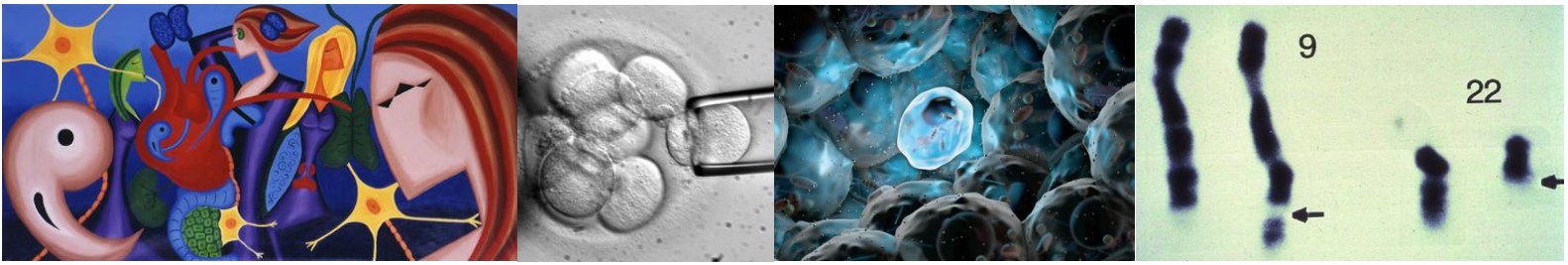
BMS2004 Biochemistry



Lecture 13

Stem cells biology

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Department of Biomedical Sciences

Final exam

- On-line exam via Zoom on/off campus (May 4th 2PM)
- Lockdown browser (1st camera) + Zoom (2nd camera)
- Closed book, 3hrs, MC, short and long answer questions
- Lecture 1 to 13 (lower % for 1-6 and higher % for 7-13)

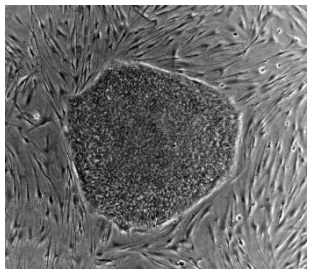
Overview

- Types and properties of stem cells
- Stem cell niches: definition and functions
- Different levels of stem cell potency
- Embryonic stem cell
- Adult stem cells

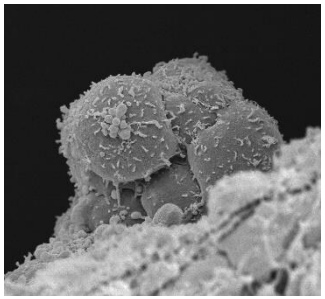
What are stem cells?

- Stem cells are undifferentiated cells that can differentiate into specialized cells and can divide (through mitosis) to produce more stem cells.
- Stem cells found in all multi-cellular organisms.
- Characteristics: ability to **renew themselves** through mitotic cell division and **differentiate** into a diverse range of specialized cell types.

Cultured Human Embryonic Stem cell colony on mouse embryonic fibroblast feeder layer



Scanning electron microscopy picture of an intra-aortic hematopoietic cluster



Embryonic stem cell harvest from blastomeres

http://www.scientificamerican.com/article/embryos-survive-stem-cell-harvest/
Wikipedia http://www.hubrecht.eu/research/robin/research.html

Stem cell types

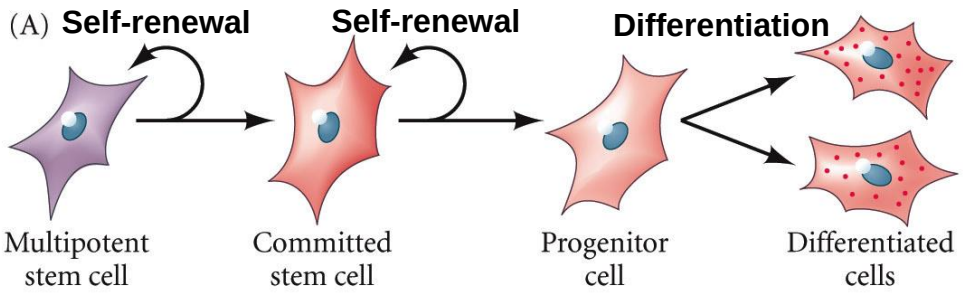
- The two broad types of mammalian stem cells are: **embryonic stem cells** and **adult stem cells** that are found in adult tissues.

- **Embryonic stem cells:** derived from the inner cell mass of mammalian blastocysts.
  - Capable of producing all the cells of the embryo (totipotent and pluirpotent)

- **Adult stem cells** are found in the tissues of organs after the organ has matured.
  - Usually involved in replacing and repairing tissues of that particular organ
  - Can form only a subset of cell types (multipotent)

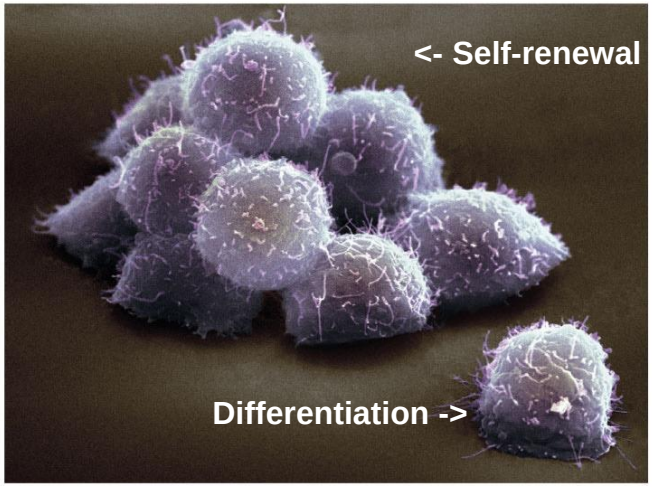
Key features of stem cell division

- **Maintain stem cell population: self-renewal.**



- **To provide a committed cell that can differentiate.**

(B)



***DEVELOPMENTAL BIOLOGY,* 9e, Part Figure III.1**

Purposes of self-renewal

- **Stem cell renews itself at each cell division (continues supply);** some stem cells divide symmetrically so that both daughter cells are stem cells.

- **Produce a daughter cell capable of responding to its environment by differentiating in a particular manner.**

- **Can be stimulated to form more cells when body is stressed by injury or environmental factors.**

Stem cell niche

- **Stem cell niche: the microenvironment where stem cells reside and maintain their stemness.**
- Extracellular matrix with growth factors.
- Allows cells residing within them **to remain relatively undifferentiated.**
- In many cases, the stem cell remains in the niche while its sister cell leaves the niche to differentiate.

Stem cell niche

Table 2. Examples of Well-Characterized Mouse Stem Cell Niches

Stem cell	Location	Supporting cells	Major signals	Stem cells/ niche	Recent References
Hematopoietic stem cells	endosteal, perivascular	osteoblasts, osteoclasts, mesenchymal progenitors, reticular cells	CXCL12; SCF; Tpo; SHH; Ang1	1	(Adams and Scadden, 2006)
Satellite muscle cell	under basal lamina on myofiber	myofiber?	Wnt; Notch; HGF; CXCL12	1	(Dhawan and Rando, 2005)
Central nervous system SVZ stem cell	SVZ	endothelial; ependymal?	SHH; Notch; Wnt; TGFα; FGF; VEGF;	many	(Doetsch, 2003)
Intestinal epithelium	base of crypt	fibroblasts?, hematopoietic cells?	Wnt; Notch; BMP	4–6	(Barker et al., 2007)
Hair follicle bulge	bulge	vascular?	Wnt; BMP; TGFß	many	(Blanpain and Fuchs, 2006)
Interfollicular epidermis	basal layer	dermis	Wnt; Notch	?	(Clayton et al., 2007)
Spermatogonial	basal layer, seminiferous tubules	Leydig, Sertoli, vascular	BMP4; BMP8b; SCF; FGF; GDNF	?	(Yoshida et al., 2007a; Yoshida et al., 2007b)

SVZ: lateral ventricle subventricular zone. Note that the critical signals that maintain mammalian stem cells and the sources of these signals are usually not sufficiently characterized to reliably categorize these niches as stromal or epithelial.

600 Cell *132*, 598–611, February 22, 2008 ©2008 Elsevier Inc.

Regulation of the stem cell niche

- Maintenance of stemness is regulated by paracrine factors (factors transfers from cell-cell) and supporting cells present at the niche.
- These factors retain the stem cells in an uncommitted state.
- Once the stem cells leave the niche, when the paracrine factors are not there, cells begin to differentiate.

Importance of stem cell niche

- Maintenance of stem cell niche is critical for our health; regulate the ratio of cell division to cell differentiation.
- **Too much stem cell differentiation: depletes stem cells and promotes the phenotype of aging.**
  - Graying of human hair: both daughters and dividing stem cells differentiate (depletion of stem cells), leaving smaller number of stem cells to make melanocytes

fabulousfaces.com

- **Too much stem cell division: can cause cancer.**
  - Myeloproliferative disease: a leukemia, stem cell niche unable to provide signals needed for proper blood cell differentiation.

www.wisegeekhealth.com

Definition of stem cell potency

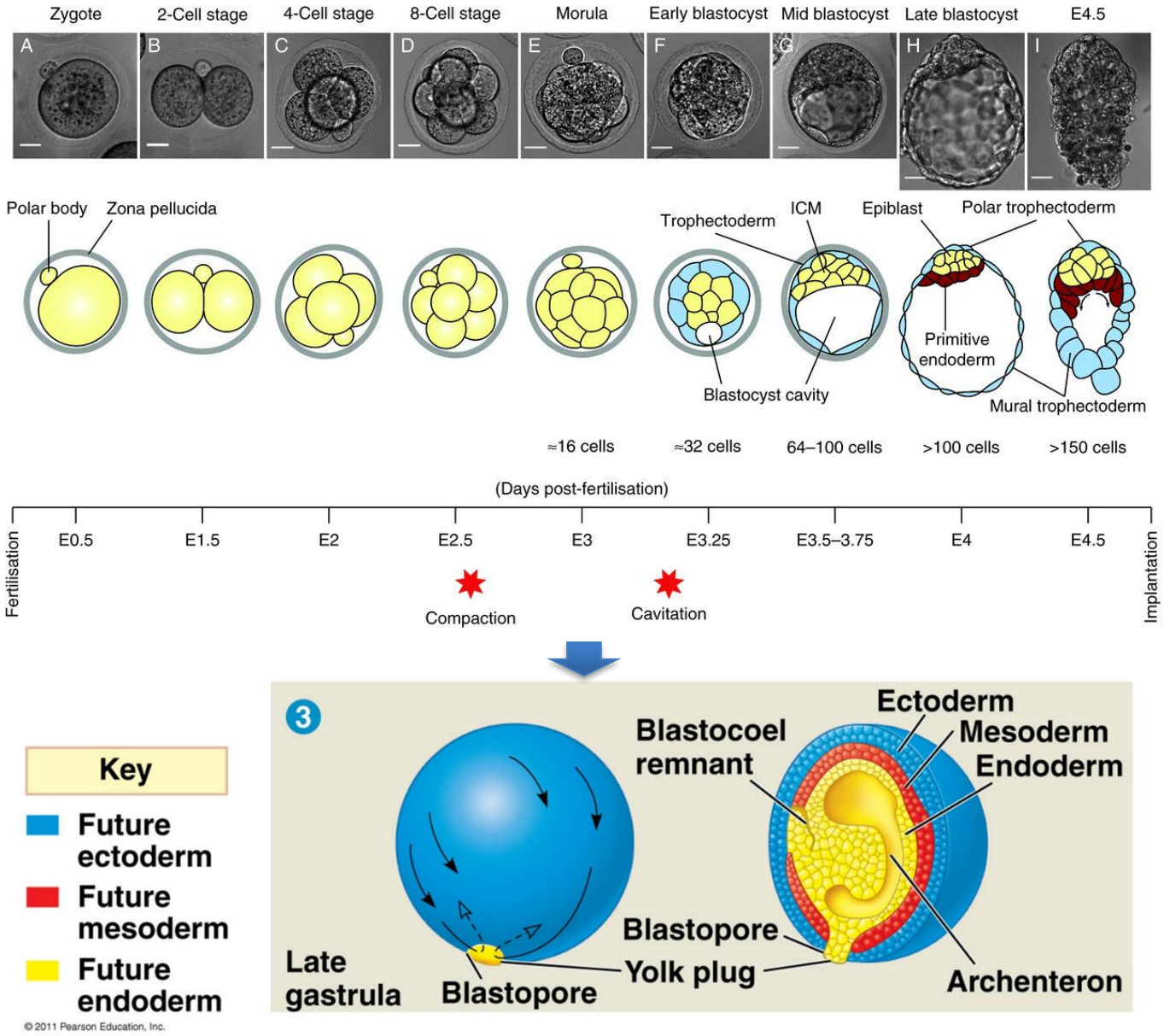
- Stem cells are able to generate numerous different types of differentiated cells but the degree varies.
- **Totipotent**: capable of forming every cell in the embryo PLUS the trophoblast cells of the placenta.
- **Pluripotent**: capable to become all the cell types of the embryo except trophoblast.
  - From inner cell mass of the mammalian blastocyst
  - Germ cells and germ cell tumors (such as teratocarcinomas) can also form pluripotent stem cells

Definition of stem cell potency

- **Multipotent**: cells commit to a relatively small subset of all the possible cells of the body.
  - Usually adult stem cells
  - Eg. hematopoietic stem cells which can form the granulocyte, platelet, and red cell lineages.

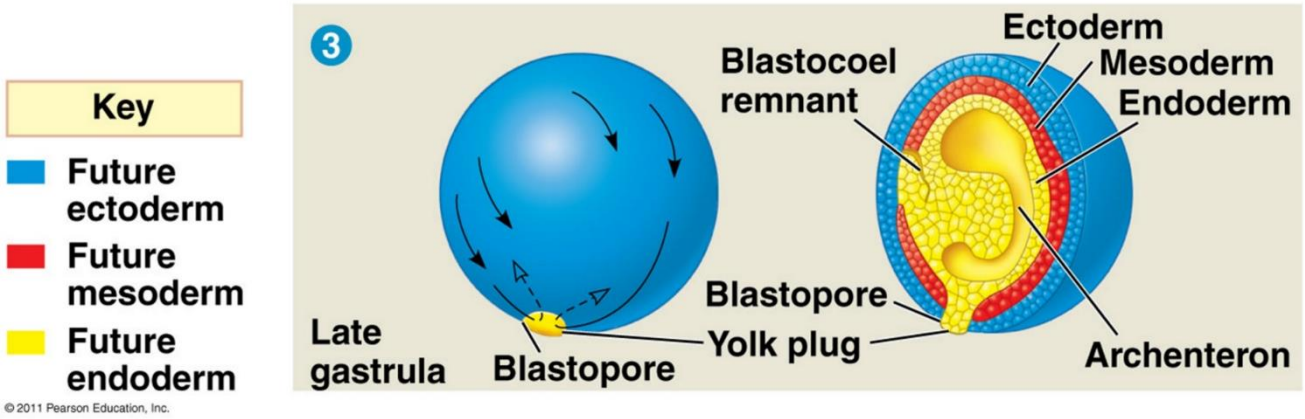
- **Unipotent**: cells found in particular tissues.
  - Involved in regenerating a particular type of cells
  - Eg. spermatogonic are stem cells which can give rise only to sperm.

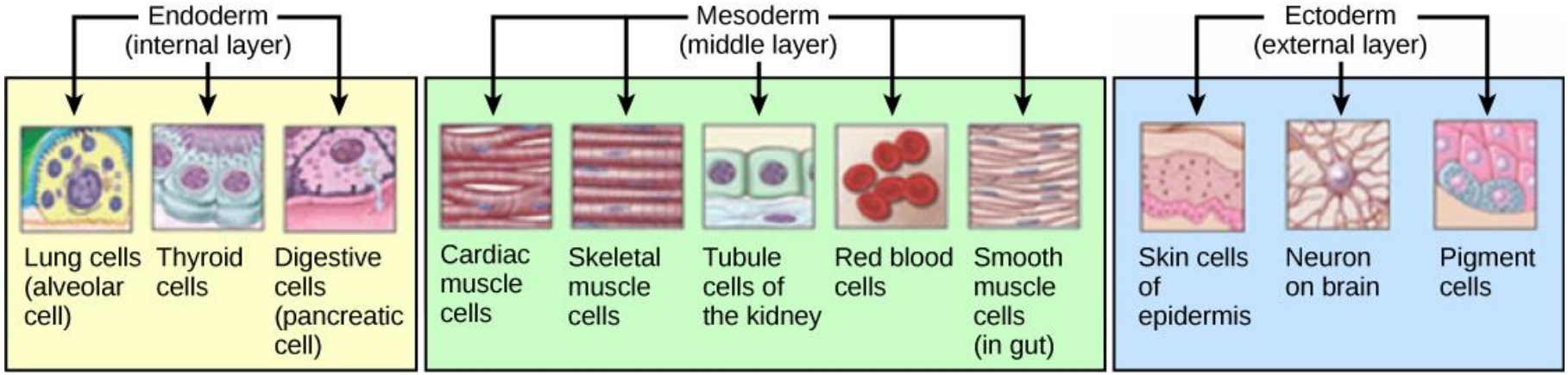
Human embryogenesis



http://www.devbio.biology.gatech.edu/?page_id=8796

Three germ layers differentiation

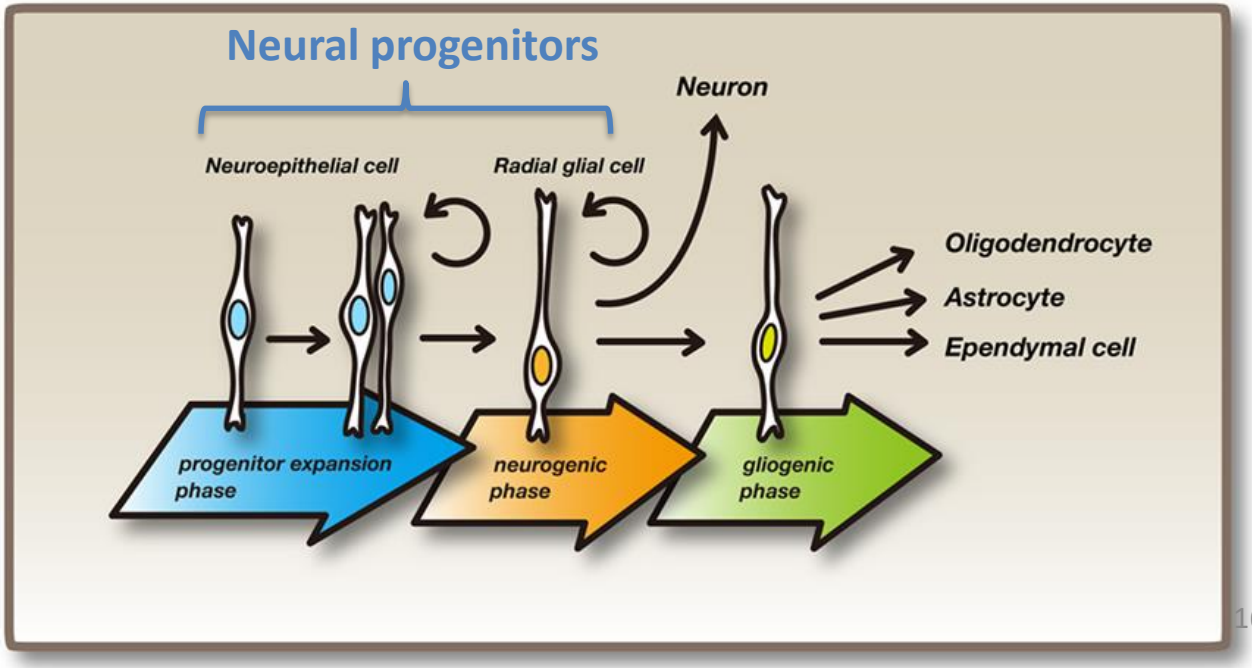


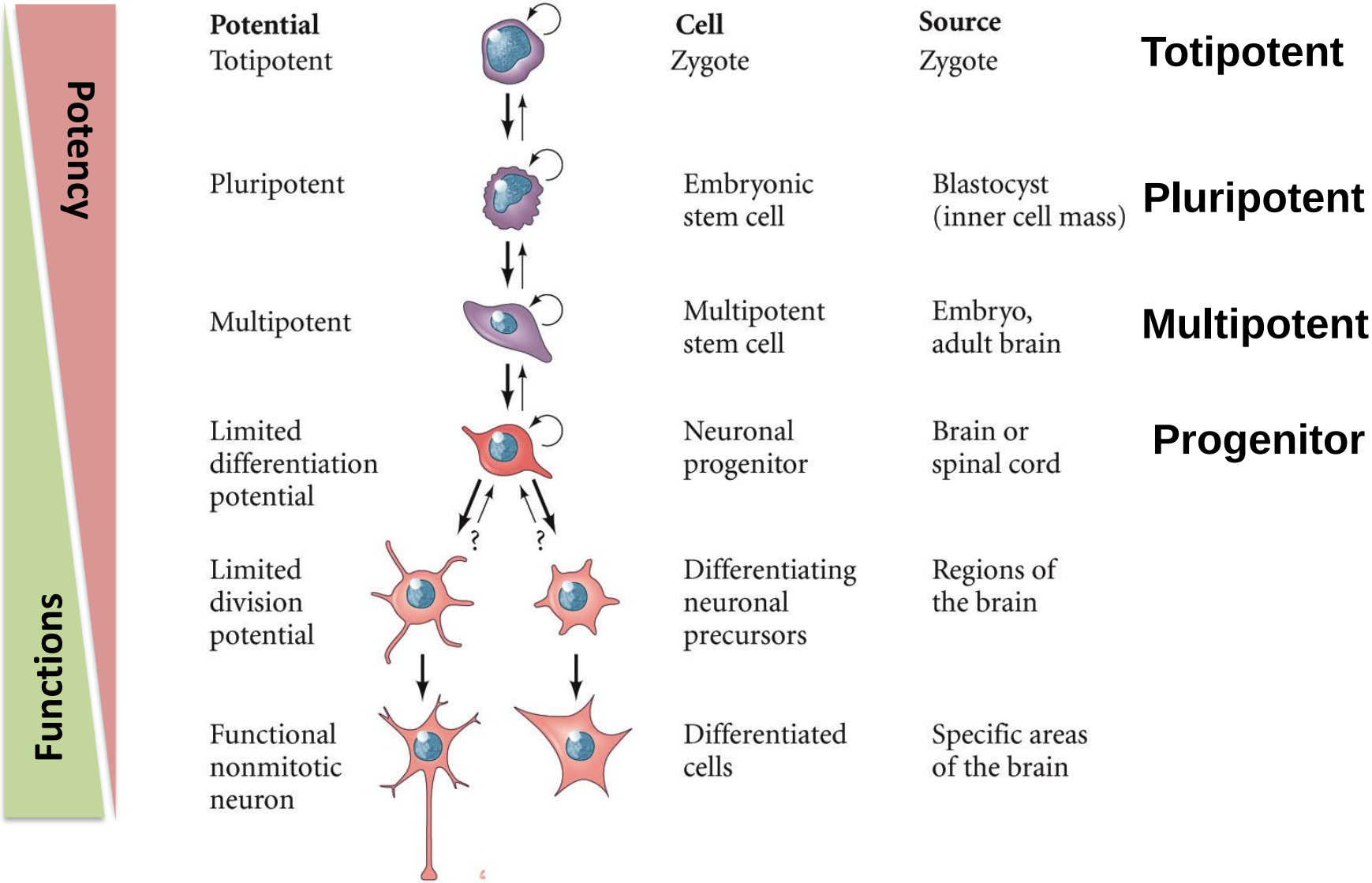


Progenitor cells

- Stem cell division generates progeny that become progenitor cells that will differentiate into specific cell types.
- Capable to divide only a few times before differentiating; NOT capable of unlimited self-renewal.

Examples: Neural stem/progenitor cells and their differentiation





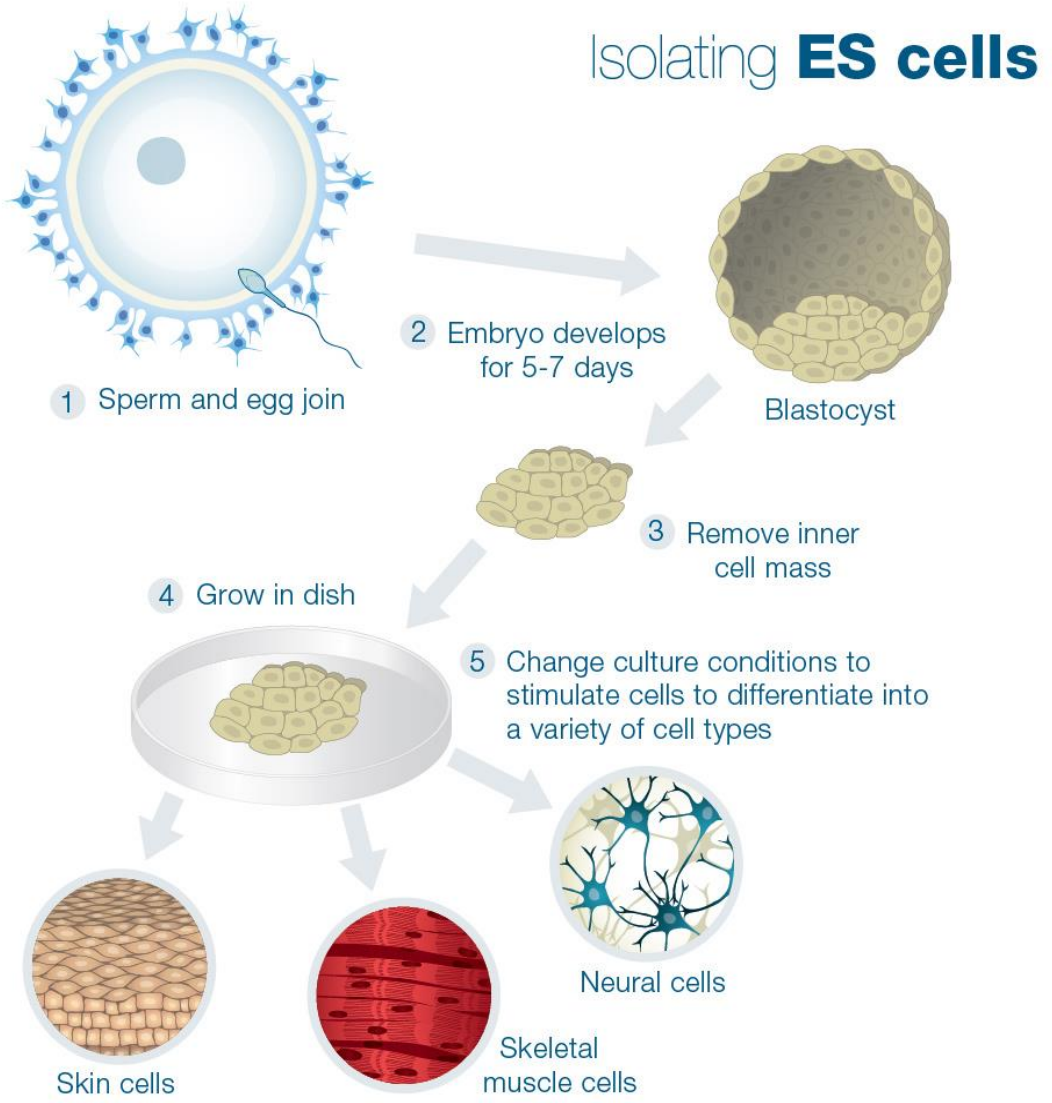
DEVELOPMENTAL BIOLOGY, 9e, Part Figure III.4

Major classes of stem cells

- Embryonic stem cells: derived from the inner cell mass of mammalian blastocysts.
  - Capable of producing all the cells of the embryo (totipotent and pluripotent)

- Adult stem cells are found in the tissues of organs after the organ has matured.
  - Usually involved in replacing and repairing tissues of that particular organ
  - Can form only a subset of cell types (multipotent)

Embryonic stem cells



http://learn.genetics.utah.edu/content/stemcells/quickref/

- Embryonic stem (ES) cells are formed as a normal part of embryonic development. They can be isolated from an early embryo and grown in a dish.
- **ES cell culture needs feeder cells** (mouse fibroblast) **or addition of certain factors** (like LIF, Leukemia inhibitory factor, for mouse stem cells) to maintain their stemness.

The first human embryonic stem cell line

Science
AAAS

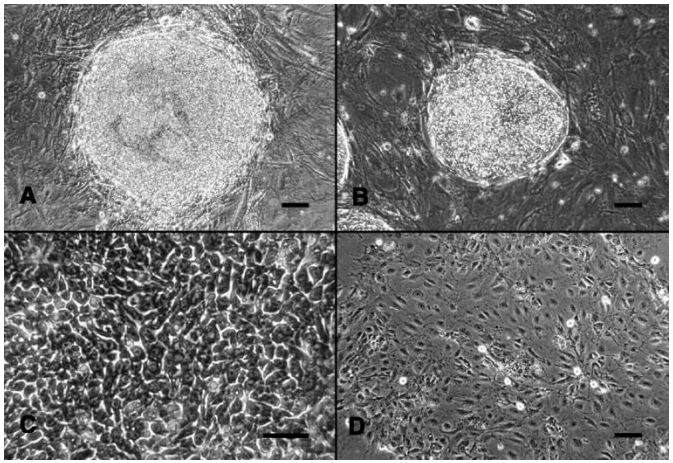
REPORTS

Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

Human blastocyst-derived, pluripotent cell lines are described that have normal karyotypes, express high levels of telomerase activity, and express cell surface markers that characterize primate embryonic stem cells but do not characterize other early lineages. After undifferentiated proliferation in vitro for 4 to 5 months, these cells still maintained the developmental potential to form trophoblast and derivatives of all three embryonic germ layers, including gut epithelium (endoderm); cartilage, bone, smooth muscle, and striated muscle (mesoderm); and neural epithelium, embryonic ganglia, and stratified squamous epithelium (ectoderm). These cell lines should be useful in human developmental biology, drug discovery, and transplantation medicine.

Derivation of the H9 cell line.

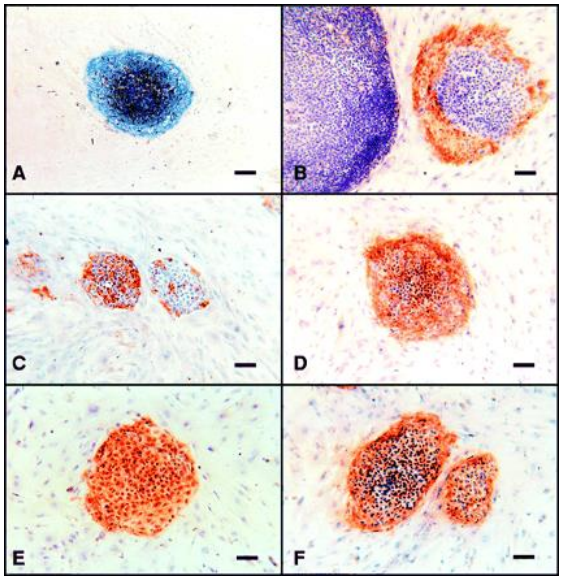


Self-renewal

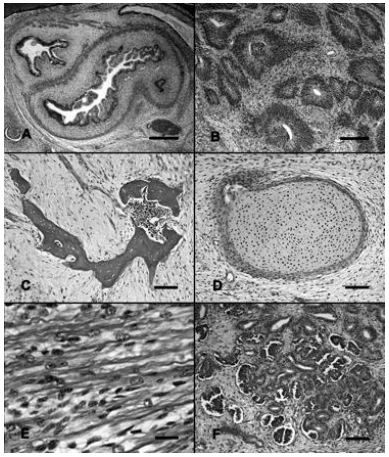
J A Thomson et al. Science 1998;282:1145-1147

The first human embryonic stem cell line

Stage-specific embryonic antigen/markers staining (SSEA)–3, SSEA-4, TRA-l-60, TRA-1-81, and alkaline phosphatase



Teratoma formed by the human ES cell lines in SCID-beige mice show different tissue types



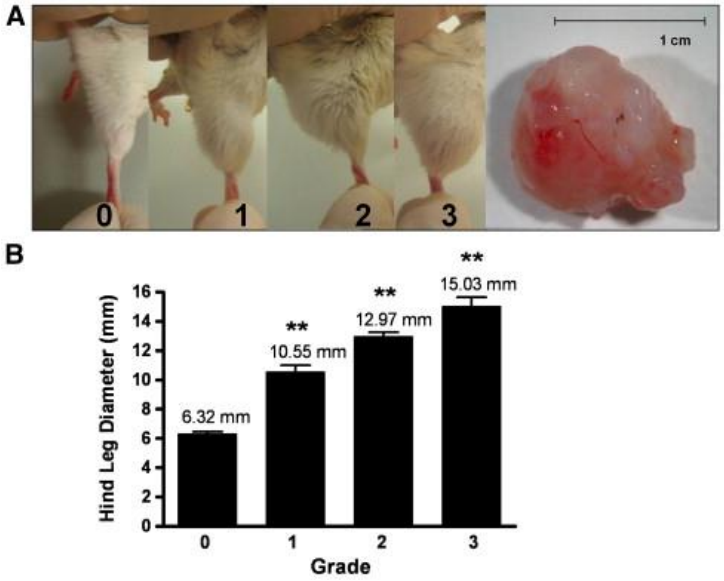
Pluripotent – differentiation

J A Thomson et al. Science 1998;282:1145-1147

Formation of teratoma

- Teratomas are benign tumors characterized by their rapid growth *in vivo* and their haphazard mixture of tissues and thus often have semi-semblances of organs, teeth, hair, muscle, cartilage and even bone.

- Injection of human ES cell lines into immunodeficient mouse and **formation of teratoma from injected stem cells can prove the pluripotency of the stem cells.**



http://www.sciencedirect.com/science/article/pii/S187350610900018X

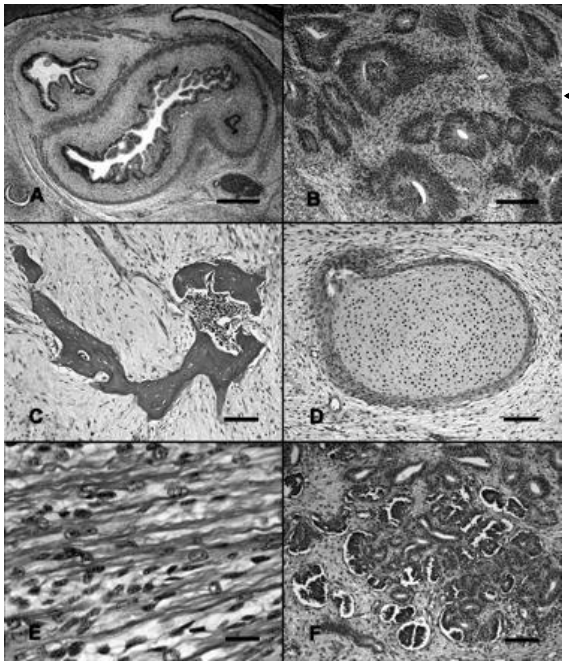
Formation of teratoma

Gutlike structures

Injection into rear leg muscles of SCID-beige mice

Bone

Striated muscle



Rosettes of neural epithelium

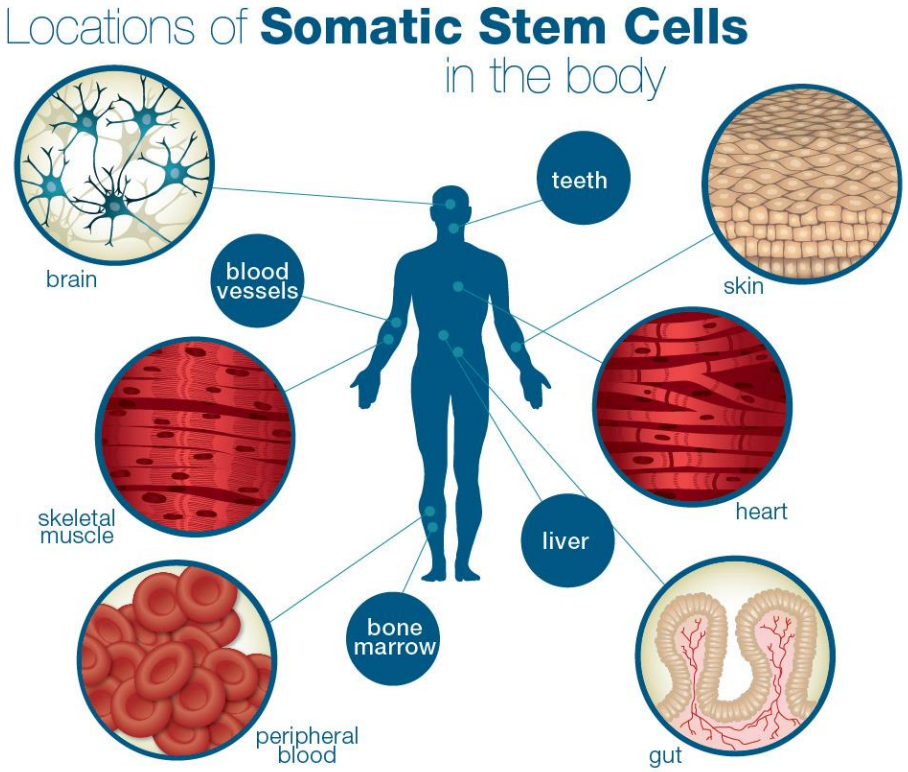
Cartilage

Tubules interspersed with structures resembling fetal glomeruli

J A Thomson et al. Science 1998;282:1145-1147

Adult stem cells

- Numerous adult organs contain committed stem cells:
  - Hematopoietic stem cells
  - Mesenchymal stem cells
  - Epidermal stem cells
  - Neural stem cells
  - Hair stem cells
  - Melanocyte stem cells
  - Muscle stem cells
  - Tooth stem cells
  - Gastrointestinal stem cells
  - Adipose tissue stem cells



http://learn.genetics.utah.edu/content/stemcells/quickref/

Properties of adult stem cells

- **Not easy to use:**
  -Difficult to isolate, often represent 1 out of every 1000 cells in an organ

- **Relatively low rate of cell division**
  -Do not proliferate readily

- **Still very useful for medical treatment**
  -40,000 bone marrow transplants using hematopoietic stem cells, saving leukemia and other blood disorder patients

Adult stem cell niche

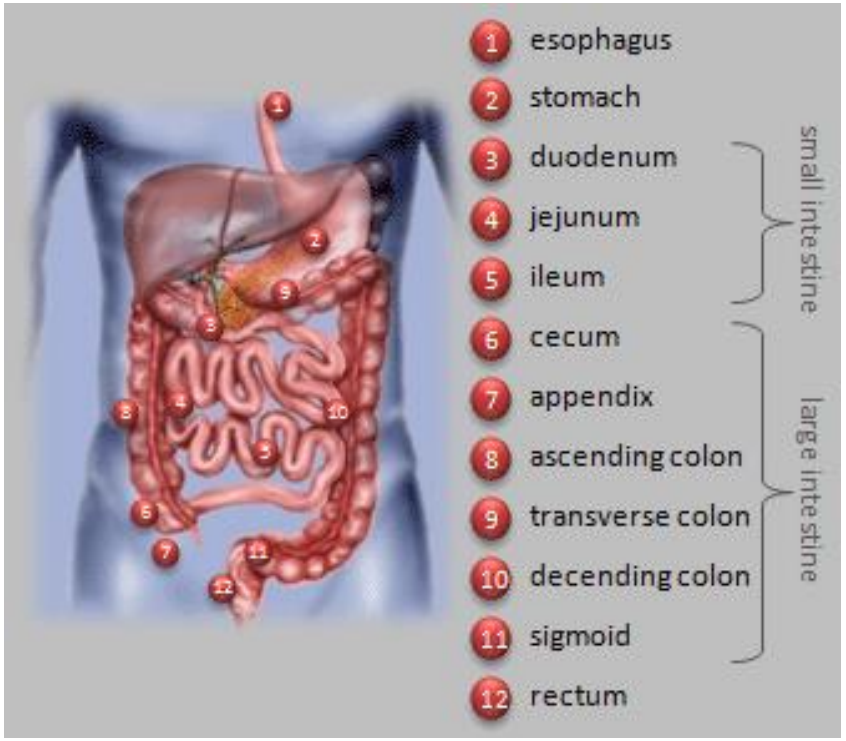
- Stem cells must maintain the long term ability to divide, how?
- Determined in large part by where the stem cells reside.
- The continuously proliferating stem cells are housed in compartments in regulatory microenvironment.

Adult stem cells

- **Gastrointestinal stem cells**

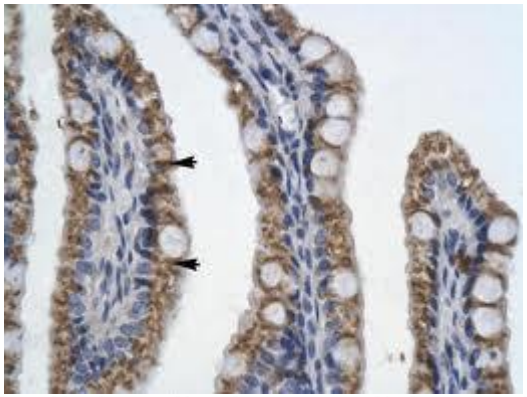
What is Gastrointestinal (GI) Tract?

- The digestive system
- All parts from mouth to the anus
- Upper GI tract
  - Esophagus, stomach and duodenum
- Lower GI tract
  - Small intestine and large intestine

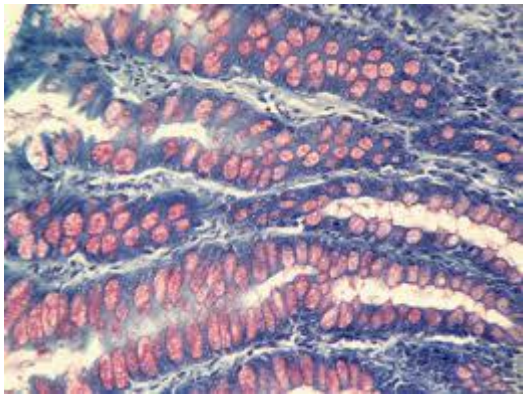


Why do we study stem cells in GI tract?

- Surface of the GI tract, is protected by the epithelium.
- Turnover of the epithelial cell lineages is very fast, 2-7 days.
- Multipotent stem cells generate all GI epithelial cell lineages.
- GI tract is a good model
  - study stem cell plasticity
  - mechanism of various differentiation pathways

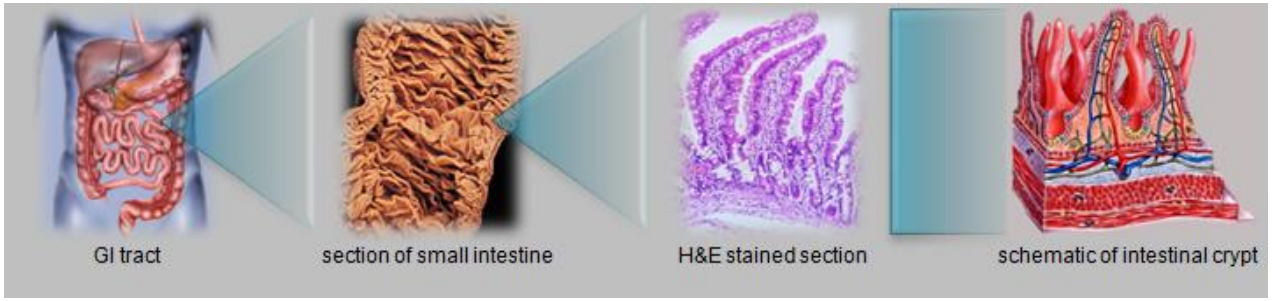


Epithelial cells in small intestine

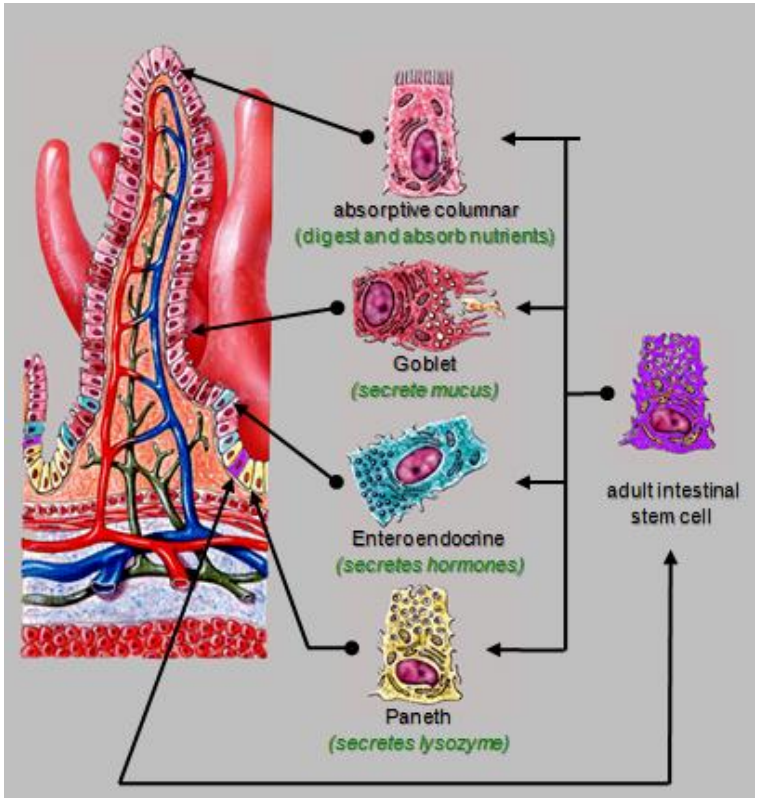


Epithelial cells in large intestine

Epithelial GI stem cells



- Adult multipotent stem cells
- Found in the base of the intestinal crypts.
- Divide and differentiate into all types of epithelial cell in GI tract.
- Provide a continual renewable source of the four types of epithelial cells.

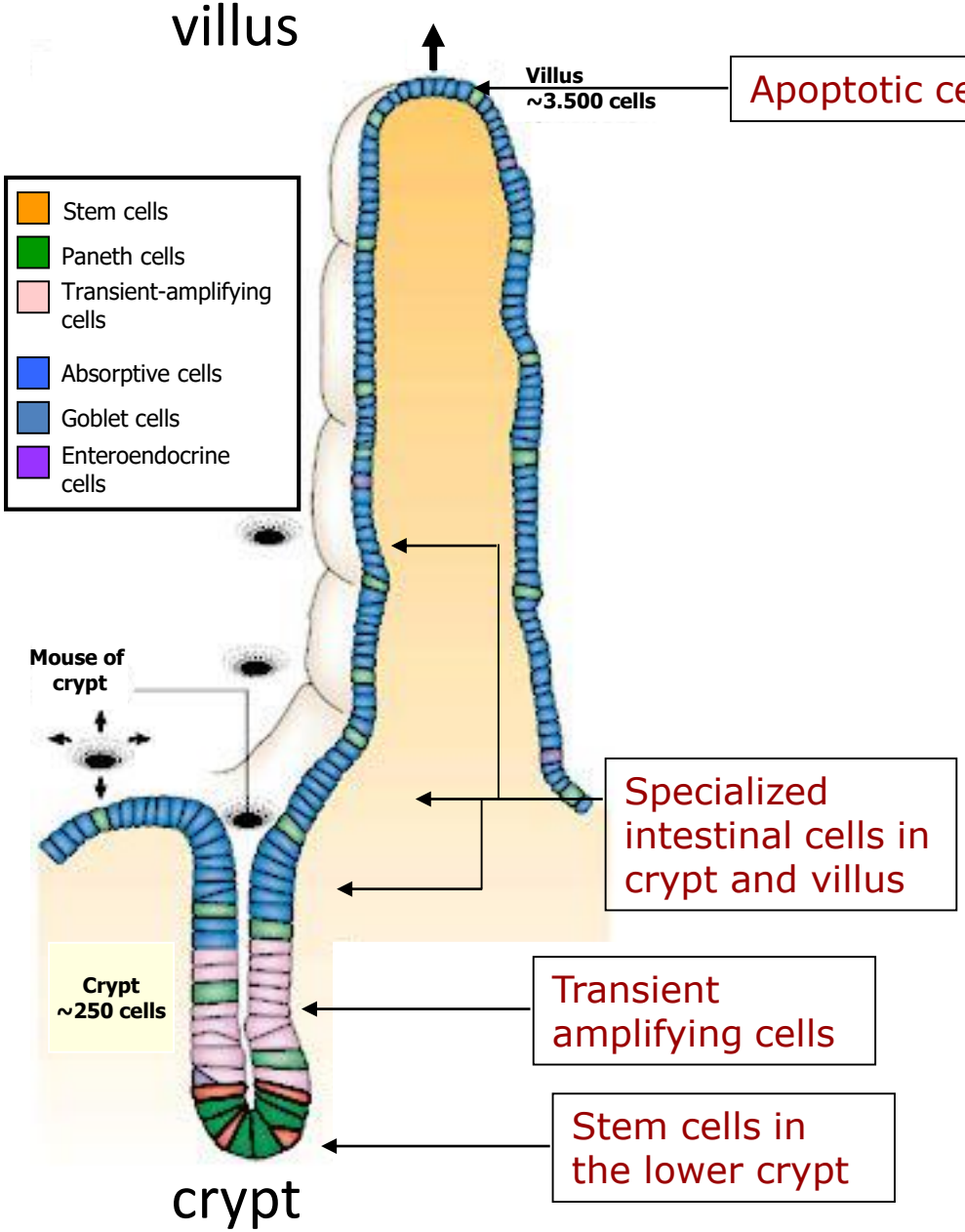


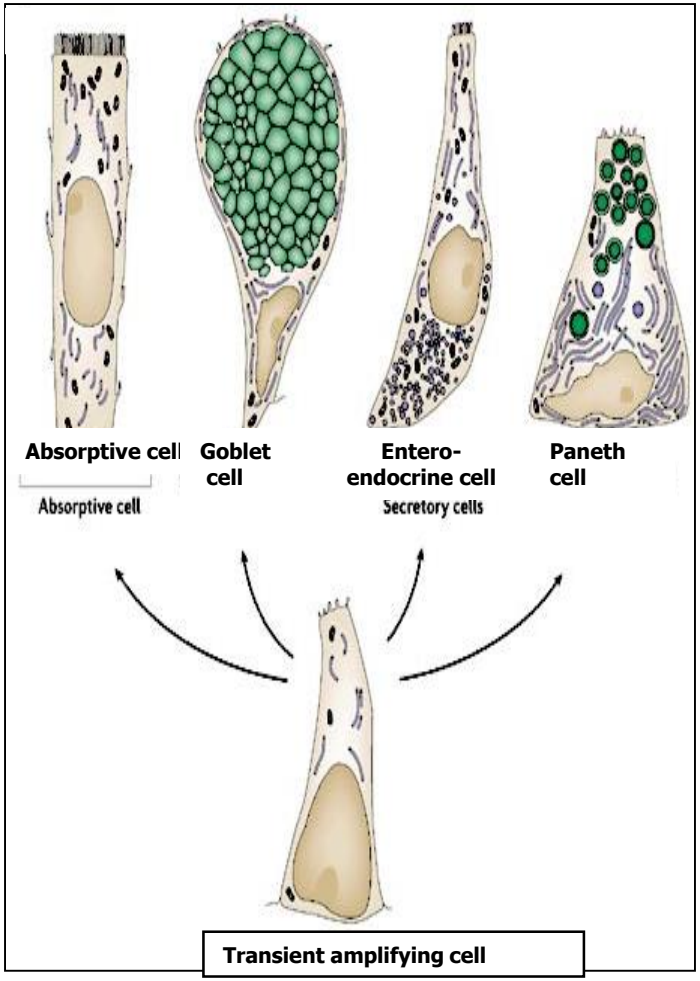
Small intestine villi

www.alamy.com - D0MCAB

library.med.utah.edu

Cell fate in small intestine



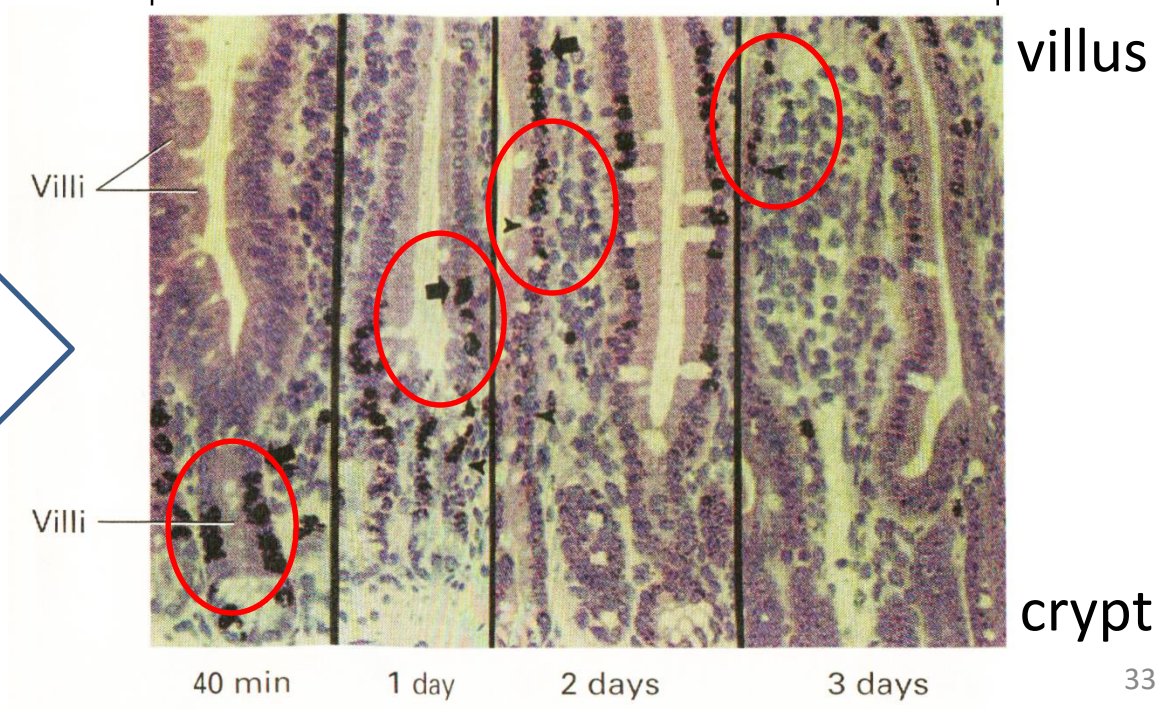


Pulse-chase experiment demonstrated the presence and the development pattern of GI stem cells

- Dividing cells (indicated by arrows), presumably stem cells, initially were present at the bottom of the villus; cells produced after stem cell division, presumably differentiated cells, were moved up from the bottom to the top of the villus.

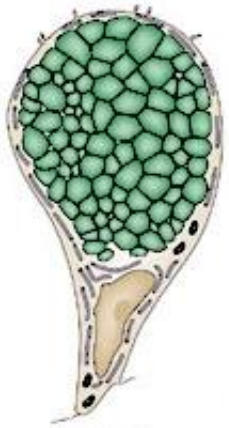
"Chase": tracing the fate of the labelled cells as times goes on

"Pulse": short period of labeling of dividing cells by labeled thymidine (nucleotide "T")



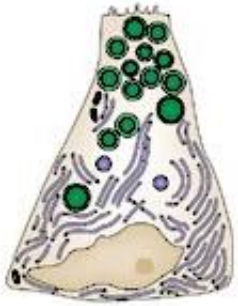
Four types of terminally differentiated cells

- Absorptive columnar cells (Enterocytes)
  - comprise the principle lineage of intestinal epithelial cells.
  - develop well-organized apical microvillus membranes in which reside proteins mediating digestion and absorption.



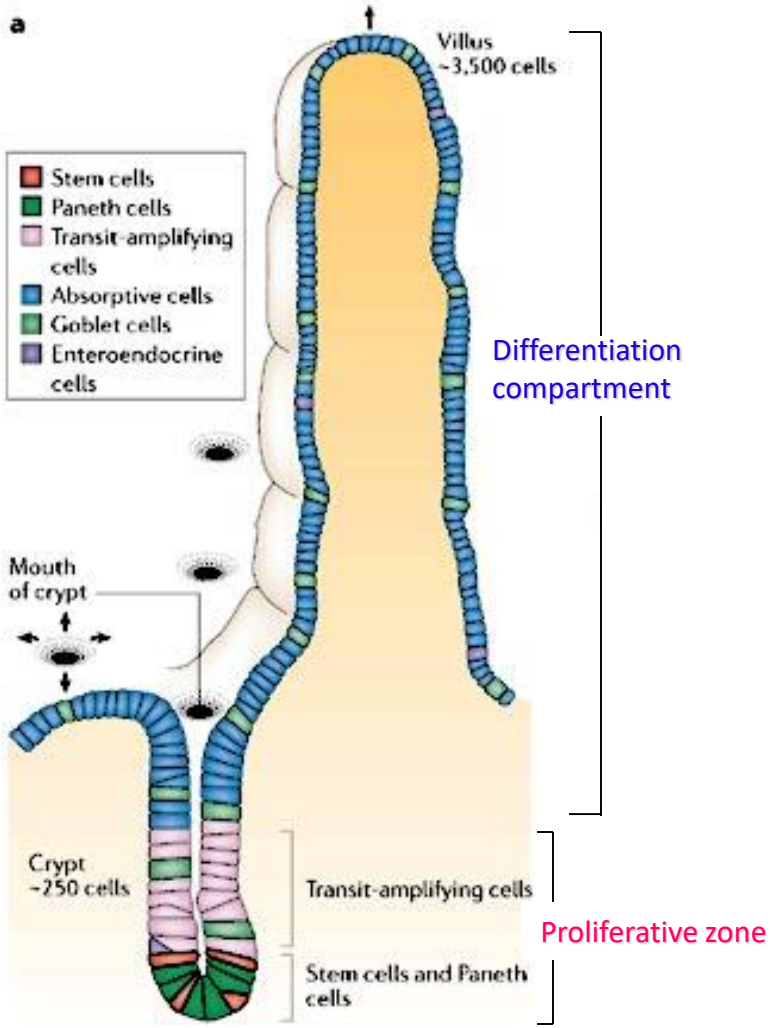
- Goblet cells
  - secrete mucin and establish the mucus layer.
  - protect the epithelium and potentiate digestion and absorption.

- Entero-endocrine cells
  - part of the neuroendocrine system, secreting local paracrine hormones.
  - supporting neuromuscular activity, secretion, and central nervous system regulation of calorie consumption.



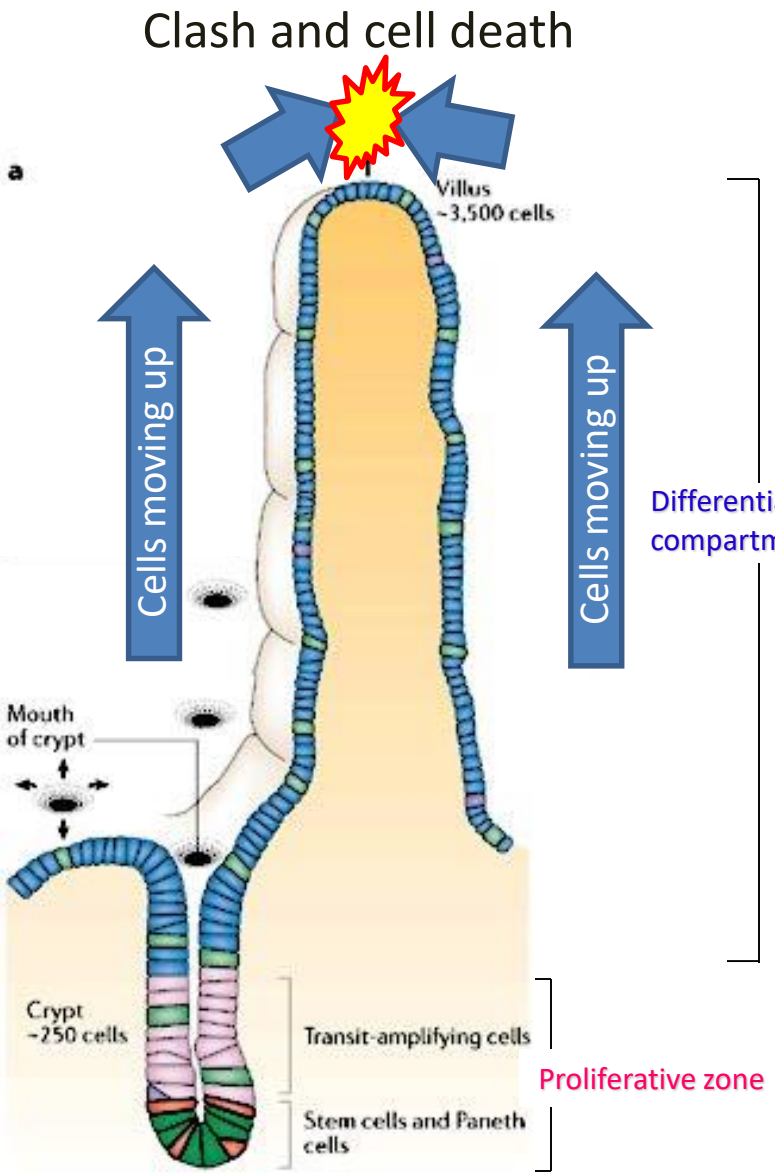
- Paneth cells
  - produced only in small intestine.
  - secrete antimicrobial peptides and growth factors.
  - contribute to the barrier defending against microbial invasion and tumorigensis.
  - Contribute to intestinal stem cell niche.

How stem cells differentiate into different types of epithelial cells



Intestinal crypt-villus axis

- Intestinal epithelium is composed of the intestinal crypt-villus axis
- It undergoes cycles (turnover time is 2-7 day) of proliferation, migration, differentiation and apoptosis
- The cycle maintain the integrity of the crypt-surface axis
- The crypt-villus axis consists of the proliferative zone and differentiation compartment



Intestinal crypt-villus axis

- In the proliferative zone
  - Stem cells give rise to rapidly proliferating transit cells
  - Transit cells:
    - migrate up the vertical axis to the differentiated compartment.
    - migrate down to the bottom of the crypt to become Paneth cells.
- In the differentiation compartment
  - Transit cells undergo nuclear and cytoplasmic reprogramming which coordinates cytostasis (inhibition of cell growth and cell death at the top of villus) and differentiation (along the path as cells moving up).

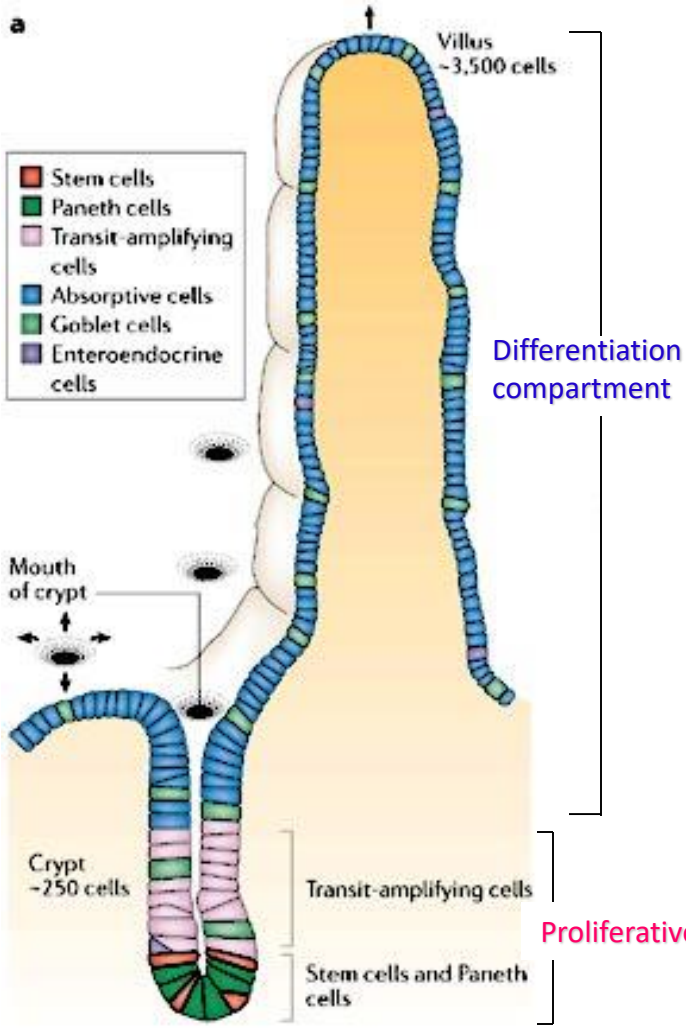
Intestinal epithelium is like a conveyor belt…but clash at the top

Proliferative zone

Differentiation compartment

http://droid-games.com
http://m.inmagine.com/

Molecular determinants of intestinal crypt-villus axis



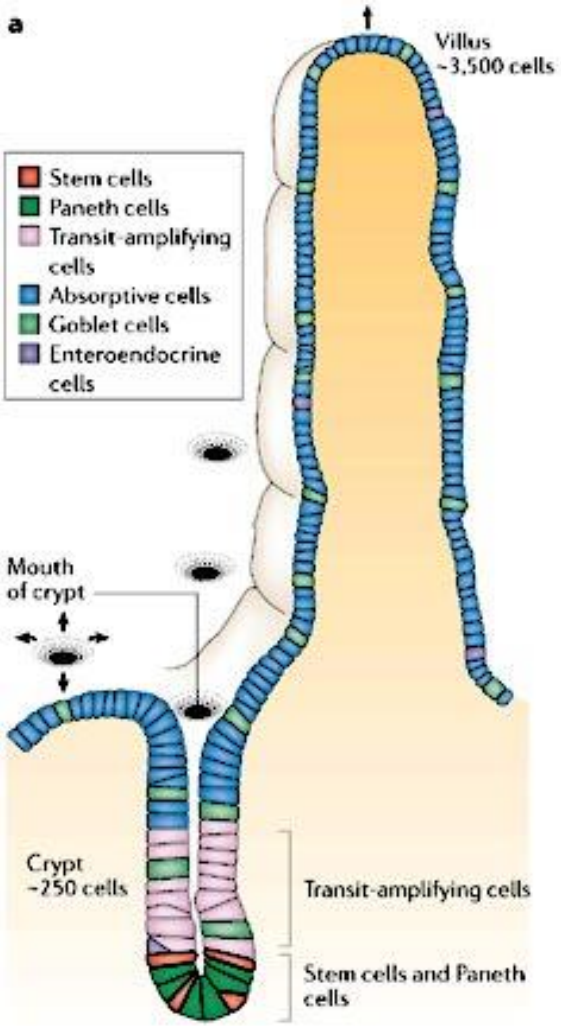
Intestinal crypt-villus axis

Apoptosis: low level of Netrin-1 (which inhibits cell death) at the top.

Differentiation: Multiple signaling pathways including the bone morphogenic proteins (BMP) and sonic hedgehog.

Stem cell maintenance (also is the stem cell niche): Wnt/β-catenin signaling pathway, which promotes stemness and cell proliferation.

Summary of GI stem cells



- Epithelial cells along the crypt-villus axis of GI tract undergo fast turnover
- Multipotent stem cells, which are at the base of the crypt, generate all GI epithelial cell lineages
- Stem cells move up along the crypt-villus axis in a way likes conveyor belt and differentiate along the way
- Multiple morphogenetic factors, eg. Wnt, BMP, Sonic hedgedog, regulate stemness and differentiation
- Lgr5 is an important component of and a marker for intestinal stem cells.

End of this lecture