BS20001 | Science of Living Systems

Cell and Developmental Biology

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Topics

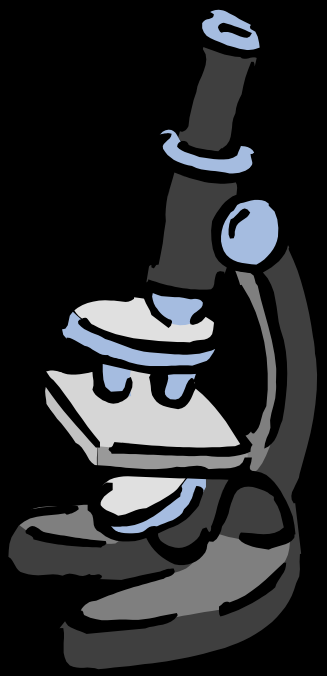
- Microscopy
- Prokaryotic and Eukaryotic Cells
- Development of Multicellular Organisms
  - Mitosis
  - Meiosis
  - Differentiation
- Stem cells and their applications

Microscopy techniques to study cell biology

Microscopes are used to observe small objects invisible to the eye.

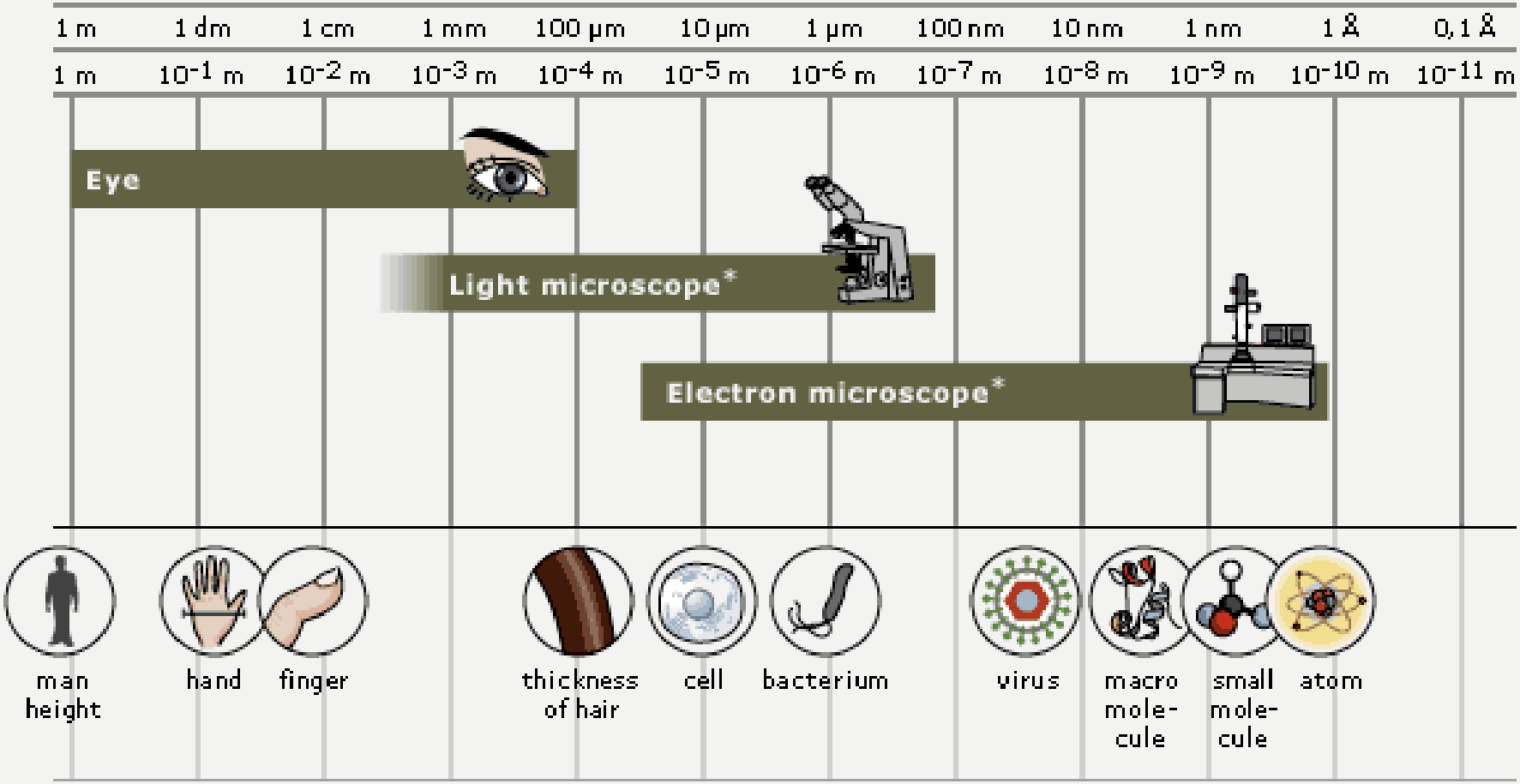
The quality of the image depends on:

- **Magnification:** the microscope's power to increase an object's apparent size
- **Resolution:** minimum distance between two distinguishable points
- **Contrast:** visible differences between different parts of the sample



Resolving Power Line

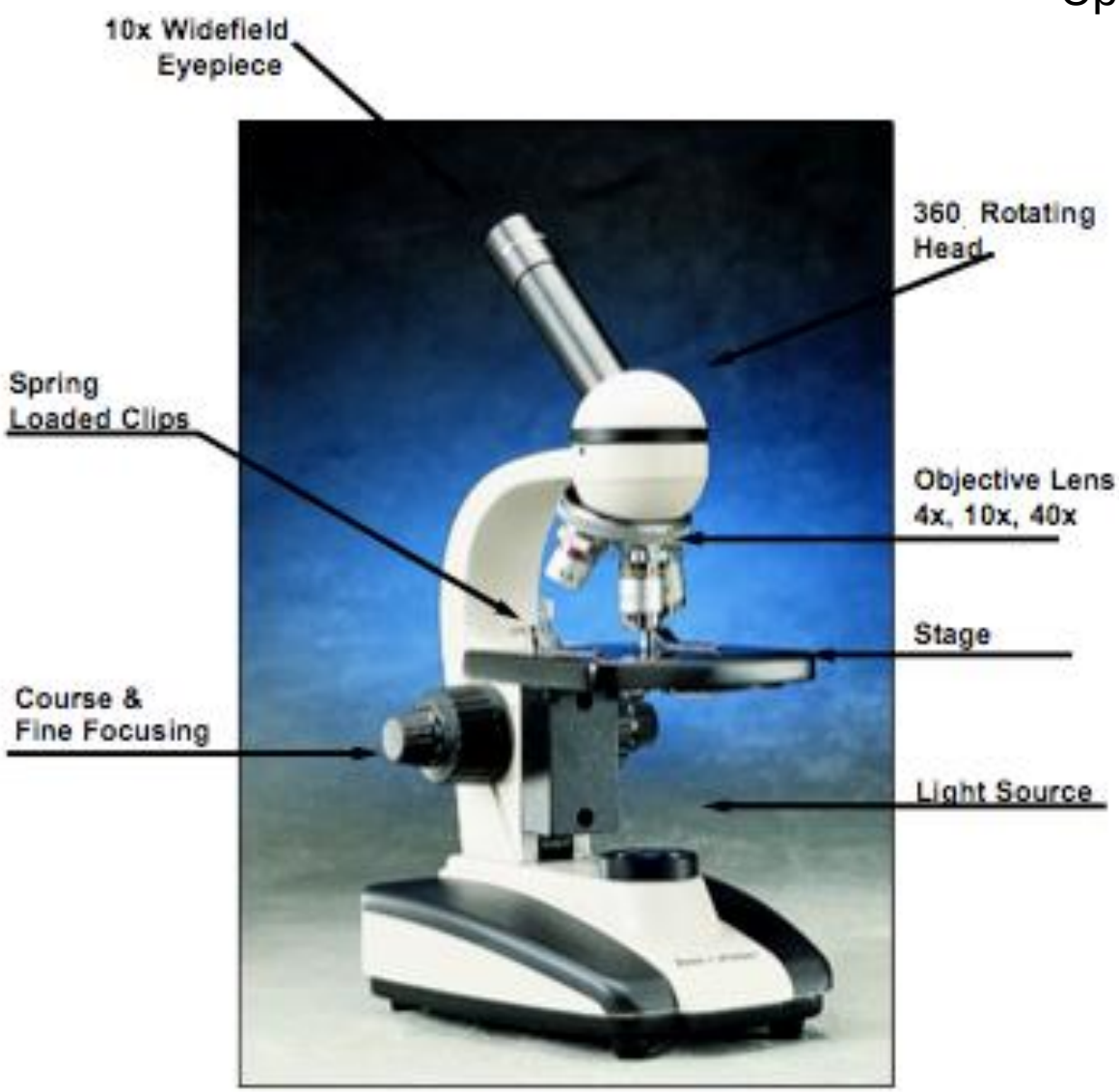
What can you see with the different types of microscopes? The human eye is capable of distinguishing objects down to a fraction of a millimeter. With the use of light and electron microscopes it is possible to see down to an angstrom and study everything from different cells and bacteria to single molecules or even atoms.

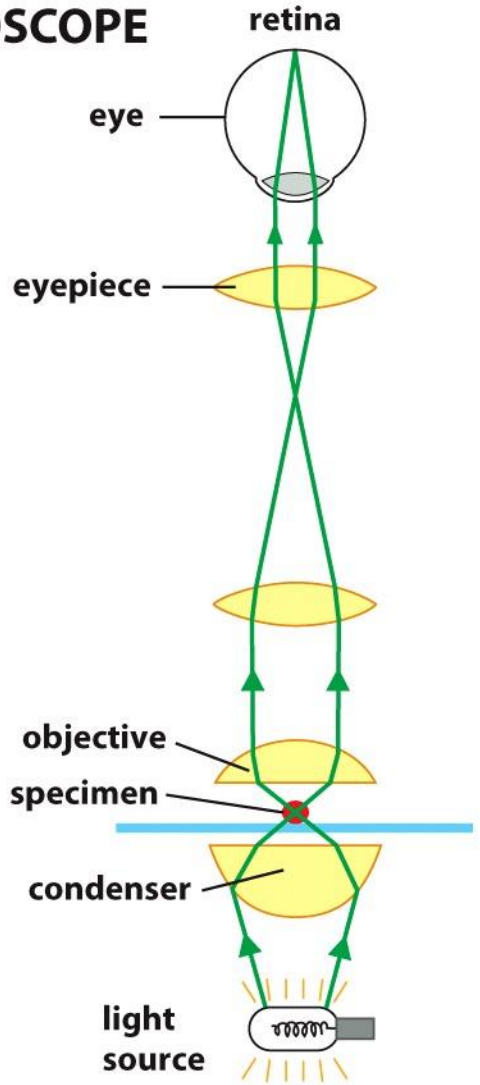


* Light microscope includes phase contrast and fluorescence microscopes. Electron microscope includes transmisson electron microscope.

THE LIGHT MICROSCOPE

Up to 0.2 μm

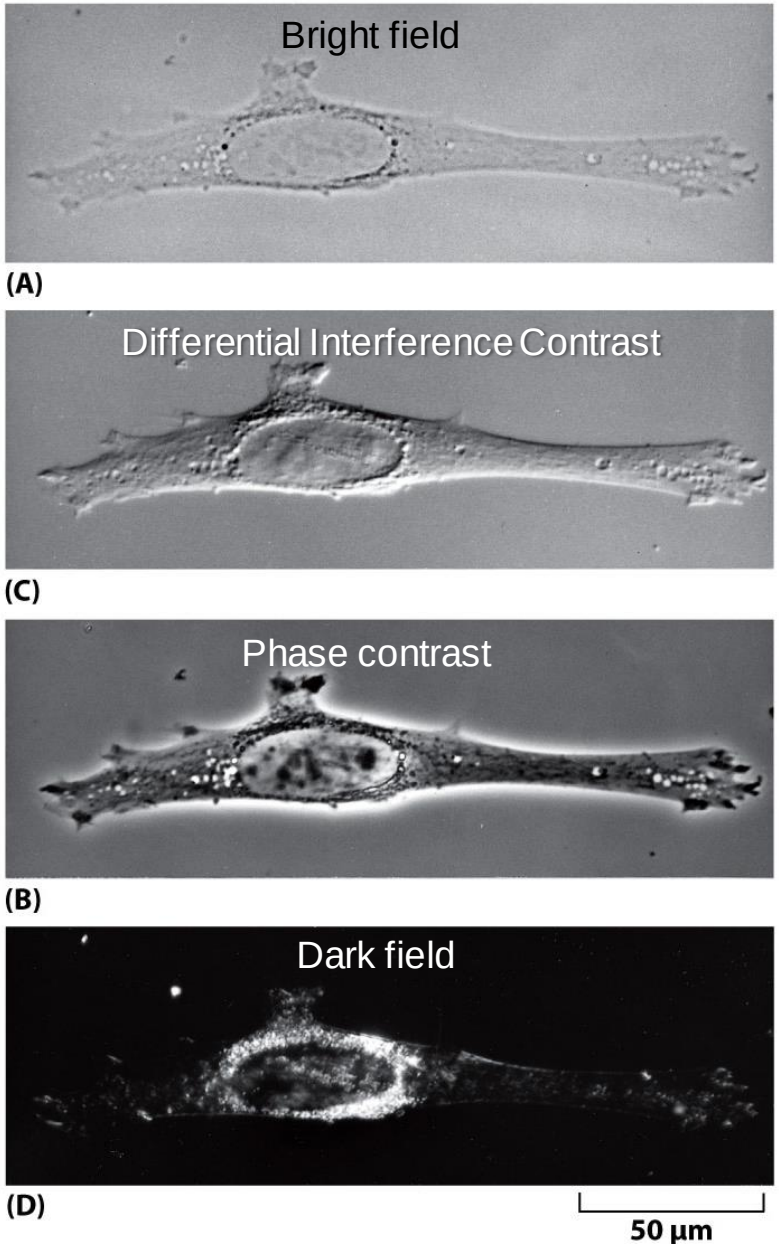




the light path in a light microscope

Panel 1-1a (part 2 of 2) Essential Cell Biology, 4th ed. (© Garland Science 2014)

Microscope Contrast



Specimen Contrast

By selective and differential staining of cellular components

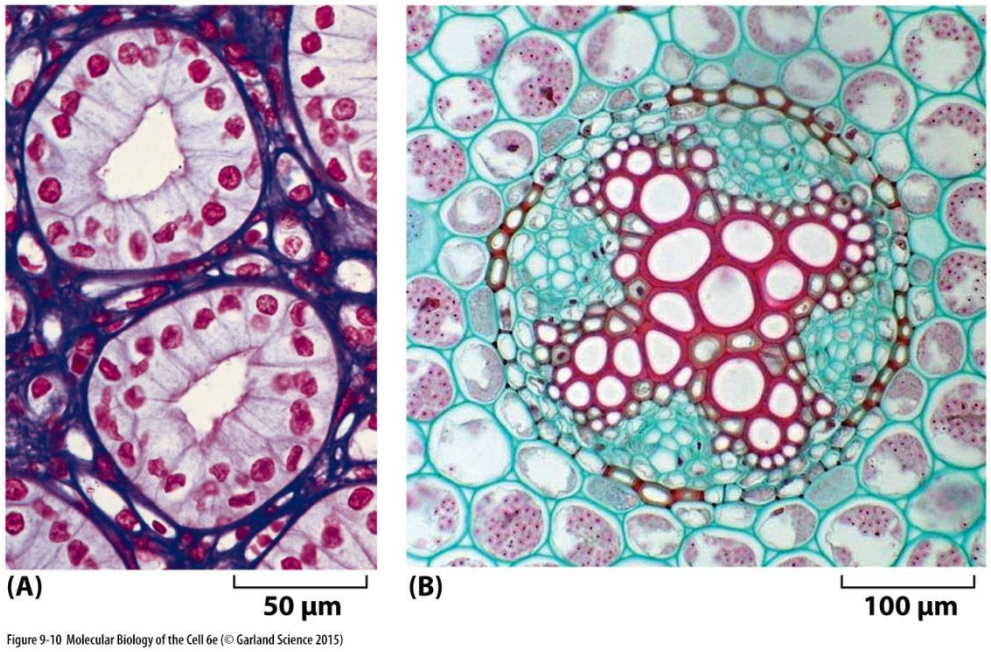


Figure 9-10 Molecular Biology of the Cell 6e (© Garland Science 2015)

Electron Microscopy

Subcellular structures are studied by electron microscopes. They are of two types:

- **Scanning Electron Microscopes (SEM)** focus a beam of electrons onto the surface of the sample and provide images that give 3D representation of the sample. SEM is used to study surface structure of objects
- **Transmission Electron Microscopes (TEM)** focus a beam of electrons through the sample. TEMs are used to study the internal structure of the cell

Electron Microscopy: SEM vs TEM

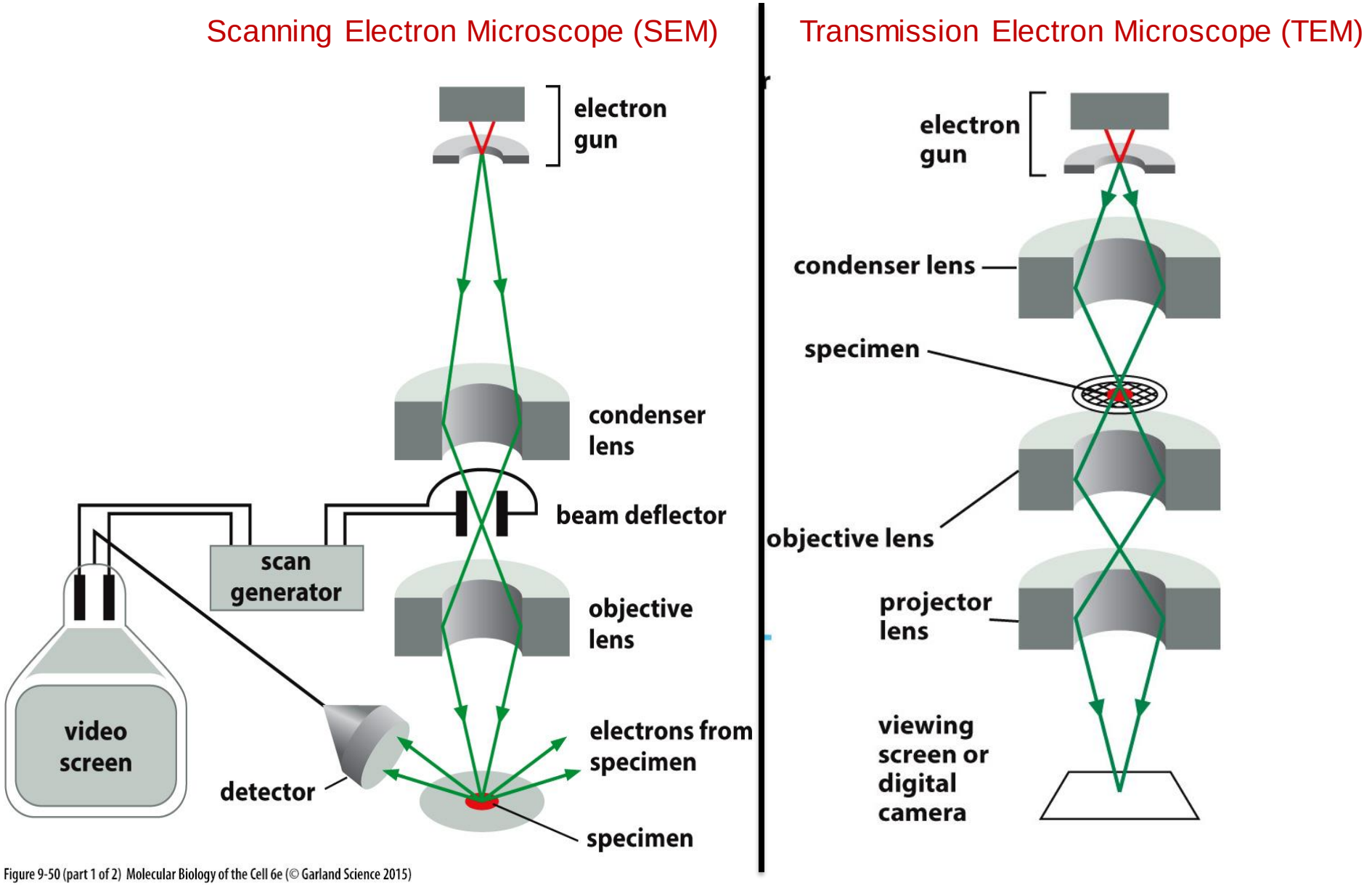


Figure 9-50 (part 1 of 2) Molecular Biology of the Cell 6e (© Garland Science 2015)

Electron Microscopy: SEM vs TEM

Scanning Electron Microscope (SEM)

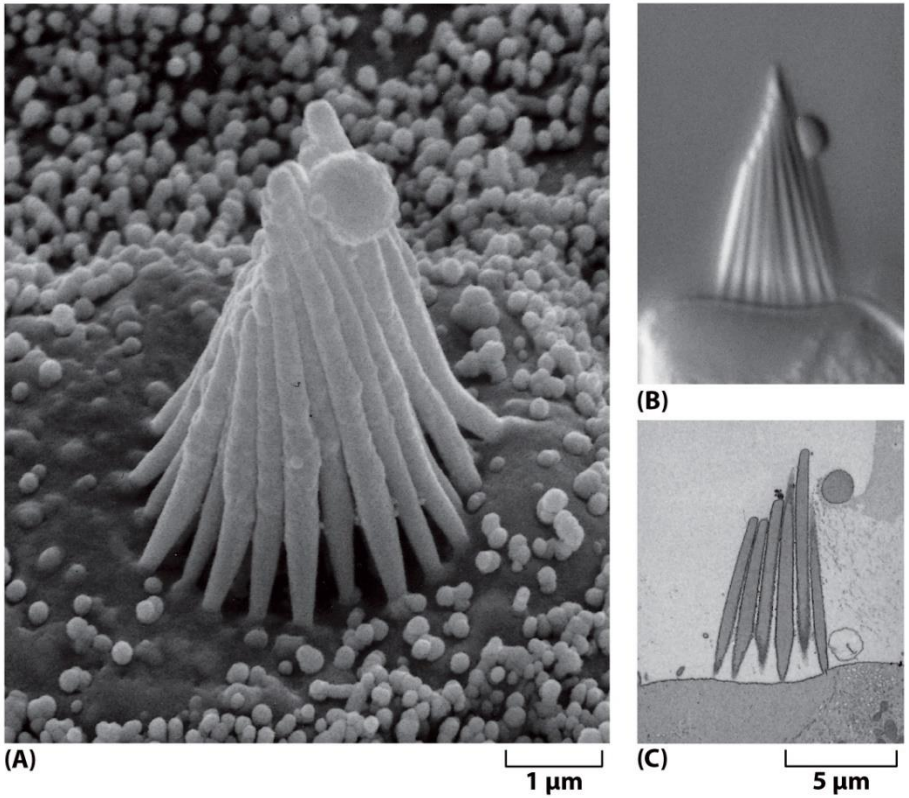


Figure 9-51 Molecular Biology of the Cell 6e (© Garland Science 2015)

Surface features

Transmission Electron Microscope (TEM)

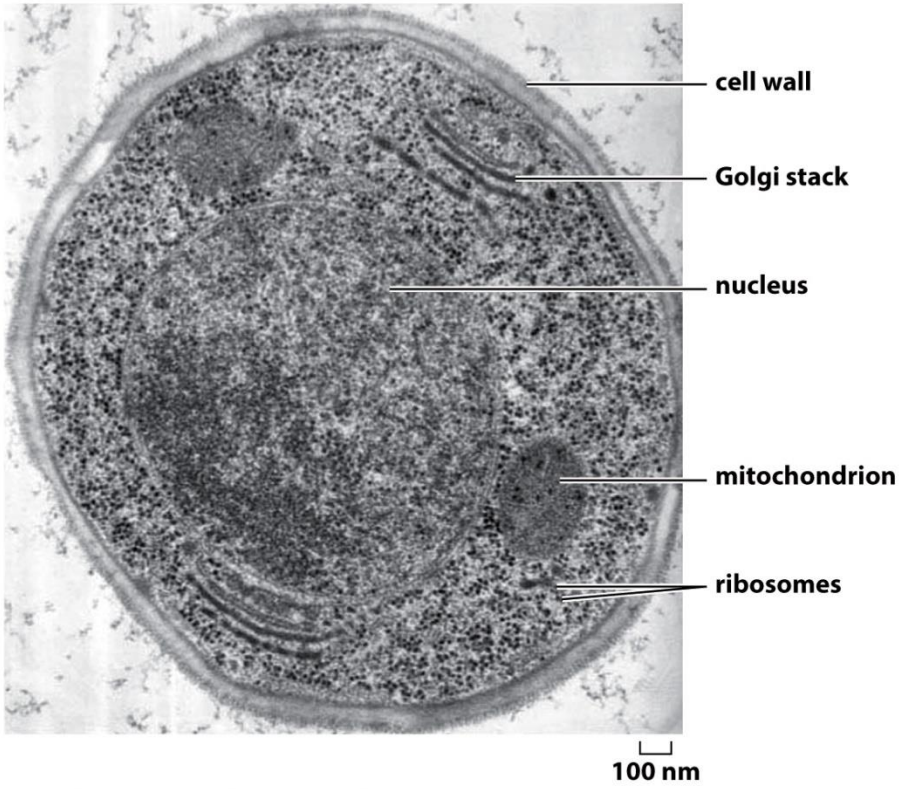


Figure 9-44 Molecular Biology of the Cell 6e (© Garland Science 2015)

Intracellular ultrastructure

Fluorescence Microscope

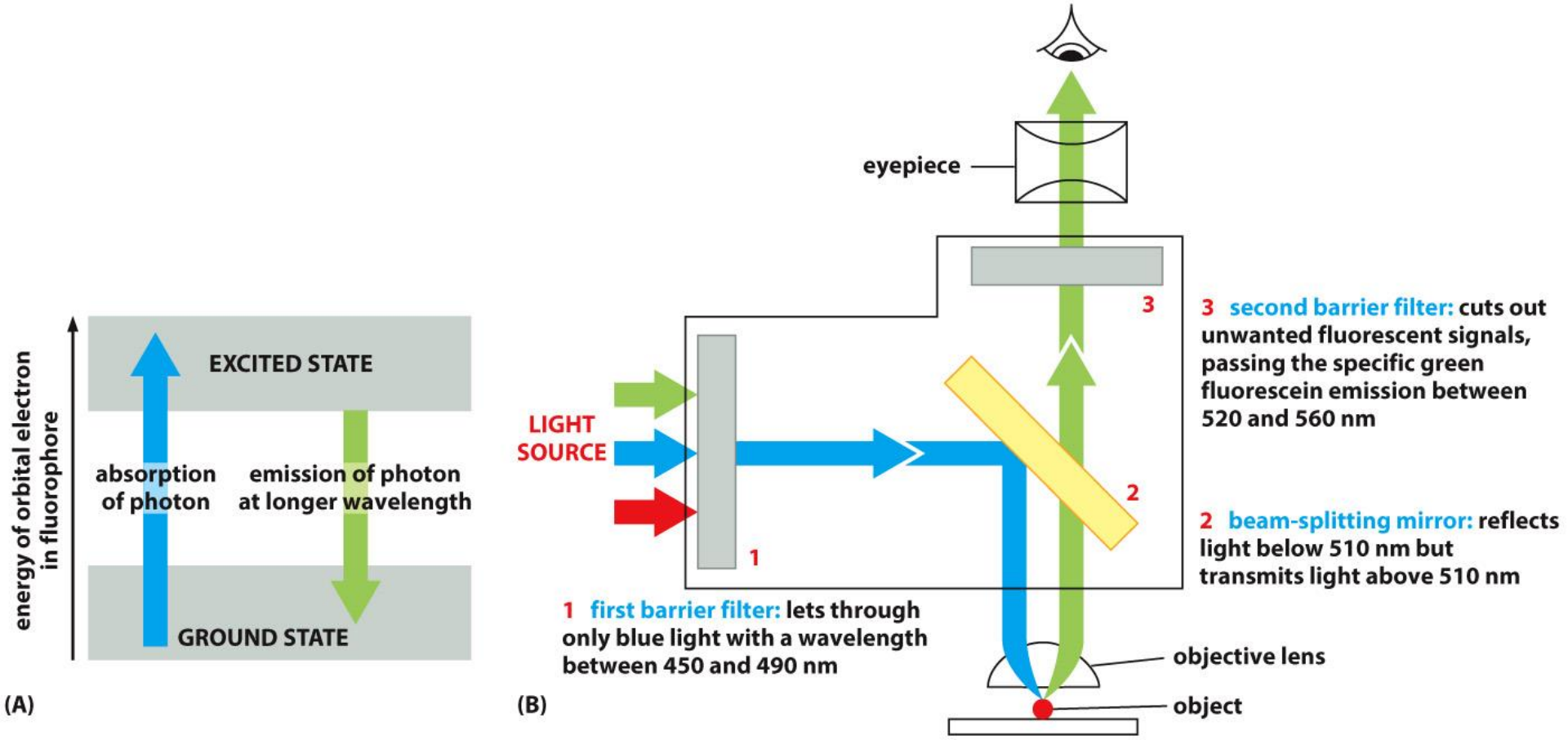
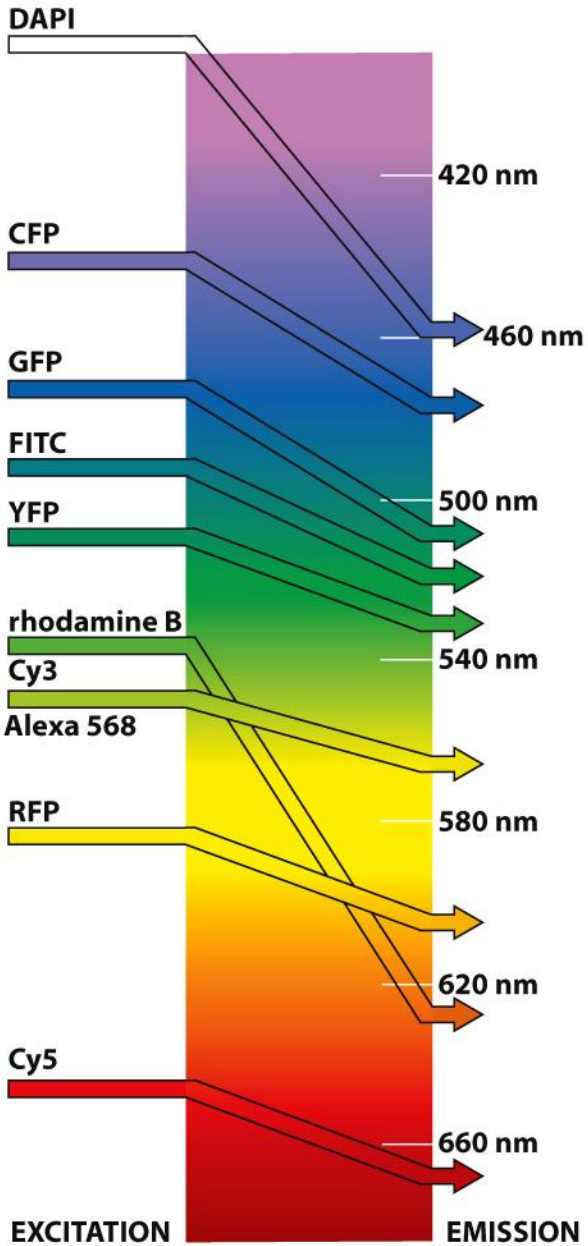


Figure 9-12 Molecular Biology of the Cell 6e (© Garland Science 2015)



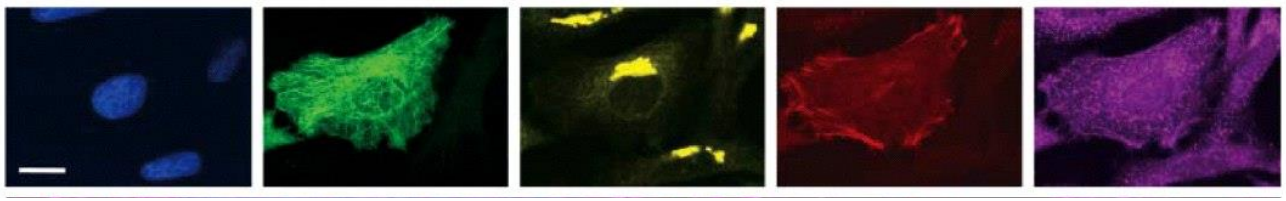
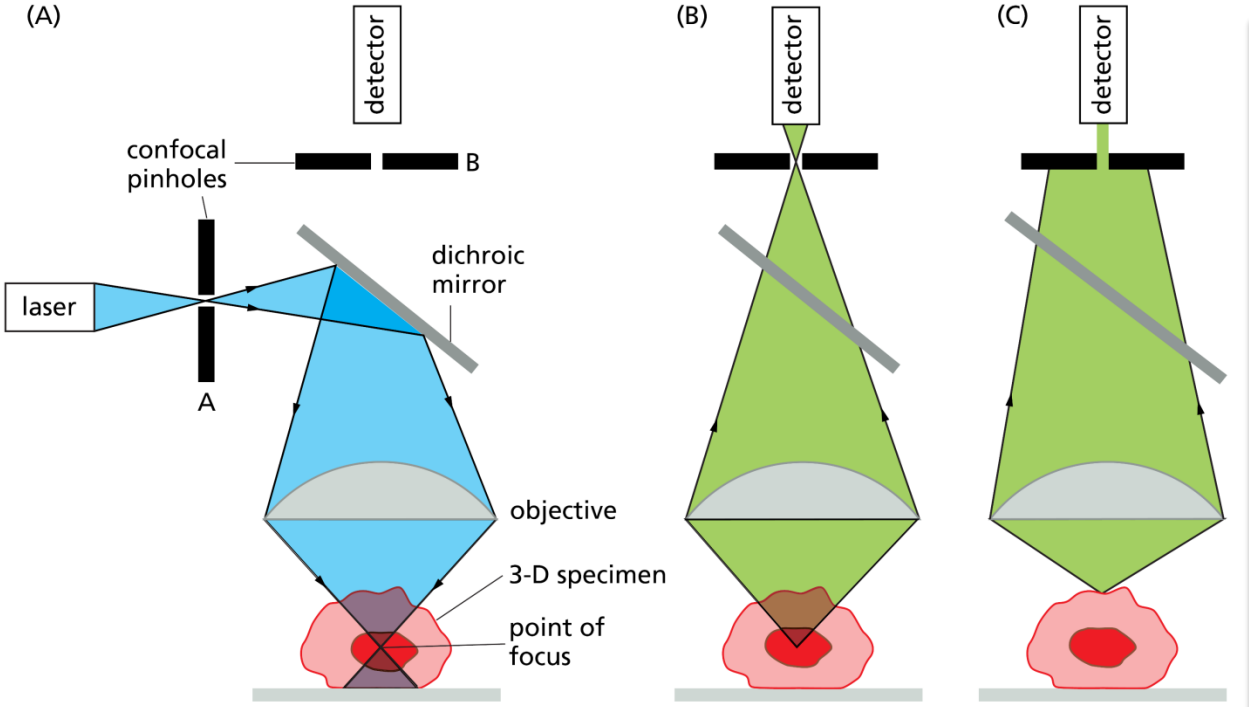


Figure 9-13 Molecular Biology of the Cell 6e (© Garland Science 2015)

Chapter 9 Opener
Molecular Cell Biology, Sixth Edition

Confocal microscope

- A pinhole focuses the illumination at a point
- Another pinhole collects emitted light (signal) only from a point (focus)
- Noise from out of focus points in specimen is excluded

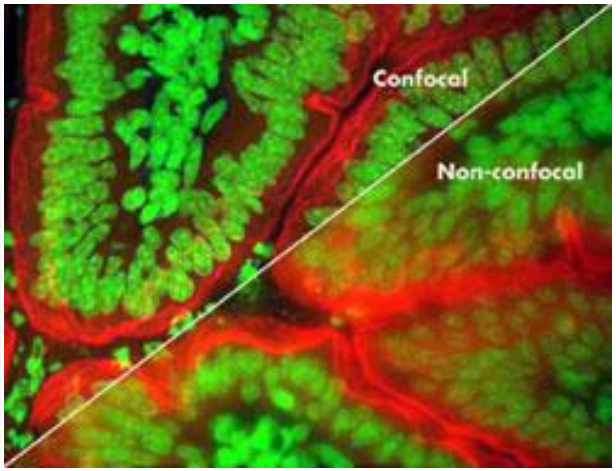


fluorescent specimen is illuminated with a focused point of light from a pinhole

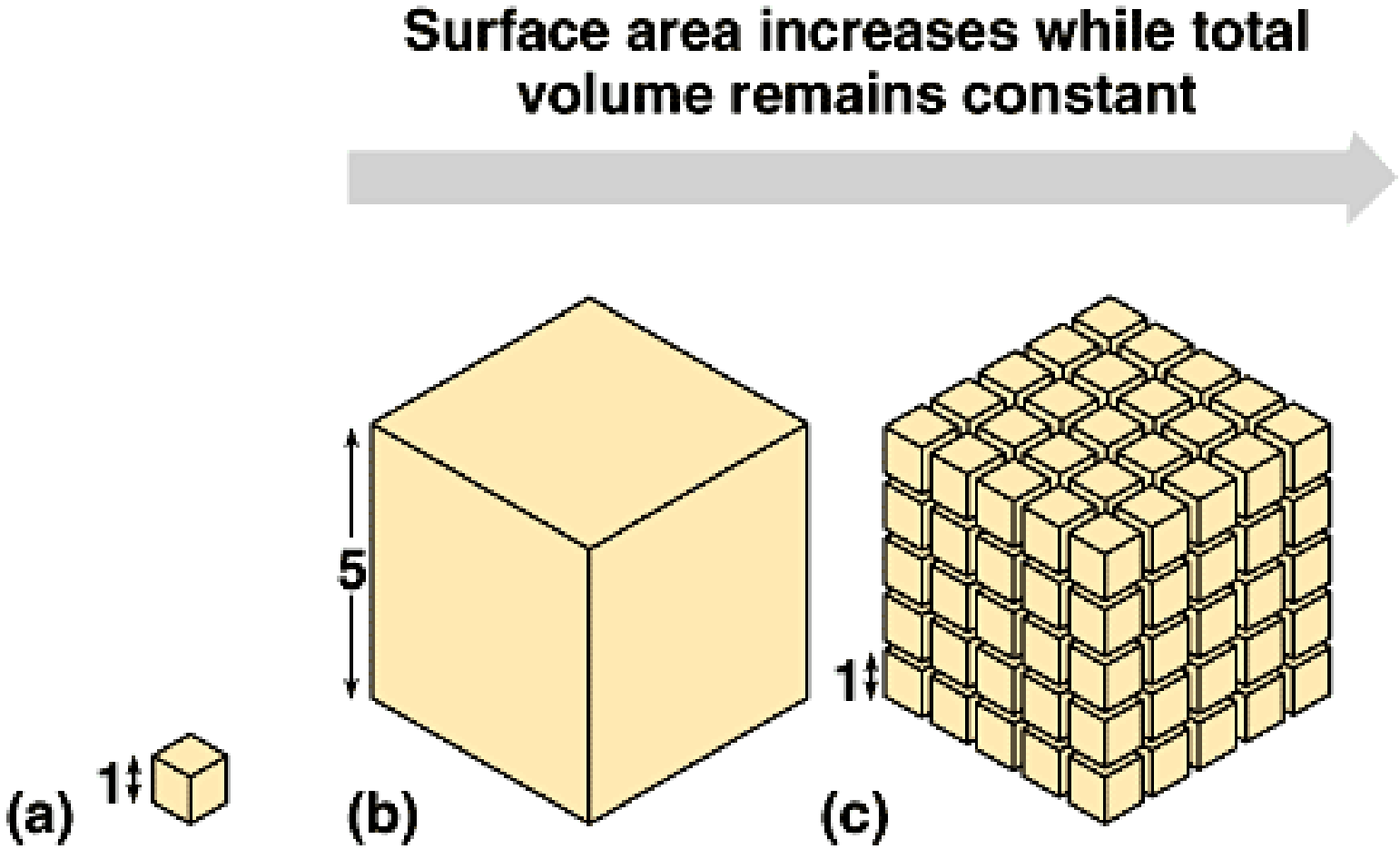
emitted fluorescent light from in-focus point is focused at pinhole and reaches detector

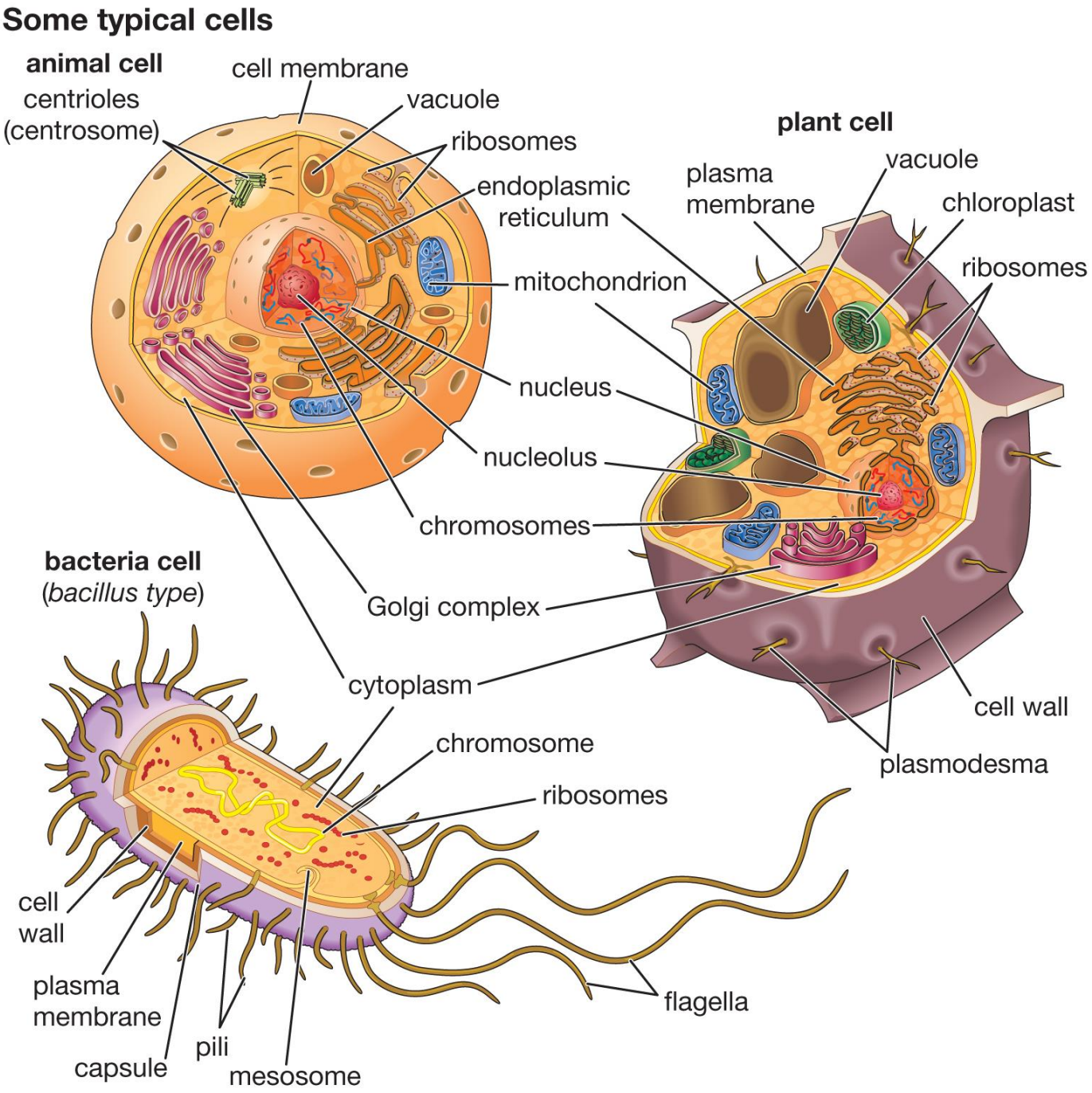
emitted light from out-of-focus point is out of focus at pinhole and is largely excluded from detector

Point of focus of illuminating light and emitted light are same



Why are Cells Small?





Prokaryotic and Eukaryotic Cells

Prokaryotic cells	Eukaryotic cells
No membrane-enclosed intracellular compartment to house genetic material (DNA)	Have well defined nucleus (membrane-enclosed intracellular compartment) to house genetic material (DNA)
Prokaryotic cells lack most of the complex membrane bound internal organelles	Eukaryotic cells have well defined and complex membrane bound internal organelles
Prokaryotic cells have a single circular chromosome	Eukaryotic cells have paired chromosomes
Prokaryotic cells lack histone proteins;	Eukaryotic cells have histone proteins
Prokaryotic cell wall has peptidoglycan.	Plant and fungal cells have both cellulose and chitin in cell wall. No such cell wall in animal cells

Bacterial Cell Wall

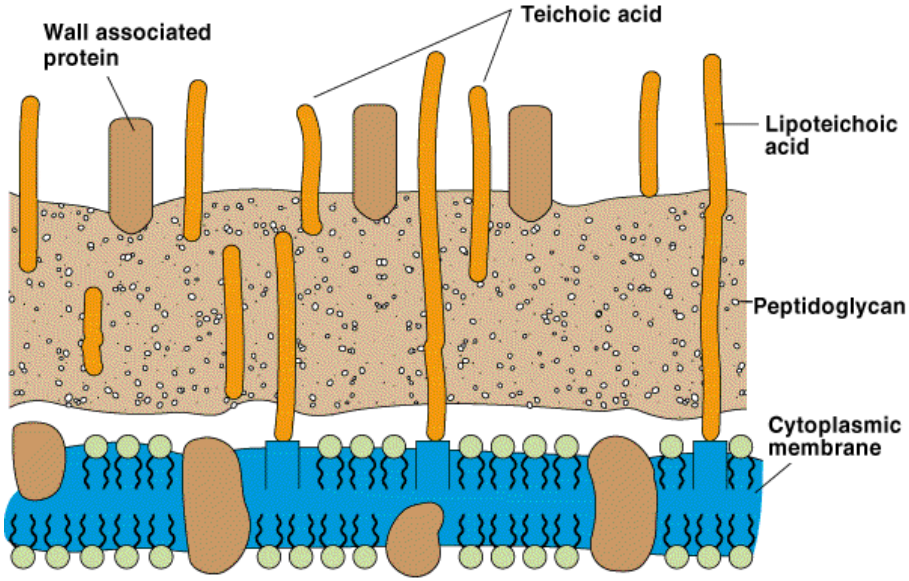
- Lies outside the cell membrane in nearly all bacteria (except mycoplasma and some archaebacteria)

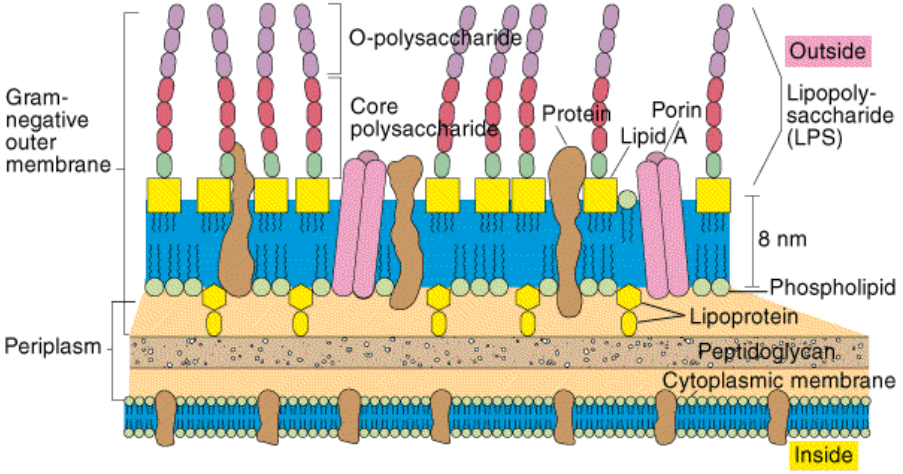
- Two important functions:

1. Maintains the characteristic shape

2. Prevents osmotic lysis

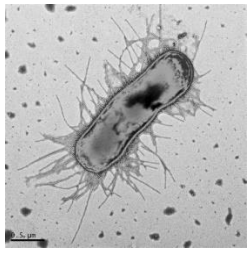
Bacterial Peptidoglycan Layer





Gram +ve
S. aureus

Gram –ve
E. coli



Components of Bacterial Peptidoglycan Layer

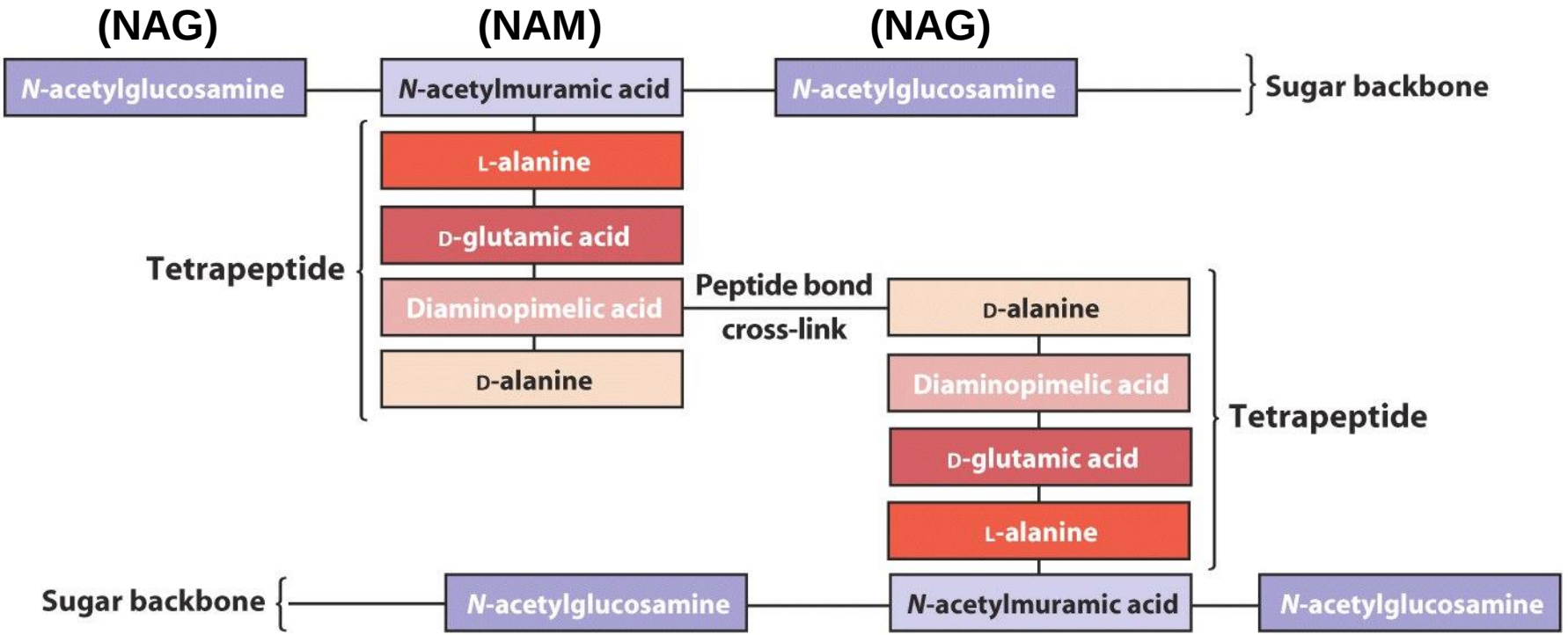
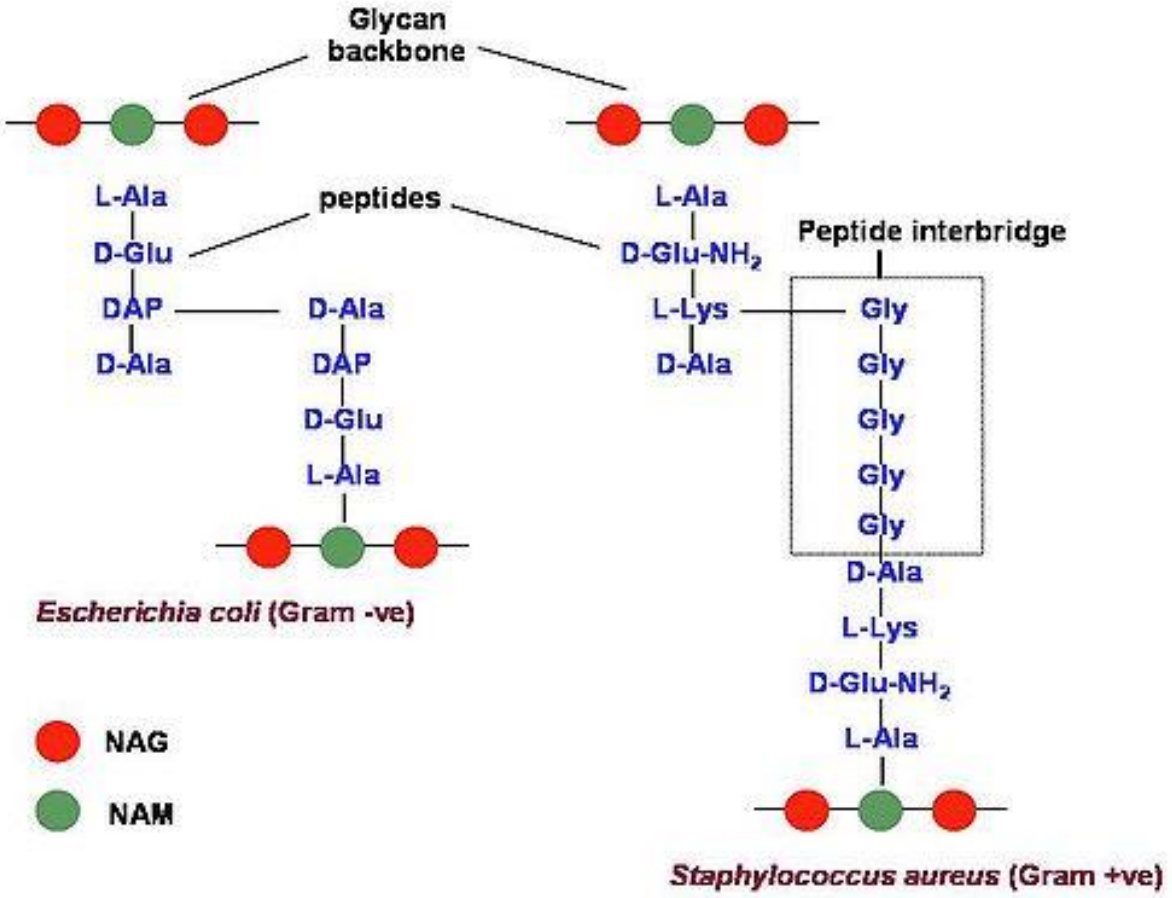


Figure 4-4a Microbiology, 6/e

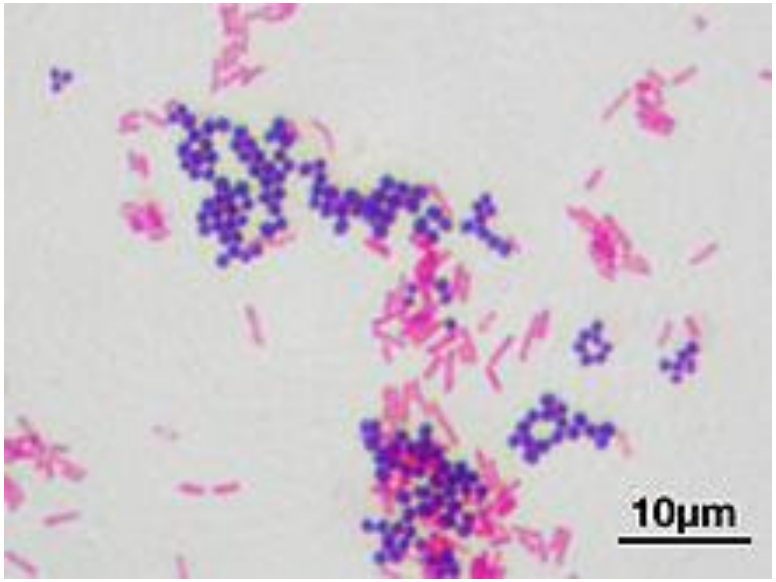
Gram –ve bacteria like *E. coli*

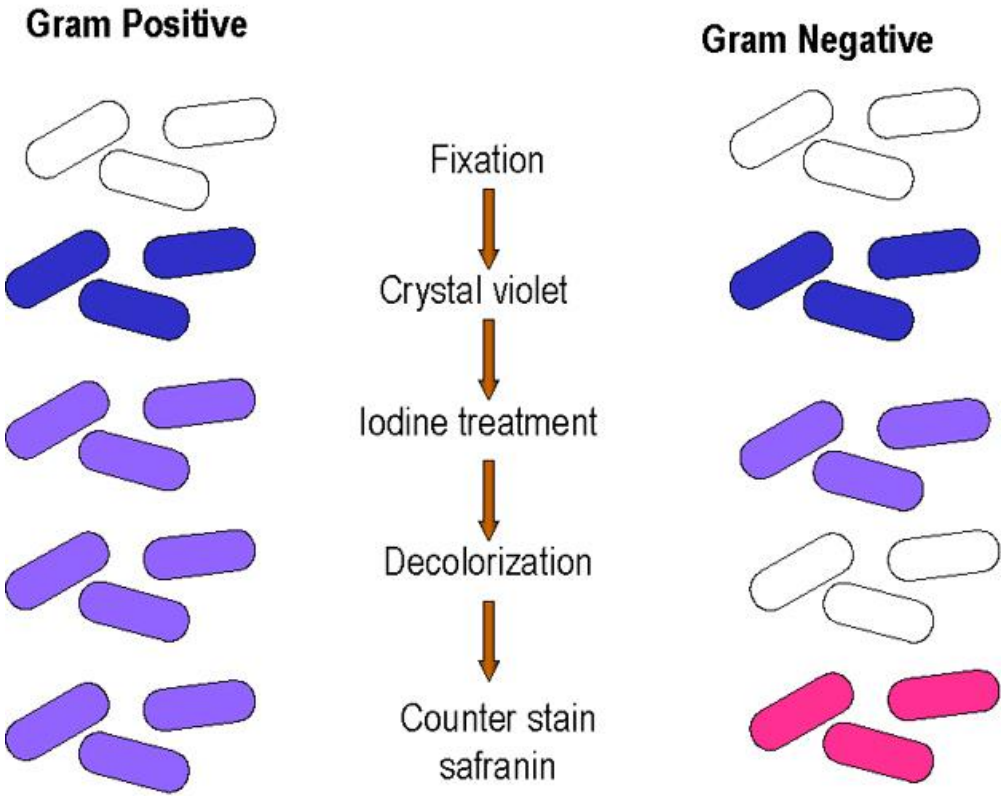
Components of Bacterial Peptidoglycan Layer

Gram+ve vs Gram-ve



Gram Staining



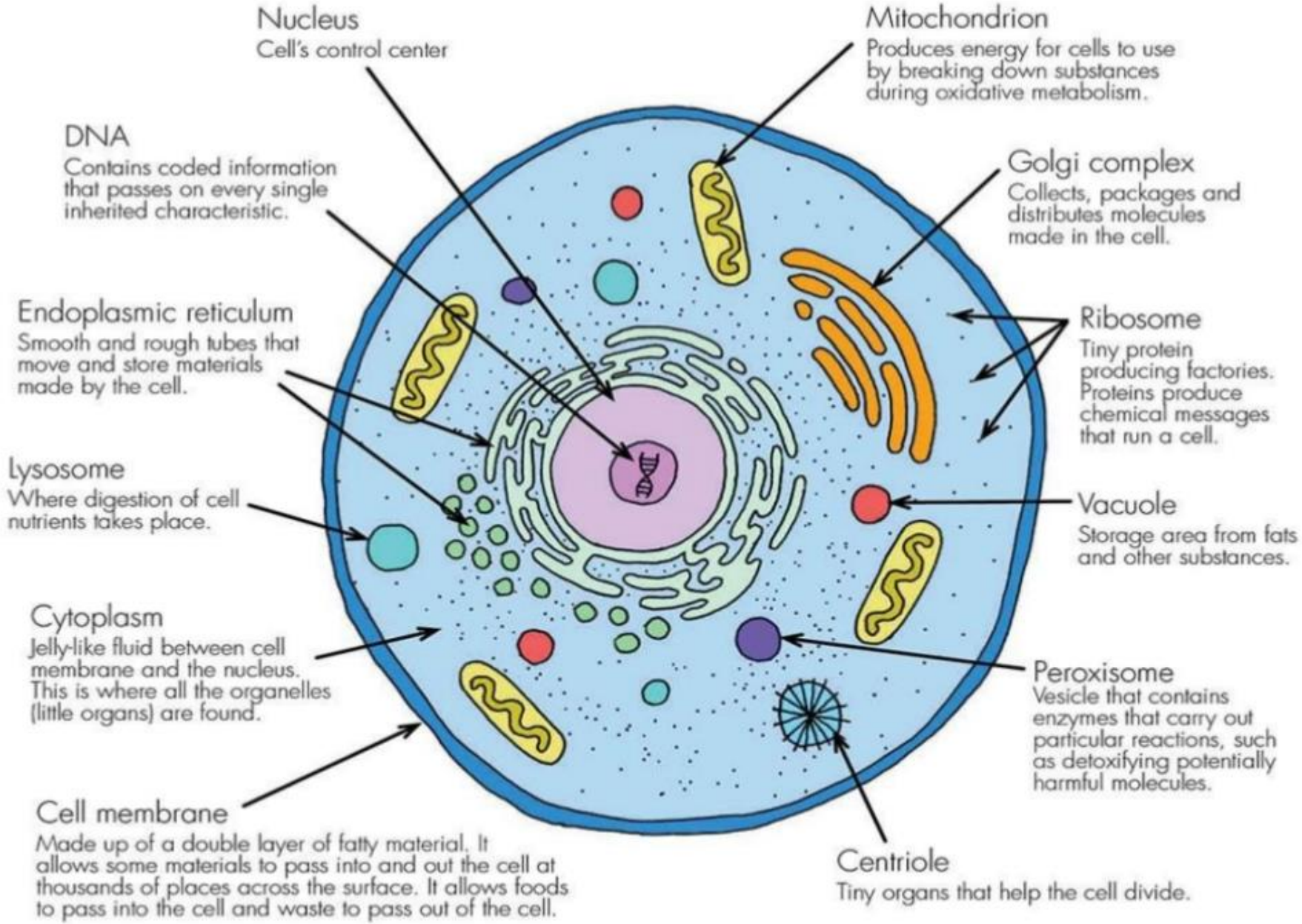


A Gram stain of mixed *Staphylococcus aureus* (Gram positive cocci) and *Escherichia coli* (Gram negative bacilli), the most common Gram stain reference bacteria

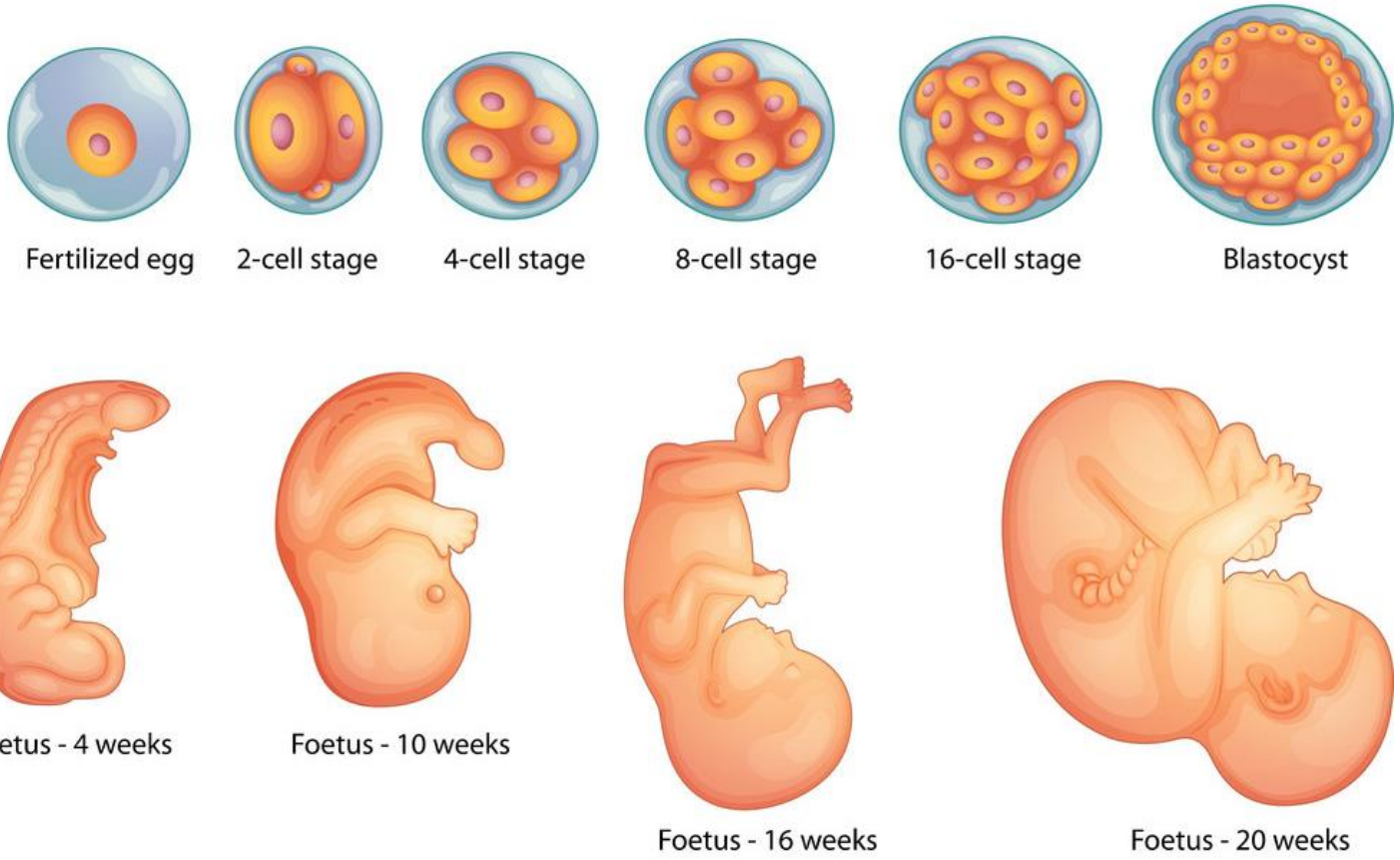
Controlling Bacteria by Damaging Cell Walls

- The antibiotic penicillin blocks the final stages of peptidoglycan synthesis
- The enzyme lysozyme, found in tears and other human body secretions, digests peptidoglycan

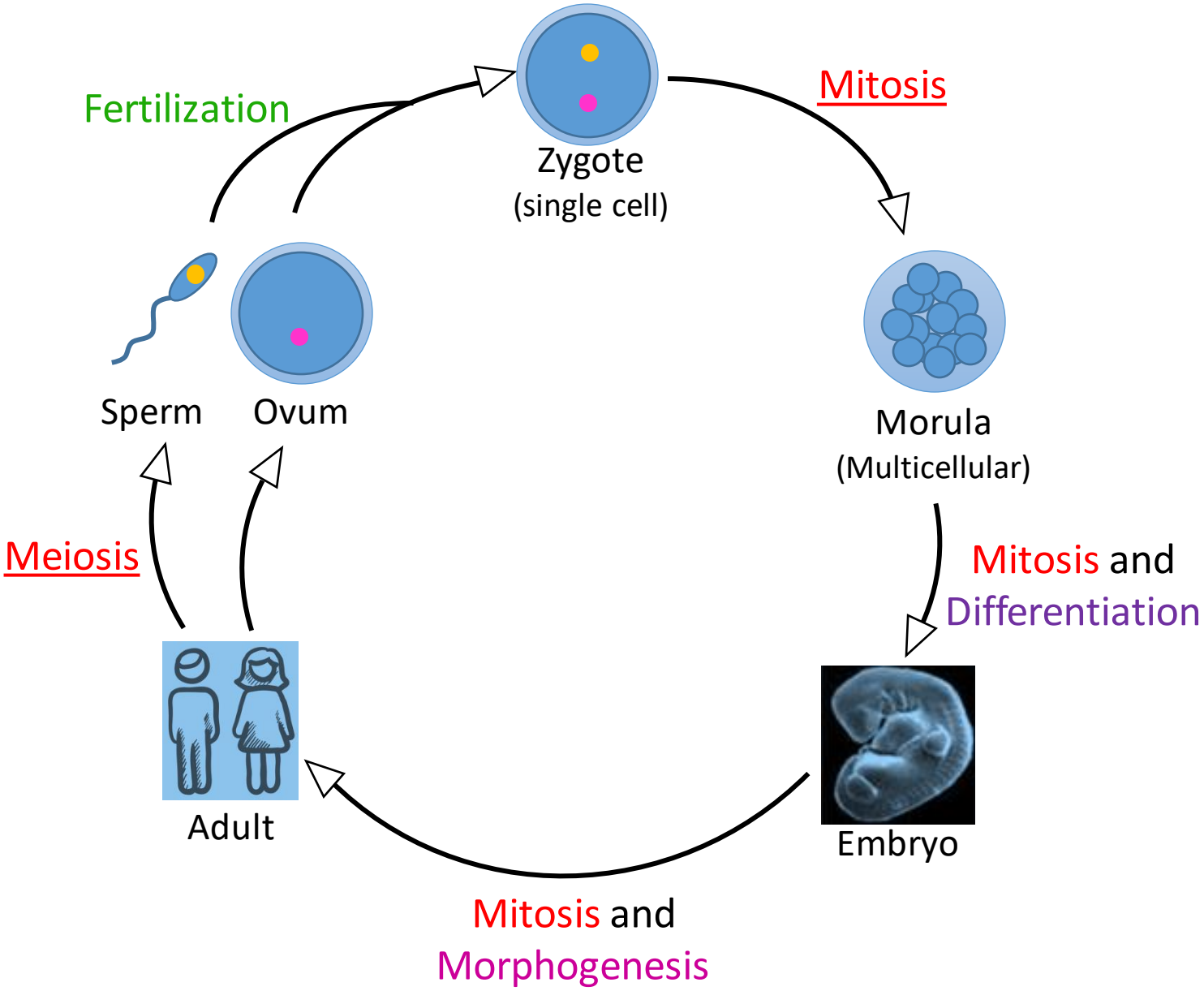
Eukaryotic cellular compartments and their functions



Development of Multicellular Organisms

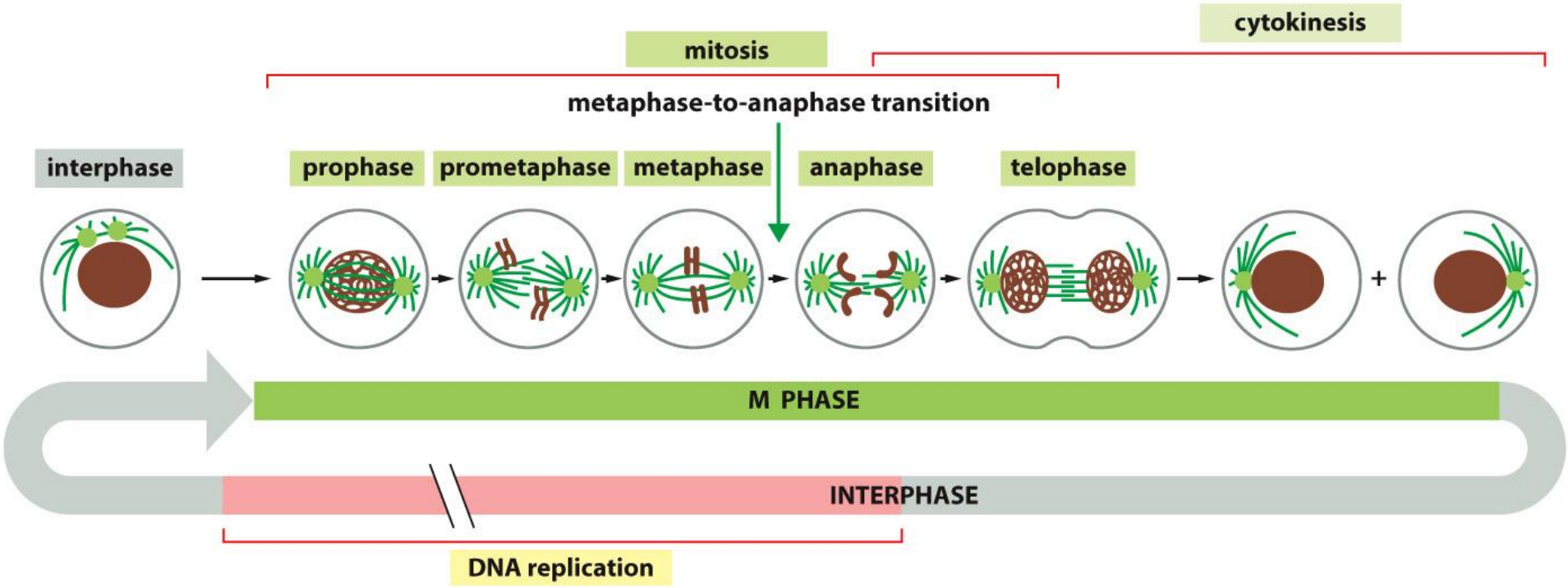


Cycle of life depends on two modes of cell division



Expansion of Zygote (single cell) to adult human (10^{13} cells):

Mitosis cell division

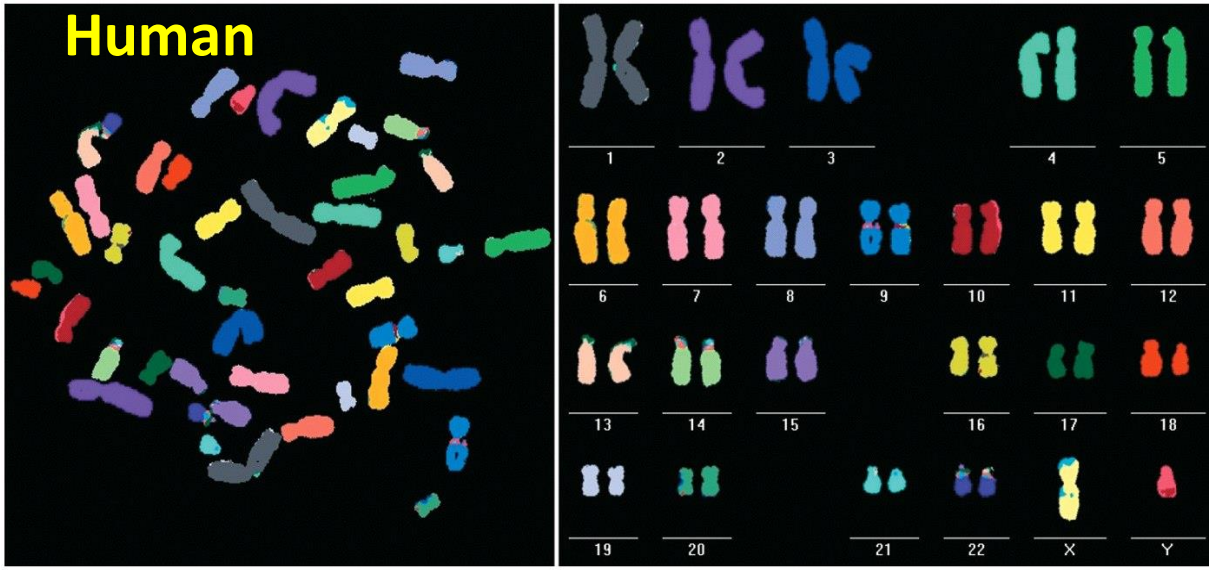


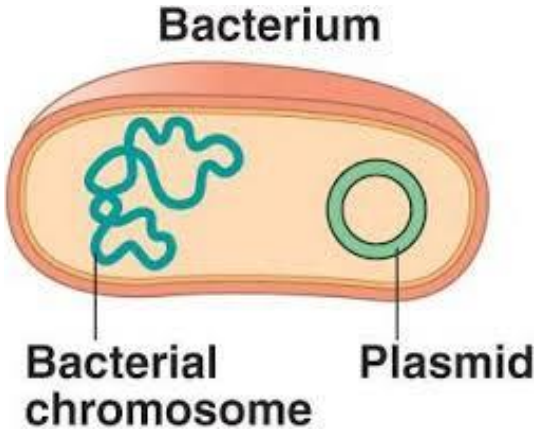
- Division of 1 parent cell produces 2 daughter cells
- Chromosome number remains same after Mitosis (2n to 2n)- equational division
- Replication doubles the genomic content (2c to 4c), Mitosis halves it (4c to 2c)

Accurate Distribution of DNA during cell division:

A challenging task considering the complexity of our genome

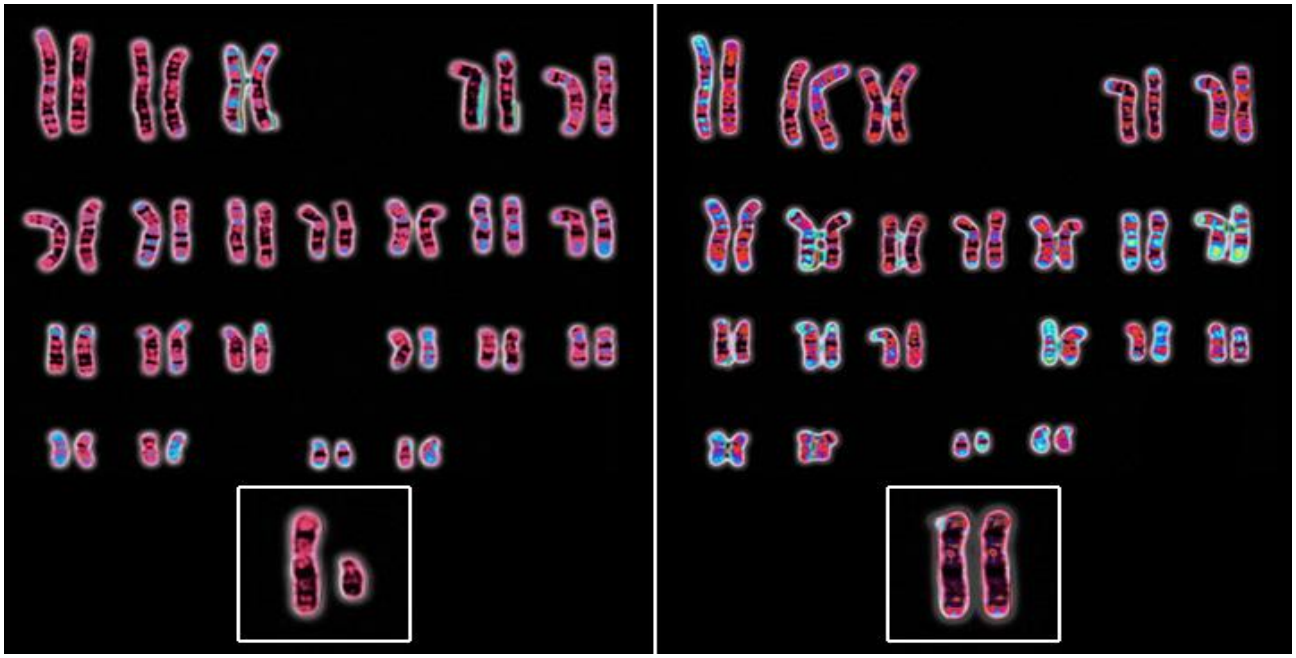
- **Genome**: The complete genetic information (i.e., total DNA content) carried by a cell or organism
- **Human genome** is divided into 23 pairs of chromosomes
- **Bacterial genome** is present in a single circular chromosome





Two copies of Genome (2n) in each body cell

Most of the higher eukaryotes are **diploid (2n)** i.e. their body (somatic) cells contain **two copies of the basic genome set** (two sets of homologous chromosomes)



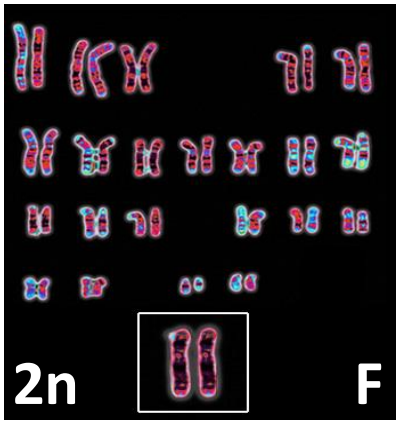
Male **Female**

One copy of Genome (n) in each Sperm/Ovum

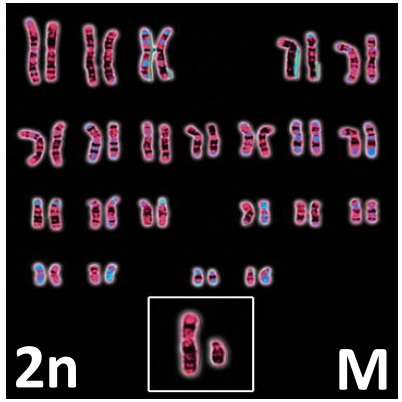
The gametes of most higher eukaryotes are **haploid (n)** i.e. these cells contain **one copy of the basic genome set** (one set of chromosomes)

Somatic Cells (2n) **Somatic Cells (2n)** **Gametes (n)**

Mitosis **Meiosis**

(22 x 2) + XX ⟵  ⟶ **22 + X** **Egg**

Mitosis **Meiosis**

(22 x 2) + XY ⟵  ⟶ **22 + X** **Sperm**

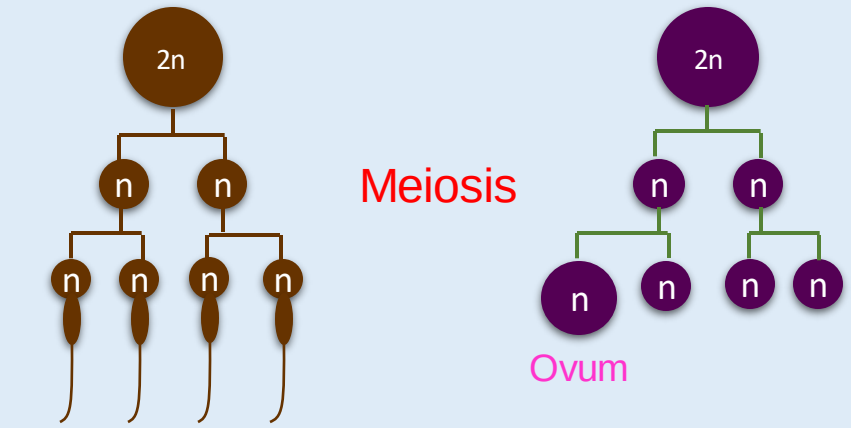
or

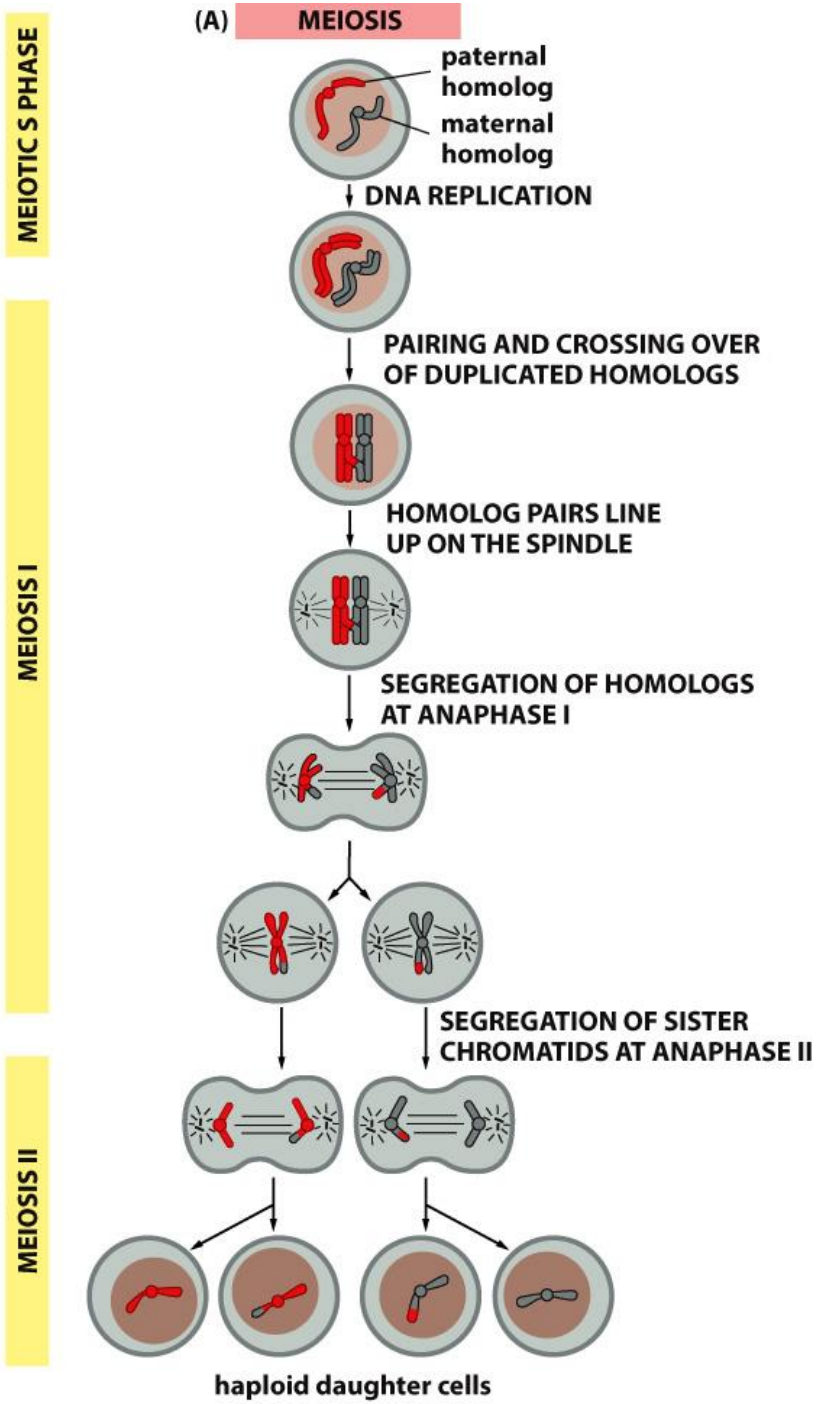
22 + Y **Sperm**

Production of sex cells (gametes):

Meiosis cell division

- Two rounds of division of 1 parent cell produces 4 daughter cells
- Chromosome number becomes half after Meiosis; i.e. diploid (2n) cell divides to generate haploid (n) gametes- reductional division
- Replication doubles the genomic content (2c to 4c), Meiosis reduces it (4c to c)





Mitosis vs Meiosis

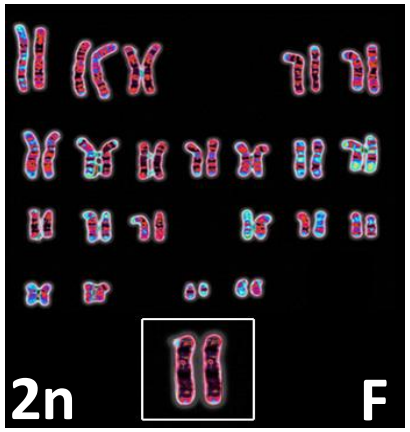
- **Mitosis (*equational division*):** Somatic (body) cells increase in number in this mode
- **Meiosis (reduction division):** Specialized diploid cells (meiocytes) undergo two sequential nuclear divisions to form four haploid gametes (sperms and eggs in plants, animals) or spores (fungi, algae).

	Mitosis		Meiosis	
	Chromosome sets	Genomic content	Chromosome sets	Genomic content
Parent cell	2n	2C	2n	2C
Genome duplication	2n	4C	2n	4C
Progeny cells	2n	2C	n	C
				

Diploid (2n) Genome arises by Fertilization

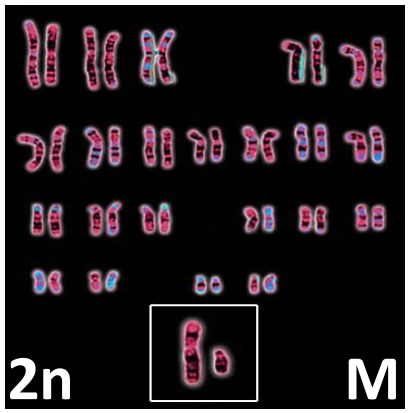
Through **Fertilization** of two haploid gametes, i.e., one genome set (n) from male gamete (i.e. sperm) and another genome set (n) from female gamete (i.e. egg).

Gamete formation (2n → n) | **Fertilization (n + n → 2n)**



Meiosis

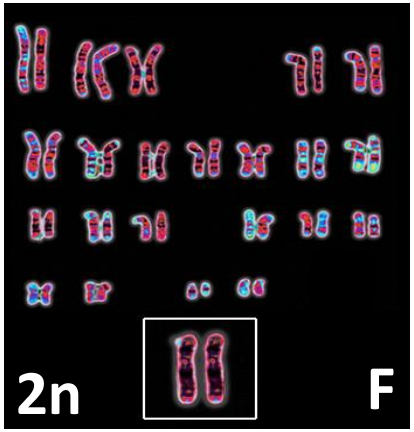
Egg: 22 + X
(n)

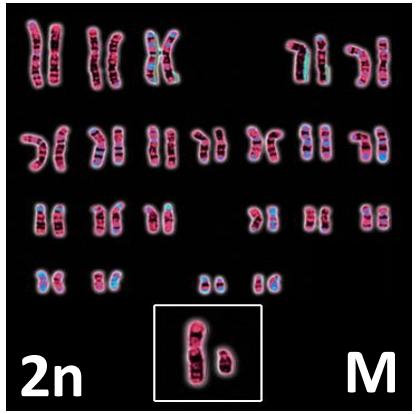


Meiosis

Sperm: 22 + X
(n)

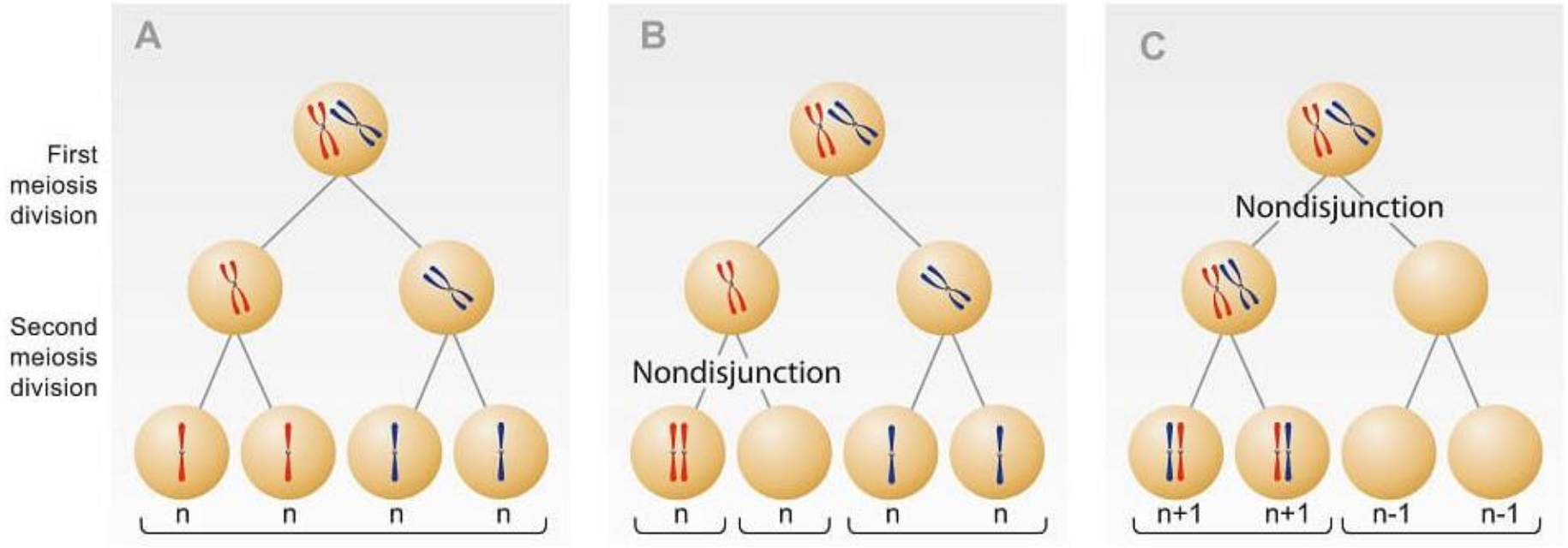
Sperm: 22 + Y
(n)





Nondisjunction

Failure of separation of homologous chromosomes or sister chromatids during meiosis

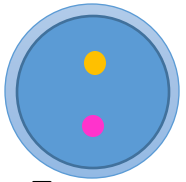


Some diseases caused due to Nondisjunction:

- **Down syndrome (trisomy 21):** It is the most common irregularity of chromosome number in humans. Children with Down syndrome have severe mental illness. Advanced maternal age is the risk factor for DS
- **Turners syndrome (X monosomy: 45, X0)**

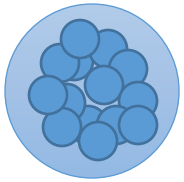
How do so many types of cells arise in our body: Differentiation

- Expression of **tissue-specific genes (TSGs)** dictate differentiation path towards a specific cell identity

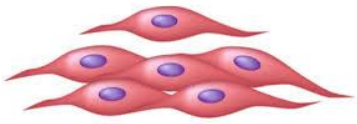


Zygote

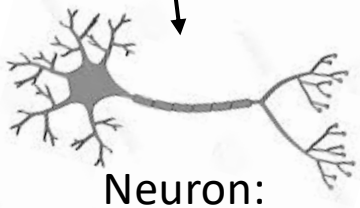
Cell division



Differentiation



Muscle:
- ✓ Myoglobin
- ✗ Neurotransmitter
- ✗ Hemoglobin



Neuron:
- ✗ Myoglobin
- ✓ Neurotransmitter
- ✗ Hemoglobin

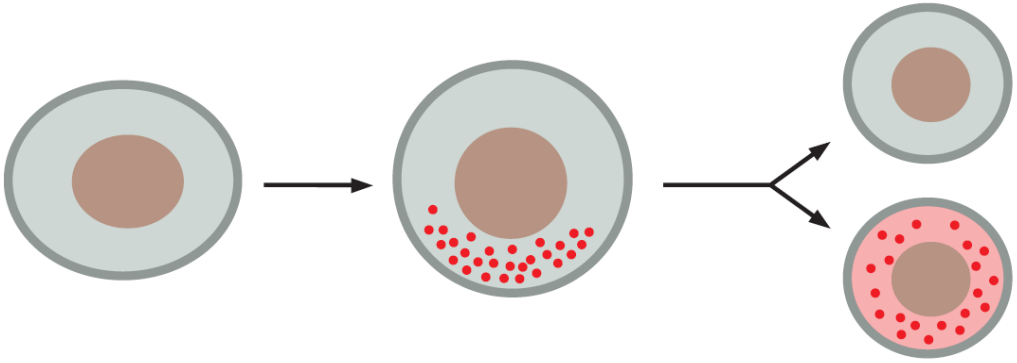
RBC:
- ✗ Myoglobin
- ✗ Neurotransmitter
- ✓ Hemoglobin

- In contrast, **House keeping genes** (coding for RNA Polymerase, Ribosomes, Cytoskeletal proteins etc.) are expressed in all cells

How do different genes express in different cells?

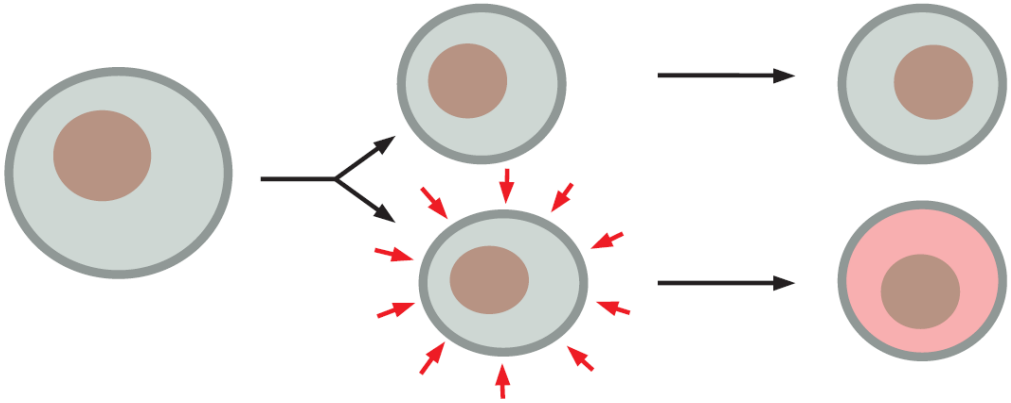
Differentiation:

Two ways of making sister cells different



1. asymmetric division: sister cells born different

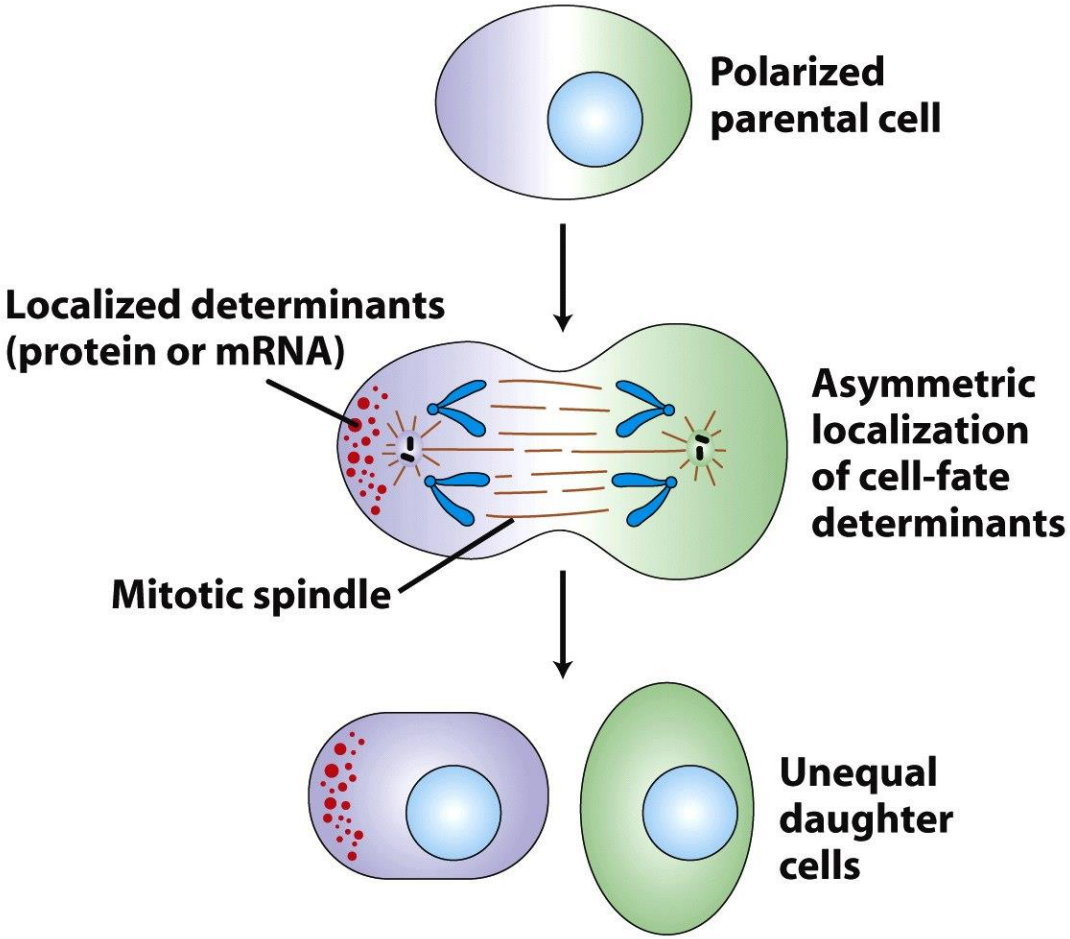
1. **Asymmetric Cell division:** Some proteins and/RNA gets asymmetrically distributed in dividing cell; after division, they distribute unevenly in the to daughter cells



2. symmetric division: sister cells become different as a result of influences acting on them after their birth

2. **Extrinsic Signal:** Neighbouring cells or secreted signalling molecules act on one of the two post-mitotic daughter cells to assign a specific identity

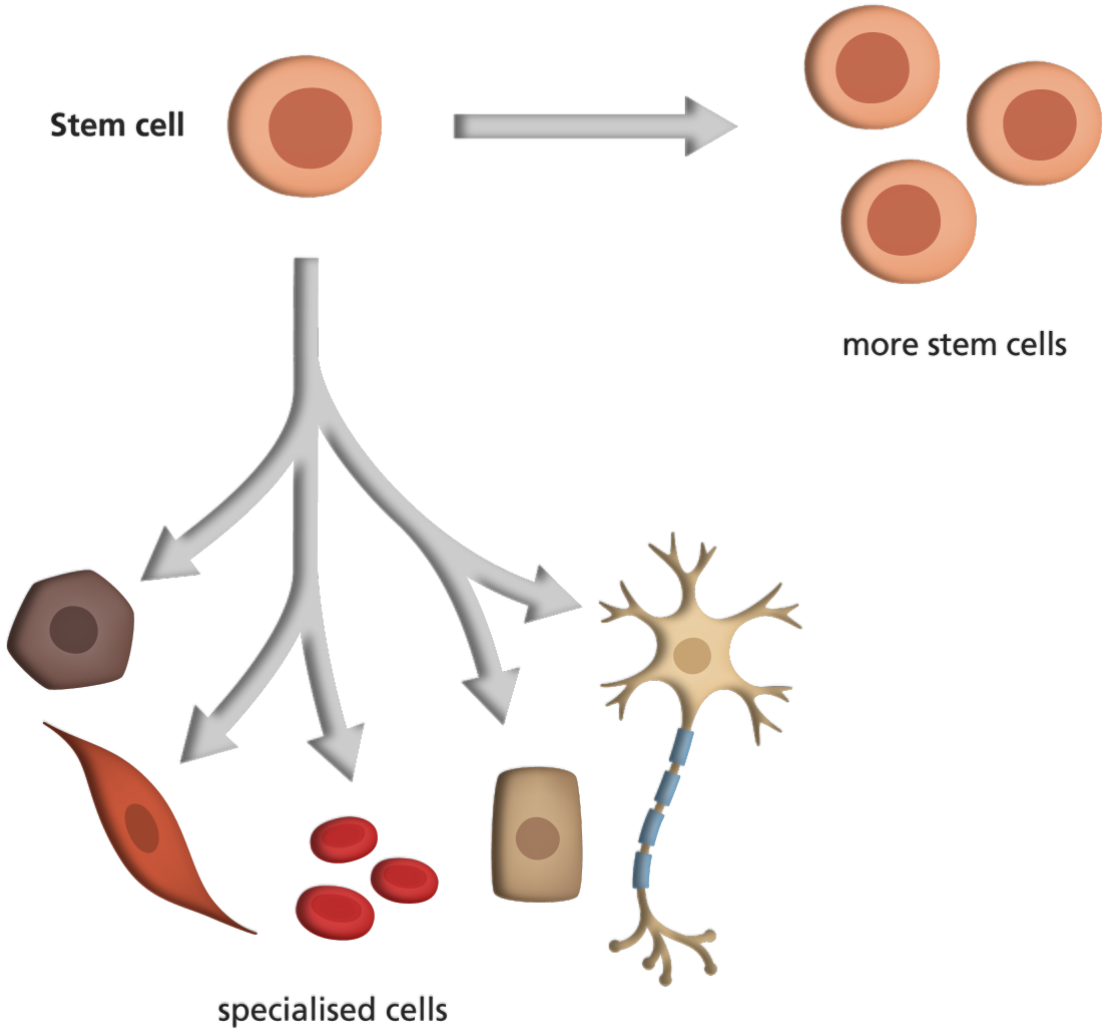
Asymmetric division leads to different cell fates



- Essential to asymmetric cell division is *polarization* of the parental cell and then differential incorporation of parts of the parental cell into the two daughters
- Some cytoplasmic components (such as mRNA or proteins) are localized in some part of the cell
- The unequal distribution of these components to the daughter cells typically result in transcription of different sets of genes
- The resulting proteins determine the cell-fate

What is a stem cell?

- **Stem cells are undifferentiated cells in multicellular organisms, which can proliferate indefinitely and generate multiple cell types**



- Stem cells are present in embryos as well as in adults
- Adult stem cells are found in:
  - Bone marrow
  - Brain
  - Gonads
  - Gut
  - Eye
  - Skin

Important properties of stem cells

1. Totipotency: Ability to give rise a new organism

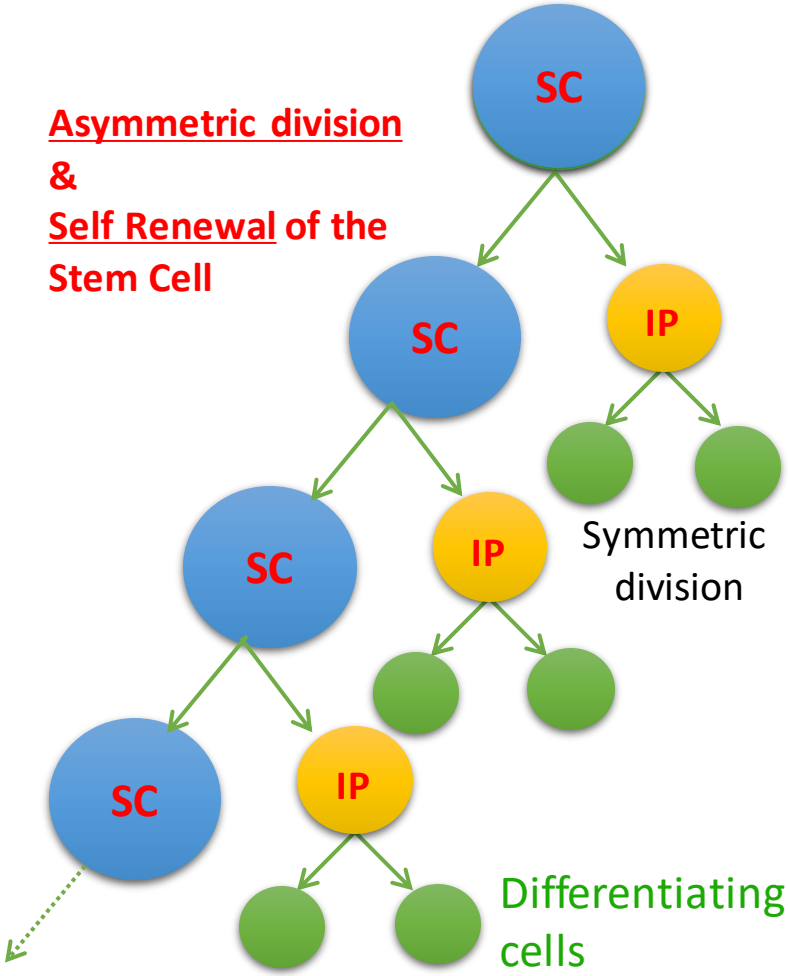
2. Pluripotency/multipotency: Ability to give rise to any/many cell types of our body

3. Self-renewal: Ability to reproduce/renew themselves repeatedly

4. Asymmetric cell division: Ability to divide asymmetrically to form one daughter stem cell identical to itself and one daughter cell that is different and usually of more restricted potential

- In this way, mitotic division of stem cells preserves a population of undifferentiated cells while steadily producing a stream of differentiating cells.

Asymmetric cell division is essential to generate different cell types in multicellular organisms



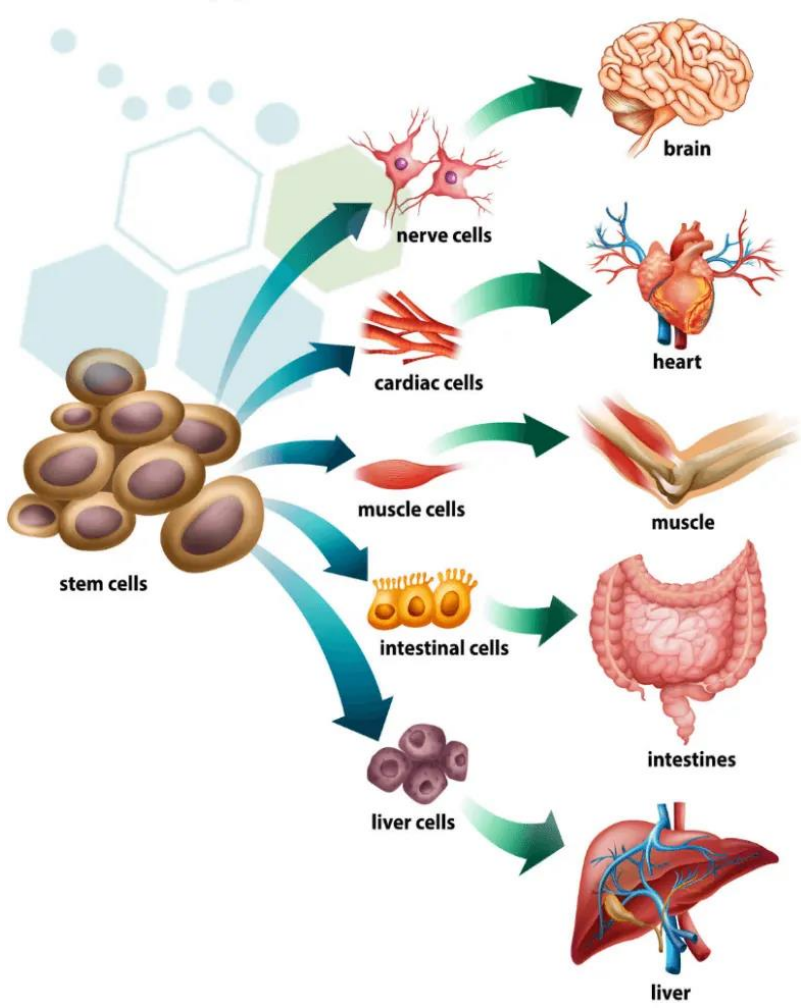
- In multicellular organisms, **stem cells** can give rise to two different cells, one that resembles the parent cell and one that does not. Such **asymmetric cell division** generates all different cell types in the body
- Daughter cells produced by such asymmetric cell division may differ in size, shape, composition of protein/RNA and most crucially in gene expression which confers different fates on the two cells
- In **symmetric cell division**, the parental cell gives rise to two daughter cells that resemble each other, at least visually

SC= stem cell
IP= intermediate progenitor

NOTE: GENOMIC CONTENT IS SYMMETRICALLY SEGREGATED EVEN IN ASYMMETRIC DIVISION

Potential Therapeutic Application of Stem Cells

STEM CELL



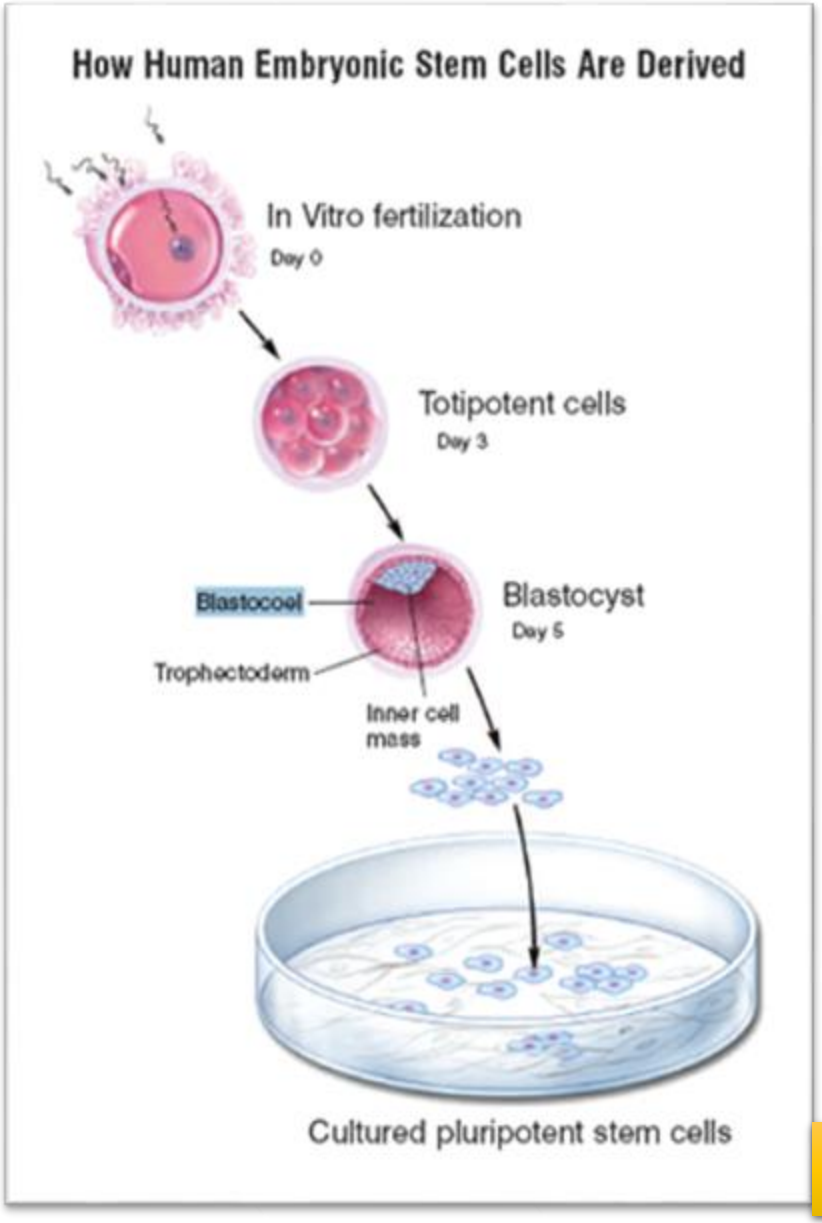
ANY TISSUE/ORGAN

TRANSPLANT INTO PATIENTS

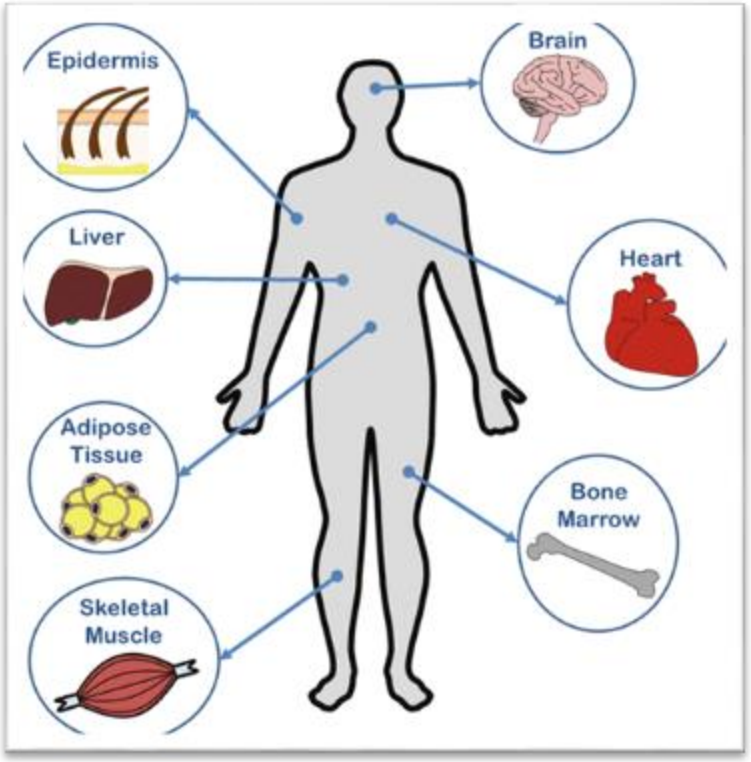
- For this purpose we need to obtain stem cells in large number.
  - Where do we find stem cells?

Sources of Stem Cells

1. Embryonic Stem (ES) Cells:



2. Adult Stem Cells:



Problem

Its extremely challenging to selectively isolate stem cells from other cells

Solution

Induced Pluripotent Stem Cells (iPSCs)

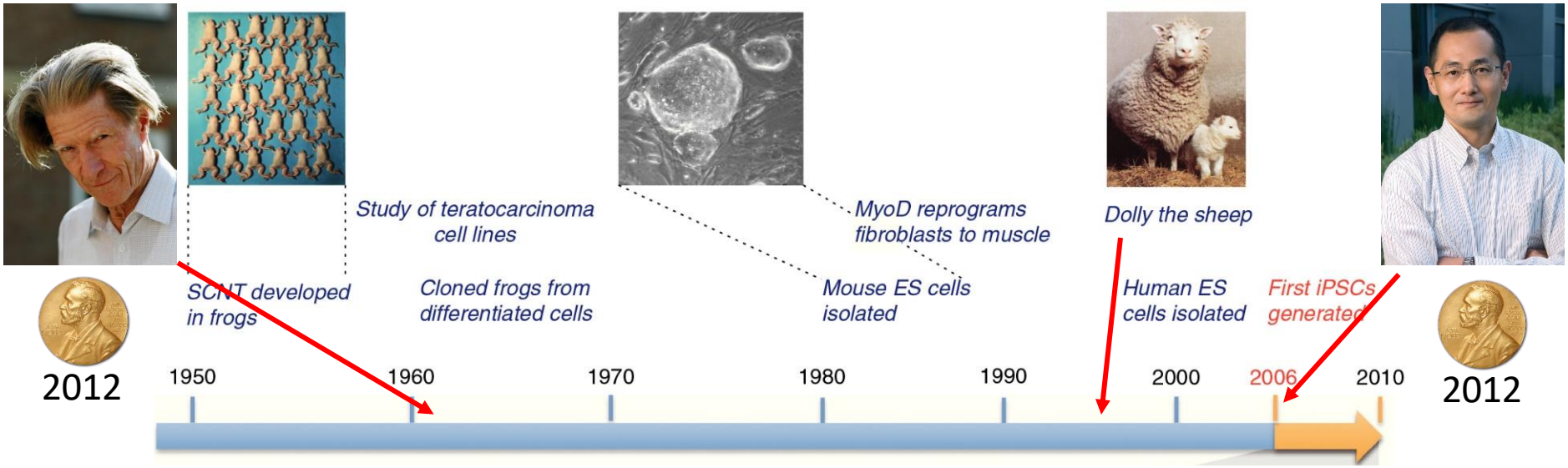
Induced pluripotency:

Pluripotency can be artificially Induced in a differentiated cell

- **Reprogramming:** Increase in potency and dedifferentiation of a somatic (differentiated) cells into **induced Pluripotent Stem Cells (iPSCs)**

- Reprogramming can be induced by:
  - Nuclear transfer
  - Cell fusion
  - Genetic manipulation
  - Overexpression of a small set of transcription factors

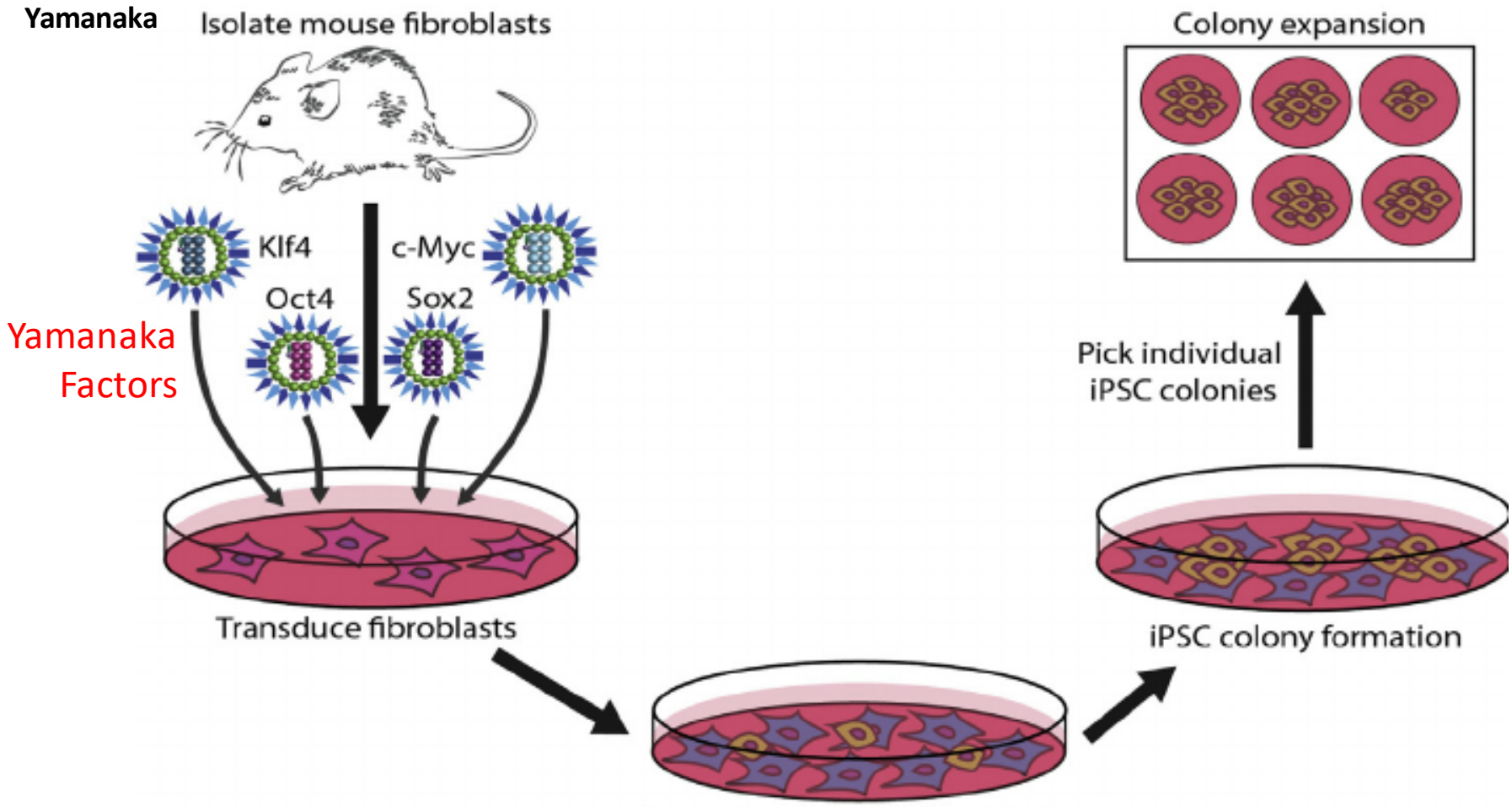
Major discoveries in reprogramming research



Shinya Yamanaka

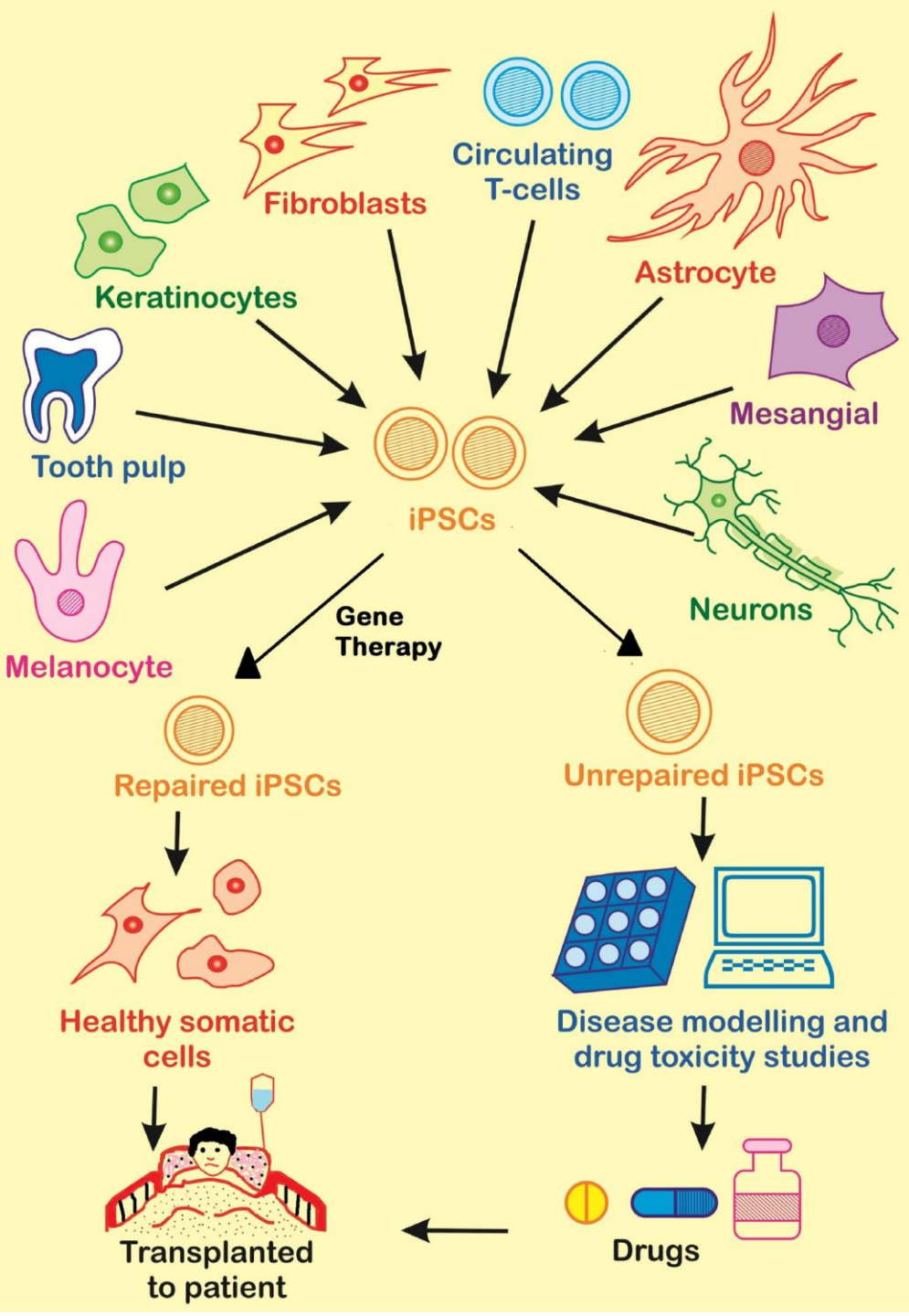
Derivation of first iPSC:

Shinya Yamanaka and Kazutoshi Takahashi (2005)

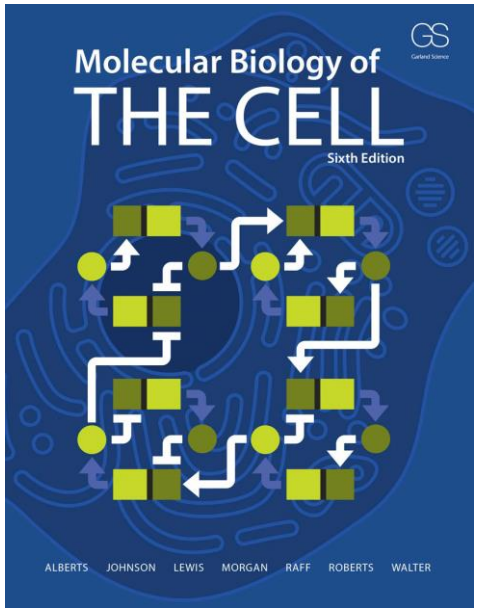


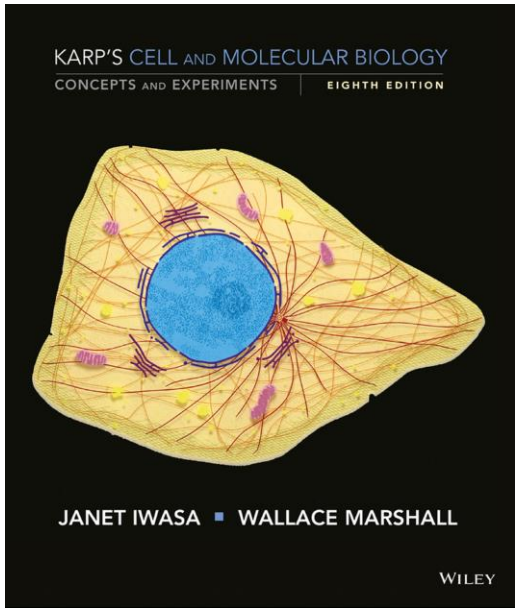
Biomedical applications of iPSCs

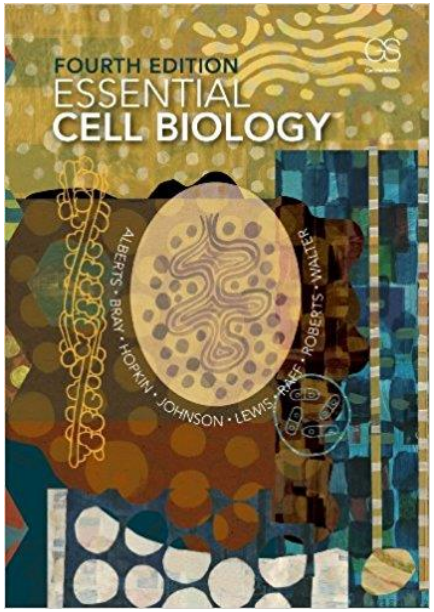
- **Regenerative medicine:** To restore or replace damaged tissue
  - For example, neuronal cells that generated from iPSCs can be used to treat Parkinson's disease patients who have lost neurons
- **Disease modelling:** iPSCs generated from patients can be used to understand the disease pathology
- **Drug discovery:** iPSCs from patients can be cultured in lab to screen for drugs that can repair the defects



Books and resources







<u>Video links</u>:

https://www.youtube.com/watch?v=URUJD5NEXC8

https://www.youtube.com/watch?v=B_zD3NxSsD8