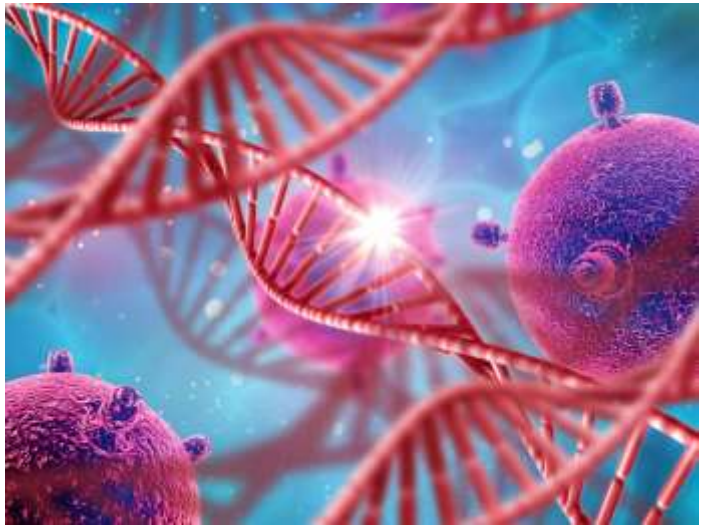
Lecture 20

General Biology & Cytology Course 2301130



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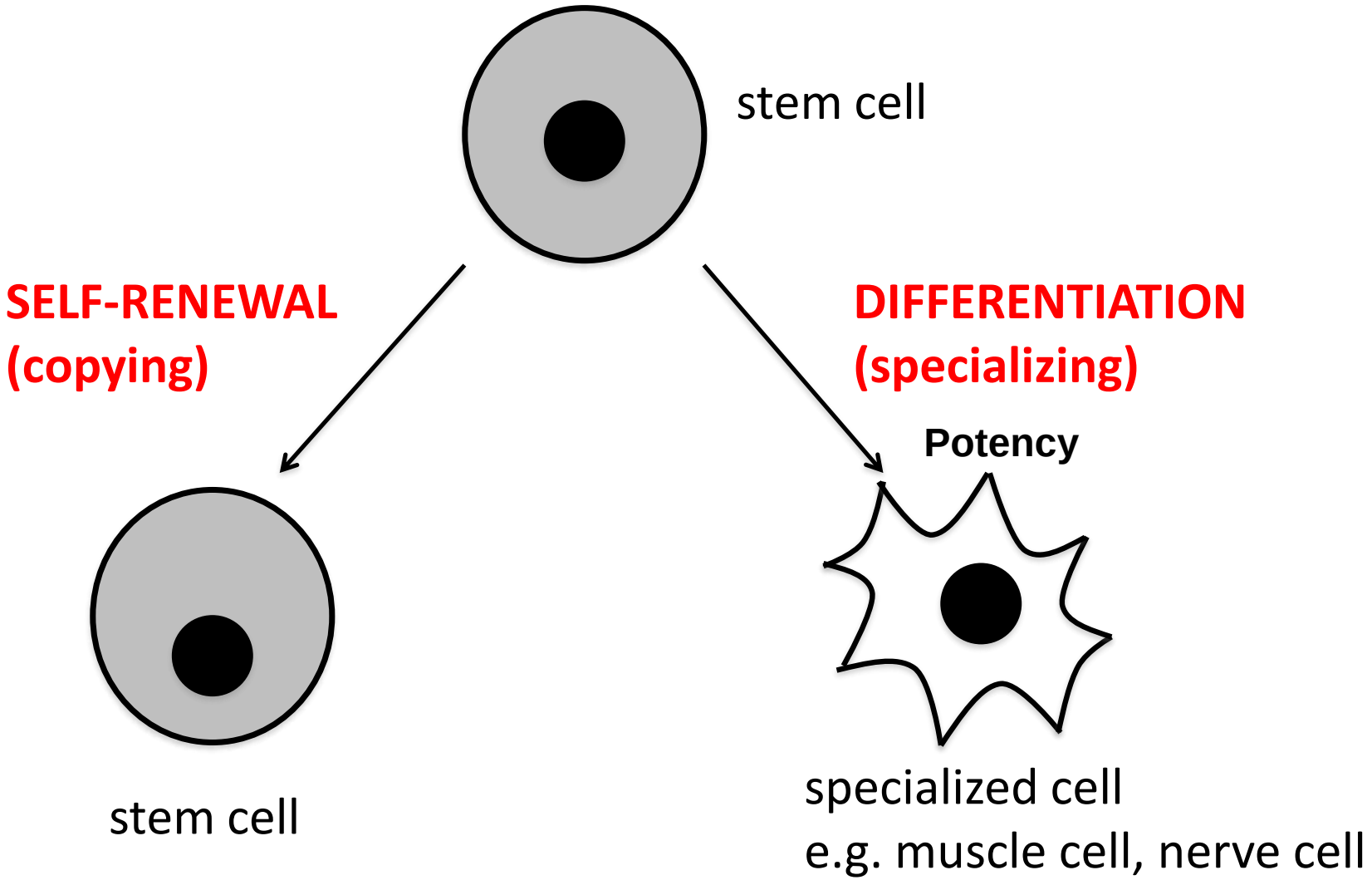
Stem cells and cell culture techniques

- Multicellular organisms are made up of various types of **specialized cells**, such as muscle, nerve, and skin cells.
- Each type of specialized cell has a unique structure and function.
- This allows each cell type to carry out a specific set of tasks needed for the survival, growth, and reproduction of the organism.

Specialized cells come from stem cells

- The cells of multicellular organisms don’t start out in their specialized forms.
- Instead, *unspecialized* cells are transformed into *specialized* cells through a highly regulated process called **cellular differentiation**.
- The unspecialized cells that undergo differentiation are called **stem cells**.

What is a stem cell?

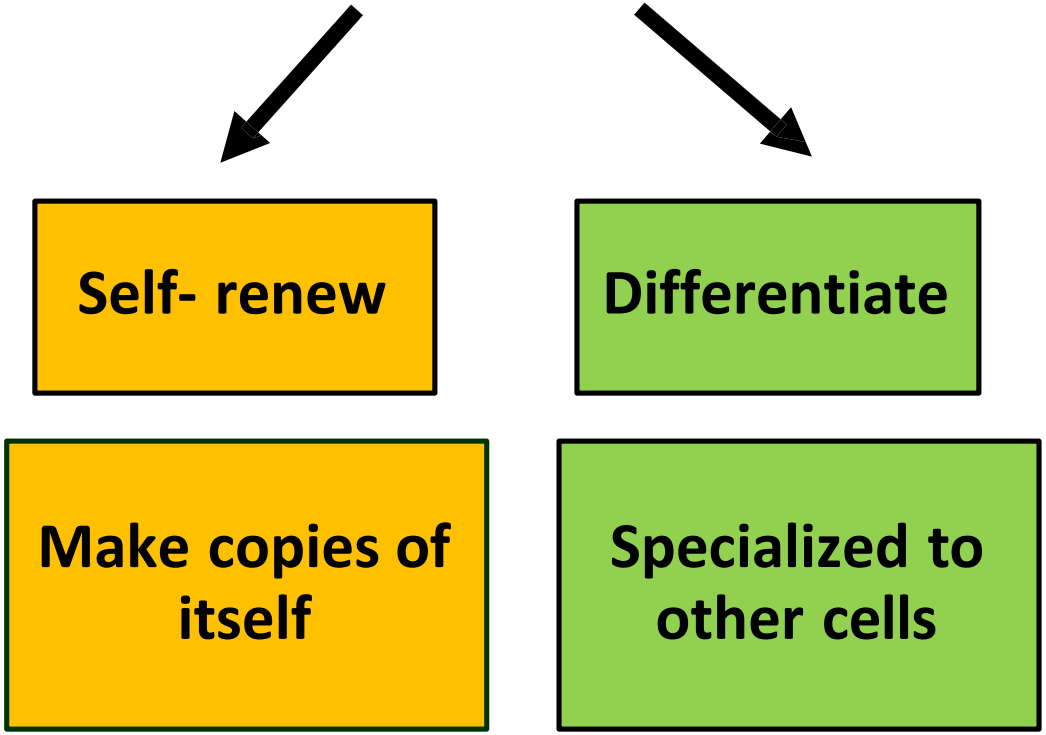


Potency: The ability to differentiate into different cell types

1. To divide and renew themselves (Self-renew) throughout life i.e. **Proliferation**

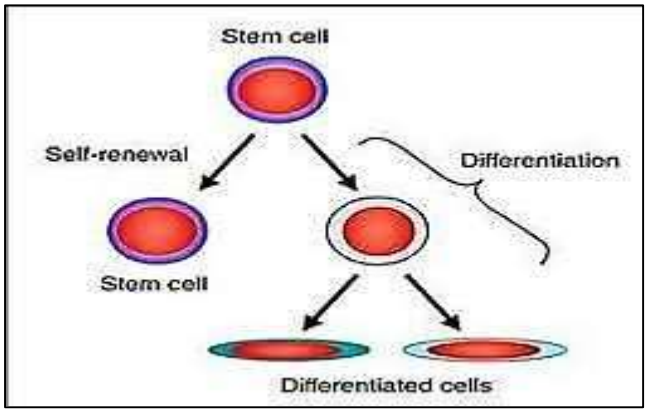
2. To develop to specialized cell types i.e. **Differentiate**

Stem cell is unique because it Can do both



A- Self renewal

- **maintain a storage** Because if they didn't copy themselves they would finish quickly. They are important for the body to use throughout our life.



B- Differentiation (Potency)

- Specialized cells are mature cells **cannot divide** or make copies of themselves, so if they damaged or die they need to be replaced so that the body can keep on working
- *Specialized*' or '*differentiated*' cells have **particular roles (Functions)** in the body e.g. blood cells, nerve cells, muscle cells

Stem cells are categorized based on their level of potency

1. **Totipotent** stem cells

- Can differentiate into all cell types of an organism, including both embryonic and extra embryonic tissues (e.g., the placenta).
- **Sources**: Zygote (fertilized egg) up to the 8-cell stage in humans.

2. **Pluripotent** stem cells

- Can differentiate into all cell types within the three germ layers (ectoderm, mesoderm, and endoderm) but not extra embryonic tissues.
- **Sources**: Cells from the inner cell mass (ICM) of a **blastocyst**.

3. **Multipotent-** stem cells

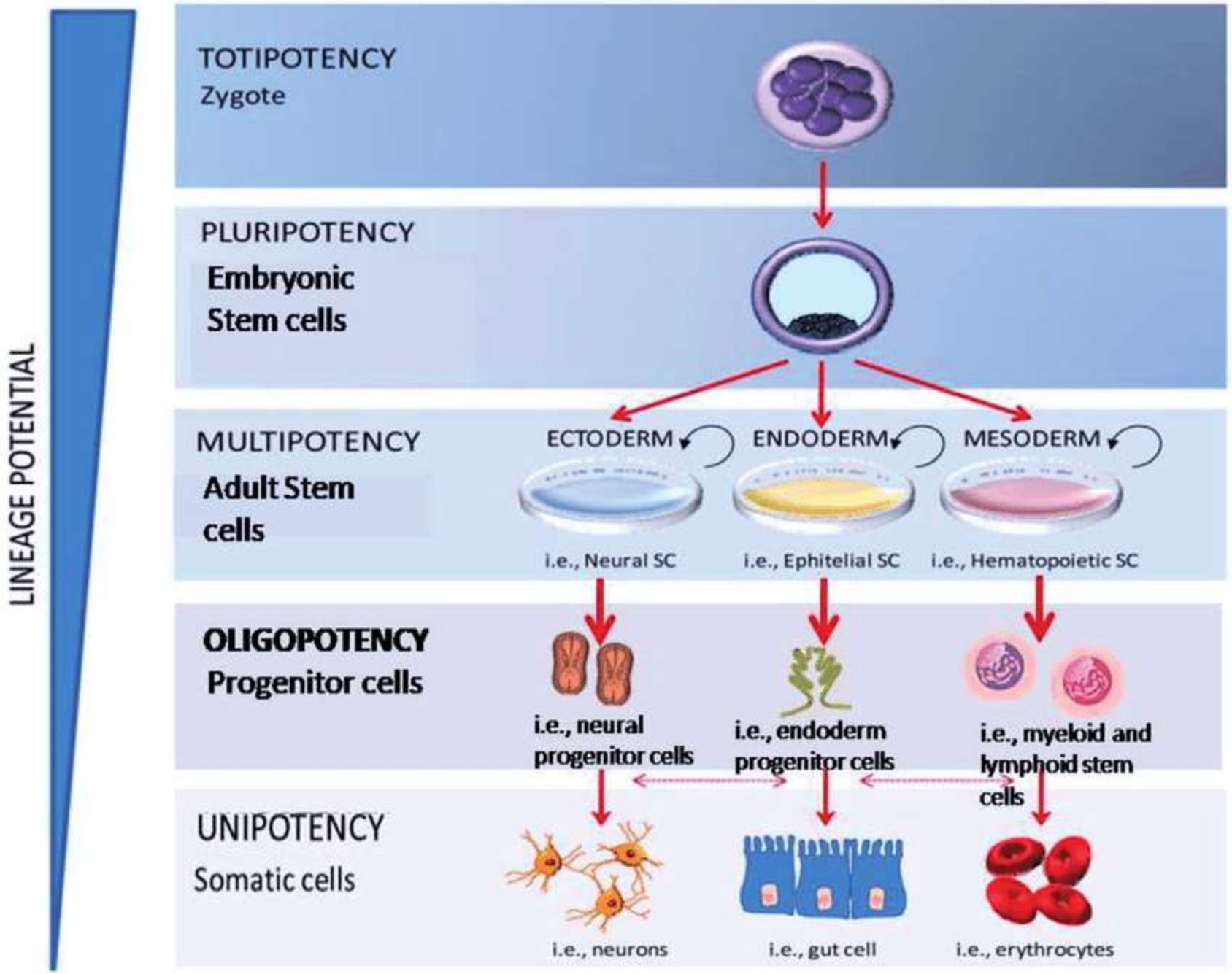
- Can differentiate into a limited range of cell types related to a specific lineage or tissue.
- **Source**: Adult stem cells (e.g., hematopoietic stem cells, mesenchymal stem cells).Cord blood stem cells. **Examples**:Hematopoietic Stem Cells

4. Oligopotent Stem Cells (similar to multipotent)

- Can give rise to only a few closely related cell types.
- **Source**: Lymphoid or myeloid progenitor cells.
- **Examples**:
  - Lymphoid Progenitor Cells: Differentiate into T-cells or B-cells.
  - Myeloid Progenitor Cells: Differentiate into monocytes or neutrophils.

5. Unipotent Stem Cells

- Can differentiate into only one type of cell but retain the ability for self-renewal.
- **Source**: Found in specific tissues (e.g., skin, liver, and muscle cells).
- **Examples**:
  - Muscle Stem Cells (**Satellite Cells**): Give rise only to skeletal muscle cells.

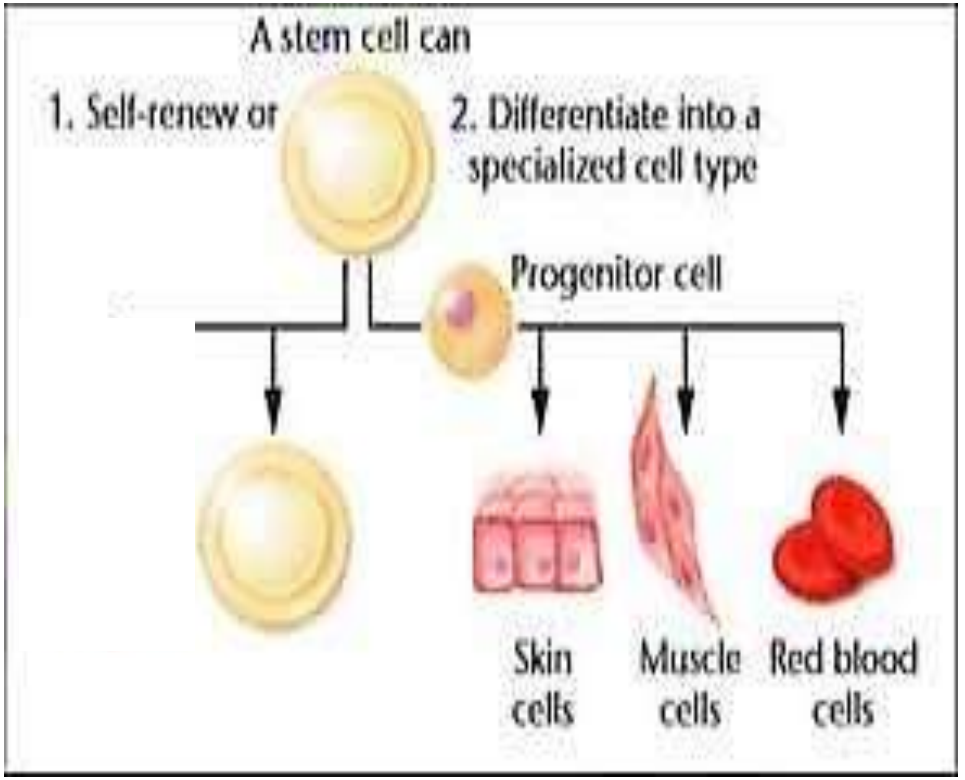

LINEAGE POTENTIAL
TOTIPOTENCY
Zygote
PLURIPOTENCY
Embryonic
Stem cells
MULTIPOTENCY
Adult Stem
cells
ECTODERM
ENDODERM
MESODERM
i.e., Neural SC
i.e., Ephitelial SC
i.e., Hematopoietic SC
OLIGOPOTENCY
Progenitor cells
i.e., neural
progenitor cells
i.e., endoderm
progenitor cells
i.e., myeloid and
lymphoid stem
cells
UNIPOTENCY
Somatic cells
i.e., neurons
i.e., gut cell
i.e., erythrocytes

Progenitor (intermediate) cells

Cells that arise from stem cells and is more specialized than stem cells but less differentiated than fully specialized / mature

Progenitor cells have limited capacity to self-renew than stem cells & they are the precursor that capable to differentiate to specific cells

They are usually **multipotent**

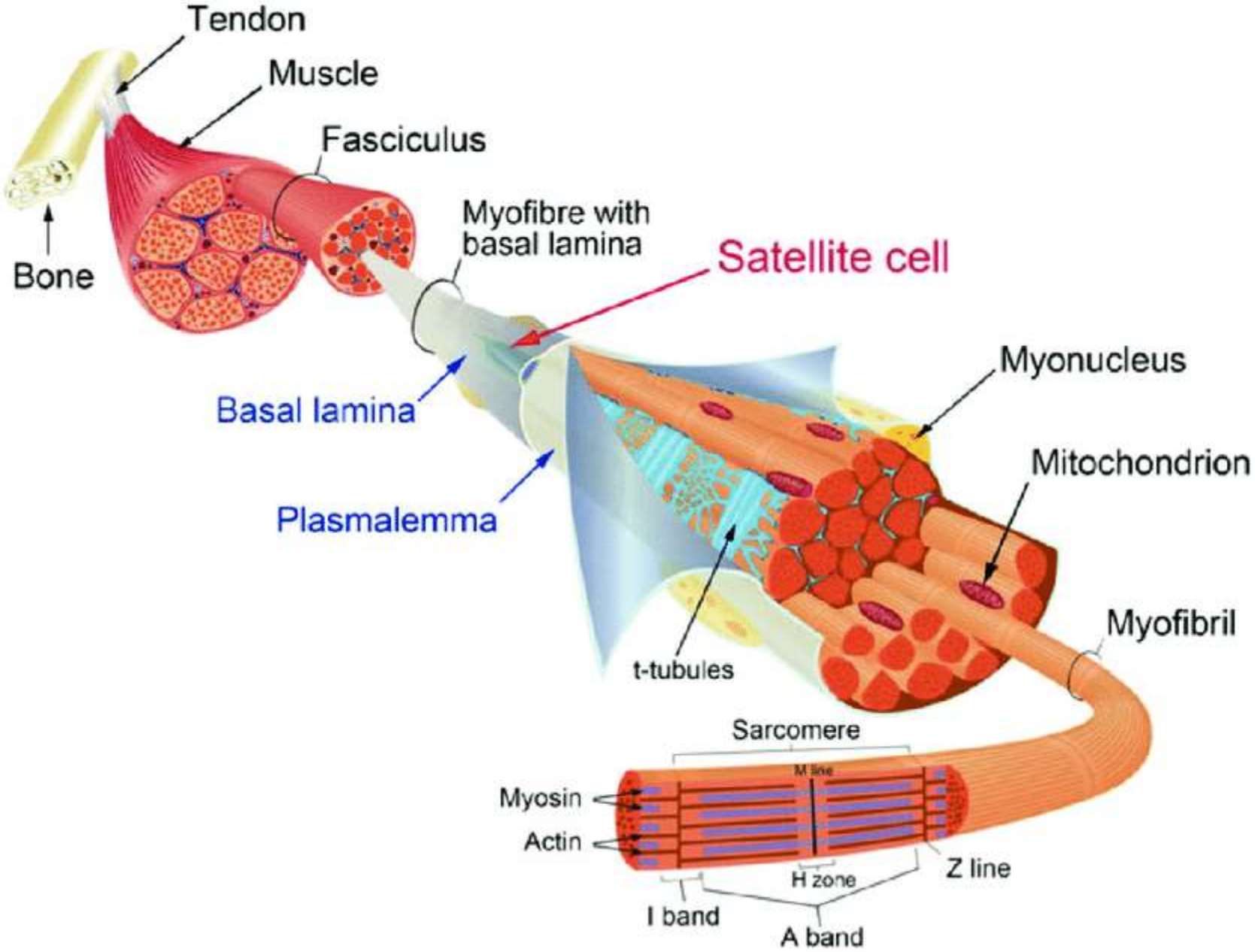


Examples:

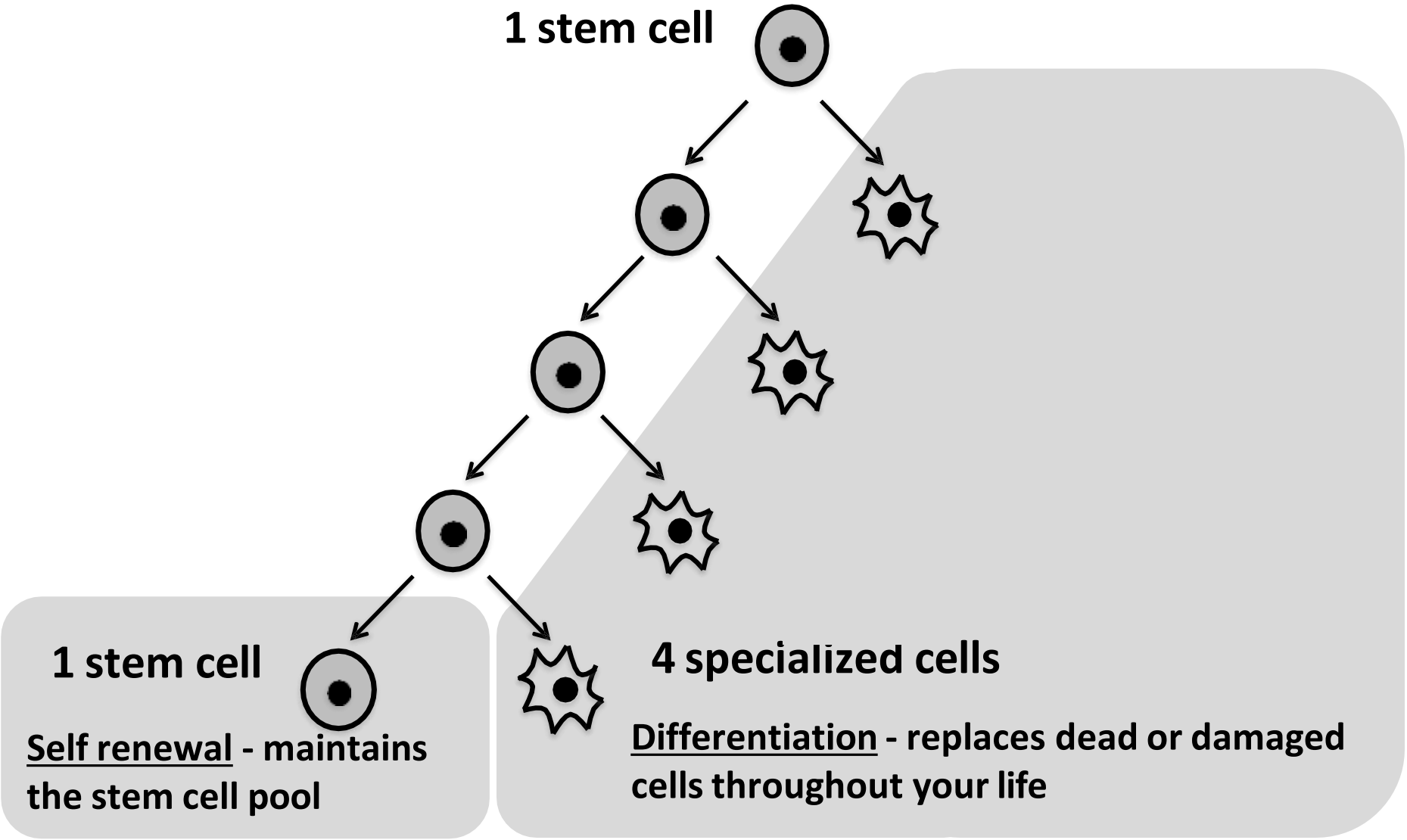
- Satellite cells
- Periosteum: on the surface of bone that develop to osteoblasts.
- Haematopoietic cells in bone marrow.

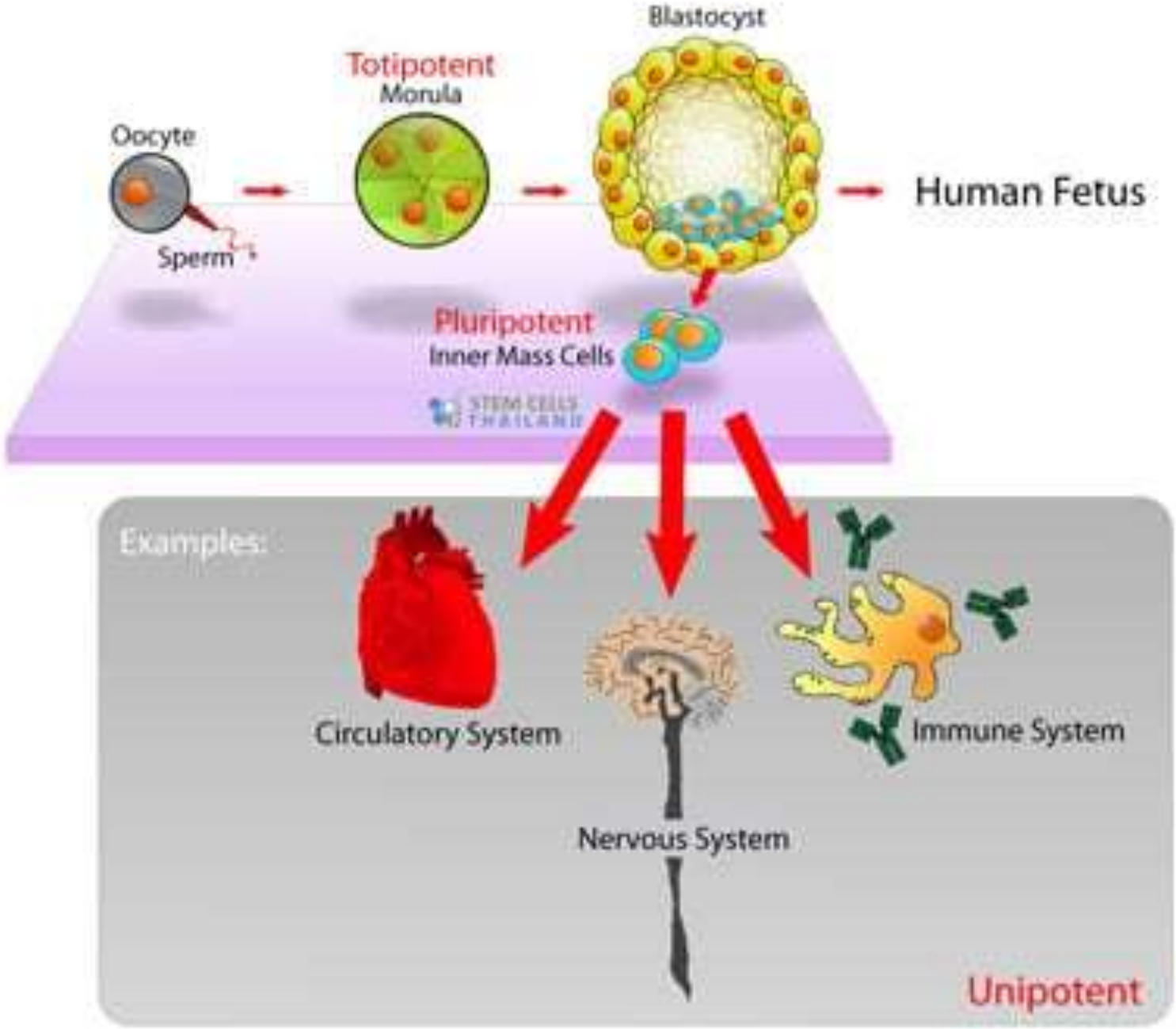
Satellite Cells

- Are a type of stem cell found in skeletal muscle tissue.
- They play a critical role in muscle growth, repair, and regeneration.
- These cells are typically inactive under normal conditions but become active when muscle damage occurs, such as after injury or exercise.
- Found between the sarcolemma (muscle cell membrane) and the basal lamina (extracellular matrix surrounding muscle fibers).
- are **unipotent stem cells**, meaning they differentiate only into muscle cells (**myocytes**).


Tendon
Muscle
Fasciculus
Bone
Myofibre with basal lamina
Satellite cell
Basal lamina
Plasmalemma
Myonucleus
Mitochondrion
Myofibril
t-tubules
Sarcomere
M line
Myosin
Actin
Z line
H zone
I band
A band

Obligate asymmetric stem cell replication




Blastocyst
Totipotent
Morula
Oocyte
Sperm
Human Fetus
Pluripotent
Inner Mass Cells
Examples:
Circulatory System
Nervous System
Immune System
Unipotent

Types of stem cells based on the origin and potency:

1. Embryonic Stem Cells (ESCs):

- **Pluripotent** cells derived from the inner cell mass of the blastocyst, meaning they can differentiate into almost any cell type in the body.
- Can develop into nearly any cell type (ectoderm, mesoderm, and endoderm-derived tissues).

2. Adult Stem Cells:

- **Multipotent** cells found in specific tissues to replace damaged cells.
- Hematopoietic stem cells (blood cells), Mesenchymal stem cells (bone, cartilage, fat)..

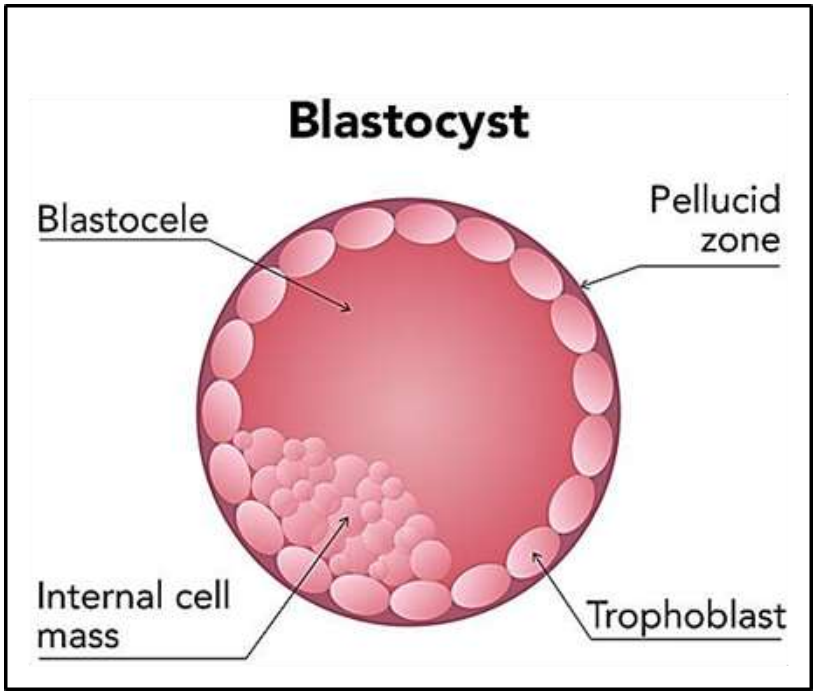
3. Induced pluripotent stem cells (iPSCs).

-Generated by reprogramming adult somatic cells to an embryonic-like state.

- Have emerged as a powerful tool in DNA technology and regenerative medicine.

EMBRYONIC STEM CELLS

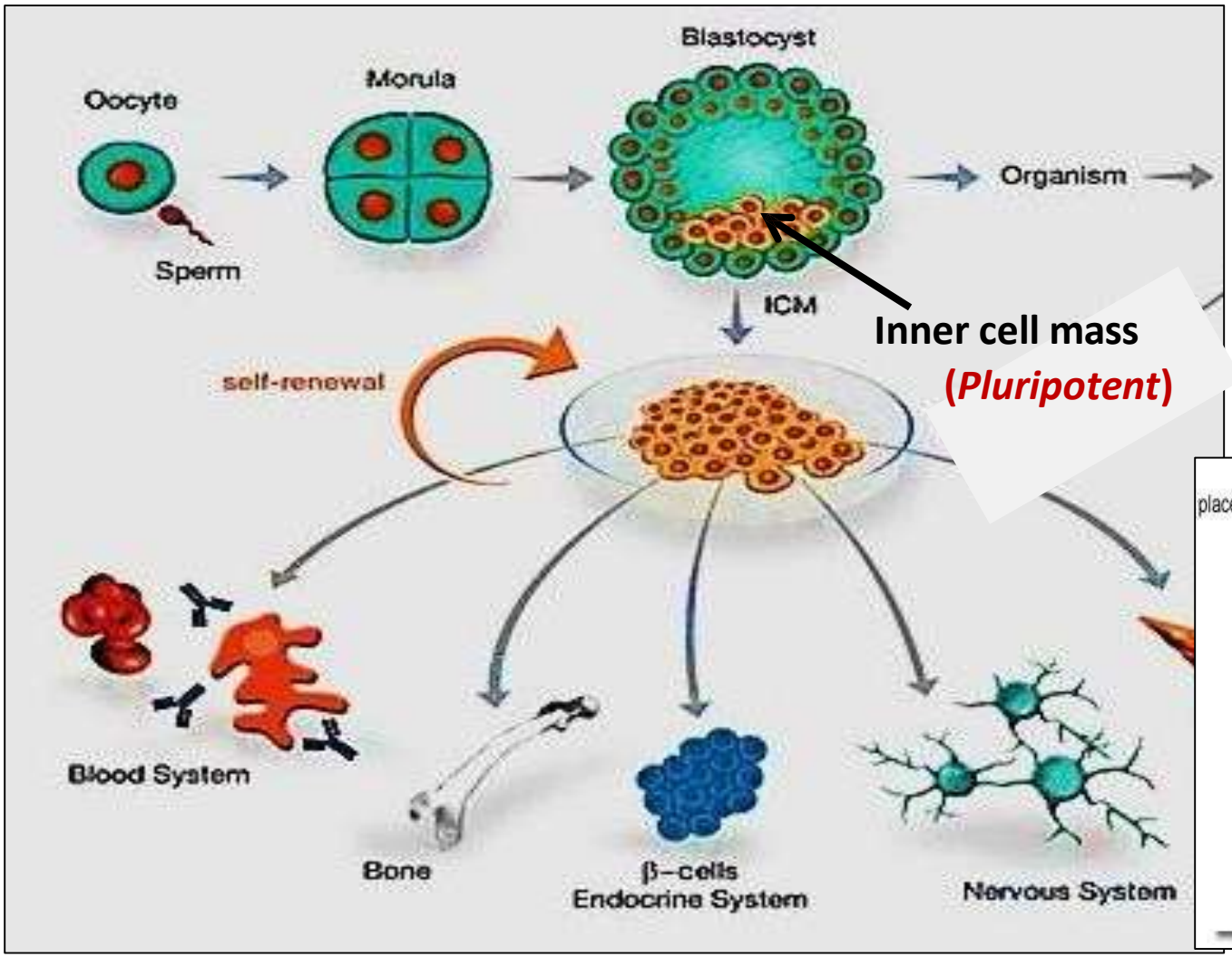
- Embryonic stem cells (ESCs) are pluripotent cells derived from the inner cell mass (ICM) of a blastocyst, an early-stage pre-implantation embryo.
- **Embryos** typically **four or five days old** and are a hollow microscopic ball of cells called the **BLASTOCYST**

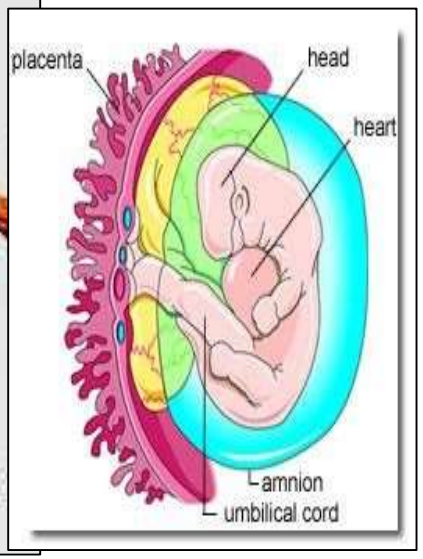


Formation of the Embryo:

- After fertilization, resulting in the formation of a **zygote**, a single-cell organism
- The zygote undergoes rapid mitotic divisions, transforming from a single cell into a multi-cellular structure called a **morula** (solid ball of cells).
- The morula develops into a **blastocyst**, a hollow structure with an outer layer of cells (**trophoblast**) and an **inner cell mass (ICM**).
- The ICM gives rise to the **embryo proper**, while the trophoblast contributes to the formation of the placenta.
- Around day 6–7 post-fertilization, the blastocyst embeds into the uterine wall.
- The implanted blastocyst continues to grow and differentiate, marking the beginning of embryonic development.

Embryonic stem cell



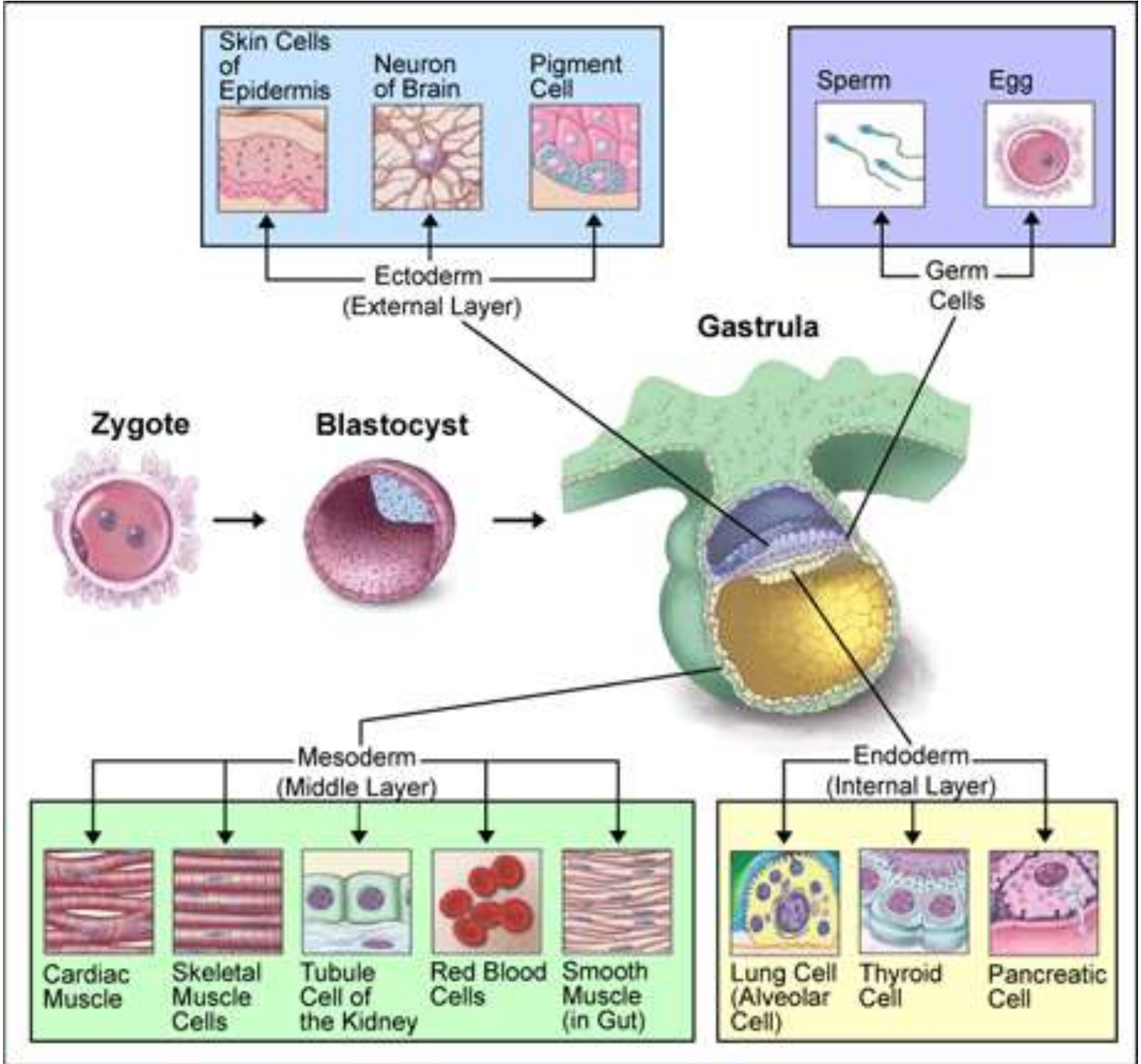


ES cells don't contribute to the extra-embryonic membranes or placenta

Con.. Germ Layer Formation (Gastrulation)

- By the third week of development, the embryo forms three primary germ layers that give rise to all body tissues and organs:

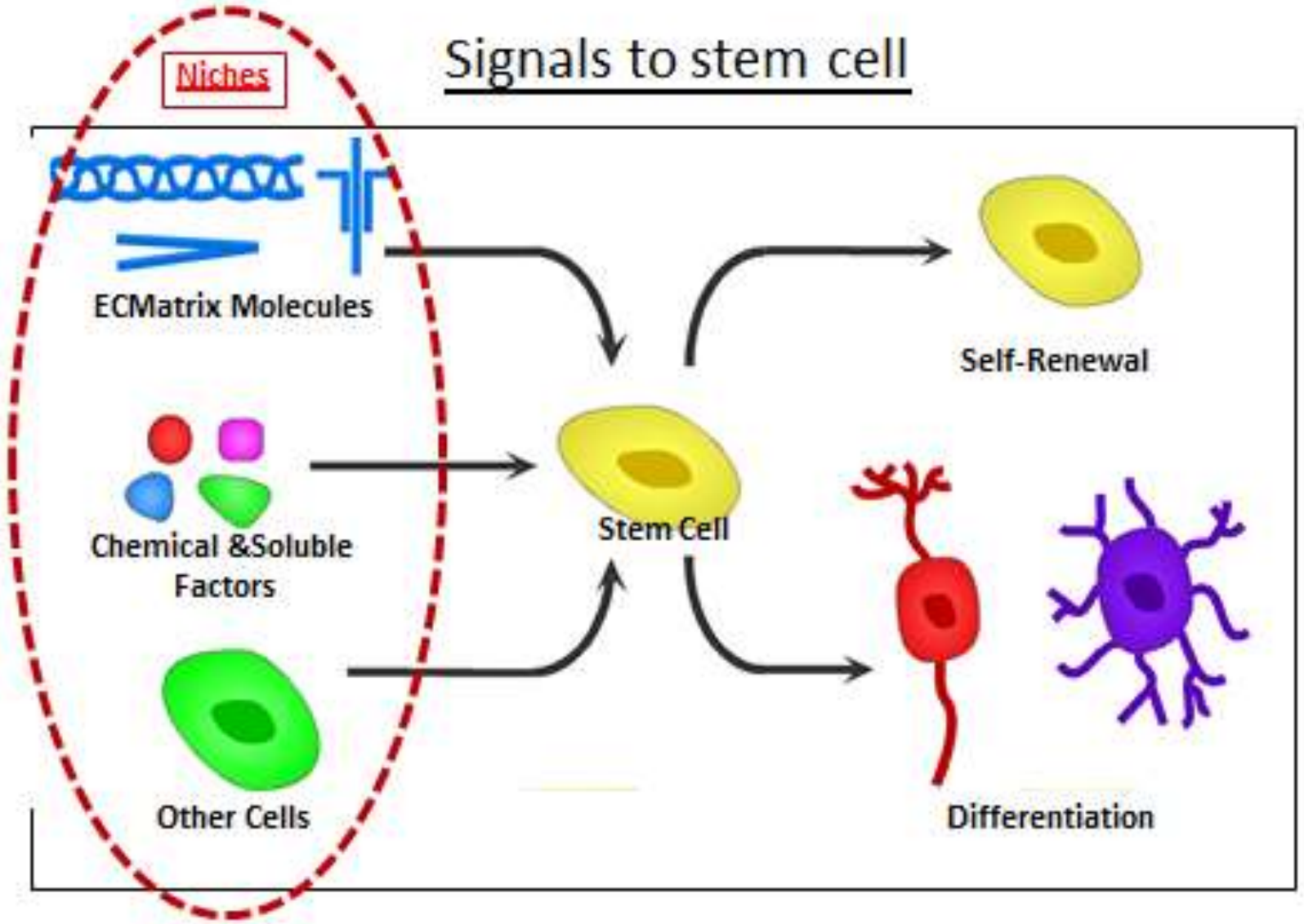
1. **Ectoderm**: Forms the nervous system, skin, and hair.
2. **Mesoderm**: Develops into bones, muscles, blood vessels, and connective tissues.
3. **Endoderm**: Produces the gut lining, lungs, liver, and pancreas.


Skin Cells of Epidermis
Neuron of Brain
Pigment Cell
Sperm
Egg
Ectoderm (External Layer)
Germ Cells
Gastrula
Zygote
Blastocyst
Mesoderm (Middle Layer)
Endoderm (Internal Layer)
Cardiac Muscle
Skeletal Muscle Cells
Tubule Cell of the Kidney
Red Blood Cells
Smooth Muscle (in Gut)
Lung Cell (Alveolar Cell)
Thyroid Cell
Pancreatic Cell

Stem Cell Niches

- A stem cell niche is a specialized microenvironment within tissues that regulates the behavior, maintenance, and fate of stem cells.
- These niches provide a protective habitat and deliver essential signals to control stem cell self-renewal, differentiation, and quiescence.
- This niches composed of cellular & non cellular components &function to :

1. Provide physical support
2. Regulate stem cell activity through signals & cell-cell interactions
3. Protect stem cells from damage & maintain their undifferentiated state


Signals to stem cell
Niches
ECMatrix Molecules
Chemical &Soluble Factors
Other Cells
Stem Cell
Self-Renewal
Differentiation

Somatic stem cell

ADULT STEM CELLS(ASC)

- Adult stem cells (ASCs), also known as somatic stem cells, are undifferentiated cells found throughout the body after development.
- They are primarily responsible for tissue repair and regeneration.
- Unlike embryonic stem cells (ESCs), ASCs are typically multipotent or unipotent, meaning they can give rise to a limited range of cell types.

Sources of Adult Stem Cells

- **Bone Marrow**: Hematopoietic and mesenchymal stem cells.
- **Brain**: Neural stem cells.
- **Skin**: Epidermal stem cells for skin and hair repair.
- **Skeletal Muscle**: Satellite cells for muscle regeneration.
- **Liver**: Oval cells aiding in liver regeneration.
- **Adipose Tissue**: Adipose-derived stem cells.
- **Dental Pulp**: Found within teeth.

Characteristics of Adult Stem Cells

1.Multipotency

1. Can differentiate into several cell types specific to their tissue of origin. For example:
    1. Hematopoietic stem cells differentiate into blood cells.
    2. Mesenchymal stem cells differentiate into bone, cartilage, and fat cells.

2.Self-Renewal

1. Can divide to produce more stem cells, maintaining their population throughout the organism's life.

3.Tissue-Specificity

1. Reside in specific tissues or organs and function to repair those areas.

4.Limited Plasticity

1. Have restricted differentiation potential compared to pluripotent cells like ESCs or induced pluripotent stem cells (iPSCs).

Applications of Adult Stem Cells

1.Medical Therapies

1. **Bone Marrow Transplants**: Hematopoietic stem cells are used to treat blood disorders like leukemia and lymphoma.
2. **Cartilage Repair**: Mesenchymal stem cells are used in orthopedic treatments.
3. **Neurological Disorders**: Neural stem cells hold promise in treating conditions like Parkinson's and spinal cord injuries.

2.Tissue Engineering

1. Adult stem cells are used in constructing new tissues for grafts, including artificial skin or bone.

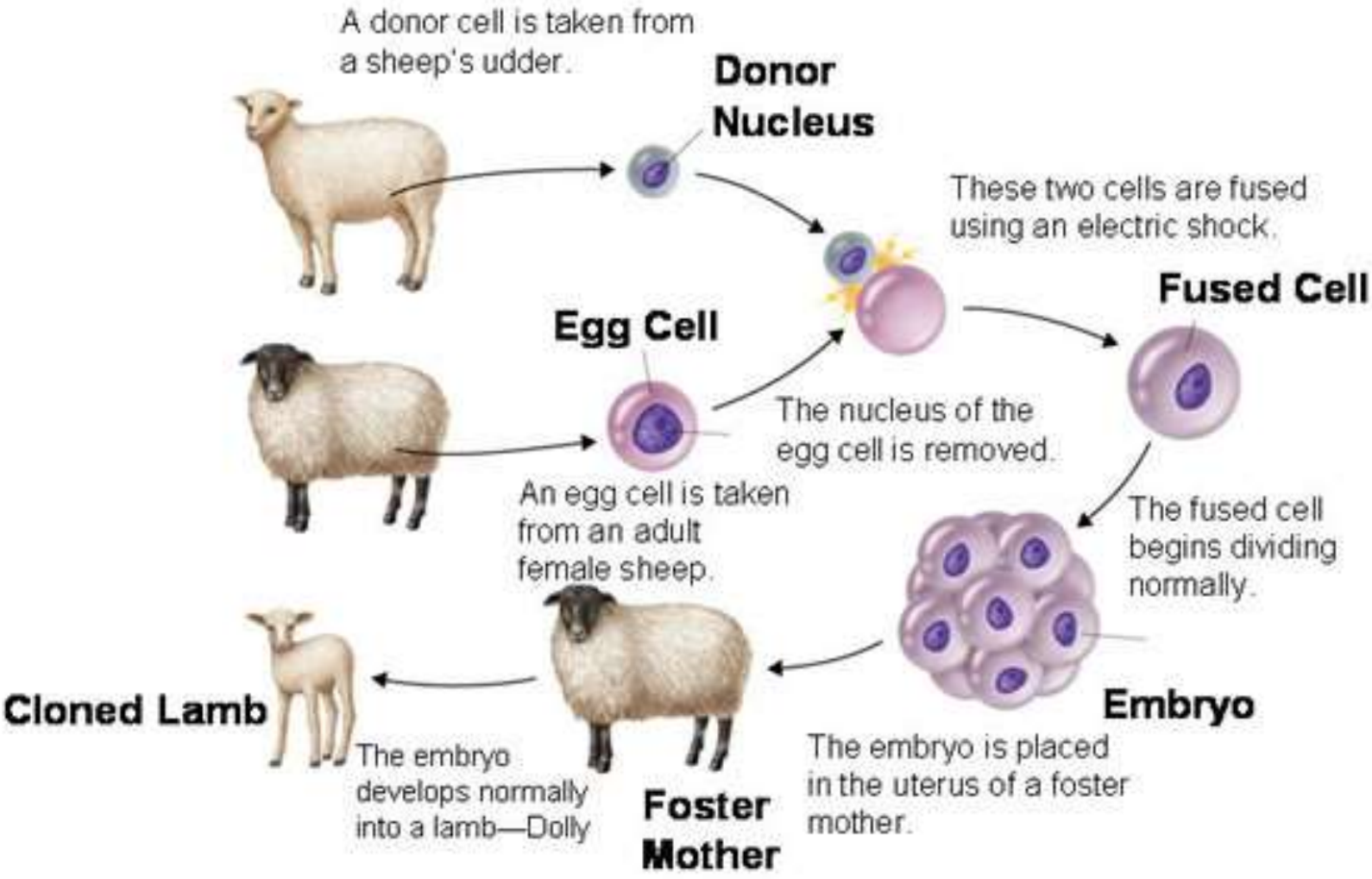
3.Drug Testing

1. Cultured ASCs serve as a model for testing new drugs or studying disease mechanisms.

Somatic Cell Nuclear Transfer (SCNT)

- Is a laboratory technique used in genetics and cloning. It involves transferring the nucleus of a somatic (body) cell into an enucleated egg cell to create a viable embryo.

reproductive cloning



Steps of SCNT

1.Isolation of Donor Somatic Cell

1. A somatic cell is collected from the individual to be cloned.
2. The nucleus of this somatic cell contains the complete set of genetic material.

2.Enucleation of the Egg Cell

1. An unfertilized egg cell is obtained from a donor.
2. Its nucleus is removed, creating an "enucleated egg."

3.Nucleus Transfer

1. The nucleus of the somatic cell is inserted into the enucleated egg using micromanipulation techniques.

4.Activation of the Egg

1. The reconstructed egg is stimulated, often with electric pulses or chemical signals, to begin cell division.
2. This mimics fertilization, initiating embryonic development.

5.Culturing and Development

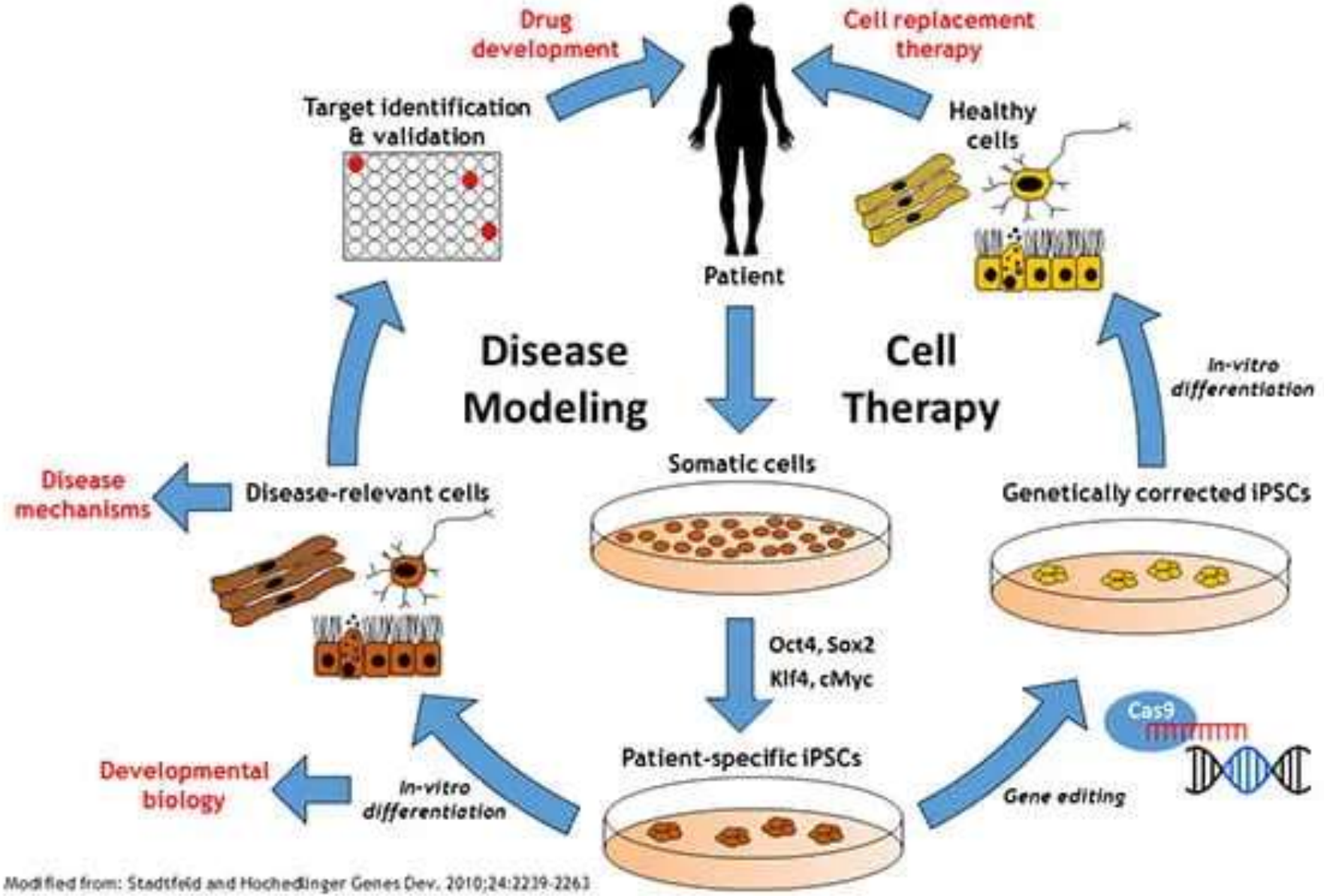
1. The egg develops into a blastocyst (an early embryonic stage) over several days in culture.

6.Application

1. In **therapeutic cloning**, stem cells are harvested from the inner cell mass of the blastocyst for research or regenerative medicine.
2. In **reproductive cloning**, the blastocyst is implanted into a surrogate mother to develop into a cloned organism.

3. Induced pluripotent stem cells (iPSCs

- iPSCs technology discovery (2006-1012) → NP
- *Concept*: mature cells can be reprogrammed to become pluripotent out need for embryo
- *Technique* : done by introduce a few specific pluripotency genes into already specialized somatic cells→ the cells will be reprogramed into an embryonic –like state (reset the somatic cell)
- *Goal*: **regenerative medicine** ….. To replace damage tissue in a given person by using pluripotent stem cells from his own body, not only the patient will **get the new tissue** he needs, but also with **NO immune- rejection complications**



Modified from: Stadtfeld and Hochedinger Genes Dev. 2010;24:2239-2263

Production Process

1. **Harvesting Somatic Cells**:
   - Skin fibroblasts or peripheral blood cells are obtained from the donor.
2. **Delivery of gene Factors**:
   - Delivered via viral vectors (e.g., retrovirus, lentivirus) or non-viral methods (e.g., mRNA, small molecules).
3. **Reprogramming**:
   - Factors alter gene expression, reverting the somatic cells to a pluripotent state.
4. **Selection and Culturing**:
   - Successfully reprogrammed iPSCs are selected and cultured under defined conditions.

Cell culture techniques

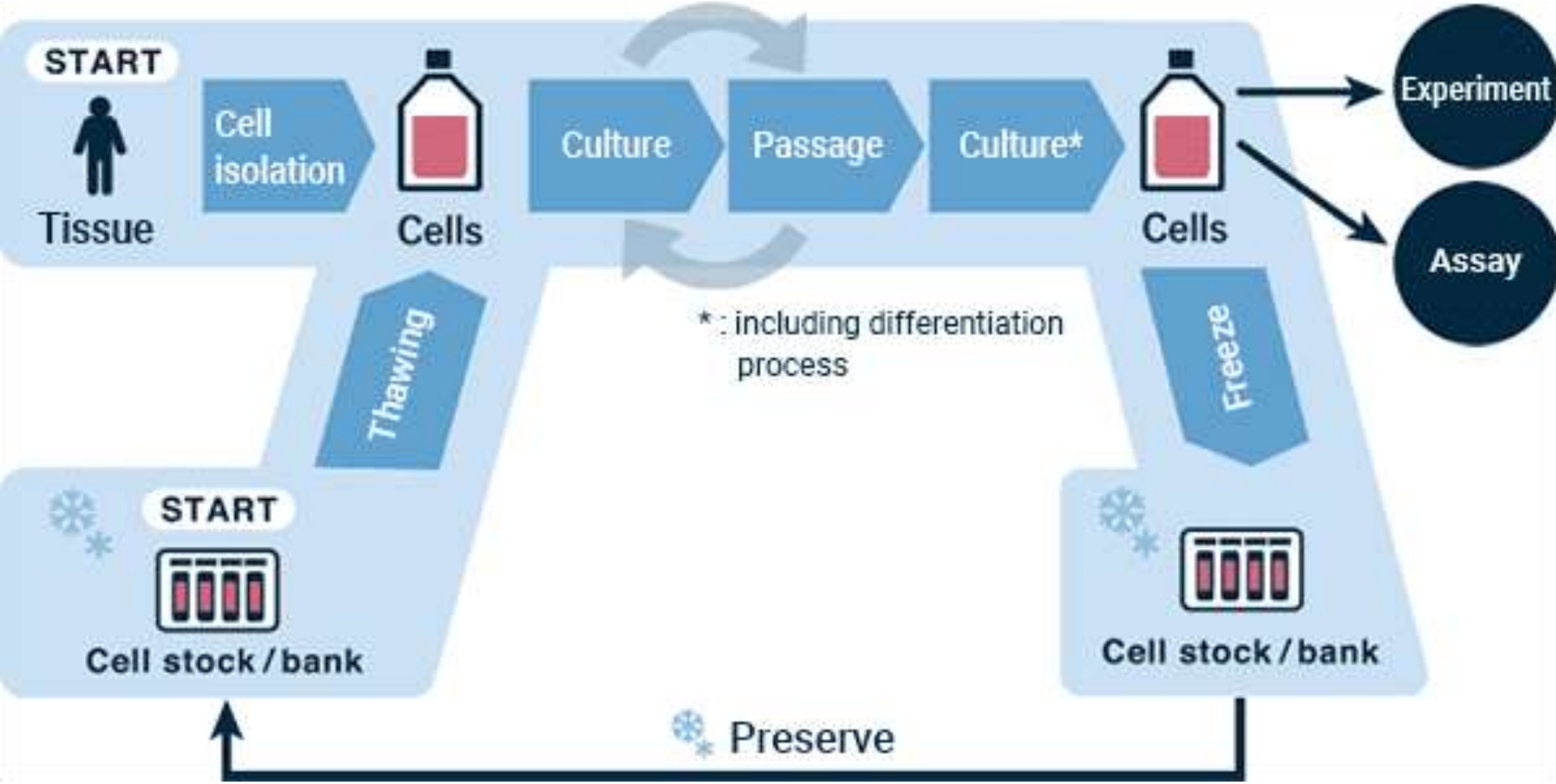
- Cell culture refers to the removal of cells from an animal and their subsequent growth in a favorable artificial environment.
- The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been established.

- **There are two methods for obtaining cells:**

✓ from a cell bank

✓ Isolating cells from donor tissue.

- When starting culture from cells obtained from a cell bank, one needs to go through the procedures of "thawing," "cell seeding" and "cell observation."



When using tissue collected from a donor, unnecessary tissue are usually removed if it is attached. There are two major methods to isolate cells from the tissue, 1- **Explant culture** 2- **Enzymatic method** by using a proteolytic enzyme solution.

Thawing

- Thawing frozen cryopreserved cells to initiate a cell culture may be thought of as "waking up the cells.“
- A vial of frozen cells obtained from a cell bank is transferred from a liquid nitrogen tank to the bench, and thawed in a 37°C water bath.
- Before ice is almost melted, medium is quickly added to dilute the cryoprotectant liquid (EX. DMSO), the cells are precipitated by centrifugation, and after removing the supernatant, fresh medium pre-heated to 37°C is added.
- The cells are then resuspended by pipetting and the number of cells/cell concentration is measured using a microscope or cell counter.

Cell Seeding

- To achieve the target cell seeding density, the amount of fresh medium required should be calculated based on the measured cell numbers.

Cell Observation

- After seeding the cells in a new culture vessel, observe the cells in the vessel with an optical microscope or other observation device in the following manner:

✓ Check that there are viable cells
✓ Check to make sure that cells are evenly distributed in the vessel
✓ Check for the presence of foreign objects other than cells
✓ Check the cell morphology

- After confirming the above, the cell culture vessel placed in a humidified CO2 incubator at 37°C and start culturing.

Culture of embryonic stem cell