

BS20001

Spring 2022

# Science of Living Systems

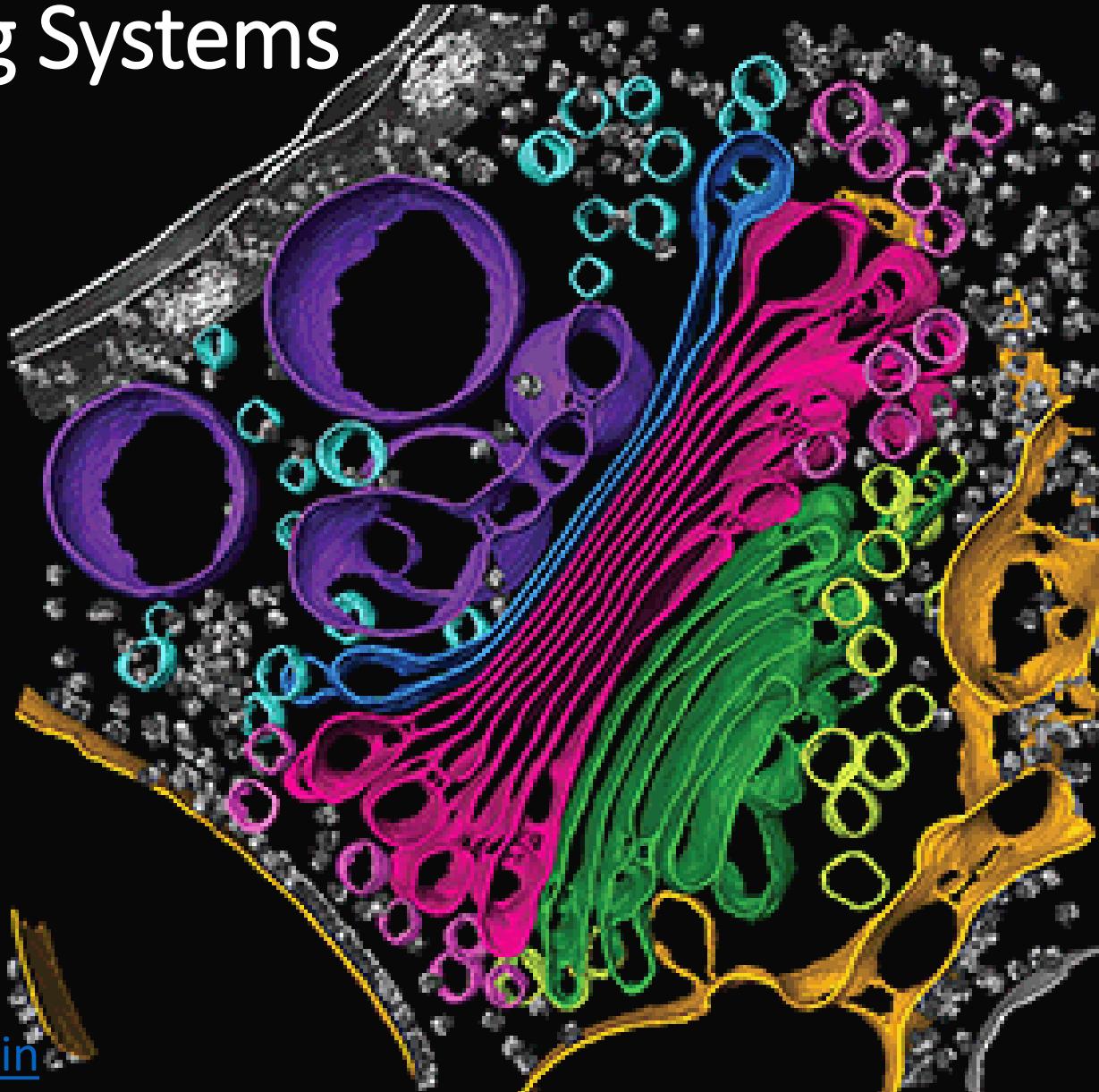
Cell and  
Developmental  
Biology

**Abhijit Das**

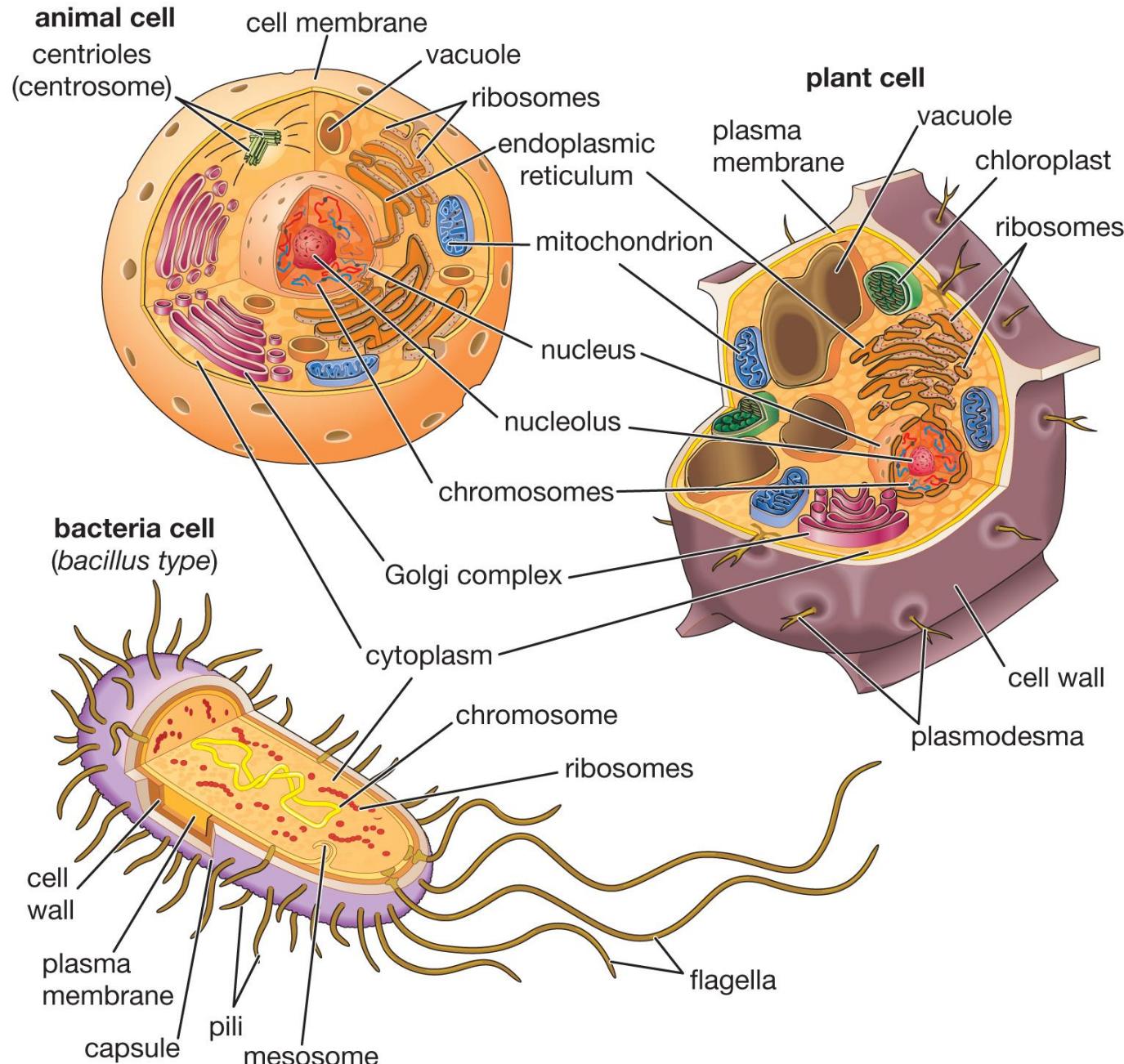
School of Bio Science

Email: [abhijit.das@iitkgp.ac.in](mailto:abhijit.das@iitkgp.ac.in)

Tel: 03222-284572

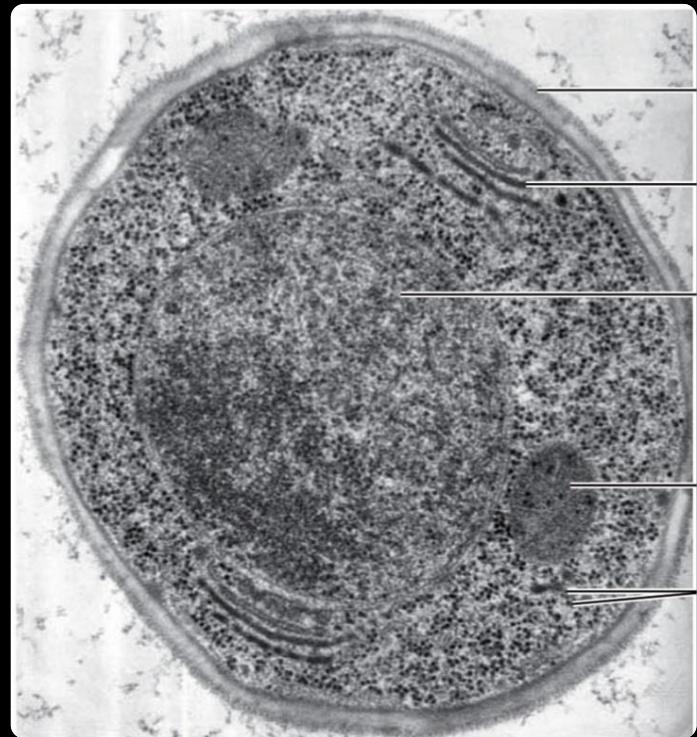
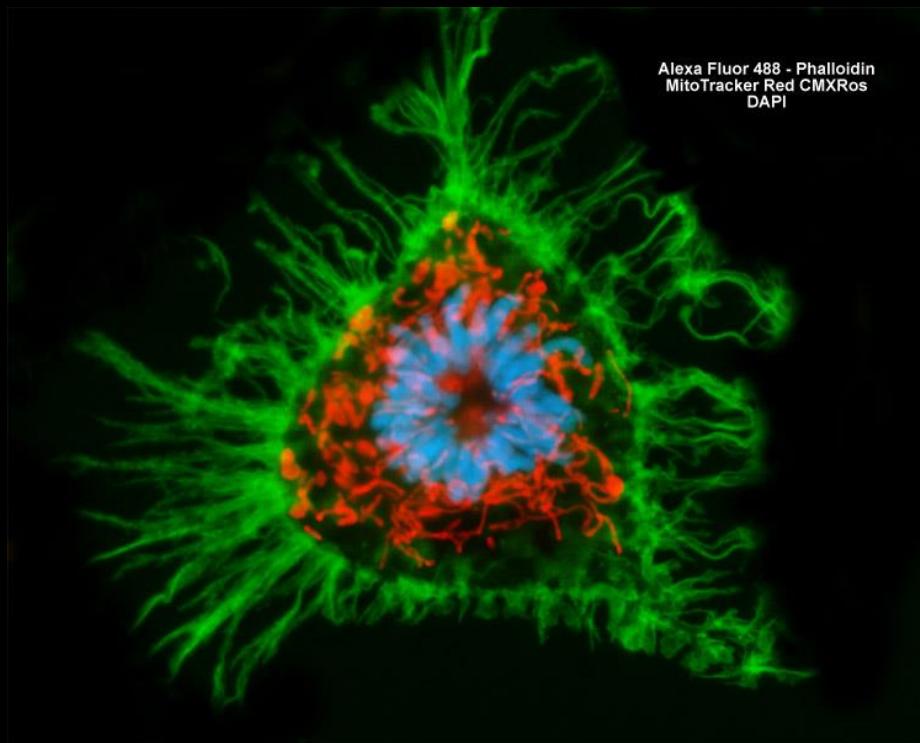


## Some typical cells



# How to study Cells?

## Cell visualization by Microscopy techniques

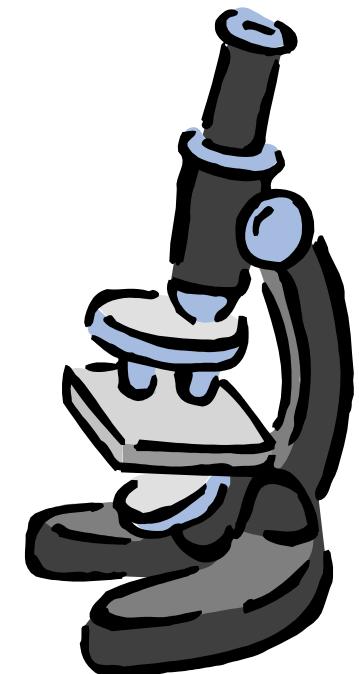


# Microscopy techniques to study cell biology

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



# Magnification

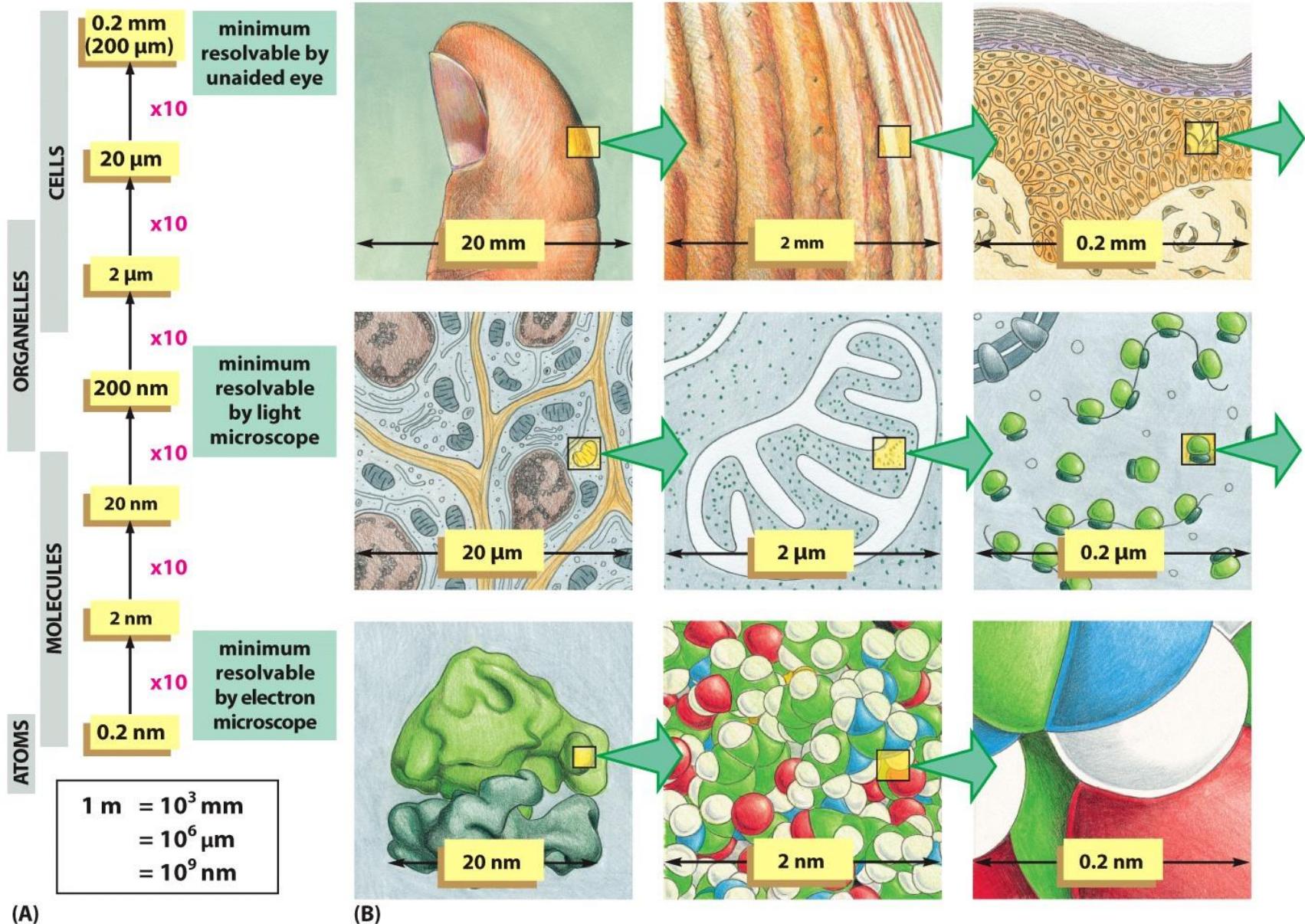
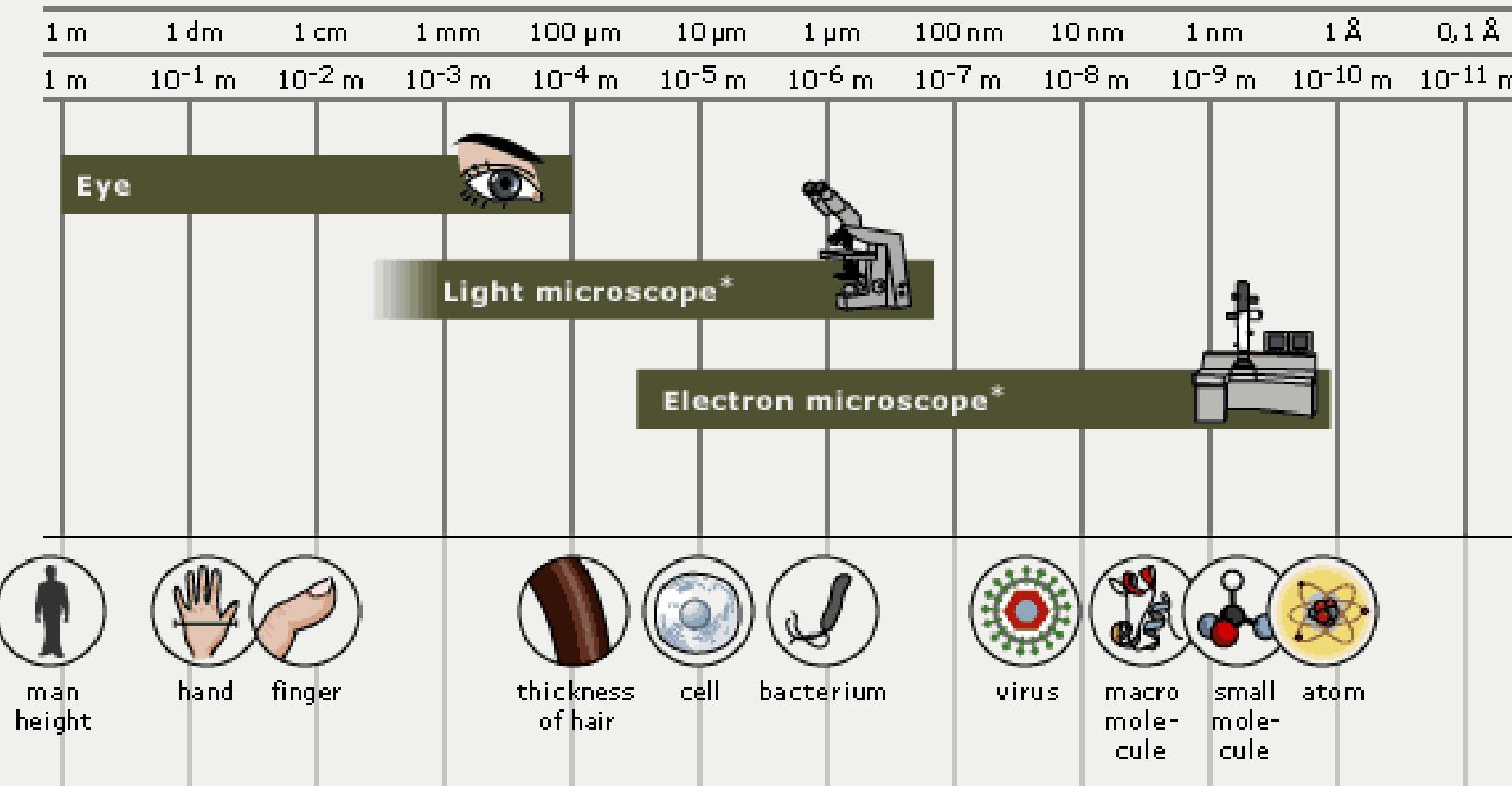


Figure 1-8 Essential Cell Biology, 4th ed. (© Garland Science 2014)

# Resolving Power Line

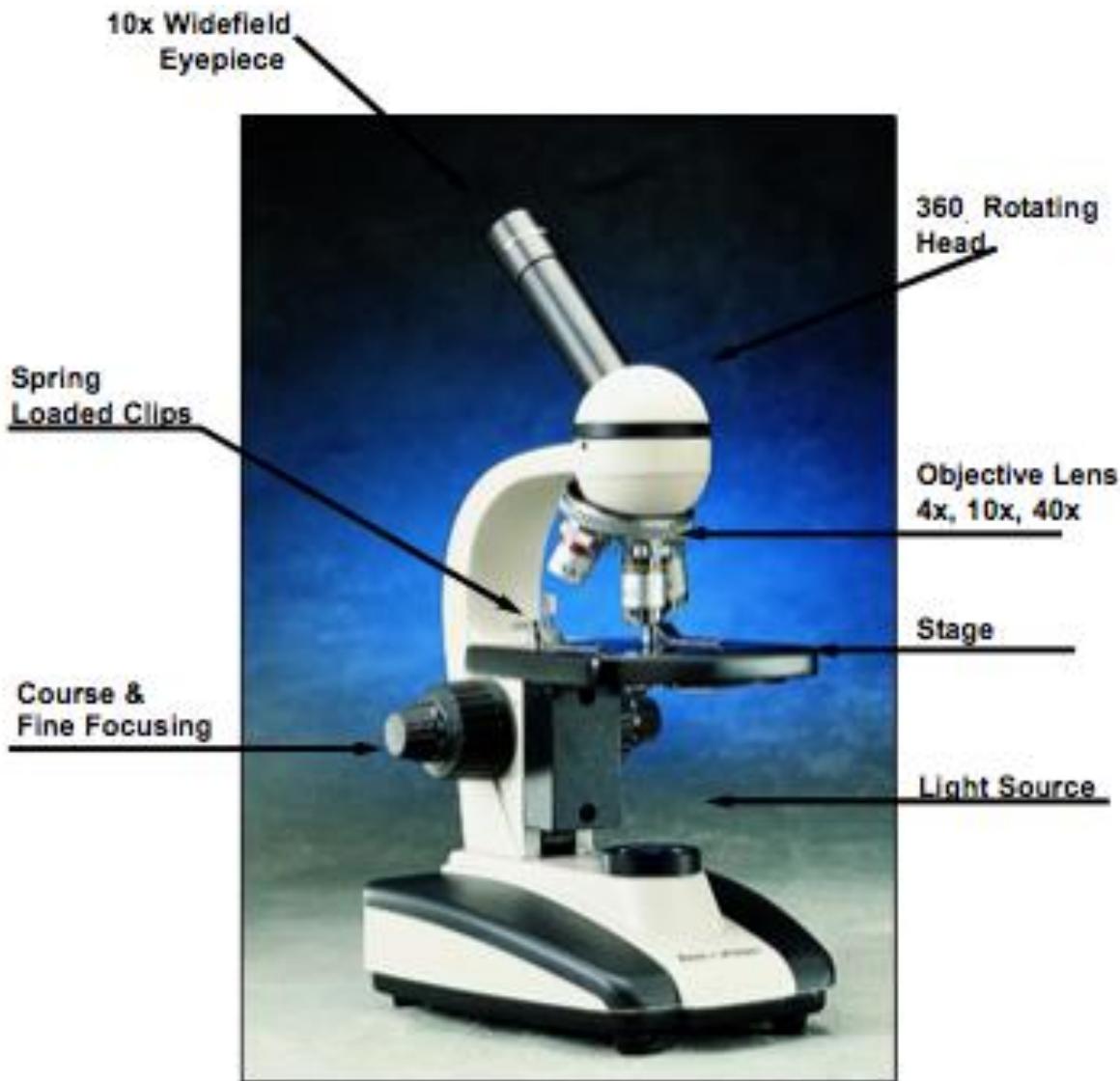
What can you see with the different types of microscopes? The human eye is capable of distinguishing objects down to a fraction of a millimeter. With the use of light and electron microscopes it is possible to see down to an angstrom and study everything from different cells and bacteria to single molecules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



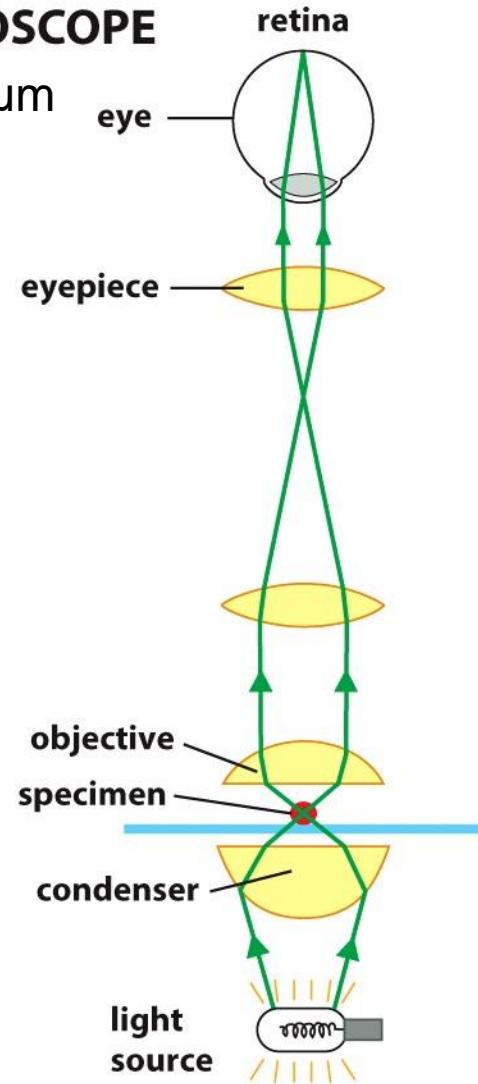
\* Light microscope Includes phase contrast and fluorescence microscopes. Electron microscope Includes transmission electron microscope.

# THE LIGHT MICROSCOPE

Resolution Up to  $0.2 \mu\text{m}$



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

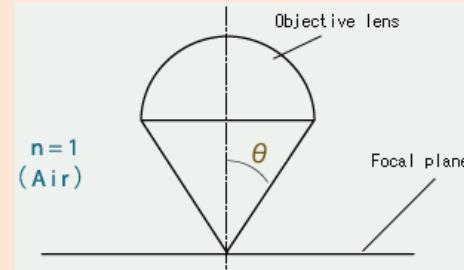


**the light path in a  
light microscope**

# Resolution: Numerical aperture and airy disc

**Numerical aperture (NA)** is the amount of light coming from the focus that the objective can collect:

$$NA = n \sin(\theta) \quad (\text{where } n = \text{refractive index of the Lens Immersion Medium} \text{ and } \theta = \text{half-aperture angle})$$



**Airy disc** is the central bright circular region of the diffraction pattern of light passing through a small circular aperture

Size of Airy disc depends on the wavelength of the light and size of the aperture

Radius  $r$  of the Airy disc ( $r$ ) = the distance between the central maxima and the first minima

$$r = 0.61 \frac{\lambda}{NA} = 0.61 \frac{\lambda}{n \sin(\theta)}$$

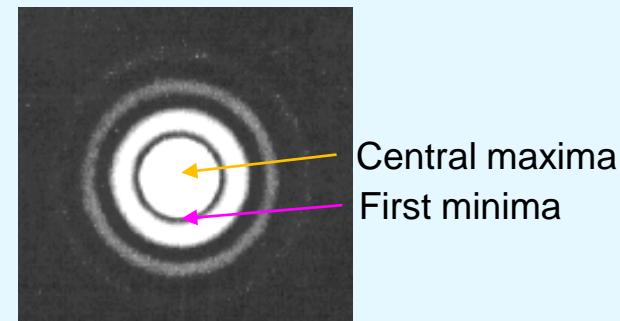
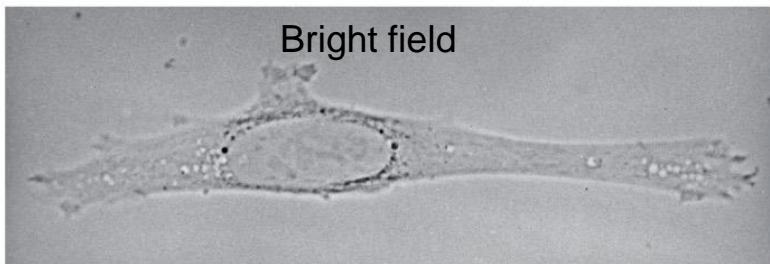


Image source: UCLA Brain Research Institute

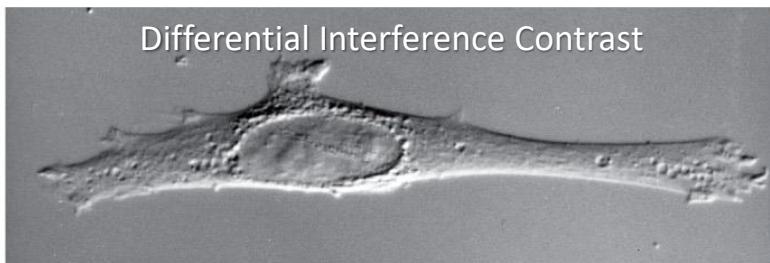
**$r$  is the resolution of a lens or microscope**

# Contrast in Microscopy

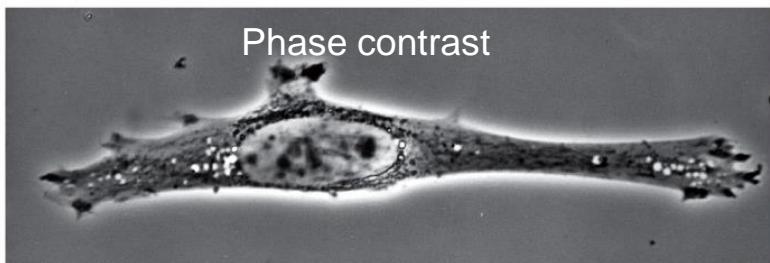
## Microscope Contrast



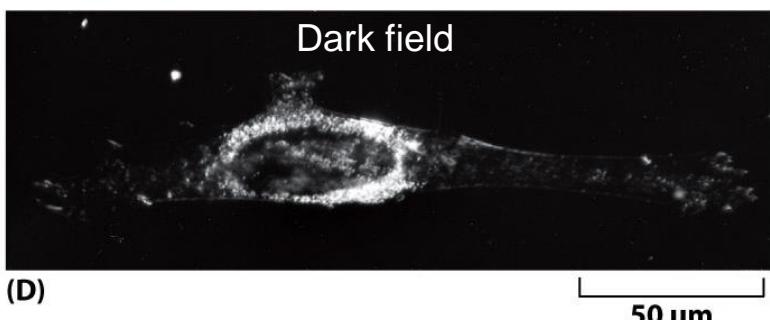
(A)



(C)



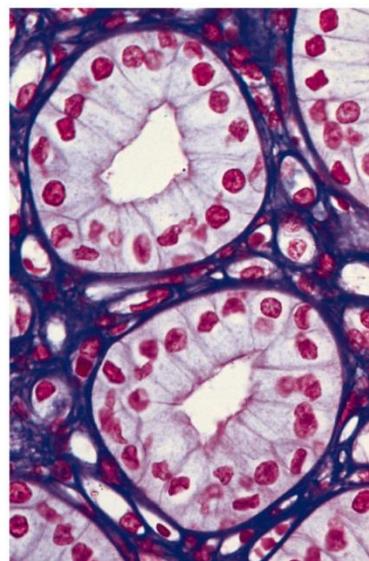
(B)



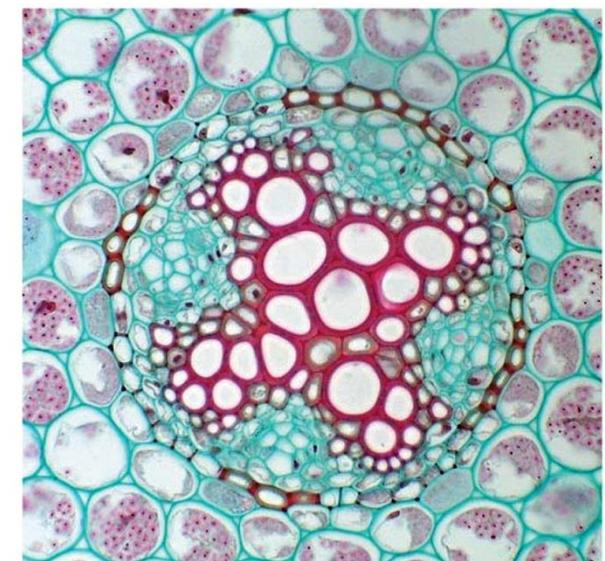
(D)

## Specimen Contrast

By selective and differential staining of cellular components



(A)



(B)

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

# **Electron Microscopy**

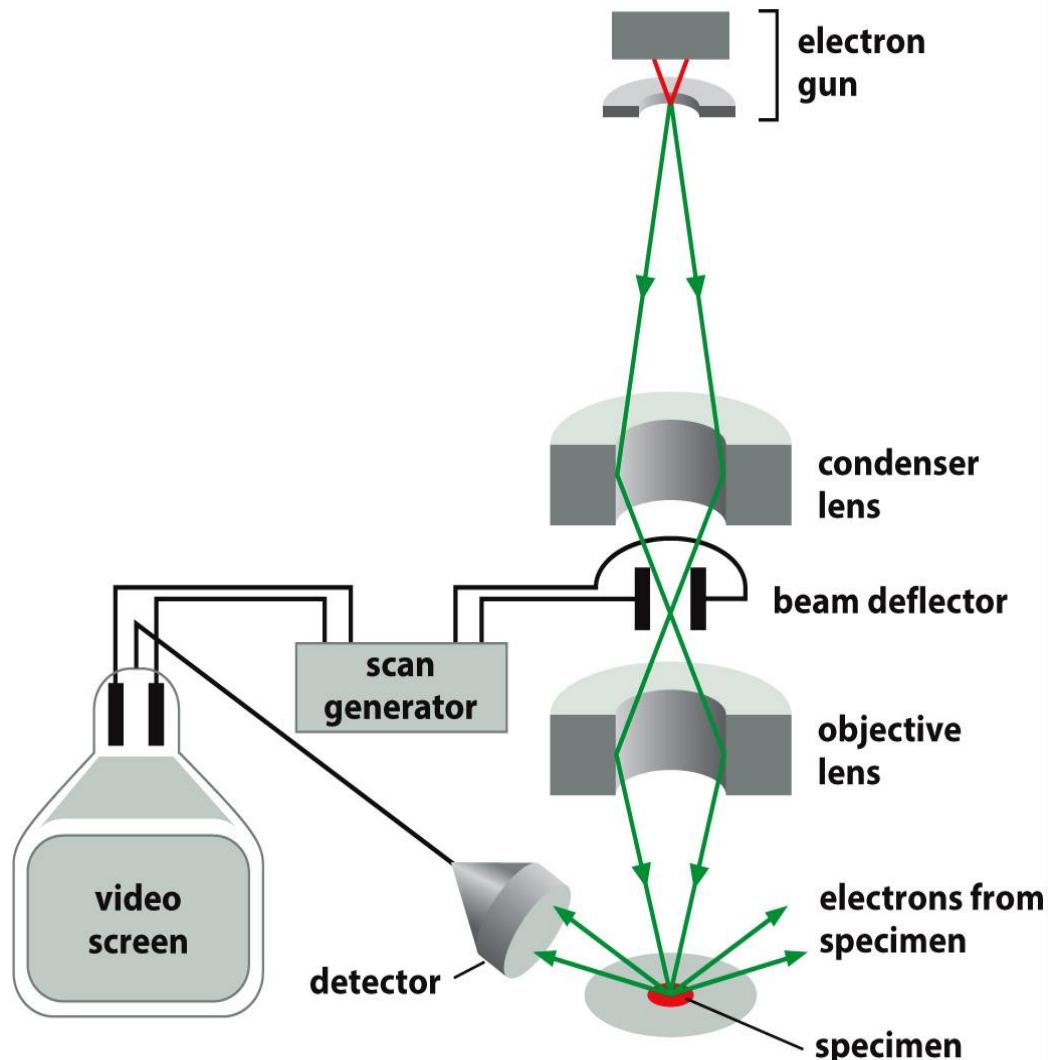
Subcellular structures are studied by electron microscopes.

They are of two types:

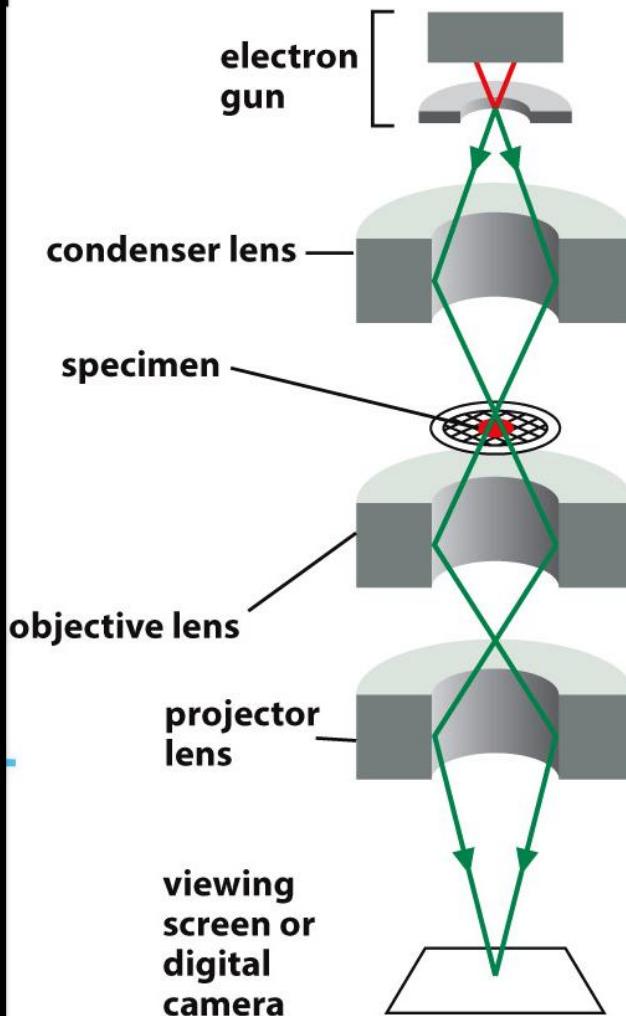
- **Scanning Electron Microscopes (SEM)** focus a beam of electrons onto the surface of the sample and provide images that give 3D representation of the sample. SEM is used to study surface structure of objects
- **Transmission Electron Microscopes (TEM)** focus a beam of electrons through the sample.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

# Electron Microscopy: SEM vs TEM

Scanning Electron Microscope (SEM)

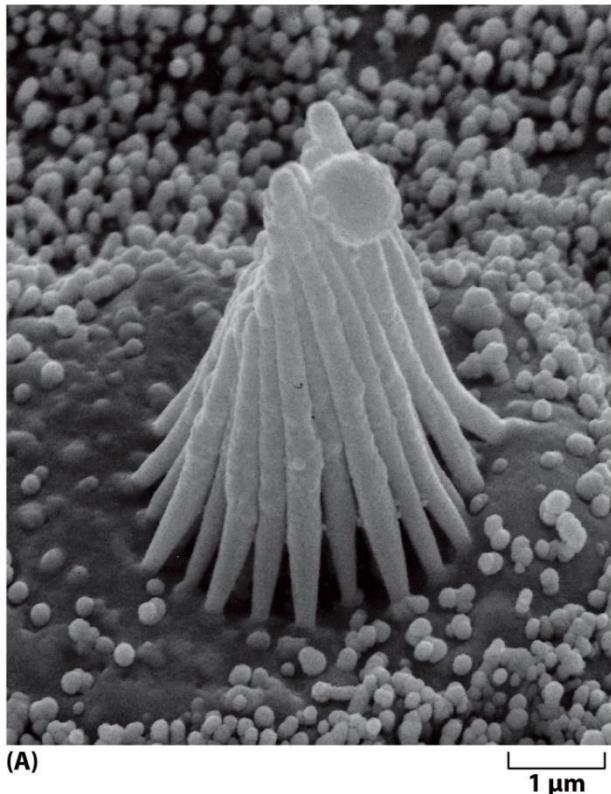


Transmission Electron Microscope (TEM)



# Electron Microscopy: SEM vs TEM

Scanning Electron Microscope (SEM)



Surface features

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

Transmission Electron Microscope (TEM)

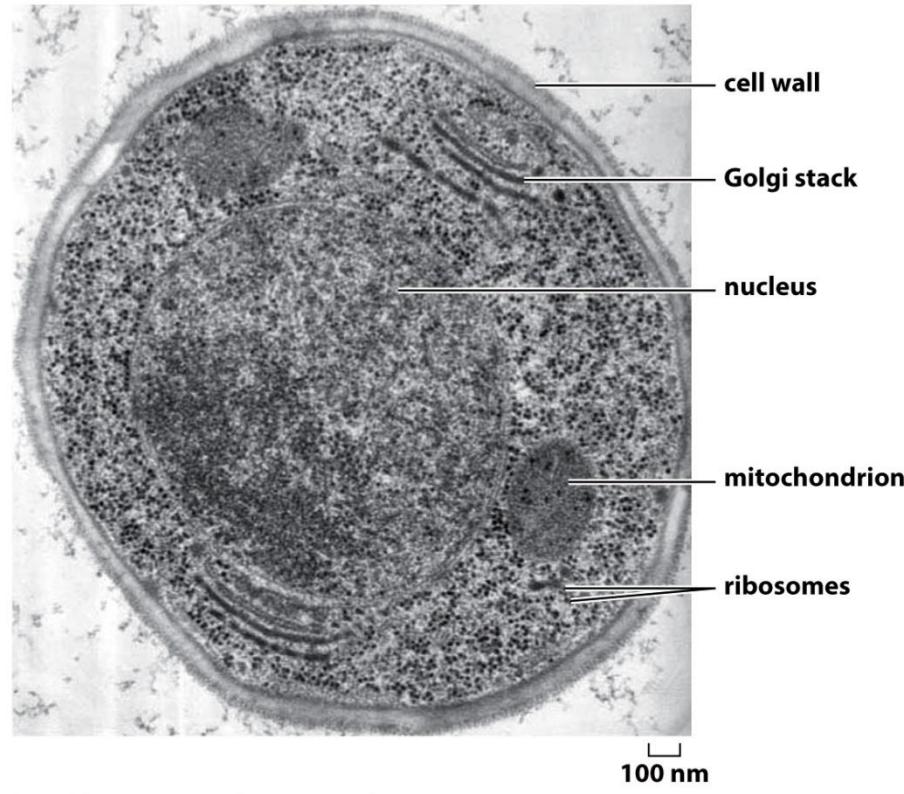


Figure 9-44 Molecular Biology of the Cell 6e (© Garland Science 2015)

Intracellular ultrastructure

# Fluorescence Microscope

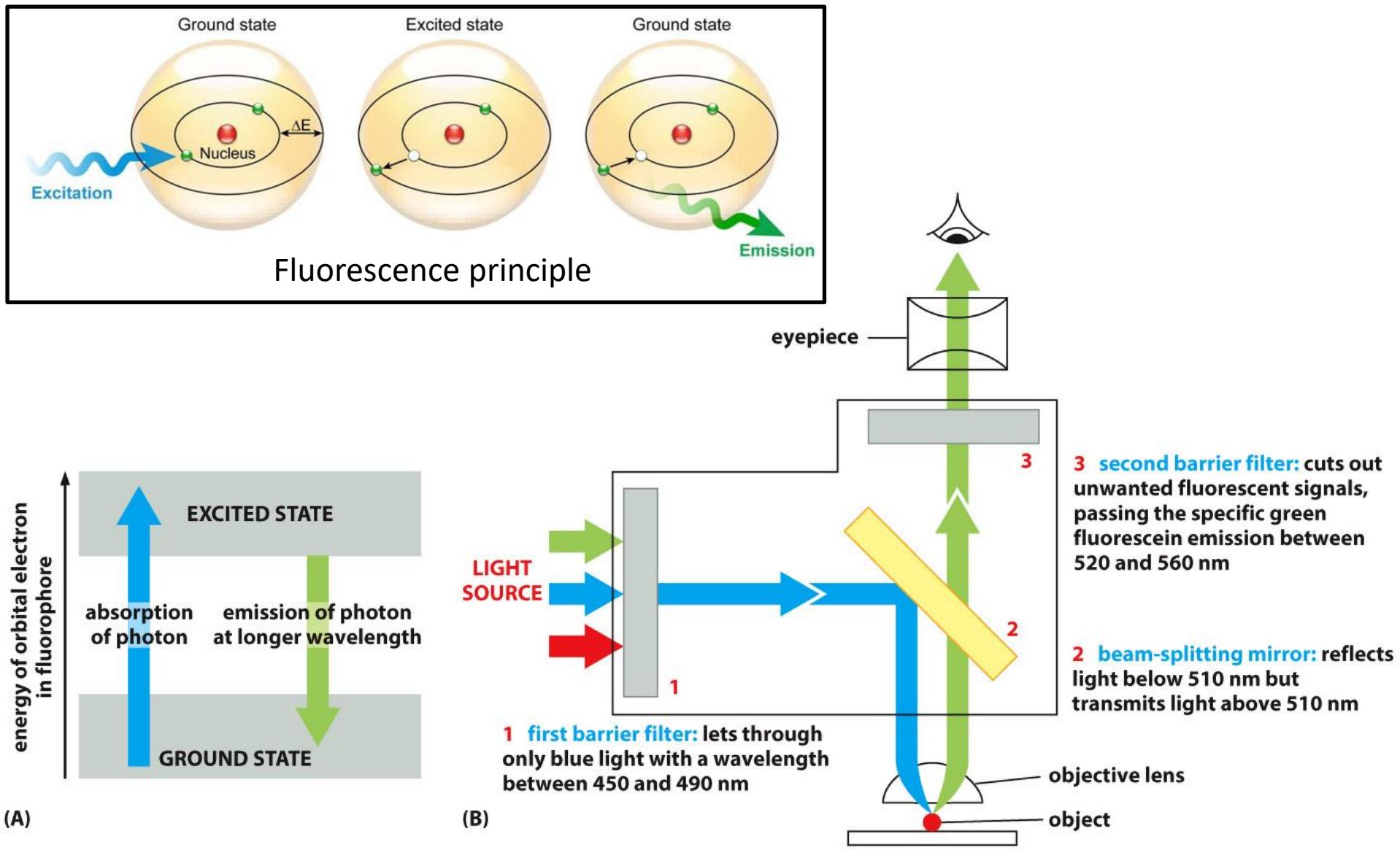
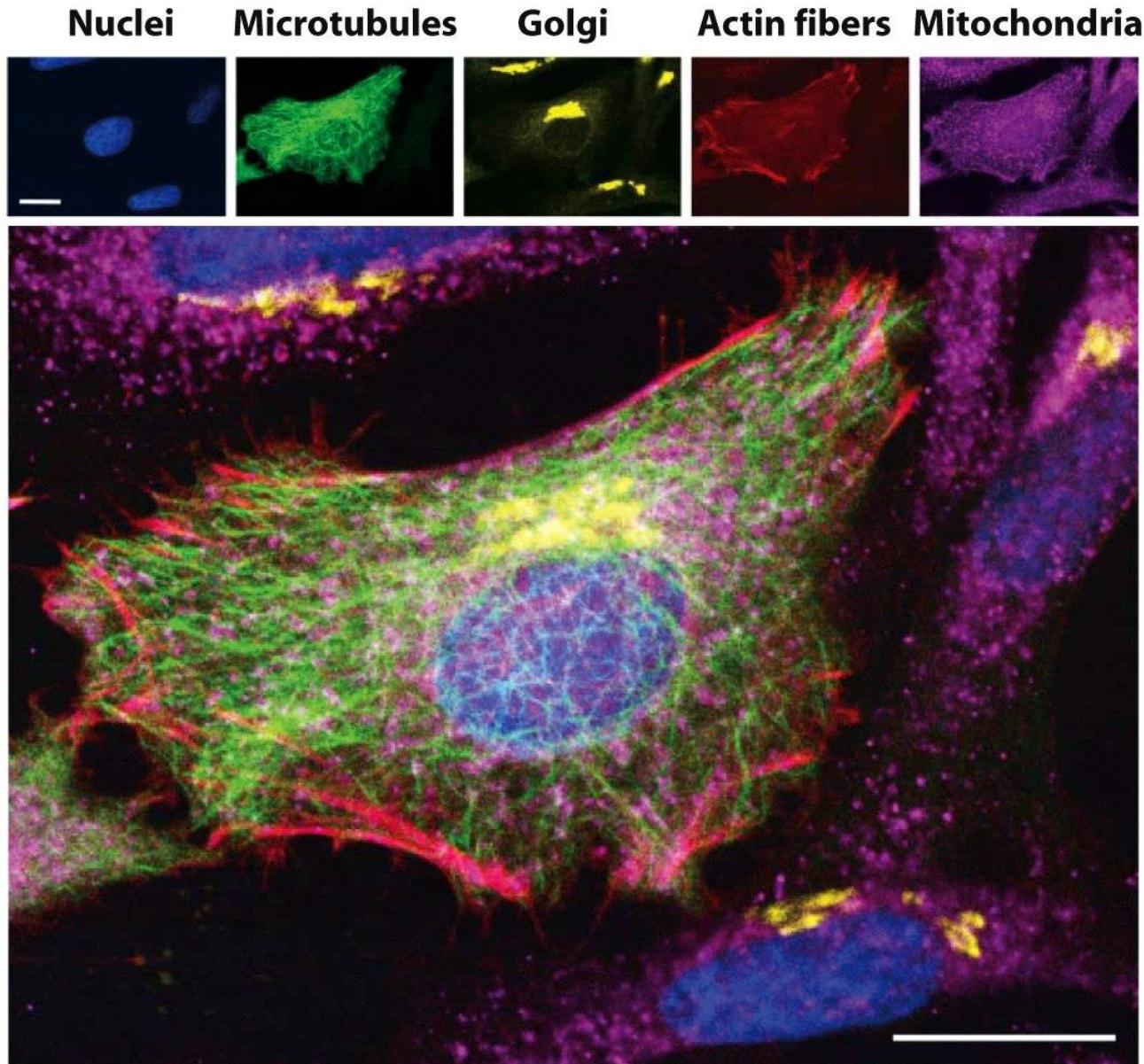
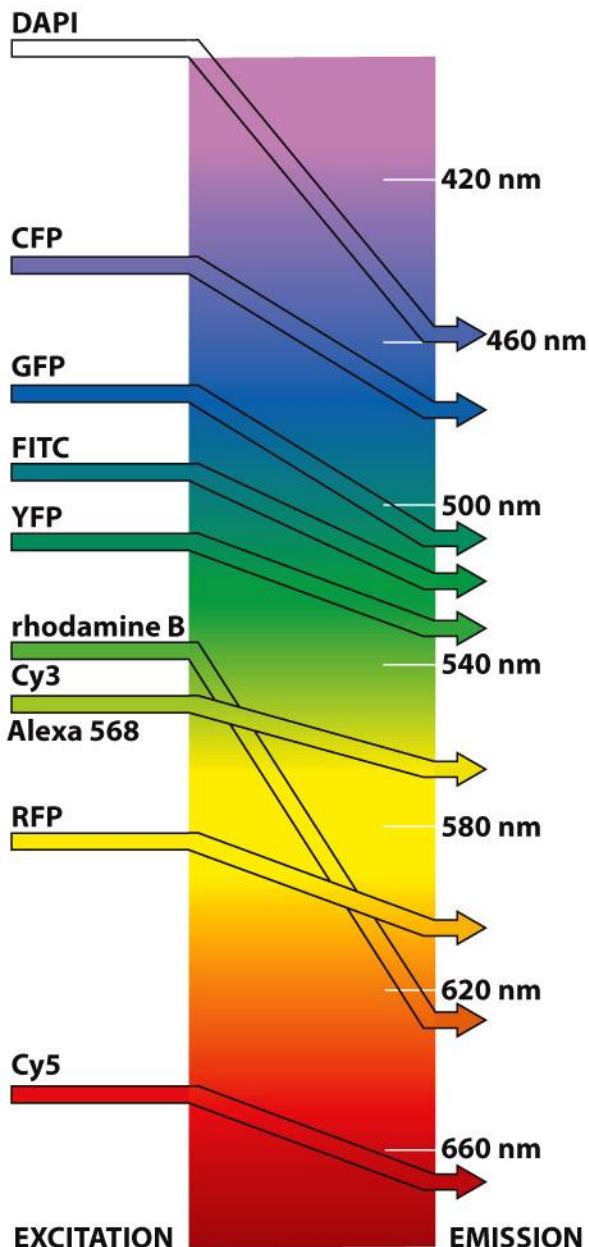


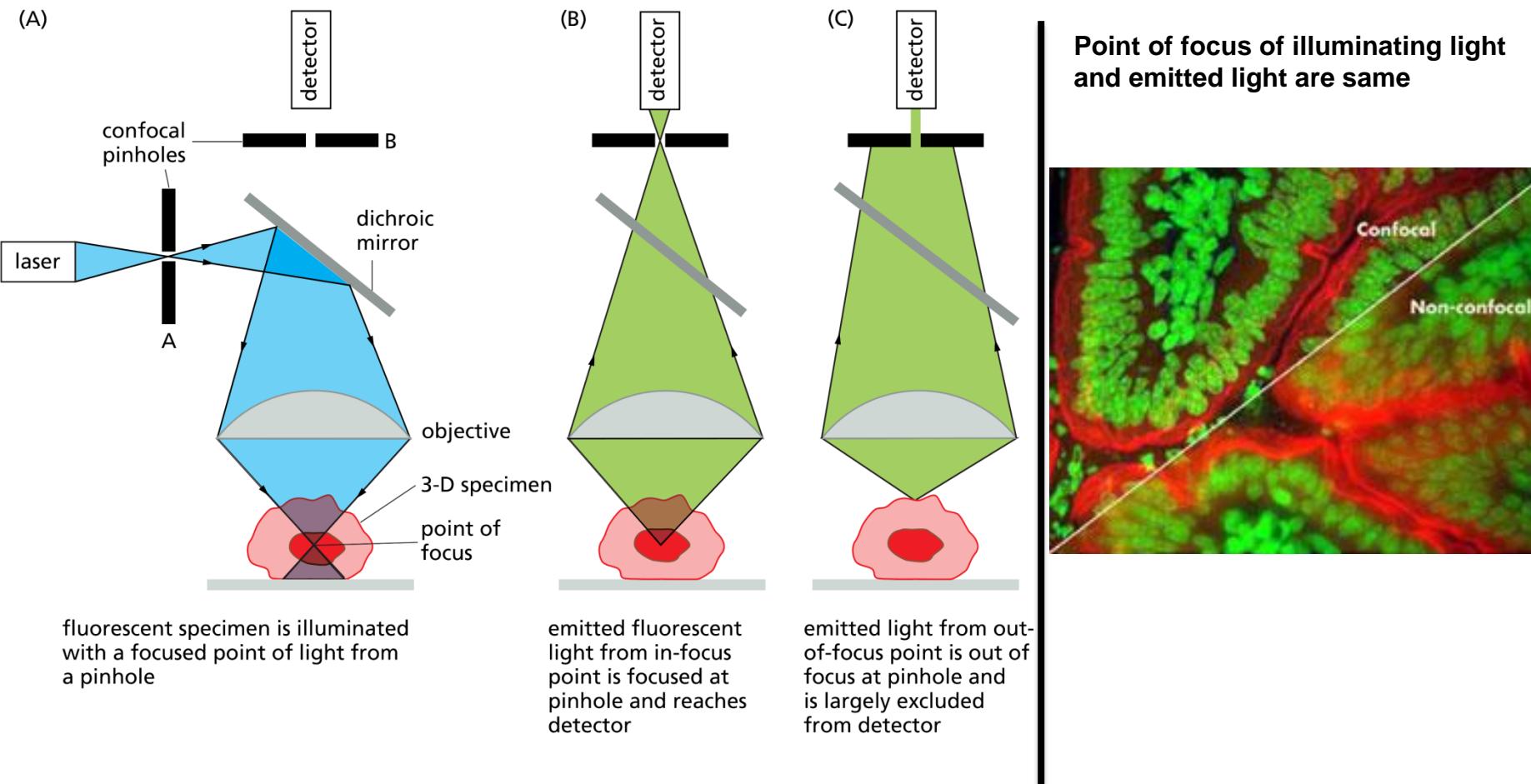
Figure 9-12 Molecular Biology of the Cell 6e (© Garland Science 2015)



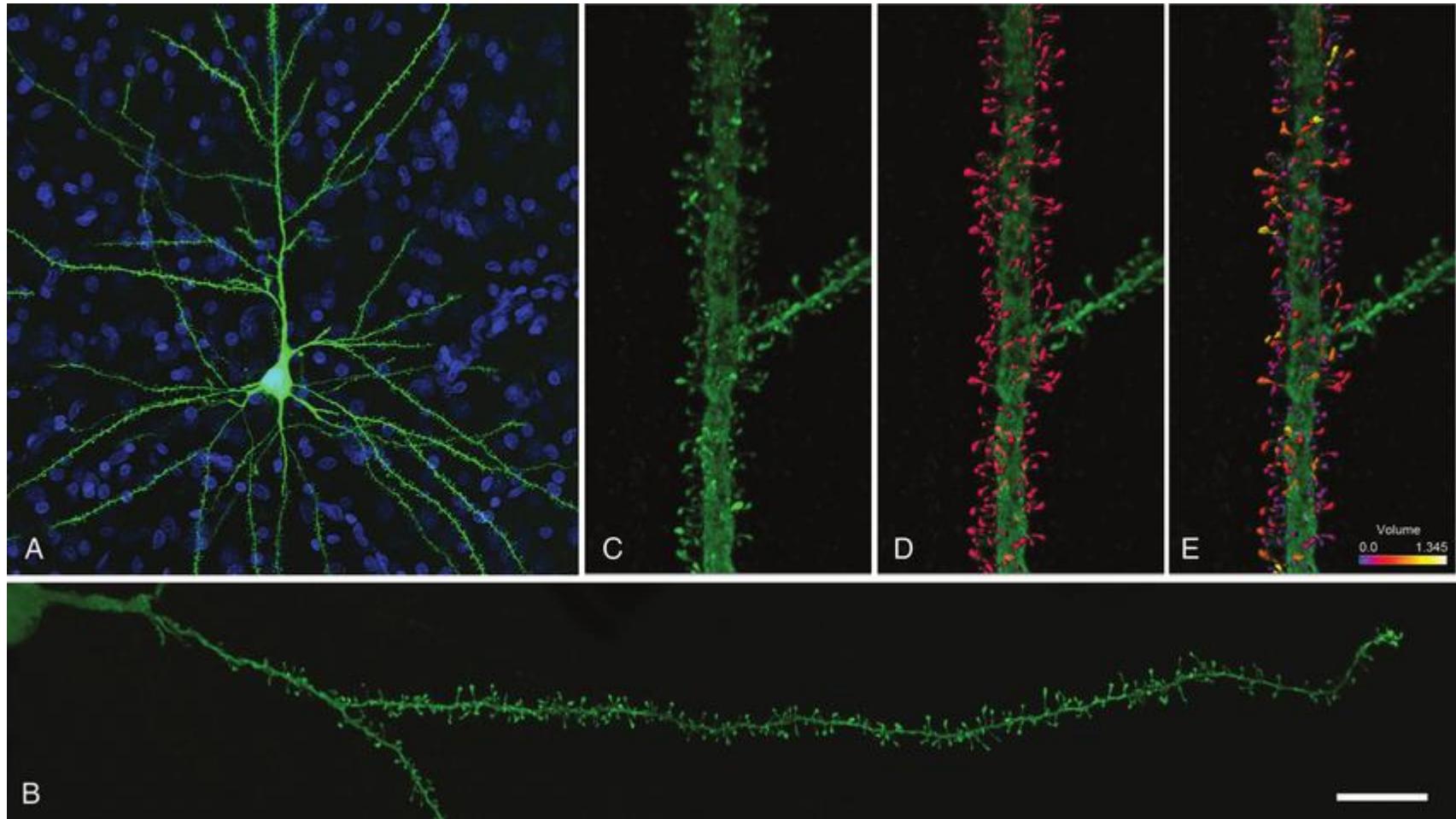
Chapter 9 Opener  
*Molecular Cell Biology, Sixth Edition*  
© 2008 W.H. Freeman and Company

# 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



# Confocal microscopic image of a neuron



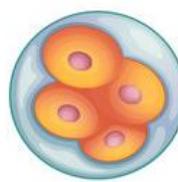
# Development of Multicellular Organisms



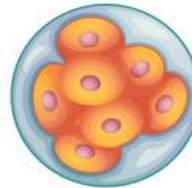
Fertilized egg



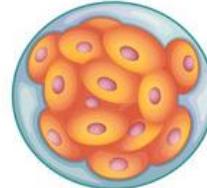
2-cell stage



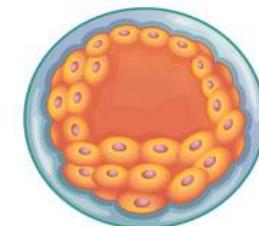
4-cell stage



8-cell stage



16-cell stage



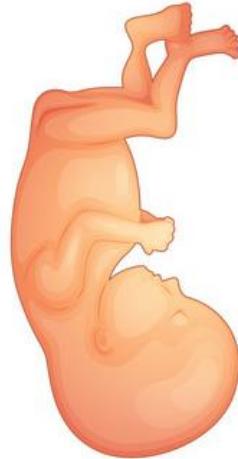
Blastocyst



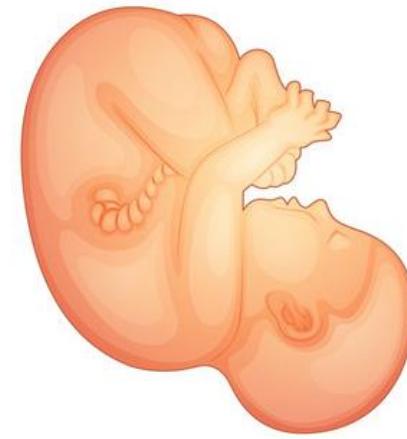
Foetus - 4 weeks



Foetus - 10 weeks

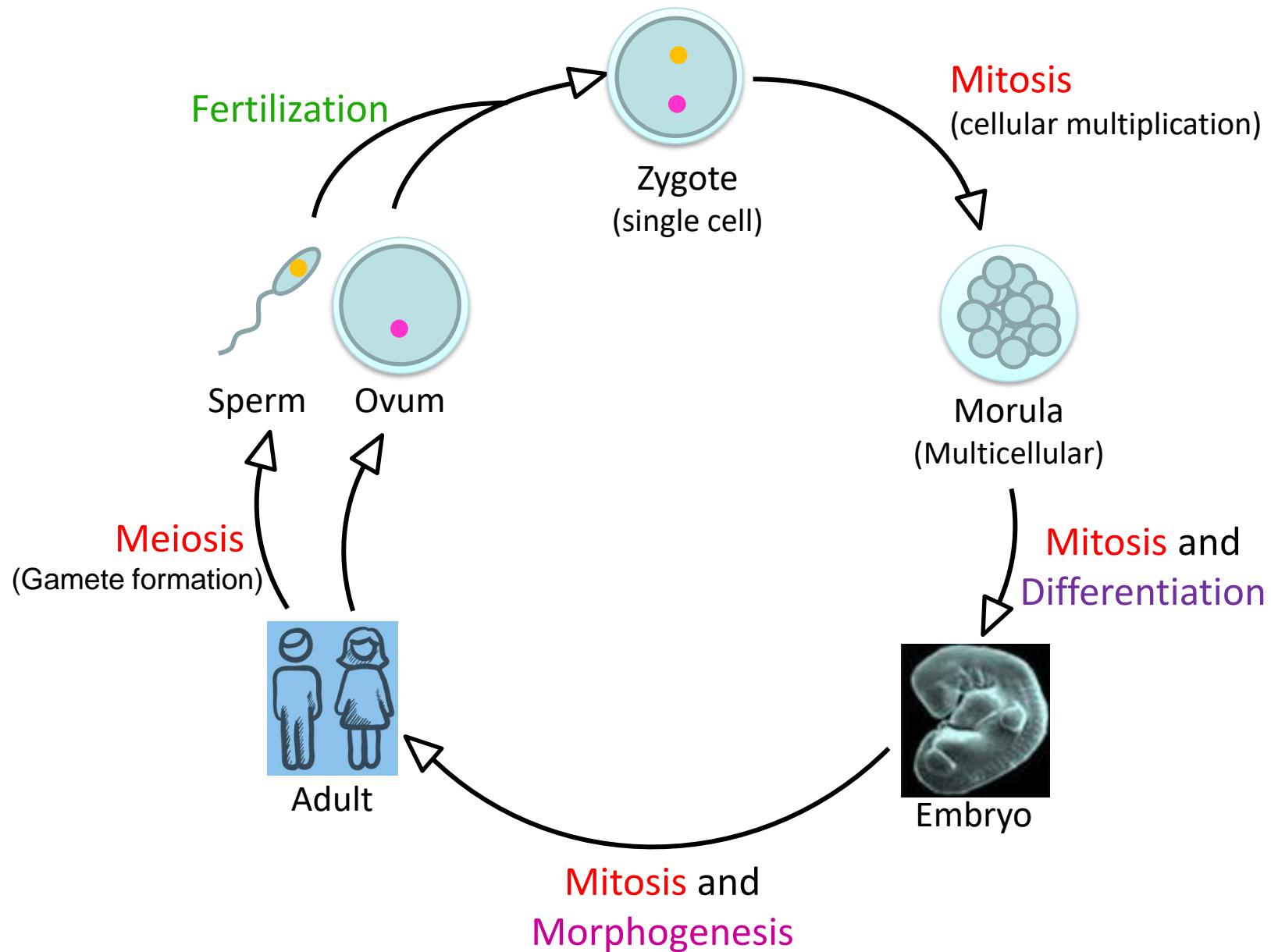


Foetus - 16 weeks



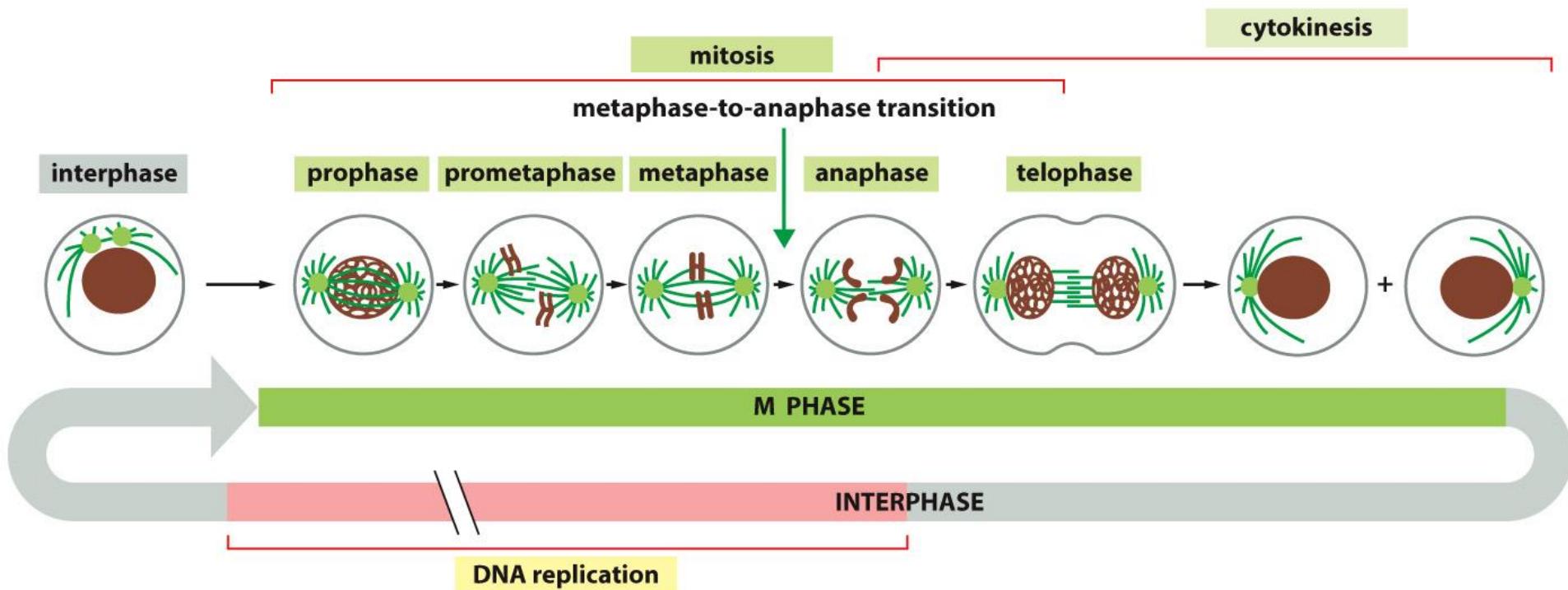
Foetus - 20 weeks

# Cycle of life depends on two modes of cell division



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

## Mitotic cell division

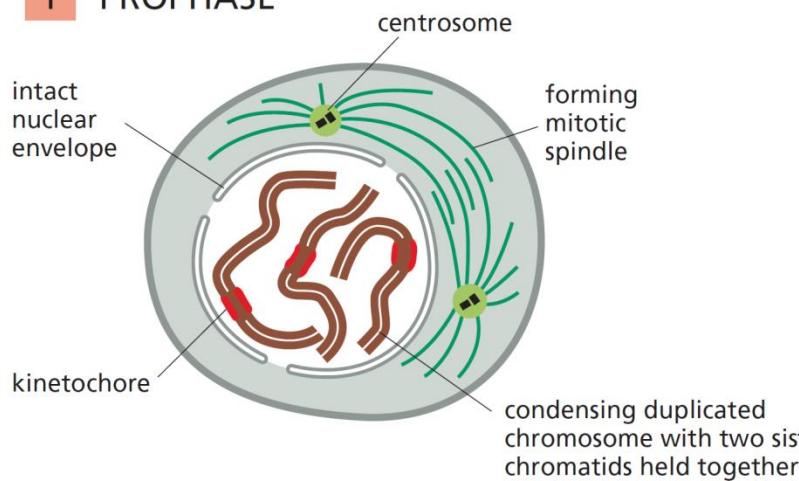


- Division of 1 parent cell produces 2 daughter cells
- Chromosome number remains same after Mitosis ( $2n$  to  $2n$ )- equational division
- Replication doubles the genomic content ( $2c$  to  $4c$ ), Mitosis halves it ( $4c$  to  $2c$ )

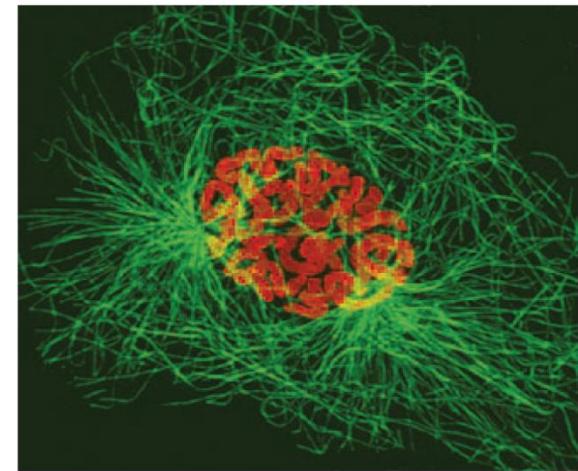
# Stages of Mitosis

## MITOSIS

### 1 PROPHASE



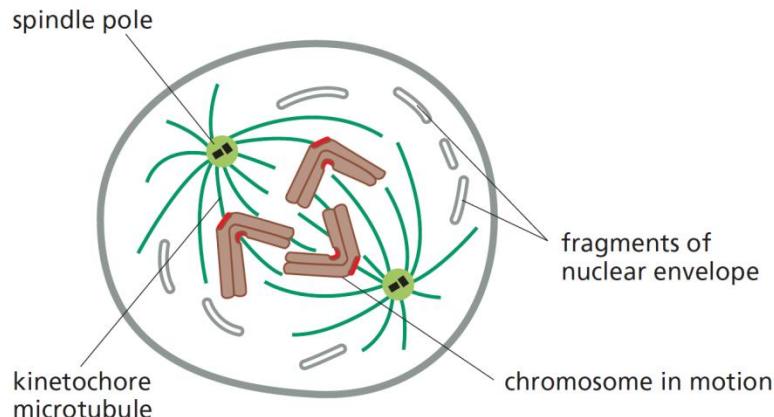
At **prophase**, the duplicated chromosomes, each consisting of two closely associated sister chromatids, condense. Outside the nucleus, the mitotic spindle assembles between the two centrosomes, which have begun to move apart. For simplicity, only three chromosomes are drawn.



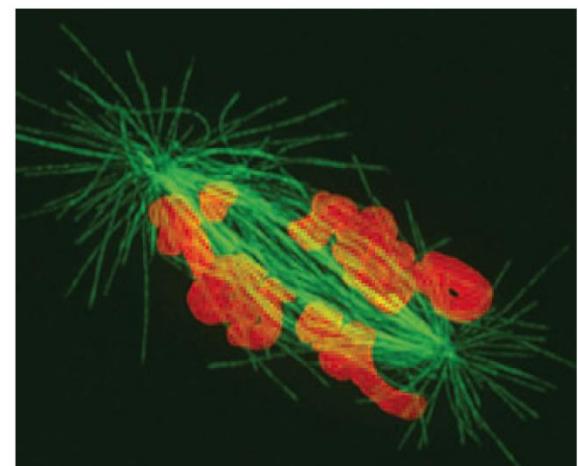
time = 0 min

## MITOSIS

### 2 PROMETAPHASE



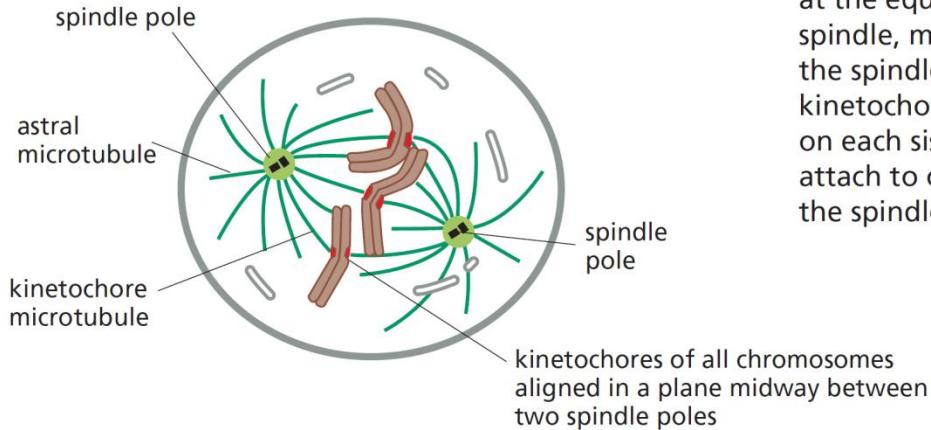
Prometaphase starts abruptly with the breakdown of the nuclear envelope. Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movement.



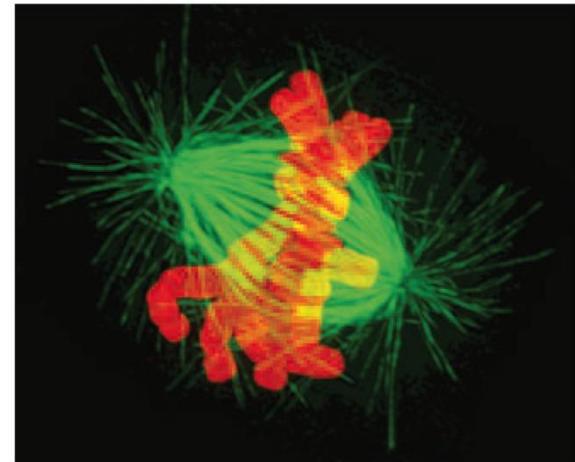
time = 79 min

## MITOSIS

### 3 METAPHASE



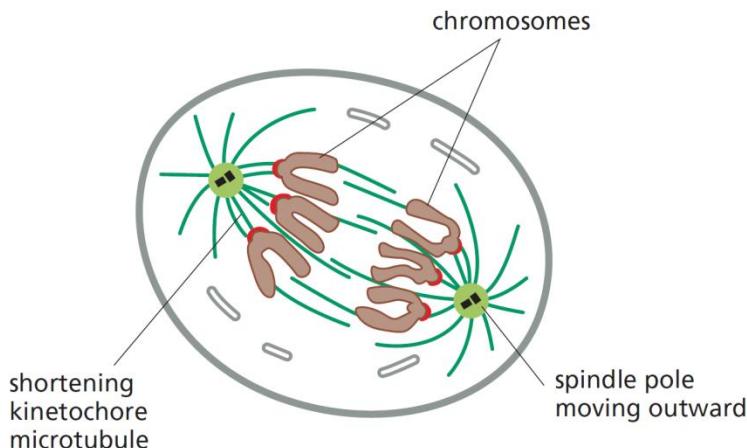
At **metaphase**, the chromosomes are aligned at the equator of the spindle, midway between the spindle poles. The kinetochore microtubules on each sister chromatid attach to opposite poles of the spindle.



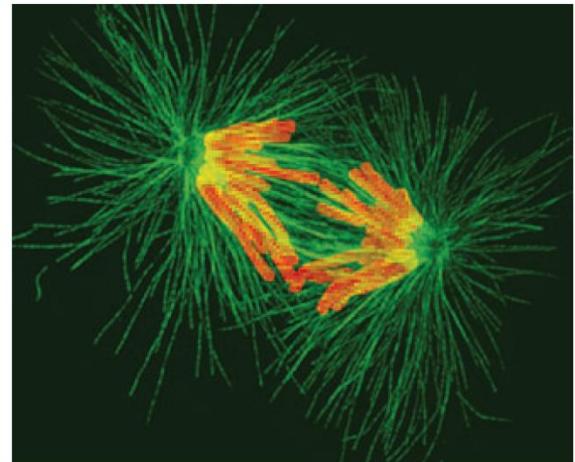
time = 250 min

## MITOSIS

### 4 ANAPHASE



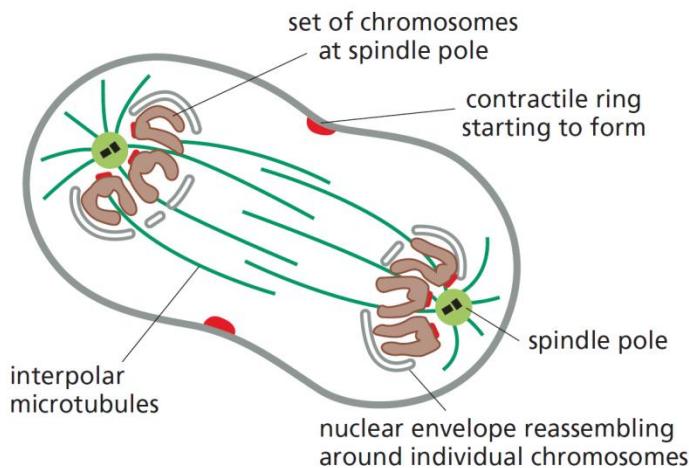
At **anaphase**, the sister chromatids synchronously separate and are pulled slowly toward the spindle pole to which they are attached. The kinetochore microtubules get shorter, and the spindle poles also move apart, both contributing to chromosome segregation.



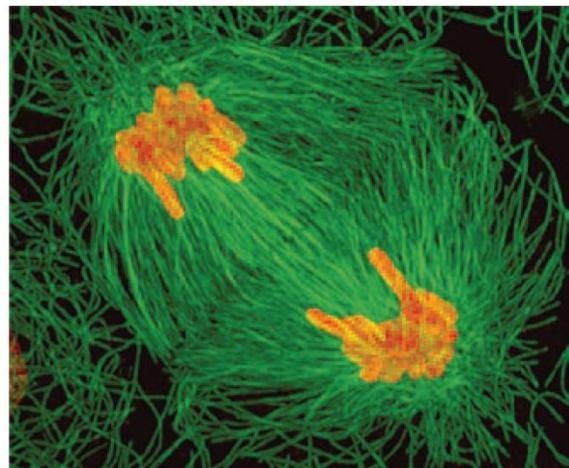
time = 279 min

## MITOSIS

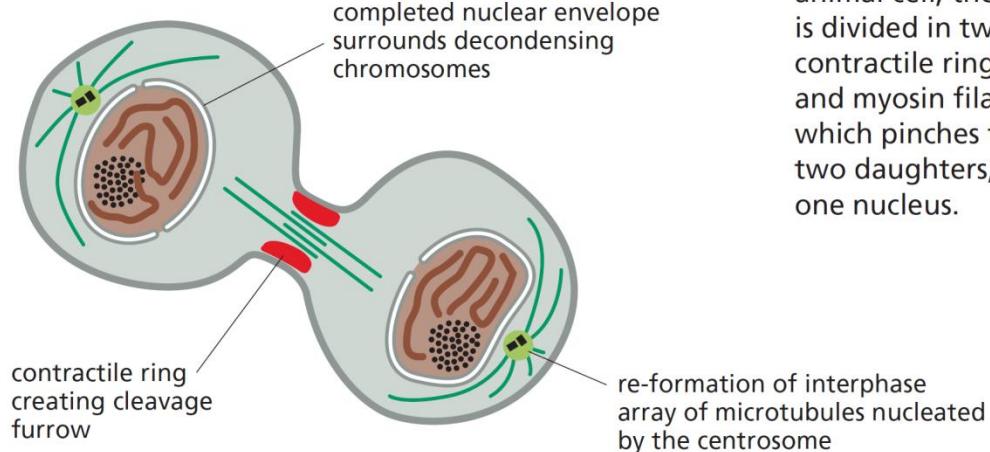
### 5 TELOPHASE



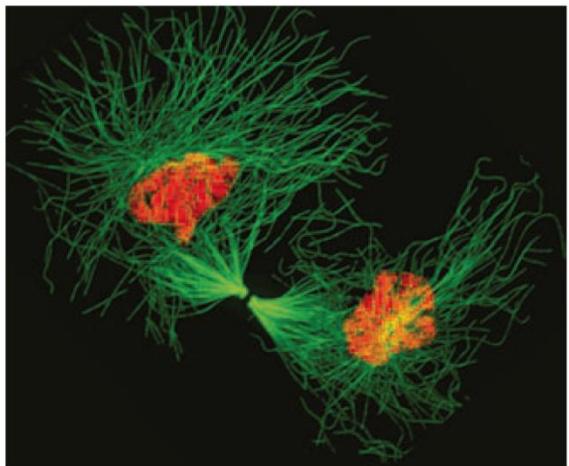
During **telophase**, the two sets of chromosomes arrive at the poles of the spindle. A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis. The division of the cytoplasm begins with the assembly of the contractile ring.



## CYTOKINESIS



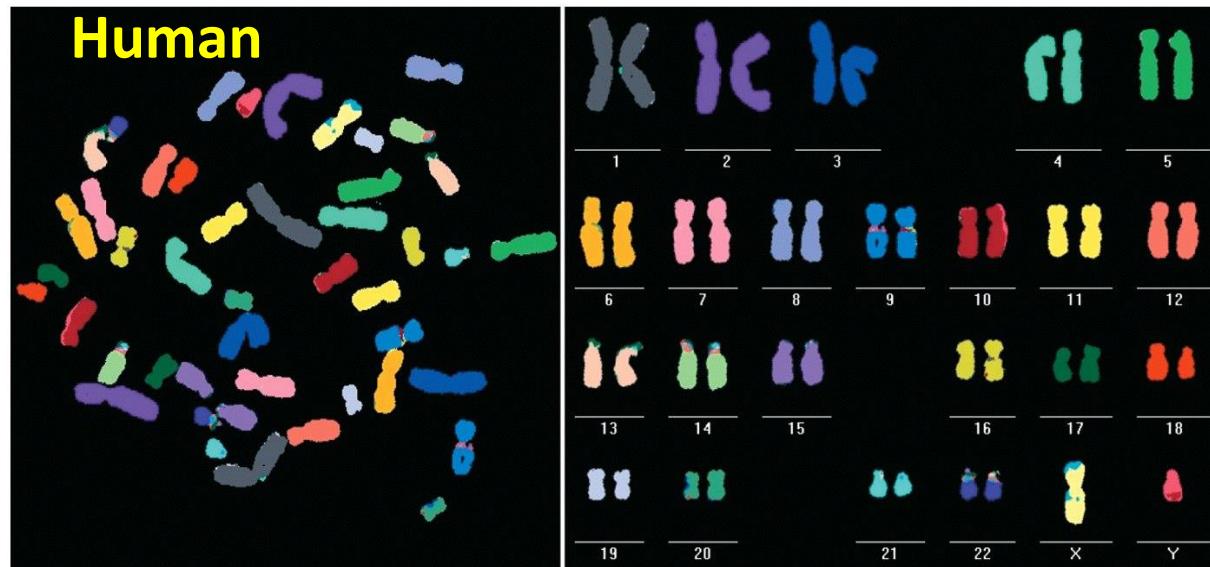
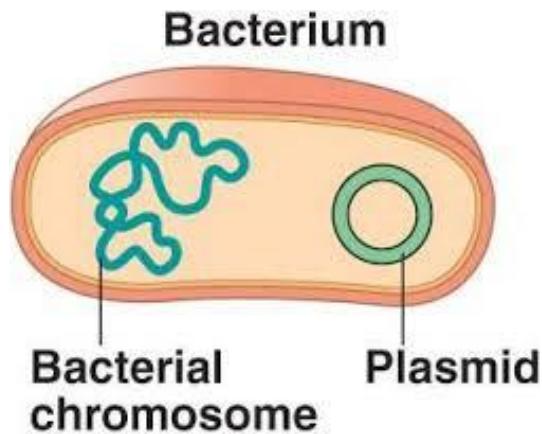
During **cytokinesis** of an animal cell, the cytoplasm is divided in two by a contractile ring of actin and myosin filaments, which pinches the cell into two daughters, each with one nucleus.



# Accurate Distribution of DNA during cell division:

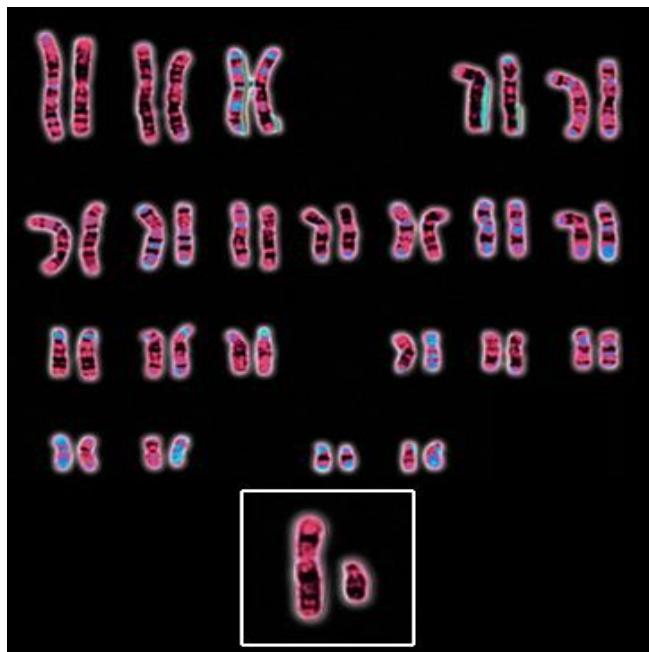
## A challenging task considering the complexity of our genome

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

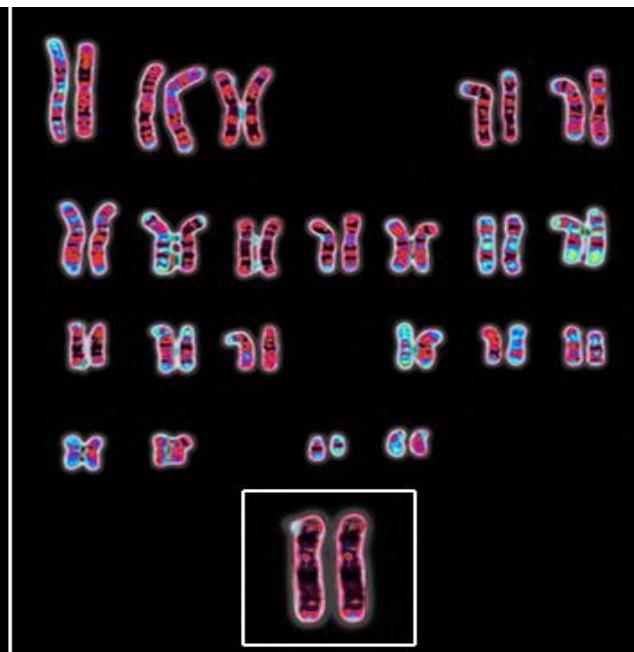


# Diploid genome (2n) in each body cell

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



Male (23,XY)

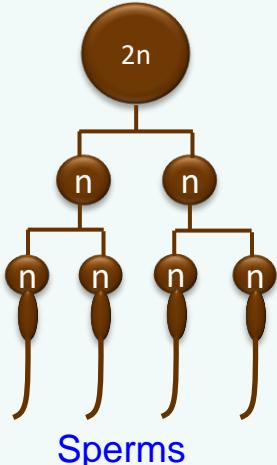


Female (23,XX)

# 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  $c$ )

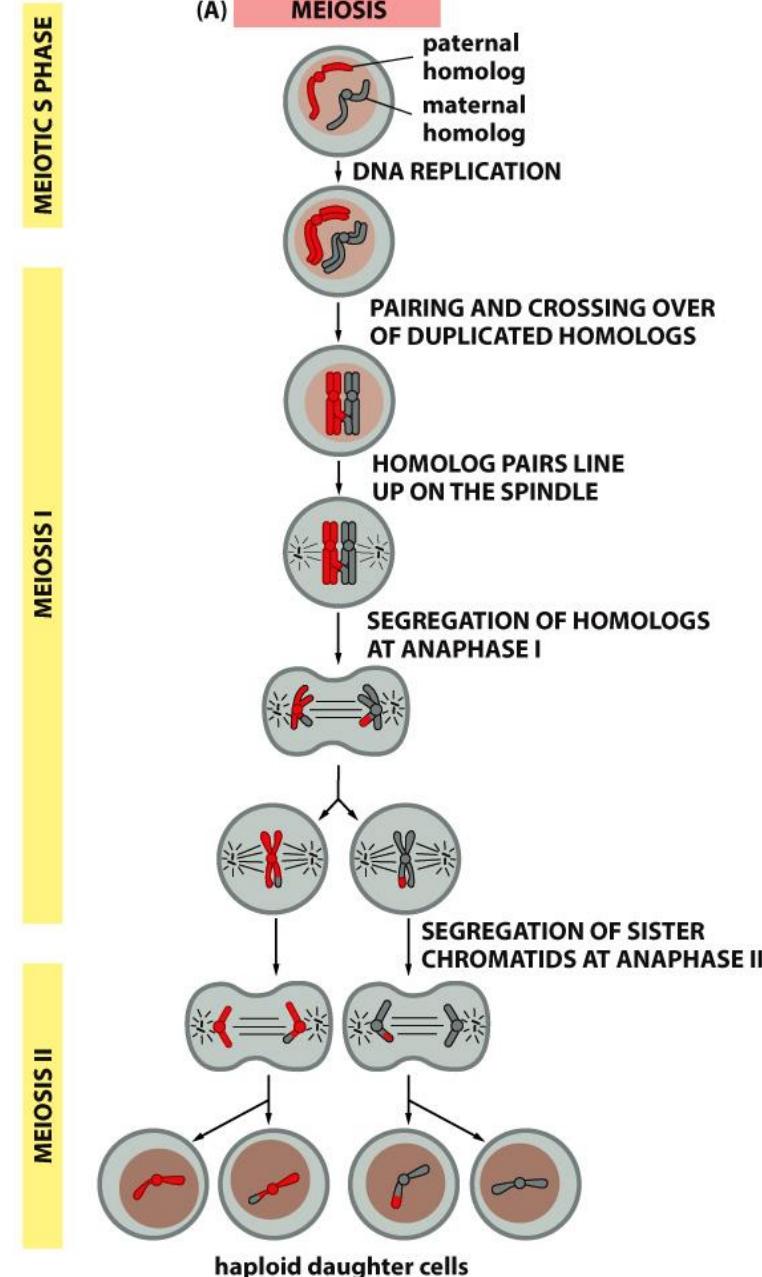
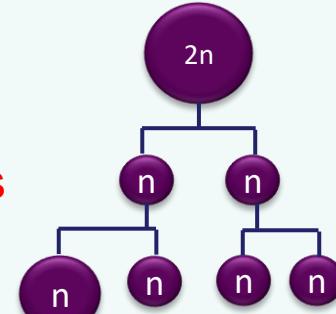
Spermatogenesis



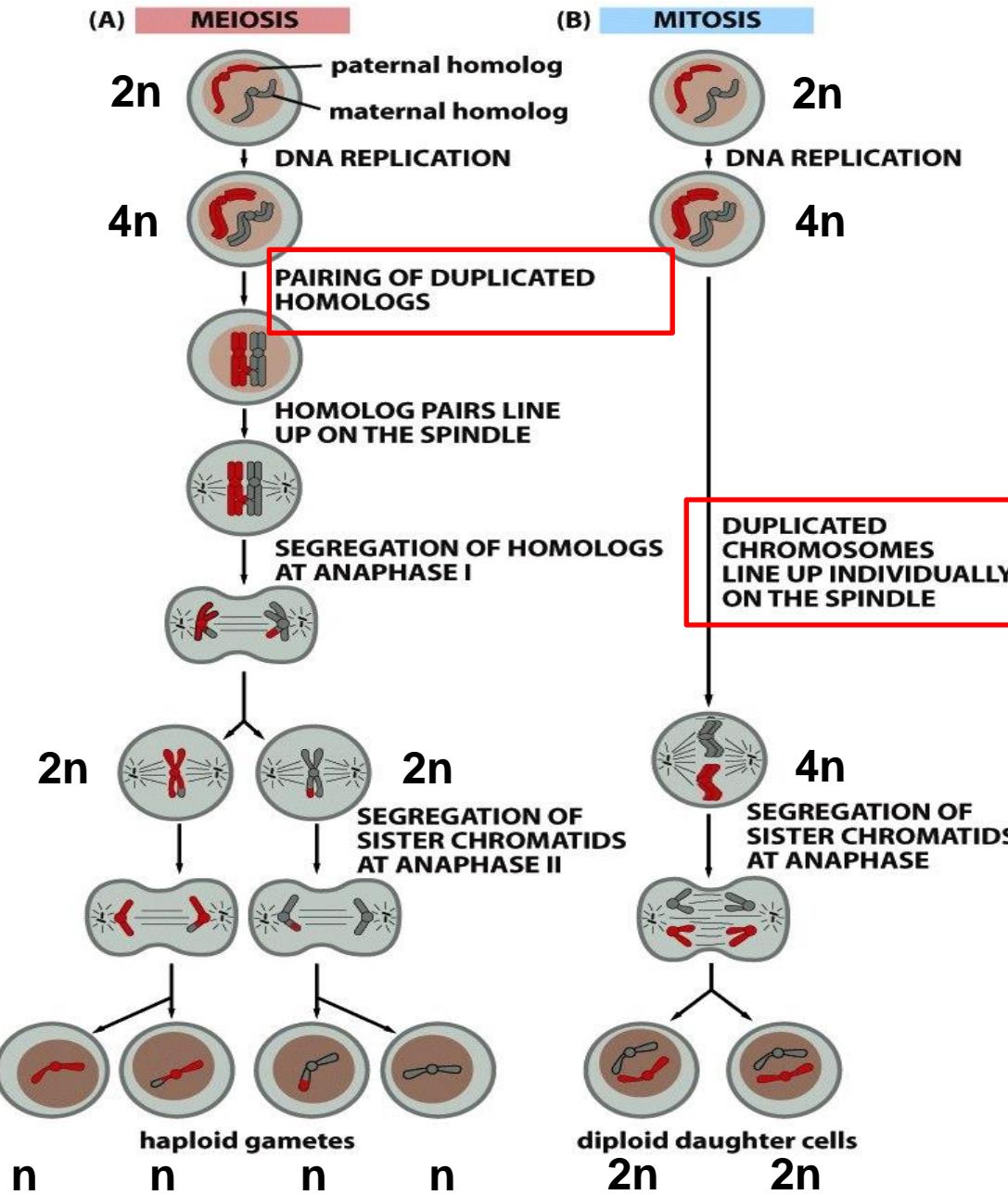
Oogenesis

Meiosis

Ovum



# Unique features of mitosis and meiosis compared



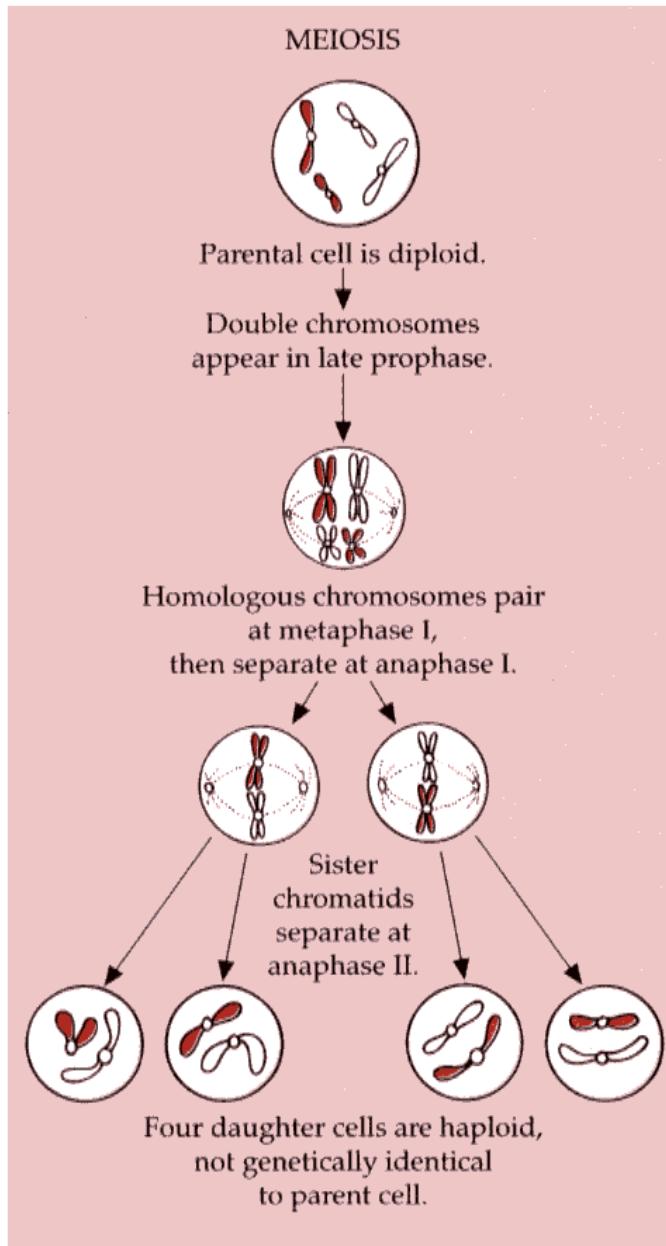
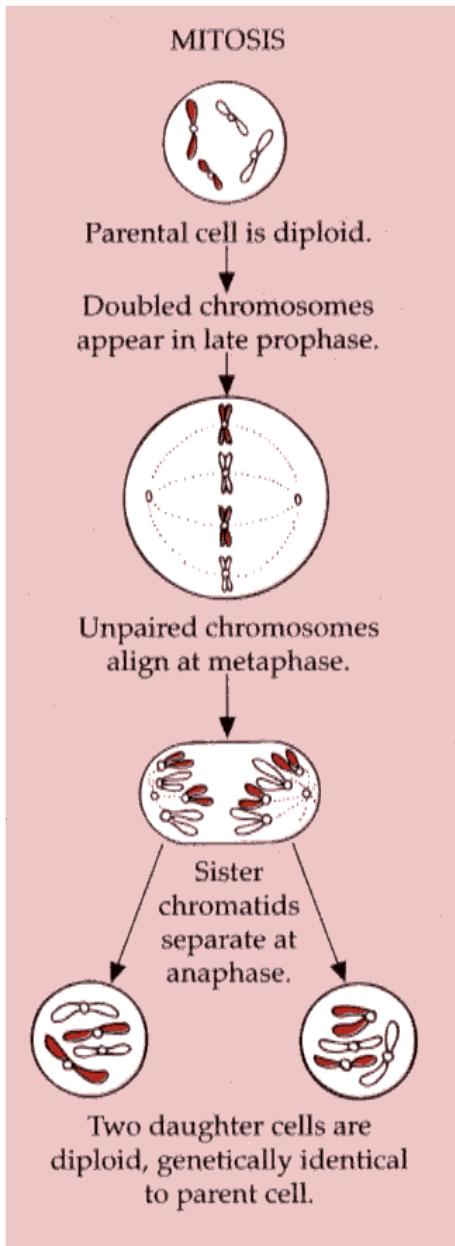
**Meiosis:** single round of chromosome duplication followed by two rounds of chromosome segregation.

1<sup>st</sup> round (Meiosis-I) segregates the homologs that pair up.

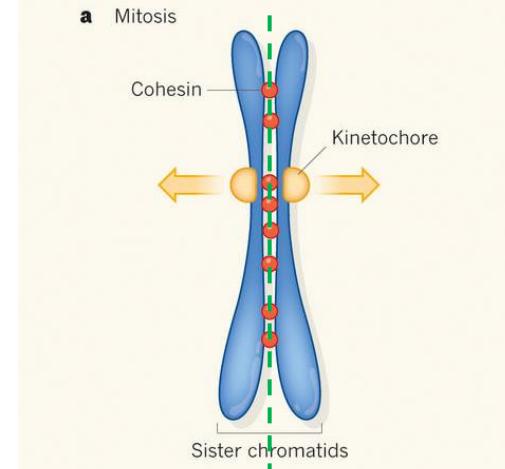
2<sup>nd</sup> round (Meiosis-II) segregates the sister-chromatids

**Mitosis:** homologs do not pair up and segregate but the sister-chromatids segregate

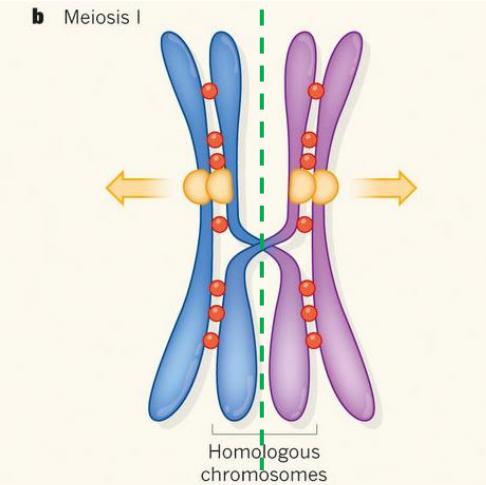
# Chromosome orientation in mitosis and meiosis



## Mitosis: Separation of chromatids



## Meiosis: Separation of homologous chromosomes



# Haploid genome ( $n$ ) in each Sperm/Ovum

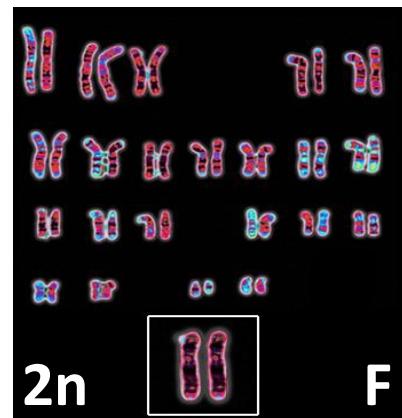
The gametes of most higher eukaryotes are **haploid ( $n$ )** i.e. these cells contain one copy of the basic genome set (one set of chromosomes)

**Somatic Cells (2n)**

$(22 \times 2) + XX$

Mitosis  
↔

**Somatic Cells (2n)**



**Gametes (n)**

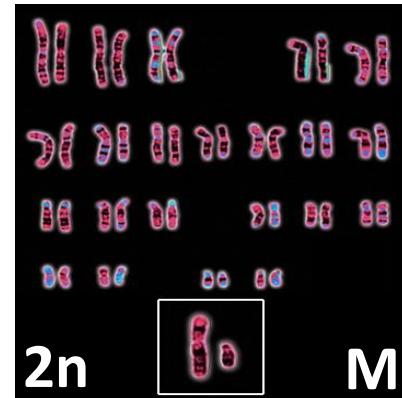
$22 + X$

Egg

Meiosis  
→

$(22 \times 2) + XY$

Mitosis  
↔



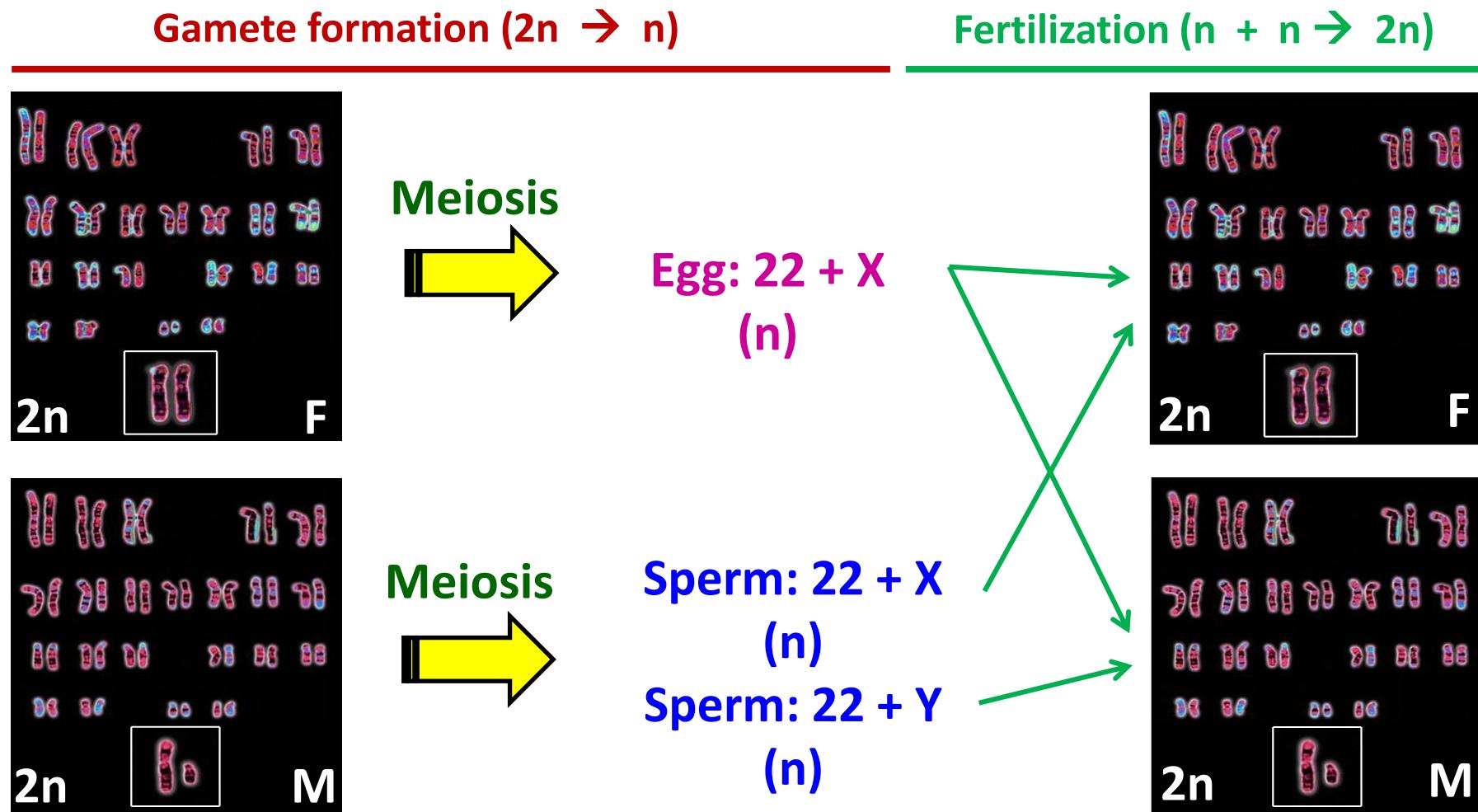
Meiosis  
→

$22 + X$   
or  
 $22 + Y$

Sperm  
Sperm

# Diploid ( $2n$ ) Genome arises during 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).



# 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
				

# Nondisjunction

A failure of separation of homologous chromosome or sister chromatids during meiosis

**Normal individual: 46,XX or 46,XY**

**Some example of Nondisjunction:**

**Down syndrome (trisomy 21: 47,XY+21 or 47,XX+21)**

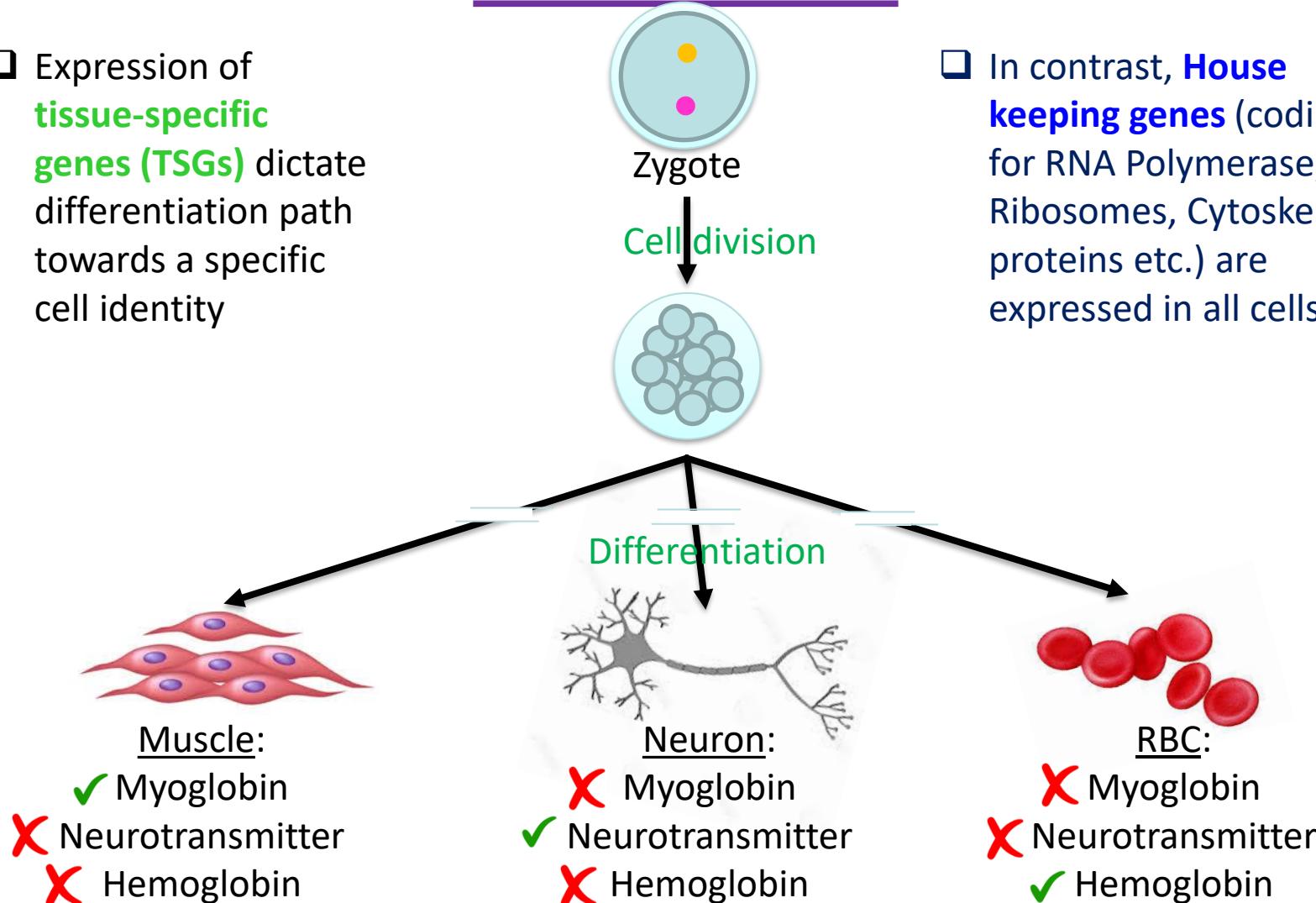
It is the most common irregularity of chromosome number in humans. Children's with Down syndrome have severe mental illness. Advanced maternal age is the risk factor for Down syndrome.

**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

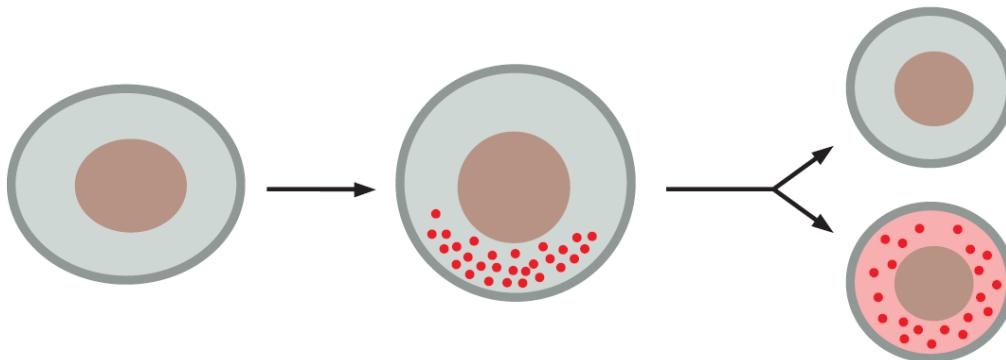
- In contrast, **House keeping genes** (coding for RNA Polymerase, Ribosomes, Cytoskeletal proteins etc.) are expressed in all cells



How do different genes express in different cells?

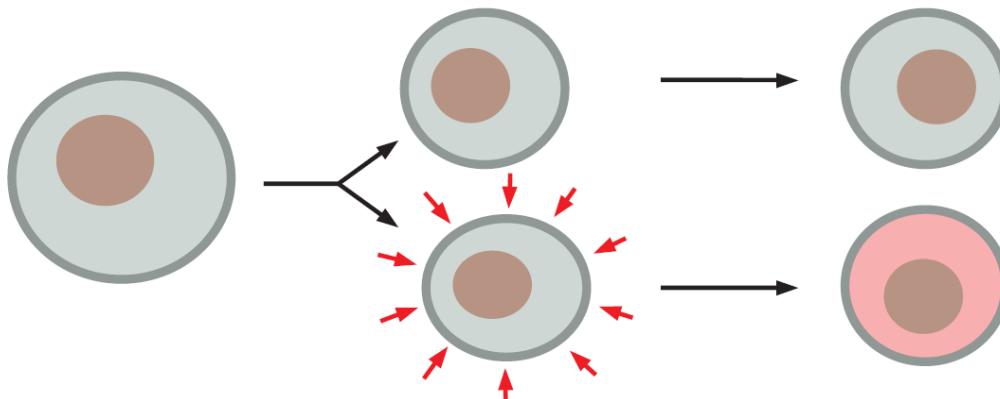
# Differentiation

## 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 two daughter cells

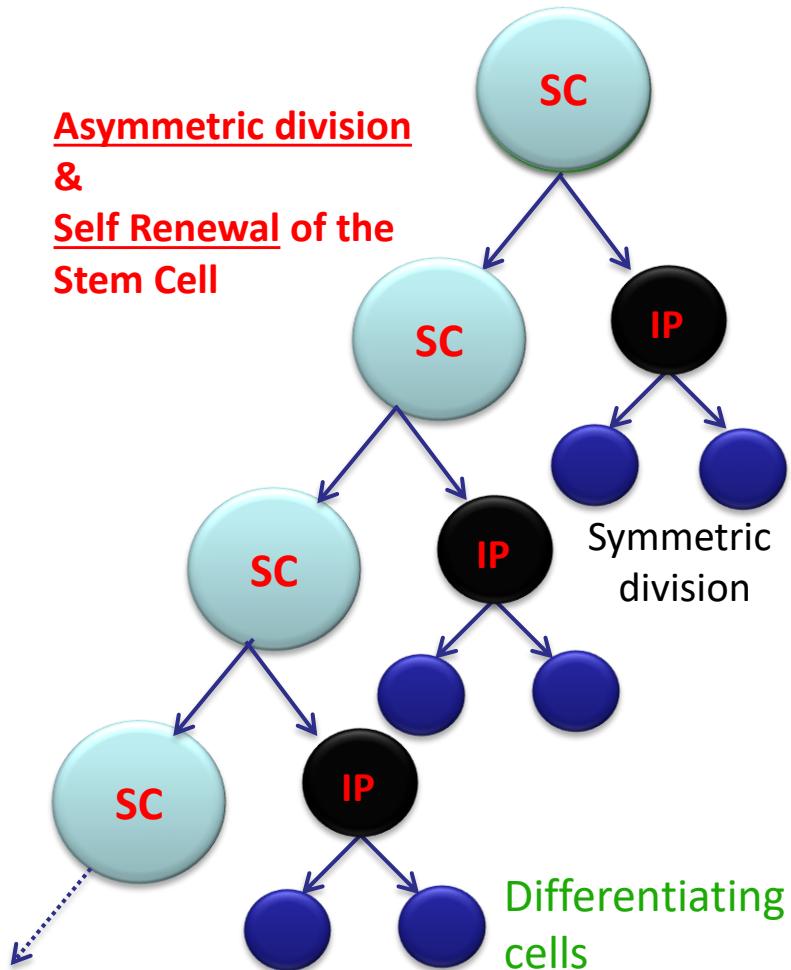


2. symmetric division: sister cells become different as a result of influences acting on them after their birth

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

Asymmetric division  
&  
Self Renewal of the Stem Cell



SC= stem cell

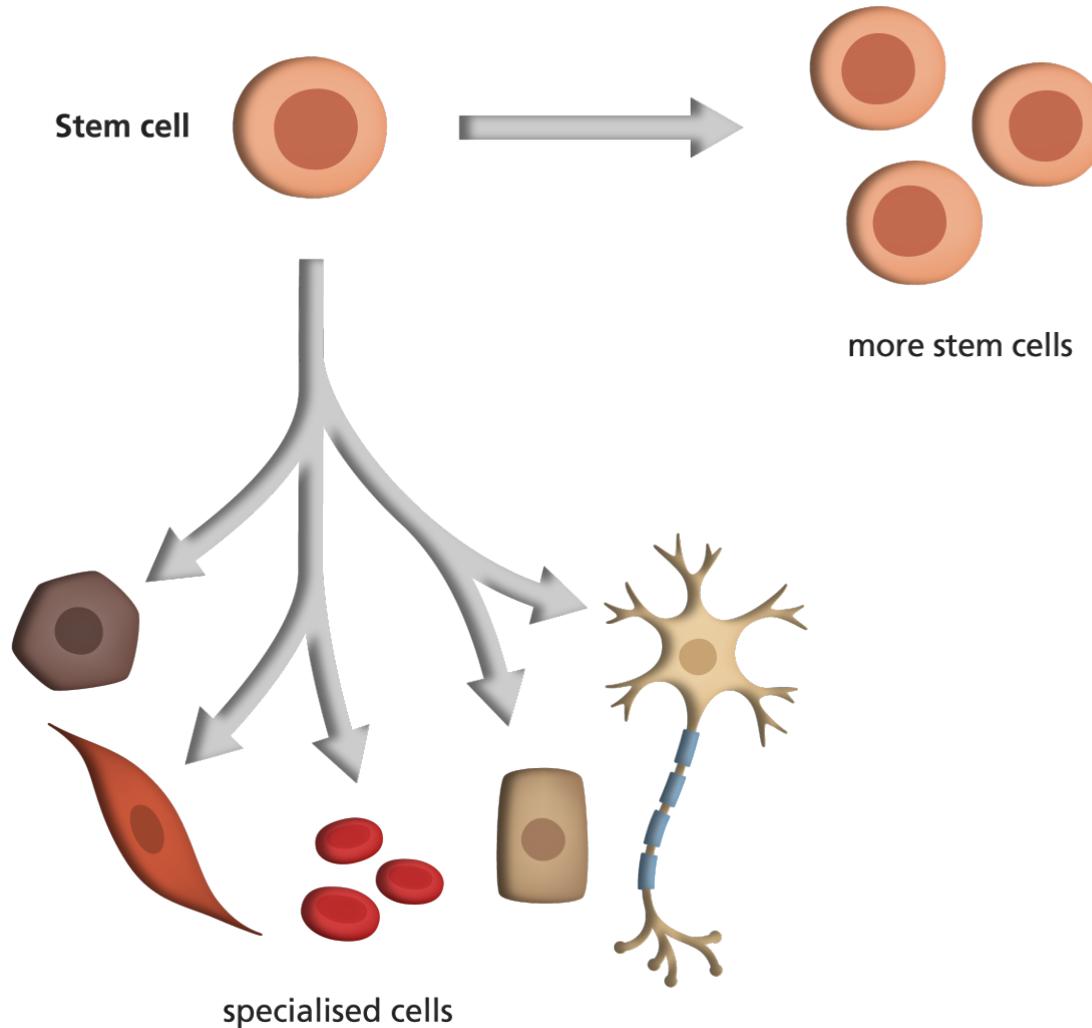
IP= intermediate progenitor

NOTE: GENOMIC CONTENT IS SYMMETRICALLY SEGREGATED  
EVEN IN ASYMMETRIC DIVISION

- 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

# 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

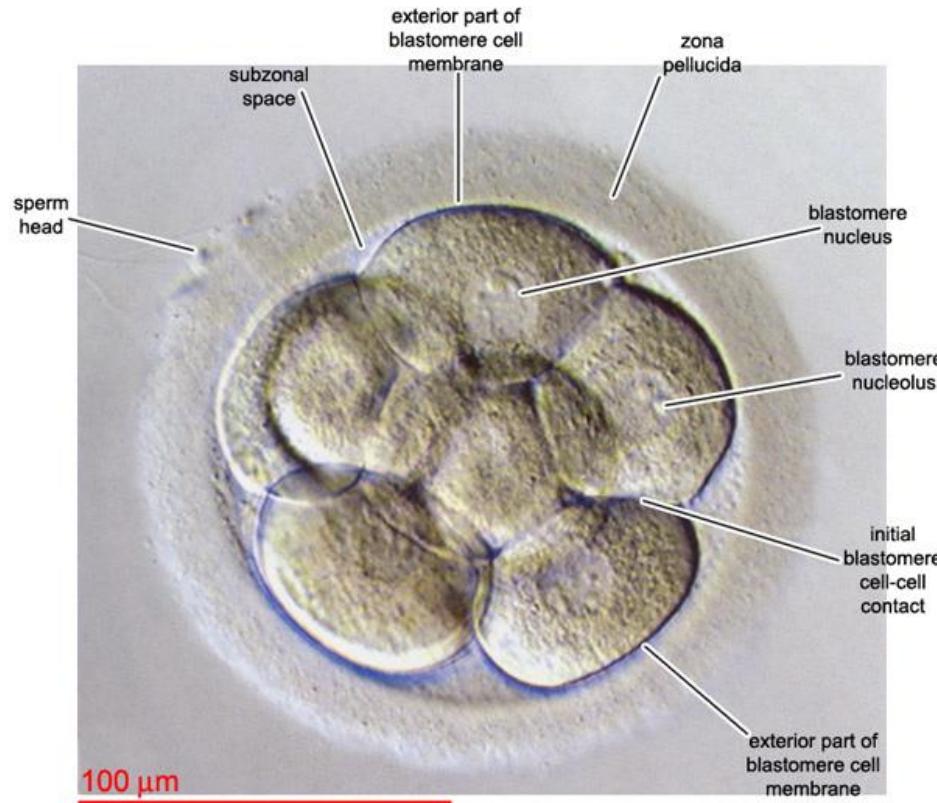
- 1. Totipotency:** Ability to give rise a new organism
- 2. Pluripotency/multipotency:** Ability to give rise to any/many cell types of our body
- 3. Self-renewal:** Ability to reproduce/renew themselves repeatedly
- 4. Asymmetric cell division:** Ability to divide asymmetrically to form one daughter stem cell identical to itself and one daughter cell that is different and usually of more restricted potential.

Asymmetric mitotic division of stem cells preserves a population of undifferentiated cells while steadily producing a stream of differentiating cells.

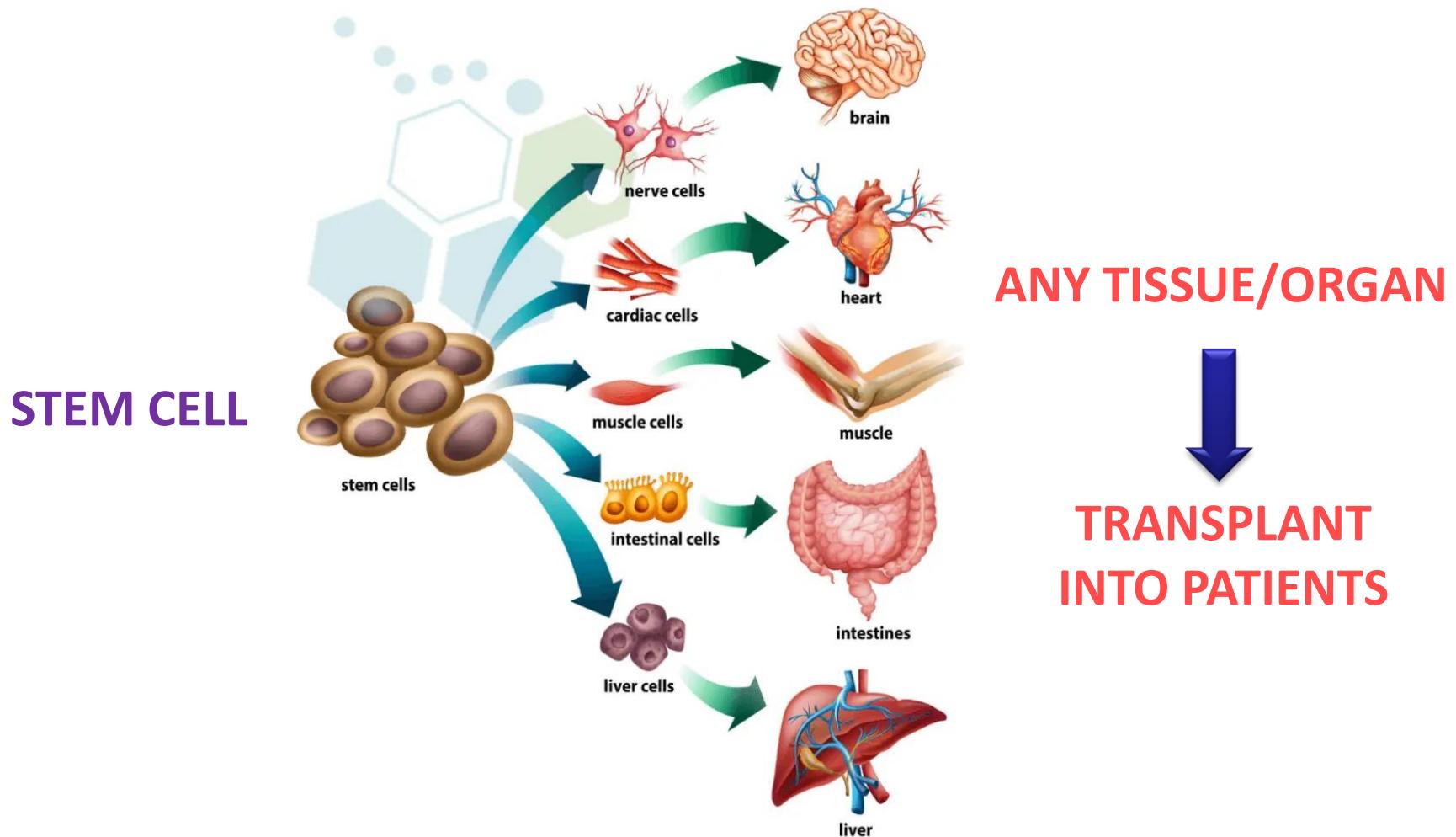
# Totipotent stem cells

Totipotent stem cells have the capacity to self-renew by dividing and give rise to an entire embryo.

A fertilized egg is a totipotent stem cell.



# Potential Therapeutic Application of Stem Cells

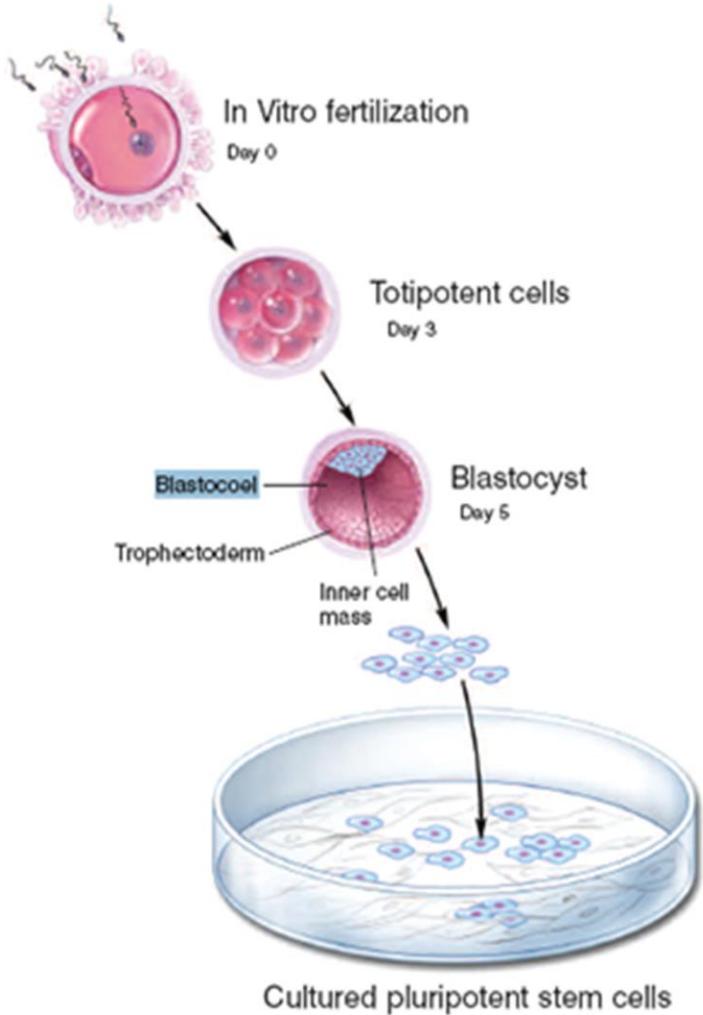


- ❑ For this purpose we need to obtain stem cells in large number.
  - Where do we find stem cells?

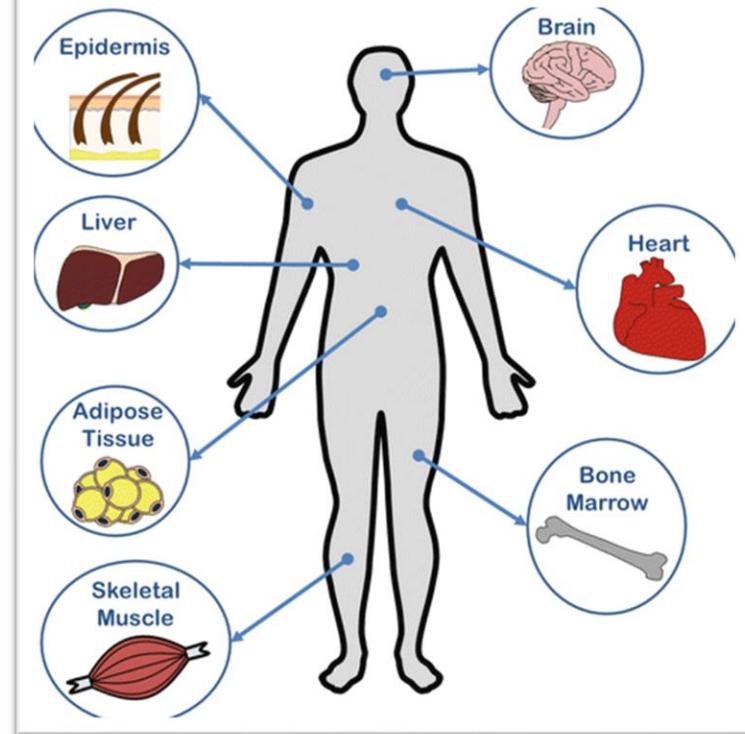
# Sources of Pluripotent Stem Cells

## 1. Embryonic Stem (ES) Cells:

### How Human Embryonic Stem Cells Are Derived



## 2. Adult Stem Cells:



**Problem**  
It's extremely challenging to selectively isolate stem cells from other cells

**Solution**

Induced Pluripotent Stem Cells (iPSCs)

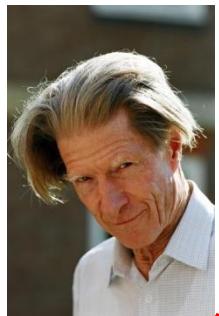
## Induced pluripotency:

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

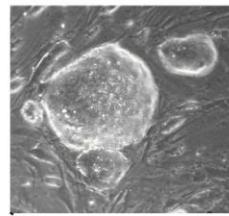
Sir John Gurdon



SCNT developed in frogs

2012

Study of teratocarcinoma cell lines  
Cloned frogs from differentiated cells

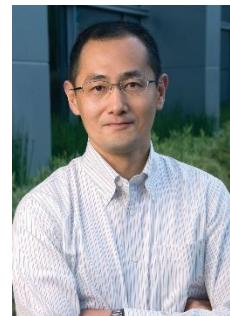


MyoD reprograms fibroblasts to muscle  
Mouse ES cells isolated



Dolly the sheep

Shinya Yamanaka



2012

Human ES cells isolated

First iPSCs generated

2006

2010

1950

1960

1970

1980

1990

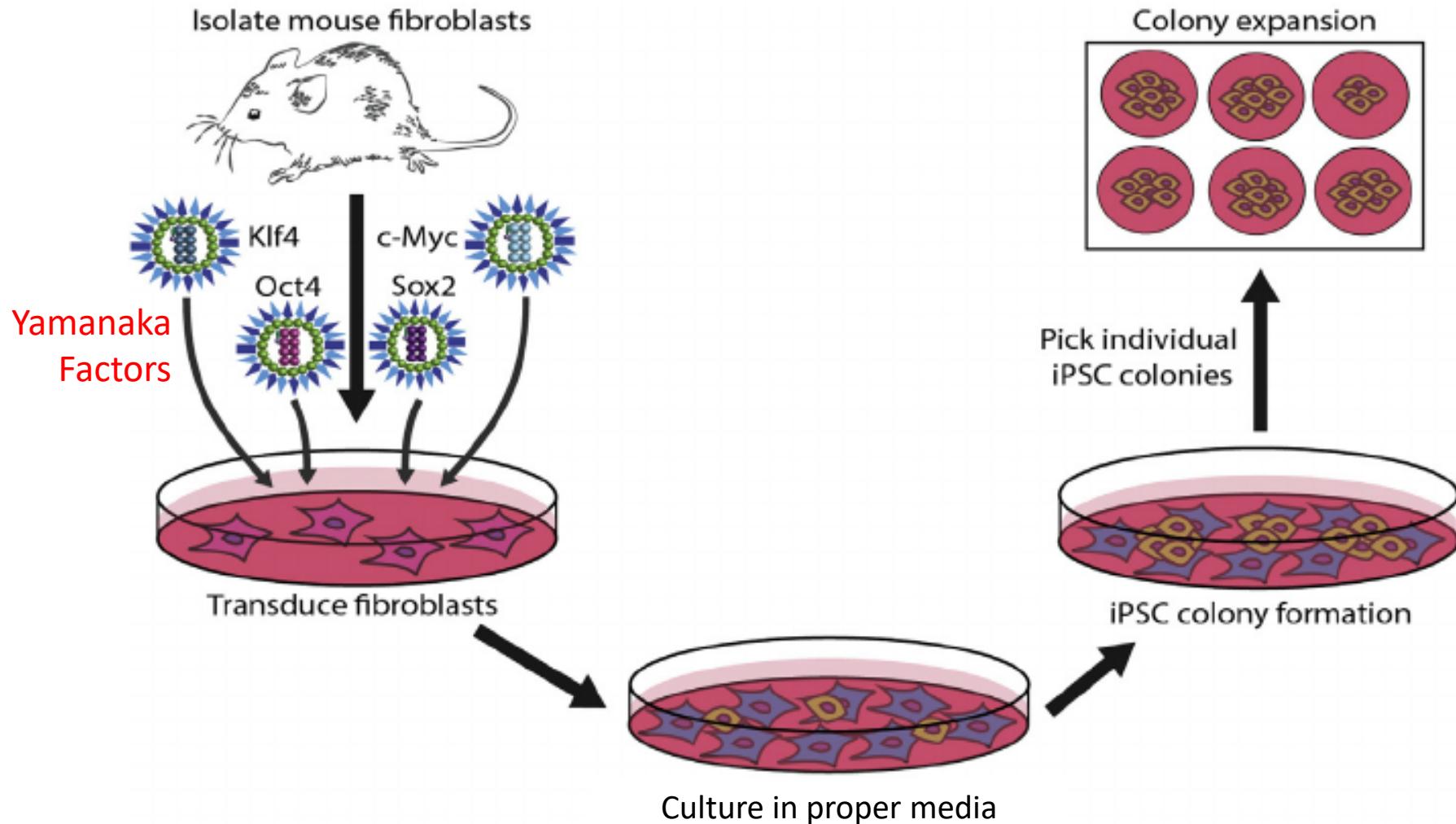
2000

2010



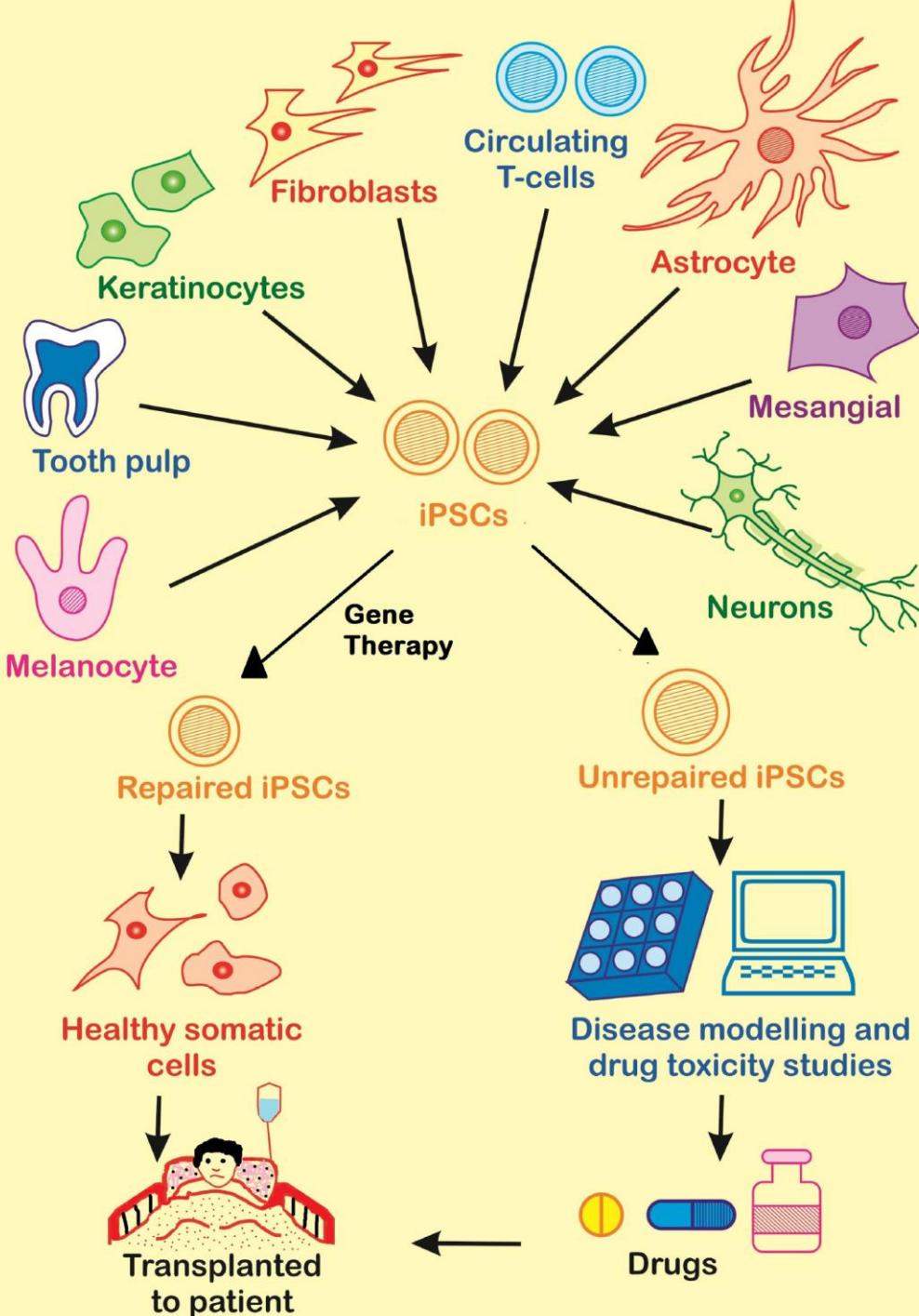
# Derivation of first iPSC:

Shinya Yamanaka and Kazutoshi Takahashi (2005)

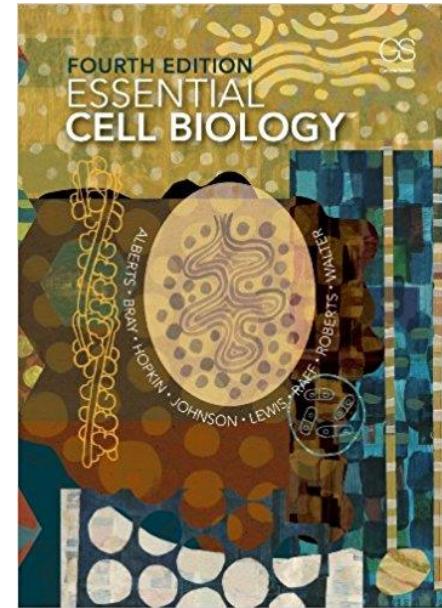
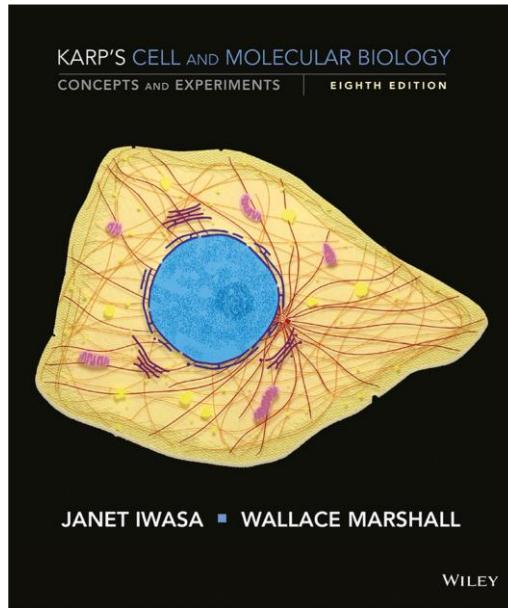
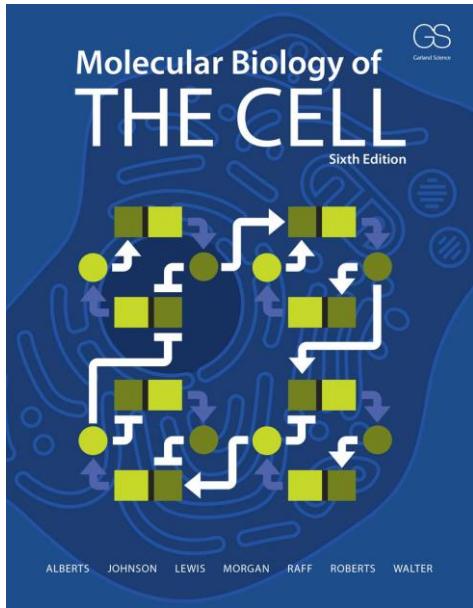


# Biomedical applications of iPSCs

- **Regenerative medicine:** To restore or replace damaged tissue
  - For example, neuronal cells 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



## Video links:

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

[https://www.youtube.com/watch?v=B\\_zD3NxSsD8](https://www.youtube.com/watch?v=B_zD3NxSsD8)