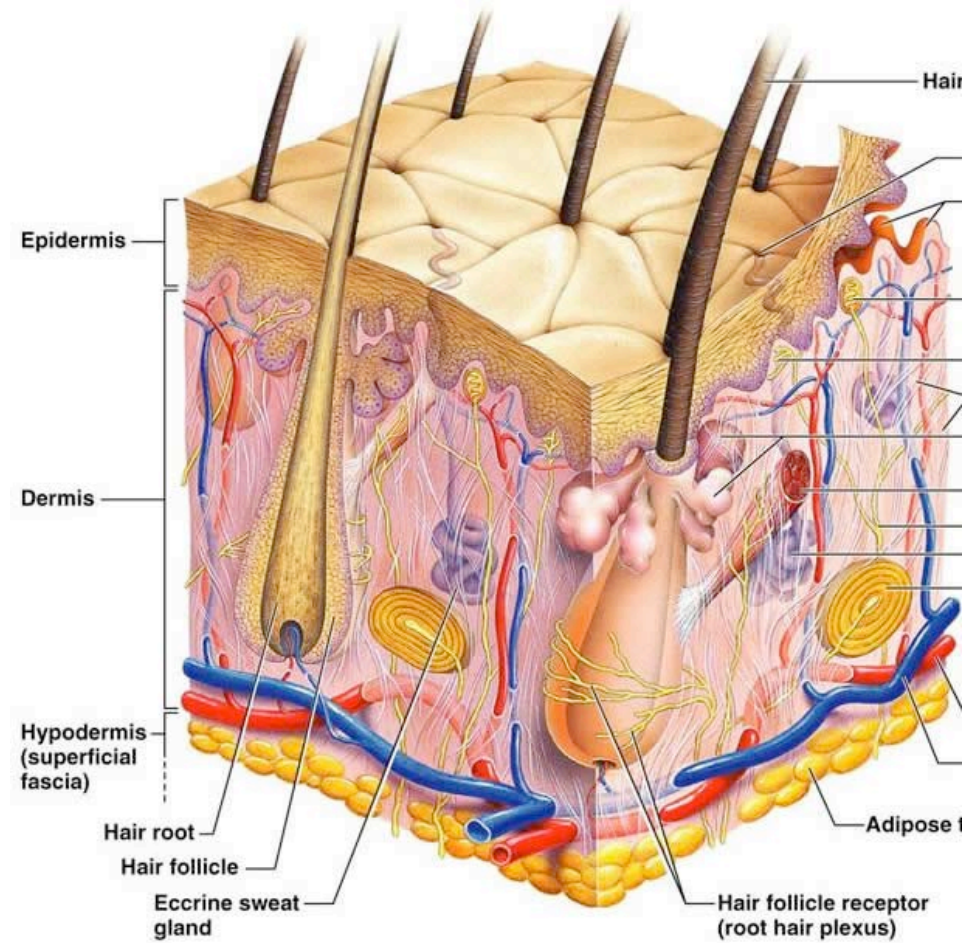
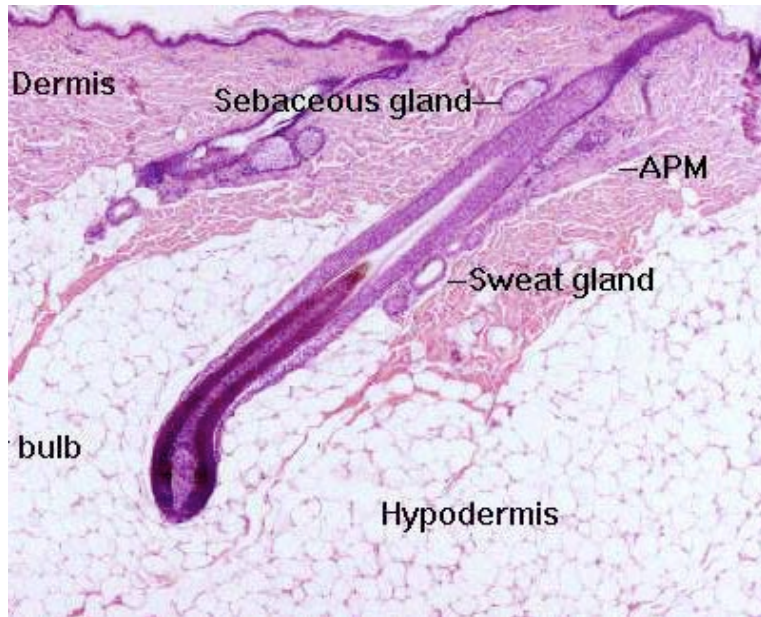


B Non-cellular niche



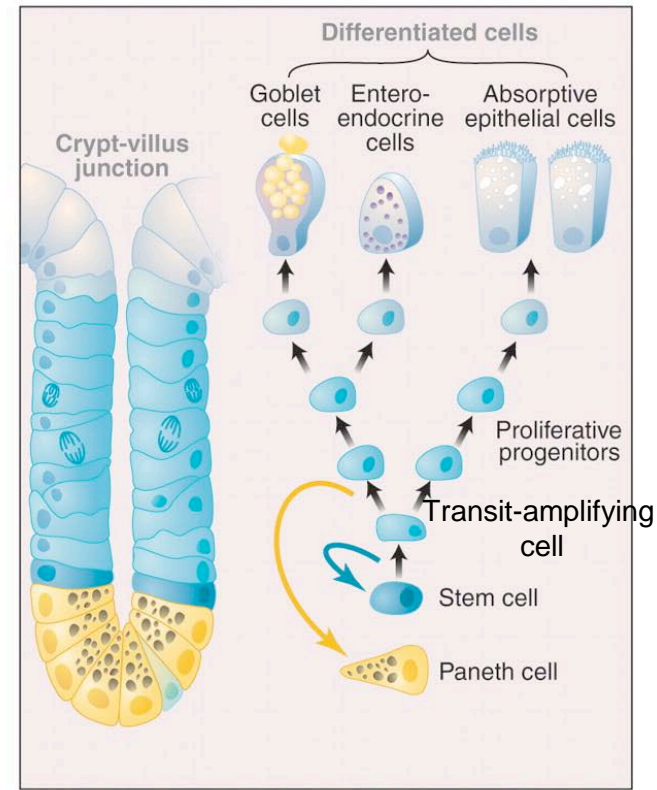
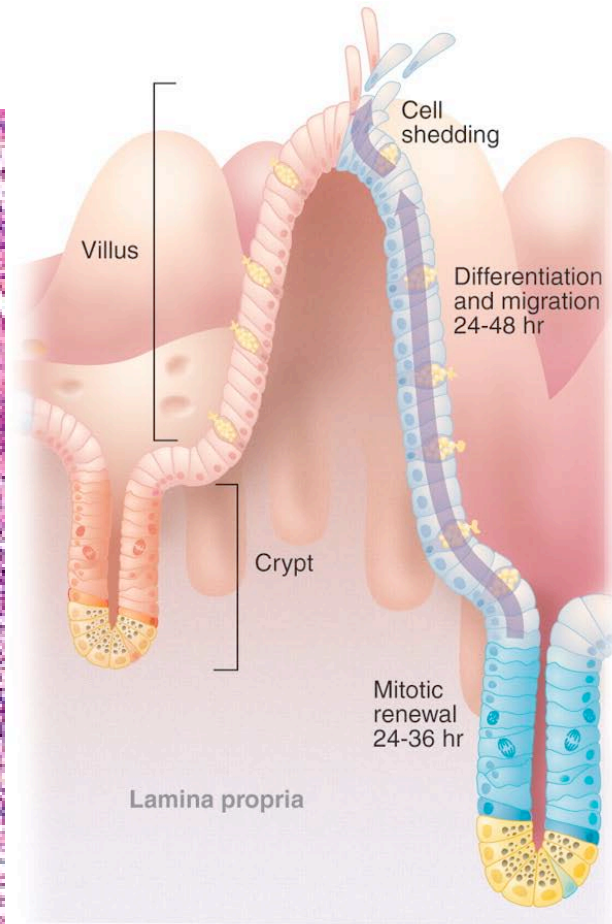
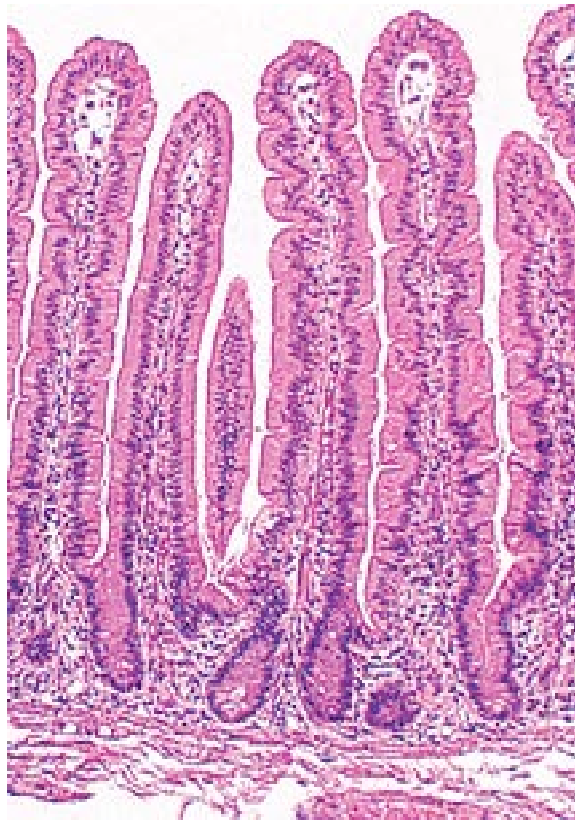
Key:  Secreted signals from niche

Epidermal Stem Cell Niches



The Intestinal Crypts Stem Cell Niche

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



The Haemopoietic Stem Cell Niche

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

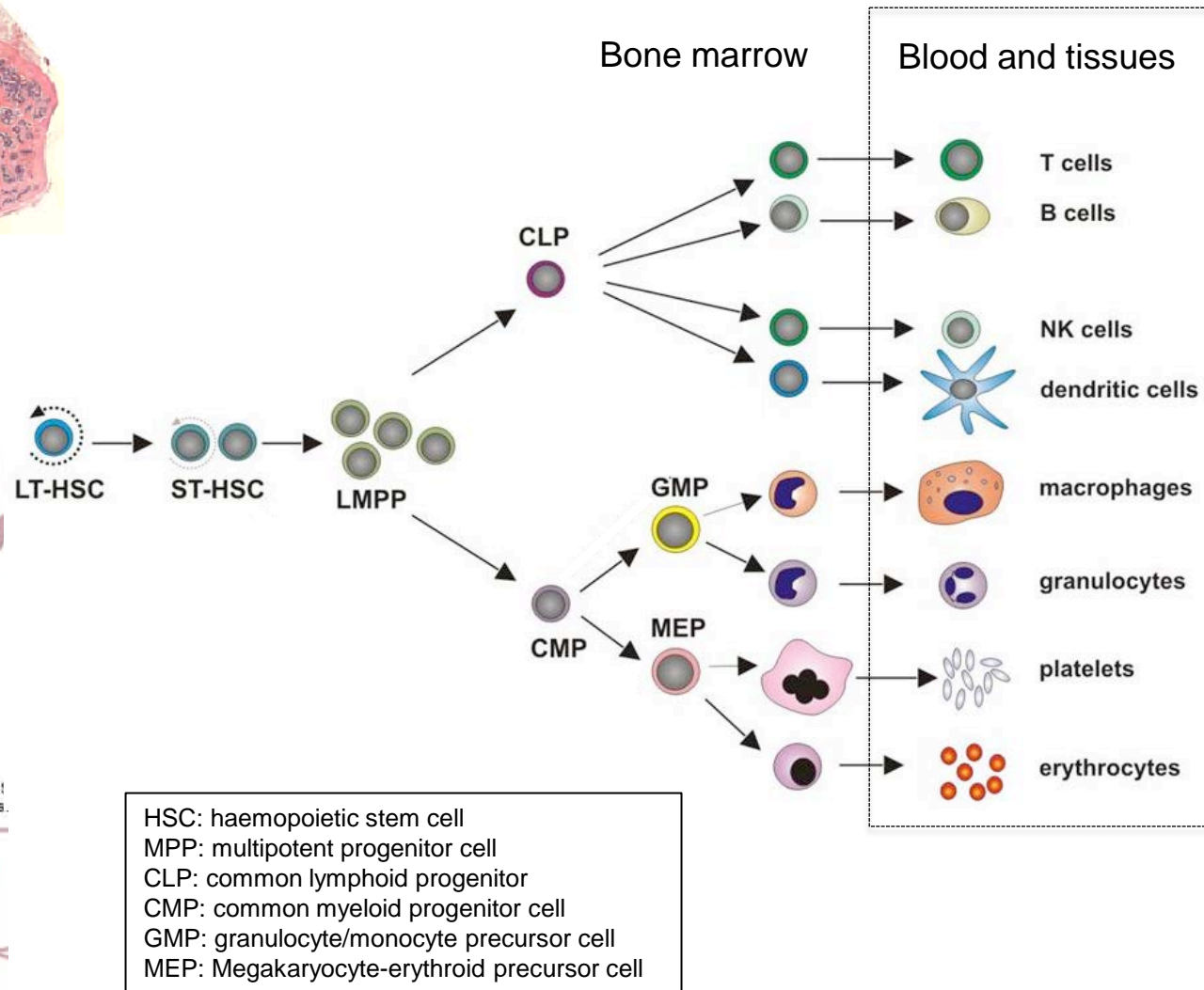
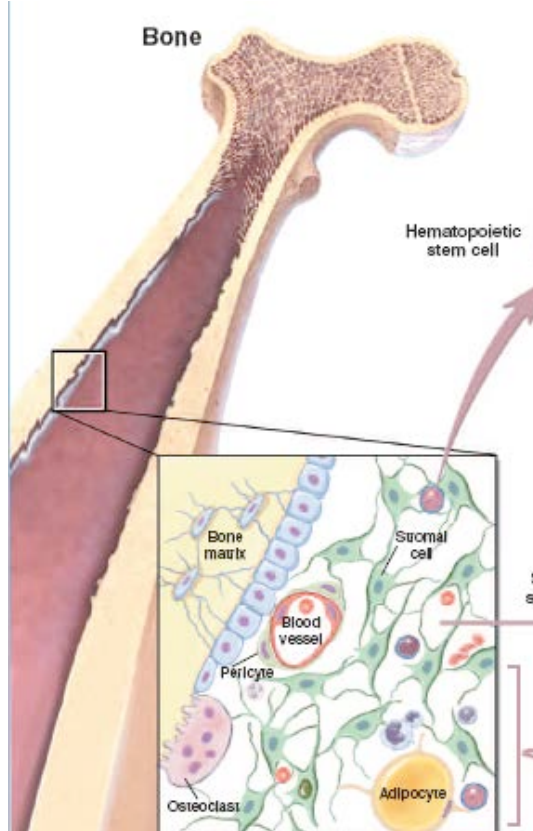
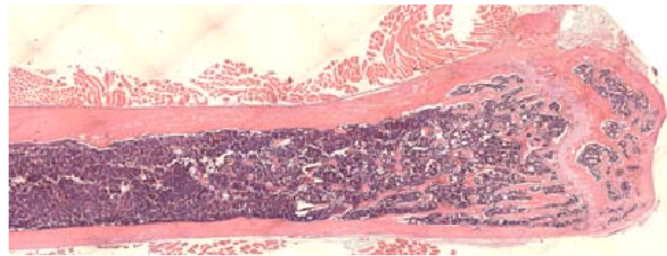
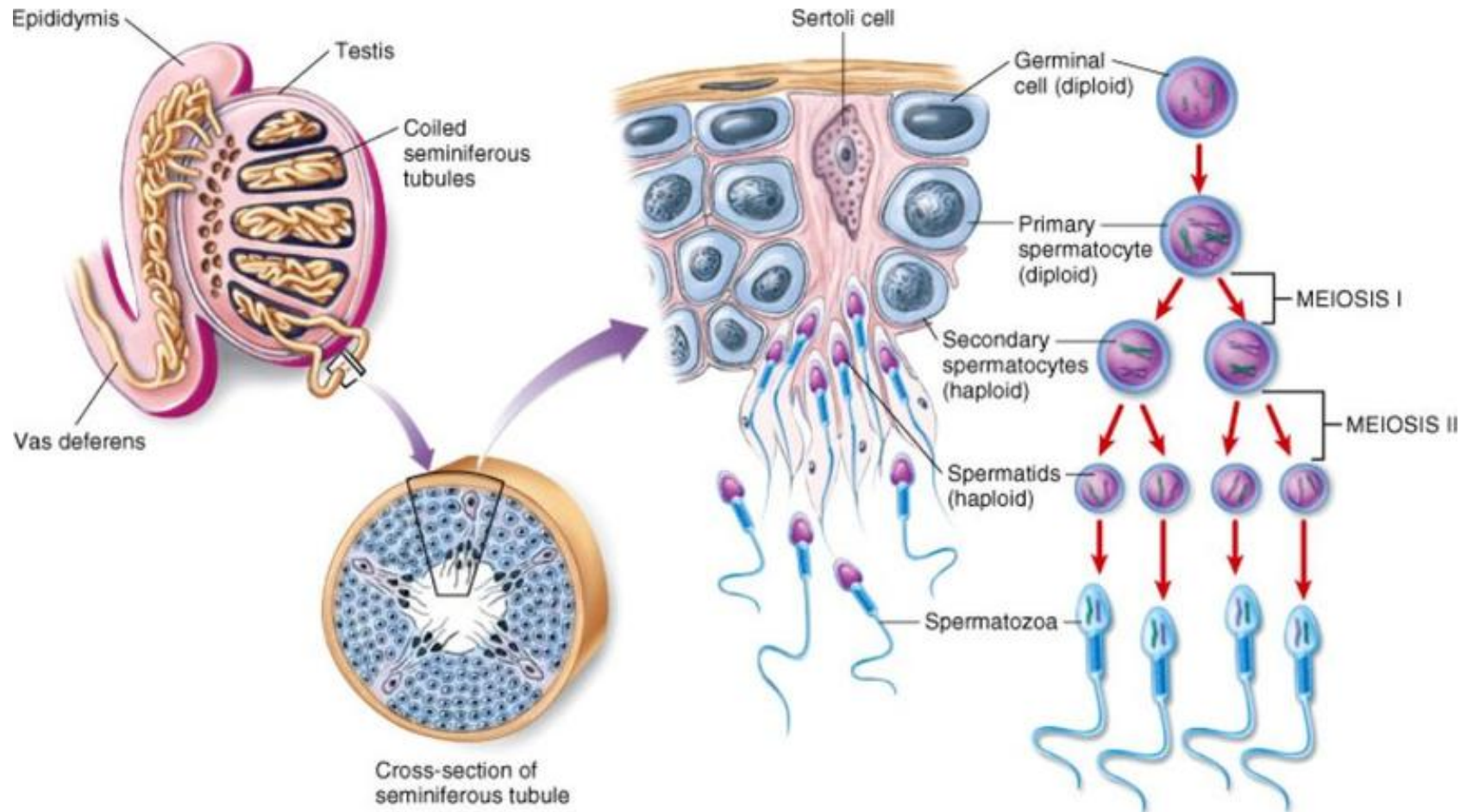


Figure 5.1. Hematopoietic Stem Cell Differentiation (2001 Terese Winslow, Lydia Kibiuk)

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?

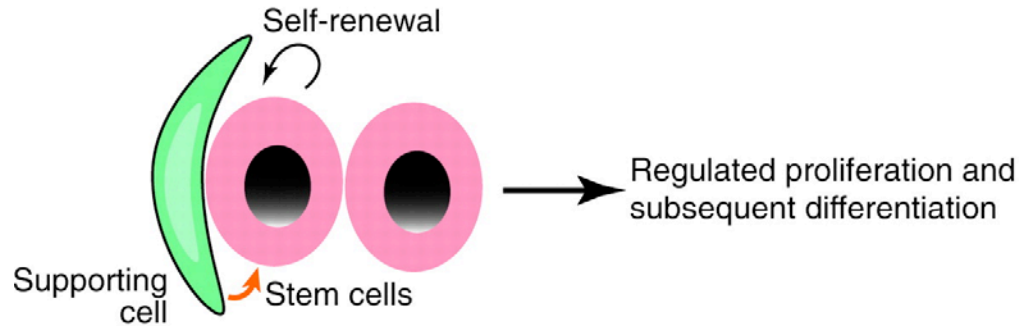
Should I generate differentiating daughter cells?



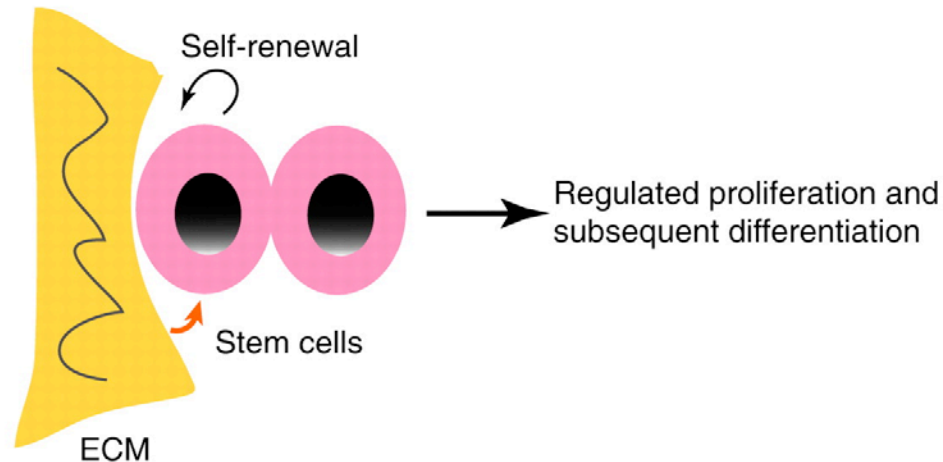
Stem cell niche:

Keeps stem cells in an undifferentiated state

A Cellular niche



B Non-cellular niche

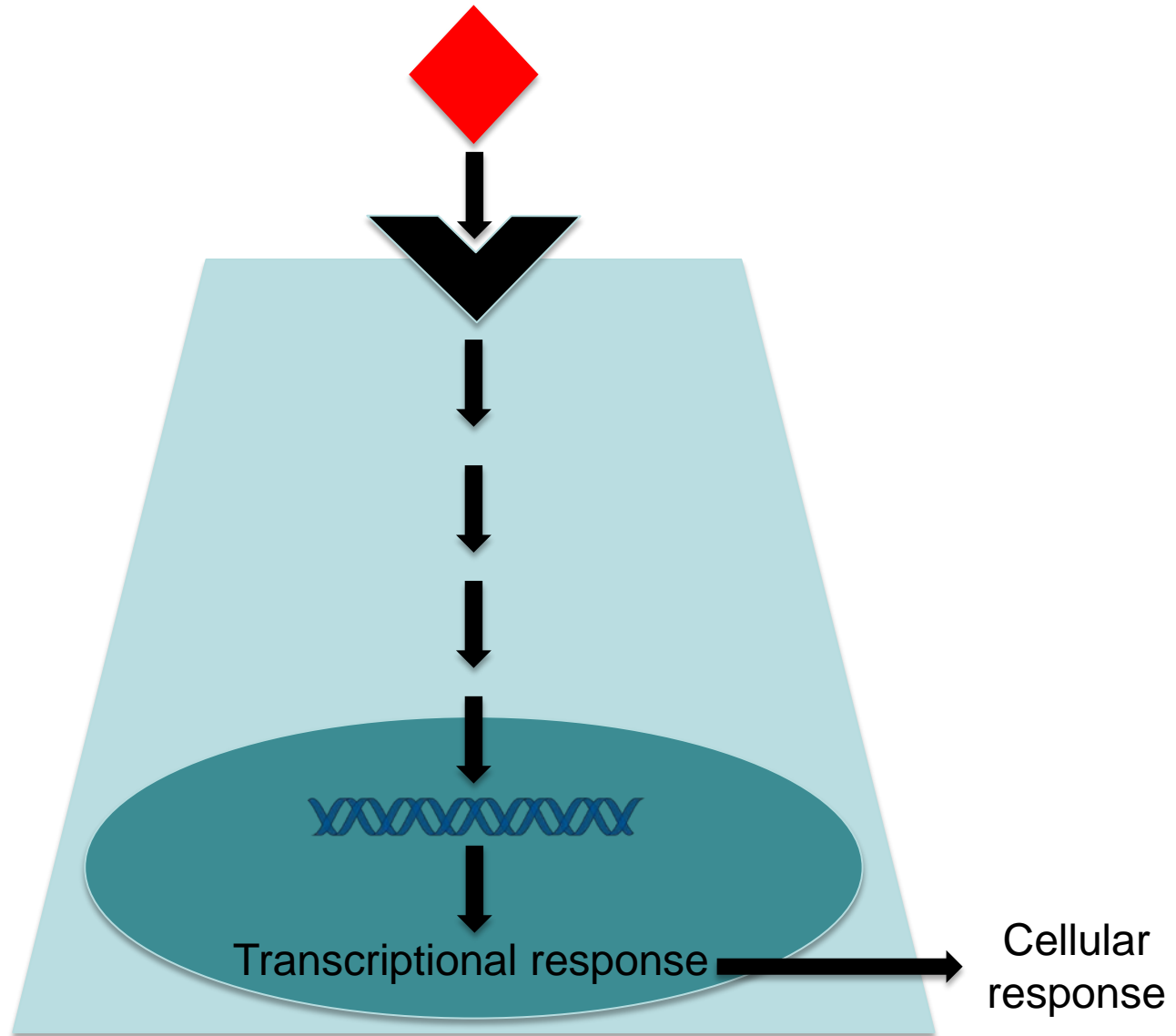


Key:  Secreted signals from niche

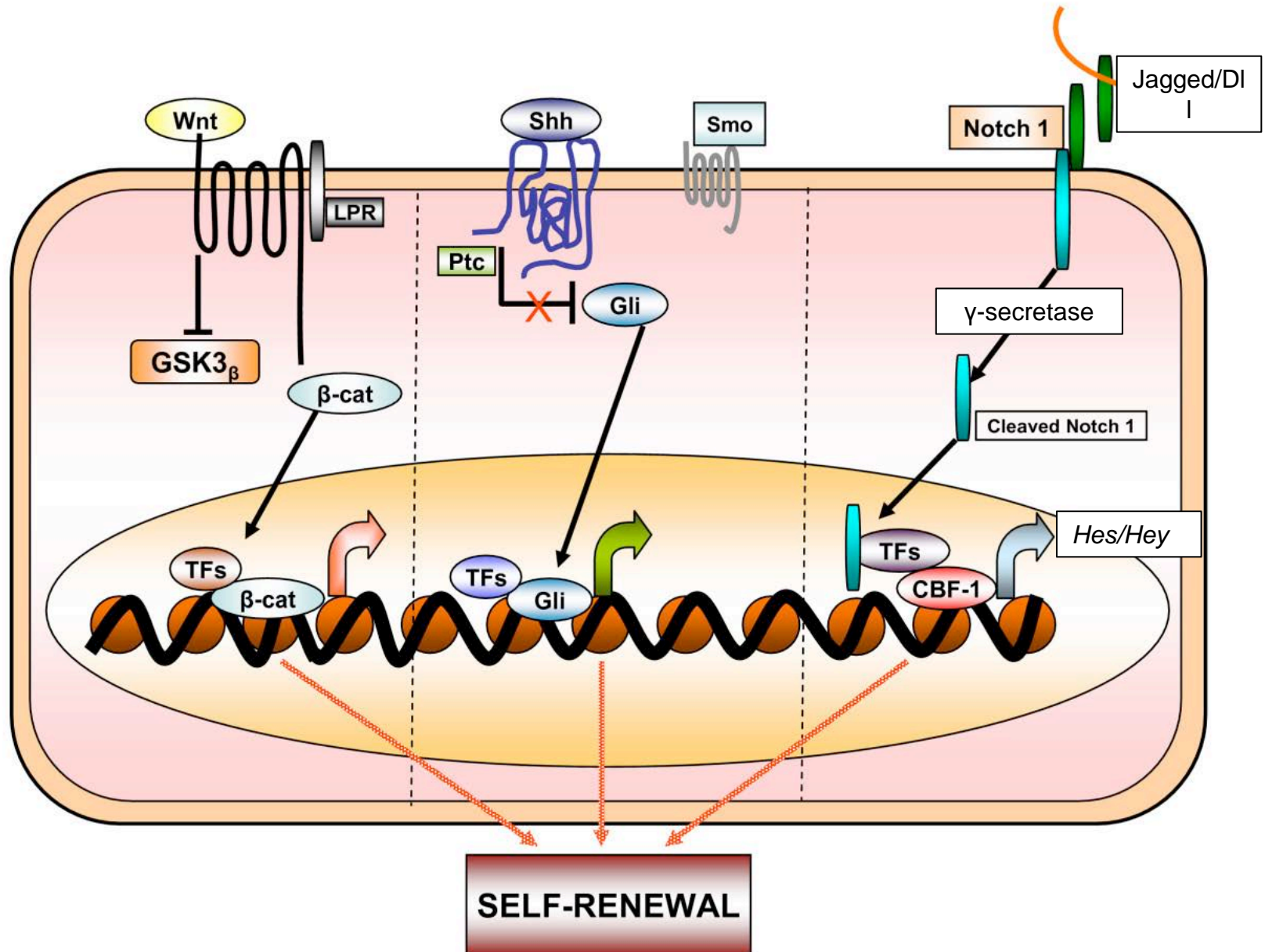


Regulation of Stem Cells

Signalling pathways

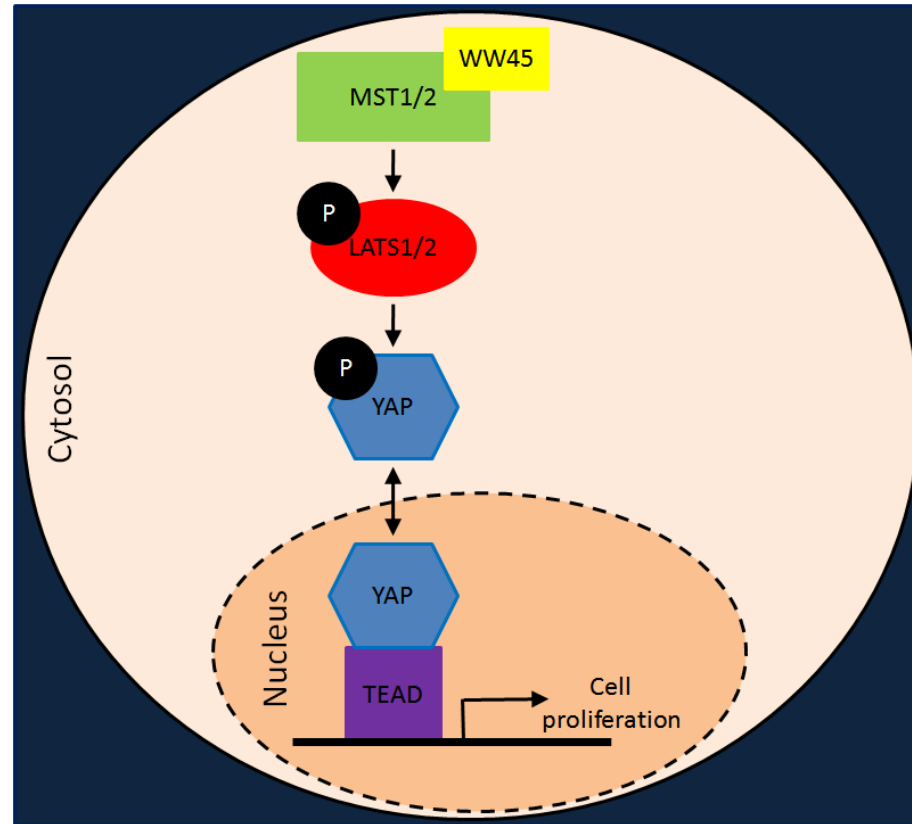


Regulation of Stem Cells

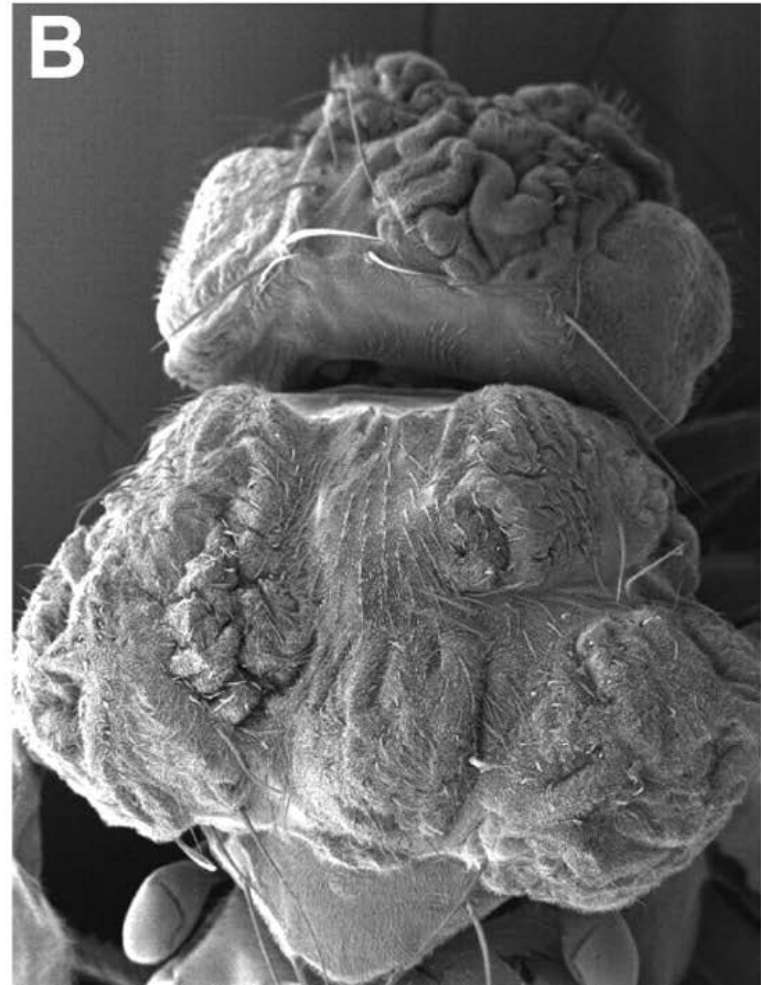
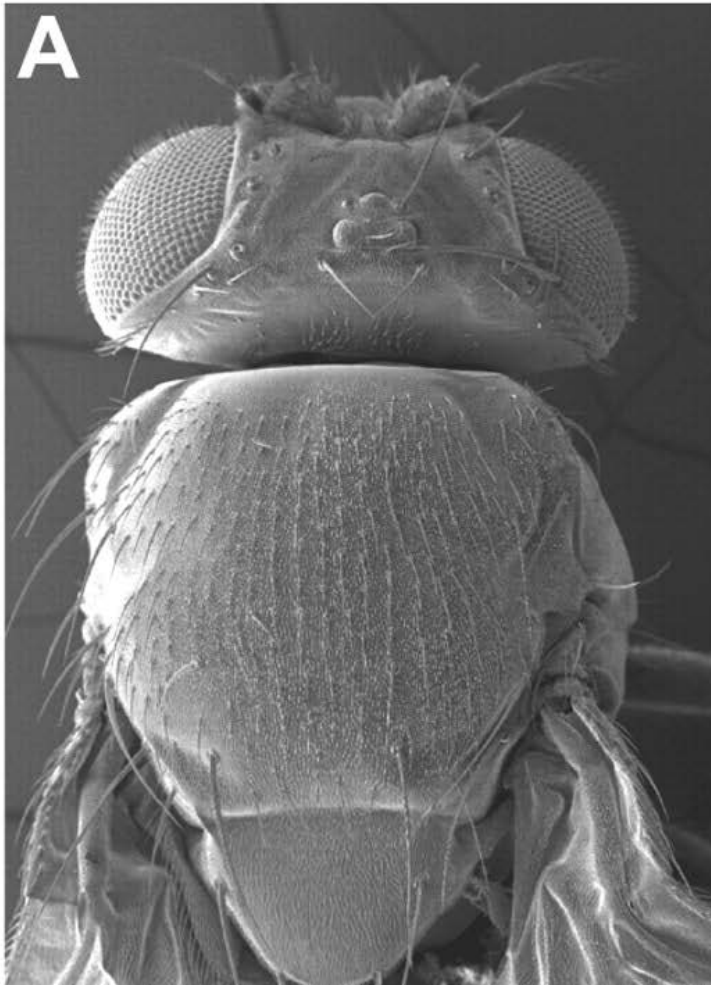


Regulation of Stem Cells

The Hippo Pathway

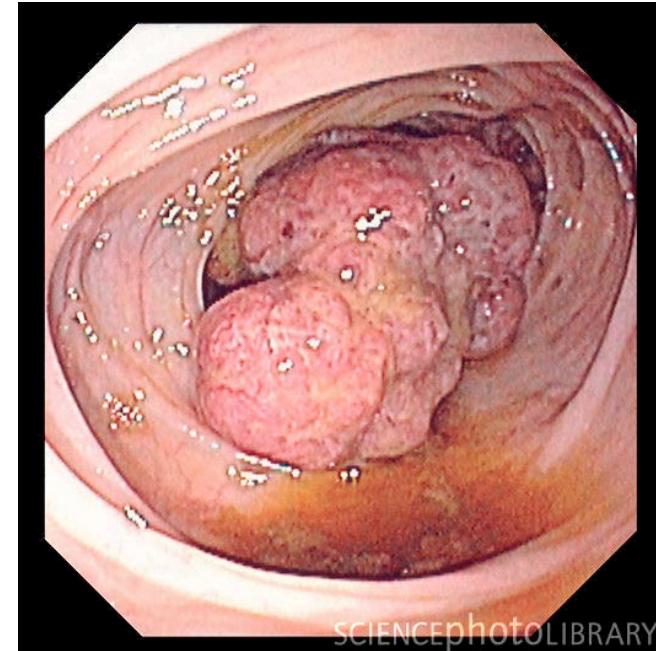
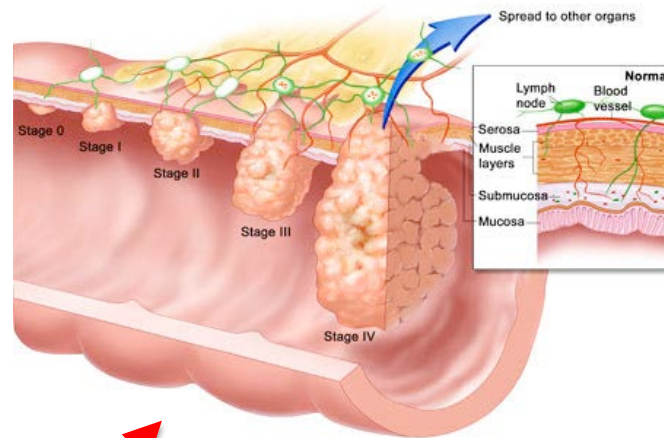
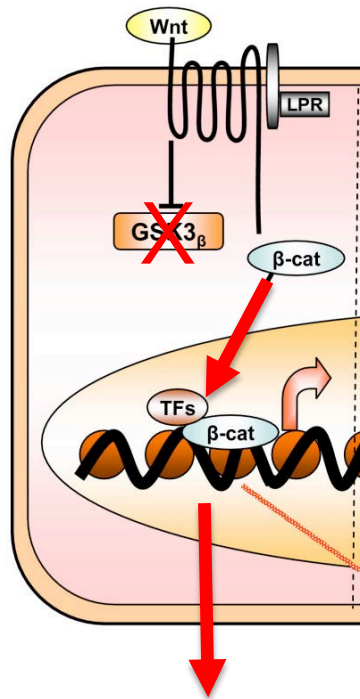


What happens if cell renewal regulation goes wrong?



Mutations in Wnt pathway result in Cancer

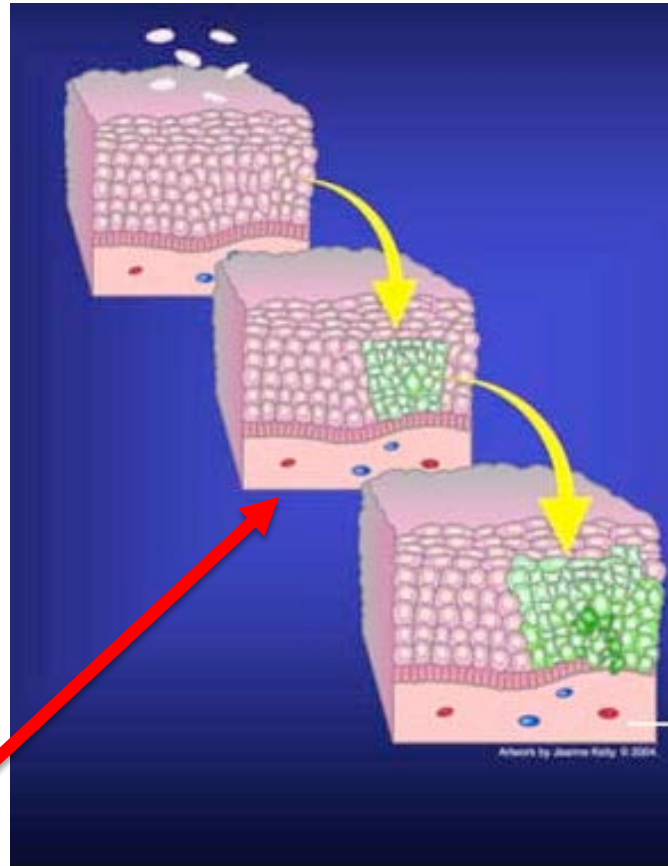
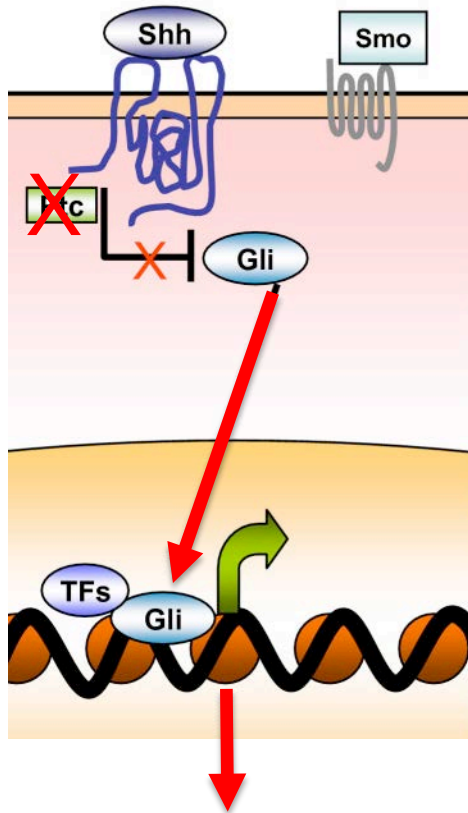
Adenomatous Polyposis Coli



hyperproliferation

Mutations in Hedgehog pathway result in cancer

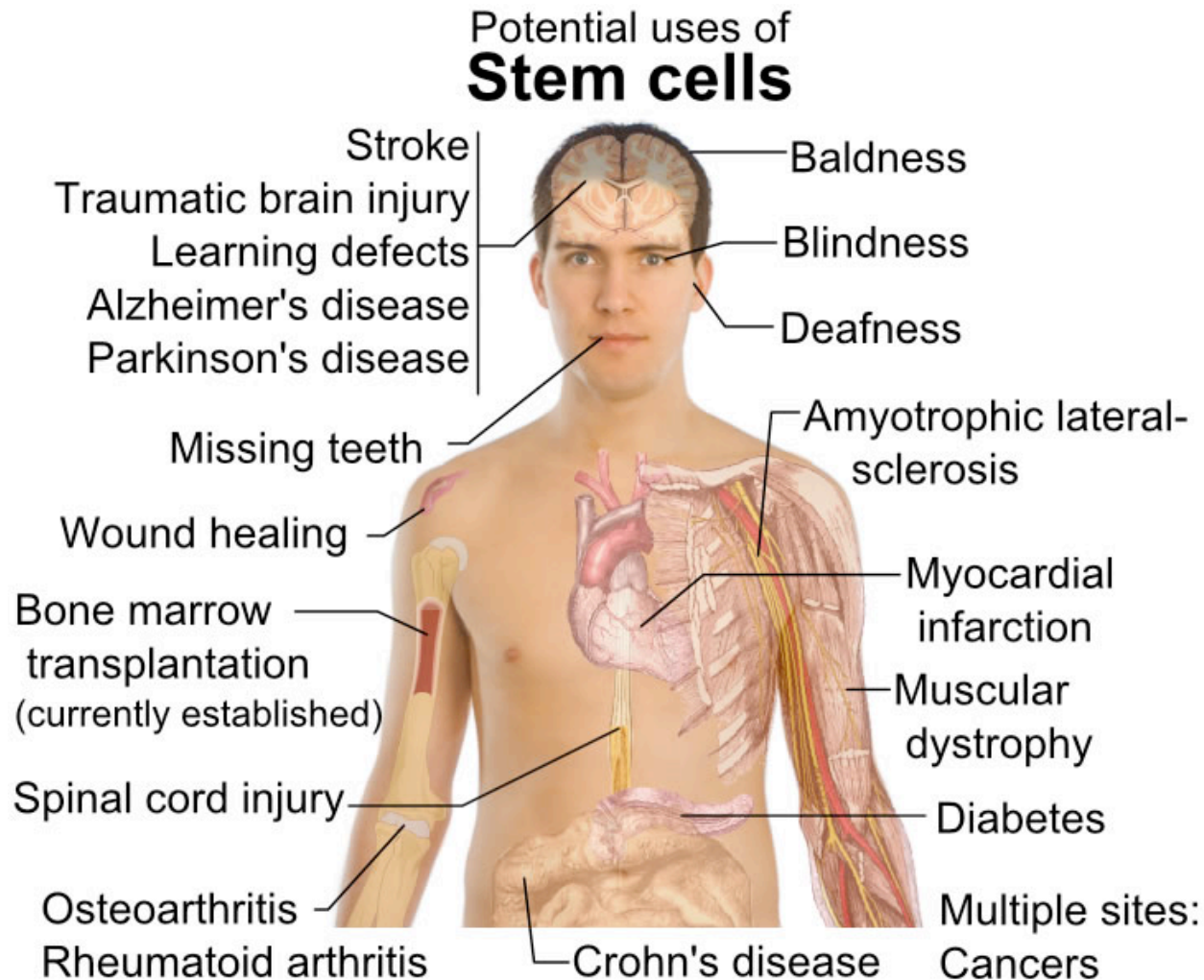
Basal Cell Carcinoma



Regenerative medicine

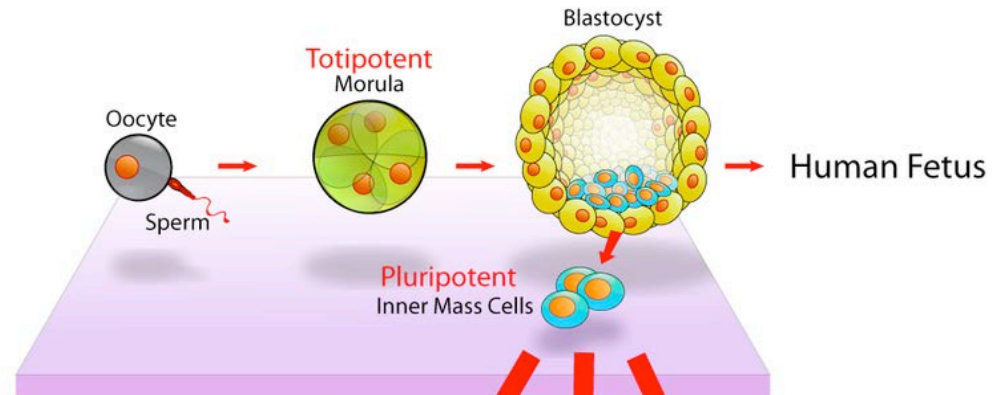
the clinical application of stem cells

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

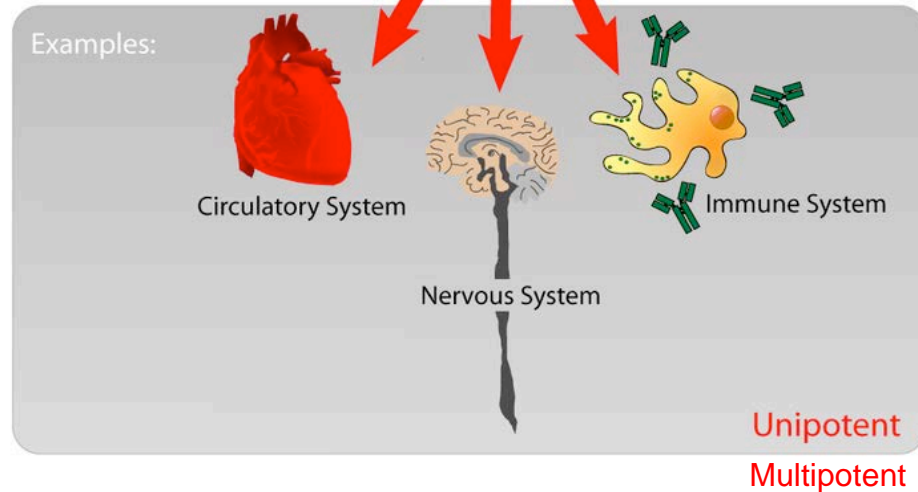


Stem Cell Sources for Regenerative Medicine

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

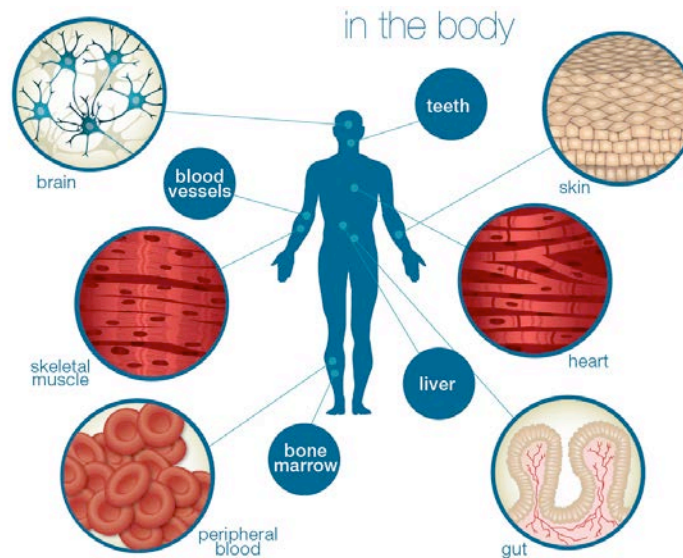
No genetic abnormalities

In vitro culture and expansion

Ethical issues

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”



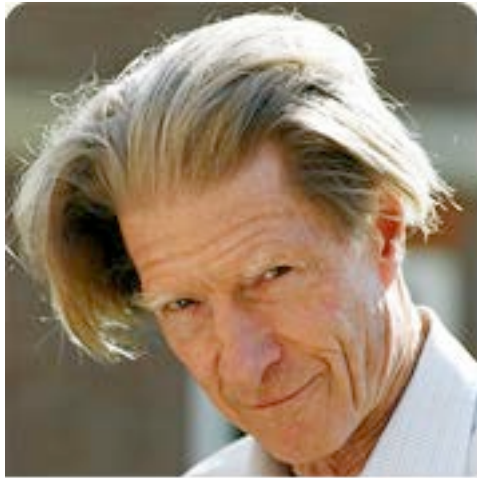
No ethical issues
Restricted plasticity
Limited quantities
Hard to identify



Somatic Cell Nuclear Transfer

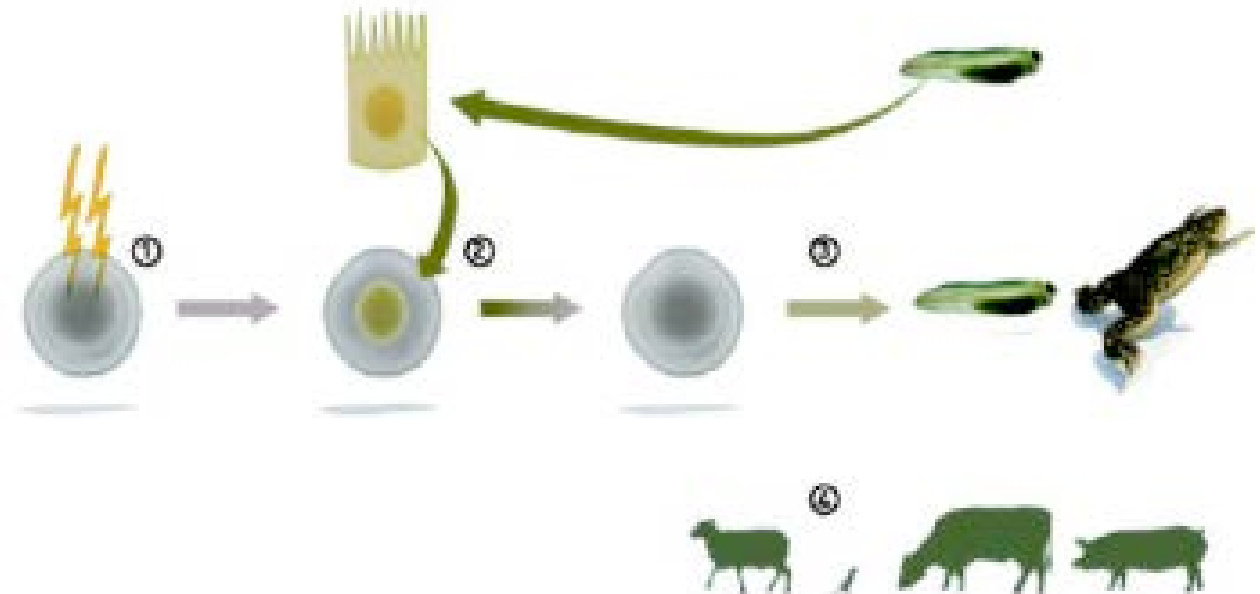
John Gurdon, 1958

The developmental potential of nuclei of differentiated cells



John Gurdon

University of Cambridge



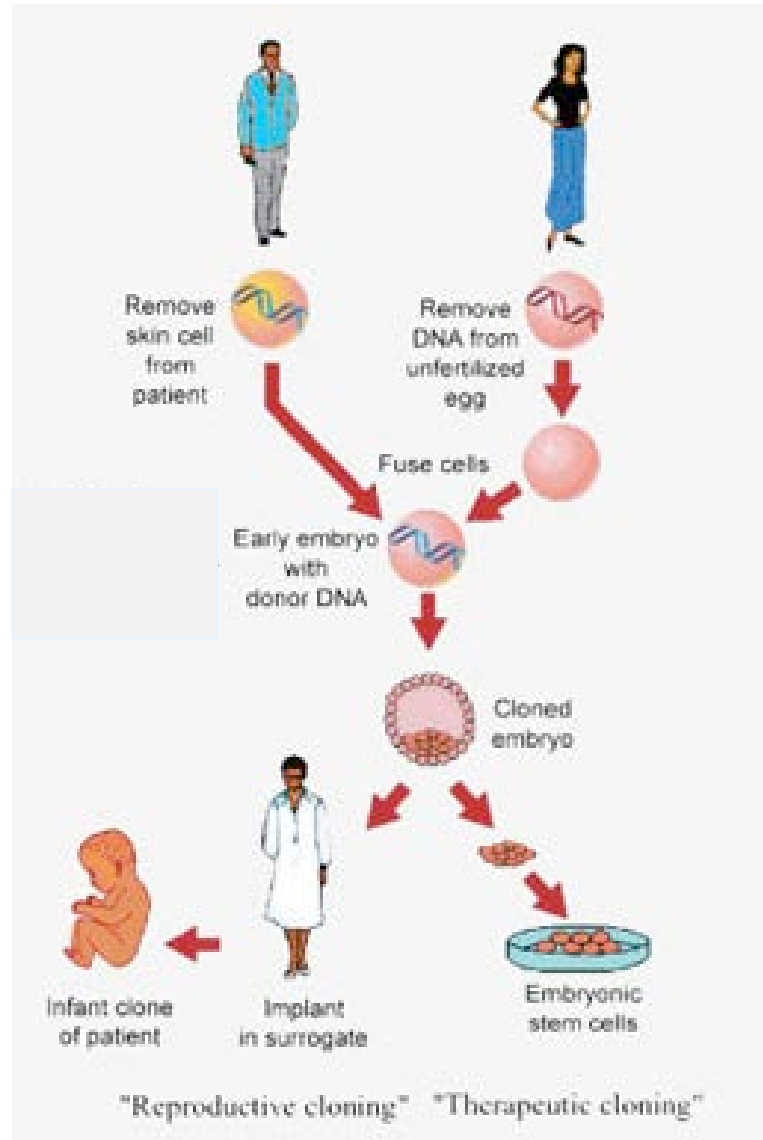


Somatic Cell Nuclear Transfer

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



Reproductive/Therapeutic Cloning



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

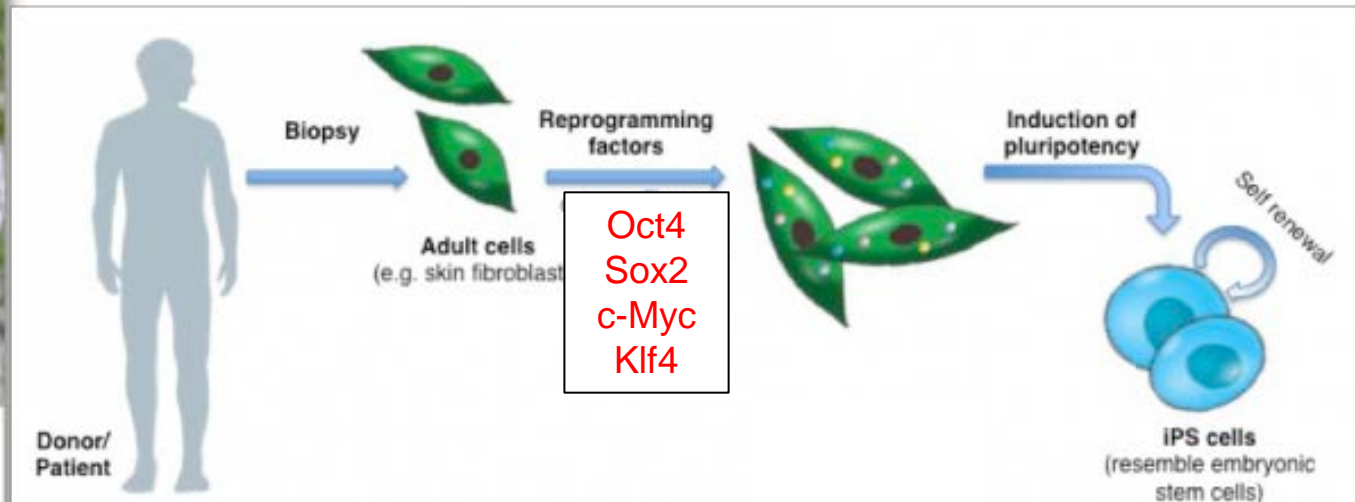
Nuclear Reprogramming

Induced pluripotency (iPS), Yamanaka, 2006

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



Shinya Yamanaka
Kyoto University

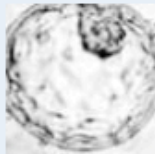

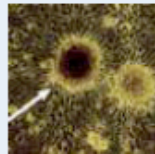


Pluripotent
Good success rates
No need for human embryos
Genetic/phenotypic abnormalities?

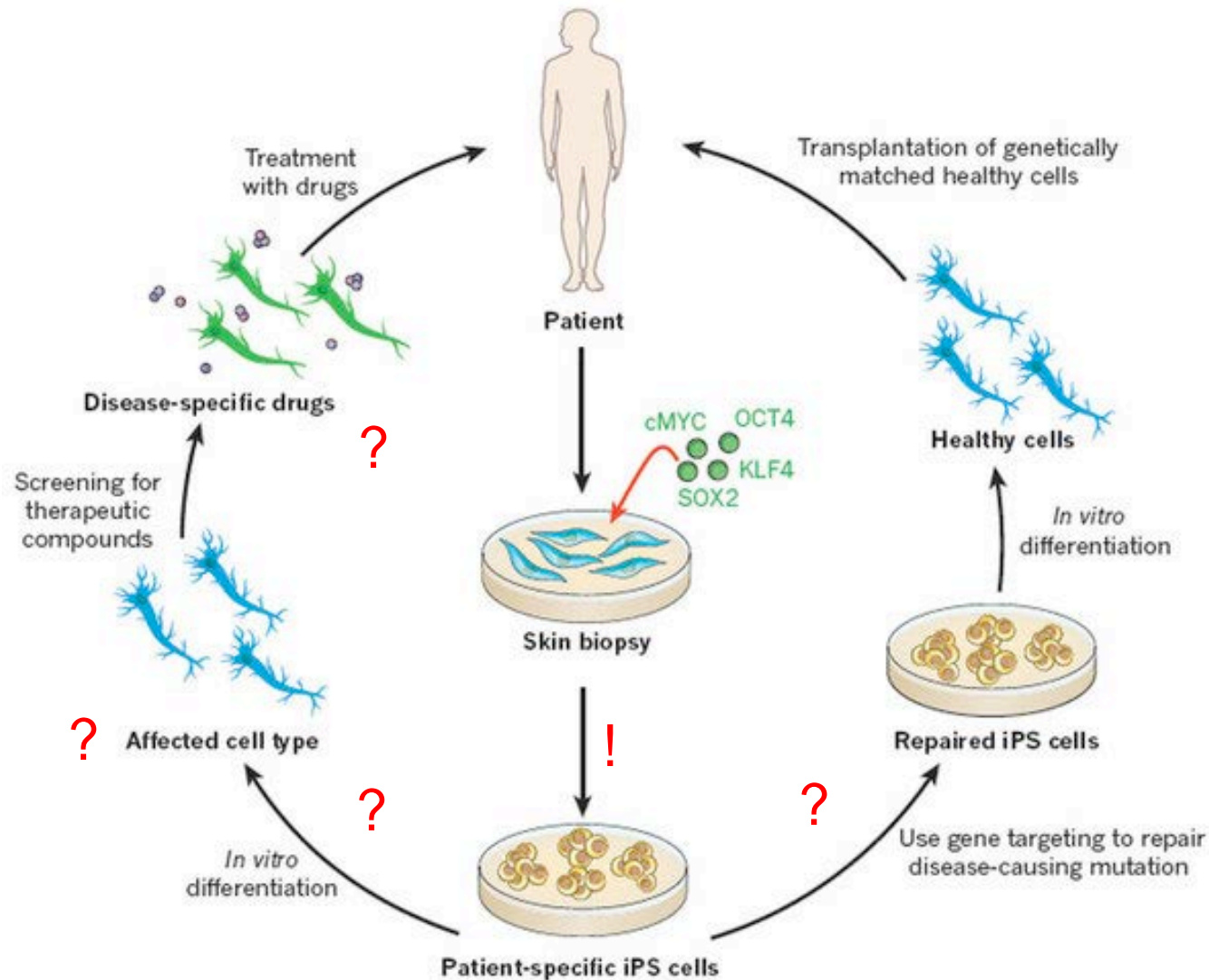
Stem Cell Sources

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	<ul style="list-style-type: none">• can produce all cell types• relatively easy to identify, isolate, maintain, and grow in the laboratory• large source of "excess" blastocysts from IVF clinics	<ul style="list-style-type: none">• can produce all cell types• relatively easy to identify, isolate, maintain, and grow in the laboratory• stem cells may be genetically matched to patient	<ul style="list-style-type: none">• demonstrated success in some treatments• stem cells may be genetically matched to patient	<ul style="list-style-type: none">- Can generate any cell type- Easy to generate, maintain and grow in lab- Perfect genetic match to patient
Limitations	<ul style="list-style-type: none">• limited number of cell lines available for federally funded research	<ul style="list-style-type: none">• risk of creating teratomas (tumors) from implanting undifferentiated stem cells	<ul style="list-style-type: none">• produce limited number of cell types• not found in all tissues• difficult to identify, isolate, maintain, and grow in the laboratory	<ul style="list-style-type: none">- May retain age of parental cell- Inheritance of mutations: teratomas
Ethical Concerns	<ul style="list-style-type: none">• destruction of human blastocysts• donation of blastocysts requires informed consent	<ul style="list-style-type: none">• destruction of human blastocysts• donation of eggs requires informed consent• concern about misapplication for reproductive cloning	<ul style="list-style-type: none">• no major ethical concerns have been raised	<ul style="list-style-type: none">- 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?

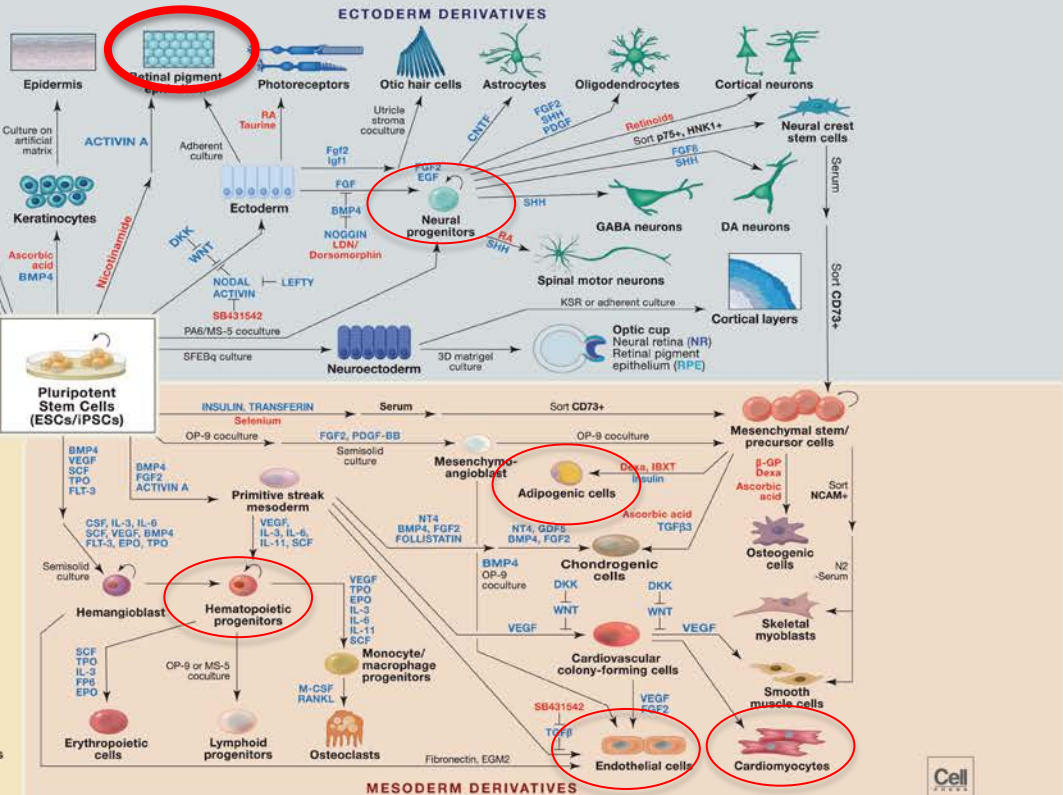
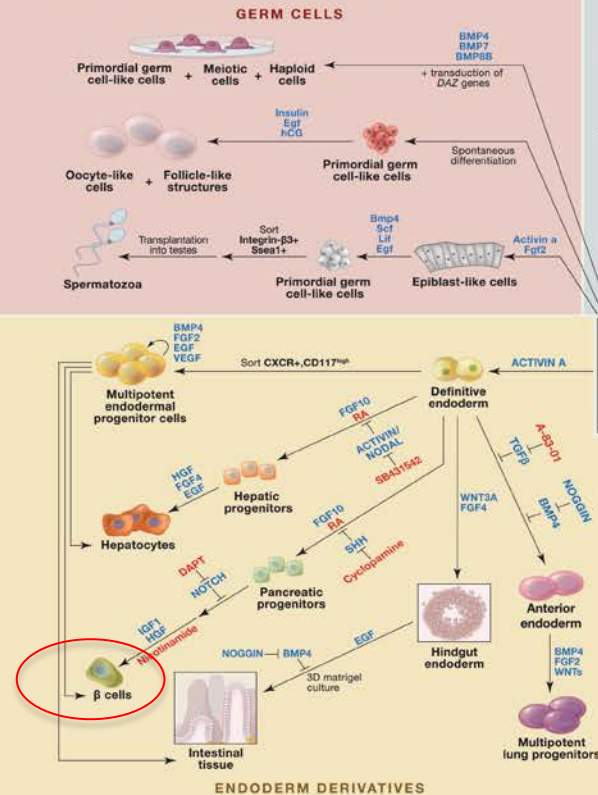
Future of Regenerative Medicine

Directed differentiation of pluripotent stem cells

SnapShot: Directed Differentiation of ESCs and iPSCs

Luis A. Williams, Brandi N. Davis-Dusenbery, and Kevin C. Eggan
HHMI, Harvard University, Cambridge, MA 02138, USA

This SnapShot was previously published in Cell 149, May 25, 2012 ©2012 Elsevier Inc. DOI 10.1016/j.cell.2012.05.015



STEMCELL Technologies is committed to making sure your research works. As scientists helping scientists, we support our customers by creating novel products with consistent, unfailing quality; and by providing unparalleled technical support.

For optimized hPSC differentiation to specific lineages, use the STEMdiff™ product line:

- For endoderm: STEMdiff™ Definitive Endoderm Kit (Catalog #05110)
- For mesoderm: STEMdiff™ Cardiomycocyte Kit (Catalog #05310)
- For ectoderm: STEMdiff™ Neural Induction Medium (Catalog #05831)
- For flexible differentiation to any germ layer: STEMdiff™ APEL™ Medium (Catalog #05210)

Other key products for maintenance and differentiation:

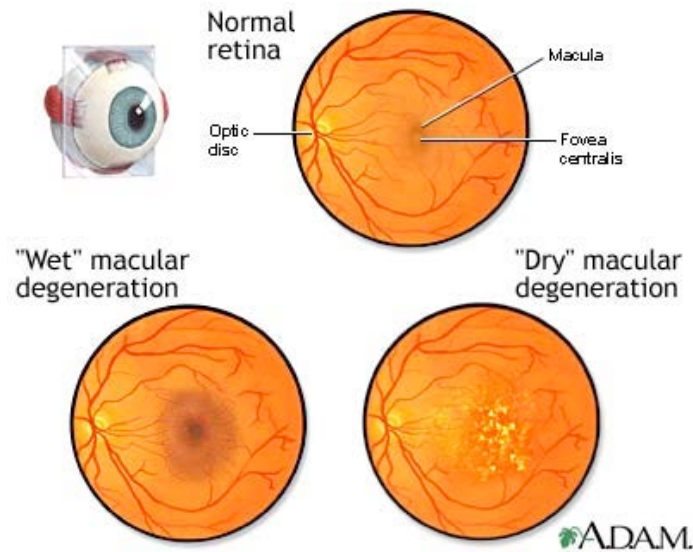
- Make size-controlled embryoid bodies and standardize your differentiation protocols with AggreWell™ plates (Catalog #27945/#27895)
- Support pluripotency with mTeSR™1, the most widely published feeder-free culture medium for hPSCs (Catalog #05850)



Scientists Helping Scientists™ | WWW.STEMCELL.COM

Stem Cell Therapy

Macular Degeneration



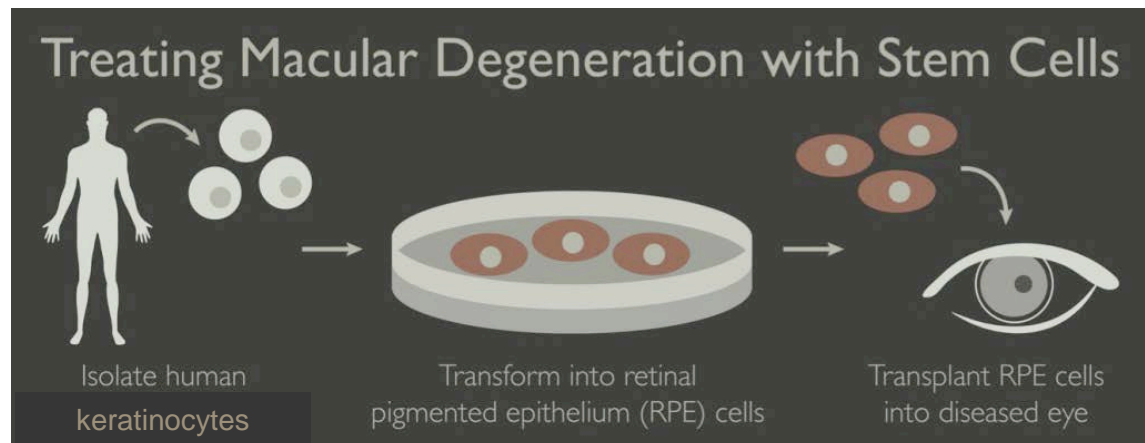
Masayo Takahashi (RIKEN)

iPS on skin cells of patient

Differentiate into retinal pigment epithelium cells

Grow in sheets to transplant in retina

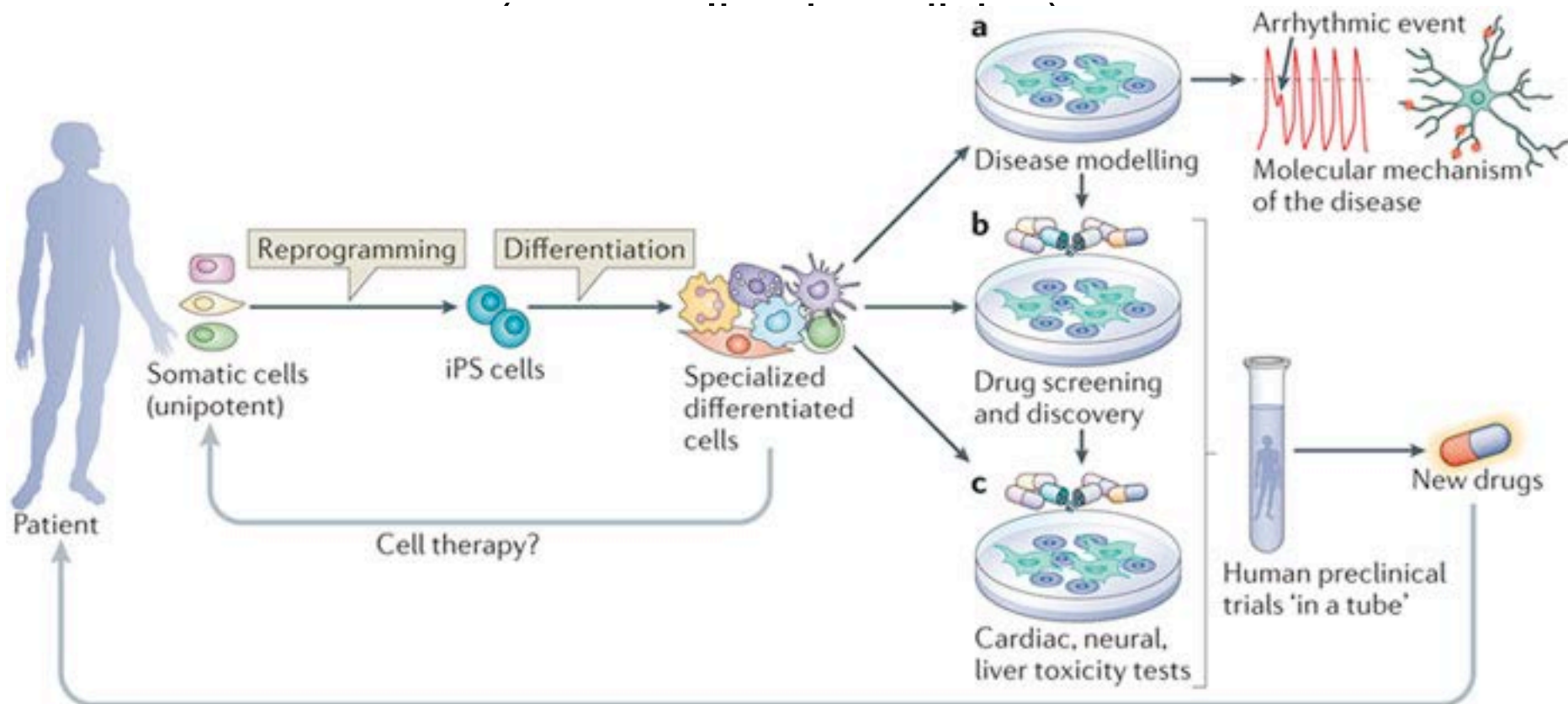
(Surgery on 12 September 2014)



Future of Regenerative Medicine

How can we cure disease?

Disease Modeling and Drug discovery



Future of Regenerative Medicine

Disease Modeling of Spinal Muscular Atrophy Mutations in *SMN1*



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:

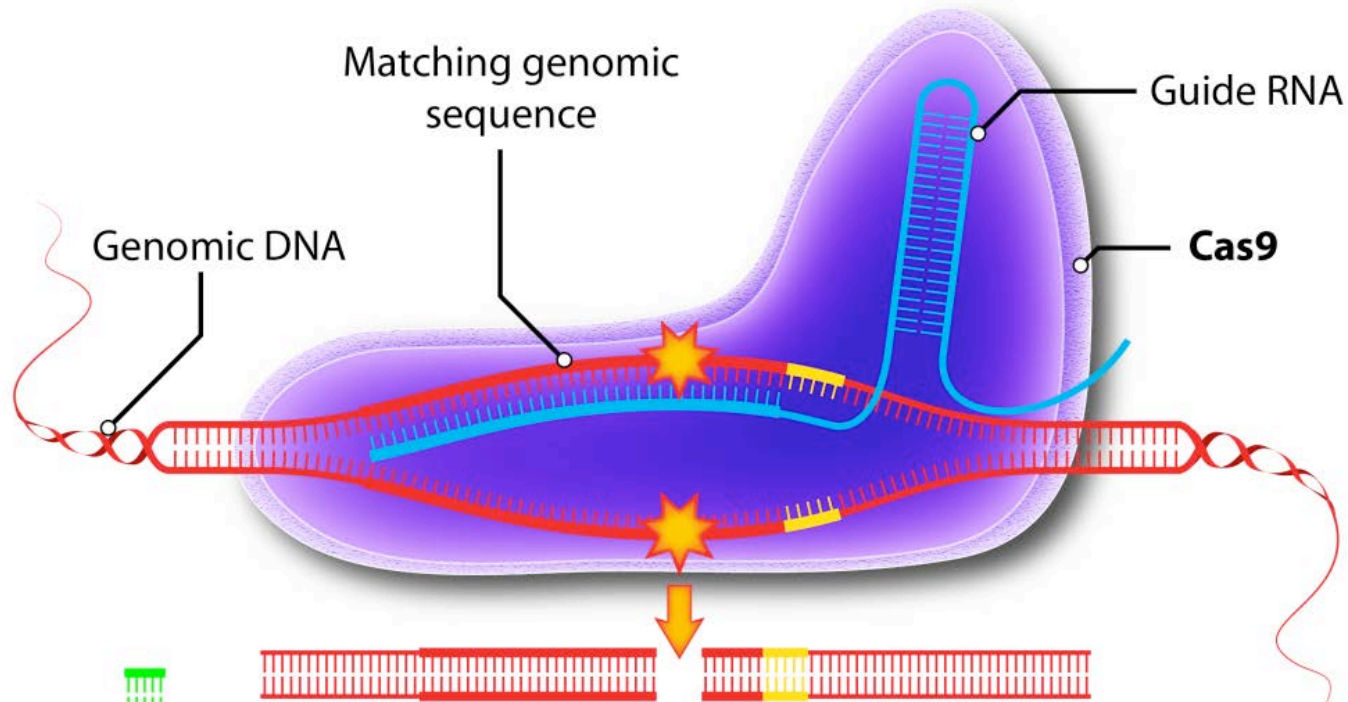
CRISPR/CAS9 genome editing



CRISPR/Cas9 Genome Engineering

(Clustered Regularly Interspaced Short Palindromic Repeats)

Guide RNA and Cas9

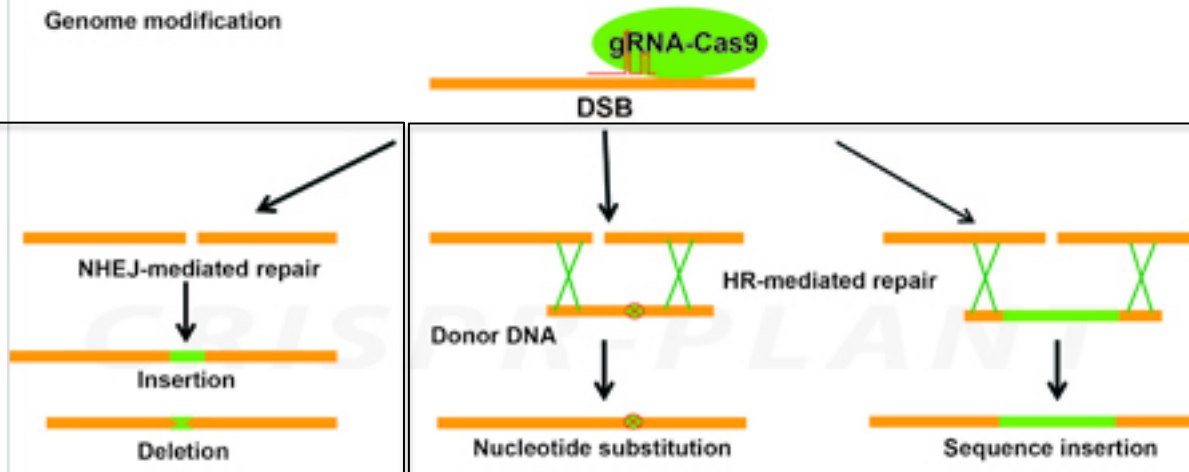




CRISPR/Cas9 Genome engineering

Repair

Genome modification



Non-homologous end joining:

Small insertion/deletion
gene disruption
(and occasional errors)

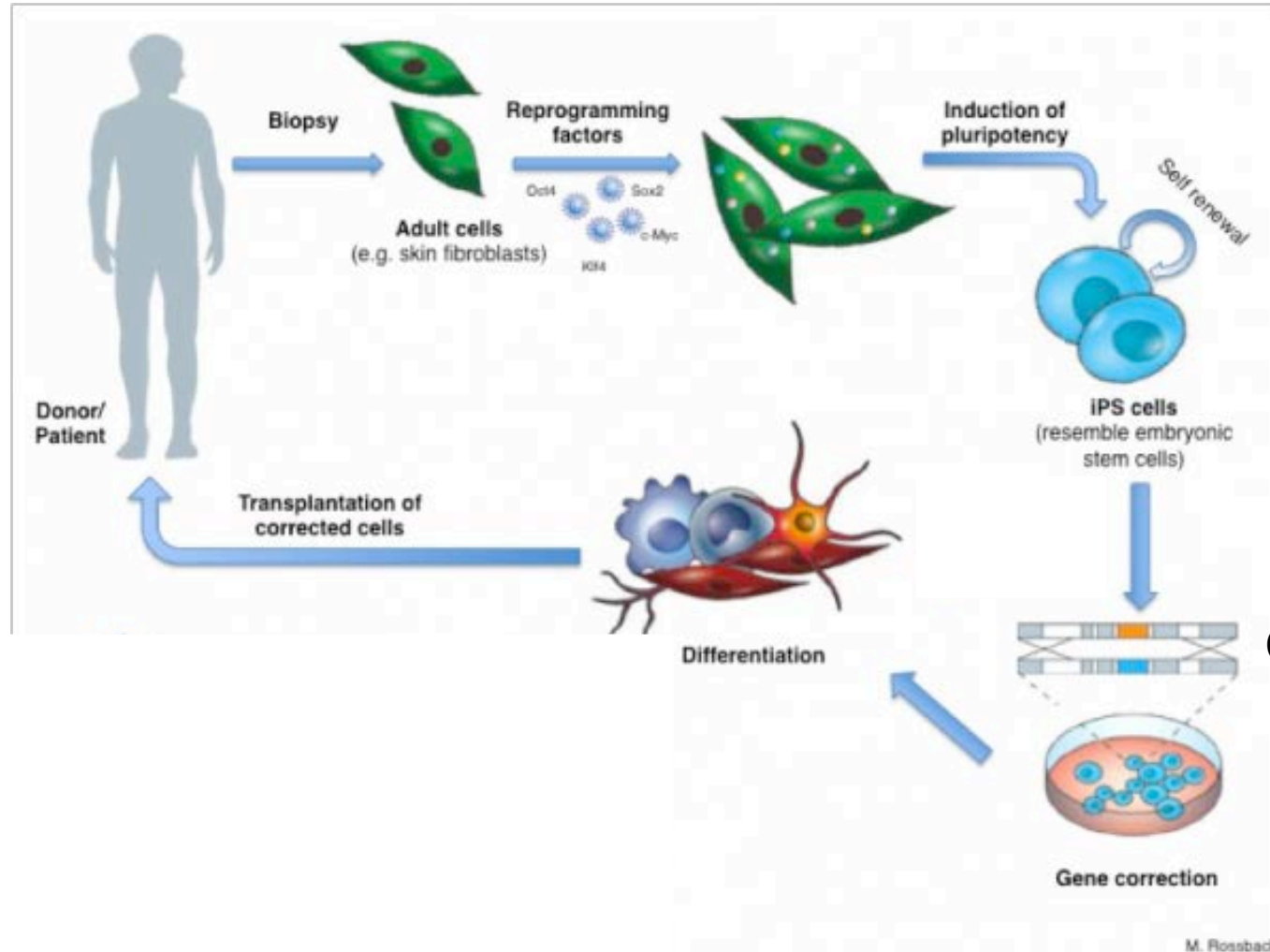
Homology-directed repair:

Provide donor template with homology arms
Gene mutation/correction/addition
(Cas9 D10A mutant)



CRISPR/Cas9 Genome engineering

Applications in Stem Cells





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

CF: accumulation of viscous mucus in pulmonary and gastrointestinal tract

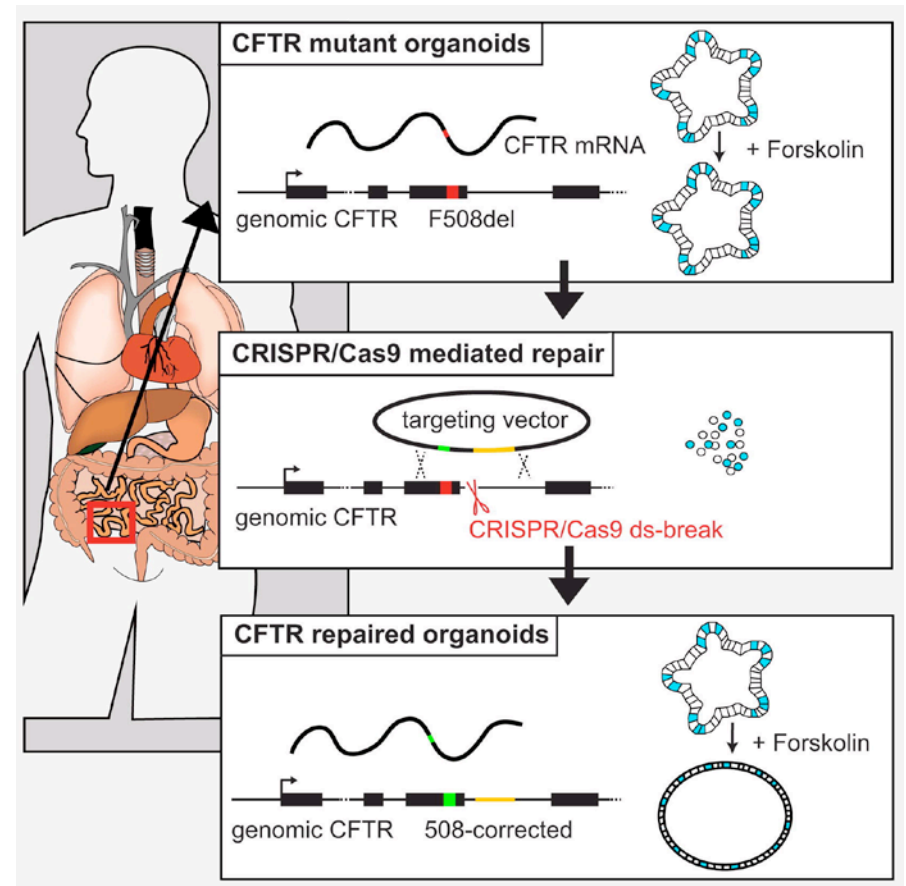
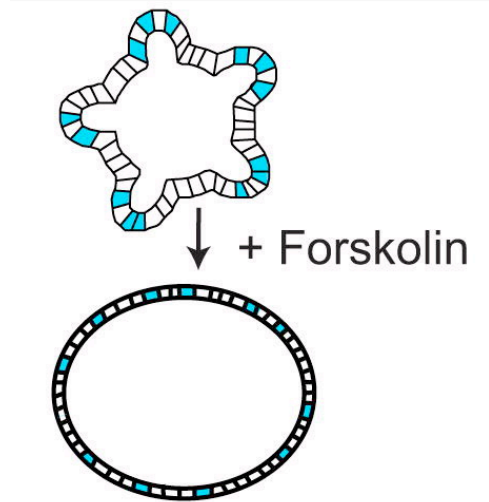
Life expectancy: 40 years

F508del in CFTR (anion channel essential for fluid and electrolyte homeostasis of epithelia)

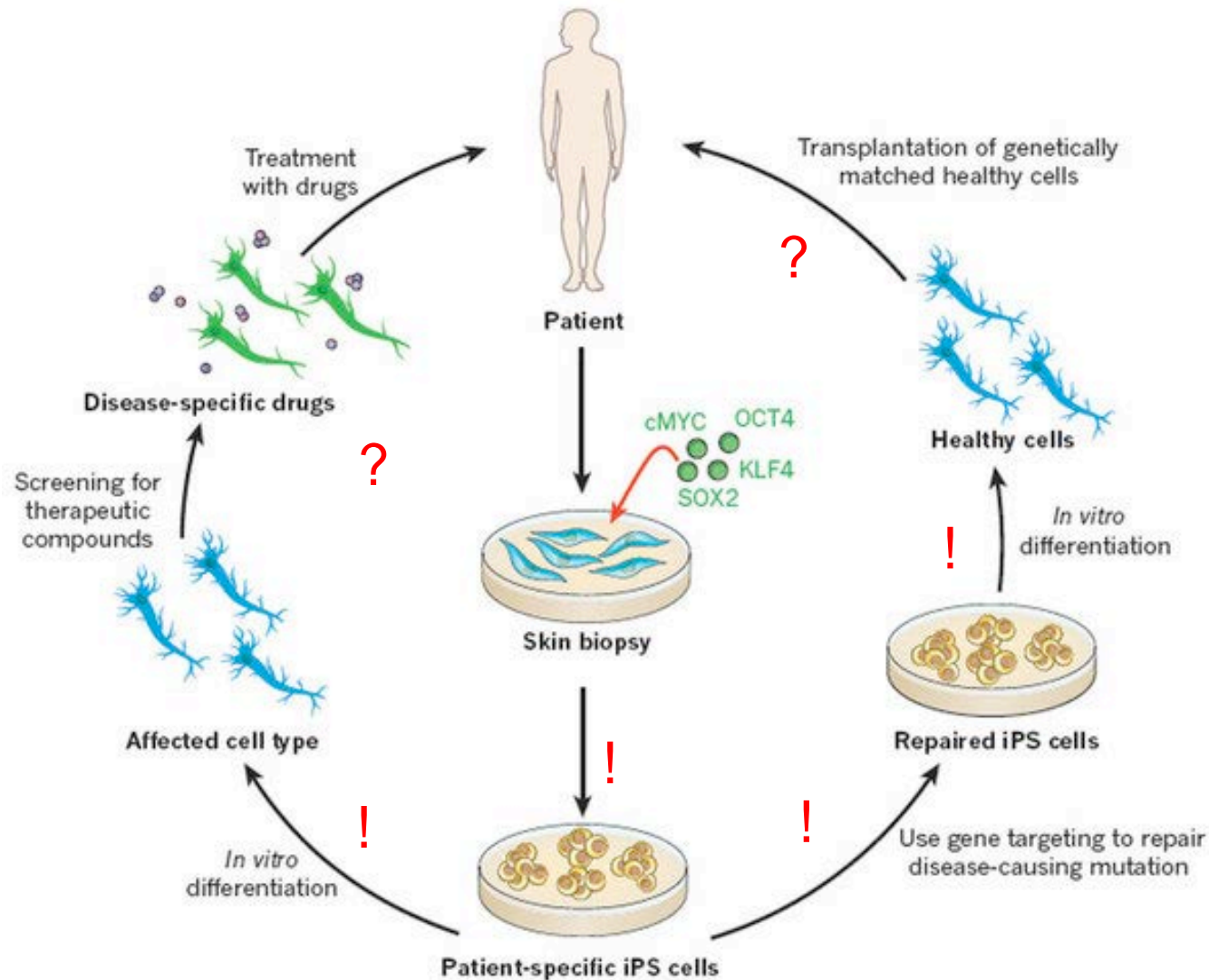
Lgr5+ intestinal stem cells -> organoids

In vitro assay in intestinal organoids:

Forskolin -> CFTR -> expansion



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



ANAT2341: Stem Cell Lab

Stem cell isolation and maintenance: Group 4

Stem cell differentiation: Group 1, 2, 6

Regenerative Medicine: Group 2, 3

Anti-cancer stem cell therapy: Group 5

Duration: 12 minutes max!

Do not discuss M&M in detail

Do not improvise your lines, take time to rehearse